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

A DIVERSIDADE DE INSETOS MADÍCOLOS EM MONTANHAS DE MATA ATLÂNTICA: UMA ABORDAGEM ECOLÓGICA E TAXONÔMICA

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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 – UFSCar, como parte dos requisitos para obtenção do Título de Doutor em ciências, área de concentração em Ecologia e Recursos Naturais.

Orientadora: Prof. Dr. Susana Trivinho Strixino

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“I have no special talent.
I am only passionately curious”

Albert Einstein

À Hilomi Shimabukuro e
Antônio Shimabukuro (in memorian)

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RESUMO

O estudo de montanhas há muito tem interessado ecologistas ao redor do mundo. Muitos fatores explicam a importância desses ecossistemas como modelos para testar padrões de variação espacial das comunidades biológicas: 1. Ao longo de gradientes altitudinais mudanças climáticas significantes são observadas em relativamente curta escala espacial; 2. Em comparação com outros gradientes espaciais, e.g. latidude, experimentos realizados em montanhas podem ser facilmente replicados; 3. O possível isolamento ou proximidade de outras montanhas, bem como os diversos eventos climáticos e geológicos que podem sofrer, garantem um elevado valor biogeográfico para as montanhas; 4. Conferem condições ideais para o estabelecimento de uma grande biodiversidade, propiciando o surgimento de novas espécies, e a sobrevivência de espécies raras e endêmicas. Considerando todos estes fatores, este estudo teve como principal objetivo avaliar padrões de variação da riqueza e da composição de espécies ao longo de gradientes altitudinais na Mata Atlântica no Sudeste do Brasil. A comunidade de insetos associada aos habitats madícolos (higropétricos) em três montanhas localizadas na Serra do Mar e na Serra da Mantiqueira, compondo um gradiente de 0 m a 2.700 m acima do nível do mar, foram os objetos de estudo do presente trabalho. Na primeira parte da tese (enfoque ecológico), foram testadas as hipóteses de variação da riqueza e da dissimilaridade de espécies ao longo de gradientes altitudinais: 1. Efeito Rapoports; 2. Efeito espacial; 3. Controle ambiental; 4. “Mountain Passes are Higher in the Tropics”; 5. Dinâmica fonte-sumidouro. Como os habitats madícolos detém uma fauna de insetos singulares, e pouco abordados na literatura, na segunda parte desta tese (enfoque taxonômico), é apresentado um *checklist* de todas as espécies de Chironomidae madícolos catalogadas no presente estudo. Além disso, novas espécies pertencentes a três gêneros de Chironomidae são descritas, e novos registros foram realizados. Os insetos madícolos são altamente diversos, e os grandes táxons que os compõem são os mesmos que compõem a comunidade de insetos aquáticos de riachos. Foi observada uma significativa variação da riqueza e da dissimilaridade ao longo dos gradientes estudados, e, além disso, estas variáveis se mostraram altamente relacionadas com a altitude. Com relação à comunidade de Chironomidae madícola muitas espécies desconhecidas para a ciência foram evidenciadas, grande parte com distribuição altitudinal restrita. Além disso, algumas espécies já conhecidas foram registradas pela primeira vez neste ambiente peculiar. Estes resultados confirmam a importância e a necessidade da conservação e monitoramento de ecossistemas de montanha, especialmente em regiões tropicais, onde os impactos climáticos e a pressão antrópica tendem a ser mais rigorosos.

ABSTRACT

Ecologists all over the world have long been interested in studying mountains. Many factors explain the importance of mountain ecosystems as models to test patterns of spatial variation of biological communities: 1. Along altitudinal gradients significant climate changes can be observed in a relatively short spatial scale; 2. Comparing to oher spatial gradients, e.g. latitude, experiments developed in mountains are easily replicable; 3. The isolation and proximity from other mountains, as well as the noticeable climatic and geologic events that they experience, ensure the high biogeographical value of mountains; 4. Mountains guarantee ideal conditions for the establishment of a remarkable biodiversity, facilitating speciation process and the survivor of rare and endemic species. Regarding these factors, the main objective of this study was to assess patterns of richness and dissimilarity variations along altitudinal gradients at Atlantic Forest from Southeast Brazil. The madicolous (hygropetric) insect community and three mountains located at Serra do Mar and Serra da Mantiqueira – compounding a gradient from 0 m to 2700 m a.s.l. 0 were the study object of this work. At the first part of the thesis (ecologic approach), important hypotheses of richness and dissimilarity' variations along altitudinal gradients have been tested: 1 Rapoport's effect; 2. Spatial effect; 3. Environmental control; 4. "Mountain Passes are Higher in the Tropics"; 5. Source-sink dynamics. As madicolous habitats shelter a peculiar fauna of insects, and poorly addressed in literature, in the second part of the thesis (taxonomic approach), a *checklist* of all madicolous Chironomidae recorded here is presented. In addition, new species belonging to three Chironomidae genera are described and new records were evidenced. Madicolous insects are extremely diverse, and most of the taxa that compound it are the same found in insect community from streams. A significant variation of richness and dissimilarity have been observed along the gradients analyzed, and further, this variables showed deeply related to the gradients' altitude. Regarding the madicolous Chironomidae community, many species unknown to science have been verified, many of them with restricted altitudinal distribution. In addition, many known species have been recorded for the first time in this peculiar habitat. These results confirm the importance and the need of conservation and monitoring of mountain ecosystems, especially at tropical region, where climatic impacts and anthropic pressure tend to be harsher.

INTRODUÇÃO

Os habitats higropétricos (Thienemann, 1905) ou madículos (do latim *madidus*: úmido, molhado) são aqueles formados por um filme de água com menos de 2 mm de espessura que percorre superfícies rochosas (Vaillant, 1956). Essas lâminas d’água podem ser mantidas pelo afloramento de água diretamente das lajes rochosas ou formadas nas margens de córregos a partir do constante umedecimento pela corrente principal (“splash zones”) (Ward, 1992). Esse ambiente confere condições ideais para a colonização de briófitas, algas, ou até mesmo plantas vasculares, e, consequentemente, favorecem o desenvolvimento de uma rica e peculiar fauna invertebrada (“madícola”) de rápida colonização, alto grau de endemismo e algumas espécies raras (Spangler, 1972). A fauna madícola é dominada pelos insetos (Oliver & Sinclair, 1989; Ward, 1992; Sinclair & Marshall, 1987; Sinclair & Saigusa, 2002). Embora alguns grupos de Coleoptera, como Hydrophilidae, e alguns Trichoptera sejam muito recorrentes nesses ambientes, a ordem Diptera costuma ser a mais representativa, com destaque para algumas famílias, como Tipulidae (Sinclair, 1988), Psychodidae, Stratiomyidae, Dixidae e Chironomidae (Hynes, 1972; Pinder, 1995). Entretanto, esse tipo de ambiente está entre os habitats menos estudados nas regiões temperadas e tropicais, tanto em abordagens ecológicas quanto faunísticas (Isaia et al., 2009).

Embora muitos organismos possam ser encontrados nesses habitats, a minoria é estritamente higropétrica (eumadícolos) (Ward 1992). Em uma lista de 47 espécies de Chironomidae, catalogadas por Oliver e Sinclair (1989), de ambientes madícolos Holárticos, apenas quatro eram restritos ao habitat e pertencentes aos gêneros *Limnophyes, Orthocladius, Syndiamesa* e *Hudsonimyia.* A associação de *Hudsonimyia*, pertencente à subfamília Tanypodinae, com esse tipo de biótopo foi também exatltada por Roback (1979). No Brasil, o primeiro registro em habitat higropétrico pertencente a este gênero foi *Hudsonimyia araxa* Silva, Wiedenbrug, Trivinho-Strixino, Oliveira & Pepinelli cujas larvas foram coletadas em rochas cobertas por algas, musgos e detritos, em regiões montanhosas do estado de São Paulo e Minas Gerais (Silva et al., 2012).

Podonomus pepinellii, a única espécie até então registrada para subfamìlia Podonominae no país, também foi encontrada em associação com rochas nas quais percorria uma fina película de água, em elevada altitude na Serra da Mantiqueira (região de Minas Gerais) (Roque & Trivinho-Strixino, 2004; Trivinho-Strixino et al., 2012). Recentemente, uma nova espécie pertencente ao gênero *Limnophyes* foi descoberta na região serrana de Santa Catarina, *Limnophyes guarani* Pinho & Andersen (Pinho & Andersen, 2015), também em associação com habitats madícolos. Assim, a importância taxonômica dos ambientes higropétricos vem sendo gradativamente ressaltada à medida que novas espécies e novos registros são reportados. Contudo, abordagens ecológicas que utilizam habitats madícolos como modelo para avaliação de hipóteses são, até onde sabemos, inexistentes.

Descobrir e entender padrões ecológicos em função de gradientes ambientais e geográficos sempre foi um assunto de grande interesse nos estudos envolvendo comunidades biológicas. As alterações na biodiversidade podem ser explicadas por múltiplos fatores intimamente dependentes da escala espacial (Whittaker et al., 2001; Hawkins et al., 2003; Rahbeck, 2005; Heino, 2009). Dentre os padrões observados em larga escala geográfica, a redução da diversidade biológica da região equatorial em direção aos pólos do planeta, por exemplo, destaca-se como um dos mais difundidos e fundamentais em ecologia e biogeografia (Willig et al., 2003; Brown & Lomolino, 1998; Baselga, 2008, 2010). Apesar disso, a variação da diversidade em função de gradientes altitudinais parece não seguir uma tendência tão clara (Jacobsen et al., 2004).

A redução da riqueza conforme o aumento da altitude já foi constatada por estudos realizados em ambientes terrestres (Rahbeck, 1995; Bruhl et al., 1999; Escobar et al., 2007) e aquáticos (Rautio, 1998; Jacobsen et al., 1997; Lencioni et al., 2011; Wang et al., 2011). Há ainda muitas evidencias de que nas altitudes intermediárias ao longo de um gradiente altitudinal podem ser encontrados os maiores valores de riqueza de espécies (Colwell & Hurtt, 1994; Holloway, 1987; Bachman et al., 2004; Tilman, 1982, Rahbek 1997; Rozensweig, 1995; Kessler 2000). Esse padrão unimodal pode ser resultante de puro efeito geométrico, o chamado MDE – "mid-domain-effect" (Colwell & Hurtt, 1994; Holloway, 1987; Bachman et al., 2004), ou relacionada a fatores ambientais, como por exemplo, a produtividade (Tilman, 1982, Rahbek 1997; Rozensweig, 1995; Kessler 2000).

Jacobsen et al. (2004) sugere algumas importantes razões que explicam a inexistência de um consenso na relação riqueza e altitude para insetos aquáticos; dentre elas destacam-se: 1. Muitos estudos têm enfocado em apenas uma determinada ordem taxonômica, o que não permite a extrapolação do padrão encontrado para toda a comunidade; 2. Evidentes relações com a altitude não podem ser esperadas de casos em que a variação da altitude não ultrapasse 500 m; 3. Áreas de estudo que envolvam corpos d'água com diferentes níveis de conservação não são adequados, pois podem mascarar o efeito da altitude; 4. O gradiente longitudinal de riqueza ao longo de um mesmo riacho pode camuflar o efeito da altitude devido a diferenças existentes entre os habitats de *rhithron* e *potamon;* 5. Impossibilidade de se obter conclusões claras quando, por dificuldades taxonômicas, os espécimes não podem ser identificados em nível específico. Portanto, todas estas questões foram levadas em conta ao se testar a relação entre a riqueza de espécies e o gradiente de altitude no presente estudo.

Diferentemente de ambientes lóticos que possuem relativamente grandes dimensões, como córregos, rios e riachos, os ambientes higropétricos podem ser considerados micro-ecossistemas; pelo fato de serem estruturalmente mais homogêneos que os habitats anteriormente citados e frequentemente isolados (Ward, 1992), os habitats madícolos seriam melhores modelos de estudo para se observar variações na comunidade habitante ao longo de gradientes altitudinais.

O estudo de gradientes altitudinais possibilita a experimentação de importantes hipóteses ecológicas que visam entender as respostas da biota frente a variações ambientais e geográficas (Darwin, 1839; Stevens, 1992; Colwell & Hurtt, 1994; Lomolino, 2001; Rahbek, 1997, 2005; Ricklefs, 2004). Além disso, a biodiversidade de montanhas há muito fascina cientistas do mundo inteiro, devido ao alto grau de endemismo e estratégias morfológicas, fisiológicas e comportamentais que as espécies habitantes podem apresentar.

Muitos estudos em ambientes aquáticos ressaltam que importantes mudanças composicionais nas comunidades de insetos aquáticos e outros macroinvertebrados são observadas ao longo de gradientes altitudinais (i.e. diversidade beta) (Heino et al., 2015; Al-Shami et al., 2013; Wang et al., 2012; Gill et al., 2014). Além disso, o recente avanço nas propostas e abordagens relativas à diversidade beta possibilitou que investigações mais completas fossem adotadas para avaliar a similaridade na composição de espécies em função de largas escalas espaciais (Diserud and Ødegaard, 2007; Baselga & Jiménez-Valverde, 2007; Baselga, 2010; Koleff et al., 2003).

Dentre as múltiplas formas de se avaliar a diversidade-beta, um dos conceitos parte do princípio de que esta é uma medida que traduz como as espécies respondem à heterogeneidade ambiental ao longo de gradientes ecológicos (Whittaker, 1960; Heino, 2009, Jurasinski et al., 2009) e pode refletir dois diferentes fenômenos: a troca de espécies e o aninhamento de espécies nas comunidades (Brendonck et al., 2014; Baselga, 2012; Dobrovolski et al., 2012; Harrison et al., 1992). O padrão de aninhamento ocorre quando as biotas dos locais com menor número de espécies representam uma porção daquelas em locais mais ricos em espécies. Há, portanto, uma perda de espécies em decorrência da ordenada desagregação das comunidades, gerada por um determinado fator no gradiente. O *turnover* por sua vez implica na substituição de algumas espécies por outras, como consequência de fatores geográficos, históricos ou ecológicos.

De maneira geral, a dissimilaridade na composição de espécies ao longo de gradientes altitudinais é controlada por fatores ambientais, espaciais (Heino et al., 2015) e históricos (como por exemplo especiação e extinção) (Graham et al., 2006). A heterogeneidade ambiental e as taxas de dispersão são dependentes da escala espacial. Como exemplo, se a distância geográfica é grande o suficiente para impedir a dispersão, a distribuição das espécies ocorre apenas em locais próximos, independente de filtros ambientais (Heino et al., 2015). Além disso, espécies com capacidade de dispersão limitada estão mais propensas aos processos vicariantes e à extinção local (MacArthur & Wilson, 1967).

De acordo com Lobo & Halftter (2000), dois processos principais podem explicar a colonização de montanhas: 1. Horizontal, quando a colonização se dá por elementos provenientes de elevadas altitudes e 2. Vertical, quando as espécies se originam de regiões vizinhas de menor altitude. Ambos os processos dependem da orientação e da localização das montanhas, que estão relacionadas ao grau de isolamento e aos processos biogeográficos inerentes a cada formação. Estas características influenciam significantemente na capacidade de algumas montanhas servirem como refúgio e “corredores” para o estabelecimento das espécies (Lobo & Halftter, 2000) e, em função disso, também refletem em diferentes padrões de dissimilaridade ao longo dos gradientes.

Em regiões tropicais, as espécies tendem a mostrar curtas distribuições altitudinais, hipótese conhecida como “Mountain Passes are Higher in the Tropics” (MPHT) (Janzen, 1967). Essa hipótese se baseia no histórico evolutivo das espécies em clima tropical, já que nesta região, poucas alterações climáticas foram observadas quando comparada com regiões temperadas, que passam por muitas oscilações de temperatura anuais (Stevens, 1989). Assim, as variações climáticas sofridas ao longo dos gradientes altitudinais tendem a exercer maior pressão sobre as espécies tropicais, e, portanto, a substituição de espécies ao longo do gradiente é intensificada. Isso pode em parte explicar a alta endemicidade observada em montanhas tropicais.

A biodiversidade de montanhas representa um potencial indicador de mudanças climáticas globais. As previsões atuais sugerem que a temperatura global média pode aumentar de 2,0 a 6,0 ºC em 2100 e que as temperaturas e taxas de precipitação serão menos constantes (Garcia et al., 2014). Consequentemente a biota de montanhas está susceptível a alterações em termos de ocorrência, distribuição, diversidade genética, entre outros. Assim sendo, entender os processos que afetam essas comunidades é uma necessidade imediata para o adequado planejamento e estratégias de conservação e monitoramento. Além disso, um dos objetivos deste trabalho foi contribuir para o conhecimento da biodiversidade do bioma Mata Atlântica, um dos mais diversos e, ao mesmo tempo, mais ameaçados do mundo sendo considerado por isso um hotspot para a conservação (Myers et al., 2000).

No presente trabalho, foram estudados os insetos madícolos em três montanhas localizadas em trechos de Mata Atlântica preservada, escolhidas de forma a preencher completamente o gradiente altitudinal existente no bioma (0 m – 2700 m). A composição e abundância dos insetos imaturos foram analisadas, sempre que possível, a cada 200 metros de altitude, sendo os organismos amostrados em campo e identificados em laboratório. A família Chironomidae, a mais abundante e diversa no presente estudo, foi analisada em todos seus estágios de desenvolvimento (larva, pupa e adulto), para fins taxonômicos. Dessa maneira, a presente tese foi organizada em duas partes, a primeira com uma abordagem ecológica, no qual foram utilizados os dados com a comunidade inteira, e a segunda com uma abordagem taxonômica, no qual apenas a família Chironomidae foi analisada.

Na primeira parte busquei analisar grandes padrões de variação de riqueza e composição de insetos madícolos ao longo dos gradientes de altitude. Para isso, apresento dois capítulos, o primeiro testando três importantes hipóteses de variação de riqueza (efeito de Rapoport, efeito espacial e controle ambiental), e o segundo testando duas hipóteses de variação da composição da comunidade (MPHT e modelo “fonte-sumidouro”). Ambos os manuscritos encontram-se nas normas da revista *Diversity and Distributions*.

A segunda parte é composta por quatro capítulos de cunho taxonômico, dos quais o primeiro agrega um *checklist* das espécies madícolas de Chironomidae encontradas no presente estudo, para o qual foram determinadas as espécies indicadoras de altitude, e acrescentadas informações a respeito da atual distribuição (altitudinal e geográfica) destas espécies. Os três demais capítulos apresentam descrições de novas espécies encontradas e novos registros realizados. De forma que, no capítulo dois, duas novas espécies de *Podonomus* são descritas; no capítulo três, quatro espécies de *Pseudochironomus* são descritas; e no capítulo quatro, três novas espécies de *Tanytarsus* são descritas. Uma vez que os artigos ainda não foram publicados, os nomes dado às espécies são provisórios. Para os quatros capítulos desta parte, a padronização segue as normas da revista *Zootaxa.*

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PARTE I

ECOLOGIA

Cap. 1 Elevational patterns of richness: "The ends are bad" for madicolous insects in the Atlantic Forest

Cap. 2 Drivers of compositional dissimilarity of madicolous insects in elevational gradients of Atlantic Forest

Elevational patterns of richness: Are the ends bad for madicolous insects in the Atlantic Forest?

ABSTRACT

Aim “Biologists have long recognized that elevational and latitudinal species-richness gradients mirror each other”. With this statement Stevens (1992) and other ecologists assumed the same hypotheses to explain richness patters in latitudinal and altitudinal gradients. However, the monotonic decrease of species towards highest altitudes is far from being a rule in elevational gradients studies, with the majority reporting an intermediate peak of richness. Here we analyze the richness pattern of madicolous insects’ community along elevational gradients. We tested three hypotheses to explain the observed richness patterns 1. Rapoport’s rule; 2. Spatial constraint (area and mid-domain effect); 3. Environmental control on richness.

Location Three mountains from the Atlantic Forest in Southeast Brazil each one with approximately 1000 m of altitudinal range, representing low, intermediate and high gradients. Thus, we were able to explored the complete altitudinal range existent in the Atlantic Forest domain (0 – 2700 m a.s.l.).

Methods Sampling were performed along a transect. All madicolous habitats available along the transects were sampled, ensuring that at least three replicates (corresponding to 0.125 m^2) in each 200 meters of elevation were obtained. Simple and multiple regressions were used in order to identify significant relations and comparison between empirical richness data and null model for MDE has been performed.

Results The complete elevational gradient showed an intermediate peak of richness. Nor Rapoport’s effect neither area had important influence over richness, but a significant effect of environmental variables and geometric constraint has been detected.

Main conclusions Environmental variables explained most of the relation between richnees and altitude, but the hard boundaries established (sea level and highest mountaintop) have constrained species distribution and contributing for the observed richness patterns, but higher explanation have been found for environmental control, where emergence rate of madicolous insects (productivity), temperature and oxygen were the main predictors.

Keywords emergence rate, spatial constraint, mountains, altitudinal pattern, climate variability, harsh conditions, hygropetric habitat, tropical forest

INTRODUCTION

Understanding ecological patterns derived from environmental and geographical gradients have been one of the main challenges in community ecology. The variation in biodiversity is explained by multiples factors related to spatial scale (Whittaker *et al.*, 2001; Hawkins *et al.*, 2003; Rahbek, 2005; Heino, 2009), so that gradients in species richness are explained by the joint effect of historical events, environmental predictors and spatial constraint (Willig *et al.*, 2003). Among the patterns observed in large geographic scale, the decrease of biological diversity from equator towards the poles, for example, is one of the most widespread and fundamental in ecology and biogeography studies (Willig *et al.*, 2003; Brown & Lomolino, 1998; Baselga, 2008, 2010).

Once Stevens (1992) has stated that "biologists have long recognized that elevational and latitudinal species-richness gradients mirror each other", most of the hypotheses about the variation in species richness along altitudinal gradients derived from the latitudinal hypotheses of richness pattern (MacArthur 1969; Wright 1983; Simpson, 1983; Brown 1988; Stevens, 1992; Whittaker *et al.*, 2001).

One of the most widespread hypotheses for the monotonic decrease in species richness along latitudinal gradients posits that species at higher latitudes can tolerate a higher range of climatic variation due to historical constrains, and therefore present wider latitudinal range – The Rapoport' s rule or effect (Stevens, 1989). This would inflate the number of species living at lower latitudes, who presents short tolerance range to environmental changing (Stevens, 1989; Blackburn & Gaston 1996). The same hypothesis has been proposed to explain altitudinal decrease in species richness (Stevens, 1992), where lowland communities have a greater number of species due to the mass effect (Shmida & Whittaker, 1981), and sink habitats (Shmida & Ellner, 1984). However, many studies do not support this hypothesis (McCain & Knight, 2013; Colwell & Hurt, 1994).

Actually, nor latitude neither altitude are directly responsible for spatial richness variation, but they are surrogates for some environmental factors that co-varies with the gradient (Rahbek, 2005). Other hypothesis predicts that harsh climate conditions observed at higher altitudes may limit the existence of most of species (Jacobsen, 2008). Thus, some studies attribute the decline in species richness to the decrease in productivity as altitude increases (Hawkins *et al.*, 2003; Orians 1969; Brown &

Lomolino 1998; MacArthur 1969). The predicted positive relation between richness and productivity take into consideration that high energy availability in most productive region would favor the occurrence of a higher number of species rather than increase the existent populations (Connel & Orians, 1964; Currie, 1991). The decrease of richness as the altitude rises has been verified in terrestrial (Rahbek, 1995; Bruhl *et al*., 1999; Escobar *et al*., 2007) and aquatic ecosystems (Rautio, 1998; Jacobsen *et al*., 1997; Lencioni *et al*., 2011).

Most of the evidences, however, indicate that higher richness occurs at intermediate altitudes when considering an elevational gradient (Colwell & Hurtt, 1994; McCain, 2004; Sánchez-Cordero, 2001; Rahbek, 2005). Many hypotheses have been extensively discussed to explain this pattern (Colwell & Hurtt, 1994; Tilman, 1982, Rahbek 1997). "The ends are bad" hypothesis states that climatic constraint is the most important factor driving richness, so the decrease of richness is due to the temperature severity at both extremities: mountaintops and lowlands, and additionally, the high predation pressure may control richness at low altitudes (McCoy, 1990). "The middle is good" hypothesis states that productivity is higher at intermediate altitudes, where photosynthesis is optimized by mild temperatures during the day, and respiratory rates are lower at the cool evenings (Janzen, 1973; Janzen *et al*., 1976).

Other widespread concept posits that in a limited spatial interval, species tend to overlap their range close to the middle of the domain, this is called the mid domain effect (MDE) (Colwell & Hurtt, 1994; Holloway, 1987; Bachman *et al*., 2004). Thus, the MDE is considered a pure geometric constraint on species richness due to the boundaries imposed to species ranges, and therefore predicts a null effect of environmental, biological, historical factors. The MDE have been evidenced in many studies (Miyamoto *et al*., 2014; Sanders 2002; McCain, 2004; Colwell *et al*., 2004; Colwell *et al*., 2005; McCain, 2003). Other hypothesis posits that the hump shape of richness in intermediate altitudes is once again associated with productivity (Tilman, 1982, Rahbek 1997; Rozensweig, 1995; Kessler 2000). In this case, richness increases as productivity increases, peaks at intermediate elevation, and decreases at most productive extremity (which most of the times refers to sea level). The decrease phase of richness in most productive areas is not enlightened yet (Rozensweig, 1995), but may be associated with covariance of populations' density or intertaxonomic competition (Rahbek, 1997).

The influence of area on richness patterns is one of the few laws in ecology (Schoener, 1976; Dodds, 2009), thus the effects of the area should be regarded in studies dealing with spatial variation in richness. However, few studies have considered the area effect in altitudinal patterns of richness (Romdal & Grytnes, 2007; Sanders, 2002; McCain, 2007). Once many mountains present a conical shape (Korner, 2000; Lomolino, 2001), species richness tends to increase towards the base, where the available area is larger. When area influence is aggregated to the analysis, an intermediate peak of richness in the altitudinal gradient can be detected (Rahbek, 1997), or even the absence of richness pattern (Lawton *et al.*, 1987).

Regarding aquatic insects communities from streams, two other important aspects can preclude the observation of altitudinal effects on richness: the extension of the elevational range studied – ranges below 500 m seems to be inadequate to demonstrate richness patterns (Jacobsen *et al.*, 2004) – and the high heterogeneity observed along longitudinal river course, so that the observed variation in richness suffers from strongly habitat influence (Harrington *et al.*, 2015; Jacobsen *et al.*, 2004). Thus, the definition of altitudinal gradient boundaries and also the choice of stable and homogeneous habitats are essential for studies dealing with richness patterns.

Madicolous habitats (Thienemann, 1905) are characterized by a thin film of water that runs over rocky surfaces (Vaillant, 1955), therefore they are spatially restricted and isolated ecosystems (Ward, 1992). Madicolous habitats are less environmentally heterogeneous than streams, what make them a good object for altitudinal gradient studies. In addition, this biotope is known to harbor a peculiar insect fauna, with many species exclusively adapted to it (Vaillant, 1955; Hynes, 1970, Trivinho-Strixino *et al.*, 2012). However, this habitat has long been neglected from faunistic and ecologic studies (Fischer *et al.*, 1998; Isaia *et al.*, 2009).

In this study, we describe richness patterns using madicolous insect community in three different 1000m-elevation gradients, represented by low, intermediate and high altitudes, and which together represents the complete altitudinal range existent in the Atlantic Forest domain (0 – 2800 m a.s.l.). We expect to find: 1. A monotonic decrease of species as altitude increases, or 2. An unimodal pattern with a peak of richness in central portion of the domain. Then, we tested three hypotheses to explain the observed richness patterns 1. Rapoport's rule; 2. Spatial constraint (area and mid-domain effect); 3. Environmental control on richness.

METHODS

Study area

The study area comprehends three mountains from the Atlantic Forest located in Southeastern of Brazil (Fig. 1). The mountains were chosen in order to represent the complete elevational range present in the Atlantic Forest, and undisturbed conditions were ensured by their localization inside conservation units.

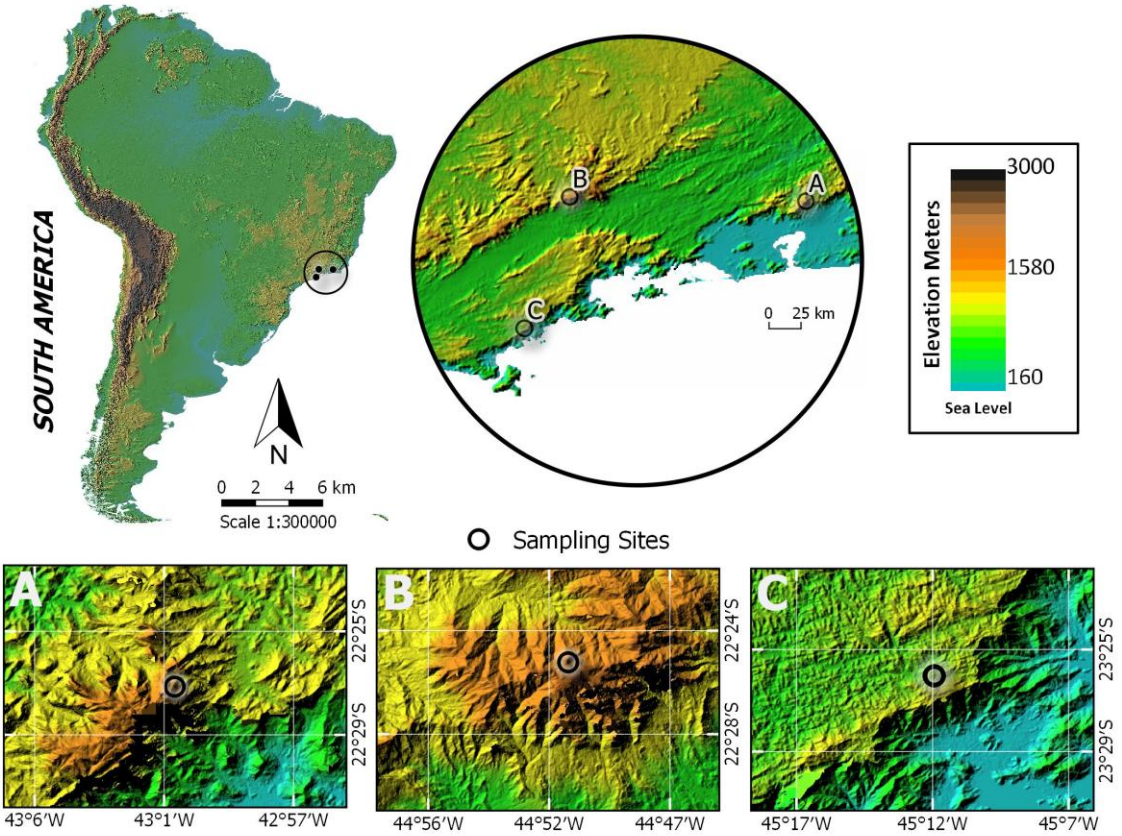

Figure 1 Localization of the study area in Southeast Atlantic Forest (Brazil). A – PS Mountain (intermediate altitudinal gradient); B – PM Mountain (high altitudinal gradient); C – C Mountain (low altitudinal gradient).

Serra do Mar State Park (PESM) is the widest conservation unit in São Paulo State, occupying approximately 315,000 ha, and sheltering the Corcovado Mountain (23˚27'876"S/45˚11'911"W), one of our objects of study. Corcovado (C) (Fig. 1c) ranges from the sea level to approximately 1100 m a.sl. and therefore represented the first altitudinal gradient analyzed here. From its base to the top, Corcovado is densely covered by ombrophilous forest that is favored by the exceptional rainfall records that strikes the region.

Serra dos Órgãos National Park (PARNASO) is situated in Rio de Janeiro State, and the rough terrain present in the region allowed us to complement our investigation in an intermediate altitudinal gradient. Pedra do Sino Mountain (PS) (Fig. 1a), situated in PARNASO (22°27'707'S/43°01'815"W), ranges from 1200 to 2200 m a.s.l. and presents four phytophysiognomies along the elevational gradient: Submountain Forest, Mountain Forest, Altimontain Forest and Campos the Altitude (similar to Andean Paramos).

Serra da Mantiqueira Environmental Protection Area (APASM) concentrates one of the largest and more important mountain ranges from eastern South America. It comprises three States (São Paulo, Rio de Janeiro and Minas Gerais) and the altitude in the region frequently exceeds 2000 m a.s.l. Inside this conservation area is located the Pedra da Mina Mountain (PM) (22˚23'955"S/44˚50'991"W) (Fig. 1b), which is the second highest peak from Atlantic Forest domain, ranging 2798 m a.s.l. (the first is at 2891 m a.s.l.), and the fourth highest from Brazil. Pedra da Mina represented the third and the highest gradient in the present study, where sampling were performed from 1600 to 2700 m a.s.l.

Serra da Mantiqueira and Serra do Mar formations, which includes also PARNASO territory, have the same geological origin that dates from Late Cretaceous - about 70 Mya (Almeida & Carneiro, 1998; Modenesi-Gautieri *et al.*, 2002), and topographic profile is derived from tectonics movements.

Sampling methods

Sampling were performed along a transect in each mountain. All madicolous habitats available along the transects were sampled, ensuring that at least three replicates in each 200 meters of elevation were obtained, and totalizing 70 sampling sites from 0 to 2700 m a.s.l. Each sampling site was delimited by the coverage area of an emergence trap (Figura 1, em anexo) – approximately 0.125 m^2 – (Shimabukuro *et al.*, 2015, anexo 1).

Emergence traps were installed directly above the madicolous habitats and remained in the field for seven days. The adults were removed from the traps after the seven days and were identified as - species level for Chironomids (Parte II – Cap. 3) and order or family for other taxa (Shimabukuro *et al.*, 2015) – and counted. However, in the present study, abundance of emergent insects was the only information on the adults' data used (estimates of productivity).

All richness analysis was performed with immature organisms' data. Larvae were collected directly from the madicolous habitats, after the removal of the traps, by washing the substrate right above them and only at that specific area. All material were conserved in absolute ethanol and analyzed under stereomicroscope and optic microscope. All immature insects sampled were identified using specialized bibliography (Domínguez & Fernández, 2009; Trivinho-Strixino, 2011; Hamada *et al*., 2014; Lecci & Froehlich, 2007; Salles *et al*., 2004; Segura *et al*., 2011) to the lowest possible taxonomic level (genus/species). Working at genus level of aquatic insects has proved to be efficient to describe ecological trends (Wiggins & Mackay, 1978).

Predictor variables

Environmental factors were measured *in situ* at each sampling site. Water variables (temperature, dissolved oxygen, pH, conductivity, depth) were measure with the help of an analytical kit of chemical and physical parameters for freshwaters (Alfakit®). Water current was estimated by mass displacement. Visual measure of canopy openness (indicating the local solar incidence) was also determined once it could indirectly affect the madicolous community. Geographic data were obtained with a GPS, and the area of each altitudinal band was calculated with the software Google Earth Pro 7.1.2.2041.

Given that productivity can be defined as a rate of energy flow into an ecosystem, and that it is hardly possible to measure in nature, indirect methods are usually adopted to describe productivity variation and its relation with richness (Mittelbach *et al*., 2001). e.g. vegetation indexes (Levanoni *et al*., 2011), evapotranspiration (Kessler, 2001), or the actual productivity of organisms at a site (Tilman *et al*., 1997). Attempting to estimate the variation in productivity of madicolous habitats along the altitudinal gradients, we adopted the emergence of madicolous insects as a proportional measure of secondary production level at each altitude (Poepperl, 2000). Once the number of emergent insects from each site is not independent from immature abundance, we calculated the emergence rate at each site by dividing the number of emergent adults by the sum of all larvae and adults observed at that site.

Data analysis

Richness patterns

The general tendency of richness variation along the entire elevation range (0-2700 m) was verified and the relationship significance was tested through regression analysis. Based on the general pattern of the observed richness variation, the existence of significant linear or unimodal relationship was tested in each mountain separately with linear regression analysis.

Rapoport's rule

To test if Rapoport's rule had any influence over the observed variation in richness patterns, we verified if the elevational range of species and the altitude was positively and significant correlated applying Spearman correlations ($\alpha = 0.05$). To calculate the range size of each species, we considered the distance between the lowest and the highest altitudinal limit where the species was recorded. Following the Steven's method (Stevens 1989), we obtained the mean elevational range of each altitude analyzed, calculating the mean altitudinal range of all species that occurred at each altitude.

Additionally and given that the species in higher altitudes present higher ranges according to Rapoport's rule, we tested whether the species in PM have significant higher mean altitudinal range than species in PS and C. We compared the mean altitudinal range in each mountain by means of ANOVA one-way with Tukey HSD test *a posteriori*.

Spatial constrains

To verify if area and geometric constrains could significant affect the richness, each gradient was divided into 200 m-altitudinal bands. Once different sampling effort have been performed among the altitudinal bands, richness have been standardized by rarefaction procedure, based on the number of individuals sampled at each altitudinal band (Gotelli & Cowell, 2011). Thus, we found the expected number of species in 60 individuals for each altitudinal band in C, 24 for PS, and 297 for PM (respective smallest number of individuals found in the altitudinal bands of each mountain). The rarefactions were performed in R Cran Project 3.0.3. (R Core Development) Software, using "Vegan" package (Oksanen *et al*., 2013). The resultant richness of each altitudinal band was then submitted to a simple linear regression analysis with the estimated area, separately for each mountain.

To test if MDE constrained the richness in each gradient, we analyzed how well the empirical richness values fitted the expected richness curve resultant from a null model. The null model, generated in Mid-Domain-Null for Excel (McCain, 2004), was based on the analytical-stochastic models of Colwell (1999) and Colwell & Hurtt (1994). Through 50000 Monte Carlo simulations (without replacement) on the empirical ranges sizes, the expected variation in richness along each mountain elevation and the complete elevational gradient were determined with 95% of confidence interval. To test if the observed richness were significantly explained by geometric constrains, simple linear regressions were applied to the empirical richness and the expected richness calculated by the null model analysis. The shared influence of area and MDE on richness patterns was also calculated through multiple regression analysis.

Environmental predictors

Multiple regressions analyses have been performed to verify the environmental contribution in shaping the observed richness patterns. Variables have been divided into two groups: elevation-dependent variables (EDV), which presents a unidirectional trend with altitude (Korner, 2007), and elevation-independent variables (EIV), in order to verify which type of variable best influence richness. Variables classified as EDV demonstrated significant relation with the altitude (linear or unimodal variation), and while EIV changed randomly along the gradient, reflecting a noisy relation. Further, we determined which environmental predictors, among all measure factors, best explained the variation in species richness. Data were log-transformed prior to regressions analyses.

