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A tale of long tails: evolution of host specialization in non-pollinating fig wasps.

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Data availability

Molecular sequences: the data that support the findings of this study will be openly available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/>, with reference numbers described in supporting information. The authors confirm that the data supporting the findings of this study are available within the article and its supporting information.

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Conflict of interest disclosure

No potential competing interest was reported by the authors.

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Abstract

1. The evolution of host specialization and feeding habits in non-pollinating fig wasps remains poorly understood.
2. This study investigates these dynamics within the wasp genus *Idarnes*, which exhibits diverse life strategies and degrees of host specificity.
3. We reconstructed the phylogeny of 57 *Idarnes* species using four genetic markers—two mitochondrial, one nuclear, and one ribosomal— and performed ancestral state reconstructions to elucidate the evolution of host specialization.
4. Our analyses reveal that host monophagy is ancestral in *Idarnes*, with oligophagy evolving independently in several lineages. Oligophagous species are more prevalent in kleptoparasitic compared to gall-inducing lineages. Early gallers—which are wasps that colonize figs during the pre-receptive stage of development—are predominantly monophagous, while some oligophagous species occur in the galler lineage that uses flower ovaries at the fig receptive phase.
5. The relative ovipositor length correlates with the number of host species visited by kleptoparasite species, suggesting morphological adaptations play a role in host specialization. In the gall-inducing lineages, the use of multiple host species seems not primarily constrained by the ovipositor size but possibly by the capacity of the wasp to induce galls in a non-typical host species.
6. This study provides valuable insights into the mechanisms driving host specialization in fig wasps, enhancing our understanding of this intricate insect-plant system.

Keywords: Pteromalidae, Ancestral state reconstruction, phylogenetic comparative methods, evolutionary ecology, *Ficus*

Resumo

Título: Uma longa história de longos ovipositores: evolução da especialização aos hospedeiros em vespas não polinizadoras do figo.

1. A evolução da especialização aos hospedeiros e hábitos alimentares em vespas não polinizadoras do figo permanece pouco compreendida.
2. Este estudo investiga essa dinâmica no gênero de vespas *Idarnes*, que exibem estratégias de vida diversificadas e graus variados de especificidade de hospedeiro.
3. Reconstruímos a filogenia de 57 espécies de *Idarnes* utilizando quatro marcadores genéticos—dois mitocondriais, um nuclear e um ribossomal—e realizamos reconstruções do estado ancestral para elucidar a evolução da especialização aos hospedeiros.
4. Nossas análises revelam que a monofagia em hospedeiros é ancestral em *Idarnes*, com a oligofagia evoluindo independentemente em diferentes linhagens. Espécies oligófagas são mais prevalentes nas linhagens cleptoparasitas em comparação com as linhagens galhadoras. As galhadoras iniciais—que são vespas 1^{ue} colonizam figueiras no estágio pré-receptivo do desenvolvimento—são predominantemente monófagas, enquanto algumas espécies oligófagas ocorrem na linhagem galhadora que utiliza ovários de flores na fase receptiva da figueira.
5. O comprimento relativo do ovipositor correlaciona-se com o número de hospedeiros utilizados por espécies cleptoparasitas, sugerindo que adaptações morfológicas desempenham um papel na especialização em hospedeiros. Nas linhagens indutoras de galhas, o uso de múltiplos hospedeiros parece não estar primariamente limitado pelo tamanho do ovipositor, mas possivelmente pela capacidade da vespa de induzir galhas em espécies de hospedeiro atípicas.
6. Este estudo fornece percepções valiosas sobre os mecanismos que impulsionam a especialização em hospedeiros em vespas de figueira,

aprimorando a compreensão deste intrincado sistema de interação inseto-planta.

Palavras-chave: Agaonidae, métodos filogenéticos comparativos, ecologia evolutiva, *Ficus*, reconstrução de estado de caráter ancestral.

Introduction

Herbivorous insects encompass over half a million described species, with most exhibiting a high degree of dietary specialization, utilizing only one or a few plant species as hosts (Hardy *et al.*, 2020). This specificity is posited to be a crucial factor in their remarkable diversity (Hardy *et al.*, 2020). Among herbivorous insects, gall inducers (with over 13,000 described species—[Calderón-Cortés, 2020](#)) are particularly noteworthy due to their role as microhabitat engineers, creating structures that support a community of associated insects and displaying diverse life histories and host specialization patterns (Sanver & Hawkins, 2000; Stone *et al.*, 2002; Stone & Schönrogge, 2003; Luz & Mendonça Jr., 2019). Macroevolutionary evidence suggests species-specific interactions promote greater diversification (Day *et al.*, 2016).

Nonetheless, numerous insects exhibit host generalism, challenging the notion that specialization is the sole pathway to diversification (Bernays & Graham, 1988; Ali & Agrawal, 2012). Ecological fitting, characterized by phenotypic flexibility and phylogenetic conservatism in resource use traits, offers a framework for understanding host switching without necessitating the evolution of novel host-use capabilities (Agosta *et al.*, 2010). This concept suggests that the utilization of an additional host may initially incur no extra cost to the insect's ability to exploit its primary host.

Ficus (Moraceae) provides a unique system for examining the dynamics of specialization and generalization within communities of chalcid (Hymenoptera: Chalcidoidea) wasps. Most fig wasps are stringent fig specialists, and their synchronous emergence from figs facilitates their collection and study (Conchou *et al.*, 2014; Farache *et al.*, 2018). Fig wasps include pollinating and non-pollinating (NPFW) species (Cook & Rasplus, 2003). Fig pollinators belong to the family Agaonidae and are involved in an obligate mutualism with *Ficus* (Cruaud *et al.*, 2012). These wasps enter the fig to facilitate pollination of pistillate flowers laying in the fig cavity, and some of them are utilized as oviposition sites. Each larva develops at the expense of one galled flower

ovule (Weiblen, 2002). The NPFWs mainly belong to Epichrysomallidae and in Pteromalidae, Sycophaginae and Pteromalinae: Otitesellini that now encompasses previous subfamilies Sycoecinae, Otitesellinae and Sycoryctinae (Burks *et al.*, 2022). Several Eurytomidae, Ormyridae, Torymidae, and even Braconidae are also members of fig wasp communities, though they are much less frequently found (Bouček, 1993; van Achterberg & Marsh, 2002; Cook & Rasplus, 2003; Wei *et al.*, 2013; Burks *et al.*, 2022; Cruaud *et al.*, 2023). While a few NPFW species enter the fig to lay eggs, most oviposit from the external fig surface, using ovipositors of varying lengths to pierce the fig wall and deposit eggs in pistillate flowers within the fig cavity at variable stages of the fig development (Cook & Rasplus, 2003).

