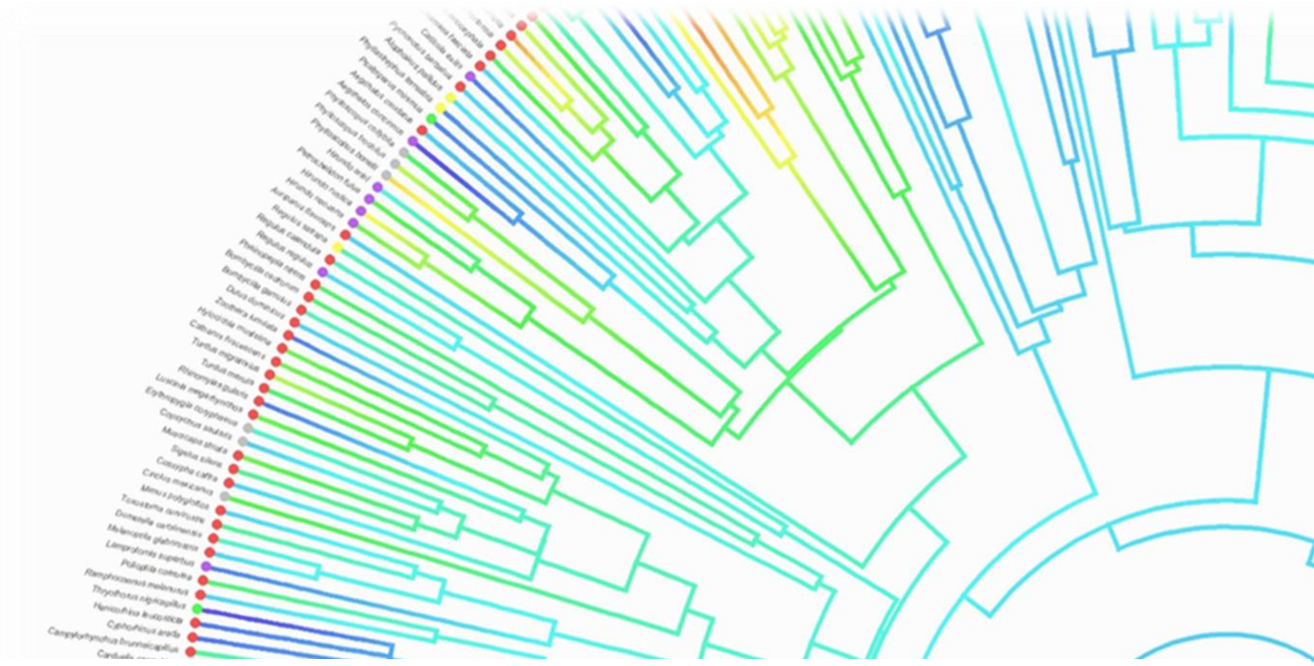
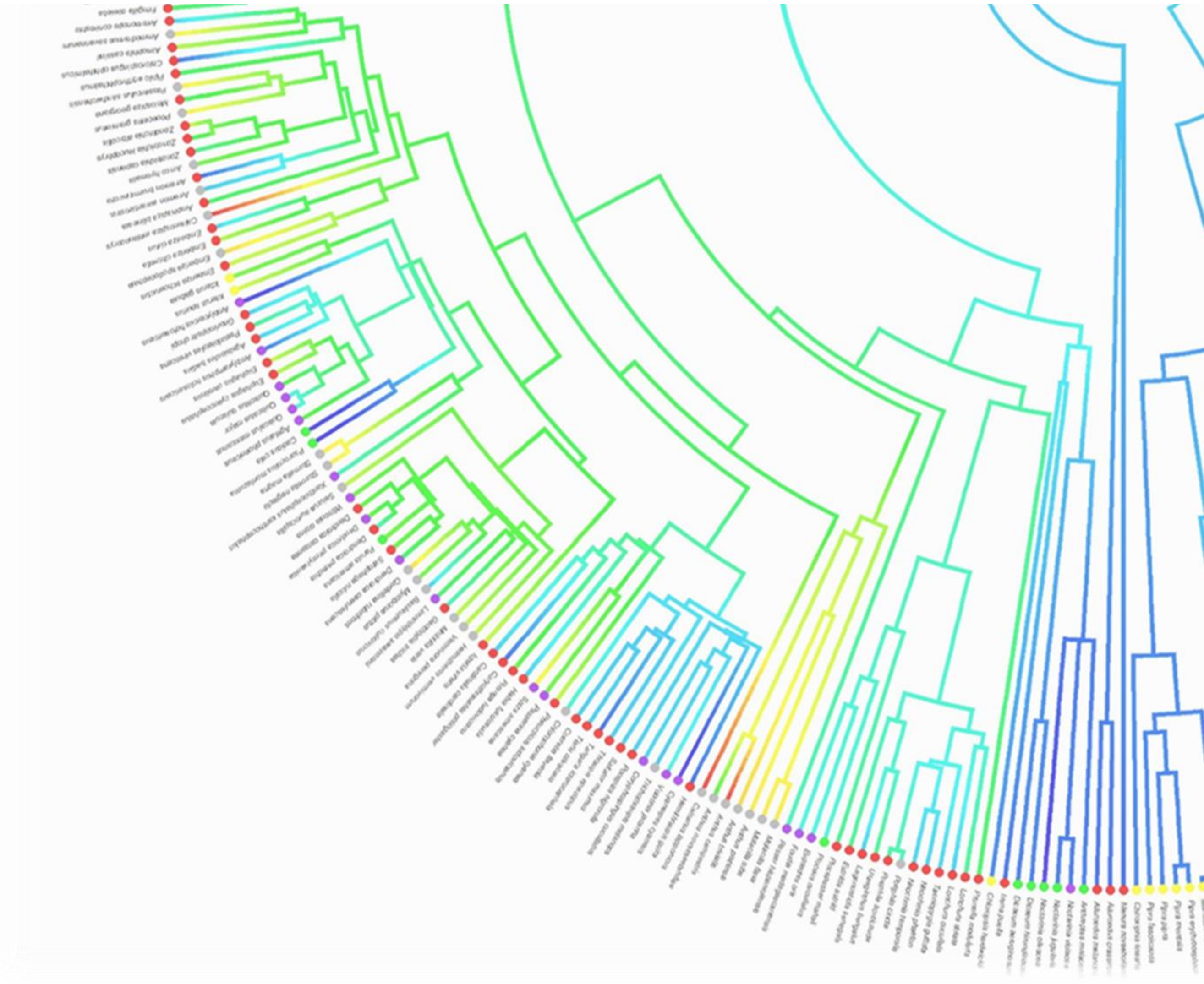


ANCESTRALIDADE E TRAJETÓRIAS EVOLUTIVAS DE NINHOS EM TRÊS FAMÍLIAS DE AVES: FURNARIIDAE, TROGLODYTIDAE E ICTERIDAE



ABRAÃO DE BARROS LEITE

UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

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São Carlos-SP
2024

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Tese apresentada ao Centro de Ciências Biológicas e da Saúde (CCBS) 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) junto ao Programa de Pós-Graduação em Ecologia e Recursos Naturais (PPGERN).

Orientador: Prof. Dr. Mercival Roberto Francisco

São Carlos-SP
2024

UNIVERSIDADE FEDERAL DE SÃO CARLOS

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

Folha de Aprovação

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O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Ecologia e Recursos Naturais.

Dedico este trabalho à minha esposa Greyce Kelly, e aos meus pais, Aparecida e Manoel Messias (*in memoriam)*

AGRADECIMENTOS

Aos membros da banca que gentilmente aceitaram o convite.

Agradeço à minha esposa Greyce Kelly por sempre acreditar em mim, e sempre me encorajar a dar o meu melhor na minha carreira acadêmica. Agradeço aos meus queridos pais Aparecida e Manoel Messias (*in memoriam)*, pessoas que me educaram com todo amor e carinho, assim me preparando para a vida da melhor maneira possível, fazendo-me tornar a pessoa de caráter que sou hoje. Já aproveito para agradecer também à minha querida avó Júlia (*in memoriam)*, uma pessoa que pude discutir sobre qualquer assunto, ela foi para mim uma grande fonte de conhecimento.

Agradeço com igual importância, ao meu sogro Gilson e a minha sogra Elaine, também muito importantes na minha vida, que tornaram este sonho possível.

Ao meu orientador Prof. Dr. Mercival Roberto Francisco, pela paciência, pela dedicação, pela confiança que depositou em mim desde o início, acreditando sempre no meu potencial, me apoiando e ensinando em todos os momentos, compartilhando assim seus conhecimentos e esclarecendo muitas dúvidas ao longo da execução deste trabalho. Quero deixar claro minha admiração pelo seu excelente profissionalismo, e minha eterna gratidão pela amizade e confiança depositada em mim.

Agradeço à Universidade Federal de São Carlos (UFSCar) – Campus São Carlos e também a todos os funcionários.

Agradeço ao Programa de Pós-Graduação em Ecologia e Recursos Naturais e também a todos os professores e funcionários da universidade que me apoiaram e me ensinaram durante todo o meu doutorado.

Agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES), pela bolsa de doutorado concedida.

Agradeço ao recurso financeiro recebido pelo Proap - Código de Financiamento 001.

Minha eterna gratidão a todos!

“Há uma tendência na natureza à progressão contínua de certos tipos de variedades que estão cada vez mais se afastando da classe original”

Alfred Russel Wallace

RESUMO

Os ninhos das aves desempenham importantes papéis para o sucesso evolutivo deste grupo de organismos. Por isto, o entendimento sobre as origens, trajetórias evolutivas e as variáveis ambientais correlacionadas com a diversificação destas estruturas são importantes para a compreensão da evolução das próprias aves. Nesta tese, subdividida em 3 capítulos, são abordados os aspectos evolutivos dos ninhos em 3 famílias de aves com distribuição principalmente no Novo Mundo. Os representantes da família Icteridae constroem uma ampla variedade de ninhos e utilizam diferentes locais para a nidificação, com algumas espécies também utilizando ninhos velhos de outras aves ou sendo parasitas de ninhos. No capítulo 1, nós avaliamos a origem e as trajetórias evolutivas dos ninhos e habitats dos Icteridae; testamos a hipótese de que o uso de ninhos velhos possa ter sido uma etapa intermediária para o surgimento do parasitismo e avaliamos a premissa de que a construção de ninhos complexos, como os ninhos fechados dependurados, está associada às aves de menor porte devido à necessidade de manipulação de materiais mais finos. O ancestral comum da família provavelmente construiu ninhos abertos, com material vegetal não fibroso e apoiados na vegetação pela parte inferior. Os tipos de fixação dos ninhos na vegetação foram mais correlacionados com os habitats do que os próprios tipos de ninhos e os materiais de construção. As premissas de que o uso de ninhos velhos possa ter sido uma etapa para a evolução do parasitismo, bem como a premissa de que ninhos complexos são construídos por espécies menores, foram rejeitadas. Os representantes da família Troglodytydae constroem ninhos abertos dentro de cavidades, ninhos fechados sobre a vegetação e alguns poucos representantes constroem ninhos fechados dento de cavidades. No capítulo 2, os tipos de ninhos dos Troglodytydae foram classificados como abertos e fechados e as cavidades ou vegetação foram consideradas como locais de nidificação. Foi observado que o ancestral comum desta família provavelmente construiu ninhos fechados dentro de cavidades e a irradiação do grupo foi caracterizada por transições para ninhos abertos dentro de cavidades ou retenção de ninhos fechados que passaram a ser construídos sobre a vegetação, havendo correlação evolutiva entre o tipo de ninho e local de nidificação. Além disso, foi observado que o uso de locais fechados para a nidificação foi correlacionado com tamanhos corporais menores, provavelmente devido à redução da competitividade pelo uso de cavidades. No capítulo 3, as aves da família Furnariidae foram utilizadas para o teste da hipótese evolutiva de que mudanças nos tipos de ninhos possam ter sido inovações chave (*key innovations*), ou seja, constituíram mudanças evolutivas que permitiram o acesso a nichos novos (estratégia de forrageio e habitat). A evolução dos tipos de ninhos foi fortemente correlacionada e precedeu as mudanças de estratégias de forrageio e de habitats, dando suporte à hipótese dos ninhos como inovações chave que contribuíram para a irradiação deste grupo. Estas foram as primeiras evidências sobre o efeito das mudanças nos tipos de ninhos sobre o forrageamento. Esses resultados contribuíram com um maior entendimento não apenas sobre a evolução das aves das famílias abordadas, mas também com a elucidação de questões relacionadas às aves em geral.

Palavras-chave: Icteridae, Troglodytidae, Furnariidae, Habitat, Reconstrução ancestral, Correlações evolutivas, Sinal Filogenético, Evolução de ninhos, Parasitismo de ninhos, Inovações chave

ABSTRACT

Bird nests play important roles in the evolutionary success of this organismal group. For this reason, understanding the origin, evolutionary trajectories and the environmental parameters correlated with nest diversification is important for the understanding of Avian evolution. In this thesis, subdivided into 3 chapters, the nest evolutionary aspects of 3 bird families with distribution mainly concentrated in the New World are addressed. The birds of the family Icteridae build many different nest types, in a variety of habitats, with some species also using old nests from other birds, and others are brood parasitic. In chapter 1, we examined the origin and evolutionary patterns of nest traits and habitat in the Icteridae. We also tested the hypothesis that the use of abandoned nests of other species (non-nest construction) is an intermediate step in the evolution of interspecific brood parasitism, and we addressed the hypothesis that the construction of complex nests is linked to small-bodied species. The common ancestor of the Icteridae likely constructed cup-shaped nest, basally supported, and built of leaves/sticks in woodlands. We detected significant evolutionary correlations between nest traits, with nest attachment modes being more often correlated with habitat than nest type and material. We rejected the idea that brood parasitism evolved from non-nest construction, and nest complexity increased with body size, likely due to shifts in nest materials permitting the construction of more resistant nest walls. Birds of the family Trogloditydae build open nests within cavities and enclosed nests on the vegetation, with some a few representatives also constructing enclosed nests within cavities. In chapter 2, we considered open and enclosed as nest types and cavity and vegetation as nest sites. We found that the common ancestor of the Troglodytidae likely constructed enclosed nests within sheltered sites (cavity or crevice), but the radiation of the group was characterized by shifts to exposed nest sites (vegetation) with retention of enclosed nests or retention of sheltered sites with nest simplification (cup nests). Nest site and nest type evolved interdependently, with habitat being poorly correlated with nest evolution. Sheltered nest sites were associated with small body size, likely to avoid competition with other animals for these places. In chapter 3, we used the family Furnariidae to test the hypothesis of nests as key innovations, i.e. the emergence of new traits that can affect transitions to new ecological niches. We addressed the hypothesis of nest type transitions as key innovations for two important proxies of niche diversification, foraging strategy and habitat. We found strong evolutionary correlations between nest type and both foraging strategy and habitat, with model selection and ancestral state reconstructions evidencing nest type shifts preceding niche transitions, confirming the idea of nests as key innovations for the irradiation of this group. This is the first evidence for nest transitions promoting new foraging strategies. These results improved the understanding not only about the evolution of the studied avian families, but also elucidated questions of broad ornithological interest.

Keywords: Icteridae, Troglodytidae, Habitat, Ancestral state reconstruction, Evolutionary correlation, phylogenetic signal, nest evolution, nest parasitism, key innovation

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AIC – Akaike Information Criterion

AICc – Akaike Information Criterion Corrected

ARD – All Rates Different

BPMMs – Bayesian Phylogenetic Mixed Models

Cis – Credibility Intervals

D – Phylogenetic Signal Value

ΔAIC – Delta Akaike Information Criterion

ER – Equal Rates

IC – Intervals Confidence

fdr – False Discovery Rate

MCMC – Markov Chain Mont Carlo

MCMCglmm – Markov Chain Mont Carlo Generalized Linear Mixed Models

Rc – Reference Value of Convergence Chain

SE – Standard Errors

SYM – Symmetric Entities

P – Significance Value

W – Statistic Value Shapiro-Wilk Test

SUMÁRIO

1. INTRODUÇÃO GERAL

1.1. As aves e os ninhos

Os ninhos para o grupo das aves têm importantes funções ecológicas, que seriam de garantir proteção para os ovos e filhotes contra predadores e contra os fatores climáticos (Mainwaring *et al*., 2014; Colombo *et al*., 2024). Durante a evolução das diferentes famílias das aves surgiram os mais variados tipos de ninhos, por exemplo, estruturas que são plataformas rudimentares construídas apenas com gravetos, até as estruturas mais elaboradas como os ninhos fechados e suspensos (Price & Griffith, 2017; Leite *et al.,* 2023). Mainwaring *et al*., (2023), em uma revisão baseando-se nas características dos ninhos, concluíram que tais estruturas são multifuncionais e que também podem apresentar uma ampla variação quanto a sua localização e formato. Além disso, a construção dos ninhos pode ser dividida em várias etapas, desde a escolha do local de nidificação, a escolha e coleta do material apropriado, e pôr fim, a fase de construção propriamente dita, uma etapa que exige da ave construtora uma alta habilidade de manipulação do material e o uso de técnicas refinadas para tecer as paredes do ninho (Collias, 1997; Hall, 2013; Fang *et al.,* 2018; Mainwaring *et al.,* 2023). Durante a irradiação das aves, os ninhos também tiveram uma grande importância no sucesso reprodutivo e na diversificação no modo de vida desse grupo, ao passo que as diferentes espécies foram se estabelecendo nos mais diversos habitats do planeta (Fang *et al.,* 2018).

1.2. Os primeiros estudos sobre a evolução dos ninhos das Aves

Ao longo de muitas décadas, os aspectos ecológicos que influenciam na construção dos ninhos vêm chamando a atenção dos ornitólogos, como por exemplo: as interações presa/predador, as características morfológicas dos parentais (tamanho corporal, tamanho cerebral, medidas de bico, medidas de tarso), a variação latitudinal do local de reprodução, o tipo de habitat de nidificação e mais recentemente as questões climáticas (Hansel, 2000; Street *et al*., 2022; Leite *et al*., 2023, Sheard *et al*., 2023; Barros-Leite & Francisco, 2024; Colombo *et al*., 2024).

Os primeiros trabalhos que focaram em entender a origem e trajetórias evolutivas dos ninhos eram principalmente descritivos, ou seja, sem abordagens analíticas e filogenéticas que pudessem quantificar as transições nas características dos ninhos que aconteceram durante a irradiação das aves (Collias, 1964;

Slagsvold, 1989). Collias (1997) apresentou uma ampla discussão descritiva sobre a origem e evolução dos ninhos, concluindo que a adaptabilidade e a variação no comportamento de construção de ninhos na ordem Passeriformes foi um dos fatores mais importantes na irradiação adaptativa e expansão dos nichos desse grupo de aves durante o período Cenozoico.

1.3. Métodos filogenéticos e as novas abordagens em estudos de evolução dos ninhos

Com o uso de dados genéticos houve um avanço no entendimento das relações filogenéticas bem como das trajetórias evolutivas dos ninhos. Winkler & Sheldon (1993), usando filogenias baseadas em sequenciamento genético, discutiram a evolução dos ninhos da família Hirundinidae baseando-se em 17 espécies dessa família, concluindo que o tipo de ninho ancestral das andorinhas eram ninhos escavados em barrancos. Também, estudos focados na família Furnariidae, um grupo com alta diversidade de tipos de ninhos, diferentes trabalhos usaram informações genéticas para analisar a diversidade estrutural dos ninhos ao longo da filogenia, como por exemplo o trabalho de Zyskowski & Prum (1999). Posteriormente, Irestedt *et al*. (2006), analisou a evolução dos furnarídeos baseando-se em uma filogenia construída a partir de DNA nuclear e mitocondrial. Os autores sugeriram que as mudanças na arquitetura do ninho tiveram um importante papel nessa família de aves, uma vez que, tais mudanças facilitaram o processo de diversificação e especialização desse grupo dentro dos novos habitats.

As novas abordagens em estudos de evolução dos ninhos foram possíveis a partir do desenvolvimento de diferentes métodos estatísticos em filogenia comparativa, os quais permitiram um melhor entendimento das relações evolutivas entre as variáveis ambientais e morfológicas frente às mudanças ocorridas nas características dos ninhos durante a irradiação das aves. Algumas destas análises envolvem estimativas de sinais filogenéticos, modelagens de reconstrução ancestral, correlações evolutivas e modelos lineares generalizados mistos com controle filogenético.

A análise de sinal filogenético baseia-se no princípio do conservatismo filogenético, ou seja, os caracteres de espécies filogeneticamente mais próximas são mais semelhantes do que os caracteres de espécies mais distantes (Garamszegi, 2014; Revell & Harmon, 2022). Portanto, as análises de sinal filogenético medem o quanto da diversificação dos caracteres é explicado pela proximidade filogenética aos invés de respostas evolutivas a variações ambientais. Os resultados são obtidos a partir de uma matriz de covariância entre as diferenças na medida de um atributo entre espécies e as distâncias filogenéticas entre elas, de maneira que se a covariação é alta em espécies filogeneticamente próximas, há um alto sinal filogenético (Blomberg *et al.,* 2002).

Na reconstrução ancestral é utilizada uma filogenia e posteriormente é selecionado o melhor modelo evolutivo para explicar os dados observados nas OTUs (*Operational Taxonomic Unit*) (Revell & Harmon, 2022). Em seguida, são estimadas as probabilidades dos nós ancestrais apresentarem os diferentes caracteres analisados. Pode-se afirmar que é um método que permite entender a evolução dos caracteres, quantificando as transformações entre os diferentes estados ao longo dos ramos de uma árvore filogenética. Existem duas aproximações para se fazer reconstruções do estado ancestral, uma delas é estimando-se o estado mais provável para cada nó numa filogenia usando uma função de Máxima Verossimilhança (Garamszegi, 2014) e a outra opção, amplamente usada atualmente, é uma aproximação Bayesiana que simula múltiplas reconstruções de estado ancestral sobre uma filogenia e estima a probabilidade posterior de cada um dos estados de caractere que podem ocorrer em cada um dos nós da filogenia (Pagel *et al*., 2004; Revell & Harmon, 2022).

A correlação evolutiva mede o quanto as transições de um caractere x depende das transições de um outro caractere y ao longo de uma filogenia (Revell & Harmon, 2022). Neste tipo de análise são utilizados quatro modelos: i) Independente: onde os caracteres não dependem um do outro para evoluir. ii) Interdependente: onde a evolução do caractere x depende da evolução do caractere y, assim como y depende da evolução de x. iii) a evolução do caractere x depende da evolução do caractere y, mas não o inverso. iv) a evolução do caractere y depende da evolução do caractere x, mas não o inverso. Assim, é possível descrever como os vários traços biológicos afetam a evolução um do outro, ou seja, como duas características tendem a evoluir juntas devido a processos como mutação, deriva genética ou seleção natural (Revell & Harmon, 2022).

Já os Modelos Lineares Generalizados Mistos (Hadfield, 2010; Garamszegi, 2014) com controle filogenético são uma extensão dos Modelos Lineares Generalizados Mistos tradicionais, mas a principal diferença consiste no teste de hipótese através da comparação entre espécies utilizando um controle filogenético. Neste caso, a filogenia é utilizada como uma variável aleatória, sendo assim possível testar se a significância de um conjunto de variáveis explanatórias persiste mesmo após a consideração da influência do efeito filogenético (Hadfield, 2010).

Além desses importantes métodos estatísticos, a plataforma digital *Birdtree* e o programa R foram importantes para o avanço no entendimento dos aspectos evolutivos na área da Ornitologia, principalmente no desenvolvimento analítico dos estudos evolutivos de ninhos. A plataforma *Birdtree* disponibiliza as informações genéticas das espécies de aves do mundo (Jetz *et al.,* 2012), as quais são utilizadas para a construção de árvores filogenéticas na própria plataforma. Por sua vez, o programa R, através dos seus pacotes como *Phytools*, *Ape* e *Ace* possibilita a realização de testes estatísticos, assim como a construção de árvores filogenéticas, as quais são úteis para a visualização das transições de caracteres ao longo dos ramos quando se utiliza o método de reconstrução ancestral.

Devido a esta versatilidade na obtenção, análise e manipulação de filogenias, praticamente todos os trabalhos atuais de ornitologia que buscam entender a ecologia evolutiva dos ninhos utilizam estas ferramentas estatísticas (Street *et al.,* 2022; Leite *et al.,* 2023; Sheard *et al.,* 2023; Colombo *et al.,* 2024). Hall *et al*. (2013), mostrou que há uma relação entre a complexidade cerebral e o tipo de ninho construído. Posteriormente, Leite *et al*. (2023), nesta mesma abordagem, mostrou que ao invés do tipo de ninho, o modo de fixação dos ninhos na vegetação estava relacionado com a complexidade cerebral nos Passeriformes. Price & Griffith (2017), usando o método de reconstrução ancestral mostrou que o ninho do tipo domo foi o ancestral para Passeriformes. Fang *et al*. (2018), utilizando o mesmo método e também o sinal filogenético, examinou a evolução dos ninhos em geral, considerando os seguintes aspectos: tipo de ninho, local de nidificação e forma de fixação, e por fim observaram que tais características não mudaram sincronicamente ao longo da filogenia das Aves, mas elas tiveram trajetórias evolutivas interdependentes, mostrando que o surgimento de ninhos abertos colaborou com a diversificação da arquitetura e permitiu a ocupação de novos locais de nidificação. Posteriormente, Medina (2022) mostrou que a evolução de ninhos fechados para ninhos abertos foi uma importante inovação (*key innovation*), resultando em um aumento na distribuição geográfica das espécies de aves do estudo. Mainwaring *et al*. (2023), fizeram inferências quanto a evolução do local e arquitetura dos ninhos de aves modernas em relação aos grupos ancestrais das aves. Sheard *et al*. (2023), utilizando um banco de dados de 5924 espécies, mostrou que o formato do bico determina o tipo de material utilizado na construção do ninho.

