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Phylogenetic relationships and biogeography of the *Ipsiura* cuckoo wasps (Hymenoptera: Chrysididae)

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Abstract

Phylogenetic studies addressing relationships among chrysidid wasps have been limited. There are no hypotheses proposed for the Neotropical lineages of Chrysidini other than the classic cladogram published in the 1990s by Kimsey and Bohart. Herein we present a cladistic analysis based on 64 morphological characters coded for 54 species of Chrysidini, 32 of them being *Ipsiura* and 22 representing *Caenochrysis*, *Chrysis*, *Exochrysis*, *Gaullea*, *Neochrysis*, and *Pleurochrysis*. The species of *Ipsiura* were recovered as monophyletic and as the sister clade of *Neochrysis* in all most parsimonious trees. We discuss the high plasticity of some morphological characters as evidenced by their high homoplasy in the phylogenetic results, and we clarify the main morphological changes inferred on the phylogenetic tree for this genus. The effects of the inferred homoplasy were evaluated under an implied weighting cladistic analysis, and from a probabilistic perspective with Bayesian inference. Those alternative strategies did not alter the general conclusions about the monophyly of *Ipsiura* or the generic relationships in Chrysidini (changes were noticed in the species-level relationships within certain parts of *Ipsiura*, where low branch support was common across all approaches). Among the species groups proposed by Linsenmaier (1985), only the *marginalis* group was recovered as monophyletic. We also evaluated the convoluted

Introduction

The Chrysidini are one of the four recognized tribes of cleptoparasitic wasps in the Chrysidinae (Kimsey & Bohart, 1991; Carpenter, 1999; Lucena & Melo, 2018). These wasps are readily diagnosed by the characteristic reduced number of external metasomal segments and their distinct rigid integument, which are thought to be associated with their life-history strategies in defence against powerful aculeate hymenopteran hosts (Bohart & Kimsey, 1982; Kimsey & Bohart, 1991; Kimsey, 1992; Lucena & Melo, 2018).

In the Neotropical region, the Chrysidini comprise 206 species distributed in nine genera (Kimsey, 2006a,b). Four of these genera are particularly diverse and primarily Neotropical, with some taxa extending as far north as the southeastern U.S.A. (Kimsey & Bohart, 1991; Kimsey, 2006b; Lucena *et al*., 2016): *Pleurochrysis* Bohart, 1966 (36 spp.), *Neochrysis* Linsenmaier, 1959 (19 spp.), *Exochrysis* Bohart, 1966 (12 spp.), and *Ipsiura* Linsenmaier, 1959 (41 spp.). These taxa are interpreted to form a monophyletic group, according to Kimsey & Bohart (1991), based on their interpretation of their putative morphological synapomorphies. The taxonomy of these genera was based on studies conducted independently and almost concomitantly by Bohart (1985), Kimsey (1985) and Linsenmaier (1985). The taxonomic discrepancies resulting from those three contributions resulted in controversies about delimitation of some genera and the taxonomic placement of many species (details discussed by Kimsey & Bohart, 1991; Linsenmaier, 1997; Lucena *et al*., 2016).

The distinction between the *Neochrysis* and its closely related genera was discussed by Bohart (1985) and Kimsey (1985). Bohart (1985) revised and elevated *Ipsiura* to genus and discussed morphological characters shared by species of the genus. Kimsey (1985) revised species of *Neochrysis*, *Pleurochrysis* and *Exochrysis*, listed generic morphological characters to distinguish among them and discussed the higher-level phylogenetic affinities among these genera including *Ipsiura*. Kimsey & Bohart (1991) recognized morphological similarities among these four genera and proposed the first comprehensive phylogenetic hypothesis for Chrysidini as a whole, focused on the suprageneric discussion. However, the phylogenetic relationships at the species level were not inferred by those authors. Linsenmaier (1985), in his revisionary study of *Neochrysis* sensu lato, divided *Neochrysis* into subgenera and species groups and provided keys for subgenera and species groups. He also listed some morphological characters to identify those groups. Linsenmaier (1985) was the first to formally suggest species groups for the taxon; he divided *Ipsiura* into four species groups based mainly on the metasomal characters, such as the number of distal teeth of tergum III: *marginalis* (two and four-toothed), *leucocheila* (four-toothed), *lateralis* (=*lata*) (six-toothed) and *genbergi* (six-toothed) species groups. Bohart such as the *Exochrysis*, *Neochrysis* and *Pleurochrysis*, are widely distributed in the Neotropical region, with many species occurring from Mexico to Argentina (Kimsey & Bohart, 1991). This distribution indicates that the Neotropics may have played an important role in the diversification of these cuckoo wasps, but this was never formally evaluated. In Brazil, the only extensive surveys on the chrysidid's local fauna was that of Adolf Ducke, who exhaustively collected in the tropical Amazonian rainforest near Belém and Óbidos (Ducke, 1904, 1907, 1911), and in several xeric localities in northeastern Brazil (Ducke, 1908, 1910; Zanella & Lucena, 2014). Given the seemingly nonhierarchical biogeographical organization of most species distributions, combined with the broad environmental tolerances of several species of *Ipsiura*, it has been hard to suggest an *a priori* close association between the geological events of formation of the Neotropical region and the diversification of this lineage of Chrysididae.