Classifying the life histories of fig wasps is a complex task. The pollinating and some NPFWs are gallers, inducing galls in plant tissues where their larvae—which are exclusively phytophagous—develop (Jansen-González *et al.*, 2012, 2014; Yadav & Borges, 2018). Other species are classified as kleptoparasites because, although phytophagous, they cannot induce their own galls (Pereira *et al.*, 2007; Elias *et al.*, 2008). Instead, they lay their eggs in galls induced by other wasp species. It remains unclear whether the larvae of these kleptoparasitic species consume the larvae of the primary gallers or cause their death through starvation. Despite this, kleptoparasites do not meet the criteria for parasitoids, as they depend on plant tissues for larval development. Sometimes, these species are referred to as inquilines; however, because they co-opt gall resources and invariably kill the host, they are more parasitic than strictly inquilinous, considering that "inquiline" implies a commensal relationship. Therefore, we refer to them as kleptoparasites (Ward *et al.*, 2024). Some fig wasp species, however, are true parasitoids, as their larvae develop exclusively at the expense of a galler immature (Elias *et al.*, 2008; Wang & Zheng, 2008; Yadav & Borges, 2018). The patterns of host specialization in NPFWs are not well understood. Host specialization can arise from codivergence between fig tree species and their associated NPFWs, as demonstrated in the Afrotropical section *Platyphyllae* (= sect. *Galoglychia*) (Jousselin *et al.*, 2008, 2006). Alternatively, specialization via host switching has been suggested for Asian *Philotrypesis* species (Jiang *et al.*, 2006). Conversely, host oligophagy is seen in some groups of NPFWs. An example is an undescribed species belonging to genus *Arachonia* (Pteromalidae: Sycoryctina), which uses four *Ficus* host species from the sections *Platyphyllae* and *Sycomorus* (McLeish *et al.*, 2012). Generally, on a given *Ficus* species, parasitoids appear to be more constrained by the

size of the parasitized wasp gall than by the species or genus of the parasitized host (Compton *et al.*, 2018; Deng *et al.*, 2021).

In the Neotropical region, several NPFW species associated with *Ficus* section *Americanae* are known to develop on multiple fig tree species (Marussich & Machado, 2007; Farache *et al.*, 2018). Section *Americanae* comprises approximately 120 species pollinated by *Pegoscapus* wasps (Berg & Villavicencio, 2004) and hosts a diverse community of section-specific genera of NPFWs. However, community structure analyses indicate that even oligophagous species tend to have preferred host trees (Farache *et al.*, 2018). Despite the extensive research on fig-wasp interactions, gaps remain in our understanding of the mechanisms driving host specialization and oligophagy within these communities. Additionally, while host codivergence and preference have been documented in some fig wasp lineages, the patterns of the evolution of host specificity within wasp lineages remain unclear.

Among these NPFWs, the genus *Idarnes* (Pteromalidae) provides an excellent opportunity to explore the relationships between feeding habits and specialization among fig wasp lineages due to the varied life strategies of its species. There are 42 described species of *Idarnes*, divided into three monophyletic groups—*incertus*, *flavicollis* and *carne*, each one having distinct life history (Bouček, 1993; Elias *et al.*, 2012; Farache *et al.*, 2017). Based on the timing of fig colonization and larval diet, *incertus*-group species are classified as early gallers, that oviposit in figs before pollination (Elias *et al.*, 2008; Cruaud *et al.*, 2011b); *flavicollis*-group species as receptive phase gallers, that oviposit at the same time as pollinators and compete with them for oviposition sites (Elias *et al.*, 2008, 2012); and *carne*-group species are kleptoparasites (Elias *et al.*, 2012). Moreover, *Idarnes* exhibits varying degrees of host specialization, with some species demonstrating strict host specificity while others utilize multiple host species (Farache *et al.*, 2018).

In this study, we reconstruct the phylogeny of 57 *Idarnes* species, perform ancestral state reconstructions to infer the evolution of host specialization, and investigate the correlation between wasp feeding habits and the degree of specialization. Our findings suggest that oligophagy evolved multiple times in *Idarnes* and that feeding habits may influence host specialization, as species in the kleptoparasitic clade are less host-specialized than those in the gall-inducing clades.

Methods

Sampling and specimen identification

We sampled adult *Idarnes* individuals emerging from 25 *Ficus* species or forms, found across multiple localities in Brazil, Costa Rica, French Guiana, Mexico, and the United States (Supplementary material 1, Table S1; additionally, Tables S2 and S3 include locality details and Genbank accession numbers for examined specimens). This sampling represents about 20% of all *Ficus* species that host *Idarnes* wasps (Berg 2007; Berg & Villavicencio 2004). While the *incertus*-group has been revised (Farache *et al.*, 2017), enabling specimens to be assigned to species, specimens from the two other species groups were mostly assigned to morphospecies based on a careful examination of morphological characters and molecular data (see ‘Species delimitation’ section).

DNA extraction amplification and sequencing

DNA was isolated from individual specimens using Qiagen extraction kits following non-destructive protocols that allow the keeping of voucher specimens. Vouchers are deposited at CBGP (Centre de Biologie pour la Gestion des Populations, Montferrier-sur-Lez, France) and RPSP insect collection (Universidade de São Paulo, Ribeirão Preto, Brazil).

Primers, PCR protocols, sequencing (Sanger), and assembly followed Cruaud *et al.* (2010) and Cruaud *et al.* (2011a). We amplified one nuclear protein-coding region (F2 copy of elongation factor-1 α , *EF-1 α*), two mitochondrial protein-coding regions (Cytochrome Oxidase I—*COI* and Cytochrome B—*Cytb*), and two regions of the *rRNA* 28S (D2–D3 and D4–D5 expansion regions). To detect frameshift mutations and premature stop codons, coding sequences were aligned (as described in ‘species delimitation’ topic) and translated to amino acids using AliView v. 1.26 (Larsson, 2014). Sequences were submitted to GenBank, and accession numbers are given in Supplementary material 1, Tables S2 & S3.

Species delimitation

COI and *Cytb* sequences (211 specimens, Supplementary material 1, Table S2) were analyzed separately using the generalized mixed Yule coalescent approach (GMYC-

Pons *et al.* 2006) to validate the morphological identification, especially for morphospecies that occurred in more than one *Ficus* species.

Sequences were aligned using MAFFT v.7.455 (Katoh & Standley, 2013) and the L-INS-i algorithm. For each region, the best codon partitioning scheme and evolutionary model available in BEAST were selected using the Bayesian Information Criterion (BIC) in PartitionFinder v.2.1.1 (Lanfear *et al.*, 2016), using the greedy algorithm (Lanfear *et al.*, 2012).