Quanto ao efeito da predação, Street *et al*. (2022) discutem que os ninhos suspensos dos Icterídeos e ploceídeos evoluíram para evitar a predação, havendo um possível *trade-off* entre o gasto energético utilizado na construção desses ninhos complexos e o sucesso reprodutivo. Além disso, Vanadizna *et al*. (2024), em um amplo estudo com 1117 espécies de 19 família de aves mostrou que a variação no tamanho dos ninhos abertos depende não apenas da predação, mas também de fatores climáticos como a temperatura média da área de reprodução das espécies. Recentemente, Colombo *et al*. (2024), também mostrou que a variação climática tem efeito na evolução dos ninhos das aves do grupo Thraupidae. Além dessas abordagens, Nagy *et al*. (2019) mostraram que há uma correlação evolutiva entre ninho e formato dos ovos de 598 espécies de aves, concluindo primeiramente que os ninhos semi-abertos são o tipo ancestral e que os locais de nidificação afetam tanto a forma quanto o volume dos ovos.

A maioria desses estudos macroevolutivos mostrou que há diferentes fatores que determinam a ancestralidade e as trajetórias evolutivas dos ninhos, mas indiretamente evidenciaram a necessidade de se considerar algumas famílias específicas neste tipo de estudo, uma vez que tais análises globais não permitem o entendimento das trajetórias evolutivas dos ninhos com características específicas que ocorrem dentro de um grupo taxonômico menor, por exemplo, a nível de família.

Portanto, o grupo dos icterídeos, furnarídeos e trogloditídeos são excelentes modelos de estudos em pesquisas focadas na ecologia evolutiva de ninhos. Esses grupos apresentam uma ampla diversidade de tipos de ninhos, diversos tipos de habitats e de materiais utilizados na construção dessas estruturas, ou seja, constroem desde ninhos simples até ninhos mais complexos utilizando fibras vegetais ou barro, além de haver alguns casos de parasitismo interespecífico de ninhos, como no gênero *Molothrus* pertencente à família Icteridae.

2. JUSTIFICATIVA

Os estudos filogenéticos comparativos que abordam os ninhos das aves têm avançado principalmente através de análises macroevolutivas que envolvem grandes conjuntos de espécies, no entanto, utilizando-se de classificações simplificadas não apenas das características dos ninhos, como também das variáveis ambientais (Medina *et al*., 2022; Sheard *et al*., 2024). Embora os avanços no conhecimento permitidos por estes trabalhos sejam fundamentais, lacunas importantes ainda existem principalmente porque muitos clados específicos não seguem os padrões evolutivos encontrados para as análises globais (Barros-Leite & Francisco, 2024) e as análises globais não permitem o entendimento das trajetórias evolutivas dos ninhos com características específicas que ocorrem dentro de cada grupo. Por isto, tanto trabalhos globais quanto trabalhos que abordam a evolução dos ninhos das famílias são necessários para um amplo entendimento das relações evolutivas entre os ninhos das aves e os parâmetros ambientais que influenciam em sua diversificação.

3. OBJETIVOS

Diante disso, os objetivos deste trabalho foram analisar a origem e as trajetórias evolutivas dos ninhos em três famílias de aves, bem como potenciais fatores correlacionados com a diversificação destas estruturas.

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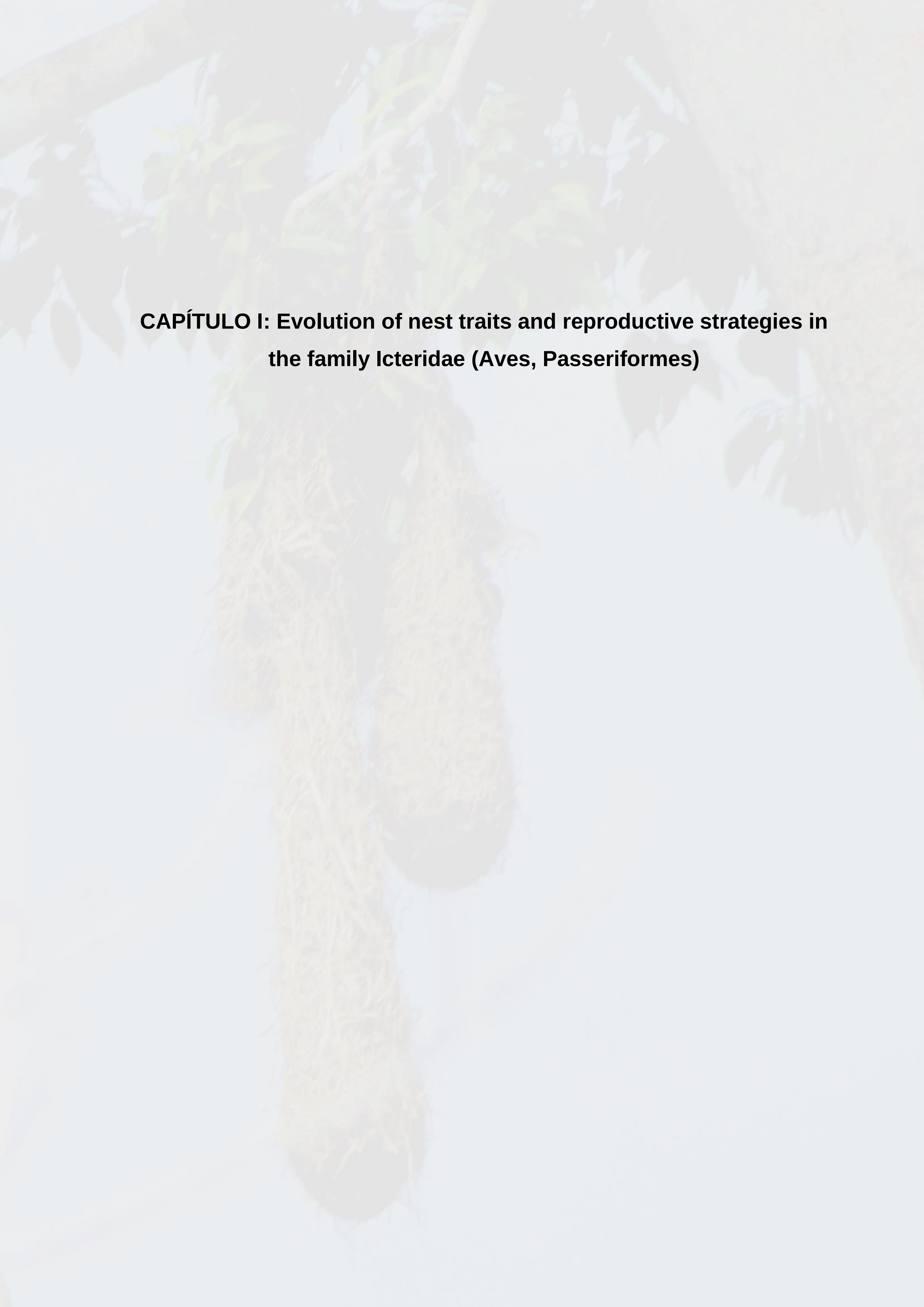
CAPÍTULO I: Evolution of nest traits and reproductive strategies in the family Icteridae (Aves, Passeriformes)



Evolution of nest traits and reproductive strategies in the family Icteridae (Aves, Passeriformes)

Abstract

Understanding the evolutionary trajectories of nest traits and reproductive strategies is important for the comprehension of avian evolution. Here, we examined the origin and evolutionary patterns of nest traits and habitat in the Icteridae. We also tested the hypothesis that the use of abandoned nests of other species (non-nest construction) is an intermediate step in the evolution of interspecific brood parasitism, and we addressed the hypothesis that the construction of complex nests is linked to small-bodied species. The common ancestor of the Icteridae likely constructed cup-shaped nest, basally supported, and built of leaves/sticks in woodlands. These traits were labile, but with strong phylogenetic signals. We detected significant evolutionary correlations between nest traits, with nest attachment modes being more often correlated with habitat than nest type and material. We suggest that the pendent enclosed nests, considered as some of the most complex structures built by animals, evolved through rim elongation of pouch-shaped nests. We rejected the idea that brood parasitism evolved from non-nest construction, and nest complexity increased with body size, likely due to shifts in nest materials permitting the construction of more resistant nest walls. Our analyses revealed unprecedented aspects of the intricate evolution of bird nest traits and reproduction.

Keywords: Ancestral state reconstruction, Brood parasitism, Correlated evolution, Nest complexity, Nest evolution, Phylogenetic comparative methods

1. Introduction

One of the most remarkable bird attributes is nest construction, an ability by which they modify the environment to increase protection for eggs and nestlings against weather conditions, predators and parasites (Hansell, 2000; Martin *et al.,* 2016). For this reason, nest construction skills are thought to be under intense selective pressures (Leite *et al.,* 2023; Mainwaring *et al.,* 2023a) and adaptations to the variety of present and past ecological conditions contributed with the high diversification of nest characteristics across species (Price & Griffith, 2017; Fang *et al.,* 2018; Mainwaring *et al.,* 2023a,b). Then, understanding the evolutionary trajectories of nest traits and their correlates is an important step for the comprehension of adaptation and evolution of birds themselves (Price & Griffith, 2017; Fang *et al.,* 2018; Mainwaring *et al.,* 2023a).

In recent years, the availability of global bird phylogenies and the development of analytical approaches permitting ancestral state reconstruction have provided researchers with the possibility to exploit the evolution of various bird reproductive parameters (Mainwaring *et al.,* 2021; Levin *et al.,* 2023; Sheard *et al.,* 2023), including nest traits (Price & Griffith, 2017; Fang *et al.,* 2018; Sheard *et al.,* 2024). Addressing nest evolutionary history is not straightforward because they have different dimensions (e.g. nest shape, diversity of nest materials, number of layers, weaving techniques, among others), but important insights about nest evolution have been provided in family-level (Fang *et al.,* 2018) and multi-species comparisons addressing nest types, nest attachment mode, nest sites and their environmental correlates across all avian phylogeny (Price & Griffith, 2017; Ocampo *et al.,* 2023; Sheard *et al.,* 2023).

Nest type can vary from scrape, in which a nest is virtually absent; platform, which is characterized by a shallow bed of nest materials; cup, which is an open nest with a well-defined rim; pouch, which is a purse-shaped nest with deep borders; enclosed, which is a roofed structure constructed by birds, with a well-defined often lateral entrance; primary cavity, which is a cavity excavated by the own nesting birds, and secondary cavity, which is a pre-existent cavity used by the birds for reproduction (Hansell, 2000; Fang *et al.,* 2018). Nest attachment is the way by which a nest is attached to the structure that holds it, and it can vary from basal, when a nest is supported mainly from the bottom; lateral, when lateral nest walls are attached to the structures supporting the nest; rim-suspended, when a nest is attached at the rim to horizontal forks or parallel branches, and top-suspended, when a nest is a pendent structure attached by its upper part to the supporting structure (Hansell, 2000; Fang *et al.,* 2018; Leite *et al.,* 2023). The ancestral of the modern birds likely laid eggs in scrape nests placed on the ground, and while scrape, platform, and cavity nests became the predominant types in non-passerine lineages, in the Passeriformes the cup and enclosed nests evolved as the most common nest types (Price & Griffith 2017; Fang *et al.,* 2018; Mainwaring *et al.,* 2023a). Except for the lineages containing swifts, treeswifts and hummingbirds, basal nest attachment mode predominates in the non-passerine birds, while a high nest attachment diversification occurred in the Passeriformes, especially in the groups constructing cup and enclosed nests (Fang *et al.,*2018; Ocampo *et al.,* 2023).

Among the bird families with which to study nest evolution, Icteridae is certainly one of the most informative ones (Fraga, 2008; Street *et al.,* 2022). It comprises about 106 species and 30 genera, found throughout the Americas, from Alaska to Tierra del Fuego, in almost all types of habitats (Winkler *et al.,* 2020). In the icterids, nest type vary from simple cups to enclosed nests with top-entrances, passing by many intermediate forms, and a few species are brood-parasitic or secondary users of abandoned nests constructed by other birds (non-nest constructors) (Fraga, 2008; Winkler *et al.,* 2020; Street *et al.,* 2022). Nest attachment modes are also highly variable, with virtually all of the categories of this trait present in this single family (Winkler *et al.,* 2020). Notably, the large pendent (top-suspended) nests constructed by the caciques and oropendolas (genera *Cacicus* and *Psarocolius*) are considered as some of the most elaborate and complex nests in nature (Street *et al.,* 2022). Fraga (2008) used a phylogeny constructed with cytochrome-b DNA sequences for 60 icterid taxa (Lanyon & Omland, 1999) to show that the pendent nests have emerged twice independently, in the clade formed by the caciques and oropendulas and in the orioles (genus *Icterus*), as well as to confirm that brood parasitism have emerged only once in the monophyletic cowbirds (genus *Molothrus*). However, modern ancestral state reconstruction methods were not available to provide the potential evolutionary trajectories. In the family-level study of Fang *et al.* (2018), the most common recent ancestor of the Icteridae likely constructed basally supported cup nests in trees or non-tree vegetation. Street *et al.* (2022) used a subset of the

Icteridae species (58 species) to test of the hypothesis that top-suspended nests evolved as an antipredatory mechanism, and for illustrative purposes ancestral state reconstruction was performed for nest attachment, confirming “basal” for the most recent common ancestor, but other nest traits were not addressed. Then, most of the evolutionary aspects involved in nest trait diversification within the family Icteridae are still poorly understood.

The presence of interspecific brood-parasitic and non-nest constructor species, as well as the contrast in complexity levels found between the simple nests of some ground-nester species and the top-suspended nests of the caciques and oropendolas, also make this bird group ideal for addressing other questions of broad interest. An aspect of avian reproduction that has long called the attention of researchers is the evolution of interspecific brood parasitism (Payne, 1977; Rothstein, 1990). Several hypotheses have been proposed to explain the contexts in which interspecific brood parasitism can result in increased reproductive output for parasitic birds (Avilés, 2019), and a number of studies have investigated the ecological and environmental conditions predicting the origin of this behavior (Kruger & Davies, 2002; Antonson *et al.,* 2020). Another important debate is whether interspecific brood parasitism has evolved from conspecific brood parasitism, or directly from non-parasitic ancestors, and the best available evidence give support to “parental care’’ being the ancestral state of most of the brood-parasitic avian lineages (Kruger & Davies, 2002). The existence of icterid birds that use the nests constructed by other species to lay their eggs and to raise their own young (non-nest constructors) poses the question of whether non-nest construction could be an intermediate step for the emergence of interspecific brood parasitism in this family. If this is true, the use of inactive nests of other species would precede the evolution of egg-laying in host active nests. Another important question regards to nest complexity and avian body size. It has been long speculated that the construction of elaborate nests, such as the top-suspended ones, could be achieved only by small bird species because they would be more capable of handling finer and more diversified nest materials (Collias, 1964; Collias, 1997; Hansell, 2000). Because Icterids are highly variable in nest characteristics and in body size (19.4 to 405.3 g), they provide an opportunity for investigating the association between body size and nest complexity.

Here, we gathered information on nearly 90% of the Icterid species and we used ancestral state reconstruction and phylogenetic signal to examine the origin and evolutionary patterns of nest type, nest attachment mode and nest material across an Icterid phylogeny. Then, we tested for correlated evolution between these traits and we addressed whether evolutionary shifts in these traits could be associated with habitat. Concomitantly, we analyzed the evolutionary trajectories of the Icteridae reproductive strategies, i.e. nest construction with parental care, non-nest construction with parental care and interspecific brood parasitism, to test the hypothesis that non-nest construction can be an intermediate step in the evolution of interspecific brood parasitism. We predicted that this idea would receive support if *Molothrus*, the typical interspecific brood-parasitic icterids, derive from non-nest constructor ancestors with parental care. Finally, we addressed whether in the icterids the construction of more complex nests is linked to small-bodied species. Because nest attachment, rather than nest type is correlated with passerine relative brain size and likely with the cognitive skills needed for nest construction, we used a nest attachment mode hierarchical classification as a proxy of variation in nest complexity (see Leite *et al.,* 2023) and body mass as a proxy of body size. Based on the observation that the icterids constructing top-suspended nests are large-bodied birds, we predicted that an opposite pattern could be found in the Icteridae.

2. Methods

2.1. Nests and bird species characteristics

To obtain information on nest type, nest attachment mode, nest material and reproductive strategy we first examined the texts available for each icterid species in Birds of the World, The Cornell Lab of Ornithology (https://birdsoftheworld.org/bow/home). Because nest attachment details are rarely provided in nest descriptions, we also analyzed pictures from the citizen science database eBird (https://ebird.org/home) and Wikiaves (https://www.wikiaves.com.br) to observe details about this trait. Secondly, for the species with no information in Birds of the World, we performed searches in Google Scholar and in the indexing base Web of Science, using the species' scientific name, the English name, as well as Icteridae as keywords, one at a time, and we checked all the literature reporting breeding information. Thirdly, especially for Neotropical species, we also analyzed local books.

We classified nest types as Cup, Pouch, Dome and Enclosed. Cup nests are open structures with a well-defined rim surrounding the incubatory chamber and Pouch differed from Cup by being purse-like structures with deeper borders (Hansell, 2000; Fang *et al.,* 2018). In the icterids, two different types of enclosed nests can be diagnosable*,* the roofed structures with lateral entrance typical of some species of *Sturnella* and the deep nests of *Cacicus*, *Psarocolius* and a few *Icterus*, which are enclosed structures with a very restricted top-entrance (Figure 1). Because these are very different structures, here we used “Dome” to describe the roofed nests with lateral entrance, and “Enclosed” to describe the structures with top-entrance. For nest attachment mode, we used the classification from Leite *et al*. (2023)*,* Basal A, Basal B, Lateral, Rim-suspended and Top-suspended. Basal A are nests placed on flat substrates, e.g. ground; Basal B are nests supported mainly from the bottom on vegetation; Lateral are nests in which vertical or horizontal branches are interwoven in lateral nest walls; Rim-suspended are nests hung by the rim and Top-suspended are pendent structures attached from the upper part (Leite *et al.,* 2023). We classified nest material into two broad categories*,* Fibers and Leaves/Sticks. This is because the nests of many species belonging mainly to the genera *Cacicus*, *Psarocolius* and *Icterus* are remarkable by their walls constructed with fine filaments that are sewed using very sophisticated techniques (see also Street *et al.,* 2022) (Figure 1), and we placed them in the “Fibers” category. Nests of other icterids are composed mainly of interlaced leaves, such as those constructed by *Chrysomus* (Figure 1), or by mixtures of leaves and harder sticks (e.g. *Quiscalus*), reason why we placed them together in the category “Leaves/Sticks”. We obtained habitat information from the dataset of Tobias *et al.* (2022), with modifications. Specifically, for species classified in “human modified” category, we used Birds of the World to obtain the original habitat. Because too few species were classified in “Shrubland” (six species), we joined them together with “Woodland”. Then, our habitat categories were*,* Forest (tall tree vegetation with closed canopy), Woodland (shrublands, savanna, cerrado, caatinga, mangrove, forest borders), Grassland (grassy vegetation) and Wetland (marshland, cattail-like vegetation, swamps, reeds). We classified the icterid reproductive strategies as nest construction with parental care (nest constructor), use of nests of other species with parental care (non-nest constructor), and interspecific brood parasitism (brood-parasitic). We collated body mass data also from Tobias *et al.* (2022).

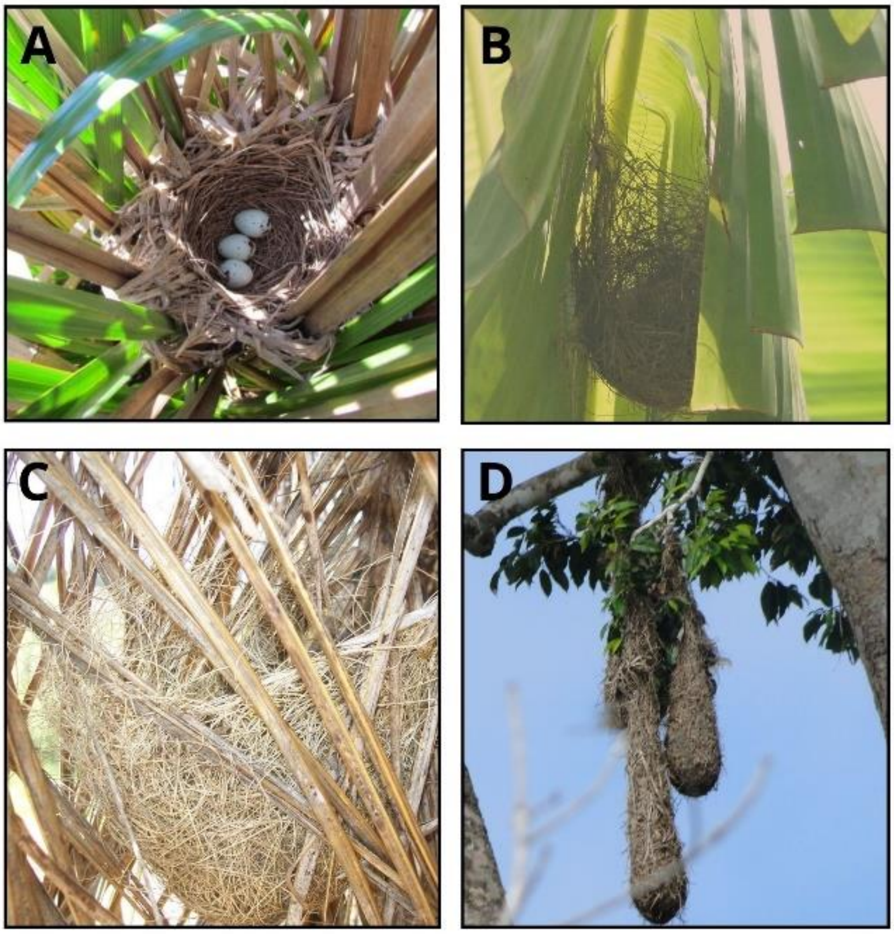


Figure 1. Nest characteristics of icterid birds. (A) Cup-shaped, laterally attached nest of the Chestnut-capped Blackbird *Chrysomus ruficapillus* built of interlaced Leaves. (B) Pouch-shaped, Rim-suspended nest of the Epaulet Oriole *Icterus cayanensis* built of fine fibers. (C) Detailed view of the fabric of a nest of *I. cayanensis*, built of fibers using advanced sewing techniques. (D) The complex Enclosed, Top-suspended nests of the caciques *Cacicus* sp. with a very restricted Top-entrance, made of fibers and considered as one of the most complex structures built by animals. Photograph (A) was a courtesy by M.C. Costa.

2.2. Phylogenetic tree

For the phylogenetic hypothesis, we generated 3,000 time-calibrated trees in birdtree.org (Jetz *et al*., 2012), with the method of Ericson as a backbone (Ericson *et al.,* 2006; Jetz *et al.,* 2012). Then, we used this dataset to select the tree with the highest sum of clades posterior probabilities (consensus tree), using the maximum clade credibility tree summarization method of the Phangorn R Package (Schliep, 2011) for use in the subsequent analyses (see also Leite *et al.,* 2023; Ocampo *et al.,* 2023). For ancestral state reconstructions of nest traits and habitat (see below), we generated consensus trees with variable sample sizes depending on data availability, and we excluded the non-nest constructor and brood-parasitic icterids. However, non-nest constructors and brood-parasitic species were maintained in the dataset used in correlated evolution analyses, as the data were binarized and they were always placed in the “Others” category (see below). We used the taxonomic classification of birdtree because overall it matched the classifications used in the most complete species-level phylogenetic work (Powell *et al.,* 2014).

2.3. Ancestral state reconstruction and phylogenetic signal

We examined the origin and evolutionary trajectory of nest type, nest attachment mode, nest material, habitat and reproductive strategy by estimating ancestral character state for the internal nodes of the phylogeny using the Maximum Likelihood procedure developed for discrete characters available in the ace-function of the ape R-package. The best model of evolution for each character was fitted using the fitDiscrete function of Geiger (Pennell *et al.,* 2014), with the best model being selected through corrected Akaike Information Criterion (AICc) and we considered three models*,* Equal Rates (ER); All-Rates Different (ARD) and Symmetric Entities (SYM).

