

Universidade Federal de São Carlos
Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

**Sistemática e biogeografia de *Labrundinia* Fittkau, 1962 (Diptera:
Chironomidae: Tanypodinae): uma abordagem morfológica e
molecular**

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Chironomidae: Tanypodinae): uma abordagem morfológica e
molecular**

Fabio Laurindo da Silva

Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos, como parte dos requisitos para obtenção do título de Doutor em Ciências, área de concentração em Ecologia e Recursos Naturais.

São Carlos - SP

2012

**Ficha catalográfica elaborada pelo DePT da
Biblioteca Comunitária/UFSCar**

S586sb Silva, Fabio Laurindo da.
Sistemática e biogeografia de *Labrundinia* Fittkau, 1962
(Diptera: Chironomidae : Tanypodinae): uma abordagem
morfológica e molecular / Fabio Laurindo da Silva. -- São
Carlos : UFSCar, 2013.
283 f.

Tese (Doutorado) -- Universidade Federal de São Carlos,
2012.

1. Insetos aquáticos. 2. Larva. 3. Pupa. 4. Adulto. 5.
Taxonomia. 6. Região neotropical. I. Título.

CDD: 574.5263 (20^a)



UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

Relatório de Defesa de Tese Candidato: Fabio Laurindo da Silva

Aos 19/12/2012, às 14:00, realizou-se na Universidade Federal de São Carlos, nas formas e termos do Regimento Interno do Programa de Pós-Graduação em Ecologia e Recursos Naturais, a defesa de tese de doutorado sob o título: Sistemática e Biogeografia de *Labrundinia Fittkau, 1962* (Diptera: Chironomidae: Tanypodinae): uma abordagem morfológica e molecular, apresentada pelo candidato Fabio Laurindo da Silva. Ao final dos trabalhos, a banca examinadora reuniu-se em sessão reservada para o julgamento, tendo os membros chegado ao seguinte resultado:

Participantes da Banca

Profa. Dra. Alaide Aparecida Fonseca Gessner
Profa. Dra. Angélica Maria Penteadó Martins Dias
Profa. Dra. Susana Trivinho Strixino
Profa. Dra. Sonia Silveira Ruiz
Profa. Dra. Sofia Wiedenbrug

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Resultado Final: Aprovado

Parecer da Comissão Julgadora*:

Aprovado com distinção e louvor

Encerrada a sessão reservada, o presidente informou ao público presente o resultado. Nada mais havendo a tratar, a sessão foi encerrada e, para constar, eu, João Augusto da Silva Affonso, representante do Programa de Pós-Graduação em Ecologia e Recursos Naturais, lavrei o presente relatório, assinado por mim e pelos membros da banca examinadora.

Profa. Dra. Alaide Aparecida Fonseca Gessner

Profa. Dra. Angélica Maria Penteadó Martins Dias

Profa. Dra. Susana Trivinho Strixino

Profa. Dra. Sonia Silveira Ruiz

Profa. Dra. Sofia Wiedenbrug

Representante do PPG: João Augusto da Silva Affonso

Não houve alteração no título da tese Houve. O novo título passa a ser:

*Obs: Se o candidato for reprovado por algum dos membros, o preenchimento do parecer é obrigatório.

Para gozar dos direitos do título de Doutor em Ecologia e Recursos Naturais, o candidato ainda precisa ter sua tese homologada pelo Conselho de Pós-Graduação da UFSCar.

*“When you have eliminated all which is impossible, then whatever remains,
however improbable, must be the truth”*

Doyle, Arthur Conan

“It is good to have an end to journey toward, but it is the journey that matters, in the end”

Hemingway, Ernest

*Este trabalho é dedicado a elas, as pequenas
Labrundínias que, apesar de sua vida efêmera e
discreta, instruíram-me e levaram a terras
longínquas. Vós não morrestes em vão!*

AGRADECIMENTOS

Aos meus orientadores, Alaide A. Fonseca Gessner e Torbjørn Ekrem, os ensinamentos e o incentivo durante todo meu doutorado.

Às professoras Sonia Silveira Ruiz e Susana Trivinho Strixino a amizade e as oportunidades.

Às amigas e parceiras de trabalho Sofia Wiedenbrug e Caroline Silva Neubern de Oliveira.

À Elisabeth Stur a calorosa recepção na Noruega.

Aos colegas de laboratório Sondre Dahle, Alyssa Anderson, Ingeborg Engh e Magni Olsen Kyrkjeide.

Aos amigos Alexey, Ana Paula, Andrea, Amanda, Ariano, Carolina, Clóvis, Eduardo, Ellen, Fabio Roque, Gisele, Ivy, Ira, Janda, Juliano, Kogilam, Lurdinha, Paula (a Filhona), Patrícia, Priscila, Magda, Manuel, Marion, Mateus, Nathan (for everything), Neusa, Roberta, Virginia e Wânia.

Aos pesquisadores Augusto Siri, Broughton Caldwell, Charles Watson e John Epler o empréstimo de material.

Aos curadores e aos responsáveis pelas Coleções de Chironomidae das instituições: Academy of Natural Sciences of Philadelphia - Jon Gelhaus, Edward B. Daeschler, Jason Weintraub e Greg Cowper; Florida State Collection of Arthropods - Jan Peters, Manuel Pescador e Kevin Lewis; Zoologische Staatssammlung München - Martin Spies e Marion Kotrba; Institut Royal des Sciences Naturelles de Belgique - Jérôme Constant; e Museum of Comparative Zoology (Harvard University) - Philip Perkins.

Aos órgãos/instituições Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Deutscher Akademischer Austauschdienst (DAAD), Programa de Pós-Graduação em Ecologia e Recursos Naturais da Universidade Federal de São Carlos (PPGERN-UFSCAR), The Academy of Natural Sciences of Philadelphia (ANSP), Center for Systematic Entomology (CSE), Museum of Comparative Zoology (MCZ - Harvard University), Research Council of Norway (RCN) e Research School in Biosystematics (ForBio) a logística e o apoio financeiro.

RESUMO

Esta pesquisa de doutorado enfocou a sistemática e a biogeografia das espécies de *Labrundinia* (Diptera: Chironomidae: Tanyptodinae), sendo que a base deste estudo incidiu no exame do material-tipo de todas as espécies descritas para o gênero, incluindo espécies depositadas em coleções nacionais e estrangeiras. Adicionalmente, trabalho de campo foi realizado com objetivo de coletar novas espécies e garantir associações seguras entre larvas, pupas e adultos. Neste estudo, foram redescritas 14 espécies conhecidas de *Labrundinia* e 25 espécies novas para a ciência foram descritas para região Neotropical. Dados morfológicos e moleculares foram utilizados para inferir relações filogenéticas entre espécies do gênero e chaves de identificação foram preparadas para os adultos machos, pupas e larvas. Com base nos resultados da filogenia e distribuição das espécies, análises biogeográficas foram conduzidas na tentativa de determinar a mais provável história biogeográfica para o grupo. As interpretações das análises filogenéticas indicam o gênero *Labrundinia* como um grupo monofilético, com presença em todas as árvores resultantes. Com base nestas reconstruções filogenéticas, análises biogeográficas foram processadas e indicam que o ancestral de *Labrundinia* teve diversificação inicial na Região Neotropical.

Palavras-Chave: insetos aquáticos, larva, pupa, adulto, ecologia, taxonomia, Neotropical, Brasil.

ABSTRACT

This doctoral research focused on the systematics and biogeography of the *Labrundinia* species (Insecta: Diptera: Chironomidae: Tanypodinae). The basis of this study rested on the examination of the type material of all previously described *Labrundinia* species, including species deposited in national and foreign museums. Field work were carried out to collect more specimens and to secure associations between larvae, pupae and adults. In this study 14 known species of *Labrundinia* were redescribed and 25 new species were described from the Neotropical region. Morphological and molecular analyses were conducted to infer phylogenetic relationships among all species of the genus and identification keys were developed for all life stages. Based on the results of the phylogeny and distribution of the species, a biogeographic analysis was performed to provide the most probable biogeographic history for the group. The interpretations of the phylogenetic results indicate *Labrundinia* as a monophyletic group. The group is shown in all resulting trees. Based on these phylogenetic reconstructions, biogeographical analyses were run and indicated *Labrundinia* ancestor as having its initial diversification in the Neotropical region.

Key-words: aquatic insects, larva, pupa, adult, ecology, taxonomy, Neotropical, Brazil.

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INTRODUÇÃO

INTRODUÇÃO GERAL

A família Chironomidae possui distribuição cosmopolita e seus representantes frequentemente dominam as comunidades de insetos aquáticos, tanto em abundância quanto em riqueza de espécies. Suas espécies ocorrem em todos os continentes, incluindo Antártica e Ilhas Oceânicas. Apesar de algumas espécies terem hábitos terrestres, semiterrestres ou semiaquáticos e marinhos, a grande maioria é dulcícola (Oliver 1971, Armitage *et. al* 1995, Ferrington 2008).

Nas últimas décadas, esse grupo vem sendo amplamente estudado (Sæther 1990, 2000a-b, Adam e Sæther 1999, Boothroyd e Cranston 1999, Ekrem 2003, Stur e Ekrem 2006, Silva *et al.* 2010, 2012, Oliveira e Silva 2011), sendo que o crescente interesse, em parte, deve-se à importância dos Chironomidae nos ambientes aquáticos (Pinder 1986), na agricultura, particularmente orizicultura (Ferrarese 1993), nos monitoramentos e avaliações ambientais (Rosenberg e Resh 1992, Armitage *et al.* 1995), nas pesquisas paleoecológicas (Eggermont *et. al* 2005, Velle *et al.* 2005, Schmidt *et. al* 2011) e na saúde pública (Cranston 1995). No Brasil, apesar do crescente número de trabalhos de cunho ecológico e taxonômico envolvendo a fauna bentônica nos últimos anos, permanece a dificuldade de identificação desses organismos. No caso dos Chironomidae, a maioria dos trabalhos reporta apenas o nível taxonômico de gênero, que, em geral, é insuficiente para conclusões ecológicas, taxonômicas, filogenéticas e evolutivas.

A família Chironomidae reúne 11 subfamílias (Sæther 2000a), compreende um total de 339 gêneros válidos até 2006 (Ferrington 2008) e as estimativas são de 15.000 espécies (Coffman 1995), distribuídas em todas as regiões zoogeográficas (Ashe *et al.* 1987, Sæther 2000b). Na região Neotropical, são registradas apenas 618 espécies para 154 gêneros (Ferrington 2008), o que representa menos da metade dos gêneros descritos mundialmente. No catálogo mundial de Chironomidae, a subfamília Tanypodinae possui 592 espécies distribuídas em 58 gêneros (Ashe e O'Connor 2012). Destes, apenas 28 são citados para a região Neotropical. Durante a última década, vários gêneros têm sido registrados, pela primeira vez para o Brasil, como *Hudsonimyia* (Silva *et al.* 2012), ou descritos como novos, como é o caso de *Guassutanypus* Roque e Trivinho-Strixino (2003), *Parapentaneura* Stur *et al.* (2006) e, tais dados sustentam a premissa de que a fauna brasileira de Chironomidae, em especial a de Tanypodinae, é pouco conhecida.

O gênero *Labrundinia* (Tanypodinae: Pentaneurini) foi estabelecido por Fittkau, em 1962, com base em *Tanypus longipalpis* Goetghebuer, e atualmente reúne 14 espécies (Silva *et. al* 2011). As espécies neotropicais de *Labrundinia* são oito: *L. fera* Roback, *L. fosteri* Roback, *L. hirsuta* Roback, *L. meta* Roback, *L. opela* Roback, *L. parabecki* Roback e *L. tenata* Roback, registradas para a Colômbia (Roback 1987); e *L. separata* Edwards, para a Argentina (Edwards 1931). Na região Neártica ocorrem quatro espécies, *L. becki* Roback, *L. johannseni* Beck e Beck, *L. neopilosella* Beck e Beck e *L. virescens* Beck e Beck (Roback 1971). *L. longipalpis* Goetghebuer representa a única espécie holártica (Silva *et al.* 2011). Além dessas, 25 novas espécies da América do Sul e Central são descritas neste trabalho de doutoramento. Até o presente estudo, nenhuma filogenia ou mesmo hipótese de monofiletismo foi proposta incluindo *Labrundinia*, assim como toda a subfamília Tanypodinae.

A maioria das descrições de *Labrundinia* está fundamentada em adultos machos, o que não contempla o reconhecimento das larvas em nível específico. Esta restrição impede o refinamento da resolução taxonômica em trabalhos de cunho ecológico e a sua compreensão em estudos de avaliações ambientais. O gênero *Labrundinia* ocupa diferentes ambientes aquáticos com características distintas e a identificação das larvas, em nível genérico, é insuficiente para maioria das propostas de biomonitoramento. Identificações, em nível específico, podem ser alcançadas através da criação individual de larvas e a sua associação com adultos machos emergidos (Ekrem *et al.* 2007). Este procedimento, no entanto, demanda tempo e nem sempre é bem-sucedido para espécies com exigências ambientais particulares. Ainda, o reconhecimento das espécies com base em machos adultos, é dificultado pela similaridade morfológica interespecífica. Neste contexto, o uso de sequências específicas de DNA para a identificação das espécies constitui uma alternativa.

Com o recente desenvolvimento da Biologia Molecular, a busca por fragmentos de DNA, contendo informações úteis à taxonomia tem resultado em vários estudos, dos quais se destaca o programa *The Barcoding of Life*, que tem como objetivo estabelecer um método padrão para identificação de espécies através do sequenciamento de um gene mitocondrial alvo, o Citocromo Oxidase Subunidade I (COI) (Hebert *et al.* 2003a, 2003b). Além do COI, outras sequências de DNA têm sido amplamente empregadas na reconstrução de filogenias em diferentes níveis taxonômicos (Ekrem e Willassen 2004, Moulton e Wiegmann 2004, Schühli *et al.* 2007). Ekrem *et al.* (2010) sugerem o uso do gene nuclear CAD ou rudimentar no entendimento das relações filogenéticas entre espécies de Chironomidae devido aos valores de divergência genética adequados que esta sequência tem demonstrado para o estudo de variações intra e interespecíficas, uma vez que esse gene possui 1/3 de sítios informativos para análise de parcimônia e, junto com os dados morfológicos, tornam-se eficazes na construção de hipóteses filogenéticas.

OBJETIVOS

Neste estudo, pretendeu-se: (1) revisar as espécies de *Labrundinia* com base em seus tipos nominais e descrever novas espécies e semaforontes; (2) elaborar chaves de identificação para os diferentes estágios de vida das espécies do gênero; (3) inferir a filogenia entre as espécies de *Labrundinia*, incluindo os grupos-irmãos postulados, assim como testar o monofiletismo do gênero; (4) apresentar evidências baseadas em dados morfológicos e sequências de genes nucleares e mitocondriais para a construção de uma hipótese filogenética para *Labrundinia*, e discutir propostas taxonômicas para as espécies do gênero; (5) realizar análises biogeográficas utilizando as metodologias da biogeografia cladística.

ESTRUTUTURA DA TESE

Esta tese é apresentada em um conjunto de três capítulos independentes, redigidos sob a forma de artigos científicos para posterior publicação nos periódicos estrangeiros ZooKeys, Canadian Entomologist e Zoological Journal of the Linnean Society, respectivamente, o que justifica a redação em inglês. O capítulo I inclui a revisão taxonômica do gênero *Labrundinia*. O capítulo II trata do uso de sequencias de DNA barcode em delimitações de espécies de Chironomidae utilizando o gênero *Labrundinia* como caso-teste. O capítulo III consiste no estudo filogenético e biogeográfico do gênero *Labrundinia* a partir de evidências morfológicas e moleculares.

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CAPÍTULO I

A TAXONOMIC REVISION OF GENUS *LABRUNDINIA* FITTKAU, 1962 (DIPTERA: CHIRONOMIDAE: TANYPODINAE)

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ABSTRACT

The species of the genus *Labrundinia* (Diptera: Chironomidae: Tanypodinae: Pentaneurini) are revised, described and figured. Keys to known adult males, pupae and larvae are provided. Fourteen previously known species are redescribed, and 25 species from Neotropical region are diagnosed and described as new to science. An emended diagnosis of the genus is presented and morphological differences to closely related species are discussed.

KEY WORDS: Pentaneurini, non-biting midges, generic diagnosis, keys, taxonomy, immature stages.

INTRODUCTION

Species of the genus *Labrundinia* are small dipterans with immatures living in a variety of unpolluted freshwater habitats (Silva *et al.* 2011). The genus belongs to the tribe Pentaneurini and was erected by Fittkau in 1962, based on the Palearctic *Tanypus longipalpis* Goetghebuer, 1921. Since then, new species have been added to the genus, mostly resulting from research activities by Elisabeth C. and William M. Beck and Selwyn S. Roback, in the 1960's and 1970's. Until the present study, there were few *Labrundinia* species known to science. The Catalogue of Neotropical and Mexican Chironomidae (Spies and Reiss 1996) listed 10 species and the later a World Catalogue of Chironomidae (Ashe and

O'Connor 2009) recorded 15 valid species. More recently, *Labrundinia maculata* Roback was placed as a junior synonym of *Labrundinia longipalpis* (Goetghebuer) (Silva *et al.* 2011), which decreases the number of valid species within the genus from 15 to 14.

Labrundinia is known from the Neotropical, Nearctic and western Palaearctic regions, with most species found in the Neotropics. The immatures live in a variety of aquatic systems, from small streams and ponds to lakes and bays, where the larvae usually live associated with aquatic macrophytes or marginal vegetation in slow flowing streams or rivers. *Labrundinia* specimens often have been reported as predators and recorded in many ecological studies (Trivinho-Strixino and Strixino 1993, Aburaya and Callil 2007, Silva and Fonseca-Gessner 2009, Maltchik *et al.* 2012). A study of the chironomid fauna associated with aquatic macrophytes, in a small reservoir in southeast Brazil, has reported *Labrundinia* specimens in high abundance associated with *Salvinia auriculata* rather than *Myriophyllum aquaticum*, which may indicate that larvae of *Labrundinia* have specific habitat preferences (Dornfeld and Fonseca-Gessner 2005).

Recent collecting in Brazil has revealed numerous new species of *Labrundinia*, which supplemented by undescribed species from several entomology collections, yielded 25 new species. The main aim of this study was to revise and describe known and previously unknown species in the genus *Labrundinia* and to present identification keys to adult males, pupae and larvae as well as an emended diagnosis of the genus.

MATERIAL AND METHODS

Field work was conducted in southeast Brazil to supplement material on loan from collections and colleagues in North America. In laboratory, larvae were isolated and kept in small vials with water for rearing purposes. The emerged adults were fixed with 96% ethanol together with their pupal and larval exuviae. Alcohol-preserved specimens were dissected, the bodies cleared in 8% KOH and slide-mounted in Euparal. Morphological terminology and abbreviations follow Sæther (1980), Kowalyk (1985), Roback (1987a,b) and Silva *et al.* (2011). Measurement methods followed Epler (1988). The adult females of *Labrundinia* are very similar and no distinct and constant morphological differences were found to provide reliable diagnoses. The material of the new species has been deposited in the Museu de Zoologia da Universidade de São Paulo (MZSP), São Paulo, Brazil, Museum of Natural History and Archaeology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway and Reference Collection of Laboratório de Entomologia Aquática (LEA), Universidade Federal de São Carlos (UFSCar), São Carlos, Brazil.

Collections holding holotype material studied here: ANSP, Academy of Natural Sciences of Philadelphia; CAS, California Academy of Sciences; CNC, Canadian National Collection, IRSNB, Institut Royal des Sciences Naturelles de Belgique; MZSP, Museu de Zoologia da Universidade de São Paulo; USNM, National Museum of Natural History; ZSM, Zoologische Staatssammlung München.

TAXONOMY

Labrundinia Fittkau

Ablabesmyia Johannsen 1905: 152. *Tanypus* Malloch 1915: 372. *Tanypus* Walley 1928: 583. *Pentaneura* Edwards 1929: 294. *Pentaneura* Johannsen 1946: 283. *Labrundinia* Fittkau 1962: 372. *Labrundinia* Beck and Beck 1966: 337. *Labrundinia* Roback 1971: 275.

Type species: *Tanypus longipalpis* Goetghebuer 1921

Other species of genus: *Labrundinia becki* Roback 1971. *L. fera* Roback 1987. *L. fosteri* Roback 1987. *L. johannseni* Beck and Beck 1966. *L. hirsuta* Roback 1987. *L. longipalpis* (Goetghebuer 1921). *L. meta* Roback 1987. *L. neopilosella* Beck and Beck 1966. *L. opela* Roback 1987. *L. parabecki* Roback 1987. *L. pilosella* Loew 1866. *L. separata* Edwards 1931. *L. tenata* Roback 1987. *L. virescens* Beck and Beck 1966.

Diagnostic characters

Male. Small to moderate sized, 1.55–3.19 mm, wing length 1.12–2.22 mm. Head with eyes bare, antenna with terminal flagellomere not offset, almost fused to the penultimate flagellomere. Thorax with 26–56 acrostichals, 8–44 dorsocentrals, 5–17 prealars and 5–14 scutellars; anepisternals, preepisternals and postnotals absent.

Wing usually with macrotrichia not much dense, except for *L. fosteri* and *L. hirsuta*, Costa short, not produced beyond R_{4+5} , ending level with or slightly beyond tip M_{3+4} ; R_{2+3} present or absent. Fore leg ratio 0.50–0.91; apices of fore and mid tibiae with a pectinate spur, with 3–5 teeth, apex of hind tibia without spur, with comb of 5–8 setae; claws slender, hook-shaped; pulvilli absent. Hypopygium with tergite IX large, convex posteriorly and with an apical row of setae; anal point small, usually covered by tergite IX; inferior volsella absent; phallapodeme straight; sternapodeme usually with anterior process.

Pupa. Small to moderately sized, 1.54–2.68 mm long. Cephalothorax with thoracic horn slender to globose; plastron plate reduced to moderate seated on a small tubercle; horn sac occupies most of lumen;

reticulation of respiratory atrium indistinct; external membrane with pale spines; basal lobe wedge-shaped; thoracic comb with 8–13 conical teeth. Abdominal segment I (A I) with scar; shagreen sparse, more conspicuous on AVII–AVIII, basally concentrated; A II may have prominent spines, sometimes with dense multi-branched spines; A VII with 2–4 lateral setae (LS); tergite VIII with projections over base of anal lobe; anal lobe long and narrow, with inner border membranous, slightly concave; macrosetae with adhesive sheaths; male genital sac almost not reaching apex of anal lobe or slightly surpassing it, sometimes reaching much beyond apex of anal lobe.

Larva: Small to moderately sized, head capsule 398–781 µm long. Head evenly coloured, sometimes may have ventral maculation; surface may be smooth or covered with spinules, lateroventral and posteroventral spine groups present or absent. Antennal rasion (AR) 1.76–3.22. Mandible strongly curved with distinct or short, subequal teeth. Ligula with 5 teeth, middle tooth usually longer than outer teeth. Paraligula bifid or multitoothed. Posterior parapod with numerous simple claws and a bifid claw with outer tooth shorter than inner tooth; subbasal seta simple or multitoothed at the base; serrated claw present or absent.

Key to adult males of the *Labrundinia* species

1. Abdomen with pale with brown transverse bands on any tergite (Figs 5L; 10L) 2
 - Abdomen brown (Figs 1L; 37L)..... 35
2. Tergite II with divided or faint transverse band (Figs 71L; 60L) or wholly pale (Fig. 31L) 3
 - Tergite II with distinct continuous transverse band (Fig. 19L) 16
3. Tergite II with divided or faint transverse band 4
 - Tergite II wholly pale 9
4. Tergite V wholly brown (Fig. 71 L) *Labrundinia* sp. 19
 - Tergite V with transverse band (Figs 5L; 62L) 5
5. Tergite V divided..... 6
 - Tergite V continuous 7
6. Wing with two band of macrotrichia.....*Labrundinia fosteri*
 - Wing not as above *Labrundinia meta*
7. Tergite V with faint band (Fig. 60L) *Labrundinia* sp. 12
 - Tergite V with distinct band (Figs 61L; 62L)..... 8
8. Tergite V with almost wholly brown; fore leg with tibial comb absent (Fig. 61G); hind leg with tibial comb with 4 setae (Fig. 61K) *Labrundinia* sp. 13

–	Tergite V with narrow transverse band; fore leg with tibial comb present (Fig. 61G); hind leg with tibial comb with 8 setae (Fig. 62K).....	<i>Labrundinia</i> sp. 14
9.	Tergite V with faint transverse band (Fig. 31L) or wholly brown(Fig. 69L)	10
–	Tergite V with continuous transverse band (Fig. 6L)	11
10.	Tergite V with faint transverse band; tergite VIII wholly pale; fore leg with tibial comb present (Fig. 31G)	<i>Labrundinia</i> sp. 1
–	Tergite V wholly brown; tergite VIII almost brown; fore leg with tibial comb absent (Fig. 69G)	<i>Labrundinia</i> sp. 17
11.	R ₂₊₃ present (Fig. 6F)	12
–	R ₂₊₃ absent (Fig. 34F)	15
12.	Tergite V with narrow band; tergite VI with arch-shaped band	<i>Labrundinia hirsuta</i> Roback
–	Tergite V ½ or over brown; tergite VI not as above	13
13.	Tergite V over ¾ brown (Fig. 28L).....	<i>Labrundinia virescens</i> Beck & Beck
–	Tergite V about ½ brown (Fig. 17L)	14
14.	Tergite VI–VIII with transverse band (Fig. 17L); anterior process of sternapodeme reduced (Fig. 17M)	<i>Labrundinia opela</i> Roback
–	Tergite VI–VIII wholly brown (Fig. 86L); anterior process of sternapodeme moderate (Fig. 86M)	<i>Labrundinia</i> sp. 25
15.	Wing length 1.36–1.53 mm; HR 1.68–1.77; anterior process of sternapodeme distinct (Fig. 34M)	<i>Labrundinia</i> sp. 2
–	Wing length 1.16 mm; HR 1.45; anterior process of sternapodeme reduced (Fig. 80M)	<i>Labrundinia</i> sp. 23
16.	Tergite V almost wholly brown or brown (Figs 59L; 72L).....	17
–	Tergite V with continuous transverse band (Figs 7L; 14L).....	21
17.	Tergite III wholly brown (Fig. 19L)	18
–	Tergite III over ¾ brown or with continuous transverse band	19
18.	Wing length 1.66–1.88 mm; hypopygium pale brown (Fig. 19M)	<i>Labrundinia pilosella</i> (Loew)
–	Wing length 1.21 mm; hypopygium pale (Fig. 72M)	<i>Labrundinia</i> sp. 20
19.	Tergite III over ¾ brown (Fig. 7L)	<i>Labrundinia johannseni</i> Beck & Beck
–	Tergite III with continuous transverse band (Fig. 14L).....	20
20.	Tergite VI wholly brown; hypopygium pale brown (Fig. 14M)	<i>Labrundinia neopilosella</i> Beck & Beck

–	Tergite VI $\frac{3}{4}$ brown; hypopygium dark brown (Fig. 59M)	<i>Labrundinia</i> sp. 11
21.	Hypopygium evenly pale or bicolored, with apical $\frac{1}{2}$ brown	22
–	Hypopygium pale brown or brown	23
22.	Tergite VI–VIII with transverse band; hypopygium with apical $\frac{1}{2}$ brown (Fig. 4M) <i>Labrundinia fera</i> Roback
–	Tergite VI–VIII wholly brown; hypopygium pale (Fig. 83M)	<i>Labrundinia</i> sp. 24
23.	Hypopygium brown; tergite VI wholly brown or with continuous transverse band	24
–	Hypopygium pale brown; tergite VI with continuous transverse band	29
24.	R ₂₊₃ absent (Fig. 74F)	25
–	R ₂₊₃ present (Fig. 70F)	27
25.	Tergite VII $\frac{3}{4}$ brown (Fig. 70L)	26
–	Tergite VII wholly brown.....	<i>Labrundinia</i> sp. 16
26	Tentorium 162–181 μ m long; wing length 1.32–1.45 mm	<i>Labrundinia</i> sp. 18
–	Tentorium 127–145 μ m long; wing length 1.01–1.17 mm	<i>Labrundinia tenata</i> Roback
27.	Tergite VI wholly brown (Fig. 22L).....	<i>Labrundinia separata</i> (Edwards)
–	Tergite VI with continuous transverse band.....	28
28.	Tergite VI with band transverse posteriorly (Fig. 77L)	<i>Labrundinia</i> sp. 22
–	Tergite VI with band transverse anteriorly (Fig. 74L)	<i>Labrundinia</i> sp. 21
29.	R ₂₊₃ present (Fig. 40F)	30
–	R ₂₊₃ absent (Fig. 10F)	31
30.	Tergite VII–VIII over $\frac{3}{4}$ brown (Fig. 40L); anal point rounded, apical edge notched (Fig. 40M)	<i>Labrundinia</i> sp. 4
–	Tergite VII–VIII wholly brown (Fig. 63L); anal point rounded, apical edge convex (Fig. 63M) <i>Labrundinia</i> sp. 15
31.	Fore leg ratio (LR) 0.56–0.68	32
–	Fore leg ratio (LR) 0.74–0.91	33
32.	Wing length 1.23–1.55 mm; anal point trapezoidal, apical edge notched (Fig. 10M) <i>Labrundinia longipalpis</i> (Goetghebuer)
–	Wing length 1.15; anal point rounded, apical edge convex (Fig. 43M)	<i>Labrundinia</i> sp. 5
33.	Wing length 1.56–1.67 mm; gonocoxite ratio 2.46–2.54 (Fig. 49M)	<i>Labrundinia</i> sp. 7
–	Wing length 1.20–1.32 mm; gonocoxite ratio 1.88–2.2	34
34.	Hypopygium ratio 1.61–1.77 (Fig. 46M)	<i>Labrundinia</i> sp. 6
–	Hypopygium ratio about 1.52 (Fig. 52M)	<i>Labrundinia</i> sp. 8
35.	R ₂₊₃ present (Fig. 55F).....	36

–	R ₂₊₃ absent (Fig. 37F)	38
36.	Tentorium 150 µm long (Fig. 55C); gonostylus with an intern depression subapically (Fig. 55M)	<i>Labrundinia</i> sp. 9
–	Tentorium 124–143 (Fig. 18C) µm long; gonostylus not as above	37
37.	Tergite II–IV with darker cruciate marks (Fig. 18L); fore leg ratio 0.77–0.81	<i>Labrundinia parabecki</i> Roback
–	Tergite II–IV evenly in colour (Fig. 1L); fore leg ratio 0.55.....	<i>Labrundinia becki</i> Roback
38.	Tentorium 90–126 µm long (Fig. 37C)	<i>Labrundinia</i> sp. 3
–	Tentorium 149–192 µm long (Fig. 56C)	<i>Labrundinia</i> sp. 10

Key to known pupae of the *Labrundinia* species

The pupae of *Labrundinia fera*, *L. fosteri*, *L. hirsuta*, *L. meta*, *L. opela*, *L. parabecki*, *L. sp. 9*, *L. sp.11*, *L. sp. 12*, *L. sp. 13*, *L. sp. 14*, *L. sp. 17*, *L. sp. 18*, *L. sp. 19* remain unknown.

1.	Thoracic horn with lateromedial papilla (Fig. 32B); preapical indentation absent	<i>Labrundinia</i> sp. 1
–	Thoracic horn with preapical papilla; preapical indentation present or absent	2
2.	Thoracic horn with preapical indentation deep, more than $\frac{2}{3}$ of the thoracic horn (Fig. 29B); aeropyle tube elongated	3
–	Thoracic horn with preapical indentation absent to moderate deep, less than $\frac{2}{3}$ of the thoracic horn; aeropyle tube elongated or short	8
3.	Shagreen on segment II sparse, with small spinules (Fig. 35D)	<i>Labrundinia</i> sp. 2
–	Shagreen on segment II with prominent spines, sometimes with dense multi-branched spines (Fig. 29D)	4
4.	Genital sac reaching or almost reaching apex of the anal lobe (Fig.78E)	5
–	Genital sac reaching much beyond apex of the anal lobe (Fig. 81E)	6
5.	Apical spines 23–29 µm long; genital sac almost reaching apex of the anal lobe (Fig.78E)	<i>Labrundinia</i> sp. 22
–	Apical spines 39–45 µm long; genital sac reaching somewhat beyond apex of anal lobe (Fig. 29E)	<i>Labrundinia virescens</i> Beck & Beck
6.	Abdominal segment VII with 3 lateral setae (Fig. 81D)	<i>Labrundinia</i> sp. 23
–	Abdominal segment VII with 4 lateral setae (Fig. 84D)	7
7.	Shagreen on segment II with prominent spines, generally bifid; apical spines 18–26 µm long.....	

	<i>Labrundinia</i> sp. 24
–	Shagreen on segment II with multi-branched spines; apical spines 46–60 µm long	
	<i>Labrundinia</i> sp. 25
8.	Thoracic horn with lateromedial indentation (Fig. 50B)	9
–	Thoracic horn without lateromedial indentation	11
9.	Segment VII with 4 lateral setae (Fig. 50D); preapical indentation absent (Fig. 50C)	
	<i>Labrundinia</i> sp. 7
–	Segment VII with 3 lateral setae (Fig. 47D); preapical indentation deeper (Fig. 47C)	10
10.	Thoracic horn ratio 1.75–2.08 (Fig. 47B)	<i>Labrundinia</i> sp. 6
–	Thoracic horn ratio 2.17–2.20 (Fig. 53D)	<i>Labrundinia</i> sp. 8
11.	Thoracic horn with preapical indentation moderate deep	12
–	Thoracic horn with preapical indentation absent.....	22
12.	Thoracic horn about 2.0–2.5 times as long as wide (Fig.38B).....	13
–	Thoracic horn about 4.0 times as long as wide (Fig.20B)	19
13.	Thoracic horn with external margin convoluted (Fig.38B).....	<i>Labrundinia</i> sp. 3
–	Thoracic horn with external margin straight (Fig.26B).....	14
14.	Abdominal segment VII with 2 lateral setae (Fig.26D)	15
–	Abdominal segment VII with 4 lateral setae (Fig.2D)	16
15.	Thoracic horn ratio 2.34 (Fig.67B); genital sac not reaching apex of the anal lobe (Fig.67E)	
	<i>Labrundinia</i> sp. 16
–	Thoracic horn ratio 2.72–3.41(Fig.26B); genital sac reaching much beyond apex of the anal lobe (Fig.26E)	<i>Labrundinia tenata</i> Roback
16.	Genital sac reaching somewhat beyond apex of anal lobe (Fig.2E)	<i>Labrundinia becki</i> Roback
–	Genital sac reaching much beyond apex of the anal lobe (Fig.57E)	<i>Labrundinia</i> sp. 10
17.	Preapical papilla ratio 0.34–0.36 (Fig.75C)	18
–	Preapical papilla ratio 0.06–0.26 (Fig.20C)	19
18.	Plastron plate seated on a small tubercle (Fig.73A)	<i>Labrundinia</i> sp. 20
–	Plastron plate seated on an enlarged tubercle (Fig.75B)	<i>Labrundinia</i> sp. 21
19.	Genital sac reaching much beyond apex of the anal lobe.....	20
–	Genital sac reaching or almost reaching apex of the anal lobe.....	21
20.	Preapical indentation moderate deep, forming a distinct diverticulum (Fig. 20C).....	
	<i>Labrundinia pilosella</i> (Loew)
–	Preapical indentation more shallow (Fig. 64C)	<i>Labrundinia</i> sp. 15

21.	Preapical indentation shallow (Fig. 44C); plastron plate seated on an enlarged tubercle (Fig. 44 B); genital sac not reaching beyond apex of the anal lobe (Fig. 44 E).....	<i>Labrundinia</i> sp. 5
–	Preapical indentation deeper (Fig. 41C); plastron plate seated on a smaller tubercle (Fig. 41B); genital sac reaching somewhat beyond apex of anal lobe (Fig. 41E)	<i>Labrundinia</i> sp. 4
22.	Apical width of thoracic horn about 1.5 times as long as basal width	23
–	Apical width of thoracic horn about 2.0–2.5 times as long as basal width	24
23.	Thoracic horn ratio 2.07–2.20 (Fig.8B); aeropyle tube short	
	<i>Labrundinia johannseni</i> Beck & Beck
–	Thoracic horn ratio 2.54–2.91 (Fig.15B); aeropyle tube elongated.....	
	<i>Labrundinia neopilosella</i> Beck & Beck
24.	Shagreen on segment II with prominent spines, generally bifid (Fig. 23D)	
	<i>Labrundinia separata</i> (Edwards)
–	Shagreen on segment II sparse, with small spinules (Fig. 11D)	
	<i>Labrundinia longipalpis</i> (Goetghebuer)

Key to known larvae of the *Labrundinia* species

The larvae of *Labrundinia fera*, *L. fosteri*, *L. hirsuta*, *L. meta*, *L. opela*, *L. parabecki*, *L. sp. 9*, *L. sp.11*, *L. sp. 12*, *L. sp. 13*, *L. sp. 14*, *L. sp. 17*, *L. sp. 18*, *L. sp. 19* remain unknown.

1.	Surface of head capsule covered with small spines or nodules (Fig. 12A)	2
–	Surface of head capsule smooth (Fig. 9A)	15
2.	Head with lateroventral spine present, one or more spines (Figs 33A; 12A)	3
–	Head with lateroventral spine group absent (Fig. 27A).....	7
3.	Lateroventral spine consist of only a single, enlarged spine	<i>Labrundinia</i> sp. 1
–	Lateroventral spine group consist of several small spines.....	4
4.	Subbasal setae of posterior parapods multitoothed (Fig. 12H); bifid claw with U-shaped lower ... indentation (Fig. 12I)	<i>Labrundinia longipalpis</i> (Goetghebuer)
–	Subbasal setae of posterior parapods simple (Fig. 65H); bifid claw with V-shaped lower indentation (Fig. 65I).....	5
5.	Head with posteroventral dark brown spot (Fig. 65A)	<i>Labrundinia</i> sp. 15
–	Head without posteroventral maculation (Fig. 42A)	6
6.	Head slender almost rectangular, as in figure 42A.....	<i>Labrundinia</i> sp. 4
–	Head not as above, broader, as in figure 45A	<i>Labrundinia</i> sp. 5
7.	Serrated claw of posterior parapods present (Fig. 27H)	8

–	Serrated claw of posterior parapods absent	11
8.	Bifid claws of posterior parapods with U-shaped lower indentation (Fig. 27J)	
 <i>Labrundinia tenata</i> Roback	
–	Bifid claws of posterior parapods with V-shaped lower indentation (Fig. 68J)	9
9.	Subbasal setae of posterior parapods multitoothed (Fig. 68I)	<i>Labrundinia</i> sp. 16
–	Subbasal setae of posterior parapods simple (Figs 48I and 54I)	10
10.	Head with posteroventral spine group present (Fig. 54A).....	<i>Labrundinia</i> sp. 8
–	Head with posteroventral spine group absent (Fig. 48 A)	<i>Labrundinia</i> sp. 6
11.	Head with ventral maculation (Fig.73B)	12
–	Head without ventral maculation (Fig. 21A)	13
12.	Head with ventral semicircular spot (Fig. 73B); bifid claws of posterior parapod with U-shaped lower indentation	<i>Labrundinia</i> sp. 20
–	Head with ventral dark area (Fig. 51A), with variable shape; bifid claw of posterior parapods with V-shaped lower indentation (Fig. 51I)	<i>Labrundinia</i> sp. 7
13.	AR 2.33–2.34; ratio between inner and outer tooth on bifid claw (B/C) 1.09–1.22; posterior margin may be darkened	<i>Labrundinia pilosella</i> (Loew)
–	AR 1.82–2.11; ratio between inner and outer tooth on bifid claw (B/C) 0.89–0.97	14
14.	AR 2.11; middle tooth of ligula slender as in figure 24F	<i>Labrundinia separata</i> (Edwards)
–	AR 1.82–1.85; middle tooth of ligula broader as in figure 76F	<i>Labrundinia</i> sp. 21
15.	Head with lateroventral spine group present (Figs 9A; 36A)	16
–	Head with lateroventral spine group absent	22
16.	Head with lateroventral spine consist of only a single, enlarged spine	<i>Labrundinia</i> sp. 2
–	Head with lateroventral spine group consist of several small spines	17
17.	Mandible with distinct teeth in size, as in figure 9E	18
–	Mandible with short, subequal teeth, as in figure 30E	20
18.	Head with distinct ventral maculation (Fig. 9A)	<i>Labrundinia johannseni</i> Beck and Beck
–	Head not as above	19
19.	Subbasal setae of posterior parapods multitoothed (Fig.16H); bifid claw with U-shaped lower indentation (Fig.16I)	<i>Labrundinia neopilosella</i> Beck & Beck
–	Subbasal setae of posterior parapods simple (Fig. 3H); bifid claw with V-shaped lower indentation (Fig. 3I).....	<i>Labrundinia becki</i> Roback
20.	Head with lateroventral spine group conspicuous, with large spines (Fig. 30A)	
 <i>Labrundinia virescens</i> Beck & Beck	
–	Head with lateroventral spine group inconspicuous, with minute spines (Fig. 82A)	21

21. Ligula 54–61 μm long (Fig. 82F) *Labrundinia* sp. 23
 – Ligula 88–89 μm long (Fig. 88F) *Labrundinia* sp. 25
22. Head with posteroventral spine group (Fig. 79A); paraligula bifid (Fig. 79F); subbasal setae of posterior parapods simple (Fig. 79H) 23
 – Head without posteroventral spine group absent (Fig. 78A); paraligula multitoothed (Fig. 58F); subbasal setae of posterior parapods multitoothed (Fig. 58H) 24
23. AR 2.80–2.93 *Labrundinia* sp. 22
 – AR 3.02–3.22..... *Labrundinia* sp. 24
24. Head with small, dark brown spots ventrally (Fig. 58A) *Labrundinia* sp. 10
 – Head not as above *Labrundinia* sp. 3

Species descriptions and redescriptions

Labrundinia becki Roback (Figs 1–3)

Labrundinia pilosella Beck and Beck (nec. Loew) 1966: 340 (description of male, female and immatures stages).

Labrundinia becki Roback 1971: 279 (redescription of male).

Labrundinia becki Roback 1987: 184 (description of immature stages).

Labrundinia becki Epler 2001: 4.48 (larvae identification key).

Labrundinia becki Jacobsen 2008: 41 (pupae identification key).

Material examined

Type: Holotype male, USA: **Florida**, Tucker, 15.iv.1952, J. R. Vockeroth (CNC).

Additional. 1 male with pupal exuvia, USA: **Florida**, Flagler County, Rayonier Forest, 27.x.1967. W. Beck; 1 larva as previous except for Leon, Polk Creek, 10 mi SW Tallahassee, 9.vi.1972; 1 pupa with larval exuviae as previous except for Little Haw Creek, 8.vi.1964. 1 male, **Georgia**, Clarke, Sways Area (near Oconee River) 24/vii/1978; 1 female with pupal and larval exuviae as previous except for Laurens County, 13.iii.1986; 1 female with pupal exuvia as previous. 1 male with pupal exuvia. 1 male with pupal exuvia, **Pennsylvania**, Wayne County, Lake Lacawac, 19.ix.1970. S.S. Roback; 1 female as previous. 1 male, **South Carolina**, Tinken Creek, Savannah River Plant, 19–20.ix.1980, P.L. Hudson; 3 larvae as previous except Horry, 1.vii.1979, T.R. White; 1 larva as previous except for Anderson, Charles Creek, 21.ix.1975, P.H. Carlson.

Diagnostic characters

Labrundinia becki differs from other *Labrundinia* species by the combination of the following characters.

Male: R_{2+3} present; abdomen wholly brown; hypopygium brown, sternapodeme with reduced anterior process. **Pupa:** thoracic horn semi-globose with preapical indentation shallow; genital sac reaching somewhat beyond apex of anal lobe. **Larva:** Surface of head capsule smooth, lateroventral spine group with several small spines; posterior parapod with subbasal seta simple and bifid claw with V-shaped lower indentation.

Redescription

Adult male (n = 5 unless otherwise stated)

Size. Total length 1.91–2.64 (4) mm. Wing length 1.23–1.35 (3) mm. Total length/wing length 1.65–1.99 (4). Wing length/length of profemur 2.24–2.79 (3).

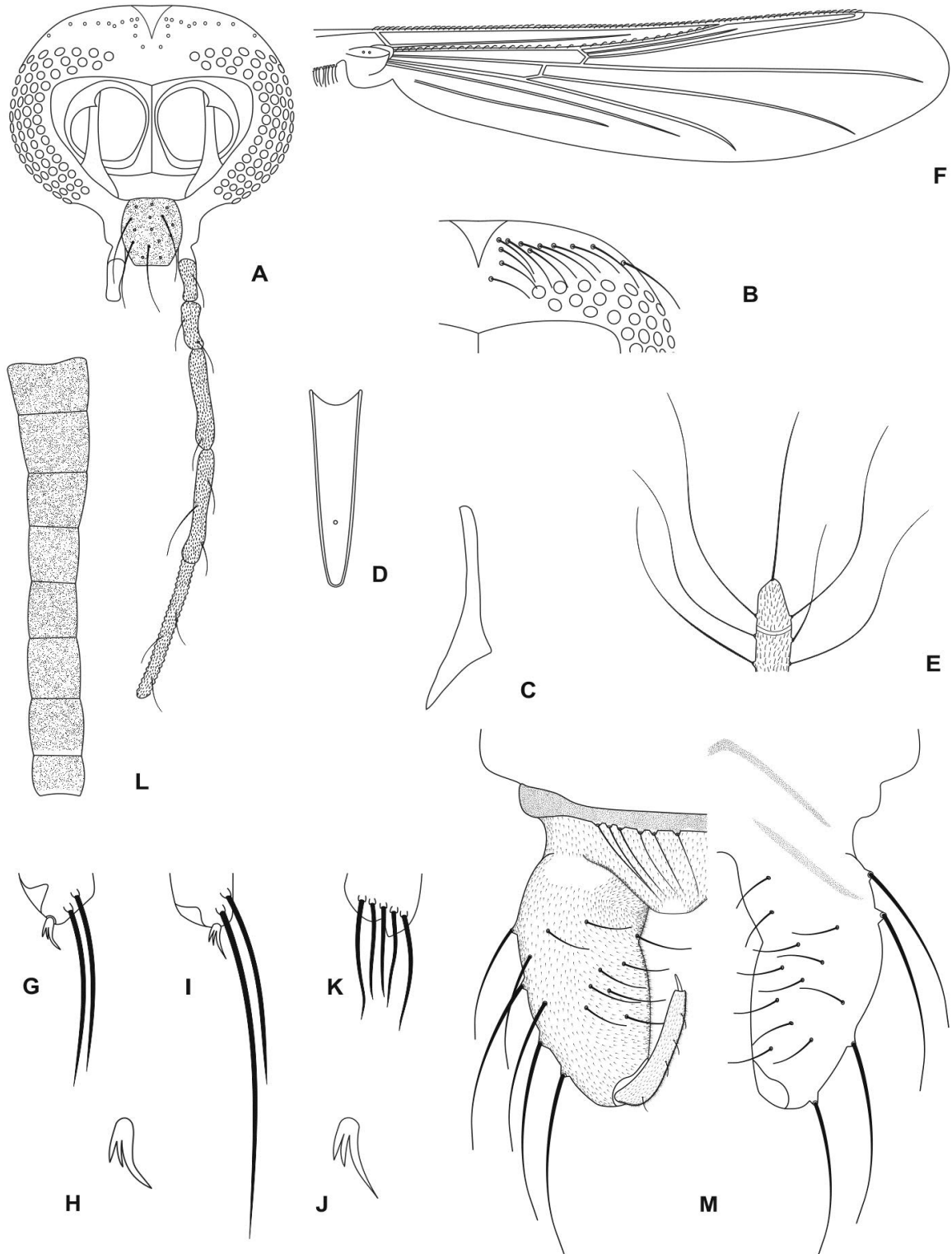
Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen wholly brown as in figure 1L. Hypopygium brown.

Head (Figs 1A–E). Temporal setae 14 (3), uniserial (Fig. 1B). Eye ratio 0.37–0.53 (2). Tentorium (Fig. 1C) 124–141(3) μm long, stipes not measurable. Clypeus 98–120 (3) μm long, 80–82 (3) μm wide at largest part, bearing 14 (3) setae. Cibarial pump (Fig. 1D) with anterior margin concave, 153–182 (2) μm long. Palpomere lengths 1–3 (in μm): 31 (1); 51(1); 98 (1). Antenna with 14 flagellomeres, AR 1.06–1.07 (2), flagellum 639–676 (2) μm long, diameter of pedicel 98–120 (2) μm , apical setae single (Fig. 1E).

Thorax. Anteprenotum with 3 (2) lateral setae. Acrostichals 36–42 (2), biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 16–26 (2), irregularly uniserial; prealars 7–9 (2); supraalars 2 (2). Anapleural suture ratio 0.41–0.49 (2). Scutellum with 9–10 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 1F). Width 0.27–0.39 (3) mm. Costa 1.04–1.15 (2) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.08–0.09 (3) mm long. VR 0.70–0.74 (2). WW 0.28–0.29 (3). Brachiolum with 2 setae (2). Squama setiferous.

Legs (Figs 1G–K). Fore leg: width at apex of tibia 31–39 μm (Fig. 1G), tibia with single, apical and pectinate spur 11–16 (2) μm long (Fig. 1H), with three teeth and two preapical setae; Ta_{1-4} without



Figures 1A–M. *Labrundinia becki* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

preapical pseudospurs. Mid leg: width at apex of tibia 39, 39 (3) μm long (Fig. 1I), tibia with single, apical and pectinate spur 24 (1) μm long with three teeth (Fig. 1J) and two preapical setae; Ta_{1-4} each with one preapical pseudospurs. Hind leg: width at apex of tibia 39 (2) μm long (Fig. 1K), tibia without spur; comb with 5 (3) setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table I.

Table I. Lengths (in μm) and proportions of leg segments in *Labrundinia becki* Roback, adult male.

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	429–546 (3)	500–531 (3)	234 (1)	109 (1)	86 (1)
p ₂	547–626 (3)	515–547 (2)	625 (1)	265 (1)	156 (1)
p ₃	477–555 (3)	656–727 (3)	672 (1)	289 (1)	195 (1)
	ta ₄	ta ₅	LR	BV	SV
p ₁	55 (1)	39 (1)	0.55 (1)	4.16 (1)	3.40 (1)
p ₂	102 (1)	78 (1)	1.14 (1)	2.98 (1)	1.88 (1)
p ₃	117 (1)	78 (1)	0.92 (1)	2.87 (1)	1.90 (1)

Hypopygium (Fig. 1M). Tergite IX arched, with 11 (4) dorsal setae. Anal point trapezoidal. Phallapodeme 45–55 μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 90–113 μm long, 47–59 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.76–2.25 (4). Gonostylus simple and slender, 55–64 μm long; megaseta enlarged, 10–14 (4) μm long. HR 1.58–1.85. HV 3.04–3.35 (3).

Adult female ($n = 2$ unless otherwise stated)

Size. Total length 1.45 mm. Wing length 1.05–1.11 mm. Total length/wing length 1.25–1.37. Wing length/length of profemur 2.50–2.76.

Coloration. Head, pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown with mesonotum dorsally dark brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs brown to pale brown. Abdomen wholly brown. Seminal capsules brown.

Head. Temporal setae 11–16, uniserial. Eyes ratio 0.78 (1). Tentorium 134 (1) μm long, stipes not measurable. Clypeus 81 (1) μm long, 74 (1) μm wide at largest part, bearing 21–26 setae. Cibarial pump as in male, 143–185 μm long. Palpomere lengths 1–5 (in μm): 22–40; 47–52; 75–85; 118–130; 167–209. Antenna with 11 flagellomeres, AR 0.42 (1), flagellum 407 (1) μm long, diameter of pedicel 60–75 μm .

Thorax. Anteprepronotum with 1 lateral seta. Acrostichals 32–34, irregularly biserial, starting close to

anteprenotum; dorsocentrals 32, irregularly uniserial; prealars 10–12; supraalars 2. Scutellum with 10 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.36–0.38 mm. Costa 0.95 (1) mm long, not produced beyond R₄₊₅. R₂₊₃ present. Base of radial sector 0.07 (1) mm long. VR 0.68 (1). WW 0.34–0.36. Brachiolum with 2 setae (2). Squama setiferous.

Legs. Fore leg: width at apex of tibia 39–44 µm, tibia with single, apical and pectinate spur 12–17 µm long, with three teeth and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Mid leg: width at apex of tibia 32–46 µm, tibia with single, apical and pectinate spur 16–19 µm long, with three teeth and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Hind leg: width at apex of tibia 40–46 µm, tibia without spur; comb with 7 setae; preapical pseudospurs on Ta₁₋₄ unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table II.

Table II. Lengths (in µm) and proportions of leg segments in *Labrundinia becki* Roback, adult female.

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	401–421	367–375	195 (1)	156 (1)	102 (1)
p ₂	485–561	438–493	445 (1)	195 (1)	140 (1)
p ₃	468–503	515–617	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	78 (1)	62 (1)	0.53 (1)	2.47 (1)	4.04 (1)
p ₂	94 (1)	78 (1)	1.02 (1)	2.69 (1)	2.07 (1)
p ₃	–	–	–	–	–

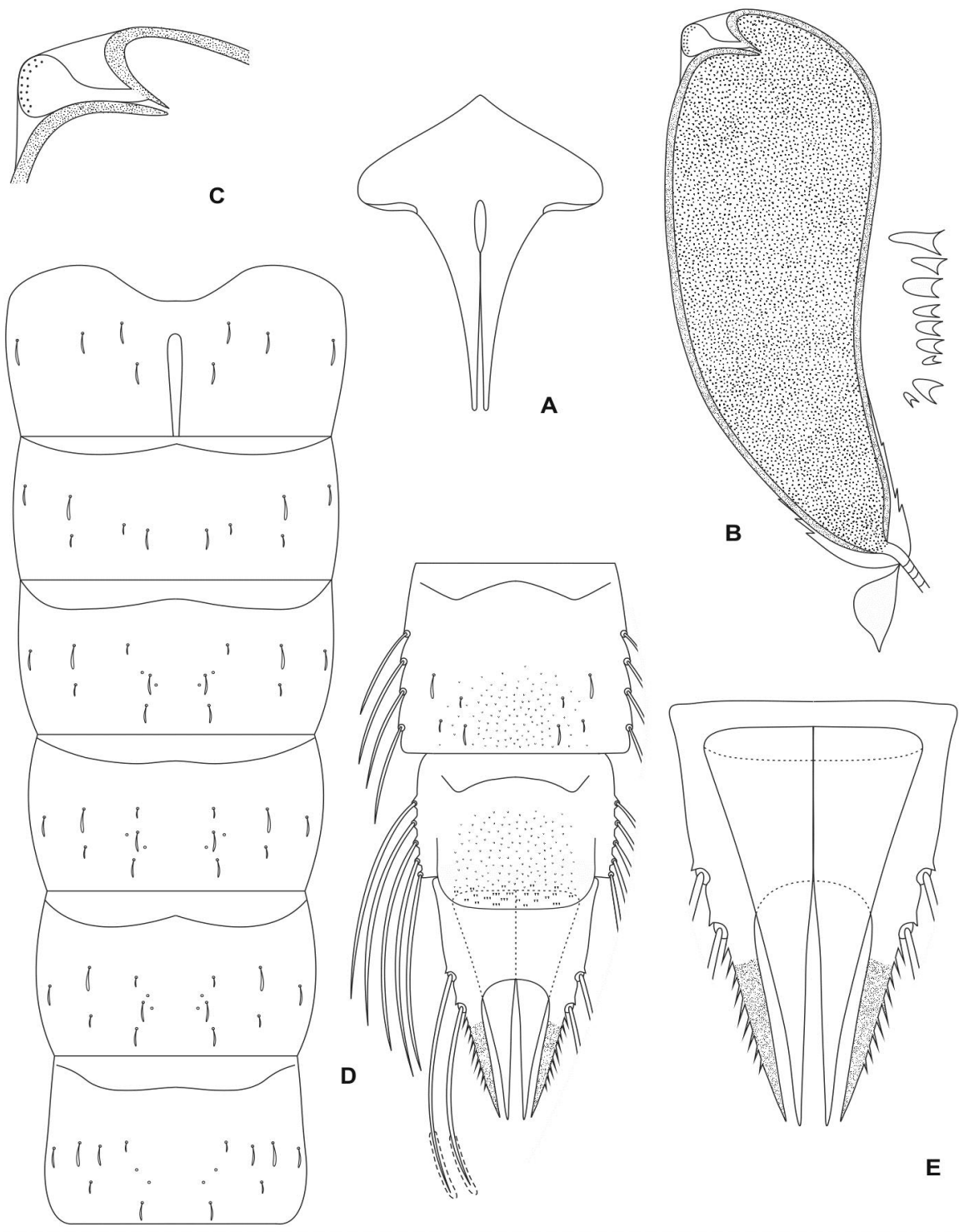
Genitalia. Gonapophysis VIII broadly rounded, 75 (1) µm long. Coxosternapodeme 85 (1) µm long. Tergite X with 10 lateroventral setae on each side. Postgenital plate rounded. Cerci oval-quadrangle, 28 (1) µm long and 26 (1) µm wide; with 4 (1) elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 104 (1) µm. Seminal capsules oval with conical shaped necks, length 45 (1) µm, maximum width 30 (1) µm. Length ratio SCa/No 0.30–0.43.

Pupa (*n* = 4 unless otherwise stated)

Size. Male abdomen 1.64–1.88 (3) mm long; female abdomen 2.33 (1) mm long.

Coloration. Exuvia and thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 2A–C). Frontal apotome as in figure 2A. Wing sheath smooth 0.70–0.79 mm long. Thoracic horn 197–248 µm long and 65–70 µm wide (Fig. 2B), preapical indentation moderate



Figures 2A–E. *Labrundinia becki* Roback, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

deep, THR 2.66–2.88 (3). Membranous preapical papilla 14–22 μm long (Fig. 2C), PTH 0.07–0.09, aeropyle tube simple, short and robust, 20–24 (2) μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 12 (3) conical teeth (Fig. 2B).

Abdomen (Figs 2D–E). Tergite I with scar, 88 (1) μm long. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 2D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 213–273 (2) μm long and 139–156 μm wide (Fig. 2E), outer margins sclerotized, with 6–10 (3) spines, longest spine 11–14 (3) μm long, inner margins of lobes membranous. ALR 1.53–1.75 (2). Genital sac elongated, reaching somewhat beyond apex of anal lobe.

4th instar larva ($n = 6$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 3A). Length 453–504 (3) μm , 257–305 (3) μm wide. Surface smooth; lateroventral spine group present but weakly developed, with 3–4 spines; posteroventral spine group absent; cephalic index 0.58–0.60 (3). Chaetotaxy as in figure 3A.

Antenna (Figs 3B–C). Length 253–282 μm , A_1 165–189 μm long, with ring organ placed 150–191 μm from base, A_2 77–84 μm long. AR 1.89–2.08. Blade and accessory blade unobserved.

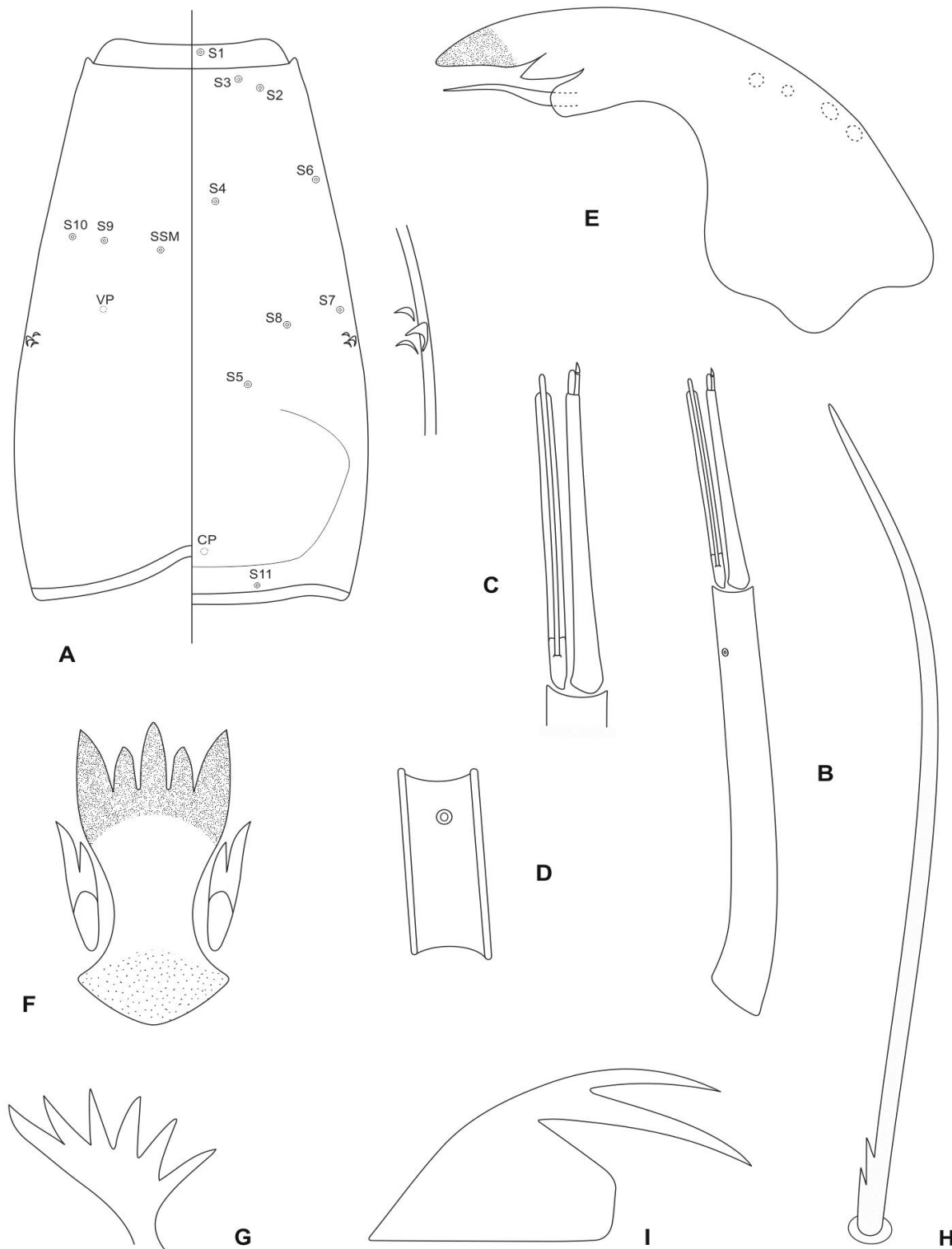
Maxilla (Fig. 3D). Basal palp segment 18–23 μm long and 7–9 μm wide, with ring organ 16–19 (3) μm from base. PR 2.33–2.89. APR 8.10–8.64.

Mandible (Fig. 3E). Length 56–71 μm , with 3 lateral setae. Sensillum campaniformium 45–53 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.59–3.26.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 3F–G). Ligula 47–58 μm long, 23–28 μm wide, with row of 5 teeth. IO 0.92–0.99, MO 1.01–1.04. Paraligula bifid, 23–29 μm long, inner tooth 18–23 μm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 3H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 129–166 μm long, 25–36 μm wide, with 7 anal setae 437–572 (4) μm long. L/W 3.98–4.60. Supraanal seta well developed. Anal tubules 129–157 (2) μm long. Posterior parapod 331–368 (4) μm long; subbasal seta simple, basal spines reduced, 1–3 spines (Fig. 3H); parapod apex with numerous simple claws, without



Figures 3A–I. *Labrundinia becki* Roback, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

serrated claw; bifid claw with V-shaped lower indentation (Fig. 3I). B/C 1.02–1.05 (4).

Labrundinia fera Roback (Fig. 4)

Labrundinia fera Roback 1987: 218 (description of male).

Material examined

Type: Holotype male, Colombia: **Departamento del Meta**, Puerto López, Laguna Mozambique, 16 km s. w. of Puerto López, 25.ii.1972, N. R. Foster (ANSP).

Diagnostic characters

Labrundinia fera differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite I brown, T II–VII with continuous brown transverse band near proximal margin, T VII–VIII almost wholly brown; hypopygium with apical 0.50 brown, sternapodeme with anterior process absent.

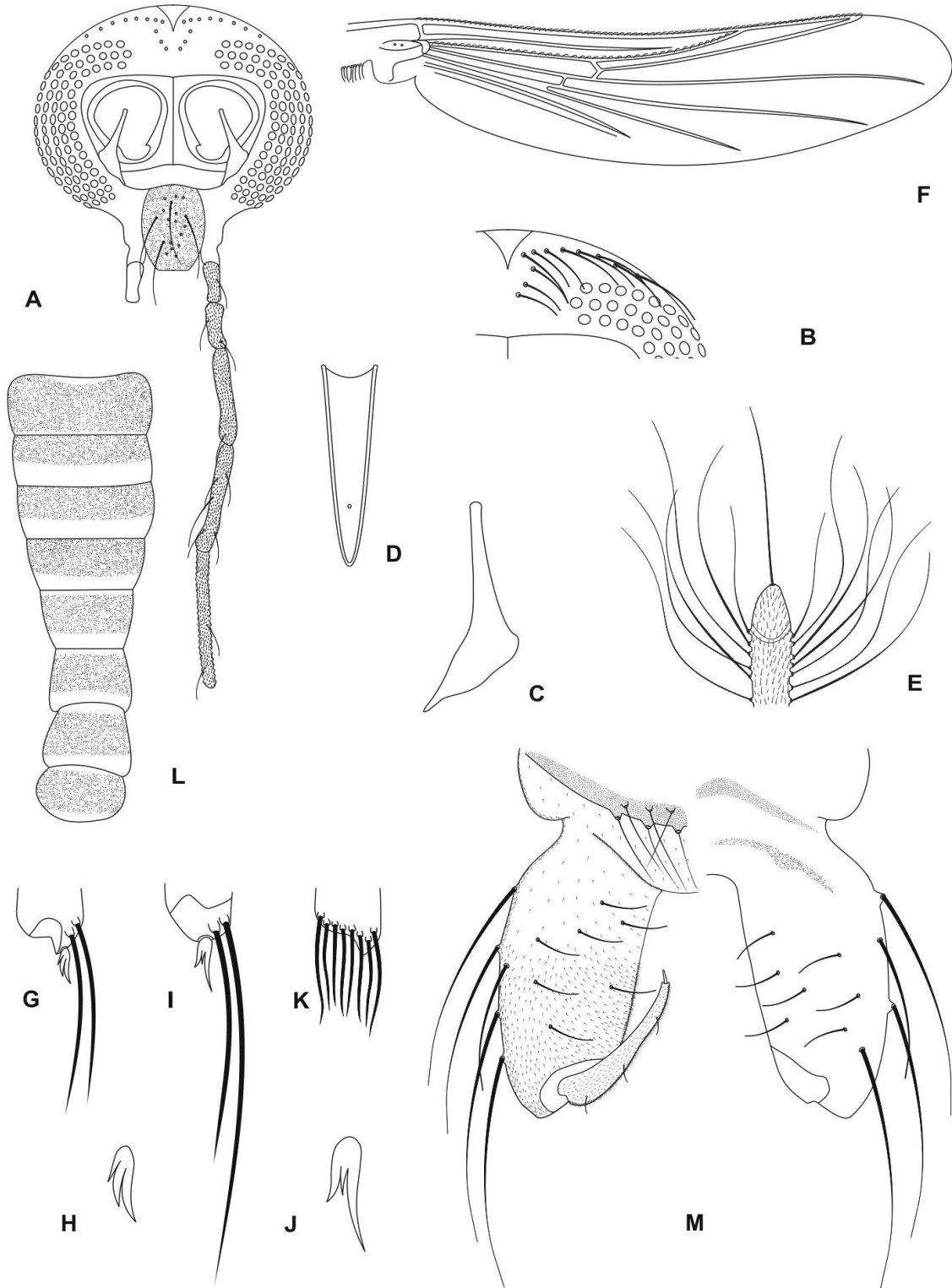
Redescription

Adult male ($n = 1$)

Size. Total length 2.35 mm. Wing length 1.18 mm. Total length/wing length 2.00. Wing length/length of profemur 2.99.

Coloration. Head dark brown with brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale; supraalar callus dark brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale. Femur I–III with apex brown. Tibia I with apex brown, Ti II–III brown. Tarsomere 1 brown, tarsomeres 2–5 pale. Abdomen pale brown with maculation as in figure 4L. Hypopygium apically brown.

Head (Figs 4A–E). Temporal setae 15, uniserial (Fig. 4B). Eye ratio 0.75. Tentorium (Fig. 4C) 129 μm long, stipes not measurable. Clypeus 153 μm long, 102 μm wide at largest part, bearing 22 setae. Cibarial pump (Fig. 1D) with anterior margin concave, unmeasured. Palpomere lengths 1–4 (in μm): 37; 53; 129; 131. Antenna with 14 flagellomeres, AR 1.14, flagellum 386 μm long, diameter of pedicel 118 μm , apical setae single (Fig. 4E).



Figures 4A–M. *Labrundinia fera* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 42, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20, irregularly uniserial; prealars 7; supraalars 3. Anapleural suture ratio 0.41. Scutellum with 10 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 4F). Width 0.36 mm. Costa 1.01 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.06 mm long. VR 0.74. WW 0.30. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 4G–K). Fore leg: width at apex of tibia 47 μm (Fig. 4G), tibia with single, apical and pectinate spur 14 μm long (Fig. 4H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 55 μm long (Fig. 4I), tibia with single, apical and pectinate spur 26 μm long with three teeth (Fig. 4J) and two preapical setae; Ta_{1-4} each with one preapical pseudospurs. Hind leg: width at apex of tibia 47 μm long (Fig. 4K), tibia without spur; comb with 8 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table III.

Table III. Lengths (in μm) and proportions of leg segments in *Labrundinia fera* Roback, adult male.

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	394	453	296	171	125
p ₂	594	468	555	250	133
p ₃	524	586	563	266	195
	ta ₄	ta ₅	LR	BV	SV
p ₁	100	66	0.65	2.50	2.85
p ₂	101	86	1.18	2.83	1.92
p ₃	125	78	0.96	2.51	1.97

Hypopygium (Fig. 4M). Tergite IX arched, with 13 dorsal setae. Anal point trapezoidal. Phallapodeme 60 μm long. Sternapodeme with anterior process absent. Gonocoxite cylindrical, 111 μm long, 59 μm wide; inferior volsella absent. GcR 1.90. Gonostylus simple and slender, 69 μm long; megaseta, 10 μm long. HR 1.59. HV 3.39.

Adult female, pupa, and larva. Unknown.

Labrundinia fosteri Roback (Fig. 5)

Labrundinia fosteri Roback 1987: 218 (description of male).

Material examined

Type: Holotype male, Colombia: **Departamento del Meta**, Puerto López, Laguna Mozambique, 16 km s. w. of Puerto López, 25.ii.1972, N. R. Foster (ANSP). Paratype: 1 male same data as holotype .

Diagnostic characters

Labrundinia fosteri differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite II–IV with divided brown transverse band near proximal margin; T V with continuous brown transverse band, T VI–VIII wholly brown; hypopygium with apical 0.50 brown, sternapodeme with anterior process absent.

Redescription

Adult male ($n = 2$ unless otherwise stated)

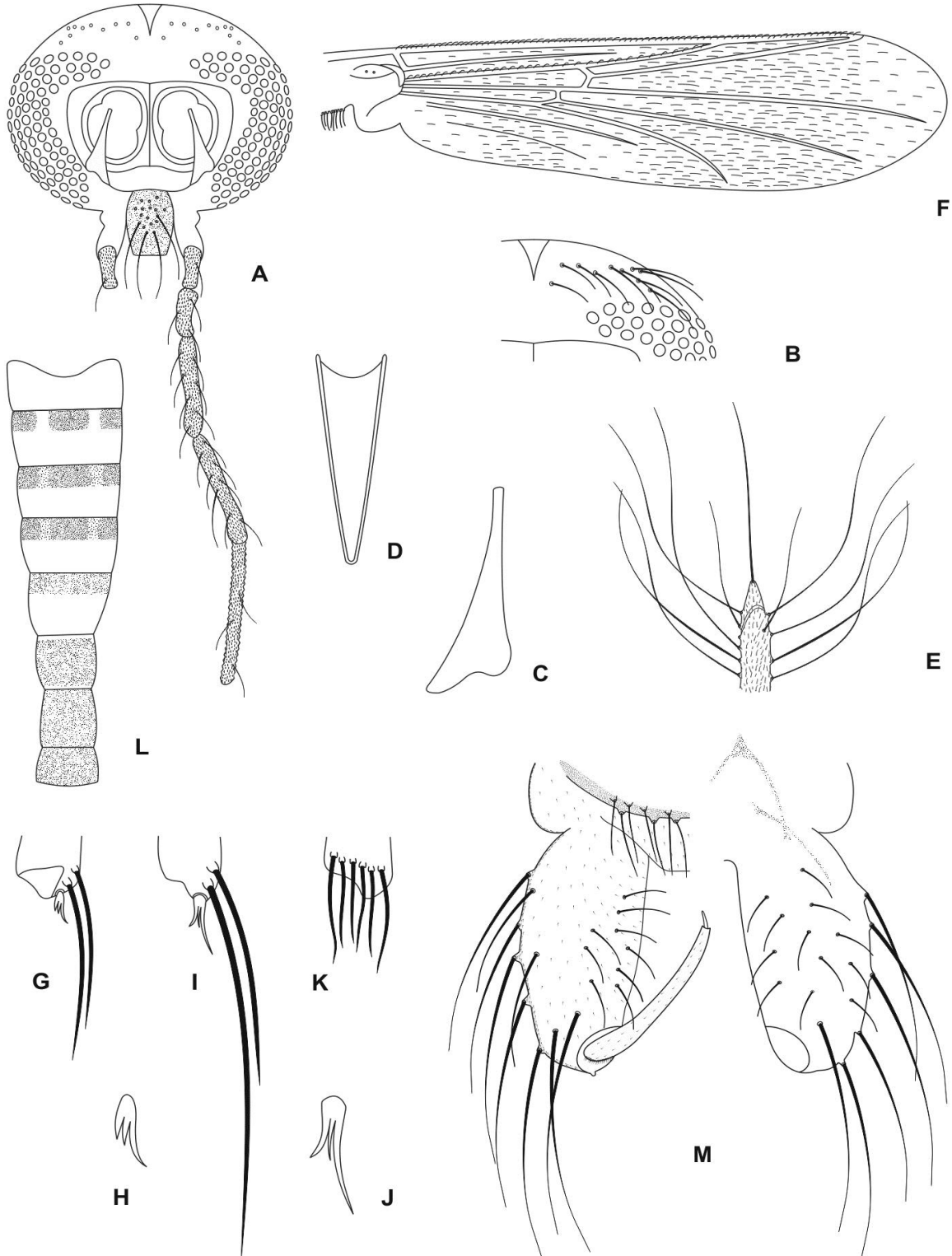
Size. Total length 2.11 (1) mm. Wing length 1.16–1.17 mm. Total length/wing length 1.79 (1). Wing length/length of profemur 2.61 (1).

Coloration. Head dark brown with brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax brown with mesonotum dorsally light brown; anteprenotum pale; supraalar callus pale brown. Wing membrane transparent with two bands present formed mostly by dark hairs, veins brown and macrotrichia on veins. Legs pale with apex brown. Tibias basally brown. Abdomen pale brown with maculation as in figure 5L. Hypopygium pale.

Head (Figs 5A–E). Temporal setae 10, uniserial (Fig. 5B). Eye ratio 1.04–1.33. Tentorium (Fig. 5C) 104–108 μm long, stipes not measurable. Clypeus 125–141 μm long, 90 μm wide at largest part, bearing 16–19 setae. Cibarial pump (Fig. 5D) with anterior margin concave, 157–182. Palpomere lengths 1–2 (in μm): 49; 102. Antenna with 14 flagellomeres, AR 1.35 (1), flagellum 700 μm long, diameter of pedicel 107 (1) μm , apical setae single (Fig. 5E).

Thorax. Anteprenotum lateral setae unobserved. Acrostichals 44 (1), biserial, diverging evenly posteriorly starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20–21, irregularly uniserial; prealars 5–7; supraalars 2. Anapleural suture ratio 0.47. Scutellum with 9–10 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 5F). Width 0.36–0.38 mm. Costa 0.99–1.01 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.06 mm long. VR 0.70–0.74. WW 0.29–



Figures 5A–M. *Labrundinia fosteri* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

0.32. Brachiolum setae unobserved. Squama setiferous.

Legs (Figs 5G–K). Fore leg: width at apex of tibia 31 (1) μm (Fig. 5G), tibia with single, apical and pectinate spur 15 (1) μm long (Fig. 5H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 31 (1) μm long (Fig. 5I), tibia with single, apical and pectinate spur 20 (1) μm long with three teeth (Fig. 5J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 31 (1) μm long (Fig. 5K), tibia without spur; comb with 6 setae; Ta_1 with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table IV.

Table IV. Lengths (in μm) and proportions of leg segments in *Labrundinia fosteri* Roback, adult male ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	445	430	215	203	97
p ₂	648	437	507	207	141
p ₃	500	481	512	234	172
	ta ₄	ta ₅	LR	BV	SV
p ₁	85	70	0.50	2.38	4.07
p ₂	94	86	1.16	3.02	2.13
p ₃	109	78	1.07	2.51	1.91

Hypopygium (Fig. 5M). Tergite IX arched, with 12 dorsal setae. Anal point trapezoidal. Phallapodeme 86–122 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 137–141 μm long, 69–71 μm wide; inferior volsella absent. GcR 1.95–2.06. Gonostylus simple and slender, 98–108 μm long; megaseta 12–14 μm long. HR 1.42–1.44. HV 2.20 (1).

Adult female, pupa, and larva. Unknown.

Labrundinia hirsuta Roback (Fig. 6)

Labrundinia hirsuta Roback 1987: 221 (description of male).

Material examined

Type: Holotype male, Colombia: **Departamento del Meta**, Puerto López, Laguna Mozambique, 16 km s. w. of Puerto López, 25.ii.1972, N. R. Foster (ANSP). Paratype: 1 male same data as holotype except for

6.iii.1971. 3 male same data as holotype except for 9.iii.1971.

Diagnostic characters

Labrundinia hirsuta differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite I–II pale, II–V with narrow, laterally tapered band T VI with U-shaped band, T VI–VIII almost wholly brown; hypopygium pale, sternapodeme with distinct anterior process.

Adult male (n = 5 unless otherwise stated)

Size. Total length 2.15–2.50 (2) mm. Wing length 1.22–1.31 (4) mm. Total length/wing length 2.04–2.15 (2). Wing length/length of profemur 2.81–3.11 (3).

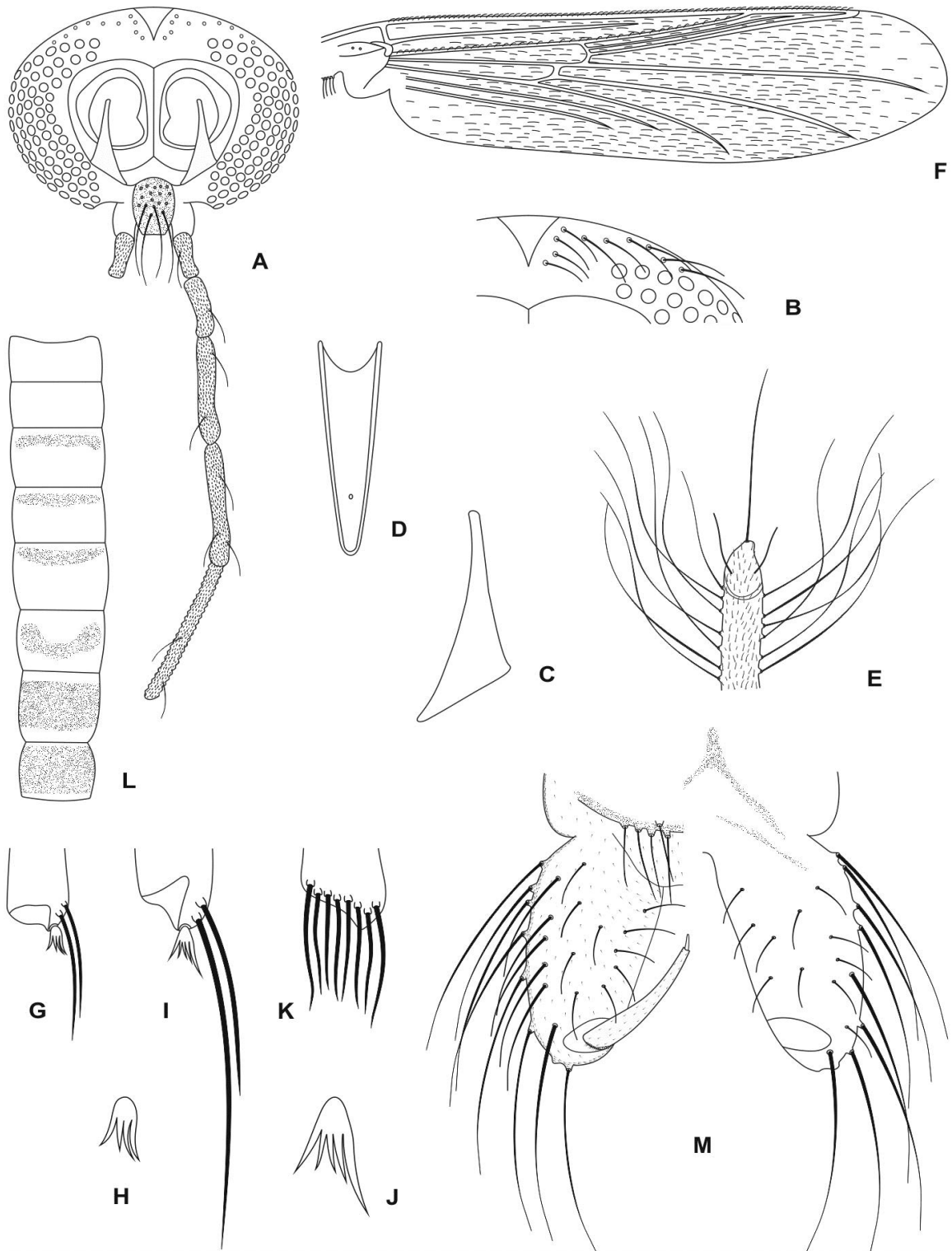
Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax dark brown; anteprenotum pale; supraalar callus dark brown. Wing membrane transparent with one median band present, about $\frac{2}{3}$, formed mostly by dark hairs, veins brown and macrotrichia on veins. Legs pale brown. Femur I with apex brown, Fe II–III brown. Tibia I with apex and base brown, Ti II pale, Ti III with apex brown. Tarsomere 1 with apex brown, tarsomeres 2–5 pale brown. Abdomen pale brown with maculation as in figure 6L. Hypopygium pale.

Head (Figs 6A–E). Temporal setae 10–15, uniserial (Fig. 6B). Eye ratio 1.18–1.50. Tentorium (Fig. 6C) 112–147 μm long, stipes not measurable. Clypeus 98–125 (4) μm long, 69–88 μm wide at largest part, bearing 16–19 setae. Cibarial pump (Fig. 6D) with anterior margin concave, 161–170 (4). Palpomere lengths 1–5 (in μm): 33–37 (4); 49–65; 118–133; 137–159 (4); 233–239 (4). Antenna with 14 flagellomeres, AR 1.52–1.55 (2), flagellum 843–857 (4) μm long, diameter of pedicel 107–119 (3) μm , apical setae single (Fig. 6E).

Thorax. Anteprenotum with 5–6 (4) lateral setae. Acrostichals 40–44 (4), biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20–22, irregularly biserial; prealars 8–9 (4); supraalars 3 (4). Anapleural suture ratio 0.46–0.52. Scutellum with 8–10 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 6F). Width 0.34–0.38 (4) mm. Costa 1.03–1.10 (4) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.05–0.06 mm long. VR 0.72–0.77 (4). WW 0.27–0.31 (4). Brachiolum with 2 setae (3). Squama setiferous.

Legs (Figs 6G–K). Fore leg: width at apex of tibia 31–39 μm (Fig. 6G), tibia with single, apical and pectinate spur 13–16 (3) μm long (Fig. 6H), with four and two preapical setae; Ta_1 with two preapical



Figures 6A–M. *Labrundinia hirsuta* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

pseudospurs. Mid leg: width at apex of tibia 31–39 (4) μm long (Fig. 6I), tibia with single, apical and pectinate spur 16–25 (3) μm long with four teeth (Fig. 6J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 39 (2) μm long (Fig. 6K), tibia without spur; comb with 8 (4) setae; Ta_1 with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table V.

Table V. Lengths (in μm) and proportions of leg segments in *Labrundinia hirsuta* Roback, adult male ($n = 5$).

	fe	ti	ta_1	ta_2	ta_3
p_1	422–481	438–481	273–297 (2)	188–195 (2)	125–141 (2)
p_2	602–688	503–523	581 (1)	259 (1)	141
p_3	523–546 (3)	643 (1)	555 (1)	281 (1)	184 (1)
	ta_4	ta_5	LR	BV	SV
p_1	94–96 (2)	63 (2)	0.55–0.62 (2)	2.42–2.62 (2)	3.02–3.62 (2)
p_2	95 (1)	78 (1)	1.16 (1)	2.97 (1)	1.95 (1)
p_3	133(1)	86 (1)	0.86 (1)	2.54 (1)	2.13 (1)

Hypopygium (Fig 5M). Tergite IX arched, with 12–13 dorsal setae. Anal point trapezoidal, slightly concave. Phallapodeme 55–67 (2) μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 137–151 μm long, 62–75 μm wide; inferior volsella absent. GcR 1.89–2.40. Gonostylus simple and slender, 86–92 μm long; megaseta, 16–18 (3) μm long. HR 1.55–1.75 (4). HV 2.44 (1).

Adult female, pupa, and larva. Unknown.

Labrundinia johannseni Beck and Beck (Figs 7–9)

Labrundinia johannseni Beck and Beck 1966: 342 (description of male, female and immatures stages).

Labrundinia johannseni Roback 1971: 279 (description of male).

Labrundinia johannseni Roback 1987: 196 (description of immature stages).

Labrundinia johannseni Epler 2001: 4.47 (larvae identification key).

Labrundinia johannseni Jacobsen 2008: 42 (pupae identification key).

Type material examined

Type: Holotype male with pupal and larval exuviae, USA: **Florida**, Flagler County, Rayonier

Ditch, 30.i.1965, W. Beck (USNM).

Additional. 1 larva, USA: **Florida**, Flagler County, Rayonier Ditch, 17.x.1967, W. Beck; 1 larva as previous except for 30.i.1965; 1 female with pupal exuvia as previous except for 27.ii.1965; 1 larva as previous except for Orange, Shinae Creek, 14.i.1958; 1 larva as previous except for Highlands, Arbuckle Creek, 3.ii.1987, R.P Rutter; 1 larva as previous except for Santa Rosa, Black river at Wright basin, 3.viii.1976, G. Bass. 1 male, **Georgia**, Decatur, Little Attapulgus Creek, Georgia Hwy 241, 7.xi.1984, B.A. Caldwell.

Diagnostic characters

Labrundinia johannseni differs from other *Labrundinia* species by the combination of the following characters. **Male:** abdominal tergite II, IV with continuous brown transverse band, T III over 0.75 brown, T V, VI–VIII almost wholly brown; hypopygium pale brown, sternapodeme with reduced anterior process. **Pupa:** Thoracic horn ovoid; preapical indentation absent; thoracic horn ratio 2.07–2.20. **Larva:** Surface of head capsule smooth with brown transverse band, lateroventral spine group present with several conspicuous spines; subbasal seta multitoothed; bifid claw with U-shaped lower indentation.

Redescription

Adult male (n = 2 unless otherwise stated)

Size. Total length 2.18–2.39 mm. Wing length 1.38–1.45 mm. Total length/wing length 1.50–1.73. Wing length/length of profemur 2.63 (1).

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally light brown; anteprenotum pale yellow; supraalar callus pale brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale. Abdomen as in figure 7L. Hypopygium pale brown.

Head (Figs 7A–E). Temporal setae 10 (1), uniserial (Fig. 7B). Eye ratio 0.94 (1). Tentorium (Fig. 7C) 171 (1) μm long, stipes not measurable. Clypeus 85 (1) μm long, 62 (1) μm wide at largest part, bearing 24 (1) setae. Cibarial pump (Fig. 7D) with anterior margin concave, 213 (1) μm long. Palpomere lengths 1–5 (in μm): 41 (1); 77 (1); 137 (1); 150 (1); 209 (1). Antenna with 14 flagellomeres, AR 1.27–1.34, flagellum 692–813 μm long, diameter of pedicel 116–118 μm , apical setae single (Fig. 7E).

Thorax. Anteprenotum lateral setae unobserved. Acrostichals 44–50, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 24–26,

irregularly uniserial; prealars 12–15; supraalars 2–3. Anapleural suture ratio 0.45–0.46. Scutellum with 10 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 7F). Width 0.38–0.39 mm. Costa 1.09–1.18 mm long, not produced beyond R₄₊₅, ending very slightly beyond tip of M₃₊₄. R₂₊₃ present, but not very discernible. Base of radial sector 0.07–0.08 mm long. VR 0.74–0.75. WW 0.26–0.28. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 7G–K). Fore leg: width at apex of tibia 39–42 µm (Fig. 7G), tibia with single, apical and pectinate spur 12–18 µm long (Fig. 7H), with three teeth and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Mid leg: width at apex of tibia 36–39 µm long (Fig. 7I), tibia with single, apical and pectinate spur 22–23 µm long with three teeth (Fig. 7J) and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Hind leg: width at apex of tibia 37–47 µm long (Fig. 7K), tibia without spur; comb with 5 setae; preapical pseudospurs on Ta₁₋₄ unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table VI.

Table VI. Lengths (in µm) and proportions of leg segments in *Labrundinia johannseni* Beck and Beck, adult male (n = 1–2 unless otherwise stated).

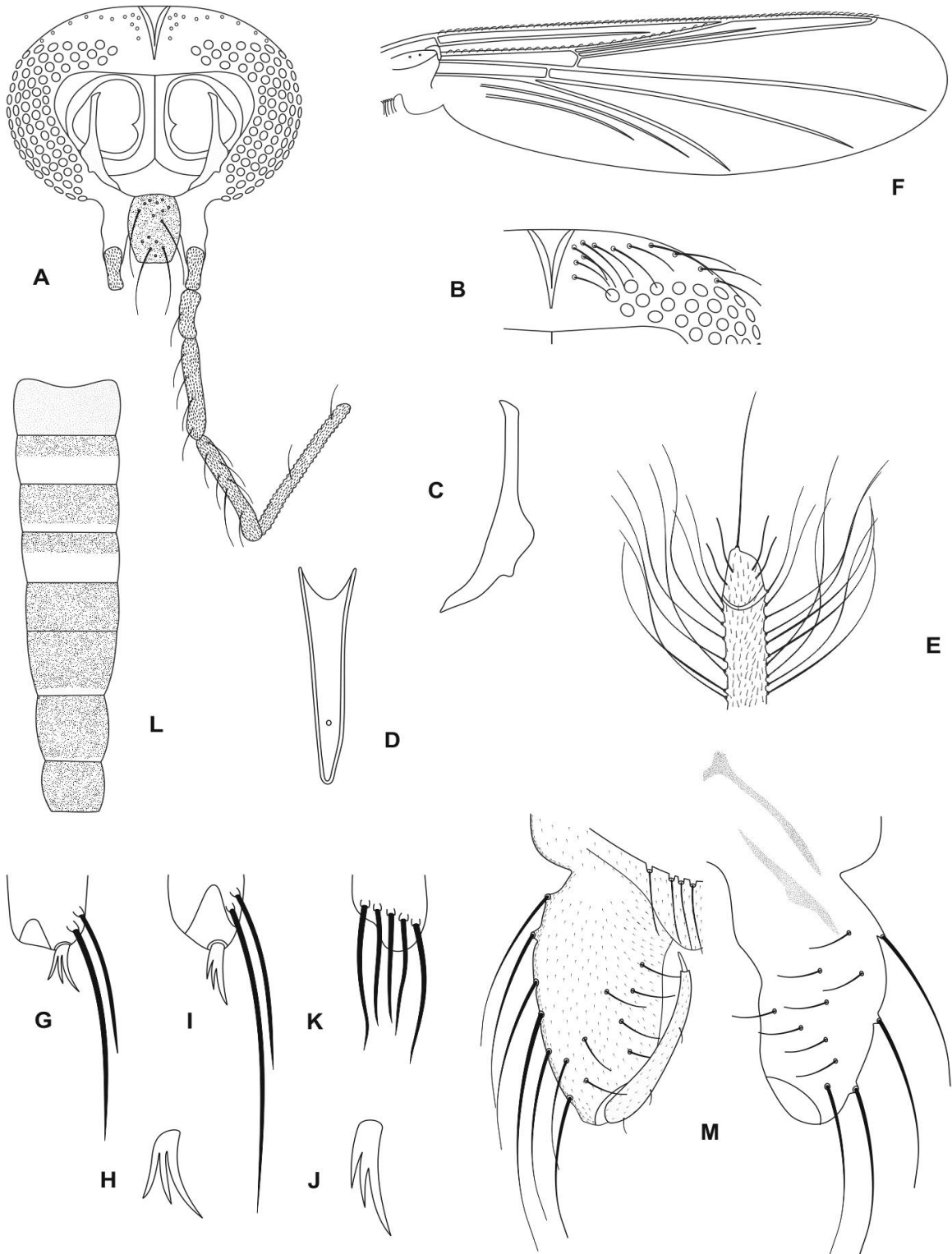
	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	399–524	429–549	313	234	180
p ₂	684–687	453–594	–	–	–
p ₃	547–590	664–810	547	273	195
	ta ₄	ta ₅	LR	BV	SV
p ₁	125	78	0.72	1.85	2.65
p ₂	–	–	–	–	–
p ₃	125	78	0.82	2.61	2.21

Hypopygium (Fig. 7M). Tergite IX arched, with 13 dorsal setae. Anal point rounded. Phallapodeme 53–56 µm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 108–124 µm long, 57 µm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.90–2.19. Gonostylus simple and slender, 74–86 µm long; megaseta 12–18 µm long. HR 1.44–1.45. HV 2.77–2.92.