In this work, we investigate the phylogenetic relationships among species of the genus *Ipsiura*, as well as among major lineages of Neotropical Chrysidini (the genera *Gaullea*, *Exochrysis*, *Neochrysis* and *Pleurochrysis*). We included novel morphological characters and formally evaluated the traditionally suggested species groups of *Ipsiura*. We discuss the morphological changes occurred within the clade *Ipsiura* and implications of new morphological characters for the interpretation of phylogenetic relationships among the Neotropical Chrysidini. Based on the phylogenetic results, we performed event-based analyses to address the historical biogeography of *Ipsiura* species. Furthermore, a discussion is presented regarding some preliminary biogeographic considerations for the taxon.

Material and methods

Material examined

The following institutions and curators provided loans of material examined during the course of this study: BME, Bohart Museum of Entomology, University of California, Davis, U.S.A. (Dr Steven Heydon); CAVS, Coleção de Abelhas e Vespas Solitárias, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Ribeirão Preto, Brazil (Dr Carlos A. Garófalo); DZUP, Coleção Entomológica 'Pe. Jesus Santiago Moure', Universidade Federal do Paraná, Curitiba, Brazil (Dr Gabriel A. R. Melo); INBPY, Museo Nacional de Historia Natural del Paraguay, San Lorenzo, Paraguay (Dr Bolívar R. Garcete-Barrett); INPA, Coleção Entomológica do Instituto Nacional de Pesquisa da Amazônia, Manaus, Brazil (Dr Márcio L. Oliveira); LEBIC, Laboratório de Ecologia e Biogeografia de Insetos da Caatinga, Universidade Federal de Campina Grande, Patos, Brazil (Dr Fernando C. V. Zanella; Mr. Pedro E. Santos-Neto); LRRP, Coleção Entomológica do Laboratório de Sistemática e Bioecologia de Parasitóides e Predadores, APTA Centro Leste, Ribeirão Preto, Brazil (Dr Nelson W. Perioto); MPEG, Museu Paraense Emílio Goeldi, UFES, Coleção Entomológica da Universidade Federal do Espírito Santo, Vitória, Brazil (Dr Marcelo T. Tavares); UNICENTRO, Laboratório de Biologia e Ecologia de Vespas e Abelhas, Universidade Estadual do Centro-Oeste, Guarapuava, Brazil (Dr Maria Luisa T. Buschini; Ms Tayane Buggenhagen).

Taxonomic sampling

We included a total of 54 terminal species, representing the genera *Caenochrysis*, *Chrysis*, *Exochrysis*, *Gaullea*, *Ipsiura*, *Neochrysis*, and *Pleurochrysis*. *Ipsiura* is the genus most densely sampled for this analysis, being represented by 32 species, which accounts for most of the known morphological diversity of the genus. Outgroups are represented by 22 species, including *Caenochrysis nigropolita* (Bischoff), *Chrysis* (two spp.), *Gaullea argentina* du Buysson, *Pleurochrysis* (seven spp.), *Neochrysis* (four spp.) and *Exochrysis* (seven spp.) (Table S1 in File S1). We used adult pinned representatives of species (Table S2 in File S1), and an effort was made to examine multiple individuals to cover intraspecific variation. Specimens of *Ipsiura* were listed in detail by Lucena *et al*. (2016). The characters presented in the matrix are considered applicable for both sexes, except when noted, because males and females of many Chrysidini are nearly identical morphologically (see List of characters in the Supplementary Material). Trees were rooted with *Caenochrysis nigropolita* as the most distant outgroup (in accordance with the phylogenetic hypothesis of Kimsey & Bohart, 1991), and the sampling of as many as 22 outgroup species was judged relevant for the establishment of the position of the *Ipsiura* root node. Species of *Caenochrysis* are readily diagnosed by the presence of the 'odd' character condition, concerning the number of distal teeth on metasomal tergum III, and the distally articulated paramere of the male genitalia (Kimsey & Bohart, 1981, 1991; Bohart & Kimsey, 1982).

Morphological study

Most males had their genitalia dissected. Specimens were rehydrated in a humid chamber overnight. The male genital capsule was removed and macerated in a 10% solution of KOH overnight, following a protocol adapted from Porto *et al*. (2016). Illustrations of male genitalia for the majority of *Ipsiura* species are presented in Lucena *et al*. (2016). Female ovipositors were dissected and cleared following the same protocols. Some specimens were kept intact due to their rarity. Fore- and hindwings of some specimens were dissected and dehydrated using a sequence of immersion in different alcohol concentrations (70–100%), followed by a bath in xylene solution and mounted in slides using the mounting medium Entellan (Germany). Photographs were taken using a Leica DFC425 camera attached to a Leica M205C stereomicroscope. Images were improved using LEICA APPLICATION SUITE software (Germany) to