Ultrametric, time-calibrated phylogenetic trees for each region (*COI* and *CytB*) were generated using Beast v.2.6.2 (Bouckaert *et al.*, 2019). To facilitate convergence, we used HKY substitution models instead of GTR models. In each analysis, we used a single strict clock. Indeed, a strict clock provided results more consistent with morphology than a relaxed clock and showed a clearly stepped difference in age of divergence within and among species sequence comparisons. We used a Yule tree prior with a birth rate parameter prior following a gamma distribution with $M = 0.001$ and $S = 1,000.0$. The tree was calibrated using node ages, with a normal prior distribution set at mean 22.5 Ma and standard deviation of 2.5 Ma for the crown *Idarnes* (Cruaud *et al.* 2011a). Default priors were used for all other parameters. Two runs of 10,000,000 generations were performed with sampling every 1000 generations. Parameter convergence was checked using Tracer v. 1.7.1 (Rambaut *et al.*, 2018), and a burn-in of 10% was applied after visually checking trace plots and effective sample sizes. The trees generated by the independent chains were merged using LogCombiner v2.6.2, and TreeAnnotator v2.6.1 was used to generate a maximum clade credibility tree with common ancestor node heights.

We used phylogeny-based species delimitation using the single threshold version of the generalized mixed Yule coalescent approach (GMYC – Pons *et al.* 2006) as implemented in the package splits (Ezard *et al.*, 2017) in R v. 3.6.3 (R Core Team, 2023).

Phylogenetic analysis

We selected one specimen of each species to infer phylogenetic relationships among *Idarnes* species prioritizing those with higher sequencing success for the targeted markers. Specimens belonging to other Sycophaginae genera (Cruaud *et al.*, 2011b,

2011a) were included as outgroups (Supplementary material 1, Table S3), and *COI*, *Cytb*, *EF-1 α* , and *28S rRNA* data were analyzed.

Sequence alignment was performed in MAFFT v.7.455, using the L-INS-I algorithm. The number of iterative refinement cycles (--maxiterate) was limited to 1000 and the gap opening penalty at group-to-group alignment (--op) was set to 3. The best partitioning scheme and evolution models for the four gene regions were selected using Bayesian Information Criterion with Partitionfinder v.2.1.1 (Supplementary material 1, Table S4).

Maximum Likelihood phylogenetic inferences were performed using RAxML version 8.2.12 (Stamatakis, 2014). To estimate node support, the GTRCAT approximation was used to generate 1,000 ML bootstrap iterations.

Bayesian phylogenetic inferences were carried out in MrBayes version 3.2.2 (Ronquist *et al.*, 2012), assuming across-partition heterogeneity in model. Parameter values were initiated with default priors; branch lengths were estimated using default exponential priors. The optimization of the posterior probability was achieved using Metropolis-coupled Markov chain Monte Carlo (MCMC). To improve the mixing of the cold chain and avoid converging on local optima, we executed two independent runs, including a cold chain and three incrementally heated chains for each run. The heating parameter was set to 0.02 to allow frequent swapping between cold and heated chains. The runs were executed for 20 million generations, and values were sampled every 1,000 generations. We also evaluated the convergence between parameters from the two chains by analyzing estimates and frequency distributions of each parameter using Tracer v. 1.7 (Rambaut *et al.*, 2018). We examined the plot of overall model likelihood against generation number to find the point where the likelihood started to fluctuate around a constant value and applied a 10% relative burn-in.

Maximum Likelihood and Bayesian analyses were performed in Cipres Science Gateway (Miller *et al.*, 2010).

Morphological and ecological traits

We recorded the number of *Ficus* hosts from which each (morpho)species of *Idarnes* was collected. Species associated with a single host were classified as monophagous, while species collected on more than one host were considered oligophagous. For each (morpho)species of *Idarnes*, we also measured body length and ovipositor sheath

length. Measurements were taken using a Leica MZ16 stereoscope connected to a digital camera and a computer running Leica Application Suite (LAS) V3.6 imaging software. One to five specimens belonging to each species were measured, totaling 176 measured specimens. The body length was estimated using the sum of the straight measurements of the head length (from the insertion of the antenna to the occipital foramen), the mesosoma length (from the neck to the end of the propodeum), and the metasoma length (from the petiole region to the insertion of the ovipositor sheaths). Ovipositor sheaths were measured using a segmented line measure tool. Measurements were taken using ImageJ v. 1.50 software (Abràmoff *et al.*, 2004). Measurement data are shown in Supplementary material 1, Table S5

Ancestral character state estimation

Ancestral state estimation of binary and multistate characters was performed by simulating stochastic character maps on the Maximum Likelihood phylogenetic tree (Huelsenbeck *et al.*, 2003), using the package *phytools* (Revell, 2012) in R v. 4.1.1 (R Core Team, 2023). We used the results of 1,000 stochastic character mappings for each trait to estimate state probabilities at each node. Model selection was based on the AIC values, calculated using the function `fitMK` in *phytools*.

For specialization as binary variable, we compared the Equal Rates (ER) against the All Rates Different (ARD) models. For the number of hosts, we compared Equal Rates (ER), Symmetrical (SYM), and All Rates Different (ARD) models, as well as custom versions of these models in which character state changes were ordered (1 host \leftrightarrow 2 hosts \leftrightarrow 3+ hosts).

For continuous characters (body length, ovipositor length, and ovipositor/body ratio), ancestral state estimation was performed using an ML approach, as implemented in the `contMap` function of *phytools*. The number of changes between different discrete character states in the simulations was estimated using the `countSimmap` function of *phytools*.

Phylogenetic regressions of specialization

To evaluate specialization as a binary character (monophagous/oligophagous) in relation to morphological and life-history variables, we employed phylogenetic logistic

regression (Ives & Garland, 2010) as implemented in the R package *phylolm* (Ho & Ane, 2014). Convergence was achieved by maximizing the penalized likelihood in the logistic regression model, using the "logistic_MPLE" method with Firth's correction. The number of hosts as a function of morphological and life-history variables was analyzed using phylogenetic general least-squares models (PGLS) using the R package *caper* (Orme *et al.*, 2018). In this analysis, the lambda parameter was optimized using Maximum Likelihood, while the kappa and delta values were kept at their default settings.

In the analyses, we encoded species' life histories in two ways: (1) as a binary variable representing kleptoparasites or gall inducers, and (2) as a three-state categorical variable representing kleptoparasites (spg *carme*), early gallers (spg *incertus*), and receptive-phase gallers (spg *flavicollis*).

For each response variable, the most plausible model(s) was selected by using the Akaike information criterion (AIC).

Since the life-history traits (galler and kleptoparasite) are phylogenetically correlated, which may impact the estimation of coefficients and p-values, we validated our results by splitting the dataset into two subsets: one for gallers and one for kleptoparasites. We then conducted phylogenetic logistic regression and PGLS separately for each subset.

Results

Species delimitation

Molecular-based analyses mostly confirmed morphological inferences. Indeed, for 54 out of 57 species, molecular inference upheld initial morphological species determination (see details in Supplementary Material 1: Supporting information: Species delimitation results and Figs S1–S3).