Adult female (n = 1 unless otherwise stated)

Size. Total length 1.24 mm. Wing length 1.16 mm. Total length/wing length 1.07. Wing length/length of profemur 3.45.

Coloration. Head, pedicel, antenna and maxillary palp pale brown. Thorax pale brown with mesonotum dorsally darker; antepnotum pale yellow; supraalar callus brown. Wing membrane transparent without



Figures 7A–M. *Labrundinia johannseni* Beck and Beck, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

spots veins pale brown; macrotrichia on veins. Legs pale. Abdomen dark brown, not very discernible. Seminal capsules brown.

Head. Temporal setae uncounted, uniserial. Clypeus 57 μm long, 48 μm wide at largest part, setae uncounted. Cibarial pump as in male, 128 μm long. Palpomere lengths 1–4 (in μm): 34; 53; 99; 128. Antenna with 11 flagellomeres, AR 0.33, flagellum 278 μm long.

Thorax. Anteprenotum lateral setae unobserved. Acrostichals 30, irregularly biserial, starting close to anteprenotum; dorsocentrals 34, irregularly uniserial; prealars 12; supraalars 2. Scutellum with 10 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.37 mm. Costa 1.00 mm long, not produced beyond R_{4+5} . R_{2+3} unobserved. Base of radial sector 0.07 mm long. VR 0.87. WW 0.32. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 23 μm , tibia with single, apical and pectinate spur 16 μm long, with two teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Mid leg: width at apex of tibia 35 μm , tibia with single, apical and pectinate spur 18 μm long, with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Hind leg: width at apex of tibia 32 μm , tibia without spur; comb setae unobserved; preapical pseudospurs on Ta_{1-4} unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table VII.

Table VII. Lengths (in μm) and proportions of leg segments in *Labrundinia johannseni* Beck and Beck, adult female.

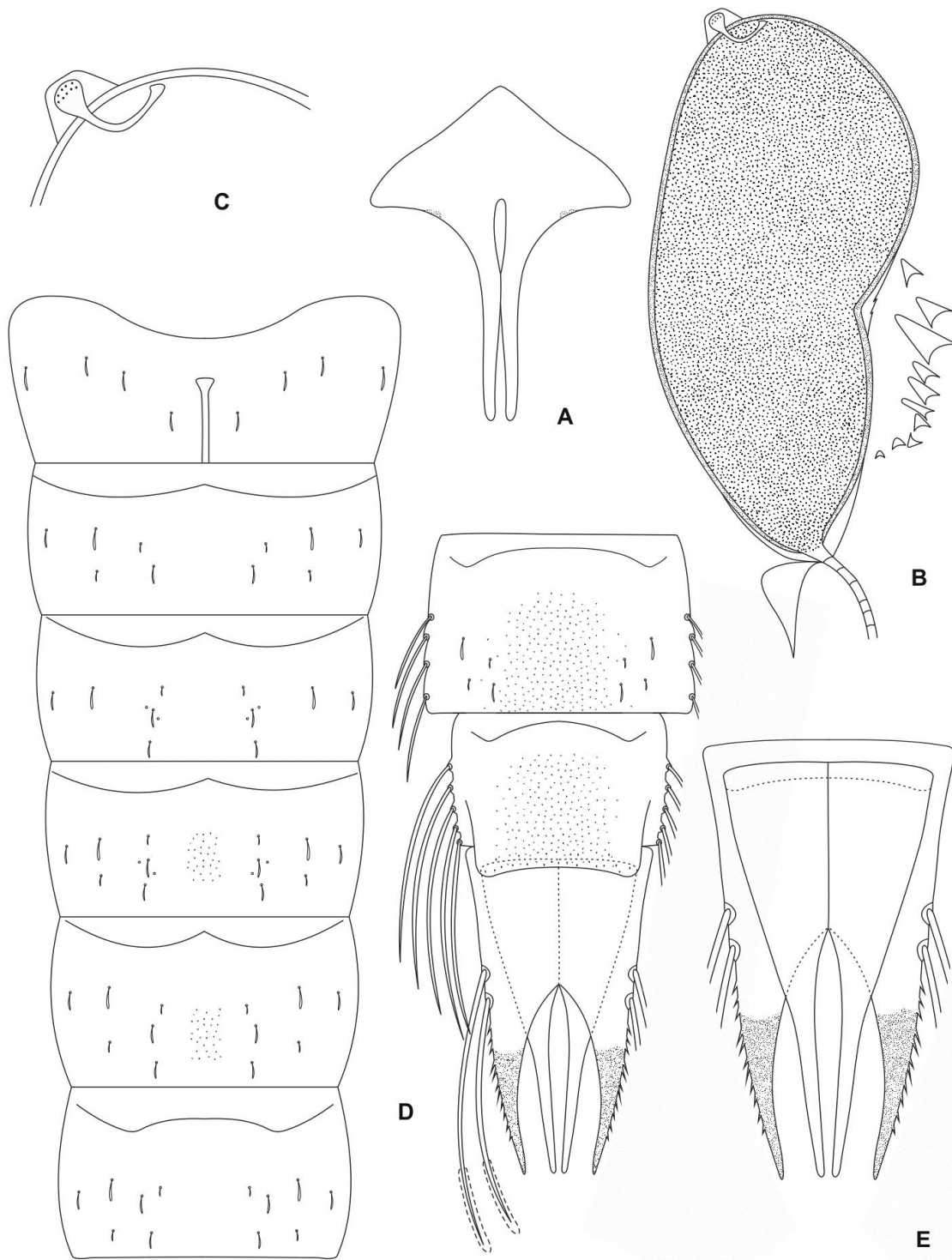
	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	336	343	245	178	118
p ₂	458	422	–	–	–
p ₃	395	537	478	225	158
	ta ₄	ta ₅	LR	BV	SV
p ₁	82	67	0.71	2.07	2.77
p ₂	–	–	–	–	–
p ₃	103	78	0.89	2.50	1.95

Genitalia. Cerci oval-quadrate. Seminal capsules oval with conical shaped necks, length 38 μm , maximum width 21 μm .

Pupa ($n = 2$ unless otherwise stated)

Size. Male abdomen 2.06 (1) mm long; female abdomen 2.00 (1) mm long.

Coloration. Exuviae and thoracic horn brown. Apex of anal lobe brown.



Figures 8A–E. *Labrundinia johannseni* Beck and Beck, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Cephalothorax (Figs 8A–C). Frontal apotome as in figure 8A. Wing sheath smooth 0.89–1.01 mm long. Thoracic horn 221–258 µm long and 106–117 µm wide (Fig.8B), preapical indentation absent, THR 2.07–2.20. Membranous preapical papilla 27 (1) µm long (Fig. 8C), PTH 0.11 (1), aeropyle tube simple, short, unmeasured; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct. Basal lobe wedge-shaped. Thoracic comb with 10 conical teeth (Fig.8B).

Abdomen (Figs 8D–E). Tergite I with scar, 129 (1) µm long. T I–III without shagreen, IV–V with a few spines medially concentrated, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 8D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 255–257 µm long and 140–162 µm wide (Fig. 8E), outer margins sclerotized, with 10–12 spines, longest spine 12–15 µm long, inner margins of lobes membranous. ALR 1.56–1.83. Genital sac elongated, almost reaching apex of anal lobe.

4th instar larva (n = 7 unless otherwise stated)

Coloration. Head pale yellow, with brown transverse band; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 9A). Length 490 (1) µm, 315 (1) µm wide. Surface smooth; lateroventral spine group present, well developed, with 10–16 spines; posteroventral spine group absent; cephalic index 0.62–0.72. Chaetotaxy as in figure 9A.

Antenna (Figs 9B–C). Length 246–288 µm, A₁ 167–198 µm long, with ring organ placed 142–185 µm from base, A₂ 69–78 µm long. AR 2.13–2.42. Blade longer than A₂ over-reached by accessory blade.

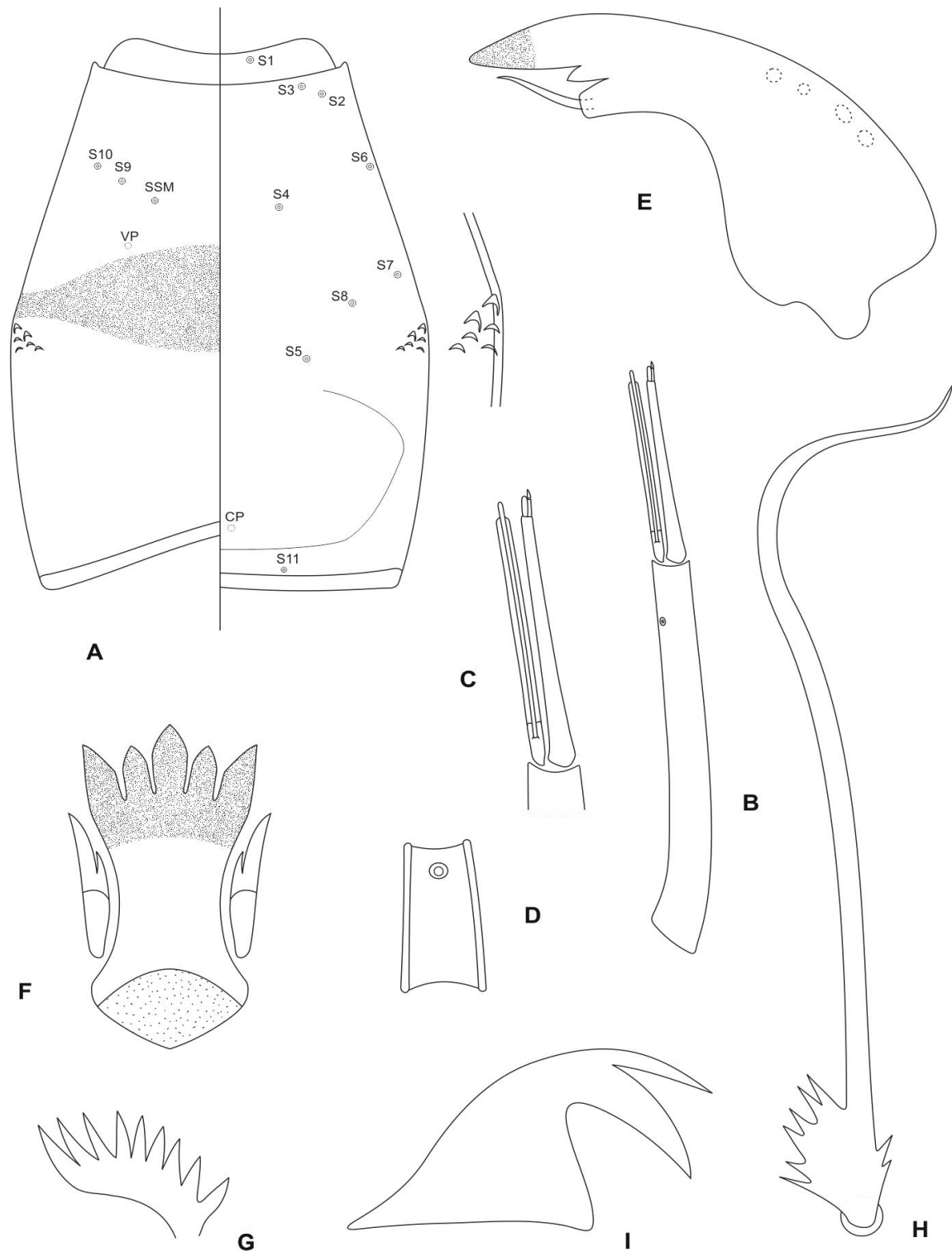
Maxilla (Fig. 9D). Basal palp segment 20–32 µm long and 7–11 µm wide, with ring organ 15–26 µm from base. PR 1.78–3.21. APR 5.98–9.25.

Mandible (Fig. 9E). Length 60–69 µm, with 3 lateral setae. Sensillum campaniformium 39–50 µm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 3.28.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 9F–G). Ligula 55–66 µm long, 25–28 µm wide, with row of 5 teeth. IO 0.80–0.98, MO 1.05–1.08. Paraligula bifid, 24–31 µm long, inner tooth 19–25 µm long, shorter than outer tooth. Pecten hypopharyngis with 7 teeth almost equal in size.

Body (Figs 9H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 137–170 µm long, 31–43 µm wide, with 7 anal setae 511–598 (6) µm long. L/W 3.60–5.42. Supraanal seta well



Figures 9A–I. *Labrundinia johannseni*, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

developed. Anal tubules 137–176 (3) μm long. Posterior parapod 343–370 (5) μm long; subbasal seta multitoothed, with basal spine group, with 7–9 spines (Fig. 9H); parapod apex with numerous simple claws, without serrated claw; bifid claw with U-shaped lower indentation (Fig. 9I). B/C 0.86–0.98 (6).

Labrundinia longipalpis (Goetghebuer) (Figs 10–12)

Tanypus longipalpis Goetghebuer 1921: 18 (description of male).

Tanypus longipalpis Goetghebuer 1927: 61 (redescription of male).

Pentaneura longipalpis Edwards 1929: 294 (description of male).

Ablabesmyia longipalpis Goetghebuer 1936: 43 (description of male, new combination).

Labrundinia longipalpis Fittkau 1962: 376 (description of male and pupa, new combination).

Labrundinia maculata Roback 1971:271 (description of male and female).

Labrundinia maculata Roback 1987a: 192 (description of immatures).

Labrundinia maculata Epler 2001: 4.46 (larvae identification key).

Labrundinia maculata Jacobsen 2008: 42 (pupae identification key).

Labrundinia maculata Silva *et al.* 2011: 294 (description of male, female and immatures, new synonym).

Material examined

Type: Labrundinia longipalpis: 3 syntype males, BELGIUM: **Flandres**, Broeck d'Overmeire, Étang d'Overmeire, 25.v.1913, M. Goetghebuer(IRSNB). *Labrundinia maculata*: Holotype male, USA: **California**, Inland Empire, Riverside County, 11–14.ix.1962, Frommer (CAS). Paratypes: 2 males and 2 females same data as holotype; 1 male and 2 females, USA: **California**, Santee-San Diego, 27.vii.1962, Perry (ANSP).

Additional: 2 pupal exuviae, CANADA: **Ontario**, Kingston, Lake near Parham, 23.vii.1980, F. Reiss (ZSM). 1 male, GREECE: **Corfu**, Temploni, 13.vi.1977, H. Malicky. (ZSM) 3 pupal exuviae, MEXICO: Laguna Alberga, 3.i.1954, L. Brundin(ZSM); 1 larva, Coahuila, ditch S. of Palau, 19/v/78, Dunn & Bereza (ANSP). 1 larva, NETHERLANDS: **Noord-Holland**, s'Graveland, 10.v.1982, H. Steenbergen (ZSM). 1 male, ROMANIA, P. Albu (ZSM). 1 male, SPAIN: **Andalusia**, Huelva, Riv. d. Tamujoso, 24–28.iv.1981, W. Schacht (ZSM). 1 pupal exuvia, SWEDEN: **Götland**, Småland, Grimsgöl Lake, 15.vi.47, L. Brundin. 1 male, USA: **California**, Orange County, Irvine, Woodbridge village, from spider webs, v.1993, M. Spies (ZSM); 1 male with larval and pupal exuviae, **Georgia**, Glynn County, pond at Plantation Village, Demere RD, St. Simons Isl., 20.ii.1992, B. A. Caldwell; 1 male with larval and pupal

exuviae as previous except for 28.xi.1990; 1 male with larval and pupal exuviae as previous except for 7.v.1991; 1 female with larval and pupal exuviae as previous except for 27.ii.1991; 1 male with pupal exuvia, 1 prepupa and 1 larva, **Kansas**, Cherokee, Short Creek, 1.6 mi W, 1.0 mi N Galen, 9.ix.86, L. C. Ferrington (ANSP); 1 male and 1 pupal exuvia, **North Carolina**, Belews Lake, 10.v.1976 (ANSP).

Diagnostic characters

Labrundinia longipalpis differs from other *Labrundinia* species by the combination of the following characters **Male**: abdominal tergite I pale brown, II–VI with continuous brown transverse band near proximal margin, T VII–VIII wholly brown; hypopygium pale brown, sternapodeme with reduced anterior process. **Pupa**: thoracic horn wedge-shaped, preapical indentation absent, external membrane with pale spinules basally concentrated. **Larva**: surface of head capsule usually covered with spinules, lateroventral spine group present but weakly developed; subbasal seta multitoothed; bifid claw with U-shaped lower indentation.

Redescription

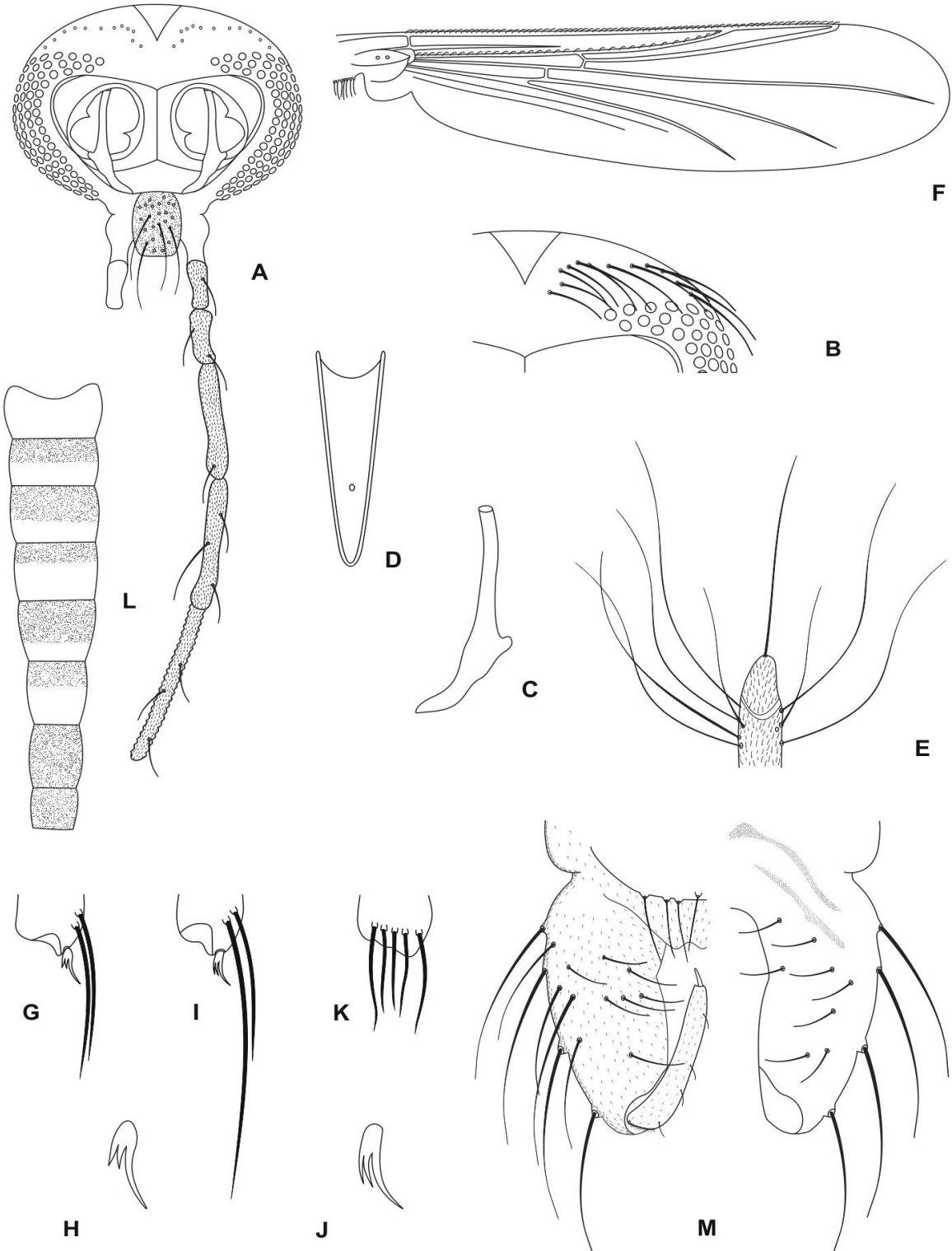
Adult male ($n = 12$ unless otherwise stated)

Size. Total length 1.95–2.61 (7) mm. Wing length 1.23–1.55 (6) mm. Total length/wing length 1.56–1.84. Wing length/length of profemur 2.43–2.90 (8).

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen pale brown with maculation as in figure 10L. Hypopygium pale brown.

Head (Figs 10A–E). Temporal setae 12–14 (11), uniserial (Fig. 10B). Eyes ratio 1.10–1.39 (7). Tentorium (Fig. 10C) 128–145 (4) μm long, stipes not measurable. Clypeus 65–100 (7) μm long, 50–88 (7) μm wide at largest part, bearing 16–24 setae. Cibarial pump (Fig. 10D) with anterior margin concave, 157–185 μm long. Palpomere lengths 1–5 (in μm): 23–50 (10); 46–82 (10); 100–129 (9), 100–149 (8); 146–237 (5). Antenna with 14 flagellomeres, AR 0.97–1.27 (10), flagellum 559–754 (10) μm long, diameter of pedicel 103–114 (10) μm , apical setae single (Fig. 10E).

Thorax. Anteprenotum with 4–9 (5) lateral setae. Acrostichals 33–48 (10), biserial, diverging evenly posteriorly starting close to anteprenotum and almost reaching scutellum; dorsocentrals 22–28 (11),



Figures 10A–M. *Labrundinia longipalpis* (Goetghebuer), adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

irregularly uniserial; prealars 8–13; supraalars 3–4 (10). Anapleural suture ratio 0.38–0.65 (7). Scutellum with 8–12 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 10F). Width 0.30–0.44 (10) mm. Costa 0.88–1.28 (10) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.04–0.09 (10) mm long. VR 0.63–0.91 (10). WW 0.26–0.31 (10). Brachiolum with 2 setae (9). Squama with 13–16 (2) setae.

Legs (Figs 10G–K). Fore leg: width at apex of tibia 31–47 (10) μm (Fig. 10G), tibia with single, apical and pectinate spur 12–17 (7) μm long (Fig. 10H), with three teeth and two preapical setae; Ta_{1-3} each with two preapical pseudospurs. Mid leg: width at apex of tibia 31–44 (11) μm long (Fig. 10I), tibia with single, apical and pectinate spur 18–23 (8) μm long with three teeth (Fig. 10J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 31–56 (10) μm long (Fig. 10K), tibia without spur; comb with 5 (11) setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table VIII.

Table VIII. Lengths (in μm) and proportions of leg segments in *Labrundinia longipalpis* (Goetghebuer), adult male (n = 10–12 unless otherwise stated).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	398–519	431–581	263–338	194–269 (6)	131–194 (6)
p ₂	406–650	414–563	400–625	206–294 (5)	140–170 (5)
p ₃	436–630	555–800	550–694	238–306 (3)	175–213 (3)
	ta ₄	ta ₅	LR	BV	SV
p ₁	94–125 (5)	69–82 (6)	0.56–0.63 (6)	2.06–2.37 (6)	2.97–3.34 (6)
p ₂	94–125 (5)	60–88 (5)	0.71–1.23 (5)	2.70–3.06 (5)	1.90–2.98 (5)
p ₃	94–138 (3)	69–94 (3)	0.90–0.99 (3)	2.72–2.87 (3)	1.84–1.97 (3)

Hypopygium (Fig. 10M). Tergite IX arched, with 10–17 (11) dorsal setae. Anal point trapezoidal, apical edge slightly notched. Phallapodeme 35–66 (11) μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 88–125 (11) μm long, 35–65 (11) μm wide, with slightly concave inner margin; dorsomedian surface with shorter marginal setae scarcely distributed; inferior volsella absent. GcR 1.80–2.60 (11). Gonostylus simple and slender, 59–80 (11) μm long; megaseta enlarged, 12–15 (11) μm long. HR 1.36–1.71 (11). HV 3.16–3.74 (7).

Adult female (n = 4 unless otherwise stated)

Size. Total length 2.03 (1) mm. Wing length 1.09–1.44 (2) mm. Total length/wing length 1.58 (1). Wing length/length of profemur 2.82 (1).

Coloration. Head, pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen brown with tergites 2, 4 and 6 lighter apically. Seminal capsules brown.

Head. Temporal setae 12–14, uniserial. Eyes bare, dorsomedian extension with 2–3 (3) terminal facets; eye ratio 0.81–1.41 (3). Tentorium 110 (1) μm long, stipes not measurable. Clypeus 83 (1) μm long, 50 (1) μm wide at largest part, bearing 20–32 setae. Cibarial pump as in male, 141–151 (2) μm long. Palpomere lengths 1–5 (in μm): 26–27 (2); 38–51 (2); 85–100 (2); 108 (1); 129 (1). Antenna with 11 flagellomeres, AR 0.35 (1), flagellum 360 (1) μm long, diameter of pedicel 50 (2) μm .

Thorax. Anteprenotum with 4 (1) lateral setae. Acrostichals 50–52 (2), irregularly biserial, starting close to anteprenotum; dorsocentrals 31–41, irregularly uniserial; prealars 12–16 (5); supraalars 3–4 (3). Scutellum with 8 (3) setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.34–0.41 (2) mm. Costa 0.93 (1) mm long, not produced beyond R_{4+5} . R_{2+3} absent. Base of radial sector 0.06 (1) mm long. VR 0.72 (1). WW 0.31–0.36 (2). Brachiolum with 2 setae (2). Squama with 14 (1) setae.

Legs. Fore leg: width at apex of tibia 39–44 (2) μm , tibia with single, apical and pectinate spur 16–18 (2) μm long, with three teeth and two preapical setae; $Ta_{1,4}$ without preapical pseudospurs. Mid leg: width at apex of tibia 31–38 (2) μm , tibia with single, apical and pectinate spur 12–23, 18 (3) μm long, with 2–3 teeth and two preapical setae; Ta_1 with one preapical pseudospur. Hind leg: width at apex of tibia 36–39 (2) μm , tibia without spur; comb with 4–5 (2) setae; Ta_1 with one preapical pseudospur. Legs with slender, hook-shaped claws. Pseudospurs indistinct. Pulvilli absent. Lengths and proportion of leg segments as in Table IX.

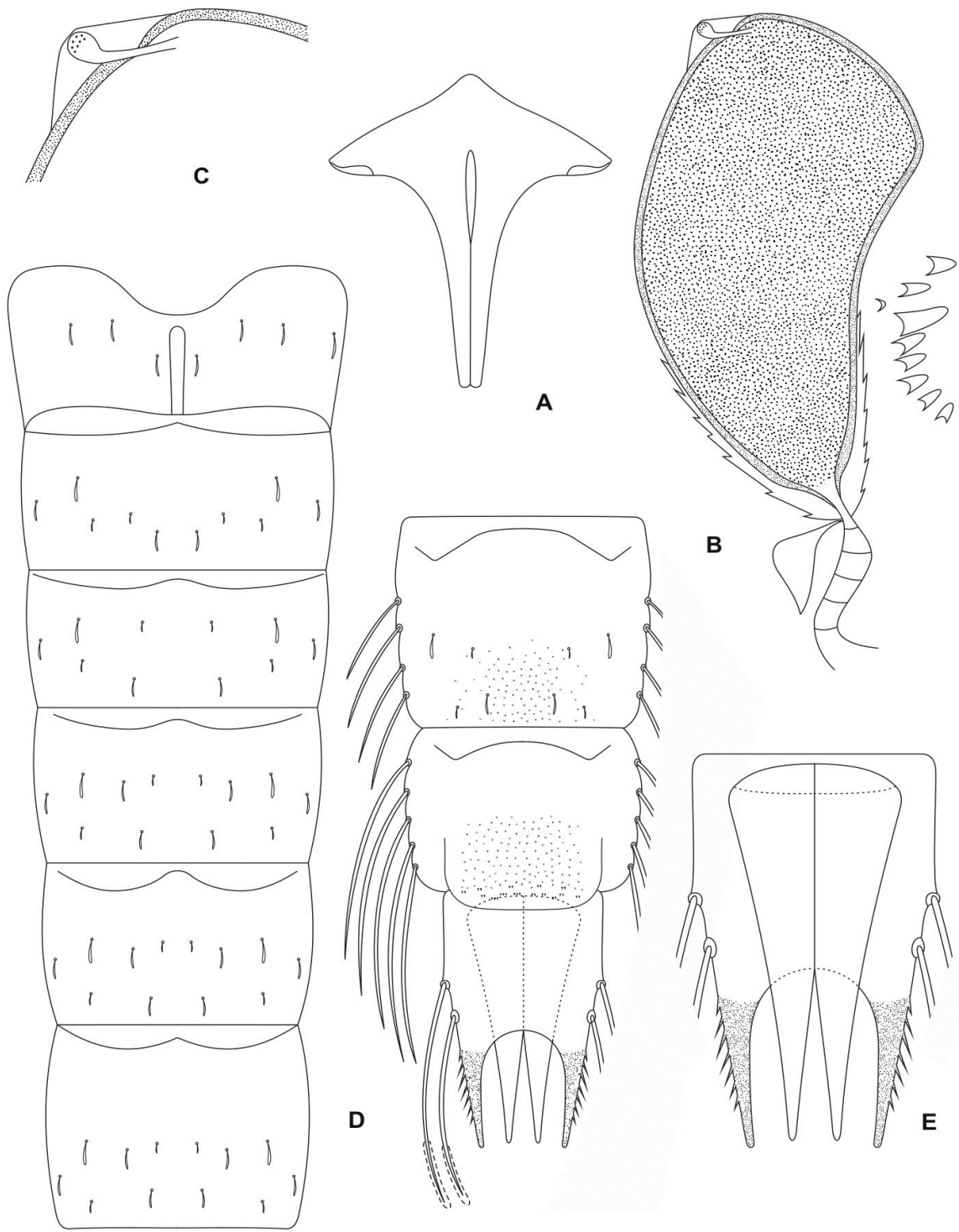
Genitalia. Gonapophysis VIII broadly rounded, 63 (1) μm long. Tergite IX 175 (1) μm long, 100 (1) μm wide at middle, without setae. Coxosternapodeme 75 (1) μm long. Tergite X with 8 lateroventral setae on each side. Postgenital plate rounded. Cerci quadrate to oval-quadrate, 38 (1) μm long and 13 (1) μm wide; with 10 setae (1). Labia with inconspicuous microtrichia. Notum length (from ramus forward) 133 (1) μm . Seminal capsules oval with conical shaped necks, length 40 (1) μm , maximum width 33 (1) μm . Length ratio SCa/No 0.30.

Pupa ($n = 12$ unless otherwise stated)

Size. Male abdomen 1.73–2.58, 1.99 (7) mm long; female abdomen 1.99–2.14, 2.08 (5) mm long.

Coloration. Exuviae pale brown. Thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 11A–C). Frontal apotome as in figure 11A. Wing sheath smooth 0.66–1.09 mm



Figures 11A–E. *Labrundinia longipalpis* (Goetghebuer), pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Table IX. Lengths (in μm) and proportions of leg segments in *Labrundinia longipalpis* (Goetghebuer), adult female (n = 1–2 unless otherwise stated).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	406–414	391–419	260	169	113
p ₂	475–547	436–550	475	206	144
p ₃	519–531	578–625	391–480	234–238	156–175
	ta ₄	ta ₅	LR	BV	SV
p ₁	94	69	0.64	2.46	3.07
p ₂	106	63	0.86	2.89	2.16
p ₃	117–119	69–70	0.68–0.84	2.59–2.78	2.18–2.84

long. Thoracic horn 211–328 μm long and 86–203 μm wide (Fig. 11B), preapical indentation absent, THR 1.61–2.64. Membranous preapical papilla 18–57 μm long (Fig. 11C), PTH 0.08–0.18, aeropyle tube simple, short and robust 28–35 (8) μm long; plastron plate much reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 10–12 conical teeth (Fig. 11B).

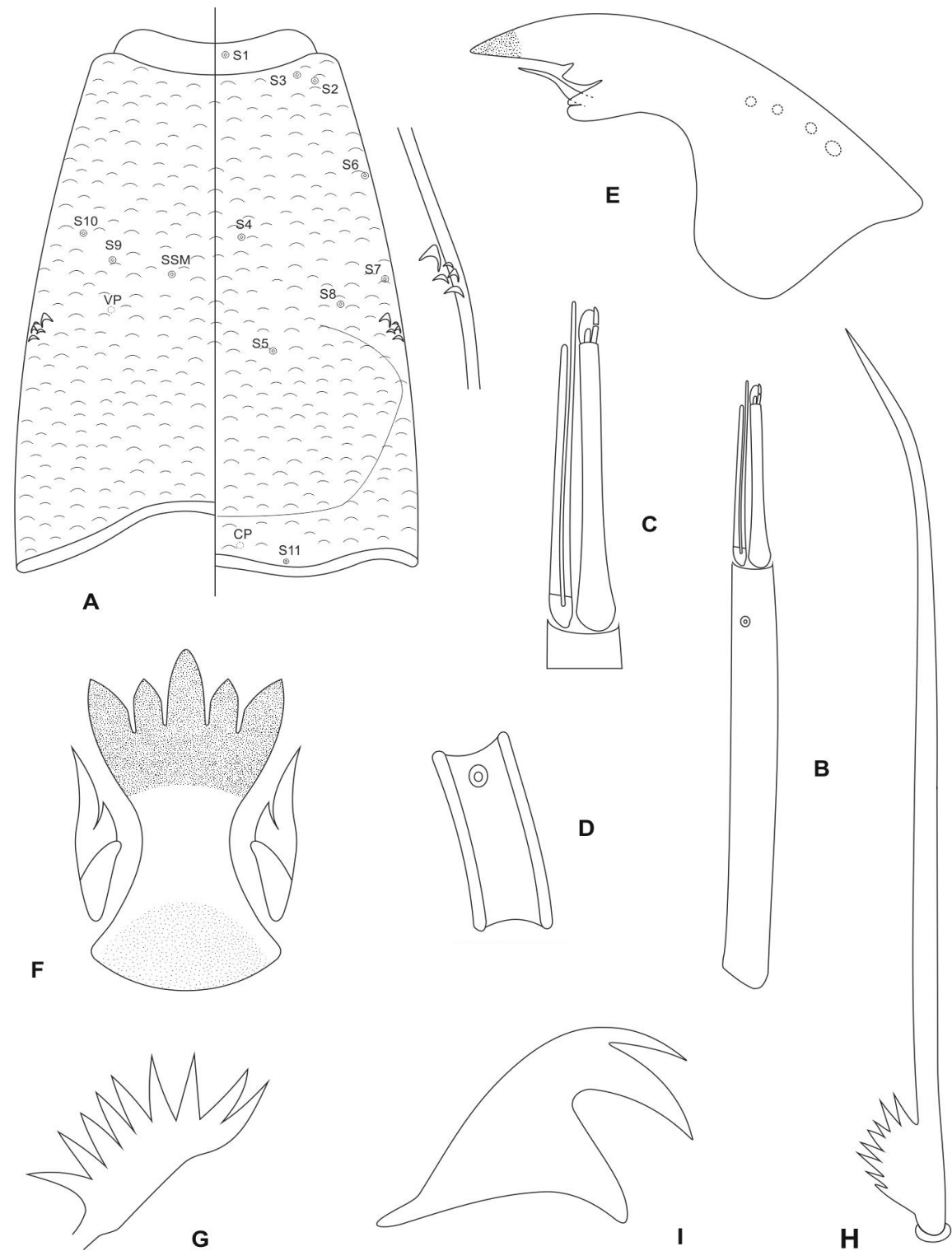
Abdomen (Figs 11D–E). Tergite I with scar, 113–143 (8) μm long. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 11D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 180–368 μm long and 150–186 μm wide (Fig. 11E), outer margins sclerotized, with 8–12 (8) spines, longest spine 8–17 (9) μm long, inner margins of lobes membranous. ALR 1.14–1.80 (9). Genital sac elongated, almost reaching apex of anal lobe.

4th instar larva (n = 8 unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 12A). Length 398–555, 491 μm , 260–406, 337 μm wide. Surface usually covered with spinules; lateroventral spine group present but weakly developed, with 5–6 (6) spines; posteroventral spine group absent; cephalic index 0.50–0.80, 0.69. Chaetotaxy as in figure 12A.

Antenna (Figs 12B–C). Length 236–284 (4) μm , A₁ 163–205 (4) μm long, with ring organ placed 125–155 (4) μm from base, A₂ 63–80 (4) μm long. AR 2.28–2.56 (4). Blade longer than A₂ over-reached by accessory blade.



Figures 12A–I. *Labrundinia longipalpis* (Goetghebuer), larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

Maxilla (Fig. 12D). Basal palp segment 20–29 μm long and 7–11 μm wide, with ring organ 15–19 μm from base. PR 2.08–3.67. APR 7.45–8.90.

Mandible (Fig. 12E). Length 63–75 μm , with 3 lateral setae. Sensillum campaniformium 43–55 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.39–3.28.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 12F–G). Ligula 51–63 (7) μm long, 23–32 (7) μm wide, with row of 5 teeth. IO 0.98–1.05 (7), MO 1.09–1.17 (7), muscle attachment 13–20 (7) μm long. Paraligula bifid, 22–27 (7) μm long, inner tooth 19–23 (7) μm long, shorter than outer tooth. Pecten hypopharyngis with 9 (5) teeth almost equal in size (Fig. 12G).

Body (Figs 12H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 109–166 μm long, 23–34 μm wide, with 7 anal setae 500–749 μm long. L/W 3.39–5.43 (7). Supraanal seta well developed. Anal tubules 163–206 (3) μm long. Posterior parapod 231–396 (3) μm long; subbasal seta multitoothed, with basal spine group, with 5–8 spines (Fig. 12H); parapod apex with numerous simple claws, without claw serrated; bifid claw with U-shaped lower indentation, lower spur arched down toward base of claw (Fig. 12I). B/C 0.87–0.95.

Labrundinia meta Roback (Fig. 13)

Labrundinia meta Roback 1987: 220 (description of male).

Material examined

Type: Holotype male, Colombia: **Departamento del Meta**, Puerto López, Laguna Mozambique, 16 km s. w. of Puerto López, 25.ii.1972, N. R. Foster (ANSP).

Diagnostic characters

Labrundinia meta differs from other *Labrundinia* species by the combination of the following characters **Male**: abdominal tergite I, VIII pale, T II, V with divided brown transverse band near proximal margin, T III–IV, VI–VII with continuous brown transverse band near proximal margin; hypopygium pale, sternapodeme with moderate anterior process.

Redescription

Adult male (n = 1)

Size. Total length 1.91 mm. Wing length 1.25 mm. Total length/wing length 1.53. Wing length/length of profemur 2.81.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax brown with mesonotum dorsally darker; anteprenotum pale; supraalar callus pale brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Femur I with apex brown, Fe II–III pale. Tibia I with apex and base brown, Ti II–III brown. Tarsomere 1 with apex brown, tarsomeres 2–5 pale brown to brown. Abdomen pale brown with maculation as in figure 13L. Hypopygium pale.

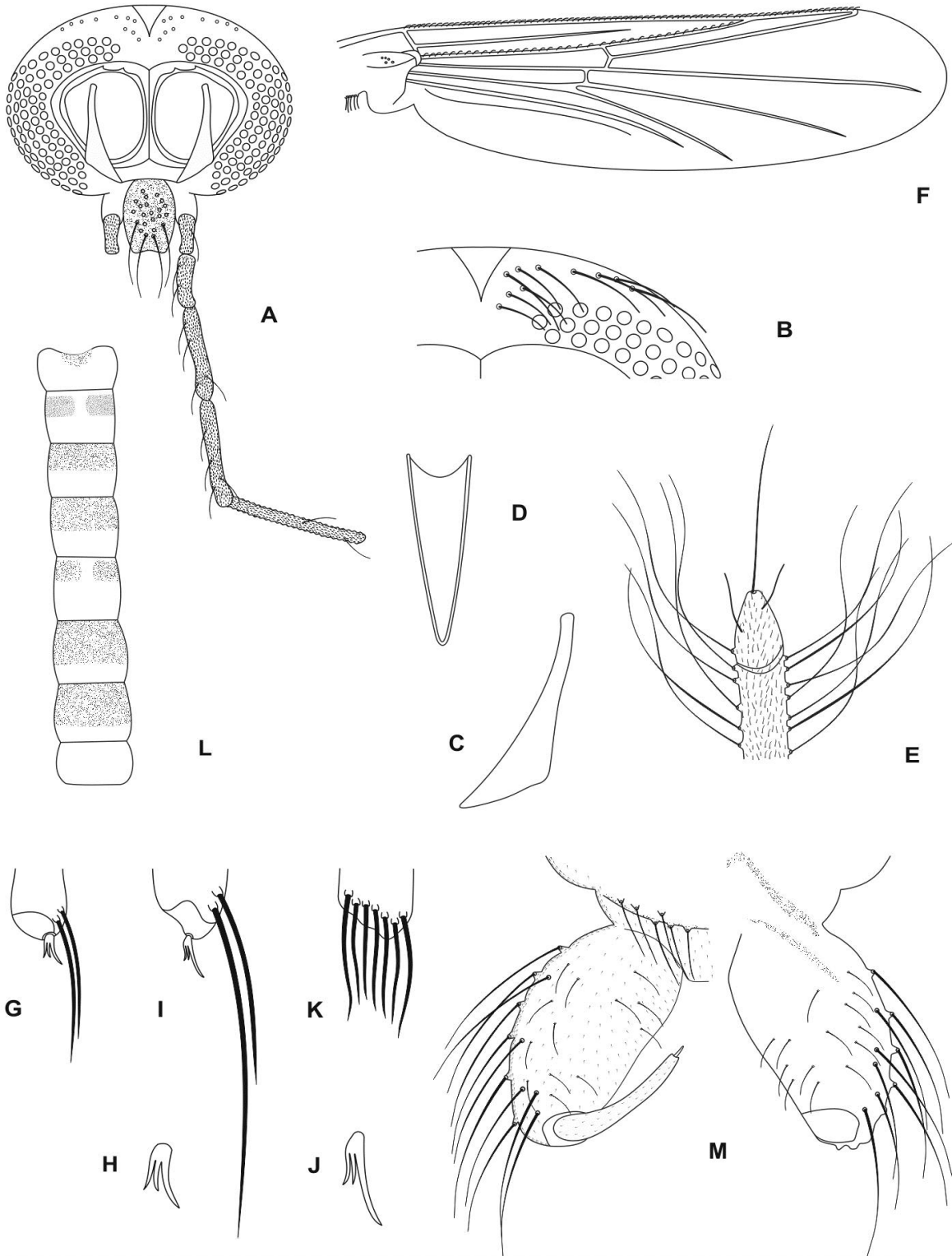
Head (Figs 13A–E). Temporal setae 11, uniserial (Fig. 13B). Eye ratio 1.16. Tentorium (Fig. 13C) 134 μm long, stipes not measurable. Clypeus 108 μm long, 78 μm wide at largest part, bearing 23 setae. Cibarial pump (Fig. 13D) with anterior margin concave, 108. Palpomere lengths 1–5 (in μm): 34; 55; 111; 145; 214. Antenna with 14 flagellomeres, AR 1.37, flagellum 427 μm long, diameter of pedicel 59 μm , apical setae single (Fig. 13E).

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 40, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 23, irregularly biserial; prealars 7; supraalars 2. Anapleural suture ratio 0.48. Scutellum with 12 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 13F). Width 0.40 mm. Costa 1.00 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.05 mm long. VR 0.87. WW 0.32. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 13G–K). Fore leg: width at apex of tibia 47 μm (Fig. 13G), tibia with single, apical and pectinate spur 16 μm long (Fig. 13H), with three and two preapical setae; Ta_1 with two preapical pseudospurs. Mid leg: width at apex of tibia 39 μm long (Fig. 13I), tibia with single, apical and pectinate spur 23 μm long with three teeth (Fig. 13J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 47 μm long (Fig. 13K), tibia without spur; comb with 7 setae; Ta_1 with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table X.

Hypopygium (Fig. 13M). Tergite IX arched, with 11 dorsal setae. Anal point trapezoidal, broad. Phallapodeme 65 μm long. Sternapodeme with moderate anterior process. Gonocoxite cylindrical, 127 μm long, 66 μm wide; inferior volsella absent. GcR 1.94. Gonostylus simple and slender, 77 μm long; megaseta, 12 μm long. HR 1.67. HV 2.49.



Figures 13A–M. *Labrundinia meta* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Table X. Lengths (in μm) and proportions of leg segments in *Labrundinia meta* Roback, adult male ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	455	461	281	219	125
p ₂	625	387	–	–	–
p ₃	531	585	578	281	180
	ta ₄	ta ₅	LR	BV	SV
p ₁	94	63	0.63	2.39	3.14
p ₂	–	–	–	–	–
p ₃	110	78	0.99	2.61	1.93

Adult female, pupa, and larva. Unknown.

Labrundinia neopilosella Beck and Beck (Figs 14–16)

Labrundinia neopilosella Beck and Beck 1966: 341 (description of male, female and immatures stages).

Labrundinia neopilosella Roback 1971: 278 (redescription of male).

Labrundinia neopilosella Roback 1987: 193 (description of immature stages).

Labrundinia neopilosella Epler 2001: 4.48 (larvae identification key).

Labrundinia neopilosella Jacobsen 2008: 42 (pupae identification key).

Material examined

Type: Holotype male with larval exuvia, USA: **Florida**, Flagler County, Rayonier Ditch, 3.vi.1964, W. Beck (USNM 69125). Paratype: 1 male with pupal and larval exuviae same data as holotype except for 27.ii.1965.

Additional. 3 larvae, USA: **North Carolina**, Forsyth, Belews Lake, 16.ix.1985, W. Beck. 1 male with pupal exuvia, **South Carolina**, Pickens, Clemson University, 16.ix.1985, P. L. Hudson; 1 larva as previous except for Anderson, Fish Pond, 25.iii.1977, P. H. Carlson and T. R. White.

Diagnostic characters

Labrundinia neopilosella differs from other *Labrundinia* species by the combination of the following

characters **Male**: abdominal tergite I pale brown II–IV with continuous brown transverse band near proximal margin, T V–VIII wholly brown; hypopygium pale brown, sternapodeme with reduced anterior process. **Pupa**: thoracic horn ovoid; preapical indentation absent; thoracic horn ratio 2.54–2.91. **Larva**: Surface of head capsule smooth, lateroventral spine group present with several conspicuous spines; subbasal seta multitoothed; bifid claw with U-shaped lower indentation.

Redescription

Adult male (n = 4 unless otherwise stated)

Size. Total length 1.55–1.95 (3) mm. Wing length 1.04–1.18 mm. Total length/wing length 1.33–1.75 (3). Wing length/length of profemur 2.80–2.85 (3).

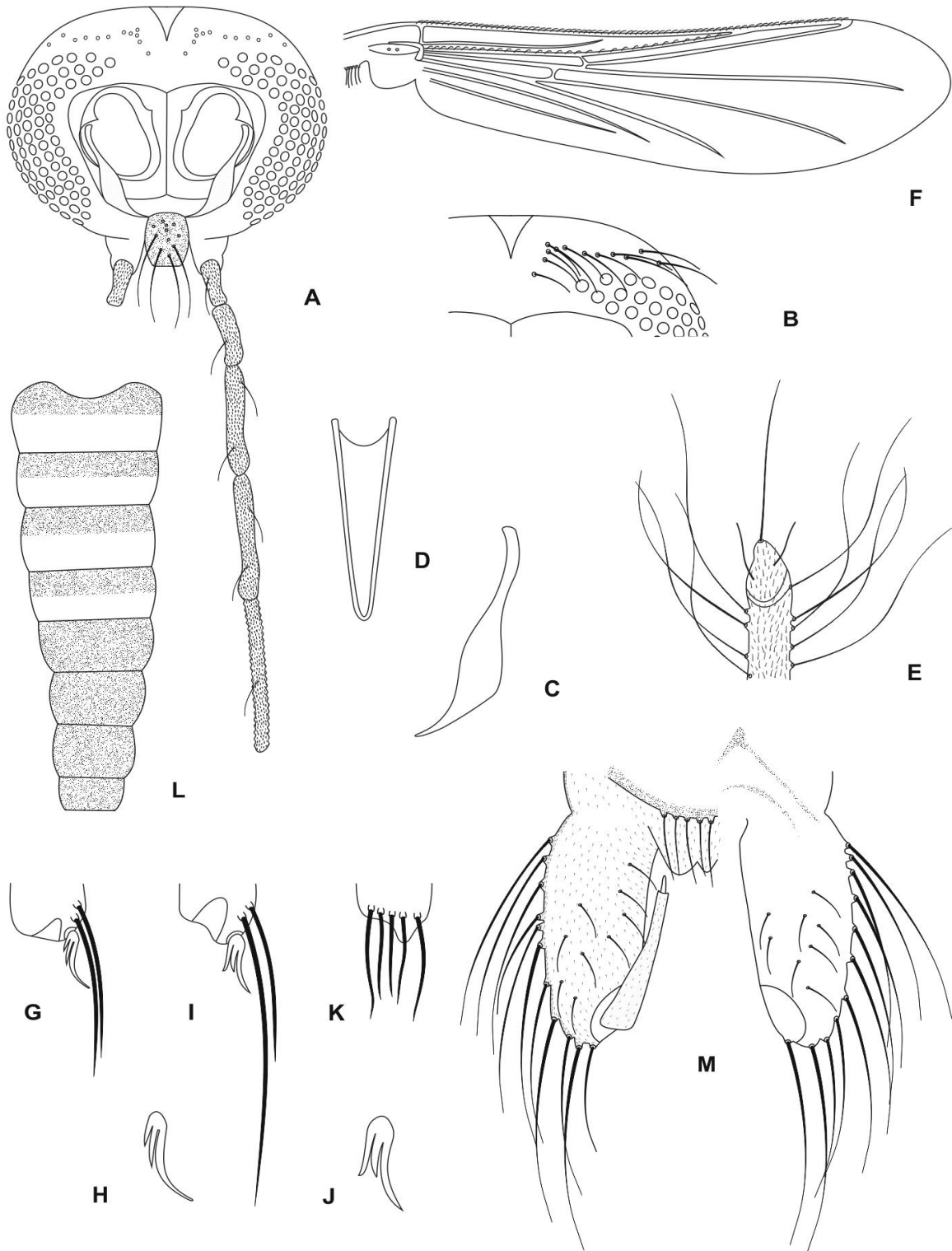
Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale brown; supraalar callus pale brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale. Femur I–III pale. Tibia I–II pale, Ti III pale brown. Tarsomere 1–5 pale to pale brown. Abdomen as in figure 14L. Hypopygium pale brown.

Head (Figs 14A–E). Temporal setae 10–14 (3), uniserial (Fig. 14B). Eye ratio 0.84–0.96 (2). Tentorium (Fig. 14C) 98–128 (2) μm long, stipes not measurable. Clypeus 58–92 (2) μm long, 53–63 (2) μm wide at largest part, bearing 12–17 setae. Cibarial pump (Fig. 14D) with anterior margin concave, 144–165 (3) μm long. Palpomere lengths 1–4 (in μm): 23–29 (3); 41–47 (3); 88–92 (3); 90–120 (2). Antenna with 14 flagellomeres, AR 1.15–1.19 (3), flagellum 582–678 (3) μm long, diameter of pedicel 96–108 μm , apical setae single (Fig. 14E).

Thorax. Anteprenotum with 3–5 (2) lateral setae. Acrostichals 30–36 (2), biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 18–26, irregularly uniserial; prealars 7–10; supraalars 3. Anapleural suture ratio 0.35–0.50 (2). Scutellum with 8–10 (3) setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 14F). Width 0.30–0.33 mm. Costa 0.79–1.00 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.05–0.07 mm long. VR 0.64–0.71. WW 0.26–0.30. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 14G–K). Fore leg: width at apex of tibia 32–39 μm (Fig. 14G), tibia with single, apical and pectinate spur 15–16 (3) μm long (Fig. 14H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 31–39 μm long (Fig. 14I), tibia with single, apical



Figures 14A–M. *Labrundinia neopilosella* Beck and Beck, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

and pectinate spur 17–18 (3) μm long with three teeth (Fig. 14J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 29–39 μm long (Fig. 14K), tibia without spur; comb with 5 (2) setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XI.

Table XI. Lengths (in μm) and proportions of leg segments in *Labrundinia neopilosella* Beck and Beck, adult male (n = 2–3 unless otherwise stated).

	fe	ti	ta_1	ta_2	ta_3
p ₁	414–453	452–484 (1)	254–274	207–218	133–145
p ₂	487–523	352–416	453–507	180–242	133–141
p ₃	469–477	484–586	460–500	219–242	148–179
	ta_4	ta_5	LR	BV	SV
p ₁	103–109	62–63	0.60–0.63	2.26–2.38	2.95–3.34
p ₂	101–102	63–70	1.30–1.38	2.68–2.69	1.68–1.74
p ₃	101–109	63–78	0.95–0.98	2.45–2.48	1.86–1.95

Hypopygium (Fig. 14M). Tergite IX arched, with 10–14 dorsal setae. Anal point rounded, notched apically. Phallapodeme 39–49 (3) μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 84–96 μm long, 41–46 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.86–2.23. Gonostylus simple and slender, 58–63 μm long; megaseta 9–12 (3) μm long. HR 1.36–1.53. HV 2.64–3.10 (3).

Adult female (n = 1)

Size. Total length 0.81 mm. Wing length 0.90 mm. Total length/wing length 0.98. Wing length/length of profemur 2.68.

Coloration. Head, pedicel, antenna and maxillary palp pale brown. Thorax pale brown with mesonotum dorsally darker; anteprotum pale brown; supraalar callus pale brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale. Abdomen wholly pale brown, not very discernible. Seminal capsules brown.

Head. Temporal setae 10, uniserial. Eyes ratio 1.04. Tentorium 107 μm long, stipes not measurable. Clypeus 74 μm long, 71 μm wide at largest part, bearing 19 setae. Cibarial pump as in male, 142 μm long. Palpomere lengths 1–5 (in μm): 34; 52; 65; 92; 147. Antenna with 11 flagellomeres, AR 0.42, flagellum 299 μm long, diameter of pedicel 49 μm .

Thorax. Anteprenotum with 6 lateral setae. Acrostichals 36, irregularly biserial, starting close to anteprenotum; dorsocentrals 32, irregularly uniserial; prealars 11; supraalars 3. Scutellum with 9 setae, scattered. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.32 mm. Costa 0.71 mm long, not produced beyond R_{4+5} . R_{2+3} absent. Base of radial sector 0.04 mm long. VR 0.82. WW 0.35. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 36 μm , tibia with single, apical and pectinate spur 14 μm long, with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Mid leg: width at apex of tibia 28 μm , tibia with single, apical and pectinate spur 18 μm long, with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Hind leg: width at apex of tibia 27 μm , tibia without spur; comb setae unobserved; preapical pseudospurs on Ta_{1-4} unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XII.

Table XII. Lengths (in μm) and proportions of leg segments in *Labrundinia neopilosella* Beck and Beck, adult female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	334	313	242	141	107
p ₂	470	361	–	–	–
p ₃	417	484	441	241	100
	ta ₄	ta ₅	LR	BV	SV
p ₁	85	62	0.77	2.25	2.67
p ₂	–	–	–	–	–
p ₃	99	77	0.91	2.59	2.04

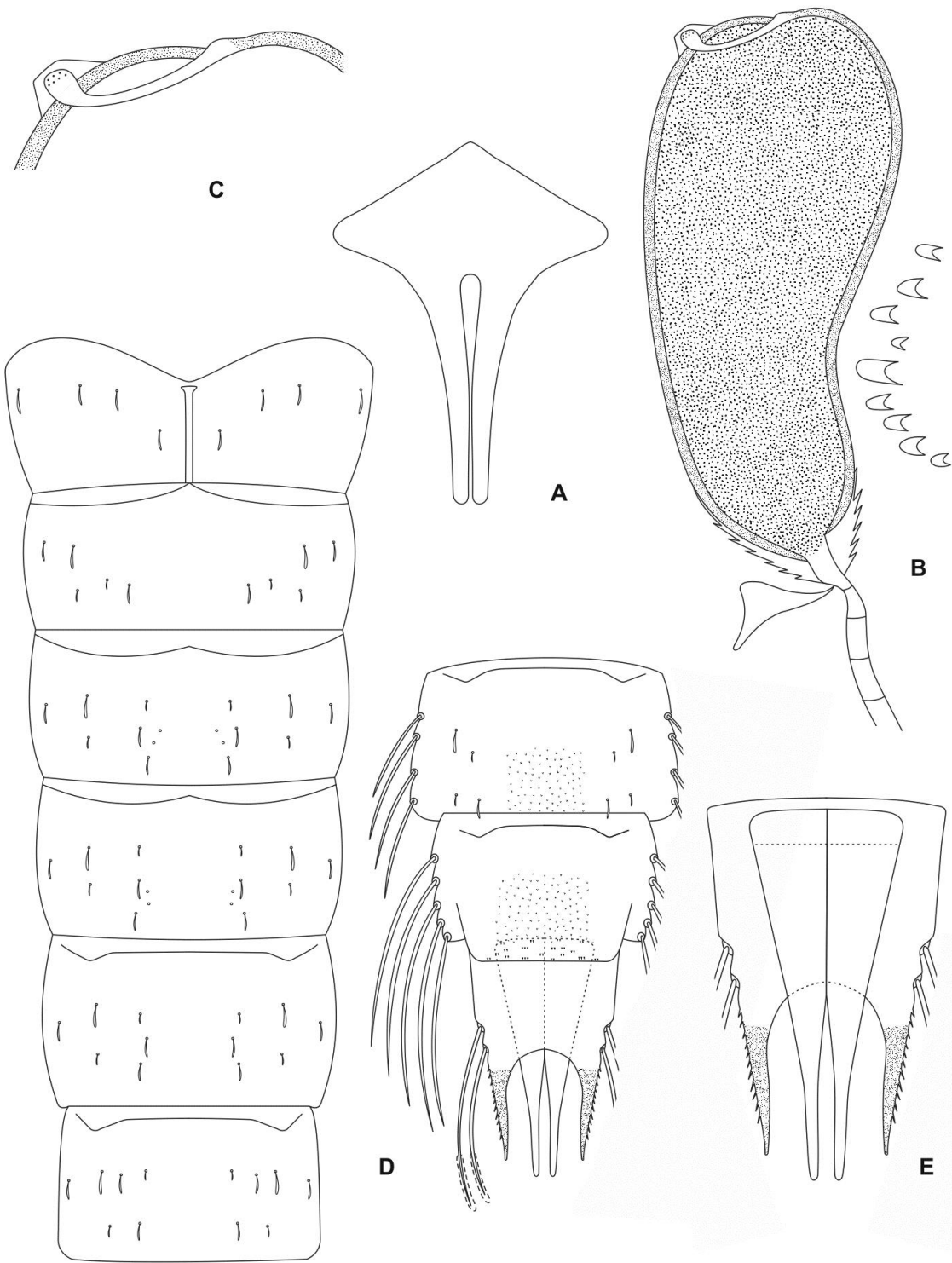
Genitalia. Gonapophysis VIII broadly rounded, 67 μm long. Coxosternapodeme 108 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 38 μm long and 21 μm wide; with 4 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 108 μm . Seminal capsules oval with conical shaped necks, length 50 μm , maximum width 30 μm . Length ratio SCa/No 0.47.

Pupa (n = 4 unless otherwise stated)

Size. Male abdomen 1.65–1.81 (3) mm long; female abdomen 1.81 (1) mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 15A–C). Frontal apotome as in figure 15A. Wing sheath smooth 0.71–0.78 (3) mm long. Thoracic horn 219–231 μm long and 76–101 μm wide (Fig.15B), preapical indentation



Figures 15A–E. *Labrundinia neopilosella* Beck and Beck, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

absent, THR 2.54–2.91. Membranous preapical papilla 22–37 μm long (Fig. 15C), PTH 0.10–0.17, aeropyle tube simple, elongated, 28–29 (2) μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct. Basal lobe wedge-shaped. Thoracic comb with 8–10 conical teeth (Fig. 15B).

Abdomen (Figs 15D–E). Tergite I with scar, 114–120 (2) μm long. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 15D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 234–258 (3) μm long and 148–171 μm wide (Fig. 15E), outer margins sclerotized, with 8 (2) spines, longest spine 14–16 (3) μm long, inner margins of lobes membranous. ALR 1.58–1.64 (3). Genital sac elongated, reaching somewhat beyond apex of anal lobe.

4th instar larva ($n = 8$ unless otherwise stated)

Coloration. Head pale yellow; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 16A). Length 438–463 (4) μm , 254–297 (4) μm wide. Surface smooth; lateroventral spine group present, well developed, with 7–10 (7) spines; posteroventral spine group absent; cephalic index 0.56–0.75. Chaetotaxy as in figure 16A.

Antenna (Figs 16B–C). Length 246–288 μm , A_1 148–189 μm long, with ring organ placed 122–154 μm from base, A_2 68–78 μm long. AR 1.85–2.13 (7). Blade longer than A_2 over-reached by accessory blade.

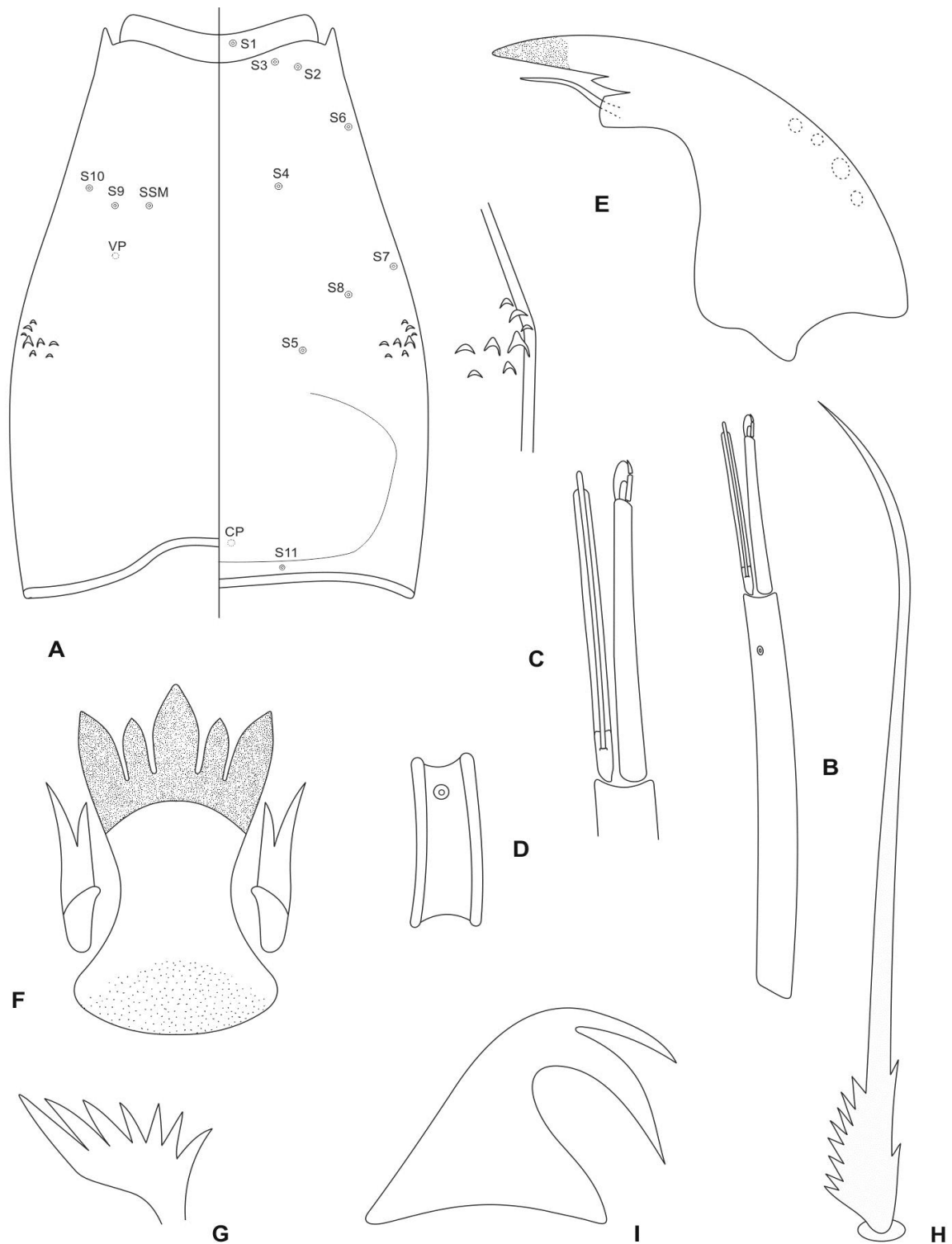
Maxilla (Fig. 16D). Basal palp segment 24–38 μm long and 8–18 μm wide, with ring organ 19–31 (6) μm from base. PR 2.14–3.20 (3). APR 4.80–7.60.

Mandible (Fig. 16E). Length 60–73 μm , with 3 lateral setae. Sensillum campaniformium 40–55 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.28–3.05.

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 16F–G). Ligula 52–65 μm long, 24–27 μm wide, with row of 5 teeth. IO 0.95–0.99 (7), MO 1.08–1.15. Paraligula bifid, 20–29 μm long, inner tooth 19–26 (7) μm long, shorter than outer tooth. Pecten hypopharyngis with 8 teeth almost equal in size.

Body (Figs 16H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 125–151 (6) μm long, 22–40 μm wide, with 7 anal setae 494–650 (5) μm long. L/W 4.60–6.00 (5). Supraanal seta well developed. Anal tubules 173–210 (4) μm long. Posterior parapod 251–251 (4) μm long; subbasal seta multitoothed, with basal spine group, with 5–9 (7) spines (Fig. 16H); parapod apex with numerous simple



Figures 16A–I. *Labrundinia neopilosella* Beck and Beck, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

claws, without serrated claw; bifid claw with U-shaped lower indentation (Fig. 16I). B/C 0.88–0.96 (7).

Labrundinia opela Roback (Fig. 17)

Labrundinia opela Roback 1987: 218 (description of male).

Material examined

Type: Holotype male, Colombia: **Departamento del Meta**, Puerto López, Laguna Mozambique, 16 km s. w. of Puerto López, 6.iii.1971, N. R. Foster (ANSP).

Diagnostic characters

Labrundinia opela differs from other *Labrundinia* species by the combination of the following characters
Male: abdominal tergite I–II pale, T III–V with continuous brown transverse band near proximal margin, T VI about 0.66 % brown, VII–VIII almost wholly brown; hypopygium pale, sternapodeme with reduced anterior process.

Redescription

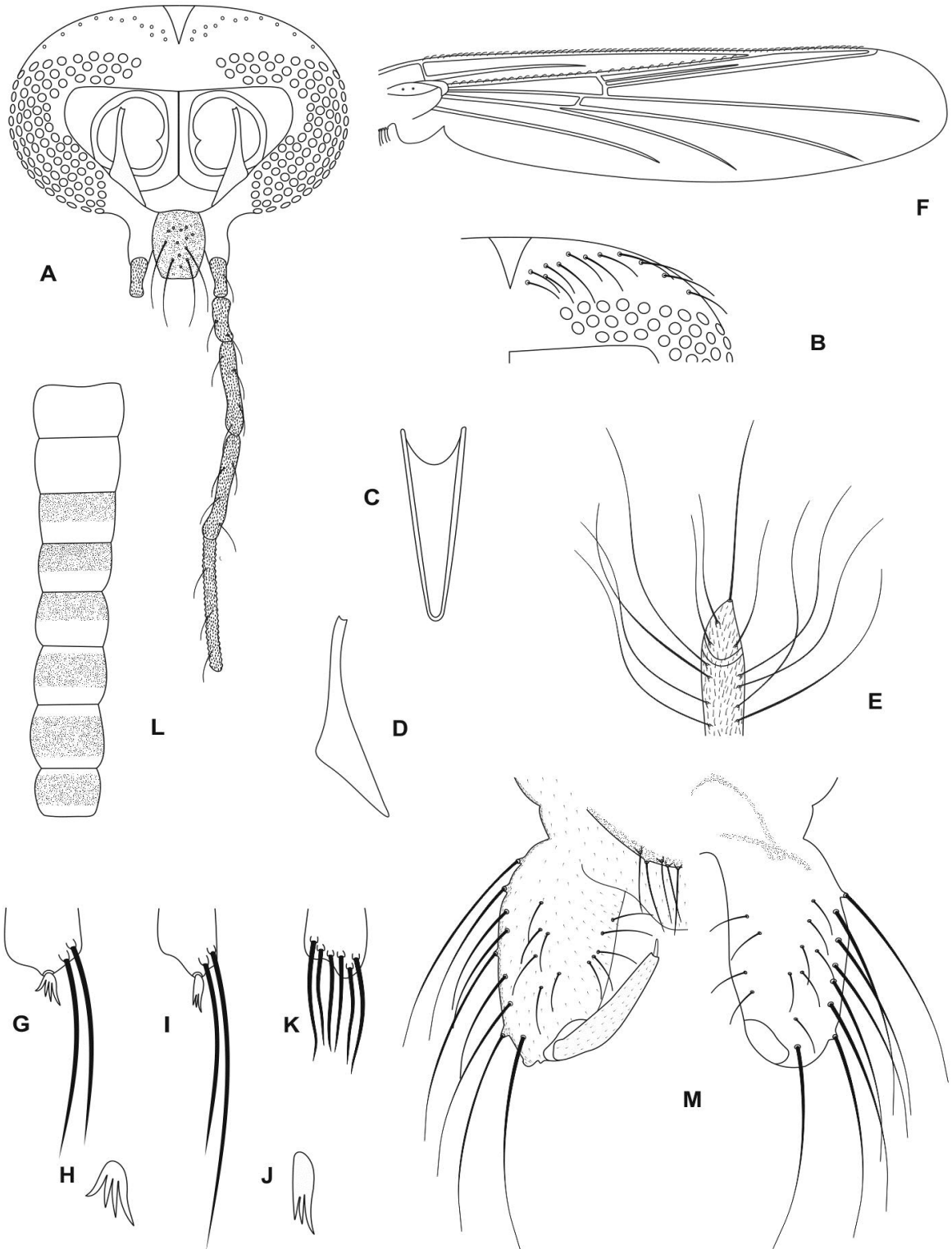
Adult male ($n = 1$ unless otherwise stated)

Size. Total length 2.17 mm. Wing length 1.20 mm. Total length/wing length 1.81. Wing length/length of profemur 3.28.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax brown with mesonotum dorsally darker; anteprenotum pale; supraalar callus dark brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Femur I–III pale. Tibia I–III pale. Abdomen pale brown with maculation as in figure 17L. Hypopygium pale.

Head (Figs 17A–E). Temporal setae 12, uniserial (Fig. 17B). Eye ratio 1.02. Tentorium (Fig. 17C) 141 μm long, stipes not measurable. Clypeus unmeasured, bearing 13 setae. Cibarial pump (Fig. 17D) with anterior margin concave, unmeasured. Palpomere lengths 1–3 (in μm): 45; 104; 116. Antenna with 14 flagellomeres, AR 1.31, flagellum 718 μm long, diameter of pedicel 98 μm , apical setae single (Fig. 17E).

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 42, biserial, diverging evenly posteriorly, starting



Figures 17A–M. *Labrundinia opela* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

close to antepnotum and almost reaching scutellum; dorsocentrals 22, irregularly uniserial; prealars 9; supraalars 2. Anapleural suture ratio 0.51. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 17F). Width 0.32 mm. Costa 0.99 mm long, not produced beyond R₄₊₅, ending very slightly beyond tip of M₃₊₄. R₂₊₃ present. Base of radial sector 0.06 mm long. VR 0.73. WW 0.27. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 17G–K). Fore leg: width at apex of tibia 31 µm (Fig. 17G), tibia with single, apical and pectinate spur 12 µm long (Fig. 17H), with four and two preapical setae; preapical pseudospurs unobserved. Mid leg: width at apex of tibia 31 µm long (Fig. 17I), tibia with single, apical and pectinate spur 20 µm long, number of teeth unobserved (Fig. 17J) and two preapical setae; preapical pseudospurs unobserved. Hind leg: width at apex of tibia 31 µm long (Fig. 17K), tibia without spur; comb with 6 setae; preapical pseudospurs unobserved. Pulvilli absent. Lengths and proportion of leg segments as in Table XIII.

Table XIII. Lengths (in µm) and proportions of leg segments in *Labrundinia opela* Roback, adult male ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	367	406	–	–	–
p ₂	547	418	–	–	–
p ₃	484	555	–	–	–
	ta ₄	ta ₅	LR	BV	SV
P ₁	–	–	–	–	–
P ₂	–	–	–	–	–
P ₃	–	–	–	–	–

Hypopygium (Fig. 17M). Tergite IX arched, with 9 dorsal setae. Anal point slightly triangular. Phallapodeme 49 µm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 125 µm long, 61 µm wide; inferior volsella absent. GcR 2.06. Gonostylus simple, concave apically, 82 µm long; megaseta, 10 µm long. HR 1.52. HV 2.64.

Adult female, pupa, and larva. Unknown.

Labrundinia parabecki Roback (Fig. 18)

Labrundinia parabecki Roback 1987: 215 (description of male).

Material examined

Type: Holotype male, Colombia: **Departamento del Meta**, Puerto López, Laguna Mozambique, 16 km s. w. of Puerto López, 25.ii.1972, N. R. Foster (ANSP). Paratype: 1 male same data as holotype.

Diagnostic characters

Labrundinia parabecki differs from other *Labrundinia* species by the combination of the following characters **Male:** abdomen wholly brown, tergite II–IV with darker cruciate marks; hypopygium brown, sternapodeme with distinct anterior process.

Redescription

Adult male ($n = 2$ unless otherwise stated)

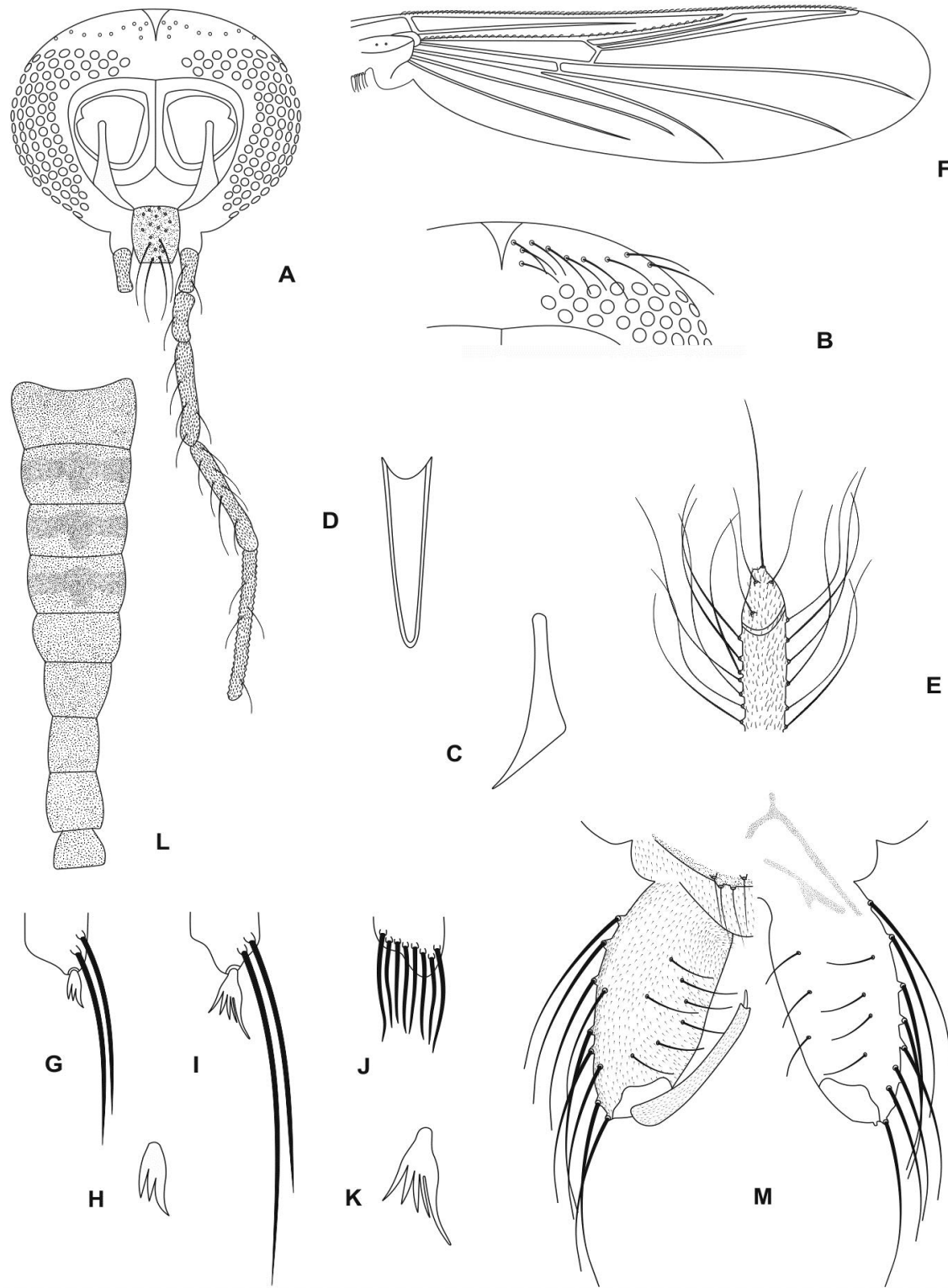
Size. Total length 2.44 (1) mm. Wing length 1.15–1.27 mm. Total length/wing length 1.91 (1). Wing length/length of profemur 3.06–3.39.

Coloration. Head dark brown with brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax brown with mesonotum dorsally darker; anteprenotum pale; supraalar callus dark brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Femur I pale, Fe II–III pale brown. Tibia I–III apex brown. Tarsomere 1–5 pale to pale brown. Abdomen brown with maculation as in figure 18L. Hypopygium brown.

Head (Figs 18A–E). Temporal setae 13, uniserial (Fig. 18B). Eye ratio 1.06–1.2. Tentorium (Fig. 18C) 137–143 μm long, stipes not measurable. Clypeus 108 (1) μm long, 76 (1) μm wide at largest part, bearing 17–18 setae. Cibarial pump (Fig. 18D) with anterior margin concave, 169 (1). Palpomere lengths 1–5 (in μm): 29–35; 55; 108–118; 147–149; 196–222. Antenna with 14 flagellomeres, AR 1.26–1.34, flagellum 720–776 μm long, diameter of pedicel 103–118 μm , apical setae single (Fig. 18E).

Thorax. Anteprenotum with 6 lateral setae. Acrostichals 42 (1), biserial, diverging evenly posteriorly starting close to anteprenotum and almost reaching scutellum; dorsocentrals 28–30, irregularly biserial; prealars 11–12; supraalars 2. Anapleural suture ratio 0.39. Scutellum with 11 (1) setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 18F). Width 0.32–0.35 mm. Costa 0.89–1.00 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.07 mm long. VR 0.68–0.77. WW 0.28–0.30. Brachiolum with 2 setae. Squama setiferous.



Figures 18A–M. *Labrundinia parabecki* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Legs (Figs 18G–K). Fore leg: width at apex of tibia 39 μm (Fig. 18G), tibia with single, apical and pectinate spur 13 μm long (Fig. 18H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 31–39 (1) μm long (Fig. 18I), tibia with single, apical and pectinate spur 22 μm long with five teeth (Fig. 18J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 39 μm long (Fig. 18K), tibia without spur; comb with 8 setae; Ta_1 with two preapical pseudospurs. Legs with slender with apical hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XIV.

Table XIV. Lengths (in μm) and proportions of leg segments in *Labrundinia parabecki* Roback, adult male ($n = 2$).

	fe	ti	ta_1	ta_2	ta_3
p_1	375	406	313–328	180–188	133
p_2	531–592	429–450	531–532	234–258	148–156
p_3	500–508	531–555	531–555	234	148–164
	ta_4	ta_5	LR	BV	SV
p_1	86	63–70	0.77–0.81	2.34–2.37	2.38–2.50
p_2	94	86	1.18–1.23	2.51–2.80	1.81–1.98
p_3	109–117	86–94	1.00	2.65–2.70	1.91–1.94

Hypopygium (Fig. 18M). Tergite IX arched, with 7–10 dorsal setae. Anal point trapezoidal. Phallapodeme 56–61 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 143–153 μm long, 61–65 μm wide; inferior volsella absent. GcR 2.35–2.36. Gonostylus simple and slender, 104–106 μm long; megaseta, 16 μm long. HR 1.35–1.47. HV 2.30 (1).

Adult female, pupa, and larva. Unknown.

***Labrundinia pilosella* (Loew) (Figs 19–21)**

Tanypus pilosellus Loew 1866: 5 (description female).

Ablabesmyia pilosella Johannsen 1905: 152 (description female, new combination).

Tanypus pilosellus Malloch 1915: 372 (description of male, female and immatures stages)

Pentaneura pilosella Johannsen 1946: 293 (description male and female, new combination)

Pentaneura pilosella Roback 1962: 253 (description of male and immatures stages).

Pentaneura pilosella Sublette 1964: 100 (description of male).

? *Labrundinia floridana* Beck and Beck 1966: 339 (description of male, female and immatures stages).

nec. *Labrundinia pilosella* Beck and Beck 1966: 340 (misdetermination of *L. pilosella* Loew, male, female and immatures stages).

Labrundinia pilosella Roback 1971: 276 (redescription of male, new combination).

Labrundinia pilosella Roback 1987: 169 (redescription of immature stages).

Labrundinia pilosella Epler 2001: 4.46 (larvae identification key).

Labrundinia pilosella Jacobsen 2008: 41 (pupae identification key).

Material examined

Type: Lectotype female, USA: **District Columbia**, O. Sacken (MCZ10370). Paralectotype: 3 females same data as lectotype.

Additional. 1 male, CANADA: **Alberta**, Bigoray river, 7.vi.1973, H. Boeger (CNC CH3104). 1 male with pupal and larval exuviae, **Northwest Territory**, Martin river, 5.vii.1973, B. Bilyj (CNC CH801.5). 1 male, **Ontario**, St. Lawrence Island. N. P (S.L.I.N.P.), Grenadier Island, 1.viii.1975, M. E. Roussel (CNC CH4076); 1 male as previous except for Gordon Island, 7.vii.1976 (CNC CH7010). 1 male as previous except for Mallorytown landing, 3.vi.1975 (CNC CH4055). 2 females (as previous except for Waterloo, Canagagigue river, 24.x.1975, B. Bilyj CNC CH2795 and CH2810). 1 female with pupal and larval exuviae, USA: **Florida**, Clay County, Peter's Creek, 7.iii.1963, W. Beck; 1 female with pupal and larval exuviae as previous except for 20.viii.1963. 1 male with pupal exuvia, **Georgia**, west of Preston, Kinchafoonee creek, 25.v.1972, S. S. Roback; 1 pupa as previous except for Girard, Savannah river, M 157, 6 mi, S. S. Roback; 1 larva as previous except for Crisp, Gum Creek, R+30, 6m. w of Corde, 31.x.1983, S. S. Roback. 1 larva, **Illinois**, Illiapolis, Ditch, Trib. Long pt., 25/ix.1984, S. S Roback. 1 male, **Minnesota**, Garrison Bay, Bay lake, 3.viii.1981, P. L. Hudson. 1 pupa, **South Carolina**, Allendale, Savannah river, station 5 M149, ca 6 mi w of Martin, 10.ix.1980, S. S. Roback; 1 larva as previous except for Colleton, Ashepoo river, close to Waterboro, 2.iv.1977, P. H. Carlson/T. R. White; 1 larva as previous except for Lexington, 28.xi.1976; 1 larva as previous except for station 4 at S.C Route 63 bridge, 21.vii.1964, S. S. Roback.

Diagnostic characters

Labrundinia pilosella differs from other *Labrundinia* species by the combination of the following characters **Male:** abdominal tergite II pale brown II, V wholly brown, T IV, VI with continuous brown transverse band near proximal margin, T VII–VIII wholly brown; hypopygium pale brown, sternapodeme with distinct anterior process. **Pupa:** thoracic horn club-shaped; preapical indentation moderate deep,

forming a distinct diverticulum; genital sac reaching much beyond apex of the anal lobe. **Larva:** surface of head capsule covered with spinules, posterior margin may be darkened, lateroventral and posteroventral spine groups absent; subbasal seta simple; bifid claw with V-shaped lower indentation.

Redescription

Adult male (n = 5 unless otherwise stated)

Size. Total length 2.70–3.19 (4) mm. Wing length 1.66–1.88 (4) mm. Total length/wing length 1.60–1.83 (4). Wing length/length of profemur 2.66–3.19.

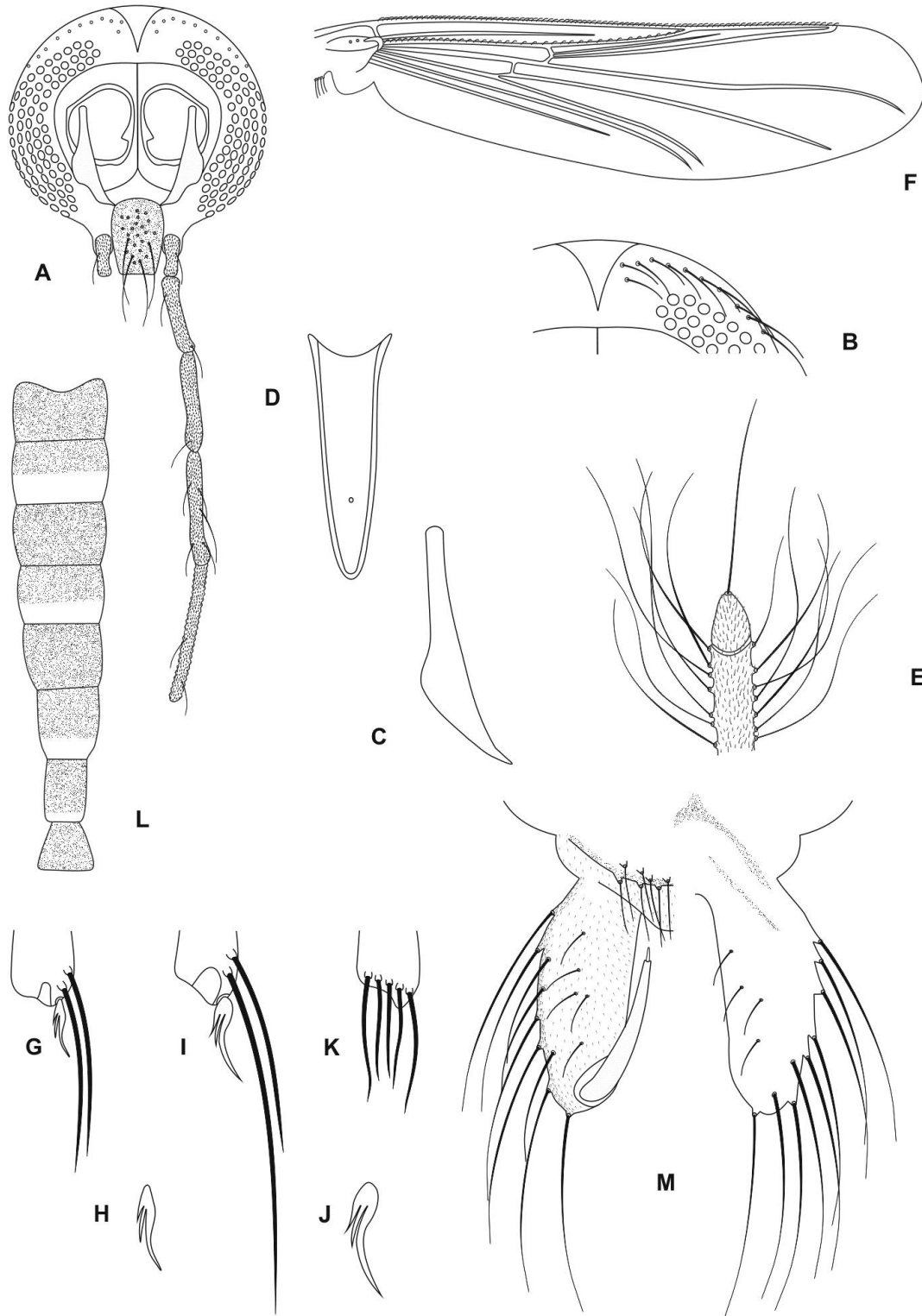
Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; antepronotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 19L. Hypopygium pale brown.

Head (Figs 19A–E). Temporal setae 13–20, uniserial (Fig. 19B). Eye ratio 1.18–1.47. Tentorium (Fig. 19C) 165–178 μm long, stipes not measurable. Clypeus 129–153 (4) μm long, 94–112 (4) μm wide at largest part, bearing 17–26 setae. Cibarial pump (Fig. 19D) with anterior margin concave, 196–216 μm long. Palpomere lengths 1–5 (in μm): 35–39; 73–88 (4); 153–171 (4); 157–176 (4); 280–310 (3). Antenna with 14 flagellomeres, AR 1.12–1.39 (4), flagellum 686–931 μm long, diameter of pedicel 120–157 (4) μm , apical setae single (Fig. 19E).

Thorax. Antepronotum with 6–9 lateral setae. Acrostichals 45–50 (4), biserial, diverging evenly posteriorly, starting close to antepronotum and almost reaching scutellum; dorsocentrals 22–34, irregularly multiserial; prealars 15–17; supraalars 3 (4). Anapleural suture ratio 0.41–0.59. Scutellum with 10–12 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 19F). Width 0.36–0.49 (4) mm. Costa 1.40–1.60 (4) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.09–0.14 mm long. VR 0.66–0.72 (4). WW 0.26–0.28 (4). Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 19G–K). Fore leg: width at apex of tibia 47 (4) μm (Fig. 19G), tibia with single, apical and pectinate spur 14–20 μm long (Fig. 19H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 39–47 (4) μm long (Fig. 19I), tibia with single, apical and pectinate spur 24–27 (4) μm long with three teeth (Fig. 19J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 47–55 (3) μm long (Fig. 19K), tibia without spur; comb with 5 (4) setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws.



Figures 19A–M. *Labrundinia pilosella* (Loew), adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Pulvilli absent. Lengths and proportion of leg segments as in Table XV.

Table XV. Lengths (in μm) and proportions of leg segments in *Labrundinia pilosella* Beck and Beck, adult male (n = 3–4 unless otherwise stated).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	570–633	703–758	390–484	305–344	211–234
p ₂	742–828	766–805	664–758	352–383	188–203
p ₃	680–758	1008–1078	766–859	336–375	234–250
	ta ₄	ta ₅	LR	BV	SV
p ₁	141–164	86–102	0.56–0.64	2.15–2.32	2.94–3.40
p ₂	117–133	86–102	0.92–0.97	2.65–2.90	2.08–2.20
p ₃	156–172	86–102	0.80–0.84	2.94–3.00	2.05–2.12

Hypopygium (Fig. 19M). Tergite IX arched, with 14 (3) dorsal setae. Anal point trapezoidal. Phallapodeme 61–82 (4) μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 145–169 (4) μm long, 67–71 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.38–2.42 (3). Gonostylus simple and slender, 84–100 μm long; megaseta 14–18 (4) μm long. HR 1.52–1.75. HV 2.87–3.20.