All regression analysis and Spearman correlation was performed in Statistica (StatSoft version 7.0).

RESULTS

9765 indidivuals from 164 taxa, including genera and species, of madicolous insects were recorded in the present study (Tabela 1, em anexo). All broad taxonomic groups frequently found in streams were also observed in madicolous habitats in this study, such as Ephemeroptera, Trichoptera, Plecoptera, Diptera, Coleoptera, Hemiptera and Odonata.

The complete elevational range of madicolous insects' richness in the Atlantic Forest showed a clear mid-elevational peak (Fig. 2a). Analyzing the altitudinal gradient in each mountain separately, we note that only PM was marked by a monotonic decrease in species richness along the altitude (Fig. 2d); PS was the only to show a hump shape in species richness, although it was not a significant relation (Fig. 2c); and the lower altitudinal range, C, showed a positive relation between richness and altitude (Fig. 2b).

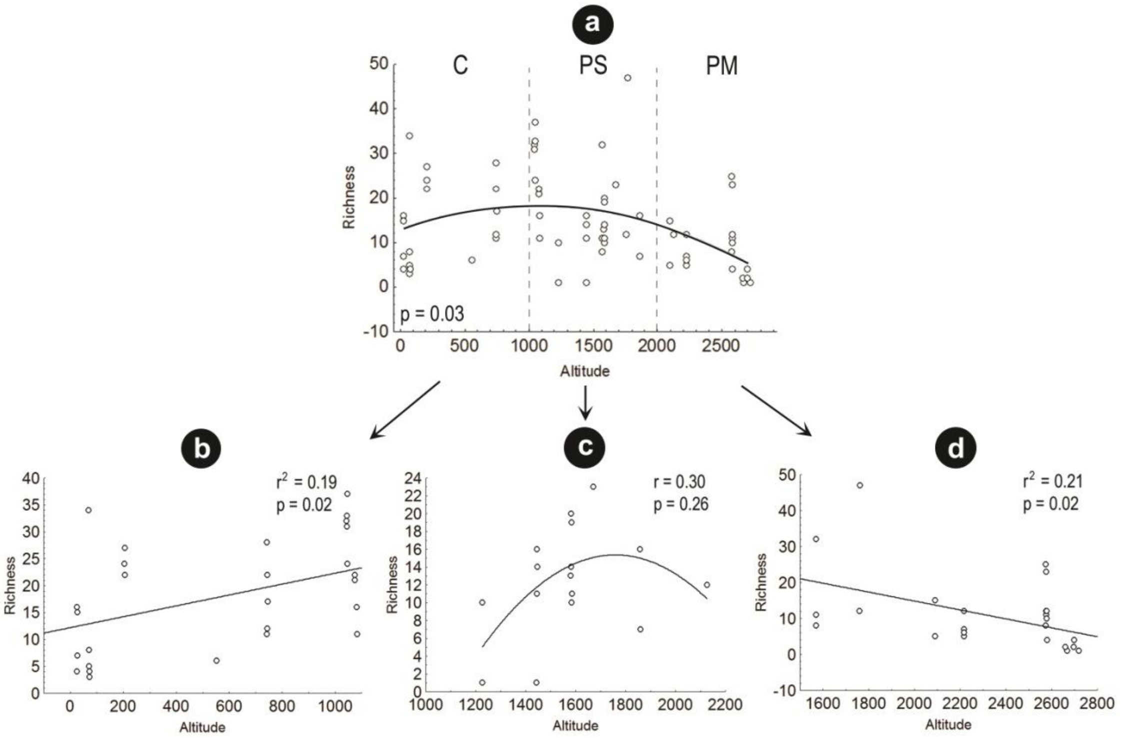

Figure 2 Relationships between richness and altitude. a. Complete range (C+PS+PM) b. Gradient at lower altitudes (C) c. Gradient at intermediate altitudes (PS) d. Gradient at higher altitudes (PM).

The validity of Rapoport's rule was not confirmed in any of the analyzed gradients (Fig. 3). The significant correlation between range size and altitude was only verified in Corcovado (Fig. 3a), where lower species ranges were predominant at higher altitudes – the opposite pattern of Rapoport's rule predictions. In PS, a tendency of lower species ranges concentration at intermediate elevations has been verified, but it was not statically detected (Fig. 3b). For PM, no pattern of range distribution was observed (Fig. 3c).

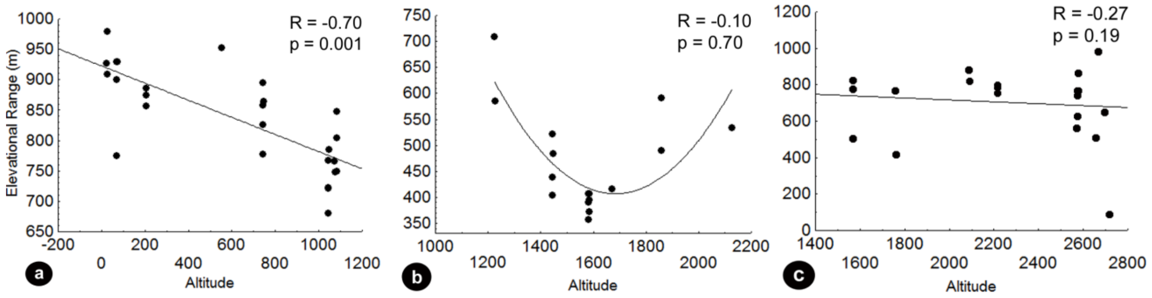

Figure 3 a. Gradient at lower altitudes (C) b. Gradient at intermediate altitudes (PS) c. Gradient at higher altitudes (PM). Relation between range size (m) and altitude (m) by Steven's method (Stevens, 1992) tested by means of Spearman correlation (α=0.05).

Comparing the mean species' range size at each gradient, a significant higher range is observed at Corcovado (Fig. 4), confirming that higher altitudes did not show any relation with higher distributional ranges of species in the present study.

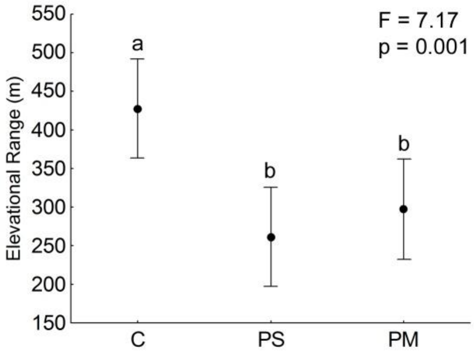

Figure 4 One-way ANOVA showing significant differences among elevational range of species in each gradient, and post-hoc Tukey HSD test evidencing the groups. C: Gradient at lower altitudes; PS: Gradient at intermediate altitudes; PM: Gradient at higher altitudes.

Temperature, dissolved oxygen and emergence rate changed spatially according to the altitudinal gradient (Fig. 5). Temperature, as expected, showed a significant negative relation with altitude (Fig. 5a). Dissolved oxygen showed a significant unimodal pattern of variation with altitude, increasing approximately until 1700 m a.s.l. and then continuously decreasing until the highest site (Fig. 5b). Emergence rate increased significantly with the increase of altitude (Fig. 5c). Solar incidence also showed a positive relation with altitude (r2= 0.28; p= 0.0001), although data fitting was not satisfactory.

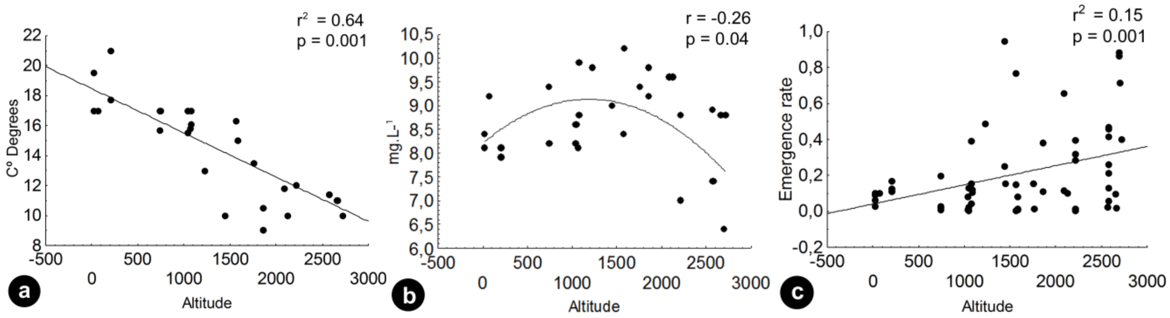

Figure 5 Elevational dependent variables (EDV) that significantly varied with altitude ($p<0.05$). a. temperature b. dissolved oxygen c. emergence rate.

In all tested cases (four elevational gradients), environmental predictors significantly influenced richness along the elevation (Table 1). We highlight the high explanation power of the environmental predictors over richness in PS (explaining more than 80% of the relation) (Table 1). Among the environmental classes tested, EDV were more important in driving richness in all gradients than EIV (Tab. 1). Area alone did not affect significantly the number of species in any of the gradients, but the shared effect with MDE had potentially influenced the richness in PM, although this relation was not significant (Table 1). This was the only case where spatial constraint showed some influence over the observed richness and this implicated in lower explanation showed by environmental predictors over richness in this locality. MDE alone possibly influenced richness patterns at the complete elevational range (C+PS+PM), and specifically at PS (Table 1). Eight from fifteen values of empirical richness considering the entire elevational range occurred within the predictions of the mid domain model (Fig. 6), and half of those at the central domain, relative to PS gradient. Almost all empirical richness points from PM showed consistent deviation from the mid-domain prediction interval.

Temperature, dissolved oxygen and emergence rate together best explained variation in richness in all gradients (Table 1), and water flow was the only EIV that contributed to the observed richness pattern in PM and PS gradient (Table 1). Water flow was relatively slow in most of sites at PM (0.11 $m.s^{-1}$ ± 0.13), PS (0.29 $m.s^{-1}$ ± 0.2) and C (0.12 $m.s^{-1}$ ± 0.09).

Table 1 Linear determination coefficients (r^2 for simple regressions and adjusted r^2 for multiple regressions) between empirical richness and environmental predictors, area and predicted richness for MDE (average of the 50000 simulations). C: Gradient at lower altitudes; PS: Gradient at intermediate altitudes; PM: Gradient at higher altitudes. Bold values highlight significant relations.

Gradient	Environmental predictors				Spatial constrains				
		Best fitting*	EDV	EIV		MDE	Area		MDE + Area
C	Ajusted-r^2	**0.61**	**0.61**	0.08	r^2	0.27	0.24	Ajusted-r^2	0.02
	p-value	**0.0004**	**0.0001**	0.17	p	0.30	0.40	p-value	0.49
PS	Ajusted-r^2	**0.82**	**0.66**	**0.42**	r^2	**0.53**	0.04	Ajusted-r^2	0.19
	p-value	**0.002**	**0.003**	**0.03**	p	**0.02**	0.75	p-value	0.40
PM	Ajusted-r^2	**0.45**	**0.41**	**0.12**	r^2	0.17	0.59	Ajusted-r^2	0.69
	p-value	**0.0001**	**0.0001**	**0.007**	p	0.48	0.07	p-value	0.08
Complete	Ajusted-r^2	**0.2**	**0.13**	**0.07**	r^2	**0.38**	0.26	Ajusted-r^2	0.14
	p-value	**0.004**	**0.02**	**0.03**	p	**0.01**	0.06	p-value	0.18

*Best fitting variables. C: EDV; PS: EDV + water flow; PM: EDV + water flow; Complete range: EDV+EIV.

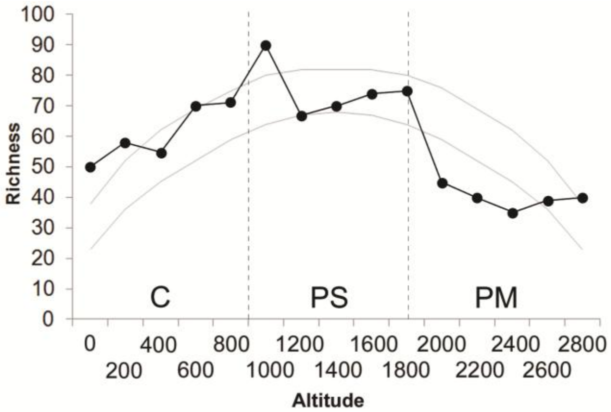

Figure 6 Empirical richness (black line with filled circles) fitting on null model (95% prediction curve without replacement), performed on Mid-domain null with 50000 simulations, regarding altitudinal bands from 0 to 2800 m a.s.l. C: Gradient at lower altitudes; PS: Gradient at intermediate altitudes; PM: Gradient at higher altitudes.

DISCUSSION

We found different relations between richness and altitude in madicolous insect community from Atlantic Forest, explained by the different elevational profiles of the mountains. However, a remarkable continuity in richness response was observed when analyzing the complete elevational range, which demonstrated a clear hump-shaped pattern. These observations confirm that the processes driving richness patterns in elevation ranges are scale dependent (Rahbek, 2005), and consequently, our choices on ranges extent and grain size can affect our conclusion regarding altitudinal patterns of species richness.

The above mentioned pattern already contradicts what is postulated in Rapoport's rule (Steven, 1989; 1992). In fact, we found no positive relation among elevational range size and elevation, even at PM where a monotonic reduction of species with increase altitude was observed. Rapoport's rule has been tested by several studies dealing with latitudinal (Colwell & Hurtt, 1994; Rohde *et al.*, 1992) and elevation gradients (Fleishman *et al.*, 1998; McCain & Knight 2013; Sanders, 2002), but most did not confirm this hypothesis for mountain gradients (McCain & Knight 2013). In addition, we found significantly higher range sizes at the low-gradient, the opposite result expected from Rapoport's rule. However, Neotropical lowland streams have passed through intense climatic variation during Pleistocene (Covich, 1988), what would explain the noticeable higher altitudinal ranges verified in C, especially at its lowest altitudes, through the theoretical basis of Rapoport's rule (Steven, 1989; 1992), but in inverse relation. Thus, we may consider that no Rapoport's rule was verified and it was evident that other processes have been driving richness along elevation gradient.

MDE test confirmed that richness has been spatially constrained at the wider scale (complete elevation range) and at PS gradient. The influence of MDE in richness pattern when considering the complete range can be supported, once we are dealing with a hard boundary (Rahbek, 1997; Colwell and Hurtt 1994). It means that species found are completely spatially limited by the lowest altitude (sea) and the highest altitude (top of the highest mountain = 2800 m a.s.l.) found at Atlantic Forest domain. Thus, the distribution of madicolous insects from Atlantic Forest are limited to this range (2800 m) and therefore are more prone to overlap their ranges in the central region of the domain (intermediate altitudes) (Lyons and Willig, 1997; Colwell and Hurtt, 1994). However, the relatively low explanation value found (less than 40%) implies that MDE was not the primary cause of richness variation along elevation gradient.

Analyzing each gradient separately we were able to demonstrate that environmental factors are significant drivers of richness patterns in elevational gradients, explaining more than 80% of the relation in the intermediate gradient. So probably, the MDE observed at this gradient had also a secondary importance, and it was a consequence of the MDE determined by the boundaries of the complete altitudinal range.

In all cases, the co-variables of altitude (EDV) were more important for the observed richness pattern than the independent variables (EIV). This emphasizes our belief that madicolous habitats are good habitats-models to test ecological patterns, once they are relatively structurally homogeneous and isolated systems (Ward, 1992). EIV are frequently responsible for the absence of richness patterns in altitudinal gradients (Jacobsen *et al*., 2004; Harrington *et al*., 2015). EIV poorly affected richness patterns in this study, however, water flow was important for PM and PS gradients. Water flow is known to influence insects' distribution in running waters (Rabeni & Minshall, 1977; Mazzucco *et al*., 2015), and although the variation of water flux in madicolous habitats of the present study was discrete, it was enough to cause variations in species richness. Among the EDV, emergence rate, dissolved oxygen and temperature joint effect best explained richness patterns.

Emergence is directly related to the energy dynamics in the ecosystem and can be used as a method for estimating secondary production of aquatic insects (Poepperl, 2000). Here, emergence rate and solar incidence were considered surrogates for biological processes related to productivity. In this study, the increase of madicolous ecosystems' productivity with altitude may be intimately related to the increase of light intensity. As the altitude increases, large trees are replaced by small vegetation and solar incidence increases for ground organisms in most tropical mountains (Homeier *et al*., 2010; Aiba & Kitayama, 1999). Light is the primary responsible for the increase of periphyton production in running waters (Elsaholi *et al*., 2011; Hill *et al*., 1995). Given that madicolous insects feed primarily on periphyton (Vaillant, 1955, 1961; Hynes, 1970; Oliver & Sinclair, 1989) is expected that a greater secondary production be observed in congruence with food availability. Decrease of ground-dwellers lichens toward low altitudes has already been evidenced in response to light reduction below forest limit (Grytnes *et al*., 2006).

Productivity can affect richness in many different ways (Whitaker, 2010), but most common patterns are: positive, when richness increases with the increase of productivity (Hawkins *et al*., 2003; Brown & Lomolino 1998; MacArthur 1969), and unimodal relation, when richness first increase with the increase of productivity and then decrease (Rahbek 1997; Rozensweig, 1995; Kessler 2000). Although we did not measure primary or secondary production in this study, we believe that emergence rate and solar incidence on madicolous ecosystems are directly related to their productivity, and consequently, with richness patterns (Hutchinson, 1959), specially explaining the initial increase in richness.

Different from most systems, where the highest productivity occurs at lowest altitudes (at the sea level), and decreases toward the mountaintop (Hawkins *et al*., 2003; Hutchinson, 1959; Orians 1969; Homeier *et al*., 2010; Aiba & Kitayama, 1999), for the small aquatic ecosystems analyzed in the present study, we found evidences indicating that productivity increases with increasing altitude. Thus, the relation between richness and productivity here is described by a hump-shaped variation, where the initial increase in richness, at low altitudes, is related to the increase of productivity – contrasting from the enigmatic and frequently found relation between high-productive lowlands and low richness values, which causes are still unknown (Rozensweig, 1995). Further, our results support that the latter decrease in richness towards higher productive altitudinal bands (top of the higher mountain), is primary a consequence of severe temperature drop at highest altitude. Temperature is world-wide accepted as one of the most important driver of richness patterns for altitudinal and latitudinal gradients (MacArthur 1969; Rahbek, 1995; Jacobsen, *et al*., 1997; Murray, 1997) Also, oxygen concentration declined monotonically from intermediate altitude toward the top, and this fact certainly negatively affected madicoulous insects' community, contributing to the observed decrease in richness at the highest altitudes of the gradient. Oxygen concentration is hardly considered a predictive variable for richness variation in elevational gradients, maybe because most of studies deal with terrestrial organisms for which this variable constantly decrease with altitude. However, for aquatic organisms the oxygen availability is extremely important (Connolly *et al*., 2004; Ward, 1992) and it is not the first time that dissolved oxygen has been depicted as the main cause of richness decrease in altitudinal gradients (Jacobsen, 2008). The increase of oxygen concentration in the first half of the domain is probably related to the solubility increase as temperature declines (Tromans, 1999), however, it reaches the peak at approximately 1700 m and then decrease, probably due to the rarefied atmospheric oxygen, which constantly decreases with altitude (Korner, 2007; Jacobsen, 2008).

The fitting of the empirical richness curve on the null model prediction help us verifying areas of significant deviation, and thus we find that empirical richness was considerably low at higher altitudinal bands (PM gradient). This fact reinforce that richness at this sites are suffering by some evident pressure that pulls it down. According to the results found, this is caused by the severe climatic conditions, especially regarding temperature and oxygen decrease, which are in accordance with the "ends are bad" hypothesis.

In this study, area did not affect significantly richness in any of the mountains, although in PM a slight relation was observed. The weak explicability of area over richness is mainly due to the non-conical shape of the mountains (Korner, 2000; Lomolino, 2001). One of the few laws in ecology is the positive relation between area and richness (Schoener, 1976; Dodds, 2009), so the conical shape of most studied mountains may lead to an erroneous monotonic decrease in richness along the elevation (Rahbek, 1997). In addition, estimating altitudinal bands would not have the same importance for madicolous insects than it represents for terrestrial organisms, once area may not be directly related to habitat availability, thus poor effect over richness is expected.

We should highlight that the factors underlining richness variation along elevation in Atlantic Forest are deeply associated with the elevational range, and thus, are scale-dependent. The aspects related to energy dynamics (productivity) and oxygen availability explain the observed patterns more than spatial constraints (area effect and MDE) or Rapoport's effect. However, the influence of MDE is undeniable, once species are limited by the elevation boundaries. Leading with the entire altitudinal range existent at Atlantic Forest through the study of three mountains relatively close allowed us to verify a hump-shaped variation of richness along elevation that would not be noticeable at other scale, and gives more power to the "the ends are bad" hypotheses.

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Drivers of compositional dissimilarity of madicolous insects in elevational gradients of Atlantic Forest

ABSTRACT

Aim A widespread hypothesis may explain species distribution at elevational gradients of tropical mountains: the “Mountain Passes are higher in the Tropics” (MPHT), while other mechanism could also have important impact over communities’ dissimilarity: e.g. the source-sink hypothesis. Both lead to two antithetic patterns of compositional dissimilarity (turnover and nestedness, respectively). In this study we aim to test the importance of both hypotheses for madicolous insects in altitudinal gradients of a preserved tropical forest.

Location We analyzed madicolous insects from altitudinal bands in two gradients (0-1500 m and 1500-2700 m) in Atlantic Forest (Southeast Brazil).

Methods All madicolous habitats available in a transect were sampled ensuring that at least three replicates (corresponding to 0.125 m^2) in each 200 meters of elevation were obtained. Total beta-diversity and the contribution of each component (turnover and nestedness) have been accessed and correlated to altitude gradients.

Results The remarkable variation in species composition showed by madicolous insects in Atlantic Forest’s mountains is primary resultant from turnover along the altitudinal gradient, what confirms the MPHT hypothesis. However, nestedness displayed some importance over the observed dissimilarity among sites. Cold-adapted groups, presented higher altitudinal ranges and variability of ranges, thus causing nested dissimilarity at the higher gardient. Colonization of autochthonomus species (from surrounding lowlands) and colonization of allochthonous species probably contributed for nestedness patterns at both gradients.

Main conclusions We support the MPHT hypothesis, though some cold-adapted groups with higher diversification at temperate climate contradicted the turnover pattern, and presented higher altitudinal range. We sugest that the short altitudinal range demonstrated by most of populations at mountaintops is resultant from horizontal colonization, while lowlands receives elements from vertical colonization, what also could lead to nested patterns.

Keywords faunal dissimilarity, climate variability, altitudinal range, mountain, mass-effect, hygropetric habitat, aquatic insects

INTRODUCTION

Ecologists have long been interested in identify large scale patterns in diversity and their causality (Huston, 1994; Brown & Lomolino, 1998). Mountains are good models to test ecological hypothesis, because they are relatively easily replicated and display significant environmental changes in a relatively short spatial scale (Janzen, 1967; MacArthur, 1972; Lomolino, 2001; Stevens, 1992; Gaston & Blackburn, 2000).

The use of beta-diversity analysis as a tool to evaluate the dissimilarity in species composition along large spatial scale has been constantly proposed in macroecology (Diserud and Ødegaard, 2007; Baselga & Jiménez-Valverde, 2007; Baselga, 2010; Koleff *et al*., 2003). Through this approach we can understand how species respond to environmental heterogeneity along ecological gradients (Whittaker, 1960; Heino, 2009; Jurasinski *et al*., 2009). Beta diversity may reflect two different phenomena: *turnover* and *nestedness* (Brendonck *et al*., 2014; Baselga, 2010, 2012; Dobrovolski *et al*., 2012; Harrison *et al*., 1992).

The *nestednesss* pattern occurs when the community with less number of species represents a subset of those with higher richness (Patterson & Atmar, 1986; Wright & Reeves, 1992; Schoener & Schoener, 1983). Thus, a “loss” of species is observed as a result of the continued desegregation of the communities’ composition along the gradient (Atmar & Patterson, 1993). The *turnover* (Whittaker 1960, 1972; Tuomisto 2010) is the replacement of species in environmental gradients, and is often a consequence of spatial factors or historical events (Qian *et al*., 2005; Baselga, 2010). Identify which process most contribute for the dissimilarity between adjacent communities in large scale can contribute to untangle ecological phenomena that lead to current biodiversity (Baselga, 2010).

Elevational richness patterns of madicolous insects in the Atlantic Forest demonstrated a peak in intermediate altitudes (Cap. 1 – Parte I), similar to other organisms groups in Neotropical mountains (Mcain, 2004; Smith *et al*., 2007; Herzog *et al*., 2005; Kessler, 2000). Differences in species richness along altitudinal gradients may be related to differences in species composition from site to site in the gradient, caused primarily by ordered loss or gain of species (Gaston & Blackburn, 2000).

One of the mechanisms frequently used to explain species loss along spatial gradients is the “source-sink” hypothesis (Janzen, 1967; Rex *et al*., 2005; Hanfling & Weetman, 2006; Moreno *et al*., 2008). This hypothesis states that poor-species sites are maintained by sites with higher richness. This was confirmed for ocean bathymetric gradients (Rex *et al*., 2005; Moreno *et al*., 2008), where impoverished abyssal zones are supported only by non-viable populations that migrate from richer bathyal areas (Moreno *et al*., 2008). For altitudinal gradients it implies in organisms flow from sites with optimal conditions to sites with suboptimal conditions, where most populations are not sustainable (Kessler *et al*., 2011; Grytnes *et al*. 2008). Thus, a constant flow from "source populations" to "sink populations" is observed (Pulliam, 1988; Shmida & Wilson, 1985; Grytnes & Mccain, 2007). Thus, communities from sink sites would represent only a small fragment of source sites.

In contrast, another explanation for current species distribution has been proposed for tropical mountains. Based on the climate variability hypothesis, which posits that thermal tolerances and range sizes are proportional to the climate variability a species has experienced (Stevens, 1989; Stevens, 1992), Janzen (1967) rose the "mountain passes are higher in the tropic" (MPHT) hypothesis. The MPHT predicts that species in tropical mountains present narrow elevational ranges due to the reduced annual climate variation in tropical regions comparing to temperate regions. Thus, environmental changes along altitudinal gradients in tropical mountains would represent an effective physiological barrier for their inhabitants, leading to a constant turnover of species (Janzen, 1967; Ghalambor *et al*., 2006).

Aquatic insects' diversity varies across latitudinal gradient, but the patterns differ among broad taxonomic groups (Vinson & Hawkins 2003; Pearson & Boyero, 2009; Boulton *et al*., 2008). Most of aquatic insect taxa are more diverse in tropical regions (Coffman & de la Rosa, 1998; Cranston *et al*., 1997; Jacobsen *et al*., 1997), such as Trichoptera (Boulton *et al*., 2008; Lake *et al*., 1986) and Chironomidae (Cranston *et al*., 1997), although some cold-adapted groups presents higher richness at mid to high latitudes, such as Plecoptera and Ephemeroptera (Pearson & Boyero, 2009; Lake *et al*., 1986). Plecoptera species are known to tolerate low temperatures, showing high diversity in mountain streams (Knight & Gaufin, 1966) and alpine lakes (Fureder *et al*., 2006). Ephemeroptera species appear to present higher diversity at mid and high latitudes (Vinson & Hawkins, 2003). Thus, according to the climate variability hypothesis (Stevens, 1989), their distribution at tropical mountains may present divergences from taxa with high diversification at low latitudes (e.g. Trichoptera).

Studies that investigated changing in aquatic insects' community along altitudinal gradient have focused on streams ecosystems (Heino *et al*., 2015; Al-Shami *et al.*, 2013; Wang *et al.*, 2012; Gill *et al.*, 2014). Nevertheless, the high connectivity among stream sites may also interfere in the species' distribution along the gradient, what implies in variation on communities' dissimilarities (Jacobsen, 2004; Harrington *et al.*, 2016). Madicolous habitats (Thienemann, 1905) are characterized by a thin film of water that runs over rocky surfaces (Vaillant, 1956), therefore they are spatially restricted and frequently isolated ecosystems (Ward, 1992). Further, madicolous habitats are less environmentally heterogeneous than streams, what make them a good object for altitudinal gradient studies. However, this type of ecosystem has been neglected from faunistic and ecologic studies (Fischer *et al.*, 1998).

Expecting to find a significant change in species composition along the altitudinal range, we investigate what was the main cause of communities' dissimilarity in the Atlantic forest, testing two widespread hypotheses for variation in species composition in elevation ranges: the "source-sink" dynamics and the "mountain passes are higher in the tropics", by means of beta-diversity analysis. Also, extending the climate variability hypothesis to elevational gradients, and regarding the differences of thermal tolerances among aquatic insects' taxonomic groups, we expected that groups with higher diversification at temperate zones would tolerate higher range of temperature variation and thus present a nested distribution, differing from taxa with higher diversification at low latitudes. We also speculate about the colonization pathways in both altitudinal gradients from Atlantic Forest.

METHODS

Study area

Three elevation gradients (Fig. 1 em Cap. 1 – Parte I) were chosen in order to represent the complete elevational range present in the Atlantic Forest. Undisturbed conditions were ensured by their localization inside conservation units. (additional information on the study area in cap. 1).

All mountains belong to Serra da Mantiqueira and Serra do Mar formations, and have the same geological origin that dates from Late Cretaceous - about 70 Mya (Almeida & Carneiro, 1998; Modenesi-Gautieri *et al.*, 2002).

Sampling methods

Sampling were performed along a transect in each mountain. All madicolous habitats available along the transects were sampled, ensuring that at least three replicates in each 200 meters of elevation were obtained, and totalizing 70 sampling sites from 0 to 2700 m a.s.l. Each sampling site was delimited by the coverage area of an emergence trap – approximately 0.125 m^2 (Figura 1, em anexo).

Madicolous insects were collected directly from the rocks by washing the substrate. All material were conserved in absolute ethanol and analyzed under stereomicroscope and optic microscope. All immature insects sampled were identified using specialized bibliography (Domínguez & Fernández, 2009; Trivinho-Strixino, 2011; Hamada *et al*., 2014; Lecci & Froehlich, 2007; Salles *et al*., 2004; Segura *et al*., 2011) to the lowest possible taxonomic level (genus/species). Trends in diversity of aquatic insect's genera at large scale appears to be equivalent to that found for species (Vinson & Hawkins, 2003). In this study we refer to taxonomic categories as species, in a general sense.

Data analysis

To test both hypotheses (source-sink and MPHT) we based on previous results on richness patterns (Cap. 1 - Parte I). Given that the peak of richness was verified at mid elevations across the complete range (0 - 2700 m a.s.l.), and therefore, richness decayed toward the lowest and the highest altitude, so the mechanisms driving communities' dissimilarity may differ in both situations (toward the sea level and the top of the highest mountain), we decide to divide the complete range into two altitudinal gradients: a lower gradient (LG) (0 – 1500 m) and a higher gradient (HG) (1500 – 2700 m). Thus, testing the ecological hypotheses we could define which processes describes diversity patterns at each altitudinal range. In order to avoid the overestimating of turnover pattern along elevational gradients, we assumed that each species occurred from the lowest to the highest altitude where it has been recorded

First, to test if the decrease in species richness from the intermediate altitudes toward the extremities was a result of source-sink dynamics, we investigated how nested the depauperate-sites (sink) were in relation to sites with higher richness (source). To detect the importance of nestedness mechanism in determining changes in insect composition, we applied a dissimilarity analysis – partitioned Sorensen beta-diversity (Baselga, 2010) – which disentangles nestedness from turnover pattern. Thus, to investigate if our data would support the MPHT, the same analysis was used in order to detect the potential species turnover in both gradients.

We used the functions “beta.multi.R” and “beta-pairwise.R” from “betapart” (Baselga, 2010) package for R software, to calculate the multiple site dissimilarity (overall dissimilarity for each gradient) and to generate a dissimilarity distance matrix, respectively (using presence/absence data). Both functions give the total beta-diversity value (Sorensen dissimilarity) and its two components: the turnover (Simpson dissimilarity) and the nestedness. With the dissimilarity matrix we verified the relationship between the observed dissimilarity and altitude through linear models. The significance of the relations was investigated by means of Mantel test, using “vegan” package (Oksanen *et al.*, 2013) in R software.

To test if mechanisms that lead to dissimilarity among sites varied among different taxa, we calculate the overall dissimilarity and the variation of the turnover of species with altitude for 4 taxonomic groups: Chironomidae, Trichoptera, Ephemeroptera and Plecoptera. We choose these taxa based on their latitudinal center of diversification, of which Chironomidae and Trichoptera are known to present higher richness at lower latitudes and Ephemeroptera and Plecoptera are known to present higher richness at highest latitudes. Although all these taxa showed high frequency in this study, Chironomidae and Trichoptera were the most abundant and presented the highest number of species in this study (Tabela 1, em anexo).

RESULTS

The relationship between compositional dissimilarity and elevation was positive for both gradients. Altitude was consistently important for faunal dissimilarity at the LG and HG, evidenced by the positive relationship between compositional dissimilarity (total beta-diversity) and altitude (Fig. 1ab). However, turnover component explained better this relationship (80% and 82% for LG and HGs, respectively). The nestedness component of beta-diversity, although positive correlated with altitude, showed a weak relation with it in both cases.

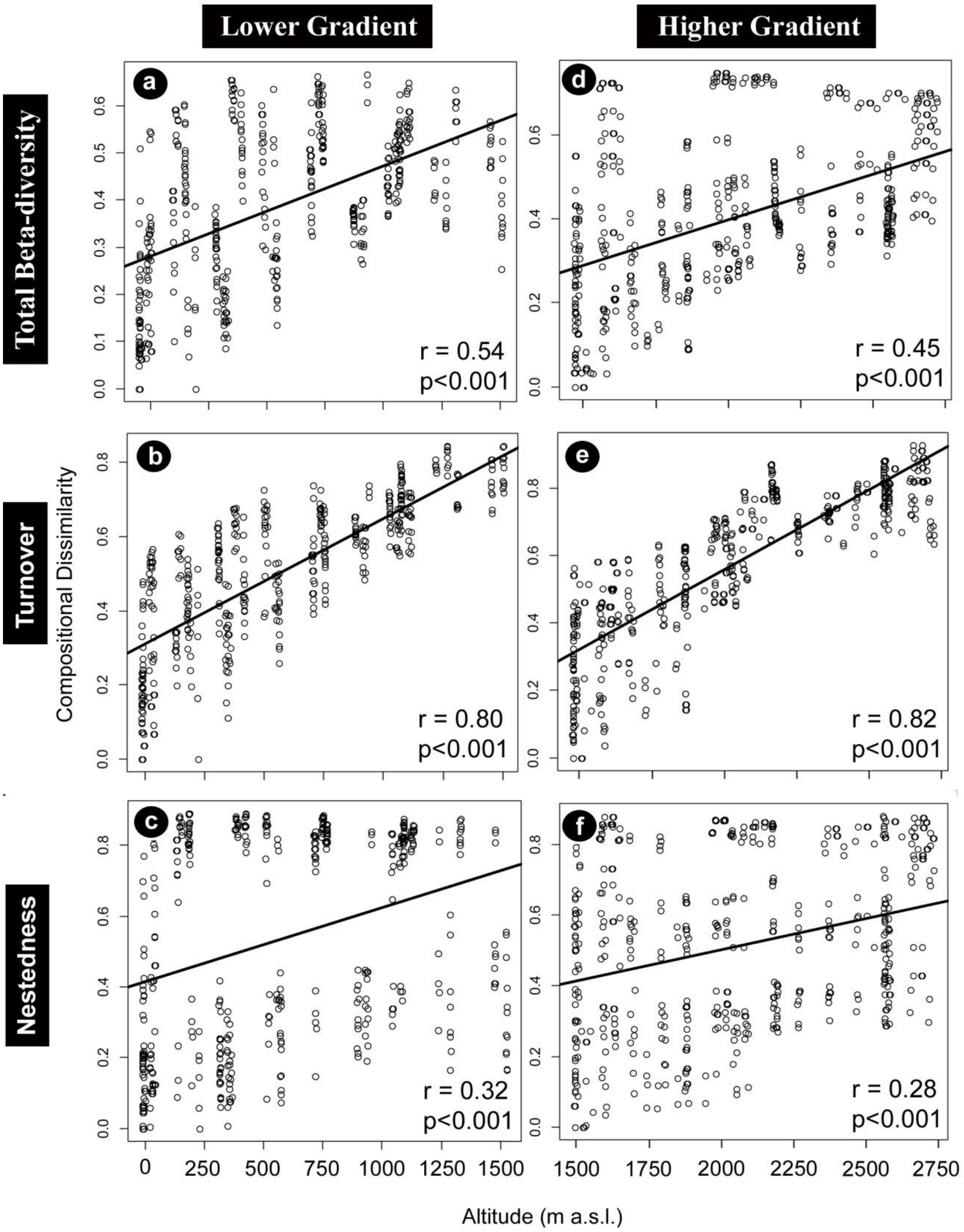

Figure 1 Relationship between compositional dissimilarity and altitude at LG (a, b and c) and HG (d, e and f). Total beta-diversity: Sorensen dissimilarity; Turnover: Simpson dissimilarity.

A high compositional dissimilarity was evidenced for all orders of madicolous insects in both gradients (Fig. 2). For all taxa at LG, compositional dissimilarity was governed by turnover process (Fig. 2A). At the HG, although the whole community was predominantly driven by turnover mechanism, Plecoptera and Ephemeroptera were driven primary by nestedness (Fig. 2B).

Significant differences among mean altitudinal range of the taxa have been detected at the LG, while differences found in the HG were expressive but not significant (p=0.08) (Fig. 3). In both gradients, Plecoptera showed a mean altitudinal range higher than the other taxa. In contrast, Trichoptera presented the lower mean among all taxa (Fig. 3). High confidence intervals evidenced for Plecoptera and Ephemeroptera indicates that the species ranges of both taxa present more variability than Chironomidae and Trichoptera's ranges. The tendency of variation among taxa was very similar between gradients, although elevation ranges are consistently higher at the lower altitudinal gradient (Fig. 3).