Terminology and abbreviations

Morphological characters discussed by Bohart (1966, 1985), Kimsey (1985), Linsenmaier (1985) and Kimsey & Bohart (1991) were examined, reinterpreted when necessary, and coded into the matrix. We use 'state' followed by numbers of the character and its alternatives to refer the character conditions discussed throughout the paper. Morphological terminology follows primarily that of Kimsey & Bohart (1991). The abbreviations F, S and T are employed for flagellomeres, metasomal sterna, and metasomal terga, respectively. MOD refers to the median ocellus diameter. TFC refers to the transverse frontal carina. MOC refers to the median ocellus carinae (=midocellar carinule of Kimsey & Bohart, 1991: 22). Secondary facial carinulae refers to the small carinules that rise supralaterally from the MOC. Cupula and foramen genitale are preferred label herein used to refer to the gonobase and genital foramen, respectively (*sensu* Michener, 1944) (see definitions in Hymenoptera Anatomy Consortium, 2015). Additionally, we use also paramere instead of gonocoxa to refer to the anatomical cluster that is composed of sclerites located distally of the cupula dorsolaterally of the volsella, and that surround the aedeagus (Hymenoptera Anatomy Consortium, 2015). When the paramere has an articulated distal part, we refer to it as harpe instead of gonostylus. Sculpturing terminology follows Harris (1979).

Phylogenetic analyses

The data matrix was submitted to heuristic cladistic analyses, with parsimony criterion using equal and implied weights of characters, implemented in the TNT software (Goloboff *et al*., 2008). The tree search parameters were as follows: traditional search with random seed set to 0, 5000 replications saving ten trees per replication. The conclusions of this research were based on the equal weights analysis but were complemented with strategies adopted to evaluate the impact of homoplasious characters on the phylogenetic conclusions, as discussed below.

Implied weights parsimony analyses were run varying the values of the *K* concavity constant from 1 to 30 (changing *K*'s values one by one). From 30 to 100, *K*-values were evaluated every five steps. The data matrix was also analysed in a probabilistic framework using a Bayesian inference as implemented in MRBAYES (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). We ran an analysis using a standard discrete model (Lewis, 2001), with and without gamma-distributed rate heterogeneity model for morphological characters. We ran the MRBAYES data block at the CIPRES gateway (Miller *et al*., 2010) for 10 × 10^6 MCMC generations with four chains and four parallel runs. We checked the parameter convergence in TRACER (Rambaut *et al*., under the unambiguous scheme. Character length and indices (steps 'L', consistency index 'CI', and retention index 'RI') are based on the strict consensus obtained for the equal weights analysis. We assessed the relative support of clades given by the data using direct and indirect (*sensu* Siddall, 2002) indices. Two indirect measures were used: (i) nonparametric bootstrap (Felsenstein, 1985), done by resampling with replacement and allowing for an evaluation of clade stability with differential weighting of characters in various pseud-replicates of the original data matrix; and (ii) GC frequencies (Goloboff *et al*., 2003), or just 'GCs', estimated as the difference in frequencies of clades present or contradicted in a symmetrical resampling procedure. For each replication of the character set, it is necessary to calculate whether the group of interest is supported by the resulting sample of characters. Any group with a frequency > 50% is certain to be supported, but clades with frequencies below this level cannot necessarily be taken as weakly supported (Goloboff *et al*., 2003). For both resampling methods, 10 000 trees were calculated from pseudoreplications of the matrix using traditional heuristic searches in TNT; node frequencies were then calculated on the strict consensus using WINCLADA. We contrasted the support estimated with indirect measures using Bremer values (Bremer, 1994) as a direct measure of support (e.g. Siddall, 2002). Stability of nodes was measured using relative node frequencies estimated from suboptimal trees with additional steps varying from 1 to 50 also using TNT. Bremer values do not have an upper bound, whereas bootstrap and GCs are shown as percentages (0–100%), and in all cases, groups are better supported to the extent that there is more character-evidence in favour of them.

Tree reconciliation

Previous to this study, no explicit phylogenetic hypothesis for *Ipsiura* was available for comparison with the present results. The taxonomic revisions by Bohart (1985) and Linsenmaier (1985) are the most comprehensive comparative studies available for the group, but they both lacked phylogenetic treatments for the taxon. We used the Linsenmaier's system of species groups to contrast with our results and to compare the morphological evidence in favour of them. We built a hypothetical phylogenetic tree using MESQUITE (Maddison & Maddison, 2017) for the Linsenmaier's species groups as they are presented in his original text: *marginalis* + (*leucocheila* + (*lateralis* + (*genbergi*))). The arrangement of terminal species into the groups follows the same order of appearance in the text, which reflected the author's views on the relative proximities of species. We optimized our morphological characters on this tree topology using WINCLADA. We pruned the species in our phylogeny to match the same taxonomic representativeness. Finally, we used TREEMAP (Page, 1995) to mirror and compare both tree topologies as a tanglegram.