Adult female (n = 5 unless otherwise stated)

Size. Total length 1.61–1.85 (3) mm. Wing length 1.06–1.42 (4) mm. Total length/wing length 1.30–1.51 (3). Wing length/length of profemur 1.88–2.23 (4).

Coloration. Head, pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus pale brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown; leg I dark brown. Abdomen wholly pale brown. Seminal capsules brown.

Head. Temporal setae 13–15, uniserial. Eyes ratio 1.03–1.12 (2). Tentorium 107 (1) μm long, stipes not measurable. Clypeus 82 (1) μm long, 66 (1) μm wide at largest part, bearing 20–31 setae. Cibarial pump as in male, 186–215 μm long. Palpomere lengths 1–5 (in μm): 35–39 (4); 67–82 (4); 120–148 (3); 127–155 (4); 153–227 (4). Antenna with 11 flagellomeres, AR 0.36 (1), flagellum 335 μm long, diameter of pedicel 55–67 μm .

Thorax. Anteprenotum with 5–7 (2) lateral setae. Acrostichals 40–48, irregularly biserial, starting close to anteprenotum; dorsocentrals 30–38, irregularly biserial; prealars 12–16 (4); supraalars 3. Scutellum with

10–12 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.48–0.57 mm. Costa 0.80 (1) mm long, not produced beyond R₄₊₅. R₂₊₃ present. Base of radial sector 0.05 (1) mm long. VR 0.69 (1). WW 0.35–0.40 (4). Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 39–47 (4) µm, tibia with single, apical and pectinate spur 14–21 µm long, with three teeth and two preapical setae; Ta₁₋₄ each with one preapical pseudospurs. Mid leg: width at apex of tibia 39–55 µm, tibia with single, apical and pectinate spur 17–23 µm long, with three teeth and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Hind leg: width at apex of tibia 39–55 µm, tibia without spur; comb with 5 (3) setae; preapical pseudospurs on Ta₁₋₄ unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XVI.

Table XVI. Lengths (in µm) and proportions of leg segments in *Labrundinia pilosella* Beck and Beck, adult female (n = 3–4 unless otherwise stated).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	414–531	438–509	328–373	195–234	151–156
p ₂	590–700	593–645	570–618	258–291	141–172
p ₃	547–594	781–812	445–664 (2)	211–3.36 (2)	156–2.19 (2)
	ta ₄	ta ₅	LR	BV	SV
p ₁	109–118	63–86	0.66–0.75	2.25–2.42	2.60–2.78
p ₂	86–118	70–93	0.95–1.28	2.83–3.03	1.88–2.23
p ₃	101–141 (2)	70–86 (2)	0.78–0.82 (2)	2.65–2.75 (2)	2.12–2.33 (2)

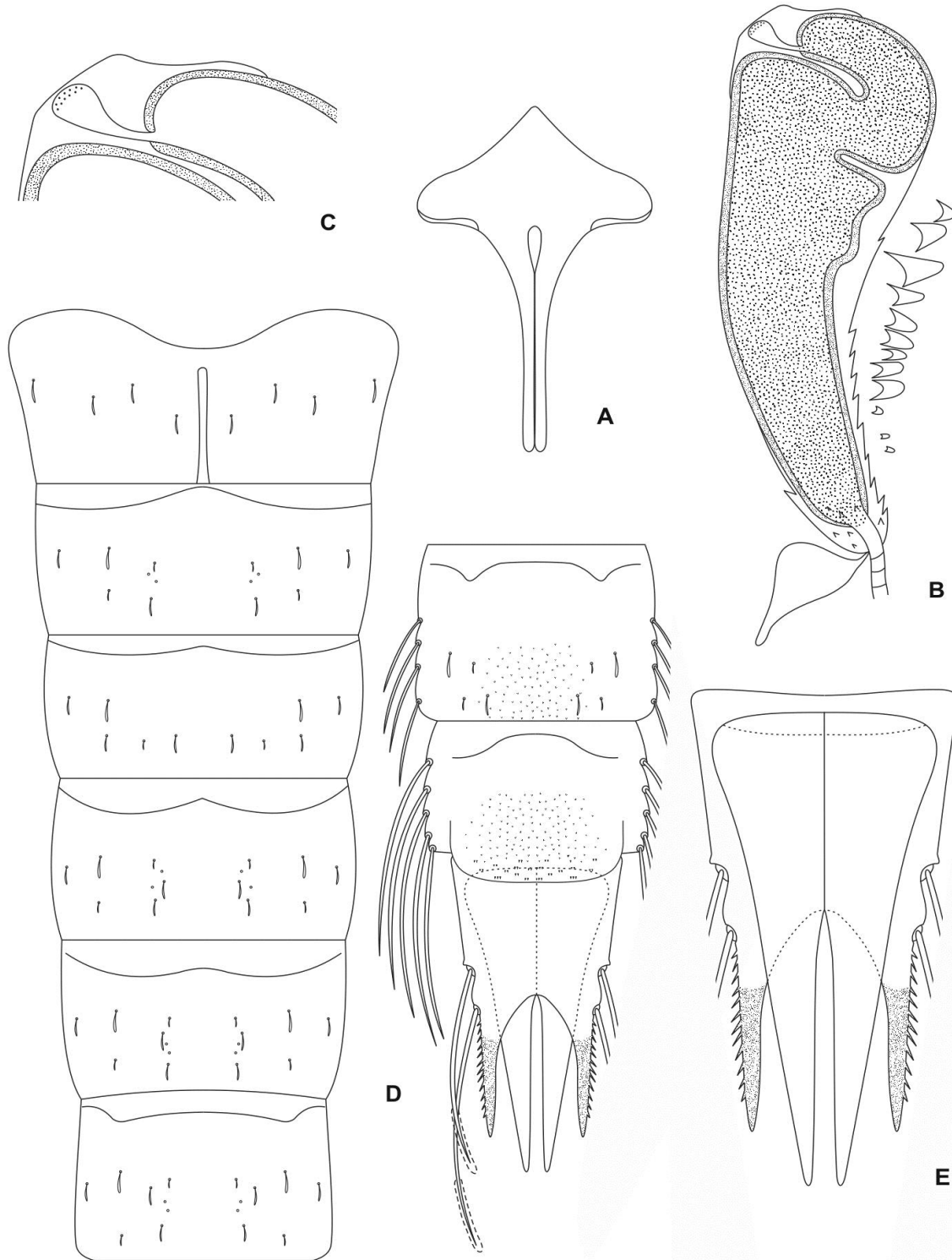
Genitalia. Gonapophysis VIII broadly rounded, 58 (1) µm long. Coxosternapodeme 66 (1) µm long. Postgenital plate rounded. Cerci oval-quadrated, 43 (1) µm long and 13 (1) µm wide. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 81 (1) µm. Seminal capsules oval with conical shaped necks, length 35–47 µm, maximum width 29–36 (2) µm. Length ratio SCa/No 0.47 (1).

Pupa (n = 4 unless otherwise stated)

Size. Male abdomen 1.91–2.25 (3) mm long; female abdomen 1.77 (1) mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 20A–C). Frontal apotome as in figure 20A. Wing sheath smooth 0.68–0.75 mm long. Thoracic horn 195–242 µm long and 63–78 µm wide (Fig. 20B), preapical indentation moderate deep, THR 2.6–3.50. Membranous preapical papilla 14–39 µm long (Fig. 20C),



Figures 20A–E. *Labrundinia pilosella* (Loew), pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

PTH 0.06–0.20, aeropyle tube simple, short, 14–29 μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct. Basal lobe wedge-shaped. Thoracic comb with 8–12 conical teeth (Fig. 20B).

Abdomen (Figs 20D–E). Tergite I with scar, 103 (1) μm long. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 20D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 250–313 (3) μm long and 141–164 (3) μm wide (Fig. 20E), outer margins sclerotized, with 11 (3) spines, longest spine 10–18 μm long, inner margins of lobes membranous. ALR 1.62–1.90 (3). Genital sac elongated, reaching much beyond apex of anal lobe.

4th instar larva ($n = 5$ unless otherwise stated)

Coloration. Head pale yellow, posterior margin may be darkened; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 21A). Length 407 (1) μm , 277 (1) μm wide. Surface covered with small spinules; lateroventral and posteroventral spine groups absent; cephalic index 0.68–0.84. Chaetotaxy as in figure 21A.

Antenna (Figs 21B–C). Length 212–271 (4) μm , A₁ 142–190 (4) μm long, with ring organ placed 144–160 (2) μm from base, A₂ 59–73 (4) μm long. AR 2.33–2.34 (2). Blade longer than A₂ over-reached by accessory blade.

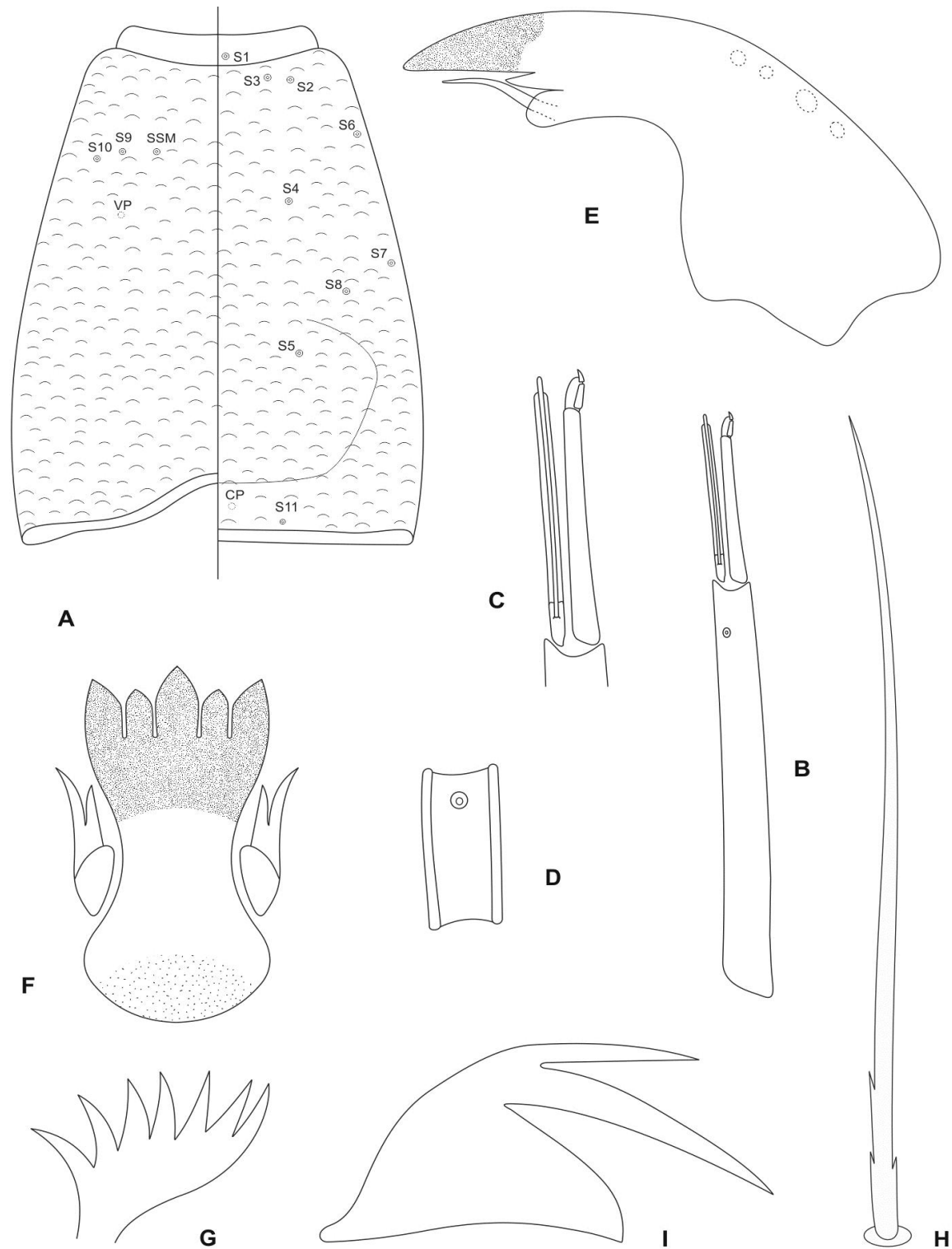
Maxilla (Fig. 21D). Basal palp segment 26–38 μm long and 8–14 μm wide, with ring organ 14–23 μm from base. PR 1.84–3.70. APR 5.05–8.62.

Mandible (Fig. 21E). Length 71–85 μm , with 3 lateral setae. Sensillum campaniformium 49–59 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.23–3.39 (3).

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 21F–G). Ligula 52–68 μm long, 23–31 μm wide, with row of 5 teeth. IO 0.94–0.97, MO 1.00–1.06. Paraligula bifid, 23–29 μm long, inner tooth 20–21 μm long, shorter than outer tooth. Pecten hypopharyngis with 7–8 teeth almost equal in size.

Body (Figs 21H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 103–127 μm long, 26–41 μm wide, with 7 anal setae 411–565 (4) μm long. L/W 2.74–4.48. Supraanal seta well developed. Anal tubules 167–176 (3) μm long. Posterior parapod 294–366 (2) μm long; subbasal seta simple, with 3–5 small teeth (Fig. 21H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 21I). B/C 1.09–1.22.



Figures 21A–I. *Labrundinia pilosella* (Loew), larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

***Labrundinia separata* (Edwards)** (Figs 22–24)

Pentaneura separata Edwards 1931: 252 (description male and female)

Labrundinia separata Siri and Donato 2011: 76 (description male, female and immatures)

Material examined

Type: Holotype male, Argentina: **Río Negro**, Lago Nahuel Huapi, 28–31.x.1926, F.W. Edwards (BMNH 950647).

Additional: 1 female with pupal and larval exuviae, Argentina: **Río Negro**, Mallín La Heladera, 10.ii.2009, A. Siri. 2 pupae as previous. 1 larva as previous. 4 males, Chile: **South**, SA 78, G. Kuschel.

Diagnostic characters

Labrundinia separata differs from other *Labrundinia* species by the combination of the following characters. **Male**: abdominal tergite I pale brown, T II–V with continuous brown transverse band near proximal margin, T VI–VIII wholly brown; hypopygium brown, sternapodeme with distinct anterior process. **Pupa**: thoracic horn wedge-shaped, preapical indentation absent; shagreen on segment II with prominent spines. **Larva**: surface of head capsule covered with spinules, lateroventral and posteroventral spine groups absent; AR about 2.11; subbasal seta simple; bifid claw with V-shaped lower indentation.

Description

Adult male ($n = 5$)

Size. Total length 2.88–3.13 mm. Wing length 1.78–2.22 mm. Total length/wing length 1.36–1.64. Wing length/length of profemur 2.36–3.19.

Coloration. Head dark brown to black with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown with mesonotum dorsally dark brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Abdomen as in figure 22L. Hypopygium brown.

Head (Figs 22–E). Temporal setae 20–27, multiserial (Fig. 22B). Eye ratio 0.81–1.45 (4). Tentorium (Fig. 22C) 155–174 μm long, stipes not measurable. Clypeus 100–165 μm long, 74–87 μm wide at largest part, bearing 15–22 setae. Cibarial pump (Fig. 22D) with anterior margin concave, 189–280 μm long.

Palpomere lengths 1–5 (in μm): 29–38; 65–88; 106–154; 112–169 (3); 231–275 (2). Antenna with 14 flagellomeres, AR 1.14–1.29 (3), flagellum 778–988 (3) μm long, diameter of pedicel 123–152 (4) μm , apical setae single (Fig. 22E).

Thorax. Anteprenotum with 4–6 (3) lateral setae. Acrostichals 34–52, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 30–42, irregularly biserial; prealars 9–17; supraalars 4–5. Anapleural suture ratio 0.40–0.56 (2). Scutellum with 10–12 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

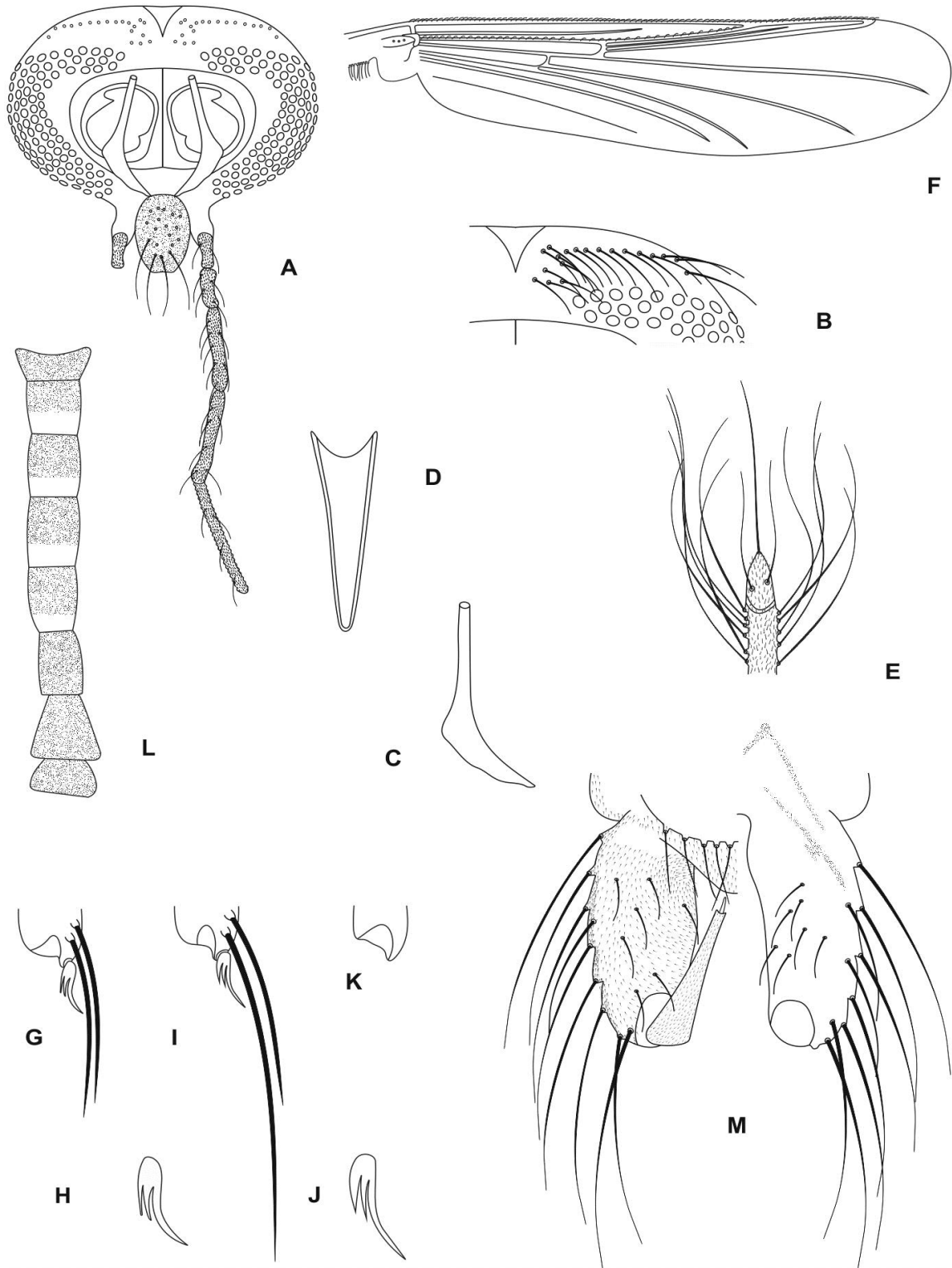
Wing (Fig. 22F). Width 0.48–0.59 mm. Costa 1.60–1.99 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.14–0.17 mm long. VR 0.61–0.71. WW 0.27–0.28. Brachiolium with 2 setae. Squama setiferous.

Legs (Figs 22G–K). Fore leg: width at apex of tibia 44–66 μm (Fig. 22G), tibia with single, apical and pectinate spur 18–28 (4) μm long (Fig. 22H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 38–60 μm long (Fig. 22I), tibia with single, apical and pectinate spur 23–30 (4) μm long with three teeth (Fig. 22J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 44–61 μm long (Fig. 22K), tibia without spur; comb indistinct; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XVII.

Table XVII. Lengths (in μm) and proportions of leg segments in *Labrundinia separata*, male ($n = 2-5$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	694–857	650–838	575–583	350–363	222–250
p ₂	694–956	813–938	325–431	200–250	131–181
p ₃	763–888	863–1219	932–1069	436–450	296–306
	ta ₄	ta ₅	LR	BV	SV
p ₁	156	75	0.69–0.70	2.72	2.93
p ₂	131–181	100–131	0.78–1.04	2.46–3.12	2.00–2.40
p ₃	180–206	131–111	0.82–1.24	2.78–2.80	2.08–2.04

Hypopygium (Fig. 22M). Tergite IX arched, with 11–16 dorsal setae. Anal point small, apical edge rounded. Phallapodeme 62–93 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 164–173 (4) μm long, 58–70 (4) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.31–2.89. Gonostylus simple and slender, 95–106 μm long; megaseta 12–15 μm long. HR 1.64–1.75. HV 2.84–3.05.



Figures 22A–M. *Labrundinia separata* (Edwards), adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia without comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Adult female (n = 1)

Size. Total length 2.55 mm. Wing length 1.57 mm. Total length/wing length 1.62. Wing length/length of profemur 3.11.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen wholly dark brown. Seminal capsules brown.

Head. Temporal setae 12, uniserial. Eyes ratio 1.93. Tentorium 100 μm long, stipes not measurable. Clypeus 99 μm long, 74 μm wide at largest part, bearing 18 setae. Cibarial pump as in male, 187 μm long. Palpomere lengths 1–5 (in μm): 43; 58; 107; 113; 244. Antenna with 11 flagellomeres, AR 0.37, flagellum 538 μm long, diameter of pedicel 55 μm .

Thorax. Anteprenotum with 4 lateral setae. Acrostichals 46, irregularly uniserial, starting close to anteprenotum; dorsocentrals 36, irregularly biserial; prealars 17; supraalars 5. Scutellum with 12 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.55 mm. Costa 0.77 mm long, not produced beyond R_{4+5} . R_{2+3} present. Base of radial sector 0.11 mm long. VR 0.63. WW 0.35. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 37 μm , tibia with single, apical and pectinate spur 17 μm , with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Mid leg: width at apex of tibia 47 μm , tibia with single, apical and pectinate spur 23 μm , with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Hind leg: width at apex of tibia 40 μm long, tibia without spur; comb with indistinct; preapical pseudospurs on Ta_{1-4} unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XVIII.

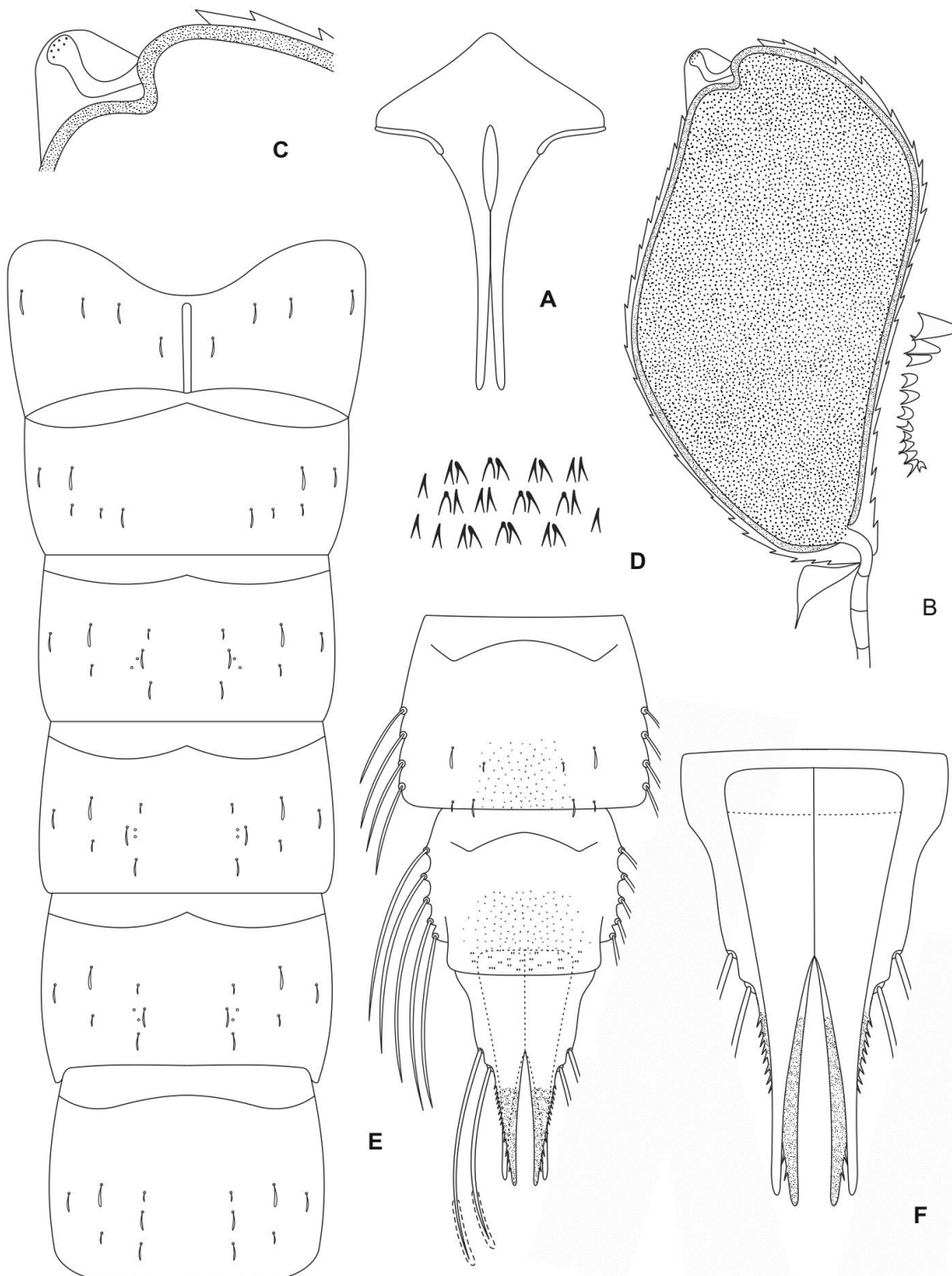
Genitalia. Gonapophysis VIII broadly rounded, 72 μm long. Coxosternapodeme 80 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 44 μm long and 17 μm wide; with 10 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 125 μm . Seminal capsules oval with conical shaped necks, length 41 μm , maximum width 37 μm . Length ratio SCa/No 0.33.

Pupa (n = 3 unless otherwise stated)

Size. Male abdomen 2.27–2.32 (2) mm long; female abdomen 2.29 (1) mm long.

Coloration. Exuviae and thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 23A–C). Frontal apotome as in figure 23A. Wing sheath smooth 0.70–0.96 mm long. Thoracic horn 285–293 μm long and 156–187 μm wide (Fig. 23B), preapical indentation



Figures 23A–F. *Labrundinia separata* (Edwards), pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Spines on segment II. **E.** Abdominal segments with chaetotaxy, dorsal aspect. **F.** Anal lobe and male genital sac, ventral aspect.

Table XVIII. Lengths (in μm) and proportions of leg segments in *Labrundinia separata*, female ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	505	540	422	260	181
p ₂	720	559	566	288	175
p ₃	636	802	699	338	233
	ta ₄	ta ₅	LR	BV	SV
p ₁	126	92	0.78	2.22	2.48
p ₂	107	92	1.01	2.78	2.26
p ₃	136	100	0.87	2.64	2.06

absent, THR 1.54–1.82. Membranous preapical papilla 45–52 μm long (Fig. 23C), PTH 0.15–0.18, aeropyle tube simple, short, 27–31 μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules, almost reaching apex of thoracic horn. Basal lobe wedge-shaped. Thoracic comb with 11–12 conical teeth (Fig. 23B).

Abdomen (Figs 23D–F). Tergite I with scar, 155–168. T I–VI without shagreen, VII–VIII with shagreen medial basal concentrated. Segment II with dense, prominent spines (Fig. 23D). Abdominal chaetotaxy as in figure 23E. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 304–349 μm long and 146–152 μm wide (Fig. 23F), outer margins sclerotized, with 13–18 spines, longest spine 20–23 μm long, inner margins of lobes membranous. ALR 1.99–2.32. Genital sac elongated, almost reaching apex of anal lobe.

4th instar larva ($n = 1$ unless otherwise stated)

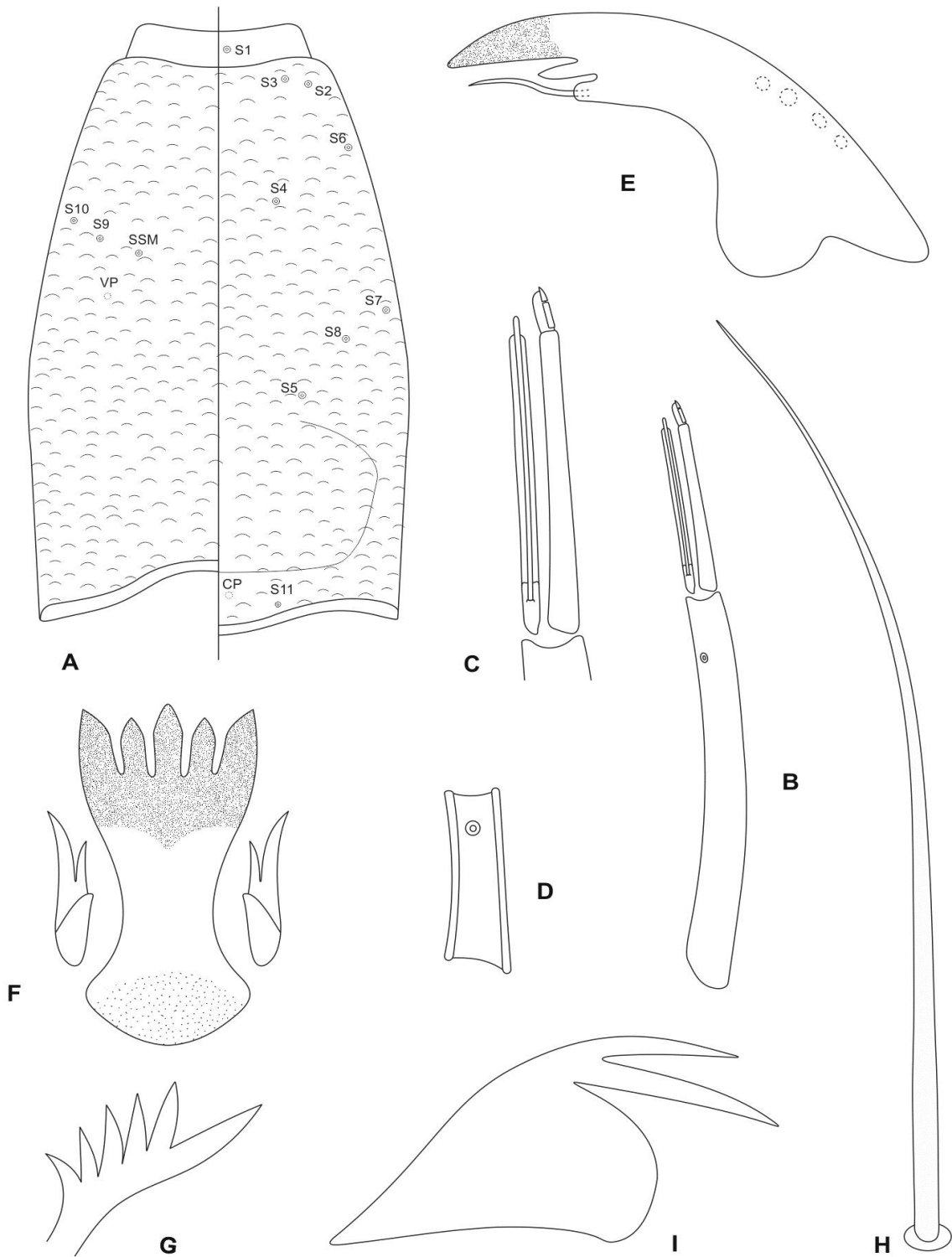
Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 24A). Length 490 μm , 316 μm wide. Surface covered with small spinules; lateroventral and posteroventral spine groups absent; cephalic index 0.65. Chaetotaxy as in figure 24A.

Antenna (Figs 24B–C). Length 279 μm , A₁ 189 μm long, with ring organ placed 160 μm from base, A₂ 79 μm long. AR 2.11. Blade 87 μm long, over-reached by accessory blade.

Maxilla (Fig. 24D). Basal palp segment 27 μm long and 7 μm wide, with ring organ 22 μm from base. PR 3.42. APR 6.94.

Mandible (Fig. 24E). Length 72 μm , with 3 lateral setae. Sensillum campaniformium 49 μm from apex,



Figures 24A–I. *Labrundinia separata* (Edwards), larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.62.

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate, 80 µm long.

Hypopharyngeal complex (Figs 24F–G). Ligula 62 µm long, 59 µm wide, with row of 5 teeth. IO 0.99, MO 1.07. Paraligula bifid, 28 µm long, inner tooth 24 µm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 24H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 146 µm long, 34 µm wide, with 7 anal setae, 589 µm long. L/W 4.29. Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod 266 µm long; subbasal seta simple, without spines (Fig. 24H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 24H). B/C 0.97 µm long.

Labrundinia tenata Roback (Figs 25–27)

Labrundinia tenata Roback 1987: 214 (description of male).

Labrundinia tenata Silva and Fonseca-Gessner 2009: 541 (redescription male and immatures).

Labrundinia tenata Trivinho-Strixino 2011: 338 (larvae identification key).

Material examined

Type: Holotype male, Colombia: **Departamento del Meta**, Puerto López, Laguna Mozambique, 16 km s. w. of Puerto López, 25.ii.1972, N. R. Foster (ANSP). Paratype: 2 males same data as holotype; 2 males same data as holotype except for 9.ii.1972.

Additional: 2 males with pupal and larval exuviae, Brazil: **São Paulo**, São Carlos, Fazzari reservoir, 22.v.2008, F. L. Silva; 1 male with pupal and larval exuviae as previous except for 14.v.2008. 1 male with pupal and larval exuviae as previous except for 27.xi.2007. 1 male with pupal and larval exuviae as previous except for 4.v.2007. 1 male with pupal exuvia as previous except for Monjolinho stream, 27.vii.2009. 1 female with pupal and larval exuviae as previous except for Valparaíso lake, 4.iv.2011. 1 female with pupal and larval exuviae as previous except for 19.iv.2011.

Diagnostic characters

Labrundinia tenata differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite I pale brown, T II–VI with continuous brown transverse band near proximal

margin, T VII–VIII wholly brown; hypopygium brown, sternapodeme rounded, with anterior process absent. **Pupa:** thoracic horn semi-globose, preapical indentation moderate deep, forming a small diverticulum; abdominal segment VII with 2 lateral setae. **Larva:** surface of head capsule covered with spinules, lateroventral spine group absent, posteroventral spine group present, with 5–13 spines; subbasal seta simple; serrated claw present; bifid claw with U-shaped lower indentation.

Redescription

Adult male (n = 11)

Size. Total length 1.64–2.29 (8) mm. Wing length 1.01–1.17 mm. Total length/wing length 1.55–1.97 (8). Wing length/length of profemur 2.43–3.12 (9).

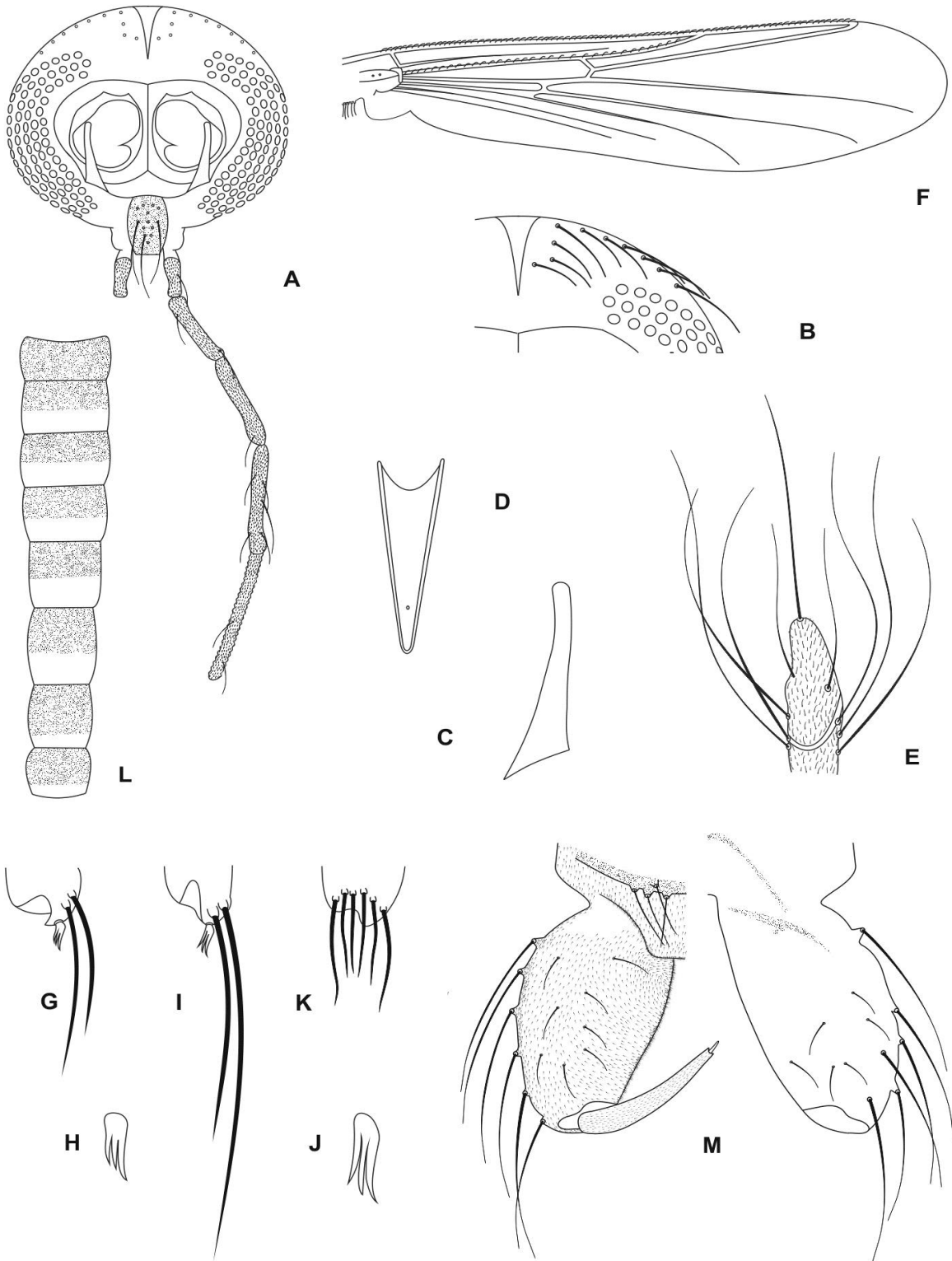
Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax pale brown with mesonotum dorsally dark brown; anteprenotum pale; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 25L. Hypopygium brown.

Head (Figs 25–E). Temporal setae 10–13, uniserial (Fig. 25B). Eye ratio 1.08–1.97 (7). Tentorium (Fig. 25C) 127–145 (5) μm long, stipes not measurable. Clypeus 63–104 (10) μm long, 49–75 (10) μm wide at largest part, bearing 9–13 (10) setae. Cibarial pump (Fig. 25D) with anterior margin concave, 132–171 μm long. Palpomere lengths 1–5 (in μm): 29–44 (8); 39–56 (8); 84–117 (8); 115–127 (5); 157–199 (4). Antenna with 14 flagellomeres, AR 1.04–1.24 (7), flagellum 599–768 (7) μm long, diameter of pedicel 92–113 (9) μm , apical setae single (Fig. 25E).

Thorax. Anteprenotum with 2–5 (9) lateral setae. Acrostichals 26–40 (7), biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 13–20 (10), irregularly uniserial; prealars 5–6 (10); supraalars 2. Anapleural suture ratio 0.33–0.54. Scutellum with 6–8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 25F). Width 0.29–0.35 (10) mm. Costa 0.89–1.04 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.06–0.09 (10) mm long. VR 0.67–0.76 (10). WW 0.28–0.31 (10). Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 25G–K). Fore leg: width at apex of tibia 30–39 (9) μm (Fig. 25G), tibia with single, apical and pectinate spur 10–14 (10) μm long (Fig. 25H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 31–46 (10) μm long (Fig. 25I), tibia with single, apical and pectinate spur 14–28 μm long with three teeth (Fig. 25J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 31–46 (10) μm long (Fig. 25K), tibia



Figures 25A–M. *Labrundinia tenata* Roback, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

with spur; comb 7 (9) setae; Ta₁₋₄ without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XIX.

Table XIX. Lengths (in μm) and proportions of leg segments in *Labrundinia tenata*, male (n = 6–10).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	353–437	344–415	242–318	139–172	116–142
p ₂	401–500	367–457	424–531	191–237	123–141
p ₃	367–466	477–580	504–549	198–245	104–175
	ta ₄	ta ₅	LR	BV	SV
p ₁	86–101	54–64	0.71–0.76	2.16–2.50	2.46–2.90
p ₂	81–94	60–87	1.01–1.22	2.68–2.79	1.67–1.92
p ₃	87–96	70–77	0.85–1.05	2.79–3.03	1.79–2.26

Hypopygium (Fig. 25M). Tergite IX arched, with 7–12 dorsal setae. Anal point trapezoidal, apical edge slightly straight. Phallapodeme 43–55 (10) μm long. Sternapodeme with anterior process absent. Gonocoxite cylindrical, 94–104 (7) μm long, 41–63 (7) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.60–2.25 (7). Gonostylus simple and slender, 61–71 (7) μm long; megaseta 10–16 μm long. HR 1.44–1.54 (7). HV 2.85–3.77 (7).

Adult female (n = 2)

Size. Total length 0.93–1.07 mm. Wing length 0.79–0.94 mm. Total length/wing length 1.14–1.17. Wing length/length of profemur 2.69–3.94.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen pale brown, with dark brown transverse bands near proximal margin, not much distinguishable. Seminal capsules brown.

Head. Temporal setae 10, uniserial. Eyes ratio 1.22 (1). Tentorium 111 μm long, stipes not measurable. Clypeus 72–88 μm long, 48–50 μm wide at largest part, bearing 15–17 setae. Cibarial pump as in male, 136–179 μm long. Palpomere lengths 1–4 (in μm): 28–33; 38–44; 80–112; 126 (1). Antenna with 11 flagellomeres, AR 0.35–0.36, flagellum 232–294 μm long, diameter of pedicel 52–56 μm .

Thorax. Anteprenotum with 1–3 lateral setae. Acrostichals 30–38, irregularly uniserial, starting close to anteprenotum; dorsocentrals 24–32, irregularly uniserial; prealars 5–8; supraalars 2. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.31–0.35 mm. Costa 0.68–0.81 mm long, not produced beyond R₄₊₅. R₂₊₃ absent. Base of radial sector 0.05 mm long. VR 0.69–0.70. WW 0.37–0.39. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 33–34 µm, tibia with single, apical and pectinate spur 16–17 µm, with three teeth and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Mid leg: width at apex of tibia 44–47 µm, tibia with single, apical and pectinate spur 17–28 µm, with three teeth and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Hind leg: width at apex of tibia 30–37 µm long, tibia without spur; comb with 7 setae; preapical pseudospurs on Ta₁₋₄ unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XX.

Table XX. Lengths (in µm) and proportions of leg segments in *Labrundinia tenata*, female (n = 1–2).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	238–295	288–357	238	103	74
p ₂	485–549	444–468	–	–	–
p ₃	340–497	408–512	290–380	134–160	88–106
	ta ₄	ta ₅	LR	BV	SV
p ₁	61	47	0.83	2.89	2.44
p ₂	–	–	–	–	–
p ₃	65–70	56–63	0.93	3.01–3.54	1.97

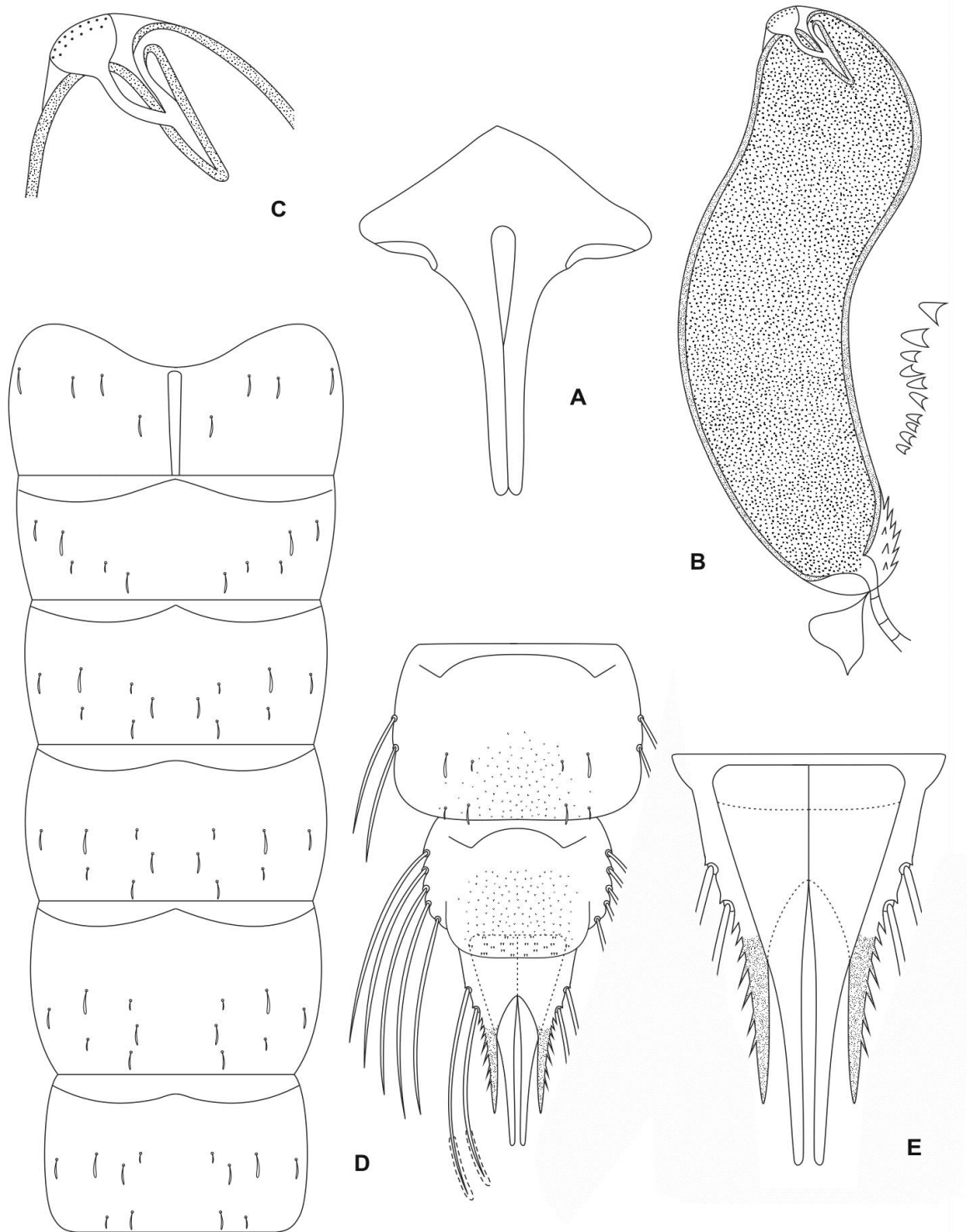
Genitalia. Gonapophysis VIII broadly rounded, 44–69 µm long. Coxosternapodeme 64–67 µm long. Postgenital plate rounded. Cerci oval-quadrangle, 33–44 µm long and 10–21 µm wide; with 8–10 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 81–82 µm. Seminal capsules oval with conical shaped necks, length 31–35 µm, maximum width 30–31 µm. Length ratio SCa/No 0.39–0.43.

Pupa (n = 8 unless otherwise stated)

Size. Male abdomen 1.54–1.95 (6) mm long; female abdomen 1.50–1.76 (2) mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 26A–C). Frontal apotome as in figure 26A. Wing sheath smooth 0.55–0.82 mm long. Thoracic horn 272–226 µm long and 60–83 µm wide (Fig. 26B), preapical indentation moderate deep, THR 2.72–3.41. Membranous preapical papilla 27–35 µm long (Fig. 26C), PTH 0.13–0.18, aeropyle tube simple, short, 17–25 µm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 8–11 conical teeth (Fig. 26B).



Figures 26A–E. *Labrundinia tenata* Roback, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Abdomen (Figs 26D–E). Tergite I with scar, 104–122. T I–VI without shagreen, VII–VIII with shagreen medial basal concentrated. Abdominal chaetotaxy as in figure 26D. Abdominal segment VII with 2 lateral setae. A VIII with 5 lateral setae. Anal lobe 215–285 μm long and 133–192 μm wide (Fig. 26E), outer margins sclerotized, with 6–10 spines, longest spine 9–15 μm long, inner margins of lobes membranous. ALR 1.05–1.16. Genital sac elongated, almost reaching much beyond apex of anal lobe.

4th instar larva ($n = 6$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 27A). Length 480–556 μm , 292–383 μm wide. Surface covered with small spinules; lateroventral spine group absent; posteroventral spine group present, with 5–13 spines; cephalic index 0.59–0.69. Chaetotaxy as in figure 27A.

Antenna (Figs 27B–C). Length 296–314 μm , A_1 197–207 μm long, with ring organ placed 170–184 (4) μm from base, A_2 87–97 μm long. AR 1.76–2.05. Blade longer than A_2 over-reached by accessory blade.

Maxilla (Fig. 27D). Basal palp segment 22–32 μm long and 6–8 μm wide, with ring organ 19–26 (5) μm from base. PR 3.72–5.02. APR 6.59–9.22.

Mandible (Fig. 27E). Length 56–74 μm , with 3 lateral setae. Sensillum campaniformium 38–46 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.64–3.73.

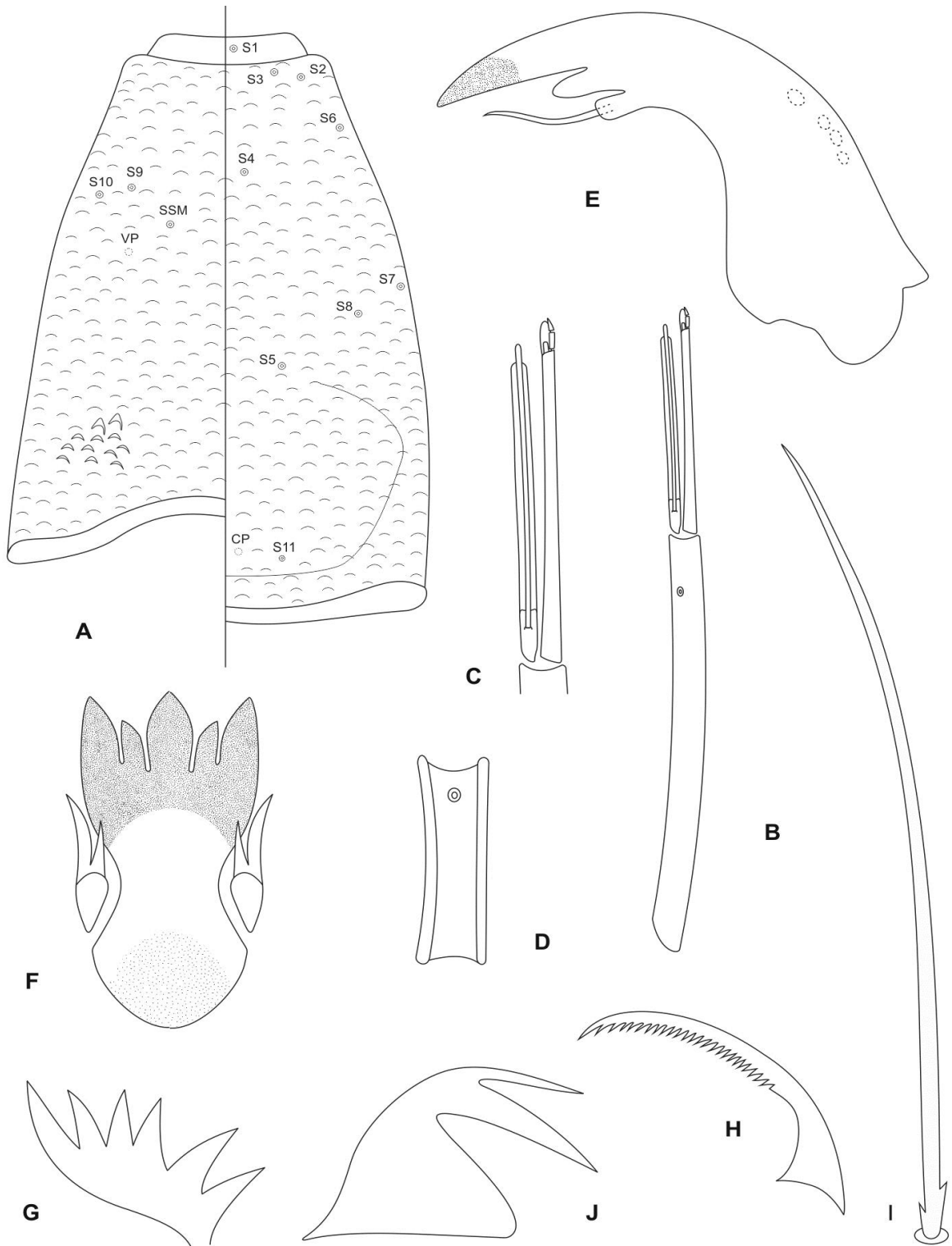
Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 27F–G). Ligula 44–65 μm long, 25–28 μm wide, with row of 5 teeth. IO 0.93–0.98, MO 1.02–1.04. Paraligula bifid, 21–27 μm long, inner tooth 16–21 μm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 27H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 129–142 μm long, 19–29 μm wide, with 7 anal setae, 404–516 (5) μm long. L/W 4.95–7.10. Supraanal seta well developed. Anal tubules 176 (1) μm long. Posterior parapod 401 (1) μm long; subbasal seta simple, with 2–5 small spines (Fig. 27H); parapod apex with numerous simple claws, with serrated claw; bifid claw with U-shaped lower indentation (Fig. 27H). B/C 0.76–1.04 (4) μm long.

Labrundinia virescens Beck and Beck (Figs 28–30)

Labrundinia virescens Beck and Beck 1966: 341 (description of male, female and immatures stages).



Figures 27A–J. *Labrundinia tenata* Roback, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Serrated claw of posterior parapod. **I.** Subbasal seta of posterior parapod. **J.** Bifid claw of posterior parapod.

Labrundinia virescens Roback 1971: 279 (description of male).

Labrundinia virescens Roback 1987: 197 (description of immature stages).

Labrundinia virescens Epler 2001: 4.48 (larvae identification key).

Material examined

Type: 1 male with pupal and larval exuviae, USA: **Florida**, Leon, Boliek's Pond, near Natural Bridge, 30.vi.1964, W. Beck (USNM 69124). *Paratypes*: 1 female with pupal and larval exuviae same data as holotype except for 5.v.1964 (USNM 69124); 1 male with pupal and larval exuviae same data as holotype.

Additional: 1 prepupa, USA: **Florida**, Leon, Boliek's Pond, 22.vii.1965, W. Beck; 1 larva as previous except for Flagler County, Rayonier Ditch, 18.vi.1964; 1 larva as previous except for Chaoborus Pond, 30.vi.1964; 1 larva, **Florida**, St. John's, Sphagnum Ditch, 17.xi.1965. 1 male with pupal and larval exuviae, **Georgia**, St. Simons Isl., Pond at Plantation village, Demere Rd. 23.xi.1990, B. A. Caldwell; 1 female as previous except for 28.xi.1990. 1 male, **North Carolina**, Belews Lake, 5.x.1976, S. S. Roback.

Diagnostic characters

Labrundinia virescens differs from other *Labrundinia* species by the combination of the following characters. **Male**: abdominal tergite I–II pale, T III–V with continuous brown transverse band near proximal margin, TVI–VIII almost wholly brown; hypopygium pale, sternapodeme with moderate anterior process. **Pupa**: thoracic horn 9-shaped, preapical indentation deep; abdominal segment VII with 2 lateral setae; segment II with dense multi-branched spines; genital sac elongated, reaching somewhat beyond apex of anal lobe. **Larva**: surface smooth; lateroventral spine group present, with 4–5 conspicuous spines; posteroventral spine group absent; mandible with short, subequal teeth.

Description

Adult male ($n = 4$)

Size. Total length 2.35–2.83 (3) mm. Wing length 1.33–1.59 mm. Total length/wing length 1.76–1.85 (3). Wing length/length of profemur 2.85–3.22.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax pale brown with mesonotum dorsally dark brown; antepronotum pale; supraalar callus

brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale. Abdomen as in figure 28L. Hypopygium pale.

Head (Figs 28A–E). Temporal setae 10 (2), uniserial (Fig. 28B). Eye ratio 0.78 (1). Tentorium (Fig. 28C) 130 (1) μm long, stipes not measurable. Clypeus 95–110 (2) μm long, 62–88 (2) μm wide at largest part, bearing 14–17 (3) setae. Cibarial pump (Fig. 28D) with anterior margin concave, 127–276 (3) μm long. Palpomere lengths 1–5 (in μm): 46 (1); 62 (1); 162 (1); 167 (1); 262 (1). Antenna with 14 flagellomeres, AR 1.47–1.50(2), flagellum 814–936 (2) μm long, diameter of pedicel 65–98 (3) μm , apical setae single (Fig. 28E).

Thorax. Antepronotum with 4 (2) lateral setae. Acrostichals 32–54 (2), biserial, diverging evenly posteriorly, starting close to antepronotum and almost reaching scutellum; dorsocentrals 27–32 (2), irregularly biserial; prealars 9–12; supraalars 3. Anapleural suture ratio 0.41–0.42 (2). Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

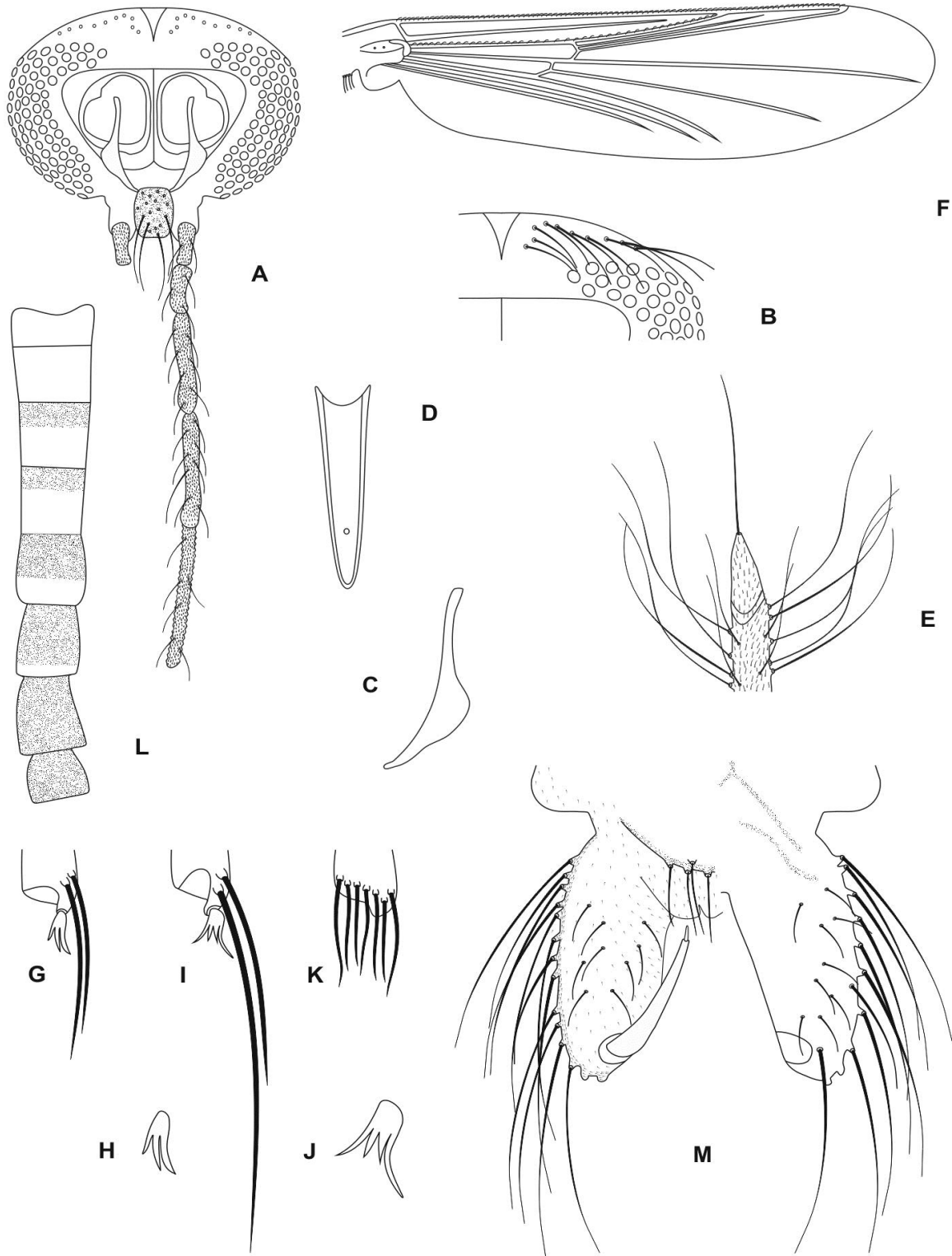
Wing (Fig. 28F). Width 0.39–0.43 mm. Costa 1.14–1.32 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.05–0.08 mm long. VR 0.74–0.79. WW 0.25–0.29. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 28G–K). Fore leg: width at apex of tibia 31–39 μm (Fig. 28G), tibia with single, apical and pectinate spur 13–21 (10) μm long (Fig. 28H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 31–43 μm long (Fig. 28I), tibia with single, apical and pectinate spur 19–29 μm long with 3–4 teeth (Fig. 28J) and two preapical setae; Ta_{1-3} each with two preapical pseudospurs. Hind leg: width at apex of tibia 31–54 μm long (Fig. 28K), tibia without spur; comb 7 setae; Ta_1 with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXI.

Table XXI. Lengths (in μm) and proportions of leg segments in *Labrundinia virescens*, male ($n = 1-3$).

	fe	ti	ta_1	ta_2	ta_3
p_1	437–549	475–545	294–383	218–255	158–187
p_2	465–712	443–589	466–714	287–332	159–175
p_3	610	796	690	325	219
	ta_4	ta_5	LR	BV	SV
p_1	125–156	70–79	0.62–0.70	2.05–2.27	2.85–3.17
p_2	107–126	87–98	1.05–1.21	2.15–2.75	1.82–1.95
p_3	152	96	0.87	2.64	2.03

Hypopygium (Fig. 28M). Tergite IX arched, with 10–14 dorsal setae. Anal point trapezoidal, apical edge slightly notched. Phallapodeme 49–73 (3) μm long. Sternapodeme with moderate anterior process.



Figures 28A–M. *Labrundinia virescens* Beck and Beck, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Gonocoxite cylindrical, 138–186 (3) μm long, 43–82 (3) μm wide, with slightly concave inner margin; inferior volsella absent. GeR 2.27–2.51 (3). Gonostylus simple and slender, 88–111 μm long; megaseta 10–16 μm long. HR 1.57–1.67 (3). HV 2.55–3.97 (3).

Adult female (n = 2)

Size. Total length 1.69–1.85 mm. Wing length 1.21–1.29 mm. Total length/wing length 1.45–1.51. Wing length/length of profemur 2.56 (1).

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs brown. Abdomen seems wholly brown, not much distinguishable. Seminal capsules brown.

Head. Temporal setae 11 (1), uniserial. Eyes ratio 1.38 (1). Tentorium 160 (1) μm long, stipes not measurable. Clypeus 84 (1) μm long, 82 (1) μm wide at largest part, bearing 22 setae. Cibarial pump as in male, 185 μm long. Palpomere lengths 1–4 (in μm): 52 (1); 53 (1); 110 (1); 114 (1). Antenna with 11 flagellomeres, AR 1.17 (1), flagellum 416 (1) μm long, diameter of pedicel 62 (1) μm .

Thorax. Anteprenotum setae unobserved. Acrostichals 42–44, irregularly uniserial, starting close to anteprenotum; dorsocentrals 36, irregularly biserial; prealars 10–11; supraalars 3. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.39–0.48 mm. Costa not produced beyond R_{4+5} , unmeasured. R_{2+3} unobserved. Base of radial sector 0.06 (1) mm long. VR 0.82 (1). WW 0.30–0.43. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 34–39 μm , tibia with single, apical and pectinate spur 19–28 μm , with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Mid leg: width at apex of tibia 46–47 μm , tibia with single, apical and pectinate spur 23–38 μm , with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Hind leg: width at apex of tibia 39–43 μm long, tibia without spur; comb unobserved; preapical pseudospurs on Ta_{1-4} unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXII.

Genitalia. Gonapophysis VIII broadly rounded, 68 (1) μm long. Coxosternapodeme 87 (1) μm long. Postgenital plate rounded. Cerci oval-quadrangle, 68 (1) μm long and 10 (1) μm wide; with 4 (1) elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 107 (1) μm . Seminal capsules oval with conical shaped necks, length 46 (1) μm , maximum width 36 (1) μm . Length ratio SCa/No 0.43(1).

Pupa (n = 4 unless otherwise stated)

Table XXII. Lengths (in μm) and proportions of leg segments in *Labrundinia virescens*, female ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	438	444	305	198	140
p ₂	632	578	625	234	156
p ₃	516	688	625	281	219
	ta ₄	ta ₅	LR	BV	SV
p ₁	108	80	0.68	2.26	2.88
p ₂	109	94	1.08	3.09	1.93
p ₃	140	86	0.91	2.51	1.91

Size. Male abdomen 2.10–2.68 (3) mm long; female abdomen 2.51(1) mm long.

Coloration. Exuviae pale brown; thoracic horn brown.

Cephalothorax (Figs 29A–C). Frontal apotome as in figure 29A. Wing sheath smooth 0.74–1.09 mm long. Thoracic horn 258–313 μm long and 131–144 μm wide (Fig. 29B), preapical indentation deep, THR 1.94–2.17. Membranous preapical papilla 50–54 μm long (Fig. 29C), PTH 0.18–0.20, aeropyle tube simple, elongated and slender, 46–55 μm long; plastron plate reduced. Horn sac moderately developed. Reticulation of respiratory atrium indistinct, external membrane with pale spinules. Basal lobe wedge-shaped. Thoracic comb with 8–10 conical teeth.

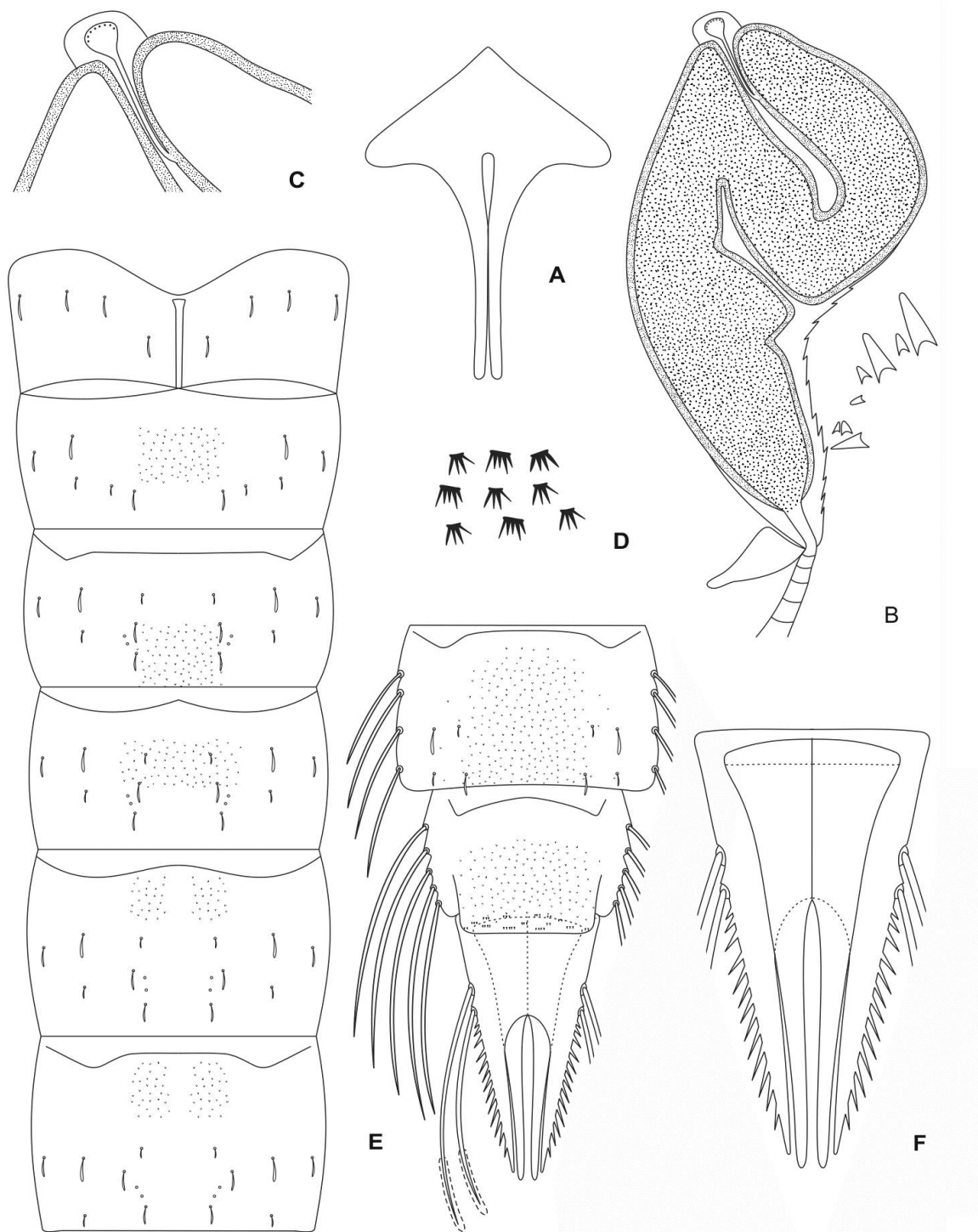
Abdomen (Figs 29D–F). Tergite I with scar, 138–163 (2). T I without shagreen, II–VI with a few spines medial apical concentrated, VII–VIII with shagreen medial concentrated. Segment II with dense multi-branched spines (Fig. 29D). Abdominal chaetotaxy as in figure 29E. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 320–441 μm long and 156–208 μm wide (Fig. 29F), outer margins sclerotized, with 12–13 spines, longest spine 39–45 μm long, inner margins of lobes membranous. ALR 1.99–2.11. Genital sac elongated, reaching somewhat beyond apex of anal lobe.

4th instar larva ($n = 7$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 30A). Length 668–683 (2) μm , 382–426 (2) μm wide. Surface smooth; lateroventral spine group present, with 4–5 spines; posteroventral spine group absent; cephalic index 0.55–0.64 (2). Chaetotaxy as in figure 30A.

Antenna (Figs 30B–C). Length 338–390 μm , A₁ 240–274 μm long, with ring organ placed 217–261 (5)



Figures 29A–F. *Labrundinia virescens* Beck and Beck, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Spines on segment II. **E.** Abdominal segments with chaetotaxy, dorsal aspect. **F.** Anal lobe and male genital sac, ventral aspect.

μm from base, A_2 86–100 μm long. AR 2.35–2.55. Blade longer than A_2 over-reached by accessory blade. *Maxilla* (Fig. 30D). Basal palp segment 35–58 μm long and 7–14 μm wide, with ring organ 27–53 (5) μm from base. PR 3.69–5.60. APR 4.30–7.32.

Mandible (Fig. 30E). Length 80–120 μm , with 3 lateral setae. Sensillum campaniformium 57–84 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.02–3.11.

Mentum and M appendage. Dorsosomal teeth reduced; pseudoradula uniformly granulate, 61–75 (2) μm long.

Hypopharyngeal complex (Figs 30F–G). Ligula 70–89 (6) μm long, 40–47 (6) μm wide, with row of 5 teeth. IO 0.88–0.93, MO 0.98–1.06. Paraligula bifid, 31–40 μm long, inner tooth 22–32 μm long, shorter than outer tooth. Pecten hypopharyngis with 7 teeth almost equal in size.

Body (Figs 30H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 216–243 μm long, 31–45 (6) μm wide, with 7 anal setae, 412–739 (6) μm long. L/W 5.35–8.76 (5). Supraanal seta well developed. Anal tubules 250–434 (5) μm long. Posterior parapod 401 (1) μm long; subbasal seta simple, with 1–2 small spines (Fig. 30H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 30H). B/C 1.22–1.29 μm long.

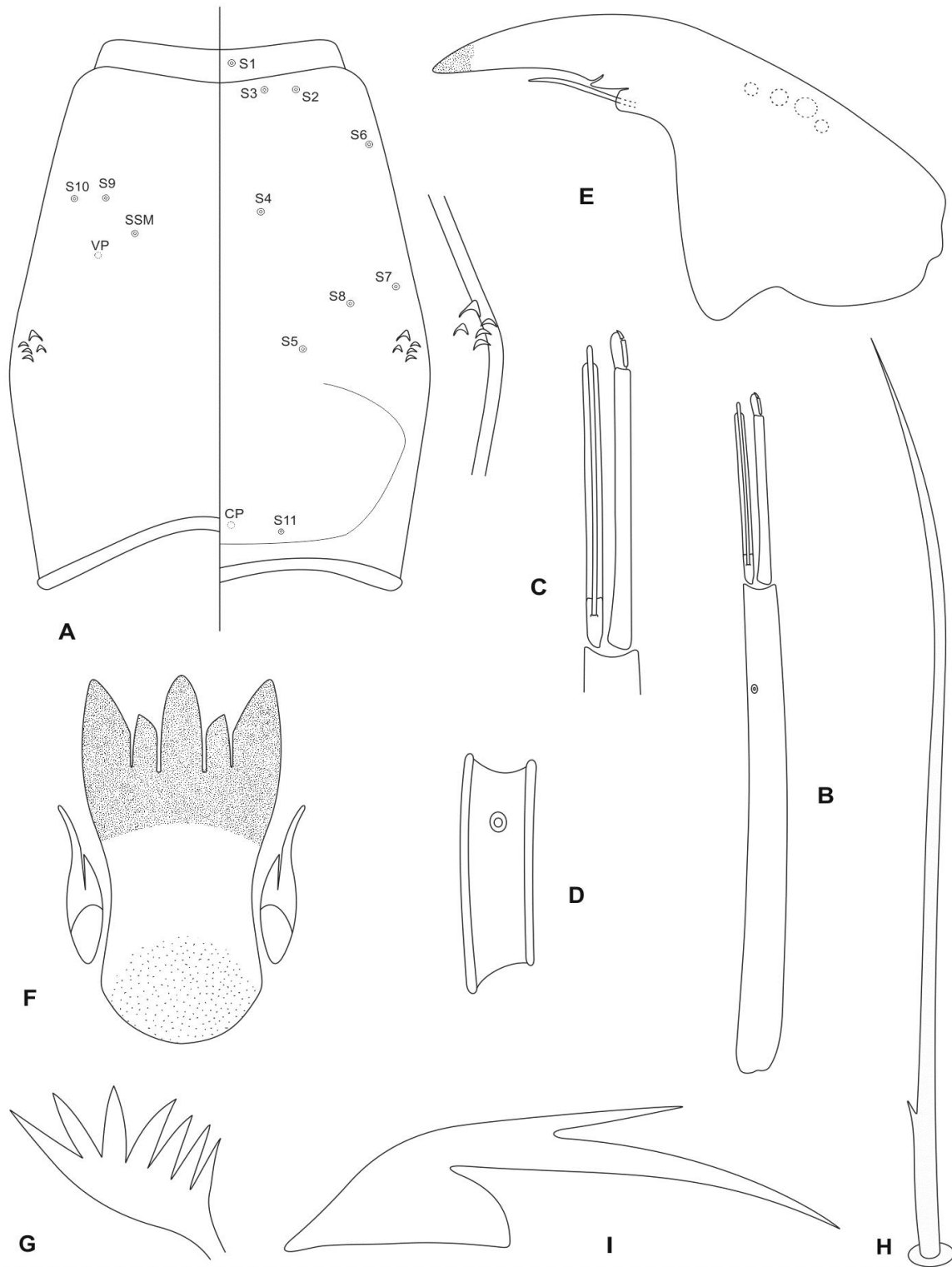
Labrundinia spec. nov. 1 (Figs 31–33)

Material examined

Type: Holotype male with pupal and larval exuviae, **Brazil**: **São Paulo**, Ribeirão Preto, Monte Alegre Lake, 23.viii.1997, H. F. Mendes (MZUSP). Paratypes: 3 males with pupal exuvia same data as holotype; 1 female with pupal exuvia same data as holotype; 2 males same data as holotype; 1 male with pupal exuvia same data as holotype except for 26.ii.1997; 2 pupa same data as holotype; 2 males same data as holotype except for 30.viii.1997; 1 male same data as holotype except for **Mato Grosso**, Nova Mutum, Fazenda Buriti, Buriti stream, 09.i.1999.

Diagnostic characters

Labrundinia sp. 1 differs from other *Labrundinia* species by the combination of the following characters. **Male**: fore leg with tibial comb; abdominal tergite I–II, VIII pale, T II–IV, VI–VII with continuous brown transverse band near proximal margin, T V with divided brown transverse band near proximal margin; hypopygium pale, sternapodeme with moderate anterior process. **Pupa**: thoracic horn light bulb-shaped,



Figures 30A–I. *Labrundinia virescens* Beck and Beck, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

preapical indentation absent; genital sac elongated, not reaching apex of anal lobe. **Larva:** surface covered with small spinules or nodules, with ventral maculation; lateroventral spine group present with one single, well developed spine; posteroventral spine group present with about 18 spines; bifid claw with U-shaped lower indentation.

Description

Adult male (n = 10)

Size. Total length 1.84–2.45 mm. Wing length 1.12–1.34 (9) mm. Total length/wing length 1.57–1.94. Wing length/length of profemur 2.01–3.05.

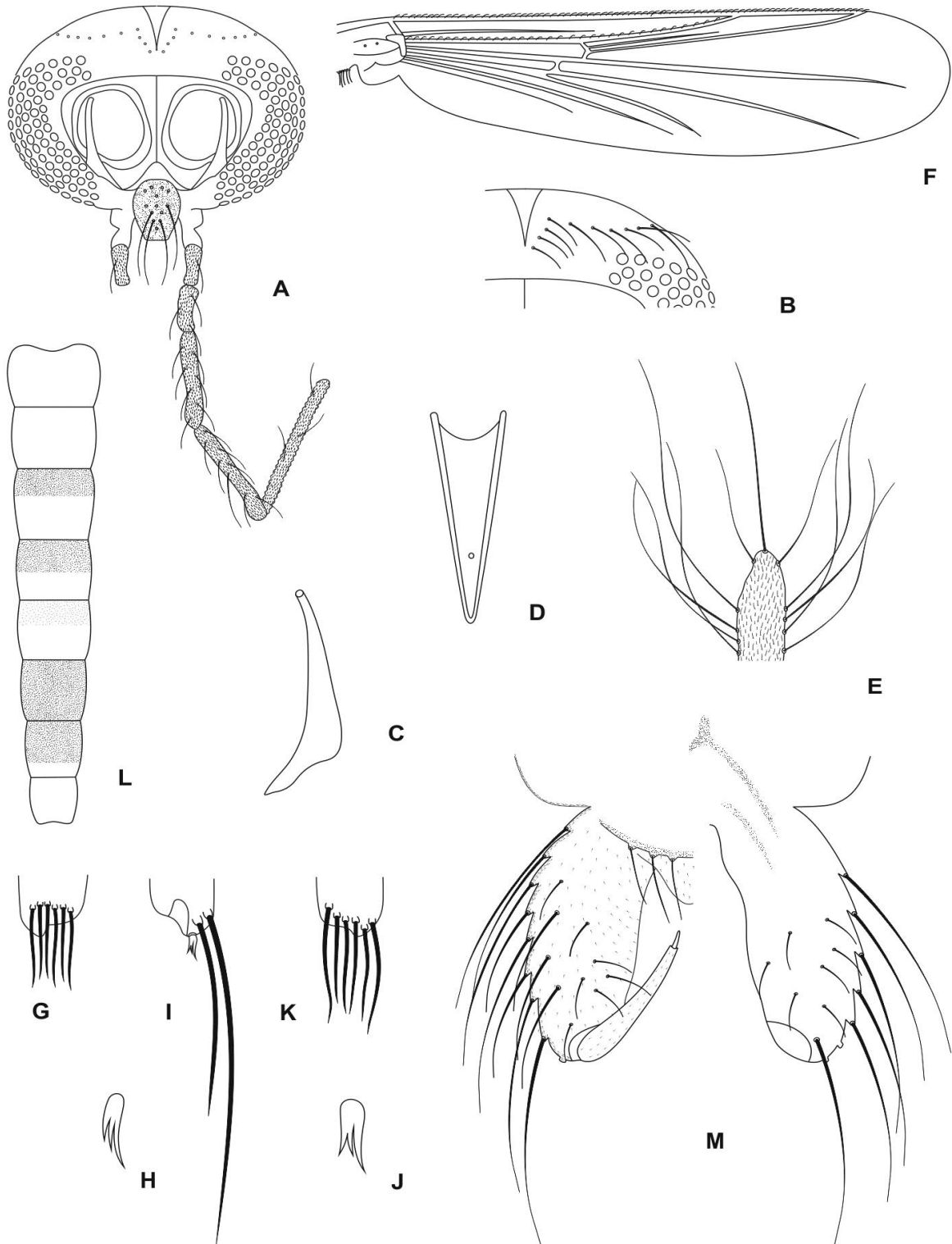
Coloration. Head yellow-brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown with mesonotum dorsally dark brown; anteprenotum yellow-brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Tibia I–III with apex brown. Abdomen as in figure 31L. Hypopygium pale.

Head (Figs 31–E). Temporal setae 10–14, uniserial (Fig. 31B). Eye ratio 1.00–1.39 (7). Tentorium (Fig. 31C) 125–146 (6) μm long, stipes not measurable. Clypeus 91–106 μm long, 58–67 μm wide at largest part, bearing 16–20 setae. Cibarial pump (Fig. 31D) with anterior margin concave, 160–201 μm long. Palpomere lengths 1–5 (in μm): 29–35; 34–49; 109–134; 111–142 (8); 197–208 (8). Antenna with 14 flagellomeres, AR 1.18–1.34 (9), flagellum 680–790 (9) μm long, diameter of pedicel 92–120 (8) μm , apical setae single (Fig. 31E)

Thorax. Anteprenotum with 2–4 (7) lateral setae. Acrostichals 34–40 (5), biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20–30 (7), irregularly biserial; prealars 7–9; supraalars 2 (9). Anapleural suture ratio 0.35–0.52. Scutellum with 8–10 (6) setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 31F). Width 0.31–0.40 mm. Costa 0.96–1.23 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.05–0.07 mm long. VR 0.71–0.78. WW 0.27–0.29. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 31G–K). Fore leg: width at apex of tibia 38–43 μm (Fig. 31G), tibia with single, apical and pectinate spur 15–21 μm long (Fig. 31H), with three teeth and two preapical setae; comb with 6 setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 38–44 μm long (Fig. 31I), tibia with single, apical and pectinate spur 20–23 (8) μm long with three teeth (Fig. 31J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 38–44 μm long (Fig. 31K), tibia without spur; comb 6 setae; Ta_{1-4} with two preapical pseudospurs. Legs with slender, hook-shaped claws.



Figures 31A–M. *Labrundinia* sp. 1, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Table XXIII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 1, male (n = 6–10).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	406–487	400–525	218–344	200–250	125–163
p ₂	581–675	381–487	575–618	231–275	118–175
p ₃	481–563	519–593	488–613	200–263	131–181
	ta ₄	ta ₅	LR	BV	SV
p ₁	94–144	63–81	0.55–0.66	2.04–2.24	2.69–3.19
p ₂	106–118	63–88	1.22–1.31	2.61–2.85	1.73–1.89
p ₃	88–118	63–93	0.92–1.05	2.59–2.94	1.81–2.15

Pulvilli absent. Lengths and proportion of leg segments as in Table XXIII.

Hypopygium (Fig. 31M). Tergite IX arched, with 10–15 (9) dorsal setae. Anal point rounded, apical edge slightly notched. Phallapodeme 51–69 (9) μm long. Sternapodeme with moderate anterior process. Gonocoxite cylindrical, 110–137 (7) μm long, 43–52 (7) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.27–2.51 (7). Gonostylus simple and slender, 75–100 μm long; megaseta 12–18 μm long. HR 1.37–1.50 (7). HV 2.40–2.77 (7).

Adult female (n = 1)

Size. Total length 1.91 mm. Wing length 0.85 mm. Total length/wing length 2.24. Wing length/length of profemur 2.66.

Coloration. Head yellow-brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum yellow-brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen pale brown, with dark brown transverse bands near proximal margin, not much distinguishable. Seminal capsules brown.

Head. Temporal setae unobserved. Eyes ratio unmeasured. Tentorium 146 μm long, stipes not measurable. Clypeus 98 μm long, 76 μm wide at largest part, bearing 21 setae. Cibarial pump as in male, 161 μm long. Palpomere lengths 1–5 (in μm): 27; 38; 91; 97; 139. Antenna with 11 flagellomeres, AR 0.37, flagellum 358 μm long, diameter of pedicel 63 μm .

Thorax. Anteprenotum with 1 seta. Acrostichals uncounted, irregularly uniserial, starting close to anteprenotum; dorsocentrals 30, irregularly biserial; prealars 9; supraalars 2. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.32 mm. Costa not produced beyond R₄₊₅, 0.74 mm long. R₂₊₃ present. Base of radial sector

0.04 mm long. VR 0.75. WW 0.37. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 29 μm , tibia with single, apical and pectinate spur 22 μm , with three teeth and two preapical setae; comb unobserved; Ta₁₋₄ without preapical pseudospurs. Mid leg: width at apex of tibia 37 μm , tibia with single, apical and pectinate spur 20 μm , with three teeth and two preapical setae; Ta₁₋₄ each with two preapical pseudospurs. Hind leg: width at apex of tibia 36 μm long, tibia without spur; comb with 6 setae; Ta₁ with preapical two pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXIV.

Table XXIV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 1, female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	285	316	225	133	84
p ₂	484	351	391	168	104
p ₃	416	419	386	163	141
	ta ₄	ta ₅	LR	BV	SV
p ₁	62	57	0.71	2.45	2.66
p ₂	94	66	1.12	2.82	2.13
p ₃	87	72	0.92	2.64	2.16

Genitalia. Gonapophysis VIII broadly rounded, 58 μm long. Coxosternapodeme 79 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 61 μm long and 12 μm wide; with 4 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 78 μm . Seminal capsules oval with conical shaped necks, length 41 μm , maximum width 26 μm . Length ratio SCa/No 0.53.

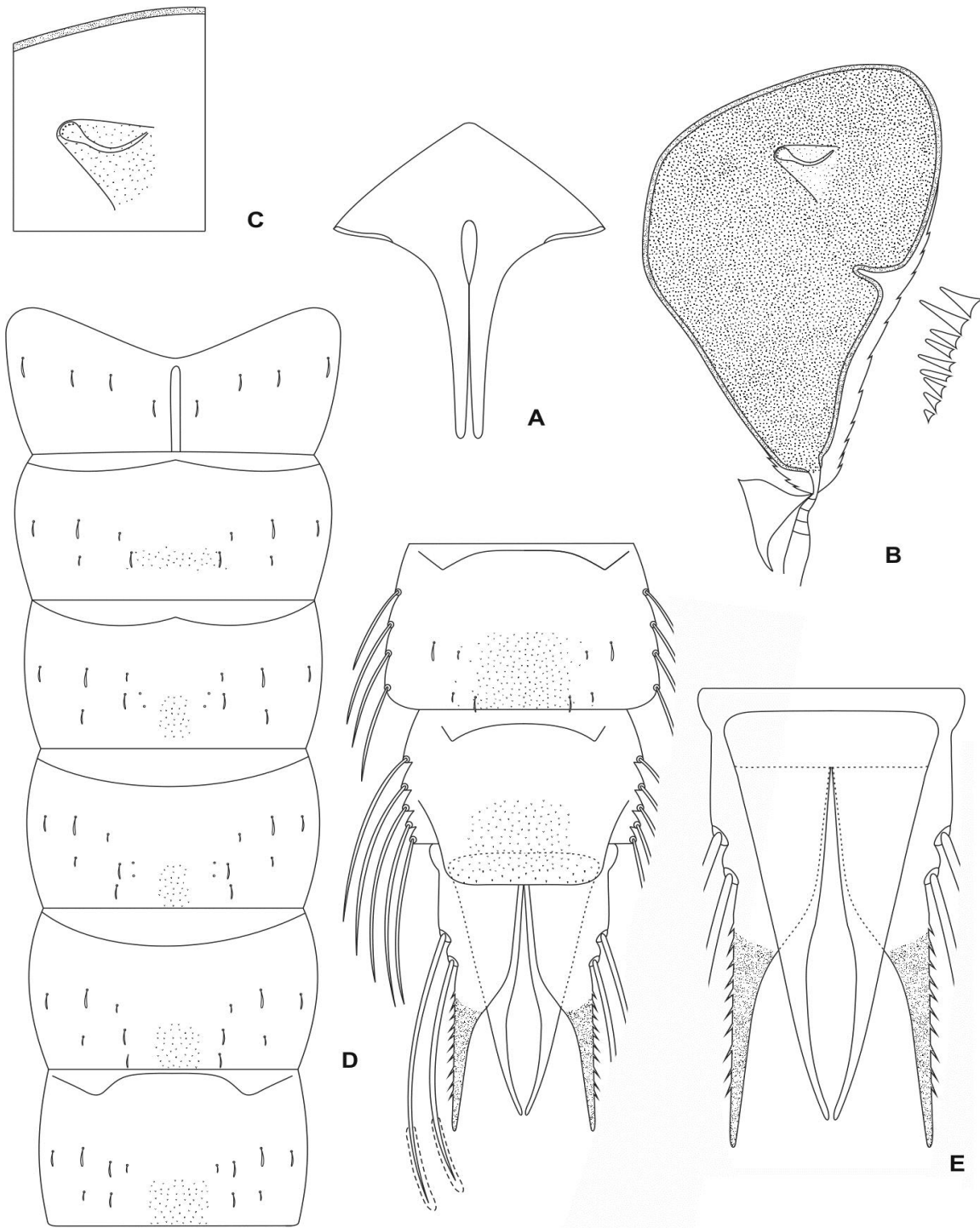
Pupa (n = 8 unless otherwise stated)

Size. Male abdomen 1.69–2.10 (6) mm long; female abdomen 2.02–2.08 (2) mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 32A–C). Frontal apotome as in figure 32A. Wing sheath smooth 0.68–0.81 mm long. Thoracic horn 263–325 (7) μm long and 181–219 (7) μm wide (Fig. 32B), preapical indentation absent, THR 1.37–1.82 (7). Membranous preapical papilla 32–66 (7) μm long (Fig. 32C), PTH 0.12–0.24, aeropyle tube simple, short, 29–45 (5) μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules. Basal lobe wedge-shaped. Thoracic comb with 8–11 conical teeth.

Abdomen (Figs 32D–E). Tergite I with scar, 112–134. T I without shagreen, II–VI with a few spines medial basal concentrated, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 32D.



Figures 32A–E. *Labrundinia* sp. 1, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 293–363 μm long and 163–194 μm wide (Fig. 32E), outer margins sclerotized, with 10–12 spines, longest spine 11–15 μm long, inner margins of lobes membranous. ALR 1.61–2.23. Genital sac elongated, not reaching apex of anal lobe.

4th instar larva (n = 1 unless otherwise stated)

Coloration. Head pale yellow, with maculation as in figure 33A; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 33A). Length 570 μm , 414 μm wide. Surface covered with small spinules or nodules; lateroventral spine group present with one single, well developed spine; posteroventral spine group present with about 18 spines; cephalic index 0.72. Chaetotaxy as in figure 33A.