We estimated D statistic (Fritz & Purvis, 2010) with the phylo.d function of the R package caper (Orme, 2018) to generate the phylogenetic signal for each of the traits. In this method developed for discrete binary data, a value of $D < 0$ suggests strong phylogenetic signal; a value $= 0$ suggests that the character states are clustered as evolving under a Brownian-motion model of evolution and a value of $D \geq 1$ suggests random distribution or overdispersion of the trait across the phylogeny. We generated 1,000 values by tip-shuffling to test whether the observed value of D differed significantly from 0 and from 1 ($P < 0.05$).

2.4. Correlated evolution analyses

We investigated the existence of correlated evolutionary changes between nest traits, as well as between nest traits and habitat, using the FitPagel function implemented in the package phytools (Revell, 2012), aiming to fit the correlated evolution procedure of Pagel (1994), under the Markov Chain model. In this method, the rates of change of the characters across the branches of the phylogenetic tree are estimated and the models of independent and of correlated evolution are fitted to the data. Because analyses of correlated evolution can be achieved only with binary traits, we binarized our traits by contrasting each category in a trait against all others. For instance, for nest type we used Cup/Others, Pouch/Others, Dome/Others and Enclosed/Others, and the same approach was used for nest attachment mode and habitat (see also Dellinger *et al.,* 2021; Busschau & Boissinot, 2022). Then, we exploited all of the possible combinations of nest traits and of nest traits with habitat, except for the combinations not found among the existent Icteridae species (e.g. Top-suspended/Others vs. Grassland/Others) or trait combinations for which one of the categories had sample sizes smaller than five. For each combination of binary trait, we tested for correlated evolution by checking whether the model of dependent evolution differed significantly from the model of independent evolution, using a likelihood ratio test with alfa = 0.05. In this step, we used False Discovery Rate (fdr) correction for multiple comparisons (Jafari & Ansari-Pour, 2019). Then, for the pairs of traits presenting significant correlation, we used AIC to select among the following models describing the relationships between the variables, interdependent; substitution rate of the state x dependent on the state of y and the model of substitution rate of the state y dependent on the state of x (Revell, 2012).

2.5. Body size and nest complexity

To test the prediction that complex nests are constructed by small-bodied species, we used the same nest attachment mode classification from above (Leite *et al.,* 2023) and we compared log-transformed body mass across categories using regular One-way ANOVA. Before performing the ANOVA, we checked for data normality with Shapiro-Wilk test and we used Tukey's post-hoc test for pairwise comparisons, with a level of significance of 0.05.

3. Results

Before the analyses, we excluded seven species from our dataset due to incomplete branch length information (Red-shouldered Blackbird *Agelaius assimilis*, Tawny-shouldered Blackbird *A. humeralis*, Yellow-winged Cacique *Cassiculus melanicterus*, Bahama Oriole *Icterus northropi*, Puerto Rican Oriole *I. portoricensis*, Variable Oriole *I. pyrrhopterus* and Pale-eyed Blackbird *Agelasticus xanthophthalmus*). The Pampas Meadowlark *Sturnella defilippii*, Cuban Blackbird

Dives atroviolaceus, Forbes's Blackbird *Curaeus forbesi*, Baudo Oropendula *Psarocolius cassini*, Black Oropendola *P. guatimozinus*, Selva Cacique *Cacicus koepckeae* and Orange-backed Troupial *I. croconotus* had no DNA information and were inserted in birdtree phylogenies based on the best available taxonomic information (Jetz *et al.,* 2012). Because these species were always assigned to the correct genus and they never implied in new mutations within their clades, we maintained them in the analyses. Virtually all clade positions matched those found in Powell *et al.* (2014), suggesting that our trees were consistent with the best resolved species-level phylogenies available for the group. The Band-tailed Cacique *Ocyalus latirostris* and the Casqued Oropendola *Clypicterus oseryi*, assigned to the *Cacicus* clade (see below), may not imply in *Cacius* polyphyly because these birds have been classified as *Cacicus* in more recent literature (e.g. Winkler *et al.,* 2020). Overall, we gathered reliable information on nest type for 87 species (40 had Cup, 23 had Pouch, 2 had Domed and 22 had Enclosed nests), nest attachment mode for 87 species (10 species presented Basal A, 15 Basal B, 16 Lateral, 24 Rim-suspended and 22 Top-suspended nests) and nest material for 87 species (46 species used Fibers and 41 used Leaves/Sticks), and all of the genera present in Powell *et al.* (2014) were represented for these variables. For habitat we gathered information for 94 species (8 occurred mainly in Grasslands, 12 in Wetlands, 46 in Woodlands and 28 in Forests), as well as for reproductive strategy (87 species were nest constructors, 2 were non-nest constructors and 5 were brood-parasitic). Open Cup nests occurred in Grasslands (n = 6), Wetlands (n = 12), Woodlands (n = 18) and Forests (n = 4); Pouch nests occurred in Woodlands (n = 18) and in Forests (n = 5); Domed nests occurred only in Grasslands (n = 2) and Enclosed nests occurred in Woodlands (n = 3) and Forests (n = 19). Nests with Basal A attachment mode occurred in Grasslands (n = 8) and Woodlands (n = 2); Basal B occurred in Woodlands (n = 12) and Forests (n = 3); Lateral occurred in Wetlands (n = 12), Woodlands (n = 3) and Forest (n = 1); Rim-suspended occurred in Woodlands (n = 19) and Forest (n = 5), and Top-suspended occurred in Woodlands (n = 3) and Forests (n = 19). Regarding to the combinations of nest type and attachment mode, Cups could have Basal A (n = 8), Basal B (n = 15), Lateral (n = 16) and Rim-suspended (n = 1) attachment modes; Pouch nests were all Rim-suspended (n = 23); Domed nests were all Basal A (n = 2) and Enclosed nests were all Top-suspended (n = 23). All of the Rim- (n = 27) and

Top-suspended nests (n = 22) were constructed of Fibers, while nests belonging to all of the other attachment categories could be categorized as Leaves/Sticks

The SYM evolutionary model was the best fitted for nest attachment mode and habitat, while the best fitted evolutionary model for nest type and reproductive strategies was ER. For nest material, ER and SYM had the similar AIC values, then we arbitrarily chose ER (Table S1). The ancestor state reconstruction analyses indicated that the common ancestor of the Icteridae had most likely a Cup-shaped nest (root probability = 0.99), basally supported (Basal B) (root probability = 0.54) and constructed of Leaves/Sticks (root probability = 0.95) in Woodlands (root probability = 0.47) (Figure 2 to Figure 6; Table S2). In the evolutionary trajectory of nest type, Cup (the ancestral form) was conserved in 46% of the extant icterid species, but it also emerged independently later from a Pouch ancestor (reversal evolution) in Orchard Oriole *I. spurious*. Pouch derived early from Cup in the *Icterus* clade, where it remained mostly conserved, and twice independently in the clade of *Lampropsar* and *Hypopyrrhus*. Domed nests derived late only once from Cup in the clade of the ground-nesters *Sturnella*. Enclosed derived early and was highly conserved in the clade formed by *Cacicus* and *Psarocolius*, and also twice independently from a Pouch ancestor within the *Icterus* clade (Figure 2). For nest attachment mode, the simpler Basal A was a derived category that emerged independently twice, one from the Basal B common ancestor of the clade containing *Sturnella* and *Dolychonix*, and another from a Lateral ancestor of the clade formed by *Agelaioides* and *Oreopsar*. Basal B was conserved in the genera *Dives*, *Nesopsar*, *Curaeus*, *Macroagelaius*, *Gymnomystax* and in the Yellow-shouldered Blackbird *Agelaius xanthomus*, but it also emerged secondarily independently three times in the Carib Grackle *Quiscalus lugubris*, the Greater Antillean Grackle *Q. niger* and likely in the Chopi Blackbird *Gnorimopsar chopi* from Lateral ancestors (reversal evolution). Lateral nests emerged independently at least six times, one in Yellow-headed Blackbird *Xanthocephalus xanthocephalus* likely from a Basal A ancestor; in the *Quiscalus* clade; in the clade formed by the Tricolored Blackbird *Agelaius tricolor* and Red-winged Blackbird *Agelaius phoeniceus*; in the Scarlet-headed Blackbird *Amblyramphus holosericeus*; in the clade formed by *Chrysomus*, *Xanthopsar* and *Pseudoleistes*; in the *Agelasticus* clade and in Yellow-billed Cacique *Amblycercus holosericeus*, all derived from Basal B ancestors. Rim-suspended emerged independently three times from Basal B ancestors. It first derived early and was mostly conserved in the big *Icterus* clade, and later in *Lampropsar* and *Hypopyrrhus*. Top-suspended emerged three times independently. It first derived early and was highly conserved in the clade formed by *Cacicus* and *Psarocolius*, and later, within the *Icterus* clade, it emerged in the Yellow Oriole *I. nigrogularis*/Altamira Oriole *I. gularis* and in the Streak-backed Oriole *I. pustulatus* (Figure 3). For nest material, Fibers was a derived trait that emerged independently four times from Leaves/Sticks, early in the clades of *Cacicus*/*Psarocolius* and *Icterus*, where they remained highly conserved, and later in *Lampropsar* and *Hypopyrrhus* (Figure 4).

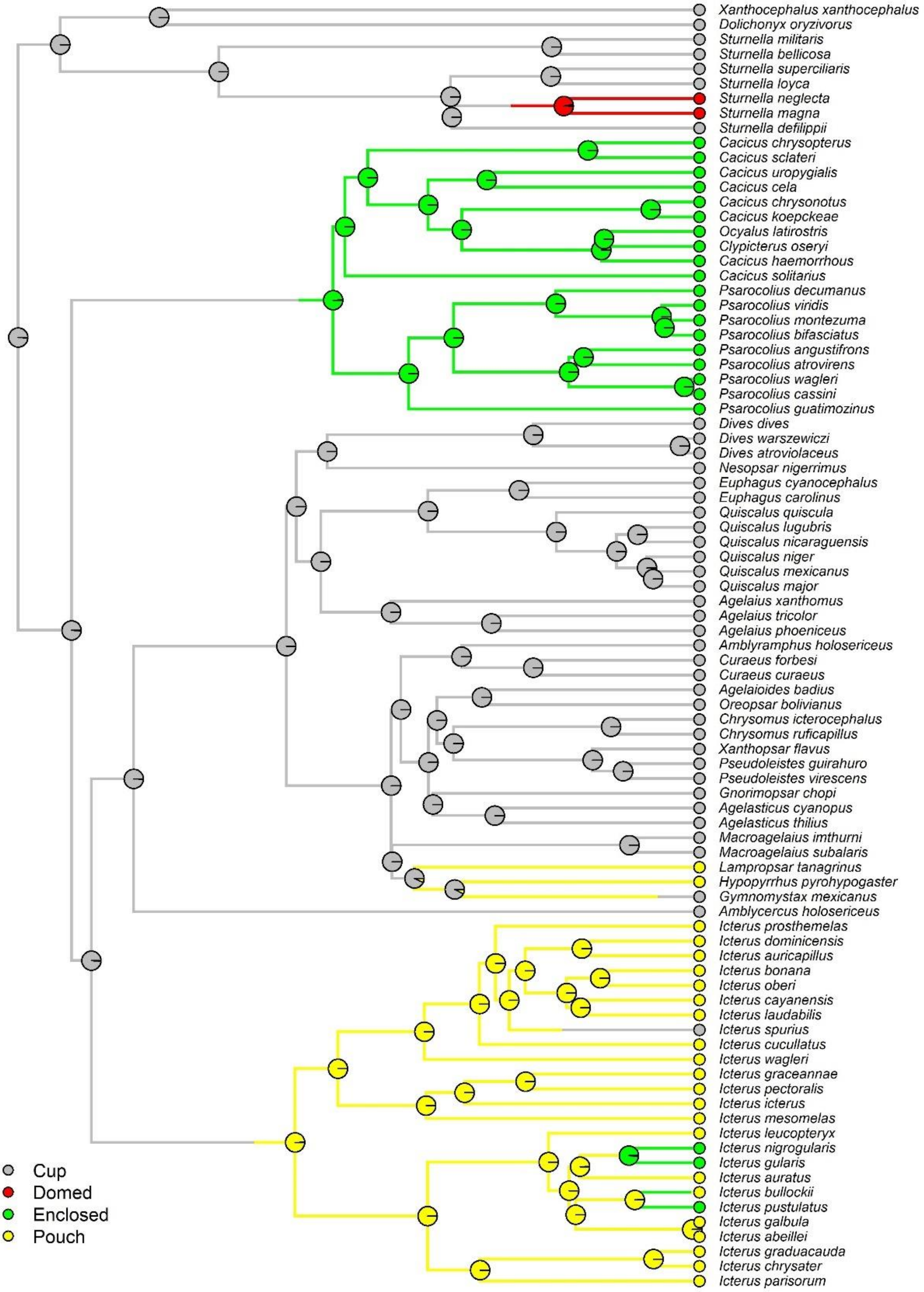


Figure 2. Consensus Phylogenie of the Icteridae (Aves, Passeriformes) depicting character state distributions across terminal nodes and ancestral states reconstructed at the internal nodes on evolutionary trajectories of nest type.

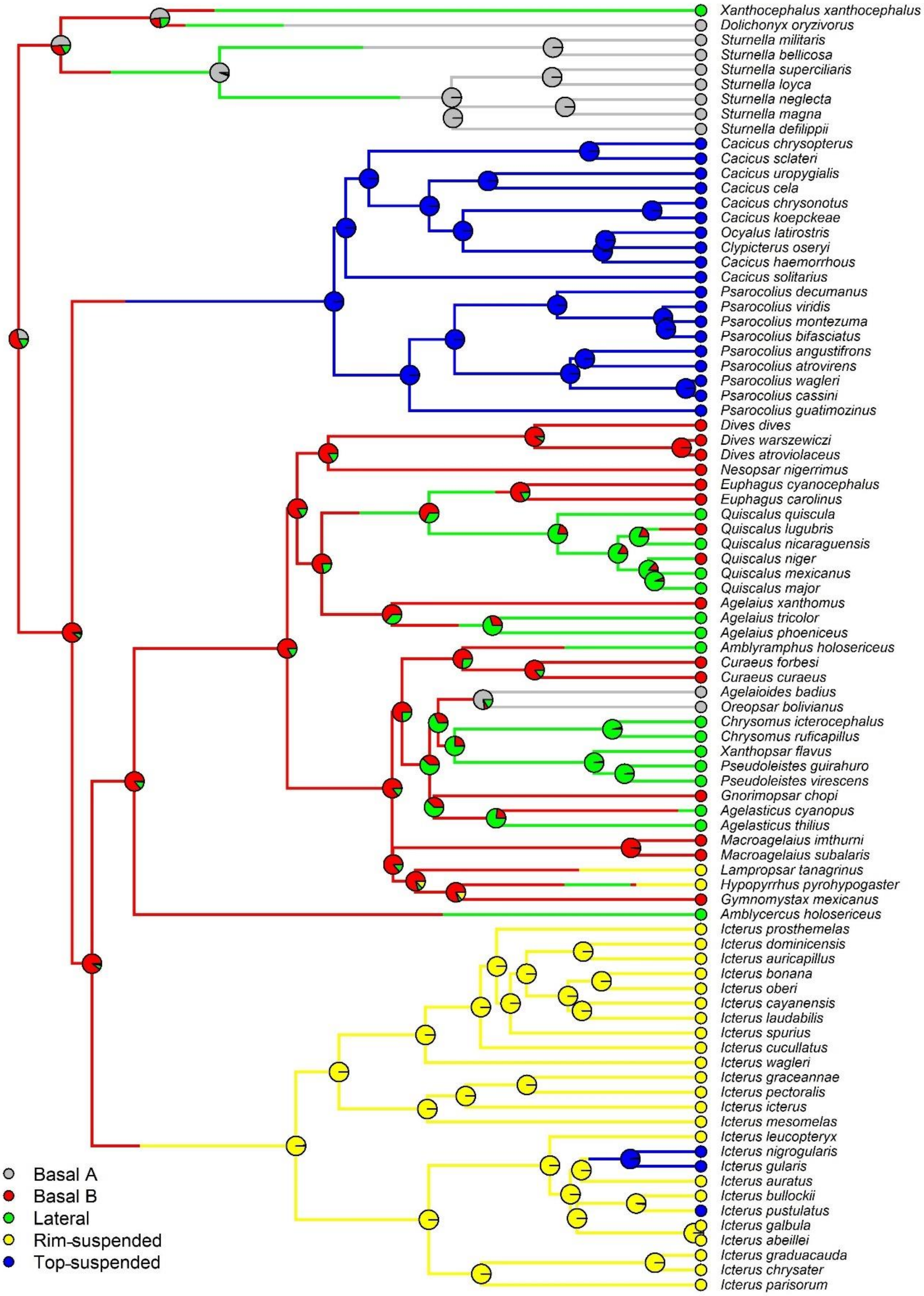


Figure 3. Consensus Phylogenie of the Icteridae (Aves, Passeriformes) depicting character state distributions across terminal nodes and ancestral states reconstructed at the internal nodes on evolutionary trajectories of nest attachment mode.

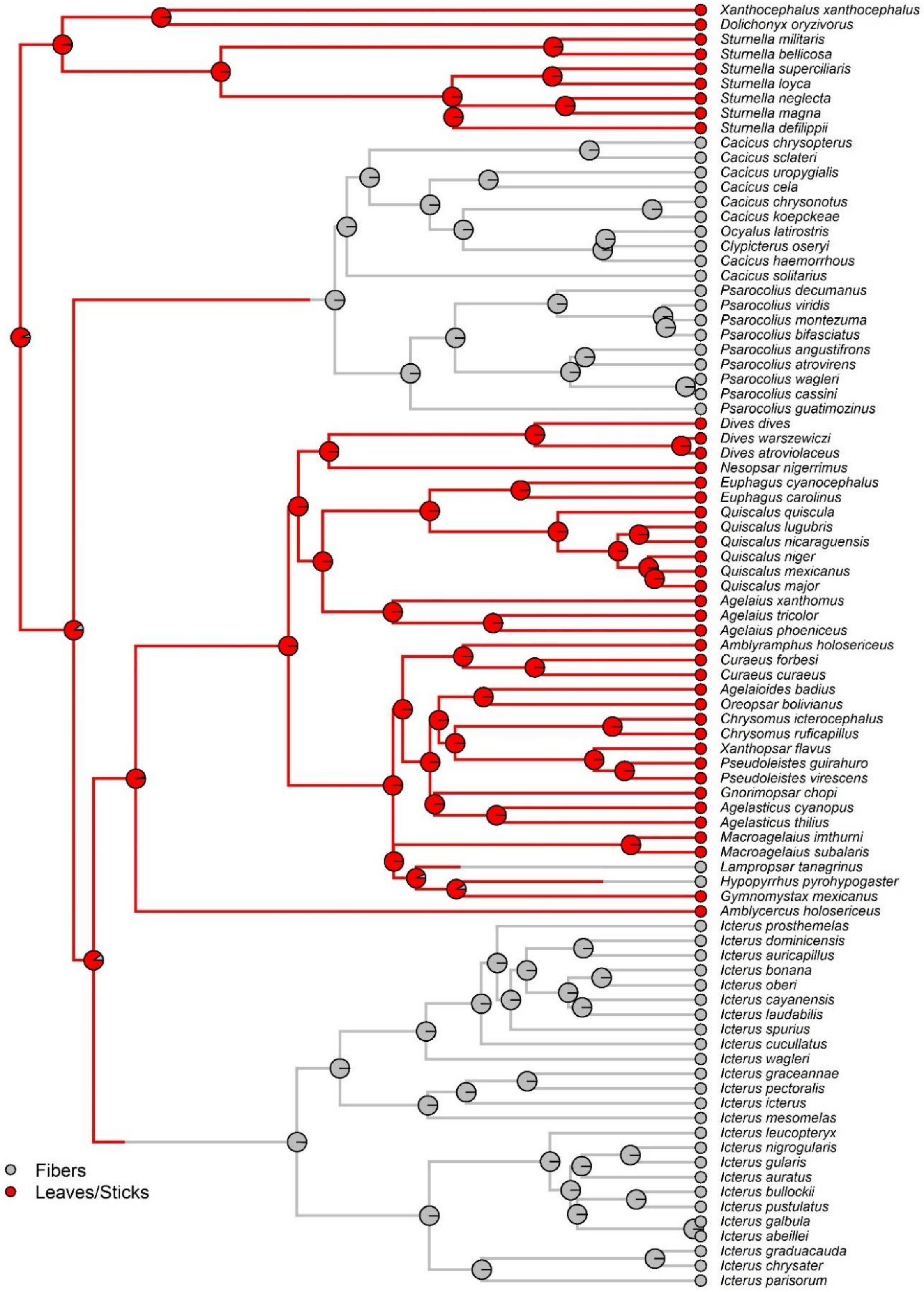


Figure 4. Consensus Phylogenie of the Icteridae (Aves, Passeriformes) depicting character state distributions across terminal nodes and ancestral states reconstructed at the internal nodes on evolutionary trajectories of nest material.

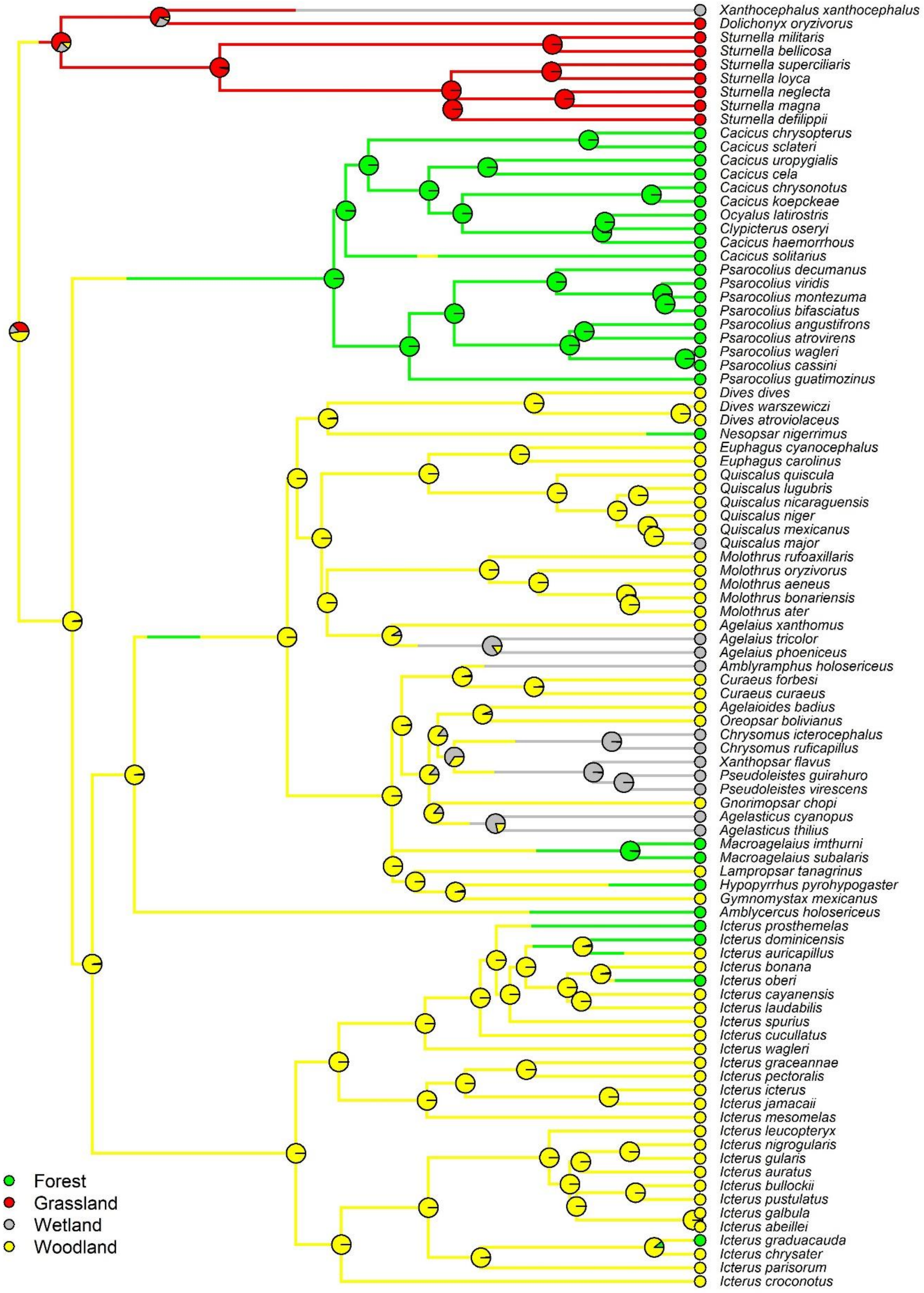


Figure 5. Consensus Phylogenie of the Icteridae (Aves, Passeriformes) depicting character state distributions across terminal nodes and ancestral states reconstructed at the internal nodes on evolutionary trajectories of habitat.