Phylogenetic analysis

The ML and Bayesian phylogenies were similar; most clades were well supported (Bootstrap support > 70% and Posterior Probabilities > 95%), with the exception of a few nodes (Figure 1). *Idarnes* was found to be monophyletic, comprising three well-supported clades corresponding to the *Idarnes* species groups *carme*, *flavicollis*, and *incertus*. *Idarnes carme*-group was retrieved as a sister group to the two other species groups (Figure 1; Bayesian inference tree in Supplementary material 1, Figure S4).

All analyzed species in the *incertus*-group are monophagous concerning the host *Ficus* species. Three out of 17 species in the *flavicollis*-group (18%) are associated with more than one host. Nine out of 28 species (32%) in the *carne*-group use more than one host (Figure 2).

Ancestral reconstruction of host specialization

The binary models of ancestral character state estimation of specialization (oligophagy/monophagy) showed similar reconstruction patterns. The ER model showed a lower AIC, but equally plausible as the ARD model ($\Delta\text{AIC} < 2$; Supplementary material 1, Table S6). For the number of hosts, the Symmetrical Ordered model showed a lower AIC, but equally plausible as the standard SYM model ($\Delta\text{AIC} < 2$; Supplementary material 1, Table S6).

According to ASR, the oligophagous habit evolved independently several times in the *I. carne* and *I. flavicollis* species groups (Figure 2). The ancestral character state estimation suggests that the ancestor of all *Idarnes* species, as well as the ancestors of each of the three species groups, were monophagous (Figure 2).

The stochastic mappings indicated that transitions from monophagy to oligophagy were more frequent than the reverse transition during the diversification of *Idarnes*. The models estimated 10 transitions (CI: 8 – 11), with nine visually inferred in Figure 2, from monophagy to oligophagy, and 1 transition (CI: 0 – 4), with none visually inferred in Figure 2, from oligophagy to monophagy (Figure 3).

Ancestral reconstruction of morphological characters

Ovipositor length correlated with body length, though the slope of this relationship differed between the *incertus*-group and other species groups. Species in *incertus*-group have the shorter relative ovipositor length (ovipositor/body length ratio < 1), often exhibiting a larger body size and shorter ovipositors compared to wasps in the *flavicollis* and *carne* groups..

The ancestral reconstruction of morphological characters showed that the ancestor of the *carne*-group had the smallest body length (1.46 ± 0.02 mm, mean \pm variance calculated using fastAnc) compared to the ancestor of *Idarnes* (1.59 ± 0.04 mm) and the remaining species groups. A longer ovipositor was observed in the ancestor of the *flavicollis*-group (3.72 ± 0.42 mm) and a shorter one in the *incertus*-group (1.95 ± 0.57

mm) (Figure 4A–B). The models indicated that the relative ovipositor length was greater in the ancestors of the *carme* and *flavicollis* species groups (respectively 2.00 ± 0.06 mm and 2.22 ± 0.07 mm) when compared to the ancestor of all *Idarnes* (1.89 ± 0.10). The ancestor of the *incertus-group* likely had the smallest relative ovipositor length (1.16 ± 0.1 ; Figure 4A–B).

Generally, the species of *carme*-group collected from a single *Ficus* species exhibited ovipositor lengths consistent with fig size, as previously described for this group by [Farache *et al.* \(2016\)](#). The exception is *I. barbiger*, collected only from *F. citrifolia* in Costa Rica. It has a long ovipositor similar in length to that of nsp09 and nsp26. When comparing the size of monophagous species in *flavicollis* and *carme* species groups associated with the same *Ficus* species in the same region, the *flavicollis* species was systematically somewhat larger and presented a longer ovipositor than *carme*-group species (Supplementary material 1, Figure S5).

Phylogenetic models of specialization

The host specialization (i.e., monophagy/oligophagy) and the number of used hosts were best explained by ovipositor/body ratio and wasp life history (i.e., galler or kleptoparasite) (Tables 1-2).

The most plausible models indicated that within both gall-inducing and kleptoparasite life histories, wasp species with relatively longer ovipositors were more likely to be oligophagous or to use more *Ficus* species (Table 3). The interaction term between ovipositor/body ratio and life history (bio1) was significant in both most plausible models (Table 3), indicating that the relationship between host specificity and ovipositor size is not constant across species life histories. This pattern is evidenced by the steeper slope between the number of hosts and ovipositor/body rate in the kleptoparasite compared to the gall-inducing species (Figure 5).

When analyzing gallers and kleptoparasites independently, we found a significant positive effect of the ovipositor-to-body ratio on specialization in the kleptoparasite subset. In contrast, the galler subset showed no significant effect of the ovipositor-to-body ratio on specialization, consistent with the findings from the complete *Idarnes* dataset (Supplementary material 1, Table S7).

Scripts for ancestral reconstruction and phylogenetic models, as well as the phylogenetic tree and dataset are available as Supplementary Material 2.

Discussion

Our reconstructions of the ancestral host spectrum show that host monophagy is ancestral in *Idarnes*, and the oligophagous trait has evolved independently in several lineages. This scenario is consistent in both types of analyses (binary and multistate). The homogeneity of relative sizes and ovipositor lengths within species groups (*incertus*-group large body size, short ovipositor, *flavicollis*-group intermediate body size, very long ovipositor, *carme*-group smallest body size, long ovipositor) supports the hypothesis that feeding habit is conserved within species groups.

Host specialization in kleptoparasites

Though few, oligophagous species were more abundant in the *carme*-group kleptoparasitic lineage than gall-inducer lineages. In the sole comparable dataset on parasitic/kleptoparasitic fig wasps in Southern Africa, 22 species of *Philotrypesis* were each associated with a single fig tree species, while only one species was associated with three host species ([supporting information from McLeish et al., 2010](#)). No oligophagous *Philotrypesis* has been observed in Asia (Jiang *et al.*, 2006). The weaker host specificity in *carme*-group could relate to the more recent radiation of *Ficus* section *Americanae* compared to the African section *Platyphyllae* and Asian *Ficus* lineages (Cruaud *et al.*, 2012). This suggests that more recently diverged *Ficus* species may share biological traits that facilitate a wasp species in utilizing them. In support of a role for age of diversification, the set of fig trees colonized by the oligophagous *Philotrypesis* species (*F. burkei*, *F. natalensis* and *F. petersii*) belong to subsection *Chlamydodora* and diverged more recently (Cruaud *et al.*, 2012). These three host species have similar fig sizes, respectively 0.4-1.1, 0.8-1.5, and 0.8-1 cm (Berg & Wiebes, 1992). In Africa, fig size largely correlates with phylogeny, with *Ficus* species presenting large figs grouped in the more distantly related section *Platyphyllae* subsection *Caulocarpae* and in subgenus *Sycomorus* (McLeish *et al.*, 2010). Hence, phylogenetic distance among *Ficus* species may limit the use of small figs by kleptoparasites usually associated with large figs. However, this is not the main pattern of oligophagy observed for *carme*-group. The relatively frequent use of several *Ficus* species by *carme*-group compared to *Philotrypesis* might be better explained by the recent radiation of *Ficus* section *Americanae*, leading to diversified fig sizes across closely related species (Cruaud *et al.*, 2012).