Biogeography

program by Ronquist, 1996). The cost of vicariance is down-weighted in relation to dispersal and extinction, which therefore favours historical vicariant explanations over dispersals for disjoint distributions. We coded the taxa as being present or absent from the following areas: (i) Mexican transition zone, (ii) Brazilian subregion Mesoamerican dominion, (iii) Pacific dominion, (iv) Boreal Brazilian dominion, (v) South Brazilian dominion, (vi) Chacoan dominion, (vii) Parana dominion, and (viii) Chacoan subregion south-eastern Amazonian dominion (Morrone, 2014). Geographic distribution of *Ipsiura* species was obtained from the labels of the material examined and listed in detail by Lucena *et al*. (2016). We used the shapefiles of Morrone's areas (Löwenberg-Neto, 2014) to determine which areas were occupied by each species. We performed separate DIVA analyses for each of the eight equally most parsimonious trees found for *Ipsiura*. We did not constrain the number of areas that could be optimized at each node by DIVA and set the program to hold as many reconstructions as possible during the search (i.e. hold = 32 767). In addition to the classic DIVA, we also performed a 'statistical-DIVA' (s-DIVA) analysis in RASP (Yu *et al*., 2015) to integrate all DIVA biogeographic reconstructions for all most parsimonious tree topologies. Alternative area regionalization and coding was done by treating adjacent areas as one unit, in which case the coding of presence and absence of taxa was redone, and DIVA rerun.

Results

Morphological study

We coded 64 morphological characters based on a comparative study of the adult external morphology and terminalia, which was complemented by characters described by Bohart (1966, 1985), Kimsey (1985), Linsenmaier (1985) and Kimsey & Bohart (1991). Among those, 13 come from the head capsule, 13 from the mesosoma, 12 from the locomotory appendices, 19 from the metasoma and seven from the male genitalia (online Supplementary Materials: List of characters; Table S1 in File S1). All characters were parsimony-informative for our data matrix.

Ipsiura monophyly

The cladistic analysis with equal weights resulted in eight equally most parsimonious trees with GC = 53, thus well supported by the characters in this study. The strict consensus of the most parsimonious trees is shown in Figs 1 and 2. The cladistic analyses under equal and implied weights (all values of *K*) have unambiguously recovered *Ipsiura* as monophyletic and as the sister group of *Neochrysis* (Figs 1, 2, S4). The Bayesian analysis also recovered the same overall result with high support values (Fig. S6).