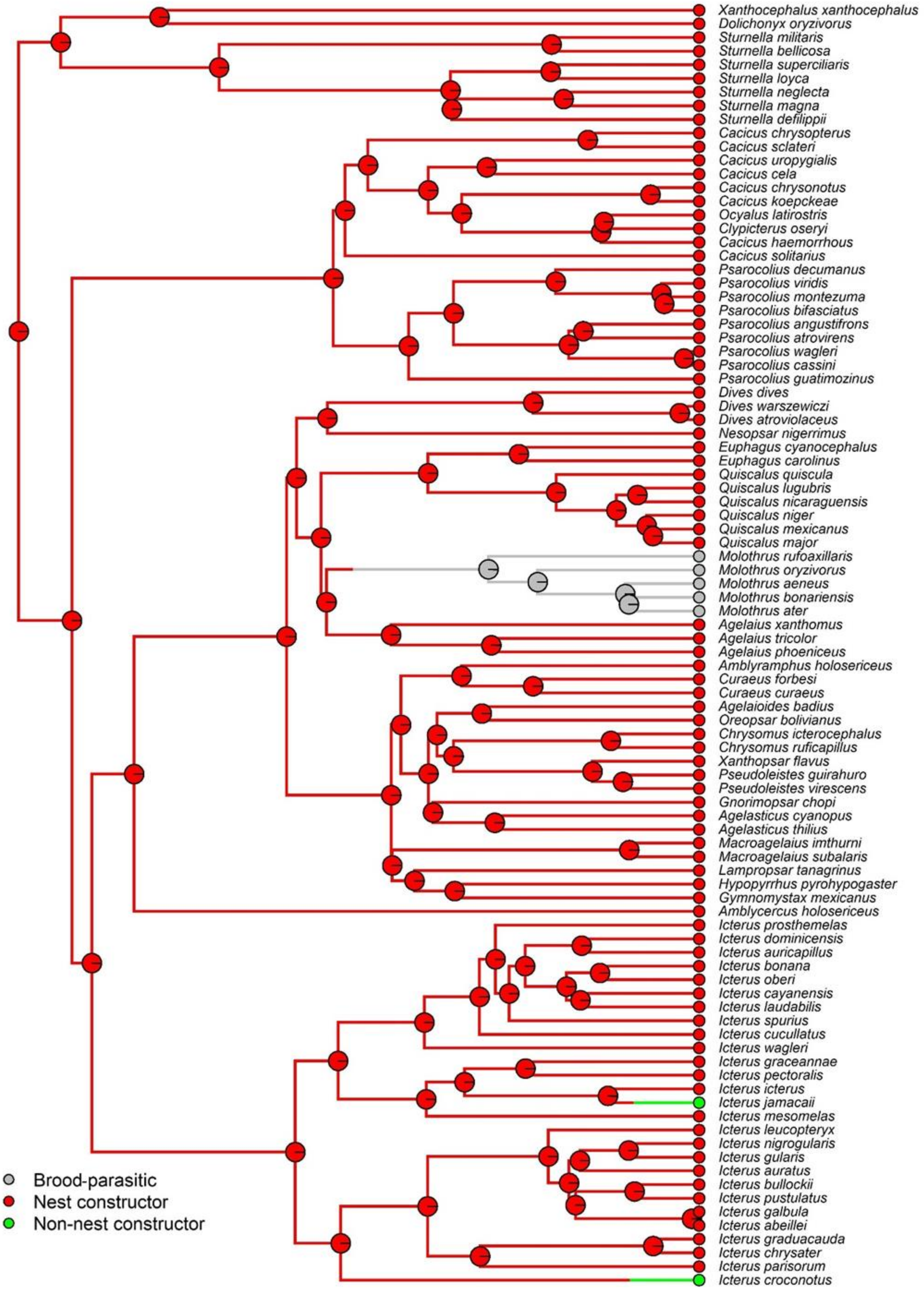


Figure 6. Consensus Phylogenie of the Icteridae (Aves, Passeriformes) depicting character state distributions across terminal nodes and ancestral states reconstructed at the internal nodes on evolutionary trajectories of reproductive strategy (nest constructor, non-nest constructor, and brood-parasitic).

For habitat, Grassland emerged early only once from the Woodland Icterid common ancestor, being relatively conserved in the clade containing *Sturnella* and *Dolichonyx*. Wetlands emerged independently six times, in *X. xanthocephalus* from a Grassland ancestor, and then in Boat-tailed Grackle *Quiscalus major*, in the clade formed by *Agelaius tricolor* and *A. phoeniceus*, in *A. holosericeus*, in the clade formed by *Chrysomus*, *Xanthopsar* and *Pseudoleistes* and in the *Agelasticus* clade, all derived from woodland ancestors. Woodland was the habitat category of the most recent common ancestor of the Icteridae, with only one independent origin and it was the predominant habitat category among the extant icterids. Forest emerged independently nine times, always from Woodland ancestors (Figure 5). Both non-nest construction and brood parasitism were derived behaviors that emerged late in the Icteridae phylogeny. Non-nest construction emerged twice independently within the genus *Icterus* (Campo Troupial *I. jamacaii* and Orange-backed Troupial *I. croconotus*), while brood parasitism emerged only once in the ancestor of *Molothrus* (Figure 6). All of the binary combinations of nest type, nest attachment mode, nest material and habitat resulted in negative D values, with strong and significant phylogenetic signals (Table S3).

3.1. Correlated evolution

We detected correlated evolution for several combinations of nest type and nest attachment mode, Cup/Others vs. Basal B/Others (with the model in which changes in nest attachment mode depends on the state of nest type best fitting the data); Pouch/Others vs. Rim-suspended/Others (with the model in which change in nest type depends on the state of nest attachment mode receiving enough AIC support); Enclosed/Others vs. Top-suspended/Others (with the models in which change in nest type depends on the state of nest attachment mode, and vice-versa, both receiving enough AIC support) (Table 1; Table S4). We found significant correlations between nest type and nest material in two cases, Cup/Others vs. Fibers/Leaves-Sticks (with the model in which change in nest type depends on the state of nest material best fitting the data), and Pouch/Others vs. Fibers/Leaves-Sticks (with the model in which change in nest material depends on the state of nest type best fitting the data) (Table 1; Table S4). We found correlations between nest attachment mode and nest material only for the combinations Rim-suspended/Others vs. Fibers/Leaves-Sticks (with the model in which change in nest material depends on the state of nest attachment best fitting the data, and the model of interdependent evolution also receiving some support).

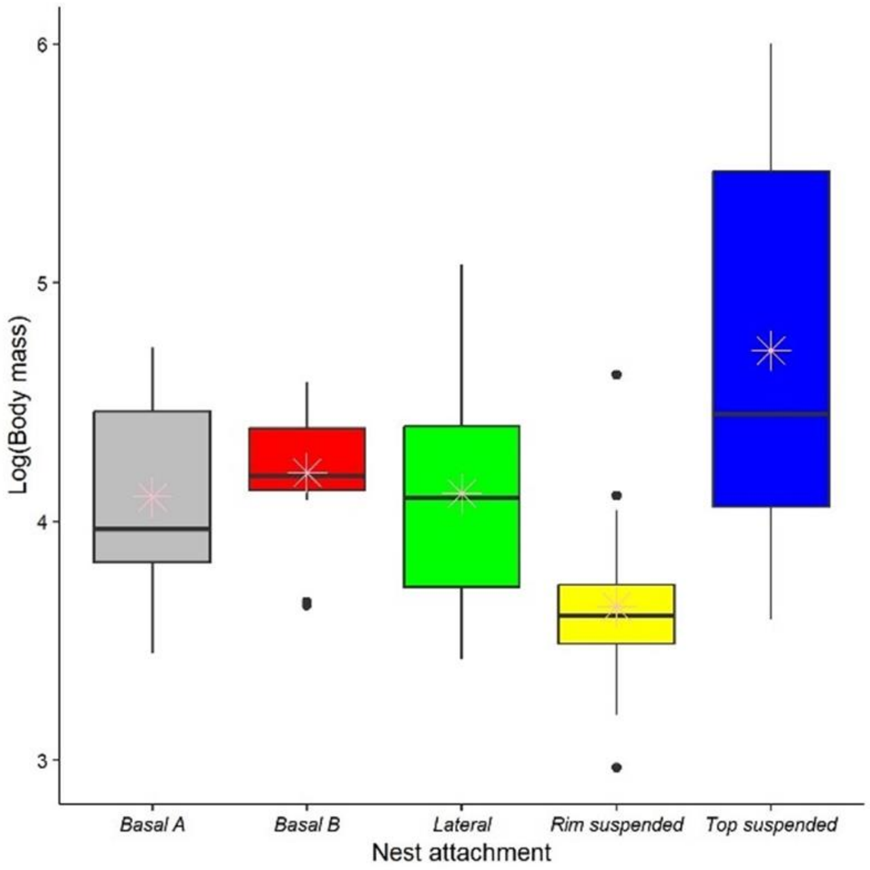
We virtually found no evidence for correlated evolution between nest type and habitat, except for a marginal result involving Pouch/Others vs. Forest/Others (P = 0.048), with the model in which habitat change depends on the state of nest type the one that best fitted the data (Table 1; Table S4). Nest attachment mode, however, often coevolved in association with habitat. Except for Basal B, the following correlations were found, Basal A/Others vs. Grassland/Others (with the model of interdependent evolution and the model in which habitat change depends on the state of nest attachment receiving enough support from AIC); Lateral/Others vs. Wetlands/Others (with the model in which habitat change depends on the state of nest attachment best fitting the data); Rim-suspended/Others vs. Woodland/Others (with the model of interdependent evolution and the model in which habitat change depends on the state of nest attachment receiving enough support from AIC); Top-suspended/Others vs. Forest/Others (with the model in which nest attachment change depends on the state of habitat best fitting the data) (Table 1; Table S4). We found no association between nest material and habitat (Table 1).

Table 1. Tests of correlated evolution for combinations of binarized nest traits and habitats of Icterid birds. P values indicate whether the model of dependent evolution differed significantly from the model of independent evolution and they were obtained using a likelihood ratio test with alfa = 0.05. The final values of significance were obtained after correction for multiple comparisons (fdr). Only the combinations present in the extant icterid species, with sample sizes higher than five were considered.

	Independent		**Dependent**			
	Log-Likelihood	AIC	Log-Likelihood	AIC	P	fdr
Nest type VS. Nest attachment						
Cup/Others vs. Basal A/Others	-37.472	82.945	-35.248	86.497	0.348	0.417
Cup/Others vs. Basal B/Others	-58.087	124.174	-49.100	114.200	**0.001**	**0.002**
Cup/Others vs. Lateral/Others	-51.181	110.362	-48.352	112.705	0.226	0.339
Pouch/Others vs. Rim-suspended	-52.864	113.729	-29.576	75.152	**<0.001**	**<0.001**
Enclosed/Others vs. Top-suspended/Others	-30.688	69.377	-15.348	46.697	**<0.001**	**<0.001**
Nest type VS. Nest material						
Cup/Others vs. Fibers/Leaves-Sticks	-35.050	78.100	-24.270	64.540	**<0.001**	**<0.001**
Pouch/Others vs. Fibers/Leaves-Sticks	-36.999	81.999	-26.900	69.800	**<0.001**	**<0.001**
Enclosed/Others vs. Fibers/Leaves-Sticks	-28.820	65.640	-25.867	67.734	0.206	0.206
Nest attachment VS. Nest material						
Basal A/Others vs. Fibers/Leaves-Sticks	-27.134	62.269	-23.904	63.808	0.167	0.417
Basal B/Others vs. Fibers/Leaves-Sticks	-40.661	89.323	-39.235	94.470	0.582	0.727

Lateral/Others vs. Fibers/Leaves-Sticks	-40.064	88.128	-38.720	93.440	0.611	0.611
Rim-suspended/Others vs. Fibers/Leaves-Sticks	-34.893	77.787	-24.995	65.990	**<0.001**	**0.002**
Top-suspended/Others vs. Fibers/Leaves-Sticks	-28.820	65.640	-25.867	67.734	0.206	0.343
Nest type VS. Habitat						
Cup/Others vs. Grassland/Others	-30.258	68.517	-29.987	75.975	0.969	0.969
Cup/Others vs. Wetland/Others	-50.566	109.133	-44.312	104.624	**0.013**	0.078
Cup/Others vs. Woodland/Others	-63.614	135.228	-61.190	138.381	0.303	0.363
Pouch/Others vs. Forests/Others	-56.494	120.989	-50.922	117.845	**0.024**	**0.048**
Pouch/Others vs. Woodland/Others	-66.855	141.711	-61.055	138.110	**0.020**	0.060
Enclosed/Others vs. Forests/Others	-44.620	97.240	-39.565	95.130	**0.038**	0.057
Nest attachment VS. Habitat						
Basal A/Others vs. Grassland/Others	-19.776	47.552	-11.069	38.139	**0.001**	**0.002**
Basal B/Others vs. Woodland/Others	-67.380	142.761	-68.429	152.858	1.000	1.000
Lateral/Others vs. Wetland/Others	-53.792	115.584	-38.141	92.282	**<0.001**	**<0.005**
Rim-suspended/Others vs. Woodland/Others	-65.282	138.564	-59.386	134.772	**0.018**	**0.030**
Top-suspended/Others vs. Forest/Others	-44.620	97.240	-39.565	95.130	**0.038**	**0.047**
Nest material VS. Habitat						
Fibers/Leaves-Sticks vs. Grassland/Others	-19.999	47.998	-18.794	53.589	0.660	0.880
Fibers/Leaves-Sticks vs. Woodland/Others	-51.237	110.474	-49.739	115.479	0.558	1.116

Fibers/Leaves-Sticks vs. Wetland/Others	-39.828	87.656	-34.598	85.196	**0.033**	0.132
Fibers/Leaves-Sticks vs. Forests/Others	-42.227	92.454	-41.634	99.269	0.880	0.880



Graphic 1. Body sizes of 88 nest constructor species of Icteridae (Aves, Passerifomes) distributed across five nest attachment categories used as proxies of nest complexity. Boxes are 25% quartiles, whiskers are 75% quartiles, asterisks are average values, horizontal lines are medians, and points are outliers.

Log-transformed body masses were normally distributed (Table S5) and the One-way ANOVA used to compare body masses across nest attachment categories was significant (F = 12.240, P = <0.0001). The Tukey post-hoc test revealed that species constructing Top-suspended nests were significantly bigger than species from all of the other nests attachment categories, and species constructing Rim-suspended nests were smaller than those constructing Basal B (Graphic 1; Table S5).

4. Discussion

One of the most remarkable characteristics of the Icteridae is the evolution of the complex Top-suspended nests. The clade formed by the caciques and oropendolas (genera *Cacicus* and *Psarocolius*) diverged early from the icterid common ancestor that most likely constructed basally-supported cup-shaped nests, being for this reason poorly informative about the evolutionary trajectory that culminated in the large top-suspended nests. However, nests with the same pattern emerged again later, at least three times independently in the clade formed by *Icterus*, shading light into the potential origins of the Enclosed Top-suspended nests of the icterids. Within the *Icterus* clade, the Enclosed Top-suspended nests always derived from Rim-suspended Pouch nests. The entrance of the icterid Enclosed Top-suspended nests is in its upper part, differing from those found in other bird families, which entrances are lateral or in the inferior part of the nest (Crook, 2008; Street *et al.,* 2022). Then, these observations are consistent with a model in which the Enclosed Top-suspended nests of the icterids have derived from the gradual elongation of the nest rim of ancestors constructing Pouch, Rim-suspended nests, an idea that is corroborated by the existence of species with intermediate nest patterns between Rim- and Top-suspended, such as the Orange Oriole *I. auratus*, the Audubon’s Oriole *I. graduacauda* and the Yelow Tailed oriole *I. mesomelas*, that in our analyses were classified as Pouch. Although this evolutionary trajectory could be traced only within the genus *Icterus*, it is possible that the same evolutionary steps have occurred in *Cacicus* and *Psarocolius*. According to Street *et al.* (2022), a potential selective force driving the evolution of Top-suspended nests is the defense against nest predators, as it could difficult the access by snakes, for instance.

Important correlations occurred between the nest architectural components we analyzed, i.e. nest type, nest attachment mode and nest material. Cup nest with basal support (Basal B) was the ancestral condition of the icterid and it persisted in many lineages. The significant evolutionary correlation between these traits (Cup vs. Basal B), with the model of nest attachment depending on nest type best fitting the data, was not expected because different nest types can be Basal B when the whole avian phylogeny is considered (Fang *et al.,* 2018). The strong evolutionary correlation between Pouch nest type and Rim-suspended nest attachment mode likely occurred because a slightly elongated rim is what gives a nest a pouch format, meaning that the pouch concept relies on the fact that a nest is Rim-suspended, and the higher support to the model of nest type change dependent on the state of nest attachment confirms this premise. It is surprising that Cup-shaped Rim-suspended nests do not occur among the Icteridae species, because it is parsimonious to consider that the Pouch nests may have evolved from rim-attached Cup nests, rather than directly from the basally supported (Basal B) cup-shaped nest of the ancestor of the *Icterus* clade. The significant correlated evolution between Top-suspended attachment mode and Enclosed nest type, with the models in which change in nest type depends on the state of nest attachment mode, and vice-versa, both receiving enough AIC support, is an evidence of the interdependence of these traits. A potential explanation is the fact that, differently from some other bird groups, the Top-suspended nests of the icterids are long pendent structures that drift in the air, and in this case the evolution of the very restricted top entrance through rim elongation, that characterizes the enclosed nests, may have evolved to avoid eggs and nestlings to fall dawn. It is worth noting that within the Icteridae there are two different types of enclosed nests, with clearly distinct origins. In addition to the top-suspended nests of the caciques, oropendolas and some orioles that likely shifted from pouch to enclosed through rim elongation, the enclosed nests of the ground-nesters Western Meadowlark *Sturnella neglecta* and Eastern Meadowlark *S. magna*, that here we classified as Domed, likely derived from Cup nests constructed on the ground (Basal A) for which a roof has evolved, with the production of a lateral entrance. This is evidence that enclosed nests not only can have distinct origins, but also that they may emerge in response to different selective pressures occurred in different environmental contexts. The significant correlations of nest material with Pouch nest type and Rim-suspended attachment mode, with the models in which change in nest material depends on the state of nest type and attachment best fitting the data, suggest that shifts from basally supported cups to Rim-suspended pouches could have been the selective force driving nets material shift from Leaves/Sticks to Fibers.

The lack of correlated evolution between nest type and habitat is evidence that the types of habitats do not constrain nest type shifts and vice-versa, which was an expected result because in the icterids Cup, Pouch and Enclosed nests can be found throughout different types of habitats. Nest attachment, however, seemed to be a limiting factor for habitat shifts, as the models in which habitat change depends on the state of nest attachment received support in most of the comparisons. Basal A evolved in association with Grasslands, a type of habitat in which bushes and trees are not available for the occupation of species with Basal B or Lateral nest attachment modes, and our ancestral state reconstruction analyses suggested that the extant icterid species constructing Basal A nests in Grasslands (genera *Sturnella* and *Dolichonyx*) evolved secondarily from an ancestor constructing Basal B nests in Woodlands. The significant evolutionary correlation between Lateral and Wetland may be explained by the fact that in Wetland nests cannot be constructed on the ground and the available structures with which to tie a nest are often cattail-like vegetation, with vertical stems providing little Basal or Top supports, which may have favored the occupancy of icterids constructing nests with Lateral attachment mode. Similar explanations also may account for the association between Rim-suspended nests and Woodlands, i.e. Woodlands may provide vegetation structures for Rim-attachment better than grasslands and wetlands, for instance, especially because the icterids constructing Rim-suspended nests (genus *Icterus*) often attach their nests to the under parts of large horizontal leaves of palms or banana trees. However, at least for the associations between Basal A/Grassland and Rim-suspended/Woodland, the explanation that habitat can precede and drive nest attachment changes cannot be discarded, as the models of interdependent evolution also received enough AIC support. The relationship between Top-suspended nests and habitat presented a different evolutionary pattern because the model in which Forest occupation preceded the evolution of the complex nests of the caciques and oropendolas was the one receiving AIC support, although the Top-suspended nests of *Icterus* likely emerged in Woodlands. This finding is corroborated by the observation that Top-suspended nests, with their long pendent attachment structures, are constructed in tall forest trees by the extent icterids.

Although basally-supported Cup is the most common nest architecture across the passerine families, the common ancestor of all passerines likely constructed basally-supported Domed nests (Fang *et al.,* 2018). It means that ancestral variations could have contributed with the various independent origins of Domed or Enclosed nests within the Icteridae family. However, Lateral and Top-suspended nest attachment modes emerged late only in a few passerine lineages and the icterids do not share common ancestors with none of these clades (Fang *et al.,* 2018), which suggests that both Lateral and Top-suspended attachment modes emerged independently within the icterids through new mutations. The negative and significant phylogenetic signals evidenced strong phylogenetic conservatism in all nest architectural characters and also in Habitat, meaning that phylogenetically close species tend to resemble each other in these characters (Blomberg & Garland, 2002). Despite the phylogenetic conservatism, except for Dome nest type; Leaves/Sticks nest material, and Grassland and Woodland habitats, all of the other categories of the analyzed traits had at least two independent origins within the icterid phylogeny, revealing certain levels of evolutionary liability.

We found an unexpected positive correlation between body size and nest complexity. This pattern likely resulted from the fact that the caciques and oropendolas, which construct the most complex nests (Enclosed Top-suspended nests), are among the biggest icterids and are among the biggest passerine birds (36.16 – 405.31g), resembling crows in body size. Other bird families in which Top-suspended nests are present are often composed of much smaller species, e.g. Ploceidae (12.5 – 76.2g) and Tyrannidae (5.0 – to 99.1g) (Tobias *et al.,* 2022), an observation that may have led previous authors to formulate the hypothesis that the evolution of complex structures could be exclusive of small-bodied species (Collias, 1964; 1997; Hansell, 2000). Although the relationship between nest complexity and body size is still to be addressed within other bird families, we see a potential explanation for the correlation observed for the Icterids. The structure of nest wall differs markedly between the icterids constructing Pouch and Enclosed Top-suspended nests in relation to the other groups. The caciques, oropendolas and orioles use fine fibers and complex weaving techniques, including elaborate stitches and knots (Fraga, 2008; Street *et al.,* 2022) to produce a resistant fabric in which sometimes eggs and young can be seem through, while in the other clades they generally use interwoven leaves in the nest wall construction. This is evidence that the capacity to stitch fine materials is not exclusive of the small passerines as previously thought. Then, the shift in nest material associated with the construction of Rim- and Top-suspended nests, and the evidence that Top-suspended nests likely evolved from the Rim-suspended ones, provide support to a model in which shifts in nest material (and in stitching techniques) associated with the construction of Pouch nests provided nest wall resistance and permitted the subsequent evolution of Top-suspended nest construction even by the biggest icterids, once the produced type of fabric would be resistant enough to hold the weight of the parental and nestling caciques and oropendolas.

Our analyses did not provide support to the hypothesis that non-nest construction could be an intermediate step for the emergence of interspecific brood parasitism in the Icteridae, once these two different behavioural patterns have emerged independently in different lineages. Previously proposed hypotheses (reviewed in Fraga, 2008) were that competition among closely related icterid species for the use of nesting cavities could have led the less competitive species to develop brood parasitism on the more competitive ones (Skutch, 1996), however, none the extant genera related with *Molothrus* are cavity nesters, which provides little support for this idea. Another hypothesis predicted that the Brown-headed Cowbird *M. ater* was adapted to forage by following Bison Herds *Bison bison* in North America before European settlement, and that brood parasitism could have emerged to copy with this nomadic behavior (Widmann, 1907). This hypothesis also did not receive support from our phylogeny because *M. ater* was not the ancestral taxa within the genus *Molothrus*. Because the hypothesis that they could derive from ancestors with conspecific brood parasitism was also rejected (Kruger & Davies, 2002), the potential intermediate behaviours existing across the evolutionary trajectory between nest constructor and brood-parasitic icterid species still remain obscure.

While the seminal backgrounds for the understanding of bird nest evolution and diversification were derived using large datasets in recent years (Price & Griffith, 2017; Fang *et al.,* 2018; Sheard *et al.,* 2024), our study show that many important aspects can be revealed with studies on specific bird families. In this context, the family Icteridae is certainly one of the most important ones. In summary, we revealed for the first time that enclosed nests, for instance, can have different origins within the same family and we suggest that the remarkable Top-suspended nests constructed by the caciques, oropendolas and a few orioles, considered some of the most complex structures constructed by animals, derive from rim elongation of pouch nests. The construction of Top-suspended nests by the large caciques and oropendolas, was likely possible due to shifts in nest material and the evolution of stitching techniques capable of generating a resistant fabric, contradicting a long-standing idea that complex nest structures could be constructed only by small birds. These are new discoveries for birds in general, and our work provide insights for the development of other studies addressing bird families, each with specific adaptations capable of revealing the intricate mechanisms behind animal construction skills.