Considering the same host tree, all *Idarnes* species in *carne*-group were smaller than those in *flavicollis*-group, suggesting they are all kleptoparasitic on the pollinating *Pegoscapus* wasps. This study did not investigate whether a single *Idarnes* species parasitizes multiple *Pegoscapus* species that may pollinate the same *Ficus* host throughout its range. However, some *carne*-group species have ranges that overlap with those of multiple *Pegoscapus* species pollinating the same *Ficus* species (R.A.S. Pereira, unpublished data). This observation aligns with the general pattern that fig wasps kleptoparasitic on the pollinators can indiscriminately parasitize any pollinator of their host *Ficus* species. For example, in Asia, the geographic distributions of *Philotrypesis* species associated with the galls of pollinators of *F. hirta* show that some *Philotrypesis* species parasitize multiple pollinator species (Deng *et al.*, 2021). Similar patterns are observed for *Philotrypesis* species parasitizing the pollinators of *F. rubiginosa* in eastern Australia (Darwell *et al.*, 2014; Darwell & Cook, 2017). In *F. microcarpa*, the same *Philotrypesis* species feeds on galls initiated by both the pollinating and the cheating *Eupristina* species. We conclude that these kleptoparasitic wasps are eclectic in terms of wasp host species but are specialists in a type of gall on a given *Ficus* species (Compton *et al.*, 2018).

Data on kleptoparasite specialization is also available for oak gall wasps, which presents a different scenario. Gall-forming cynipid wasps produce a variety of gall morphologies on different plant organs (Ward *et al.*, 2020), unlike a series of closely related agaonid wasps, which produce morphologically identical galls while pollinating the same *Ficus* species. If we define specialization as the use of a single gall type on a single host-plant species, then while *Idarnes carne*-group are oligophagous among kleptoparasitic fig wasps, they are still more specialized than *Synergus* on oak gall wasps in the New World. More than half of *Synergus* species (18 out of 31) are reared from the galls of more than one gall wasp species (Ward *et al.*, 2024).

It has been proposed that parasites select their host species based on the fitness benefits they provide (Singer, 2008). Specifically, the host species a parasite uses tend to offer similar and high fitness advantages, meaning the parasite achieves equally good reproductive success and survival on all these hosts. These benefits are greater than those provided by other potential hosts that the parasite does not use (Singer, 2008). Alternatively, the ecological fitting hypothesis offers a dynamic perspective on how organisms adapt to new hosts. It suggests that an organism can successfully colonize a new host due to its pre-existing traits when encountering a new condition. These traits

can be co-opted to establish a new fit interaction with the host, without the need for new adaptations (Janzen, 1985; Agosta *et al.*, 2010; Gil-Tapetado *et al.*, 2021). Thus, for *carne*-group, selecting an alternative less rewarding host or a host more challenging to reach before competitors may simply result from a wasp encountering this alternative host by chance at the appropriate stage. Choosing an alternative host can help avoid the costs of searching for a better one.

Host specialization in gallers

Among gall-inducing *Idarnes*, only some oligophagous species occurred in the lineage using receptive flowers (i.e., *flavicollis*-group). All species in the early galler lineage (i.e., *incertus*-group) are monophagous. The biology of the agaonid pollinators can provide insights into the higher host specificity observed in the receptive phase gallers, as both pollinators and *flavicollis*-group wasps use the plant ovules at the same stage, thus they are probably subjected to similar selective pressures. In some cases, pollinators can successfully initiate galls and develop in non-host species; in others, they cannot. For instance, *Elisabethiella stuckenbergi* managed to thrive in *F. lutea*, a fig tree belonging to the same section as its usual host (Compton, 1990). Conversely, *Kradibia tentacularis* fails to gall ovules in *F. asperifolia*, a non-host species in the same subgenus as its usual host (Ghana *et al.*, 2015). In this latter case, eggs were deposited in the ovules, but the initial swelling of the ovule stimulated by the ovipositing wasp did not occur (Ghana *et al.*, 2015).

Gallers of receptive figs face several challenges in colonizing multiple *Ficus* species. Firstly, they must be attracted by the scents emitted by receptive figs of different species, which are typically highly species-specific (Souto-Vilarós *et al.*, 2018). Secondly, the ovipositing female must be capable of inducing the initial rapid swelling of the ovules following oviposition. Finally, the larva must complete gall induction and thrive by feeding on the plant tissues (Jansen-González *et al.*, 2012). The similarity of receptive fig scents may facilitate the recognition of closely related non-host *Ficus* species (Deng *et al.*, 2022). Species of *flavicollis*-group using more than one host were only recorded in southern Brazil. This may relate to more intensive sampling in this region. Alternatively, it could relate to regional ecological conditions. Fig densities are overall low in southern Brazil (Coelho *et al.*, 2014), which could favor wasps using

several host species, in a classical ecological process involving host density-dependent niche expansion (e.g. Soler et al., 2009).

The role of ovipositor length and wasp life history

Wasps belonging to *carme*-group oviposit by inserting their ovipositors straight through the plant tissues (Elias et al., 2012). A longer ovipositor is required to reach the galls in *Ficus* presenting larger figs, as in other NPFW lineages (Yu et al., 2018). In most cases, the oligophagous species use *Ficus* hosts presenting different fig sizes, for which different lengths of ovipositors are predicted and observed in monophagous species. Several potential costs are associated with ovipositors longer than necessary. They could include reduced flight prowess, a higher risk of predation, a higher risk of ovipositor damage, and a longer host handling time. Hence, wasps using figs of different sizes are maladapted to the smaller figs they use compared with specialist wasps. However, individual fig trees generally produce synchronized crops, and only a few trees within a population present figs at the appropriate stage for wasp oviposition at a given time (Bronstein et al., 1990). A wasp that encounters a non-optimal host tree bearing figs at the appropriate stage for oviposition may achieve higher reproductive success ovipositing on that tree rather than leaving the tree in search of a hypothetical, optimal host tree presenting figs at the appropriate stage.

Conversely, the relative ovipositor size and the number of used hosts were not correlated in the gall-inducing species. The early gallers of the *incertus*-group are mostly host-specific. Only one species has been reported using two hosts (*I. americanae* Farache & Rasplus, 2018), but this host sharing has not yet been confirmed by molecular markers. Early gallers oviposit at the beginning of fig development, when the fig wall is thinner and fig florets are still developing. Some species are thought to oviposit in the fig wall instead of within fig pistillate flowers (Bronstein, 1999; Ghara et al., 2014). Therefore, ovipositor size may not constrain the occupation of multiple hosts. Another factor, such as the species-specific wasp venom associated with gall induction, is likely responsible for the host specificity observed in early gall inducers (Elias et al., 2018).