Antenna (Figs 33B–C). Length 274 μm , A₁ 199 μm long, with ring organ placed 166 μm from base, A₂ 66 μm long. AR 2.60. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 33D). Basal palp segment 32 μm long and 8 μm wide, with ring organ 28 μm from base. PR 4.4. APR 6.12.

Mandible (Fig. 33E). Length 69 μm , with 3 lateral setae. Sensillum campaniformium 51 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.89.

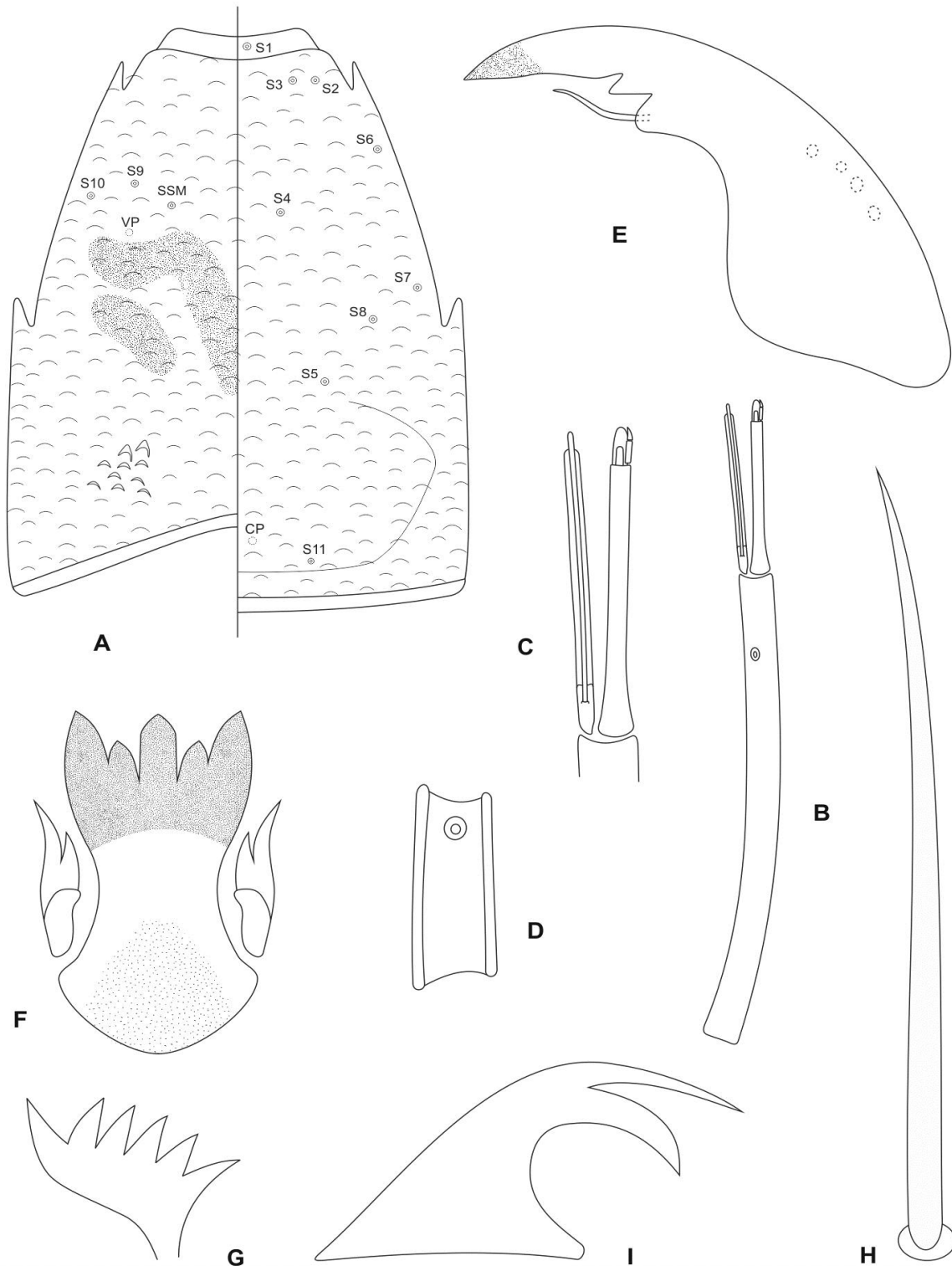
Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate, 48 μm long.

Hypopharyngeal complex (Figs 33F–G). Ligula 56 μm long, 26 μm wide, with row of 5 teeth. IO 0.94, MO 0.96. Paraligula bifid, 23 μm long, inner tooth 21 μm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 33H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 98 μm long, 29 μm wide, with 7 anal setae, 489 μm long. L/W 3.34. Supraanal seta well developed. Anal tubules unobserved. Posterior parapod 458 μm long; subbasal seta simple, without spines (Fig. 33H); parapod apex with numerous simple claws, without serrated claw; bifid claw with U-shaped lower indentation (Fig. 33H). B/C 0.84 μm long.

Remarks: This is the *Labrundinia* sp. 1 (description of male and immatures stages) of Silva (2009).

***Labrundinia* spec. nov. 2** (Figs 34–36)



Figures 33A–I. *Labrundinia* sp. 1, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, São Carlos, Rubi lake, 5.vi.2008, F. L. Silva (MZUSP). *Paratypes:* 1 male and 1 female with pupal and larval exuviae same data as holotype except for Canchin reservoir, 25.vii.2007; 1 male with pupal and larval exuviae same data as holotype except for Monjolinho stream, 29.ii.2009; 1 male with pupal and larval exuviae same data as holotype except for Ribeirão Preto, Monte Alegre Lake 30.viii.1997; 1 larva as previous except for 12.viii.1997; 1 larva same data as holotype except for Luiz Antonio, Óleo lake, 8.iii.1994, S. T Strixino; 1 larva same data as holotype except for Corumbataí, F. O. Roque, 6.ix.2005.

Diagnostic characters

Labrundinia sp. 2 differs from other *Labrundinia* species by the combination of the following characters. **Male:** R₂₊₃ absent; abdominal tergite I–II, VIII pale, T II–VI with continuous brown transverse band near proximal margin, T VII–VIII almost wholly brown; hypopygium pale, sternapodeme with distinct anterior process. **Pupa:** thoracic horn 9-shaped, preapical indentation deep; shagreen on segment II sparse, with small spinules. **Larva:** surface smooth; lateroventral spine group present with one single, well developed spine; posteroventral spine group absent; bifid claw with V-shaped lower indentation.

Description

Adult male ($n = 4$)

Size. Total length 2.42–2.46 (2) mm. Wing length 1.36–1.53 mm. Total length/wing length 1.62–1.82 (2). Wing length/length of profemur 2.57–3.08.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown with mesonotum dorsally dark brown; anteprenotum brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Femur I–III brown. Tibia I–III with apex brown. Abdomen as in figure 34L. Hypopygium pale.

Head (Figs 34–E). Temporal setae 11–15, uniserial (Fig. 34B). Eye ratio 1.08–1.46 (3). Tentorium (Fig. 34C) 141 (1) μm long, stipes not measurable. Clypeus 101–114 μm long, 60–82 μm wide at largest part, bearing 12–16 setae. Cibarial pump (Fig. 34D) with anterior margin concave, 185–225 μm long. Palpomere lengths 1–5 (in μm): 42–52; 50–71; 124–137; 104–158 (3); 251 (1). Antenna with 14

flagellomeres, AR 1.28–1.43 (3), flagellum 887–898 (3) μm long, diameter of pedicel 117 (1) μm , apical setae single (Fig. 34E).

Thorax. Anteprenotum with 5–8 (3) lateral setae. Acrostichals 36–46 (3), biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 24–26 (3), irregularly biserial; prealars 11–12; supraalars 2. Anapleural suture ratio 0.40–0.41 (2). Scutellum with 8–9 (6) setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 34F). Width 0.40–0.44 mm. Costa 1.16–1.29 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.05–0.07 mm long. VR 0.72–0.82. WW 0.29–0.30. Brachiolum with 2 setae. Squama setiferous.

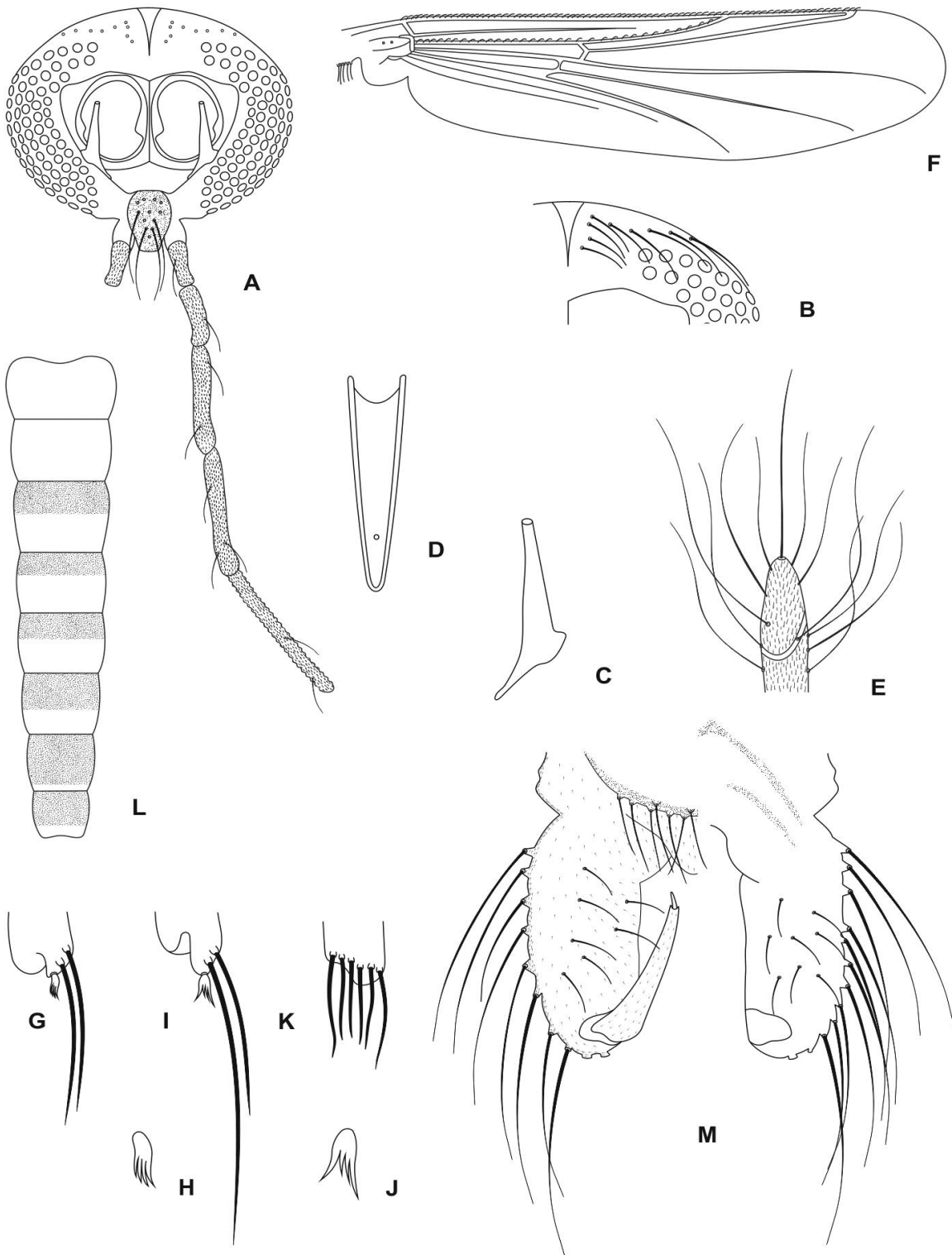
Legs (Figs 34G–K). Fore leg: width at apex of tibia 38–44 μm (Fig. 34G), tibia with single, apical and pectinate spur 13–16 μm long (Fig. 34H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 38–44 μm long (Fig. 34I), tibia with single, apical and pectinate spur 21–33 μm long with three teeth (Fig. 34J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 38–44 μm long (Fig. 34K), tibia without spur; comb 7 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXV.

Table XXV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 2, male ($n = 3-4$).

	fe	ti	ta_1	ta_2	ta_3
p_1	475–581	500–563	319–350	219–250	131–188
p_2	650–725	500–593	650–713	263–313	156–169
p_3	550–588	719–750	663–744	275–331	188–231
	ta_4	ta_5	LR	BV	SV
p_1	106–131	69–81	0.61–0.65	2.20–2.60	2.95–3.32
p_2	113–119	88–100	1.12–1.38	2.79–3.04	1.64–1.85
p_3	125–150	88–106	0.92–0.99	1.87–2.77	1.82–1.99

Hypopygium (Fig. 34M). Tergite IX arched, with 10–15 (3) dorsal setae. Anal point small, apical edge rounded. Phallapodeme 63–67 (2) μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 148–155 (2) μm long, 58–60 (2) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.53–2.59 (2). Gonostylus simple and slender, 88 (2) μm long; megaseta 15–17 μm long. HR 1.68–1.77 (2). HV 2.76–3.08 (2).

Adult female ($n = 1$)



Figures 34A–M. *Labrundinia* sp. 2, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Size. Total length 1.79 mm. Wing length 1.30 mm. Total length/wing length 1.37. Wing length/length of profemur 2.61.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax brown with mesonotum dorsally darker; anteprenotum brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Femur I-III brown. Tibia I-III with apex brown. Abdomen seems wholly brown, not much distinguishable. Seminal capsules brown.

Head. Temporal setae 10, uniserial. Eyes ratio 1.48. Tentorium unmeasured, stipes not measurable. Clypeus 83 μm long, 82 μm wide at largest part, bearing 17 setae. Cibarial pump as in male, 166 μm long. Palpomere lengths 1–5 (in μm): 28; 48; 133; 142; 184. Antenna with 11 flagellomeres, AR 0.35, flagellum 420 μm long, diameter of pedicel 75 μm .

Thorax. Anteprenotum with 8 setae. Acrostichals 38, irregularly uniserial, starting close to anteprenotum; dorsocentrals 24, irregularly biserial; prealars 15; supraalars 2. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

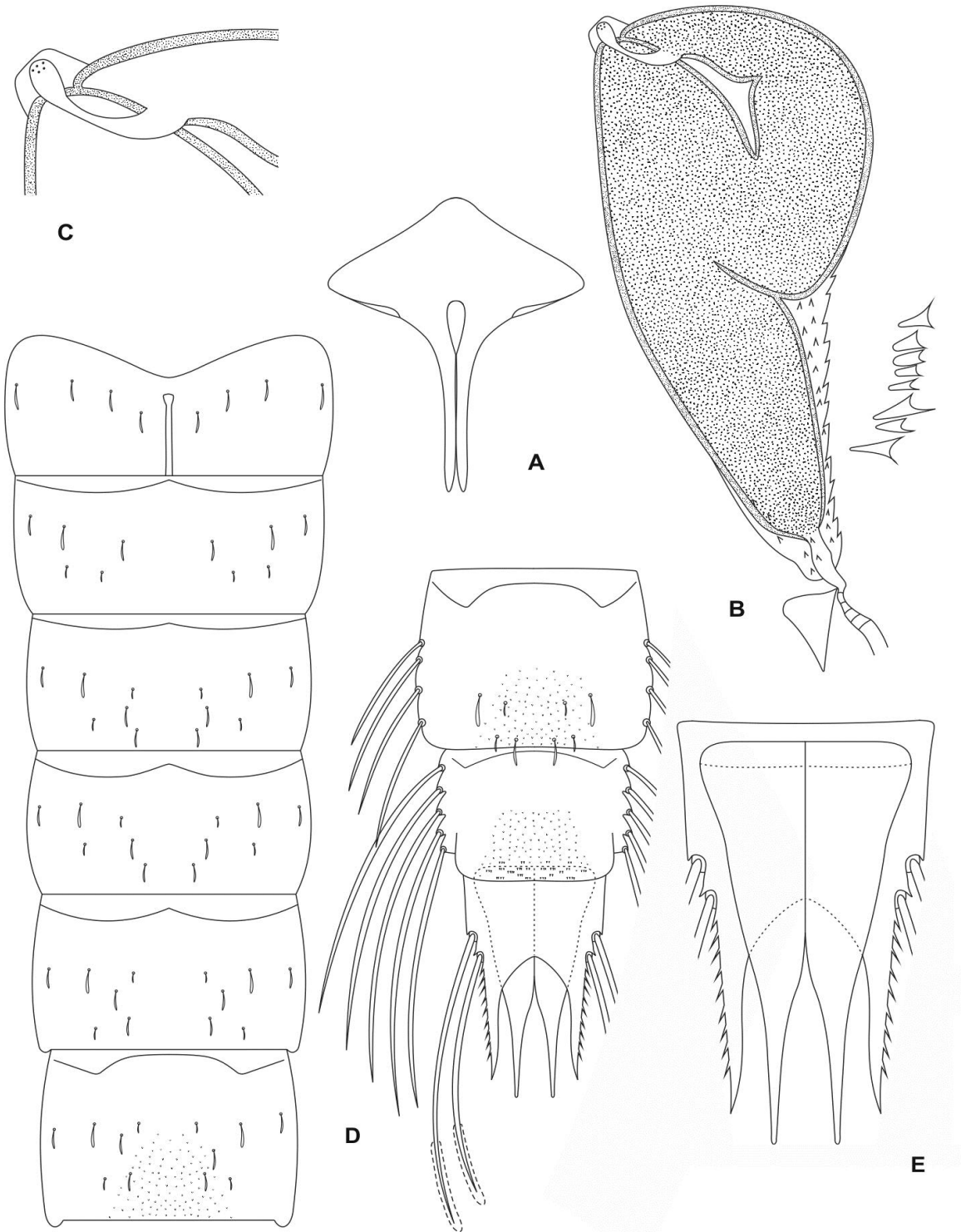
Wing. Width 0.50 mm. Costa not produced beyond R_{4+5} , 1.15 mm long. R_{2+3} absent. Base of radial sector 0.05 mm long. VR 0.76. WW 0.38. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 39 μm , tibia with single, apical and pectinate spur 13 μm , with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 48 μm , tibia with single, apical and pectinate spur 31 μm , with three teeth and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 44 μm long, tibia without spur; comb unobserved; Ta_1 without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXVI.

Table XXVI. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 2, female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	487	429	333	169	132
p ₂	659	582	657	250	151
p ₃	681	561	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	81	75	0.78	2.75	2.78
p ₂	102	90	1.13	3.19	1.89
p ₃	–	–	–	–	–

Genitalia. Coxosternapodeme 99 μm long. Postgenital plate rounded. Cerci oval-quadrate, 51 μm long



Figures 35A–E. *Labrundinia* sp. 2, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

and 62 μm wide; with 4 elongated setae, not much distinguishable. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 66 μm . Seminal capsules oval with conical shaped necks, length 35 μm , maximum width 32 μm . Length ratio SCa/No 0.49.

Pupa (n = 5 unless otherwise stated)

Size. Male abdomen 2.16–2.59 (4) mm long; female abdomen 2.54 (1) mm long.

Coloration. Exuviae pale brown; thoracic horn brown.

Cephalothorax (Figs 35A–C). Frontal apotome as in figure 35A. Wing sheath smooth 0.90–1.08 mm long. Thoracic horn 256–300 μm long and 119–150 μm wide (Fig. 35B), preapical indentation deep, THR 2.00–2.32. Membranous preapical papilla 22–41 μm long (Fig. 35C), PTH 0.07–0.16, aeropyle tube simple, short, 31 μm long; plastron plate reduced. Horn sac moderately developed. Reticulation of respiratory atrium indistinct, external membrane with pale spinules. Basal lobe wedge-shaped. Thoracic comb with 9–11 conical teeth.

Abdomen (Figs 35D–E). Tergite I with scar, 117–143. T I–V without shagreen, VI–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 35D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 338–412 μm long and 156–212 μm wide (Fig. 35E), outer margins sclerotized, with 9–10 spines, longest spine 14–18 μm long, inner margins of lobes membranous. ALR 1.56–2.16. Genital sac elongated, not reaching much beyond apex of anal lobe.

4th instar larva (n = 7 unless otherwise stated)

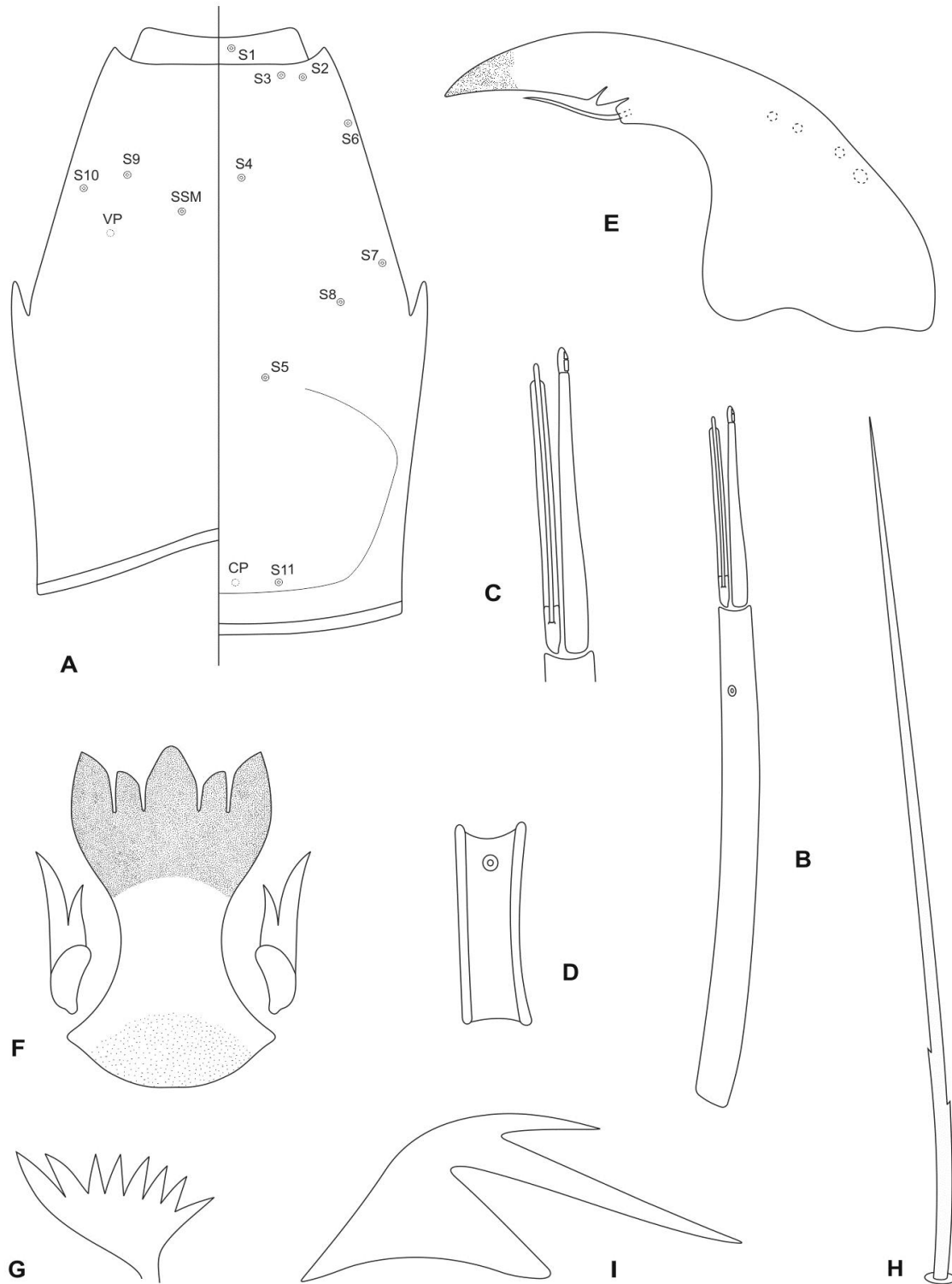
Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 36A). Length 718–781 (6) μm , 507–547 (2) μm wide. Surface smooth; lateroventral spine group present with one single, well developed spine; posteroventral spine group absent; cephalic index 0.67–0.72. Chaetotaxy as in figure 36A.

Antenna (Figs 36B–C). Length 524–582 (6) μm , A₁ 230–258 (6) μm long, with ring organ placed 166 μm from base, A₂ 84–93 (6) μm long. AR 2.38–2.82 (6). Blade longer than A₂; accessory blade 94–100 (5).

Maxilla (Fig. 36D). Basal palp segment 29–44 μm long and 8–12 μm wide, with ring organ 29–38 μm from base. PR 2.42–5.27. APR 5.77–8.96.

Mandible (Fig. 36E). Length 64–76 (6) μm , with 3 lateral setae. Sensillum campaniformium 39–51 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory



Figures 36A–I. *Labrundinia* sp. 2, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

tooth present, AMD 3.58–3.84.

Mentum and M appendage. Dorsosomal teeth reduced; pseudoradula uniformly granulate, 45–56 (2) μm long.

Hypopharyngeal complex (Figs 36F–G). Ligula 62–81 (6) μm long, 34–42 (6) μm wide, with row of 5 teeth. IO 0.92–0.99, MO 0.99–1.03. Paraligula bifid, 32–35 μm long, inner tooth 24–29 μm long, shorter than outer tooth. Pecten hypopharyngis with 7 teeth almost equal in size.

Body (Figs 36H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 179–213 (6) μm long, 23–36 (6) μm wide, with 7 anal setae, 410–760 (6) μm long. L/W 5.84–8.24 (6). Supraanal seta well developed. Anal tubules 229–271 (4) μm long. Posterior parapod 373–498 (3) μm long; subbasal seta simple, with a few small spines (Fig. 36H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 36H). B/C 1.13–1.31 μm long.

Remarks: This is the *Labrundinia* sp. 2 (description of male and immatures stages) of Silva (2009) and *Labrundinia* sp. 5 of Trivinho-Strixino (2011) (larvae identification key).

***Labrundinia* spec. nov. 3** (Figs 37–39)

Material examined

Type: Holotype male with pupal and larval exuviae, **Brazil: São Paulo**, São Carlos, Fazzari reservoir, 21.v.2007, F. L. Silva (MZUSP). Paratypes: 1 male with pupal and larval exuviae same data as holotype except for Ecological Park, 23.v.2008; 2 males with pupal and larval exuviae same data as holotype except for Canchin reservoir, 2.ii.2009; 2 males and 1 female with pupal exuvia as previous except for Canchin reservoir, 2.ii.2009; 1 male with pupal and larval exuviae same data as holotype except for Ribeirão Preto, Monte Alegre Lake, 7.iv.1998, H. F. Mendes; 1 male with pupal and larval exuviae as previous except for Ribeirão Preto, Monte Alegre Lake, 22.v.2000, H. F. Mendes; 1 pupa with larval exuvia and 3 larvae as previous except for 12.vii.1997; 1 male with pupal and larval exuviae same data as holotype except for Pirassununga, CEPTA, 10.ix.20010. 1 male with pupal exuvia as previous.

Diagnostic characters

Labrundinia sp. 3 differs from other *Labrundinia* species by the combination of the following characters.

Male: tentorium 90–126 μm long; R_{2+3} absent; abdomen wholly brown; hypopygium brown, sternapodeme with distinct anterior process. **Pupa:** thoracic horn semi-globose, external margin convoluted, preapical

indentation moderate deep. **Larva:** surface smooth, lateroventral and posteroventral spine groups absent; paraligula multitoothed; subbasal seta multitoothed; bifid claw with V-shaped lower indentation.

Description

Adult male (n = 11)

Size. Total length 1.86–2.36 (9) mm. Wing length 1.06–1.36 mm. Total length/wing length 1.51–1.96 (9). Wing length/length of profemur 2.05–3.30.

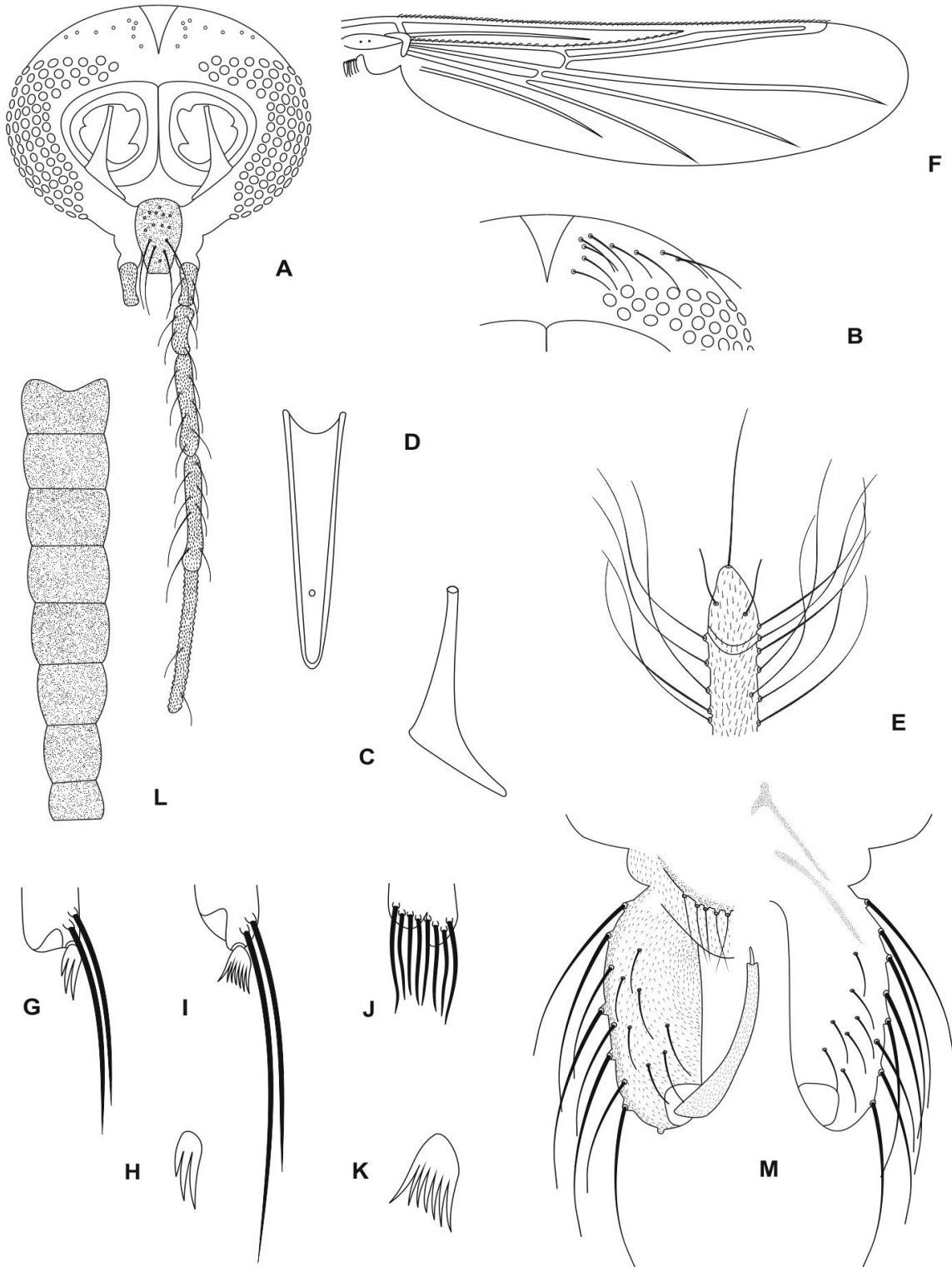
Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown with mesonotum dorsally dark brown; anteprenotum brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Abdomen as in figure 37L. Hypopygium brown.

Head (Figs 37–E). Temporal setae 11–15, uniserial (Fig. 37B). Eye ratio 1.04–1.38 (8). Tentorium (Fig. 37C) 90–126 (8) μm long, stipes not measurable. Clypeus 78–107 μm long, 46–70 μm wide at largest part, bearing 14–22 setae. Cibarial pump (Fig. 37D) with anterior margin concave, 153–204 μm long. Palpomere lengths 1–5 (in μm): 29–43 (6); 38–59 (6); 85–117 (6); 111–157 (3); 207–237 (2). Antenna with 14 flagellomeres, AR 1.35–1.54 (6), flagellum 740–860 (6) μm long, diameter of pedicel 116–142 (4) μm , apical setae single (Fig. 37E).

Thorax. Anteprenotum with 2–4 (7) lateral setae. Acrostichals 42–46 (7), biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 24–36 (9), irregularly uniserial; prealars 9–14 (10); supraalars 2–3. Anapleural suture ratio 0.33–0.49 (7). Scutellum with 8–10 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 37F). Width 0.32–0.43 mm. Costa 0.88–1.17 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.06–0.90 mm long. VR 0.68–0.78. WW 0.29–0.32. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 37G–K). Fore leg: width at apex of tibia 30–39 μm (Fig. 37G), tibia with single, apical and pectinate spur 13–20 μm long (Fig. 37H), with 3–5 teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 31–38 μm long (Fig. 37I), tibia with single, apical and pectinate spur 14–26 μm long with 3–7 teeth (Fig. 37J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 32–38 μm long (Fig. 37K), tibia without spur; comb 8 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXVII.



Figures 37A–M. *Labrundinia* sp. 3, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Table XXVII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 3, male (n = 8–11).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	400–518	372–494	318–454	190–259	114–143
p ₂	501–640	456–505	513–564	192–258	110–148
p ₃	492–556	441–581	534–569	236–263	133–175
	ta ₄	ta ₅	LR	BV	SV
p ₁	85–101	56–85	0.72–0.82	2.28–2.87	2.16–3.05
p ₂	94–119	72–88	1.07–1.17	2.34–2.91	1.97–2.17
p ₃	113–120	72–95	0.97–1.06	2.53–2.83	1.90–2.03

Hypopygium (Fig. 37M). Tergite IX arched, with 8–12 dorsal setae. Anal point broad, apical edge rounded. Phallapodeme 53–70 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 123–160 (10) μm long, 65–90 (10) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.75–2.15 (10). Gonostylus simple and slender, 94–123 μm long; megaseta 15–17 μm long. HR 1.23–1.52 (10). HV 1.88–2.29 (8).

Adult female (n = 1)

Size. Total length 1.43 mm. Wing length 1.02 mm. Total length/wing length 1.41. Wing length/length of profemur 2.25.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs brown.. Abdomen wholly brown. Seminal capsules brown.

Head. Temporal setae 12, uniserial. Eyes ratio 1.04. Tentorium 137 μm long, stipes not measurable. Clypeus 85 μm long, 64 μm wide at largest part, bearing 21 setae. Cibarial pump as in male, 193 μm long. Palpomere lengths 1–4 (in μm): 32; 49; 100; 154. Antenna with 11 flagellomeres, unmeasured, diameter of pedicel 65 μm .

Thorax. Anteprenotum with 2 setae. Acrostichals 60, irregularly uniserial, starting close to anteprenotum; dorsocentrals 35, irregularly uniserial; prealars 17; supraalars 2. Scutellum with 10 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.40 mm. Costa not produced beyond R₄₊₅, 0.88 mm long. R₂₊₃ absent. Base of radial sector 0.07 mm long. VR 0.60. WW 0.39. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 34 μm , tibia with single, apical and pectinate spur 14 μm , with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Mid leg: width at apex of tibia 38 μm , tibia with single, apical and pectinate spur 21 μm , with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Hind leg: width at apex of tibia 46 μm long, tibia without spur; comb with 8 setae; preapical pseudospurs on Ta_{1-4} unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXVIII.

Table XXVIII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 3, female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	452	355	–	–	–
p ₂	562	450	–	–	–
p ₃	478	517	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

Genitalia. Gonapophysis VIII broadly rounded, 61 μm long. Coxosternapodeme 70 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 51 μm long and 14 μm wide; with 9 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 93 μm . Seminal capsules oval with conical shaped necks, length 38 μm , maximum width 32 μm . Length ratio SCa/No 0.41.

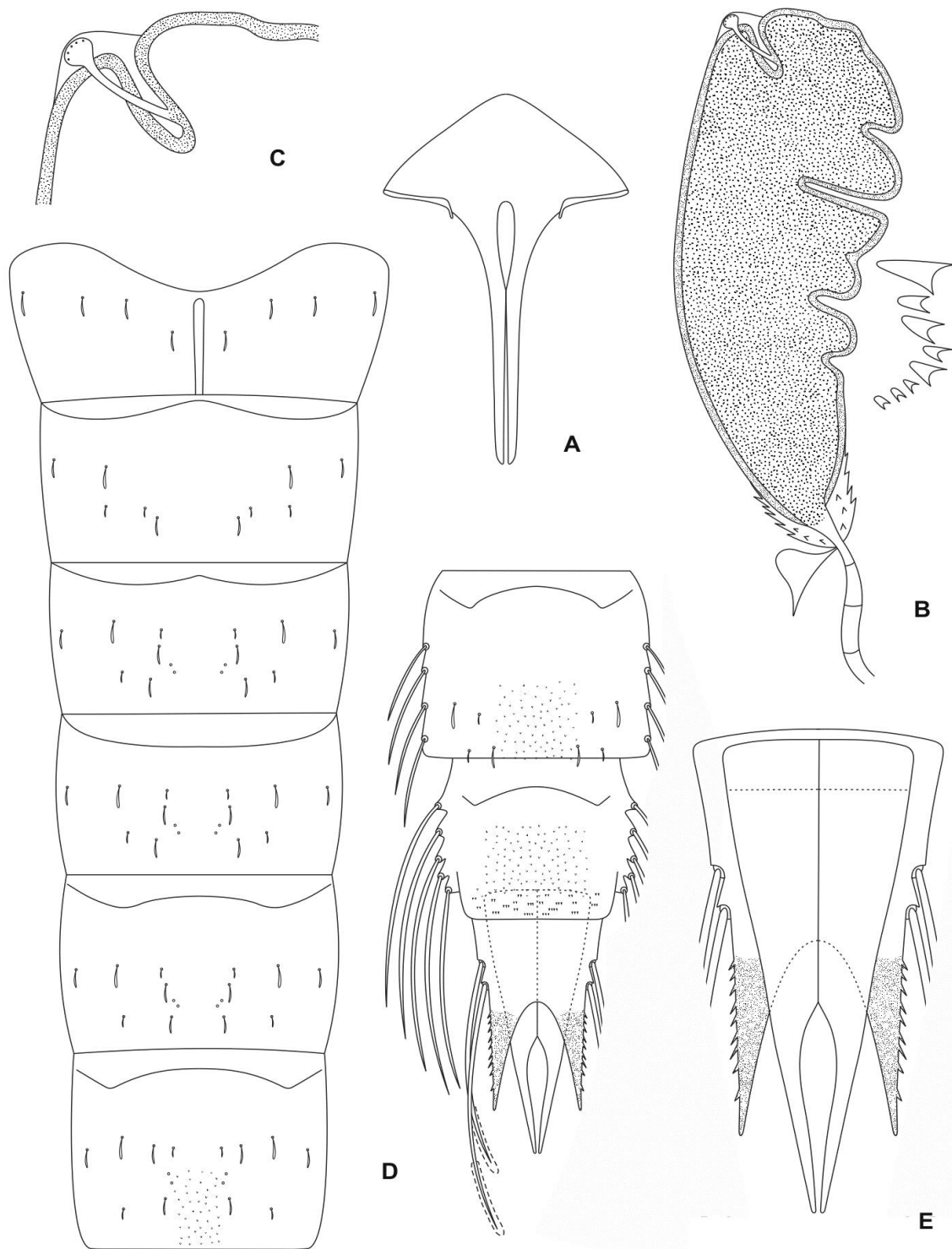
Pupa (n = 13 unless otherwise stated)

Size. Male abdomen 1.67–2.31 (12) mm long; female abdomen 2.20 (1) mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 38A–C). Frontal apotome as in figure 38A. Wing sheath smooth 0.81–1.08 mm long. Thoracic horn 216–350 μm long and 80–138 μm wide (Fig. 38B), preapical indentation moderate deep, THR 1.93–2.89. Membranous preapical papilla 22–40 (10) μm long (Fig. 38C), PTH 0.06–0.16 (10), aeropyle tube simple, short, 22–33 (9) μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules. Basal lobe wedge-shaped. Thoracic comb with 7–8 conical teeth.

Abdomen (Figs 38D–E). Tergite I with scar, 104–148. T I–V without shagreen, VI–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 38D. Abdominal segment VII with 4 lateral setae.



Figures 38A–E. *Labrundinia* sp. 3, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

A VIII with 5 lateral setae. Anal lobe 258–356 μm long and 138–198 μm wide (Fig. 38E), outer margins sclerotized, with 8–14 spines, longest spine 9–15 μm long, inner margins of lobes membranous. ALR 1.55–2.30. Genital sac elongated, reaching much beyond apex of anal lobe.

4th instar larva (n = 11 unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 39A). Length 412–640 μm , 235–316 μm wide. Surface smooth; lateroventral and posteroventral spine groups absent; cephalic index 0.48–0.65. Chaetotaxy as in figure 39A.

Antenna (Figs 39B–C). Length 315–358 (8) μm , A₁ 217–260 (8) μm long, with ring organ placed 197–217 (6) μm from base, A₂ 79–89 (8) μm long. AR 2.26–2.66 (6). Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 39D). Basal palp segment 21–31 μm long and 7–10 μm wide, with ring organ 20–24 (8) μm from base. PR 2.20–4.14. APR 7.82–11.6.

Mandible (Fig. 39E). Length 55–72 μm , with 3 lateral setae. Sensillum campaniformium 38–55 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 3.29–4.38.

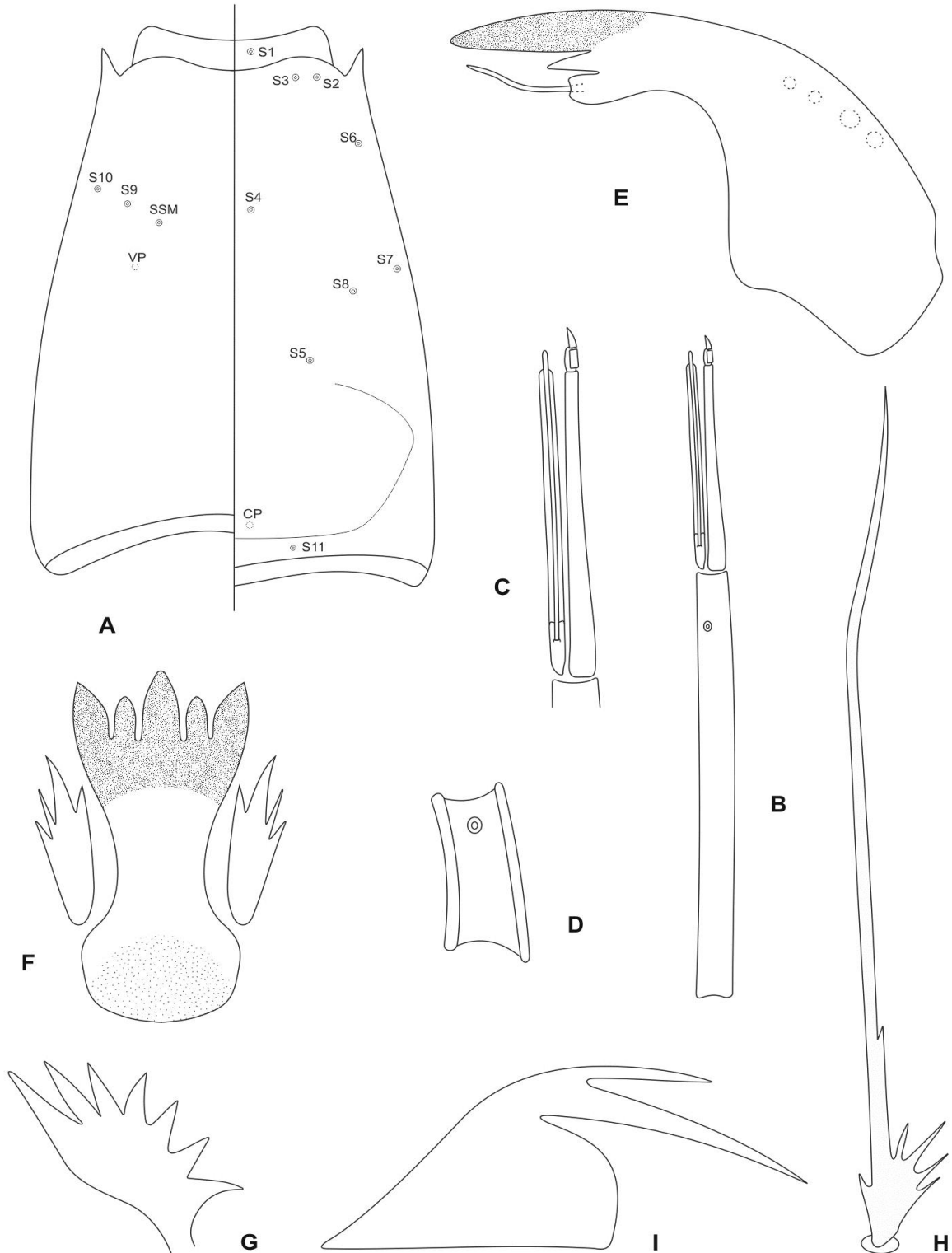
Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 39F–G). Ligula 45–60 μm long, 25–27 μm wide, with row of 5 teeth. IO 0.95–1.02, MO 1.00–1.08. Paraligula multitoothed, 19–30 μm long, inner tooth 15–28 μm long, shorter than outer tooth. Pecten hypopharyngis with 7–8 teeth almost equal in size.

Body (Figs 39H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 140–204 μm long, 24–37 μm wide, with 7 anal setae, 377–561 μm long. L/W 4.56–7.24. Supraanal seta well developed. Anal tubules 235–241 (2) μm long. Posterior parapod 294–328 (3) μm long; subbasal seta multitoothed, with basal spine group, with 4–6 spines (Fig. 39H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 39H). B/C 1.10–1.35 (10) μm long.

Labrundinia spec. nov. 4 (Figs 40–42)

Material examined



Figures 39A–I. *Labrundinia* sp. 3, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

Type: Holotype male with pupal and larval exuviae, BRAZIL: São Paulo, Ribeirão Preto, Monte Alegre Lake, 15.viii.1997, H. F. Mendes (MZUSP).

Diagnostic characters

Labrundinia sp. 4 differs from other *Labrundinia* species by the combination of the following characters. **Male:** abdominal tergite I–VIII with continuous brown transverse band near proximal margin; hypopygium pale brown, sternapodeme with moderate anterior process; anal point rounded, apical edge notched. **Pupa:** thoracic horn club-shaped, preapical indentation moderate deep, preapical papilla ratio 0.19; genital sac elongated, reaching somewhat beyond apex of anal lobe. **Larva:** head slender almost rectangular, surface covered with small spinules, lateroventral spine group present but weakly developed, with 3–4 spines, posteroventral spine group absent; subbasal seta simple; bifid claw with V-shaped lower indentation.

Description

Adult male ($n = 1$)

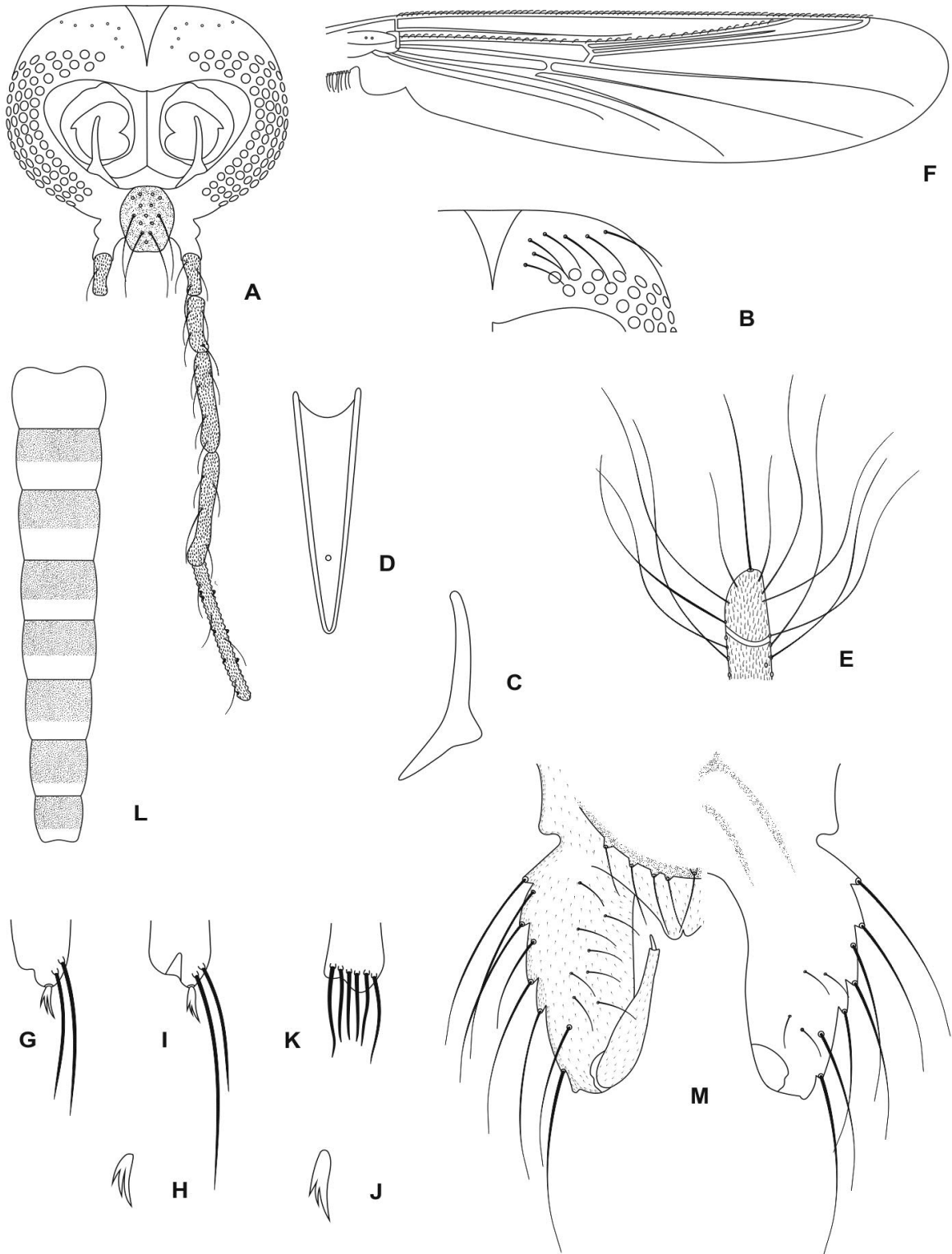
Size. Total length 2.00 mm. Wing length 1.19 mm. Total length/wing length 1.68. Wing length/length of profemur 3.10.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale brown; supraalar callus pale brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 40L. Hypopygium pale brown.

Head (Figs 40A–E). Temporal setae 10, uniserial (Fig. 40B). Eye ratio 1.17. Tentorium (Fig. 40C) 117 μm long, stipes not measurable. Clypeus 76 μm long, 46 μm wide at largest part, bearing 15 setae. Cibarial pump (Fig. 40D) with anterior margin concave, 173 μm long. Palpomere lengths 1–5 (in μm): 31; 50; 87; 118; 211. Antenna with 14 flagellomeres, AR 1.16, flagellum 689 μm long, diameter of pedicel 96 μm , apical setae single (Fig. 40E).

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 26, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 16, irregularly uniserial; prealars 6; supraalars 2. Anapleural suture ratio 0.69. Scutellum with 5 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 40F). Width 0.34 mm. Costa 1.02 mm long, not produced beyond R_{4+5} , ending very slightly



Figures 40A–M. *Labrundinia* sp. 4, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.08 mm long. VR 0.74. WW 0.28. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 40G–K). Fore leg: width at apex of tibia 32 μm (Fig. 40G), tibia with single, apical and pectinate spur 12 μm long (Fig. 40H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 33 μm long (Fig. 40I), tibia with single, apical and pectinate spur 14 μm long with three teeth (Fig. 40J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 35 μm long (Fig. 40K), tibia without spur; comb with 6 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXIX.

Table XXIX. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 4, adult male ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	384	443	294	196	134
p ₂	526	454	583	247	130
p ₃	464	598	560	233	153
	ta ₄	ta ₅	LR	BV	SV
p ₁	101	65	0.66	2.26	2.81
p ₂	88	84	1.29	2.84	1.68
p ₃	106	72	0.94	2.87	1.90

Hypopygium (Fig. 40M). Tergite IX arched, with 10 dorsal setae. Anal point rounded, apical edge slightly notched. Phallapodeme 37 μm long. Sternapodeme with moderate anterior process. Gonocoxite cylindrical, 89 μm long, 33 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.64. Gonostylus simple and slender, 60 μm long; megaseta 10 μm long. HR 1.50. HV 3.37.

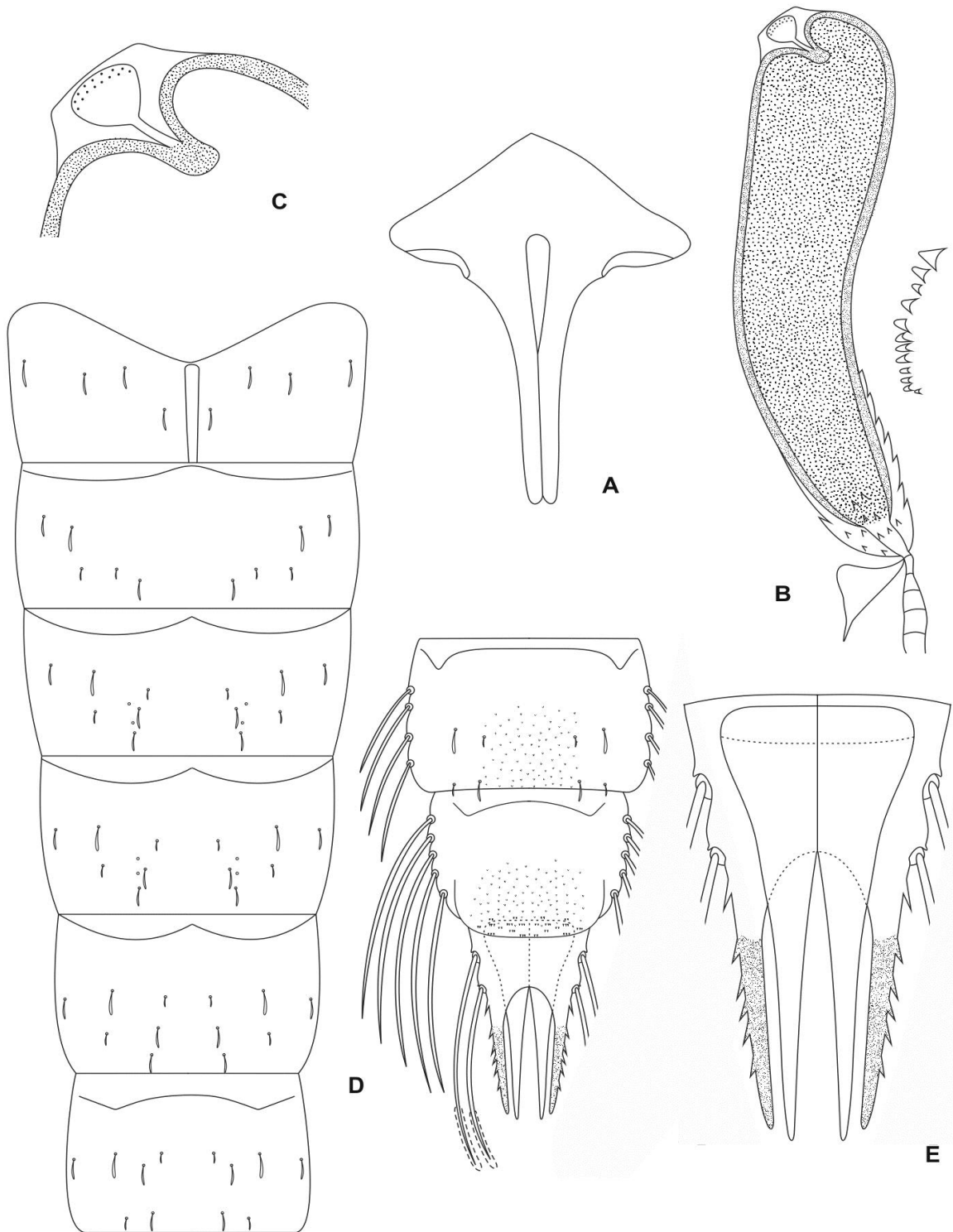
Pupa ($n = 1$)

Size. Male abdomen 1.60 mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 41A–C). Frontal apotome as in figure 41A. Wing sheath smooth 0.71 mm long. Thoracic horn 184 μm long and 46 μm wide (Fig. 41B), preapical indentation moderate deep, THR 3.96. Membranous preapical papilla 36 μm long (Fig. 41C), PTH 0.19, aeropyle tube simple, short, 17 μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium.

Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated.



Figures 41A–E. *Labrundinia* sp. 4, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Basal lobe wedge-shaped. Thoracic comb with 13 conical teeth.

Abdomen (Figs 41D–E). Tergite I with scar, 118 μm long. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 41D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 269 μm long and 133 μm wide (Fig. 41E), outer margins sclerotized, with 9 spines, longest spine 12 μm long, inner margins of lobes membranous. ALR 2.00. Genital sac elongated, reaching somewhat beyond apex of anal lobe.

4th instar larva (n = 1)

Coloration. Head pale yellow, posterior margin may be darkened; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 42A). Length 489 μm , 249 μm wide. Surface covered with small spinules; lateroventral spine group present but weakly developed, with 3–4 spines; posteroventral spine group absent; cephalic index 0.51. Chaetotaxy as in figure 42A.

Antenna (Figs 42B–C). Length 262 μm , A₁ 172 μm long, with ring organ placed 134 μm from base, A₂ 75 μm long. AR 1.91. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 42D). Basal palp segment 20 μm long and 8 μm wide, with ring organ 9 μm from base. PR 2.50. APR 8.60.

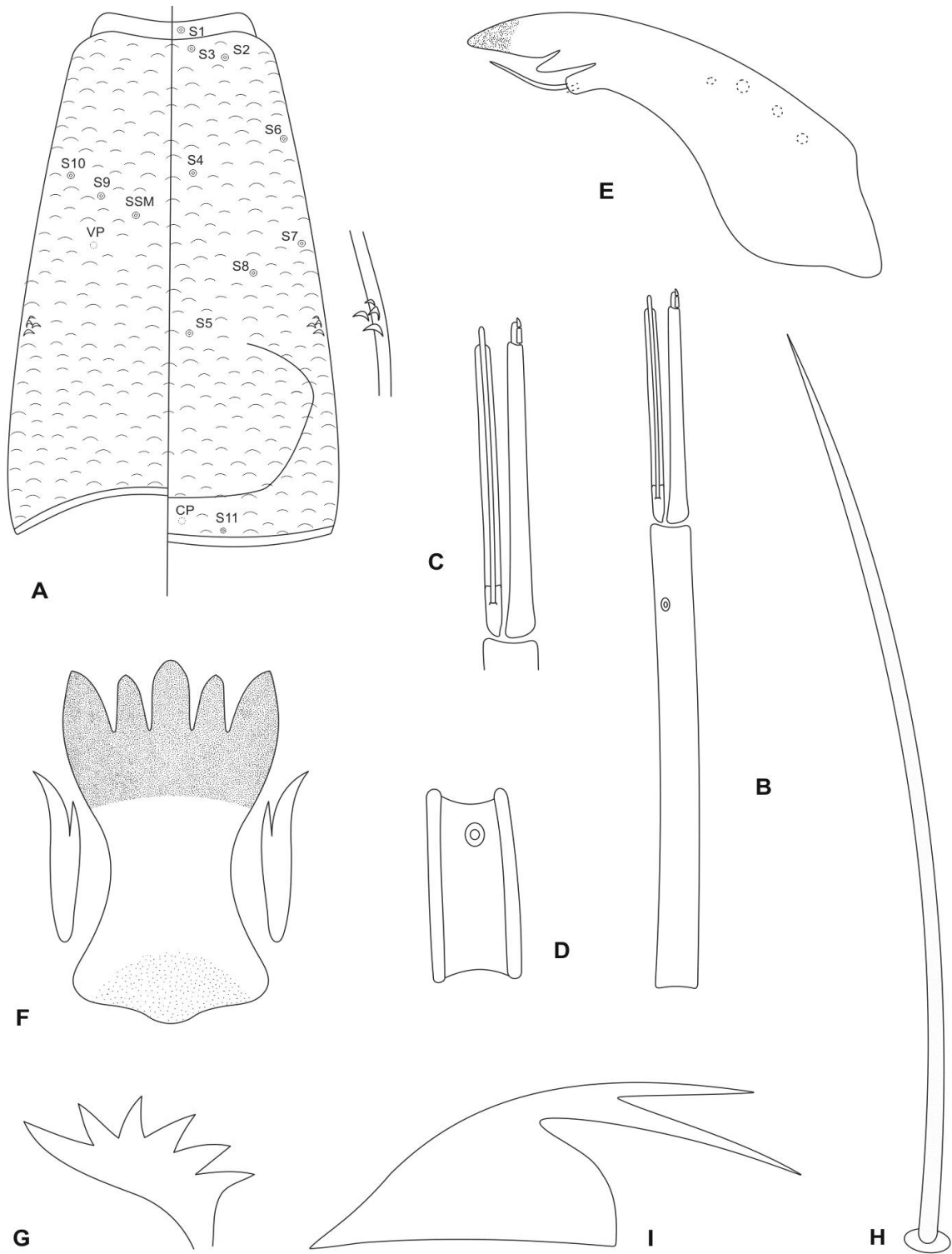
Mandible (Fig. 42E). Length 63 μm , with 3 lateral setae. Sensillum campaniformium 45 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.73.

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 42F–G). Ligula 46 μm long, 21 μm wide, with row of 5 teeth. IO 0.99, MO 1.02. Paraligula bifid, 23 μm long, inner tooth 18 μm long, shorter than outer tooth. Pecten hypopharyngis with 8 teeth almost equal in size.

Body (Figs 42H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 112 μm long, 25 μm wide, with 7 anal setae 300 μm long. L/W 4.44. Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod 331 μm long; subbasal seta simple, without teeth (Fig. 42H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 42I). B/C 1.10.

Remarks: This is the *Labrundinia* sp. 3 (description of male and immatures stages) of Silva (2009).



Figures 42A–I. *Labrundinia* sp. 4, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

Adult female. Unknown.

***Labrundinia* spec. nov. 5** (Figs 43–45)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, São Carlos, Fazzari stream, 25.viii.2008, F. L. Silva (MZUSP). Paratype: 1 male with pupal and larval exuviae same data as holotype except for Canchin reservoir, 5.vi.2007.

Diagnostic characters

Labrundinia sp. 5 differs from other *Labrundinia* species by the combination of the following characters. **Male:** R₂₊₃ absent; fore leg ratio 0.61–0.68; abdominal tergite II–VIII with continuous brown transverse band near proximal margin; hypopygium pale brown, sternapodeme rounded, with anterior process absent. **Pupa:** thoracic horn club-shaped, preapical indentation shallow, plastron plate seated on an enlarged tubercle. **Larva:** surface covered with small spinules, lateroventral spine group present but weakly developed, with 3–4 spines, posteroventral spine group absent; subbasal seta simple; bifid claw with V-shaped lower indentation.

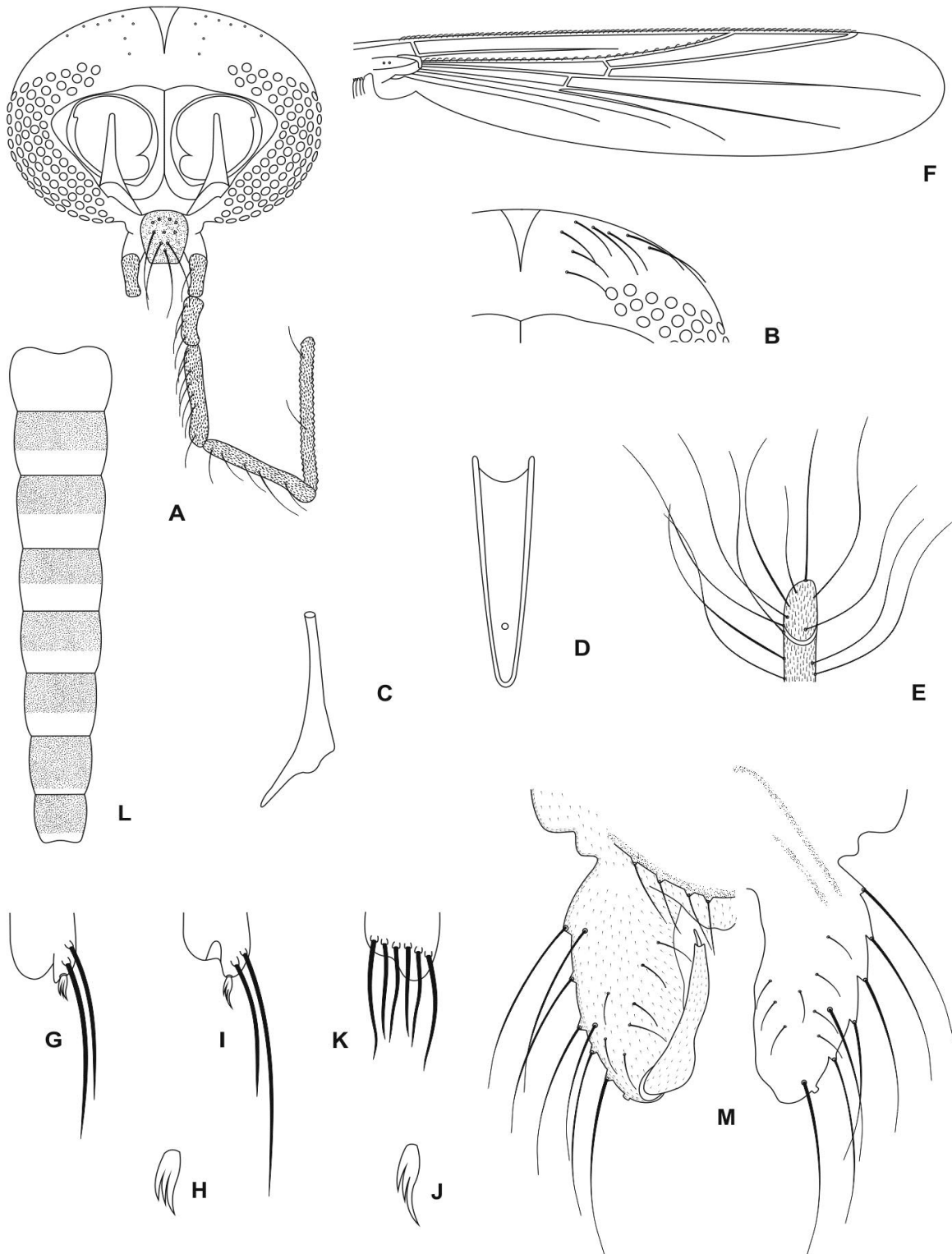
Description

Adult male (*n* = 2)

Size. Total length 2.18 (1) mm. Wing length 1.15 (1) mm. Total length/wing length 1.90. Wing length/length of profemur 3.11.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp brown. Thorax brown with mesonotum dorsally dark brown; antepronotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 43L. Hypopygium pale brown.

Head (Figs 43–E). Temporal setae 10, uniserial (Fig. 43B). Eye ratio 1.08–1.25. Tentorium (Fig. 43C) 134 (1) μ m long, stipes not measurable. Clypeus 79–84 μ m long, 56–58 μ m wide at largest part, bearing 10 setae. Cibarial pump (Fig. 43D) with anterior margin concave, 160–175 μ m long. Palpomere lengths 1–5 (in μ m): 33–34; 44–54; 77–112; 116 (1); 211 (1). Antenna with 14 flagellomeres, AR 1.17 (1), flagellum



Figures 43A–M. *Labrundinia* sp. 5, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

761 μm long, diameter of pedicel 124 μm , apical setae single (Fig. 43E).

Thorax. Anteprepronotum with 2–3 lateral setae. Acrostichals 32–40, biserial, diverging evenly posteriorly, starting close to anteprepronotum and almost reaching scutellum; dorsocentrals 18–22, irregularly biserial; prealars 8; supraalars 2. Anapleural suture ratio 0.47 (1). Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 43F). Width 0.31 (1) mm. Costa 0.97 (1) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.08 (1) mm long. VR 0.71 (1). WW 0.28 (1). Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 43G–K). Fore leg: width at apex of tibia 31–37 μm (Fig. 43G), tibia with single, apical and pectinate spur 10–17 μm long (Fig. 43H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 31–35 μm long (Fig. 43I), tibia with single, apical and pectinate spur 14–20 μm long with three teeth (Fig. 43J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 32–34 μm long (Fig. 43K), tibia without spur; comb with 6 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXX.

Table XXX. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 5, male ($n = 2$).

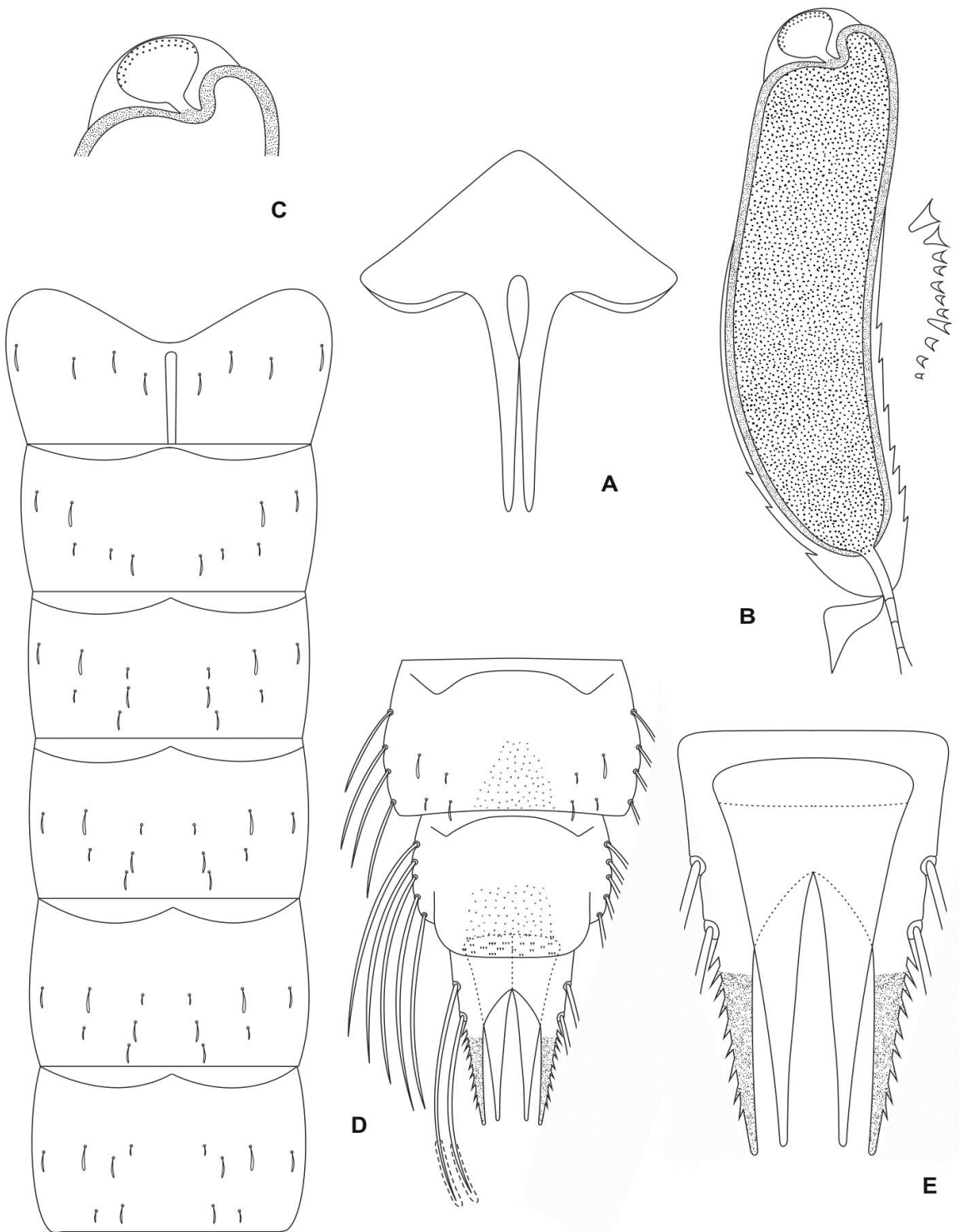
	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	369–413	461–500	282–338	204–274	142–182
p ₂	519–611	470–561	575–650	247–283	138–150
p ₃	459–554	602–744	556–680	238–290	158–176
	ta ₄	ta ₅	LR	BV	SV
p ₁	117–126	63–70	0.61–0.68	1.92–2.12	2.70–2.94
p ₂	82–101	71–87	1.16–1.22	2.91–2.94	1.72–1.80
p ₃	105–118	73–84	0.92–0.93	2.82–2.96	1.90–1.91

Hypopygium (Fig. 43M). Tergite IX arched, with 9 dorsal setae. Anal point rounded, apical edge convex. Phallapodeme 43 μm long. Sternapodeme with anterior process absent. Gonocoxite cylindrical, 91–95 μm long, 33–35 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.57–2.89. Gonostylus simple and slender, 62–67 μm long; megaseta 10–14 μm long. HR 1.42–1.47. HV 3.41–3.85.

Pupa ($n = 2$ unless otherwise stated)

Size. Male abdomen 1.73–1.97 mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.



Figures 44A–E. *Labrundinia* sp. 5, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Cephalothorax (Figs 44A–C). Frontal apotome as in figure 44A. Wing sheath smooth 0.81–0.90 mm long. Thoracic horn 169–213 μm long and 43–53 μm wide (Fig. 44B), preapical indentation moderate deep, THR 3.92–4.02. Membranous preapical papilla 34–44 μm long (Fig. 44C), PTH 0.16–0.26, aeropyle tube simple, short, 13–19 μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 9–11 conical teeth.

Abdomen (Figs 44D–E). Tergite I with scar, 110–140. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 44D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 253–278 μm long and 128–132 μm wide (Fig. 44E), outer margins sclerotized, with 9–14 spines, longest spine 10–11 μm long, inner margins of lobes membranous. ALR 1.98–2.10. Genital sac elongated, almost reaching beyond apex of anal lobe.

4th instar larva ($n = 2$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 45A). Length 471–498 μm , 281–284 μm wide. Surface covered with small spinules; lateroventral spine group present but weakly developed, with 3–4 spines; posteroventral spine group absent; cephalic index 0.56–0.60. Chaetotaxy as in figure 45A.

Antenna (Figs 45B–C). Length 273–278 μm , A_1 178–185 μm long, with ring organ placed 156–159 μm from base, A_2 85–86 μm long. AR 1.87–1.98. Blade longer than A_2 over-reached by accessory blade.

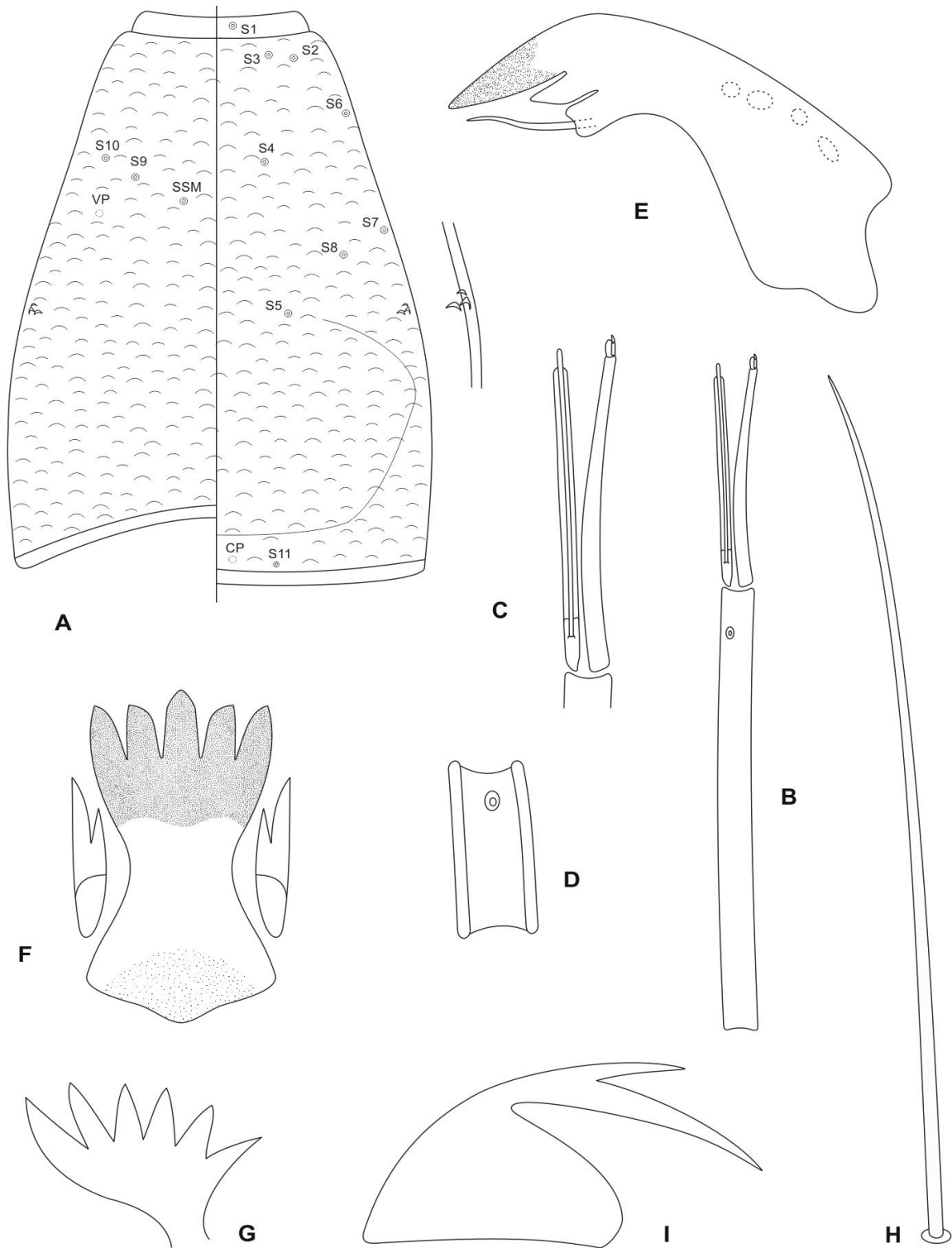
Maxilla (Fig. 45D). Basal palp segment 20–24 μm long and 7–9 μm wide, with ring organ 16 μm from base. PR 2.62–3.00. APR 7.51–9.16.

Mandible (Fig. 45E). Length 61–64 μm , with 3 lateral setae. Sensillum campaniformium 39–43 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.88–2.91.

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 45F–G). Ligula 44–51 μm long, 24–25 μm wide, with row of 5 teeth. IO 0.98–0.99, MO 1.01–1.02. Paraligula bifid, 22–26 μm long, inner tooth 17–19 μm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 45H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 113–119 μm long, 21–28 μm wide, with 7 anal setae, 333–347 μm long. L/W 4.09–5.71. Supraanal seta well developed.



Figures 45A–I. *Labrundinia* sp. 5, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

Anal tubules unmeasured. Posterior parapod unmeasured; subbasal seta simple, without spines (Fig. 45H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 45H). B/C 1.10–1.15 μm long.

Remarks: This is the *Labrundinia* sp. 4 (description of male and immatures stages) of Silva (2009).

Adult female. Unknown.

***Labrundinia* spec. nov. 6** (Figs 46–48)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, Gália, Caetetus Ecological Station, 1st dam before the Barreiro reservoir, 08.vii.2008, F. L. Silva & J. F. Nunes (MZUSP). Paratypes: 3 males with pupal and larval exuviae same data as holotype except for, Canchin reservoir, 6.i.2009. 1 male with pupal and larval exuviae same data as holotype except for, 2.ii.2009. 1 female with pupal exuvia same data as holotype except for, 2.ii.2009.

Diagnostic characters

Labrundinia sp. 6 differs from other *Labrundinia* species by the combination of the following characters. **Male:** abdominal tergite II–VI with continuous brown transverse band near proximal margin, T VII–VIII almost wholly brown; hypopygium pale brown, sternapodeme with much reduced anterior process, hypopygium ratio 1.61–1.77. **Pupa:** thoracic horn kidney-shaped, preapical indentation reduced, thoracic horn ratio 1.75–2.08. **Larva:** Surface covered with small spinules; lateroventral and posteroventral spine groups absent; subbasal seta simple; serrated claw present; bifid claw with V-shaped lower indentation.

Description

Adult male ($n = 5$)

Size. Total length 1.89–2.34 mm. Wing length 1.20–1.32 mm. Total length/wing length 1.53–1.71. Wing length/length of profemur 2.94–2.97 (4).

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary

palp brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale brown; supraalar callus pale brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 46L. Hypopygium pale brown.

Head (Figs 46E). Temporal setae 11–12 (4), uniserial (Fig. 46B). Eye ratio 0.89–0.90 (2). Tentorium (Fig. 46C) 141–158 (4) μm long, stipes not measurable. Clypeus 71–100 (4) μm long, 57–59 (4) μm wide at largest part, bearing 11–16 (4) setae. Cibarial pump (Fig. 46D) with anterior margin concave, 177–204 (4) μm long. Palpomere lengths 1–5 (in μm): 34–43 (4); 50–65 (4); 114–137 (3); 130–146 (4); 170 (1). Antenna with 14 flagellomeres, AR 1.09–1.27, flagellum 676–820 μm long, diameter of pedicel 103–124 μm , apical setae single (Fig. 46E).

Thorax. Anteprenotum with 1–2 lateral setae. Acrostichals 30–38, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 16–22, irregularly uniserial; prealars 7; supraalars 2. Anapleural suture ratio 0.47–0.61. Scutellum with 6–8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 46F). Width 0.32–0.37 (4) mm. Costa 0.98–1.16 (4) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.07–0.09 (4) mm long. VR 0.72–0.81 (3). WW 0.27–0.28 (4). Brachiolum with 2 setae. Squama setiferous.

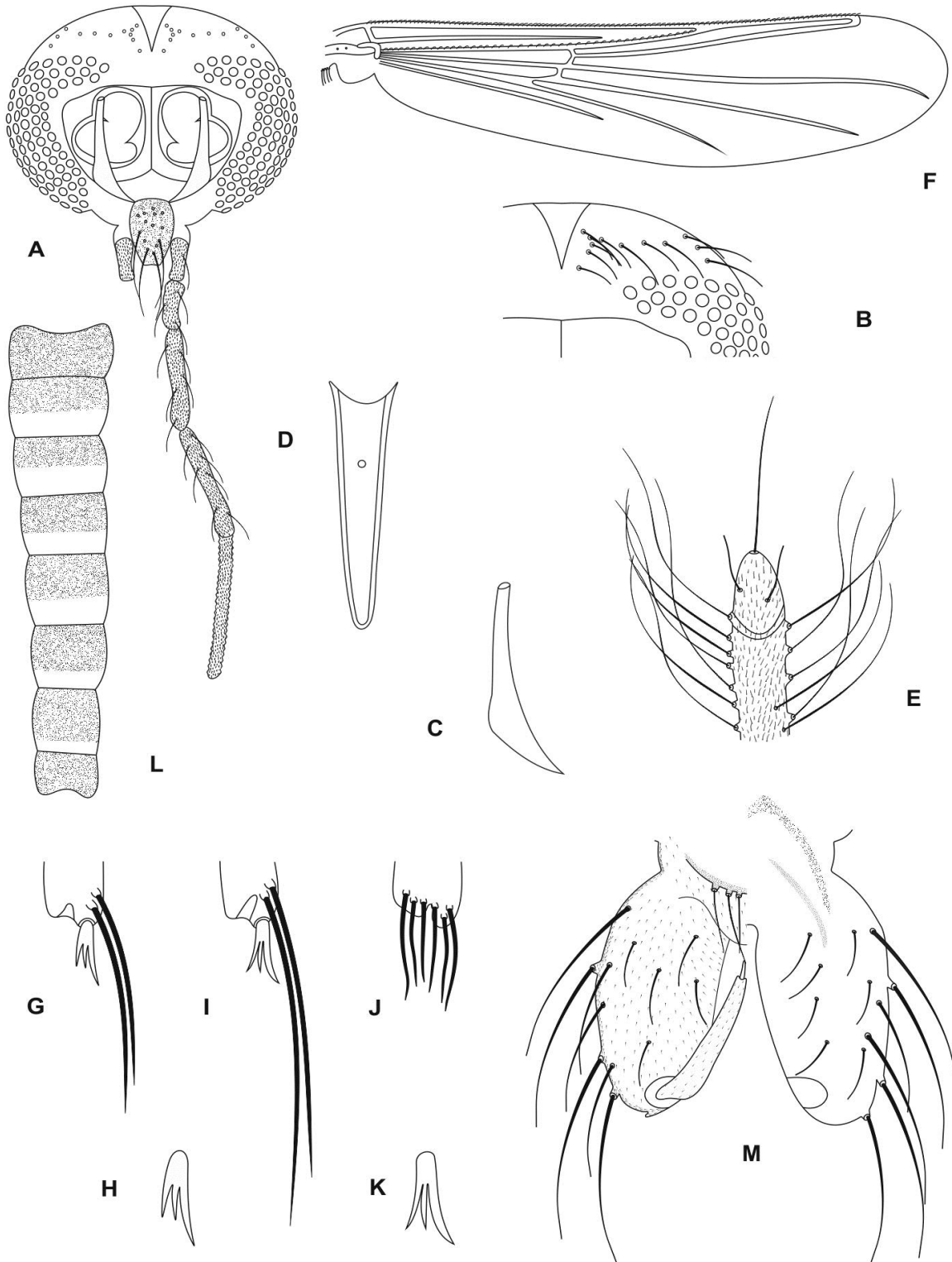
Legs (Figs 46G–K). Fore leg: width at apex of tibia 32–41 (4) μm (Fig. 46G), tibia with single, apical and pectinate spur 14–17 (4) μm long (Fig. 46H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 30–37 μm long (Fig. 46I), tibia with single, apical and pectinate spur 18–23 μm long with three teeth (Fig. 46J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 33–37 μm long (Fig. 46K), tibia without spur; comb with 6 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXI.

Hypopygium (Fig. 46M). Tergite IX arched, with 8–11 dorsal setae. Anal point rounded, apical edge slightly convex. Phallapodeme 44–54 μm long. Sternapodeme with much reduced anterior process. Gonocoxite cylindrical, 94–106 (4) μm long, 45–56 (4) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.88–2.10 (4). Gonostylus simple and slender, 53–69 μm long; megaseta 9–12 μm long. HR 1.61–1.77. HV 3.30–3.55.

Adult female ($n = 1$)

Size. Total length 1.31 mm. Wing length 1.03 mm. Total length/wing length 1.27. Wing length/length of profemur 2.81.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary



Figures 46A–M. *Labrundinia* sp. 6, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

palp pale brown. Thorax brown with mesonotum dorsally darker; antep pronotum pale brown; supraalar callus pale brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen brown. Seminal capsules brown.

Head. Temporal setae 10, uniserial. Eyes ratio 0.93. Tentorium 137 μm long, stipes not measurable. Clypeus 77 μm long, 57 μm wide at largest part, bearing 18 setae. Cibarial pump as in male, 193 μm long. Palpomere lengths 1–4 (in μm): 35; 48; 98; 124. Antenna with 11 flagellomeres, unmeasured.

Thorax. Antep pronotum with 2 lateral setae. Acrostichals 37, irregularly biserial, starting close to antep pronotum; dorsocentrals 30, irregularly uniserial; prealars 10; supraalars 2. Scutellum with 10 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.38 mm. Costa 0.91 mm long, not produced beyond R_{4+5} . R_{2+3} absent. Base of radial sector 0.06 mm long. VR 0.68. WW 0.38. Brachiolum with 2 setae. Squama setiferous.

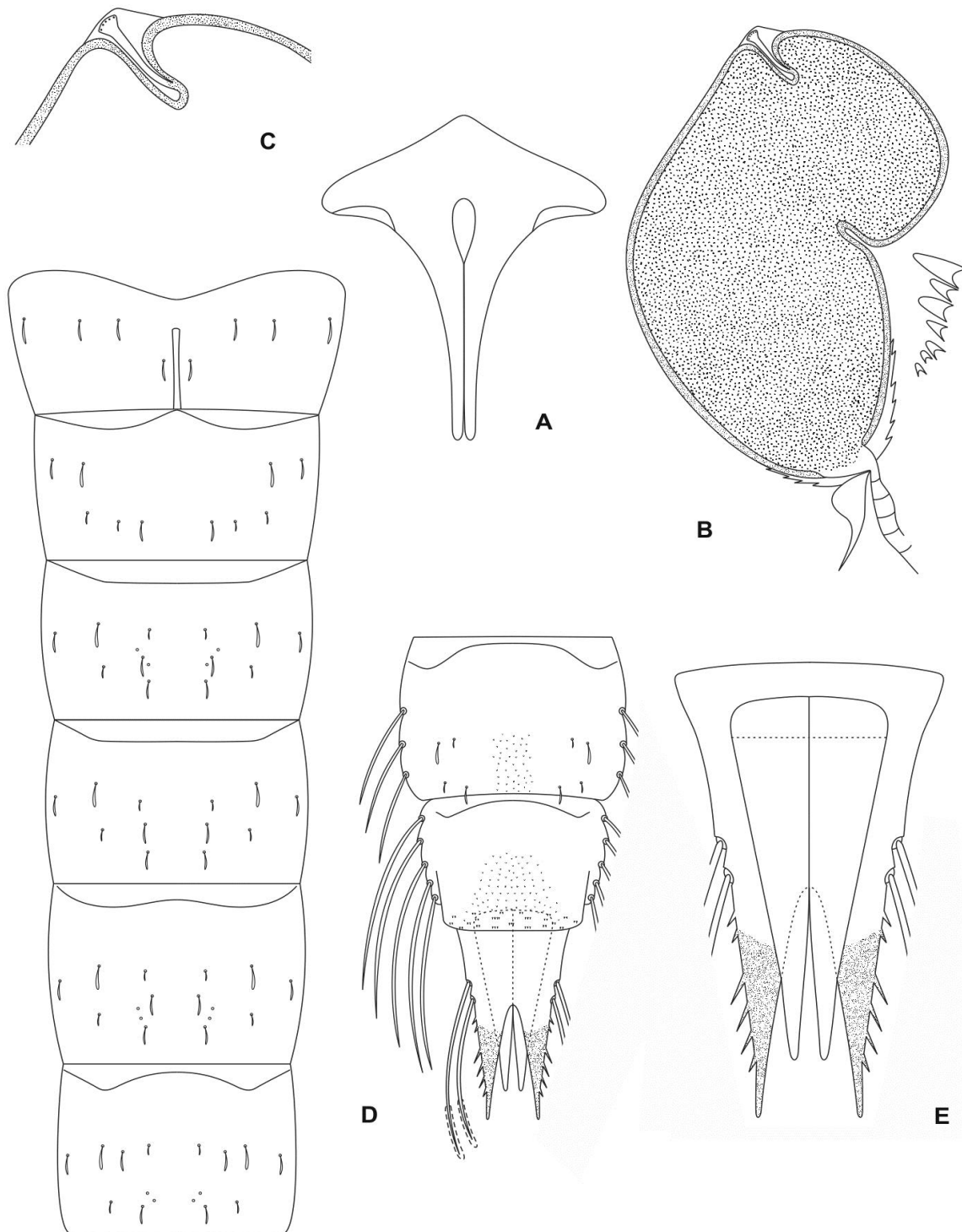
Legs. Fore leg: width at apex of tibia 42 μm , tibia with single, apical and pectinate spur 18 μm long, with three teeth and two preapical setae; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 34 μm long, tibia with single, apical and pectinate spur 20 μm long with three teeth and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 43 μm long, tibia without spur; comb with 5 setae; Ta_{1-4} each without two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXII.

Table XXXII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 6, female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	366	383	349	165	122
p ₂	540	411	494	242	163
p ₃	456	545	560	206	128
	ta ₄	ta ₅	LR	BV	SV
p ₁	92	75	0.91	2.42	2.13
p ₂	118	90	1.20	2.35	1.92
p ₃	84	80	1.02	3.13	1.78

Genitalia. Gonapophysis VIII broadly rounded, 51 μm long. Coxosternapodeme 59 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 53 μm long and 15 μm wide; with 5 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 96 μm . Seminal capsules oval with conical shaped necks, length 46 μm , maximum width 28 μm . Length ratio SCa/No 0.48.