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6. Supplementary materials

Table S1. Best fitted evolutionary models for nest type, nest attachment mode, nest material, habitat, and reproductive strategy of birds from the family Icteridae. The candidate models were Equal Rates (ER); All-Rates Different (ARD), and Symmetric Entities (SYM), which were selected using Akaike Information Criterion (AICc) for posterior use in ancestral state estimates.

Nest type			
Model	Log-Likelihood	AIC	AICc
ER	**-38.810**	**79.621**	**79.668**
ARD	-33.650	91.301	95.517
SYM	-36.195	84.391	85.441
Nest attachment			
Model	Log-Likelihood	AIC	AICc
ER	-73.860	149.720	149.767
ARD	-55.045	150.090	162.817
SYM	**-59.800**	**139.600**	**142.495**
Nest material			
Model	Log-Likelihood	AIC	AICc
ER	**-15.815**	**33.631**	**33.678**
ARD	-13.718	31.437	31.580
SYM	**-15.815**	**33.631**	**33.678**
Habitat			
Model	Log-Likelihood	AIC	AICc
ER	-67.420	136.841	136.884

ARD	-55.504	135.009	138.860
SYM	**-60.414**	**132.829**	**133.795**
Reproductive strategies			
Model	Log-Likelihood	AIC	AICc
ER	**-16.121**	**34.243**	**34.286**
ARD	-15.325	42.651	43.616
SYM	-15.879	37.758	38.024

Table S2. Scaled likelihoods indicating the most likely state of nest type, nest attachment mode, nest material, habitat, and reproductive strategy for the root of an Icteridae consensus phylogeny, obtained through ancestral character state estimates.

Nest type				
Cup	Domed	Enclosed	Pouch	
0.995	0.001	0.002	0.002	
Nest attachment				
Basal A	**Basal B**	Lateral	Rim-suspended	Top-Suspended
0.278	**0.544**	0.166	0.008	0.001
Nest material				
Fibers	**Leaves/Sticks**			
0.050	**0.950**			
Habitat				
Forest	Grassland	Wetland	**Woodland**	
0.004	0.361	0.160	**0.475**	
Reproductive strategies				
Nest constructor	Non-nest constructor	Brood-parasitic		
0.998	0.001	0.001		

Table S3. Estimates of phylogenetic signal for binarized icterid nest traits and habitat using D statistic. Observed D values were compared with distributions of D values generated under the phylogenetic randomness model (D = 1; no phylogenetic signal) and Brownian motion model (D = 0; phylogenetic signal). P values indicate whether observed D values have differed significantly from 1 and 0.

<table>
<tr><th colspan="4">Nest type</th></tr>
<tr><td></td><td>D-Value</td><td>Random (P)</td><td>Brownian motion (P)</td></tr>
<tr><td>Cup/Others</td><td>-0.882</td><td>0.000</td><td>0.997</td></tr>
<tr><td>Domed/Others</td><td>-2.569</td><td>0.003</td><td>0.894</td></tr>
<tr><td>Enclosed/Others</td><td>-1.206</td><td>0.000</td><td>1.000</td></tr>
<tr><td>Pouch/Others</td><td>-0.619</td><td>0.000</td><td>0.954</td></tr>
<tr><th colspan="4">Nest attachment</th></tr>
<tr><td></td><td>D-Value</td><td>Random (P)</td><td>Brownian motion (P)</td></tr>
<tr><td>Basal A/Others</td><td>-1.376</td><td>0.000</td><td>0.996</td></tr>
<tr><td>Basal B/Others</td><td>-0.316</td><td>0.000</td><td>0.792</td></tr>
<tr><td>Lateral/Others</td><td>-0.430</td><td>0.000</td><td>0.855</td></tr>
<tr><td>Rim-suspended/Others</td><td>-0.752</td><td>0.000</td><td>0.979</td></tr>
<tr><td>Top-suspended/Others</td><td>-1.257</td><td>0.000</td><td>1.000</td></tr>
<tr><th colspan="4">Nest material</th></tr>
<tr><td></td><td>D-Value</td><td>Random (P)</td><td>Brownian motion (P)</td></tr>
<tr><td>Fibers/Leaves - Sticks</td><td>-1.008</td><td>0.000</td><td>0.998</td></tr>
<tr><th colspan="4">Habitat</th></tr>
<tr><td></td><td>D-Value</td><td>Random (P)</td><td>Brownian motion (P)</td></tr>
<tr><td>Grassland/Others</td><td>-0.617</td><td>0.000</td><td>0.962</td></tr>
<tr><td>Wetland/Others</td><td>-0.583</td><td>0.000</td><td>0.959</td></tr>
<tr><td>Woodland/Others</td><td>-0.568</td><td>0.000</td><td>0.962</td></tr>
<tr><td>Forest/Others</td><td>-0.632</td><td>0.000</td><td>0.964</td></tr>
</table>

Table S4. Selection of the best fitted Pagel’s models of correlated evolution for pairs of binarized nest and habitat traits of icterid birds, using Akaike Information Criterion (AIC) and ΔAIC.

Nest type VS. Nest attachment			
MODEL	Log-Likelihood	AIC	ΔAIC
Nest attachment (Basal B) and nest type (Cup) evolved independently	-58.087	124.174	13.328
Nest attachment (Basal B) and nest type (Cup) evolved interdependently	-49.100	114.200	3.354
Nest type (Cup) depends on nest attachment (Basal B)	-55.511	123.022	12.176
Nest attachment (Basal B) depends on nest type (Cup)	-49.423	110.846	0.000
Nest attachment (Rim-suspended) and nest type (Pouch) evolved independently	-52.864	113.729	42.119
Nest attachment (Rim-suspended) and nest type (Pouch) evolved interdependently	-29.576	75.152	3.542
Nest type (Pouch) depends on nest attachment (Rim-suspended)	-29.805	71.610	0.000
Nest attachment (Rim-suspended) depends on nest type (Pouch)	-30.264	72.528	0.918
Nest attachment (Top-suspended) and nest type (Enclosed) evolved independently	-30.688	69.377	26.687
Nest attachment (Top-suspended) and nest type (Enclosed) evolved interdependently	-15.348	46.697	4.007
Nest type (Enclosed) depends on nest attachment (Top suspended)	-15.345	42.690	0.000
Nest attachment (Top-suspended) depends on nest type (Enclosed)	-15.345	42.690	0.000
Nest type VS. Nest material			
MODEL	Log-Likelihood	AIC	ΔAIC
Nest material (Fibers/Leaves-Sticks) and nest type (Cup) evolved independently	-35.050	78.100	17.990
Nest material (Fibers/Leaves-Sticks) and nest type (Cup) evolved interdependently	-24.270	64.540	4.430
Nest material (Fibers/Leaves-Sticks) depends on nest type (Cup)	-25.232	62.465	2.355
Nest type (Cup) depends on Nest materials (Fibers/Leaves-Sticks)	-24.055	60.110	0.000
Nest material (Fibers/Leaves-Sticks) and nest type (Pouch) evolved independently	-36.999	81.999	13.595

Nest material (Fibers/Leaves-Sticks) and nest type (Pouch) evolved interdependently	-26.900	69.800	1.396
Nest material (Fibers/Leaves-Sticks) depends on nest type (Pouch)	-28.202	68.404	0.000
Nest type (Pouch) depends on Nest material (Fibers/Leaves-Sticks)	-35.102	82.205	13.801
Nest attachment VS. Nest material			
MODEL	Log-Likelihood	AIC	ΔAIC
Nest material (Fibers/Leaves-Sticks) and nest attachment (Rim-suspended) evolved independently	-34.893	77.787	13.671
Nest material (Fibers/Leaves-Sticks) and nest attachment (Rim-suspended) evolved interdependently	-24.995	65.990	1.874
Nest material (Fibers/Leaves-Sticks) depends on nest attachment (Rim-suspended)	-26.058	64.116	0.000
Nest attachment (Rim-suspended) depends on Nest material (Fibers/Leaves-Sticks)	-32.961	77.922	13.806
Nest type VS. Habitat			
MODEL	Log-Likelihood	AIC	ΔAIC
Habitat (Forest) and nest type (Pouch) evolved independently	-56.494	120.989	5.097
Habitat (Forest) and nest type (Pouch) evolved interdependently	-50.922	117.845	1.953
Nest type (Pouch) depends on habitat (Forest)	-55.667	123.334	7.442
Habitat (Forest) depends on nest type (Pouch)	-51.946	115.892	0.000
Nest attachment VS. Habitat			
MODEL	Log-Likelihood	AIC	ΔAIC
Habitat (Grassland) and nest attachment (Basal A) evolved independently	-19.776	47.552	9.413
Habitat (Grassland) and nest attachment (Basal A) evolved interdependently	-11.069	38.139	0.000
Nest attachment (Basal A) depends on habitat (Grassland)	-14.933	41.866	3.727
Habitat (Grassland) depends on nest attachment (Basal A)	-11.069	38.848	0.709
Habitat (Wetland) and nest attachment (Lateral) evolved independently	-53.792	115.584	31.175
Habitat (Wetland) and nest attachment (Lateral) evolved interdependently	-38.141	92.282	7.873
Nest attachment (Lateral) depends on habitat (Wetland)	-39.660	91.321	6.912
Habitat (Wetland) depends on nest attachment (Lateral)	-36.204	84.405	0.000
Habitat (Woodland) and nest attachment (Rim-suspended) evolved independently	-65.282	138.564	3.792

Habitat (Woodland) and nest attachment (Rim-suspended) evolved interdependently	-59.386	134.772	0.000
Nest attachment (Rim-suspended) depends on habitat (Woodland)	-63.182	138.364	3.592
Habitat (Woodland) depends on nest attachment (Rim-suspended)	-62.190	136.381	1.609
Habitat (Forest) and nest attachment (Top-suspended) evolved independently	-44.620	97.240	4.988
Habitat (Forest) and nest attachment (Top-suspended) evolved interdependently	-39.565	95.130	2.878
Nest attachment (Top-suspended) depends on habitat (Forest)	-40.126	92.252	0.000
Habitat (Forest) depends on nest attachment (Top-suspended)	-42.691	97.383	5.131

Table S5. Results of Shapiro-Wilk tests of normality for Icteridae log-transformed body mass data across different nest attachment classifications.

Variable	W-value	P-value
Basal A	0.933	0.476
Basal B	0.900	0.095
Lateral	0.946	0.427
Rim-suspended	0.929	0.096
Top-suspended	0.926	0.088

CAPITULO II: Nest site is correlated with nest type and body size in Troglodytidae passerines

Nest site is correlated with nest type and body size in Troglodytidae passerines

Abstract

Nest characteristics are highly variable in the Passeriformes, but works on specific lineages showed that the evolutionary patterns of passerine nests, and their correlates, are mixed and need further clarification. Here, we used phylogenetic comparative methods to address the evolutionary patterns of nest type, nest site and habitat in the Troglodytidae, a passerine group with diversified nest and habitat characteristics. The common ancestor of the Troglodytidae likely constructed enclosed nests within sheltered sites (cavity or crevice), but the radiation of the group was characterized by i) shifts to exposed nest sites (vegetation) with retention of enclosed nests or ii) retention of sheltered sites with nest simplification (cup nests). Nest site and nest type presented strong phylogenetic conservatism and evolved interdependently, while habitat was poorly correlated with nest evolution. A phylogenetic mixed modelling approach showed that sheltered nest sites were associated with small body size, likely to avoid competition with other animals for these places. With these results, we improve the understanding of nest characters evolution in the Troglodytidae and we reveal evolutionary aspects not observed so far for passerine birds.

Keywords: Nest evolution; Correlated evolution; Ancestral state reconstruction; Phylogenetic signal; Phylogenetic comparative methods

1. Introduction

Understanding the history of phenotypic variation between organisms, and how they correlate with environmental factors are among the main interests of Evolution (Cornwell & Nakagawa, 2017). In addition to morphological, physiological and behavioural aspects, in some animal groups the evolutionary trajectories also can be reflected in the structures they construct (Mainwaring *et al.,* 2023). Bird nests play crucial roles in reproduction and survival, providing protection and suitable microhabitats for eggs and nestlings, being for these reasons under strong selective pressures (Hansell, 2000; Martin *et al.,* 2017). While in most bird orders nests can be simple scrapes or platforms, in the passerines nest architecture reached the highest diversification, and to some extent nest innovations have contributed with the expansion of this bird group to new habitats (Fang *et al.,* 2018; Medina *et al.,* 2022; Ocampo *et al.,* 2023).

Nest type and nest site are important adaptive components of bird reproduction (Fang *et al.,* 2018; Medina *et al.,* 2022; Ocampo *et al.,* 2023). In the passerine birds, nest types often vary from cups, which are open nests with well-defined rim, to enclosed nests, which are roofed structures, usually presenting a lateral entrance (Hansell, 2000). Compared to open nests, enclosed nests can provide important thermal benefits (Brightsmith, 2005; Martin *et al.,* 2017), but they take longer to be constructed and are associated with smaller geographic ranges (Medina *et al.,* 2022). Nest site is the location where a nest is placed, and can vary from ground, tree and non-tree vegetation, cavities, crevices, banks and cliffs (Fang *et al.,* 2018). Nesting in cavities, for instance, also may provide protection against weather conditions, but adaptation to use secondary-cavities, i.e. the dependence on pre-existing cavities, can impose limitations because these nest sites are scarce (Wesolowsky, 2002; Cockle *et al.,* 2010). Then, it was the evolution of open nests constructed on vegetation that was associated with the big passerine radiation (Price & Griffith, 2017; Fang *et al.,* 2018). Yet, transitions back to the states of ancestral clades can occur in passerine nest components (Fang *et al.,* 2018; Ocampo *et al.,* 2023), and the correlates of nest type and nest site shifts are still generally obscure.

The insectivorous passerines of the family Troglodytidae (wrens) can provide researchers with cues about the potential trajectories of nest evolution and their correlates because nests in this family vary from simple cups to enclosed nests, constructed in tree branches, non-tree vegetation, secondary cavities, crevices and rock (Sick, 1997; Winkler *et al.,* 2020). Furthermore, this predominantly New World bird family is composed of approximately 86 species and 19 genera, with representatives occurring across a variety of habitats, including tropical forests, woodlands, deserts, grasslands and marshlands (Winkler *et al.,* 2020). Here, we used ancestral state reconstruction and phylogenetic signal to address the origin, trajectory and evolutionary patterns of nest type, nest site and habitat in the Troglodytidae. We then used evolutionary correlation analyses to test whether nest type evolved in association with nest site and whether nest site evolved in association with habitat. Because the categories of nest sites are widespread across many different habitats, we do not expect to see correlation between nest site and habitat, but because constructing enclosed nests within cavities and crevices could be a waste of energy because these sheltered sites are already roofed and concealed, we predict that nest type is associated with nest site. Based on the observation that the extant Troglodytidae using sheltered nest sites (secondary cavities, crevices or rocks) are very small birds, we also used phylogenetic mixed modeling to test for the first time the prediction that sheltered nest sites can be associated with small body sizes in a bird lineage.

2. Methods

2.1. Nest and species traits

We obtained nest type and nest site information primarily from Birds of the World, the Cornell Lab of Ornithology (https://birdsoftheworld.org/bow/home). Because most Central and South American species are known from only a few nests, whenever possible we compared author’s descriptions with pictures available in Birds of the World, and in citizen science datasets such as eBird (https://ebird.org) and Wikiaves (https://www.wikiaves.com.br) to check for potential inconsistencies. For species with no information in Birds of the World, we searched for articles using Google Scholar and the indexing base Web of Science, with the species’ scientific and English name as keyword. Especially for Neotropical species, we also consulted non-indexed texts, such as books and articles from local journals.

We classified nest type using two broad categories, cup and enclosed (see also (Ocampo *et al.,* 2023)). Cup nests are open structures with a well-defined rim (Hansell, 2000). This is the most common form of open nest in the order Passeriformes, and must represent well these birds because other forms of open nests, such as platform, are rare in the passerines (Fang *et al.,* 2018; Leite *et al.,* 2024). Enclosed nests are structures constructed by the parental individuals, with a roof and a lateral entrance (Hansell, 2000; Fang *et al.,* 2018). We classified nest sites, characterized as the location in which a nest is placed, as exposed (tree and non-tree vegetation) and sheltered (secondary cavity, crevice, tunnel, buildings, roofed cliff or rock fissure) (Alerstam & Hogstedt, 1981). This is because a single species could use different types of vegetation, e.g. bushes, vines, and trees, making the classification of a specific vegetation type difficult. Similarly, many species were generalist in relation to the sheltered sites they used. Although some works have considered ‘’cavity’’ as a nest type (e.g. (Fang *et al.,* 2018)), the Troglodytidae can construct both cup and enclosed nests inside cavities or crevices, then here we considered cavity as a nest site. We categorized as sheltered one species that could construct its nests in both sheltered and exposed sites (*Troglodytes troglodytes*). We obtained habitat and body mass information from a recent dataset, with habitats classified as forest, woodland, shrubland, wetland, grassland and rock (Tobias *et al.,* 2022). Then, for the analyses, habitat was binarized as forest (forest and woodland) and non-forest (shrubland, wetland, grassland and rock).

2.2. Phylogenetic hypothesis

We generated a sample of 10,000 time-calibrated trees from birdtree.org (Jetz *et al.,* 2012) using the method of Ericson as a backbone for phylogenetic reconstruction and we considered only species with DNA information (Ericson *et al.,* 2006; Jetz *et al.,* 2012). Then, we used the maximum clade credibility tree summarization method implemented in the Phangorn R Package (Schliep, 2011) to select the tree with the highest sum of clades posterior probabilities from the tree dataset (O’Reilly & Donoghue, 2018) and we used this consensus tree in all of the subsequent analyses (Ocampo *et al.,* 2023; Leite *et al.,* 2024).

2.3. Ancestral state reconstruction and phylogenetic signal

To address the origin and evolutionary trajectory of nest type, nest site and habitat, we estimated ancestral character state for each ancestral node of the phylogeny using the Maximum Likelihood reconstruction procedure for discrete characters of the Phytools package (Revell, 2012) (ancr-function) (Revell, 2024). To achieve this purpose, we first fitted the best model of evolution for each character by addressing three models, Equal Rates (ER); All-Rates Different (ARD) and Symmetric Entities (SYM). Because different models presented similar likelihoods to fit the data (see Results), we used a model averaging procedure recommended for these cases (Revell, 2024).

We estimated the phylogenetic signal for each of the characters with D statistic (Fritz & Purvis, 2010), using the phylo.d function of the R package caper (Orme, 2018). This method is suitable for discrete binary data, with a value of $D < 0$ indicating stronger phylogenetic signal than expected by Brownian-motion model of evolution; a value = 0 indicating that the character states are distributed as if they have evolved under a Brownian-motion; $D = 1$ indicating random distribution and $D \geq 1$ indicating that species sharing the same traits are less related than predicted by chance. We tested whether D differed significantly from 0 and from 1 ($P < 0.05$) by comparing the observed value with a distribution of 1,000 values generated by tip-shuffling, which is particularly useful when values between 0 and 1 occur.

2.4. Correlated evolution analyses

We tested for the occurrence of correlated evolutionary changes between nest type and nest site and between nest site and habitat. To achieve this purpose, we used the FitPagel function of the package Phytools (Revell, 2012) to fit the correlated evolution model of (Hadfield, 2010) with fitMK method. This procedure estimates the rates of change of the characters across the branches of a phylogenetic tree and models of independent and of correlated evolution are fitted to the data. First, we tested for evidence of correlated evolution across the target combinations of binary traits by addressing whether the model of dependent evolution differed significantly from the model of independent evolution using a likelihood ratio test with alfa = 0.05. Then, for the pairs of combinations presenting significant correlation we addressed the types of relationships by comparing the models, interdependent, substitution rate of the state x dependent on the state of y, and the model of substitution rate of the state y dependent on the state of x, using Akaike Information Criterion (AIC).

2.5. Phylogenetic mixed modelling

To test whether nesting in sheltered sites could be associated with reduced body size we computed a Markov Chain Monte Carlo (MCMC) Bayesian Phylogenetic Mixed Modeling (BPMM), as implemented in the MCMCglmm v2.20 R package (Hadfield, 2010). We performed a logistical modelling using sheltered/exposed (1 and 0) as the response variable, log transformed body mass (g) as the predictive variable and the consensus phylogeny as a random factor. The MCMC was programmed to 1010,000 iterations, with a burn-in of 10,000 and a thinning interval of 1000 iterations, resulting in a posterior distribution of 1000 samples.

To assess chain convergence, we i) visually inspected the trace plots of the model variables using the plotTrace function of the plotMCMC R package (Magnusson & Stewart, 2020), ii) checked whether the autocorrelation factor was <0.1 with the autocorr.diag function of the Coda R package (Plummer *et al.,* 2006), and iii) generated a Gelman–Rubin statistic through adopting scaling reduction factors (Rc) < 1.1 as a threshold (Gelman & Rubin, 1992), using the function gelman.diag of the Coda R package. The autocorr.diag function contrasts the dependence of parameter values between samples drawn across serial segments within a chain, with high autocorrelation values indicating slow mixing and slow chain convergence. The Gelman–Rubin procedure investigates chain convergence by comparing the variations of parameter values within and between chains, and we generated Rc values for each variable using 10 replicates of the same model.

3. Results

Of the 86 species of Troglodytidae, 53 had DNA information in birdtree and nesting data in the literature, being used in our analyses. Of these species, 45 constructed enclosed nests (85.0 %) and eight constructed cup nests (15.0 %) (Figure 1). Of the species constructing enclosed nests, 43 placed them on exposed sites, one placed them within sheltered sites (*Thryotorus ludovicianus*), and one could construct nests both on exposed and within sheltered sites (*Troglodytes troglodytes*). All of the species constructing cup nests placed them within sheltered sites. Species constructing enclosed nests occurred in forest (n = 23), woodland (n = 11), shrubland (n = 9) and wetland (n = 2), and species constructing cup nests occurred in forest (n = 4), shrubland (n = 2) and rock (n = 2). ER and SYM evolutionary models had similar likelihoods to fit the data for the three variables (nest type, nest site and habitat), then we used model averaging for ancestral character state estimates (Table S1). The evolutionary history reconstruction revealed that the common ancestor of the Troglodytidae likely built enclosed nests (root probability = 0.976) placed in sheltered site (root probability = 0.997) (Figure 2) in forest habitat (root probability = 0.578) (Figure S1). We found that shifts from enclosed to cup nests and from sheltered to exposed nest sites occurred each five times independently (Figure 2). For Habitat, nine independent shifts occurred from forest to non-forest, and we reveal two potential reversals in *Campylorhynchus chiapensis* and *T. troglodytes* (Figure S1). Both nest type (D = -1.65, P(random) < 0.001, P(Brownian) = 0.980) and nest site (D = -1.20, P(random) < 0.001, P(Brownian) = 0.958) presented negative D values and strong phylogenetic signal. Habitat presented a positive D value that diverged significantly from random, but not from Brownian distribution across the phylogeny (D = 0.11, P(random) = 0.005, P(Brownian) = 0.450).

Figure 1. Nest type and nest site of two species of Troglodytidae, (A) enclosed nest of *Cantorchilus leucotis* in an exposed site on the vegetation (Photo credits, Dáfini

Letícia Bruno); (B) nest of *Troglodytes aedon*, constructed in a sheltered site, i.e. inside an artificial horizontal cavity made of bamboo; (C) details of *T. aedon* nest inside the bamboo; (D) nest of *T. aedon* extracted from the cavity after fledging, evidencing its cup type embedded in a substantial base of twigs.