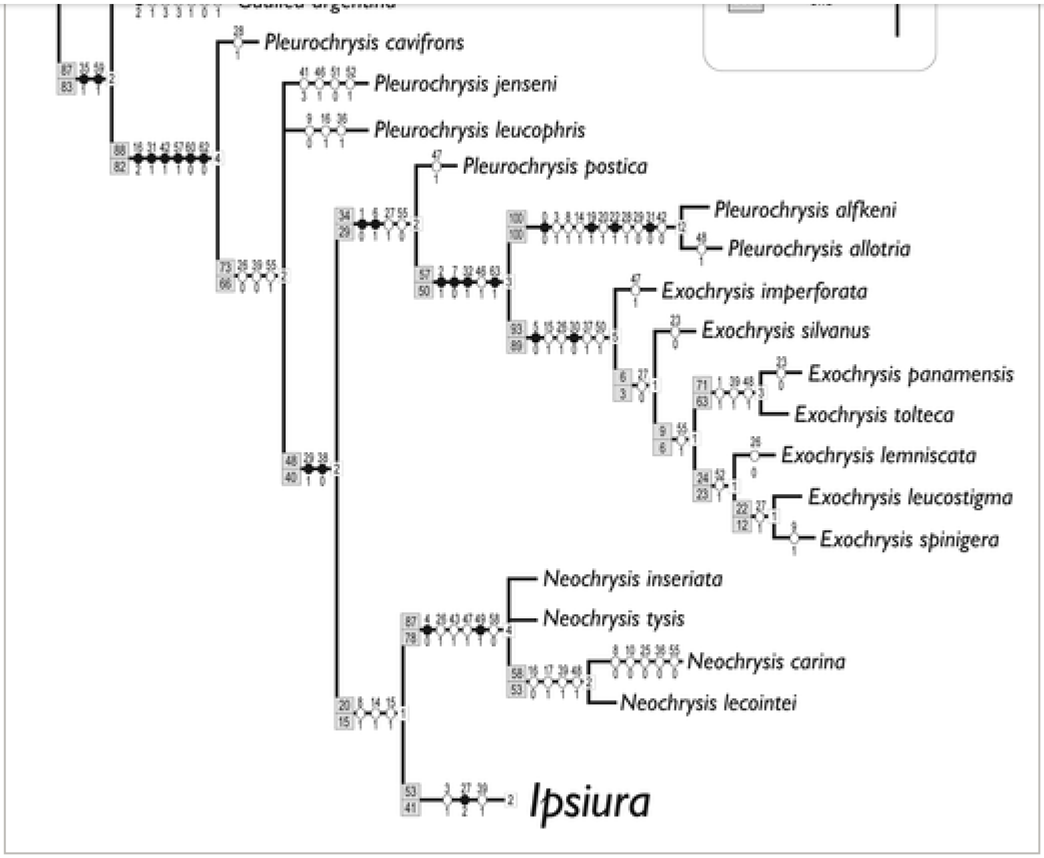

Figure 1

Open in figure viewer | PowerPoint

Strict consensus of the eight most parsimonious trees of 64 morphological characters with equal weights (L = 209, consistency index = 37, retention index = 78) for *Ipsiura* and other Neotropical Chrysidini taxa (Chrysididae). Node support estimated using GC frequencies (GC), bootstrap frequencies (Boot), and with Bremer values (BS). Character state transformations are depicted as solid (unequivocal changes) and empty (reversed or multiple changes) circles; only unambiguous transformations are shown. Phylogenetic relationships among *Ipsiura* species are shown in Fig. 2.

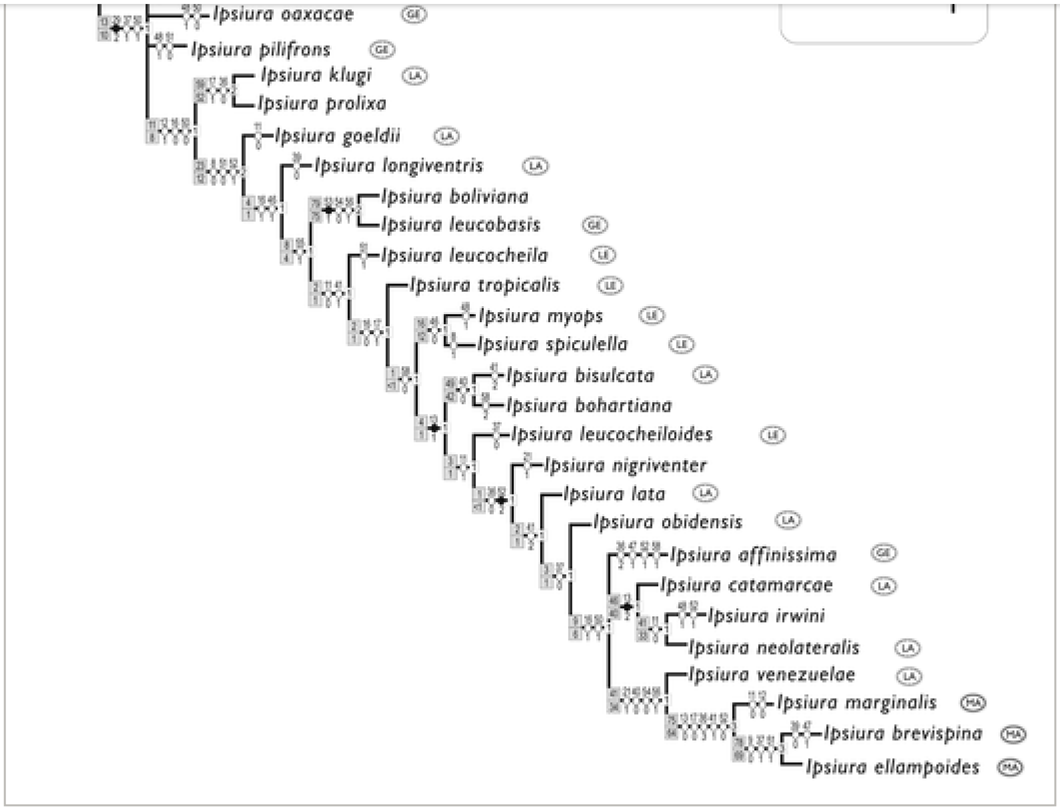

Figure 2

Open in figure viewer | PowerPoint

Strict consensus of the eight most parsimonious trees for the species of *Ipsiura* (Chrysididae: Chrysidini). Node support estimated using GC frequencies (GC), bootstrap frequencies (Boot), and with Bremer values (BS). Character state transformations are depicted as solid (unequivocal changes) and empty (reversed or multiple changes) circles; only unambiguous transformations are shown. Letters within circles indicate association of a species to one of Linsenmaier's species groups (GE, LA, LE, and MA refer to the *genbergi* group, *lateralis* group, *leucocheila* group and *marginalis* group, respectively); species not accompanied by a letter code were not part of this species group arrangement.

Ipsiura is supported by three character transformations, one of them unique to the group: the lateral margin of pronotum with strongly produced carina (state 27-2, Fig. 4O). This character has been recognized for a long time as a distinctive feature of the species in the genus (Linsenmaier, 1959, 1985; Bohart, 1966, 1985; Kimsey, 1985; Kimsey & Bohart, 1991). Other taxa (e.g. *Pleurochrysis alfkeni*, *Pleurochrysis allotria* and *Pleurochrysis postica*), as well as species of *Exochrysis*, have a sharp, somewhat produced, irregular carina on the lateral margins of pronotum (state 27-1, Fig. 4M, P), but distinctly arranged and herein interpreted as not the basolateral translucent margin on T2 (as in Fig. 8D), but it is interpreted as having been secondarily reversed in *I. brevispina* and *I. longiventris* (state 39-1). According to Kimsey & Bohart (1991: 278), *Ipsiura* is supported by two synapomorphies, the lateral pronotal carina and the exposed male S4 (Kimsey & Bohart, 1991: 278–281). Although the lateral pronotal carina is unique to *Ipsiura*, the male S4 largely exposed and forming a green metallic shield (states 43-0, 45-0) are interpreted as plesiomorphic and also observed in species of *Pleurochrysis*. In the most parsimonious scenarios (using equal or implied weights of characters), as well as using a likelihood framework under the Bayesian posterior probability criterion (with and without gamma), the general higher-level phylogenetic scenario recovered for the Neotropical Chrysidini was the same (Figs 1, S4, S6).