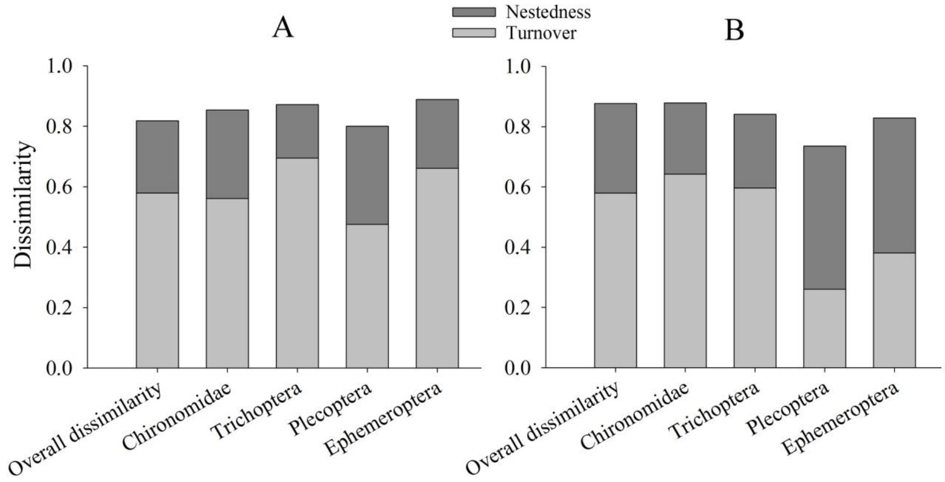

Figure 2 Sorensen dissimilarity considering multiple-site dissimilarities of each broad taxonomic group and overall community, where A: lower gradient and B: higher gradient. Gray stacks shows relative contribution of turnover and nestedness components into each category.

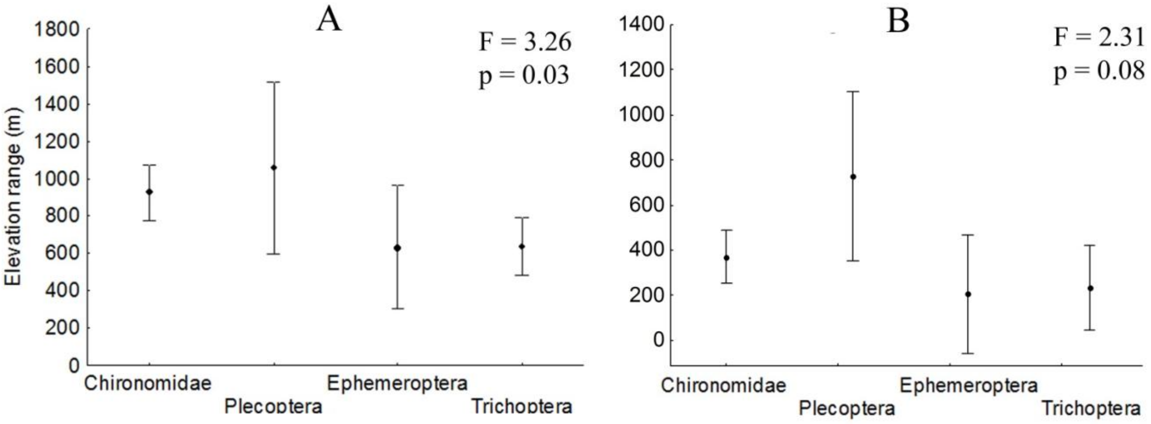

Figure 3 Mean altitudinal range with 95% confidence interval of each taxonomic group analyzed at lower (A) and higher gradient (B), compared by means of ANOVA (F and p-values).

Turnover mechanism described significantly the change in species composition of Chironomidae and Trichoptera with altitude (Fig. 4 a,b,c,d). On the other hand, this mechanism was weakly responsible for compositional changes in Plecoptera and Ephemeroptera along both gradients (Fig. 4 e,f,g,h).

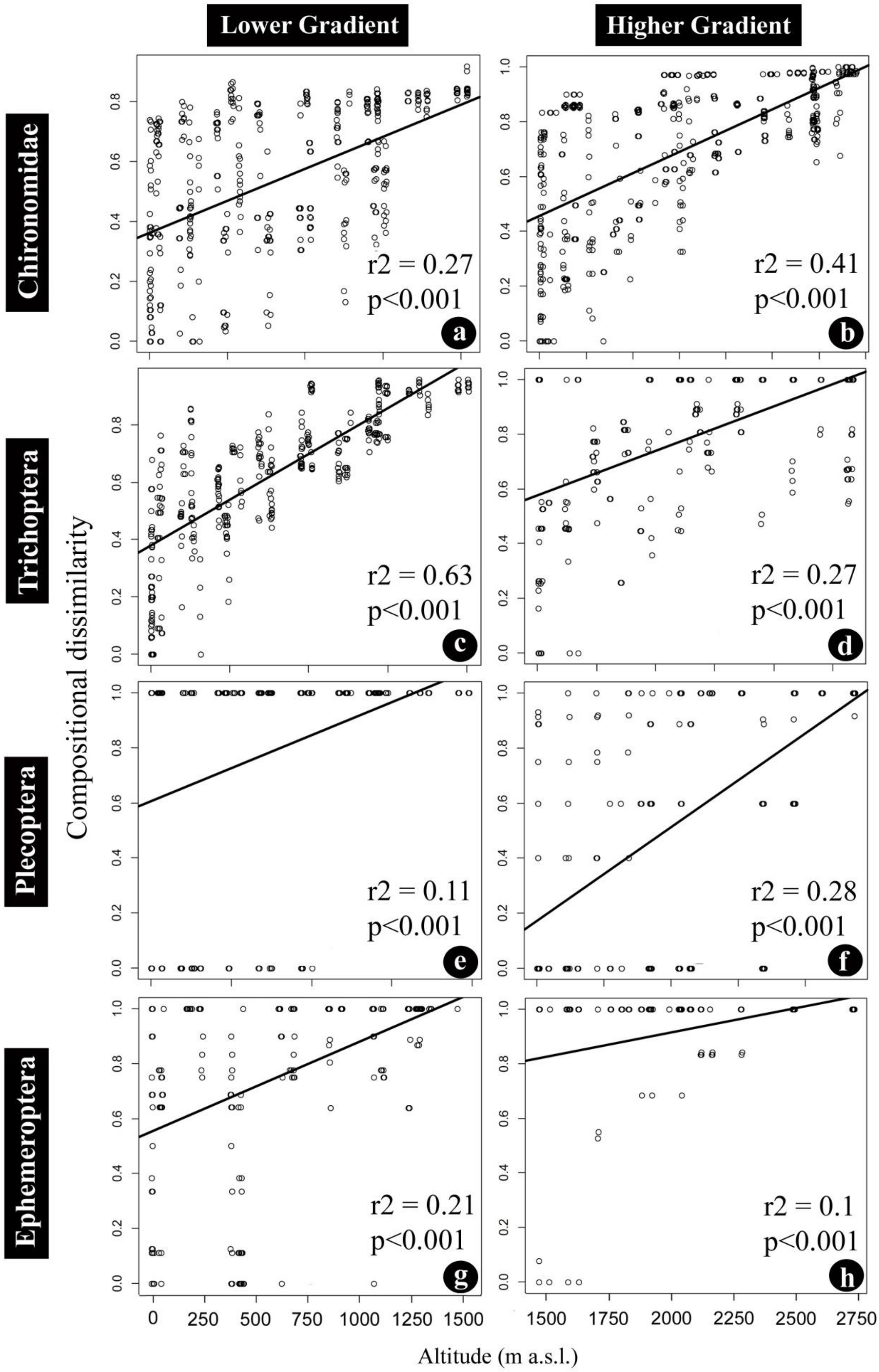

Figure 4 Relationship between turnover (Simpson dissimilarity) and altitude depicted by each broad taxonomic group: a-b. Chironomidae; c-d. Trichoptera; e-f. Plecoptera; g-h. Ephemeroptera, at lower gradient (left) and higher gradient (right).

DISCUSSION

High beta-diversity values have already been verified in aquatic macroinvertebrate communities along altitudinal gradients (Finn *et al*., 2013; Wnag *et al*., 2012; Jacobsen, 2003). As expected, a high dissimilarity was observed among madicolous community along the altitudinal ranges. In turn, the overall turnover of species verified along the altitudinal ranges allowed us to discard the hypothesis of source-sink dynamics, at least as a primary mechanism controlling species distribution. The turnover of species explained most of the dissimilarity verified at the LG and at HG. Furthermore, this mechanism was significantly related to altitude, indicating an intensification of the replacement of species with this variable. These results consequently support the MPHT hypothesis (Janzen, 1967; Ghalambor *et al*., 2006).

Dealing with mountains' ecosystems means that remarkable environmental changes are observed in a relatively small spatial scale (Stevens, 1992; Janzen, 1967; Graves, 1988). According to the MPHT hypothesis (Janzen, 1967), for inhabitants of tropical mountains, these climatic variations are more important because they represent effective barriers to the development of species. The background for this hypothesis is that species at lower latitudes did not experienced strong climatic variation in their life-history than did species from higher latitudes (climate variability hypothesis) (Stevens, 1989; Stevens, 1992), so they present narrow thermal tolerances, what in turn may limit their dispersal ability, allowing isolation and speciation process (Janzen, 1967; Ghalambor *et al*., 2006). Thus, species are replaced among the altitudinal bands.

Although changes along altitude were consistently explained by turnover, an important portion of the overall dissimilarity was due to nestedness (about 29% of overall dissimilarity at LG and 34% at HG). We sugest that different process, regarding the LG and the HG, explain these observed patterns.

The higher variability of ranges showed by Plecoptera and Ephemeroptera, and also the predominance of nestedness component characterizing their dissimilarity at HG confirmed our expectations. Also, Ephemeroptera and especially Plecoptera did not confirm the MPHT hypothesis. We speculate that taxa with higher diversification in temperate zones, such is the case of Plecoptera and Ephemeroptera (Pearson & Boyero, 2009; Lake *et al*., 1986), react differently to temperature variations at tropical mountains. Also based on climate variability hypothesis (Stevens, 1989; Stevens, 1992), we sugest that most of species from these taxa have physiological adaptations that somehow, possibly due to genetic predisposition, enable them to cope with high temperature variation at altitudinal gradients, and thus present higher altitudinal ranges, even at tropical latitudes. This explains why the dissimilarity at the HG was not entirely described by turnover patterns, and, once the number of Plecoptera and Ephemeroptera species is lower than the other groups, nested patterns represented only a small portion of the overall observed dissimilarity.

Few groups have highest richness at higher latitudes, but it is frequently observed for some taxa, such as Plecoptera (Pearson & Boyero, 2009; Lake *et al*., 1986, Vinson & Hawkins, 2003). Lower abundance and diversity of Plecoptera genera have been observed at tropical streams, while the peak of richness appears to occur at latitudes 40ºN and 40ºS (Vinson & Hawkins, 2003). In addition, Plecoptera species are frequently found at high altitude in mountains (Knight & Gaufin, 1966; Fureder *et al*., 2006). Different from other taxa, the highest richness of Plecoptera species occurred between 1900 and 2500 m a.s.l. in this study, but the occurrence at lower and higher altitudes (suboptimal conditions), may indicate the existence of source-sink dynamics in their distribution, where the optimal conditions can be found where richness peaked (1900 – 2500 m, in this study). Ephemeroptera have peaks of richness at lower latitudes comparing to Plecoptera (Vinson & Hawkins, 2003), thus the altitudinal range depicted by Ephemeroptera species were lower than Plecoptera species, what resulted in a slightly lower importance of nestedness for the former.

The intensification of turnover pattern with altitude in both gradients indicates that species at higher altitudes are possibly restricted to the mountaintops. In fact, many studies have noticed that species at highest altitudes have limited range and make up a peculiar community compared to communities at lower altitudes (Finn *et al*., 2013; Harrington *et al*., 2016; Cap. 1 – Parte I). Further, new and endemic species are frequently reported at mountaintops in Neotropical region (Fiaschi & Pirani, 2009; Chaves *et al*., 2015; Dumasa & Nessimian, 2012; Trivinho-Strixino *et al*., 2012; Froehlich, 2011; Finn, *et al*., 2012). In addition, this evidence may indicate that the species' distribution in the current study were essentially resultant from horizontal colonization (Lobo & Halftter, 2000).

Contrary to the HG, the peak of richness at the LG occurred at the highest altitudes and progressively decreased towards sea level (Cap.1 – Parte I). Once the source-sink dynamics predicts that impoverished sites can be sustained only by some tolerant species that migrate from sites with highest richness (Pulliam, 1988; Grytnes &

Mccain, 2007; Kessler *et al*., 2011; Grytnes *et al*., 2008), is unlikely that this process is driving dissimilarity along the LG. Most of species at the top of the LG showed small ranges (Fig. 2, em Cap. 1 – Parte I) confirmed also by the turnover-altitude relation (Figure 1b), what indicates that this species have narrow tolerances for the environmental changes and probably did not migrate from lowlands. We believe that the highest altitudes at this gradient shelter few species from lower altitudes (with higher altitudinal ranges) and receive substantial horizontal colonization from cold-stenothermic species that live at higher altitudes close to it (Figura 2, em anexo). The combination of both mechanism of colonization, in addition to the favorable conditions at highest altitudes in this gradient (Cap. 1 – Parte I) resulted in the peak of richness observed. Thus, nestedness here may be resultant from this mixed mechanisms of species distribution, where some species are widely distributed along gradient (originated by vertical distribution) and other are more restricted to highest altitudes (originated by horizontal distribution).

At mountains with recent formation (e.g. Andes), and highly isolated, species' colonization occurred from low altitudinal region towards the top (Escobar *et al*., 2005; Lobo & Halfftter, 2000), so higher altitudes control the establishment and survivor of species from lower altitudes, and species replacement uses to be reduced at the altitudinal gradient (Lobo & Halfftter, 2000). The formation of the mountains in this study is resultant from tectonics movements during Late Cretaceous - about 70 Mya (Almeida & Carneiro, 1998; Modenesi-Gautieri *et al*., 2002), so considerable climatic changes during this period could have affected local biodiversity, leading to the high species richness and compositional changes among sites.

The pool of species living at the mountaintop of the HG probably are also maintained by horizontal colonization of cold-stenothermic species living in mountaintops nearby (Figura 2, em anexo), once most species from lower altitudes are unable to tolerate the harvest condition imposed by the extremely high altitude. Species with restricted altitudinal range occurring in isolated mountains are more prone to suffer from local extinction (MacArthur & Wilson, 1967) and isolated mountains are hardly refuge zones for allochthonous elements during climate change (Lobo & Halfftter, 2000). These few populations found at highest altitudes in this study depend on horizontal colonization from the abundant mountain ranges found at Serra da Mantiqueira formations, confirmed by the widespread distribution of some species into Atlantic Forest mountaintops (Trivinho-Strixino *et al*., 2012; Safford, 2007). Once the colonization at these mountains appears to be caused rather by horizontal than vertical forces, and consequently the species' substitution were strongly related to altitudinal variation, we believe that historical events had an important causality over current biodiversity.

The remarkable variation in species composition showed by madicolous insects in Atlantic Forest's mountains is primary resultant from replacement of species along the altitudinal ranges, what confirms the MPFT hypothesis. However, nesting process displayed some importance over the observed dissimilarity among sites. At the HG, nestedness mechanism was probably caused by cold-adapted groups with higher altitudinal ranges, which distribution is probably determined by higher thermal tolerances and isolated populations at mountaintops (originated from horizontal colonization). At the LG, nestedness mechanism is resultant from the colonization of autochthonomus species (from surrounding lowlands) and colonization of allochthonous species that come from horizontal colonization, thus not resultant from source-sink dynamics. These results highlight also the urgent need of conservation of mountains ecosystems, which harbor a unique pool of species, many of them very susceptible to environmental impacts especially climate changes.

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PARTE II

TAXONOMIA

Cap. 1 Madicolous Chironomidae (Diptera: Insecta) from Brazilian Atlantic Forest: a checklist with notes on altitudinal distribution

Cap. 2 Two new species of *Podonomus* (Diptera: Chironomidae: Podonominae) from mountains of southeastern Brazil

Cap. 3 New species of the genus *Pseudochironomus* Malloch 1915 (Diptera: Chironomidae) from Brazilian highlands

Cap. 4 Tanytarsini from madicolous habitats in Southeasth Brazil: new species and new records

Madicolous Chironomidae (Diptera: Insecta) from Brazilian Atlantic Forest: a checklist with notes on altitudinal distribution

Abstract

Madicolous habitats are defined by thin layer of water that runs over rocky surfaces. Although they can harbor peculiar Chironomidae species, evidenced mainly by some sparse taxonomic studies, little is known about the identity and ecology of madicolous Chironomidae in Neotropical region. The main purpose of this research was to reveal the madicolous Chironomidae from the Atlantic Forest and contribute towards the ecological knowledge of the species. The sampling was performed with emergence traps from 0 to 2700 m a.s.l. We found 61 species and morphospecies were recorded, of which only 19 were known to science. Most of these have their geographic and altitudinal range extended, while other showed significant specificity for an altitudinal band (as evidenced by the indicator species analysis). In view of the great diversity evidenced by madicoulous habitats, this and other semi-aquatic ecosystems should not be overlooked. Moreover, the biodiversity in mountainous region from Atlantic Forest, one of the richest in the world, suffers from intense environmental and human pressure, what makes the study and conservation of the area an urgent need.

Key words: non-biting midges, hygropetric habitats, semi-aquatic habitats, Tropical Forests, mountains

Introduction

Madicolous habitats are characterized by thin layer of water that frequently flows over rocky surfaces, and for this reason they are also called hygropretric habitats. The first to use the term was Thienemann in 1909, when he analyzed the hygropetric entomofauna in Central Europe. After, some catalogues of madicoulous fauna were done in North America (Sinclair & Marshall, 1987) and Europe (Bertrand, 1948; Vaillant, 1956). But lately, most of the progress involving the study of madicolous organisms is resultant from taxonomic works (Sinclair, 1988; Cranston 1998; Roque & Trivinho-Strixino, 2004; Pinho & Andersen, 2015), which emphasize that this habitat can harbor a rich and, not uncommonly, an endemic fauna. In South America, madicolous habitats have recently provided remarkable discoveries on the occurrence of insects, including new records (Roque & Trivinho-Strixino 2004; Short *et al*. 2013; Pinho & Andersen 2015) and new species (Pepinelii *et al*. 2009; Silva *et al*. 2012; Trivinho-Strixino *et al*. 2012; Miller & Montano 2014).

In natural ecosystems, madicolous insects can live in the shoreline of streams or in isolated overflowing groundwater. Additionally, when robust water bodies, such as streams and lakes, are hardly found, e.g. in mountaintops, madicolous biotopes can be the only source of permanent water that allows the establishment and survivor of aquatic and semi-aquatic insects, contributing for the maintenance of biodiversity in natural systems. The truly inhabitants (eumadicoles) present morphological and physiological adaptations that favors their survivor in such a specific environmental condition. Once the insects, especially dipterans, are the predominant group among madicolous organisms, they use to have flattened bodies, and their larval stages, may present strong locomotors appendages (Trivinho-Strixino *et al*. 2012) or even be constructors of portable cases, to avoid water carrying (Fittkau & Reiss 1998).

The Chironomidae family is one of the most diverse within Diptera. The estimated number of species reaches 20,000 (Coffman 1995), but only 6,000 species, approximately, have been described. This remarkable evolutionary success allowed them to occur in all zoogeographic regions in the world, including Antarctica, and also to tolerate the harshest environmental conditions (Sugg *et al*. 1983; Linevich 1971; Watanabe *et al*. 2006; Andersen *et al*. 2016). Although the immature stages of most the known species show high dependence of water (Ferrington 2008), some of them are considered semi-aquatic or terrestrial, and some species have even been recorded in artificial madicolous systems (Hamerlik *et al.* 2010).

A high diversity of chironomids is expected to occur in natural madicolous habitats from tropical regions, nevertheless this biotope has been neglected in freshwater researches, which make difficult to estimate the diversity supported by this habitat and also the identity of its inhabitants. Furthermore, concerning the taxonomy of chironomids, most of the descriptions are based only on adults (male), so the habitats, behavior, and other ecological information of the species, related to larval stages, are disregarded.

Despite the significant progress on Chironomidae research in the last decade, most of the registered species are still concentrated in Neartic and Paleartic regions, which emphasize the urgent need of studies in Tropical region that presents potentially higher diversity. Here we provide the first checklist of the madicolous Chironomidae species from Atlantic Forest– one of the richest hotspot in the world, and still the most affected by the habitat loss (Myers *et al*. 2000) – with notes on their distribution in the altitudinal gradients and other ecological features.

Material and Methods

Study Area

The exceptional biodiversity verified in the Brazilian Atlantic Forest is mainly due to the environmental heterogeneity resulted from its singular geographical characteristics. It comprehends a high variation in latitudinal (originally from parallels

3º to 30º) and altitudinal ranges (0 to 2892 m.a.s.l.), which generates a diverse forest composition and a wide variety of habitats (Ribeiro *et al*. 2009). Also, the high precipitation rates recorded annually (about 1400 mm) (Forti *et al*. 2005) allied to the abundant and easily found overflowing groundwater provide a perfect condition for the maintenance of different kinds of water bodies, in especial madicolous habitats, that can be observed even in upmost sites.

This study was conducted inside three conservation units from the Atlantic Forest located in Southeastern of Brazil: Serra do Mar State Park (PESM), Serra dos Órgãos National Park (PARNASO) and Serra da Mantiqueira Environmental Protection Area (APASM) (mean distance between those areas: 185 km). These conservation units was chosen due to the utmost variation in topographic profile they present, which allowed us to explore madicolous Chironomidae communities in a wide range of altitude (from 0 to 2700 m a.s.l.).

PESM is the widest continuous protected area inside the Atlantic Forest (332,000 ha). It comprehends all costal region of São Paulo State, and therefore, includes stretches at the sea level, and also some peaks, that elevate the altitudinal range up to 1270 m a.s.l. The vegetation present in the region includes: mangroves, resting (costal dunes), costal vegetation, ombrophilous dense forest and campos de altitude (high altitude-vegetation composed predominantly by grass, shrub and herbaceous). In this locality, we explored sites between 0-1100 m a.s.l. (23˚26'782"S, 45˚11'567"W).

PARNASO is located in the mountainous region of the Rio de Janeiro State, where it occupies 20,024 ha. The relief in the region is marked with slopes, which enable to find a high altitudinal gradient. The upmost site in the park reaches 2263 m a.s.l., and the vegetation changes according to the elevation: submontane forest, montane forest, misty forest and campos de altitude. In this park, we explored intermediate altitudes, between 1200-2100 m a.s.l. (22°27'707'S, 43°01'815"W).

APASM includes three states from the Southeast: São Paulo, Rio de Janeiro and Minas Gerais, comprising an area of 421,804 ha. It harbors two of the five highest mountains in Brazil, including the culminant site at 2798 m a.s.l. (Pedra da Mina Mountain). The vegetation in the region create a mosaic of phytophysiognomies, composed by upper-montane forests, araucaria forest and campos de altitude. In this locality we collected at sites from 1700 to 2700 m a.s.l. (22˚25'51"S/44˚50'590"W).

Sampling

We sampled madicolous habitats every 200-300 m along the gradient, with at least three replicates in each altitudinal band.

The adults' sampling was performed with emergence traps (Shimabukuro *et al*. 2015), that stayed in the field during 7 days. The advantage of using this type of trap is the guarantee that the emergent adults really belong to the interested habitat, once the immature have completed their development at that specific site. In addition, the isolation provided by this trap, prevents the invasion by foreign specimens. Larvae, pupae and exuvie were also collected from the substrate below the traps with hand net. Organisms were preserved in absolute ethanol, slide mounted with Euparal and analyzed in optic microscopy. Male were used for identification in species level. When the characterization of the specimens did not match any species' description, probably representing new species, we designate them as morphotypes.

Data analysis

We provide a list with all the species and morphotypes found; the respective development stage analyzed; the type of madicolous habitat and the locality where the specimens were recorded. When the development stage consists only of adults (A), it does not mean that immature were not collected, but the stages' association were not established yet, otherwise the species development stage is stated as "all" (descriptions on next chapters). In addition, some genera were recorded only in larval instars (L).

Further, for each species we add a set of data from the literature, which includes previous information on their geographical distribution, altitudinal records, habitats and which development stages and sexes are already known. Our data, consisting on additional information about the ecology and distribution of each species, is presented as "Remarks" after the previous cited data. In addition, we included some information about the morphospecies (probably new species) found, denominated into the text as "unknown species".

In order to test the degree of affinity of each species with the altitude they occurred, we applied an analysis of indicator species (Dufrêne &. Legendre 1997), from which we obtained the "indicator value" (IndVal) also the "p-value" for each species; the IndVal varies from 0 to 1.0, and higher values indicate a more expressive altitudinal representation; significant indicator species presented $p<0.05$, although we considered $p=0.06$ species with high altitudinal specificity; $0.06<p<0.2$ species with median altitudinal specificity; and $p>0.2$ species with low altitudinal specificity. This analysis was performed in R Cran Project 3.0.3 (R Core Development) software, using "labdsv" package (Roberts 2007).

Results

In Table 1 we provide a checklist based on adult males and larvae sampled from madicolous habitats in the present study. A total of 62 species, including known species (20) and morphospecies (42), were recorded. Regarding the 20 recognized species, 16 of them had their altitudinal records extended (Figure 1).

Only six from the 62 species recorded were significantly considered indicators of altitude, and all of them are still unknown to science (Figure 2).

TABLE 1. Taxa recorded in madicolous habitats from Atlantic Forest in Southeastern Brazil. DS: development stage; A: adults; L: larvae; SE: stream edges; RS: rocky seepages; L: low altitudinal band, which comprehend the sampling sites in PESM; I: intermediate altitudinal band, which comprehend the sampling sites in PARNASO; H: high altitudinal band, which comprehend the sampling sites in APASM.

	DS	Habitat		Altitudinal Band		
		SE	RS	L	I	H
PODONOMINAE						
Podonomini						
Podonomus pepinellii Roque & Trivinho-Strixino, 2004	A	x	x		x	x
Podonomus sp. 1**	All	x	x			x
ORTHOCLADIINAE						
Orthocladiini						
Lipurometriocnemus biancae Andersen, Pinho & Mendes, 2016	A	x			x	x
Lipurometriocnemus sp. 1*	A		x			x
Urubicimbera montana Andersen, Mendes & Pinho, 2015	A		x			x
Urubicimbera sp. 1*	A	x	x			x
Pseudosmittia catarinense Andersen, Sæther & Mendes, 2010	A		x			x
Limnophyes guarani Pinho & Andersen, 2015	A	x	x			x
Limnophyes gercinoi (Oliveira, Messias & Santos, 1995)	A	x		x	x	
Limnophyes sp. 1*	A	x			x	
Cricotopus sp 1*	A	x				x
Cricotopus sp 2*	A	x			x	x
Cricotopus sp 3*	A	x		x		
Cricotopus sp 4*	A	x		x		x
Cricotopus sp 5*	A	x				x
Rheocricotopus sp. 1*	A		x			x
Rheocricotopus sp. 2*	A	x			x	

	DS	Habitat		Altitudinal Band		
		SE	RS	L	I	H
Metriocnemus sp. 1*	A		x			x
Parametriocnemus sp. 1*	A	x				x
Parametriocnemus sp. 2*	A	x		x		x
Parametriocnemus sp. 3*	A	x		x	x	
Parakiefferiella strixinorum Wiedenbrug & Andersen, 2002	A	x		x		
Parakiefferiella sp. 1*	A	x		x		
Bryophenocladius carus Roback 1962	A	x		x		
Bryophenocladius sp. 1*	A	x		x		
Caaporangombera intervales Andersen, Pinho & Mendes, 2015	A	x		x		
Paraphaenocladius	L	x	x	x	x	x
Antilocladius	L	x		x	x	x
Psectrocladius	L	x	x		x	x
Lopescladius	L	x		x		x
Nanocladius	L	x				x
Thienemannia	L	x	x		x	x
Gymnometriocnemus	L	x			x	x
Gênero 1 Prox. *Lipurometriocnemus*	A		x			x
Gênero 2 Prox *Thalassosmittia*	A	x			x	
Corynoneurini						
Corynoneura unicapsulata Wiedenbrug & Trivinho-Strixino, 2011	A	x				x
Corynoneura hermanni Wiedenbrug & Trivinho-Strixino, 2011	A	x	x		x	x
Corynoneura septadentata Wiedenbrug & Trivinho-Strixino, 2011	A	x	x	x	x	
Corynoneura sertaodaquina Wiedenbrug & Trivinho-Strixino, 2011	A	x		x		
Onconeura japi Wiedenbrug, Mendes, Pepinelli & Trivinho-Strixino, 2009	A	x				x
Onconeura oncovolsella Wiedenbrug, Mendes, Pepinelli & Trivinho-Strixino, 2009	A	x			x	
Onconeura sp. 1*	A	x			x	
Onconeura sp. 2*	A	x			x	
Onconeura sp. 3*	A	x		x		
Thienemanniella sp. 1*	A	x				x
Ubatubaneura	L	x		x		
CHIRONOMINAE						
Tanytarsini						
Tanytarsus giovannii Sanseverino & Trivinho-Strixino, 2010	A	x				x
Tanytarsus sp. 1*	A	x				x
Tanytarsus sp. 2*	A	x				x
Tanytarsus sp. 3*	A	x				x
Tanytarsus sp. 4*	A	x		x		
Tanytarsus sp. 5*	A	x			x	
Tanytarsus sp. 6*	A	x			x	
Paratanytarsus silentii Trivinho-Strixino, 2010**	All	x		x	x	
Rheotanytarsus	L	x	x	x	x	x
Constempellina	L	x		x		x
Stempelinella	L	x		x		
Chironomini						
Nilothauma sp. 1*	A	x				x
Polypedilum (s. str.) solimoes Bidawid-Kafka, 1996	A	x				x
Polypedilum (s. str.) sp 1*	A	x				x

	DS	Habitat		Altitudinal Band		
		SE	RS	L	I	H
Polypedilum (s. str.) sp 2*	A	x		x		
Polypedilum (s. str.) sp 3*	A	x			x	
Polypedilum (Pentapedilum) sp 1*	A	x		x		
Polypedilum (Pentapedilum) sp 2*	A	x			x	
Polypedilum (Tripodura) sp. 1*	A	x		x		
Gênero 3	A	x		x	x	
Lauterborniella sp. 1*	A	x		x		
Oukuriella sublettei Messias & Oliveira, 1999	A	x		x		
Stenochironomus sp. 1*	A	x		x		
Stenochironomus sp. 2*	A	x		x		
Chironomini 1	L	x		x	x	x
Phaenospectra	L	x		x		x
Beardius	L	x		x		x
Chironomus	L	x		x		
Pseudochironomini						
Pseudochironomus sp. 1**	All	x				x
Riethia	L	x	x		x	x
TANYPODINAE						
Pentaneurini						
Hudsonimyia caissara Silva, Wiedenbrug, Trivinho-Strixino, Oliveira & Pepinelli, 2012	A	x		x		
Hudsonimyia sp. 1*	A	x		x		
Pentaneura	L	x	x	x	x	x
Parapentaneura	L	x	x	x	x	
Ablabesmyia	L	x				x
Larsia	L	x	x	x		x
Gr. *Thienemanimyia*	L	x		x		x
Nilotanypus	L	x				x
Macropelopini						
Alotanypus	L	x				x
Procladini						
Djalmabatista	L	x				x

*species unknown to science

**Preliminary description on next chapters. For *P. silentii*, larva and pupa are described.

Data from the 24 genera recorded, and their respective species and morphospecies cited previously:

Subfamily Podonominae

Podonomus Philippi, 1865

40 valid species

Southern Hemisphere

Running water and tarn inhabitants

P. pepinellii Roque & Trivinho-Strixino, 2004

Distribution. BRAZIL - Mantiqueira and Espinhaço mountain ranges: Monte Verde, Minas Gerais State (22° 53′9.6″S, 46°1′55.2″W); Campos do Jordão, São Paulo State (22°46′1.2″S, 45°31′15.6″W); Teresópolis, Rio de Janeiro State (22°27′3.6″S, 43°0′50.4″ W); Alto Caparaó, Minas Gerais State (20°25′12″S, 41°50′45.6″W).

Elevation: 1275-1815 m a.s.l.

Habitats. Pupae found in a first-order stream; larvae found in madicolous habitats.

Known stages: L, P, M, F.

References. Roque & Trivinho-Strixino 2004; Trivinho-Strixino *et al*. 2012.

Remarks. In this study we found larvae living on marginal rocks of a low order stream and in isolated rocky seepages, extending the altitudinal records up to 2700 m a.s.l. Environmental characterization: Water temperature varying from 10-22 ºC; dissolved oxygen 6.4-9.0 $mg.l^{-1}$; pH 5.0-6.0; very slow water flow; vegetal coverage completely absent (shrub-herbaceous vegetation). *P. pepinellii* was found in PARNASO (Rio de Janeiro State) and APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.25; p=0.32).

Unknown species: *Podonomus* sp. 1 (Cap. 2 – Parte II). Locality: APASM. Altitudinal range: 2200-2700 m a.s.l. Significant altitudinal specificity (IndVal: 0.41; **p=0.04).**

Subfamily Orthocladiinae

***Lipurometriocnemus* Saether, 1981**

4 valid species

Holartic, Neartic and Neotropical

Unknown, probably semi-aquatic and terrestrial

L. biancae Andersen, Pinho & Mendes, 2016

Distribution. BRAZIL - Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07’37”S, 49°28’47”W).

Elevation: 1670 m a.s.l.

Habitats. Male collected in malaise trap in cloud forest.

Known stages: M.

References. Andersen *et al*. 2016.

Remarks. In this study *L. biancae* was found on marginal rocks of low order streams and small waterfalls, extending the altitudinal records from 1570-2575 m a.s.l. Environmental characterization: Water temperature varying from 11-22 ºC; dissolved oxygen 7.4-10.0 mg.l^{-1}; pH 4.5-6.4; slow to fast flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in PARNASO (Rio de Janeiro State) and APASM (Minas Gerais State), the northernmost records. Low altitudinal specificity (IndVal: 0.16; p=0.39).

Unknown species: *Lipurometriocnemus* sp. Locality: APASM. Altitudinal record: 2700 m a.s.l. Low altitudinal specificity (IndVal: 0.2; p=0.45).

***Urubicimbera* Andersen, Mendes & Pinho, 2015**

1 valid species

Neotropical

Unknown habitats

***U. montana* Andersen, Mendes & Pinho, 2015**

Distribution. BRAZIL - Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07’37”S, 49°28’47”W).

Elevation: 1670 m a.s.l.

Habitats. Male collected in malaise trap in cloud forest.

Known stages: M.

References. Andersen *et al*. 2015.

Remarks. In this study *U. montana* was found on rocky seepages, from 2200-2700 m a.s.l, expanding its altitudinal range. Environmental characterization: Water temperature varying from 21.3-27.6 ºC; dissolved oxygen 6.4-7.0 mg.l^{-1}; pH 6; slow flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in APASM (Minas Gerais State), extending the geographical records to northernmost. Median altitudinal specificity (IndVal: 0.26; p=0.13).

Unknown species: *Urubicimbera* sp. 1. Locality: APASM. Altitudinal range: 2575-2700 m a.s.l. Significant altitudinal specificity (IndVal: 0.91; **p=0.002).**

***Pseudosmittia* Edwards, 1932**

98 valid species

Worldwide

Aquatic, terrestrial and semi-terrestrial habitats

***P. catarinense* Andersen, Saether & Mendes, 2010**

Distribution. BRAZIL - Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07’32”S, 49°29’38”W).

Elevation: 1822 m a.s.l.

Habitats. Male collected in malaise trap in cloud forest, close to small stream.

Known stages: M.

References. Andersen *et al*. 2010.

Remarks. In this study *P. catarinense* was found on rocky seepages, at 2200 m a.s.l, extending slightly it altitudinal occurrence. Environmental characterization: Water temperature 27.6 ºC; dissolved oxygen 7.0 mg.l^{-1}; pH 6; slow flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in APASM

(Minas Gerais State), extending the geographical records to northernmost. Low altitudinal specificity (IndVal: 0.17; p=0.84).

Limnophyes **Eaton, 1875**

90 valid species

Worldwide

Aquatic, terrestrial and semi-terrestrial habitats

L. guarani Pinho & Andersen, 2015

Distribution. BRAZIL - Serra do Corvo Branco, Grão-Pará, Santa Catarina State (28°03’21”S, 49°22’00”W).

Elevation: 1241 m a.s.l.

Habitats. Madicolous habitat.

Known stages: L, P, F, M

References. Pinho & Andersen 2015.

Remarks. In this study *L. guarani* was found on rocky seepages and also on marginal rocks of first order streams, from 1570-2700 m a.s.l, extending the altitudinal range of the species. Environmental characterization: Water temperature 16.3-22 ºC; dissolved oxygen 6.4-8.4 $mg.l^{-1}$; pH 5.5-6.4; slow to fast flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in APASM (Minas Gerais State), extending the geographical records to northernmost. Median altitudinal specificity (IndVal: 0.19; p=0.18).

L. gercinoi (Oliveira, Messias & Santos, 1995)

Distribution. BRAZIL - Parque João Paulo II, Curitiba, Paraná State; UCAD, Florianópolis, Santa Catarina State; Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07’32”S, 49°29’38”W); Nova Teutônia, Santa Catarina State (27°11’S, 52°23’W).

Elevation: 300-1822 m a.s.l.

Habitats. Adults collected with entomological net and malaise trap close to stream.

Known stages: F, M.

References. Oliveira *et al*. 1995; Spies & Reiss 1996; Mendes, *et al*. 2007; Roque *et al*. 2007.

Remarks. In this study *L. gercinoi* was found on marginal rocks of small streams, from 1080-1445 m a.s.l. Environmental characterization: Water temperature 10.0-16.1 ºC; dissolved oxygen 8.1-9.9 $mg.l^{-1}$; pH 5; slow to fast flowing; partial canopy (about 50-70% covered). The species was found in PARNASO (Rio de Janeiro State), PESM (São Paulo State) extending the geographical records to northernmost. Low altitudinal specificity (IndVal: 0.19; p=0.2).

Unknown species: *Limnophyes* sp. 1. Locality: PARNASO. Altitudinal range: 1445-2125 m a.s.l. Low altitudinal specificity (IndVal: 0.11; p=0.87).