The receptive phase gallers (i.e., *flavicollis*-group) showed a higher ovipositor/body ratio but no clear relationship with the number of hosts they use. In contrast with *carme*-group, they have a complex oviposition mechanism, in which the ovipositor is inserted

through the flower stigma and style, mimicking the oviposition strategy of the pollinators (Elias *et al.*, 2012; Jansen-González *et al.*, 2012). Thus, their relatively longer ovipositors may be related to this oviposition mechanism. Data from *F. petiolaris* show that *flavicollis*-group species primarily exploit the flowers closest to the fig lumen (those with shorter styles) most accessible for oviposition. In contrast, *carme*-group species occupy the pollinator galls located closer to the fig wall, which are more accessible to their ovipositors (Satler *et al.*, 2020). Although the relatively longer ovipositors might allow *flavicollis*-group wasps to exploit a broader spectrum of *Ficus* species with varying fig sizes, the success of host utilization likely involves additional factors beyond access to flower ovaries. Species-specific volatile cues associated with wasp attraction during the receptive phase (Chen *et al.*, 2009; Proffit *et al.*, 2018, 2020; Zeng *et al.*, 2018) and the potentially fine-tuned adaptations required to induce galls in the ovary tissues (Elias *et al.*, 2012; Jansen-González *et al.*, 2012, 2014) may constrain oligophagy in the receptive-phase galler species.

Conclusions

Our study reveals that host monophagy is ancestral in *Idarnes*, with the oligophagous trait evolving independently in several lineages. Among intrageneric *Idarnes* lineages, oligophagous species are more frequent in the *carme*-group than gall-inducer lineages (i.e., *flavicollis* and *incertus* species groups). Early galler species (i.e., *incertus*-group) are predominantly monophagous, whereas some oligophagous species occur in the receptive phase galls (i.e., *flavicollis*-group). Our findings emphasize the complex interplay of evolutionary history, ecological factors, and species-specific adaptations in shaping the host utilization patterns of *Idarnes* wasps. This study contributes to a deeper understanding of the dynamics of specialization and oligophagy in fig wasps and their host plants, offering valuable insights into the evolutionary processes governing these intricate mutualistic and parasitic relationships.

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Figures

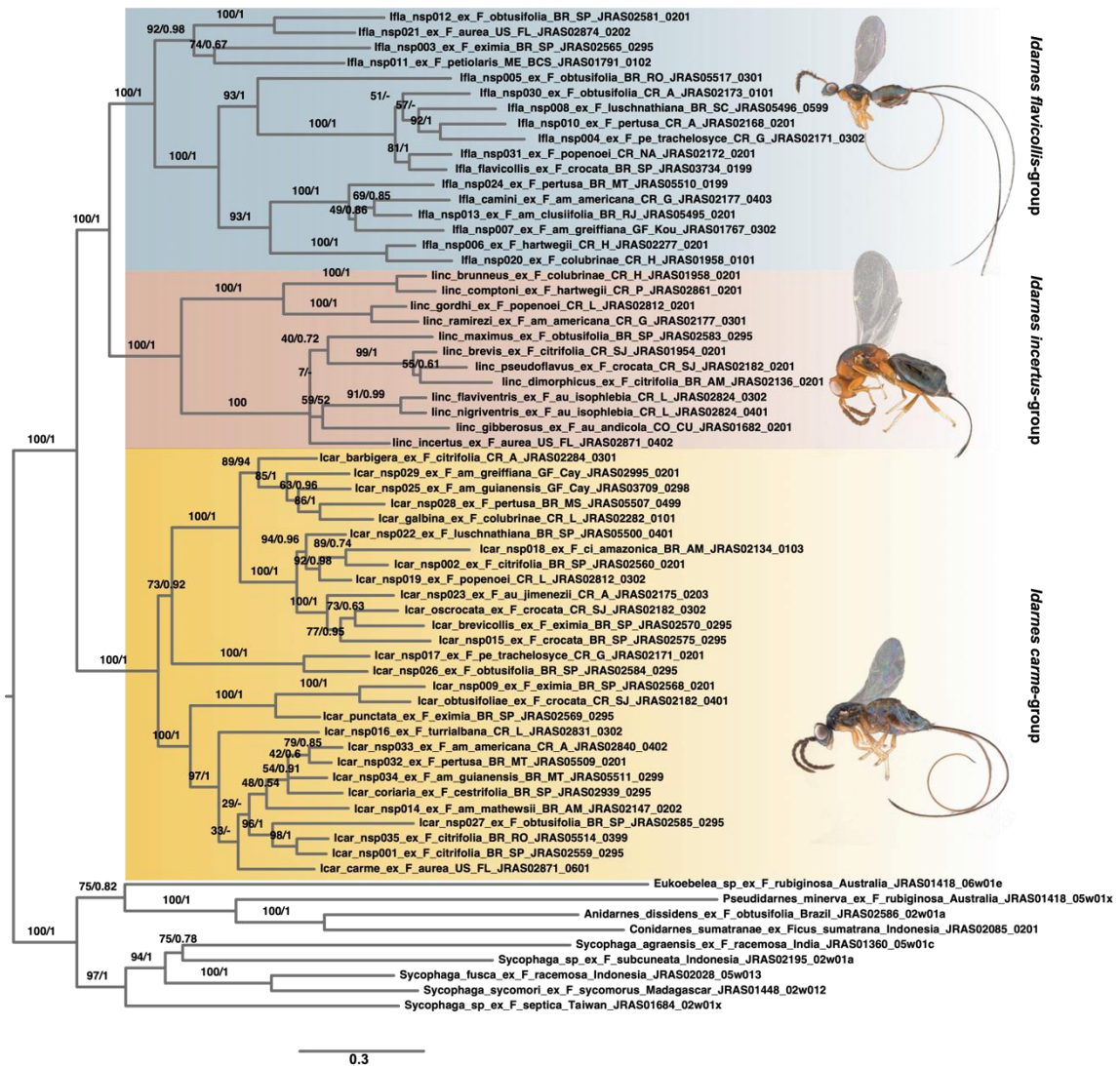


Figure 1: Phylogram (ML) showing the relationships within *Idarnes*. Node support show ML Bootstrap support/Posterior Probabilities (PP). Abbreviations in tip labels include: Icar = *Idarnes carme*-group; Ifla = *Idarnes flavicollis*-group; linc = *Idarnes incertus*-group; BR = Brazil; CR = Costa Rica; GF = French Guiana; ME = Mexico; US = USA Country abbreviations are followed by standard state/province abbreviations and collection codes. F am = *Ficus americana* species complex; F au = *Ficus aurea* species complex; F ci = *Ficus citrifolia* species complex. Dashes (“-”) in PP indicate that the clade was not retrieved in Bayesian phylogenetic analysis.