Species groups

Among the species groups recognized by Linsenmaier (1985), only the *marginalis* group was fully recovered in the analysis (Fig. 2, 11A–C). As originally proposed, this group included species that have indistinct or very obtuse distal teeth on T3 (Fig. 8S), elongated metanotum, and long *M* distal to the discoidal cell of the forewing (Fig. 7E). The *marginalis* group originally included the following species: *Neochrysis* (*Ipsiura*) *brevispina*, *Neochrysis* (*Ipsiura*) *ellampoides* and *Neochrysis* (*Ipsiura*) *marginalis*. Subsequently, Linsenmaier (1997) also described *Neochrysis* (*Ipsiura*) *surinamensis* in the *marginalis* group. This species group is the one clade to have numerous distinct synapomorphies compared with the other groups in *Ipsiura* (Fig. 2). A close phylogenetic relationship is proposed between this clade and the T3 six-toothed species *I. venezuelae* (=*schlaeflei*). Linsenmaier (1985) treated the latter species as related to the *lateralis* group (=*lata*), based on the number of distal teeth on T3. The clade *I. venezuelae* + (*I. marginalis* + (*I. brevispina* + *I. ellampoides*)) form a distinct group, supported by four unequivocal character transformations (states 21-1; 40-0; 54-0; 56-1). However, it is not clear to which *Ipsiura* taxa they are closely related phylogenetically, as shown in the strict consensus tree (Fig. 2).

Species in the *marginalis* group share with the clade *I. leucobasis* + *I. boliviana*, a heavily sclerotized female T6 and serrated T5, which form part of the 'ovipositor tube' (Fig. 9F–I) (states 54-0 and 56-1). Bohart (1985) suggested these traits would be associated with the life-history strategies for piercing and boring nests. Under implied weights of characters (*K*'s intervals = 1–7), the cladistic analyses have recovered a close association of the *marginalis* group with *I. leucobasis* + *I. boliviana* supported by these character states (Fig. S4). Interestingly, character 56 has many missing entries, which could influence the implied weight of characters analysis by inflation of weights (because they necessarily display less homoplasy) (Goloboff, 2014). However, it does not hold true for the state 54-0 (see Table S1 in File S1). While state 56-1 is nonrelated Chrysidini groups (such as some species of *Chrysis* and *Stilbum cyanurum*), which can be interpreted as a form of convergence probably due to a similar biology. Hosts of *I. boliviana*, *I. leucobasis*, and of *I. brevispina*, *I. ellampoides*, *I. marginalis* and *I. venezuelae* are still unknown. Under all *K*-values used in implied weights analyses, as well as with Bayesian inference, we have recovered the same general pattern found under equal weights parsimony, and fewer conflicting points (Figs 2, S4, S6).

Using a hypothetical phylogenetic tree that represents the species groups of Linsenmaier (1985), many of the nodes are not supported by the characters proposed in this study (Fig. 11A). Our cladistic hypothesis convincingly shows that these character states are highly homoplasious, and they fail to support the recognition of these species groups as natural groups. The *genbergi* group was partially recovered in a polytomy at the base of the *Ipsiura* clade (Fig. 2). The *genbergi* group had been proposed by Linsenmaier (1985: 474) based on the character states of the hidden pit row, and the small and well-separated S2 spots. Some of these species are indeed closer to each other phylogenetically in comparison to any other *Ipsiura* species, but their interrelationships remain unresolved (see Fig. 2, *I. frieseana*, *I. genbergi*, *I. lilloi*, *I. oaxacae*, and *I. pilifrons*). The *leucocheila* and *lateralis* (=*lata*) species groups of Linsenmaier (1985) were not even partially recovered (Fig. 11A–B). As initially proposed, the *leucocheila* group would be supported primarily by the linear gena, round metanotum, four apical teeth on T3 and round S2 spots (Linsenmaier, 1985: 468). According to Linsenmaier (1985), the *lateralis* group included species with a short *M* vein distal to the discoidal cell, narrow gena, lateral whitish spots on metasoma, and a well-developed transverse frontal carina.