***Cricotopus* van der Wulp, 1874**

180 valid species

Worldwide

Running and standing water

Unknown species: *Cricotopus* sp. 1. Locality: APASM. Altitudinal record: 2575 m a.s.l.. Low altitudinal specificity (IndVal: 0.17; p=0.85); *Cricotopus* sp. 2 Locality: APASM and PARNASO. Altitudinal range: 1445-1570 m a.s.l. Low altitudinal specificity (IndVal: 0.15; p=0.47); *Cricotopus* sp. 3 Locality: PESM. Altitudinal range: 70-1075 m a.s.l. Low altitudinal specificity (IndVal: 0.13; p=0.58); *Cricotopus* sp. 4 Locality: PESM. Altitudinal range: 70-200 m a.s.l.. Significant altitudinal specificity (**IndVal: 0.71; p=0.003)**; *Cricotopus* sp. 5 Locality: APASM. Altitudinal range: 1750 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p=1.0).

***Rheocricotopus* Brundin, 1956**

69 valid species

Except Antarctica and Oceania

Mostly rheophilic

Unknown species: *Rheoricotopus* sp. 1 Locality: APASM. Altitudinal record: 2200 m. a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.85); *Rheoricotopus* sp. 2. Locality: PARNASO. Altitudinal range: 1580-1670 m a.s.l. Median altitudinal specificity (IndVal: 0.29; p=0.11).

***Metriocnemus* van der Wulp, 1874**

67 valid species

Worldwide

Mosses, phytotelmata, springs, ditches, streams, lakes and rock pools

Unknown species: *Metriocnemus* sp. 1 Locality: APASM. Altitudinal record: 2200 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.84).

***Parametriocnemus* Goetgebuer, 1932**

34 valid species

Worldwide

Springs, streams and rivers

Unknown species: *Parametriocnemus* sp. 1. Locality: APASM. Altitudinal record: 2575 m a.s.l. High altitudinal specificity (IndVal: 0.33; p=0.06); *Parametriocnemus* sp. 2. Locality: PESM and APASM. Altitudinal range: 25-1570 m a.s.l. Low altitudinal specificity (IndVal: 0.12; p=0.70); *Parametriocnemus* sp. 3. Locality: PESM and APASM. Altitudinal range: 25-1445 m a.s.l. Low altitudinal specificity (IndVal: 0.11; p=0.76).

Parakiefferiella **Thienemann, 1936**

44 valid species

Worldwide

Running and standing waters

P. strixinorum **Wiedenbrug & Andersen, 2002**

Distribution. BRAZIL - Taquara, Rio Grande do Sul State (29°46'S, 50°53'W); São Francisco de Paula, Rio Grande do Sul State (29°26'S, 50°35'W); Bom Jesus, Rio Grande do Sul State (28°40'S, 50°26'W).

Elevation: 600-1000 m a.s.l.

Habitats. Stream.

Known stages: P, M.

References. Wiedenbrug & Andersen 2002

Remarks. In this study *P. strixinorum* was found on marginal rocks of small streams, at 1045 m a.s.l, extending slightly the altitudinal range of the species. Environmental characterization: Water temperature 17 ºC; dissolved oxygen 8.6 mg.l^{-1}; pH 5; fast flowing; reduced vegetal canopy (less than 20% covered). The species was found in PESM (São Paulo State) extending the geographical records to northernmost. Low altitudinal specificity (IndVal: 0.17; p=0.85).

Unknown species: *Parakiefferiella* sp. 1. Locality: PESM. Altitudinal record: 70 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p=0.33).

Bryophaenocladius **Thienemann, 1934**

115 valid species

Worldwide

Terrestrial and semi-terrestrial, few aquatic

B. carus **(Roback, 1962)**

Distribution. BRAZIL – Parque Estadual Intervales, Iporanga, São Paulo State (24°30'S, 48°37'W); PANAMA, Canal Zone, Curundu, Holbrook Air Force Base.

Elevation: 20-100 m a.s.l.

Habitats. Mosses on tree barks.

Known stages: L, P, F, M.

References. Roback 1962; Sæther 1976; Spies & Reiss 1996; Sæther 1981; Wang *et al.* 2006; Donato 2011.

Remarks. In this study *B. carus* was found on marginal rocks of small streams, at 1075 m a.s.l., extending the altitudinal range of the species. Environmental characterization: Water temperature 15.8 ºC; dissolved oxygen 8.1 mg.l^{-1}; pH 5; moderate to fast flowing; dense vegetal canopy (more than 70% covered). The species was found in PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.17; p=0.85).

Unknown species: *Bryophaenocladius* sp. 1. Locality: PESM. Altitudinal record: 1075 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.87).

***Caaporangonbera* Andersen, Pinho & Mendes, 2015**

4 valid species

Brazil, Atlantic Forest

Unknown, but possibly terrestrial or semi-terrestrial

C. intervales Andersen, Pinho & Mendes, 2015

Distribution. BRAZIL – Parque Estadual Intervales, Ribeirão Grande, São Paulo State (24°15’S, 48°10’W).

Elevation: 500 m a.s.l.

Habitats. Unknown, but possibly terrestrial or semi-terrestrial

Known stages: M.

References. Andersen *et al*. 2015.

Remarks. In this study *C. intervales* was found on marginal rocks of small streams, at 740 m a.s.l, extending slightly the altitudinal range of this species. Environmental characterization: Water temperature 15.7 ºC; dissolved oxygen 9.4 mg.l^{-1}; pH 5.5; very slow flowing; partial vegetal canopy (50% covered). The species was found in PESM (São Paulo State). Median altitudinal specificity (IndVal: 0.33; p=0.14).

***Corynoneura* Thienemann, 1934**

About 96 valid species

Worldwide

Running and stagnant waters

C. unicapsulata Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL – Parque Estadual do Jaraguá, São Paulo State (23°27´S, 46° 45´W) Serra do Japi, Jundiaí, São Paulo State (23°14´30´´S, 46° 57´16´´W); Paraisópolis, Minas Gerais State (22°39´54.81”S, 45°55´38.29”W); São Luís do Purunã, Paraná State (25°27.180´S, 49°43.435´W); BRAZIL, Serra da Bodoquena, Mato Grosso do Sul State (20°41´49´´S, 56°52´54´´W); Alto Paraíso de Goiás, Goias State (14°9′34.92″S, 47°35′37.32W); COSTA RICA, Caccao, Guanacaste.

Elevation: 750-1370 m a.s.l.

Habitats. Litter in stones marginal to small streams.

Known stages: L, P, F, M.

References. Wiedenbrug & Trivinho-Strixino 2011; Wiedenbrug *et al.* 2012

Remarks. In this study *C. unicapsulata* was found on marginal rocks of small streams, at 2575 m a.s.l, extending its altitudinal occurrence. Environmental characterization: Water temperature 22 ºC; dissolved oxygen 7.4 mg.l^{-1}; pH 5.5; slow flow to stagnant; vegetal canopy absent (shrub-herbaceous vegetation. The species was found in APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.17; p=0.85).

C. hermanni Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL –Ubatuba, São Paulo State (23°30.468´S, 45°11.923´W and 23°30.789´S, 45°14.442´W)

Elevation: 0-60 m a.s.l.
Habitats. Litter standing near the water surface from small streams.
Known stages: L, P, F, M.
References. Wiedenbrug & Trivinho-Strixino 2011; Wiedenbrug *et al*. 2012.
Remarks. In this study *C. hermanni* was found on marginal rocks of small streams and rocky seepages (large exposed rock in the middle of the forest), at 1570-1580 m a.s.l, extending the altitudinal occurrence of this species. Environmental characterization: Water temperature 15-16.3 ºC; dissolved oxygen 8.4-10.2 mg.l^{-1}; pH 5.0-6.4; slow to fast flowing; vegetal canopy completely absent. The species was found in APASM (Minas Gerais State) and PARNASO (Rio de Janeiro State), extending slightly the geographical distribution of this species. Low altitudinal specificity (IndVal: 0.29; p=0.23).

C. septadentata Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL – Parque Estadual do Jaraguá, São Paulo State (23°27´S, 46° 45´W), Nova Friburgo, Rio de janeiro State, Rio Boa Vista; Bocaina de Minas, Minas Gerais State (22°19´S, 44°34´W); Serra do Japí, Jundiaí, São Paulo State (23°14´30´´S, 46°57´16´´W); Serra da Bodoquena, Mato Grosso do Sul State (20°41´49´´S, 56°52´54´´W).
Elevation: 700-1200 m a.s.l.
Habitats. Litter near the water surface of a small shallow stream.
Known stages: L, P, F, M.
References. Wiedenbrug & Trivinho-Strixino 2011; Wiedenbrug *et al*. 2012
Remarks. In this study *C. sepadentata* was found on marginal rocks of small streams and on rocky seepages (large exposed rock in the middle of the forest), from 1045-1580 m a.s.l, extending slightly the altitudinal range of this species. Environmental characterization: Water temperature 15-17 ºC; dissolved oxygen 8.6-10.2 mg.l^{-1}; pH 5; moderate to fast flowing; vegetal canopy reduced or absent (less than 30% covered). The species was found in PARNASO (Rio de Janeiro State) and PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.09; p=0.88).

C. sertaodaquina Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL – PESN, Ubatuba, São Paulo State (23°30.789´S, 45°14.442´W; 23°31.068´S, 45°14.845´W; 23°31.231´S, 45°14.625´W); Alto Paraíso de Goiás, Goiás State (14°9′34.92″S, 47°35′37.32W); Serra da Bodoquena, Mato Grosso do Sul State (20°41´49´´S, 56°52´54´´W), São Simão, São Paulo State; São Luís do Purunha, Paraná State (25°27.180´S, 49°43.435´W).
Elevation: 20-1020 m a.s.l.
Habitats. Surface of stones of shallow fast flowing waters and also in litter near the water surface of streams.
Known stages: L, P, F, M.
References. Wiedenbrug & Trivinho-Strixino 2011; Wiedenbrug *et al*. 2012.
Remarks. In this study *C. sertaodaquina* was found on marginal rocks of small streams, at 70 m a.s.l. Environmental characterization: Water temperature 17 ºC; dissolved oxygen 9.2 mg.l^{-1}; pH 5.5; wet substrate, without any flow; reduced vegetal

canopy (about 20% covered). The species was found in PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.25; p=0.32).

Onconeura **Andersen & Seather, 2005**

8 valid species

Neotropical and Neartic

Running and stagnant water

O. japi Wiedenbrug, Mendes, Pepinelli & Trivinho-Strixino, 2009

Distribution. BRAZIL, Serra do Japi, Jundiaí, São Paulo State (23°14´38´´S, 06°57´02´´W); PESM, Ubatuba, São Paulo State (23°30.46'S, 45°11.923'W and 23°30.789´S, 45°14.442´W).

Elevation. 1058 m a.s.l.

Habitats. Litter below a waterfall of a first-order stream

Known stages: L, P, F, M.

References. Wiedenbrug *et al.* 2009

Remarks. In this study *O. japi* was found on marginal rocks of small streams, at 1570 m a.s.l, extending the altitudinal record of the species. Environmental characterization: Water temperature 16.3 ºC; dissolved oxygen 8.4 mg.l^{-1}; pH 6.4; slow flowing; vegetal canopy completely absent. The species was found in APASM (Minas Gerais State), extending the geographical distribution of the species. Low altitudinal specificity (IndVal: 0.14; p=1.0).

O. oncovolsella Wiedenbrug, Mendes, Pepinelli & Trivinho-Strixino, 2009

Distribution. BRAZIL – PESM, Ubatuba, São Paulo State (23°31.068´S, 45°14.845´W; 23°31.087´S, 45°14.621´W; 23°30.789´S, 45°14.442´W); São Francisco de Paula, Rio Grande do Sul State.

Elevation: 23-61 m a.s.l.

Habitats. Surface of stones in fast flowing waters

Known stages: L, P, F, M.

References. Wiedenbrug *et al.* 2009

Remarks. In this study *O. oncovolsella* was found on marginal rocks of small streams, at 1445 m a.s.l, extending the altitudinal record of the species. Environmental characterization: Water temperature 10 ºC; dissolved oxygen 9 mg.l^{-1}; pH 5; fast flowing; partial vegetal canopy (50% covered). The species was found in PARNASO (Rio de Janeiro State). Low altitudinal specificity (IndVal: 0.25; p=0.31).

Unknown species: *Onconeura* sp. 1. Locality: PARNASO. Altitudinal record: 1445 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p=0.30); *Onconeura* sp. 2 Locality: PARNASO. Altitudinal record: 1445 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p=0.32); *Onconeura* sp. 3 Locality: PESM. Altitudinal record: 1085 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.83).

***Thienemanniella* Kieffer, 1911**

53 valid species

Worldwide

Running and stagnant water

Unknown species: *Thienemanniella* sp.1. Locality: APASM. Altitudinal record: 1570 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p=1.00).

Subfamily Chironominae

***Tanytarsus* van der Wulp, 1874**

More than 300 valid species

Worldwide

Aquatic and terrestrial

***T. giovannii* Sanseverino & Trivinho-Strixino, 2010**

Distribution. BRAZIL – São Carlos, São Paulo State (21°59’10’’S, 47°52’32’’W; 21° 58’16’’S, 47°53’14’’W; 21°59'21.4"S, 47°51'14.2" W); Corumbá, Mato grosso do Sul State (19°34′30.06″S, 57°00′52.4″W).
Elevation: 90-850 m a.s.l.
Habitats. Low-order streams and eutrophic lake
Known stages: L, P, M.
References. Sanseverino & Trivinho-Strixino 2010; Trivinho-Strixino *et al*. 2015.
Remarks. In this study *T. giovannii* was found on marginal rocks of small streams, at 2575 m a.s.l, extending the altitudinal range of the species. Environmental characterization: Water temperature 22 ºC; dissolved oxygen 7.4 mg.l^{-1}; pH 5.5; fast flowing; vegetal canopy absent. The species was found in APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.17; p=0.84).
Unknown species: *Tanytarsus* sp. 1. Locality: APASM. Altitudinal record: 2575 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.82); *Tanytarsus* sp. 2. Locality: APASM. Altitudinal record: 2575 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.85); *Tanytarsus* sp. 3. Locality: APASM. Altitudinal record: 1570 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p=1.00); *Tanytarsus* sp. 4. Locality: PESM. Altitudinal record: 25 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p=0.33). *Tanytarsus* sp. 5. Locality: PARNASO. Altitudinal record: 2120 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.85); *Tanytarsus* sp. 6 Locality: PARNASO. Altitudinal record: 1445 m a.s.l. Significant altitudinal specificity (**IndVal: 0.5; p=0.02).**

***Paratanytarsus* Thienemann & Bause, 1913**

45 valid species

Worldwide

Running and stagnant water

***P. silentii* Trivinho-Strixino, 2010**

Distribution. BRAZIL – Parque Estadual do Jaraguá, São Paulo State (23°24’ S e 45°44’ W).

Elevation: 800 m a.s.l.

Habitats.

Known stages: M.

References. Trivinho-Strixino 2010.

Remarks. In this study *P. silentii* was found on marginal rocks of small streams, from 200-1445 m a.s.l, extending the altitudinal range of the species. Environmental characterization: Water temperature varying from 10-21 ºC; dissolved oxygen 7.9-9.9 mg.l^{-1}; pH 5-5.5; slow to fast flowing; occurring in scarce vegetal canopy to dense coverage (30-70% covered). The species was found in PARNASO (Rio de Janeiro State) and PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.24; p=0.25).

Nilothauma **Kieffer, 1921**

43 valid species

Worldwide

Running and stagnant water

Unknown species: *Nilothauma* sp. 1 Locality: APASM. Altitudinal record: 1570 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p=1.00).

Polypedilum **Kieffer, 1912**

More than 440 valid species

Worldwide

Standing and running water

P. solimoes Bidawid-Kafka, 1996

Distribution. BRAZIL – Tarumã River, Amazonas State; Florianópolis, Santa Catarina State (27°28'05''S, 48°22'58''W), UCAD, Santa Catarina State (27°31'51"S, 48°30'44"W), Santinho Beach, Santa Catarina State (27°27'S, 48°23'W).

Elevation: 20-80 m a.s.l.

Habitats. Adults collected close to a large Amazonian river. Larva found in leaf packs and detritus associated with the following bromeliad species: *Aechmea lindeni* (E. Morren) Baker, *Canistrum lindenii* (Regel) Mez, *Neoregelia laevis* (Mez) L.B. Smith, *Nidularium innocentii* Lem., *Vriesea philippocoburgii* Wawra, and *V. vagans* (L.B. Smith) L.B. Smith.

Known stages: L, P, F, M.

References. Bidawid-Kafka 1996; Pinho *et al*. 2013.

Remarks. In this study *P. solimoes* was found on marginal rocks of small stream, at 1570 m a.s.l, extending the elevation records of this species. Environmental characterization: Water temperature 16.3 ºC; dissolved oxygen 8.4 mg.l^{-1}; pH 6.4; slow flowing; vegetal canopy absent. The species was found in APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.14; p=1.0).

Unknown species: *Polypedilum* (s. str.) sp. 1. Locality: APASM. Altitudinal record: 1570 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p=1.00); *Polypedilum* (s. str.) sp. 2 Locality: PESM. Altitudinal record: 1080 m a.s.l. High altitudinal specificity (IndVal: 0.33; p=0.06); *Polypedilum* (s. str.) sp. 3. Locality: PARNASO. Altitudinal record: 2125 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.84); *Polypedilum* (*Pentapedilum*) sp. 1; Locality: PESM. Altitudinal record: 200 m a.s.l. Median altitudinal specificity (IndVal: 0.33; p=0.15). *Polypedilum* (*Pentapedilum*) sp. 2. Locality: PARNASO. Altitudinal record: 2125 m a.s.l. Low altitudinal specificity (IndVal: 0.17; 6 p=0.85). *Polypedilum* (*Tripodura*) sp. 1. Locality: PESM. Altitudinal record: 25 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p=0.33).

Lauterborniella **Thienemann & Bause 1913**

1 valid species

Holartic, Neartic and Neotropical

Standing water

Unknown species: *Lauterborniella* sp. 1. Locality: PESM. Altitudinal record: 1080 m a.s.l. Significant altitudinal specificity (IndVal: 0.5; **p=0.02**).

Oukuriella **Epler, 1986**

22 valid species

Neotropical

Associated with freshwater sponges and submerged wood

O. sublettei **(Roback, 1962)**

Distribution. BRAZIL – Paru do Oeste River, Missao Cururu, Amazonas State; Parque Estadual de Campos do Jordão, São Paulo State (22º41’40”S, 45º27’36”W).

Elevation: 20-1600 m a.s.l.

Habitats. Larvae found in submerged wood in a rocky first-order stream.

Known stages: L, P, M.

References. Messias & Oliveira 1998; Fusari *et al*. 2013; Bellodi *et al*. 2016

Remarks. In this study *O. sublettei* was found on marginal rocks of small streams, at 745 m a.s.l. Environmental characterization: Water temperature 17 ºC; dissolved oxygen 8.2 mg.l^{-1}; pH 5.5; fast flowing; dense vegetal canopy (more than 80% covered). The species was found in PESM (São Paulo State). Median altitudinal specificity (IndVal: 0.33; p=0.14).

Stenochironomus **Kieffer, 1919**

93 valid species

Worldwide

Miners of living or dead vegetal tissue

Unknown species: *Stenochironomus* sp. 1. Locality: PESM. Altitudinal record: 1075 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.84); *Stenochironomus* sp. 2. Locality: PESM. Altitudinal record: 70 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p=0.32).

Pseudochironomus Thienemann, 1934

13 valid species

Neartic, Paleartic, Neotropical

Running and standing water

Unknown species: *Pseudochironomus* sp. 1. Locality: APASM. Altitudinal record: 2575 m a.s.l. Significant altitudinal specificity (IndVal: 0.67; **p=0.003**).

Subfamily Tanypodinae

Hudsonimyia Roback, 1979

115 valid species

Neartic and Neotropical

Madicoulous

H. caissara Silva, Wiedenbrug, Trivinho-Strixino, Oliveira & Pepinelli, 2012

Distribution. BRAZIL, Ubatuba, São Paulo State, (23◦30.468' S, 45◦11.923' W)
Elevation: 0 m a.s.l.
Habitats. Few larvae found on leaf litter in shallow-water streams flowing over granite outcrops.
Known stages: L, P, M.
References. Silva *et al*. 2012.
Remarks. In this study *H. caissara* was found on marginal rocks of a small stream, at 200 m a.s.l. extending slightly the altitudinal records of this species. Environmental characterization: Water temperature 21 ºC; dissolved oxygen 7.9 mg.l^{-1}; pH 5.5; fast flowing; sparse vegetal canopy (less than 30% covered). The species was found in PESM (São Paulo State). Median altitudinal specificity (IndVal: 0.33; p=0.13).

Unknown species: *Hudsonimyia* sp.1. Locality: PESM. Altitudinal record: 1080 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p=0.85).

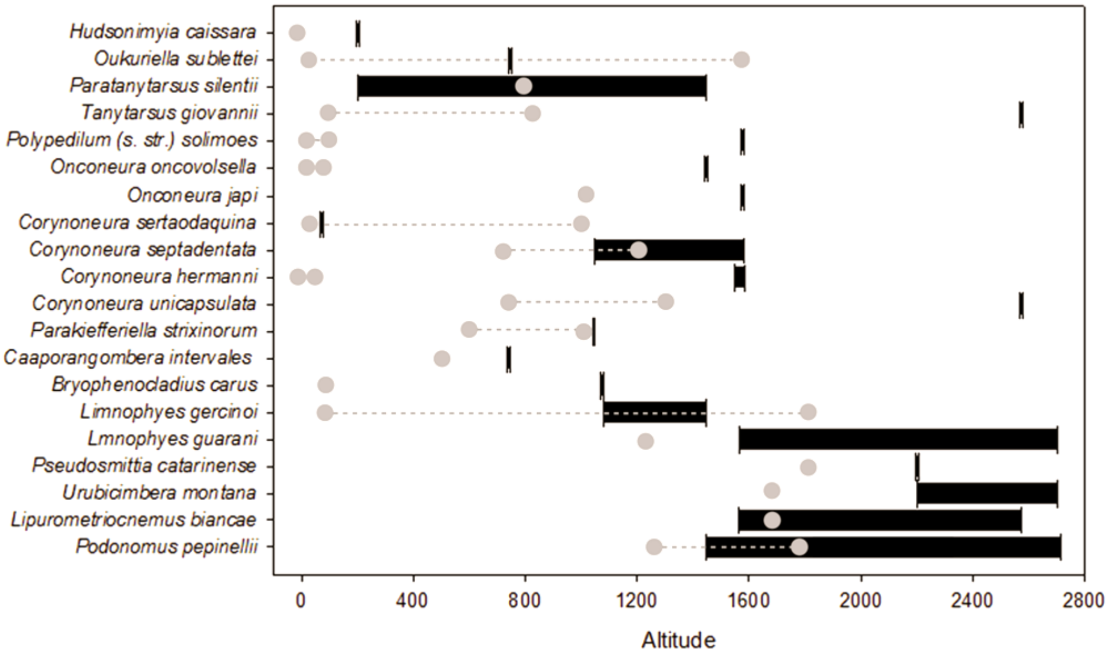

Figure 1. Previous altitudinal records (from literature) – in gray – and altitudinal records from this research – in black - of each species found in madicolous habitats in the present study.

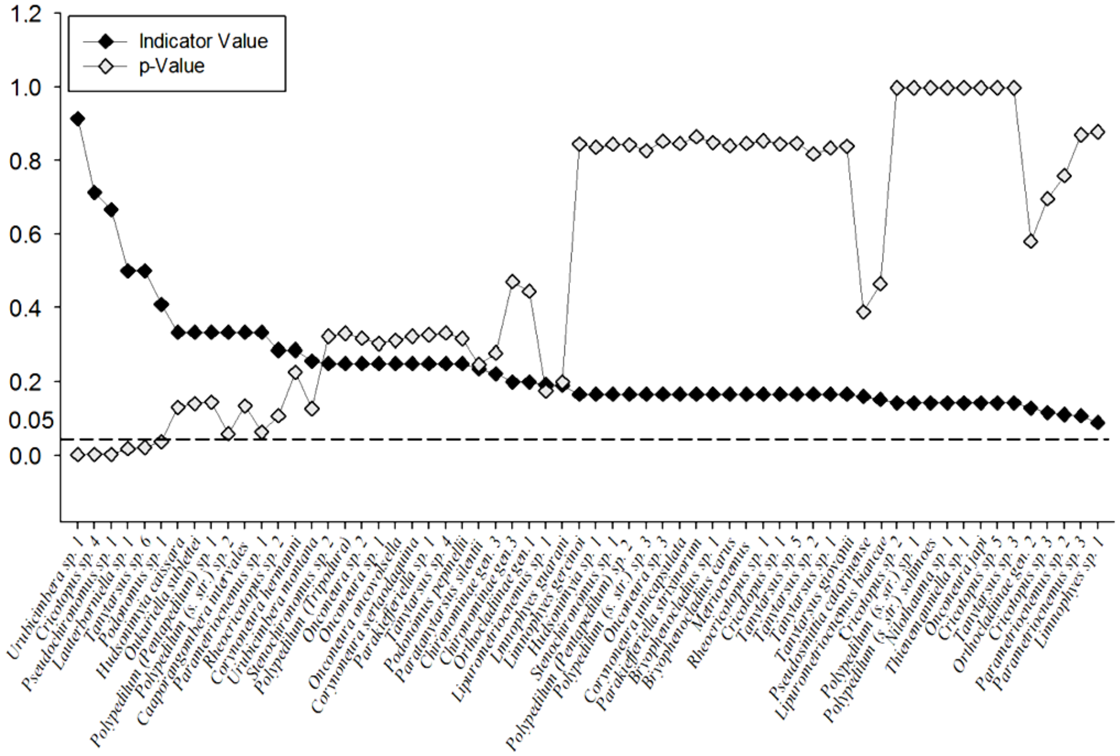

Figure 2. Indicator and fidelity values obtained for each species and morphospecies found in the present study.

Discussion

Different from other aquatic water bodies whose Chironomidae fauna have already been quite investigated in Atlantic Forest (Henriques-Oliveira *et al*. 2003; Roque *et al*. 2007; Silveira *et al.* 2015), madicolous habitats have never been formally studied, and therefore, their inhabitants were completely obscure. Here, a remarkable diversity of Chironomidae living in madicolous habitats were revealed, putting in evidence 62 species, of which only 20 were known to science. It means that about 69% of the species are probably new, what confirms that much less attention has been given on the study of semi-aquatic and terrestrial species. And despite this, evidences from chironomids preserved in ambar reveals that semi-aquatic and terrestrial life-styles were common since late Eocene (about 40 million years ago) (Zelentsov *et al*. 2012), raising the importance of madicolous habitats on the evolutionary history of many Chironomidae taxa.

The community was predominantly composed by Orthocladiinae subfamily (37 spp.), followed by Chironominae (22 spp), Podonominae and Tanypodinae (2 spp each). Within all Chironomidae subfamily, Orthocladiinae harbors the highest number of semi-aquatic species of midges (Andersen *et al.* 2010), what explains their notable richness in madicolous habitats. Orthocladiinae was also predominant within madicolous Chironomidae found in Southern Ontario (Sinclair & Marshall 1987), representing 10 of 14 genera recorded, and 5 of them were also recorded in this study: *Parakiefferiella, Metriocnemus, Parametriocnemus, Thienemanniella* and *Limnophyes,* demonstrating that these genera can be well-adapted to this habitat.

Only two of the species verified here were previously known to occur in madicolous habitats (*Podonomus pepinellii* and *Limnophyes guarani*). *Podonomus* larvae are predominantly found in streams and other fast flowing running waters, but it is not uncommon to see them exposed in the edge of streams (Brundin 1966). *Podonomus pepinellii* and all its morphotypes found in the highlands of Atlantic Forest are associate with madicolous habitas (Trivinho-Strixino *et al.* 2012), occurring in rocky seepages and in shoreline of streams. Most of the remaining species were considered stream-dweller, although some have been found in habitats probably very close to madicolous, such as those from Corynoneurini tribe and *Hudsonimyia caissara.* The larvae of *Hudsonimyia caissara* were originally found in low abundance (two specimens) in leaf litter of a mountain stream (Silva *et al*. 2012), possibly an inhabitant of the stones in the stream's edge. Further, it is very plausible that some stream-dweller species can tolerate both conditions: living on the main channel or in the edges of stream. This ability demonstrated by some species (Vaillant 1955; Sinclair & Marshall 1987) indicates that a richer fauna is expected to occur in marginal stream rocks comparing to isolate seepages.

It is not surprising that members of *Hudsonimyia, Bryophaenocladius, Metriocnemus, Limnophyes* and *Pseudosmittia* have been found in madicolous habitats during this study. These genera are known to have larval instars associated with semi-aquatic and terrestrial conditions: Roback (1979) was the first to verify *Hudsonimyia* larvae living on a thin layer of current water with periphyton and moss; species of *Metriocnemus* exhibit an extremely broad range of habitats within Chironomidae (Cranston & Judd 1987), but one species *M. hygropetricus* Kieffer received this name after being found in natural rock seepages as well as in artificial madicoulous habitats; most of *Limnophyes* larvae are semiaquatic (Saether 1990), and recently a new species of this genus – *Limnophyes guarani* Pinho & Andersen, 2015 - has been recorded on madicolous habitats in South of Brazil. The new records verified in this study, confirm the association of *Limnophyes guarani* with this habitat; *Pseudosmittia* larvae are largely terrestrial or semi-terrestrial, however this was the first time that a *Pseudosmittia* species from Neotropical region have been recorded in madicolous habitat.

Although many species were expected to occur in this research, other was completely intriguing, such as species of *Stenochironomus* and *Oukuriella*. Both genera are known to be highly habitat-specialized, the first as a vegetal miner (Epler *et al*. 2013) and the second in association with sponge or wood debris, although the habitat of basal groups in the phylogeny of *Oukuriella* could not be defined (Fusari *et al*. 2014). *Oukuriella sublettei*, recorded in this study, has recently been associated to submerged wood found in first order streams with rocky beds (Bellodi *et al*. 2016). Possibly, their presence in the marginal rocks of the stream was accidental, once only one specimen has been found. The same is expected for both *Stenochironomus* spp. found. Their reduced size indicates that they are possibly leaf miners, so they probably emerged from vegetal detritus that was on the rock by chance, where the emergence trap has been installed. Anyway, the emergence of these taxa in such conditions is an interesting report once they were able to complete their development in a thin layer of water, contrary to previous observations, when they used to stay submerged in the streams.

Once the procedure of rearing immature specimens to obtain the adults are most of the time unsuccessful due to their nature requirements (Ekrem *et al.* 2007), descriptions are frequently based only on adults collected with methods that preclude the knowledge of immature' habitat. Some species recorded in this study are only known by the adults, who were previously sampled with malaise or light traps, such was the case of *Lipurometriocnemus biancae*, *Urubicimbera montana*, *Pseudosmittia catarinense*, *Caaporangombera intervales* and *Paratanytarsus silentii* (although the immature of *P. silentii* have already been recognized in the current material, and descriptions are given on chapter 3). Through the use of emergence traps we can assure that the immature of these and the other species found in this study are madicolous inhabitants, what represents a significant advance on the ecological knowledge of the species. Although, the association and description of the immature is still necessary, and should be done with the help of molecular tools, such as DNA barcode. Further, even for those species which immature are known, and have been collected in usually explored habitats (e.g. streams, lakes and reservoirs), the first record of them in madicolous habitats, such a peculiar environment, represents a remarkable note on their success in colonizing a wide range of habitats.

The altitudinal range of 16 known species has been extended with the new records provided by this research. Some species seems to tolerate a wide altitudinal range, such as *Limnophyes gercinoi,* while other presented a narrow range, e.g. *Hudsonimyia caissara.* The altitudinal range is somewhat related to the extent of the geographical distribution of each species, therefore, species widely distributed are expected to occur in a wider range of altitude comparing to those with limited distribution. The indicator's analysis evidenced that all species significantly associated with their respective altitudinal band are unknown to science. *Urubicimbera* sp. 1, and *Podonomus* sp. 1, represented significantly the highest sites in this study (> 2600 m a.s.l.); *Pseudochironomus* sp. 1 was a significant indicator of the following altitudinal band (about 2500 m a.s.l.); *Tanytarsus* sp. 6, *Lauterborniella* sp. 1 and *Cricotopus* sp. 4 were significant indicators of 1500, 1100 and 200 m a.s.l., respectively. Regarding the narrow altitudinal range performed by these unknown species, it seems quite reasonable that they should present a high endemicity degree.

The locality with higher altitudes (APASM) demonstrated a higher number of species. 31% of the possible new species occurred above 2.100 m a.s.l. which highlights the gap existent on the taxonomic knowledge of the mountaintops' fauna. This is due to the low accessibility to these areas, which also hampers the sampling strategies. Nevertheless, the study in mountain region is urgently needed, especially when we are leading with one of the most threatened biomes in the world (Ribeiro *et al*. 2009). Mountains have been suffering from several types of environmental impacts, but the most alarming today is the climate change (Burke 2003). Current forecasts suggest that rainfall will be less constant and temperature will raise 2.0 to 6.0 º C by 2100 (Garcia et al., 2014), strongly affecting the flora and fauna, in special those living in small water bodies with high exposure to environmental pressure. Once we know that mountain species present a narrow range of tolerance to environmental conditions, and many of them are rare and endemic, thus very sensitive to environmental changes, the conservation of these areas are unquestionable.

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Two new species of *Podonomus* (Diptera: Chironomidae: Podonominae) from mountains of southeastern Brazil

Abstract

Two new species of *Podonomus* from Southeastern Brazil are revealed. The adult and immature stages of *Podonomus* **sp. n. 1** and the male adult and the pupa of *Podonomus* **sp. n. 2** were described based in specimens collected from mountaintops. DNA barcoding of *Podonomus* **sp. n. 1** was analyzed to investigate the molecular divergence of *Podonomus* species in Brazil, and as a tool to associate different development stages and sexes. The immature stages of *Podonomus* **sp. n. 1** and *Podonomus* **sp. n. 2** are madiculous inhabitants.

Keywords: Podonominae, madicolous, Atlantic Forest, Serra da Mantiqueira, Neotropical region

Introduction

The distribution of Podonominae is deeply marked by disjunction and bipolarity involving north and south hemisphere. Most of the known species are restricted to South America, which harbours the largest diversity of Podonominae found in the Austral portion of the globe (Brundin 1966). *Podonomus* is one of the richest genera (Ashe & O'Connor, 2009) with 42 valid species, 37 recorded in the Neotropical region.

Thanks to the extensive study developed by Brundin (1996), most of the current known Podonomus' species came to light. Brundin was able to find 38 new species in his monography, describing 29 of them, almost all recorded in the south region of South America, especially Patagonia. After Brundin's monography, only a few studies have incremented our knowledge on Neotropical *Podonomus*, including two species described from Equador (Roback, 1970), one species from Brazil (Roque & Trivinho-

Strixino, 2004, Trivinho-Strixino *et al*. 2012) and two from Argentina (Siri & Donato, 2012).

Podonomus, as other Podonominae genera, are commonly cold-stenothermic organisms, and their occurrence in tropical areas is typically constrained by the altitude, with the majority of species restricted to high mountains (Brundin, 1966; Roback, 1970). Until the present paper, the only recognized species from Brazil was *Podonomus pepinellii* (Roque & Trivinho-Strixino, 2004), recorded in every mountain investigated in the southeastern part of the country above 1200 m a.s.l. (Trivinho-Strixino *et al*. 2012).

Although Brazilian mountains do not present a remarkable elevation range as do the Andes Mountains (about 7000 m a.s.l. in Aconcagua), most of the higher mountains reaches more than 2000 m a.s.l., allowing the existence of more than one *Podonomus* species (Trivinho-Strixino *et al*. 2012).

Previous investigations on Chironomidae in Brazilian mountains have demonstrated two important facts about the distribution of Podonominae: the first is that *P. pepinellii* Roque & Trivinho-Strixino is a widely distributed species, occurring from south to the northeastern mountains of Brazil; and the second is that there are possibly more than one species occurring in those mountains (Trivinho-Strixino *et al*. 2012). DNA barcoding analyses have indicated the existence of at least two other *Podonomus* species in the Southeastern mountains (Trivinho-Strixino *et al*. 2012), however because the DNA extraction was only based on larval tissues, further morphological scrutiny of the specimens did not show sufficient disparity to differentiate the species. In this current paper we were able to find the pupae, female and male of at least one of the unknown species revealed by Trivinho-Strixino *et al*. (2012), and then look for morphological evidences in adults and pupae.

Here we provide the description of two new species of *Podonomus* which were found in the highest mountain ranges of Southeastern Brazil, in the Atlantic Forest domain.

Methods and terminology

The examined material is resultant from several field expeditions to southeastern mountains of Brazil. All mountains are situated in the Atlantic Forest domain, however the specimens were collected from sites located at the top of these mountains, where "Campos de Altitude" vegetation is predominant. This vegetation is characterized by grass and shrub formations, similar to the Andean Páramos (Safford, 2007). Sampling sites were characterized by thin layers of groundwater that overflows in granitic substrate, and also marginal rocks in splashing areas of small streams, called hygropretric or madiculous biotopes (Vaillant, 1956).

The adults were collected with emergence traps specifically adapted for madicolous habitats (Shimabukuro *et al*. 2015). The traps stayed in the field for seven days. Immatures and exuviae that remained in the substratum were also collected. All specimens were preserved in ethanol 98% to enable molecular analysis. DNA barcoding was used to enable association between life stages and to investigate the divergence among *Podonomus* species.

The specimens were mounted on slides with Euparal. The terminology follows Sæther (1977, 1980). Measurements are given as ranges. The number of specimens examined is given in parenthesis when it differs from the "n" stated at the beginning of description.

The material examined is deposited in the Reference Collection of the Laboratório de Ecologia de Insetos Aquáticos (LEIA) of the Universidade Federal de São Carlos (UFSCar), São Paulo State, Brazil.