Pupa (n = 6 unless otherwise stated)



Figures 47A–E. *Labrundinia* sp. 6, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Size. Male abdomen 1.76–2.41 mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 47A–C). Frontal apotome as in figure 47A. Wing sheath smooth 0.74–1.01 mm long. Thoracic horn 247–307 μm long and 124–169 μm wide (Fig. 47B), preapical indentation moderate deep, THR 1.75–2.08. Membranous preapical papilla 21–38 μm long (Fig. 47C), PTH 0.07–0.14, aeropyle tube simple, short, 17–26 (3) μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 7–9 conical teeth.

Abdomen (Figs 47D–E). Tergite I with scar, 108–136. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 47D. Abdominal segment VII with 3 lateral setae. A VIII with 5 lateral setae. Anal lobe 282–305 (5) μm long and 131–176 (5) μm wide (Fig. 47E), outer margins sclerotized, with 6–11 spines, longest spine 20–23 (5) μm long, inner margins of lobes membranous. ALR 2.06–2.30 (5). Genital sac elongated, not surpassing apex of anal lobe.

4th instar larva (n = 5 unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 48A). Length 434–525 μm , 271–323 μm wide. Surface covered with small spinules; lateroventral and posteroventral spine groups absent; cephalic index 0.59–0.65. Chaetotaxy as in figure 48A.

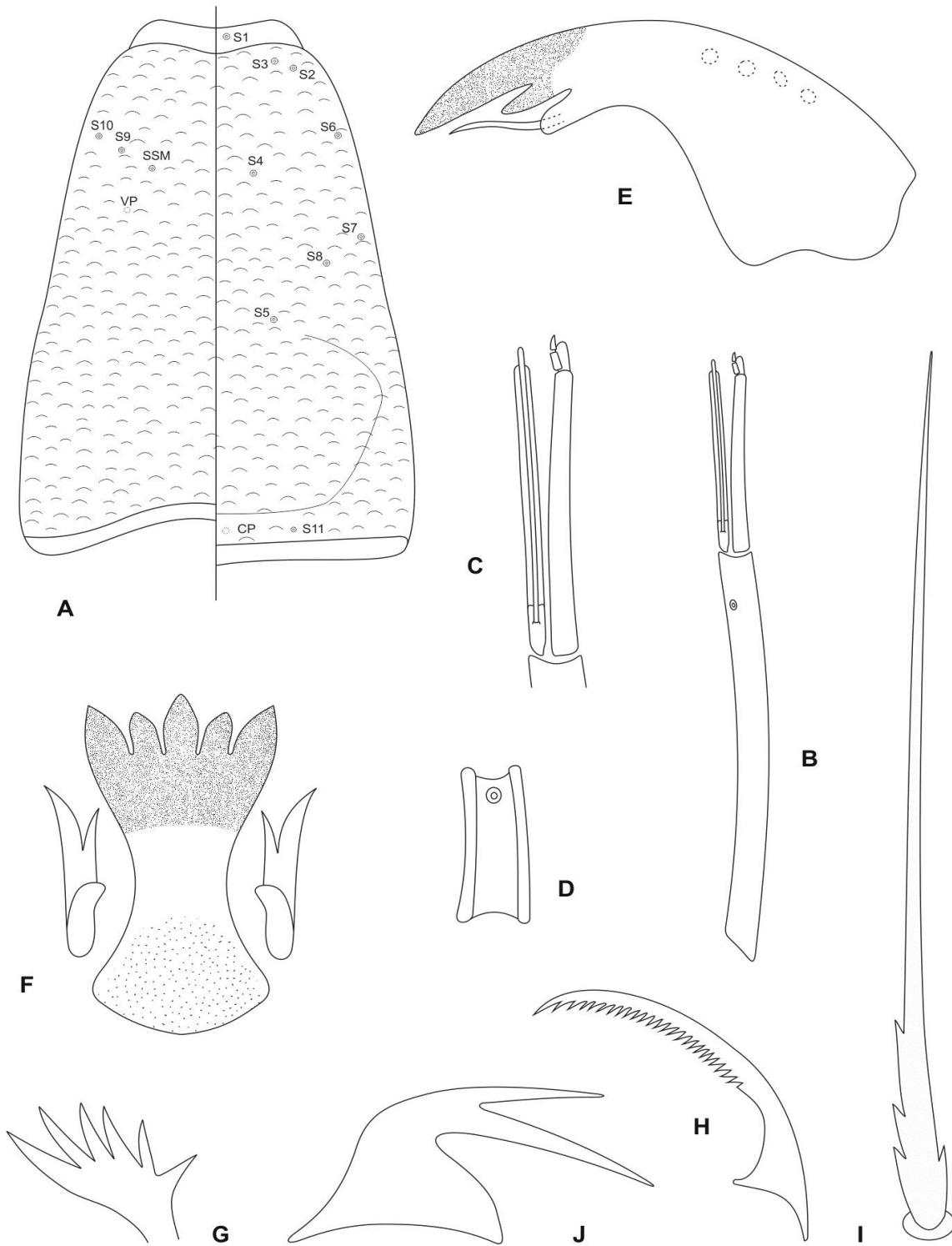
Antenna (Figs 48B–C). Length 253–285 μm , A₁ 170–195 μm long, with ring organ placed 172 (1) μm from base, A₂ 70–80 μm long. AR 1.93–2.15. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 48D). Basal palp segment 22–30 μm long and 7–9 μm wide, with ring organ 14–17 (2) μm from base. PR 2.68–4.15. APR 5.88–7.84.

Mandible (Fig. 48E). Length 61–85 μm , with 3 lateral setae. Sensillum campaniformium 42–59 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.25–2.85.

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 48F–G). Ligula 52–62 μm long, 25–26 μm wide, with row of 5 teeth. IO 0.95–0.99, MO 1.01–1.07. Paraligula bifid, 24–29 μm long, inner tooth 19–22 μm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.



Figures 48A–J. *Labrundinia* sp. 6, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Serrated claw of posterior parapod. **I.** Subbasal seta of posterior parapod. **J.** Bifid claw of posterior parapod.

Body (Figs 48H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 137–165 (4) μm long, 28–29 μm wide, with 7 anal setae, 379–448 μm long. L/W 4.83–5.64 (4). Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod unmeasured; subbasal seta simple, with 3–4 small spines (Fig. 48H); parapod apex with numerous simple claws, with serrated claw; bifid claw with V-shaped lower indentation (Fig. 48H). B/C 1.13–1.18 (2) μm long.

Labrundinia spec. nov. 7 (Figs 49–51)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, Gália, Caetetus Ecological Station, Barreiro reservoir, 08.vii.2008, F. L. Silva & J. F. Nunes (MZUSP). Paratypes: 2 males with pupal and larval exuviae same data as holotype. 1 female with pupal and larval exuviae same data as holotype.

Diagnostic characters

Labrundinia sp. 7 differs from other *Labrundinia* species by the combination of the following characters. **Male**: wing length 1.56–1.67 mm; abdominal tergite II–V with continuous brown transverse band near proximal margin, T VI–VIII almost wholly brown; hypopygium pale brown, sternapodeme with moderate anterior process. **Pupa**: thoracic horn kidney-shaped, preapical indentation very reduced; abdominal segment VII with 4 lateral setae. **Larva**: surface covered with small spinules, with ventral maculation; lateroventral and posteroventral spine groups absent; subbasal seta simple; serrated claw absent; bifid claw with V-shaped lower indentation.

Description

Adult male ($n = 3$)

Size. Total length 2.57–3.04 mm. Wing length 1.56–1.67 mm. Total length/wing length 1.61–1.87. Wing length/length of profemur 2.81–3.16.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp brown. Thorax brown with mesonotum dorsally dark brown; antepnotum pale brown; supraalar callus

brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 49L. Hypopygium pale brown.

Head (Figs 49–E). Temporal setae 12, uniserial (Fig. 49B). Eye ratio 0.97–1.01. Tentorium (Fig. 49C) 149–179 μm long, stipes not measurable. Clypeus 88–114 μm long, 66–71 μm wide at largest part, bearing 13–17 setae. Cibarial pump (Fig. 49D) with anterior margin concave, 216–232 μm long. Palpomere lengths 1–4 (in μm): 42–47; 60–95; 131–151; 159–165. Antenna with 14 flagellomeres, AR 1.44–1.50, flagellum 904–945 μm long, diameter of pedicel 144–154 μm , apical setae single (Fig. 49E).

Thorax. Anteprepronotum with 2–4 lateral setae. Acrostichals 34–40, biserial, diverging evenly posteriorly, starting close to anteprepronotum and almost reaching scutellum; dorsocentrals 22, irregularly biserial; prealars 7–9; supraalars 2. Anapleural suture ratio 0.38–0.45. Scutellum with 9–10 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

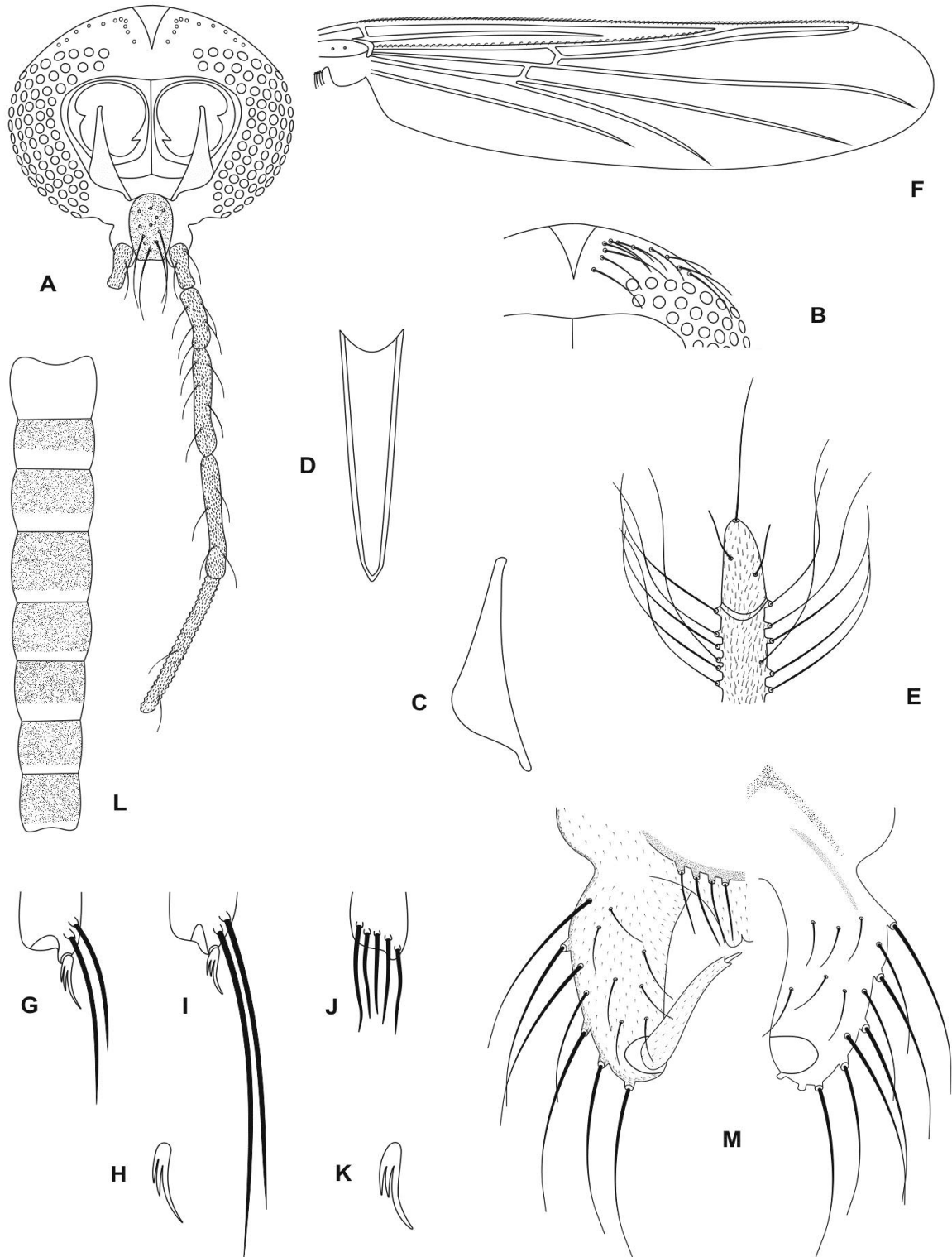
Wing (Fig. 49F). Width 0.44–0.55 mm. Costa 1.37–1.48 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.08–0.09 mm long. VR 0.70–0.75. WW 0.27–0.30. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 49G–K). Fore leg: width at apex of tibia 44–47 μm (Fig. 49G), tibia with single, apical and pectinate spur 16–25 μm long (Fig. 49H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 42–50 μm long (Fig. 49I), tibia with single, apical and pectinate spur 25–29 μm long with three teeth (Fig. 49J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 38–49 (2) μm long (Fig. 49K), tibia without spur; comb with 5 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXIII.

Table XXXIII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 7, male (n = 2–3).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	529–562	609–623	495–523	313–330	232–239
p ₂	656–694	588–599	773–862	340–402	193–211
p ₃	636–649	799–826	822–846	388–392	247–251
	ta ₄	ta ₅	LR	BV	SV
p ₁	130–155	87–97	0.81–0.85	2.10–2.12	2.24–2.30
p ₂	128–132	94–101	1.31–1.43	2.46–2.69	1.47–1.65
p ₃	154–156	89–107	1.02–1.03	2.54–2.59	1.74–1.75

Hypopygium (Fig. 49M). Tergite IX arched, with 10 dorsal setae. Anal point small, apical edge slightly notched. Phallapodeme 52–56 μm long. Sternapodeme with moderate anterior process. Gonocoxite cylindrical, 112–116 μm long, 44–48 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.46–



Figures 49A–M. *Labrundinia* sp. 7, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

2.54. Gonostylus simple and slender, 74–77 μm long; megaseta 12 (2) μm long. HR 1.50–1.52. HV 3.40–4.09.

Adult female (n = 1)

Size. Total length 1.89 mm. Wing length 1.14 mm. Total length/wing length 1.66. Wing length/length of profemur 2.51.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen pale brown, with brown transverse bands near proximal margin. Seminal capsules brown.

Head. Temporal setae 10, uniserial. Eyes ratio 1.50. Tentorium 141 μm long, stipes not measurable. Clypeus 104 μm long, 84 μm wide at largest part, bearing 25 setae. Cibarial pump as in male, 219 μm long. Palpomere lengths 1–5 (in μm): 41; 80; 129; 150; 241. Antenna with 11 flagellomeres, AR 0.91, flagellum 331–319 μm long, diameter of pedicel 55 μm .

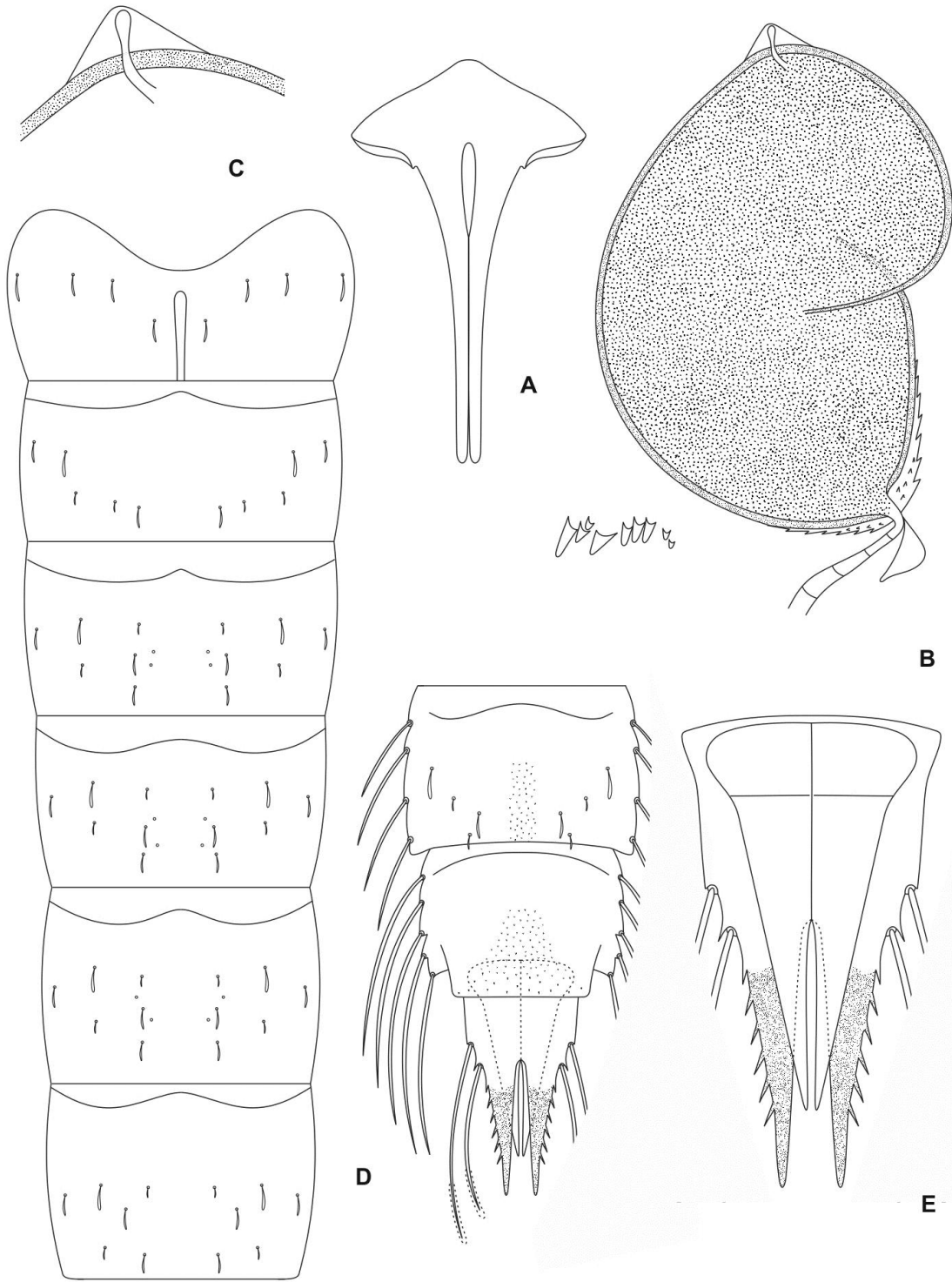
Thorax. Anteprenotum with 2 lateral setae. Acrostichals 44, irregularly biserial, starting close to anteprenotum; dorsocentrals 40, irregularly biserial; prealars 11; supraalars 2. Scutellum with 12 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.50 mm. Costa 1.14 mm long, not produced beyond R_{4+5} . R_{2+3} absent. Base of radial sector 0.07 mm long. VR 0.70. WW 0.44. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 48 μm , tibia with single, apical and pectinate spur 21 μm , with three teeth and two preapical setae; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 46 μm long, tibia with single, apical and pectinate spur 26 μm long with three teeth and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 42 μm long, tibia without spur; comb with 5 setae; Ta_{1-4} each without two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXIV.

Genitalia. Gonapophysis VIII broadly rounded, 46 μm long. Coxosternapodeme 91 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 22 μm long and 13 μm wide; with 5 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 103 μm . Seminal capsules oval with conical shaped necks, length 44 μm , maximum width 36 μm . Length ratio SCa/No 0.42.

Pupa (n = 4 unless otherwise stated)



Figures 50A–E. *Labrundinia* sp. 7, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Table XXXIV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 7, female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	455	507	415	257	171
p ₂	651	581	739	306	170
p ₃	581	728	721	328	212
	ta ₄	ta ₅	LR	BV	SV
p ₁	120	78	0.82	2.20	2.31
p ₂	116	81	1.27	2.93	1.67
p ₃	138	79	0.99	2.68	1.81

Size. Male abdomen 2.59–2.64 (3) mm long; female abdomen 2.04 (1) mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Abdomen with median brown maculation. Apex of anal lobe brown.

Cephalothorax (Figs 50A–C). Frontal apotome as in figure 50A. Wing sheath smooth 0.89–1.09 mm long. Thoracic horn 329–379 μm long and 197–220 μm wide (Fig. 50B), preapical absent, THR 1.67–1.72. Membranous preapical papilla 41–53 μm long (Fig. 50C), PTH 0.12–0.16, aeropyle tube simple, short, 23–38 μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 8–9 conical teeth.

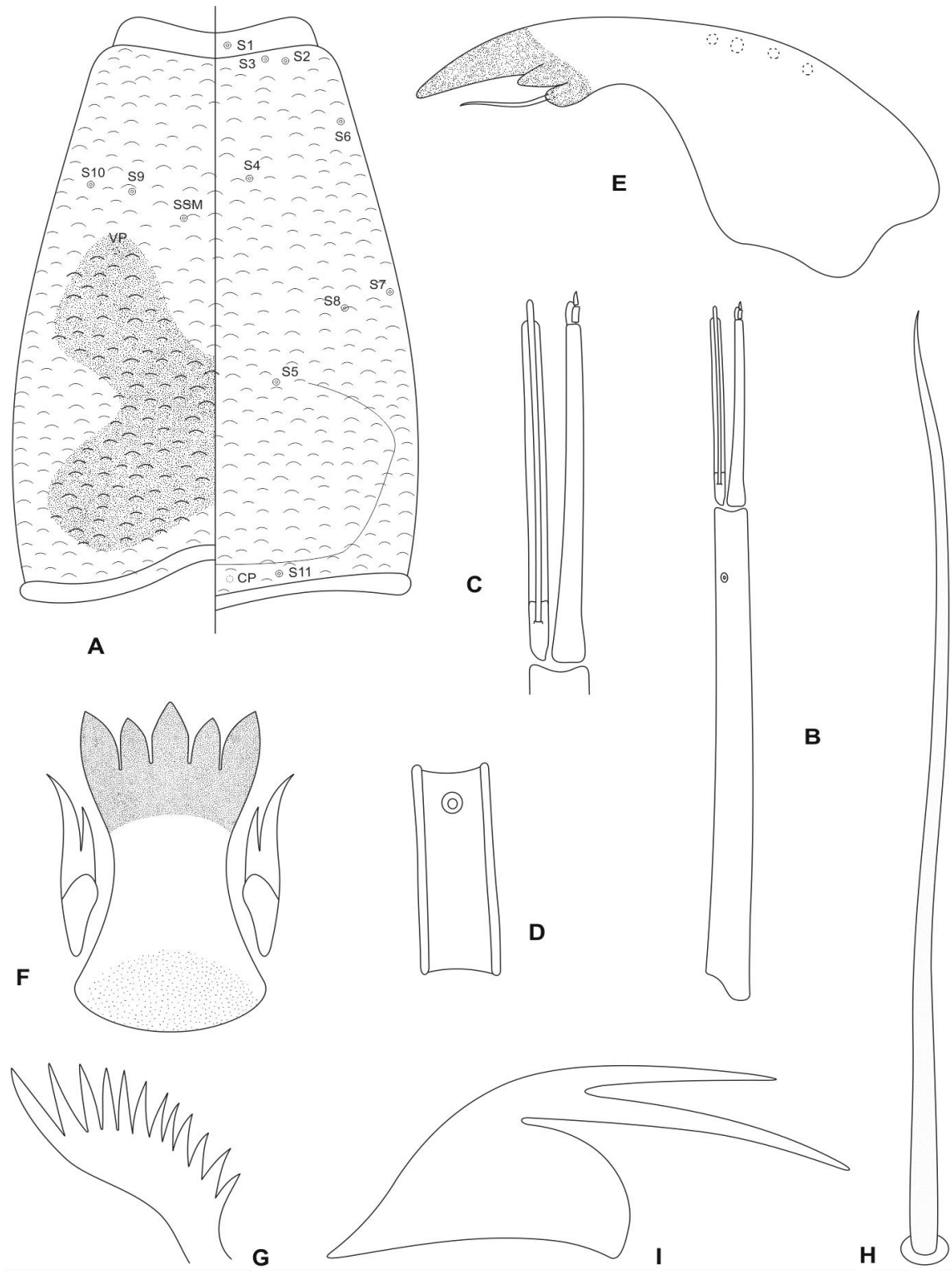
Abdomen (Figs 50D–E). Tergite I with scar, 163–180 (2). T I–VI without shagreen, VII–VIII with shagreen medial basal concentrated. Abdominal chaetotaxy as in figure 50D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 361–409 μm long and 188–224 μm wide (Fig. 50E), outer margins sclerotized, with 6–9 spines, longest spine 19–29 μm long, inner margins of lobes membranous. ALR 1.65–2.18. Genital sac elongated, not surpassing apex of anal lobe.

4th instar larva (n = 4 unless otherwise stated)

Coloration. Head pale yellow, with brown maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 51A). Length 526–563 μm , 359–369 μm wide. Surface covered with small spinules; lateroventral and posteroventral spine groups absent; cephalic index 0.64–0.69. Chaetotaxy as in figure 51A.

Antenna (Figs 51B–C). Length 320–335 μm , A₁ 230–240 μm long, with ring organ placed 174–204 μm



Figures 51A–I. *Labrundinia* sp. 7, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

from base, A₂ 85–88 µm long. AR 2.24–2.54. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 51D). Basal palp segment 24–27 µm long and 9–10 µm wide, with ring organ 18–21 µm from base. PR 2.72–3.08. APR 8.48–9.38.

Mandible (Fig. 51E). Length 63–82 µm, with 3 lateral setae. Sensillum campaniformium 41–56 µm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.81–3.52.

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 51F–G). Ligula 53–56 µm long, 26–31 µm wide, with row of 5 teeth. IO 0.98–0.99, MO 1.00–1.06. Paraligula bifid, 30–31 µm long, inner tooth 25–30 µm long, shorter than outer tooth. Pecten hypopharyngis with 11 teeth almost equal in size.

Body (Figs 51H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 163–208 µm long, 34–40 µm wide, with 7 anal setae, 564–723 (3) µm long. L/W 4.65–5.68. Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod unmeasured; subbasal seta simple, without spines (Fig. 51H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 51H). B/C 1.11–1.21 (3) µm long.

***Labrundinia* spec. nov. 8** (Figs 52–54)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, Gália, Caetetus Ecological Station, Barreiro reservoir, 08.vii.2008, F. L. Silva & J. F. Nunes (MZUSP). Paratypes: 1 female with pupal and larval exuviae same data as holotype; 1 male same data as holotype except for São Carlos, Valparaíso lake, 27.iv.2010, F. L. Silva. 1 pupa and larval exuvia same data as holotype.

Diagnostic characters

Labrundinia sp. 8 differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite II–VI with continuous brown transverse band near proximal margin, T VII–VIII wholly brown; hypopygium pale brown, sternapodeme with anterior process absent, hypopygium ratio about 1.50. **Pupa**: thoracic horn kidney-shaped, preapical indentation reduced, thoracic horn ratio 2.17–2.20. **Larva**: surface covered with small spinules; lateroventral and posteroventral spine groups absent; subbasal seta simple; serrated claw present; bifid claw with V-shaped lower indentation.

Description

Adult male (n = 2)

Size. Total length 1.80 (1) mm. Wing length 1.25 (1) mm. Total length/wing length 1.47 (1). Wing length/length of profemur 2.78 (1).

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale. Thorax pale brown with mesonotum dorsally dark brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 52L. Hypopygium pale brown.

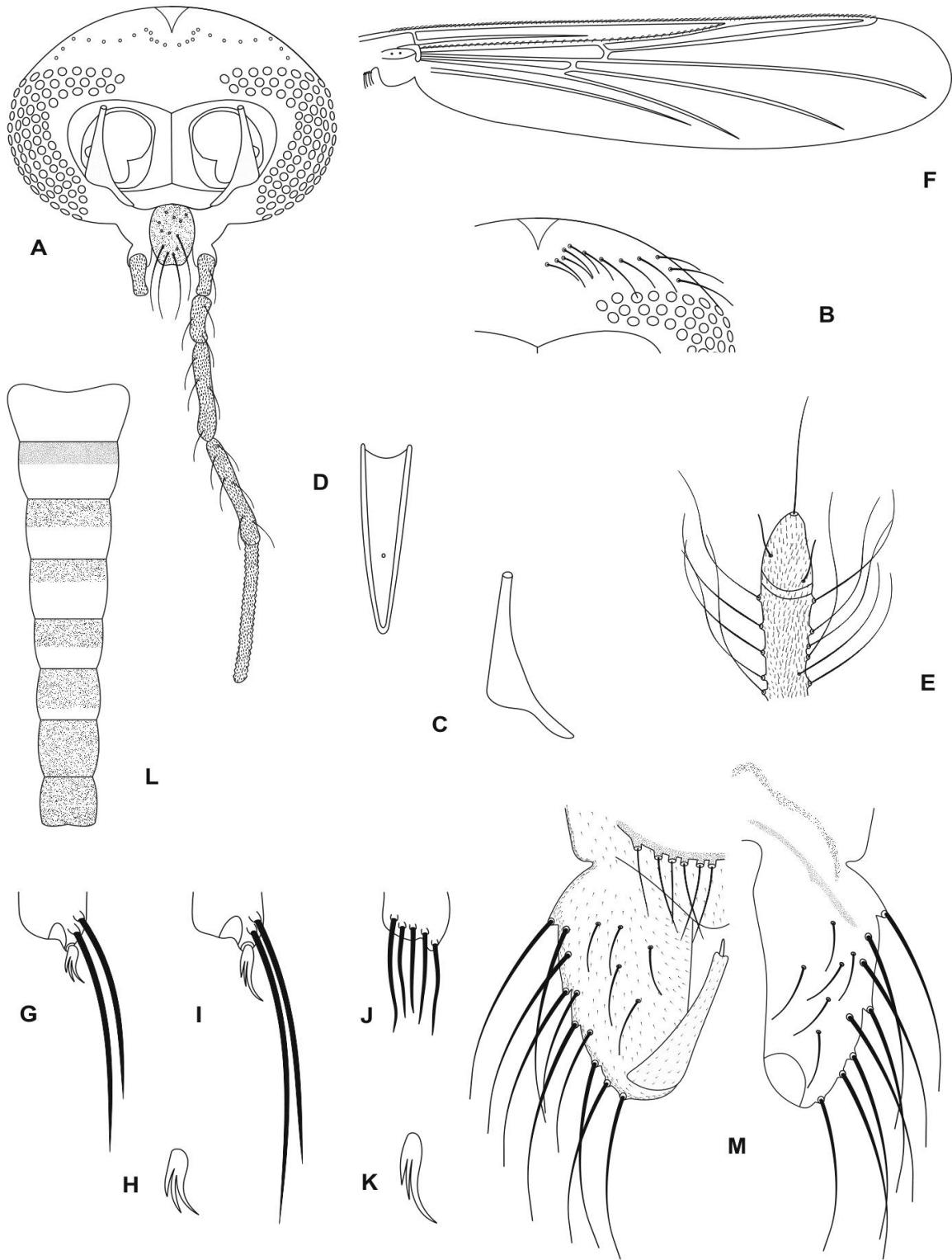
Head (Figs 52–E). Temporal setae 12–13, uniserial (Fig. 52B). Eye ratio 0.79 (1). Tentorium (Fig. 52C) 169 μm long, stipes not measurable. Clypeus 81–90 μm long, 54–56 μm wide at largest part, bearing 11–13 setae. Cibarial pump (Fig. 52D) with anterior margin concave, 150–205 μm long. Palpomere lengths 1–5 (in μm): 27–43; 45–56; 63–107; 88–144, 225 (1). Antenna with 14 flagellomeres, AR 1.21–1.29, flagellum 703–725 μm long, diameter of pedicel 121–147 μm , apical setae single (Fig. 52E).

Thorax. Anteprenotum with 3–5 lateral setae. Acrostichals 42–46, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20–22, irregularly uniserial; prealars 7–8; supraalars 2. Anapleural suture ratio 0.39. Scutellum with 8–9 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 52F). Width 0.33 (1) mm. Costa 1.08 (1) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.08 (1) mm long. VR 0.77 (1). WW 0.27 (1). Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 52G–K). Fore leg: width at apex of tibia 35–40 μm (Fig. 52G), tibia with single, apical and pectinate spur 14–18 μm long (Fig. 52H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 38–40 μm long (Fig. 52I), tibia with single, apical and pectinate spur 16–22 μm long with three teeth (Fig. 52J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 38–39 μm long (Fig. 52K), tibia without spur; comb with 5 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXV.

Hypopygium (Fig. 52M). Tergite IX arched, with 9–10 dorsal setae. Anal point rounded, apical edge slightly convex. Phallapodeme 51–54 μm long. Sternapodeme with anterior process absent. Gonocoxite cylindrical, 113 (1) μm long, 51(2) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.20 (1). Gonostylus simple and slender, 66–74 μm long; megaseta 12–14 μm long. HR 1.52 (1). HV 2.76 (1).



Figures 52A–M. *Labrundinia* sp. 8, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Table XXXV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 8, male ($n = 2$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	423–449	502–503	370–418	241–249	165–192
p ₂	434–676	484–539	652–696	282–302	155–168
p ₃	538–573	636–703	674–706	284–287	177–205
	ta ₄	ta ₅	LR	BV	SV
p ₁	123–131	82–91	0.74–0.83	2.02–2.16	2.22–2.57
p ₂	114–113	73–114	1.29–1.35	2.52–2.73	1.41–1.75
p ₃	125–123	78–79	1.00–1.06	2.79–2.85	1.74–1.81

Adult female (n = 1)

Size. Total length 0.70 mm. Wing length 1.15 mm. Total length/wing length 1.66. Wing length/length of profemur 2.82.

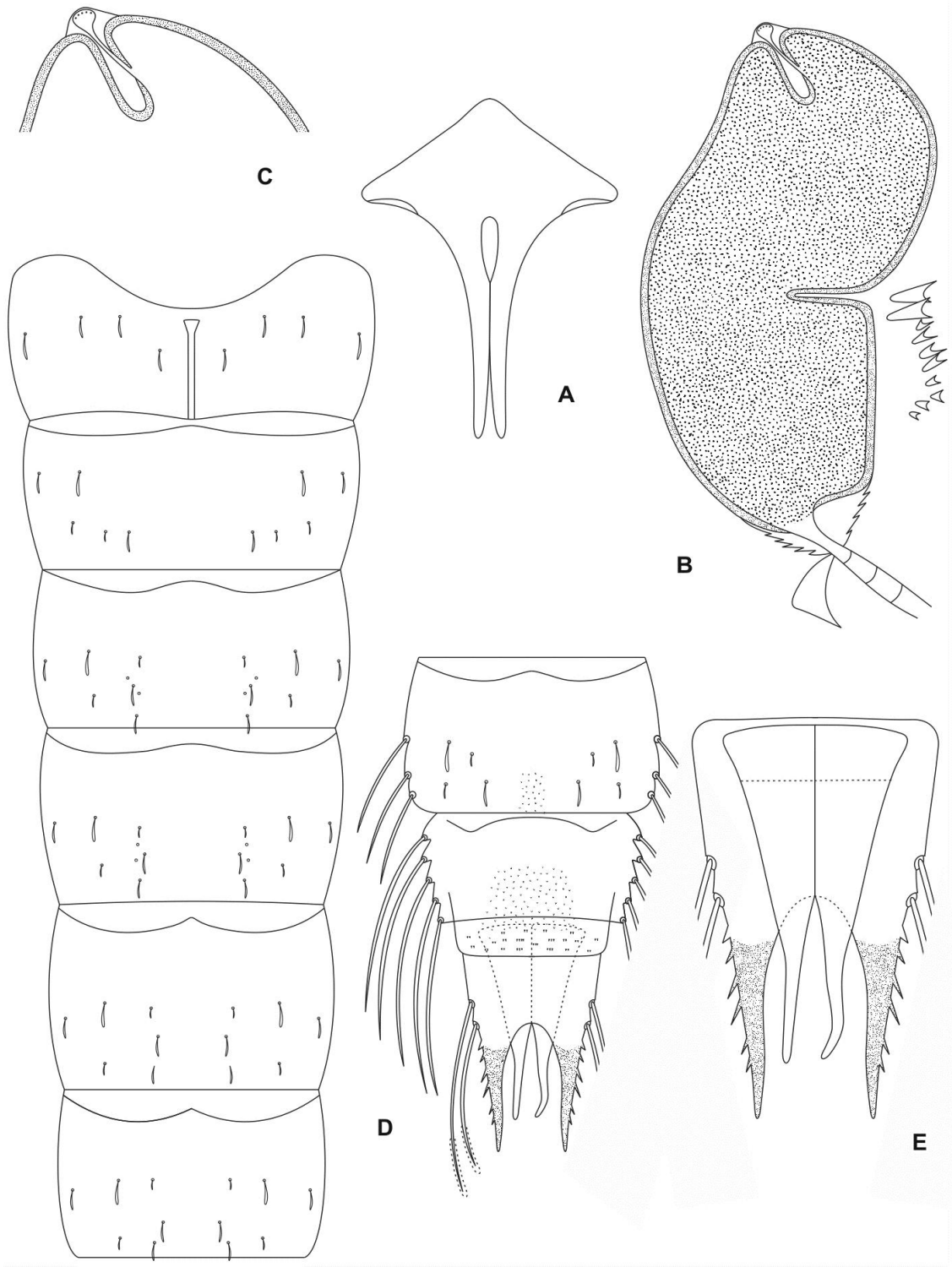
Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Femur I brown, Fe II–III pale brown. Tibia I apex brown, Ti II–III pale brown. Tarsomere 1–5 brown to pale brown. Abdomen seems wholly brown. Seminal capsules brown.

Head. Temporal setae 12, uniserial. Eyes ratio 1.08. Tentorium 166 μm long, stipes not measurable. Clypeus 95 μm long, 64 μm wide at largest part, bearing 22 setae. Cibarial pump as in male, 220 μm long. Palpomere lengths 1–4 (in μm): 46; 62; 116; 143. Antenna with 11 flagellomeres, AR 0.37, flagellum 357 μm long, diameter of pedicel 54 μm .

Thorax. Anteprenotum with 3 lateral setae. Acrostichals 44, irregularly uniserial, starting close to anteprenotum; dorsocentrals 30, irregularly biserial; prealars 10; supraalars 2. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.42 mm. Costa 0.99 mm long, not produced beyond R₄₊₅. R₂₊₃ absent. Base of radial sector 0.09 mm long. VR 0.63. WW 0.37. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 39 μm , tibia with single, apical and pectinate spur 19 μm , with three teeth and two preapical setae; Ta₁₋₄ each without two preapical pseudospurs. Mid leg: width at apex of tibia 43 μm long, tibia with single, apical and pectinate spur 22 μm long with three teeth and two preapical setae; Ta₁₋₄ each with two preapical pseudospurs. Hind leg: width at apex of tibia 45 μm long, tibia without spur; comb with 5 setae; Ta₁₋₄ each without two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXVI.



Figures 53A–E. *Labrundinia* sp. 8, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Genitalia. Gonapophysis VIII broadly rounded, 59 μm long. Coxosternapodeme 73 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 38 μm long and 12 μm wide; with 5 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 105 μm . Seminal capsules oval with conical shaped necks, length 35 μm , maximum width 31 μm . Length ratio SCa/No 0.33.

Table XXXVI. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 8, female ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	408	433	375	194	137
p ₂	549	467	578	244	153
p ₃	494	621	568	247	153
	ta ₄	ta ₅	LR	BV	SV
p ₁	106	75	0.86	2.38	2.25
p ₂	104	82	1.24	2.74	1.76
p ₃	108	77	0.92	2.88	1.96

Pupa ($n = 3$ unless otherwise stated)

Size. Male abdomen 2.05 (1) mm long; female abdomen 2.01–2.27 (2) mm long.

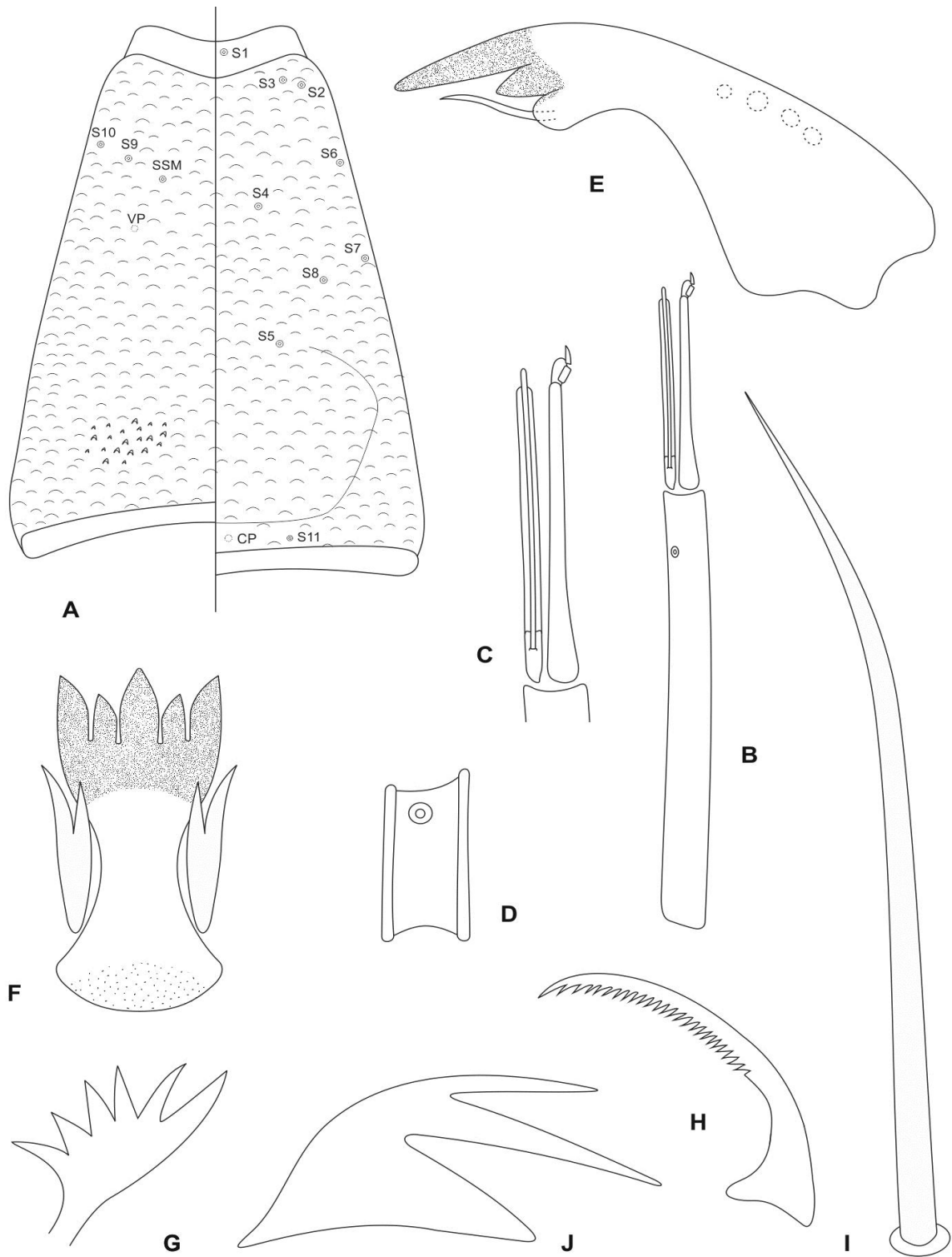
Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 53A–C). Frontal apotome as in figure 53A. Wing sheath smooth 0.84–0.81 mm long. Thoracic horn 271–295 μm long and 121–136 μm wide (Fig. 53B), preapical indentation moderate deep, THR 2.17–2.24. Membranous preapical papilla 35–49 μm long (Fig. 53C), PTH 0.11–0.18, aeropyle tube simple, short, 35–36 (2) μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 9–10 conical teeth.

Abdomen (Figs 53D–E). Tergite I with scar, 114–166. T I–VI without shagreen, VII–VIII with shagreen medial basal concentrated. Abdominal chaetotaxy as in figure 53D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 349–361 μm long and 167–190 μm wide (Fig. 53E), outer margins sclerotized, with 6–8 spines, longest spine 22–29 μm long, inner margins of lobes membranous. ALR 1.83–2.14. Genital sac elongated, not surpassing apex of anal lobe.

4th instar larva ($n = 3$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment



Figures 54A–J. *Labrundinia* sp. 8, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Serrated claw of posterior parapod. **I.** Subbasal seta of posterior parapod. **J.** Bifid claw of posterior parapod.

brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 54A). Length 520–583 μm , 314–325 μm wide. Surface covered with small spinules; lateroventral spine group absent and posteroventral spine group present; cephalic index 0.55–0.63. Chaetotaxy as in figure 54A.

Antenna (Figs 54B–C). Length 293–296 μm , A_1 195–198 μm long, with ring organ placed 166–180 μm from base, A_2 84–90 μm long. AR 1.95–2.09. Blade longer than; accessory blade 91–102.

Maxilla (Fig. 54D). Basal palp segment 30–32 μm long and 7–10 μm wide, with ring organ 25–28 μm from base. PR 3.09–4.16. APR 6.01–6.63.

Mandible (Fig. 54E). Length 73–84 μm , with 3 lateral setae. Sensillum campaniformium 54–58 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.33–2.71.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 54F–G). Ligula 58–67 μm long, 27–28 μm wide, with row of 5 teeth. IO 0.98–0.99, MO 1.04–1.10. Paraligula bifid, 27–33 μm long, inner tooth 23–25 μm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 54H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 157–185 μm long, 33–35 μm wide, with 7 anal setae, 524–602 μm long. L/W 4.50–5.44. Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod 469–534 μm long; subbasal seta simple, without spines (Fig. 54H); parapod apex with numerous simple claws, with serrated claw; bifid claw with V-shaped lower indentation (Fig. 54H). B/C 1.11–1.14 (2) μm long.

***Labrundinia spec. nov.* 9** (Fig. 55)

Material examined

Type: Holotype male, BRAZIL: Amazonas, Manacapuru, Cabaliana lake, 16.vi.1971, F. Reiss (ZSM).

Diagnostic characters

Labrundinia sp. 9 differs from other *Labrundinia* species by the combination of the following characters.

Male: abdomen wholly brown; hypopygium brown, gonostylus with a concave depression apically, sternapodeme with reduced anterior process.



Figures 55A–M. *Labrundinia* sp. 9, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Apex of mid tibia. **I.** Apex of hind tibia with comb. **J.** Abdominal coloration pattern, dorsal aspect. **K.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Description

Adult male (n = 1)

Size. Total length 2.33 mm. Wing length 1.14 mm. Total length/wing length 2.04. Wing length/length of profemur 1.77.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax golden brown; antepnotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Abdomen as in figure 55J. Hypopygium brown.

Head (Figs 55A–E). Temporal setae 10, uniserial (Fig. 55B). Eye ratio 1.12. Tentorium (Fig. 55C) 150 μm long, stipes not measurable. Clypeus 68 μm long, 58 μm wide at largest part, bearing 13 setae. Cibarial pump (Fig. 55D) with anterior margin concave, 136 μm long. Palpomere lengths 1–3 (in μm): 31; 54; 81. Antenna with 14 flagellomeres, AR 1.35, flagellum 805 μm long, diameter of pedicel 91 μm , apical setae single (Fig. 55E).

Thorax. Antepnotum lateral setae unobserved. Acrostichals 40, biserial, diverging evenly posteriorly, starting close to antepnotum and almost reaching scutellum; dorsocentrals 22, irregularly biserial; prealars 7; supraalars unobserved. Anapleural suture ratio 0.33. Scutellars unobserved. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 55F). Width 0.32 mm. Costa 1.00 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.06 mm long. VR 0.78. WW 0.28. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 55G–I). Fore leg: width at apex of tibia 43 μm (Fig. 55G), tibial spur and preapical setae unobserved; preapical pseudospurs unobserved. Mid leg: width at apex of tibia 37 μm long (Fig. 55H), tibial spur and preapical setae unobserved; preapical pseudospurs unobserved. Hind leg: width at apex of tibia 38 μm long (Fig. 55I), tibia without spur; comb with apparently 4 setae; preapical pseudospurs unobserved. Lengths and proportion of leg segments as in Table XXXVII.

Hypopygium (Fig. 55K). Tergite IX arched, with 10 dorsal setae. Anal point rounded apically. Phallapodeme 52 μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 116 μm long, 52 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.23. Gonostylus with a concave depression apically, 79 μm long; megaseta 12 μm long. HR 1.47. HV 2.95.

Adult female, pupa, and larva. Unknown.

Table XXXVII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 9, adult male (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	642	473	–	–	–
p ₂	608	493	–	–	–
p ₃	529	655	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

Labrundinia spec. nov. 10 (Figs 56–58)

Material examined

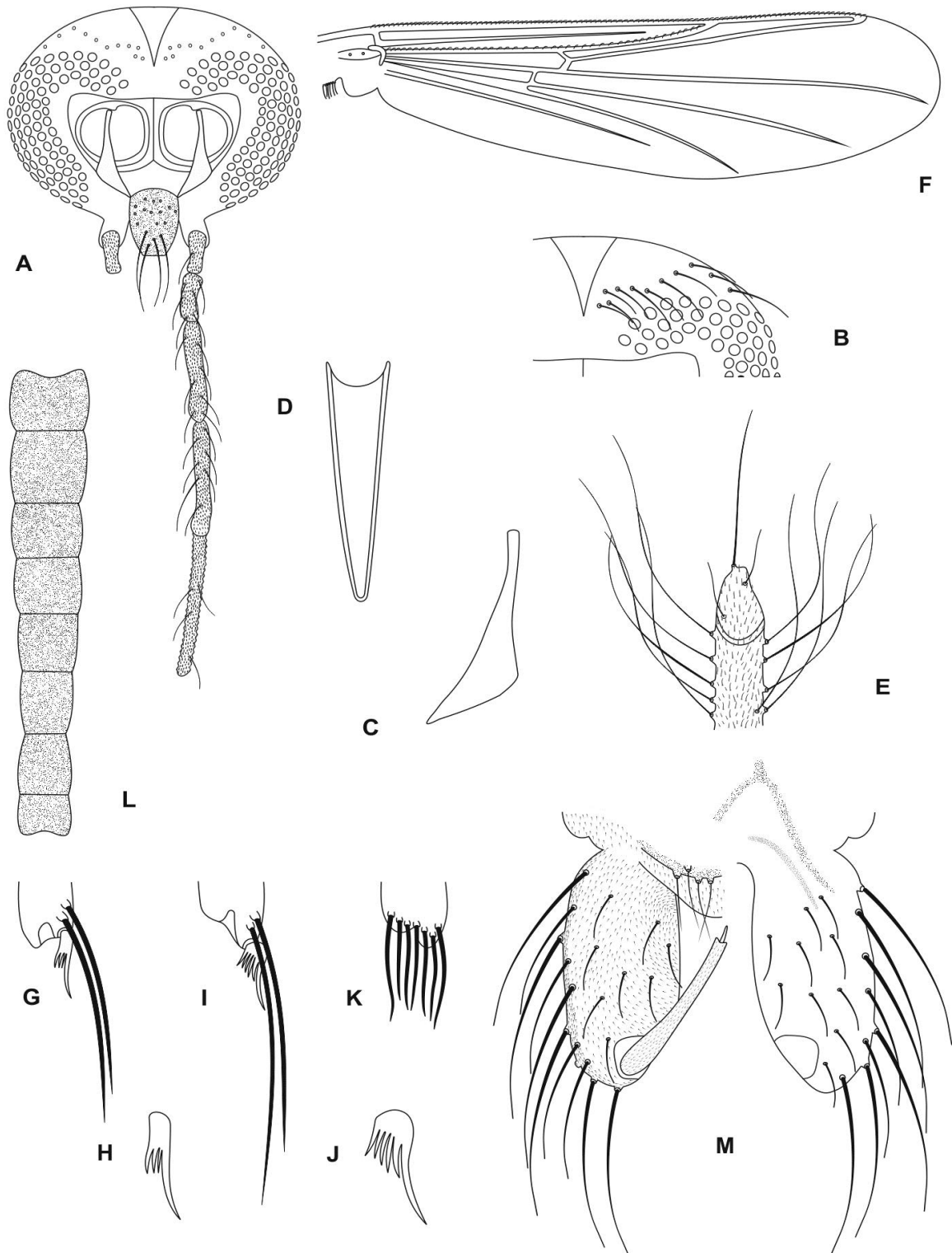
Type: Holotype male with pupal and larval exuviae, **Brazil: São Paulo**, São Carlos, Ecological Park, 19.iv.2010, F. L. Silva (MZUSP). *Paratypes:* 1 male with pupal and larval exuviae same data as holotype; 1 male with pupal and larval exuviae same data as holotype except for Canchin reservoir, 25.vii.2007; 1 female with pupal and larval exuviae same data as holotype except for Monjolinho stream, 11.iv.2011; 2 pupa with larval exuvia same data as holotype.

Diagnostic characters

Labrundinia sp. 10 differs from other *Labrundinia* species by the combination of the following characters. **Male:** tentorium 149–192 μm long; abdomen wholly brown; hypopygium brown, sternapodeme with reduced anterior process. **Pupa:** thoracic horn semi-globose, preapical indentation moderate deep; abdominal segment VII with 4 lateral setae genital sac elongated, reaching much beyond apex of anal lobe. **Larva:** surface smooth with small, dark brown spots ventrally, lateroventral and posteroventral spine groups absent; paraligula multitoothed; subbasal seta simple; bifid claw with V-shaped lower indentation.

Description

Adult male (n = 3)



Figures 56A–M. *Labrundinia* sp. 10, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Size. Total length 2.18–2.54 mm. Wing length 1.22–1.31 mm. Total length/wing length 1.75–2.04. Wing length/length of profemur 2.95–3.01.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown with mesonotum dorsally dark brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 56L. Hypopygium brown.

Head (Figs 56–E). Temporal setae 12, uniserial (Fig. 56B). Eye ratio 0.84–0.85 (2). Tentorium (Fig. 56C) 149–192 μm long, stipes not measurable. Clypeus 76–87 μm long, 55–72 μm wide at largest part, bearing 14–16 setae. Cibarial pump (Fig. 56D) with anterior margin concave, 183–191 μm long. Palpomere lengths 1–5 (in μm): 41–45 (2); 47–52 (2); 98–105 (2); 147–156 (2); 211 (1). Antenna with 14 flagellomeres, AR 1.21–1.29, flagellum 790–797 μm long, diameter of pedicel 125–129 (2) μm , apical setae single (Fig. 56E).

Thorax. Anteprenotum with 2 (2) lateral setae. Acrostichals 38–55, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 22–26, irregularly uniserial; prealars 8–9; supraalars 2. Anapleural suture ratio 0.48–0.53. Scutellum with 10–11 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 56F). Width 0.36–0.39 mm. Costa 1.05–1.16 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.07–0.09 mm long. VR 0.69–0.76. WW 0.30–0.32. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 56G–K). Fore leg: width at apex of tibia 31–37 μm (Fig. 56G), tibia with single, apical and pectinate spur 18–19 μm long (Fig. 56H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 32–38 μm long (Fig. 56I), tibia with single, apical and pectinate spur 23–30 μm long with three teeth (Fig. 56J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 34–42 μm long (Fig. 56K), tibia without spur; comb with 7 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXVIII.

Hypopygium (Fig. 56M). Tergite IX arched, with 8 dorsal setae. Anal point small, apical edge rounded. Phallapodeme 58–71 (2) μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 143–154 μm long, 72–75 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.93–2.12. Gonostylus simple and slender, 97–106 μm long; megaseta 15–16 μm long. HR 1.46–1.48. HV 2.21–2.40.

Adult female ($n = 1$)

Table XXXVIII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 10, male (n = 2–3).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	400–436	385–430	304–340	181–188	128–137
p ₂	584–616	456–493	480–487	253–275	131–145
p ₃	526–545	519–561	546–563	252–257	166–168
	ta ₄	ta ₅	LR	BV	SV
p ₁	86–98	56–58	0.75–0.79	2.50–2.51	2.95–3.07
p ₂	93–100	75–82	0.98–1.07	2.65–2.75	2.13–2.29
p ₃	109–112	88–91	1.00–1.05	2.57–2.69	1.93–1.97

Size. Total length 1.29 mm. Wing length 0.81 mm. Total length/wing length 1.48. Wing length/length of profemur 2.76.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen seems wholly dark brown. Seminal capsules brown.

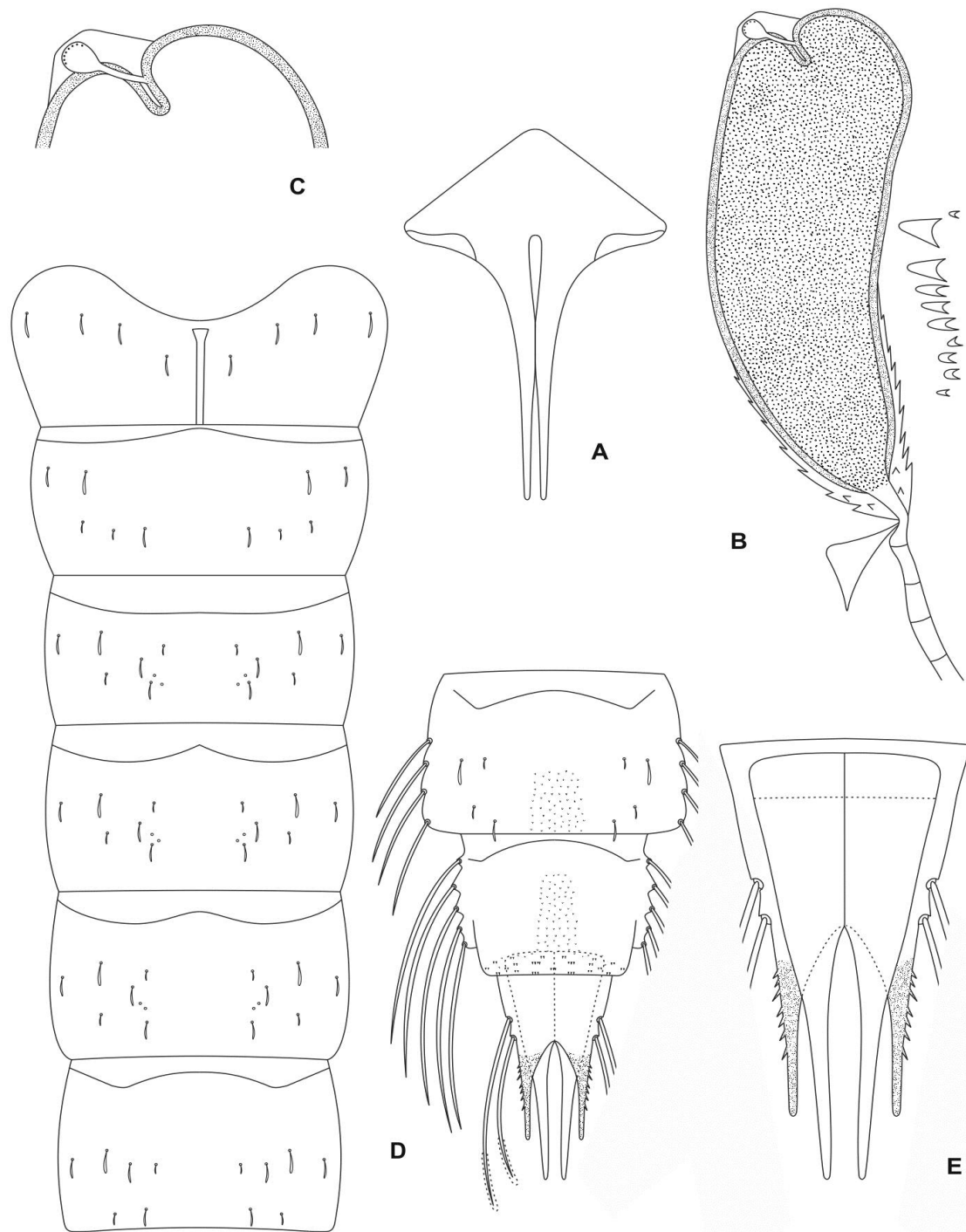
Head. Temporal setae 12, uniserial. Eyes ratio 0.94. Tentorium 149 μm long, stipes not measurable. Clypeus 85 μm long, 61 μm wide at largest part, bearing 19 setae. Cibarial pump as in male, 174 μm long. Palpomere lengths 1–3 (in μm): 31; 44; 85. Antenna with 11 flagellomeres, AR 0.34, flagellum 333 μm long, diameter of pedicel 51 μm .

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 35, irregularly uniserial, starting close to anteprenotum; dorsocentrals 35, irregularly biserial; prealars 15; supraalars 3. Scutellum with 10 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.33 mm. Costa 0.77 mm long, not produced beyond R₄₊₅. R₂₊₃ absent. Base of radial sector 0.04 mm long. VR 0.69. WW 0.38. Brachiolium with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 36 μm , tibia with single, apical and pectinate spur 15 μm , with three teeth and two preapical setae; preapical pseudospurs on Ta₁₋₄ unobserved. Mid leg: width at apex of tibia 44 μm long, tibia with two preapical setae, apical spur unobserved; preapical pseudospurs on Ta₁₋₄ unobserved. Hind leg: width at apex of tibia 41 μm long, tibia without spur; comb with 7 setae; preapical pseudospurs on Ta₁₋₄ unobserved. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XXXIX.

Genitalia. Gonapophysis VIII broadly rounded, 63 μm long. Coxosternapodeme 69 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 35 μm long and 11 μm wide; with 4 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 90 μm . Seminal capsules oval with



Figures 57A–E. *Labrundinia* sp. 10, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Table XXXIX. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 10, female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	315	305	262	128	101
p ₂	495	369	–	–	–
p ₃	439	488	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	63	59	0.86	2.51	2.37
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

conical shaped necks, length 36 μm , maximum width 28 μm . Length ratio SCa/No 0.40.

Pupa (n = 6 unless otherwise stated)

Size. Male abdomen 1.81–2.19 (4) mm long; female abdomen 1.71 (1) mm long.

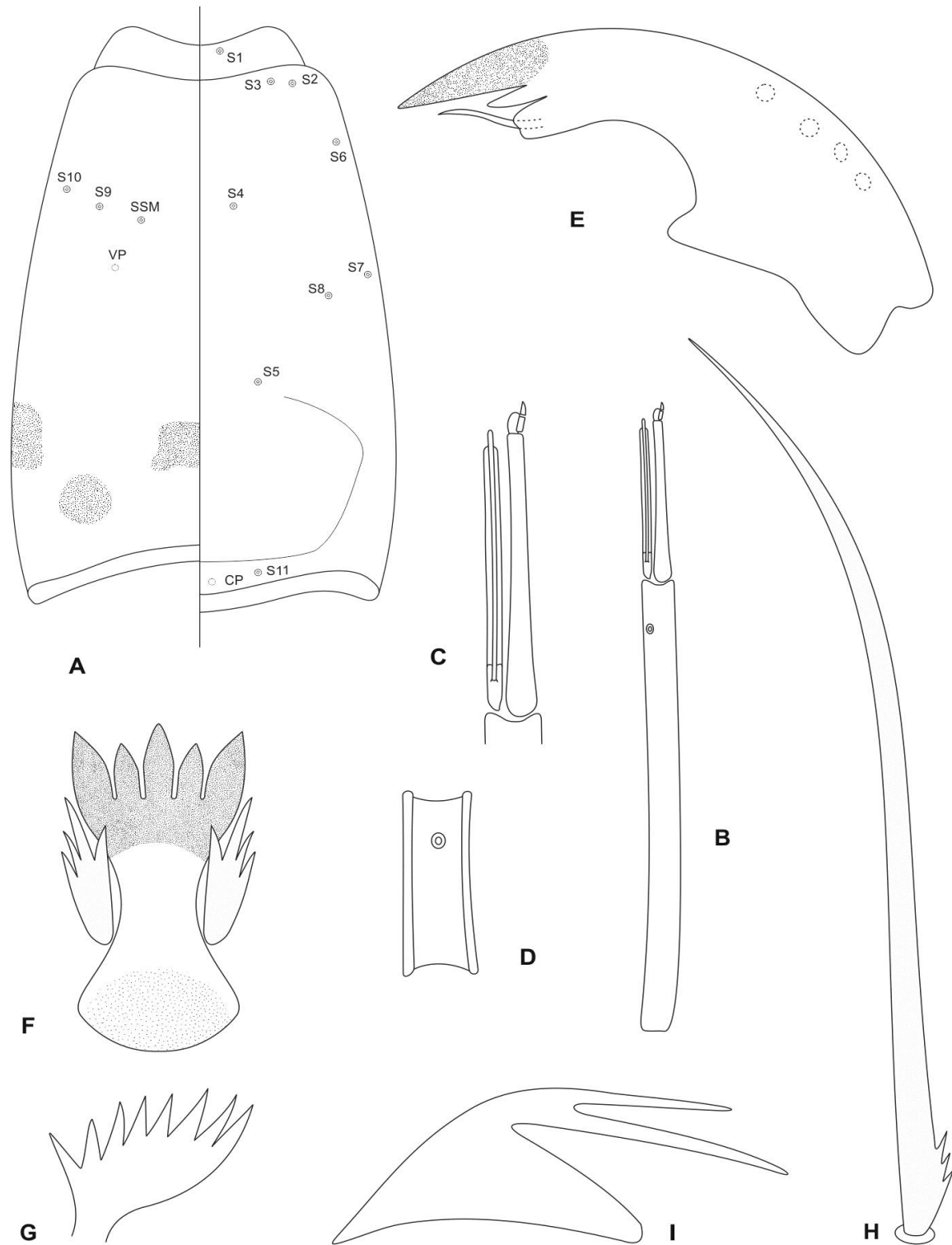
Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 57A–C). Frontal apotome as in figure 57A. Wing sheath smooth 0.70–0.96 mm long. Thoracic horn 203–239 μm long and 73–87 μm wide (Fig. 57B), preapical indentation moderate deep, THR 2.57–2.81. Membranous preapical papilla 23–43 μm long (Fig. 57C), PTH 0.09–0.17, aeropyle tube simple, short, 18–28 μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 8–10 conical teeth.

Abdomen (Figs 57D–E). Tergite I with scar, 114–132. T I–VI without shagreen, VII–VIII with shagreen medial basal concentrated. Abdominal chaetotaxy as in figure 57D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 263–318 μm long and 142–185 μm wide (Fig. 57E), outer margins sclerotized, with 8–10 spines, longest spine 10–14 μm long, inner margins of lobes membranous. ALR 1.48–2.20. Genital sac elongated, reaching much beyond apex of anal lobe.

4th instar larva (n = 6 unless otherwise stated)

Coloration. Head pale yellow, with small, dark brown spots ventrally; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.



Figures 58A–I. *Labrundinia* sp. 10, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

Head (Fig 58A). Length 464–514 μm , 283–317 μm wide. Surface smooth; lateroventral and posteroventral spine groups absent; cephalic index 0.56–0.67. Chaetotaxy as in figure 58A.

Antenna (Figs 58B–C). Length 311–348 μm , A_1 219–244 μm long, with ring organ placed 197–220 (4) μm from base, A_2 83–89 μm long. AR 2.32–2.45. Blade longer than A_2 over-reached by accessory blade.

Maxilla (Fig. 58D). Basal palp segment 23–32 μm long and 7–11 μm wide, with ring organ 19–26 (5) μm from base. PR 2.82–3.28. APR 7.31–10.2.

Mandible (Fig. 58E). Length 63–86 μm , with 3 lateral setae. Sensillum campaniformium 42–59 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.56–3.89.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 58F–G). Ligula 41–58 μm long, 22–26 μm wide, with row of 5 teeth. IO 0.92–0.98, MO 1.00–1.04. Paraligula multitoothed, 19–27 μm long, inner tooth 13–19 μm long, shorter than outer tooth. Pecten hypopharyngis with 8 teeth almost equal in size.

Body (Figs 58H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 146–171 (5) μm long, 20–33 (5) μm wide, with 7 anal setae, 518–552 (3) μm long. L/W 4.47–7.48 (5). Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod 324 (1) μm long; subbasal seta multitoothed, with basal spine group, with 3–4 (5) spines (Fig. 58H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 58H). B/C 1.02 (1) μm long.

Remarks: This is the *Labrundinia* sp. 9 of Trivinho-Strixino (2011) (larvae identification key).

Labrundinia spec. nov. 11 (Fig. 59)

Material examined

Type: Holotype male, BRAZIL: **Mato Grosso**, Upper Xingu river area, Culuene river ~100 km down stream of confluence 7 Setembro river with Culuene river, 30.viii.1965, E. J. Fittkau (ZSM). Paratype: 1 male same data as holotype.

Diagnostic characters

Labrundinia sp. 11 differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite II–IV with continuous brown transverse band near proximal margin, T V–VIII almost wholly brown; hypopygium dark brown, sternapodeme with anterior process absent.

Description

Adult male (n = 2 unless otherwise stated)

Size. Total length 2.20–2.38 mm. Wing length 1.21–1.31 mm. Total length/wing length 1.81. Wing length/length of profemur 2.57.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax golden brown with mesonotum dorsally light brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 59L. Hypopygium dark brown.

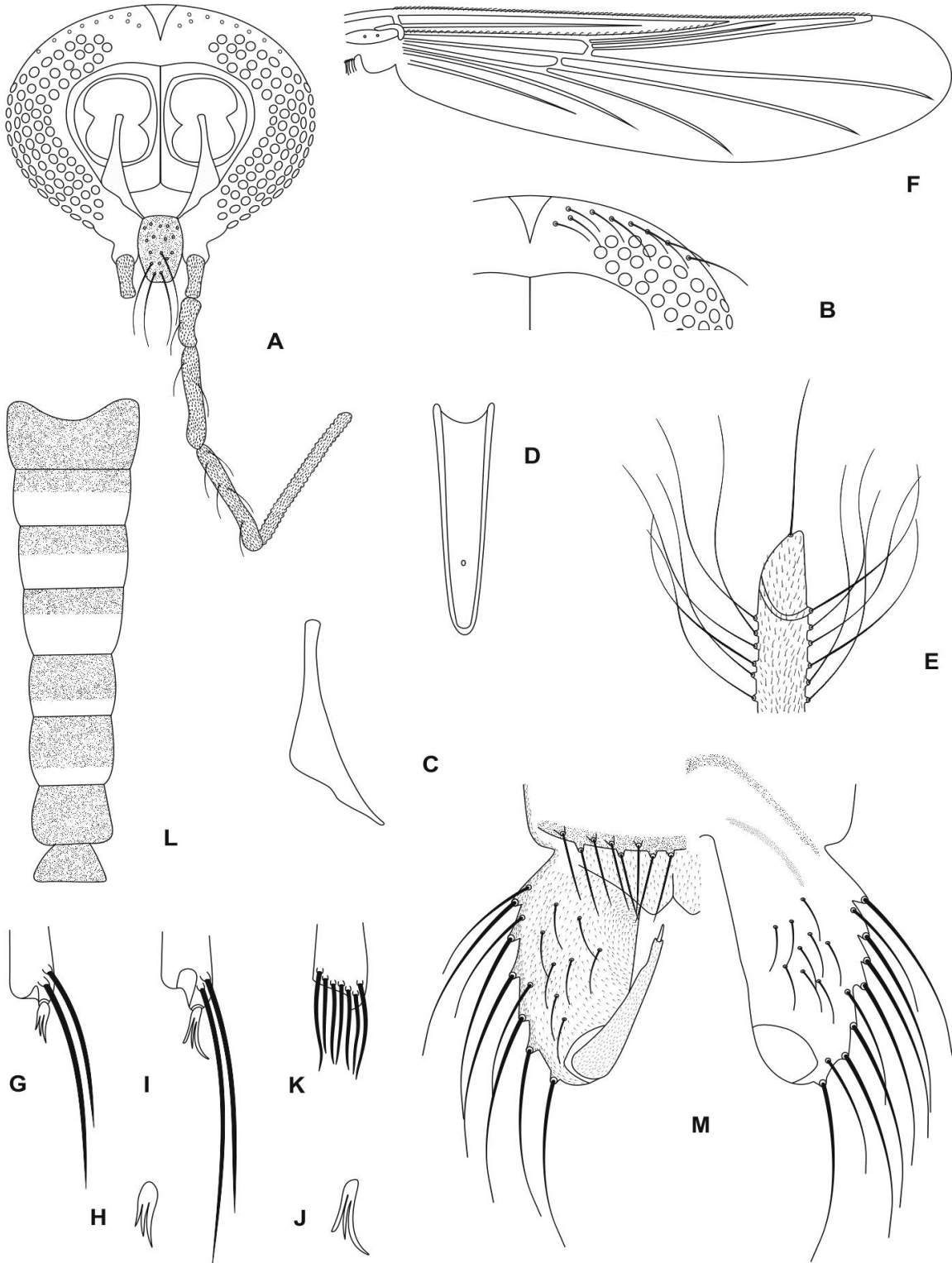
Head (Figs 55A–E). Temporal setae 10, uniserial (Fig. 59B). Eye ratio 1.12–1.40. Tentorium (Fig. 59C) 153–1.73 μm long, stipes not measurable. Clypeus 77–84 μm long, 63–70 μm wide at largest part, bearing 16–18 setae. Cibarial pump (Fig. 59D) with anterior margin concave, 177–208 μm long. Palpomere lengths 1–5 (in μm): 34–41; 42–51; 103–132; 131–165; 213–263. Antenna with 14 flagellomeres, AR 1.42–1.43, flagellum 714–839 μm long, diameter of pedicel 127–133 μm , apical setae single (Fig. 59E).

Thorax. Anteprenotum with 4 (1) lateral setae. Acrostichals 34–44, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 24–32, irregularly biserial; prealars 8–11; supraalars 2. Anapleural suture ratio 0.41 (1). Scutellum with 10–11 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 59F). Width 0.36–0.37 mm. Costa 1.00–1.13 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.08 mm long. VR 0.74 (1). WW 0.28. Brachiolium with 2 setae. Squama setiferous.

Legs (Figs 59G–K). Fore leg: width at apex of tibia 37–38 μm (Fig. 59G), tibia with single, apical and pectinate spur 13–18 μm long (Fig. 59H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 34–36 μm long (Fig. 59I), tibia with single, apical and pectinate spur 19–22 μm long with three teeth (Fig. 59J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 43–44 μm long (Fig. 59K), tibia without spur; comb with 7 setae; Ta_{1-4} each with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XL.

Hypopygium (Fig. 59M). Tergite IX arched, with 16–20 dorsal setae. Anal point trapezoidal, apical edge very notched. Phallapodeme 42–48 μm long. Sternapodeme with anterior process absent. Gonocoxite cylindrical, 93–112 μm long, 52–59 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 1.77–1.88. Gonostylus simple and slender, 63–74 μm long; megaseta 9–10 μm long. HR 1.47–1.50. HV 3.20–3.48.



Figures 59A–M. *Labrundinia* sp. 11, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Table XL. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 11, adult male (n = 1–2).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	511–473	491–439	–	–	–
p ₂	582–697	437–498	738	281	164
p ₃	571–499	610–674	591	731	323
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	103	101	1.48	2.98	1.62
p ₃	216	140	0.88	1.30	2.11

Adult female, pupa, and larva. Unknown.

Labrundinia spec. nov. 12 (Fig. 60)

Material examined

Type: Holotype male, BRAZIL: **São Paulo**, São Carlos, Monjolinho stream, associated with *Mysiophylum aquaticum*, 12.iii.2008, F. L. Silva (MZUSP). Paratype: 1 male same data as holotype.

Diagnostic characters

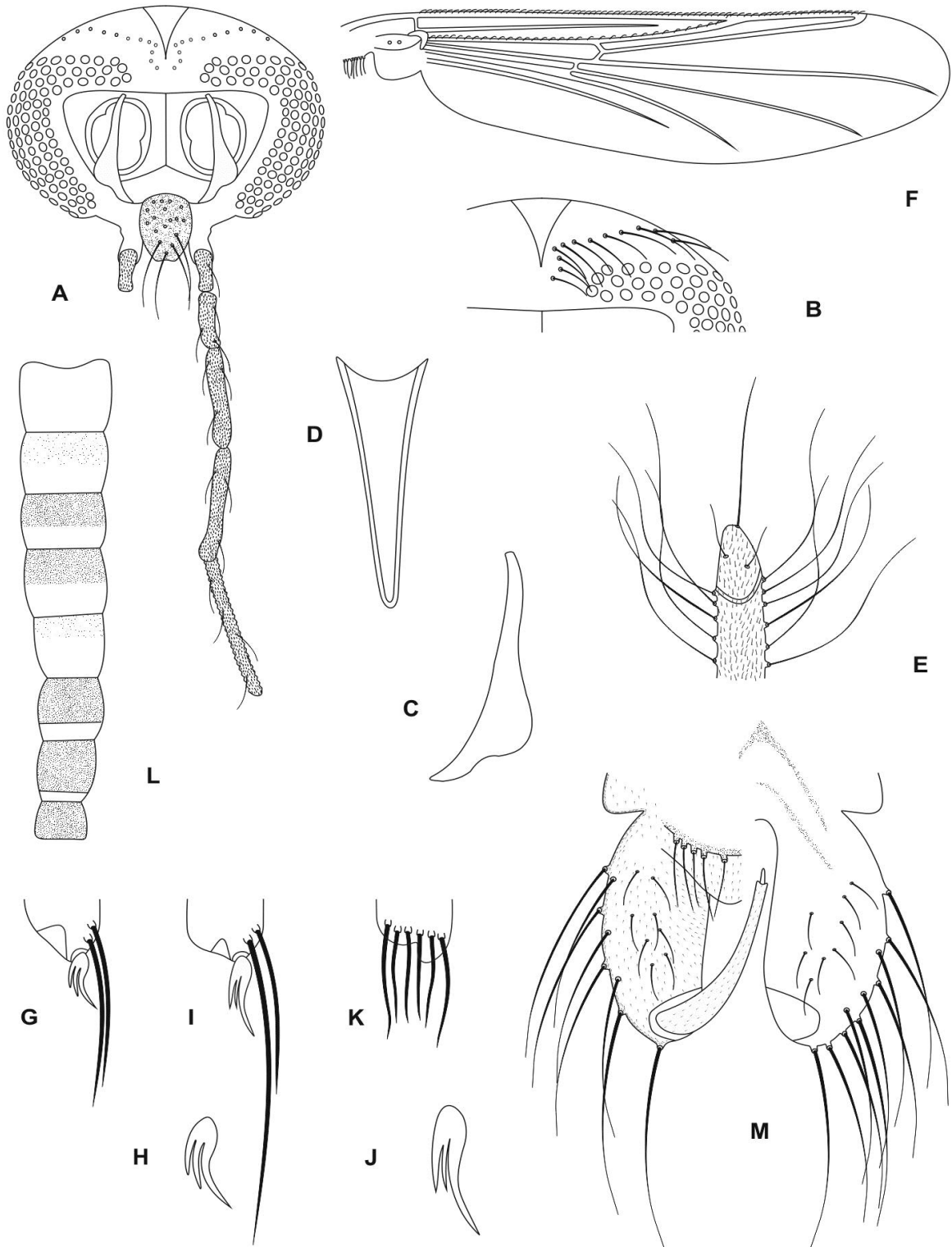
Labrundinia sp. 12 differs from other *Labrundinia* species by the combination of the following characters. **Male:** abdominal tergite II, V with divided transverse band near proximal margin, T III–IV, VI with continuous transverse band near proximal margin, VII–VIII almost wholly brown; hypopygium pale brown, sternapodeme with distinct anterior process.

Description

Adult male (n = 2 unless otherwise stated)

Size. Total length 2.30–2.51 mm. Wing length 1.42–1.44 mm. Total length/wing length 1.61–1.74. Wing length/length of profemur 2.69–2.73.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale brown; supraalar



Figures 60A–M. *Labrundinia* sp. 12, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Tibia I brown, Ti II–III apically brown. Abdomen as in figure 60L. Hypopygium pale brown.

Head (Figs 60A–E). Temporal setae 12–18, uniserial (Fig. 60B). Eye ratio 0.83–0.85. Tentorium (Fig. 60C) 161–1.69 μm long, stipes not measurable. Clypeus 90–101 μm long, 63–76 μm wide at largest part, bearing 18–19 setae. Cibarial pump (Fig. 60D) with anterior margin concave, 200–213 μm long. Palpomere lengths 1–5 (in μm): 37–40; 57–59; 129–138; 138–154; 138–150. Antenna with 14 flagellomeres, AR 1.13–1.34, flagellum 824–835 μm long, diameter of pedicel 153–159 μm , apical setae single (Fig. 60E).

Thorax. Antepnotum with 3 lateral setae. Acrostichals 48–54, biserial, diverging evenly posteriorly, starting close to antepnotum and almost reaching scutellum; dorsocentrals 30, irregularly biserial; prealars 9–11; supraalars 3. Anapleural suture ratio 0.39–0.46. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 60F). Width 0.42 (1) mm. Costa 1.21–1.25 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.07–0.08 mm long. VR 0.78–0.81. WW 0.29 (1). Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 60G–K). Fore leg: width at apex of tibia 42–45 μm (Fig. 60G), tibia with single, apical and pectinate spur 19–22 μm long (Fig. 60H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 42–49 μm long (Fig. 60I), tibia with single, apical and pectinate spur 25–32 μm long with three teeth (Fig. 60J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 43–46 μm long (Fig. 60K), tibia without spur; comb with 6 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XLI.

Table XLI. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 12, adult male (n = 1–2).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	518–534	521–537	298	267	155
p ₂	689–710	646	317	189	146
p ₃	587–607	612–629	670	299	208
	ta ₄	ta ₅	LR	BV	SV
p ₁	118	75	0.55	2.20	3.54
p ₂	146	93	1.23	2.52	1.91
p ₃	141	93	1.06	2.57	1.85

Hypopygium (Fig. 60M). Tergite IX arched, with 10–12 dorsal setae. Anal point rounded. Phallapodeme 61–73 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 137–150 μm long,

65–66 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.07–2.30. Gonostylus simple and slender, 100–108 μm long; megaseta 19–22 μm long. HR 1.37–1.39. HV 2.12–2.51.

Adult female, pupa, and larva. Unknown.

***Labrundinia* spec. nov. 13** (Fig. 61)

Material examined

Type: Holotype male, PANAMA: **Barro Colorado**, Barro Colorado Island, 10.iii.1986, H. Malicky (ZSM).

Diagnostic characters

Labrundinia sp. 13 differs from other *Labrundinia* species by the combination of the following characters.

Male: R₂₊₃ present; abdominal tergite II with divided transverse band near proximal margin, T III–VI with continuous transverse band near proximal margin, VII–VIII almost wholly brown; hypopygium brown, sternapodeme with reduced anterior process.

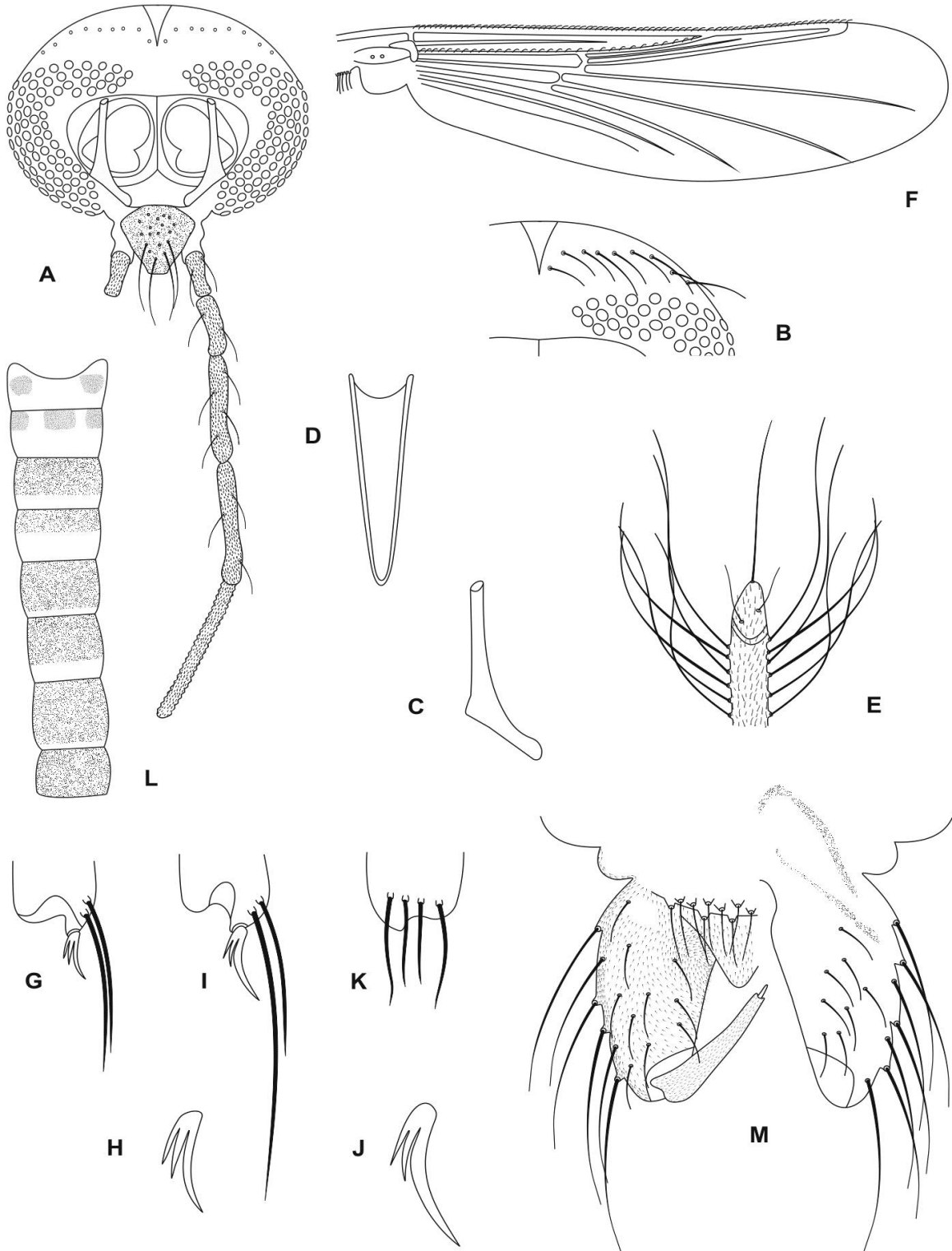
Description

Adult male ($n = 1$)

Size. Total length 1.94 mm. Wing length 0.97 mm. Total length/wing length 2.01. Wing length/length of profemur 2.32.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 61L. Hypopygium brown.

Head (Figs 61A–E). Temporal setae 10, uniserial (Fig. 61B). Eye ratio 0.86. Tentorium (Fig. 61C) 155 μm long, stipes not measurable. Clypeus 67 μm long, 71 μm wide at largest part, bearing 18 setae. Cibarial pump (Fig. 61D) with anterior margin concave, 148 μm long. Palpomere lengths 1–5 (in μm): 33; 44; 98; 104; 195. Antenna with 14 flagellomeres, AR 1.06, flagellum 590 μm long, diameter of pedicel 99 μm , apical setae single (Fig. 61E).



Figures 61A–M. *Labrundinia* sp. 13, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Thorax. Anteprenotum with 3 lateral setae. Acrostichals 40, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 26, irregularly biserial; prealars 9; supraalars 3. Anapleural suture ratio 0.44. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 61F). Width 0.30 mm. Costa 0.80 mm long, not produced beyond R₄₊₅, ending very slightly beyond tip of M₃₊₄. R₂₊₃ present. Base of radial sector 0.05 mm long. VR 0.76. WW 0.30. Brachiolium with 2 setae. Squama setiferous.

Legs (Figs 61G–K). Fore leg: width at apex of tibia 40 µm (Fig. 61G), tibia with single, apical and pectinate spur 16 µm long (Fig. 61H), with three teeth and two preapical setae; preapical pseudospurs unobserved. Mid leg: width at apex of tibia 36 µm long (Fig. 61I), tibia with single, apical and pectinate spur 18 µm long with three teeth (Fig. 61J) and two preapical setae; preapical pseudospurs unobserved. Hind leg: width at apex of tibia 39 µm long (Fig. 61K), tibia without spur; comb with 4 setae; preapical pseudospurs unobserved. Pulvilli absent. Lengths and proportion of leg segments as in Table XLII.

Table XLII. Lengths (in µm) and proportions of leg segments in *Labrundinia* sp. 13, adult male (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	417	439	–	–	–
p ₂	503	404	–	–	–
p ₃	446	543	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

Hypopygium (Fig. 61M). Tergite IX arched, with 10 dorsal setae. Anal point triangular, apical edge slightly rounded. Phallapodeme 54 µm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 97 µm long, 45 µm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.13. Gonostylus simple and slender, 58 µm long; megaseta 11 µm long. HR 1.67. HV 3.35.

Adult female, pupa, and larva. Unknown.

***Labrundinia* spec. nov. 14** (Fig. 62)

Material examined

Type: Holotype male, NICARAGUA: Masaya, Las Flores, iv.1993, C. Lecog & I. Cantamessa (ZSM).
Paratypes: 3 males same data as holotype.

Diagnostic characters

Labrundinia sp. 14 differs from other *Labrundinia* species by the combination of the following characters.

Male: fore leg with tibial comb present; abdominal tergite II, V with divided transverse band near proximal margin, T III–IV, with continuous transverse band near proximal margin, VI–VIII almost wholly brown; hypopygium pale brown, sternapodeme with moderate anterior process.

Description

Adult male (n = 4 unless otherwise stated)

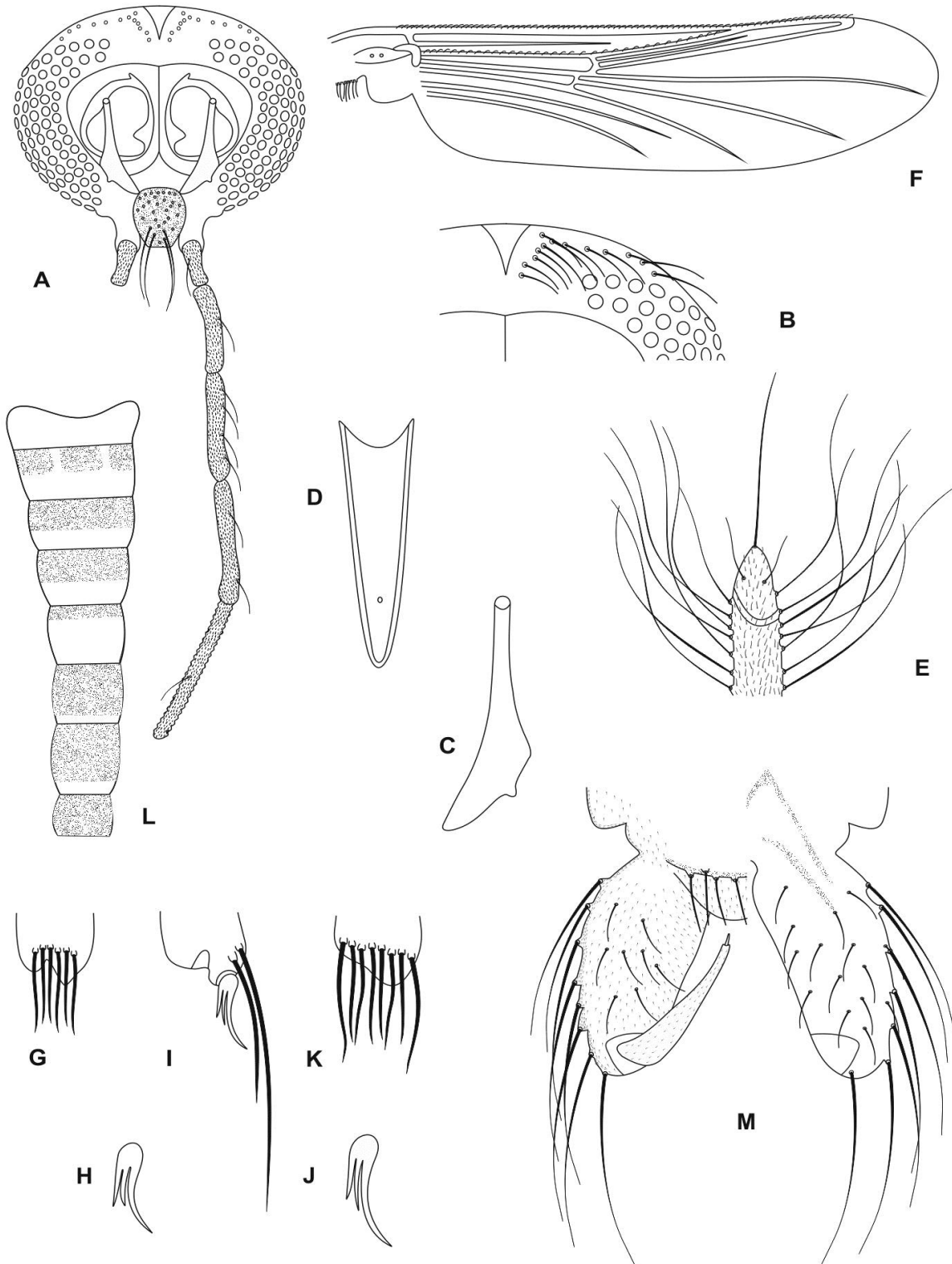
Size. Total length 2.42–2.79 mm. Wing length 1.25–1.31 mm. Total length/wing length 1.88–2.01. Wing length/length of profemur 2.50–2.63.