Biogeography

Most species of *Ipsiura* have widespread distributions (see detailed maps by Lucena *et al*., 2016), being distributed in more than one of the areas herein considered (Fig. 12). There are also multiple cases of redundant distributions (areas harbouring more than one taxon, i.e. sympatric taxa), and it is known that redundant areas and the widespread taxa are responsible for making historical biogeographical reconstructions challenging (Nelson & Platnick, 1981; Page, 1988). Although event-based methods are less sensitive to redundancies (e.g. Sanmartín & Ronquist, 2002) than to traditional cladistic biogeographical approaches (e.g. Page, 1988), widely distributed taxa are a problem. In our case, the number and extent of widespread taxa were so pervasive that not even DIVA was able to generate decisive reconstructions. When running DIVA with the individual eight most parsimonious trees, there were > 25 000 equally likely reconstructions per tree (the simplest result consisted of 25 680 optimal ancestral were run with alternative area coding, considering more extensive areas as units, the outcome was even more ambiguous for all nodes in the tree (results not shown).

Discussion

Species groups in *Ipsiura*

Linsenmaier's species groups were established based on overall morphological similarities among species. He gave particular importance to the transverse frontal carina (character 08), lateral facial carinulae (character 12), and metasomal characters, such as the number of distal teeth on T3 (character 41), the pit row (character 47) and the size of the S2 spots (character 52). However, these morphological characters are now shown to have greater plasticity than anticipated, with numerous transformations, especially the metasomatic ones (character 08, L = 7, CI = 14, RI = 64, character 12, L = 3, CI = 33, RI = 71; character 41, L = 9, CI = 33, RI = 72; character 47, L = 7, CI = 14, RI = 33; and character 52, L = 8, CI = 25, RI = 72). Typically, taxonomic diagnoses of Chrysidini as a whole are largely based on metasomal traits, such as the number of distal teeth on T3 (character 41); presence/absence and development of the pit row (character 47); and presence/absence of the lateral whitish spot on T3 (character 48) (Kimsey & Bohart, 1991: 34). Phylogenetic relationships among *Ipsiura* species based mostly on these traits were not recovered in our analyses. Metasomal characters are still useful for species recognition and assembly of identification keys (e.g. Lucena *et al*., 2016), but they need to be evaluated with caution and in consonance with other characters in the context of phylogenetic discussions.

The species groups of Linsenmaier (1985) reflect the effort to improve the taxonomy of *Ipsiura*. However, most groups are now reinterpreted as being artificial assemblages, and our analyses have contributed in demonstrate how plastic some of the morphological characters are in a cladistic perspective (Fig. 11A–B). The scenario for the interpretation of morphological evolution within *Ipsiura* became considerably more complicated than in the previous views, which were based almost exclusively on the number of distal teeth of metasoma.

Phylogenetic relationships within *Ipsiura* and morphological transitions

Some of the metasomatic characters seem to change very quickly in the phylogeny. The pit row for example, has great variability in development and arrangements that make it difficult to code all possible states. Therefore, we chose to circumscribe variation in pit rows in a simpler arrangement, considering presence/absence only, even though this resulted in seven this case (Fig. 8E–S), despite the assumed functional importance of metasomal traits and presumed constraints in their morphological variation (e.g. Kimsey & Bohart, 1991; Yamada, 1991; Niehuis & Wägele, 2004).

A major group within *Ipsiura* includes most of the species in the genus, except *I. cooperi*, *I. covillei* and *I. obidana*, and it is well supported by an unequivocal character, the width of the pronotum clearly exceeding the width of the head (state 29-2, Fig. 7C, D, see also Fig. S3C). Two other characters, although nonexclusive, also support this clade (Fig. 2). Important morphological changes have occurred within the group, which includes transformations that occurred at least twice on the fore femur. Many species have a distinct carina ventrally on the middle of fore femur (state 13-1), which resembles a flattened area on outer surface (Fig. 4R, U). This character state is found with some degree of variation in *I. bisulcata*, *I. bohartiana*, *I. leucocheiloides*, *I. nigriventer*, *I. lata*, *I. obidensis*, *I. affinissima*, and *I. venezuelae*. Other species have an alternative state of this character, where the ventral carina protrudes ventrally forming a 'tooth' distally, as in *I. catamarcae* and *I. neolateralis* (and in some specimens of *I. irwini*) (state 13-2, Fig. 4S,V). Despite substantial advances, there are persistent zones of topological uncertainty in the phylogenetic relationships within the clade *Ipsiura*, especially those concerning the placement of *marginalis* group and the monophyly and interrelationships of species which compose the *genbergi* group (see Figs 2, S4, S6).