For molecular analysis, we used legs of the adults and abdominal tissue of larvae removed from voucher specimens preserved in ethanol. DNA was extracted, amplified and sequenced following the protocols of the Canadian Centre for DNA Barcoding. Sequence information and detailed specimens records are available on Barcode of Life Database in the project files "Aquatic insects from Inselbergs in Brazil (AIIB)". Sequences have been submitted to GenBank. Kimura's two-parameter model of base substitution (Kimura, 1980) was used to calculate genetic distances in MEGA 5 software (Tamura *et al*. 2011) and NJ trees were produced by using BOLD and MEGA 5 software. The DNA barcoding methods are well covered by Trivinho-Strixino et al. (2012). We analyzed the same database containing 39 specimens and one additional specimen of P. pepinelli, plus 6 new specimens (Table 1).

Podonomus **sp. n. 1**

Type material. Holotype: male adult, Brazil, Minas Gerais State, Passa-Quatro, Serra da Mantiqueira, Pedra da Mina Mountain, 22˚25'51"S/44˚50'590"W, 2.700m a.s.l., 14.x.2014, madicolous habitat, leg. E.M. Shimabukuro, G. Perbiche-Neves & V.S. Saito (N1-29, LEIA-UFSCar). Paratypes: 3 males (N1-30-32, LEIA-UFSCar), 2 females (N1-33-34, LEIA-UFSCar), 3 pupal exuviae (N1-35-37, LEIA-UFSCar), and 1 pharate pupa with female (N1-38, LEIA-UFSCar), same data as holotype. Two males from Espirito Santo State, Serra do Caparaó, Pico da Bandeira, Vale Verde, 41°50′45.6″S/20° 25′ 12″W, 1276 m a.s.l. 18.ix.2012, leg. M. Pepinelli (N1-39-40, LEIA-UFSCar).

TABLE 1. List of specimens of *Podonomus* with mtDNA COI sequences (DNA Barcoding) from Passa-Quatro, Minas Gerais, in Southeast Brazil.

Taxon	Locality	Collection Reference	Coordinates	Altitute m a.s.l.	GenBank accession numbers
Podonomus **sp. n. 1**	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 24/Oct/2014, Shimabukuro, E. M.	MPBIM-291	22°25'41"S 44°50'38"W	2575	
Podonomus **sp.n. 1**	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 29/Jul/2014, Shimabukuro, E. M.	MPBIM-330	22°46'50"S 43°23'06"W	1581	
Podonomus pepinellii	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 24/Oct/2014, Shimabukuro, E. M.	MPBIM-289	22°25'41"S 44°50'38"W	2575	
Podonomus pepinellii	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 24/Oct/2014, Shimabukuro, E. M.	MPBIM-305	22°25'51"S 44°50'59"W	2698	
Podonomus pepinellii	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 24/Oct/2014, Shimabukuro, E. M.	MPBIM-307	22°25'51"S 44°50'59"W	2698	
Podonomus pepinellii	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 24/Oct/2014, Shimabukuro, E. M.	MPBIM-309	22°25'51"S 44°50'59"W	2698	

Diagnostic characters. The new species can be separated from other *Podonomus* species by combination of the following characters. Male. Presence of a large and rounded swell entirely covered by microtrichia in the subapical region of the gonostylus. Dorsomedian surface of gonocoxite with a group of stout setae. Female. Posterior margin of cerci obliquely cut, presenting 4 setae in the distal extremity of cercus: two large setae in the outer lobe and the inner lobe with upper larger setae and lower smaller setae. Pupae. Small size, less than 2 mm. Lateral processes of segments III and IV curved. Large ventral lamella on segment II. Five wavy setae on each side of segment IX. Long plastron plate with proximal margin beginning in the middle of respiratory atrium. Respiratory atrium with a lateral margin strongly curved distally.

Descriptions.

Male (n=6). Total length 1.18–1.38 mm. Wing length 1.63–1.70 mm. Total length/wing length 0.7–0.86. Wing length/length of profemur 2.2–3.0.

Colouration: Black; wings light brown; legs uniformly dark brown

Head. Antenna with 14 flagellomeres. AR 0.43. Presence of scapal setae 62–66 μm (3) on pedicel, and a strong seta under pedicel, on tentorium, 62–77 µm. Last flagellomere (1) 61.5 µm; XIII flagellomere (1) 169.2 µm. Temporal setae 12–21. Clypeus with 2–7 setae. Palp segment lengths (II-V) 28–62, 94–125, 72–81, 66–94. Third palpomere with 3–4 sensilla clavata (3) 26–38 μm long. Eyes bare.

Thorax. 16–25 acrostichals in mid scutum, 11–24 dorsocentrals, 7–17 antepronotals, 3–6 prealars, 1–3 supraalars, scutellum with 3–6 setae, 2–4 preescutelars.

Wing: Membrane with a patch of perpendicular setae on r cell. VR 0.89–0.92 (2). Costal extension (4) 90.7–126.1 μm long. r_1 cell (4) 77–86 μm wide, r_{4+5} cell (4) 112–132 μm wide. R with 9–14 setae, R_1 with 1–6, R_{4+5} with 0–3 setae. Brachiolum and squama with 3–5 setae each.

Legs. Spur of fore tibia 46–49 μm long, spurs of mid tibia 26–31 μm and 31–46 μm long, spurs of hind tibia 31–41 μm and 77–89 μm long. Comb (4) with 10–13 setae. Lengths and proportions of legs as in Table 2.

TABLE 2. Lengths (in μm) of legs of *Podonomus* **sp. n. 1**, male (n = 6).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	569–744	615–731	313–406	169–246	92–107	62–76	44–62	0.43–0.63
II	646–800	630–700	292–323	138–184	61–92	44–62	46–61	0.42–049
III	707–794	784–863	310–446	181–246	75–123	46–62	37–87	0.37–0.53

Hypopygium (Figure 1a). Gonocoxite 127–161 μm long with a group of stout setae in dorsomedian extension, and strong setae externally. Phallapodeme (4) 52–89 μm long; transverse sternapodeme (3) 50–59 μm long. Gonostylus 46–58 μm long (Figure 1b). True subapical lobe absent, only a large swell covered by microtrichia present; P, x and y setae not conspicuous; long apical lobe 12–15 μm with a t setae of normal type 13–25 μm.

Female (n = 2). Total length 1.2–1.4 mm. Wing length 1.6–1.7 mm. Total length/wing length 0.71–0.85. Wing length/length of profemur 2.9–3.5.

Colouration: Black; wings light brown; legs uniformly dark brown.

Head. Antenna with 9 flagellomeres (Figure 1c), last flagellomere 92 µm; I to VIII flagellomeres 261–277 μm. Temporal setae 7–8. Clypeus with 3 setae. Palp segment lengths (II–V): 26–31, 43–94, 35–57, 37–46. Third palpomere with 2–3 sensilla clavata in apical third; 26–28 μm long. Eyes bare.

Thorax. 20–26 acrostichals, 3 antepronotals, 24–27 dorsocentrals, 21–24 prealars, 1 supraalar; scutellum with 7–8 setae, preescutelars 4–5 setae.

Wing (Figure 1d). Costal extension 66–77 μm long. R with 15–17 setae. R_1 distally swollen with approximately 30 setae. R_{4+5} with 23–32 setae. Brachiolum with 2 setae and squama with 3–4 setae. r_1 cell 118–125 μm wide. r_{4+5} cell 142–155 μm wide.

Legs. Spur of fore tibia 43 μm long, spurs of mid tibia 26–31 and 35–38 μm long, spurs of hind tibia 37–38 μm and 75–80 μm long. Long setae on outer margin of hind tibia 108–129 μm. Comb with 8–9 setae. Lengths and proportions of legs as in Table 3.

TABLE 3. Lengths (in μm) of legs of *Podonomus* **sp. n**. **1**, female (n = 2).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	492–569	523–554	231–292	123–169	77	46	77–62	0.43–0.56
II	477–600	477–523	277–292	138–185	77–92	46–62	62–77	0.53–0.61
III	646–677	677–682	277–292	169–185	92	46	62	0.41–0.42

Genitalia (Figure 1e). Cercus 108–117 µm long, with 4 setae in the apex. Postgenital plate 86–108 µm long. Seminal capsules 31–32 µm long. Notum 98–111 µm long.

Pupa (n = 3). Total length 1,7 (1) mm. Exuviae brownish.

Cephalothorax (Figure 2a). Frontal apotome (Figure 2d). Thoracic horn (Figure 2e-f) 343–364 µm long and 184–195 µm wide. Plastron plate triangular 231–249 µm long, atrium 197–231 µm long. Shorter margin of respiratory atrium with distal portion strongly curved. Dc1 23–49 µm long, Dc2 (2) 15–33 µm long, Dc3 (1) 35 µm long. Sa (x) 7–24 µm long. Longest median antepronotal 31–52 µm long. Lateral antepronotal 29–63 µm long. Precorneals 5 µm long. Distance between Dc1 and Dc2 18–30 µm, between Dc2 and Dc3 17–29 µm, between Dc3 and Sa 183–207 µm (Figure 2a).

Abdomen (Figures 2b). Ventral lamella present. Segment II to VIII with lateral processes. III and IV segments with lateral processes slightly curved. Tergite I–II bare, T III–VIII with fine and sparse spinules, without coarse shagreen. T IX with relative coarse anterior spinules. Sternite I–II bare; S III–VIII with fine, sparse spinules; S IX with anterior spinules. 6–9 lateral wavy setae on segment VIII, lengths (in µm) of L1 to L7 as: 338–400, 338–346, 331–338, 338–374, 300–338, 292 (1), 331 (1). Anal lobe (Figure 2c) 215–226 µm long with 5 lateral wavy satae; macrosetae with distinct sizes, the smaller with 42 µm long, and the larger with 87 µm long. Genital sac of male (1) 238 µm reaching apex of anal lobe; presence of a large anal spur 9 µm.

Notes on distribution and biology of the species.

Podonomus **sp. n. 1** was found in madicolous habitats from high mountains in southeastern Brazil. The species was recorded in temporary films of water that percolates exposed rocks above 2700 m a.s.l. in Pedra da Mina Mountain. At the same locality, *Podonomus* **sp. n. 1** was found in marginal rocks of a first order stream, situated at 2500 m a.s.l., constantly wet by the water current. In Pico da Bandeira (Serra do Caparaó), *Podonomus* **sp. n. 1** was found in the marginal bedrock on the side of the main water channel of Rio Caparaó. Podonomus were collected at 1270 m a.s.l., however it is important to mention we only collected a few specimens in that altitude, and Rio Caparaó headwater is located at Pico do Calçado about 2800 m a.s.l.

The interesting aspect involving the distribution of *Podonomus* **sp. n. 1** is that the species occurs precisely at the two highest mountains from the southeast: Pico da Bandeira (2892 m a.s.l.) and Pedra da Mina (2798 m a.s.l.), the third and the fourth highest mountains from Brazil, respectively. Thus the elevation may have some influence on the distribution of this species.

Taxonomic discussion.

The male hypopygium of *Podonomus* **sp. n. 1** is very similar to that of *Podonomus albinervis* Edwards, both presenting a pronounced subapical swelling covered with microtrichia, and gonocoxite with an excavated area dorsomedially. However, they differ by the presence of a group of strong setae in the dorsomedial excavated region in *Podonomus* **sp. n. 1** gonocoxite. In addition, *P. albinervis* present a whitish area that crosses the middle of the wing, which is not observed in *Podonomus* **sp. n. 1.** *Podonomus* **sp. n. 1** also shares many similarities with *Podonomus setosus* Brundin and *P. caranqui* Roback, especially by the presence of those conspicuous group of stout setae in the gonocoxite, but differ from the former by the presence of a notable rounded swelling in the gonostylus, and from *P. caranqui* by the presence of microtrichia covering the swelling.

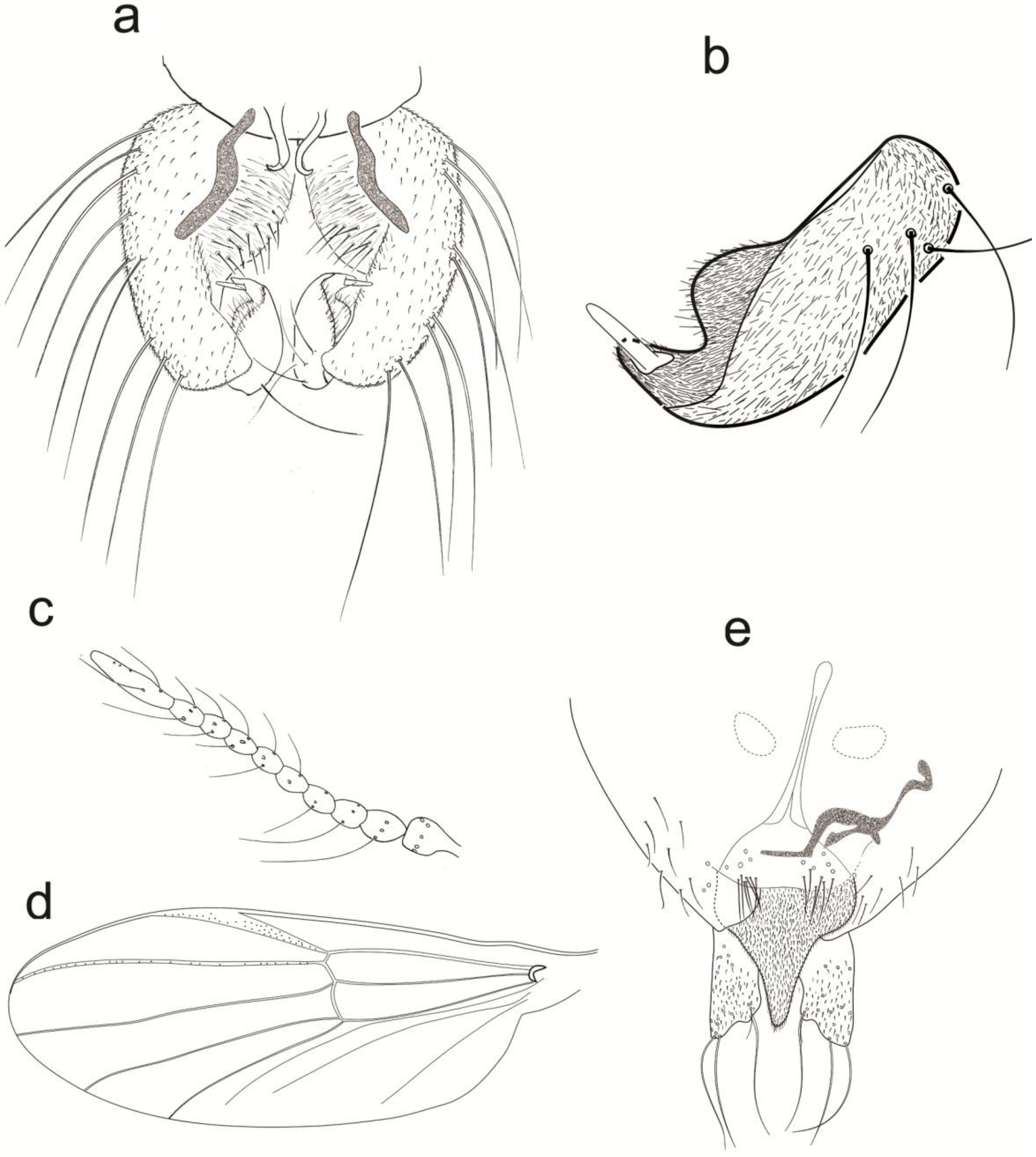


FIGURE 1. a-e. *Podonomus* **sp. n**. **1**. a-b. male. a. hypopygium. b. gonostylus. c-e. female. c. antenna. d. wing. e. genitalia.

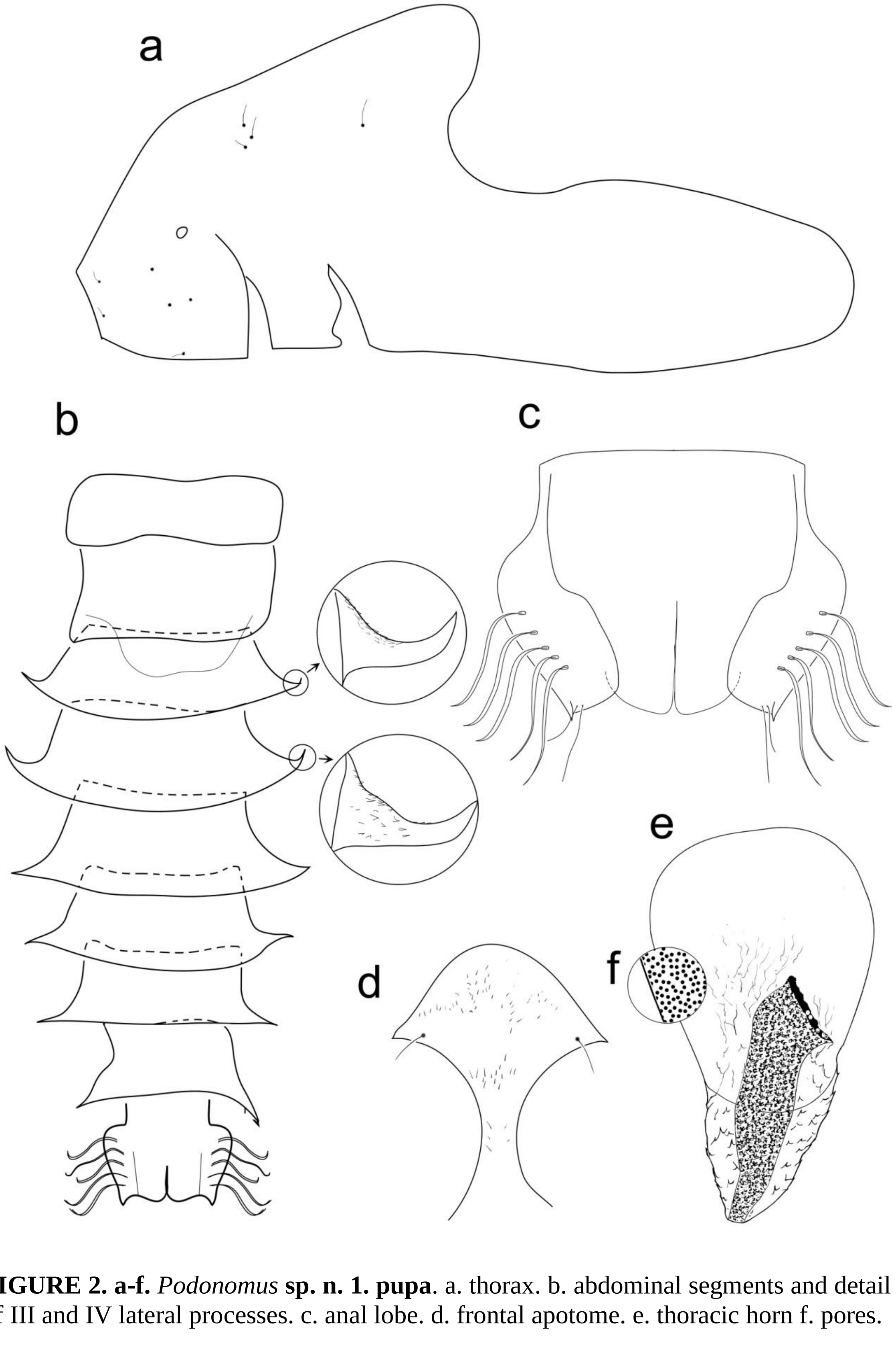


FIGURE 2. a-f. ***Podonomus*** **sp. n. 1. pupa**. a. thorax. b. abdominal segments and detail of III and IV lateral processes. c. anal lobe. d. frontal apotome. e. thoracic horn f. pores.

Although most of *Podonomus* spp females are superficially described, the female of *Podonomus* **sp. n. 1** is similar to *P. pepinellii* and *P. besti* Brundin, differing from the former by the number of setae in R_{4+5} and by the distal shape of cerci, which is obliquely cut in the *Podonomus* **sp. n. 1**, and from the last by the number segments in antenna, once *P. besti* presents 11 flagellomeres while *Podonomus* **sp. n. 1** has only 9.

The pupa of *Podonomus* **sp. n. 1** resembles *P. pepinellii* as both present a large and rounded plastron plate with small pores closely arranged. *P. pepinellii* also presents a lamella on SII and 5 wavy setae in the anal segment. Nonetheless *Podonomus* **sp. n. 1** can be separated from *P pepinellii* by the following characteristics: presence of slightly curved lateral processes on III and IV segments; proximal margin of plastron plate starting in the middle of respiratory atrium; shorter border of respiratory atrium with a distal portion strongly curved.

Podonomus **sp. n. 1** do not fit perfectly in any of the five groups created by Brundin. Although the gonostylus of the male is similar to those found in *albinervis* group, without a true subapical lobe, and presenting only a swelling covered by microtrichia, the size of the swelling seems to be more prominent than in the other species of the group, except *P. caranqui*. This fact would transfer the species for the *decarthrus* group, although species in *decarthrus* group never have the swelling quite covered with microtrichia. On the other hand, the female features seem to fit well in the *decarthrus* group, once the posterior margin of the cerci is obliquely cut and deeply sinuous, also presenting 4 setae apically. Finally, the suspicion that *Podonomus* **sp. n. 1** belongs to the *decarthrus* group was also not supported by pupal characters, especially diverging in the number of wavy setae on abdominal segment IX (only five in *Podonomu* **sp. n. 1**) and the presence of a large lamella in the segment II.

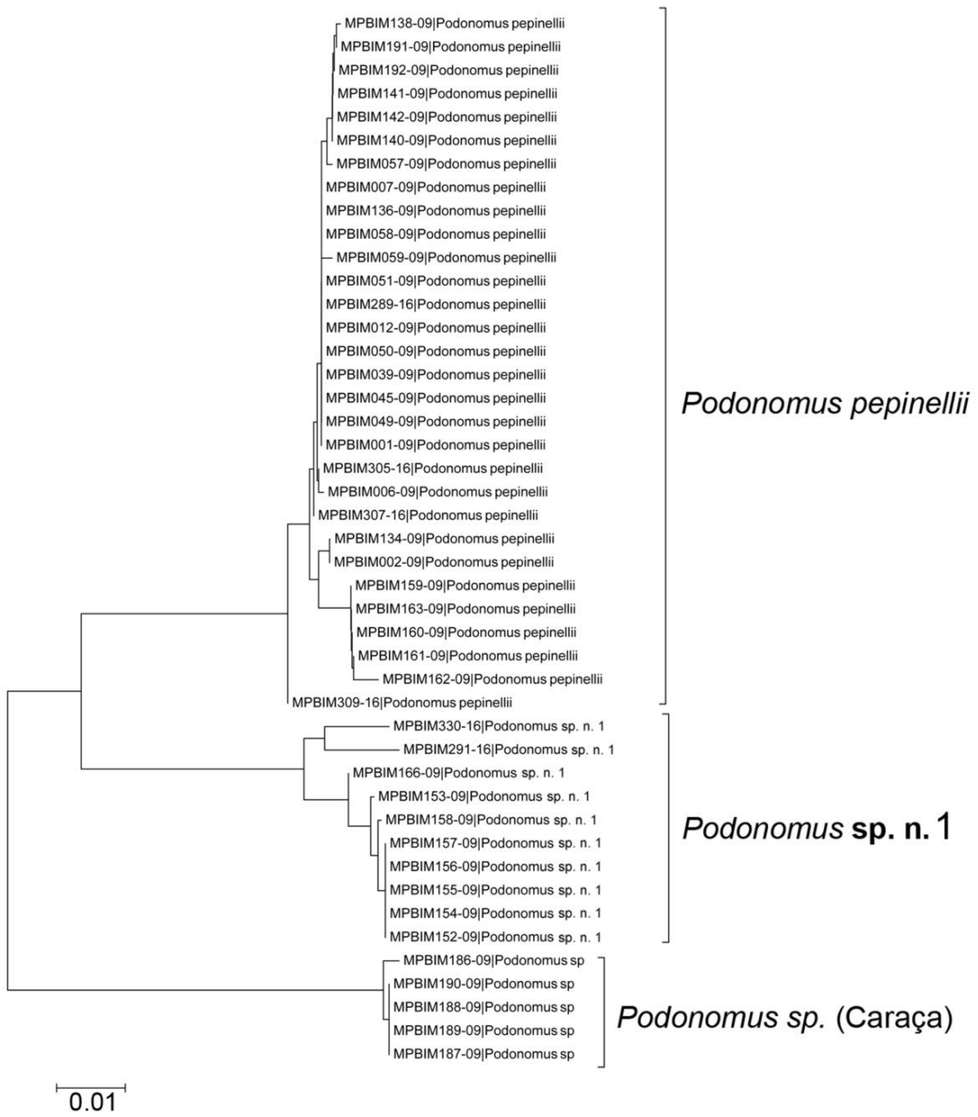
DNA Barcoding

Analysis of DNA barcodes have been proving effective for the identification of *Podonomus* species in Brazil (Trivinho-Strixino *et al*. 2012). The six new sequences combined with a previously published dataset on *Podonomus* specimens (Trivinho-Strixino *et al*. 2012) confirmed that *Podonomus* **sp. n. 1** is molecularly distinct from *P. pepinellii* and *Podonomus sp*. (Figure 3). Maximum intraspecific genetic divergence was 1.13% within *P. pepinellii* (n=27) species, 2.6% within *Podonomus* **sp. n. 1** (n=9) and 0.3% within *Podonomus sp*. (Caraça, n=5). Among the three distinct groups formed, the mean distance between *Podonomus* **sp. n. 1**and *P. pepinellii* was 8.0%, between *Podonomus* **sp. n. 1** and *Podonomus sp*. was 10.26% and between *P. pepinellii* and *Podonomus sp*. was 10.25%.

Podonomus sp. n. 2

Type material. Holotype: male adult with pupal exuvia, Brazil, Rio de Janeiro State, Itatiaia, Pico das Agulhas Negras, first–order stream/ madiculous habitat, 22°22′55.2″S/44°40′40.8″W, 2.419 m a.s.l. 10.vii.2012., leg. M. Pepinelli (N1-31, LEIA-UFSCar).

Diagnostic characters. The new species can be separated from other *Podonomus* species by the combination of the following characters. Male. Complete absence of subapical lobe and also without any trace of swelling. Gonostylus long with 66 µm and relatively slender, entirely covered by microtrichia. Antennal plume normally developed. Pupae: large ventral lamella on segment II. Five wavy setae on each side of segment IX. Lateral processes of all segments straight. Presenting two equal macrosetae in each side of the anal lobe. Presence of a large anal spur distally in anal lobe.



Fugure 3. Kimura 2-parameter neighbor joining tree of *Podonomus* species.

Descriptions.

Male (n = 1). Total length 1.28 mm. Wing length 1.18 mm. Total length/wing length. 1.08. Wing length/length of profemur 2.4.

Colouration: Black; wings light brown; legs uniformly dark brown

Head. Antenna with 14 flagellomeres, AR 0.4. Presence of scapal setae 46 µm and strong setae under pedicel 100 µm. Last flagellomere 46.15 µm; 13th flagellomere: 138.5 µm. Temporal setae 19. Clypeus with 4 setae. Palp segment lengths (II–V): 43, 92, 51, 63. Third palpomere with 4 sensilla clavata; 12–25 µm long. Eyes bare.

Thorax (Figure 4d). 21 acrostichals, 6 antepronotals, 28 dorsocentrals, 7–17 antepronotals 20 prealars, scutellum with 4 setae, 4 preescutelars.

Wing: Membrane with a patch of perpendicular setae on r cell. r_1 cell 83 µm wide, r_{4+5} cell 118 µm wide. R with 20 setae. R_1 with 2. R_{4+5} without setae. Brachiolum with 4 and squama with 5 setae.

Legs. Spur of fore tibia 42 µm long (Figure 4b.1). Spurs of mid tibia 23 µm and 27 µm long (Figure 4b.2). Spurs of hind tibia 36 µm and 69 µm long (Figure 4b.3). Long setae on tibia 160 µm long. Comb with 8 setae. Lengths and proportions of legs as in Table 4.

TABLE 4. Lengths (in µm) and proportions of legs of *Podonomus* **sp. n**. **2**, male (n=6).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	492	523	292	185	77	46	76	0.56
II	492	446	262	154	92	62	46	0.59
III	677	677	292	200	77	46	62	0.43

Hypopygium (Figure 4a). Gonocoxite 138 µm long. Phallapodeme 83 µm long; transverse sternapodeme 49 µm long. Gonostylus 66 µm long (Figure 4c); subapical lobe and swelling absent; P, x and y setae present but not conspicuous; short apical lobe 6 µm long, tooth (t setae) of normal type 12 µm.

Pupa (n = 1). Total length 1.84 mm. Exuviae brownish.

Cephalothorax. Frontal setae (Figure 4g). Thoracic horn (Figure 4h) 263 µm long and 162 µm wide. Plastron plate quadrangular 143 µm long, atrium 155 µm long. Dc1 35 µm long, Dc2 29 µm long, Dc3 42 µm long. Sa 11 µm long. Longest median antepronotal 28 µm long. Lateral antepronotal 22 µm long. Precorneals 5 µm long. Distance between Dc1 and Dc2 8 µm, between Dc2 and Dc3 58 µm, between Dc3 and Sa 129 µm.

Abdomen. Tergite I bare (Figure 4e), T II–VIII with fine and small spinules sparsely distributed. TII–TVII presenting two small patches of shagreen with coarse spinules. T

IX with anterior shagreen. Sternite I–II bare (Figure 4f); Presence of lamella on II segment (Figure 4f). S III–VIII with fine, sparse median spinules; S IX with anterior spinules. 7 lateral wavy setae in segment VIII. Lengths (in μm) of L1 to L7 as: 362, 355, 354, 354, 323, 292, 315 μm. Anal lobe 208 μm long with 5 lateral wavy setae (Figure 4i). Two macrosetae on lobo anal with similar size, 41.5 and 46.1 μm long. Anal spur 9 μm.

Notes on distribution and biology of the species.

Podonomus **sp. n. 2** is only known from the type–locality, a madicolous habitat located at 2400 m a.s.l. in Pico das Agulhas Negras. This is the third highest mountain in Southeastern of Brazil, after Pico das Bandeiras and Pedra da Mina, and the fourth highest in the country, reaching 2790 m a.s.l. in its summit.

Taxonomic discussion.

The male of *Podonomus* **sp. n. 2** resembles *P. inermis* Brundin, *P. nordenskjold* Brundin and *P. quito* Roback due to the complete absence of subapical lobe and swelling, and the elongated shape of the gonostylus. However, *Podonomus* **sp. n. 2** differs from *P. inermis* by the format of R4+5, which is not S–shaped, and by the markedly smaller size of the wing; the species also differs from *P. nordenskijold* by the normally developed antennal plume. The differentiation from *P. quito* is given by the size of the gonocoxite and gonostylus, which are longer in *Podonomus* **sp. n. 2** Once the *P. quito* male was described based on a pharate pupae, a detailed comparison is precluded by the lack of information on some structures, for example wings and legs. Analyzing legs size of the female, we suppose that the legs of *Podonomus* **sp. n. 2** may be distinctly longer than *P. quito*.

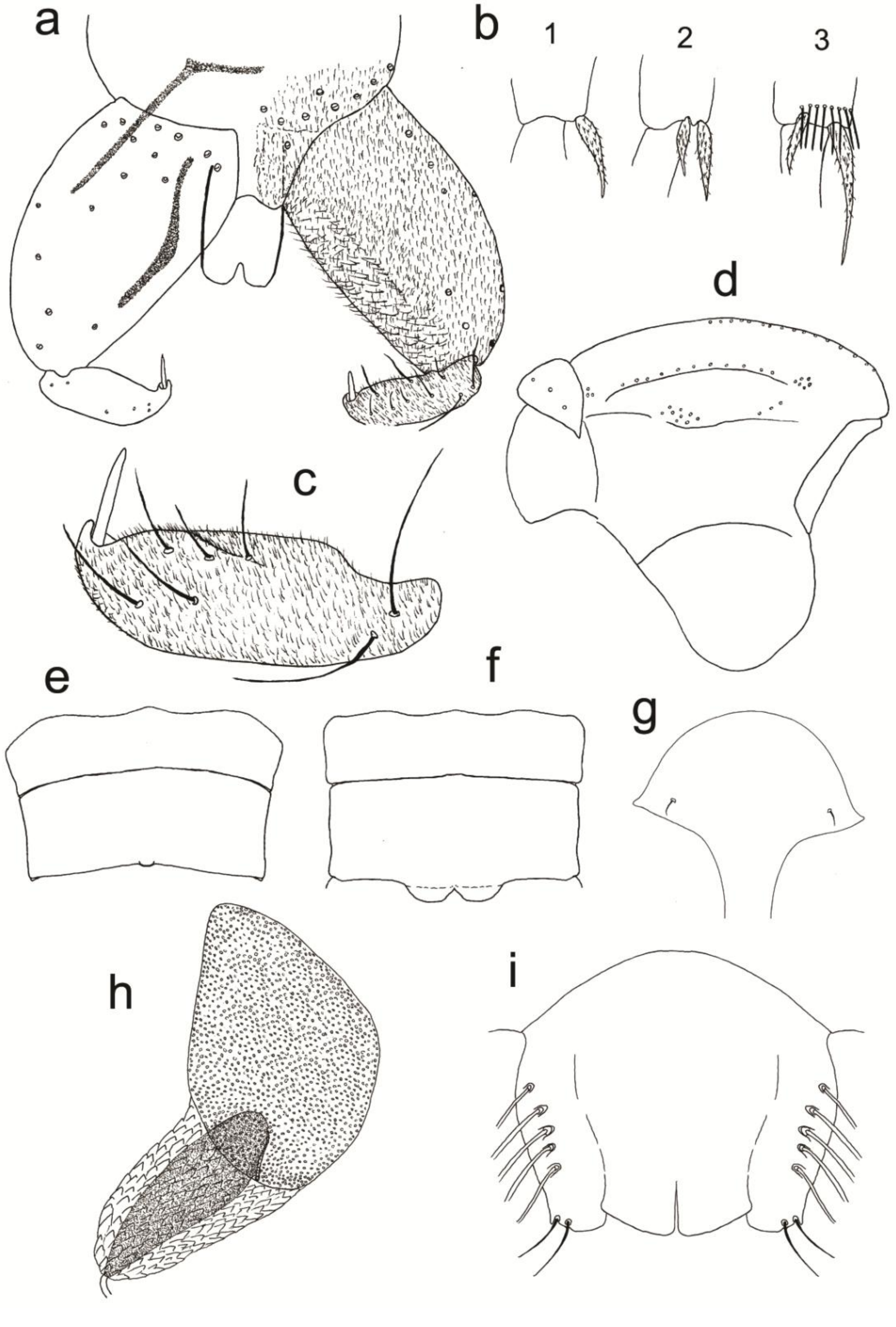


FIGURE 4.a-i. *Podonomus* **sp. n. 2.** a-d. **male adult.** a. hypopygium. b. tibial spurs. 1. front. 2. mid. 3. hind. c. gonostylus. d. lateral view of scutum. e-i. **pupa** e. tergites I–II. f. sternites I–II. g. frontal apotome h. thoracic horn i. anal lobe.

Taxonomic discussion.

On the other hand, the differences between *Podonomus* **sp. n. 2** and *P. quito* become evident when comparing the morphology of the pupa. *Podonomus* **sp. n. 2** presents a shorter and wider respiratory atrium, quadrangular plastron plate, and only 5 wavy setae on anal segment, while *P. quito* presents a thinner and larger respiratory atrium, approximately with the same size as the thoracic horn, an oval plastron plate, and 14 wavy setae on anal segment. Also, the pupa of *Podonomus* **sp. n. 2** is completely different from all the pupae described by Brundin, sharing more similarities with *P. pepinellii* and *Podonomus* **sp. n. 1** sp. n. It differs from *P. pepinellii* by the shape of plastron plate, which is conical in *P. pepinellii,* by equal–sized megasetas in lobo anal, and by the larger size of anal spur. The pupae of *Podonomus* **sp. n. 2** can be differentiated from *Podonomus* **sp. n. 1** because it does not show the abruptly curvature in the distal margin of the atrium.

Podonomus **sp. n. 2** also does not fit completely in any *Podonomus* group species created by Brundin. The male fits perfectly in the *albinervis* group, once it do not present any trace of swelling or subapical lobe in his gonostylus but this placement is discarded by morphological features of the pupae. The known pupae from *albinervis* group present 11–30 wavy setae, and *Podonomus* **sp. n. 2** presents only 5, and also the appearance of the cephalic horn is completely different from those observed in *albinervis* pupae.

Acknowledgements

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New species of the genus *Pseudochironomus* Malloch 1915 (Diptera: Chironomidae) from Brazilian highlands

Abstract

Four new species of *Pseudochironomus* Malloch were recorded in Brazilian highlands, three new species from Atlantic forest and one from Amazon Forest. Adult male and immature stages from *Pseudochironomus* **sp. n**. **1** and *Pseudochironomus* **sp. n. 2** and the adult male of *Pseudochironomus* **sp. n**. **3** and *Pseudochironomus* **sp. n. 4** are described and illustrated. Adult male and female and the larva of *Pseudochironomus* **sp. n**. **1** were linked using DNA barcode. The presence of thoracic acrosticals in the species described here is a remarkable characteristic that distinguishes this group of *Pseudochiromus* species from other species.

Key words: Pseudochironomini, Neotropical region, low-order streams, mountain, tropical forests.

Introduction

Most of the current known species of *Pseudochironomus* are concentrated in Neartic region, with about 13 described species (Saether, 1977). In South America, only two species have been previously recorded: *P. viridis* Kieffer (1925) from Argentina (Kieffer, 1925; Paggi & Rodriguez-Garay, 2015) and Peru (Roback, 1960), and recently, *P. richardsoni* Malloch, only known from Neartic region, have also been recorded in Argentina (Paggi & Rodriguez-Garay, 2015).

Although the existence of the genus *Pseudochironomus* in Brazil have been evidenced by the observation of larvae by many researchers (Suriano & Fonseca-Gessner, 2004; Trivinho-Strixino, 2011; Rosa et al. 2013), these is the first time that the species come to light through the examination of pupa and adults.

Pseudochironomus is a plesiomorphous genus among Chironomidae and is an inhabitant of a large variety of habitats that goes from littoral zone of oligotrophic lakes to large rivers (Saether, 1977). In South America, the immature stages of *Pseudochironomus* seem to be rather associated to well preserved streams, some in mountaneous regions (Paggi & Rodriguez-Garay, 2015; Suriano & Fonseca-Gessner, 2004).

In the present study, four new species of *Pseudochironomus* from Brazil are described, expanding the diversity of the genus for Neotropical region.