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally light brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Tibia I pale brown, Ti II–III apically brown. Abdomen as in figure 62L. Hypopygium pale brown.

Head (Figs 62A–E). Temporal setae 12–13, uniserial (Fig. 62B). Eye ratio 1.09–1.29 (3). Tentorium (Fig. 62C) 152–169 μm long, stipes not measurable. Clypeus 81–101 μm long, 60–78 μm wide at largest part, bearing 23–28 setae. Cibarial pump (Fig. 62D) with anterior margin concave, 189–205 μm long. Palpomere lengths 1–5 (in μm): 30–45 (3); 52–59 (3); 119–124 (3); 134–164 (3); 113–164 (3). Antenna with 14 flagellomeres, AR 1.15–1.19 (3), flagellum 693–797 μm long, diameter of pedicel 124–147 μm , apical setae single (Fig. 62E).

Thorax. Anteprenotum with 3–7 lateral setae. Acrostichals 64–66, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 36–44, irregularly biserial; prealars 9–12; supraalars 3. Anapleural suture ratio 0.37–0.50. Scutellum with 14 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 62F). Width 0.39–0.43 mm. Costa 1.01–1.11 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.05–0.07 mm long. VR 0.74–0.82. WW 0.30–0.33. Brachiolum with 2 setae. Squama setiferous.



Figures 62A–M. *Labrundinia* sp. 14, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Legs (Figs 62G–K). Fore leg: width at apex of tibia 38–42 μm (Fig. 62G), tibia with single, apical and pectinate spur 18–24 μm long (Fig. 62H), with three teeth and two preapical setae; comb with 6 setae; Ta₁₋₄ without preapical pseudospurs. Mid leg: width at apex of tibia 34–45 μm long (Fig. 62I), tibia with single, apical and pectinate spur 22–28 μm long with three teeth (Fig. 62J) and two preapical setae; Ta₁₋₄ each with two preapical pseudospurs. Hind leg: width at apex of tibia 37–44 μm long (Fig. 62K), tibia without spur; comb with 8 setae; Ta₁₋₄ each with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XLIII.

Table XLIII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 14, adult male (n = 3–4).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	482–524	462–508	334	243	150
p ₂	636–684	444–532	589–678	270–283	146–163
p ₃	566–603	643–745	572–671	282–292	186–220
	ta ₄	ta ₅	LR	BV	SV
p ₁	89	78	0.66	2.42	3.06
p ₂	102–115	78–84	1.19–1.37	2.75–2.97	1.76–1.96
p ₃	123–131	83–103	0.87–0.96	2.60–2.76	1.89–2.14

Hypopygium (Fig. 62M). Tergite IX arched, with 11–13 dorsal setae. Anal point rounded, apical edge convex. Phallapodeme 72–79 μm long. Sternapodeme with moderate anterior process. Gonocoxite cylindrical, 141–142 (2) μm long, 64–73 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.22–2.26 (2). Gonostylus simple and slender, 94–98 μm long; megaseta 16 μm long. HR 1.43–1.51. HV 2.54–2.79.

Adult female, pupa, and larva. Unknown.

***Labrundinia* spec. nov. 15** (Figs 63–65)

Material examined

Type: Holotype male with pupal and larval exuviae, **Brazil: São Paulo**, São Carlos, Monjolinho stream, 20.iv.2011, F. L. Silva (MZUSP). Paratypes: 1 male with pupal and larval exuviae same data as holotype. 2 females with pupal and larval exuviae same data as holotype except for 11.iv.2011. 3 larvae same data as holotype except for 11.iv.2011.

Diagnostic characters

Labrundinia sp. 15 differs from other *Labrundinia* species by the combination of the following characters.

Male: R₂₊₃ present; abdominal tergite I pale brown, T II–VI with continuous brown transverse band near proximal margin, T VII–VIII wholly brown; hypopygium brown, sternapodeme with reduced anterior process; anal point rounded, apical edge convex. **Pupa:** thoracic horn club-shaped, preapical indentation moderate deep; preapical papilla ratio 0.13–0.19; genital sac elongated, reaching much beyond apex of anal lobe. **Larva:** surface covered with small spinules, with small, posteroventral dark brown spot, lateroventral spine group present but weakly developed, with 5–6 spines, posteroventral spine group absent; subbasal seta simple; bifid claw with V-shaped lower indentation.

Description

Adult male (n = 2 unless otherwise stated)

Size. Total length 1.82–2.10 mm. Wing length 1.05–1.20 mm. Total length/wing length 1.74. Wing length/length of profemur 2.89–3.13.

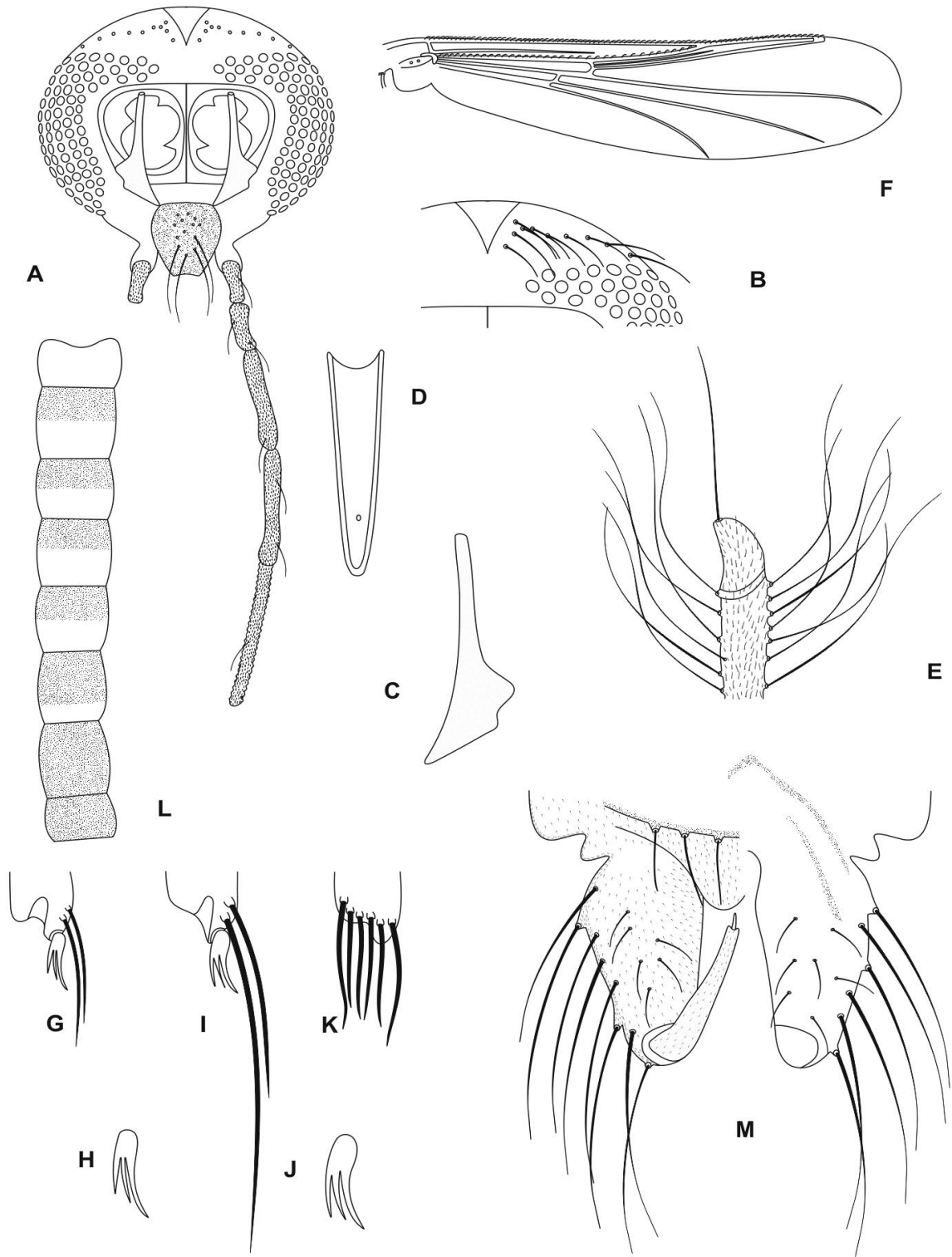
Coloration. Head brown with dark brown occipital margin; pedicel, antenna and maxillary palp brown. Thorax brown with mesonotum dorsally darker; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 63L. Hypopygium pale brown.

Head (Figs 63A–E). Temporal setae 12, uniserial (Fig. 63B). Eye ratio 0.81–0.85. Tentorium (Fig. 63C) 139 (1) μm long, stipes not measurable. Clypeus 78–85 μm long, 50–59 μm wide at largest part, bearing 13 setae. Cibarial pump (Fig. 63D) with anterior margin concave, 174–155 μm long. Palpomere lengths 1–5 (in μm): 24–26; 39–46; 68–98; 90 (1); 153 (1). Antenna with 14 flagellomeres, AR 1.21 (1), flagellum 668 μm long, diameter of pedicel 102–119 μm , apical setae single (Fig. 63E).

Thorax. Anteprenotum with 4 lateral setae. Acrostichals 34–40, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20, irregularly biserial; prealars 10–11; supraalars 2. Anapleural suture ratio 0.46. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 63F). Width 0.29–0.33 mm. Costa 0.89–1.03 mm long, not produced beyond R₄₊₅, ending very slightly beyond tip of M₃₊₄. R₂₊₃ present. Base of radial sector 0.07–0.09 mm long. VR 0.68–0.75. WW 0.27–0.28. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 63G–K). Fore leg: width at apex of tibia 29–31 μm (Fig. 63G), tibia with single, apical and



Figures 63A–M. *Labrundinia* sp. 15, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

pectinate spur 10–11 μm long (Fig. 63H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 29–33 μm long (Fig. 63I), tibia with single, apical and pectinate spur 17–20 μm long with three teeth (Fig. 63J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 32 μm long (Fig. 63K), tibia without spur; comb with 6 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XLIV.

Table XLIV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 15, adult male ($n = 1-2$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	335–418	403–466	281	192	136
p ₂	446–550	402–475	573	241	123
p ₃	403–488	523–609	547	238	149
	ta ₄	ta ₅	LR	BV	SV
p ₁	101	67	0.60	2.35	3.15
p ₂	89	76	1.20	3.02	1.79
p ₃	110	71	0.90	2.90	2.00

Hypopygium (Fig. 63M). Tergite IX arched, with 8 dorsal setae. Anal point rounded, apical edge convex. Phallopodeme 46–49 μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 84–100 μm long, 38–46 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.16–2.19. Gonostylus simple and slender, 56–70 μm long; megaseta 11–12 μm long. HR 1.45–1.50. HV 3.03–3.33.

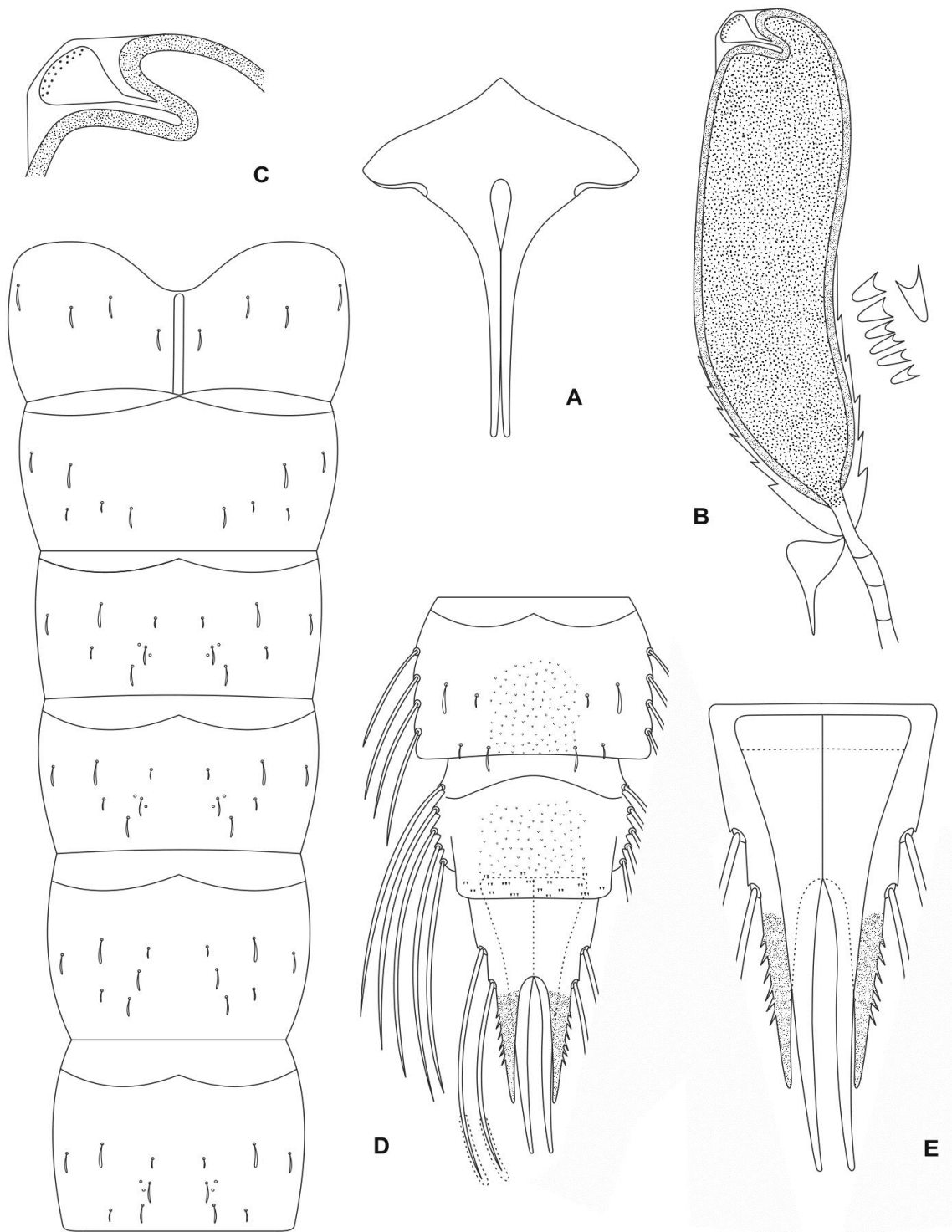
Adult female ($n = 2$ unless otherwise stated)

Size. Total length 1.82–2.01 mm. Wing length 0.83–0.87 mm. Total length/wing length 1.74. Wing length/length of profemur 2.74.

Coloration. Head, pedicel, antenna and maxillary palp brown. Thorax pale brown with mesonotum dorsally darker; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen wholly brown. Seminal capsules brown.

Head. Temporal setae 10–12, uniserial. Eyes ratio 0.97 (1). Tentorium 121–124 μm long, stipes not measurable. Clypeus 69–75 μm long, 53–62 μm wide at largest part, bearing 15–18 setae. Cibarial pump as in male, 159–164 μm long. Palpomere lengths 1–5 (in μm): 30–39; 54–56; 70–84; 100 (1); 193 (1). Antenna with 11 flagellomeres, AR 0.38–0.39, flagellum 331–319 μm long, diameter of pedicel 52–53 μm .

Thorax. Anteprenotum with 4 lateral setae. Acrostichals 36–42, irregularly biserial, starting close to



Figures 64A–E. *Labrundinia* sp. 15, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

antepronotum; dorsocentrals 20–22, irregularly biserial; prealars 9; supraalars 2. Scutellum with 10–11 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.32 mm. Costa 0.72–0.74 mm long, not produced beyond R_{4+5} . R_{2+3} present. Base of radial sector 0.05 mm long. VR 0.64–0.66. WW 0.37–0.38. Brachiolum with 2 setae (2). Squama setiferous.

Legs. Fore leg: width at apex of tibia 30 (1) μm , tibia with single, apical and pectinate spur 16 μm long, with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Mid leg: width at apex of tibia 32–34 μm , tibia with single, apical and pectinate spur 17–19 μm long, with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Hind leg: width at apex of tibia 31–36 μm , tibia without spur; comb with 6 setae; preapical pseudospurs on Ta_{1-4} unobserved. All legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XLV.

Table XLV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 15, adult female (n = 1–2).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	318	293	–	–	–
p ₂	442–444	365–384	–	–	–
p ₃	395–397	442–468	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

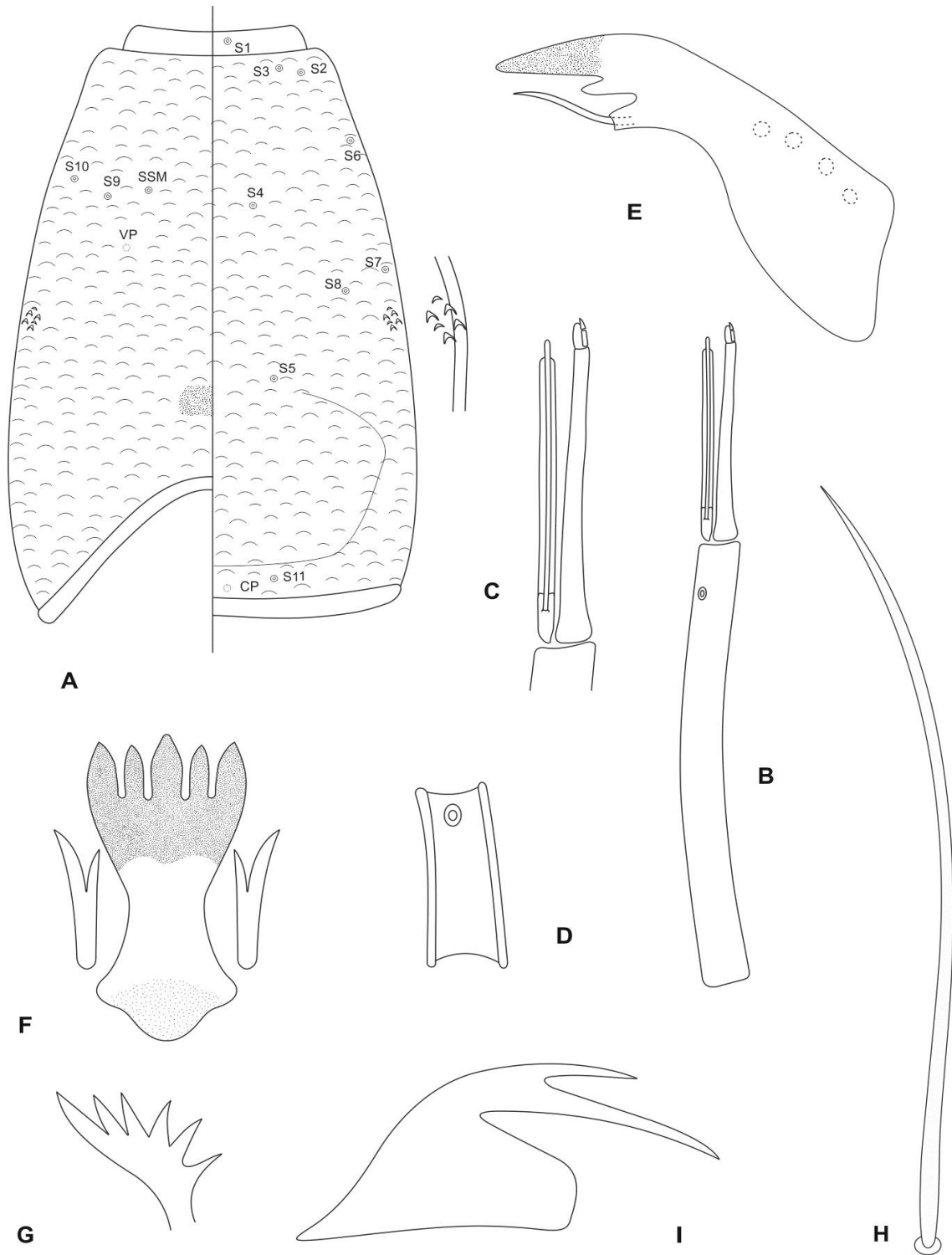
Genitalia. Gonapophysis VIII broadly rounded, 60–73 μm long. Coxosternapodeme 50–60 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 43–51 μm long and 14–15 μm wide; with 5 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 78–81 μm . Seminal capsules oval with conical shaped necks, length 34–38 μm , maximum width 34–36 μm . Length ratio SCa/No 0.44–0.47.

Pupa (n = 4 unless otherwise stated)

Size. Male abdomen 1.63–1.76 (2) mm long; female abdomen 1.53–1.60 (2) mm long.

Coloration. Exuviae pale brown; thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 64A–C). Frontal apotome as in figure 64A. Wing sheath smooth 0.62–0.79 mm long. Thoracic horn 184–206 μm long and 48–59 μm wide (Fig. 64B), preapical indentation moderate deep, THR 3.27–3.63. Membranous preapical papilla 22–39 μm long (Fig. 64C), PTH 0.13–0.19, aeropyle



Figures 65A–I. *Labrundinia* sp. 15, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

tube simple, short and robust, 14–21 μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 11 conical teeth. *Abdomen* (Figs 64D–E). Tergite I with scar, 104–137 μm long. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 64D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 193–258 μm long and 115–134 μm wide (Fig. 64E), outer margins sclerotized, with 5–9 spines, longest spine 8–12 μm long, inner margins of lobes membranous. ALR 1.67–1.93. Genital sac elongated, reaching much beyond apex of anal lobe.

4th instar larva ($n = 7$ unless otherwise stated)

Coloration. Head pale yellow, with small, posteroventral dark brown spot; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 65A). Length 416–456 μm , 282–304 μm wide. Surface covered with small spinules; lateroventral spine group present but weakly developed, with 5–6 spines; posteroventral spine group absent; cephalic index 0.61–0.69. Chaetotaxy as in figure 65A.

Antenna (Figs 65B–C). Length 235–248 μm , A_1 156–167 μm long, with ring organ placed 137–148 μm from base, A_2 66–76 μm long. AR 1.76–2.15. Blade longer than A_2 over-reached by accessory blade.

Maxilla (Fig. 65D). Basal palp segment 23–39 (6) μm long and 7–10 (6) μm wide, with ring organ 18–27 (6) μm from base. PR 2.64–3.76 (6). APR 4.45–7.35 (6).

Mandible (Fig. 65E). Length 55–68 (6) μm , with 3 lateral setae. Sensillum campaniformium 42–57 (6) μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.30–2.93.

Mentum and M appendage. Dorsomentary teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 65F–G). Ligula 49–62 μm long, 22–24 μm wide, with row of 5 teeth. IO 0.96–0.99, MO 1.03–1.09. Paraligula bifid, 23–30 μm long, inner tooth 17–22 μm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 65H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 113–132 (6) μm long, 26–32 μm wide, with 7 anal setae 329–372 (2) μm long. L/W 3.57–4.94. Supraanal seta well developed. Anal tubules 377 (1) μm long. Posterior parapod 259–305 (5) μm long; subbasal seta simple, basal spines reduced, without spines (Fig. 65H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 65I). B/C 1.10–1.15 (5).

Labrundinia spec. nov. 16 (Figs 66–68)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva (MZUSP). *Paratypes:* 2 larvae same data as holotype except for vii.1998, S. T. Strixino

Diagnostic characters

Labrundinia sp. 16 differs from other *Labrundinia* species by the combination of the following characters. **Male:** wing length 1.15 mm; R_{2+3} absent; abdominal tergite II–VI with continuous brown transverse band near proximal margin, T VII–VIII wholly brown; hypopygium brown, sternapodeme with distinct anterior process. **Pupa:** thoracic horn semi globose; abdominal segment VII with 2 lateral setae; genital sac not reaching apex of the anal lobe. **Larva:** surface covered with small spinules; lateroventral and posteroventral spine groups absent; subbasal seta multitoothed; serrated claw present; bifid claw with V-shaped lower indentation.

Description

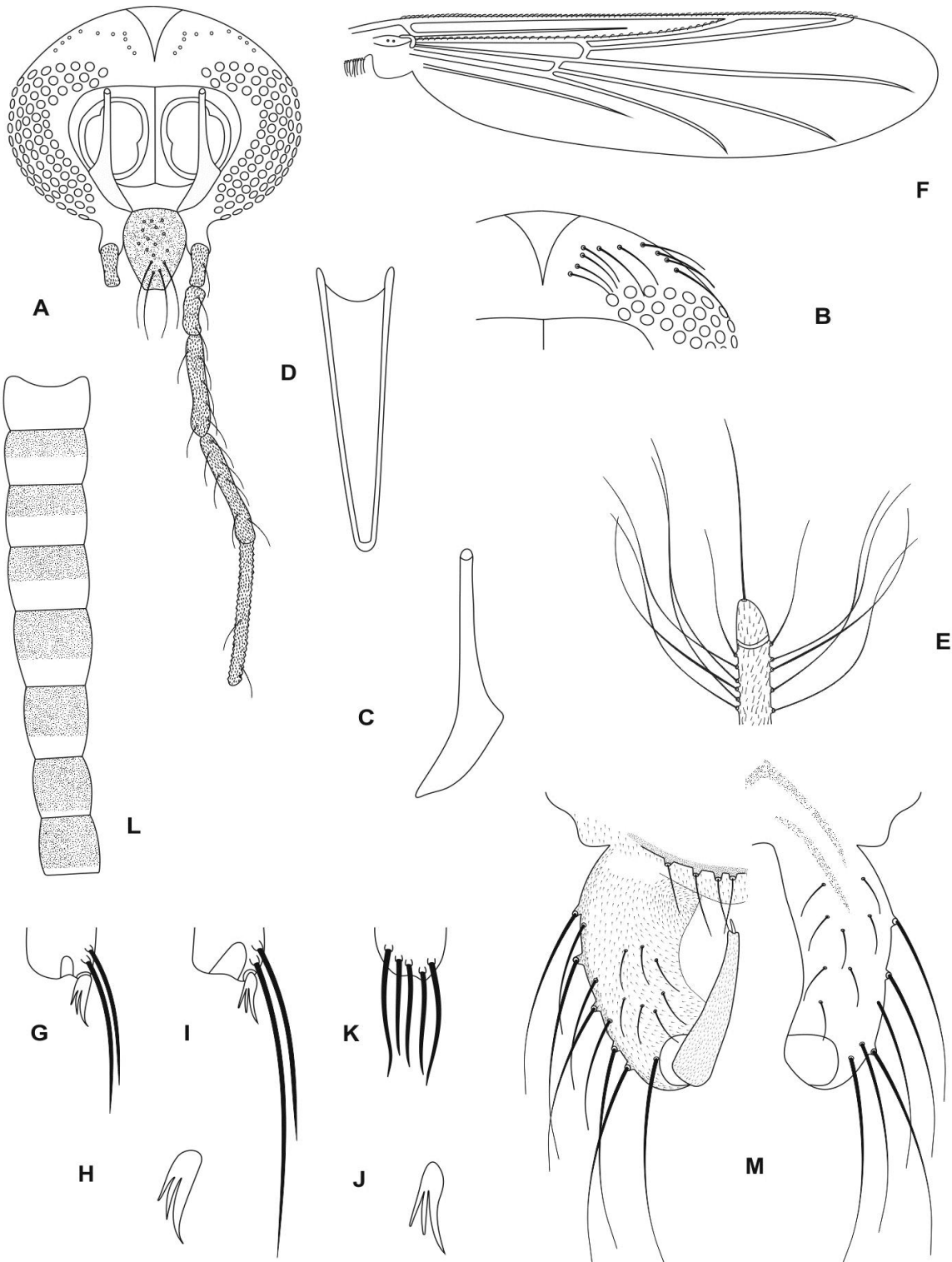
Adult male ($n = 1$)

Size. Total length 2.13 mm. Wing length 1.15 mm. Total length/wing length 1.91. Wing length/length of profemur 2.99.

Coloration. Head brown with dark brown occipital margin; pedicel, antenna and maxillary palp brown. Thorax brown with mesonotum dorsally darker; anteprenotum brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Abdomen as in figure 66L. Hypopygium brown.

Head (Figs 66A–E). Temporal setae 10, uniserial (Fig. 66B). Eye ratio 0.88. Tentorium (Fig. 66C) 142 μ m long, stipes not measurable. Clypeus 103 μ m long, 57 μ m wide at largest part, bearing 14 setae. Cibarial pump (Fig. 66D) with anterior margin concave, 173 μ m long. Palpomere lengths 1–2 (in μ m): 33; 53. Antenna with 14 flagellomeres, flagellum with apical setae single, unmeasured (Fig. 66E).

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 46, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 22, irregularly biserial; prealars 9;



Figures 66A–M. *Labrundinia* sp. 16, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

supraalars 2. Anapleural suture ratio 0.44. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 66F). Width 0.32 mm. Costa 0.94 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.07 mm long. VR 0.77. WW 0.28. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 66G–K). Fore leg: width at apex of tibia 33 μm (Fig. 66G), tibia with single, apical and pectinate spur 14 μm long (Fig. 66H), with three teeth and two preapical setae; Ta_{1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 30 μm long (Fig. 66I), tibia with single, apical and pectinate spur 15 μm long with three teeth (Fig. 66J) and two preapical setae; Ta_{1-4} without preapical pseudospurs. Hind leg: width at apex of tibia 31 μm long (Fig. 66K), tibia without spur; comb with 5 setae; Ta_{1-4} each with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XLVI.

Table XLVI. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 16, adult male ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	374	406	299	188	136
p ₂	450	415	–	–	–
p ₃	422	502	480	234	160
	ta ₄	ta ₅	LR	BV	SV
p ₁	90	66	0.74	2.24	2.60
p ₂	–	–	–	–	–
p ₃	104	71	0.96	2.47	1.93

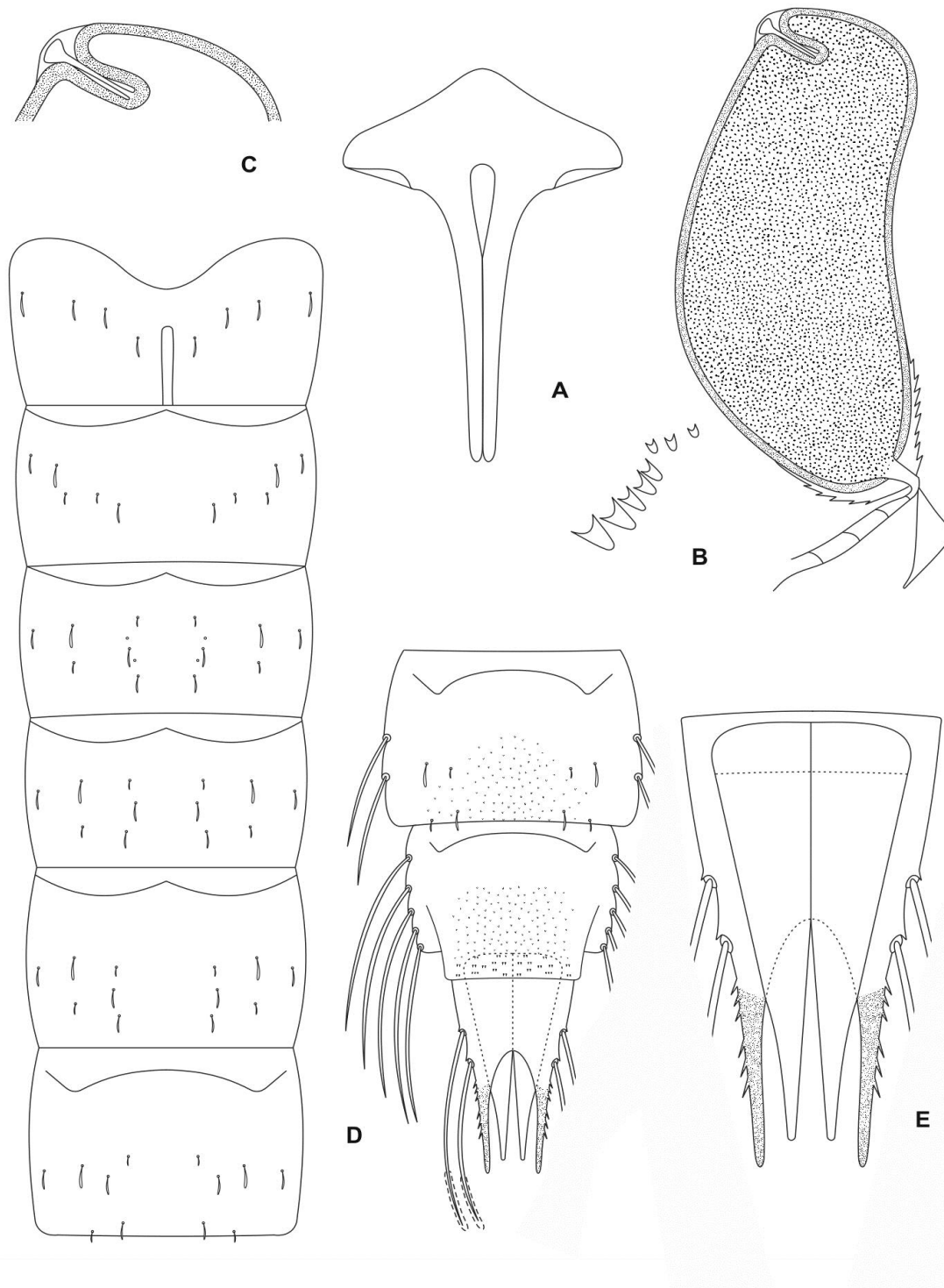
Hypopygium (Fig. 66M). Tergite IX arched, with 12 dorsal setae. Anal point rounded, apical edge convex. Phallapodeme 50 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 106 μm long, 51 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.07. Gonostylus simple and slender, 66 μm long; megaseta 15 μm long. HR 1.61. HV 3.21.

Pupa ($n = 1$)

Size. Male abdomen 1.86 mm long.

Coloration. Exuviae and thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 67A–C). Frontal apotome as in figure 67A. Wing sheath smooth 0.79 mm long. Thoracic horn 220 μm long and 94 μm wide (Fig. 67B), preapical indentation moderate deep, THR 2.34. Membranous preapical papilla 24 μm long (Fig. 67C), PTH 0.11, aeropyle tube simple, short and robust,



Figures 67A–E. *Labrundinia* sp. 16, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

20 µm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 9 conical teeth.

Abdomen (Figs 67D–E). Tergite I with scar, unmeasured. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 67D. Abdominal segment VII with 2 lateral setae. A VIII with 5 lateral setae. Anal lobe 268 µm long and 132 µm wide (Fig. 67E), outer margins sclerotized, with 7 spines, longest spine 17 µm long, inner margins of lobes membranous. ALR 2.02. Genital sac elongated, almost reaching apex of anal lobe.

4th instar larva ($n = 3$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 68A). Length 438–496 µm, 256–301 µm wide. Surface covered with small spinules; lateroventral and posteroventral spine groups absent; cephalic index 0.59–0.61. Chaetotaxy as in figure 68A.

Antenna (Figs 68B–C). Length 302 (1) µm, A₁ 194–201 µm long, with ring organ placed 169–185 (2) µm from base, A₂ 95–101 µm long. AR 1.99–2.04. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 68D). Basal palp segment 23–25 µm long and 8 µm wide, with ring organ 18–20 µm from base. PR 2.96–3.08. APR 8.10–8.64.

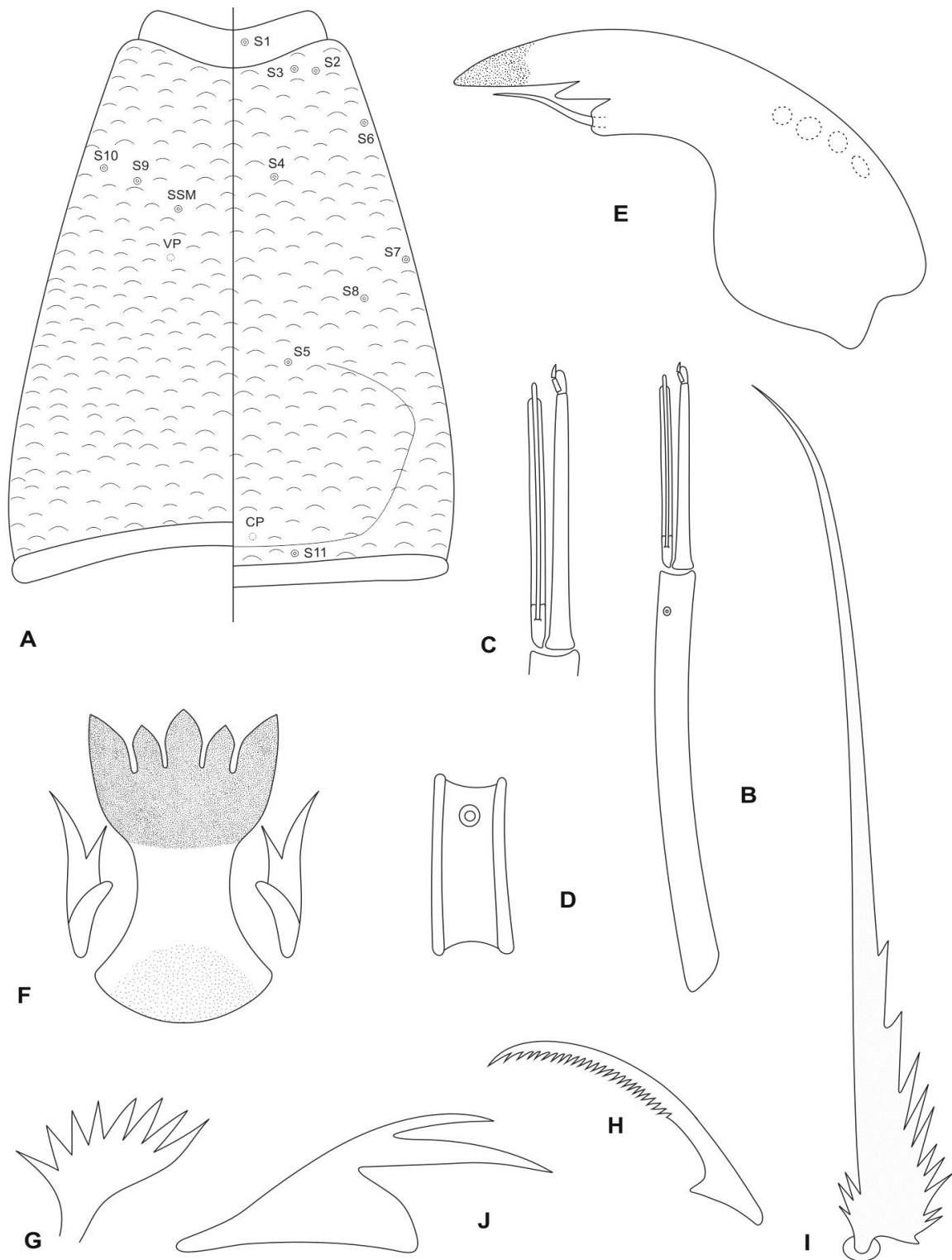
Mandible (Fig. 68E). Length 60–65 µm, with 3 lateral setae. Sensillum campaniformium 37–45 µm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 3.11–3.30.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 68F–G). Ligula 46–53 µm long, 23–26 µm wide, with row of 5 teeth. IO 0.97–0.99, MO 1.04–1.08. Paraligula bifid, 25–28 µm long, inner tooth 20–23 µm long, shorter than outer tooth. Pecten hypopharyngis with 8 teeth almost equal in size.

Body (Figs 68H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 128–140 µm long, 25–32 µm wide, with 7 anal setae 369–454 (2) µm long. L/W 4.04–5.44. Supraanal seta well developed. Anal tubules 194 (1) µm long. Posterior parapod 200–268 µm long; subbasal seta multitoothed, with basal spine group, with 12–13 spines (Fig. 68H); parapod apex with numerous simple claws, with serrated claw; bifid claw with V-shaped lower indentation (Fig. 68I). B/C 1.04–1.06 (2).

Remarks: This is the *Labrundinia* sp. 2 of Trivinho-Strixino (2011) (larvae identification key).



Figures 68A–J. *Labrundinia* sp. 16, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Serrated claw of posterior parapod. **I.** Subbasal seta of posterior parapod. **J.** Bifid claw of posterior parapod.

***Labrundinia* spec. nov. 17** (Fig. 69)

Material examined

Type: Holotype male, Nicaragua: **Solentiname Islands**, San Juan river, 30.vii.1989, C. Lecog & I. Cantamessa (ZSM).

Diagnostic characters

Labrundinia sp. 17 differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite I–II, IV pale, T III, V wholly brown, VI–VIII almost wholly brown; hypopygium pale brown, sternapodeme with distinct anterior process.

Description

Adult male ($n = 1$)

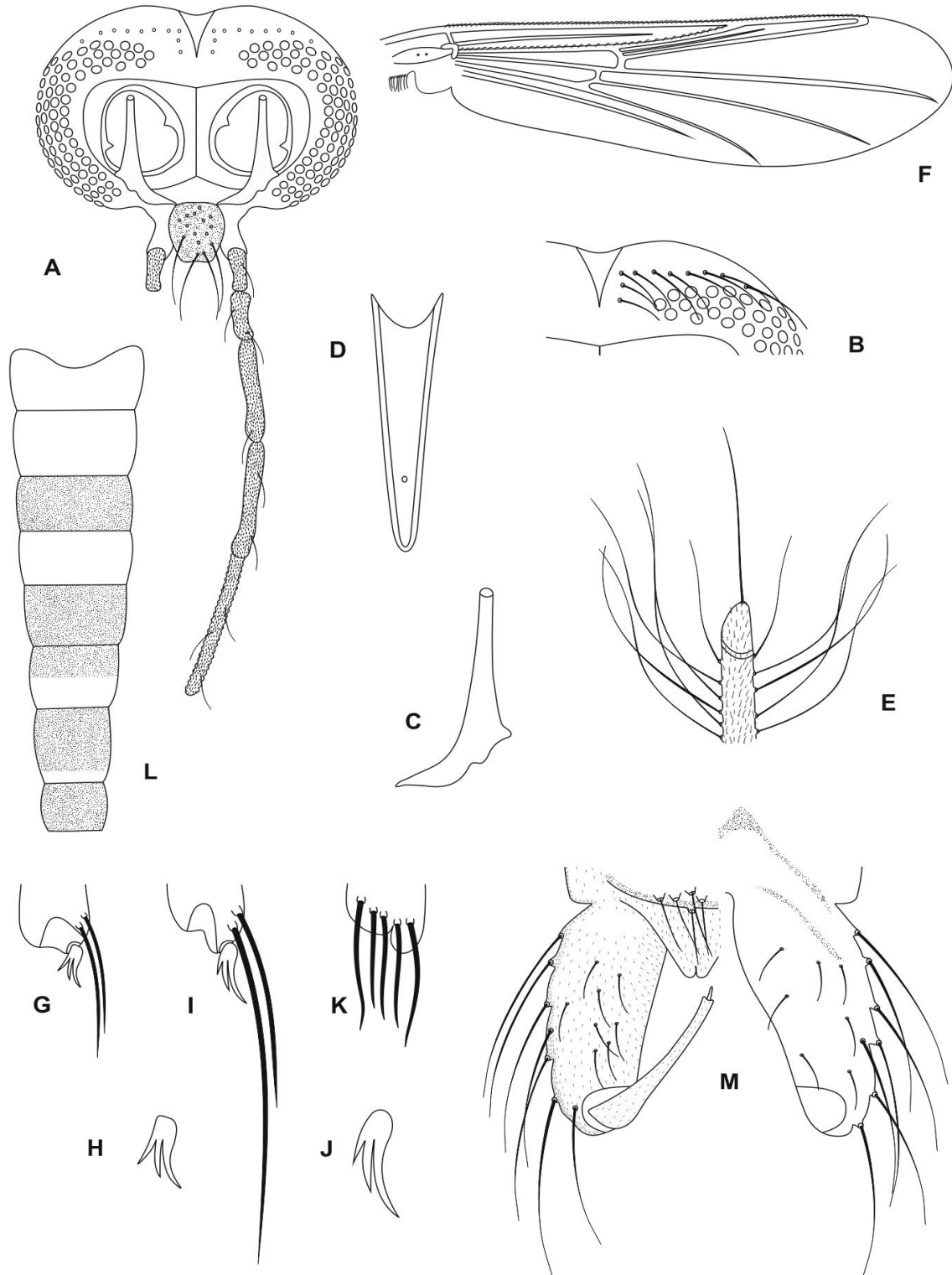
Size. Total length 2.10 mm. Wing length 1.10 mm. Total length/wing length 1.93. Wing length/length of profemur 2.64.

Coloration. Head pale brown with dark brown occipital margin; pedicel, antenna and maxillary palp brown. Thorax wholly brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Abdomen as in figure 69L. Hypopygium pale brown.

Head (Figs 69A–E). Temporal setae 10, uniserial (Fig. 69B). Eye ratio 1.17. Tentorium (Fig. 69C) 136 μm long, stipes not measurable. Clypeus 95 μm long, 61 μm wide at largest part, bearing 14 setae. Cibarial pump (Fig. 69D) with anterior margin concave, 166 μm long. Palpomere lengths 1–3 (in μm): 37; 51; 115. Antenna with 14 flagellomeres, AR 1.00, flagellum 662 μm long, diameter of pedicel 109 μm , apical setae single (Fig. 69E).

Thorax. Antepnotum lateral setae unobserved. Acrostichals 38, biserial, diverging evenly posteriorly, starting close to antepnotum and almost reaching scutellum; dorsocentrals 30, irregularly biserial; prealars 13; supraalars 2. Anapleural suture ratio 0.42. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 69F). Width 0.34 mm. Costa 0.91 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.05 mm long. VR 0.75. WW 0.32. Brachiolum with 2 setae. Squama setiferous.



Figures 69A–M. *Labrundinia* sp. 17, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Legs (Figs 69G–K). Fore leg: width at apex of tibia 30 μm (Fig. 69G), tibia with single, apical and pectinate spur 16 μm long (Fig. 69H), with three teeth and two preapical setae; preapical pseudospurs unobserved.. Mid leg: width at apex of tibia 36 μm long (Fig. 69I), tibia with single, apical and pectinate spur 24 μm long with three teeth (Fig. 69J) and two preapical setae; preapical pseudospurs unobserved. Hind leg: width at apex of tibia 42 μm long (Fig. 69K), tibia without spur; comb with 5 setae; preapical pseudospurs unobserved. Lengths and proportion of leg segments as in Table XLVII.

Table XLVII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 17, adult male (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	409	476	–	–	–
p ₂	552	463	–	–	–
p ₃	502	645	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

Hypopygium (Fig. 69M). Tergite IX arched, with 12 dorsal setae. Anal point triangular, apical edge very notched. Phallapodeme 118 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 120 μm long, 46 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.60. Gonostylus simple and slender, 81 μm long; megaseta 12 μm long. HR 1.49. HV 2.58.

Adult female, pupa, and larva. Unknown.

***Labrundinia* spec. nov. 18** (Fig. 70)

Material examined

Type: Holotype male, BRAZIL: **São Paulo**, São Carlos, Monjolinho stream, associated with *Mysiophylum aquaticum*, 12.iii.2008, F. L. Silva (MZUSP). Paratype: 1 male same data as holotype.

Diagnostic characters

Labrundinia sp. 18 differs from other *Labrundinia* species by the combination of the following characters.

Male: wing length 1.32–1.45 mm; R₂₊₃ absent; abdominal tergite II–VI with continuous brown transverse

band near proximal margin, T VII–VIII wholly brown; hypopygium brown, sternapodeme with distinct anterior process.

Description

Adult male (n = 2 unless otherwise stated)

Size. Total length 2.48–2.56 mm. Wing length 1.32–1.45 mm. Total length/wing length 1.76–1.88. Wing length/length of profemur 2.93–3.18.

Coloration. Head pale brown with dark brown occipital margin; pedicel, antenna and maxillary palp pale brown. Thorax wholly brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Abdomen as in figure 70L. Hypopygium brown.

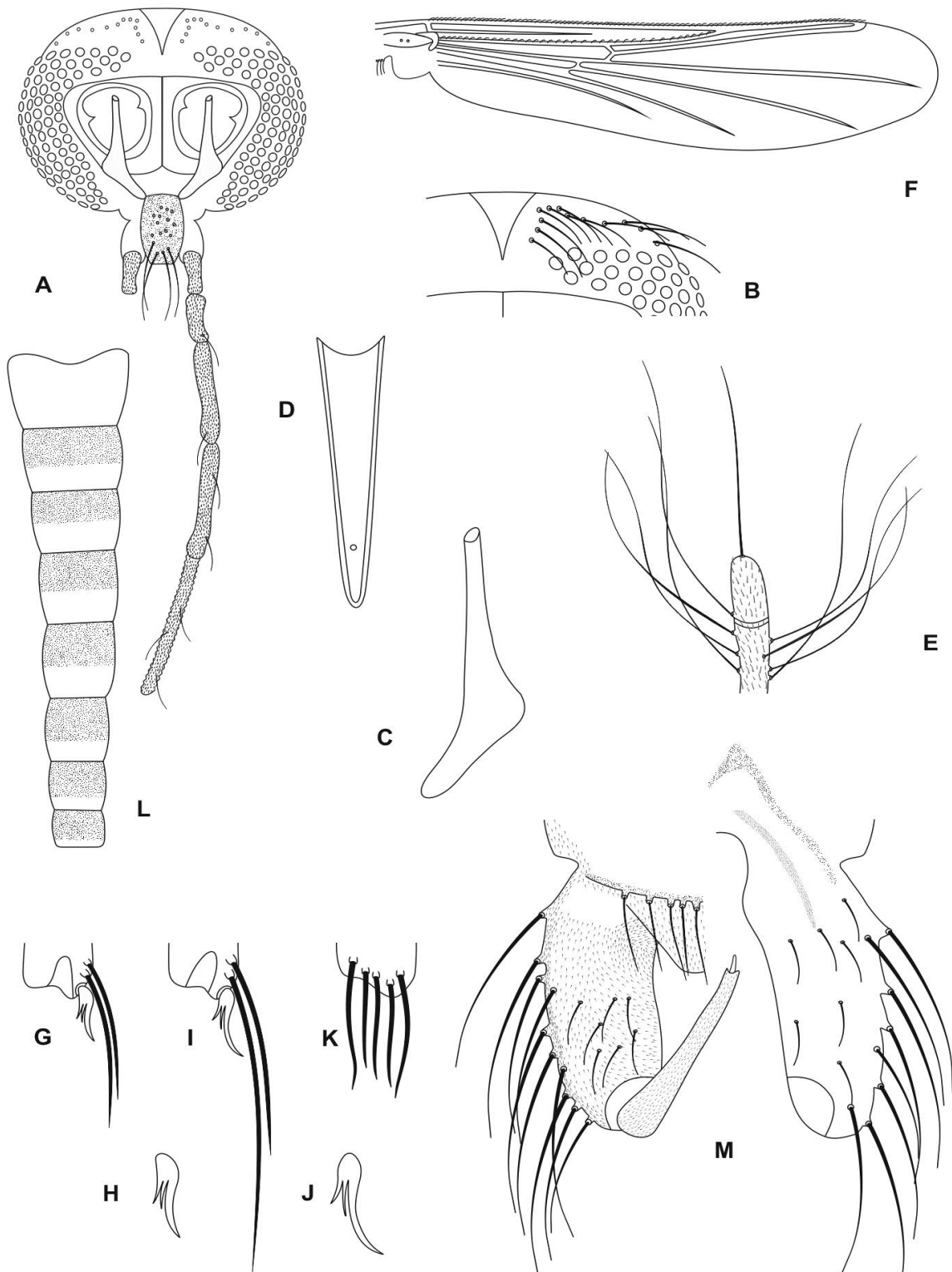
Head (Figs 70A–E). Temporal setae 14, uniserial (Fig. 70B). Eye ratio 0.71–0.74. Tentorium (Fig. 70C) 162–181 μm long, stipes not measurable. Clypeus 106 μm long, 70 μm wide at largest part, bearing 21 setae. Cibarial pump (Fig. 70D) with anterior margin concave, 204 μm long. Palpomere lengths 1–4 (in μm): 25 (1); 55 (1); 117 (1); 143 (1). Antenna with 14 flagellomeres, AR 1.22 (1), flagellum 738 (1) μm long, diameter of pedicel 117 (1) μm , apical setae single (Fig. 70E).

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 44–48, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 22–24, irregularly biserial; prealars 9; supraalars 2. Anapleural suture ratio 0.48–0.55. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 70F). Width 0.34–0.39 mm. Costa 1.20–1.26 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.10–0.13 mm long. VR 0.74–0.76. WW 0.26–0.27. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 70G–K). Fore leg: width at apex of tibia 34–48 μm (Fig. 70G), tibia with single, apical and pectinate spur 19–22 μm long (Fig. 70H), with three teeth and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Mid leg: width at apex of tibia 30–36 μm long (Fig. 70I), tibia with single, apical and pectinate spur 22–34 μm long with three teeth (Fig. 70J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 34–45 μm long (Fig. 70K), tibia without spur; comb with 5 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XLVIII.

Hypopygium (Fig. 70M). Tergite IX arched, with 12 dorsal setae. Anal point broad, apical edge convex. Phallapodeme 45–46 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 111–112 μm long, 48–51 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.28–



Figures 70A–M. *Labrundinia* sp. 18, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

2.34. Gonostylus simple and slender, 69 µm long; megaseta 12 µm long. HR 1.60–1.61. HV 3.58–3.68.

Table XLVIII. Lengths (in µm) and proportions of leg segments in *Labrundinia* sp. 18, adult male (n = 2).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	451–456	488–522	410–448	222–256	166–198
p ₂	501–560	669–682	672–717	264–304	171–200
p ₃	474–515	637–682	677–715	280–294	191–205
	ta ₄	ta ₅	LR	BV	SV
p ₁	128–143	76–77	0.84–0.86	2.12–2.27	2.93–3.18
p ₂	109–130	81–91	1.00–1.05	2.65–2.99	1.65–1.83
p ₃	131–138	74–88	1.05–1.06	2.62–2.66	1.64–1.67

Adult female, pupa, and larva. Unknown.

***Labrundinia* spec. nov. 19** (Fig. 71)

Material examined

Type: Holotype male, COSTA RICA: **Puntarenas**, Osa, Riyito river, i.1991, W. Riß (ZSM). Paratype: 2 male same data as holotype.

Diagnostic characters

Labrundinia sp. 19 differs from other *Labrundinia* species by the combination of the following characters.

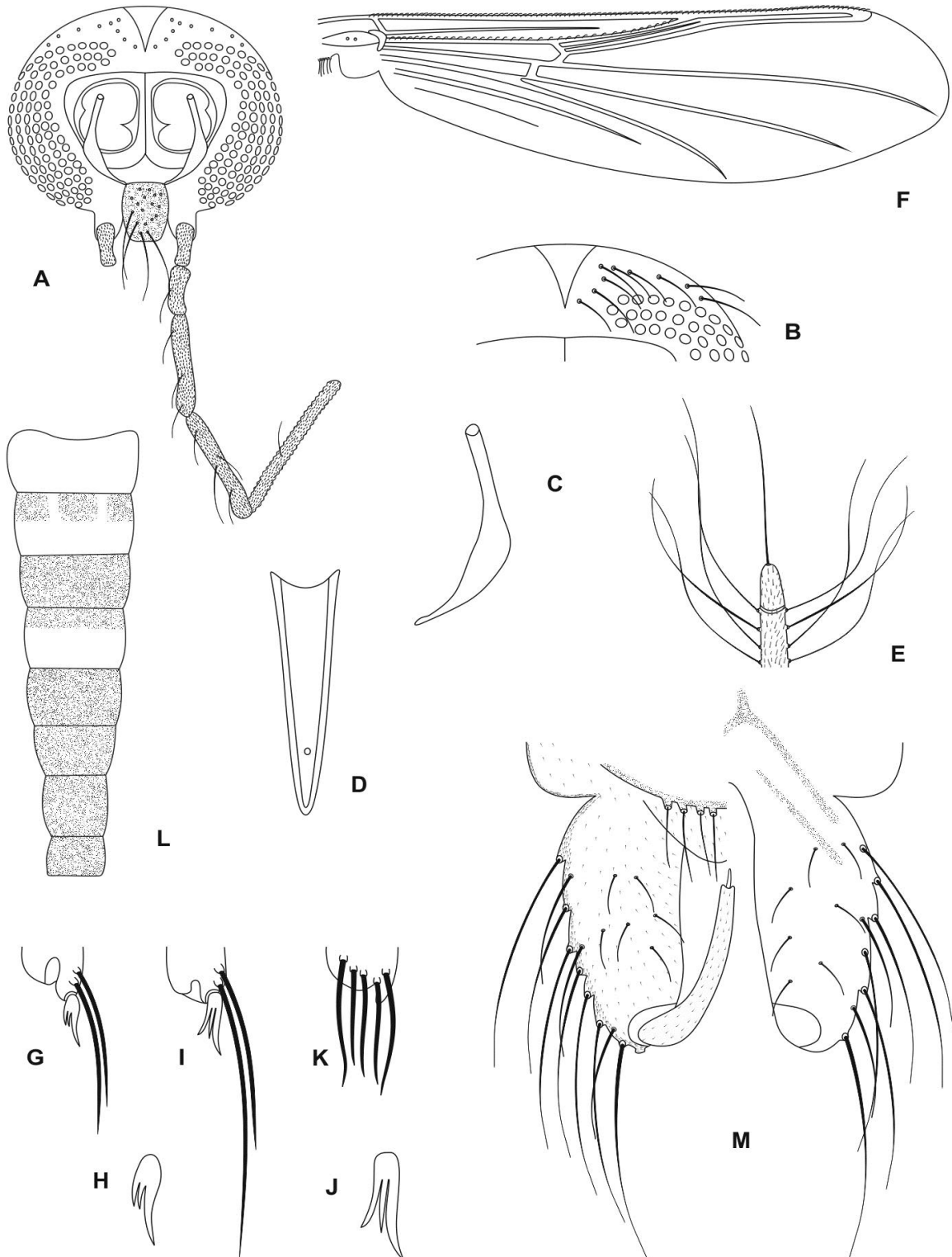
Male: abdominal tergite I pale, T II, IV with divided brown transverse band near proximal margin, T III, V–VIII wholly brown; hypopygium pale, sternapodeme with distinct anterior process.

Description

Adult male (n = 3 unless otherwise stated)

Size. Total length 1.86–1.95 mm. Wing length 0.93–1.01 mm. Total length/wing length 1.77–2.01. Wing length/length of profemur 2.58–2.87.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp



Figures 71A–M. *Labrundinia* sp. 19, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Femur I pale brown with apex brown, Fe II–III brown. Tibia I with apex brown, Ti II–III brown. Tarsomeres 1–5 brown. Abdomen as in figure 71L. Hypopygium pale.

Head (Figs 71–E). Temporal setae 10, uniserial (Fig. 71B). Eye ratio 0.90–0.97 (2). Tentorium (Fig. 71C) 118(1) μm long, stipes not measurable. Clypeus 73–83 μm long, 62–77 μm wide at largest part, bearing 15–17 setae. Cibarial pump (Fig. 71D) with anterior margin concave, 154–165 μm long. Palpomere lengths 1–5 (in μm): 32–36 (2); 47–50 (2); 88–116 (2); 117–126 (2); 199 (1). Antenna with 14 flagellomeres, AR 0.78–0.85, flagellum 585–608 μm long, diameter of pedicel 99–101 μm , apical setae single (Fig. 71E).

Thorax. Anteprenotum with 3 lateral setae. Acrostichals 44–46, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20–22, irregularly biserial; prealars 9–11; supraalars 2. Anapleural suture ratio 0.35–0.45. Scutellum with 6–7 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 71F). Width 0.30–0.32 mm. Costa 0.82–0.91 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.05 mm long. VR 0.75–0.78. WW 0.28–0.32. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 71G–K). Fore leg: width at apex of tibia 30–32 μm (Fig. 71G), tibia with single, apical and pectinate spur 15–16 μm long (Fig. 71H), with three teeth and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Mid leg: width at apex of tibia 31–35 μm long (Fig. 71I), tibia with single, apical and pectinate spur 16–20 μm long with three teeth (Fig. 71J) and two preapical setae; Ta_{1-4} without preapical pseudospurs. Hind leg: width at apex of tibia 37–40 μm long (Fig. 71K), tibia without spur; comb with 5 setae; Ta_{1-4} without preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table XLIX.

Table XLIX. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 19, adult male (n = 2–3).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	359–399	369–444	227–256	167–191	103–113
p ₂	526–553	382–422	–	–	–
p ₃	434–488	531–582	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	78–93	55–57	0.59–0.61	2.35–2.36	3.14–3.21
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

Hypopygium (Fig. 71M). Tergite IX arched, with 10–11 (2) dorsal setae. Anal point rounded, apical edge convex. Phallapodeme 39–40 (2) μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 84–91 μm long, 38–43 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.10–2.21 (2). Gonostylus simple and slender, 65–68 μm long; megaseta 9–11 μm long. HR 1.25–1.35. HV 2.82–2.89.

Adult female, pupa, and larva. Unknown.

***Labrundinia spec. nov.* 20** (Figs 72–73)

Material examined

Type: Holotype male, Brazil: **São Paulo**, São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva (MZUSP). Paratypes: 1 pharate pupa with larval exuvia same data as holotype. 1 female with larval exuvia same data as holotype.

Diagnostic characters

Labrundinia sp. 20 differs from other *Labrundinia* species by the combination of the following characters. **Male:** wing length 1.21 mm; abdominal tergite I pale, T II, IV, VI with continuous brown transverse band near proximal margin, T III, VII–VIII wholly brown; hypopygium pale, sternapodeme with distinct anterior process. **Pupa:** thoracic horn club-shaped, preapical papilla ratio 0.34, plastron plate seated on a small tubercle. **Larva:** surface covered with small spinules, with ventral semicircular spot; lateroventral and posteroventral spine groups absent; subbasal seta simple; bifid claw with U-shaped lower indentation.

Description

Adult male ($n = 1$)

Size. Total length 2.21 mm. Wing length 1.21 mm. Total length/wing length 1.83. Wing length/length of profemur 2.49.

Coloration. Head dark brown to black with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax wholly dark brown; antepnotum pale; supraalar callus brown. Wing

membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in figure 72L. Hypopygium pale.

Head (Figs 72–E). Temporal setae 11, uniserial (Fig. 72B). Eye ratio 1.58. Tentorium (Fig. 72C) 144 μm long, stipes not measurable. Clypeus 76 μm long, 67 μm wide at largest part, bearing 17 setae. Cibarial pump (Fig. 72D) with anterior margin concave, 180 μm long. Palpomere lengths 1–5 (in μm): 35; 57; 115; 120; 204. Antenna with 14 flagellomeres, AR 1.08, flagellum 742 μm long, diameter of pedicel 123 μm , apical setae single (Fig. 72E).

Thorax. Anteprenotum lateral setae unobserved. Acrostichals 44, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20, irregularly biserial; prealars 8; supraalars 2. Anapleural suture ratio 0.31. Scutellum with 7 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

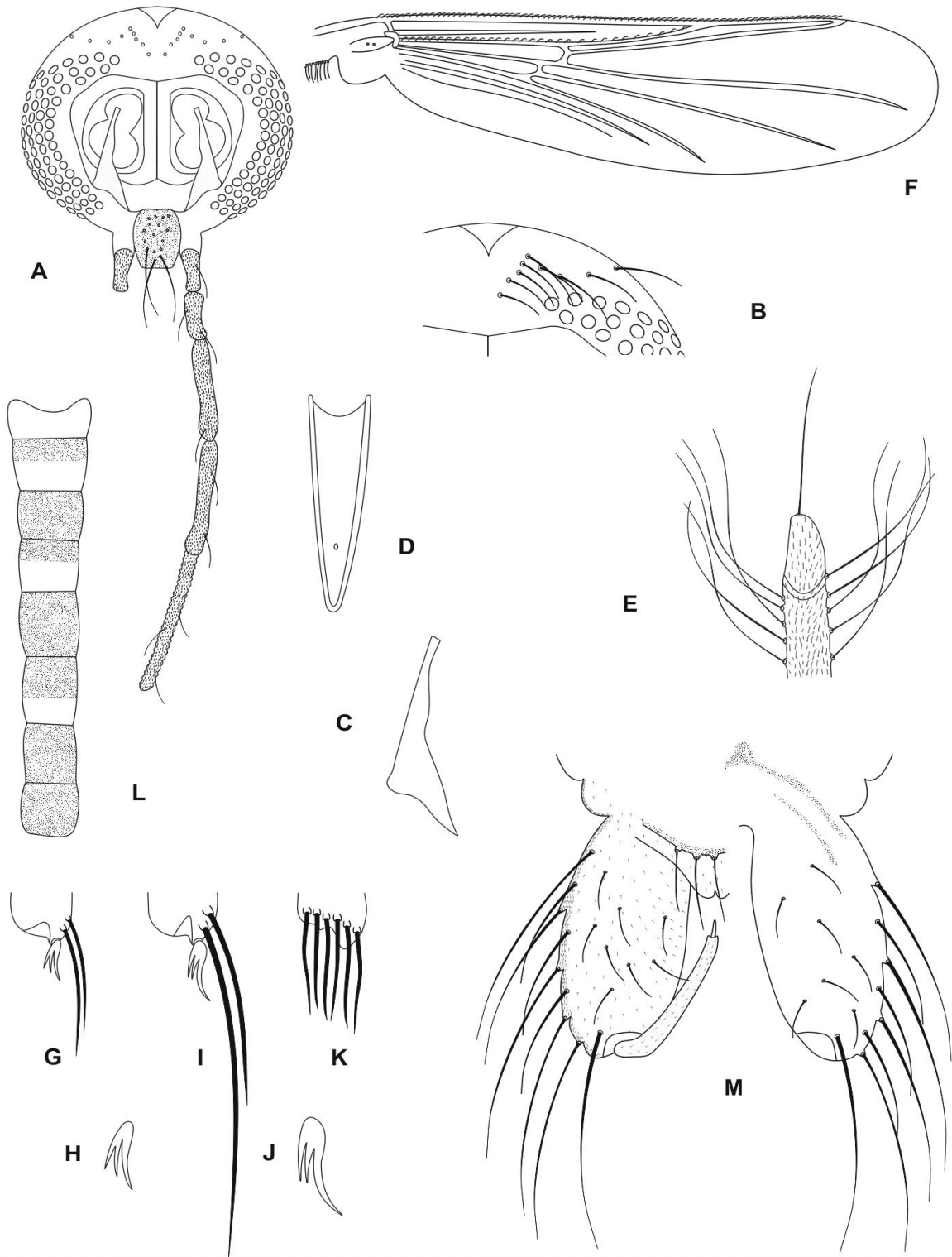
Wing (Fig. 72F). Width 0.36 mm. Costa 1.00 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.05 mm long. VR 0.75. WW 0.30. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 72G–K). Fore leg: width at apex of tibia 32 μm (Fig. 72G), tibia with single, apical and pectinate spur 10 μm long (Fig. 72H), with three teeth and two preapical setae; preapical pseudospurs unobserved. Mid leg: width at apex of tibia 35 μm long (Fig. 72I), tibia with single, apical and pectinate spur 21 μm long with three teeth (Fig. 72J) and two preapical setae; preapical pseudospurs unobserved. Hind leg: width at apex of tibia 32 μm long (Fig. 72K), tibia without spur; comb with 6 setae; preapical pseudospurs unobserved. Lengths and proportion of leg segments as in Table L.

Table L. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 20, adult male ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	405	446	–	–	–
p ₂	553	447	–	–	–
p ₃	484	609	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–

Hypopygium (Fig. 72M). Tergite IX arched, with 9 dorsal setae. Anal point triangular, apical edge slightly notched. Phallapodeme 41 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 122 μm long, 56 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.19. Gonostylus simple and slender, 74 μm long; megaseta 11 μm long. HR 1.66. HV 3.00.



Figures 72A–M. *Labrundinia* sp. 20, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Adult female (n = 1)

Size. Total length 1.38 mm. Wing length 0.94 mm. Total length/wing length 1.47. Wing length/length of profemur 2.78.

Coloration. Head dark brown to black with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax wholly dark brown; antepnotum pale; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen pale brown, with brown transverse bands near proximal margin. Seminal capsules brown.

Head. Temporal setae 10, uniserial. Eyes ratio 1.37. Tentorium 126 μm long, stipes not measurable. Clypeus 71 μm long, 59 μm wide at largest part, bearing 15–18 setae. Cibarial pump as in male, 182 μm long. Palpomere lengths 1–4 (in μm): 33; 43; 92; 106. Antenna with 11 flagellomeres, AR 0.42, flagellum 325 μm long, diameter of pedicel 57 μm .

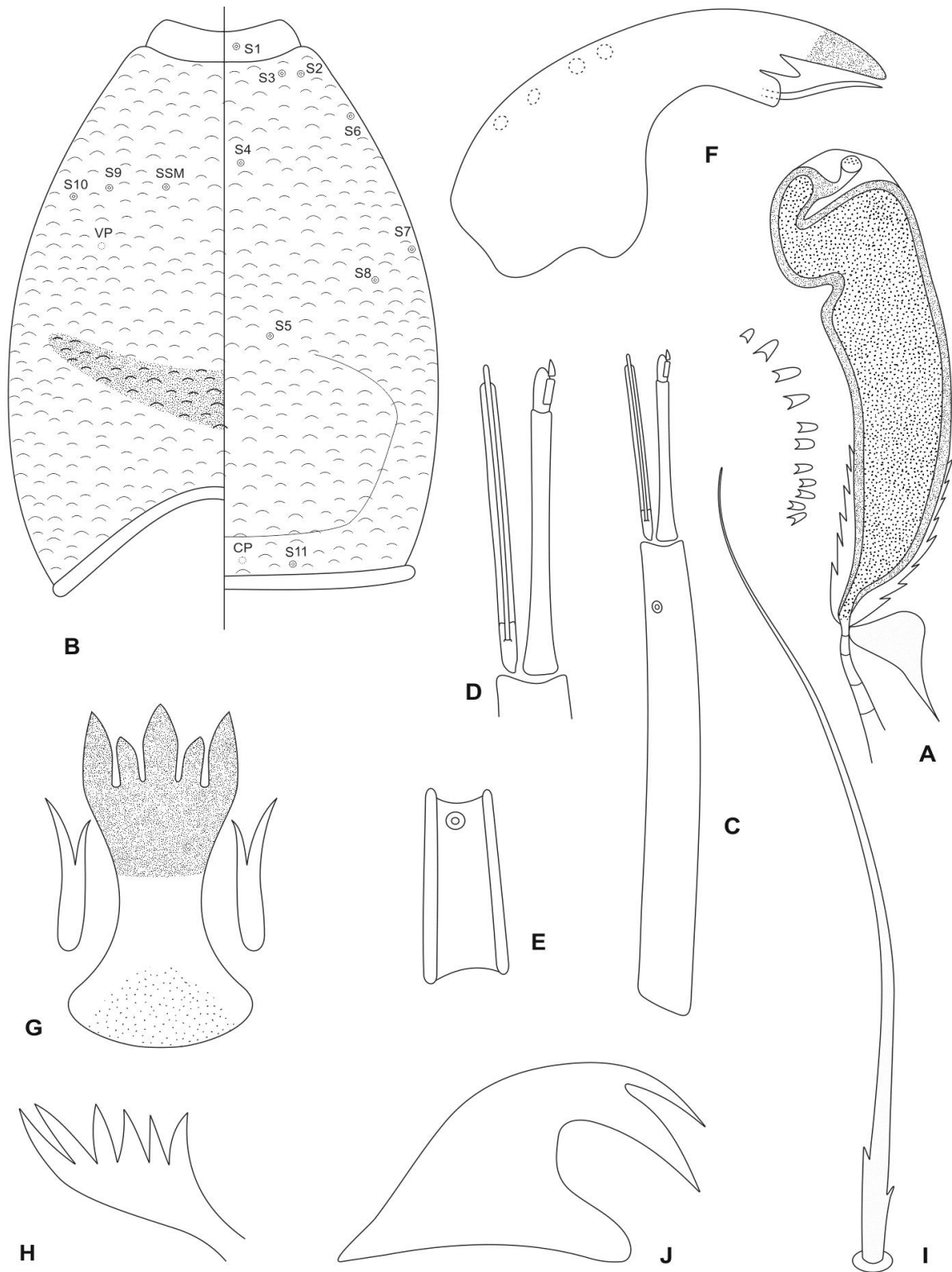
Thorax. Antepnotum lateral setae unobserved. Acrostichals 44, irregularly biserial, starting close to antepnotum; dorsocentrals 20, irregularly biserial; prealars 11; supraalars 2. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.36 mm. Costa 0.78 mm long, not produced beyond R_{4+5} . R_{2+3} present. Base of radial sector 0.05 mm long. VR 0.74. WW 0.39. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 31 μm , tibia with single, apical and pectinate spur 15 μm long, with three teeth and two preapical setae; preapical pseudospurs on $T_{a_{1-4}}$ unobserved. Mid leg: width at apex of tibia 35 μm , tibia with single, apical and pectinate spur 17 μm long, with three teeth and two preapical setae; preapical pseudospurs on $T_{a_{1-4}}$ unobserved. Hind leg: width at apex of tibia 38 μm , tibia without spur; comb with 6 setae; preapical pseudospurs on $T_{a_{1-4}}$ unobserved. Lengths and proportion of leg segments as in Table XLI.

Table XLI. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 20, adult female (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	337	328	–	–	–
p ₂	463	401	–	–	–
p ₃	427	492	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	–	–	–	–	–
p ₃	–	–	–	–	–



Figures 73A–J. *Labrundinia* sp. 20, pupa (A) and larva (B–J). **A.** Thoracic horn with basal lobe and thoracic comb. **B.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **C.** Antenna. **D.** Apex of antenna. **E.** Maxillary palp. **F.** Mandible. **G.** Ligula and paralingula. **H.** Pecten hypopharyngis. **I.** Subbasal seta of posterior parapod. **J.** Bifid claw of posterior parapod.

Genitalia. Gonapophysis VIII broadly rounded, 52 µm long. Coxosternapodeme 71 µm long. Postgenital plate rounded. Cerci oval-quadrangle, 37 µm long and 14 µm wide; with 8 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 70 µm. Seminal capsules oval with conical shaped necks, length 32 µm, maximum width 30 µm. Length ratio SCa/No 0.46.

Pupa (n = 1)

Size. Male abdomen unmeasured.

Coloration. Thoracic horn brown.

Cephalothorax (Figs 73A). Thoracic horn 138 µm long and 35 µm wide (Fig. 73A), preapical indentation moderate deep, THR 3.91. Membranous preapical papilla 46 µm long, PTH 0.34, aeropyle tube simple, short and robust, 11 µm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 11 conical teeth.

Abdomen. Missing.

4th instar larva (n = 2 unless otherwise stated)

Coloration. Head pale yellow, with ventral semicircular spot; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 73B). Length 453–458 µm, 316–365 µm wide. Surface covered with small spinules; lateroventral and posteroventral spine groups absent; cephalic index 0.70–0.80. Chaetotaxy as in figure 73B.

Antenna (Figs 73C–D). Length 234–242 µm, A₁ 166–172 µm long, with ring organ placed 141–147 µm from base, A₂ 58–59 µm long. AR 2.43–2.45. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 73E). Basal palp segment 23–24 µm long and 7–8 µm wide, with ring organ 19 µm from base. PR 2.80–3.22. APR 7.02–7.57.

Mandible (Fig. 73F). Length 66–72 µm, with 3 lateral setae. Sensillum campaniformium 52–54 µm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.40–2.51.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 73G–H). Ligula 54–59 µm long, 26 µm wide, with row of 5 teeth. IO 0.95–0.98, MO 1.05–1.07. Paraligula bifid, 25–27 µm long, inner tooth 19 µm long, shorter than outer

tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 73I–J). Without lateral fringe. Anterior parapods with simple claws. Procercus 88–92 μm long, 26–27 μm wide, with 7 anal setae 380 (1) μm long. L/W 3.27–3.53. Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod 223 (1) μm long; subbasal seta simple, with 2–3 small spines (Fig. 73I); parapod apex with numerous simple claws, without serrated claw; bifid claw with U-shaped lower indentation (Fig. 73J). B/C 0.90–1.04.

***Labrundinia spec. nov.* 21** (Figs 74–76)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, São Carlos, Espiraiado stream, 19.v.2011, S. Wiedenbrug (MZUSP). Paratypes: 1 male same data as holotype. 2 females with pupal and larval exuviae same data as holotype. 1 larva same data as holotype except for Fazzari stream, vii.2007, S. T. Strixino.

Diagnostic characters

Labrundinia sp. 21 differs from other *Labrundinia* species by the combination of the following characters.

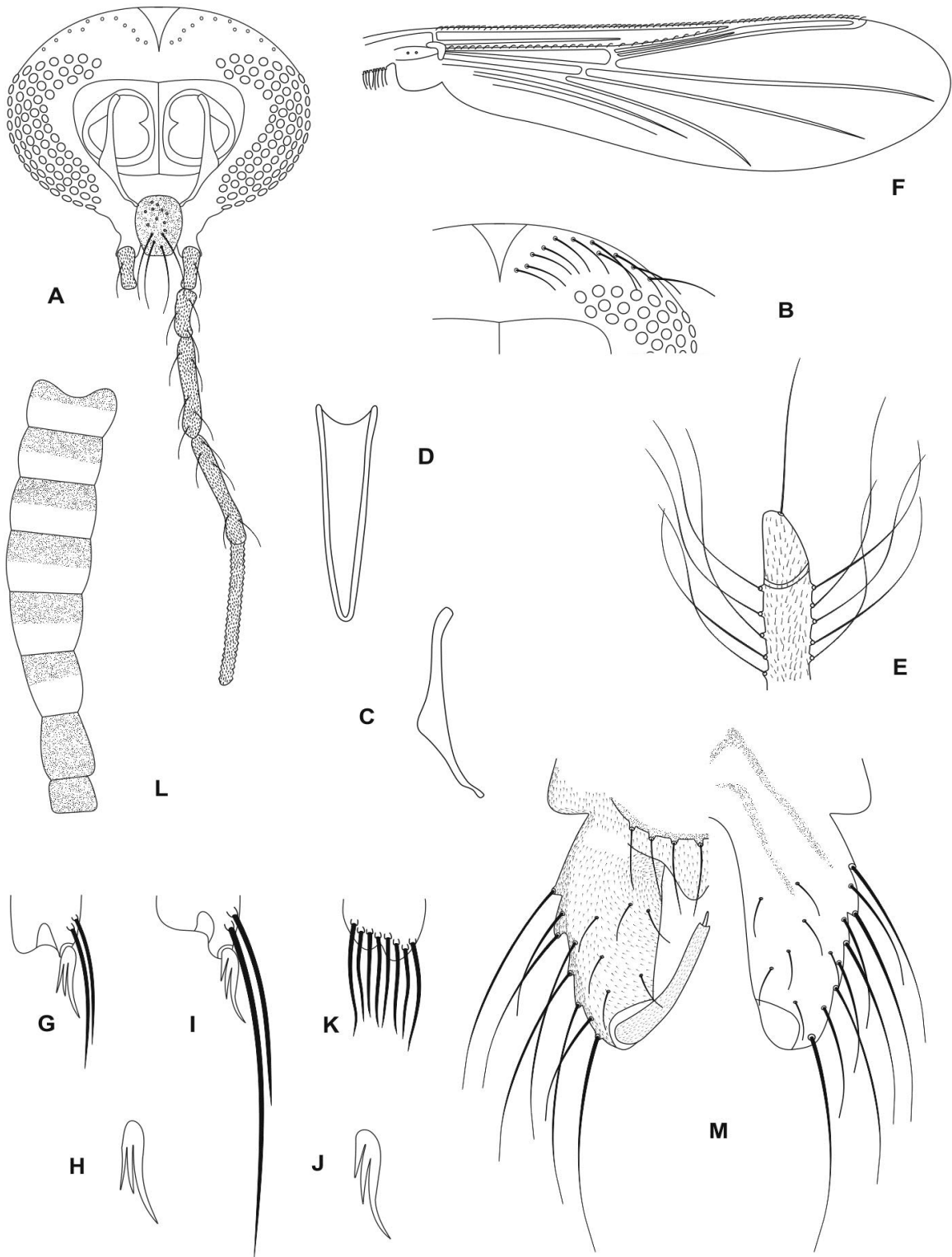
Male: R_{2+3} present; abdominal tergite I–VI with continuous brown transverse band near proximal margin, T VII–VIII wholly brown; hypopygium brown, sternapodeme with distinct anterior process. **Pupa**: thoracic horn club-shaped, preapical papilla ratio 0.36, plastron plate seated on an enlarged. **Larva**: surface covered with small spinules; lateroventral and posteroventral spine groups absent; AR 1.82–1.85; ratio between inner and outer tooth on bifid claw (B/C) 0.89–0.96.

Description

Adult male ($n = 2$ unless otherwise stated)

Size. Total length 2.15–2.27 mm. Wing length 1.20 (1) mm. Total length/wing length 1.90 (1). Wing length/length of profemur 3.04.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax wholly brown; antepnotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs pale brown. Abdomen as in



Figures 74A–M. *Labrundinia* sp. 21, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

figure 74L. Hypopygium brown.

Head (Figs 74E). Temporal setae 12, uniserial (Fig. 74B). Eye ratio 1.12–1.24. Tentorium (Fig. 74C) 113 (1) μm long, stipes not measurable. Clypeus 80–87 μm long, 50–56 μm wide at largest part, bearing 13–14 setae. Cibarial pump (Fig. 74D) with anterior margin concave, 151–164 μm long. Palpomere lengths 1–3 (in μm): 29–36; 38–44; 83–94. Antenna with 14 flagellomeres, AR 0.88 (1), flagellum 681 (1) μm long, diameter of pedicel 118–121 μm , apical setae single (Fig. 74E).

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 40–44, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 24–26, irregularly biserial; prealars 7; supraalars 2. Anapleural suture ratio 0.46–0.48. Scutellum with 7–8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 74F). Width 0.37 (1) mm. Costa 1.02 (1) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.09 (1) mm long. VR 0.70 (1). WW 0.32 (1). Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 74G–K). Fore leg: width at apex of tibia 32 (1) μm (Fig. 74G), tibia with single, apical and pectinate spur 10 (1) μm long (Fig. 74H), with three teeth and two preapical setae; preapical pseudospurs unobserved. Mid leg: width at apex of tibia 31 μm long (Fig. 74I), tibia with single, apical and pectinate spur 15 (1) μm long with three teeth (Fig. 74J) and two preapical setae; preapical pseudospurs unobserved. Hind leg: width at apex of tibia 29–37 μm long (Fig. 74K), tibia without spur; comb with 7–8 setae; preapical pseudospurs unobserved. Lengths and proportion of leg segments as in Table LII.

Table LII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 21, adult male (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	394	387	302	206	129
p ₂	558	398	307	162	96
p ₃	439	530	582	249	163
	ta ₄	ta ₅	LR	BV	SV
p ₁	84	62	0.78	2.25	3.04
p ₂	73	70	1.17	2.27	1.97
p ₃	109	73	1.10	2.61	1.67

Hypopygium (Fig. 74M). Tergite IX arched, with 9 dorsal setae. Anal point trapezoidal, apical edge slightly concave. Phallapodeme 42–46 μm long. Sternapodeme with distinct anterior process. Gonocoxite cylindrical, 84–101 μm long, 43–47 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.00–2.18. Gonostylus simple and slender, 56–74 μm long; megaseta 15 (1) μm long. HR 1.38–1.49. HV 3.09–3.80 (1).

Adult female (n = 1)

Size. Total length 1.50 mm. Wing length 0.97–0.99 mm. Total length/wing length 1.49. Wing length/length of profemur unmeasured.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax wholly brown; antepnotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs pale brown. Abdomen brown. Seminal capsules brown.

Head. Temporal setae 12, uniserial. Eyes ratio 1.08 (1). Tentorium and stipes unmeasured. Clypeus 59 (1) μm long, 83 (1) μm wide at largest part, bearing 19 (1) setae. Cibarial pump as in male, 171 (1) μm long. Palpomere lengths 1–3 (in μm): 26; 41; 93. Antenna with 11 flagellomeres, not measurable.

Thorax. Antepnotum with 2 lateral setae. Acrostichals 42–44, irregularly biserial, starting close to antepnotum; dorsocentrals 24–30, irregularly biserial; prealars 7–8; supraalars 2. Scutellum with 8–9 setae across disc. Anepisternals, preepisternals and postnotals absent.

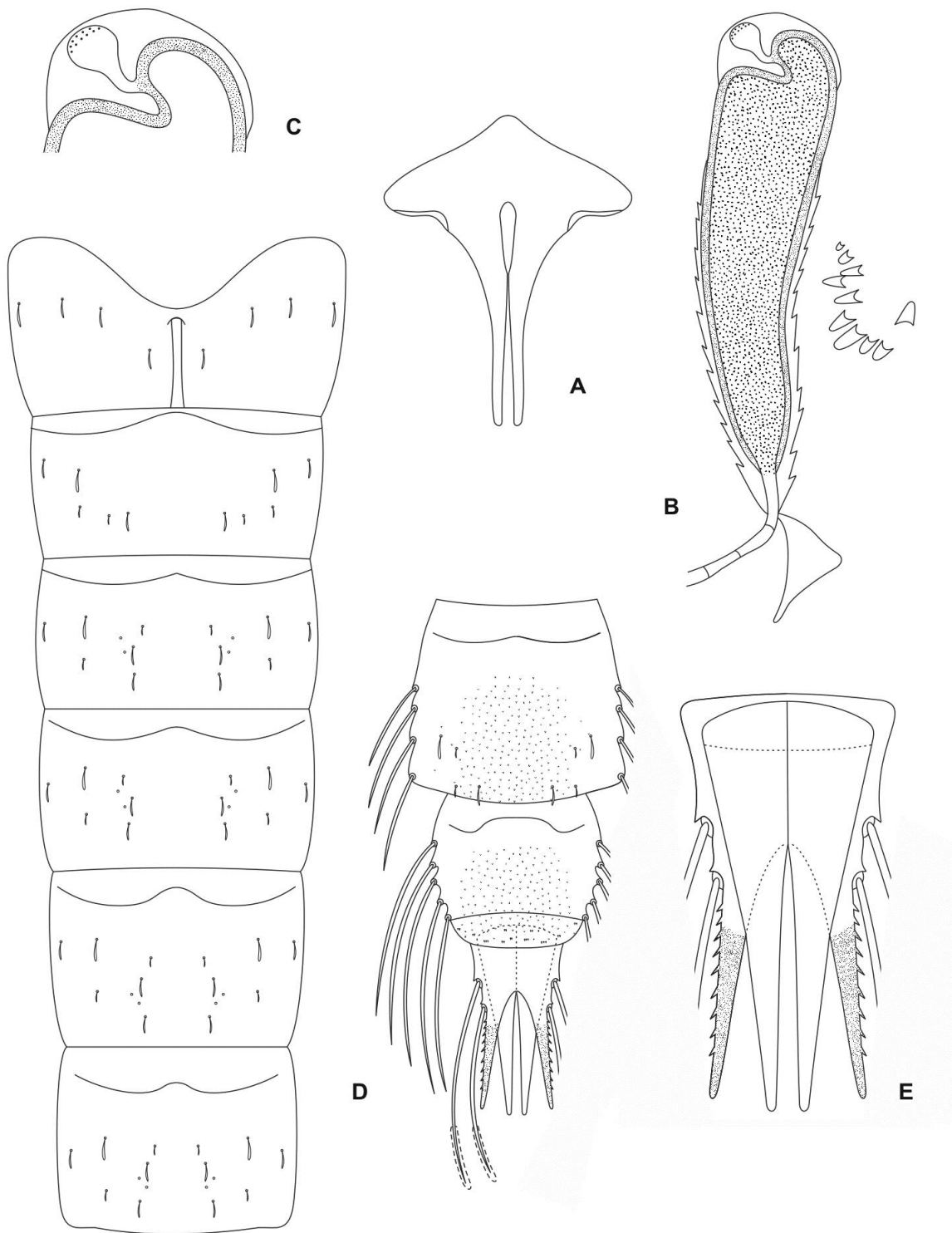
Wing. Width 0.38 mm. Costa 0.85–0.87 mm long, not produced beyond R_{4+5} . R_{2+3} present. Base of radial sector 0.06 mm long. VR 0.66–0.69. WW 0.38. Brachiolum with 2 setae. Squama setiferous.

Genitalia. Gonapophysis VIII broadly rounded, 73 (1) μm long. Coxosternapodeme 63 (1) μm long. Postgenital plate rounded. Cerci oval-quadrangle, 39 (1) μm long and 13 (1) μm wide; with 5 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 72 (1) μm . Seminal capsules oval with conical shaped necks, length 44 (1) μm , maximum width 38 (1) μm . Length ratio SCa/No 0.62 (1).

Legs. Fore leg: missing. Mid leg: width at apex of tibia 35–37 μm , tibia with single, apical and pectinate spur 16 (1) μm long, with three teeth and two preapical setae; preapical pseudospurs on Ta_{1-4} unobserved. Hind leg: width at apex of tibia unmeasured, tibia without spur; comb with 7 setae; preapical pseudospurs on Ta_{1-4} unobserved. Lengths and proportion of leg segments as in Table LIII.

Table LIII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 21, adult female (n = 1–2).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	–	–	–	–	–
p ₂	489–496	380–403	461	210	144
p ₃	436–439	467–474	–	–	–
	ta ₄	ta ₅	LR	BV	SV
p ₁	–	–	–	–	–
p ₂	91	73	1.14	2.62	1.95
p ₃	–	–	–	–	–



Figures 75A–E. *Labrundinia* sp. 21, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Abdominal segments with chaetotaxy, dorsal aspect. **E.** Anal lobe and male genital sac, ventral aspect.

Pupa (n = 3 unless otherwise stated)

Size. Male abdomen 1.53 (1) mm long; female abdomen 1.72–1.79 (2) mm long.

Coloration. Exuviae and thoracic horn brown. Apex of anal lobe brown.

Cephalothorax (Figs 75A–C). Frontal apotome as in figure 75A. Wing sheath smooth 0.69–0.77 mm long. Thoracic horn 181–280 μm long and 46–56 μm wide (Fig. 75B), preapical indentation moderate deep, THR 3.66–3.97. Membranous preapical papilla 65 (1) μm long (Fig. 75C), PTH 0.36 (1), aeropyle tube simple, short and robust, 19–22 (2) μm long; plastron plate reduced. Horn sac well developed, completely filling respiratory atrium. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 11 conical teeth.

Abdomen (Figs 75D–E). Tergite I with scar, 109–131. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Abdominal chaetotaxy as in figure 75D. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 229–238 (1) μm long and 130–133 μm wide (Fig. 75E), outer margins sclerotized, with 9–10 spines, longest spine 10–12 μm long, inner margins of lobes membranous. ALR 1.79–2.22. Genital sac elongated, almost reaching apex of anal lobe.