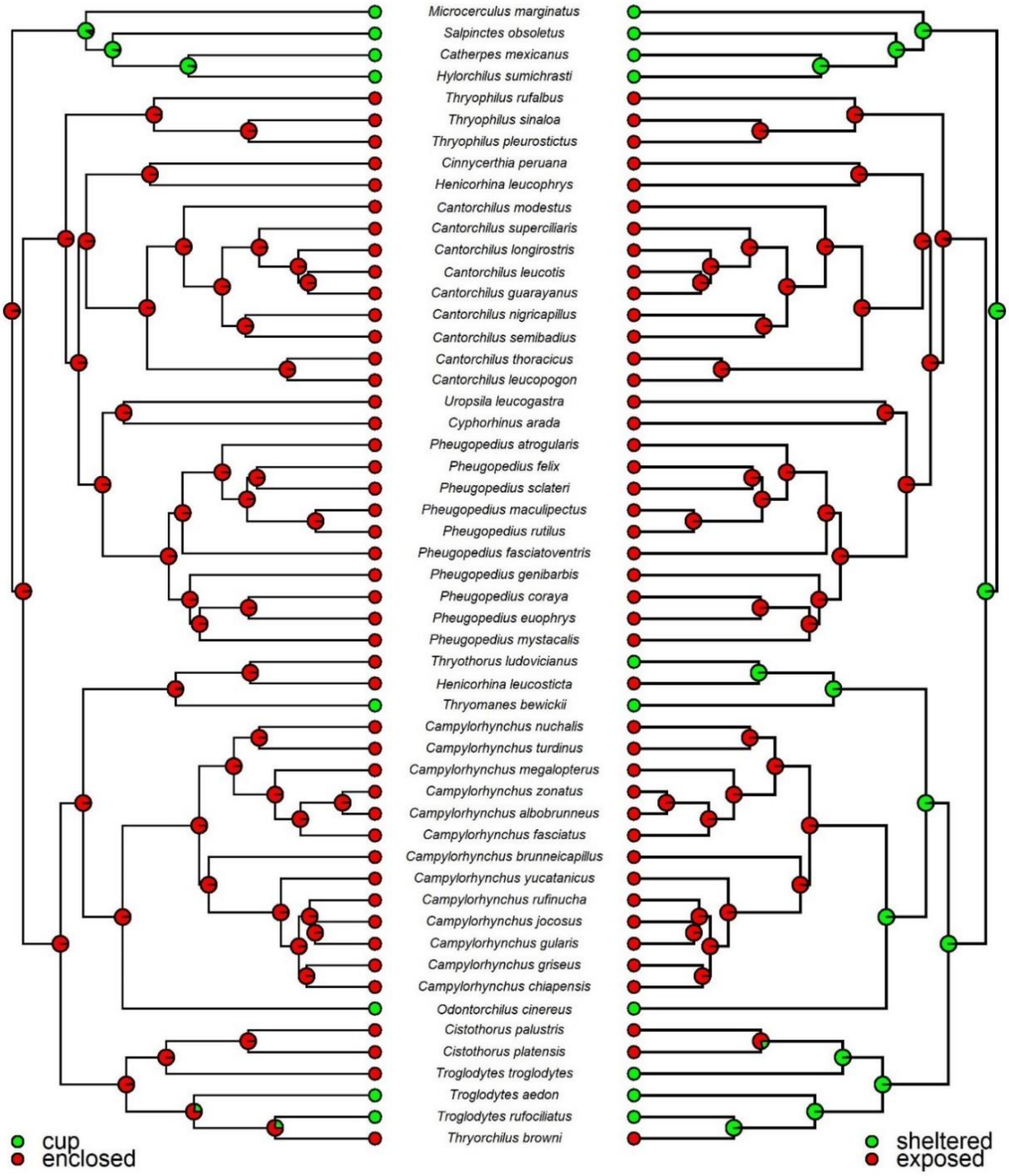

Figure 2. Character state distributions and ancestral state estimates for nest type (cup and enclosed) and nest site (sheltered and exposed), across a consensus phylogeny of the Troglodytidae (Aves, Passeriformes).

We found evidence for correlated evolution between nest type and nest site (independent, Log-Likelihood −31.461, AIC 70.921, dependent, Log-Likelihood

−19.41, AIC 54.811; likelihood ratio test p < 0.001), with enclosed nests being more common in exposed and cup nests being exclusive from sheltered sites. The best fit model was the one of nest type dependent on the state of nest site, but the model of nest site dependent on the state of nest type also received strong support (ΔAIC < 2) (Table 1), suggesting that nest site could influence nest type evolution, and vice-versa. There was no evidence for correlated evolution between nest site and habitat (independent, Log-Likelihood −43.251, AIC 94.492, dependent, Log-Likelihood −41.793, AIC 99.581; likelihood ratio test p = 0.571).

Table 1. Comparisons of Pagel's models of correlated evolution for pairs of Troglodytidae binary traits using Akaike Information Criterion (AIC) and ΔAIC.

Binary traits	**Model**	Log-Likelihood	**AIC**	**ΔAIC**
Nest site Vs. nest type	Independent	-31.461	70.921	19.717
	Interdependently	-19.411	54.811	3.607
	Nest site depend on nest type	**-19.692**	**51.393**	**0.189**
	Nest type depend on nest site	**-19.604**	**51.204**	**0.000**

We found a significant negative correlation between sheltered nest site and log-transformed body mass (Intercept, posterior mean = 5.71, 95% CI = -0.39 – 12.21, Posterior Probability = 0.076; log-transformed body mass, posterior mean = -2.34, 95% CI = -4.58 – -0.14, Posterior Probability = 0.036). The autocorrelation estimate was < 0.02, the Rc value did not reach 1.1 and the trace plot showed that the MCMC chain converged satisfactorily (Graphic S1), evidencing that chain mixing, burn-in and chain lengths were adequate.

4. Discussion

The evolutionary trajectory of the Troglodytidae was characterized by important transitions in both nest type and site, despite the strong phylogenetic conservatism observed for these traits. The most recent common ancestor of all passerines likely constructed enclosed nests on the vegetation (exposed nest site) (Price & Griffith, 2017; Fang *et al.,* 2018). Although it is also the most common pattern observed for the extant Troglodytidae, the ancestor of the passerine clade gathering the Troglodytidae, Sittidae, Certhiidae and Polioptilidae nested within secondary cavities (Fang *et al.,* 2018). Then, the individual patterns of nest type and nest site we observed are not innovations of the Troglodytidae, but the potential construction of enclosed nest within sheltered site in the most recent common ancestor was unexpected because these sites are already protected and the construction of enclosed nests is energetically costly (Barve & Mason, 2015; Medina *et al.,* 2022). Overall, cavities are scarce, generating competition, but they provide increased nest antipredatory protection and are associated with larger species that are more capable of competing for them (Li & Martin, 1991; Wesolowski, 2002; Cockle *et al.,* 2010; Vanadzina *et al.,* 2024). Enclosed nests built on the vegetation do not provide increased antipredatory protection and are associated with smaller species, likely because they provide thermal benefits for small birds that lose heat easier (Martin *et al.,* 2017). While woodpecker holes, for instance, are well protected (Cockle *et al.,* 2011; Zenil-Ferguson *et al.,* 2023), the Troglodytidae are generalist, using also crevices, cliffs or banks, that are not so closed. Then, it is possible that some ancestral lineages have benefited from enclosed nest construction even within these sheltered places, as a consequence of small body size and the use of sheltered sites of lower quality.

However, the pattern observed for the ancestral node was retained in only one species, with the radiation of the Troglodytidae characterized mainly by, i) transitions from sheltered to exposed nest sites with the retention of enclosed nests or ii) retention of sheltered nest sites associated with nest simplification, i.e. shifts from enclosed to cup nests, suggesting adaptive benefits of these patterns in relation to the ancestral form. Reduction of competition can be a potential explanation for nest site shifts from sheltered to exposed (see also (Joyce, 1993)) and an evidence is the negative correlation we found between the use of sheltered nests sites and body mass. Although it contradicts the general tendency of larger birds using sheltered nest sites (Li & Martin, 1991; Vanadzina *et al.,* 2024), our finding is consistent with a model in which only smaller species could have retained the sheltered nest sites by using the smaller cavities or crevices to avoid competition. Indeed, the representatives of the genus *Troglodytes* are smaller than any other cavity-nester species from Neotropical avian communities (Cockle *et al.,* 2010). Thermal benefits could account for the retention of enclosed nests after shifts to exposed nest sites and an evidence is the fact that transitions to cup nests, that are less energetically costly but more exposed to weather conditions, occurred only in the few Troglodytidae that retained sheltered nest sites.

An important question is whether transitions from nest sites that provide easy nest support from below (ground, cliffs and roles) to the vegetation could occur primarily for cup nests because they are simpler, facilitating the evolution of the mechanisms needed to attach a nest to the vegetation. If this is true, the construction of cup nest would be a necessary evolutionary step for shifts between sheltered to exposed nest sites, and enclosed nests would emerge secondarily on the vegetation through the evolution of roof construction. However, the lack of cup-nester ancestors for the species constructing enclosed nests on the vegetation, the observation of *T. troglodytes* constructing enclosed nests both in exposed and sheltered sites, and also of *T. cobby* that was not included in our analyses due the lack of DNA information, revealed that transitions to vegetation can be direct even for birds constructing enclosed nests. These transitions could be constrained, however, by the costs of increased nest predation in exposed nest sites, but at least in some species of *Campylorhynchus*, this cost can be balanced by nest construction in association with aggressive wasps (Barker, 2017; Winkler *et al.,* 2020).

The significant evolutionary correlation between nest type and nest site corroborated the family-level results found for the whole Avian phylogeny (Fang *et al.,* 2018), which together with the strong phylogenetic conservatism, suggest that one or both of these characters may have important adaptive functions. For birds in general, nest type shifts most likely preceded nest site shifts, meaning that evolutionary changes in nest type permitted the expansion to new nesting sites (Fang *et al.,* 2018). Our results differed in that either ways were possible in the Troglodytidae, and the lack of evidence for coevolution between nest site and habitat suggests that changes in nest architecture are more associated with specific nest sites found within the habitats than with the habitats *per se* (see also (Barve & Mason, 2015; Ocampo *et al.,* 2023)).

Knowledge on the phylogenetic relationships of the Troglodytidae has advanced mainly at genus-level (Barker, 2017), and the assignment of the two species of *Henicorhina* to two different clades suggests that potential taxonomic problems may still occur in this group at the level of species. For *Odontorchilus cinereus*, nest site information was reliable and contributed with one of the observed transitions, but nest type was not explicitly described and based on an available picture of a congener's nest (Johnson, 2017) we assumed that it builds cup nests. Although it is still to be confirmed, we noticed that when this genus is removed the significance of the results does not change (data not shown). A total of 33 species were not included in our analyses because of the lack of DNA or nesting information, although the only genus not analyzed here was the monotypic *Ferminia*. It is possible that future analyses with more complete phylogenetic and ecological information can improve our results, but here we reveal unprecedented nest evolutionary patterns for the Troglodytidae and we contribute with information new to the Passeriformes.

5. References

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6. Supplementary materials

Table S1. Results of the analyses performed to select the best fitted evolutionary models for nest type, nest site and habitat for ancestral character state estimation in Troglodytidae passerines. Candidate models were Equal Rates (ER); All-Rates Different (ARD) and Symmetric Entities (SYM), with the best model being selected through corrected Akaike Information Criterion (AIC) and AIC Corrected (AICc).

Nest Type		
Model	Log-Likelihood	AIC
ER	**-17.215**	**36.431**
SYM	**-17.215**	**36.431**
ARD	-16.861	37.722
Nest Site		
Model	Log-Likelihood	AIC
ER	**-20.315**	**42.630**
SYM	**-20.315**	**42.630**
ARD	-14.598	33.197
Habitat		
Model	Log-Likelihood	AIC
ER	**-30.037**	**62.075**
SYM	**-30.037**	**62.075**
ARD	-28.649	61.298

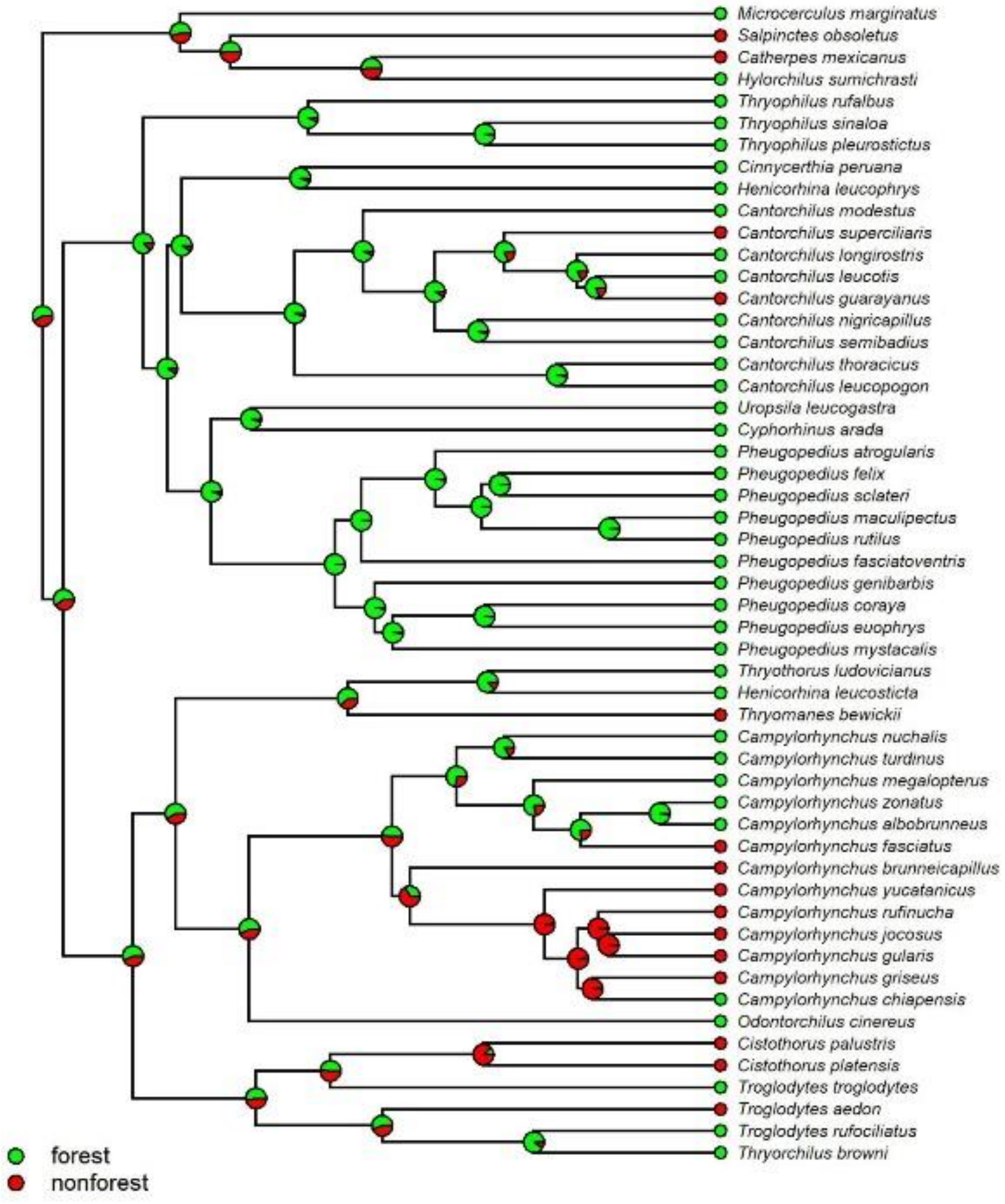
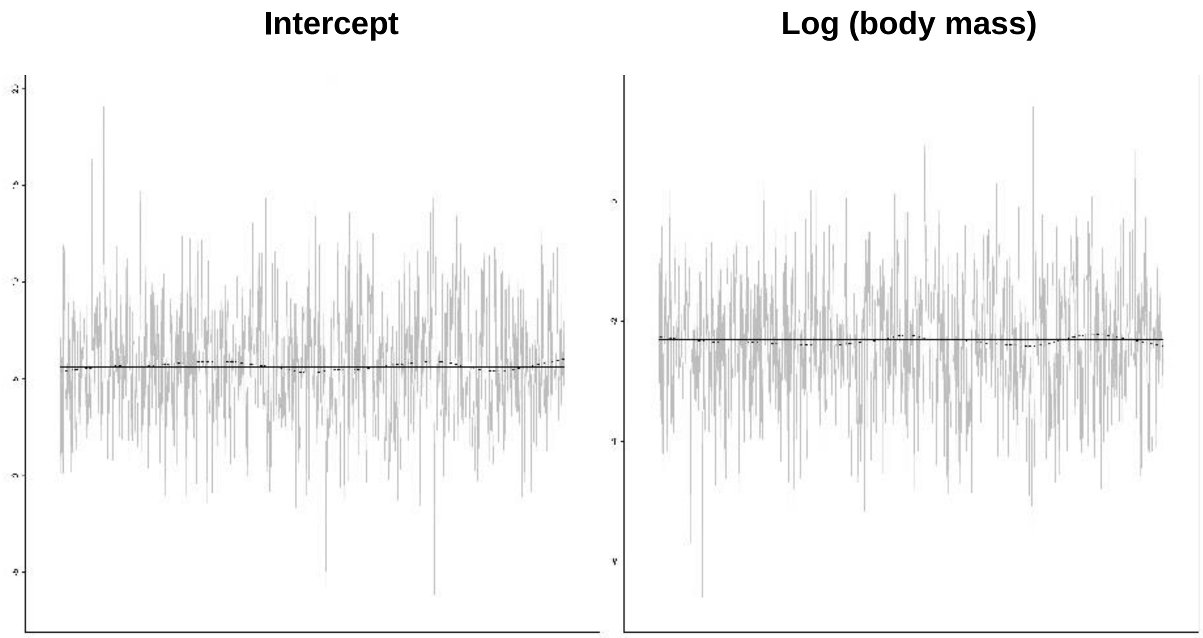
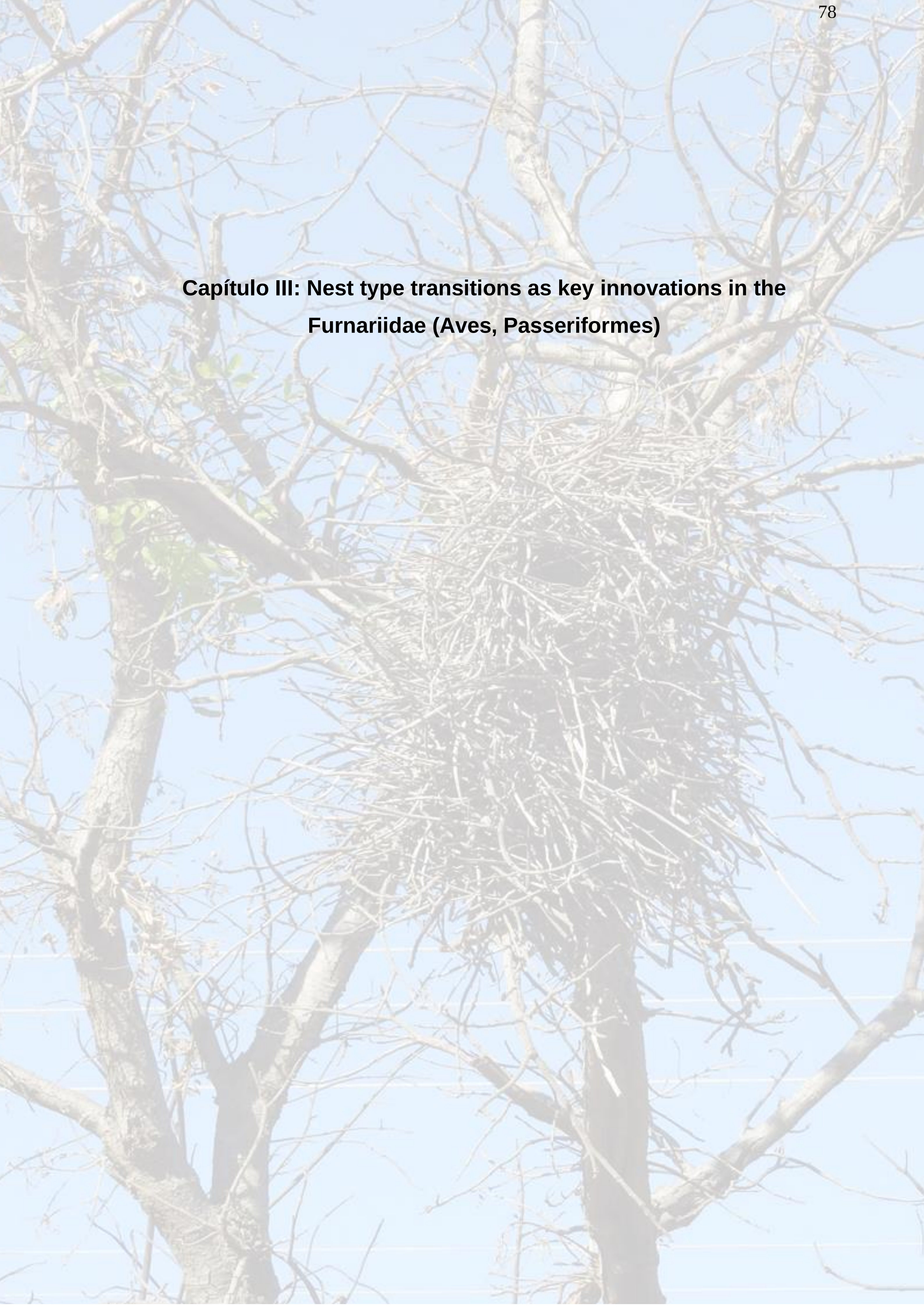


Figure S1. Character state distributions and ancestral state estimates for habitat across a consensus phylogeny of the Troglodytidae (Aves, Passeriformes).



Graphic S1. Trace plots depicting parameter value distributions across Markov Chain iterations in the MCMCglmm model.

Capítulo III: Nest type transitions as key innovations in the Furnariidae (Aves, Passeriformes)



Nest type transitions as key innovations in the Furnariidae (Aves, Passeriformes)

Abstract

Studies on key innovations aim to understand how the evolution of new traits can affect transitions to new ecological niches. Passerine nest shifts were shown to be key innovations for the exploitation of new temperature niches, but its relationships with other important niche dimensions is still obscure. Here, we addressed the hypothesis of nest type transitions as key innovation for two important proxies of niche diversification, foraging strategy and habitat, in one of the biggest passerine radiations, the Neotropical Furnariidae. We found strong evolutionary correlations between nest type and both foraging strategy and habitat, with model selection and ancestral state reconstructions evidencing nest type shifts preceding niche transitions. Despite strong phylogenetic conservatism in these parameters, our data provided support for the key innovation theory. Although the correlation with habitat was expected, we provide the first evidence for nest transitions as key innovations promoting new foraging strategies. Because the ancestor of the furnariids likely foraged on tree trunks and constructed simple vegetation beds within tree cavity, shifts to nests constructed on the vegetation or ground using large amounts of nest material may have contributed with the capacity to exploit also other foraging strategies.

Keywords: Adaptive radiation, Niche divergence, Nest evolution, Ancestral state reconstruction, Correlated evolution, Phylogenetic conservatism

1. Introduction

Key innovation is an evolutionary hypothesis that refers to the emergence of traits that provides a lineage with the capacity to exploit a previously inaccessible ecological state, interacting with the environment in a novel way (Miller, 1949; Miller & Stroud, 2022; Miller *et al*., 2023). Then, the main purpose of studies on key innovations is comprehending how the evolution of new traits can affect transitions to new ecological niches, including new resources and new habitats (Miller *et al*., 2023). Although in recent years many studies have compared rates of species accumulation between clades with and without certain traits, a key innovation is not necessarily a diversifying trait, i.e. a trait that increases clade radiation (Miller & Stroud, 2022; Miller *et al*., 2023), and a reliable way of investigating key innovation is using phylogenetic comparative methods for addressing correlations between the evolution of a trait and transitions in ecological aspects (Miller & Stroud, 2022; Miller *et al*., 2023). While the studies have traditionally focused on morphological features (Miller & Stroud, 2022; Blaimer *et al*., 2023), behavioral changes and objects manipulations also can be important key innovations (for review see: Napier, 1960; Irestedt *et al*., 2006), and highly diversified organismal groups are suitable for hypothesis testing.

Nest building can be among the most complex behavioral aspects displayed by animals, and because their construction is often energetically and time costly, they must play important adaptive functions (Fang *et al*., 2018; Healey *et al*., 2023; Sheard *et al*., 2023). In birds, nests can vary from simple scrapes placed on the ground to enclosed structures built with use of sophisticated sewing techniques (Mainwaring *et al*., 2014; Leite *et al*., 2023; Mainwaring *et al*., 2023; Sheard *et al*., 2023). Then, in recent years various studies have applied phylogenetic comparative methods to address the origin, evolution, and the environmental parameters associated with nest diversification. Although discussions about the function of bird's nests as key evolutionary innovations are not new (Winkler & Sheldon, 1993; Greeney, 2009), this idea was tested with the use of phylogenetic comparative methods only recently (Medina, 2019; Medina, 2022; Sheard *et al*., 2023). In a broad study using over 3,000 passerine species, strong correlated evolution was found between niche width (narrow/wide) and a nest type classification (open/domed), with niche width representing values below or above the median of the loads of a Principal Component analysis derived from temperature data. Open-nest constructors had wider temperature niches and geographic ranges, and transitions from domed to open nests overall preceded the transitions to wide niches, providing support to the key innovation theory, although speciation rates did not differ between the clades with divergent nest types (Medina *et al*., 2022). However, it is still not known whether nest type transitions could have contributed as key innovations also for the exploitation of other important niche dimensions, such resources accessibility and occupation of new habitats.