Material and Methods

The examined material was obtained from expeditions to some of Brazilian mountains. The new species here described were recorded in Southeast of Brazil, in preserved areas of the Atlantic Forest, and from the extreme North of the country, in the Amazon Forest.

The immature stages were sampled on marginal rocks (madicolous biotopes) and on main channel of low-order streams, with hand net and surber collectors. The adults were obtained with emergence traps (Shimabukuro et al., 2015) and with light traps. Specimens preserved in ethanol 98% were submitted to molecular analysis. DNA barcoding was used to enable the association among stages.

The specimens were mounted in slides with Euparal. The terminology follows Sæther (1977, 1980). Measurements are given as ranges. The number of specimens examined is given in parenthesis when it differs from the stated number at the beginning of description.

***Pseudochironomus* sp. n. 1**

Type material. Holotype male BRAZIL, MG, Passa-Quatro, Pedra da Mina Mountain, Vale do Ruah, first order stream, 22°24'697"S 44°50'930"W, 2575 m a.s.l., 14.x.2014, E.M. Shimabukuro, G. Perbiche-Neves & V.S. Saito (I1 – 15). Paratypes: three male (I1 – 16-18), three female (I1 – 19-21), three pupae exuviae (I1 – 22-23) and three larvae, same data as holotype, except for 5.viii.2013 (I1 – 24). Additional material examinated: one male, one female and 5 larvae, same data as holotype.

Diagnostic characters. Large species**.** Thorax with dark brown marks. The adult males are separable from the other species of the genus except *Pseudochironomus* **sp. n. 2**, *Pseudochironomus* **sp. n**. **3** and *Pseudochironomus* **sp. n. 4** by the presence of acrosticals. Tergite IX wide, with a notch, and overlaping approximately the half of inferior vorsella. Gonostylus broad and short. Median volsella enlarged and somewhat flattened, presenting one or more thin and long setae on it. Superior vorsella bare, but presenting only one long seta. Inferior vorsella foot-like. Pars ventralis completely divided. Presence of dark brown mark in tibial connection with femur.

Female. The female of *Pseudochironomus* **sp. n**. **1** is characterized by having dark brown marks on thorax, as male. 2–5 acrostichals; 8–9 antepronotals; 14–22 dorsocentrals; 6–7 prealars; and 29 scutellars; R4+5 with 21–36, absent proximally; and squama with 27–39 setae. Cerci 174–245 µm long.

Pupa. The exuvie of *Pseudochironomus* **sp. n**. **1** is remarkably darkened; it presents few and sparse spines on both tubercles of sternite I; tergite I bare. Thoracic horn 356–406 µm long. Median and posterior precorneals arising from an apparently tough protuberance. Anal lobe with 28-33 double fringe of taeniate setae.

Larva. Mentum, claws of posterior parapods, cephalic capsule and mandible teeth except apical tooth, very darkened. Mentum with 11 teeth. Five median teeth forming a concise and somewhat isolate group, the three median higher than the other teeth, and the second lateral very small and fused with the first lateral. Fifth and sixth lateral teeth flattened and fused, sometimes presenting a small fissure. Basal segment of antenna slightly smaller than premandible. AR=1.41–1.89.

Description. Male (n =4)

Total length 4.23–5.20 mm; wing length 3.00–3.34 mm; total length / wing length 1.27–1.61; wing length/ length of profemur 2.29–2.88.

Coloration: Tergite light brown with pale areas in the insertion of setae; sternite pale. Thorax pale but marked with dark brown. Wing hyaline with yellow veins. Femurs yellow, tibiae and tarsi all with a pale light brown coloration. All legs with a dark brown mark in tibial connection with femur.

Head. AR=0.30–0.34. Antenna length: 988–1000 µm. Last flagellomere length 737–769 (3); more than 20 temporal setae. Clypeus with 18–21 setae. Palpomere lengths (µm) 47–81; 81–113; 188–219, 250–278; 294–363. Third palpomere with a slight fingerlike process.

Thorax. Acrosticals*:* 5–9 (2); Antepronotals 8–10, small; dorsocentrals 13–18 in a single row, arising from pale areas; prealars 5–7; scutellum with 26–30 setae, irregularly distributed in double row.

Wing. Brachiolum with 1–3 setae, R with 15–20 setae, R1 with 5–9, R4+5 2–7 apical setae. Squama with 26–32 setae.

Legs. Spur of foretibia 59–72 µm long; spurs of midtibia 81–100 µm and 81–106 µm long; spurs of hind tibia 84–106 µm and 94–116 µm long. Length of legs and LR proportion as in Table 1.

Abdomen. Dorsally dark brown with pale circles in the base of setae, which are homogeneously distributed in all the segments. Ventrally pale, except segments VI and VII, which are similar to dorsal coloration pattern.

Hypopygium (Fig. 1a). Posterior margin of tergite IX with median notch with 20–22 setae (Fig.1c). Tergite IX broad and long, reaching the half of inferior volsella (Fig.1a). Laterosternite IX with 6–14 setae. Phallapodeme 100–112 µm long. Transverse sternapodeme 89–121 µm long. Superior volsella (Fig. 1b) 114–138 µm long curved, apically concave ending in two points, one 25 µm long and the other 9 µm long; inferior volsella foot shaped 89–97 µm long and completely hairy, with microtrichia and long setae; longest apical setae 46 µm long; median volsella 15–16 µm long bears 1–4 apical setae 26–40 µm, and 1 setae above median vorsella 31 µm long. All setae on median volsella long and thin. Gonocoxite 226–246 µm long. Gonostylus 145–208. HR = 1.1-1.6. Pars ventralis completely divided into two, 69–85 µm long and 9–17 µm wide lobes (Fig 1d).

TABLE 1. Lengths (in µm) of legs and LR proportion of *Pseudochironomus* **sp. n**. **1** (male, n = 4).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	1123–1307	1385–2615	1231–1492	585–661	492–569	369–446	215–277	0.57-0.93
II	1384–1569	1307–1507	692–753	353–384	276–323	184–199	138–153	0.49-0.53
III	1399–1584	1523–1723	908–1000	507–554	384–431	215–276	154–200	0.58-0.60

Female (n = 3)

Total length 4.5–5.5 mm. Wing length 3.1–3.5 mm.

Colour as male. Legs as male.

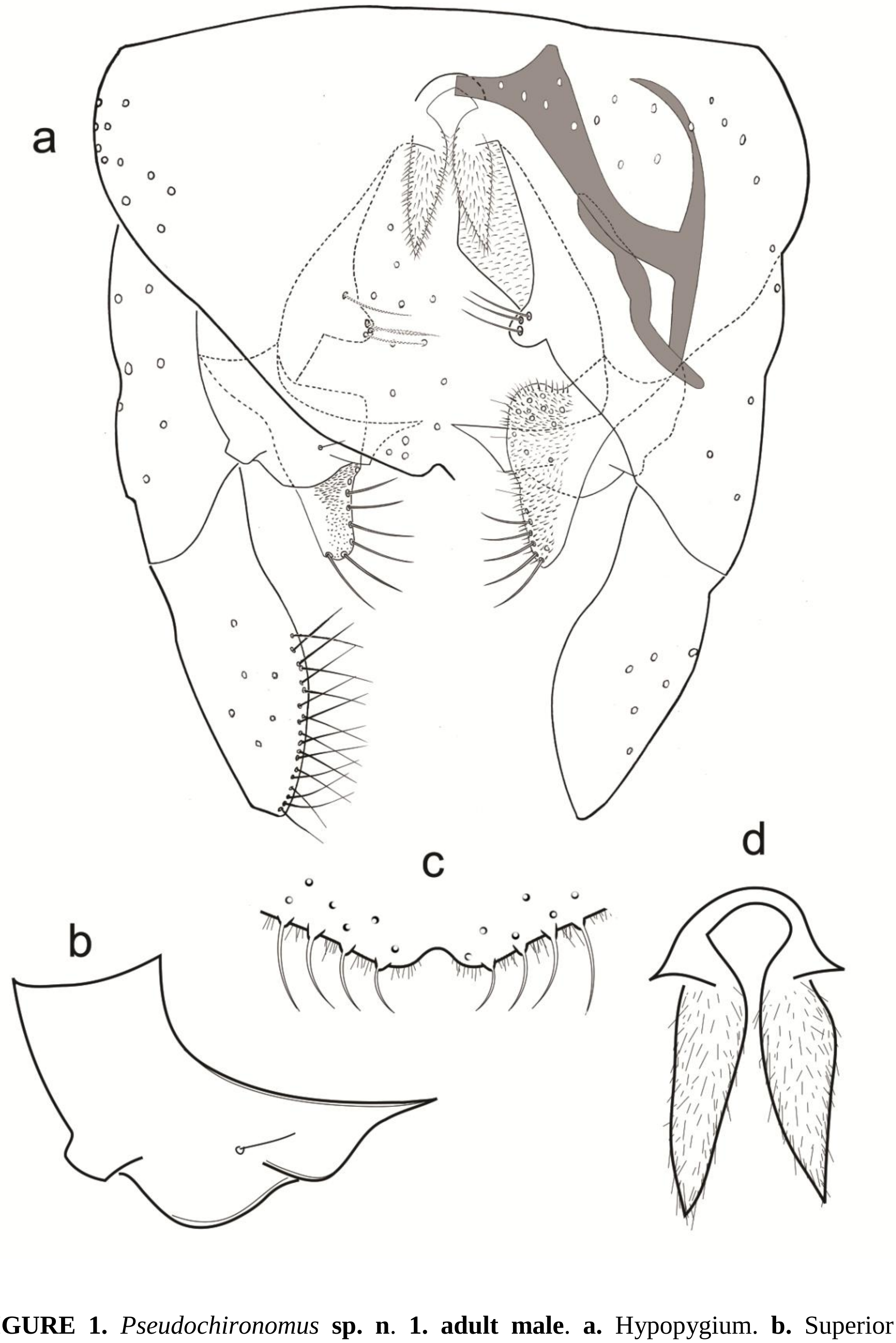

FIGURE 1. *Pseudochironomus* **sp. n**. **1. adult male**. **a.** Hypopygium. **b.** Superior volsella. **c.** Margen of tergite IX. **d.** Pars ventralis.

Head. Antenna length (µm): 403-447. AR 0.87–0.93. Last flagellomere 215–231 µm long. Temporals 30–32 (2). Clypeus with 18–23 setae. Length of palp segments 2–5 (µm): 94–112; 144–225; 250–269; 350–375 (1). Slightly fingerlike process on third palpomere.

Thorax chaetotaxy. Acrostichals 2–5, antepronotals 8–9, dorsocentrals 14–22, prealars 6–7, scutellars 29.

Wing. Brachiolum with 2 setae. R with 22–25; R1 with 11–15; R4+5 with 21–36, lacking sate proximally in R4+5. Squama with 27–39 setae.

Legs. Tibial spur lengths (µm) front 57–71; middle 86–93 and 92–105, hind 89–103 and 106–110. Length and LR proportion of legs as in Table 2.

Genitalia: Gca VIII strong, rounded posteriorly. S VIII bearing 24–28 setae irregularly distributed at each side. GPVIII 58–88 (2) µm long. Seminal capsule 128–146 µm. Notum 149–175 µm long. Gonocoxite IX 41–61 µm long. Postgenital plate 28–54 µm long. Cercus 174–245 µm long.

TABLE 2. Lengths (in µm) of legs and LR proportion of *Pseudochironomus* **sp. n**. **1** (female, n = 3).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	1123–1261	1461–1615	1215–1446	569–631	461–538	354–415	199–292	0.83-0.90
II	1384–1446	1369–1538	661–723	338–385	246–276	169–215	123–154	0.45-0.50
III	1399–1554	1538–1662	846–954	461–538	338–415	215–231	169–200	0.56-0.58

Exuvie (n = 3)

Total length 4.83–5.83 mm. Exuviae dark brown.

Cephalotorax. Frontal setae absent. Thoracic horn (Fig. 2f), 356–406 µm long, smooth. Three precorneals, median and posterior arising from a tough protuberance (Fig. 2f); anterior precorneal 131–189 µm, median 192–230 µm, posterior 65–111 µm long; 2

antepronotals, one with 172–223 µm and the other with 194–265 µm, 4 dorsocentrals, 1st 69–92 µm, 2nd 54–155 µm, 3rd 108–123 µm, 4th 61–89, 1st separated from 2nd by 35–46 µm; 3rd separated from 4th by 55–81 µm; and 2nd from 4th by 528–578 µm.

Abdomen (Fig 2a-b). TI without shagreen. TII with 67–90 posterior hooklets; TII–TVI covered with shagreen, and TVII–TVIII with shagreen on anterior margin; SI with one pair of tubercles with few spines (Fig. 2c); TIII with anterior band of strong spinules. Conjunctives III/IV to IV/V with 6 rows of sclerotized spinules. TIII–TVI sometimes presenting posterior dark spots. TV-TVIII with D1 setae of normal type (Fig. 2e). Pedes spurii A absent. Segment VIII with 4–8 comb–like caudolateral spines (Fig. 2d) the longest with 25–31 µm long. Segment II and III with 1 small L setae and second dorsolateral setae, segment IV only with one L seta. Pedes spurii B present on segment II and III. Segment V–VIII with 3, 4, 4, 5 taeniate setae. Anal lobe 338–369 µm long, with 28-33 double fringe of taeniate setae and a pair of dorsal setae.

Larvae (n = 3)

Coloration. Head brown; mentum, mandibular teeth except apical tooth blackish, claws on parapods very strong and darkned.

Head. Head width: 615–877 µm. Antenna with 5 segments (Fig. 3c), length of antennal segments (µm) 123–131; 28 (2); 17–21 (2); 11–14 (2); 6–9 (2). AR = 1.41–1.89. Blade 69–89 (2) µm long. Lauterborn organ 8 (2) µm. Premandible 140–181 µm long (Fig. 3g). Pecten epipharyngis 51–54 µm long, with approximately 22 teeth (Fig. 3e). Mentum 246–277 µm long, with 11 teeth (Fig. 3a). Five median teeth forming a concise and somewhat isolate group, the three median higher than the other teeth and the second lateral very small and fused with the first lateral. Fifth and sixth lateral tooth very flattened and fused, sometimes presenting a small fissure. Mentum/head width: 0.28–

0.45. Distance between submental setae: 194–243 μm. Ventromental plates 38–48 μm wide. Dorsal sclerites of head as in figure 3b. Mandible 238–300 μm long (Fig. 3d).

Abdomen. Length 9–10 mm. Procercus with 6–8 setae (Fig. 3f). Anal seta 656–712 μm long. Subbasal seta on procercus 100–128 μm. Anal tubules short, 329–385 μm long. Subasal seta on parapod 138–151 μm long. Parapods claws very strong and black.

Remarks. *Pseudochironomus* **sp. n**. **1** adults were collected with emergence traps in marginal rocks of a small mountain stream, situated at 2500 m. a.s.l. Larvae and pupae exuviae were collected right above the traps in the same locality. Cold and crystalline water; pH: 5.5 and dissolved oxygen: 7.4. Vegetation predominantly composed by natural shrub and grass field (Campos de Altitude vegetation).

***Pseudochironomus* sp. n. 2**

Type material. Holotype male, BRAZIL, SP, Campos do Jordão, Galharada Stream, 3rd order, 22°41”.29’S, 45°27”.42´W, 1584 m a.s.l., 14.ii.2002 (I1 – 25). M. T. Suriano. Paratype: two male, same data as holotype (I1 – 26-27), one male, same data as holotype, except for 05.xi.2001 (I1 – 28), one pupa exuvia with larva’s cephalic capsule, same date as holotype, except for 13.ix.2001 (I1 – 29), one pupa exuvia with larva’s cephalic capsule, same date as holotype, except for 12.xi.1999 (I1 – 30), and one pupa exuvie from BRAZIL, SP, Campos do Jordão, Campo do Meio Stream, 2nd order, 22°41”.35’S, 45°29”23´W, 1500 m a.s.l., 13.ix.2001 (I1 – 31), M. T. Suriano. Five larvae, same data as holotype, except for 9.xi.2001 (I1 – 32).

Diagnostic characters. The adult males are separable from the other species of the genus except *Pseudochironomus* **sp. n**. **1**, *Pseudochironomus* **sp. n**. **3** and *Pseudochironomus* **sp. n**. **4** by the presence of acrosticals. Short tergite IX, presenting a terminal notch, and never reaching the inferior vorsella. Gonostylus broad and short.

Median volsella fine, carrying only one strong and long seta. Presence of another seta right above the median volsella. Superior vorsella bare, but presenting one long seta. Inferior vorsella foot–like. Pars ventralis completely divided.

Pupa. Exuviae light brown; sternite I with small spines on lateral tubercles; tergite I with two patches of shagreen. Thoracic horn 169–312 µm long. Median and posterior precorneals arising from an apparently tough protuberance. TII with 61–78 posterior hooklets. Segment VIII with 4–7 caudolateral spines. TVI, TVII and TVIII with broadened D1 setae. Anal lobe with 22-30 taeniate setae.

Larva. Mentum, Claws and cephalic capsule brown. Mentum with 11 teeth; fifth and sixth lateral teeth not fused; sixth lateral tooth small, almost the half of fifth tooth's width. Basal segment of antenna slightly smaller than premandibule. AR=1.33–1.67.

Description. Male (n =4)

Total length 3.63–4.15 mm; wing length 2.68–2.83 mm; total length / wing length 1.28–1.51; wing length/ length of profemur 2.23–2.63.

Coloration: Thorax with brown marks. Tergites with brown bands and pale spots in the insertion of setae. Femur yellow, tarsi with intense dark brown color.

Head. AR= 0.3 (1). Antenna length: 1040 (1) µm. Last flagellomere length 800–831 µm. Clypeus with 24–29 setae. Palpomere –lengths (µm) 63–75; 63–125; 188–200, 244–281; 313–375. Third palpomere with a slight fingerlike process.

Thorax. Acrosticals*:* 5 (3); Antepronotals 4–8 (3), small; dorsocentrals 13–17 (3) in a single row, arising from pale areas; prealars 5–6 (3); scutellum with 19–33 (3) setae, double row.

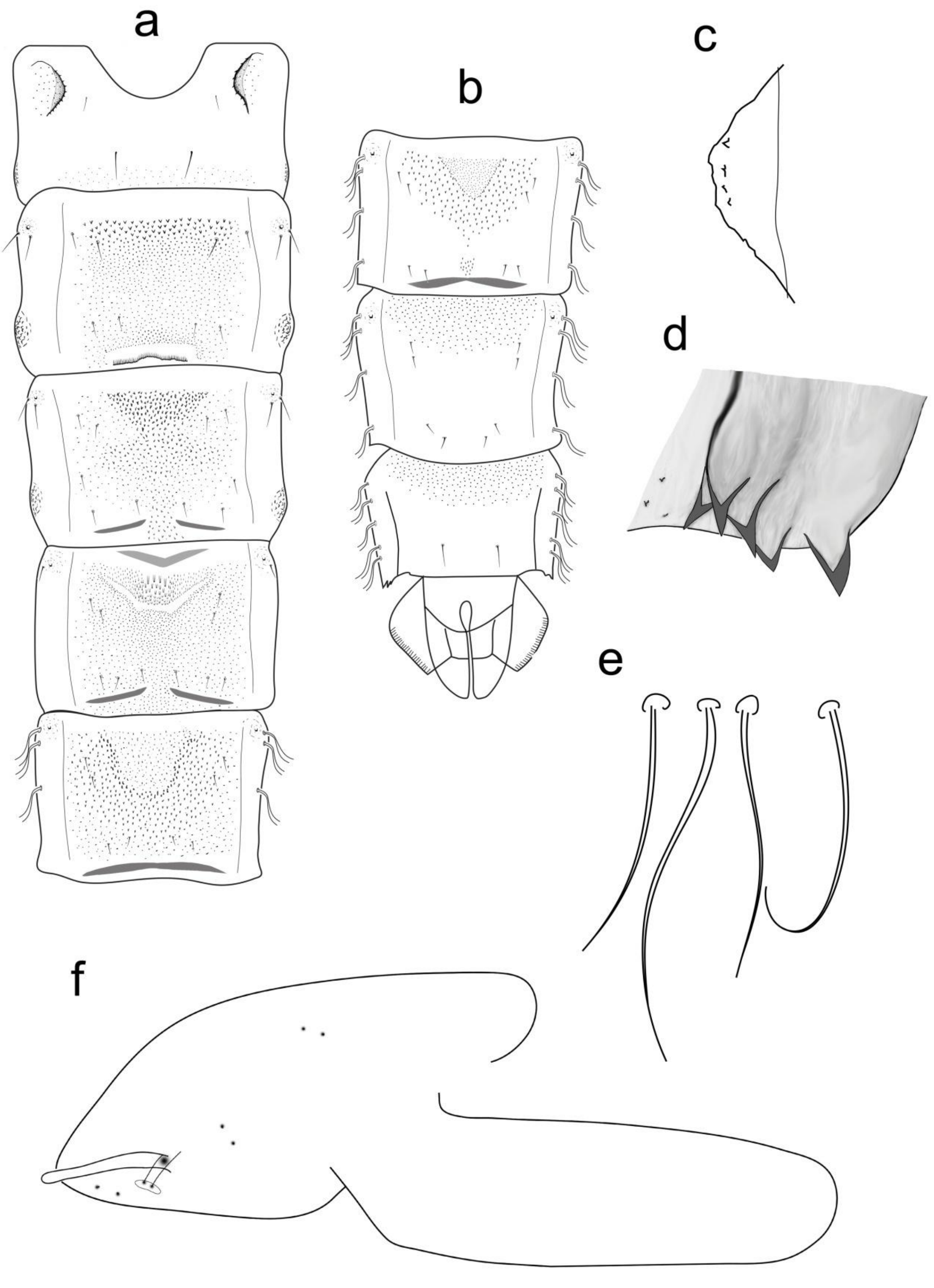

FIGURE 2. *Pseudochironomus* **sp. n**. **1. pupa**. **a.** Abdome, TI-TV **b.** Abdome, TV-TVIII and anal lobe. **c.** Tubercle from SI. **d.** Caudolateral spines on segment VIII **e.** From left to right: D1 setae from TV-TVIII. **f.** Thorax.

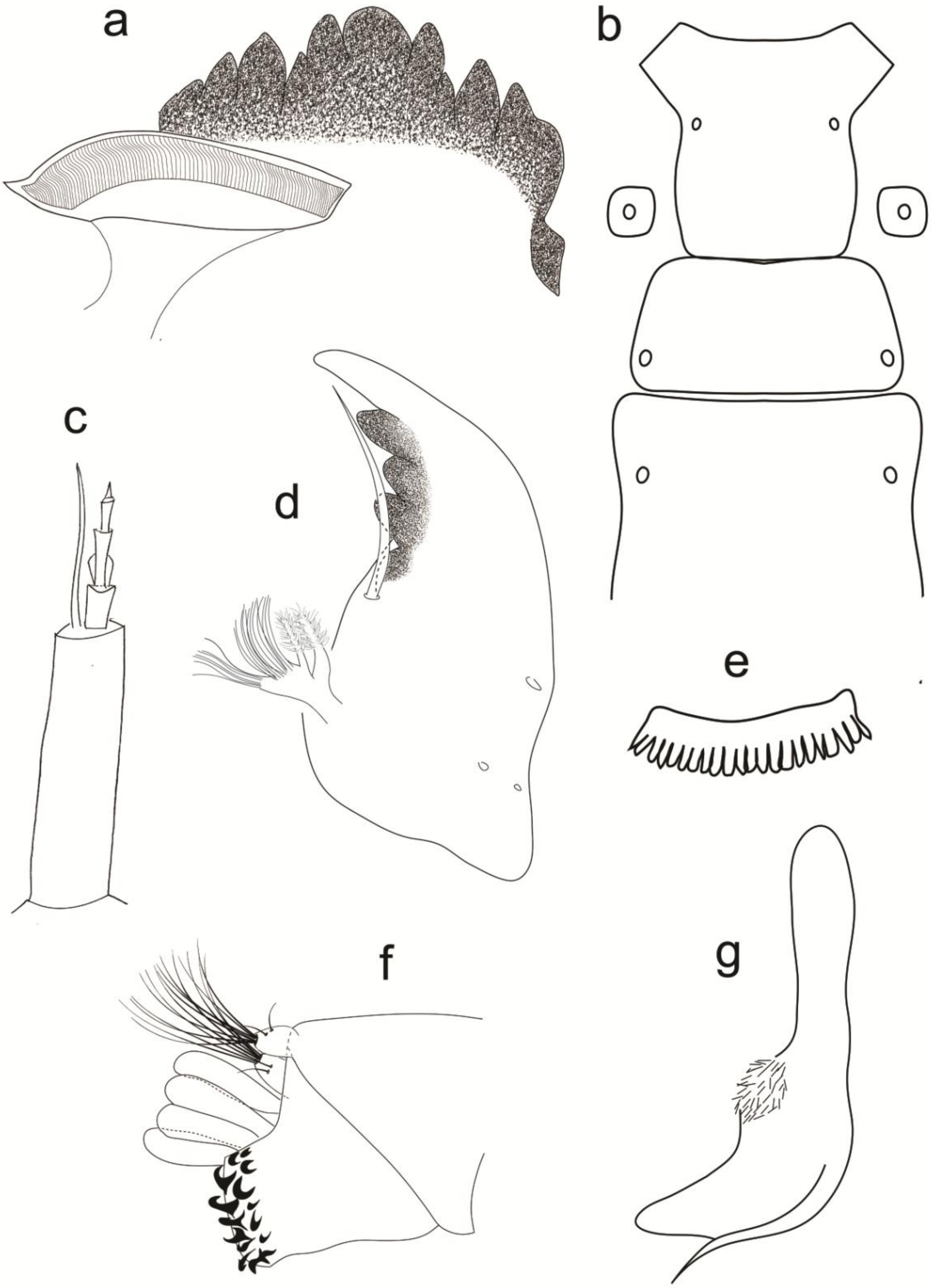

FIGURE 3. *Pseudochironomus* **sp. n**. **1. larva. a.** Mentum and ventromental plate. **b.** Dorsal sclerites of head. **c.** Antenna. **d.** Mandible. **e.** Pecten epipharyngis. **f.** Last segment of abdomen (procercus, anal tubules and parapods). **g.** premandible.

Wing. Brachiolum with 2 setae, R with 21–28 setae, R1 with 9–12, R4+5 5–8 apical setae. Squama with 25–30 setae.

Legs. Spur of foretibia 63–75 µm long; spurs of midtibia 66–94 µm and 81–93 µm long; spurs of hind tibia 59–91 µm and 81–100 µm long. Length of legs and LR proportion as in Table 3.

Abdomen. Dark brown colored with pale circles in the base of setae which are homogeneously distributed in all the segments.

Hypopygium (Fig. 3a). Posterior margin of tergite IX with median notch (Fig. 3c) with 8–15 setae. Laterosternite IX with 8–11 (3) setae. Phallapodeme 108–115 µm long. Transverse sternapodeme 137–155 µm long. Superior volsella (Fig. 3b) 108–126 µm long curved, apically concave ending as two points, with one point 26 µm and the other 9 µm long and seta 31 µm; inferior volsella foot shaped, 95–106 µm long, longest apical setae on inferior vorsella with 32–46 µm long, median volsella 18–22 µm long bears 1 apical setae 38–49 µm, and another similar seta above median volsella. Gonocoxite 198–254 µm long. Gonostylus 157–197 µm. HR = 1.2-1.3. Pars ventralis (Fig. 3d) completely divided into two 68–77 (2) µm long and 11–12 (2) µm wide lobes.

TABLE 3. Lengths (in µm) of legs and LR proportion of *Pseudochironomus* **sp. n. 2** (male, n = 4).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	1077–1231	1462–1615	1354–1492	600–662	492–554	338–446	231–262	0.90-0.93
II	1277–1385	1338–1415	662–723	308–338	246–277	154–169	108–123	0.48-0.51
III	1323–1415	1415–1585	846–892	338–508	277–385	169–215	123–138	0.51-0.57

Pupa (n = 3)

Total length 4.9–5.7 mm. Exuviae brownish.

Cephalotorax (Fig 5f). Frontal setae absent. Thoracic horn, 169–312 µm long, smooth. Three precorneals, median and posterior arising from a protuberance; Anterior precorneal 100–114 (2) µm, median 157–180 µm, posterior 55–138 µm long; 2 antepronotals, one with 114–131 µm and the other with 197–209 µm, dorsocentrals, 1st 66–123 (2) µm, 2nd 64–183 , 3rd 56–128 (2) µm, 4th 77–85 (2), distance from 1st and 2nd 29–65 µm long; distance from 3rd and 4th 77–85 µm; distance from 2nd and 3rd 428–497 µm.

Abdomen (Fig. 5a-b). TI with two patches of shagreen. TII with 61–78 posterior hooklets; TII–TVI covered with shagreen, and TVII–TVIII with shagreen on posterior margin; TV-TVIII with wide D1 setae (Fig 5e). TIII with anterior band of strong spinules; Conjunctives III/IV to IV/V with 6 rows of sclerotized spinules. TIII–TVI sometimes presentig posterior dark spots. SI with one pair of tubercles with many small spines (Fig. 5c); Pedes spurii A absent. Segment VIII with 4–7 comb–like caudolateral spines (Fig. 5d) 25–28 µm long. Segment II and III with 1 small L setae and a second dorsolateral setae, segment IV only with one L seta. Pedes spurii B present on segment II and III but very slight. segment V–VIII with 3, 4, 4, 5 L taeniate setae. Anal lobe 261–338 µm long, with 22-30 double fringe of taeniate setae and a pair of dorsal setae.

Larval exuviae (n = 4)

Coloration. Head brown; mentum, mandibular teeth except apical tooth and occipital margin dark brown.

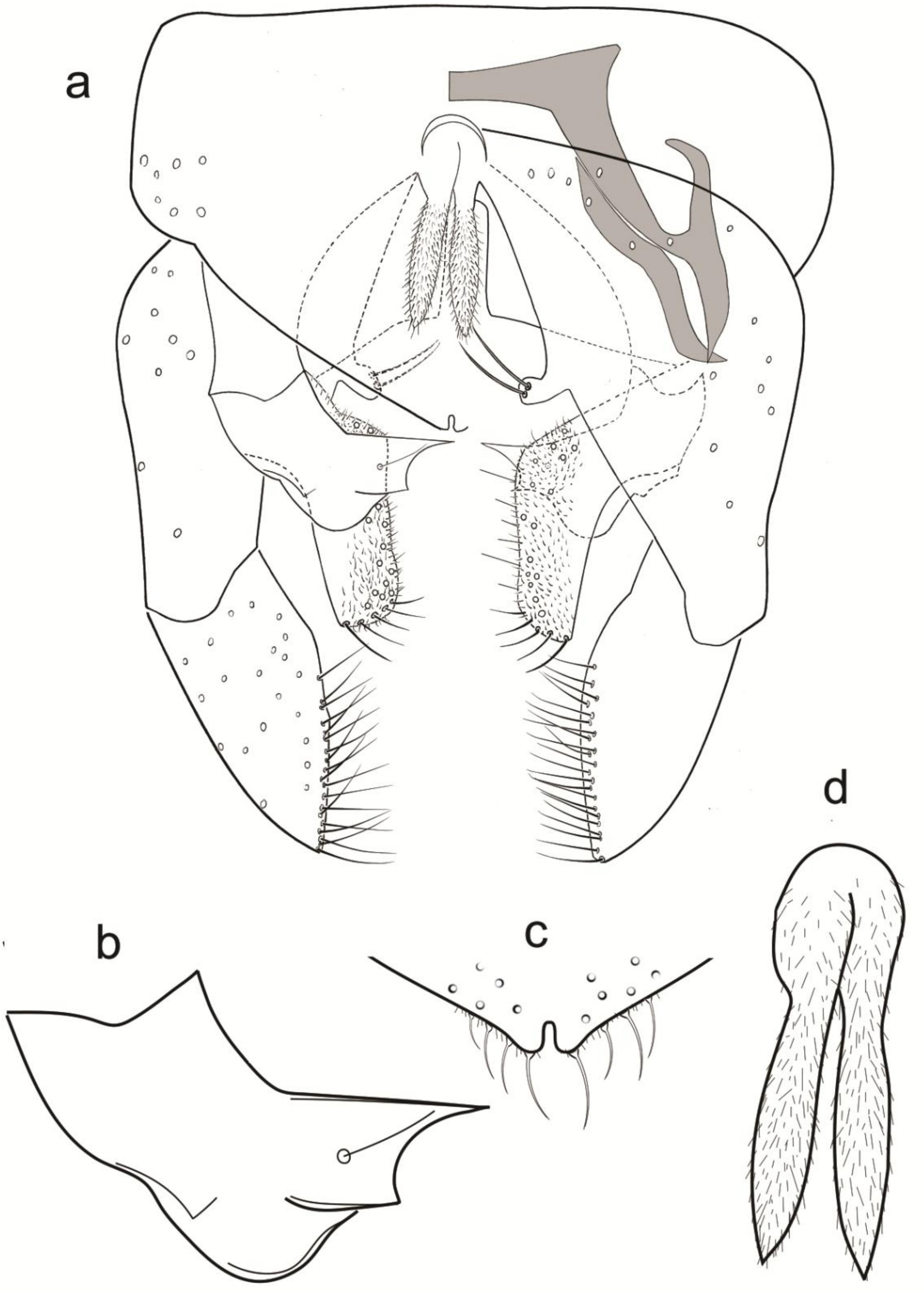

FIGURE 4. *Pseudochironomus* **sp. n. 2**. **adult male. a**. Hypopygium. **b.** Superior volsella. **c.** Margen of tergite IX. **d.** Pars ventralis.

Head. Head width: 462–692 (2) µm. Antenna with 5 segments, length of antennal segments (µm) 107–112; 20–28; 12–21; 11–15; 5–8 (Fig. 6b). AR = 1.33–1.67. Blade 66–85 µm long. Lauterborn organ 9–11 µm. Premandible 118–131 µm long (Fig. 6f). Pecten epipharyngis 34–40 µm long (Fig. 6d). Mentum 154–200 µm long (Fig. 6a), with median tooth and 5 lateral teeth, second lateral tooth very small and fused with the first lateral tooth, fifth and sixth tooth not fused; sixth tooth small, with approximately the half of fifth tooth's width. Mentum/head ratio: 0.27–0.34. Distance between submental setae: 151–200 µm. Ventromental plates 35–40 µm wide. Mandible 168–226 µm long (Fig. 6e). Dorsal sclerites of head as in figure 6c.

Abdomen. Length 9–10 mm. Procercus with 8 setae. Anal seta 394–625 (2) µm long. Subbasal seta on procercus 74–88 (2) µm. Anal tubules, 192–254 (2) µm long. Subasal seta on parapod 34–80 (2) µm long. Parapods claws brownish.

Remarks. Larvae were collected in mountain streams from Southeastern Brazil, with low depth and transparent water, dissolved oxygen 8.6-10.4 mg.l^{-1} and pH about 5-6. The altitude in sampling sites was higher than 1500 m a.s.l.

***Pseudochironomus* sp. n. 3**

Type material. Holotype male, BRAZIL, Roraima State, Serra da Mocidade, 01°42".225'S, 61°47".079´W, light trap close to the stream, 996 m a.s.l., 27.i.2016. J.M.C. Nascimento (I1 – 33). Paratype: one male, same data as holotype, except for 29.i.2016 (I1 – 34), and one male, same data as holotype, except for 02.ii.2016 (I1 – 35)

Diagnostic characters. Small species. The adult males are separable from the other species do genus except *Pseudochironomus* **sp. n**. **1**, *Pseudochironomus* **sp. n. 2**, *Pseudochironomus* **sp. n**. **4** by the presence of acrosticals. Tergite IX with protruding end projecting from the tergite, and terminal notch. Gonostylus short and broad. Median vorsella thin with only one seta, and another seta right above the volsella. Superior vorsella bare, but presenting two long setae. Inferior vorsella foot–like. Pars ventralis completely divided.

Description. Male (n =3 except when otherwise stated)

Total length 2.6–3.8 mm; wing length 2.0–2.6 mm; total length / wing length 1.32–1.48; wing length/ length of profemur 1.98–2.18.

Coloration: General coloration brown. Tergites brown with pale spots in the insertion of setae. Femur yellow, tibia and tarsi brown. Thorax with dark brown marking.

Head. AR= 0.62 (1). Antenna length: 1031 µm. Last flagellomere length 800 µm; 34–37 temporal setae. Clypeus with 23–31setae. Palpomere 2-5 lengths (µm) 75–113; 175–213; 200–262; 306–319. Third palpomere with a slight fingerlike process.

Thorax. Acrosticals*:* 3–6; Antepronotals 6–11, small; dorsocentrals 11–13 in a single row, arising from pale areas; prealars 3–5; scutellum with 19–26 setae, double row.

Wing. Brachiolum with 2 setae, R with 19–25 setae, R1 with 6–11, R4+5 0–14, proximally absent in the vein. Squama with 23–27 (2) setae.

Legs. Spur of foretibia 31–58 long; spurs of midtibia 55–72 and 65–75 µm long; spurs of hind tibia 66–81 and 71–85 long. Length of legs and LR proportion as in Table 4.

Abdomen. Dark brown with pale areas in the base of setae which are homogeneously distributed in all the segments.