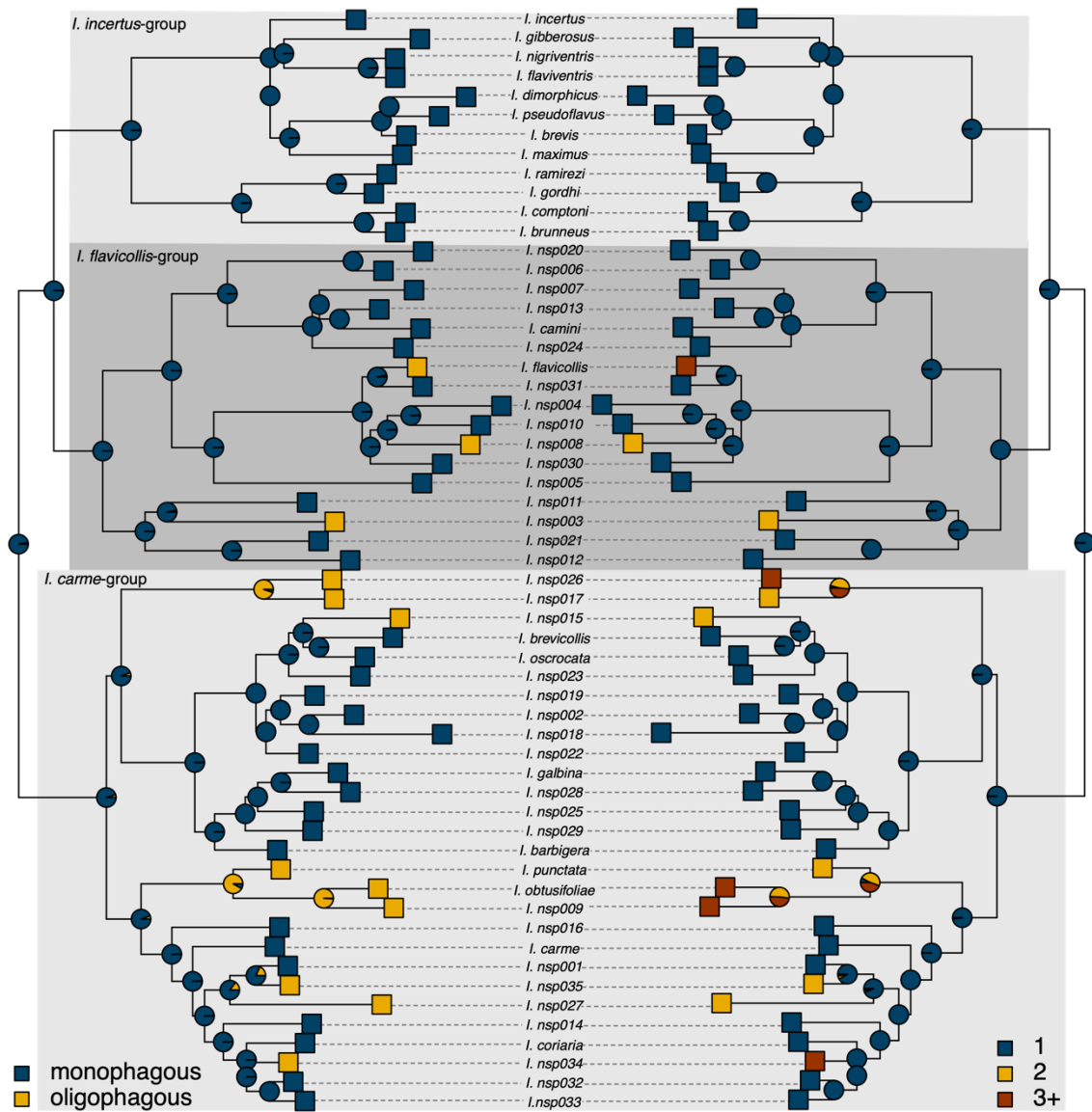


Figure 2 Ancestral state reconstructions (SIMMAP) of specialization (left; monophagous|oligophagous) and number of hosts (right; one, two, or three or more hosts).

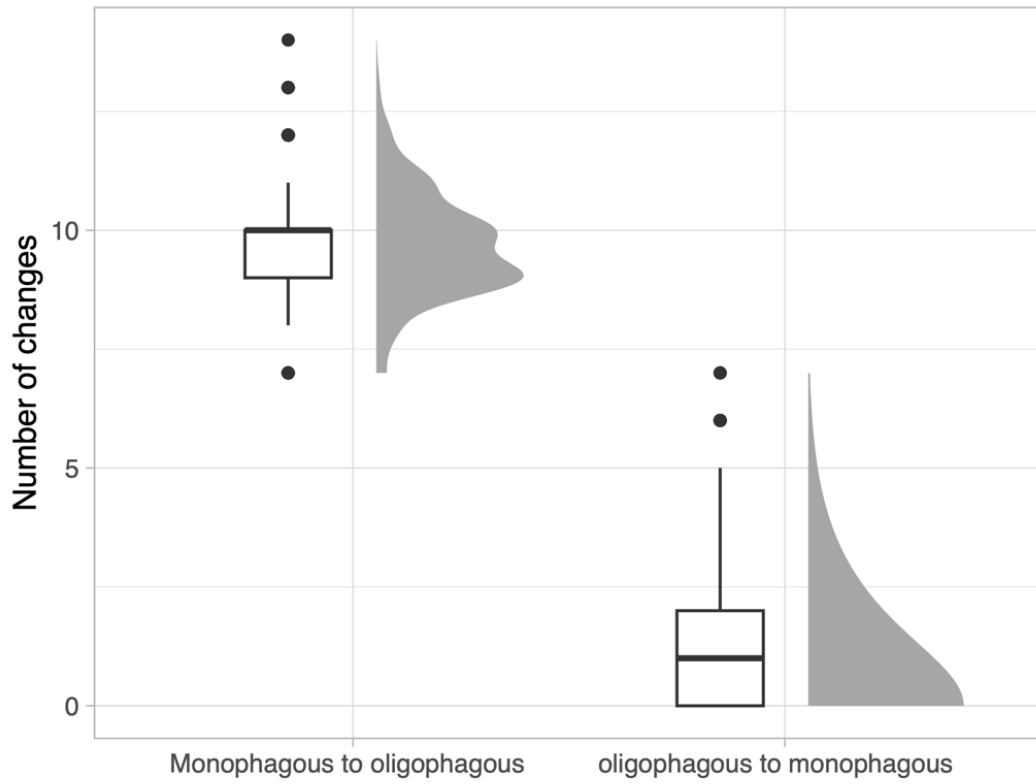


Figure 3: Distribution of character changes between monophagous and oligophagous states on 1000 stochastic character map reconstructions of character states on the *Idarnes* ML phylogeny.