The Neotropical passerines of the family Furnariidae (ovenbirds, woodcreepers, and allies) represent one of the biggest continental avian radiations, with three subfamilies, three tribes, 71 genera, and about 236 species that are characterized by outstanding diversifications in morphology, behavior, and habitat (Ridgely & Tudor, 1994; Derryberry *et al*., 2011; Seeholzer *et al*., 2017; Winkler *et al*., 2020). These birds are widespread across deserts, dunes, xeric vegetation, marshlands, coastal and riverine areas to tropical forests. Although they are predominantly insectivores, with the largest species also consuming small vertebrates, they developed remarkable morphological adaptations to forage in different vegetation structures (Ridgely & Tudor, 1994; Sick, 1997; Winkler *et al*., 2020; Leblanc *et al*., 2023). The woodcreepers (Dendrocolaptinae), for instance, are mostly scansorial birds with rigid modified tails adapted to provide bird’s support for foraging in bark fissures of tree trunks. The leaftossers (Sclarurinae) are predominantly ground birds that forage on the leaf litter, while the Furnariinae compose a big group with highly diversified foraging niches, from grasslands to bromeliads at the top of trees in tropical forests (Ridgely & Tudor, 1994; Sick, 1997; Winkler *et al*., 2020). The Furnariinae diversification also involves nest characteristics (Zyskowsky & Prum, 1999; Irestedt *et al*., 2006). The different lineages can be adapted to nest in tree cavity, burrows excavated in banks or on the ground, or in enclosed nests with lateral entrances constructed on the vegetation (Zyskowsky & Prum, 1999). High levels of specialization were reached in some clades, such as the Hornero (genus *Furnarius*) that construct highly resistant oven-shaped nests made of mud, and the representatives of the genus *Phacelodomus*, which construct very large enclosed pendant structures made of hard sticks (Schunck & Piacentini, 2021). For this reason, it has been hypothesized that nest architectural diversification may have contributed with the expansion of the furnariids to different habitats and for the evolution of morphological adaptations to forage in different types of vegetation structures, contributing with their remarkable radiation (Fjeldsa *et al*., 2005; Irestedt *et al*., 2006). However, the evolutionary relationships between nest type, habitat, and foraging traits have not been addressed using modern phylogenetic comparative methods. Zyskowski & Prum (1999) analyzed 24 nest characters of 168 species to generate a Furnariidae nest-based cladistic phylogenetic hypothesis. Irestedt *et al*. (2006) constructed a genus level phylogeny based on mitochondrial and nuclear DNA sequences and mapped the main nesting strategies in the DNA-based phylogeny, suggesting that cavity nesting is the plesiomorphic trait and that shifts from cavity nesting to nest construction on the vegetation occurred at least times independently, but ancestral state reconstructions were not performed. In the family-level ancestral state reconstruction work of Fang *et al*. (2018), the most common ancestor of the Furnariidae most likely nested in secondary cavity, i.e. cavities already available in the environment, but nest site reconstruction was equivocal, with similar likelihood of being tree cavity or cliffs/banks. In two studies investigating parameters related with the rates of species accumulation in the furnariids, ecomorphological characteristics and climatic niche divergences were both shown to play important roles in the big furnariid radiation (Derryberry *et al*., 2011; Seeholzer *et al*., 2017), but potential key innovations behind these observations were not addressed.

Here, we tested whether nest type transitions could have acted as key innovations for two important proxies of niche diversification, foraging strategy and habitat. To achieve this purpose, we used phylogenetic comparative methods to reconstruct the evolutionary trajectory of furnariid nest types, foraging strategy, and habitat across the nodes of a furnariid phylogeny and we addressed the evolutionary correlations between these variables, as well as their levels of phylogenetic conservatisms (phylogenetic signals). We predict that nest type shifts could have been key innovations in the furnariid if they are evolutionarily correlated and preceded shifts in foraging strategy and/or habitat, with nest type being characterized by low phylogenetic conservatism. On the other hand, if evolutionary correlations occur and nest type is characterized by strong phylogenetic conservatism, then nest type evolution could have acted as a constraint for niche shifts. Alternatively, nest type shifts may not be evolutionarily correlated with foraging strategy and habitat changes, meaning that niche diversification is poorly influenced by nest evolution.

2. Methods

2.1. Nest and bird data

For obtaining nest architectural information, we first compiled the data from three main sources, Birds of the World, the Cornell Lab of Ornithology (https://birdsoftheworld.org/bow/home); the general review on furnariid nests by Zyskowski & Prum (1999), and the review on the breeding biology of the tribe Philydorini by Cockle & Bodrati (2017). Second, especially for South American species, we checked a number of non-indexed books and articles from local journals. Third, we performed searches in Google Scholar and in the indexing base Web of Science using the species popular and scientific names, and fourth, for the species with no information in the above sources, we analyzed nest images from the citizen science repositories eBird (https://ebird.org) and Wikiaves (https://www.wikiaves.com.br).

Overall, furnariid nests have three main architectural patterns, tree cavity, i.e. cavities that are already available in the environments, often in decaying trunks; burrows, excavated on the ground or cliffs, and enclosed nests constructed on the vegetation, usually with a lateral entrance (Figure 1). However, even within the same pattern, nests can have many other dimensions, including different types of nest material, shapes, appendices, nest lining patterns, and nest attachment modes, but details are lacking for many species, hindering the inclusion of these traits in nest type categorizations. While cavity and burrow have been treated as nest types in some works (Fang *et al*., 2018; Medina *et al*., 2022; Mainwaring *et al*., 2023), for the furnariid it is not so simple because they can construct simple cups or elaborate oven-shaped enclosed structures inside burrows or rock crevices. Furthermore, burrows can be excavated by the nesting birds, being part of the structure constructed by them, or they can use pre-existent burrows, with parental individuals being responsible only for the lining of the incubatory chamber. For this reason, to perform ancestral state reconstructions (see below) we created a bidimensional classification capable of addressing the variations that can occur within the same type of external structure, and of reflecting the parts that are really constructed by the parental individuals (see Ocampo *et al*., 2023 for another bidimensional nest type classification). Then, we classified nest types as*,* burrow bed (burrow excavated by the parental birds with a simple bed of vegetation in the incubatory chamber); burrow enclosed (burrow excavated by the parental birds containing an oven-shaped enclosed structure inside); secondary burrow bed (use of pre-existent burrow where parental birds construct only a simple bed of vegetation in the incubatory chamber); rock bed (construction of a simple bed of vegetation within a rock crevice); rock enclosed (construction of an enclosed, roofed structure within a rock crevice); secondary cavity bed (use of pre-existent holes in decaying trunks, tree knotle, or woodpecker holes, lined with a simple bed of vegetal materials), and vegetation enclosed (elaborate enclosed nests constructed by the parental individuals on the vegetation, with a lateral entrance). Because of the outstanding characteristics of the nests of *Furnarius*, the oven-shaped structures constructed of mud, we also considered it in an independent category (clay enclosed). Although it has been reported that at least one species of woodcreeper, the White-Throated Treerunner *Pygarrhichas albogularis*, can excavate decaying trunks to build its nests (Altamirano *et al*., 2024), it was an isolated observation so we considered all of the furnariids nesting within tree cavities as users of pre-existent holes.

For habitat, we first compiled the classifications provided in the dataset of Tobias *et al*. (2022), i.e. forest, woodland, shrubland, grassland, riverine, coastal, rock, and wetland. Then, because many of these categories had too few representatives, and due to the fact that many species can occur in more than one of these habitat categories, we grouped habitat classification into forest (forest and woodland) and non-forest (shrubland, grassland, riverine, rock, wetland, and coastal) for the analyses. For foraging strategy, we used the classification from the dataset of Tobias & Pigot (2019), with modifications. In this dataset, the furnariids were classified in the categories Barkgleaning, ArborealGleaning, Groundforaging, ForagingGeneralist, and AerialSallying. Here, we changed the word ArborealGleaning by Vegetationgleaning, because many of the furnariids forage on vegetation that is not necessarily arboreal. Furthermore, we changed the classification of Plain-brown Woodcreeper *Dendrocincla fuliginosa*, Tawny-winged Woodcreeper *D. anabatina* and White-chinned woodcreeper *D. merula* from AerialSallying to Barkgleaning, and the classification of Amazonian Barred-

Woodcreeper *Dendrocolaptes certhia*, Black-banded Woodcreeper *D. picumnus*, Planalto Woodcreeper *D. platyrostris*, Northern Barred-Woodcreeper *D. sanctithomae* from ForagingGeneralist also to Barkgleaning. This is because all of these species forage in tree trunks, and AerialSallying, for instance, is the classification commonly used for birds that capture insects in the air, which is not commonly observed in these furnariids. Then, our final foraging strategy classification was, Barkgleaning, Vegetationgleaning, and Groundforaging

2.2. Phylogenetic hypothesis and ancestral state reconstruction

To construct our phylogenetic hypothesis, we first obtained a sample of 3,000 Ericson backbone time-calibrated trees from birdtree.org (Ericson *et al.,* 2006; Jetz *et al.,* 2012), in which only species with DNA and nest information were included. Then, for the use in the statistical analyses we generated a maximum clade credibility tree using the summarization method implemented in the Phangorn R Package (Schliep, 2011), which selects the tree with the highest sum of clades posterior probabilities from the tree dataset (see also Leite *et al.,* 2023; Ocampo *et al.,* 2023). To infer about the origin and evolutionary trajectories of nest type, foraging strategy, and habitat, we generated ancestral character states for each node of the phylogeny using the Maximum Likelihood reconstruction procedure developed for discrete characters of the ancr function implemented in Phytools (Revell, 2024). We performed these estimates with the use of the best fitted model of evolution, selected with corrected Akaike Information Criterion (AICc) among Equal Rates (ER), All-Rates Different (ARD), and Symmetric Entities (SYM) models. For scientific nomenclature we followed Birds of the World, the Cornell Lab of Ornithology (https://birdsoftheworld.org/bow/home)

2.3. Phylogenetic signal and correlated evolution analyses

To estimate the phylogenetic signal for each of the three characters, we used the D statistic, developed for binary categorical data (Fritz & Purvis, 2010), implemented in the phylo.d function of the R package caper (Orme, 2018). A value of $D < 0$ indicates stronger phylogenetic signal than expected under the Brownian motion model of evolution; a value of 0 indicates that the character states are distributed as expected under Brownian motion; $D = 1$ indicates random distribution; and $D \geq 1$ indicates that species sharing a trait are less related than predicted by chance. We compared the observed D value with a distribution of 1000 values generated by tip-shuffling to test if D differed significantly from 0 and from 1 ($p < 0.05$).

We tested whether nest type could evolve in association with foraging strategy and habitat by fitting a model of correlated and a model of independent evolution to the data, and by testing whether these models have differed using a likelihood ratio test with alfa = 0.05. We used the model of correlated evolution of Pagel (1994) available in the package Phytools (FitPagel function) (Revell, 2012). Because analyses of correlated evolution were developed for binary categorical traits, we reduced the nest type categories used in ancestral state reconstructions to only three main general patterns for correlated evolution analyses. Then, nest categorizations were Cavity/Others, Enclosed/Others, and Burrow/Others. Similarly, foraging strategies were Barkgleaning/Others, Vegetationgleaning/Others, and Groundforaging/Others (see also Dellinger *et al.,* 2021; Busschau & Boissinot, 2022). For the correlations involving nest type and foraging strategy, we created hypotheses based on microhabitat proximity, i.e. Cavity, present in tree trunks, was associated with Barkgleaning; Burrow, constructed on the ground or banks, was associated with Groundforaging, and Enclosed nests, constructed on the vegetation, was associated with Vegetationgleaning. For the correlations between nest type and habitat, all of the three simplified nest categories were contrasted against the binary habitat classification (Forest/Non-Forest).

3. Results

Of the approximately 169 furnariid species, all species had DNA information in birdtree, and with complete nesting information. Of the 169 species used in the analyses, 58 nested in Cavity, 37 in Burrow, and 71 constructed Enclosed nests. Only 3 species used rocky environment to constuct nest, here classified as Rock Bed (n=2), and Rock enclosed (n=1). Of the species nesting within Cavity, 45 were BarkGleaners (77.58 %) and all occurring in Forest habitat (100%). Of the species nesting within Burrow, 27 were classified as GroundForaging (72.97%) and 5 occurred in Forest habitat (18.51%), and of the birds constructing Enclosed nests, 43 were classified in VegetationGleaning (60.56%), with 19 inhabiting Non-Forest habitat (44.18%). The best fitted evolutionary model for nest type was ER, and SYM was the best fitted model for foraging strategy and habitat (Table S1). The most recent common ancestor of the Furnariidae most likely nested within Cavity (root probability = 0.93) (Figure 2), was BarkGleaner (root probability = 0.34) (Figure 3), and inhabited Forest (root probability = 0.98) (Figure 4).

Across the furnariid radiation, the ancestral nest type (secondary cavity) was conserved in at least in seven divergent lineages. Transition from cavity to secondary burrow occurred only once (Figure 5), early in the furnariid radiation in the clade of *Geositta*, where it remained conserved. Burrow excavation and the construction of enclosed nests on the vegetation emerged later in the furnariid radiation. The construction of enclosed nests on the vegetation emerged at least five times independently, deriving four times from cavity and once from Burrow. ‘’Excavated burrow bed’’ emerged independently four times, twice from secondary cavity bed; once from an ancestor using secondary burrows, and once from an ancestor constructing more likely enclosed nests. Burrow enclosed emerged only once very recently in Sharp-tailed Streamcreeper *Lochmias nematura*, from an ancestor constructing enclosed nests on the vegetation. Rock bed and Rock enclosed also were recent innovations in the furnariids, with Rock enclosed emerging only once from Cavity, and Rock bed emerging independently twice in the clade of *Cinclodes* from a Burrow bed ancestor. The Clay enclosed nests of *Furnarius* emerged only once, more likely from an ancestor constructing enclosed nest (Figure 2 and Figure 5).

All of the trait categories within our variables showed strong phylogenetic signal, with negative D values (Table S2). All of the hypothesized evolutionary correlations between nest type and foraging strategy were highly significant, with the models of interdependent, and of foraging strategy shift dependent on nest type shift, receiving AIC support (Table 1). For habitat correlations, Cavity was highly correlated with Forest, and Enclosed was highly correlated with Non-forest, with the models of interdependent evolution receiving AIC support, but Burrow had no significant correlation with habitat (Table 1).

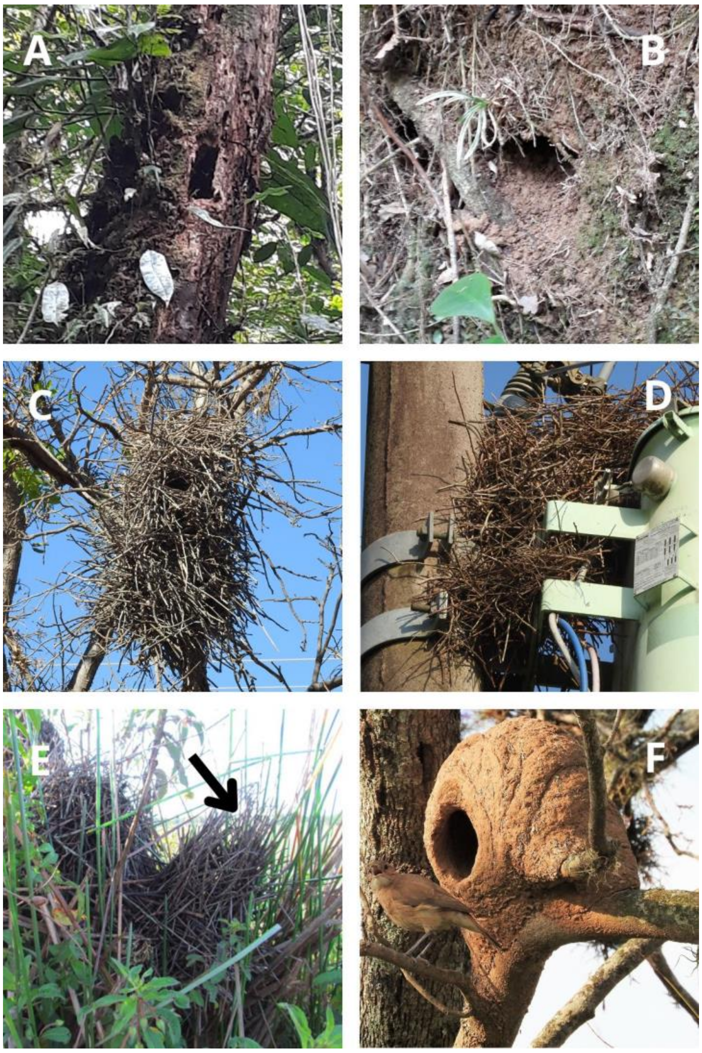


Figure 1. General nest patterns of the Furnariidae (A) Natural tree hole in decaying trunk typically used by the secondary cavity nesters of the subfamily Dendrocolaptinae (B) Burrow excavated in a river bank by the White-eyed Foliage-gleaner *Automolus leucophthalmus* (C) Enclosed nest constructed on the vegetation by Thornbirds (genus *Phacellodomus*) (D) Enclosed nest constructed in a urban structure by the Firewood-gatherer *Anumbius annumbi* (E) Enclosed nest of the Yellow-chinned Spinetail *Certhiaxis cinnamomeus*, with the arrow indicating the tubular entrance (F) Clay enclosed nest built by Rufous Hornero *Furnarius rufus*. Images C, D, and F are courtesies from Dafini L. Bruno, and image E is a courtesy from Mariellen C. Costa.

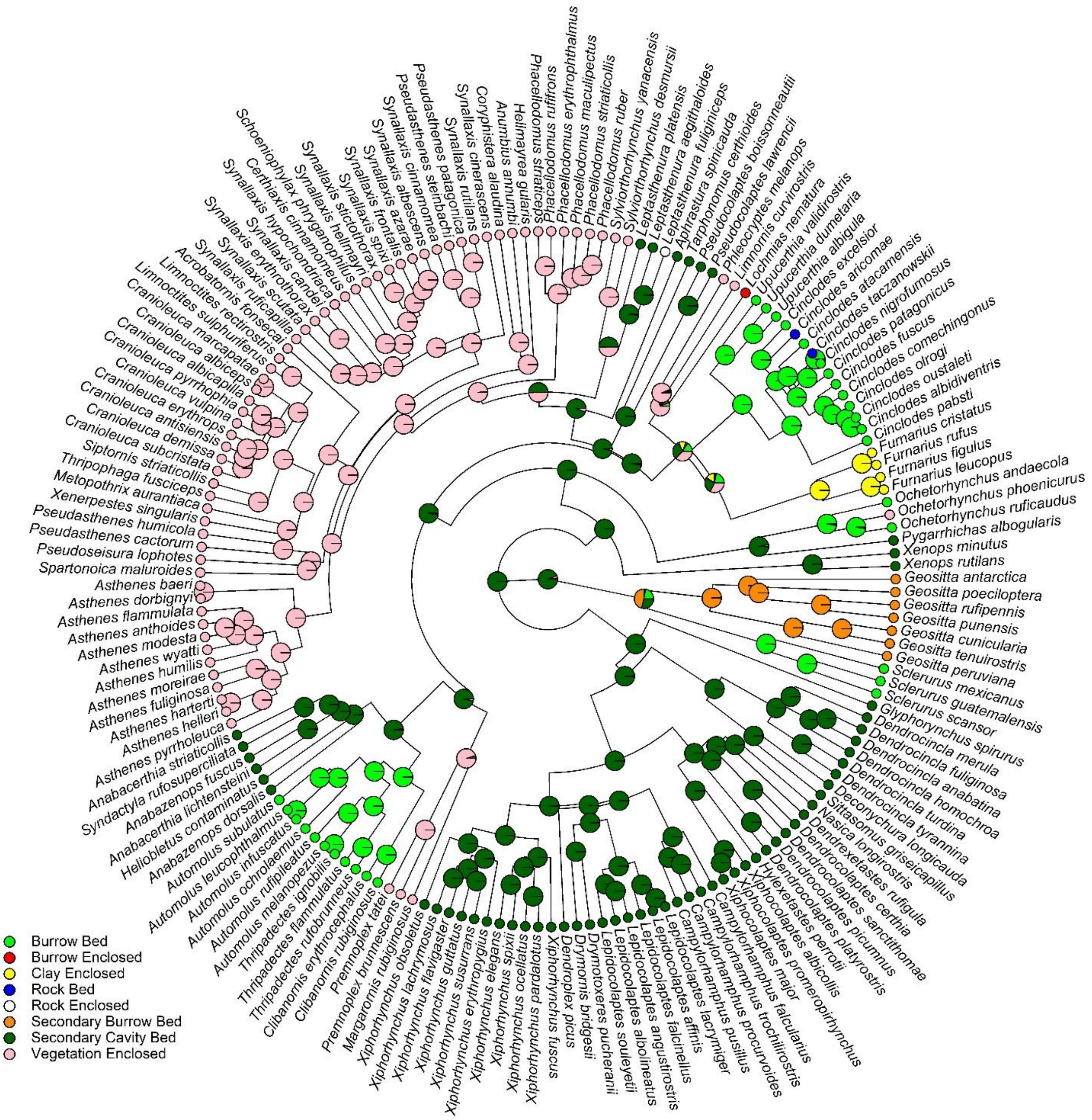


Figure 2. Phylogeny of 169 Furnariidae species constructed based on a consensus tree derived from 3,000 topologies obtained from birdtree.org, showing ancestral stated reconstructions for nest type.

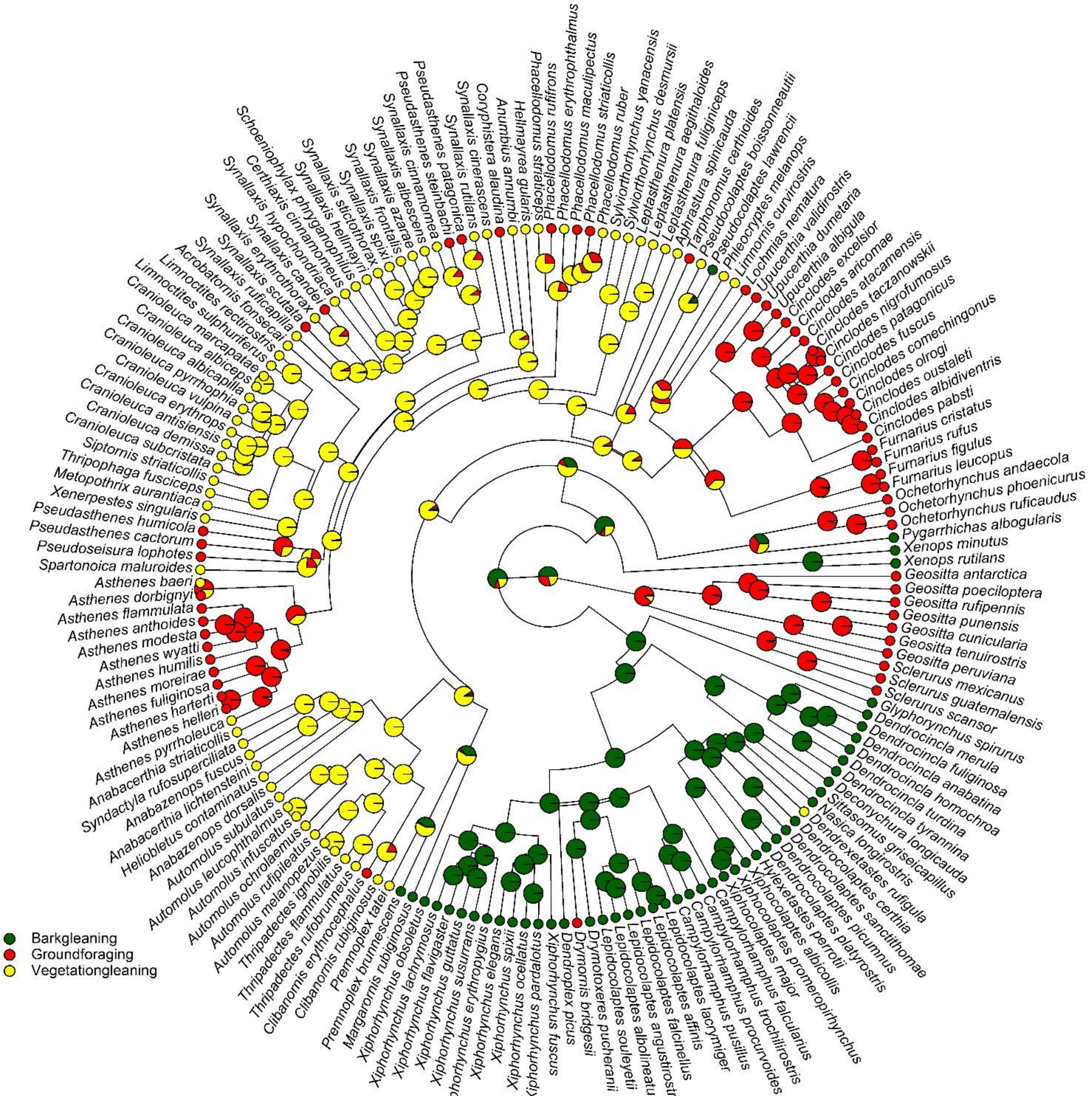


Figure 3. Phylogeny of 169 Furnariidae species constructed based on a consensus tree derived from 3,000 topologies obtained from birdtree.org, showing ancestral stated reconstructions for foraging strategy.