4th instar larva (n = 4 unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

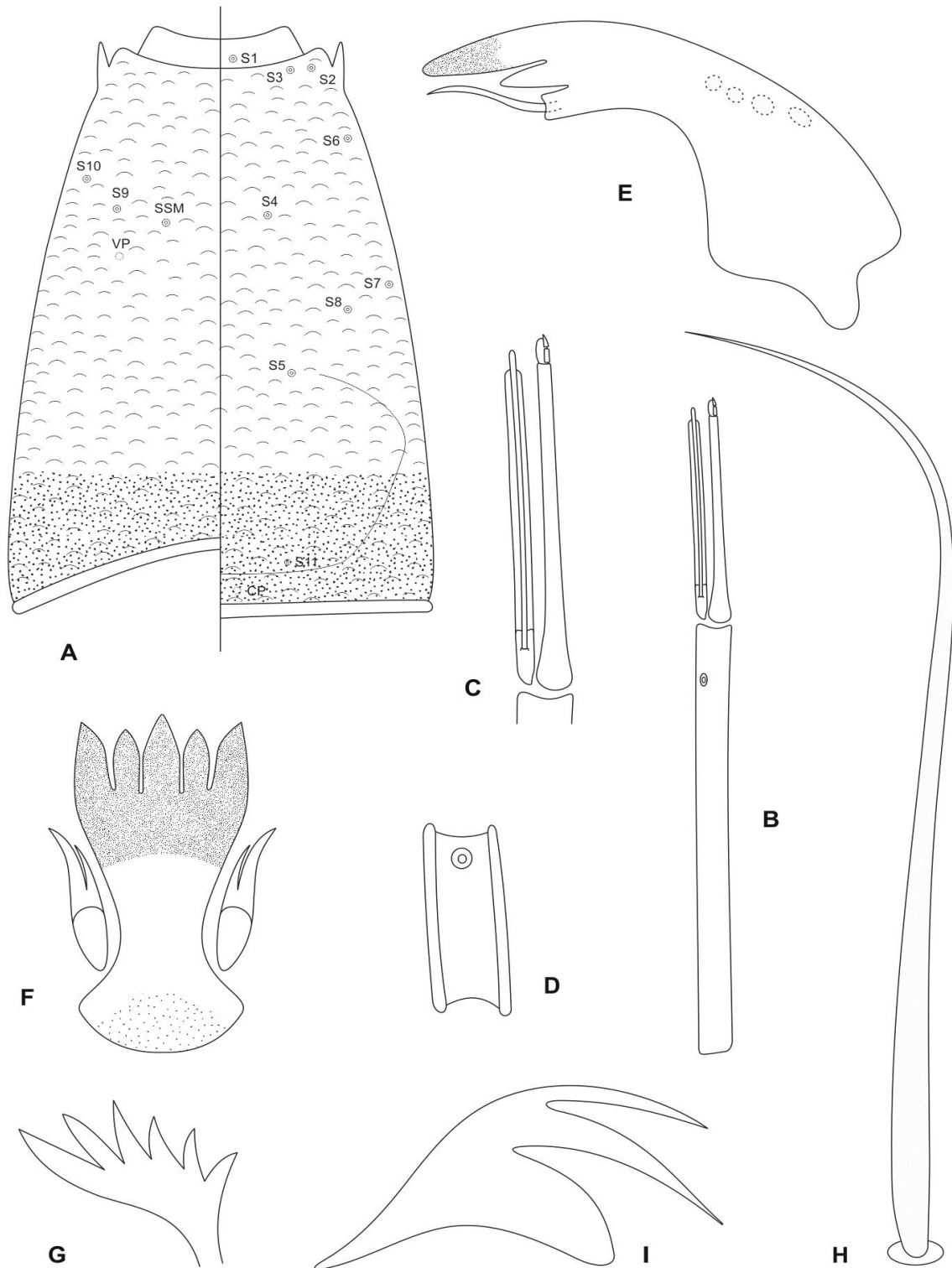
Head (Fig 76A). Length 413–436 μm , 244–258 μm wide. Surface covered with small spinules; lateroventral and posteroventral spine groups absent; cephalic index 0.59–0.60. Chaetotaxy as in figure 76A.

Antenna (Figs 76B–C). Length 266–277 (2) μm , A₁ 171–180 (2) μm long, with ring organ placed 155–166 (2) μm from base, A₂ 83–87 (2) μm long. AR 1.82–1.85 (2). Blade longer than A₂, 84–90 (2); accessory blade 86–92 (2).

Maxilla (Fig. 76D). Basal palp segment 19–20 μm long and 7–8 μm wide, with ring organ 14–17 μm from base. PR 2.43–2.69. APR 8.90–9.38 (2).

Mandible (Fig. 76E). Length 48–55 μm , with 3 lateral setae. Sensillum campaniformium 34–38 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 3.58–3.61 (2).

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.



Figures 76A–I. *Labrundinia* sp. 21, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod.

Hypopharyngeal complex (Figs 76F–G). Ligula 37–45 µm long, 19–21 µm wide, with row of 5 teeth. IO 0.98–0.99, MO 1.02–1.03. Paraligula bifid, 19–21 µm long, inner tooth 15–18 µm long, shorter than outer tooth. Pecten hypopharyngis with 6 teeth almost equal in size.

Body (Figs 76H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 107–117 (3) µm long, 21–24 µm wide, with 7 anal setae 285–332 (3) µm long. L/W 4.77–5.15 (3). Supraanal seta well developed. Anal tubules 194 (1). Posterior parapod 327–328 (2) µm long; subbasal seta simple, without spines (Fig. 76H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 76H). B/C 0.89–0.96.

Remarks: This is the *Labrundinia* sp. 8 (description of male and immatures stages) of Trivinho-Strixino (2011) (larvae identification key).

Labrundinia spec. nov. 22 (Figs 77–79)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **Paraná**, Ponta Grossa, Vila Velha State Park, 2.iv.2008, C. S. N. Oliveira. Paratypes: 1 pharate male data as holotype; 1 pharate female with larval exuvia same data as holotype.

Diagnostic characters

Labrundinia sp. 22 differs from other *Labrundinia* species by the combination of the following characters. **Male:** abdominal tergite I, VI, VIII wholly brown, II–V, VII with continuous brown transverse band near proximal margin; hypopygium brown, sternapodeme with reduced anterior process. **Pupa:** thoracic horn 9-shaped; shagreen on segment II with prominent spines; apical spines 23–29 µm long; genital sac almost reaching apex of the anal lobe. **Larva:** surface smooth; lateroventral spine group absent, posteroventral spine group present with 20–22 small spines; AR 2.80–2.93; subbasal seta simple; bifid claw brown, with U-shaped lower indentation.

Description

Adult male ($n = 2$ unless otherwise stated)

Size. Total length 2.42 mm. Wing length 1.36 (1) mm. Total length/wing length 1.77 (1). Wing length/

length of profemur 3.10 (1).

Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax wholly brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Abdomen as in figure 77L. Hypopygium brown.

Head (Figs 77–E). Temporal setae 12, uniserial (Fig. 77B). Eye ratio 1.17 (1). Tentorium (Fig. 77C) 154–160 μm long, stipes not measurable. Clypeus 73–82 μm long, 65–66 μm wide at largest part, bearing 11–13 setae. Cibarial pump (Fig. 77D) with anterior margin concave, 177–180 μm long. Palpomere lengths 1–5 (in μm): 35 (1); 49 (1); 83 (1); 95 (1); 170 (1). Antenna with 14 flagellomeres, AR 1.05, flagellum 761 (1) μm long, diameter of pedicel 125 (1) μm , apical setae single (Fig. 77E).

Thorax. Anteprenotum with 2–3 lateral setae. Acrostichals 48–52, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 16–20, irregularly uniserial; prealars 7–8; supraalars 3. Anapleural suture ratio 0.43–0.45. Scutellum with 6 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 77F). Width 0.39 (1) mm. Costa 1.13 (1) mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.06 (1) mm long. VR 0.74 (1). WW 0.28 (1). Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 77G–K). Fore leg: width at apex of tibia 36 (1) μm (Fig. 77G), tibia with single, apical and pectinate spur 17 (1) μm long (Fig. 77H), with three teeth and two preapical setae; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 34 (1) μm long (Fig. 77I), tibia with single, apical and pectinate spur 24 (1) μm long with three teeth (Fig. 77J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 43 (1) μm long (Fig. 77K), tibia without spur; comb with 5 setae; Ta_{1-4} each without two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table LIV.

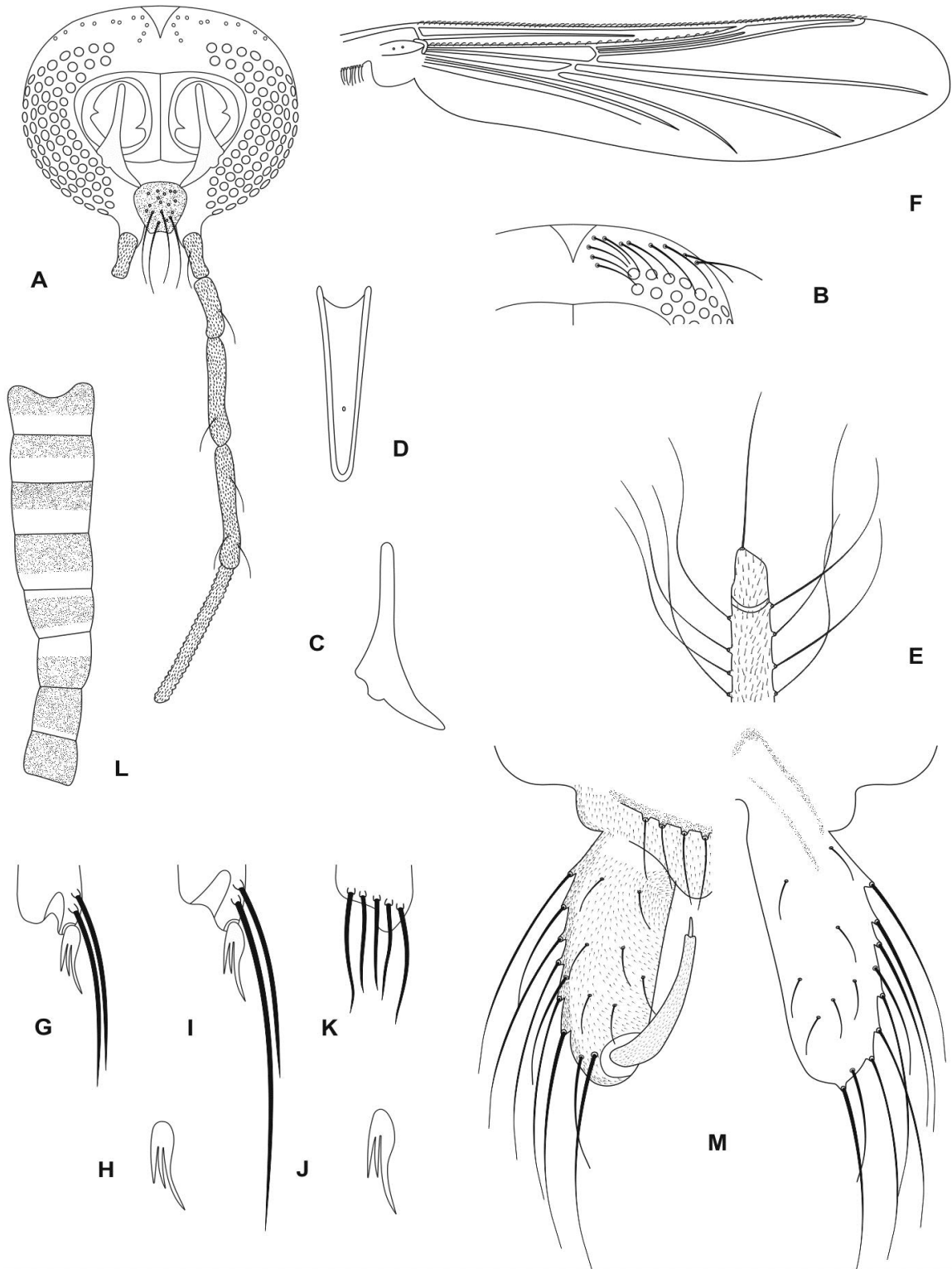
Hypopygium (Fig. 77M). Tergite IX arched, with 9–10 dorsal setae. Anal point trapezoidal, apical edge slightly concave. Phallapodeme 49–59 μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 110 (1) μm long, 45 (1) μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.49 (1). Gonostylus simple and slender, 65–84 μm long; megaseta 9–11 μm long. HR 1.71 (1). HV 3.74 (1).

Adult female ($n = 1$)

Size. Total length 2.41 mm.

Coloration as in male except for abdomen wholly brown. Seminal capsules brown.

Genitalia. Gonapophysis VIII broadly rounded, 55 μm long. Coxosternapodeme 52 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 31 μm long and 15 μm wide; with 6 elongated setae. Labia with



Figures 77A–M. *Labrundinia* sp. 22, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

inconspicuous microtrichia. Notum length (from ramus forward) 69 μm . Seminal capsules oval with conical shaped necks, length 40 μm , maximum width 20 μm . Length ratio SCa/No 0.58.

Table LIV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 22, adult male (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	438	456	315	226	142
p ₂	566	457	557	274	146
p ₃	552	618	647	288	195
	ta ₄	ta ₅	LR	BV	SV
p ₁	107	85	0.69	2.16	2.83
p ₂	98	83	1.22	2.62	1.84
p ₃	121	86	1.05	2.63	1.81

Pupa (n = 2 unless otherwise stated)

Size. Male abdomen 2.16–2.17 mm long.

Coloration. Exuviae and thoracic horn brown.

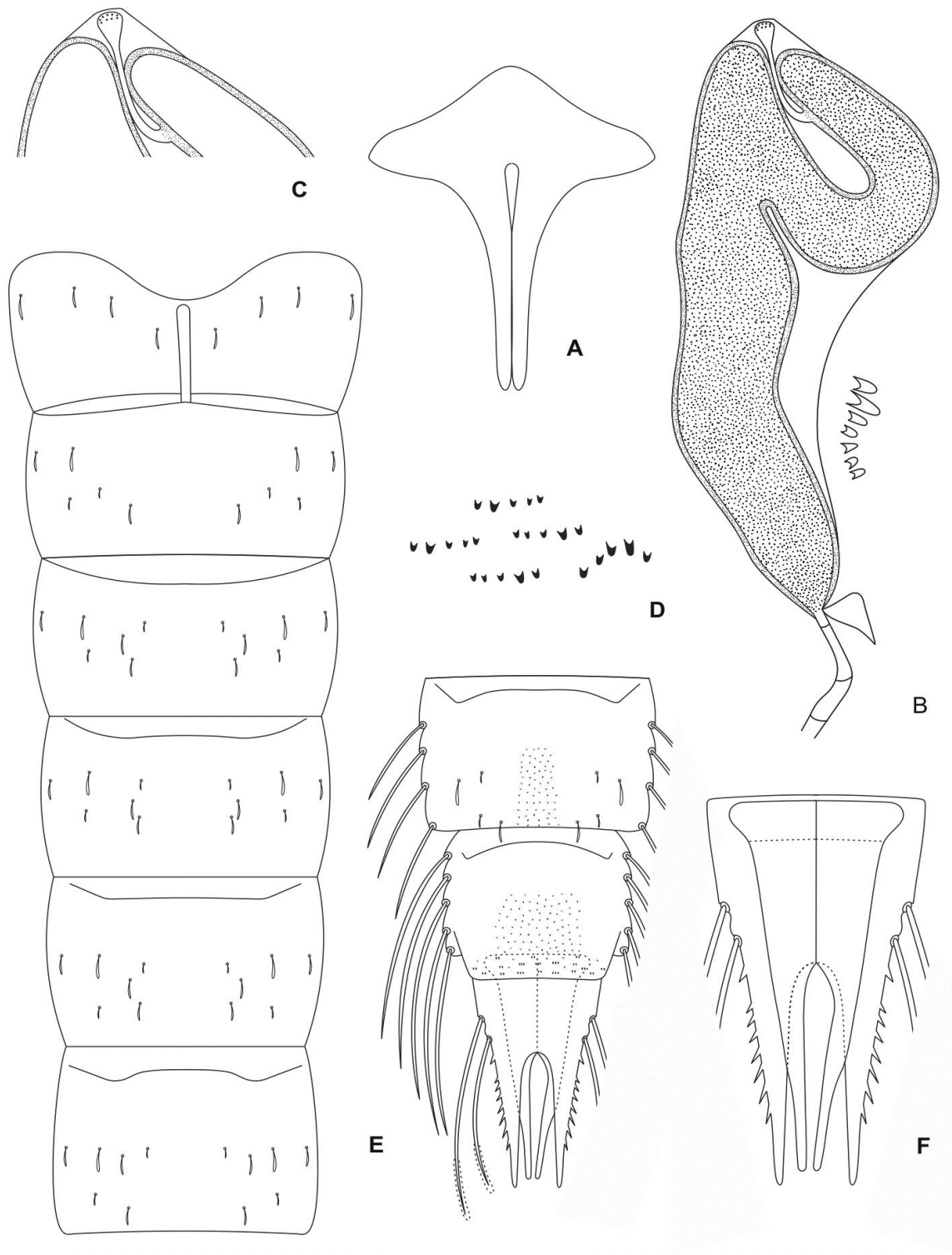
Cephalothorax (Figs 78A–C). Frontal apotome as in figure 78A. Wing sheath smooth 0.70–0.87 mm long. Thoracic horn 231–267 μm long and 100–120 μm wide (Fig. 78B), preapical indentation deep, THR 2.23–2.31. Membranous preapical papilla 31–32 μm long (Fig. 78C), PTH 0.12–0.13, aeropyle tube simple, elongated and slender, 53 (1) μm long; plastron plate reduced. Horn sac moderately developed. Reticulation of respiratory atrium indistinct, spinules on external membrane unobserved. Basal lobe wedge-shaped. Thoracic comb with 9 (1) conical teeth.

Abdomen (Figs 78D–F). Tergite I with scar, 111–133. T I–VI without shagreen, VII–VIII with shagreen basally concentrated. Segment II with prominent spines (Fig. 78D). Abdominal chaetotaxy as in figure 78E. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 338–401 μm long and 149–163 μm wide (Fig. 78F), outer margins sclerotized, with 10 spines, longest spine 23–29 μm long, inner margins of lobes membranous. ALR 2.08–2.68. Genital sac elongated, almost reaching apex of anal lobe.

4th instar larva (n = 2 unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow to pale brown except for bifid claw brown.

Head (Fig 79A). Length 550–602 μm , 338–385 μm wide. Surface smooth; lateroventral spine group absent;



Figures 78A–F. *Labrundinia* sp. 22, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Spines on segment II. **E.** Abdominal segments with chaetotaxy, dorsal aspect. **F.** Anal lobe and male genital sac, ventral aspect.

posteroventral spine group present with 20–22 small spines; cephalic index 0.61–0.64. Chaetotaxy as in figure 79A.

Antenna (Figs 79B–C). Length 333–378 μm , A_1 226–245 μm long, with ring organ placed 203–222 μm from base, A_2 71–76 μm long. AR 2.80–2.93. Blade and accessory blade unmeasured.

Maxilla (Fig. 79D). Basal palp segment 34–35 μm long and 7–9 μm wide, with ring organ 26–28 μm from base. PR 3.93–4.77. APR 6.56–7.02.

Mandible (Fig. 79E). Length 48–51 μm , with 3 lateral setae. Sensillum campaniformium 41–44 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 4.79–4.75.

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate, 118 (1) μm .

Hypopharyngeal complex (Figs 79F–G). Ligula 57–61 μm long, 31–36 μm wide, with row of 5 teeth. IO 0.96–0.97, MO 1.02–1.01. Paraligula bifid, 29–30 μm long, inner tooth 21–22 μm long, shorter than outer tooth. Pecten hypopharyngis with 7 teeth almost equal in size.

Body (Figs 79H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 195–204 μm long, 29–30 μm wide, with 7 anal setae 518–525 μm long. L/W 6.68–6.88. Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod unmeasured; subbasal seta simple, without spines (Fig. 79H); parapod apex with numerous simple claws, without serrated claw; bifid claw with U-shaped lower indentation (Fig. 79H). B/C 1.25.

Labrundinia spec. nov. 23 (Figs 80–82)

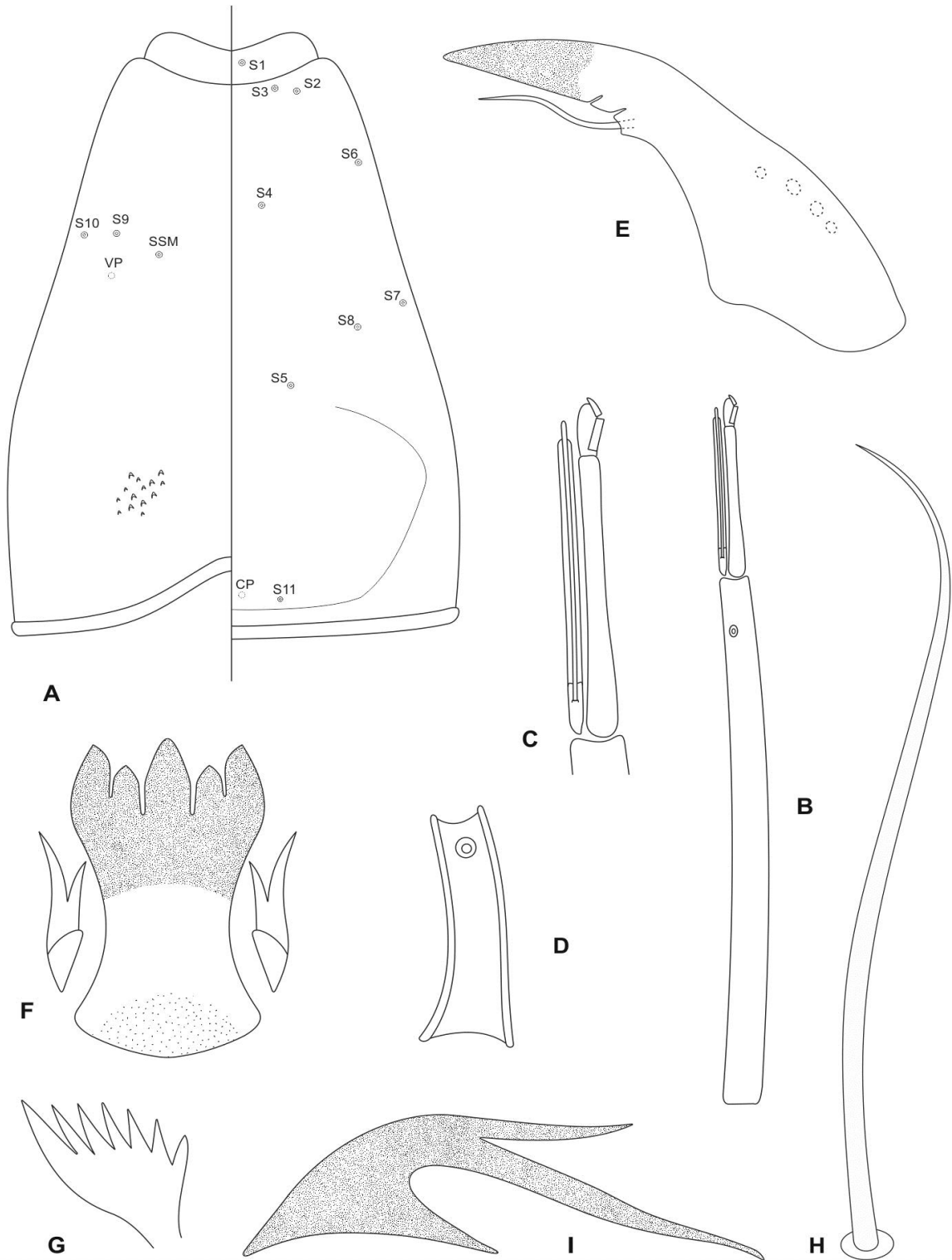
Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, Luiz Antonio, Beija-Flor reservoir, 19.v.2011, S. Wiedenbrug (MZUSP). Paratypes: 1 pupa with larval exuvia same data as holotype except for Óleo lake, 7.i.2011; 1 pupa and 1 larva same data as holotype except for Óleo lake, 7.i.2011, S. T. Strixino.

Diagnostic characters

Labrundinia sp. 23 differs from other *Labrundinia* species by the combination of the following characters.

Male: wing length 1.16 mm; R_{2+3} absent; abdominal tergite I–II pale, T II–VII with continuous brown transverse band near proximal margin, T VIII wholly brown; hypopygium pale, sternapodeme with reduced anterior process. **Pupa**: thoracic horn 9-shaped; shagreen on segment II with prominent spines;



Figures 79A–I. *Labrundinia* sp. 22, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod

abdominal segment VII with 3 lateral setae; genital sac reaching much beyond apex of the anal lobe.

Larva: surface smooth, lateroventral spine group present, but weakly developed, with 3 spines, posteroventral spine group absent; ligula slender, 54–61 μm long; subbasal seta simple; bifid claw, with V-shaped lower indentation.

Description

Adult male (n = 1)

Size. Total length 2.42 mm. Wing length 1.16 mm. Total length/wing length 2.09. Wing length/length of profemur 3.06.

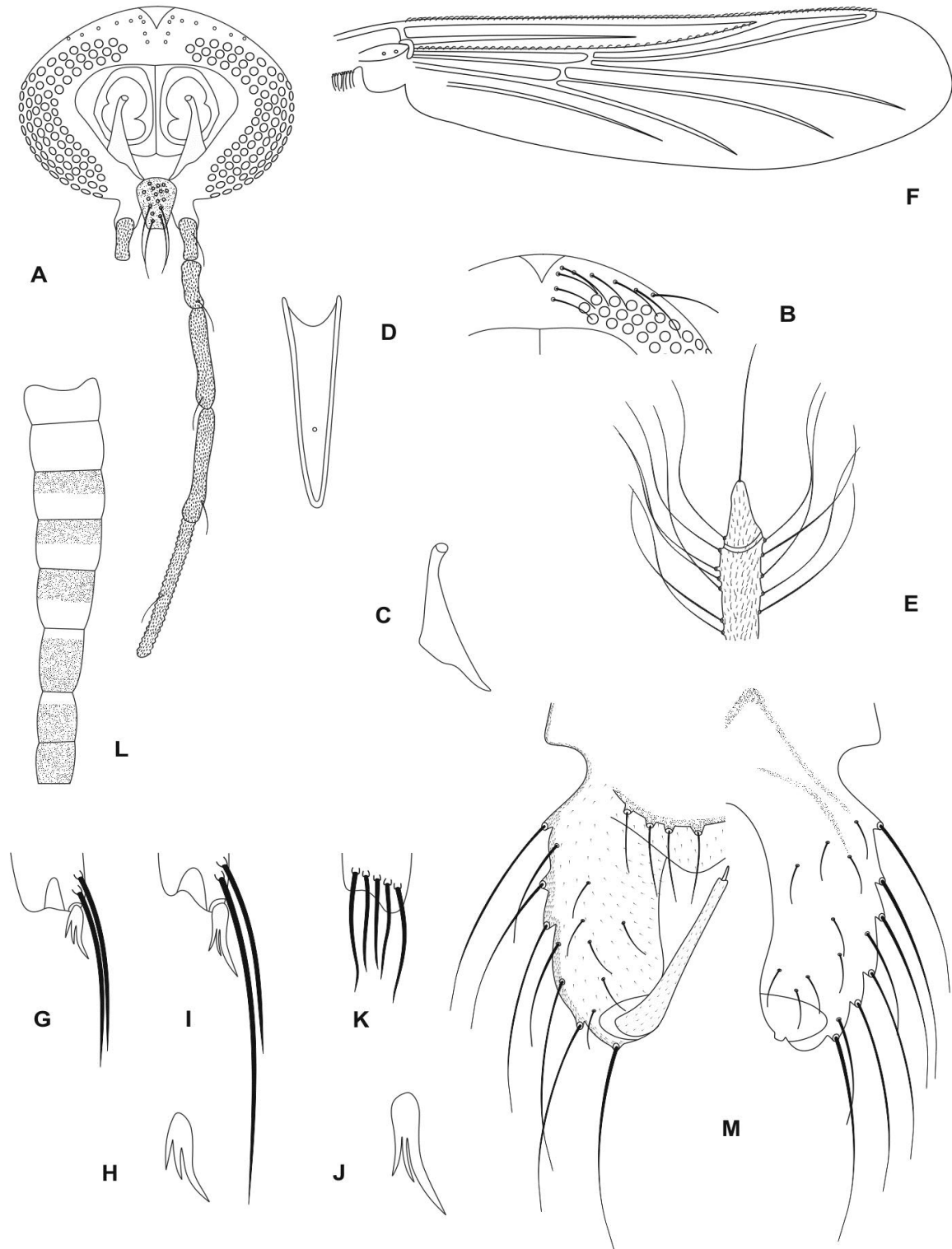
Coloration. Head pale brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp brown. Thorax brown with mesonotum dorsally pale brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Femur I–III pale brown. Abdomen as in figure 80L. Hypopygium pale.

Head (Figs 80–E). Temporal setae 10, uniserial (Fig. 80B). Eye ratio 1.13. Tentorium (Fig. 80C) 85 μm long, stipes not measurable. Clypeus 69 μm long, 63 μm wide at largest part, bearing 10 setae. Cibarial pump (Fig. 80D) with anterior margin concave, 188 μm long. Palpomere lengths 1–4 (in μm): 31; 46; 98; 110. Antenna with 14 flagellomeres, AR 1.30, flagellum 739 μm long, diameter of pedicel 138 μm , apical setae single (Fig. 80E).

Thorax. Anteprenotum with 3 lateral setae. Acrostichals 44, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 20, irregularly biserial; prealars 10; supraalars 2. Anapleural suture ratio 0.48. Scutellum with 7 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 80F). Width 0.33 mm. Costa 1.00 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} absent. Base of radial sector 0.05 mm long. VR 0.81 (1). WW 0.28. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 80G–K). Fore leg: width at apex of tibia 39 μm (Fig. 80G), tibia with single, apical and pectinate spur 14 μm long (Fig. 80H), with three teeth and two preapical setae; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 34 μm long (Fig. 80I), tibia with single, apical and pectinate spur 24 μm long with three teeth (Fig. 80J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 39 μm long (Fig. 80K), tibia without spur; comb with 7 setae; Ta_{1-4} each without two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table LV.



Figures 80A–M. *Labrundinia* sp. 23, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

Table LV. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 23, adult male ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	378	412	206	219	80
p ₂	502	374	513	243	110
p ₃	442	556	546	260	164
	ta ₄	ta ₅	LR	BV	SV
p ₁	69	54	0.50	2.37	3.85
p ₂	93	77	1.37	2.65	1.71
p ₃	106	73	0.98	2.56	1.83

Hypopygium (Fig. 80M). Tergite IX arched, with 13 dorsal setae. Anal point rounded, apical edge convex. Phallapodeme 48 μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 103 μm long, 40 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.60. Gonostylus simple and slender, 71 μm long; megaseta 12 μm long. HR 1.45. HV 3.40.

Pupa ($n = 3$ unless otherwise stated)

Size. Male abdomen 2.12–2.52 mm long; female abdomen 2.26 mm long.

Coloration. Exuviae pale brown. Thoracic horn brown.

Cephalothorax (Figs 81A–C). Frontal apotome as in figure 81A. Wing sheath smooth 0.78–0.81 mm long. Thoracic horn 253–281 μm long and 119–144 μm wide (Fig. 81B), preapical indentation deep, THR 1.96–2.12. Membranous preapical papilla 38–46 μm long (Fig. 81C), PTH 0.15–0.18, aeropyle tube simple, elongated and slender, 46–47 (2) μm long; plastron plate reduced. Horn sac moderately developed. Reticulation of respiratory atrium indistinct, external membrane with pale spinules. Basal lobe wedge-shaped. Thoracic comb with 9–11 conical teeth.

Abdomen (Figs 81D–F). Tergite I with scar, 110–149 (2). T I–VI without shagreen, VII–VIII with shagreen medial basal concentrated. Segment II with prominent spines (Fig. 81D). Abdominal chaetotaxy as in figure 81E. Abdominal segment VII with 3 lateral setae. A VIII with 5 lateral setae. Anal lobe 339–375 μm long and 152–157 μm wide (Fig. 81F), outer margins sclerotized, with 7–8 spines, longest spine 18–24 μm long, inner margins of lobes membranous. ALR 2.16–2.23. Genital sac elongated, reaching much beyond apex of anal lobe.

4th instar larva ($n = 3$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment pale brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig. 82A). Length 519–607 μm , 313–372 μm wide. Surface smooth; lateroventral spine group present, but weakly developed, with 3 spines; posteroventral spine group absent; cephalic index 0.60–0.61. Chaetotaxy as in figure 82A.

Antenna (Figs 82B–C). Length 307–344 μm , A_1 216–249 μm long, with ring organ placed 194 (2) μm from base, A_2 81–84 μm long. AR 2.36–2.64. Blade longer than A_2 over-reached by accessory blade.

Maxilla (Fig. 82D). Basal palp segment 32–33 μm long and 6–7 μm wide, with ring organ 21–26 μm from base. PR 4.57–4.64. APR 6.76–7.63.

Mandible (Fig. 82E). Length 53–57 (2) μm , with 3 lateral setae. Sensillum campaniformium 33–37 (2) μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 4.11–4.17.

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 82F–G). Ligula 54–61 μm long, 28–32 μm wide, with row of 5 teeth. IO 0.93–0.99, MO 1.01–1.05. Paraligula bifid, 26–29 μm long, inner tooth 20–24 μm long, shorter than outer tooth. Pecten hypopharyngis with 7 teeth almost equal in size.

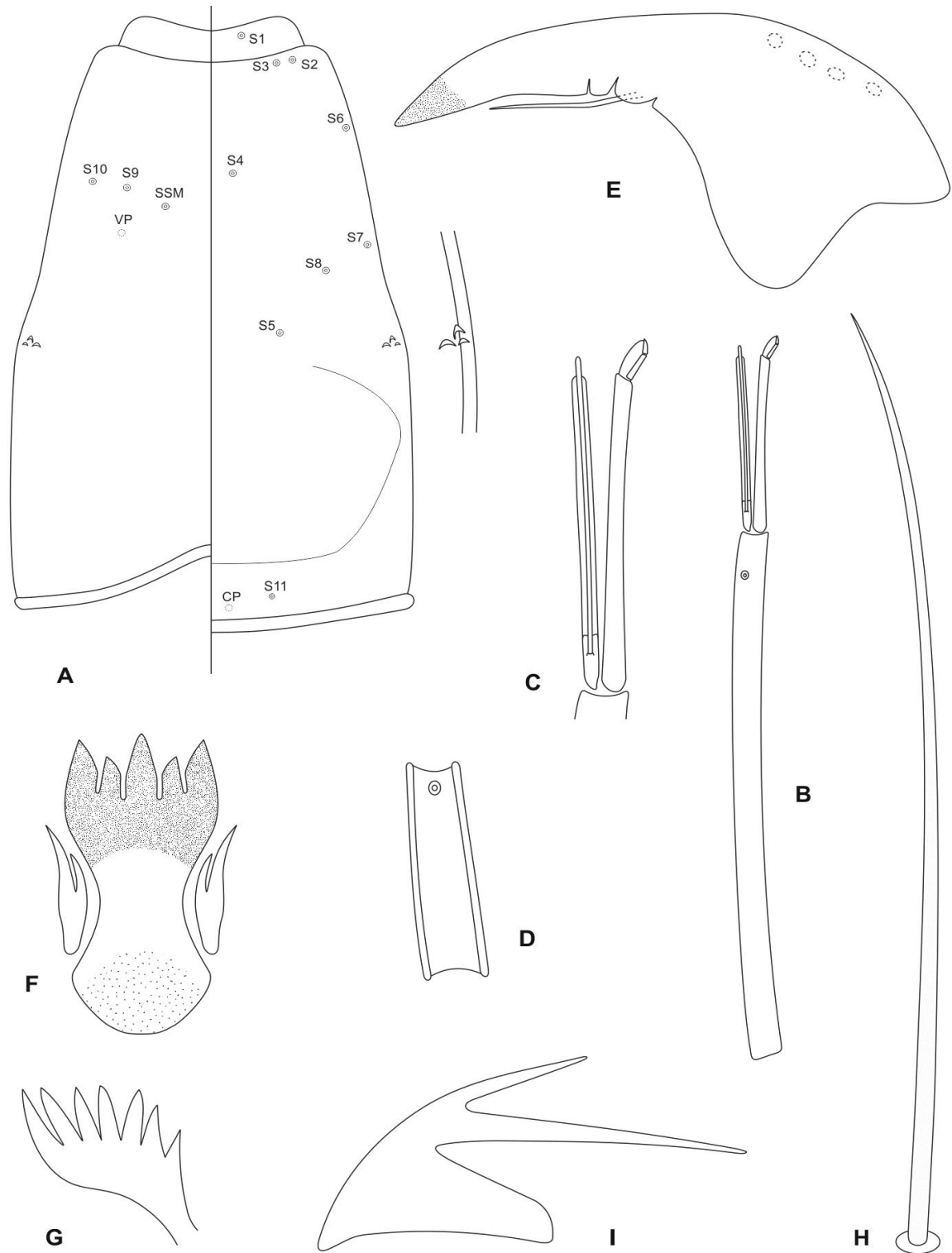
Body (Figs 82H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 237–244 (2) μm long, 27–41 (2) μm wide, with 7 anal setae 537–584 (2) μm long. L/W 5.73–8.91 (2). Supraanal seta well developed. Anal tubules 277 (1) μm long. Posterior parapod 351–430 μm long; subbasal seta simple, without spines (Fig. 82H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 82H). B/C 1.26–1.30 μm long.

Remarks: This is the *Labrundinia* sp. 6 of Trivinho-Strixino (2011) (larvae identification key).

***Labrundinia* spec. nov. 24** (Figs 83–85)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, São Carlos, Mayaca lake, 20.iv.2011, F. L. Silva (MZUSP). Paratypes: 1 male with pupal and larval exuviae same data as holotype; 1 male same data as holotype male except for 11.iv.2011. 1 female with pupal and larval exuviae same data as holotype except for 11.iv.2011; 1 female with pupal exuvia same data as holotype; 1 pharate male with larval exuvia same data as holotype.



Figures 82A–I. *Labrundinia* sp. 23, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod

Diagnostic characters

Labrundinia sp. 24 differs from other *Labrundinia* species by the combination of the following characters.

Male: abdominal tergite I–II pale, T III–VIII with continuous brown transverse band near proximal margin; hypopygium pale, sternapodeme with reduced anterior process. **Pupa:** thoracic horn 9-shaped; shagreen on segment II with prominent spines; apical spines 18–26 μm long; genital sac reaching much beyond apex of the anal lobe. **Larva:** surface smooth; lateroventral spine group absent, posteroventral spine group present with 14–22 small spines; AR 3.02–3.22; subbasal seta simple; bifid claw brown, with U-shaped lower indentation.

Description

Adult male (n = 3 unless otherwise stated)

Size. Total length 2.56–2.68 mm. Wing length 1.31–1.40 mm. Total length/wing length 1.86–1.97. Wing length/length of profemur 2.69–3.03.

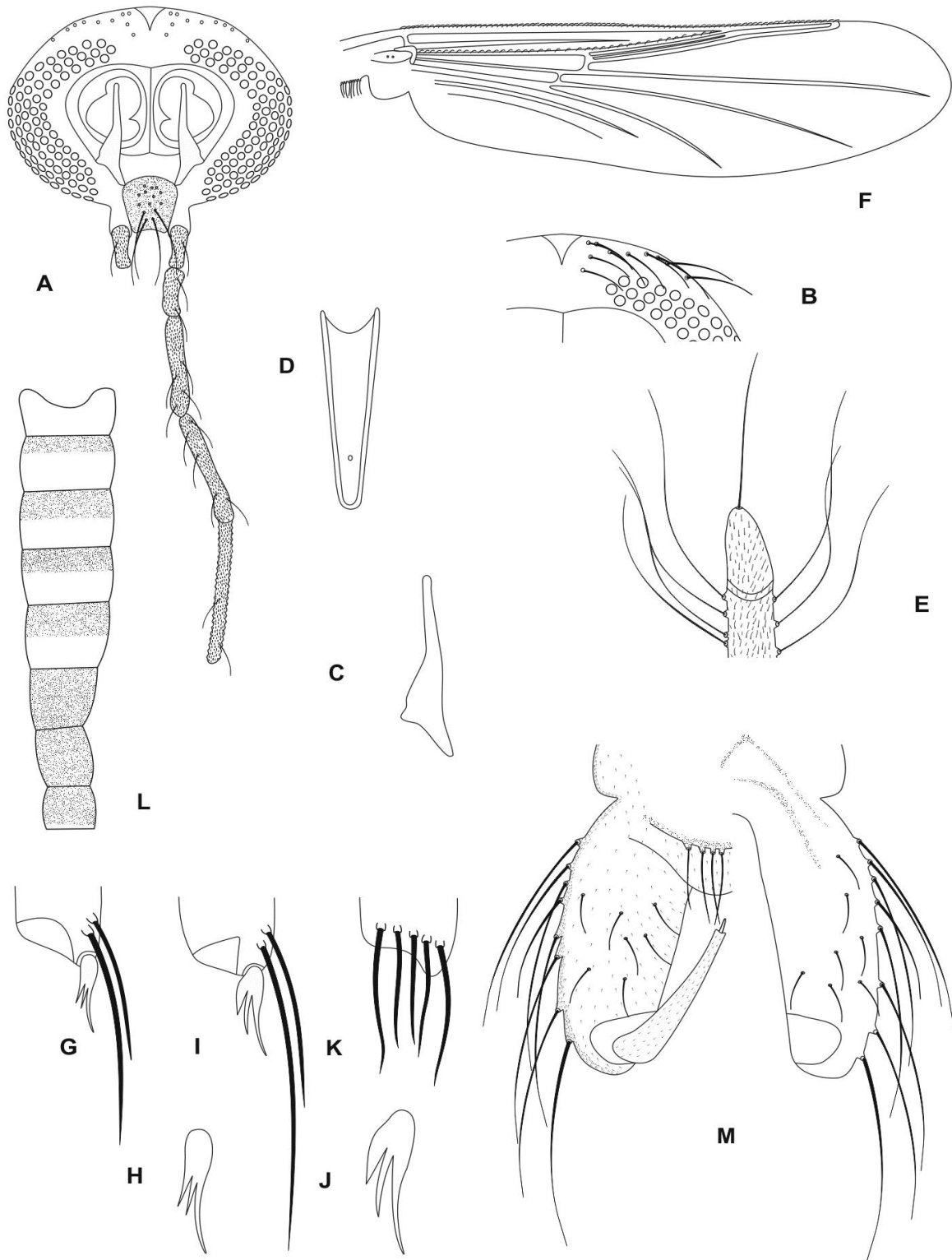
Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp brown. Thorax wholly brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Femur I–III pale brown. Tibia I brown, Ti II pale brown, Ti III pale brown with apex brown. Tarsomere 1–5 brown. Abdomen as in figure 83L. Hypopygium pale.

Head (Figs 83–E). Temporal setae 11–12, uniserial (Fig. 83B). Eye ratio 1.04–1.10. Tentorium (Fig. 83C) 157–166 μm long, stipes not measurable. Clypeus 71–73 (2) μm long, 62–66 μm wide at largest part, bearing 10–16 setae. Cibarial pump (Fig. 83D) with anterior margin concave, 154–193 μm long. Palpomere lengths 1–4 (in μm): 26–38; 50–62; 116–131; 124–145. Antenna with 14 flagellomeres, AR 1.21–1.32, flagellum 797–856 μm long, diameter of pedicel 144–165 μm , apical setae single (Fig. 83E).

Thorax. Anteprenotum with 4 lateral setae. Acrostichals 54–62, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 21–29, irregularly biserial; prealars 12–13; supraalars 3. Anapleural suture ratio 0.45–0.54. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

Wing (Fig. 83F). Width 0.39–0.42 mm. Costa 1.10–1.18 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.06–0.07 mm long. VR 0.75–0.78. WW 0.30. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 83G–K). Fore leg: width at apex of tibia 37–38 μm (Fig. 83G), tibia with single, apical and



Figures 83A–M. *Labrundinia* sp. 24, adult male. **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Apex of fore tibia. **H.** Fore tibial spur. **I.** Apex of mid tibia. **J.** Mid tibial spur. **K.** Apex of hind tibia with comb. **L.** Abdominal coloration pattern, dorsal aspect. **M.** Hypopygium, left: ventral aspect, right: dorsal aspect.

pectinate spur 15–19 μm long (Fig. 83H), with three teeth and two preapical setae; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 42–44 μm long (Fig. 83I), tibia with single, apical and pectinate spur 19–29 μm long with three teeth (Fig. 83J) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 38–44 μm long (Fig. 83K), tibia without spur; comb with 5 setae; Ta_{1-4} each with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table LVI.

Table LVI. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 24, male ($n = 1-2$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	513–519	466–475	321–344	211–231	143–147
p ₂	663–694	443–456	625–680	294–313	156–199
p ₃	531–581	487–669	581–694	281	147
	ta ₄	ta ₅	LR	BV	SV
p ₁	106–110	73–75	0.68–0.69	2.25–2.50	2.79–2.96
p ₂	100–127	75–78	1.37–1.54	2.46–2.76	1.53–1.84
p ₃	97	78	0.97	2.83	1.94

Hypopygium (Fig. 83M). Tergite IX arched, with 9–12 dorsal setae. Anal point trapezoidal, apical edge slightly concave. Phallapodeme 54–60 μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 151–153 μm long, 65–67 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.27–2.34. Gonostylus simple and slender, 84–89 μm long; megaseta 11–14 μm long. HR 1.69–1.80. HV 2.90–3.20.

Adult female ($n = 2$ unless otherwise stated)

Size. Total length 1.51–1.69 mm. Wing length 1.16 mm. Total length/wing length 1.30–1.46. Wing length/length of profemur 1.22–1.35.

Coloration. Head dark brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax wholly brown; antepnotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs brown. Femur I–III pale brown. Tibia I brown, Ti II pale brown, Ti III pale brown with apex brown. Tarsomere 1–5 brown. Abdomen pale brown, with dark brown transverse bands near proximal margin, not much distinguishable. Seminal capsules brown.

Head. Temporal setae 12–14, uniserial. Eyes ratio 1.38 (1). Tentorium 132–149 μm long, stipes not measurable. Clypeus 84–91 μm long, 68–69 μm wide at largest part, bearing 16–18 setae. Cibarial pump

as in male, 198–205 µm long. Palpomere lengths 1–5 (in µm): 34–45; 51–60; 117–118; 198 (1); 218 (1). Antenna with 11 flagellomeres, AR 0.35–0.40, flagellum 438–452 µm long, diameter of pedicel 58–63 µm.

Thorax. Anteprenotum with 4 lateral setae. Acrostichals 50–52, irregularly biserial, starting close to anteprenotum; dorsocentrals 34–36, irregularly biserial; prealars 12; supraalars 3. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.43–0.46 mm. Costa 0.99–1.00 mm long, not produced beyond R_{4+5} . R_{2+3} present. Base of radial sector 0.04–0.05 mm long. VR 0.62–0.75. WW 0.37–0.39. Brachiolum with 2 setae. Squama setiferous.

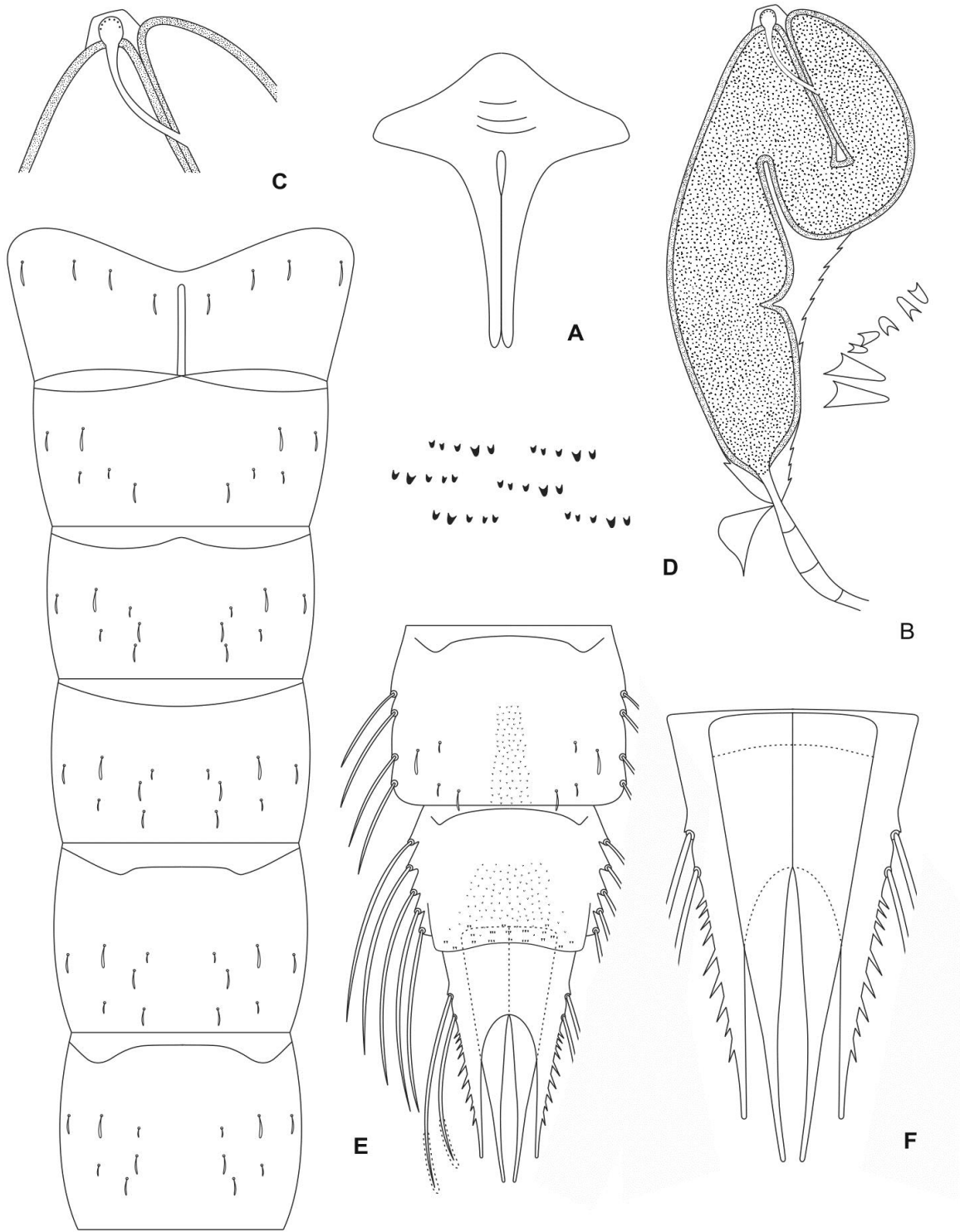
Legs. Fore leg: width at apex of tibia 38 µm, tibia with single, apical and pectinate spur 18–23 µm long, with four teeth and two preapical setae; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 44–56 µm, tibia with single, apical and pectinate spur 14–25 µm long, with four teeth and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 44 µm, tibia without spur; comb with 5 setae; Ta_{1-4} each with two preapical pseudospurs. Lengths and proportion of leg segments as in Table LVII.

Table LVII. Lengths (in µm) and proportions of leg segments in *Labrundinia* sp. 24, female (n = 1–2).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	318–375	381	300	175	113
p ₂	594–600	475–481	588–594	238–244	125–138
p ₃	506–513	581–625	575	244	131
	ta ₄	ta ₅	LR	BV	SV
p ₁	88	69	0.79	2.38	2.52
p ₂	88–113	69–75	1.23–1.24	3.02–3.10	1.81–1.83
p ₃	94	81	0.99	3.02	1.89

Genitalia. Gonapophysis VIII broadly rounded, 51–57 µm long. Coxosternapodeme 63–81 µm long. Postgenital plate rounded. Cerci oval-quadrangle, 54–66 µm long and 17–18 µm wide; with 8–10 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 97–117 µm. Seminal capsules oval with conical shaped necks, length 40–48 µm, maximum width 29–45 µm. Length ratio SCa/No 0.41.

Pupa (n = 5 unless otherwise stated)



Figures 84A–F. *Labrundinia* sp. 24, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Spines on segment II. **E.** Abdominal segments with chaetotaxy, dorsal aspect. **F.** Anal lobe and male genital sac, ventral aspect.

Size. Male abdomen 2.26–2.44 (3) mm long; female abdomen 2.21–2.33 (2) mm long.

Coloration. Exuviae and thoracic horn brown.

Cephalothorax (Figs 84A–C). Frontal apotome as in figure 84A. Wing sheath smooth 0.80–0.93 mm long. Thoracic horn 231–300 μm long and 100–131 μm wide (Fig. 84B), preapical indentation deep, THR 2.21–2.40. Membranous preapical papilla 28–40 μm long (Fig. 84C), PTH 0.09–0.13, aeropyle tube simple, elongated and slender, 45–72 (4) μm long; plastron plate reduced. Horn sac moderately developed. Reticulation of respiratory atrium indistinct, external membrane with pale spinules. Basal lobe wedge-shaped. Thoracic comb with 7–9 conical teeth.

Abdomen (Figs 84D–F). Tergite I with scar, 130–138 (4). T I–VI without shagreen, VII–VIII with shagreen medial basal concentrated. Segment II with prominent spines (Fig. 84D). Abdominal chaetotaxy as in figure 84E. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 331–344 (4) μm long and 194–213 (4) μm wide (Fig. 84F), outer margins sclerotized, with 10–12 spines, longest spine 18–26 μm long, inner margins of lobes membranous. ALR 1.56–1.75 (4). Genital sac elongated, reaching much beyond apex of anal lobe.

4th instar larva (n = 4 unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow to pale brown except for bifid claw brown.

Head (Fig 85A). Length 506–600 μm , 319 (3) μm wide. Surface smooth; lateroventral spine group absent; posteroventral spine group present with 14–22 (3) small spines; cephalic index 0.49–0.63. Chaetotaxy as in figure 85A.

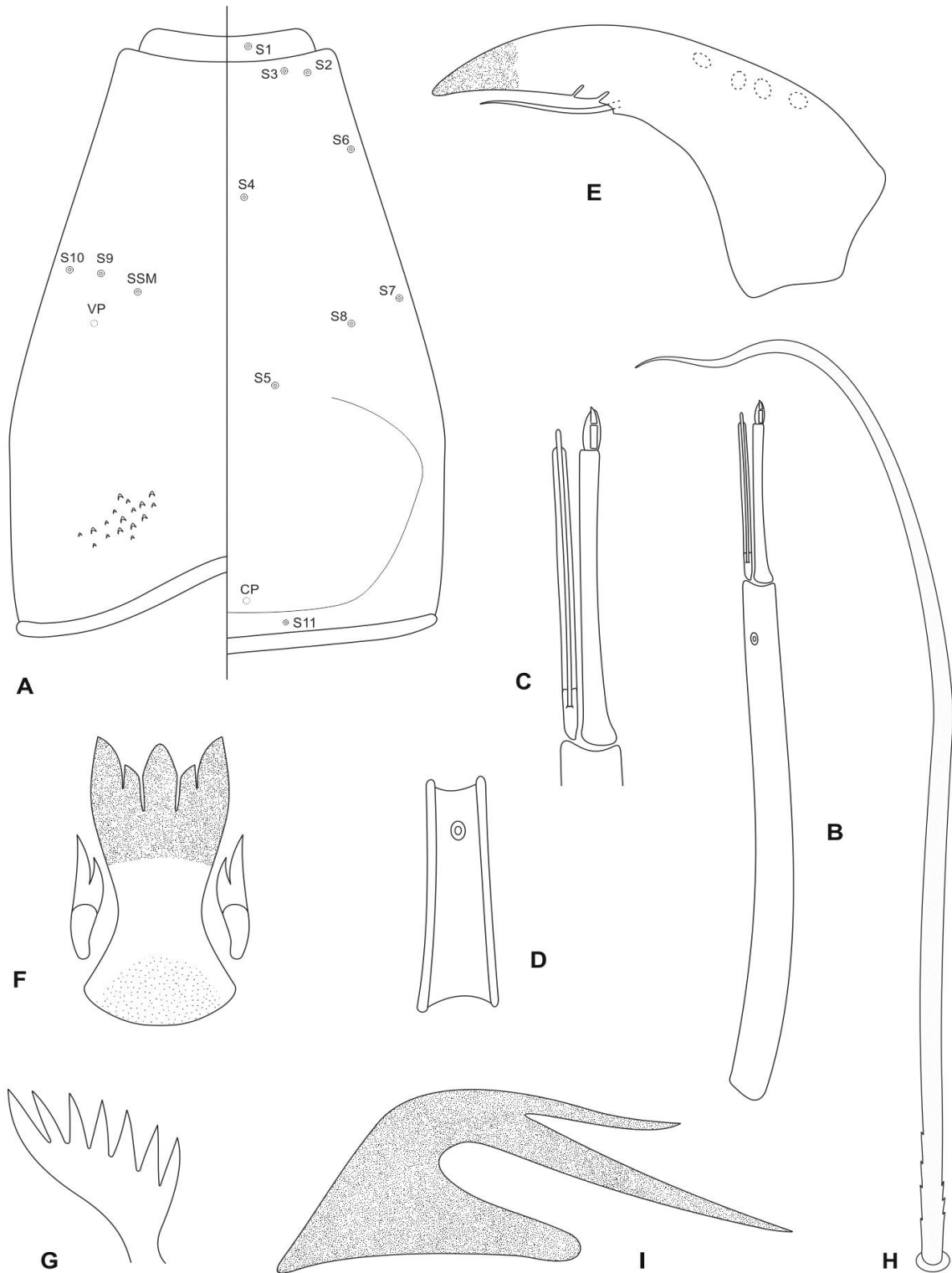
Antenna (Figs 85B–C). Length 320–335 (3) μm , A₁ 240–256 (3) μm long, with ring organ placed 217–228 (2) μm from base, A₂ 65–72 μm long. AR 3.02–3.22. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 85D). Basal palp segment 31–33 μm long and 7–8 μm wide, with ring organ 20–26 μm from base. PR 3.94–4.34. APR 7.30–7.74.

Mandible (Fig. 85E). Length 63–80 μm , with 3 lateral setae. Sensillum campaniformium 50–59 μm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 3.39–3.80 (3).

Mentum and M appendage. Dorsomenta teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 85F–G). Ligula 61–73 μm long, 28–32 μm wide, with row of 5 teeth. IO 0.84–0.93, MO 0.99. Paraligula bifid, 27–31 μm long, inner tooth 20–27 μm long, shorter than outer



Figures 85A–I. *Labrundinia* sp. 24, larva. **A.** Head with chaetotaxy, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paralingula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod

tooth. Pecten hypopharyngis with 7 teeth almost equal in size.

Body (Figs 85H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 192–213 (3) μm long, 31–32 (3) μm wide, with 7 anal setae 381–184 (2) μm long. L/W 5.95–6.80 (3). Supraanal seta well developed. Anal tubules 351 (1) μm long. Posterior parapod 451 (1) μm long; subbasal seta simple, without conspicuous spines (Fig. 85H); parapod apex with numerous simple claws, without serrated claw; bifid claw with U-shaped lower indentation (Fig. 85H). B/C 1.23–1.26 μm long.

Remarks: This is the *Labrundinia* sp. 11 of Trivinho-Strixino (2011) (larvae identification key).

Labrundinia spec. nov. 25 (Figs 86–88)

Material examined

Type: Holotype male with pupal and larval exuviae, Brazil: **São Paulo**, Luiz Antônio, Beija-Flor reservoir, viii.2011, S. Wiedenbrug (MZUSP). Paratypes: 1 female with pupal and larval exuviae same data as holotype except for São Carlos, Mayaca lake, 11.iv.2011, F. L. Silva.

Diagnostic characters

Labrundinia sp. 25 differs from other *Labrundinia* species by the combination of the following characters.

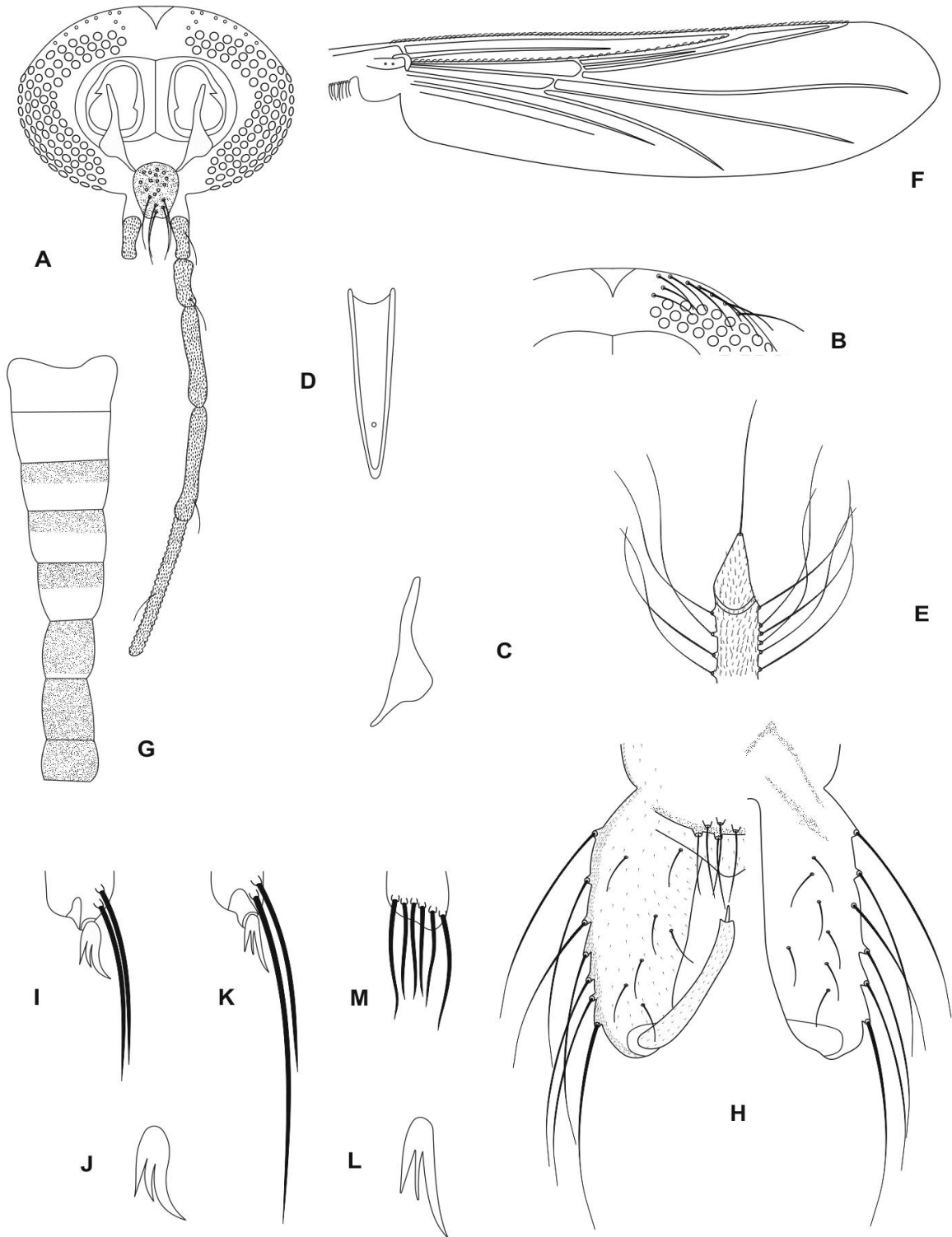
Male: R_{2+3} present; abdominal tergite I–II pale, T III–VI with continuous brown transverse band near proximal margin, T VII–VIII; hypopygium pale, sternapodeme with moderate anterior process. **Pupa:** thoracic horn 9-shaped; shagreen on segment II with multi-branched spines; apical spines 46–60 μm long; genital sac reaching much beyond apex of the anal lobe. **Larva:** surface smooth, lateroventral spine group present, but weakly developed, with 3 spines; posteroventral spine group absent; ligula stout, 88–89 μm long; subbasal seta simple; bifid claw, with V-shaped lower indentation.

Description

Adult male ($n = 1$)

Size. Total length 2.57 mm. Wing length 1.35 mm. Total length/wing length 1.90. Wing length/length of profemur 3.05.

Coloration. Head brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp



Figures 86A–M. *Labrundinia* sp. 25, adults male (A–H) and female (I–M). **A.** Head, frontal view. **B.** Temporal setae. **C.** Tentorium. **D.** Cibarial pump. **E.** Apex of antenna. **F.** Wing. **G.** Abdominal coloration pattern, dorsal aspect. **H.** Hypopygium, left: ventral aspect, right: dorsal aspect. **I.** Apex of fore tibia. **J.** Fore tibial spur. **K.** Apex of mid tibia. **L.** Mid tibial spur. **M.** Apex of hind tibia with comb.

brown. Thorax wholly brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots, veins pale brown and macrotrichia on veins. Legs brown. Femur I–III pale brown. Tibia I brown, Ti II pale brown, Ti III pale brown with apex brown. Tarsomere 1–5 brown. Abdomen as in figure 86G. Hypopygium pale.

Head (Figs 86–E). Temporal setae 11, uniserial (Fig. 86B). Eye ratio 1.01. Tentorium (Fig. 86C) 141 μm long, stipes not measurable. Clypeus 71 μm long, 77 μm wide at largest part, bearing 14 setae. Cibarial pump (Fig. 86D) with anterior margin concave, 191 μm long. Palpomere lengths 1–3 (in μm): 33; 55; 123. Antenna with 14 flagellomeres, AR 1.42, flagellum 838 μm long, diameter of pedicel 90 μm , apical setae single (Fig. 86E).

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 48, biserial, diverging evenly posteriorly, starting close to anteprenotum and almost reaching scutellum; dorsocentrals 18, irregularly uniserial; prealars 8; supraalars 3. Anapleural suture ratio 0.28. Scutellum with 8 setae across disc and numerous fine anterior setae. Anepisternals, preepisternals and postnotals absent.

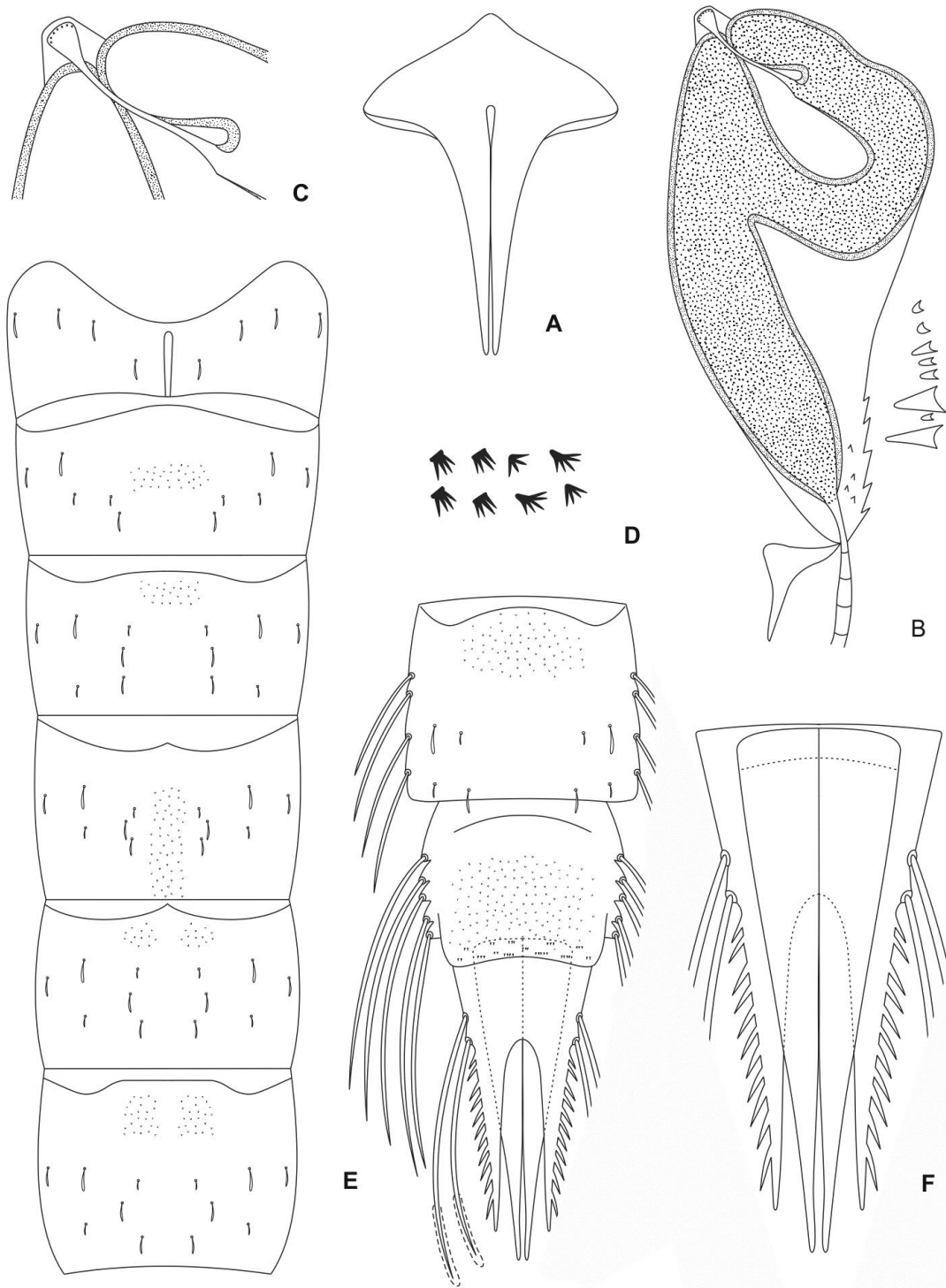
Wing (Fig. 86F). Width 0.40 mm. Costa 1.16 mm long, not produced beyond R_{4+5} , ending very slightly beyond tip of M_{3+4} . R_{2+3} present. Base of radial sector 0.06 mm long. VR 0.76. WW 0.29. Brachiolum with 2 setae. Squama setiferous.

Legs. Fore leg: width at apex of tibia 33 μm , tibia with two preapical setae, apical spur unobserved; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 32 μm long, tibia with two preapical setae, apical spur unobserved; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 39 μm long, tibia without spur; comb with 6 setae; Ta_{1-4} each with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table LVIII.

Table LVIII. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 25, male (n = 1).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	442	449	355	242	167
p ₂	603	435	641	289	190
p ₃	514	557	614	273	185
	ta ₄	ta ₅	LR	BV	SV
p ₁	116	89	0.79	2.02	2.51
p ₂	132	84	1.48	2.42	1.62
p ₃	130	83	1.10	2.51	1.74

Hypopygium (Fig. 86H). Tergite IX arched, with 8 dorsal setae. Anal point rounded, apical edge slightly notched. Phallapodeme 47 μm long. Sternapodeme with moderate anterior process. Gonocoxite cylindrical,



Figures 87A–F. *Labrundinia* sp. 25, pupa. **A.** Frontal apotome. **B.** Thoracic horn with basal lobe and thoracic comb. **C.** Apex of thoracic horn showing preapical papilla. **D.** Spines on segment II. **E.** Abdominal segments with chaetotaxy, dorsal aspect. **F.** Anal lobe and male genital sac, ventral aspect.

158 μm long, 55 μm wide, with slightly concave inner margin; inferior volsella absent. GcR 2.87. Gonostylus simple and slender, 97 μm long; megaseta 15 μm long. HR 1.63. HV 2.65.

Adult female (n = 1)

Size. Total length 1.67 mm. Wing length 1.31 mm. Total length/wing length 1.27. Wing length/length of profemur 2.83.

Coloration. Head dark brown with dark brown occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax wholly brown; anteprenotum pale brown; supraalar callus brown. Wing membrane transparent without spots veins pale brown; macrotrichia on veins. Legs brown. Femur I–III pale brown. Tibia I brown, Ti II pale brown, Ti III pale brown with apex brown. Tarsomere 1–5 brown. Abdomen brown. Seminal capsules brown.

Head. Temporal setae 12, uniserial. Eyes ratio 1.71. Tentorium 149 μm long, stipes not measurable. Clypeus 97 μm long, 78 μm wide at largest part, bearing 24 setae. Cibarial pump as in male, 226 μm long. Palpomere lengths 1–4 (in μm): 49; 69; 155; 160. Antenna with 11 flagellomeres, AR 0.36, flagellum 494 μm long, diameter of pedicel 69 μm .

Thorax. Anteprenotum with 2 lateral setae. Acrostichals 56, irregularly biserial, starting close to anteprenotum; dorsocentrals 36, irregularly uniserial; prealars 13; supraalars 3. Scutellum with 8 setae across disc. Anepisternals, preepisternals and postnotals absent.

Wing. Width 0.49 mm. Costa 1.14 mm long, not produced beyond R_{4+5} . R_{2+3} present. Base of radial sector 0.04 mm long. VR 0.68. WW 0.38. Brachiolum with 2 setae. Squama setiferous.

Legs (Figs 86I–M). Fore leg: width at apex of tibia 31 μm (Fig. 86I), tibia with single, apical and pectinate spur 19 μm long (Fig. 86J), with four teeth and two preapical setae; Ta_{1-4} each without two preapical pseudospurs. Mid leg: width at apex of tibia 31 μm long (Fig. 86K), tibia with single, apical and pectinate spur 29 μm long with three teeth (Fig. 86L) and two preapical setae; Ta_{1-4} each with two preapical pseudospurs. Hind leg: width at apex of tibia 38 μm long (Fig. 86M), tibia without spur; comb with 5 setae; Ta_{1-4} each with two preapical pseudospurs. Legs with slender, hook-shaped claws. Pulvilli absent. Lengths and proportion of leg segments as in Table LIX.

Genitalia. Coxosternapodeme 95 μm long. Postgenital plate rounded. Cerci oval-quadrangle, 40 μm long and 14 μm wide; with 8 elongated setae. Labia with inconspicuous microtrichia. Notum length (from ramus forward) 105 μm . Seminal capsules oval with conical shaped necks, length 34 μm , maximum width 46 μm . Length ratio SCa/No 0.32.

Pupa (n = 2 unless otherwise stated)

Table LIX. Lengths (in μm) and proportions of leg segments in *Labrundinia* sp. 25, female ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃
p ₁	463	444	331	225	150
p ₂	675	569	725	306	163
p ₃	613	669	681	319	206
	ta ₄	ta ₅	LR	BV	SV
p ₁	131	75	0.75	2.12	2.74
p ₂	125	81	1.27	2.92	1.72
p ₃	138	81	1.02	2.64	1.88

Size. Male abdomen 2.46 (1) mm long; female abdomen 2.57 (1) mm long.

Coloration. Exuviae and thoracic horn brown.

Cephalothorax (Figs 87A–C). Frontal apotome as in figure 87A. Wing sheath smooth 0.83–0.81 mm long. Thoracic horn 284–338 μm long and 131–156 μm wide (Fig. 87B), preapical indentation deep, THR 2.16. Membranous preapical papilla 45–49 μm long (Fig. 87C), PTH 0.15–0.16, aeropyle tube simple, elongated and slender, 49–53 μm long; plastron plate reduced. Horn sac moderately developed. Reticulation of respiratory atrium indistinct, external membrane with pale spinules basally concentrated. Basal lobe wedge-shaped. Thoracic comb with 10–12 conical teeth.

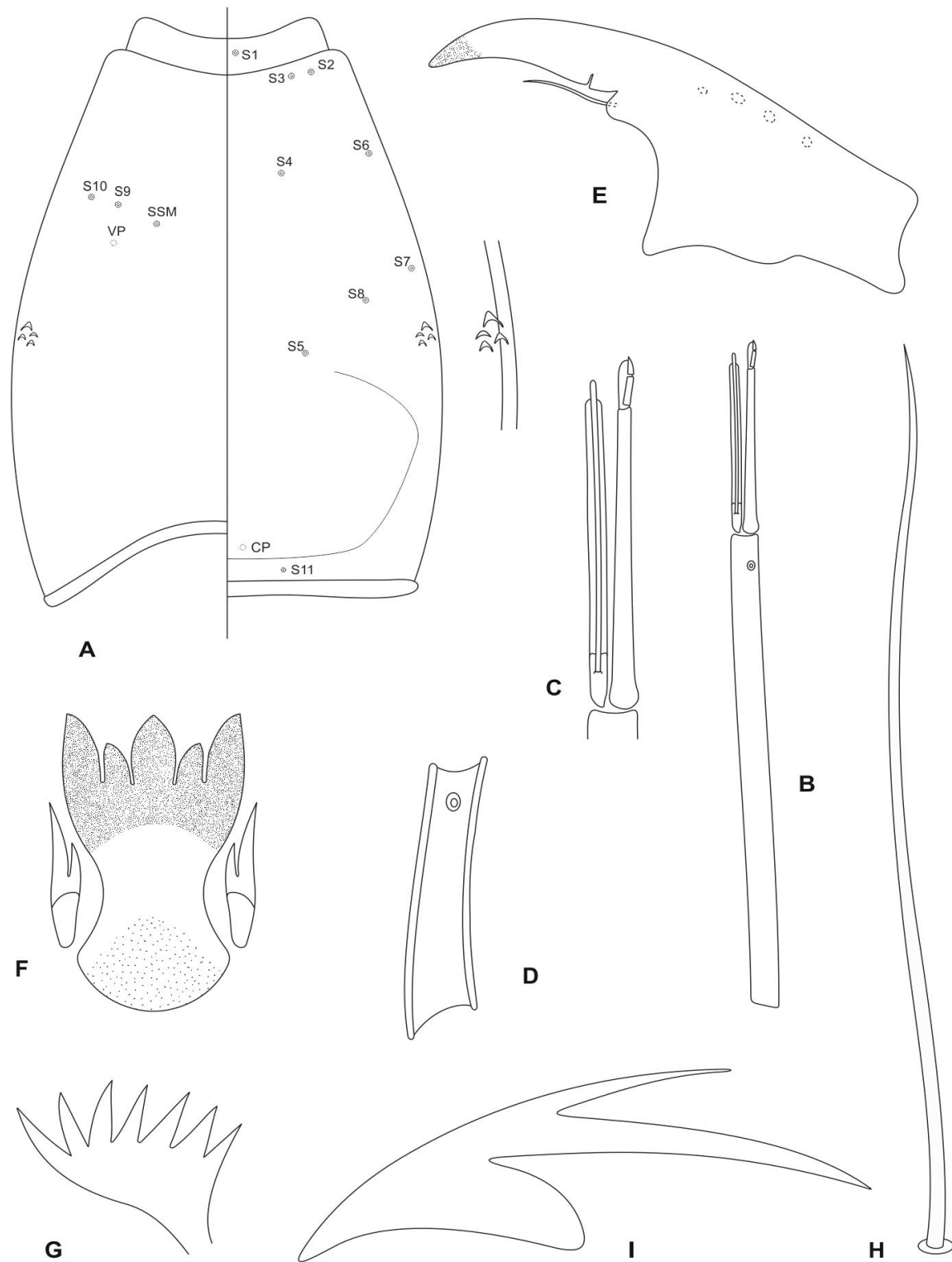
Abdomen (Figs 87D–F). Tergite I with scar, 106 (1). T I without shagreen, II–III, V–VI with a few spines medial apical concentrated, IV, VII–VIII with shagreen medial concentrated. Segment II with dense multi-branched spines (Fig. 87D). Abdominal chaetotaxy as in figure 87E. Abdominal segment VII with 4 lateral setae. A VIII with 5 lateral setae. Anal lobe 417–438 μm long and 178–188 μm wide (Fig. 87F), outer margins sclerotized, with 13 spines, longest spine 46–60 μm long, inner margins of lobes membranous. ALR 2.34. Genital sac elongated, reaching much beyond apex of anal lobe.

4th instar larva ($n = 2$ unless otherwise stated)

Coloration. Head pale yellow, without maculation; postoccipital margin brown. Second antennal segment brown; distal tooth of mandible and apex of ligula brown. Abdomen pale yellow; procercus and anal setae pale brown. Posterior parapod claws all pale yellow.

Head (Fig 88A). Length 650 (1) μm , 475 (1) μm wide. Surface smooth; lateroventral spine group present, but weakly developed, with 3 spines; posteroventral spine group absent; cephalic index 0.73 (1). Chaetotaxy as in figure 88A.

Antenna (Figs 88B–C). Length 390 (1) μm , A₁ 275 (1) μm long, with ring organ placed 247 (1) μm from



Figures 88A–I. *Labrundinia* sp. 25, larva. **A.** Head with chaetotaxy and detail of lateroventral spine group, left: ventral aspect, right: dorsal aspect. **B.** Antenna. **C.** Apex of antenna. **D.** Maxillary palp. **E.** Mandible. **F.** Ligula and paraligula. **G.** Pecten hypopharyngis. **H.** Subbasal seta of posterior parapod. **I.** Bifid claw of posterior parapod

base, A₂ 104 (1) µm long. AR 2.37. Blade longer than A₂ over-reached by accessory blade.

Maxilla (Fig. 88D). Basal palp segment 41–46 µm long and 8–10 µm wide, with ring organ 35 (1) µm from base. PR 4.13–5.63. APR 5.99 (1).

Mandible (Fig. 88E). Length 89–100 µm, with 3 lateral setae. Sensillum campaniformium 70 (1) µm from apex, basal tooth bifid, with seta subdentalis projecting from sloping end towards apical tooth, accessory tooth present, AMD 2.76 (1).

Mentum and M appendage. Dorsomental teeth reduced; pseudoradula uniformly granulate.

Hypopharyngeal complex (Figs 88F–G). Ligula 88–89 µm long, 43–44 µm wide, with row of 5 teeth. IO 0.90–0.93, MO 0.97–0.99. Paraligula bifid, 33 µm long, inner tooth 22–24 µm long, shorter than outer tooth. Pecten hypopharyngis with 7 teeth almost equal in size.

Body (Figs 88H–I). Without lateral fringe. Anterior parapods with simple claws. Procercus 228–256 µm long, 31–38 µm wide, with 7 anal setae unmeasured. L/W 6.67–7.28. Supraanal seta well developed. Anal tubules unmeasured. Posterior parapod unmeasured; subbasal seta simple, without spines (Fig. 88H); parapod apex with numerous simple claws, without serrated claw; bifid claw with V-shaped lower indentation (Fig. 88H). B/C 1.23–1.26 µm long.

REMARKS

The abdominal coloration pattern is the most important character used to distinguish the males of *Labrundinia*, whereas the thoracic horn shape and the length of genital sac are the main diagnostic characters for the pupae. Larvae can typically be distinguished by features on cephalic head such as maculation, and lateroventral and posteroventral groups of spines. Although these features appear to be consistent and sufficient to distinguish most *Labrundinia* species, it can be difficult to establish a reliable species threshold in cases where a considerable level of morphological variation is present within a species, or close related species exhibit high degree of similarity. Therefore, a few comments on selected species are considered helpful:

1. *Labrundinia floridana* – Roback (1971) placed *L. pilosella* as a senior synonym of *L. floridana* Beck and Beck due to the absence of distinguishable features on the adult males. Later, the comparison of the reared material of *L. pilosella* with that of *L. floridana* revealed consistent dissimilarities between the immatures. The issue arises from the fact that the larva described for *L. floridana* has a cephalic posterior margin noticeably darker, which does not agree with the material previously attributed to *L. pilosella*. We have examined material from both species, and, although no conclusion has been achieved, we believe to be dealing with two distinct species.

2. *Labrundinia maculata* – This species, which is known from Nearctic region, was placed as a junior synonym of Palaearctic *Labrundinia longipalpis* (Silva *et al.* 2011). The synonymization was based on the high level of similarities exhibited by all of the major life stages. The North American larvae differ from the European larvae by having surface of head capsule covered with spinules. Recently, the examination of extra material has also indicated that specimens ascribed to *L. maculata* may have a narrower brown transverse band on tergite VI than that of *L. longipalpis*. Despite these minor differences, we regard *L. longipalpis* as *L. maculata* as synonyms.

3. *Labrundinia tenata* – originally established based on specimens from Colombia, its immatures were described from material sampled in Southeast Brazil (Silva and Fonseca-Gessner 2009). This tentative association was based on abdominal coloration pattern and on the shape of the sternapodeme: rounded and without anterior process. Even though the Colombian specimens seem larger and darker, we have not been able to find any consistent difference that would justify a separate species name for the Brazilian specimens.

4. *Labrundinia* sp. 1 – described from Southeast Brazil, its pupal stage is characterized by a distinctive light bulb-shaped thoracic horn without preapical indentation. Jacobsen (2008) has illustrated and keyed the pupa of an undescribed species from Florida (USA), which appears to be identical to *L.* sp. 1. In rearing, the larva of this specimen was ascribed to the larval type called *L.* sp. B (Epler 2001), which also seems to match with *L.* sp. 1 except for having a bifid claw with V-shaped lower indentation. Although these specimens may represent one species, we hesitate to ascribe *L.* sp. 1 to *L.* sp. B on the absence of additional adult male characters.

ACKNOWLEDGMENTS

The authors extend their thanks to Augusto Siri, Broughton Caldwell, Caroline S. N. Oliveira, Charles Watson, John Epler, Humberto Fonseca Mendes, Martin Spies, Sofia Wiedenbrug and Susana Trivinho Strixino for providing us with important material. F. L. Silva received financial support from the National Council for Scientific and Technological Development (CNPq proc. 141092/2009–2). Financial support was also given by the Research Council of Norway (RCN), through a Personal Mobility Grant.

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CAPÍTULO II

DNA BARCODES FOR SPECIES DELIMITATION IN CHIRONOMIDAE (DIPTERA): A CASE STUDY ON THE GENUS *LABRUNDINIA*

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ABSTRACT

In this study, we analysed the applicability of DNA barcodes for delimitation of 79 specimens of 13 species of non-biting midges in the subfamily Tanypodinae from São Paulo State, Brazil. Our results support DNA-barcoding as an excellent tool for species identification and for solving taxonomic conflicts in this genus. Molecular analysis of COI gene sequences yielded taxon identification trees, supporting 13 cohesive species clusters, of which three similar groups were posteriorly linked to morphological variation at the larval and pupal stage, while another one anteriorly described by means of morphology was linked to molecular markers. We found a distinct barcode-gap, and in some species great interspecific pairwise divergences (up to 19.3%) were observed, which permitted identification of all analysed species. The results also indicated that barcodes can be used to associate life stages of chironomids since COI was easily amplified and sequenced from different life stages with universal barcode primers.

KEY WORDS: DNA-barcoding, DNA markers, chironomid, Tanypodinae, Neotropical, Brazil

INTRODUCTION

Non-biting midges of the genus *Labrundinia* (Diptera: Chironomidae: Tanypodinae) are minute dipterans (1–2.5 mm), currently comprising 14 species, of whose all except *L. longipalpis* (Goetghebuer,

1921) are known only from the Americas (Ashe and O'Connor 2009, Silva *et al.* 2011). The genus was erected by Fittkau (1962) based on the Palearctic species *Tanypus longipalpis*, which has immatures that live in a variety of unpolluted water bodies from small streams and ponds to lakes and bays (Silva *et al.* 2011). Morphology-based species identifications of *Labrundinia* usually are considerably difficult or even impossible, particularly for the adults. The few features used to separate the species show considerable intraspecific divergence. For example, male abdominal coloration, an important diagnostic species character, has an extensive variation within the genus.

The descriptions of *Labrundinia* species currently available are based mainly on males and do not include diagnostic characters for larvae and pupae at the species-level. Association of chironomid immature stages can be achieved by individual rearing of larvae and collecting cast larval and pupal skins to establish the associations between each life stage (e.g. Ekrem *et al.* 2007). This procedure is time-consuming, however, and is not always successful for species with special environmental requirements. Thus, life stage associations based on genetic similarity of short DNA fragments (so-called DNA-barcodes) has become increasingly popular. Several studies have recognized the benefit of short DNA sequences in associating immature stages with adult stages (e.g. Hebert *et al.* 2004a, Thomas *et al.* 2005, Ekrem *et al.* 2010a, Stur and Ekrem 2011, Silva *et al.* 2012). DNA barcoding previously has been used to differentiate six Australian species within a genus of the subfamily Tanypodinae (*Procladius*, Carew *et al.* 2011).

Tanypodinae is the third most diverse subfamily after Chironominae and Orthocladiinae, respectively (Ashe and O'Connor 2009). It was established by Thienemann and Zavřel (1916) based on the immature stages and its monophyly is strongly supported, with Podonominae as its sister-group (Cranston *et al.* 2012). Larvae of *Labrundinia* species are typical of Tanypodinae, which are often reported as having predatory habits, comparative morphological studies on larval head capsules (Gouin 1959, Bryce and Hobart 1972) indicate that the features distinguishing the Tanypodinae larvae from those of the other subfamilies were regarded as adaptations for predation.

The method of DNA barcoding employs standardized genomic fragments to enable species identification and discovery of cryptic taxa (Hebert *et al.* 2003, Kress *et al.* 2005, Savolainen *et al.* 2005). Supporters of this technique argue that a short standardized fragment of DNA can be used to recognize taxa as well as increase the speed, objectivity, and efficiency of species identification (Meyer and Paulay 2005). Initial tests of genetic barcoding using mitochondrial markers on animals have shown that a 650-bp fragment of the mitochondrial gene, cytochrome c oxidase subunit I (COI) is usually effective as a barcode sequence, providing more than 95% species-level resolution (Hebert *et al.* 2003, 2004a, Hajibabaei *et al.* 2006, Smith *et al.* 2006). Nevertheless, despite these highly encouraging prerogatives, the success rate of this approach relies particularly on the delineation between intra- and interspecific

genetic divergence (Meyer and Paulay 2005, Hebert *et al.* 2004b). On the other hand, intra- and interspecific DNA sequence variation depends directly on the evolutionary and biogeographical relationships of the group in question, which might differ severely from one population or region to another (Kirkendale and Meyer 2004).

Regarding identification of insects, DNA barcoding seems to experience a number of drawbacks (Virgilio *et al.* 2010). Recent speciation, incomplete lineage sorting and the regular interspecific hybridization in numerous insect taxa, as well their often high degree of infection by endosymbiotic bacteria such as *Wolbachia* (Funk and Omland 2003, Whitworth *et al.* 2007, Footitt and Adler 2009) may all negatively affect the performance of DNA barcoding in insects (Virgilio *et al.* 2010). Undoubtedly, and perhaps more importantly, the reliability of DNA barcoding in insects might be challenging since this group include several hundreds of thousands of described species and probably millions still undescribed (Footitt and Adler 2009). This enormous species richness definitely affects the comprehensibility of the DNA barcode sequences libraries to adequately represent the immense insect taxonomic diversity (Virgilio *et al.* 2010).

In this study, we evaluate the feasibility of using DNA barcodes for species delimitation in *Labrundinia* using the standard barcode-fragment of COI amplified with the universal primers of Folmer *et al.* (1994), LCO1490 and HCO2198. We also investigate whether partial COI gene sequences can be employed to associate life stages of species within this genus. We choose to focus on species of *Labrundinia* because this genus currently is being revised by the first author and material of numerous species is available from different localities in São Paulo State, Brazil. All unnamed species referenced in this study will be described in a future taxonomic manuscript, and published elsewhere.

MATERIAL AND METHODS

The taxa included in this study were selected to represent as many of the known morphotypes in *Labrundinia* as possible. Field work was conducted in São Paulo State, Brazil, without any design to test sampling regime or spatial distribution. Larvae and pupae were collected from aquatic systems <200km apart, using a hand-net. Some larvae and pupae were isolated in small vials covered with nylon screen and reared in the laboratory to obtain emerged adults. Various aquatic macrophyte species were collected and placed in a plastic tray in order to obtain adults emerged. Immature chironomids were preserved in absolute ethanol while imagines were kept in slightly dilute ethanol (~80–85%) to avoid breakage. More than one life stage was sequenced from all the included species (Appendix). Different morphotypes were recognized based on variation in all observable morphological traits such as coloration, genital structures, shapes of pupal thoracic horn and larval claws.

Ethanol-preserved specimens were dissected under a stereo microscope, and the wings, one pair of legs and the antennae were mounted in Euparal on microscope slides. DNA was extracted from the remaining body parts in a buffered solution with the enzyme proteinase-K. DNA extraction, PCR and bi-directional sequencing were performed at the Canadian Centre for DNA Barcoding (CCDB), using standard protocols (<http://ccdb.ca/page/research/protocols>). Other specimens were analysed in the molecular lab at the Museum of Natural History in Trondheim, where DNA extraction followed the tissue protocol using a GeneMole robot (MoleGenetics). Each PCR was made with addition of 2 µl DNA template, 2.5 µl 10× Ex Taq Buffer, 2.0 µl MgCl₂ in 25 µM concentration, 2 µl of dNTPs in 10 mM concentration, 1 µl of each of the suggested standard barcode primers (Folmer *et al.* 1994) LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAA TCA-3') in 10 µM concentration, 0.2 µL HotStarTaq DNA Polymerase (Qiagen), and 14.3 µL of ddH₂O for a total reaction volume of 25 µl. Amplifications for the COI region were performed in a thermocycler with an initial denaturation step of 95 °C for 15 min, followed by 5 cycles of 94 °C for 30 s, 45 °C for 30 s, 72 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 51 °C for 30 s, 72 °C for 1 min, and one cycle at 72 °C for 5 min, then held at 4 °C.

The PCR products were purified using ExoSAP-IT (USB Products). Purified products were sequenced in both directions using BigDye (Perkin-Elmer) termination reactions and analysed on ABI 3730 genetic analysers. Sequences were assembled and edited using DNA BASER Sequence Assembler 3.2.4 (Heracle Software, Germany), checked for stop-codons and aligned on amino acids using *default ClustalW options* (Thompson *et al.* 1999) as implemented in MEGA 5.03 (Tamura *et al.* 2011). The alignment was trivial as no indels were observed in the sequences.