Hypopygium (Fig. 7a). Posterior margin of tergite IX with median notch (Fig. 7c) with 8–9 (2) setae and projecting from the tergite. Laterosternite IX with 4–13 setae. Phallapodeme 67–99 µm long. Transverse sternapodeme 92–134 µm long. Superior vol-

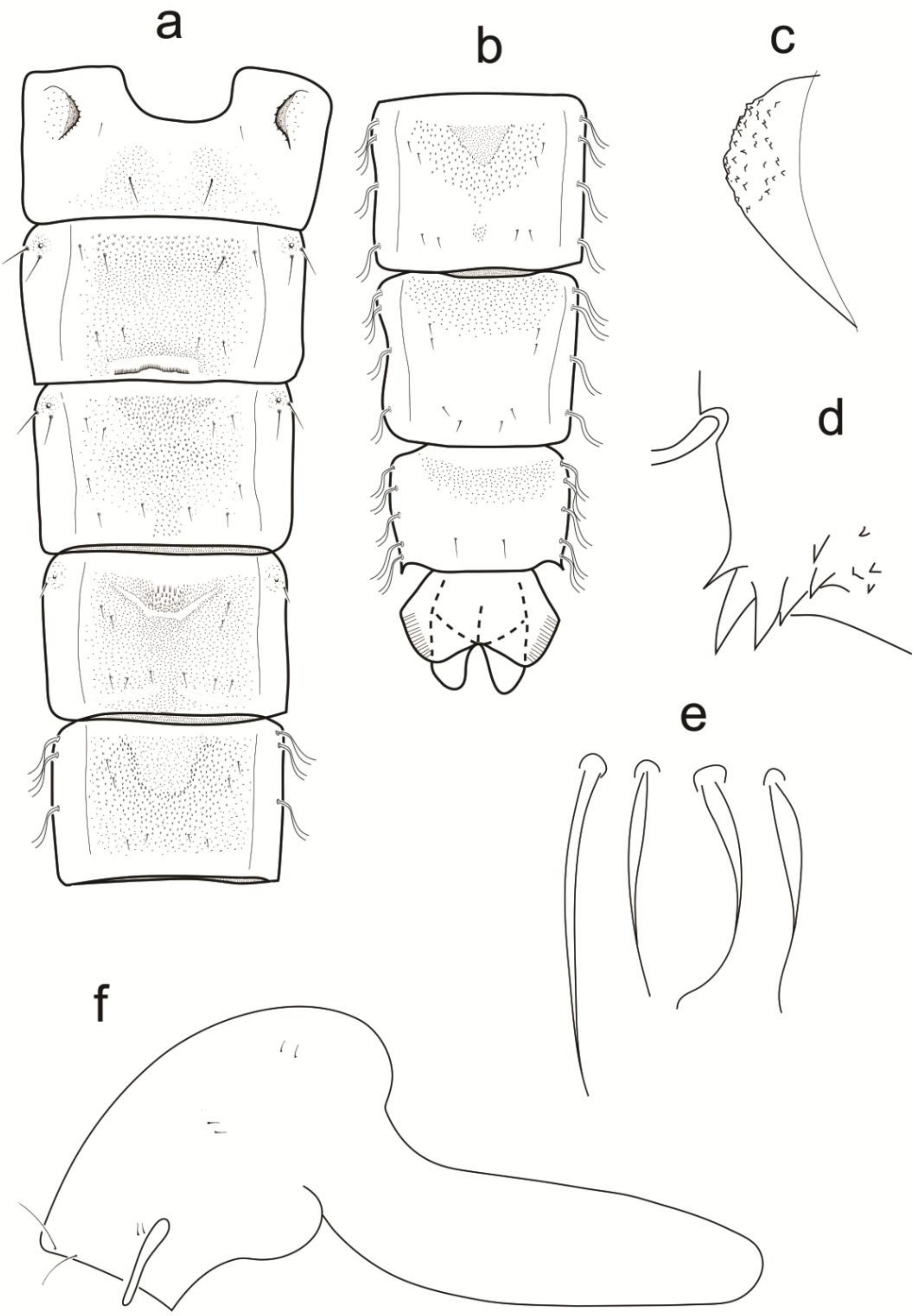

FIGURE 5. *Pseudochironomus* **sp. n. 2. pupa**. **a.** Abdome, TI-TV. **b.** Abdome, TV-TVIII and anal lobe. **c.** Tubercle from SI. **d.** Caudolateral spines on segment VIII **e.** From left to right: D1 setae from TV-TVIII. **f.** Thorax.

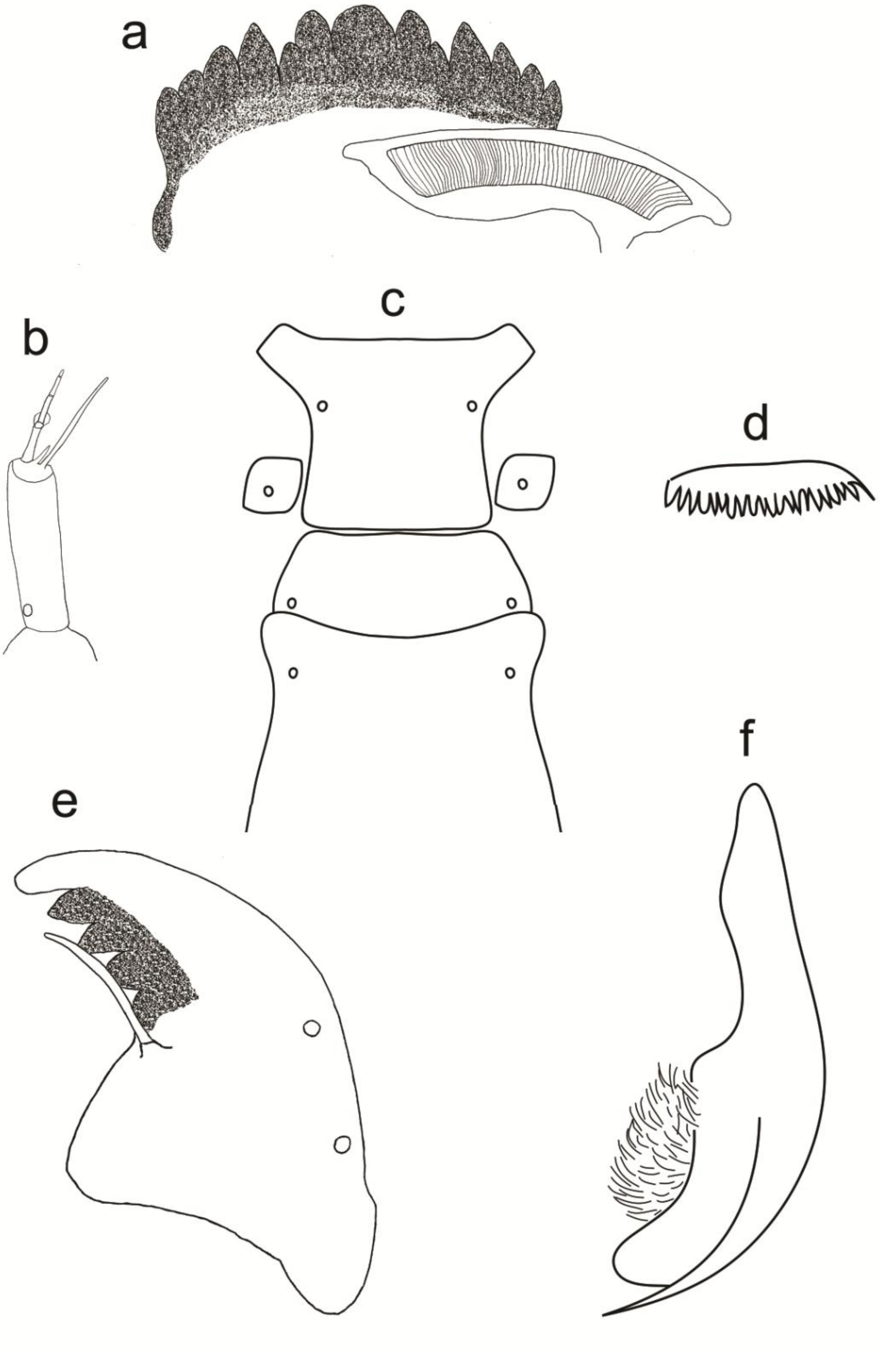

FIGURE 6. *Pseudochironomus* **sp. n. 2**. **larva. a.** Mentum and ventromental plate. **b.** Antenna. **c.** Dorsal sclerites of head **d.** Pecten epipharyngis. **e.** Mandible. **f.** premandible.

sella (Fig. 7b) 68–94 μm long curved, apically concave ending as two points, with one point 15 μm and the other with 8 μm long, with two setae: superior seta with 29 μm and the inferior with 25 μm; inferior volsella foot shaped, 78–92 μm long, with microtrichia and long setae, longest apical setae 25–30 μm long, median volsella 12–18 μm long bears 1 apical setae 38–40 μm, and 1 seta above median volsella 23–25 μm long. Gonocoxite 105–148 μm long. Gonostylus 55–85 μm. HR = 1.7-1.9. Pars ventralis completely divided (Fig. 7d) into two, 66–74 μm long and 12–15 μm wide lobes.

TABLE 4. Lengths (in μm) of legs and LR proportion of *Pseudochironomus* **sp. n**. **3** (male, n = 3).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	908–1308	1215–1600	1200–1631	538–723	446–615	369–461	185–231	0.99-1.02
II	1046–1431	1077–1400	585–769	292–385	277–323	123–169	123–138	0.54-0.55
III	1077–1415	1231–1600	692–1015	431–523	277–415	169–215	92–154	0.56-0.63

Remarks. The male adults were collected with light traps close to streams from a mountainous region in Amazon, at 1000 m a.s.l. approximately, and characterized by dense canopy cover. Water temperature was about 19.5ºC, pH: 5.39-6.75 and conductivity 12 μS.cm^{-1}.

Pseudochironomus sp. n. 4

Type material. Holotype male, BRAZIL, SP, Campos do Jordão, Galharada Stream, 3rd order, 22°41”.29’S, 45°27”.42’W, 1584 m a.s.l., 09.xi.1999. M. T. Suriano. Paratype: three male, same data as holotype.

Diagnostic characters. Small species. The adult males are separable from the other species of the genus except *Pseudochironomus* **sp. n**. **1**, *Pseudochironomus* **sp. n. 2** and *Pseudochironomus* **sp. n**. **3** by the presence of acrosticals; Short inferior vorsella.

Terminal notch lacking on tergite IX, margin of TIX rounded and continuous. Gonostylus long and narrow. Pars ventralis leaf–shaped, completely divided.

Description. Male (n = 4)

Total length 2.89–3.40 mm; wing length 2.01–2.38 mm; total length / wing length 1.31–1.46; wing length/ length of profemur 2.33–2.53.

Coloration: Abdomen with brown bands on tergites. Wing hyaline with yellow veins. Femur yellow, tibia and tarsi dark brown, thorax uniformly brown, without marking.

Head. Last flagellomere length 612–662 µm; Clypeus with 13–14 setae. Palpomere – lengths (µm) 37–59; 50–78; 116–156, 153–203, 225–291. Third palpomere with a slight fingerlike process.

Thorax. Acrosticals*:* 0–2; Antepronotals 2–3, small; dorsocentrals 11–13 in a single row, arising from pale areas; prealars 3–4; scutellum with 11–16 setae, simple to double.

Wing . Brachiolum with 2–3 setae, R with 16–21 setae, R1 with 2–7, R4+5 9–14 apical setae. Squama with 7–15 setae.

Legs. Spur of foretibia 44–53 µm long; spurs of midtibia 41–53 and 53–59 µm long; spurs of hind tibia 41–62 µm and 44–62 µm long. Length of legs and LR proportion as in Table 5.

Hypopygium (Fig. 8a). Tergite IX without median notch (Fig. 8c) ending in a continuous and rounded margin with 8–21 setae. Laterosternite IX with 4–6 setae. Phallapodeme 55–80 µm long. Transverse sternapodeme 77–89 µm long. Superior volsella (Fig. 8b) 72–91 µm long curved, apically concave ending as two points, the longest with 23 µm long; inferior volsella 43–54 µm long, with microtrichia and long

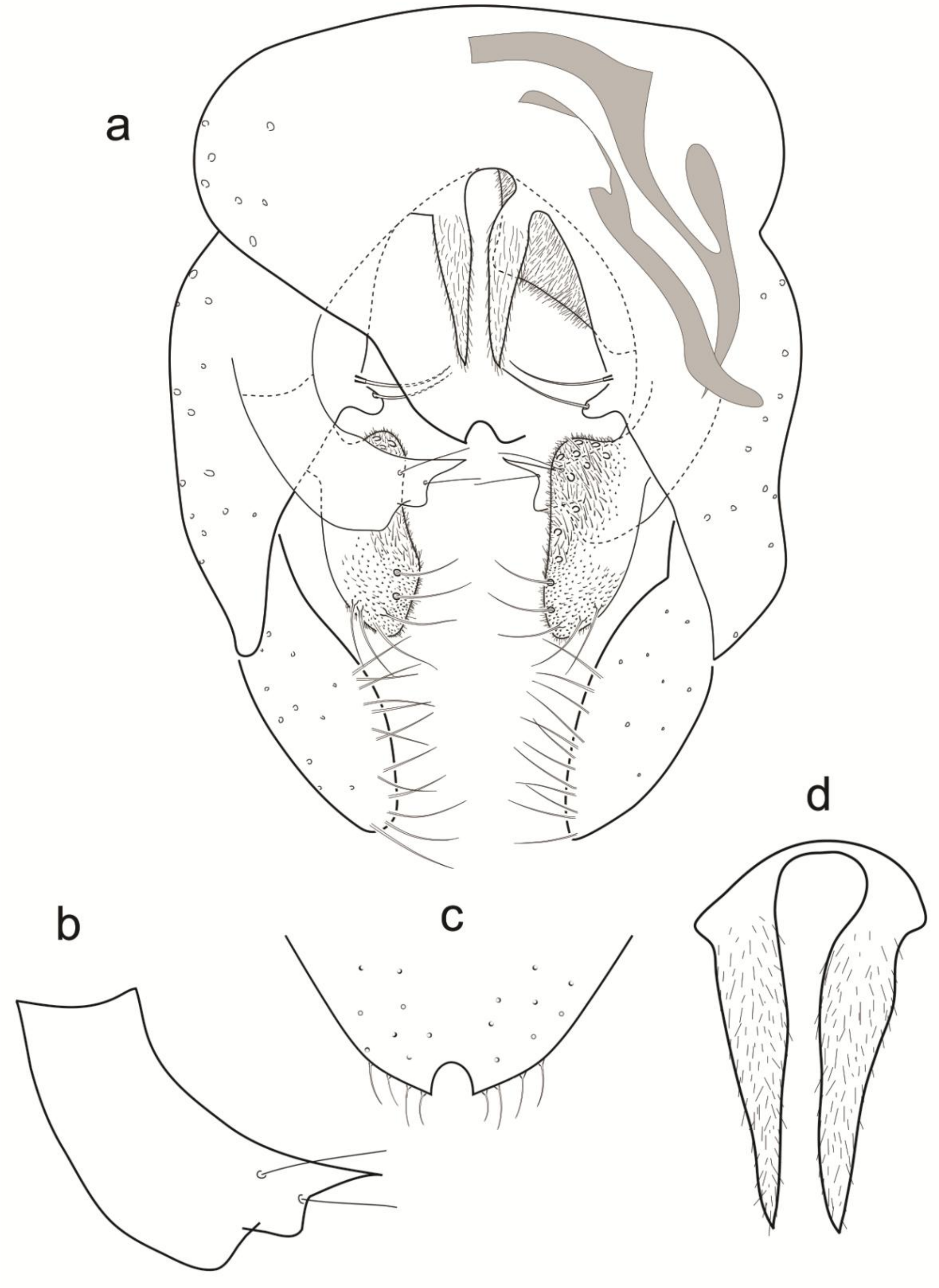

FIGURE 7. *Pseudochironomus* **sp. n**. **3**. **adult male**. **a.** Hypopygium. **b.** Superior volsella. **c.** Margen of tergite IX. **d.** Pars ventralis.

setae, longest apical setae 29–58 µm long, median volsella 12–17 µm long bears 1 apical setae 17–25 µm, and one subapical seta. Gonocoxite 143–207 µm long. Gonostylus 155–169 µm. HR = 0.9-1.2. Pars ventralis completely divided (Fig. 8d) into two 58–78 µm long and 22–25 µm wide lobes.

TABLE 5. Lengths (in µm) of legs and LR proportion of *Pseudochironomus* **sp. n. 4** (male, n = 4).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	815–985	1108–1231	969–1215	446–523	338–415	277–353	138–185	0.94-1.07
II	908–1092	908–1077	446–585	231–292	185–231	123–138	62–123	0.47-0.55
III	923–1123	1046–1231	631–815	338–462	246–277	138–185	108–123	0.60-0.66

Remarks. Adults were collected close to low-depth streams in mountain region of Southeastern Brazil. The altitude in sampling sites was higher than 1500 m a.s.l.

Discussion

All adult male described here are morphologically closer to *P. fulviventris* (Johannsen) and *P. viridis* due to the coloration patterns and the presence of a finger–like lobe on third palpomere, but, they differ from the former by having a terminal notch on tergite IX and from both by having acrosticals. Although *Pseudochironomus* **sp. n. 4** also lacks the terminal notch on tergite XI, which separate it from the other species described here, the small body size and the presence of acrosticals differs it from *P. fulviventris* and *P. viridis.*

Pseudochironomus **sp. n**. **1** also share some similarities with *P. crassus* Townes specially because of the very dark marks on thorax and the presence of a broad gonostylus, but they differ in coloration pattern of the legs, in the number of dorsocentrals, and again, by the presence of acrosticals in *Pseudochironomus* **sp. n**. **1**.

The presence of acrosticals seems to be a characteristic exclusive to the species described in the present study. In addition, other characters make them very close morphologically, especially *Pseudochironomius* **sp. n. 1**, *Pseudochironomus* **sp. n. 2** and *Pseudochironomus* **sp. n. 3**: superior vorsella ending in two parallel–sided points and presenting at least one long seta; inferior vorsella long and foot–like; tergite IX with a terminal notch; and the presence of a completely divided pars ventralis. Although Seather (1977) stated that a wide variation in the shape of pars ventralis is possible within the same species.

Pseudochironomus **sp. n**. **1** differs from *Pseudochironomus* **sp. n. 2** in body size; size and shape of tergite IX, which is wider in *Pseudochironomus* **sp. n**. **1***;* and by the format of median vorsella, number and thickness of setae on it. *Pseudochironomus* **sp. n**. **1** and *Pseudochironomus* **sp. n. 2** differs from *Pseudochironomus* **sp. n. 3**, by the small body size of *Pseudochironomus* **sp. n. 3**, and also due to the additional setae present on superior vorsella in this species. In addition, *Pseudochironomus* **sp. n**. **3** presents the terminal region of TIX protruding, forming a caudal projection, and presents no marking in thorax.

Most of the females of *Pseudochironomus* remains unknown or were not completely described yet. A detailed description of *P. fulviventris* is given by Seather (1977), and although it presents some similarities with *Pseudochironomus* **sp. n**. **1**, they differ in coloration, once *Pseudochironomus* **sp. n**. **1** presents a very dark brown thoracic marking and also presents few acrosticals. They also differ in the size of the cerci, slightly smaller in *Pseudochironomus* **sp. n**. **1**.

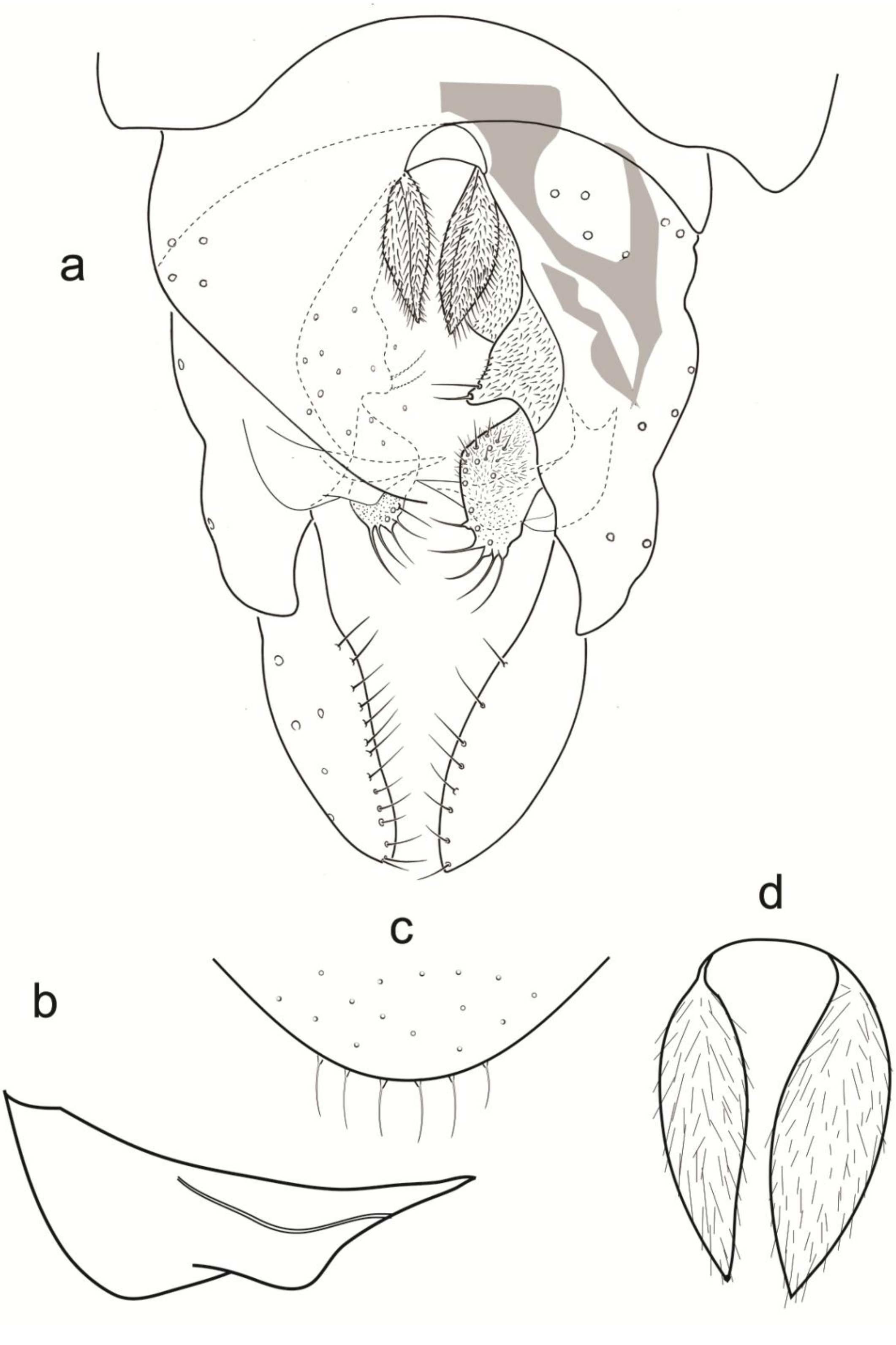

FIGURE 8. *Pseudochironomus* **sp. n. 4. adult male**. **a.** Hypopygium. **b.** Superior volsella. **c.** Margen of tergite IX. **d.** Pars ventralis.

Pupa of *Pseudochironomus* **sp. n**. **1** is similar to *P. richardsoni* Malloch due to the presence of one pair of tubercles in SI, same color and number of caudolateral spines, but they differ in size of thoracic horn, which is more than two times larger in *P. richarsoni* and divided in two branches. Also, *Pseudochironomus* **sp. n**. **1** presents more than 15 taeniate setae on anal lobe.

The pupa of *Pseudochironomus* **sp. n. 2** resembles the pupa of *Pseudochironomus* **sp. n**. **1** and also *P. richardsoni.* It differs from *Pseudochironomus* **sp. n**. **1** by the number of spines in the tubercles of SI, which are more numerous in *Pseudochironomus* **sp. n. 2.** Also, D1 setae on TVI, TVII and TVIII are approximately two times wider in *Pseudochironomus* **sp. n. 2***,* and contrary to *Pseudochironomus* **sp. n**. **1**, the species present two patches of shagreen in TI. *Pseudochironomus* **sp. n. 2**, as *Pseudochironomus* **sp. n**. **1**, differs from *P. richardsoni* in size and shape of thoracic horn.

Regarding, the format of the mentum and number of theeth, the larva of *Pseudochironomus* **sp. n**. **1** and *Pseudochironomus* **sp. n. 2** are similar to *P. articaudus* Saether, but they differ in AR ratio and in the size of basal segment of antenna, which are much higher in *Pseudochironomus* **sp. n**. **1** and *Pseudochironomus* **sp. n. 2**. The larvae of *Pseudochironomus* **sp. n**. **1** and *Pseudochironomus* **sp. n. 2** can be distinguished by the darker color of mentum and claws in *Pseudochironomus* **sp. n**. **1**. Also, the last sixth tooth is smaller than the fifth and it is not fused in *Pseudochironomus* **sp. n. 2**.

Ecological comments

Saether (1977) stated that immature stages of *Pseudochironomus* are inhabitants of edges of oligotrophic lakes an also large and slow-flowing rivers. In this study they were recorded in the main channel and in marginal rocks (madicolous habitat) of first-order streams. Although the larva and the pupa of two species are unknown (*Pseudochironomus* **sp. n. 4** and *Pseudochironomus* **sp. n. 3**), adults of both species were collected close to low order streams, what probably indicates that the immature stages inhabits that locals. Further, all species described here were found in undisturbed streams of high elevation areas from Southeast and North of Brazil. The high endemicity degree found in Brazilian mountains has been highlighted by studies with several animal groups (Ribeiro et al. 2015; Chaves et al. 2015; Fouquet et al, 2015; Nogueira et al., 2011; Carneiro et al. 2014), thus many Chironomidae species unknown to science are expected to occur in high altitudinal regions.

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Tanytarsini (Diptera: Chironomidae) from madicolous habitat in Southeast Brazil: new species and new records

Abstract

Tanytarsini (Diptera: Chironomidae: Chironominae) collected from madicolous habitats in Brazil are analyzed, and three new species of *Tanytarsus* van der Wulp are described and illustrated: *Tanytarsus* **sp. n. 1** and *Tanytarsus* **sp. n 2**. as adult male and *Tanytarsus* **sp. n. 3** as male and female. New records of another Brazilian *Tanytarsus* species are also presented, and immature stages of *Paratanytarsus silentii* Trivinho-Strixino are described.

Key words: *Tanytarsus* species; Brazilian chironomids; higropetric insects

Introdution

Among the Chironominae genera registered in Brazil, 22% of the recorded species belongs to the Tanytarsini tribe: 27 to the genus *Tanytarsus*, 28 to *Caladomyia* and 8 to other genera (Mendes & Pinho 2014); more recently, other 5 new species of *Tanytarsus* from Brazil were described (Trivinho Strixino *et al*. 2015). To date there are no reports of representatives of this tribe living in madicolous habitat and / or high-altitude areas. As result of this PhD study, 3 new *Tanytarsus* species living in madicolous habitats from high altitudes in Southeast Brazil (> 2,000 m a.s.l.) are presented. One of them is distinct in having hairy eyes and unusual cephalic structures (prominent, sclerosed processes). Additionally, new records of *Tanytarsus giovanni* Sanseverino & Trivinho-Strixino, 2010, *Tanyarsus digitatus* Sanseverino, 2006 and *Paratanytarsus silentii* Trivinho-Strixino, 2010 are given, and descriptions of immature stages (larva and pupa exuviae) of the latter species are provided.

Methods and Terminology

The adults were collected with emergence traps specifically adapted for madicolous habitats (Shimabukuro *et al*. 2015). The immatures of *Paratanytarsus silentii* were sampled on marginal rocks (madicolous biotopes) and on main channel of low-order streams, with hand net and Surber collectors in the same locality of the adults.

The specimens examined were slide-mounted in Euparal. The general terminology follows Sæther (1977, 1980) and Langton (1994). Measurements are given as the value of the holotype, followed by the range and the number of specimens examined in parenthesis if it differs from the number (n) stated at the beginning of description.

The material examined is deposited in the Reference Collection of the Laboratório de Ecologia de Insetos Aquáticos (LEIA) of the Universidade Federal de São Carlos (UFSCar), São Paulo State, Brazil.

DNA barcoding

40 specimens of the genus *Tanytarsus* were submitted to molecular analysis. We used legs of the adults and abdominal tissue of larvae removed from voucher specimens preserved in ethanol. Specimens were previously morphologically identified. We adopted the same procedure described by Trivinho-Strixino *et al*. (2012). For the samples, the primers LepF (5-ATTCAACCAATCATAAAGATATTG-3) and LepR (5-TAAACTTCTGGATGTCCAAAAAATC-3) amplified the target 658-bp fragment of COI. Sequences were obtained by using either ABI 377 or ABI 3730 sequencers (Applied Biosystems). Sequences were edited and assembled by using SEQUENCHER (Gene Codes, Ann Arbor, MI). Sequences were then aligned and edited manually. DNA

was extracted, amplified and sequenced following the protocols of the Canadian Centre for DNA Barcoding. Sequence information and detailed specimens records are available on Barcode of Life Database in the project files “Aquatic insects from Inselbergs in Brazil (AIIB)”. Sequences have been submitted to GenBank. Kimura’s two-parameter model of base substitution (Kimura, 1980) was used to calculate genetic distances in MEGA 5 software (Tamura *et al*. 2011) and NJ trees were produced by using BOLD and MEGA 5 software.

Tanytarsus sp. n. 1

Type material: Holotype male, Brazil, Minas Gerais State, Pedra da Mina Mountain, 22˚24'697"S/44˚50'930"W 2,218 m a.s.l., small pool, leg E.M. Shimabukuro, G. Perbiche-Neves. 8.viii.2013 (A3-42, LEIA-UFSCar). Paratype male, Brazil, Rio de Janeiro State, Teresópolis, Sino Mountain, 22°27'605'S/43°01'689"W 2,126 m a.s.l. small rock under a fountain, leg E.M. Shimabukuro, G. Perbiche-Neves. 29.vii.2014 (A3-43, LEIA-UFSCar).

Additional material: 1 male, Brazil, Rio de Janeiro State, Teresópolis, Sino Mountain, 22°26'848'S/43°00'798"W 1444 m a.s.l. collected in marginal rock of a stream, leg E.M. Shimabukuro, G. Perbiche-Neves. 29.vii.2014 (A3-44, LEIA-UFSCar).

Diagnostic characters: *Tanytarsus* **sp. n. 1** can be separated from other *Tanytarsus* species by the combination of the following characters: anal point thin, without crests and with four long setae on each side. Superior volsella almost squared with rounded posterior projection; digitus finger-like, extending beyond median margin of superior volsella.

Description

Adult male (n = 2). Small, total length 2.50 mm. Wing length 1.77–1.95 mm.

Coloration: Head, flagellum and maxillary palp yellowish. Thorax, abdomen and legs yellow.

Head: AR 0.63. Total length of antenna 906 μm. Eyes with large dorsal extension; temporals 11; clypeus with 9 setae. Palpomeres 2–5 lengths: 31, 112, 137, 225 μm.

Thorax: Length 0.87 mm. Scutal tubercle absent; acrostichals 16–17, biserial, beginning near antepronotum; dorsocentrals 8–9; prealar 1; scutellars 5.

Wing: Width 0.51 mm; VR 1.24. Brachiolum with 1 seta, Sc bare, all wing veins and cells setose.

Legs: Fore leg tibia bearing single spur, 21μm (1) long. Mid leg tibia bearing two pectinate spurs, 27(1) and 34 (1) μm long and two separated combs. Hind leg tibia bearing two pectinate spurs, 37 (1) and 41 (1) μm long and two separated combs. Lengths and proportions of legs as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments in *Tanytarsus* **sp. n. 1**, male (n = 2).

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	784–846	384–415	969	446	353	276	123	2.33
II	784	569	369	215	138	107	77	0.65
III	846	754–815	523–538	307–338	246–307	153–169	107–138	0.66–0.69

Hypopygium (Fig. 1): Tergite IX without apical and median setae. Anal tergal bands separated (V-type). Anal point 40–48 μm long, with 4 long lateral setae on each side; anal crests absent. Superior volsella semi quadrate with rounded posterior projection, 6 setae on dorsal surface and 1 ventral seta on anterior margin. Digitus finger-like, extending slightly beyond median margin of superior volsella. Median volsella short, 16 μm long with several simple setae. Inferior volsella 93 μm long, straight. Gonocoxite 769–74 μm long; gonostylus 106–111 μm long; hypopygium ratio (HR) 0.64–0.68.

Figure 1. *Tanytarsus* **sp. n. 1.** Hypopygium, left dorsal view, right dorsal view, tergite IX removed.

Pupa and larva. Unknown.

Remarks

Tanytarsus **sp. n. 1** is related to some Neotropical species reviewed by Sanseverino (2006) thanks to the absence of anal crests. It resembles *T. fastigatus* Reiss, 1972 by the possession of lateral setae on anal point, but differs by the anal tergal bands, which is not of V-type but join in the middle of tergite in *T. fastigatus*. *Tanytarsus* **sp. n. 1** is also related to *T. jatai* Trivinho-Strixino *et al*. 2015 by the presence of lateral setae on anal point, but it is separated by the absence of a small triangular subapical lateral projection at apex of the anal point, which is otherwise present in *T.jatai*. The shape of superior volsella are sufficient to differentiate these three species.

The type materials were collected with Shimabukuro´s trap (on a rock constantly wet by a fountain and on small rocky pool) at approximately 2,000 meters a.s.l.

***Tanytarsus* sp. n. 2**

Type material: Holotype male, Brazil, Minas Gerais State, Passa-Quatro, Pedra da Mina Mountain, 22˚25'410"S/44˚50'380"W 2575 m a.s.l., madicolous habitat, on rocks at the banks of a stream, leg E.M. Shimabukuro, G. Perbiche-Neves, V. Saito. 14.x.2014 (A3-40, LEIA-UFSCar).

Diagnostic characters. *Tanytarsus* **sp. n. 2** can be distinguished from other *Tanytarsus* species by the combination of the following characters: abdominal tergite without setae; anal point large, without crests but with setae on median part. Superior volsella semi oval with short projection on distal posterior margin; digitus very long, finger-like, extending beyond posterior margin of superior volsella.

Description

Adult male (n = 1). Small, total length 2.51 mm. Wing length 1.69 mm.

Coloration: Head, flagellum and maxillary palp yellowish. Thorax, abdomen and legs yellow.

Head: AR 0.30. Total length of antenna 744 µm. Eyes without dorsomedian extension; temporals 7; clypeus with 10 setae. Palpomeres deformed and not measured.

Thorax: Length 0.77 mm. Scutal tubercle absent; acrostichals 7, biserial, beginning near antepronotum; dorsocentrals 6; prealar 1; scutellars 4.

Wing: 0.42 mm wide, VR= 1.25. Wing membrane with few setae on distal portion. All wing veins setose.

Legs: Fore leg tibia bearing single spur, 28 µm long. Mid leg tibia bearing two pectinate spurs, 18; 26 µm long and two separated combs. Hind leg tibia bearing two pectinate spurs, 26; 32 µm long and two separated combs. Lengths and proportions of the legs as in Table 2.

TABLE 2. Lengths (in µm) and proportions of leg segments in *Tanytarsus* **sp. n. 2**, male.

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	775	419						
II	713	538	306	169	106	69	50	0.42
III	831	783	619	219	200	125	112	0.79

Hypopygium (Fig. 2a): Tergite IX without apical and median setae. Orolateral spine of laterosternite IX absent. Anal tergal bands V-type. Anal point 80 µm long, rounded at tip and with 6 spines in anteromedian part; anal crests absent. Superior volsella 89 µm long and 46 µm wide (Fig. 2b). Digitus elongate 94 µm long, extending beyond posterior margin of superior volsella (Fig. 2b). Median volsella as in Figure 2c. Gonocoxite length 77 µm long; gonostylus 103 µm long, somewhat elongate and slender; hypopygium ratio (HR) 0.74.

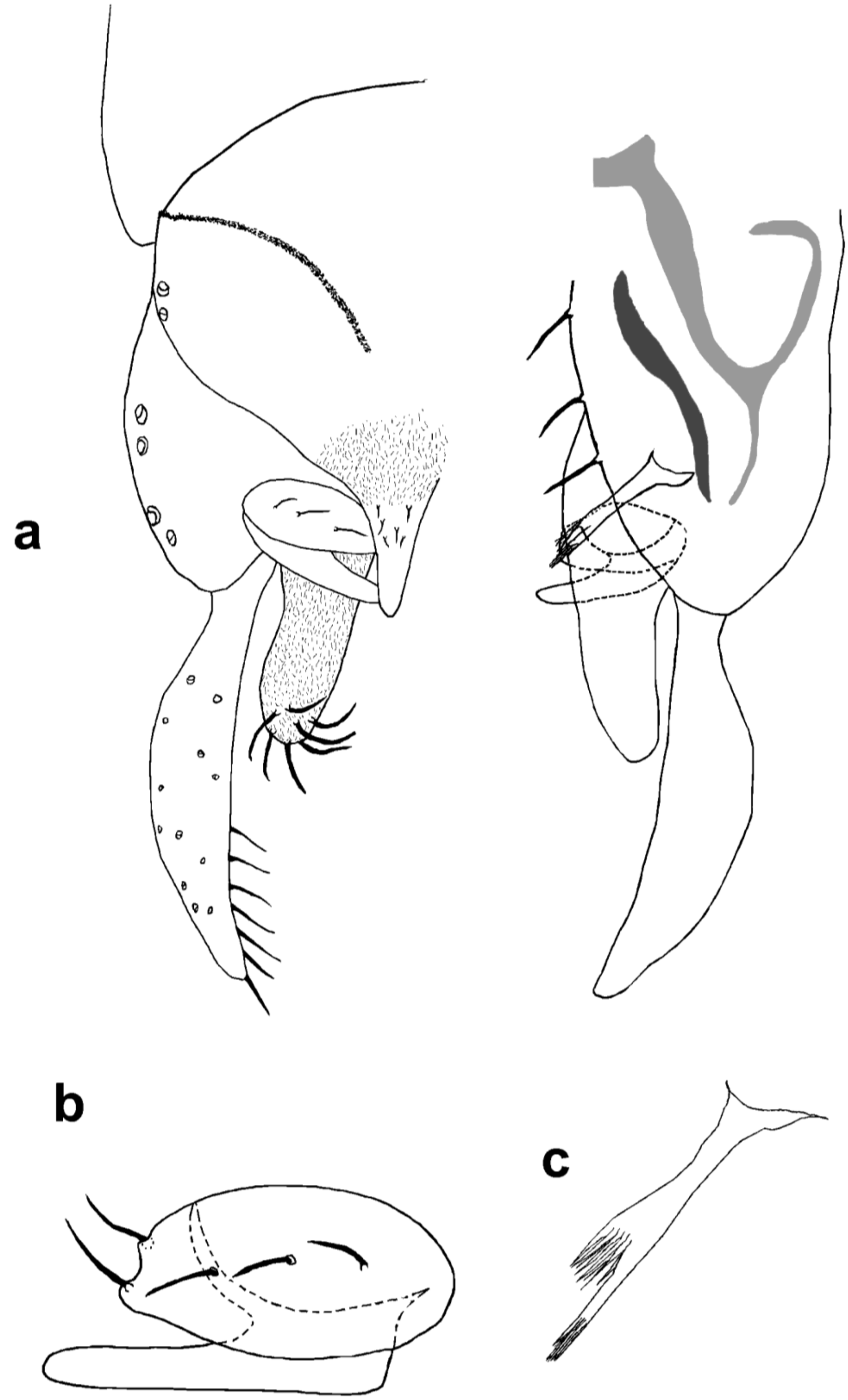

Figure 2. *Tanytarsus* **sp. n. 2 a**. Hypopygium, left dorsal view, right dorsal view, tergite IX removed. **b.** Median volsella. **c.** Superior volsella.