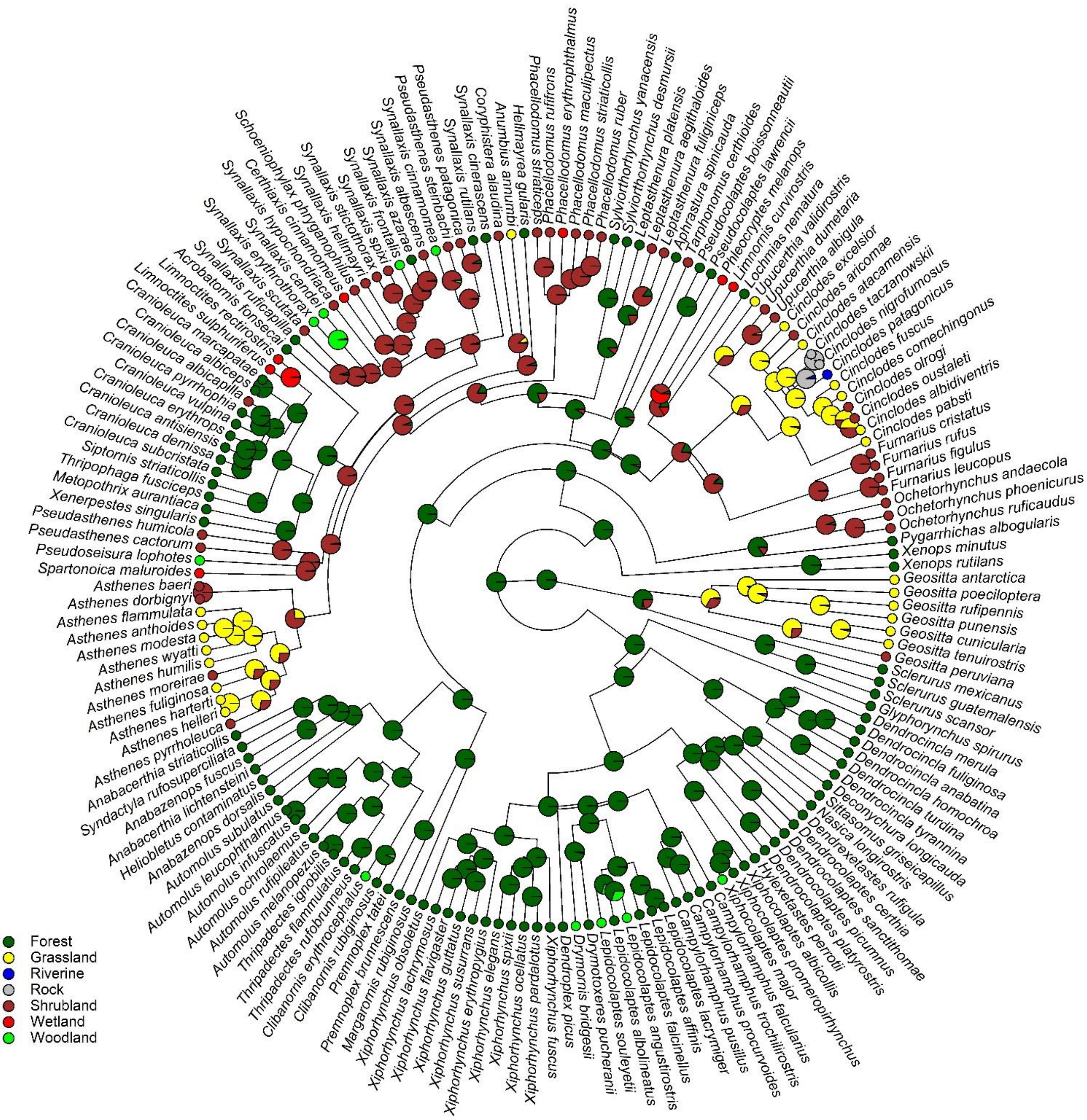


Figure 4. Phylogeny of 169 Furnariidae species constructed based on a consensus tree derived from 3,000 topologies obtained from birdtree.org, showing ancestral stated reconstructions for habitat.

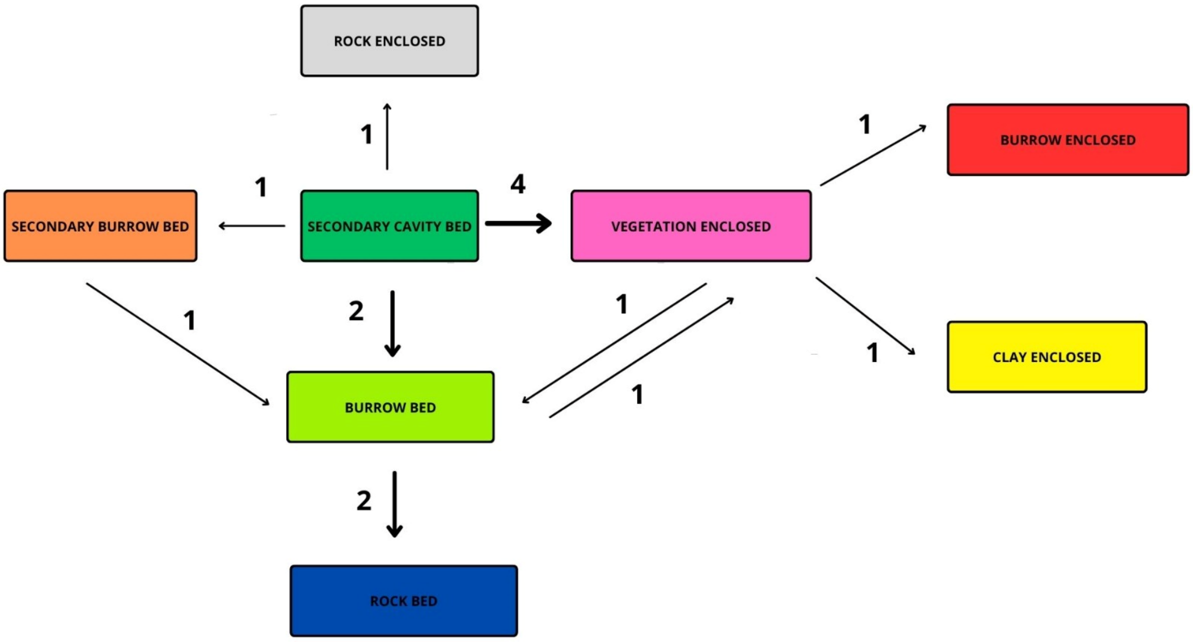


Figure 5. Flowchart representing the numbers of transitions between the eight nest type categories used in ancestral state reconstructions for the Furnariidae. Arrows widths correspond to the total numbers of transitions, with secondary cavity bed being the most likely nest type of the most recent common ancestor.

Table 1. Results of the tests for correlated evolution between pairs of binarized traits. Bold letters indicate the models receiving AIC support.

Nest type Cavity & Foraging Strategy Barkgleaning (P = 0.001)			
Models	Log-Likelihood	AIC	ΔAIC
Independent	58.709	125.421	10.061
Interdependently	**-49.680**	**115.360**	**0.000**
Nest type Cavity dependent on Foraging Strategy Barkgleaning	-53.001	118.000	2.640
Foraging Strategy Barkgleaning dependent on Nest Type Cavity	**-52.568**	**117.142**	**1.782**
Nest type Burrow & Foraging Strategy Groundforaging (P < 0.001)			
Models	Log-Likelihood	AIC	ΔAIC
Independent	-106.946	221.885	26.741
Interdependently	**-89.615**	**195.212**	**0.068**
Nest type Burrow dependent on Foraging Strategy Groundforaging	-101.312	214.622	19.478
Foraging Strategy Groundforaging dependent on Nest Type Burrow	**-91.573**	**195.144**	**0.000**
Nest type Burrow & Habitat (P= 0.084)			
Models	Log-Likelihood	AIC	ΔAIC
Independent	-96.304	200.610	1.589
Interdependently	-92.204	200.413	1.392
Nest type Burrow dependent on Habitat	-96.199	204.402	5.381
Habitat dependent on Nest Type Burrow	-93.499	199.021	0.000

Nest type Enclosed & Foraging Strategy Vegetationgleaning (P = 0.003)			
Models	Log-Likelihood	AIC	ΔAIC
Independent	-96.191	200.382	7.891
Interdependently	**-88.247**	**192.491**	**0.000**
Nest type Enclosed dependent on Foraging Strategy Vegetationgleaning	-92.247	196.493	4.002
Foraging Strategy Vegetationgleaning dependent on Nest Type Enclosed	-91.259	194.522	2.031
Nest type Cavity & Habitat (P < 0.001)			
Models	Log-Likelihood	AIC	ΔAIC
Independent	-91.016	190.032	12.351
Interdependently	**-80.842**	**177.681**	**0.000**
Nest type cavity dependent on Habitat	-84.105	180.212	2.531
Habitat dependent on Nest Type cavity	-84.206	180.415	2.734
Nest type Enclosed & Habitat (P < 0.001)			
Models	Log-Likelihood	AIC	ΔAIC
Independent	-89.830	187.662	11.589
Interdependently	**-80.033**	**176.073**	**0.000**
Nest type Enclosed dependent on Habitat	-84.859	181.721	5.648
Habitat dependent on Nest Type Enclosed	-85.589	183.184	7.111

4. Discussion

Our main findings were the strong evolutionary correlations between nest type and both foraging strategy and habitat in the Furnariidae phylogeny, providing the first evidence for nest transitions as key innovations for foraging strategy and habitat in an avian group. For foraging strategy, the models of interdependent evolution and the models of nest type shifts preceding foraging strategy shifts received AIC support in virtually all of the comparisons. The interdependently models reflect the close association between these variables and the models of foraging strategy shifts dependent on nest type shifts suggest that nest type diversification emerged before and could have worked as releases for foraging strategy diversification. Although the relationships between habitat and nest type have been exploited in other works (Fang *et al*., 2018; Barros-Leite & Francisco, 2024), to our knowledge, this is the first work providing evidence for the association between nest type and bird foraging strategy, and we see one potential explanation for this evolutionary relationship in the Furnariidae. Empirical studies have suggested that cavity nests, the ancestral form in the furnariids, provide increased clutch survival, but on the hand, pre-existent cavities can be scarce, with secondary cavity-nester species like the furnariini facing strong competition for these nest sites (Martin & Li, 1992; Brigthsmith *et al*., 2005; Wiebe, 2011; Barve & Mason, 2015). For this reason, it is possible that competition reduction could have been an important selective pressure for nest type shifts from tree cavities to enclosed nests constructed on the vegetation, for instance. Shifts from tree cavity nests, which in many species are lined with pieces of bark from the own tree trunks, to enclosed nests constructed on the vegetation, required not only changing nesting microhabitats, but also required gathering other types of nest materials in large amounts, such as the sticks that are broadly used in enclosed nest construction and are found on the foliage or on the floor. Then, the shifts in nest microhabitat, associated with the search for new nest materials could have influenced in the exploitation of niches other than tree trunks favoring the emergence of new foraging niches and foraging strategies, i.e. gleaning on the vegetation. Similarly, the evolutionary transitions from cavity or enclosed nests to burrows required the exploitation of niches associated to the soil, such as banks and forest floor, which could have in many cases mediated the evolutionary shifts from bark or vegetation gleaning to ground foraging. Burrows emerged different times from cavity and from enclosed nests. While the selective pressure accounting for transitions from cavity to burrow also could be competition avoidance, explaining the transitions from enclosed nests to burrows is more difficult because the construction of both of these nest types seem to be energetically costly and there is no background information to infer about the implications in terms of nest survival (Medina *et al*., 2022; Mainwaring *et al*., 2023).

For habitat, not all of the evolutionary correlation comparisons were significant. The strong evolutionary correlation between cavity nests and forest was expected because decaying trunks, suitable for cavity formation, do not occur in many of the other specific habitats for which the furnariids radiated, such as marshlands, grasslands, rock, and even shrublands, here grouped in non-forest habitat (Wiebe, 2011; Barve & Mason, 2015; Altamirano, 2024). On the other hand, the strong evolutionary correlation between enclosed nests and habitat likely occurred because this type of nest was the predominant form across the different non-forested habitats. Burrows emerged in both forest and non-forest habitats but the transitions were not associated with habitat type, meaning that they have evolved independently from each other, with burrows not working as evolutionary constraints or releases for habitats shifts, and vice-versa (Ocampo *et al*., 2023). Despite the strong correlations observed between cavity and enclosed nest with forest and non-forest habitat, respectively, the predictive model selection procedures did not permit to infer about the specific evolutive direction of this relationships, if habitat was dependent on nest type, or nest types was depended on habitat during the evolutionary trajectory. In the ancestral state reconstructions using the non-simplified habitat and nest type classifications, the enclosed nests of *Sylviorthorhynchus* and of the clade *Margarornis/Premnoplex*, as well as the burrow nests of *Sclerurus* and of the clade *Clibanornis/Thripadectes/Automolus* emerged within forest habitat from cavity nests (the ancestral form), indicating that at least in these lineages nest type shift preceded habitat shift, but these relationships are not clear in the other transitions of the phylogeny, possibly because of the lack of existent taxa with transitional characteristics, and this is what may have resulted in AIC support only to the interdependent relationships.

All of the variables presented strong phylogenetic signal (phylogenetic conservatism), meaning that closely related furnariid species tend to be similar in nest type, habitat, and foraging strategy. Although strong phylogenetic conservatism suggest an evolutionary pattern in which trait evolution is driven mostly by phylogenetic proximity than by evolutionary responses to environmental pressures (Blomberg & Garland, 2002; Losos, 2008), some of the nest type shifts seemed to have provided important opportunities for the diversification of certain lineages, while others radiated despite the retention of the ancestral nest type. The Dendrocolaptinae, for instance, is a highly speciose clade which experienced an incredible radiation despite the retention of the ancestral nest type, habitat, and foraging strategy. In this clade, there is no evidence for nest type shifts acting as key innovations, and niche diversification and competition reduction may have occurred mainly through changes in characters such as body size and beak morphology, permitting the exploitation of different types of preys in tree trunks and the use cavities of different sizes for nesting (Feducia, 1973; Bledsoe *et al*., 1993; Raikow, 1994). With rare exceptions, the diversification of the Dendrocolaptinae occurred within forested habitats and the strong phylogenetic signal of cavity nests suggest that nest type conservatism was an evolutionary constraint for the radiation of the Dendrocolaptinae. This could be a potential explanation for the great diversification in sizes and beak shapes of the Dendrocolaptinae, while in the explosive radiation of the Synalaxinii, for instance, the organisms are way more homogenous in size and beak shape because enclosed nests are more flexible and permitted radiation through the exploitation of tropical habitats (Martin *et al*., 2017; Street *et al*., 2022; Perez *et al*., 2023).

Our findings matched the family level study of Fang *et al*. (2018) in that secondary cavity was the nest type of the common ancestor of the Furnariidae, which is shared with the family Formicariidae. Our birdtree-based phylogeny also matched the most species-level recent phylogenies available in the literature, with the Sclerurinae (*Geositta* and *Sclerurus* clade) deriving the two other subfamilies of the furnariids, the Dendrocolaptinae and the Furnariinae. Within Furnariinae, the most complex of the three subfamilies, our phylogeny also matched the literature in that *Xenops*, *Ochetorhynchus* and *Pygarrhichas* were ancestors of the tree tribes within Furnariinae (Synalaxini, Philydorini, and Furnarini) and in our phylogeny all of the genera were assigned to the correct tribes (Irestedt *et al*., 2009). We found it was not a problem because the ancestral nodes of the three tribes had the same types of nests, but the independent shift from tree cavity to vegetation enclosed occurred within the Philydorini was questionable. These divergent results likely did not occur because of differences in the raw data, since the genes used in birdtree and in Derryberry *et al*. (2011), for instance, are virtually the same. Rather, the differences may have been cause by the smaller number of species and genera used here due to the lack of nesting information.

Most of the observed nest transitions were direct, but interesting intermediate evolutionary steps could be noticed in a few lineages. For instance, the construction of enclosed nest within a burrow by *Lochmias* can be an intermediate evolutionary step of the transition between enclosed nest construction on the vegetation and excavated burrow. This pattern was unexpected because the long tunnels built by *Lochmias* are already well-protected and roof construction seems to be an energetic waste (See also Leite & Francisco, 2024). A potential explanation is the recent transition from trees to burrows, with the retention of the enclosed nest due to the phylogenetic influence of a recent enclosed-nester ancestor. The origin of burrow excavation in *Sclerurus* from an ancestor that most likely used secondary burrows suggest that in this clade the use of burrows available in the environment could have been an intermediate step for the transition from the tree cavity nests of the ancestor of the Furnariidae to the excavated burrows constructed by the *Sclerurus*. The clay nests of the Horneros (genus *Furnarius*) are among the most remarkable structures constructed by animals. They represent a recent innovation of the furnariids, which emerged only once and was conserved in the genus, evidencing its adaptability. However, its origin was equivocal. Although it has derived more likely from enclosed nests constructed on the vegetation, the Horneros are assigned to a clade in which burrow is the predominant nest type, and both burrow and cavity presented only slightly smaller likelihoods of being the nest type of the most recent common ancestor of the clade *Furnarius/Cinclodes/Upucerthia*. Then, the best available clue about the evolutionary trajectory that culminated in the complex Hornero nests comes from *Limnornis*. The later is a marshland species that is part of an ancestor clade closely related to *Furnarius/Cinclodes/Upucerthia*, which constructs enclosed nests made of flexible fibers in cattail-like vegetation and it is the only species in the clade using an amount of mud to reinforce the nest walls, that are posteriorly covered with leaves (see Di Giacomo, 1998). The nests of the Horneros are also built of mud cemented together with vegetal fibers, and despite the fact that they are not related to marshlands, they have to seek for moisturized soil for nest construction (personal observation). Although analyzing nest material is beyond the scope of this manuscript, we cannot discard the possibility that the Hornero nest pattern derived from a gradual increase in the use of mud by an ancestor constructing enclosed nests in marshlands, where mud is abundant, but this idea is still to be confirmed, e.g. by the inclusion of more species in the phylogeny when more nesting information is available.

In conclusion, our work expanded the knowledge on the implications of nest transitions for avian evolution by evidencing that they can be key innovations for parameters not previously addressed, i.e. foraging strategy and habitat. Because the rate of lineage accumulation in the big furnariid radiation is correlated with ecomorphological foraging adaptations and climatic niche diversification (Irestedt *et al*., 2006), it is possible that nest type shifts have also contributed with the Furnariidae radiation. At the same time, the high phylogenetic conservatism of nest type, foraging strategy and habitat observable in the subfamily Dendrocolaptinae also evidenced that nest transitions were key innovations for some lineages but not for others. This means that the interpretation of key innovations can be influenced by the scale of the analyses, and we suggest that the proper interpretation of this theory may rely not only on global studies, but also on investigations of specific groups with specific natural histories and evolutionary trajectories. Future researches are still needed because furnariid nests have other important dimensions such as nest material, nest attachment mode or the presence other structures such as tunnels, which were not addressed here because of the lack of detailed information for most taxa. Another obscure aspect that deserves investigation is the evolution of very big enclosed nests, much bigger than the incubating individuals, but natural history information is still a limiting factor for evolutionary analyses.

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6. Supplementary materials

Table S1. Results of evolutionary model selections using Equal Rates (ER), All-Rates Different (ARD), and Symmetric Entities (SYM) models, with bold letters representing the models receiving AIC support.

Nest Type		
Model	Log-Likelihood	AIC
ER	**-86.660**	**175.6322**
ARD	-61.477	234.89
SYM	-75.906	207.821
Foraging strategy		
Model	Log-Likelihood	AIC
ER	-98.311	198.632
ARD	-87.695	187.385
SYM	**-90.634**	**187.271**
Habitat		
Model	Log-Likelihood	AIC
ER	-18.625	374.494
SYM	**-153.873**	**349.742**
ARD	-140.061	364.127

Table S2. Phylogenetic signal results using a Fritz and Purvis'D statistic for categorical variables. P values denote if the D statistic significantly differs from 0 (Brownian motion) or 1(randomly distributed). *P<0.05, **P<0.01, ***P<0.001. For a strong phylogenetic signal, D should be significantly <1 but not significantly >0.

	D - Value	P value D different from random phylogenetic distribution (no signal)	P value D different from Brownian motion (strong signal)
Nest type Burrow	-0.777	0.000	0.996
Nest type Cavity	-0.913	0.000	1.000
Nest type Enclosed	-0.893	0.000	1.000
Foraging Strategy Barkgleaning	-0.807	0.000	0.995
Foraging Strategy Groundforaging	-0.279	0.000	0.865
Foraging Strategy Vegetationgleaning	-0.307	0.000	0.902
Habitat Forest/Non - forest	-0.398	0.000	0.943

CONSIDERAÇÕES FINAIS

O presente estudo evidenciou correlações evolutivas até então desconhecidas entre características dos ninhos e aspectos ecológicos em três famílias de Aves com distribuição geográfica principalmente no Novo Mundo. Os resultados obtidos mostraram que as trajetórias evolutivas da arquitetura dos ninhos não seguiram um padrão único, com os fatores ecológicos afetando as diferentes linhagens de maneira distinta.

No Capítulo I, foi demonstrado que a complexidade do ninho não está ligada à massa corporal das aves, refutando assim a hipótese de que ninhos complexos são construídos apenas por passeriformes de pequeno porte. Esses ninhos considerados complexos são construídos por *Cacicus* e *Psarocolius* com materiais fibrosos e resistentes através de aprimoradas técnicas para tecer as finas paredes destas estruturas. Provavelmente isso permitiu que estas espécies grandes de icterídeos usem com sucesso essa arquitetura de ninho. Além disso, é importante ressaltar que houve pelo menos duas origens evolutivas para ninhos fechados, inicialmente a partir de ninhos basais como ocorreu em algumas espécies de *Sturnella*, e uma outro trajetória evolutiva a partir de ninhos do tipo bolsa e suspensos como identificado no clado de *Icterus*. Quanto ao efeito do habitat, essa variável foi importante devido ao uso da vegetação disponível para a ancoragem do ninho. Por exemplo, a ocorrência de juncos em brejos favoreceu a construção de ninhos ancorados lateralmente. Já os ninhos suspensos foram correlacionados evolutivamente com habitats mais arbóreos (bosques e florestas), onde os ninhos suspensos são fixados pelo topo ou pelas bordas tanto em folhas quanto em galhos. Desta forma, os tipos de vegetação presentes nos habitats de nidificação influenciaram mais nos modos de fixação do que nos tipos de ninhos construídos.

No Capítulo II, a trajetória evolutiva dos Troglodytidae foi caracterizada por importantes transições entre o local de nidificação e o tipo de ninho construído. Além disso, a variável tipo de ninho se correlacionou com a massa corporal dos Troglodytidae, resultando em uma tendência de espécies menores frequentemente usarem as cavidades secundárias para a nidificação, enquanto espécies maiores comumente construírem ninhos fechados e expostos na vegetação. A diminuição dessa massa corporal possivelmente proporcionou essa versatilidade no uso de cavidades secundárias de forma generalista em algumas espécies de Trogodytidae, podendo assim evitar a competição com outras espécies de aves maiores e que também nidificam em cavidades, como por exemplo as espécies de Psitacídeos, Piciformes e Trogoniformes. No entanto, durante as transições evolutivas, a construção de ninhos abertos e abrigados não foi uma etapa necessária para o surgimento de ninhos fechados e expostos na vegetação.

O Capítulo III deixou evidente a grande diversidade de arquitetura de ninhos presente nos Furnariídeos. Nesta família, houve uma importante correlação evolutiva entre o local de nidificação e as estratégias de forrageio. Tais correlações foram significativas nos seguintes casos: forrageio em troncos de árvores em espécies que nidificam em ocos de troncos em decomposição; forrageio no chão para espécies que escavam buracos para nidificar no chão ou em barrancos e construção de ninhos sobre a vegetação para espécies que forrageiam na folhagem. Embora essas correlações evolutivas tenham mostrado pela primeira vez que a variável tipo de ninho é uma *key innovation* na família Furnariidae, a interpretação dessa hipótese depende do nível taxonômico do estudo, uma vez que os representantes da subfamília Dendrocolaptinae, por exemplo, apresentam padrões altamente conservados de tipo de ninho, estratégia de forrageio e habitat. Em geral, cada capítulo mostrou de uma forma inovadora como a ancestralidade e as trajetórias evolutivas dos ninhos podem variar, e como os fatores ecológicos, comportamentais e de habitat se correlacionam evolutivamente entre si de uma maneira exclusiva em cada família. Também é importante ressaltar que algumas espécies foram excluídas das análises devido à falta de informações de ecologia reprodutiva, evidenciando a grande carência de estudos de história natural em Ornitologia com foco em ecologia reprodutiva. É importante que novos trabalhos abordem as trajetórias evolutivas dos aspectos relacionados à nidificação das aves pertencentes a outras famílias, utilizando além dos parâmetros ecológicos aqui considerados, outras variáveis ecológicas, comportamentais e morfológicas. Desta forma, será possível um melhor entendimento sobre a evolução das próprias Aves, as quais ao longo de milhões de anos foram influenciadas por diferentes pressões evolutivas que ocorrem nos habitats de nidificação.