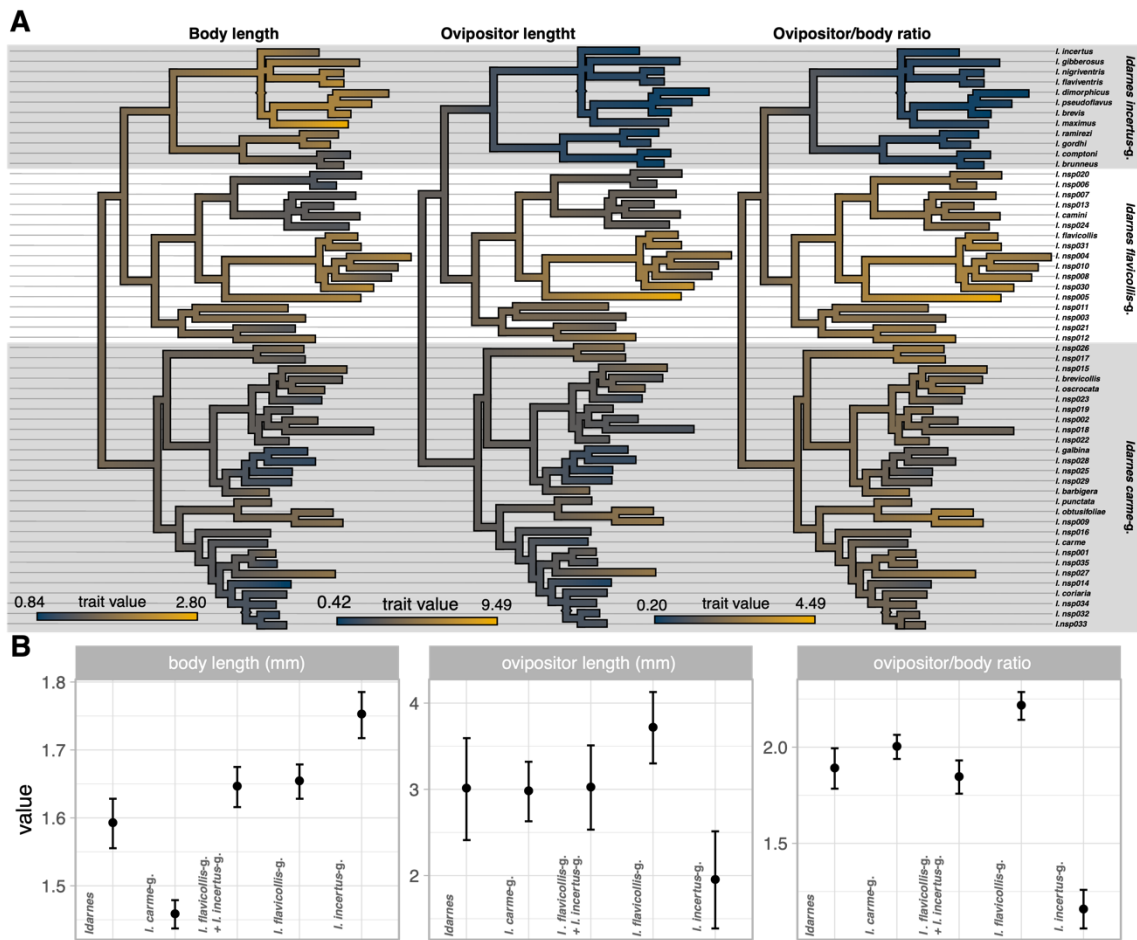


Figure 4: Morphometric characters of *Idarnes* species. A: continuous trait evolution maps for body length, ovipositor length and ovipositor/body ratio. B: average and variance of body length, ovipositor length and ovipositor/body ratio for ancestral nodes variances calculated using fastAnc phytools function. Abbreviations: g. = group.

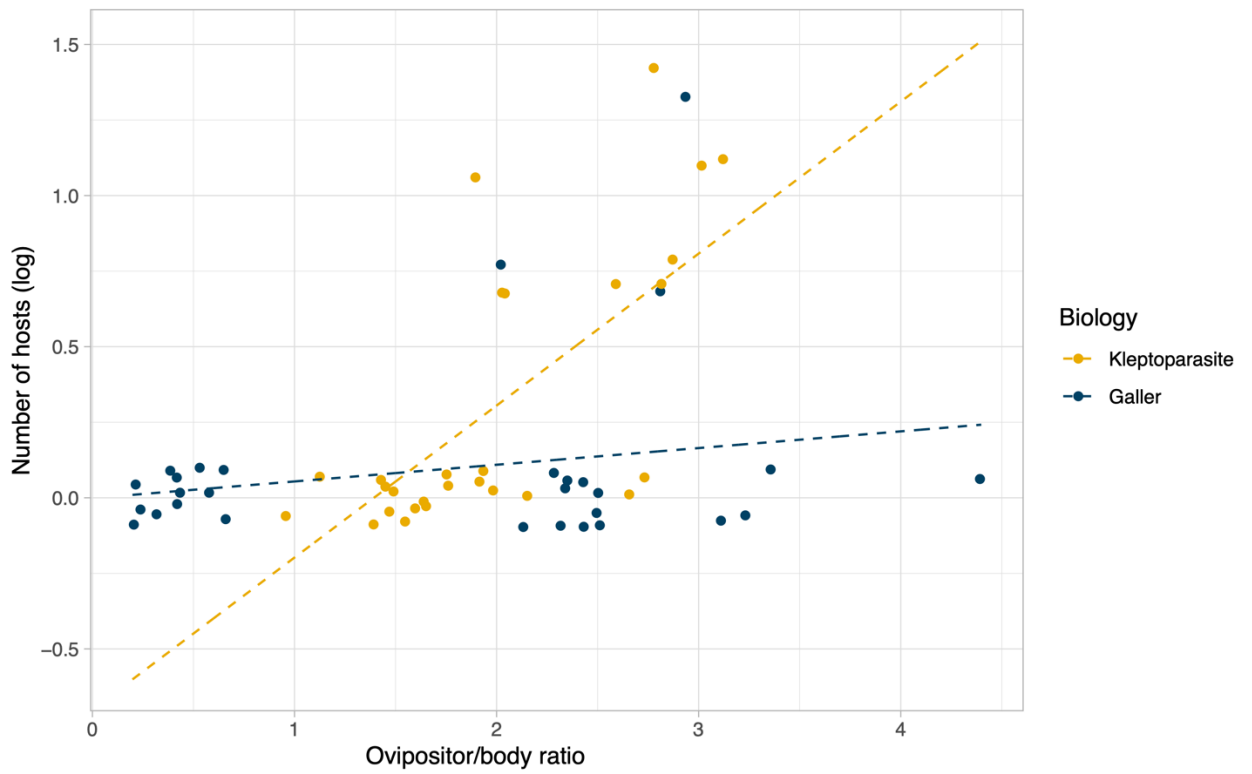


Figure 5 Number of used hosts as a function of the relative ovipositor length in 57 *Idarnes* species, according to species life histories (i.e., galler or kleptoparasite). The y-axis is jittered to facilitate the visualization of data points.