Remarks

Tanytarsus **sp. n. 2** also shares the absence of anal crest and V-type anal bands with other Neotropical species reviewed by Sanseverino *op .cit.* The main differential characteristic is the setae in the middle of the anal point, the shape of digitus which extends below the lower margin of superior volsella and the shape of median volsella.

The type material was collected with emergence traps at the edges of a first order stream at approximately 2,500 m a.s.l.

***Tanytarsus* sp. n. 3**

Type material: Holotype male, Brazil, Minas Gerais State, Passa-Quatro, Pedra da Mina Mountain, 22˚25'410"S/44˚50'380"W 2575 m a.s.l., madicolous habitat, rock marginal to a stream, leg E.M. Shimabukuro, G. Perbiche-Neves, V. Saito. 14.x.2014 (A3-37, LEIA-UFSCar). 1 Paratype female, same data as holotype (A3-38, LEIA-UFSCar).

Diagnostic characters. The male of *Tanytarsus* **sp. n. 3** can be distinguished from other *Tanytarsus* species by the combination of the following characters: hairy eyes; strong sclerosed process in the head, which differs from current known frontal tubercles. Anal tergite bands separate; anal point with crests. Hypopygium with wide and robust gonocoxite and gonostylus.

Description

Adult male (n = 1) Small, total length 2.72 mm. Wing length 1.66 mm.

Coloration: Head and thorax brown, flagellum and maxillary palp, abdomen and legs yellowish in slide preparation.

Head: Antenna 681 µm long, with 12 flagellomere. AR 0.35. Eyes with large dorsomedian extension and hairy (Figs 3a, b); temporal 6; clypeus with 10 setae. Prominent cephalic process 48 µm long and 35 µm large (Fig.3a). Palpomeres 2–4 with 28, 109, 63 µm.

Thorax: Length 0.72 mm. Scutal tubercle absent; acrostichals 6, biserial, dorsocentrals 6, prealar 1, scutellars 4.

Wing: 0.42 mm width, VR= 1.48. Wing membrane with setae on distal portion. All wing veins setose.

Legs: Fore leg bearing single tibial spur, 28 µm long. Mid leg tibia bearing two pectinate spurs, 15; 34 µm long and two separated combs. Hind leg tibia bearing two tibial pectinate spurs, 15; 36 µm long and two separated combs. Lengths and proportions as in Table 3.

TABLE 3. Lengths (in µm) and proportions of leg segments in *Tanytarsus* **sp. n. 3**, male.

	fe	ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR
I	675	394	618	344	256	181	106	1.57
II	681	506	281	163	135	87	50	0.56
III	750	625	387	225	206	119	81	0.62

Hypopygium (Fig. 4a): Tergite IX without apical and median setae. Orolateral spine of laterosternite IX absent. Anal tergal bands V-type. Anal point 51µm long, large and rounded at tip; anal crests present. Superior volsella near 50 µm long and 40 µm wide. Digitus short, extending weakly beyond the inner of margin of superior volsella. Median volsella as Figure 4. Gonocoxite length 62 µm long; gonostylus 100 µm long, somewhat elongate and slender; hypopygium ratio (HR) 0.62.

Adult female (n=1). Small. Wing length 1.60 mm.

Colouration: Head and thorax brown; abdomen and legs yellowish in slide preparation.

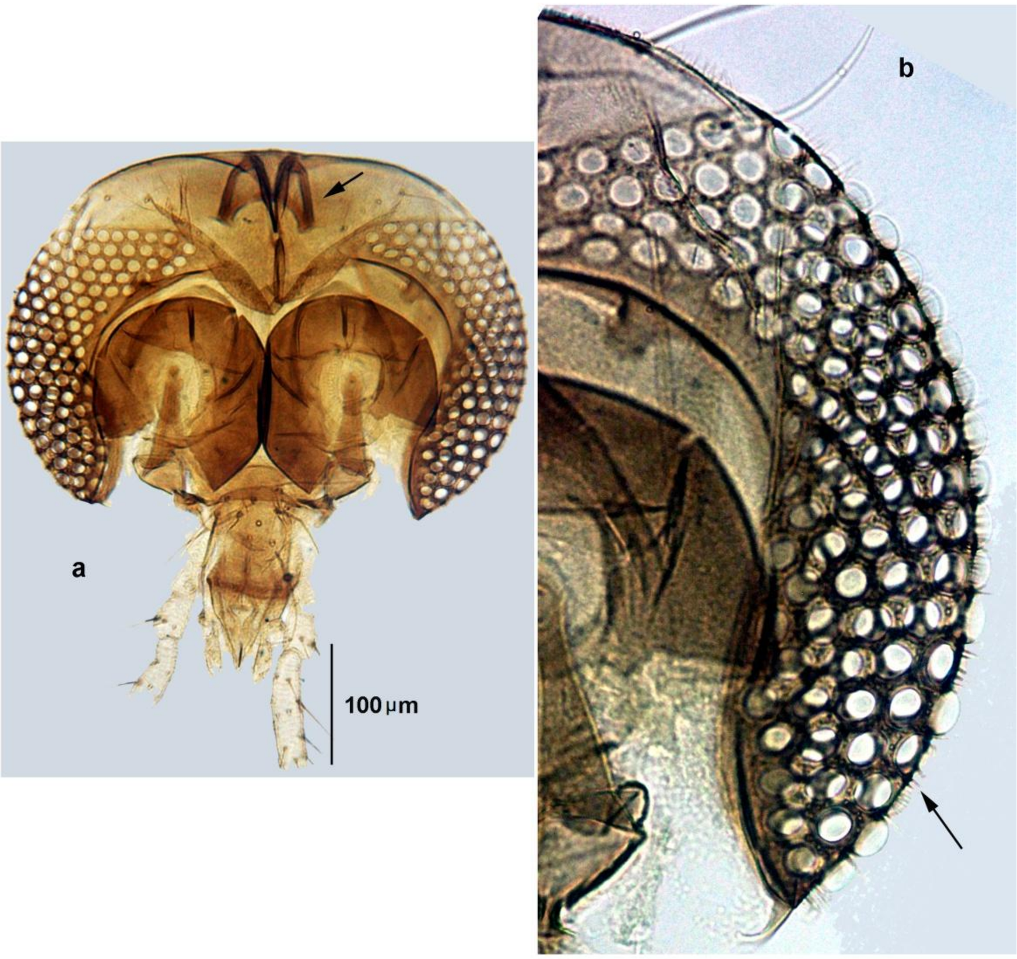

Figure 3. *Tanytarsus* **sp. n. 3 a.** Head, with indication of dorsal process. **b.** Detail of the eyes view with indication of the hairs.

Head: Antenna lost. Eyes hairy with short dorsomedian extension; temporal 6; clypeus with 13 setae Palpomeres 2–5 with 15, 81, 81, 125 μm.

Thorax: Length 1.92 mm. Scutal tubercle absent; acrostichals 4, dorsocentrals 9, prealar 1, scutellars 3.

Wing: 0.47 mm width, VR= 1.61. Wing membrane with setae on distal portion. All wing veins setose.

Legs: Fore leg bearing single tibial spur, 26 µm long. Mid leg tibia bearing two tibial pectinate spurs, 21; 31 µm long and two separated combs. Hind leg tibia bearing two pectinate spurs, 28; 34 µm long and two separated combs. Lengths segments distorted and not measured.

Genitalia (Fig.4b). Sternite VIII bearing 12 setae distributed regularly, on each side. GcaVIII curved, running diagonally to posterior corner of SVIII. SVIII form a short floor under anterior part of vagina; posteromedian contour of SVIII rounded. GpVIII long, and covered with caudolateral microtrichia. Notum long, 143 µm, 5 times longer than free rami. Seminal vesicles ovoid, near 65 µm long, without neck. Spermathecal duct not discernible in slide preparation. GcIX without setae. Postgenital plate short, triangular, slightly larger than long. Cerci near 54 µm long.

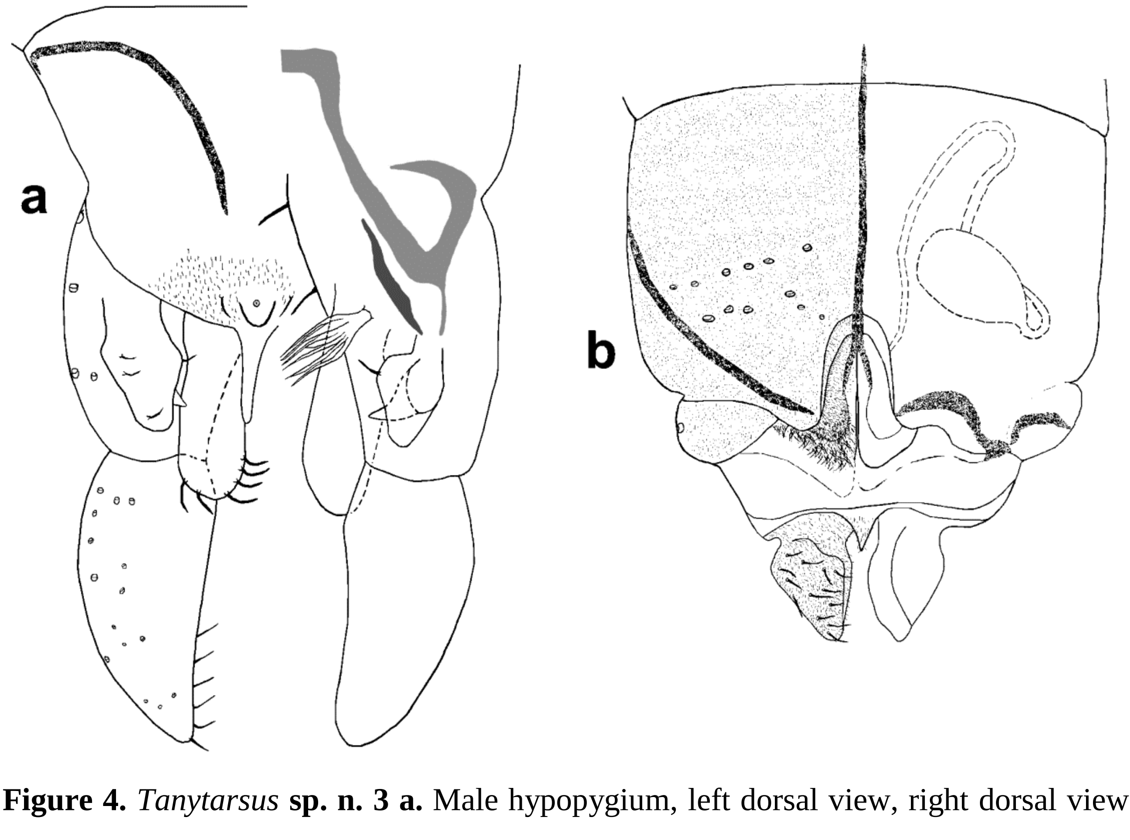

Figure 4. *Tanytarsus* **sp. n. 3 a.** Male hypopygium, left dorsal view, right dorsal view, tergite IX removed. **b.** Female genitalia, ventral view

Remarks

The main characteristics that differentiate *Tanytarsus* **sp. n. 3** are the hairy eyes present in both male and female head (uncommon among Tanytarsini tribe and not yet registered in *Tanytarsus*) and the large sclerotic process in male's head that does not appear to be the frontal tubercle. In addition, the presence of the robust hypopygium with strong gonocoxite and gonostylus seems also unusual. The presence of anal tergal bands V-type and anal crest with only 1 spine present in *Tanytarsus* **sp. n. 3** is also observed in *T. monospinosus* Ekrem & Reiss 1999 and *T. jacaretingensis* Sanseverino 2006 but the hairy eyes in *Tanytarsus* **sp. n. 3** is sufficient to differentiate from these species.

Paratanytarsus silentii Trivinho-Strixino, 2010

The male of *P. silentii* was previously collected by F. Roque in 2000 in a little stream called Córrego do Silêncio located at Parque Estadual do Jaraguá, in São Paulo city. Until this date the immature stages were unknown. Here we describe the pupal exuviae and IV instar larvae, and report new occurrences for the species.

Material examinated: Three 4th instar larvae, Brazil, São Paulo State, Ubatuba, Concorvado Peak, , 23˚27'876"S/45˚11'911"W 206 m a.s.l., lateral of a small waterfall, leg E.M. Shimabukuro, G. Perbiche-Neves. 27.x.2013 (A3-31, A3-32, A3-33, LEIA-UFSCar). 2 pupal exuviae in the same slide preparation (A3-30, LEIA-UFSCar) and 2 male of the same locality (A3-34, A3-35, LEIA-UFSCar).

Other records: 1 male, Brazil, São Paulo State, Ubatuba, Concorvado Peak, 23˚26'823"S/45˚11'585’’W 1082 m a.s.l., small current of water in a narrow rocky channel, leg E.M. Shimabukuro, G. Perbiche-Neves. 27.x.2013. 1 male, Brazil, Rio de janeiro State, Teresópolis, Sino Mountain, 22°26'848'S/43°00'798"W 1444 m a.s.l. marginal rock of a stream, leg E.M. Shimabukuro, G. Perbiche-Neves. 29.vii.2014

Description

Pupa (n = 2). Length of abdomen 2.27 (1) mm.

Cephalothorax. Frontal setae elongate, slender, 57 µm long; cephalic tubercles absent. Frontal apotome smooth (Fig. 5b). Thoracic horn elongate, c. 295 µm long, with fringe of setae (Fig. 5a). Thorax with weak granulation close to anterior median suture; scutal tubercle absent (Fig. 5a). Wing sheath with short nose; pearl row absent. Chaetotaxy of thorax: 3 precorneals (Pc 1-3) and 1 lateral antepronotal (Lap. 3) present on each side. Dc 1-4 situated in two groups, widely separated.

Abdomen. Tergite I without shagreen; II with shagreen in all extension, hardly anteriorly; III with median longitudinal patches of shagreen, hardly anteriorly; IV and V with lateral paired patches of long spines, and 2 circular field of short spines anteriorly in addition to 3–4 long spines (Fig. 5c); VI to VIII without shagreen. Hook row continuous, occupying 1/2 width of segment II. Pedes spurii B short. Segment VIII with posterolateral combs consisting of 2–3 marginal brown teeth. Segments I–IV with 0, 3, 3, 1 L setae, respectively; segments IV–VIII with 1, 3, 4, 4 and 5 taeniae seta. Anal lobe with about 28–30 taeniate fringe setae and 1 distal large dorsal taeniae on each side.

4th instar larva (n =3). Small, total length 4.53 mm [3.92–4.58].

Head. Length 317 µm, width 232 µm (1). Clypeal seta (S3) simple.

Antenna (Fig. 5e). With 5 segments placed on prominent pedestal, 41 µm long (1); basal segment 115 µm [108–121], longer than flagellum and slightly bent outside; basal ring organ near base; seta 24 µm long in distal ½ part; segment 2 (32 µm) slightly longer than segments 3–5 (12, 8, 5 µm); Lauterborn organs short, pedicels near 22 µm long, slightly shorter than segments 3–5. Pecten epipharyngis consisting of 3 distally serrated scales (Fig. 5d). Premandible with 2 teeth (Fig. 5f); brush well developed.

Mandible (Fig Xg) 86 µm long with dorsal, apical and two inner teeth brown.

Mentum (Fig. 5h) 68 µm long [58–77] with median tooth slightly notched laterally and 5 pairs of lateral teeth all brownish. Ventromental plates slightly longer than mentum close together medially.

Abdomen. Anal tubules slightly shorter (near 100 µm long) than parapods. Claws of posterior parapods simple. Eleventh abdominal with a posterior dorsal hump (Fig. 5i).

Remarks

The main characteristics that differentiate the *P. silentii* pupal exuviae from *P corbii* Trivinho-Strixino, 2010 is the absence of cephalic tubercles and the posterior pair of long spines bands on tergite III in the former. The larvae differentiate by the short pedicels of Lauterborn organs and the unusual presence of a dorsal hump on eleventh abdominal segment.

New *Tanytarsus* records: *Tanytarsus giovanni* Sanseverino & Trivinho-Strixino, 2010 male, Brazil, Minas Gerais State, Passa-Quatro, Pedra da Mina Mountain, 22˚25'410"S/44˚50'380"W 2575 m a.s.l., madicolous habitat, rock marginal to a stream, leg E.M. Shimabukuro, G. Perbiche-Neves, V. Saito. 14.x.2014 (A3-46, LEIA-UFSCar). *Tanyarsus digitatus* Sanseverino, 2006 1 male, Brazil, Rio de Janeiro State, Teresópolis, Sino Mountain, 22°26'848"S/43°00'798"W, 1444 m a.s.l. marginal rock of a stream, leg E.M. Shimabukuro, G. Perbiche-Neves. 29.vii.2014 (A3-47, LEIA-UFSCar); 1 male, Brazil, São Paulo State, Ubatuba, Concorvado Peak, 23˚28'20.72"S/45˚11'38.43"W 25 m a.s.l., lateral of a small waterfall, leg E.M. Shimabukuro, G. Perbiche-Neves. 27.x.2013 (A3-48, LEIA-UFSCar).

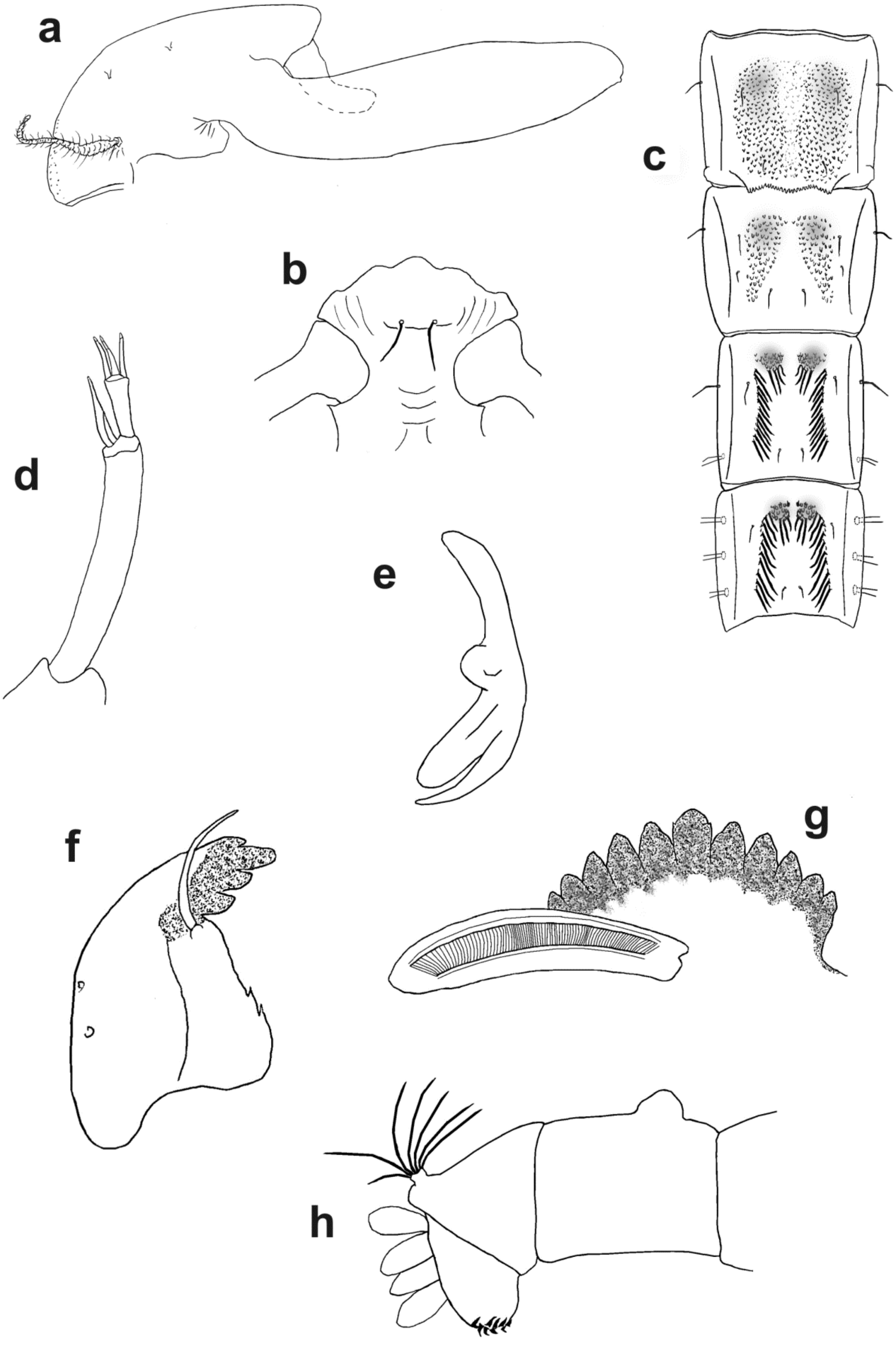

Figure 5. *Paratanytarsus silentii.* **a-c**; Pupa; **d-i**. Larva. **a.** Thorax lateral view. **b**. Frontal apotome. **c.** Abdominal segments II-V. **d.** Pecten epipharyngis. **e**. Antenna. **f.** Premandible. **g.** Mandible. **h.** Mentum. **i.** Abdomen posterior.

DNA barcoding

We were able to obtain only four sequences from the 40 specimens submitted for sequencing, one Male of *Tanytarsus* **sp. n. 1** (number of specimen BOLD), one Male of *T. giovannii* (number of specimen BOLD), and two larvae from unknown *Tanytarsus* sp. (BOLD Numbers). The interspecific divergence between *T. giovannii* and *Tanytarsus* **sp. n. 1** was approximately 25.5%, between *T. giovannii* and *Tanytarsus* sp. was 20.5% and between *Tanytarsus* **sp. n. 1** and *Tanytarsus* sp. was 22% (Figure 6). These results agree with the maximum distance expressed within the Tanytarsini tribe (25%) reported by Ekren et al. (2007). DNA barcode has been proving to be highly effective in separating Chironomidae species (Ekren et al. 2007; Trivinho-Strixino et al. 2012; Silva et al. 2012). The minimum interspecific distance for *Podonomus* species from Brazilian mountains was 10.3%, while the maximum intraspecific genetic divergence was 2.5% (Trivinho-Strixino et al. 2012). For *Hudsonimyia* species, also from Brazilian mountains, the maximum intraspecific divergence was 0.92%, and the minimum distance between groups was 11.26% (Silva et al. 2012). Although we obtained only a limited number of sequences in this study, the high molecular divergences found (over 20%) among the taxonomic units (morphological species) are an additional evidence of the differences among the species. Information on the specimens is presented in table 4.

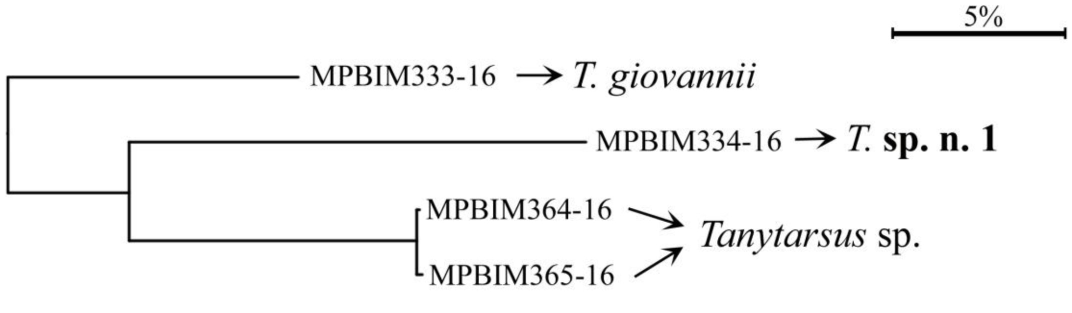

Figure 6. The neighbor-joining tree of *Tanytarsus* specimens.

TABLE 4. List of specimens of *Tanytarsus* with mtDNA COI sequences (DNA Barcoding) in the present study.

Taxon	Locality	Collection Reference	Coordinates	Altitute m a.s.l.	GenBank accession numbers
Tanytarsus giovannii male	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 8/Oct/2014	MPBIM-333	22˚25'41"S 44˚50'38"W	2575	KY810489
Tanytarsus **sp. n.1** male	Brazil, Rio de janeiro, Teresópolis, Pedra do Sino, unnamed site, 29/Jul/2014	MPBIM-334	22°27'605'S 43°01'689"W	1444	KY810488
Tanytarsus sp. larva	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 8/Oct/2014	MPBIM-364	22˚24'535"S 44˚50'813"W	2100	KY810490
Tanytarsus sp. larva	Brazil, Minas Gerais, Passa Quatro, Pedra da Mina, unnamed site, 8/Oct/2014	MPBIM-365	22˚24'535"S 44˚50'813"W	2100	KY810491

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CONCLUSÕES E CONSIDERAÇÕES FINAIS

Importantes mudanças foram observadas na comunidade de insetos madícolos nos gradientes de altitude analisados. A riqueza variou consistentemente ao longo dos três gradientes analisados, de maneira que cada gradiente/montanha demonstrou um padrão diferente de variação conforme a altitude se elevava. Ao analisar todo o patamar altitudinal – de 0 a 2700 m – foi possível verificar um padrão unimodal de variação da riqueza com a altitude, de maneira que o pico de riqueza foi encontrado exatamente em altitudes intermediárias, em torno de 1500 m acima do nível do mar. Ao testar as prováveis causas dessa variação, chegou-se a conclusão de que as mudanças climáticas e energéticas ao longo do gradiente foram consideravelmente sentidas pela comunidade de insetos madicolos. As variáveis ambientais mais importantes para o padrão observado na riqueza foram: temperatura, oxigênio, luminosidade e produtividade (medida através da taxa de emergência de insetos madícolos). Entretanto, o fato de se ter lidado com os limites altitudinais regionais (em termos de bioma), pode ter intensificado a importância do efeito de domínio central (mid-domain effect), e assim as espécies tenderam a sobrepor sua ocorrência na região central do gradiente, contribuindo também para o padrão observado. A dissimilaridade geral na composição de espécies entre faixas altitudinais foi consideravelmente elevada (cerca de 80% de dissimilaridade total), e também se intensificou significativamente ao longo desta. Os dados apresentados aqui confirmam a hipótese de que as espécies em montanhas tropicais tendem a apresentar uma curta faixa de distribuição altitudinal (“Mountain Passes are Higher in the Tropics” - MPHT). Consequentemente, para a maior parte da comunidade, houve uma substituição de espécies ao longo do gradiente. Porém, para alguns grupos taxonômicos no gradiente mais elevado, o *turnover* de espécies teve menor importância e o padrão de aninhamento determinou grande parte da dissimilaridade composicional entre as faixas altitudinais. Assim, partindo-se do princípio da variabilidade climática entre as linhagens de insetos aquáticos, sugere-se que táxons cujos centros de diversificação localizam-se em regiões temperadas possuem espécies com grande variabilidade de extensão altitudinal, que resultam primariamente em um aninhamento composicional ao longo do gradiente mais elevado. Devido à localização e formação dessas montanhas é possível que, tanto o processo de colonização vertical quanto o horizontal influenciem a biodiversidade local, o que por sua vez pode explicar a elevada complexidade composicional da comunidade de insetos madicolos ao longo da altitude. Muitas das espécies de topo se mantêm nessa curta faixa altitudinal, o que também foi confirmado no *checklist* das espécies de Chironomidae. Além disso, ficou evidente que as regiões mais elevadas possuem uma grande porcentagem de espécies ainda não conhecidas (mais de 30% das novas espécies encontram-se acima de 2.100 m de altitude), e que por estarem em regiões altamente susceptíveis às mudanças climáticas podem se extinguir antes mesmo de serem descobertas e descritas. Os habitats madícolos se apresentaram altamente diversos, com mais de 160 gêneros e espécies (aproximadamente 60 apenas na família Chironomidae) e possivelmente, com elevado grau de endemismo. A escassez de estudos em habitats madícolos também contribuiu para o elevado número de novas espécies evidenciado, e, apesar de neste estudo apenas nove espécies terem sido descritas, muitas outras ainda aguardam para serem apresentadas à ciência. Assim, este estudo ressalta a importância ecológica e taxonômica dos habitats madícolos em regiões montanhosas de Mata Atlântica, e que por tal motivo merecem atenção particular frente às mudanças globais.

ANEXOS

Tabela 1. Lista de táxons registrados no presente estudo. PESM: Parque estadual da Serra do mar (0 – 1.100 m), PARNASO: Parque Nacional da Serra dos Órgãos (1.200 – 2.100 m); APASM: Área de Proteção Ambiental da Serra da Mantiqueira (1.600 – 2.700).

Ordem	Família	Gênero/spécie	PESM	PARNASO	APASM
Diptera					
	Chironomidae				
		Podonomus	-	+	+
		Paraphaenocladius	+	+	+
		Thienemannia	-	+	+
		Caladomyia	+	+	+
		Rheotanytarsus	+	+	+
		Chironomini 1	+	+	+
		Corynoneura	+	+	+
		Ubatubaneura	+	-	-
		Tanytarsus	+	+	+
		Onconeura	+	+	+
		Riethia	-	-	+
		Manoa	+	+	-
		Cricotopus	+	+	+
		Cricotopus sp. 2	+	-	-
		Cricotopus sp. 3	+	-	-
		Thienemanimyia	+	+	+
		Pseudochironomini 1	-	-	+
		Pentaneura	+	+	+
		Parapentaneura	+	+	-
		Ablabesmyia	-	-	+
		Pseudochironomini 2	-	-	+
		Parametriocnemus	+	+	+
		Antilocladius	+	+	+
		Larsia	+	+	+
		Djalmabatista	-	+	+
		Hudsonimyia	+	+	+
		Psectrocladius	-	+	+
		Lmnophyes	+	+	+
		Cricotopus sp. 4	-	+	-
		Constempellina	+	+	-
		Stempelinella	+	+	-
		Phaenospectra	+	+	-
		Lopescladius	+	+	-
		Nanocladius	-	+	-
		Paratanytarsus	-	+	-
		Thienemanniela	+	+	-
		Polypedilum	+	+	-
		Gymnometriocnemus	-	+	-
		Nilotanypus	-	+	+
		Guassutanypus	-	+	-
		Lauterborniella	+	-	-
		Paratendipes (?)	+	+	+
		Beardius	+	-	+
		Chironomus	+	-	-
		Orthocladiinae 1	+	+	-
		Metriocnemus	-	+	-
		Pseudochironomini 3	-	+	+
		Orthocladiinae 2	-	-	+
		Nilothauma	-	-	+
	Simuliidae				
		Lutzsimulium flavopubescens	+	-	+
		Lutzsimulium sp. 1	-	+	+

Ordem	Família	Gênero/espécie	PESM	PARNASO	APASM
		Simulium sp. 1	+	+	+
		Simulium sp. 2	+	+	-
	Ceratopogonidae				
		Dasyhelea	+	+	-
		Atrichopogon	+	+	-
		Forcipomyidae	+	+	-
	Stratiomyidae		-	-	+
	Dixidae		+	-	-
	Canacidae		+	-	-
	Empididae		+	+	+
	Dolichopodidae		+	-	+
	Tipulidae		+	+	+
	Phoridae		+	-	-
	Tabanidae		+	+	-
	Psychodidae				
		Pericoma	+	+	-
		Maruina	+	+	+
		Psychodidae gen. 1	+	+	-
	Athericidae		-	-	+
	Thaumaleidae (?)		+	+	-
Plecoptera					
		Tupiperla	-	+	+
		Guaranyperla	-	+	+
		Gripopterix	+	+	+
		Paragripopteryx	+	+	+
		Kempnyia	+	+	-
		Macrogynoplax	+	-	-
		Anacroneura	+	-	+
		Enderleina	-	+	-
Coleoptera					
		Hydrophilidae	+	-	+
		Gênero M	+	-	+
		Gênero X	+	-	+
		Scirtidae	+	+	-
		Phanocerus	+	+	-
		Neoelmis	+	+	-
		Promorese	-	+	-
		Heterelmis	+	+	-
		Psephenus	-	+	-
		Psephenops	+	-	-
		Lutrochidae	-	+	-
		Xenelmis	+	+	-
		Microcylloepus	+	-	+
		Torridincolidae	+	-	+
		Macrelmis	+	-	-
		Pitlodactylidae	+	-	-
		Austrolimnius	+	-	-
		Stegoelmis	+	-	-
		Enochrus	-	-	+
		Hexanchorus	-	-	+
Ephemeroptera					
		Ephemeroptera	+	-	-
		Libelulinae	+	-	-
		Campylocia	-	+	-
		Massartella	-	+	-
		Askola	-	+	-
		Leptophebiidae	+	+	-
		Apobaetis	-	+	-
		Cryptonynpha	+	-	-
		Americabaetis	+	+	+

Ordem	Família	Gênero/espécie	PESM	PARNASO	APASM
		Tupiara	+	+	-
		Dactylophlebia	+	-	-
		Paracloedes (?)	+	+	+
		Cloedes	+	-	-
		Baetodes	+	-	+
		Microphlebia	+	-	-
		Tricorythopsis	+	-	+
Trichoptera					
		Leptohyphidae	+	-	-
		Farrodes	+	-	-
	Xiphocentronidae		+	-	-
	Hydrobiosidae		+	-	-
		Nyctiophylax	+	-	-
		Smicridea	+	+	+
		Chimarra	+	-	-
		Hydroptila	+	-	+
		Cernotina	-	-	+
		Metrichia	+	+	-
		Anastomoneura	-	-	+
		Oxyethira	-	-	+
		Cynellus	+	-	-
		Polypectropus	+	-	-
		Leptonema	+	-	-
		Leptoceridae (Gênero A)	-	+	-
		Leptoceridae X	-	+	-
		Oecetis	-	+	-
		Nectopsyche	+	+	-
		Phylopotamidae	+	-	-
		Phyloicus	-	+	-
		Neotrichia	+	-	-
		Ochrotrichia	+	+	+
		Hydroptilidae 1	+	-	-
		Hydroptilidae 2	+	-	-
		Helichopsyche	+	+	+
		Alisotrichia	+	+	+
		Cerasmatrichia	+	-	-
		Hydrobiosidae gênero X	+	+	+
	Hydrobiosidae		+	-	-
	Anomalopsychidae		+	+	-
		Prox Anomalopsychidae	+	-	-
		Marilia	+	+	-
		Leucotrichia	+	-	-
		Polycentropodidae sp 1	+	-	-
		Flintiella	+	-	-
		Glossomatidae	+	-	-
		Tricplectides	-	+	-
		Polyplectropus	-	+	-
		Notidobiella	+	-	-
		Grumichella	+	+	-
Hemiptera					
		Neotrephes	-	+	-
		Limnocoris	+	-	-
Lepidoptera					
		Pretrophila	+	+	-
		Parapoynx	+	-	-
Odonata					
	Megapodagrionidae		+	-	-
	Coenagrionidae		+	-	-
		Argia	-	+	-
		Perithemis	+	-	-

Figura 1. Alguns habitats madícolos do presente estudo. a. c. d. Serra do Mar (Pico do Corcovado) à 1.100 m de altitude; b. Serra do Mar (Pico do Corcovado) 200 m de altitude; e. g. Serra dos Órgãos (Pedra do Sino) à 1.500 m de altitude. f. casulo de Trichoptera em habitat higropétrico; h. Serra da Mantiqueira (Pedra da Mina) à 2.100 m de altitude; i. Serra da Mantiqueira (Pedra da Mina) à 2.500 m de altitude; j. k. ambiente higropétrico congelado à 2.700 m de altitude; i. rocha com organismos madícolas, dentre eles *Podonomus* sp.

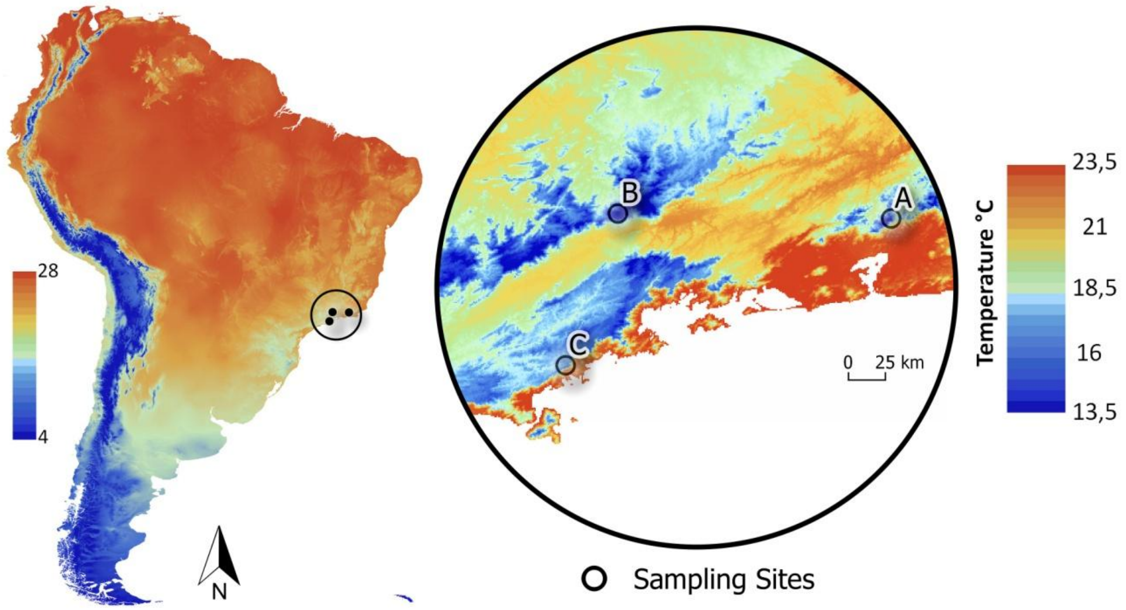

Figura 2. Mapa com a alocalização das areas de estudo, evidenciando perfis climáticos de cada região.