Neighbour-joining (NJ) and maximum likelihood trees (ML) were produced in MEGA 5.03 (Tamura *et al.* 2011), using Kimura 2-parameter (K2P) (Kimura 1980) and GTR+G+I (Lanave *et al.* 1984) models, respectively. Bootstrap-analysis used 1000 pseudoreplicates (Felsenstein 1985). Pairwise sequence divergences within and between genetic clusters were calculated under a K2P model in MEGA. The analysis of intra- and interspecific genetic distances were based on the K2P (Kimura 1980) and maximum composite likelihood (Kimura 1980) models and were as the best-fit models of nucleotide substitution and base frequencies also calculated with MEGA 5.03. Intra- and interspecific distances were plotted as a histogram using PAST version 2.14b (Hammer *et al.* 2001). A summary of species sequenced and their respective voucher and GenBank accession numbers is provided in Table 1. All specimens and DNA barcodes are deposited in the Barcode of Life Data Systems (boldsystems.org, Ratnasingham and Hebert 2007) in the project Neotropical Tanypodinae (NEOTA).

RESULTS

Partial COI gene-sequences were obtained from 79 specimens of 13 species (Appendix). The Folmer-primers worked well on templates from all tested species and no difference was observed in amplification or sequencing success with regard to different life stages. The aligned sequences in the majority (98.7%) were 657 bp long with 209 variable sites (31.8%), of which 200 (95.6%) were potentially parsimony-informative. Most variable sites occurred in the third codon-position (Table 1). The sequences were heavily AT-biased, specifically in third position which exhibited a combined average AT-composition of 89.9% (Table 1). A hierarchic likelihood ratio test of aligned sequences in MEGA 5.03 returned the general time reversible model with invariable sites and gamma correction for rate heterogeneity (GTR+G+I) as the best model (-lnL = 4110.404, BIC = 9991.709, AIC = 8531.870).

Table 1 – Variable and informative sites and average nucleotide composition in the analyzed COI gene sequences.

Nucleotide position	% Variable sites	% Informative sites	% Adenine	% Cytosine	% Guanine	% Thymine
1 st	13.69	12.5	26.6	15.6	30.7	26.8
2 nd	0.91	0.5	13.6	26.5	15.9	43.7
3 rd	80.8	87	45.6	7.20	2.71	44.3
All	31.8	95.6	28.6	16.4	16.5	38.3

So far we have come across 12 new, undescribed *Labrundinia* species. In addition, *Labrundinia tenata*, previously described by means of morphology (Roback 1987, Silva and Fonseca-Gessner 2009) was successfully sequenced. DNA barcodes were produced from all life stages.

The pairwise distances for the analysed *Labrundinia* specimens, produced by both K2P and maximum composite likelihood models, showed distinctly larger interspecific than intraspecific divergences. Thus, there were distinct barcode gaps (Fig. 1). There were no identical gene sequences between species, and all species were distinguishable by genetic distances. Average intra- and interspecific K2P-distances for all analysed *Labrundinia* species were 0.91 % and 14.53%, respectively. Maximum intraspecific divergence was observed in *Labrundinia* sp. 2 (4.96 %), followed by *Labrundinia* sp. 25 (4.03 %) and *Labrundinia* sp. 10 (3.55 %). Lowest interspecific distances were found between *Labrundinia* sp. 10 and *Labrundinia* sp. 15 (average 11.8%), followed by *Labrundinia* sp. 10 and *Labrundinia* sp. 25 (average 12.1%). Intra- and interspecific distances produced by maximum composite likelihood model provided similar results.

The specimens identified as *Labrundinia tenata* were divided into two separate barcode clusters. Nucleotide sequences of these specimens differed by minimum 2.5% (Table S1) and in up to 22 nucleotide

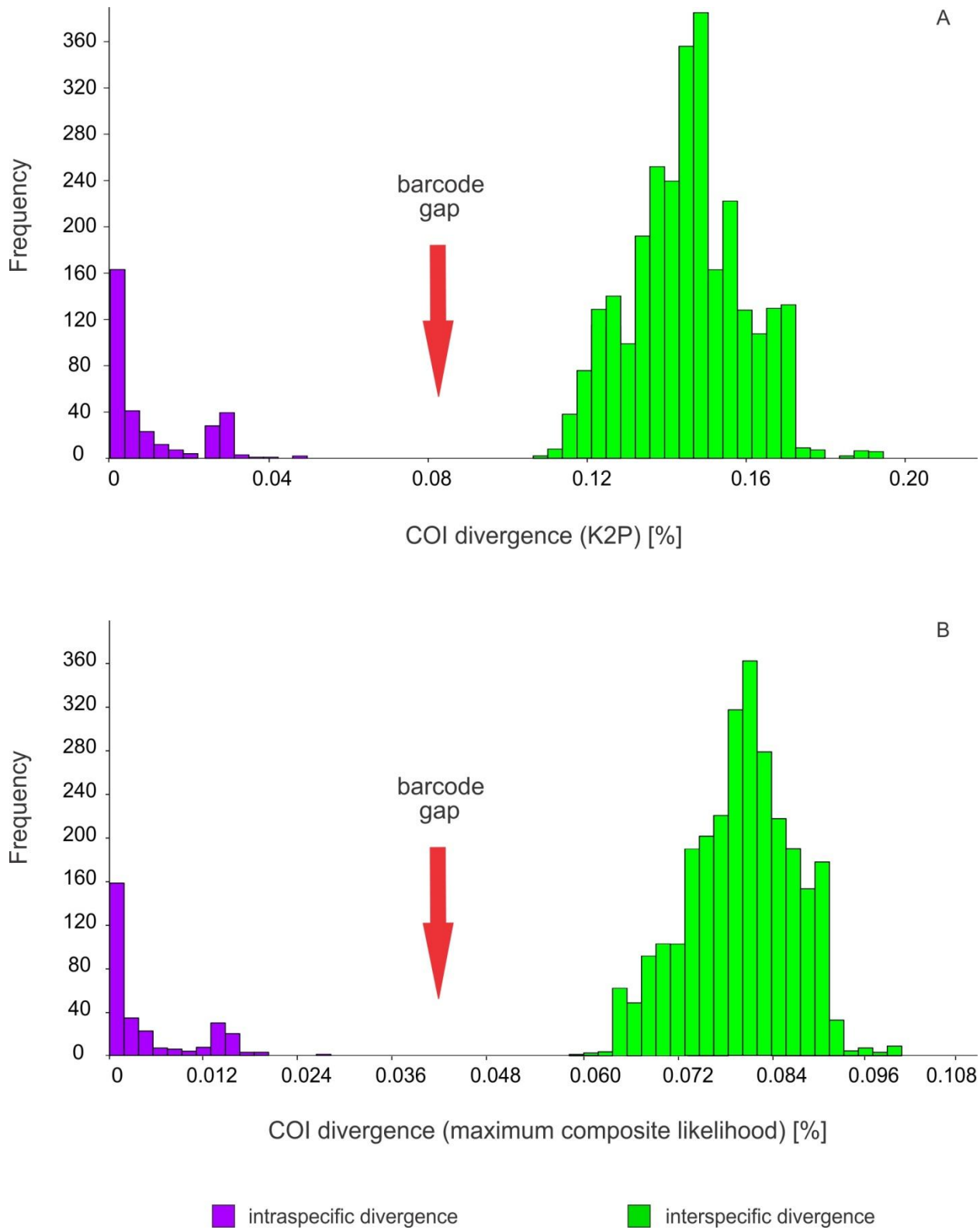


Figure 1. Histogram of the calculated intra- and interspecific distances of partial COI sequences for the analysed *Labrundinia* specimens: A. Kimura 2-parameter. B. Maximum composite likelihood.

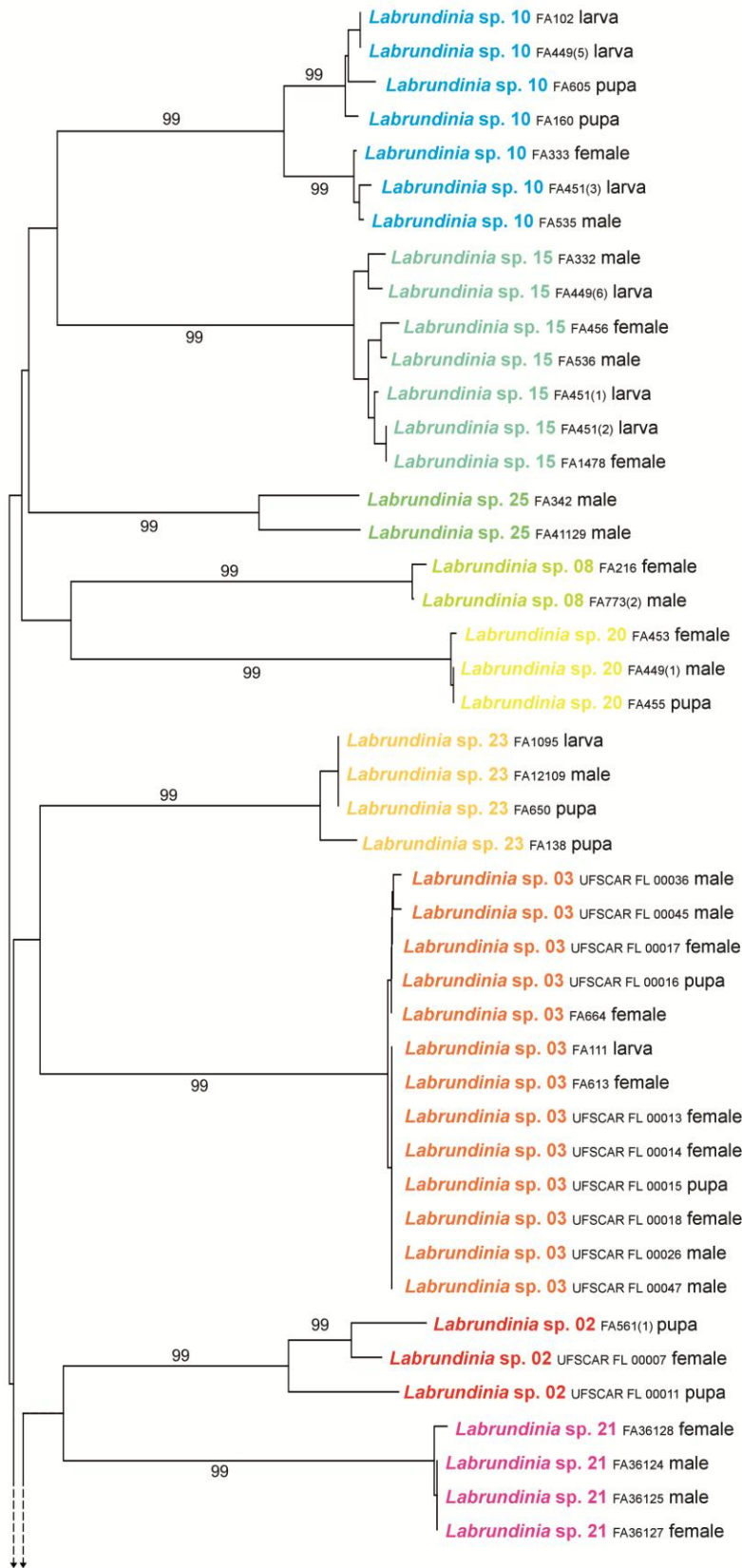
sites, but we could not find morphological characters that differentiate specimens belonging to these clusters. The diverging specimens were collected from four ecosystems in São Paulo State, three in São Carlos municipality and one in Luiz Antonio municipality (Appendix). All specimens from one cluster were collected at the São Carlos Ecological Park, so in a sense there was some geographical structure in the clustering even though the localities in São Carlos are not more than 8.5 km from each other. Equally, the specimens identified as *Labrundinia* sp. 10 also formed two distinct groups, even if no morphological differences could be observed. Nucleotide sequences of these specimens diverged by minimum 2.7% (Table S1) and in up to 25 nucleotide sites. In this case, all of these diverging specimens were collected in the same geographical locality (Ecological Park/Monjolinho stream).

DNA barcode sequences also indicated that three of the clusters were related species. This was subsequently confirmed by morphological analysis of the immature stages: *Labrundinia* sp. 8 resembled *Labrundinia* sp. 6 in the presence of a serrated claw in the larva and in the male abdominal coloration, but differed in the pupal thoracic horn ratio (length/width) that was higher in *Labrundinia* sp. 8. Nucleotide sequences of these specimens differed by minimum 15.5% (Table S1) and in up to 90 nucleotide sites. *Labrundinia* sp. 23 and *Labrundinia* sp. 25 were similar to *Labrundinia* sp. 2 in the male abdominal coloration, but diverged distinctly in the size and shape of the pupal thoracic horn, and in the number of spines that constitutes the lateroventral spine group (LVS) of larval head. Nucleotide sequences of these specimens differed by minimum 12.6% (Table S1) and in up to 133 nucleotide sites.

A taxon identification tree (ID-tree) demonstrated that a substantial barcode divergence exists between all the analysed species of *Labrundinia*. For easier comparison with other DNA-barcode studies, we here present only the taxon ID-trees produced by NJ analysis, using the K2P model (Fig. 2). NJ analyses, using maximum composite likelihood yielded identical ID-trees, whereas ML trees (using K2P and GTR+G+I models of substitution) only differ in the placement of *Labrundinia* sp. 25, which groups with *Labrundinia* sp. 10 as its closest cluster. Bootstrap support exhibited minor variation among all analyses, but were always 98% or higher for all species clusters.

DISCUSSION

Perhaps the most critical parameter for the success of large scale DNA-barcoding are highly functional primers leading to successful PCR (Hajibabaei *et al.* 2005). We amplified partial COI gene sequences from 13 *Labrundinia* species with an amplification success rate of 100%, exceeding the desirable 95% limit for large scale DNA-barcoding (Hajibabaei *et al.* 2005) DNA barcoding focuses on species delineation and identification, and not on phylogenetic inference. Moreover, COI has been found to be too variable for reliable phylogenetic analysis in Chironomidae (Ekrem *et al.* 2007; Ekrem *et al.*



a

Fig. 1 (continued)

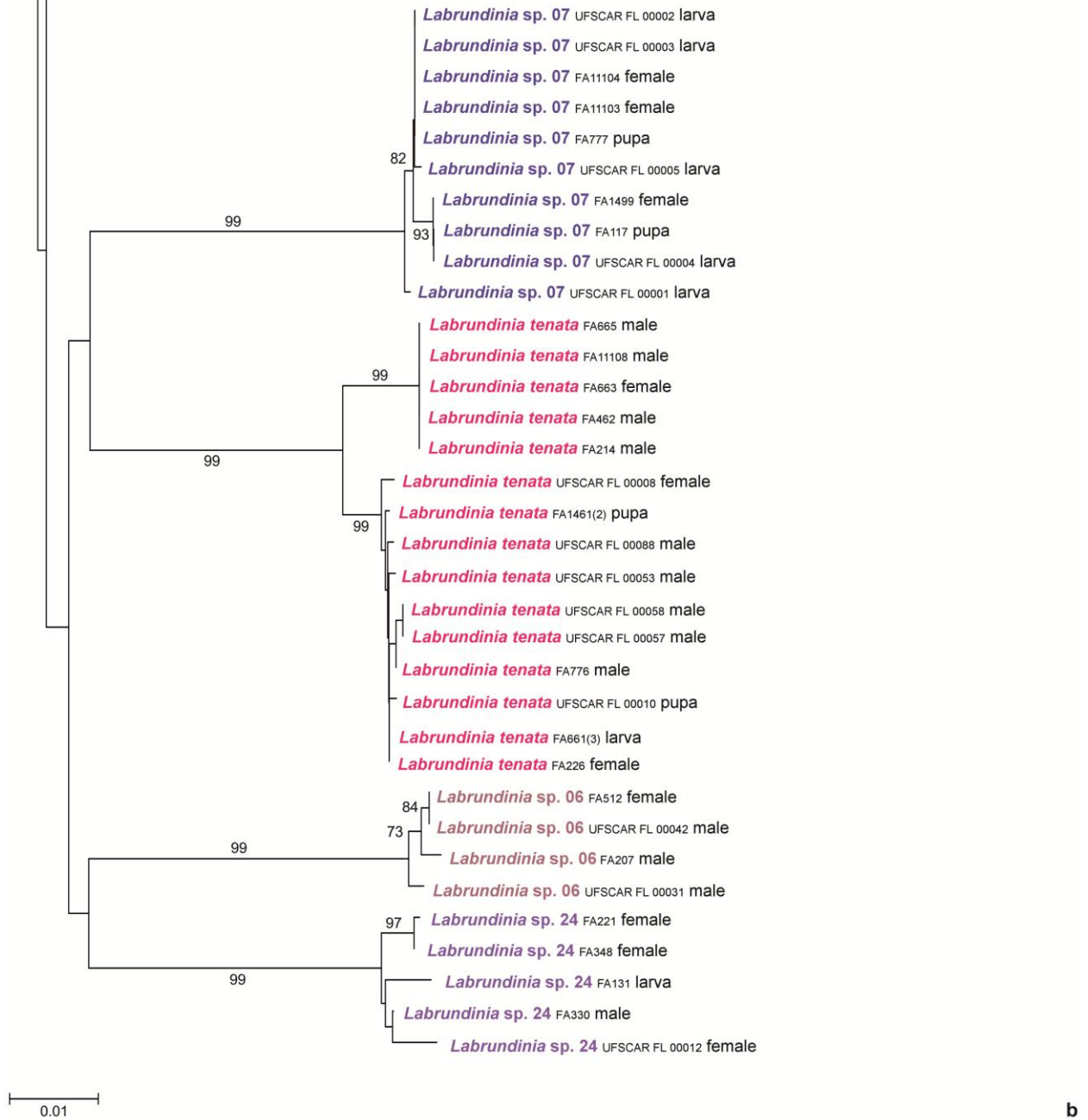


Figure 2. Neighbor joining ID-tree based on partial COI sequences (DNA barcodes) and the Kimura 2-parameter substitution model. Numbers on branches are bootstrap values >70%. DNA barcodes enabled association of different life stages. Colours denote different morphological species.

2010b). However, the taxon ID-tree based on NJ-analysis of pair-wise COI distances (Fig. 2) can be useful as a graphical representation of the genetic differences between sequences and clusters of sequences in the dataset. In this ID-tree (which is not a representation of the most probable phylogeny of the included taxa), it is noticeable that there is a substantial barcode divergence between all the analysed species of *Labrundinia*.

The use of DNA barcodes also enables the association of different life stages based on genetic similarity of phenotypic characteristics (Hebert *et al.* 2003). In many chironomids, descriptions and diagnoses are largely or entirely based on a single life stage or even a single sex of a life stage. Consequently, incomplete knowledge of the life stages of a species precludes the use of morphological characters and natural history information that might be of particular interest for testing ecological, phylogenetic and evolutionary hypotheses. The successful life stage associations made in this as well as previous studies (Carew *et al.* 2005, 2007, 2011, Sinclair and Gresens 2008, Wiedenbrug *et al.* 2009, Ekrem *et al.* 2010a, Stur and Ekrem 2011, Silva *et al.* 2012) show that similar success may be expected in the association of adults and immatures of other chironomids (and other insects), which enables a complete taxonomic description of species where rearing is difficult or even impossible.

We observed low levels of intraspecific divergence within the species analysed. This result is perhaps not surprising given that most of the specimens from a certain species were sampled in the same locality. Nevertheless, our results clearly demonstrate the potential of using DNA-barcodes in the identification of chironomids, at least in a local geographical scale. Moreover, the average intraspecific variation reported here is similar to other studies of insects. Average intraspecific variation of 0.9% (Ekrem *et al.* 2007) and 2.32% (Sinclair and Gresens 2008) in Chironomidae; 2.76% (Hebert *et al.* 2004a) and 0.46% (Hajibabaei *et al.* 2006) in Lepidoptera; and 1.1% in mayflies (Ball *et al.* 2005) have been recorded. The aforementioned results definitely indicate that a single threshold value for species delimitation is not appropriate. Even though recent efforts have been made to overcome this drawback (e.g. ABGD - Automatic Barcode Gap Discovery for primary species delimitation (Puillandre *et al.* 2011), character-based methodologies (DeSalle *et al.* 2005, DeSalle 2007) and BIN - Barcode Index Numbers (Ratnasingham and Herbert, pers. comm. 2011)), additional studies will be required to determine the best method for species delineation using DNA barcodes.

According to Aliabadian *et al.* (2009), the barcoding gap, i.e., the difference between intra- and interspecific distances, allows for identification success in distance-based barcoding. Our data show that the distribution of intra- and interspecific divergences in *Labrundinia* exhibit a clear barcoding gap which may be considered as a predictor of the identification success. Nonetheless, DNA barcodes were not enough for precise identification in all cases. For example, *Labrundinia tenata* specimens separated into two distinct groups, having nucleotide sequence divergences by minimum 2.5% and no observable morphological

differences. Based on the suggested threshold of 2–3% applied to distinguish species (Hebert *et al.* 2003), the two clusters are in fact one species. Similar results were reported to *Labrundinia* sp. 10. Using DNA barcodes, Ekrem *et al.* (2007) also obtained two separate clusters of *Micropsectra notescens* specimens (Chironominae), even though no distinct and constant morphological differences separating the clusters could be found. In contrast, Sinclair and Gresens (2008), found one cluster represented by two species of *Cricotopus* (Orthoclaadiinae). The specimens had less than 3% COI sequence divergence, but presented consistent morphological differences. These results indicate that we need further studies with nuclear DNA markers, morphological and ecological data in order to elucidate relationships between specimens that currently appear to belong to *Labrundinia tenata* and *Labrundinia* sp. 10.

DNA-barcodes are argued to be valuable in species identification of taxonomic groups that are difficult to identify using morphology and Hebert *et al.* (2003, 2004a) stressed that DNA barcoding permits the assignment of unidentified specimens to known species as well as to identify species new to science. Our findings ratify this assertion as *Labrundinia* sp. 8, *Labrundinia* sp. 23 and *Labrundinia* sp. 25 were unveiled as new, undescribed, species only after their significantly different and deeply divergent barcode-clusters were discovered. These species differ based mainly on the pupal thoracic horn, which seems to provide good diagnostic characters for many *Labrundinia* species, showing consistent patterns of interspecific variation. However, before the DNA barcode analyses, the pupal thoracic horn differences were treated as subtle morphological characters. Similarly, Carew *et al.* (2011) reported to separate apparently cryptic species of *Procladius* (Tanypodinae) by means of morphological characters in the immature stages only after the analysis of DNA barcode data. In a more general perspective, our results support DNA barcoding using COI as a promising approach for accurate interpretation of morphological variations within non-biting midges in the subfamily Tanypodinae.

The DNA-barcoding approach has been argued to be imprecise for consistent species delimitations by several authors (e.g. DeSalle *et al.* 2005, Will *et al.* 2005, Rubinoff *et al.* 2006, Ebach 2011), mostly due to general methodological reservations. The presence of mitochondrial pseudogenes (Bensasson *et al.* 2001) or of incomplete lineage sorting may lead in species-level paraphyly and polyphyly (Funk and Omland 2003), blurring delineation of species by monophyletic barcode clusters criteria (Ekrem *et al.* 2010a). Nevertheless, none of these drawbacks seems to be issue using COI sequences in species-level identification of Chironomidae (Carew *et al.* 2005, 2007, Sinclair and Gresens 2008, Ekrem *et al.* 2010b and this study).

CONCLUSIONS

In our study, analysis of DNA barcode sequences yielded taxon identification trees, supporting 13

cohesive species clusters, of which three similar groups were posteriorly linked to morphological distinctness in the immature stages. DNA barcodes also assisted in associating different life stages. We found a distinct barcode-gap and great interspecific pairwise divergences were observed, which allowed identification of all analysed species. Our specimens were sampled in a geographically fairly small region, however, and additional specimens and species must be examined to evaluate whether *Labrundinia* species can be reliably distinguished using DNA barcodes on broad geographical scale (compare Bergsten et al. 2012). Moreover, although DNA Barcode worked well for *Labrundinia*, inclusion of sequence data from extra nuclear markers is recommended in order to strengthen species-delimitation results. Finally, efforts should be made to obtain specimens of *Labrundinia longipalpis* from the Palearctic as knowledge of their genetic distance to the New World species to and North American populations of *L. longipalpis* can be essential to understand the species limits within the genus.

ACKNOWLEDGMENTS

We would like to thank Paul Hebert and the staff at the Biodiversity Institute of Ontario, University of Guelph, Canada for sequencing some of the specimens used in this study. Sequencing in Guelph was supported from Genome Canada through a grant by the Ontario Genomics Institute to Paul Hebert. The authors are indebted to Mateus Pepinelli for his assistance, processing some samples in Canada. Thanks also to Caroline Silva Neubern de Oliveira, Sofia Wiedenbrug and Susana Trivinho Strixino for providing us with important material. F. L. Silva received financial support from the National Council for Scientific and Technological Development (CNPq proc. 141092/2009-2). Financial support was also given by the Research Council of Norway (RCN) through a Personal Mobility Grant.

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Appendix – List of analysed specimens with associated sample localities in São Paulo State, Brazil, voucher reference numbers and GenBank accessions. “FA” indicates samples processed by Fabio Laurindo da Silva; “UFSCAR FL” indicates samples processed by Mateus Pepinelli.

Taxon	Locality	Voucher number	Accession number
<i>Labrundinia tenata</i>	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA214	JX887530
<i>Labrundinia tenata</i>	São Carlos, Valparaíso lake, 26.xi.2011, F. L. Silva	FA226	JX887529
<i>Labrundinia tenata</i>	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA462	JX887538
<i>Labrundinia tenata</i>	São Carlos, Fazzari reservoir, 27.iv.2010, F. L. Silva	FA661(3)	JX887537
<i>Labrundinia tenata</i>	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA663	JX887536
<i>Labrundinia tenata</i>	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA665	JX887535
<i>Labrundinia tenata</i>	São Carlos, Valparaíso lake, 04.iv.2011, F. L. Silva	FA776	JX887534
<i>Labrundinia tenata</i>	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA11108	JX887533
<i>Labrundinia tenata</i>	São Carlos, Fazzari reservoir, 27.iv.2010, F. L. Silva	FA1461(2)	JX887532
<i>Labrundinia tenata</i>	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00008	GI299026199
<i>Labrundinia tenata</i>	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00010	GI299026201
<i>Labrundinia tenata</i>	Luis Antonio, Beija-Flor reservoir, 09.xii.2008, F. L. Silva	UFSCAR FL 00053	GI299026279
<i>Labrundinia tenata</i>	Luis Antonio, Beija-Flor reservoir, 09.xii.2008, F. L. Silva	UFSCAR FL 00057	GI299026285
<i>Labrundinia tenata</i>	Luis Antonio, Beija-Flor reservoir, 09.xii.2008, F. L. Silva	UFSCAR FL 00058	GI299026287
<i>Labrundinia tenata</i>	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00088	GI299026313
<i>Labrundinia</i> sp. 02	São Carlos, Fazzari reservoir, 27.iv.2010, F. L. Silva	FA561(1)	JX887483
<i>Labrundinia</i> sp. 02	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00007	GI299026197
<i>Labrundinia</i> sp. 02	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00011	GI299026203
<i>Labrundinia</i> sp. 03	São Carlos, Fazzari stream, 27.iv.2010, F. L. Silva	FA111	JX887484
<i>Labrundinia</i> sp. 03	São Carlos, Fazzari stream, 10/09/2010, F. L. Silva	FA613	JX887486
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	FA664	JX887485
<i>Labrundinia</i> sp. 03	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00013	GI299026207
<i>Labrundinia</i> sp. 03	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00014	GI299026209
<i>Labrundinia</i> sp. 03	São Carlos, Fazzari reservoir, 06.xii.2008, F. L. Silva	UFSCAR FL 00015	GI299026211
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00016	GI299026213
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00017	GI299026215

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Appendix (continued)

Taxon	Locality	Voucher number	Accession number
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00018	GI299026217
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00026	GI299026233
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00036	GI299026247
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00045	GI299026263
<i>Labrundinia</i> sp. 03	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00047	GI299026267
<i>Labrundinia</i> sp. 06	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	FA207	JX887489
<i>Labrundinia</i> sp. 06	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	FA512	JX887487
<i>Labrundinia</i> sp. 06	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00031	GI299026237
<i>Labrundinia</i> sp. 06	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	UFSCAR FL 00042	GI299026259
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	FA117	JX887490
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	FA777	JX887494
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	FA11103	JX887493
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	FA11104	JX887492
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	FA1499	JX887491
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	UFSCAR FL 00001	GI299026187
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	UFSCAR FL 00002	GI299026189
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	UFSCAR FL 00003	GI299026191
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	UFSCAR FL 00004	GI299026193
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	UFSCAR FL 00005	GI299026195
<i>Labrundinia</i> sp. 08	São Carlos, Valparaíso lake, 26.xi.2011, F. L. Silva	FA216	JX887496
<i>Labrundinia</i> sp. 08	São Carlos, Valparaíso lake, 04.iv.2011, F. L. Silva	FA773(2)	JX887495
<i>Labrundinia</i> sp. 10	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA102	JX887503
<i>Labrundinia</i> sp. 10	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA160	JX887501
<i>Labrundinia</i> sp. 10	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA333	JX887497
<i>Labrundinia</i> sp. 10	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA449(5)	JX887502
<i>Labrundinia</i> sp. 10	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA451(3)	JX887498
<i>Labrundinia</i> sp. 10	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA535	JX887499

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Appendix (continued)

Taxon	Locality	Voucher number	Accession number
<i>Labrundinia</i> sp. 10	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	FA605	JX887500
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA332	JX887504
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA449(6)	JX887507
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA451(1)	JX887506
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA451(2)	JX887505
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA456	JX887509
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA536	JX887510
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA1478	JX887508
<i>Labrundinia</i> sp. 20	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA449(1)	JX887511
<i>Labrundinia</i> sp. 20	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA453	JX887512
<i>Labrundinia</i> sp. 20	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	FA455	JX887513
<i>Labrundinia</i> sp. 21	São Carlos, Espraiado stream, 19.viii.2011, S. Wiedenbrug	FA36124	JX887517
<i>Labrundinia</i> sp. 21	São Carlos, Espraiado stream, 19.viii.2011, S. Wiedenbrug	FA36125	JX887516
<i>Labrundinia</i> sp. 21	São Carlos, Espraiado stream, 19.viii.2011, S. Wiedenbrug	FA36127	JX887515
<i>Labrundinia</i> sp. 21	São Carlos, Espraiado stream, 19.viii.2011, S. Wiedenbrug	FA36128	JX887514
<i>Labrundinia</i> sp. 23	Luis Antonio, Óleo reservoir, 07.i.2011, S. T. Strixino	FA138	JX887521
<i>Labrundinia</i> sp. 23	Luis Antonio, Óleo reservoir, 07.i.2011, S. T. Strixino	FA650	JX887518
<i>Labrundinia</i> sp. 23	Luis Antonio, Óleo reservoir, 07.i.2011, S. T. Strixino	FA1095	JX887520
<i>Labrundinia</i> sp. 23	Luis Antonio, Óleo reservoir, 05.i.2011, S. Wiedenbrug	FA12109	JX887519
<i>Labrundinia</i> sp. 24	São Carlos, Mayaca pond, 11.iv.2011, F. L. Silva	FA131	JX887525
<i>Labrundinia</i> sp. 24	São Carlos, Mayaca pond, 20.iv.2011, F. L. Silva	FA221	JX887524
<i>Labrundinia</i> sp. 24	São Carlos, Mayaca pond, 11.iv.2011, F. L. Silva	FA330	JX887522
<i>Labrundinia</i> sp. 24	São Carlos, Mayaca pond, 11.iv.2011, F. L. Silva	FA348	JX887523
<i>Labrundinia</i> sp. 24	Luis Antonio, Beija-Flor reservoir, 09.xii.2008, F. L. Silva	UFSCAR FL 00012	GI299026205
<i>Labrundinia</i> sp. 25	São Carlos, Mayaca pond, 11.iv.2011, F. L. Silva	FA342	JX887527
<i>Labrundinia</i> sp. 25	Luis Antonio, Beija-Flor reservoir, 05.i.2011, S. Wiedenbrug	FA41129	JX887526

CAPÍTULO III

PHYLOGENY AND BIOGEOGRAPHY OF SPECIES OF *LABRUNDINIA* FITTKAU, 1962 (DIPTERA: CHIRONOMIDAE): EVIDENCE FROM MORPHOLOGICAL AND MOLECULAR ANALYSES

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ABSTRACT

Phylogenetic relations among known *Labrundinia* (Diptera: Chironomidae) species are inferred using the nuclear protein-coding gene CAD and morphological characters. Both maximum parsimony and Bayesian analyses suggest that the genus is monophyletic and robust species groups in the molecular phylogenies are supported by morphological characters. The historical biogeography of *Labrundinia* is analysed based on the phylogenetic reconstructions. Brooks Parsimony (BPA) and dispersal-vicariance (DIVA) analyses both favoured the *Labrundinia* ancestor as having its initial diversification in the Neotropical region and that current presence in the Holarctic region is due to later dispersal.

KEY WORDS: Pentaneurini, Tanypodinae, Bayesian inference, parsimony, CAD

INTRODUCTION

The dipteran family Chironomidae probably originated in the middle Triassic approximately 248-210 million years ago (Cranston *et. al* 2010). It comprises at least 10,000 species in more than 400 genera (Armitage *et. al* 1995, Sæther *et. al* 2000). Chironomidae is the most widespread of all aquatic insect

families, occurring in all biogeographical regions of the world. The immature stages of most species occur in freshwater, but many terrestrial or marine species are known (Sæther and Ekrem 2003). Although the distribution of the species in many genera is relatively well known, detailed analyses of distribution patterns and historical biogeography are rare in chironomids, especially from Neotropical region. Regarding phylogenetics, a first attempt to study the relationships between groups in Chironomidae was made by Goetghebuer (1914), but it was not until Brundin's monograph (1966) that species level phylogenies of chironomid midges were produced (Ekrem 2003). Since then, several groups have been investigated (e.g. Brundin and Sæther 1978, Sæther 1971, 1977, 1983, 1990, 2000, Adam and Sæther 1999, Boothroyd and Cranston 1999, Ekrem 2003, Stur and Ekrem 2006, Fu *et al.* 2010). However, none have so far produced a phylogenetic hypothesis at the species level within subfamily Tanypodinae.

Tanypodinae is third most speciose subfamily in the Chironomidae, with species distributed widely across most of the globe, occupying a diverse array of habits including small streams and ponds to lakes and bays (Silva *et al.* 2011). Generally regarded as predators, the larval feeding apparatus differs fully from other Chironomidae, with the strong development of premental structures as ligula and paraligulae (Cranston 1995). This subfamily was erected by Thienemann and Zavřel (1916) primarily on the basis of immature stages (Cranston 1995) and their monophyly is well-supported, with Podonominae as its sister-group (Cranston *et al.* 2012). Postulated internal relationships at the generic level in Tanypodinae derive from Fittkau (1962), who erected the tribes Anatopyniini, Macropelopiini and Pentaneurini. Roback and Moss (1978) established tribes Procladiiini and Natarsiini with an unusual phenetic method to chironomid systematics (Cranston 2012).

The Pentaneurini genus *Labrundinia* was erected by Fittkau (1962) based on *Tanypus longipalpis* Goetghebuer, designated as the type species. It comprises 39 species, most of them described in a separate paper (Silva *et al.* in preparation). Except for *Labrundinia longipalpis*, all species have been described from areas outside the Palearctic region: five from North America, four from Central America and 29 from South America. Internal relationships among *Labrundinia* species were postulated by Roback (1987a), who recognized four groups of species based on morphological characters of immatures: *Labrundinia pilosella* group, *L. maculata* group, *L. neopilosella* group and *L. virescens* group. To date, no phylogeny or even hypothesis of monophyly have been proposed for *Labrundinia*. Fittkau (1962) suggested that the genus is closely related to the *Zavrelimyia* group, in particular *Paramerina*, but morphological analyses of new species in *Labrundinia* indicate the presence of characters that contradict this argument.

Recently, several phylogenetic analyses of various groups in the family Chironomidae have been published based on morphological characters (e.g. Cranston *et al.* 2002, Ekrem 2003, Sæther 2000, Fu *et*

al. 2010). However, in most cases the results have been ambiguous, and parsimony trees have been characterised by polytomies and low node support (Ekrem *et al.* 2010a). On the other hand, molecular characters in phylogenetic analyses of chironomids have an obvious advantage and their use has notably grown over the years (e.g. Cranston *et al.* 2002, Ekrem and Willassen 2004, Allegrucci *et al.* 2006, Stur and Ekrem 2006, Martin *et al.* 2007). Silva *et al.* (in revision) analysed the applicability of DNA-barcodes in species delimitation and the life stage association of *Labrundinia*. The molecular analysis produced monophyletic groups which were entirely congruent with the morphological features, suggesting that barcodes can be used routinely in species delimitation and the identification of chironomids.

Additional studies also recognize the effectiveness of DNA barcodes in species delimitation and identification (Carew *et al.* 2003, Ekrem *et al.* 2010b, Stur and Ekrem 2011, Silva *et al.* 2012), but the variability of this gene is too high to derive phylogenies (Ekrem *et al.* 2007, 2010a). Ekrem *et al.* (2010a) tested the utility of three mitochondrial (COI, COII, 16S) and two nuclear (CAD and EF-1 α) markers in low-level phylogenetic reconstruction in Chironomidae. They found that partial CAD (*rudimentary*) sequences performed best and had the strongest phylogenetic signal in a dataset consisting of species in the genus *Micropsectra* and related genera (Ekrem *et al.* 2010a).

The primary purpose of this study is to resolve phylogenetic relationships of *Labrundinia* species using the nuclear protein-coding gene CAD, as well as morphological characters. Furthermore, the congruence of the internal groups postulated by Roback is examined. Based on the phylogenetic reconstructions obtained, hypotheses to explain biogeographical patterns of major clades are presented. This study provides the most complete cladistic treatment of a Pentaneurini genus to date.

MATERIAL AND METHODS

Taxon sampling

The taxa included in this study were selected with the aim of getting representatives from as many of the known morphotypes in *Labrundinia* as possible. Fieldwork was conducted in southeast Brazil. The larvae and pupae were collected using a hand-net in different aquatic systems. Some larvae and pupae were isolated in small vials covered with nylon screen and reared in laboratory to obtain adult life stages. Various aquatic macrophyte species were collected and placed in a plastic tray in order to obtain adults emerged. Immature chironomids were preserved in 96% pure ethanol while imagines were kept in slightly diluted ethanol (~80–85%) to avoid breakage.

Different morphotypes were recognized based on variation in all observable morphological traits such as coloration, genital structures, shapes of pupal thoracic horn and larval claws. After DNA

extraction, the exoskeleton was mounted in Euparal on microscopy slides with the corresponding wings, legs and antennae. Identifications were made based on taxonomic revisions, original descriptions (Fittkau 1962, Beck and Beck 1966, Roback 1971, 1987a-b, Epler 2001, Jacobsen 2008, Silva and Fonseca-Gessner 2009, Siri and Donato 2011, Silva *et. al* 2011) and eventually examination of type material. All reference material for the molecular data presented in this paper is deposited in the Museum of Natural History and Archaeology, Norwegian University of Science and Technology in Trondheim.

Genetic procedures

DNA was extracted from single individuals, mostly larvae but also pupae and adult males and females (for details see Appendix). DNA extraction and PCR were performed in the molecular lab at the Museum of Natural History in Trondheim. DNA extraction followed the tissue protocol using a GeneMole robot (MoleGenetics). Each PCR was made with the addition of 2 µl DNA template, 2.5 µl 10 × Ex Taq Buffer, 2.0 µl MgCl₂ in 25 µM concentration, 2 µl of dNTPs in 10 mM concentration, 1 µl of each of the CAD primers 54F (5'- GTNGTNTTYCARACNGGNATGGT-3') and 405R (5'- GCNGTRTGYTCNGGRTGRAAYTG-3') (Moulton and Wiegmann 2004) in 10 µM concentration, 0.2 µL HotStarTaq DNA Polymerase (Qiagen), and 14.3 µL of ddH₂O for a total reaction volume of 25 µl. The PCR had 5 cycles of 30 s annealing at 57 °C, 5 cycles of 30 s annealing at 52 °C and 35 cycles of 30 s annealing at 51 °C in a typical touch-down procedure. The PCR products were purified using ExoSAP-IT (USB Products) and shipped to MWG Eurofins for bi-directional sequencing. Sequences were assembled and edited using DNA BASER Sequence Assembler 3.2.4 (Heracle Software, Germany), and aligned in MEGA 5.03 (Tamura *et al.* 2011).

Phylogenetic analyses

The morphological data matrix (table 1) was constructed using Mesquite 2.75 (Maddison and Maddison 2004) and mostly based on observations by the first author. The species used as outgroup in phylogenetic analyses were a priori chosen based on morphological similarities and existing classification (Fittkau 1962, Roback and Moss 1978). Additionally, Basic Local Alignment Search Tool (BLAST) was conducted using MEGA 5.03, in order to find close matches to *Labrundinia* sequences. The following genera were included: *Ablabesmyia* Johannsen, 1905; *Australopelopia* Cranston, 2000; *Conchapelopia* Fittkau, 1962; *Fittkauimyia* Karunakaran, 1969; *Larsia* Fittkau, 1962; *Monopelopia* Fittkau, 1962; *Pentaneura* Philippi, 1865; *Procladius* Skuse, 1889 and *Zavreliomyia* Fittkau, 1962. Despite that *Labrundinia maculata* was placed as a junior synonym of *Labrundinia longipalpis* by Silva *et al.* (2011),

they were treated as a separate species in order to investigate the validity of this synonymization. The final dataset consisted of 49 terminals: 40 ingroup terminals and 9 outgroup terminals.

The best-fit models of nucleotide substitution and nucleotide statistics were calculated with MEGA 5.03. The phylogenetic analyses were conducted using PAUP* 4.10b (Swofford 1998), MrBayes 3.2.1 (Ronquist *et al.* 2012) and MEGA 5.03. Parsimony analysis used 10000 random replicates in heuristic searches, TBR branch swapping and multrees option in effect. All characters were treated as unordered. Successive approximation weighting was performed using the rescaled consistency index and a weighting scheme up to 1000. RC was preferred over CI or RI because it is believed to be less sensitive to autapomorphies (Farris 1969, Carpenter 1988). Bayesian analyses were run with four chains for 3 million generations with a print frequency of 1000, sample frequency of 500, and a burn in of 600 sampled trees (30 000 generations). MrBayes' default priors were used. The trace files were then examined with the program Tracer 1.4.1 (Rambaut and Drummond 2008) to determine the point of MCMC convergence (burn-in) and to evaluate the effective sample size (ESS) of the parameter estimates. Maximum likelihood (1000 bootstrap replicates) reconstruction was performed using MEGA 5.03. FigTree ver.1.3.1 (Rambaut 2009) was used to display the resulting trees.

List of characters

The character choice is largely based on first author observations. In some instances it was necessary to rely on published species descriptions. Although we attempted to keep the number of autapomorphies low, some single character states are listed for certain species to indicate differences to other terminal taxa.

Adult males

Head. Very little difference was found among the species included in this study. All species have 14 flagellomeres and an antennal ratio which ranges between 1.00–1.55. There are numerous features without clear interspecific variation, such as the number of temporal and clypeal setae.

1. Dorsomedian eye elongation absent or reduced (0); well developed to moderately developed (1).
2. Tentorium 92–120 μm long (0); 130–141 (1); (2) 154–174.
3. Antennal ratio 1.00–1.35 (0); 1.40–1.55 (1); 2.30–2.80 (2).

Thorax. The chaetotaxy of the thorax seems to be fairly stable among the species included and apparently depends on size. Nonetheless, the disposition of the posterior dorsocentrals divides most of species into two groups by uniserial or biserial to multiserial distributions. All species have two or three supraalars, except for *L. separata* that has 3–5 supraalars; coded as polymorphic for this species.

4. Dorsocentrals uniserial posteriorly (0); bi- to multiserial posteriorly (1).
5. Prealars uniserial (0); bi- to multiserial (1).
6. Supraalar(s) absent (0); 1 (1); 2 (2); 3 (3); 5 (4); 8–10 (5).

Wing. The shape of the wing and the venation is very constant among the included species; costa is short, not produced beyond R_{4+5} , ending level with or slightly beyond tip M_{3+4} . The presence or absence of R_{2+3} can separate all species in two groups.

7. Wing length 1.00–1.30 μm long (0); 1.40–2.70 (1).
8. R_{2+3} absent (0); present (1).

Legs. The species included exhibit large variation in the number of setae on the tibial combs, as well as in the length and number of teeth in the spurs. These characters do not possess any distinct interspecific variation. However, the fore leg ratio can place most of species into two groups by an LR less than 0.66 or more than 0.72. Although inclusion resulted in six taxa with question marks, this character was kept in the analyses.

9. Fore leg ratio (LR) 0.50–0.66 (0); 0.72–0.90 (1); higher than 1.00 (2).
10. Hind tibial spur absent (0); present (1).

Abdomen. Most characters with sufficient variation are found on the male abdomen and relates to abdominal coloration. Thus, the abdomen as such is considered as more important in the analyses. On the other hand, the hypopygium, which has traditionally been used to separate species in Chironomidae, seems to be very similar in all *Labrundinia*. As result only a few characters derived from genital structures have been included in this study.

11. Abdomen evenly coloured (0); with coloration pattern (bands) (1).
12. Abdominal segment II evenly clear (0); with band (1); with faint band or spots.
13. Abdominal segment III evenly dark (0); with band (1); evenly clear (2).
14. Abdominal segment V evenly dark (0); with band (1); with faint band or spots.
15. Abdominal segment VI evenly dark (0); with band (1).
16. Abdominal segment VIII evenly clear (0); with band (1).
17. Tergite IX with distal margin straight to weakly concave (0); with distal margin strongly convex (1).
18. Gonocoxite ratio 1.85–2.20 (0); 2.30–2.60 (1).
19. Hypopygium ratio 1.40–1.50 (0); 1.60–1.85 (1).
20. Hypopygium coloration pale (0); pale brown (1); brown (2); bicolour (3).
21. Anal point simple (0); notched (1).

Pupa

Pupal features appear to be important for phylogenetic studies in Chironomidae, especially those regarding subfamily Tanypodinae. The majority of variable characters are found on the thoracic horn and seem to be intraspecifically constant. Additionally, the size of the genital sac, if it surpasses the length of anal lobe or not, shows interspecific variation and should be well suited for phylogenetic studies.

22. Wing pads veins distinctly pigmented (0); uncoloured (1)
23. Thoracic horn 9-shaped (0); kidney-shaped (1); semi-globose (2); club-shaped (3); hatched-shaped (4); light bulb-shaped (5); ovoid (6); elongate (7).
24. Groove of thoracic horn absent (0); deep (1); moderately deep (2).
25. Aeropyle tube of thoracic horn elongated and/or sinuate (0); short simple (1).
26. Respiratory sac of thoracic horn well developed, filling respiratory atrium (0); moderately developed (1).
27. Second tooth of thoracic comb smaller than first one (0); same size or bigger (1).
28. Hump on segments III–VI absent (0); present (1).
29. Shagreen on tergite II, composed by prominent spinules (0); absent or reduced (1).
30. Segment VII with 2 L setae (0); 3 L setae (1); 4 L setae (2).
31. Tergite VIII with projections over base of anal lobe absent (0); present (1).
32. Anal lobes long and narrow (0); relatively short and broad (1).
33. Anal lobe evenly coloured (0); apex darker (1)
34. Apical spines of anal lobe absent (0); 8–17 μm long; 20–32 (2); 40–60 (3).
35. Genital sac of the male not reaching apex of anal lobe (0); reaching somewhat beyond lobe (1); reaching much beyond anal lobe (2).

Larva

Only a few larval characters showed sufficient interspecific distinction to be included in the analyses. Nevertheless, these features seem to be intraspecific stable and suitable. All species can be placed into two groups by the surface of the head capsule, which can be smooth, with spinules or nodules (crenulation). The presence/absence of lateroventral and posteroventral spine groups appears to be very important to the phylogenetic analyses in *Labrundinia*.

36. Crenulation absent (0); present (1).
37. Lateroventral spur group (LVS) absent (0); one single large spine (1); several small spines (2).
38. Antenna (AR) 1.80–2.20 (0); 2.30–2.80 (1); 3.00–4.00 (2); more than 5.00 (3).
39. Mandible with well-developed teeth (0); with very short teeth (1).
40. Paraligula bifid (0); multitoothed (1).
41. Serrated claw of posterior parapods absent (0); present (1).

42. Subbasal setae of posterior parapods simple with reduced spines (0); multitoothed (flag-like) (1).
43. Bifid claw of posterior parapods with inner tooth longer than outer tooth absent (0); present with lower indentation U-shaped (1); present with lower indentation forming V-angle (2).
44. Bifid claw of posterior parapods with inner tooth very elongated (0); not elongated (1).
45. Ratio between inner and outer tooth on larval bifid claw (B/C) 0.85–1.05 (0); 1.10–1.30 (1).

Biogeographical analyses

Biogeographical patterns of extant *Labrundinia* species were analysed in five ecoregions, based initially on a vicariance model (Amorim 2012), modified and complemented with additional areas: Nearctic and southern America (Morrone 2006). In order to reconstruct the historical biogeography of the lineage, two methods were used: the discovery-based method Brooks parsimony analysis (BPA) and the event-based method dispersal-vicariance analysis (DIVA). The biogeographical analyses used the complete tree distribution obtained from Bayesian analysis of the combined molecular and morphological data sets (Figure 2A).

Primary BPA (Brooks *et. al* 2001) was used to estimate general area relationships among ecoregions based on distributional data of *Labrundinia*. BPA combines phylogenetic information from multiple taxa into a single composite matrix, and then uses maximum parsimony to find the shortest tree(s) that are consistent with this matrix (Brooks and McLennan 2001). It differentiates among potential episodes of vicariant, peripheral isolates and sympatric speciation, without regard for relative range sizes (post-speciation range expansion or contraction) or degree of geographical overlap among sister species (McLennan and Brooks 2002). The function of primary BPA is to determine whether there is a common pattern among areas (Brooks *et al.* 2001). A matrix of the area was constructed with the patterns of distribution of 40 species of *Labrundinia*. BPA was implemented in PAUP* 4.0b10, using 10000 random replicates in heuristic searches, TBR branch swapping and multrees option in effect. Bremer support analysis was performed by using heuristic searches in PAUP*.

To account for phylogenetic uncertainty and uncertainty in area optimization, DIVA was implemented in RASP (Reconstruct Ancestral State in Phylogenies), a software that statistically evaluates the alternative ancestral ranges at nodes based on a set of trees (Yu *et al.* 2011). RASP assumes that geographic distributions can be the result of both dispersal and vicariance events. DIVA analyses depend on two key assumptions relative to the area definition: (1) the distribution of any group may be explained by fragmentation through vicariance, dividing the biota into areas of endemism (Bremer, 1992; Porzeczanski and Cracraft 2005); (2) species ranges are evenly conservative across evolutionary time (Losos and Glor 2003). Assuming these premises, there is information about the ancestral area in the deep

branches of a cladogram (Bremer 1995). The analysis was conducted allowing for a maximum of two areas in ancestral ranges. This implies that dispersal and vicariance events occurred between two single areas. The default settings of the RASP program were used, where vicariance events cost zero but dispersal and extinction events cost one per unit area. The optimal (and most parsimonious) ancestral reconstruction of the DIVA model is the one with the lowest cost. Statistical dispersal-vicariance topology (derived from DIVA, Ronquist 1997, 2001) was used for this analysis. Despite the possibility of more complex events, this configures a simple cladistic procedure for estimating the historical linkage of areas, which is usually an unknown parameter (Porzecanski and Cracraft 2005).

RESULTS AND DISCUSSION

Dataset properties

Material suitable for genetic analyses was obtained for 13 of the 40 *Labrundinia* species included in the morphological character matrix. The aligned CAD sequences were 899 bp long with 524 variable sites (58.3%), of which 424 (80.9%) were potentially parsimony-informative. Most variable sites occurred in the third codon-position (Table 2). The sequences were heavily AT-biased, specifically in third position, which exhibited a combined average AT-composition of 64.0% (Table 2). A hierarchic likelihood ratio test of aligned sequences in MEGA 5.03 returned the general time reversible model with invariable sites and gamma correction for rate heterogeneity (TN93+G+I) as the best model ($-\ln L = 10197.728$, $BIC = 20867.947$, $AIC = 20491.705$).

Table 2 – Variable and informative sites and average nucleotide composition in the analyzed CAD gene sequences.

Nucleotide position	% Variable sites	% Informative sites	% Adenine	% Cytosine	% Guanine	% Thymine
1 st	26.5	12.5	33.6	17.3	28.1	21.0
2 nd	17.9	0.5	35.2	19.2	18.1	27.5
3 rd	55.5	87	27.8	17.5	18.5	36.2
All	58.2	95.6	32.2	18.0	21.6	28.2

The morphological matrix included 45 characters (21 from adults, 14 pupal and 10 larval). Only a few species presented one morphological characters varying within population, this was scored as polymorphic character (table 1). The combined data matrix comprised 49 terminal taxa each with 944 characters; out of the total number of characters in the data matrix 53.2% were scored as missing. Despite

the elevated uncertainty, one reason for merging data matrices is that a combined analysis allows including more known species, which is particularly important in biogeographical studies such as the one presented here, where only 1/3 of the known species have material suitable for genetic analyses.

Phylogenetic patterns

In this study the monophyly of *Labrundinia* is well established. The group is recovered in all trees resulting from a reduced taxon-character matrix, or from a reweighted morphology matrix, with a number of morphological modifications recognized as synapomorphies: apex of hind tibia without spur and tergite IX with distal margin strongly convex in the male adults; thoracic horn-plastron plate reduced, seated on a small tubercle, reticulation of respiratory atrium indistinct; pupal tergite VIII with projections over base of the anal lobe; pupal anal lobe long and narrow, with inner border membranous, slightly concave; larval mandible strongly curved with distinct or short, subequal teeth; pseudoradula slightly broadened posteriorly; median tooth of the ligula usually longer than the inner teeth; bifid claw on posterior parapod with outer tooth shorter than inner tooth in the known larvae.

Bayesian analyses of the CAD gene sequences and the combined molecular and morphological data sets for 22 taxa yielded almost identical trees: *Labrundinia* came out containing three major species groups (Figure 1A). However, only two clades are supported by more than 50% of the bootstrap replicates: *L. sp. 02 + L. sp. 23 + L. sp. 24 + L. sp. 25* (67%) and *L. sp. 06 + L. sp. 07 + L. sp. 08* (83%). The Bayesian tree resulting from combined molecular and morphological data sets (Figure 1B) only differs in the placements of *L. sp. 20* which groups with *L. sp. 02 + L. sp. 23 + L. sp. 24 + L. sp. 25* as its closest relative. A parsimony analysis of the morphological data matrix alone yielded 9 trees of 148 steps (CI = 0.43, RI = 0.68). The strict consensus tree shows no resolution except for two clades of two species each (*L. sp. 06 + L. sp. 08* and *L. sp. 03 + L. sp. 10*). An analysis based on an RC-reweighted morphological character matrix returned three trees (CI = 0.43, RI = 0.68) of 150 steps. The strict consensus tree shows that the species in *Labrundinia* form a monophyletic group (Figure 1C). In all trees resulting from a reduced taxon-character matrix, members which key to the *virescens*-group (Roback 1987a) are all present in one monophyletic clade: *L. sp. 02 + L. sp. 23 + L. sp. 24 + L. sp. 25*.

A Bayesian analysis of the combined morphology and molecular data matrices for 40 *Labrundinia* species resulted in a fully solved consensus tree with four major species groups (Figure 2A). It groups *L. longipalpis* and *L. maculata* as sister species, and places with *L. fera* within *neopilosella*-group, suppressing the *maculata* group (Roback 1987a). The *virescens*-group (Roback 1987a) is expanded with the inclusion of *L. fosteri + L. hirsuta + L. opela + L. sp. 02 + L. sp. 22 + L. sp. 23 + L. sp. 24 + L. sp. 25*. The clade containing species which key to the *virescens*-group is monophyletic and presented posterior

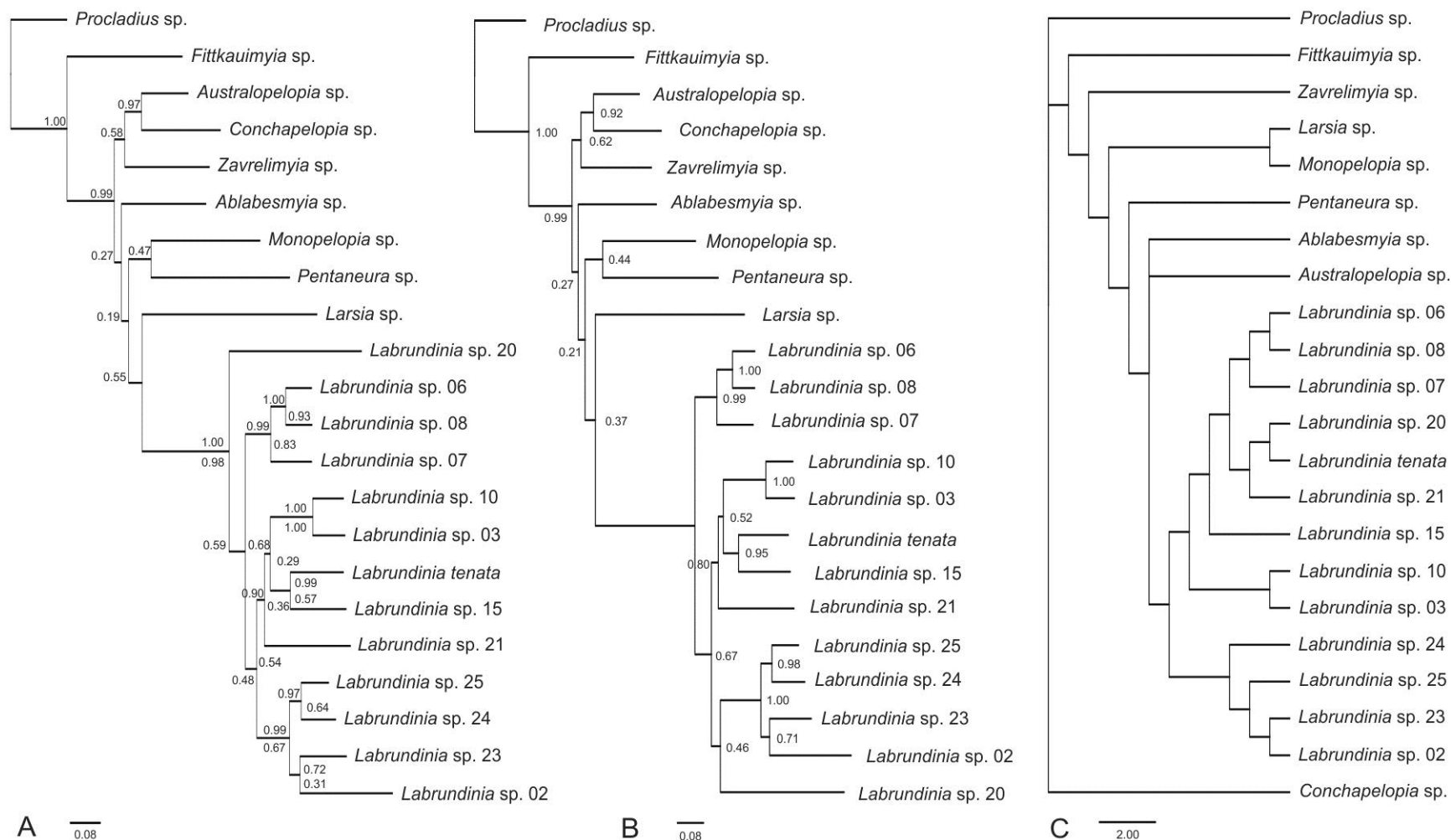


Figure 1. Phylogenetic hypotheses for the *Labrundinia* species, only including species with sequenced CAD. A, result from Bayesian analysis of CAD gene sequences. Posterior probabilities shown above branches, bootstrap support below branches from maximum likelihood analysis of the same data set. B, result from Bayesian analysis of the combined molecular and morphological data sets. Posterior probabilities on branches. C, result from parsimony analysis of the morphological data set, with characters weighted according to their rescaled consistency indices.

probability of 45%. The postulated *pilosella* species-group (Roback 1987a) was originally formed by *L. pilosella* and *L. becki*, but in our study *L. becki* grouped with *L. parabecki* + *L. sp. 03* + *L. 09* + *L. sp.10*. Despite this clade to present low posterior probability (24%), its monophyly and morphological support allows us to erect a new group of species: the *becki* species-group. On the other hand, *L. pilosella* grouped with *L. sp. 17* + *L. sp. 19*. *L. sp. 20*, and although this clade exhibit a higher posterior probability (61%), we hesitate to include the newest species within *pilosella* species group, since only *L. sp. 20* has immatures stages described. The Bayesian tree also includes a monophyletic group which comprises *L. sp. 06* + *L. sp 07* + *L. sp. 08*. This clade is supported by 86% of the posterior probability, which permit us to postulate another new group of species: the *reniformis*-species group.

A parsimony analysis of the morphological character matrix for 40 *Labrundinia* species (45 parsimony informative characters) yielded 359 trees of 251 steps (CI = 0.28, RI = 0.65). In the strict consensus tree, *Labrundinia* appears as a monophyletic group, with *becki*, *neopilosella* and *reniformis* groups present. Members that key to *virescens*-group are present in the basal polytomy together with the remaining species. An analysis based on an RC-reweighted matrix resulted in 9 trees of 254 steps (3 steps longer than the tree from the unweighted parsimony analysis), all of which show *Labrundinia* as a monophyletic group. The strict consensus tree is well resolved, and the *becki*-, *neopilosella*-, *reniformis*- and *virescens*- groups are clearly recognizable as monophyletic clades in the tree (Figure 2B).

Analyses on a matrix including all morphological characters of species with described pupal and larval stages were also attempted. The parsimony analysis based on an RC-reweighted matrix for 26 *Labrundinia* species yielded one tree of 222 steps (4 steps longer than the tree from the unweighted matrix), the resulting tree (CI = 0.31, RI = 0.65) is fully solved with members which key to *becki*-, *neopilosella*-, *reniformis*- and *virescens*- groups also forming monophyletic clusters (Figure 3A). Excluding larval characters from the parsimony analysis, thus leaving 35 parsimony informative characters in the data matrix, resulted in three trees of 176 steps on an RC-reweighted matrix (3 steps longer than the tree from the unweighted matrix). The strict consensus tree (CI = 0.32, RI = 0.66) has slightly less resolution, nonetheless the *becki*-, *neopilosella*-, *reniformis*- and *virescens*- groups are also easily noticed (Figure 3B).

In this study, the Bayesian tree (Figure 2A) was chosen as preferred due its increased number of informative characters. Based on this tree, the following groups of species can be designated for *Labrundinia*:

1. The *becki* species-group

All species are in the male adults characterized by abdomen evenly coloured, brown to dark brown, hypopygium elongated and gonocoxite ratio values ranging between 1.93–2.12; in the known pupae by

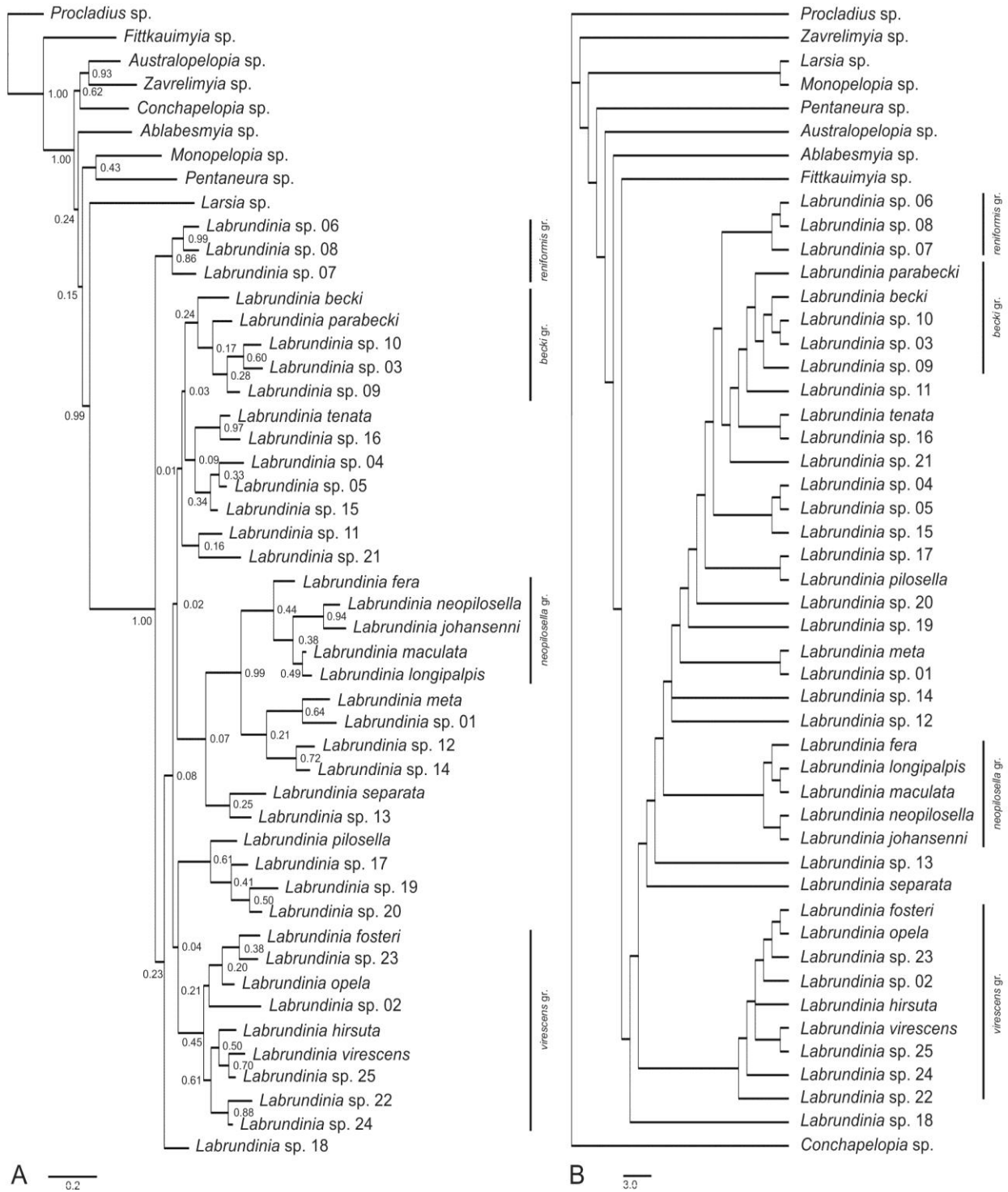


Figure 2. Phylogenetic hypotheses for the *Labrundinia* species. A, result from Bayesian analysis of the combined molecular and morphological data sets. Posterior probabilities shown on branches. B, result from parsimony analysis of the morphological data set, with characters weighted according to their rescaled consistency indices.

thoracic horn semi-globose and genital sac not reaching much beyond apex of anal lobe (except in *L. becki*); in the known larvae by surface of head capsule smooth, without spinules or nodules, posteroventral spine group absent and bifid claw with V-shaped lower indentation. The species included are: *L. becki*, *L. parabecki*, *L. sp. 03*, *L. sp. 09* and *L. sp. 10*. Roback ascribed *L. becki* and *L. pilosella* to the same group of species (1987a). However, in our study these two species have not shown close relationship.

2. The *neopilosella* species-group

The males in this group are characterized by dorsocentrals uniserial posteriorly, R_{2+3} absent (except in *L. johansenni*) and hypopygium pale brown (except for *L. fera*, bi-coloured). The known pupae have thoracic horn semi-globose (except for *L. longipalpis*) and genital sac not reaching the apex of the anal lobe (except in *L. neopilosella*). All known larvae have the lateroventral spine group present, subbasal seta multitoothed and bifid claw with U-shaped lower indentation. The species included are: *L. fera*, *L. neopilosella*, *L. johanseni* and *L. longipalpis*.

Silva *et. al* (2011) placed *L. maculata* as a new junior synonym of *L. longipalpis*, since there is no morphological difference that would justify separating species name were observable. The placement of *L. longipalpis* as sister species to *L. maculata* reinforces the proposed synonymization. However, the inclusion of *L. longipalpis* in the *neopilosella* species group is still somewhat dubious since the species possesses a wedge-shaped thoracic horn.

3. The *reniformis* species-group

All species share the following character combination: prealars uniserial, R_{2+3} absent and hypopygium pale brown in the adult male; thoracic horn kidney-shaped and abdominal segment VII with 3 lateral setae (except for *L. sp. 07*) in the known pupae; surface of head covered with small spinules or nodules, lateroventral and posteroventral spine groups absent, serrated claw present (except for *L. sp. 07*) and bifid claw with V-shaped lower indentation in the known larvae. The species included are: *L. sp. 06*, *L. sp. 07* and *L. sp. 08*.

4. The *virescens* species-group

Most of the species in this large group share the following male features: Abdominal tergite II pale or with faint band (except for *L. sp. 22* and *L. sp. 24*) and hypopygium pale (except for *L. sp. 24*). The known pupae have thoracic horn nine-shaped, with deep preapical indentation, elongated aeropyle tube and a moderately developed horn sac. The known larvae are characterized by having a smooth surface of the head capsule, without spinules or nodules; mandible with very short teeth and bifid claw of posterior parapods with inner tooth very elongated. The species included are: *L. fosteri*, *L. hirsuta*, *L. opela*, *L. virescens*, *L. sp. 02*, *L. sp. 22*, *L. sp. 23*, *L. sp. 24* and *L. sp. 25*.

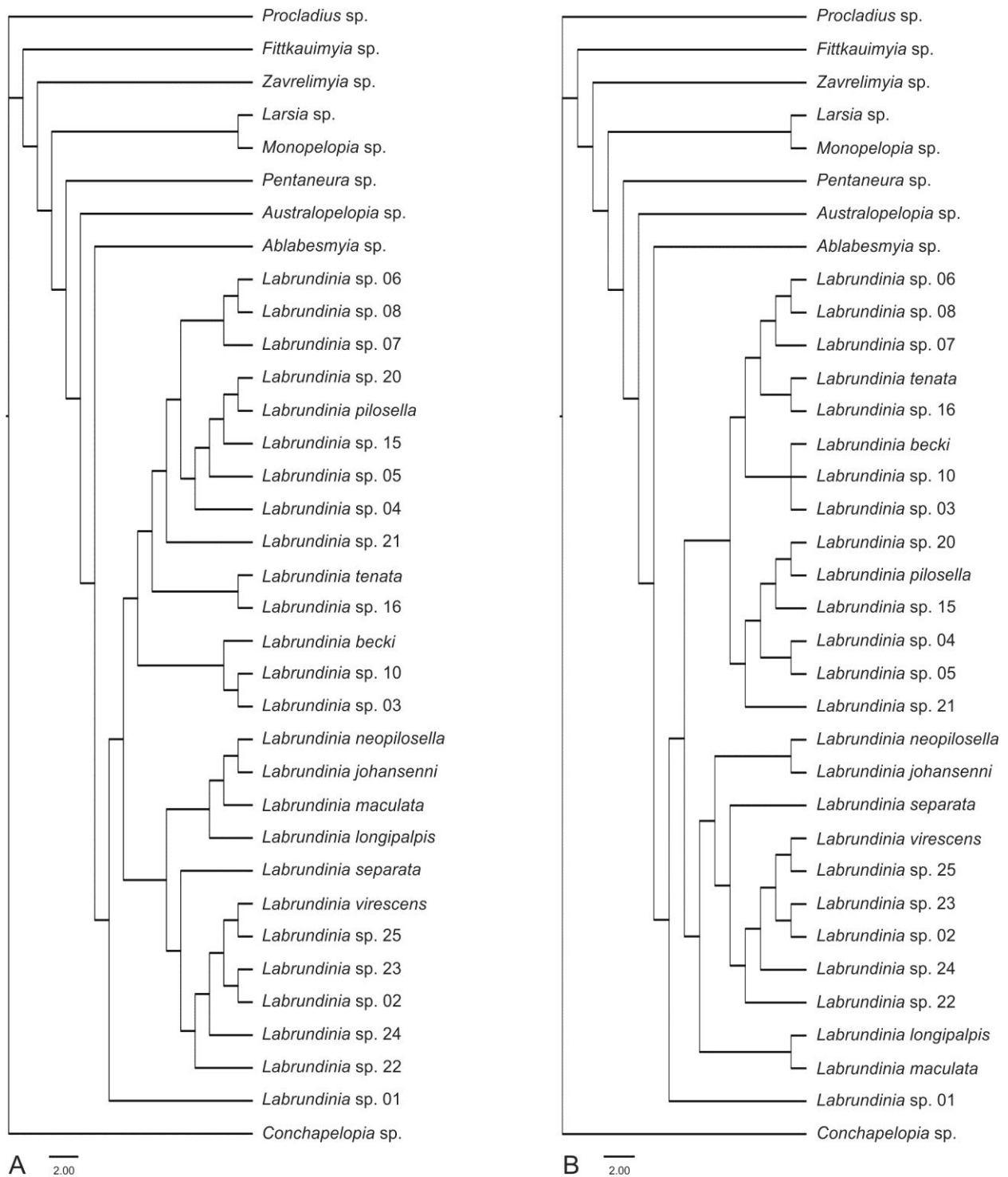


Figure 3. Phylogenetic hypotheses for the *Labrundinia* species. A, result from parsimony analysis on taxa with described male, pupa and larva only, with characters weighted according to their rescaled consistency indices. B, result from parsimony analysis on taxa with described male and pupa only, with characters weighted according to their rescaled consistency indices.

Doubtful placements

The species *L. separata* takes a position close to *L. sp. 13*. However, the wedge-shaped thoracic horn and absent preapical indentation have much more in common with *L. longipalpis* in the *neopilosella* group, this doubtful placement is possible due to a combination of several male features, almost certainly wrongly placed, such as tentorium and wing length, dorsocentrals distribution and fore leg ratio. The immatures of *L. sp. 13* is not yet known, and its morphology will probably assist in the accurate placement of *L. separata*.

In conclusion, the monophyly of the *Labrundinia* including all known species with the currently available characters is verified. The group is recovered from all analyses with Bayesian inference and parsimony methods. *Labrundinia* was recognizable in all trees resulting from a reduced taxon-character matrix, or from a reweighted morphology matrix. However, it is difficult to determine the accurate relationship among terminal taxa in data set with this many uncertain character states. Further analyses including the species with missing sequences will be necessary to reconstruct an unambiguous phylogeny of *Labrundinia*. Moreover, additional sampling ought to be conducted to find the unknown larvae and pupae in order to verify the robustness suggested by the characters of immature life stages in some of the major clades.

Biogeographical analyses

The area cladogram shown in Figure 4A was obtained by simply replacing the taxon names in the preferred tree (Figure 2A) with name of the area in which they occur. According to the area cladogram, *Labrundinia* could have its initial diversification in the Neotropical region, with one group dispersing to the Nearctic and western Palearctic, and *L. separata* dispersing into the temperate Neotropical region. Despite the errors regarding assumption 0 analyses, widely discussed by Humphries and Parenti (1999) and Ebach *et al.* (2003), Brooks Parsimony Analyses (BPA) was chosen due to its simplicity and feasibility, as well as its applicability to the dataset. The BPA analyses of the *Labrundinia* species yielded one tree of 87 steps (CI = 0.91, RI = 0.77), the resulting tree (Figure 4B) shows that distribution of *Labrundinia* has two main components, one in Neotropical region and another in the Nearctic region, confirming the area cladogram predictions. Furthermore, the North Amazonian region is more closely related to the Atlantic Forest region than to the Central America, which was expected due to geographical proximity between these components.

The resulting cladogram shows a sequence of vicariant events from South to North. The first disjunction of Neotropical component separates southern South America from all other northern America

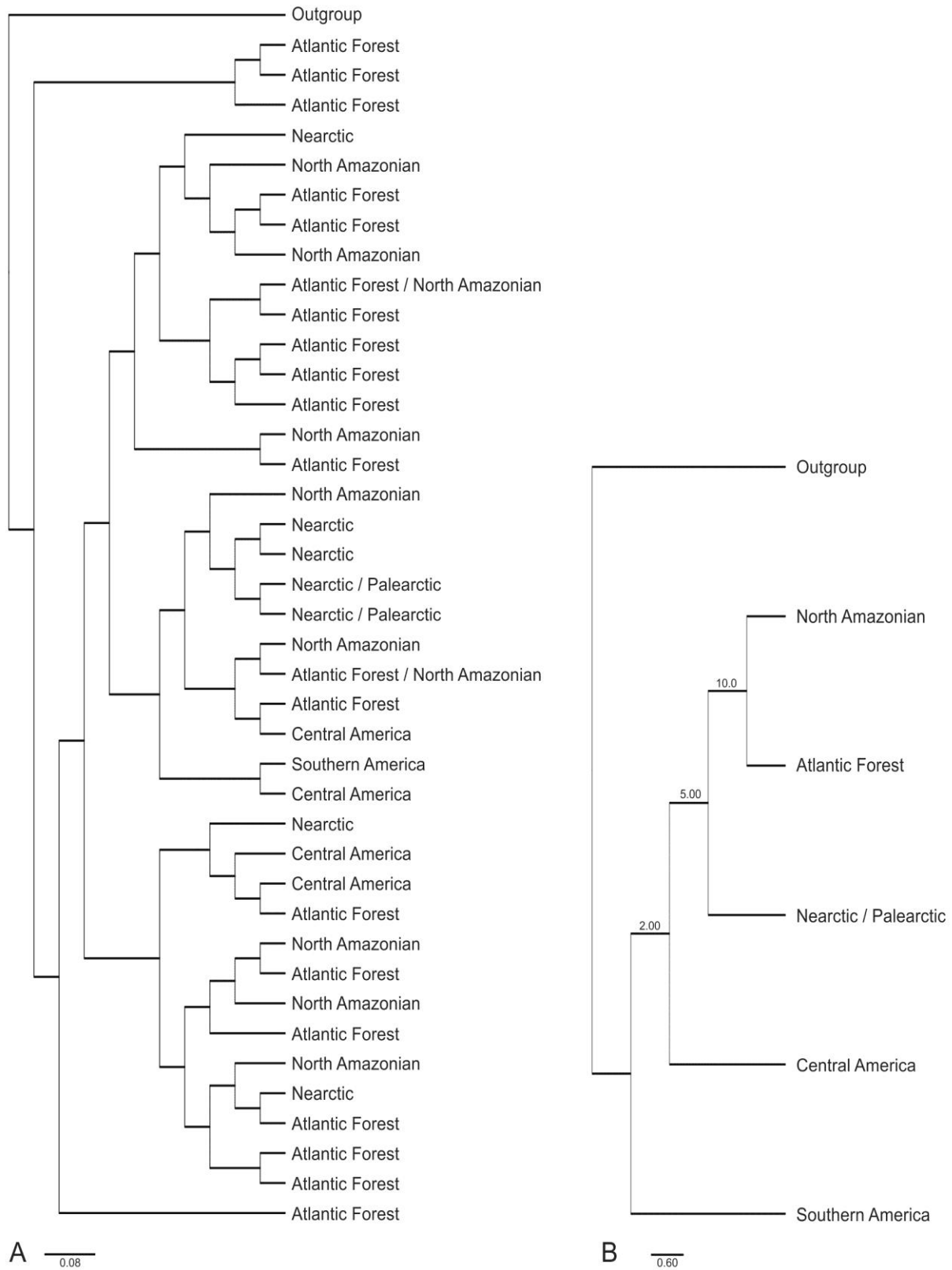


Figure 4. Cladograms for *Labrundinia* species. A, area cladogram based on tree in Figure 2A. B, result from Primary Brooks Parsimony Analysis. Bremer support shown on branches.

areas. However, it should be emphasized that the basal position of southern South America in the cladogram could be due to presence of only one endemic species recorded in this area, which might generate insufficient information to relate it to the northern areas, such as North Amazonian and Atlantic Forest. The following division was the separation between Central America and northern South America. The disjunction of northern South America into North Amazonian and Atlantic Forest, and their relationship with Nearctic component still demands a more detailed study to be completely understood. However, even though the sequence of separation of areas is not precisely the same, these patterns are similar to the ones found to several groups of Neotropical dipterans (Ditomyiidae, Sciaridae and Scatopsidae) and monkeys (Callitrichidae) (Amorim and Pires 1996) or papilionid butterflies (Lepidoptera) (Racheli and Racheli 2004).

The historical biogeography of *Labrundinia* species was also investigated using dispersal–vicariance analysis. Geographical distribution was coded as 6 states for *Labrundinia*, enabling radiation and diversification patterns to be assessed at fine resolution within the New World, where most of the subject taxa have restricted ranges. These states did not necessarily define political boundaries but harbour at least one endemic taxon. As usually observed in DIVA analyses (Ronquist 1997, Sanmartín 2003, Nylander *et al.* 2008, Wikström *et al.* 2010), the ancestral area reconstructions show wide distributions towards the root nodes. Despite these uncertainties, as well as uncertainties in the inferred relationships, the analyses provide clear indications about the biogeographical origins of the genus *Labrundinia*.

DIVA results suggest that a complex biogeographic history shaped the current distribution of *Labrundinia*. The optimal reconstruction (Figure 5) inferred at least 20 dispersal internal events to explain the present distribution of *Labrundinia* and favoured its ancestor as having originated in Neotropical region (node 2-8 and 18). The analysis of the four main components the distribution of *Labrundinia* (Figure 5) shows that the most favoured reconstructions for clade 1, Atlantic Forest (D: 100 %) is asserts as ancestral area. For clade 2 Atlantic Forest/North Amazonian provinces (D and C 50%) are asserted as ancestral areas. The ancestral reconstructions for clades 3 and 4 are remarkably ambiguous, but they favour Atlantic Forest and Central America.

The identification of multiple alternative reconstructions of ancestral areas at deeper nodes (6, 19 and 28) makes the identification of additional, unambiguous biogeographical events difficult. However, the data from DIVA can be used to summarize the overall pattern of biogeographical history in the group (Sedano and Burns 2010). All events, especially those occurring at an early point in the history of the group, have the Neotropical region as ancestral area, indicating the importance of this area in the diversification of genus *Labrundinia*. Most of species in the Neotropical region are distributed in only one biogeographical province. However, *L. sp. tenata* and *L. sp. 01* are recorded in the Atlantic Forest + North

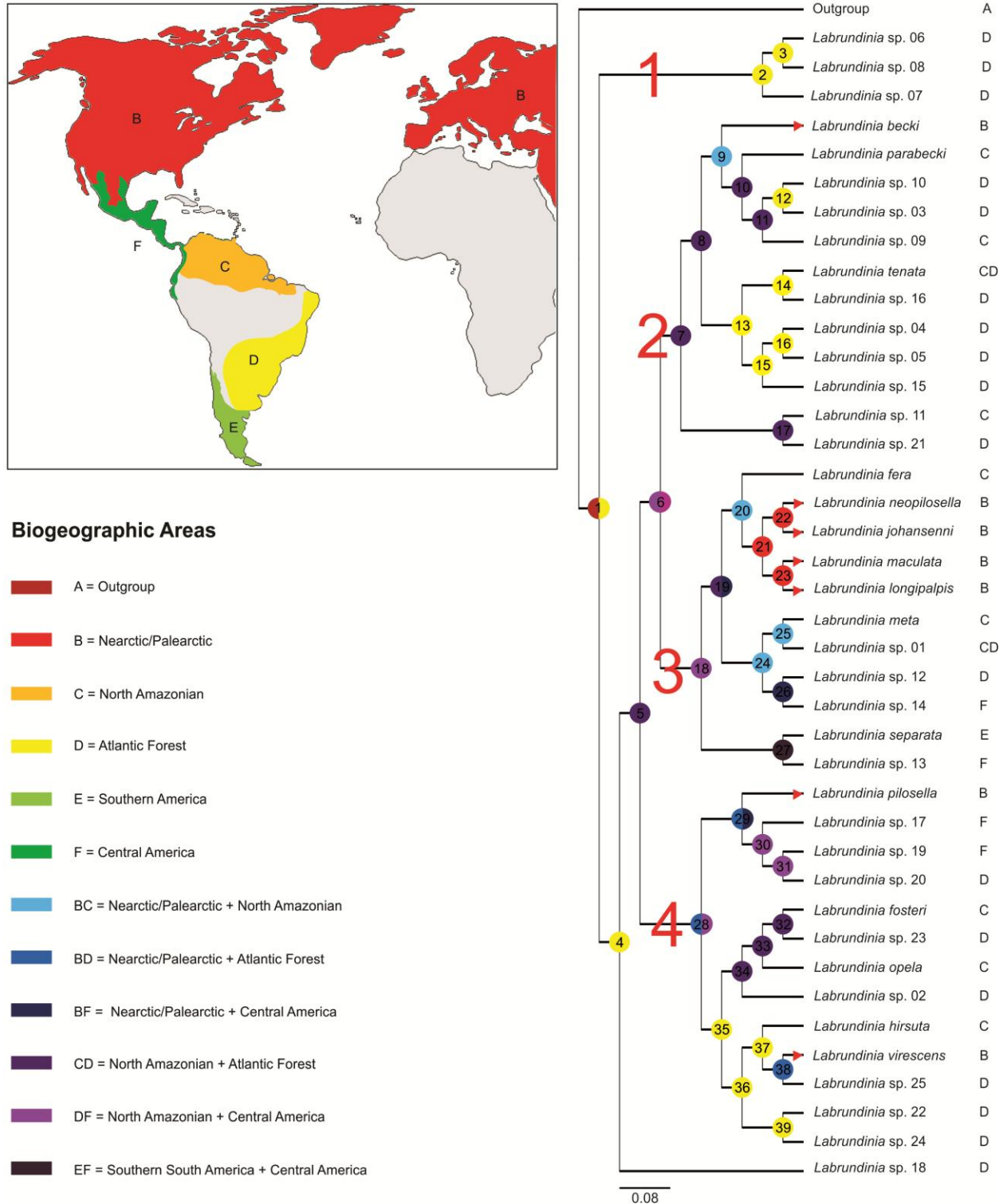


Figure 4. Statistical Dispersal-Vicariance Analysis (S-DIVA) optimizations of ancestral distributions in *Labrundinia*. Nodes are coloured according to the optimal reconstructions. Upper-case letters after taxon names indicate taxon distributions. Arrows represent main dispersal events between the defined regions. Red numbers indicates the key components of *Labrundinia* distribution.

Amazonian. *Labrundinia separata* is the only species endemic to southern South America, distributed from Argentinian Pampas to Valdivian Forest. The Nearctic species showed exclusive distribution in this region except for *L. longipalpis* which has been reported from the Palearctic region. However, the molecular relationship between Nearctic and Palearctic specimens of *L. longipalpis* remains unclarified (Silva *et al.* in revision). Our results suggest multiple colonization events from the Neotropics to the Nearctic region. Similar pattern was also found in diplopterous wasps (Bequaert 1940), toads (Pauly *et. al* 2004) and curculionid beetles (Lanteri 1990).

Unlike the Nearctic region, the South American continent is not a proper biogeographical unit (Amorim 2001, 2012), and it should be pointed out that the diversity of *Labrundinia* species is not evenly distributed in this continent. Therefore, the distributional results presented here probably reflect the lack of collection data. Several biogeographical inferences can be made from biogeographical analysis of *Labrundinia* species distributions and genealogical relationships. Nonetheless, as collections, especially from Central and South America are lacking, further inventories are required to improve the understanding of possible areas of endemism. Conclusive biogeographical analyses must assume that all areas involved have been sampled sufficiently so that absence from an area is not likely to be caused by incomplete knowledge of that area. For this reason, the results presented herein must be regarded as tentative.

ACKNOWLEDGMENTS

The authors extend their thanks to Caroline Silva Neubern de Oliveira, Humberto Fonseca Mendes, Sofia Wiedenbrug and Susana Trivinho Strixino for providing us with important material. We also thank the University of Oslo Biportal for providing a platform for running the phylogenetic software MrBayes and Paup*. F. L. Silva received financial support from the National Council for Scientific and Technological Development (CNPq proc. 141092/2009-2). Financial support was also given by the Research Council of Norway (RCN) through a Personal Mobility Grant.

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APPENDIX. List of species used in molecular analyses, with associated sample localities, voucher reference numbers and GenBank accessions.

Abbreviations: M = Male, Female = F, P = pupa, L = larva.

Taxon	Locality	Life stage	Voucher number	Accession number
<i>Labrundinia tenata</i>	São Carlos, Ecological Park, 19.iv.2010, F. L. Silva	F	FA663	JX887536
<i>Labrundinia</i> sp. 02	São Carlos, Fazzari reservoir, 27.iv.2010, F. L. Silva	P	FA561(1)	JX887483
<i>Labrundinia</i> sp. 03	Brazil: São Carlos, São Paulo, Canchin reservoir, F. L. Silva	F	FA664	JX887485
<i>Labrundinia</i> sp. 06	São Carlos, Canchin reservoir, 02.ii.2009, F. L. Silva	M	FA512	JX887487
<i>Labrundinia</i> sp. 07	Gália, Caetetus reservoir, 08.07.2008, F. L. Silva & J. F. Nunes	P	FA777	JX887494
<i>Labrundinia</i> sp. 08	São Carlos, Valparaíso lake, 26.xi.2011, F. L. Silva	F	FA216	JX887496
<i>Labrundinia</i> sp. 10	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	L	FA451(3)	JX887498
<i>Labrundinia</i> sp. 15	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	L	FA449(6)	JX887507
<i>Labrundinia</i> sp. 20	São Carlos, Monjolinho stream, 11.iv.2011, F. L. Silva	F	FA453	JX887512
<i>Labrundinia</i> sp. 21	São Carlos, Espraiado stream, 19.viii.2011, S. Wiedenbrug	F	FA36128	JX887514
<i>Labrundinia</i> sp. 23	Luis Antonio, Óleo reservoir, 07.i.2011, S. T. Strixino	P	FA650	JX887518
<i>Labrundinia</i> sp. 24	São Carlos, Mayaca pond, 11.iv.2011, F. L. Silva	L	FA131	JX887525
<i>Labrundinia</i> sp. 25	São Carlos, Mayaca pond, 11.iv.2011, F. L. Silva	M	FA342	JX887527
<i>Ablabesmyia</i> sp.	Australia: New South Wales, Molonglo River, P. S. Cranston	L	N110	Gi352741155
<i>Australopelopia</i> sp.	Australia: Queensland, Mt. Lewis, Windmill Creek, P. S. Cranston	L	FNQ10-12	Gi 352741181
<i>Conchapelopia</i> sp.	USA: California, Mailliard Redwoods State Forest, P.	L	CAM07	Gi 352741214
<i>Fittkauimyia</i> sp.	Australia: Northern Territory, Magela Creek, P. S. Cranston	L	AUNT14	Gi 352741251
<i>Larsia</i> sp.	Brazil: São Carlos, São Paulo, Monjolinho stream, F. L. Silva	M	FA3349(2)	
<i>Monopelopia</i> sp.	Brazil: São Carlos, São Paulo, Valparaíso lake, F. L. Silva	F	FA3370	
<i>Pentaneura</i> sp.	Brazil: São Carlos, São Paulo, Espraiado stream, S. Wiedenbrug	L	FA33113	
<i>Procladius</i> sp.	Australia: Melbourne, Victoria, Moonee Ponds, M. E. Carew	L	A04Pr11	Gi 329458631
<i>Zavrelimyia</i> sp.	USA: California, Mailliard Redwoods State Forest, P. S. Cranston	L	CAM08	Gi 352741447

CONSIDERAÇÕES FINAIS

→ Neste estudo, são redescritas 14 espécies conhecidas para o gênero *Labrundinia*, incluindo dados de imaturos, e 25 novas espécies são descritas para região Neotropical. Todas as espécies foram ilustradas. Tais dados sustentam a premissa de que a fauna brasileira de Chironomidae, em especial a de Tanypodinae, é pouco conhecida.

→ Análises de “DNA barcode” revelaram o uso do fragmento COI como uma excelente ferramenta para a identificação de espécies e para a resolução de conflitos taxonômicos no gênero *Labrundinia*. Os resultados também indicaram que “DNA barcode” pode ser usado para associar diferentes estágios de vida de Chironomidae, visto que o COI foi facilmente amplificado e sequenciado a partir de material biológico proveniente de diferentes semaforontes.

→ Análises de máxima parcimônia (MP) e de inferência bayesiana (IB) a partir matrizes (combinada para MP e particionada na IB) compostas por caracteres morfológicos e moleculares indicaram a monofilia de *Labrundinia* e permitiram o estabelecimento de 4 grupos de espécies: *becki*, *neopilosella*, *reniformis* e *virescens*.

→ Análises em biogeografia histórica, com abordagens baseadas em padrão (*pattern-based*) e em evento (*event-based*), sugerem que o ancestral de *Labrundinia* possui diversificação inicial na região Neotropical, sendo que a presença de espécies na região Holártica deve-se a dispersões tardias.