Higher-level phylogenetic relationships in Chrysidini

A sister-group relationship between *Ipsiura* and the *Neochrysis* was recovered for the first time. Previously, Kimsey & Bohart (1991)'s phylogenetic hypothesis had placed *Ipsiura* as sister to *Exochrysis*, based on the facial carina, having prominent subsidiary carinules that extend from the TFC around the median ocellus forming an arc ('MOC'). Several intermediate character states contradict the previous importance given to this character which are present in taxa outside the *Ipsiura* + *Exochrysis* [e.g. *Neochrysis carina* (Fig. 4D), *Pleurochrysis cavifrons*, *P. jenseni* (Fig. 4E), and *P. leucophris* (Fig. 4F)]. In our analysis, the morphological variation in MOC was reinterpreted in characters 08–12, which do not support the close relationship between *Exochrysis* and *Ipsiura*. The clade *Ipsiura* + *Neochrysis* is supported by three unambiguous characters. Two of them involve the presence of micropores set in deep foveae on the proximal area of the fore and hind femora (e.g. Fig. 5B, C). Character 15-1, hind femur with basal foveae (Fig. 5F–K), had been indicated by Kimsey (1985) as a putative synapomorphy shared by *Exochrysis*, *Ipsiura* and *Neochrysis* (see also Kimsey & Bohart, 1991: 278–280). Character states 14-1 and 15-1 are shared by the clade (*Neochrysis* + *Ipsiura*), although they are also found independently and alternately in (*Pleurochrysis alfkeni* + *P. allotria*) (sharing the state 14-1) and assumed to form a clade based on similarities of forewing venation (state 18-1, Fig. 6C–J), propodeal enclosure, shape of the propodeal angles (state 31-1) and presence of whitish maculations (state 39-0, state 48-0; Kimsey & Bohart, 1991). Indeed, the Rs of the forewing and the propodeal angles are very distinctive characters for those taxa, although only the propodeal angles were informative to support the clade (Fig. 1). Characters from the male genital capsule also support a shared phylogenetic ancestry among taxa of the *Neochrysis* lineage. In these taxa, the cupula is strongly produced ventrally (state 60-0) and the position of the foramen genitale is perpendicular to the elements of the capsule (state 62-0). This peculiar character combination suggests a rotation of 180° of the genital capsule when everted, a morphological condition not previously documented in any other Chrysidini.

Another major group composed by (*P. postica* + ((*P. alfkeni* + *P. allotria*) + (*Exochrysis*))) is well supported based on three unequivocal characters: the relative position of the tentorial fovea (state 06-1, Fig. 3A–C); the strongly convergent inner ocular margins (state 01-0, Fig. S1); and the sharp lateral margin of pronotum (state 27-1, Fig. 4M, P). *Exochrysis* is undoubtedly well supported by six unequivocal characters (states 05-0; 15-1; 26-1; 30-0; 37-1; 50-1, see 'List of characters') and it is sister group of the clade *P. alfkeni* + *P. allotria*. This finding sheds light on the most controversial groups of the Neotropical Chrysidini. *Pleurochrysis* Bohart, 1966 has been for a long time assumed to be an artificial assemblage (Kimsey, 1985; Kimsey & Bohart, 1991), and our analysis reinforced such views. Some lineages of *Pleurochrysis* show a close relationship to well-established genera such as *Ipsiura*, *Exochrysis* and *Neochrysis*. In this scenario, the enigmatic '*alfkeni*-complex' has been problematic for a long time (Ducke, 1902; Kimsey, 1985; Linsenmaier, 1985; Kimsey & Bohart, 1991). *Pleurochrysis alfkeni* (Ducke), *P. allotria* (Linsenmaier) and *P. adnexa* (Linsenmaier) form a complex of species very similar to each other morphologically, but with no clear phylogenetic relationships with other groups of Chrysidini. These uncertainties led to a complex taxonomic history, starting with the placement of *Chrysogona alfkeni* Ducke (1902) into *Pleurochrysis* (Kimsey, 1985). Subsequently, Linsenmaier (1985) described two new species related to this complex and proposed the subgenus *Neochrysis* (*Exsecochrysis*) to house these species. He designated *N.* (*Exs.*) *gracilia* as the type and moved *C. alfkeni* (Ducke) to *N.* (*Exs.*). Kimsey & Bohart (1991: 523) synonymized *N.* (*Exs.*) *gracilia* under *P. alfkeni* (Ducke) and moved the species described by Linsenmaier (1985) to *Pleurochrysis*. Similarities on the male genitalia now confirm the close phylogenetic affinity of the '*alfkeni*-complex' with the major Neotropical Chrysidini clade, more specifically with *Exochrysis*. Characters supporting this affinity include the characteristic long slender elements of the male genitalia (as in the Fig. 10C), the ventrally produced cupula (as in the Fig. 10B, D), and the perpendicular position of the foramen genitale (Fig. 10D). Additionally, this clade (*P. allotria* + *P. alfkeni*) has controversial morphology, comprising the substantial reduction of the irregular carina (state 28-1, Fig. 4P), and lack of the facial carinae (states 08-1 and 10-1). Currently, a closer phylogenetic relationship with *Exochrysis* is better supported by the morphological evidence, mainly based on the unusual microstructures of the genal fovea (Fig. 3J–O). These species form a clade and need to be comprehensively revised and have their classification improved in future taxonomic works. Despite the weight of evidence pointing to *Pleurochrysis* being an artificial assemblage of species, a revised classification to improve the treatment of this genus and consequences to Chrysidini as a whole cannot be proposed as yet. Additional Chrysidini taxa not yet studied phylogenetically will undoubtedly help to improve the systematics of *Pleurochrysis* in the future.

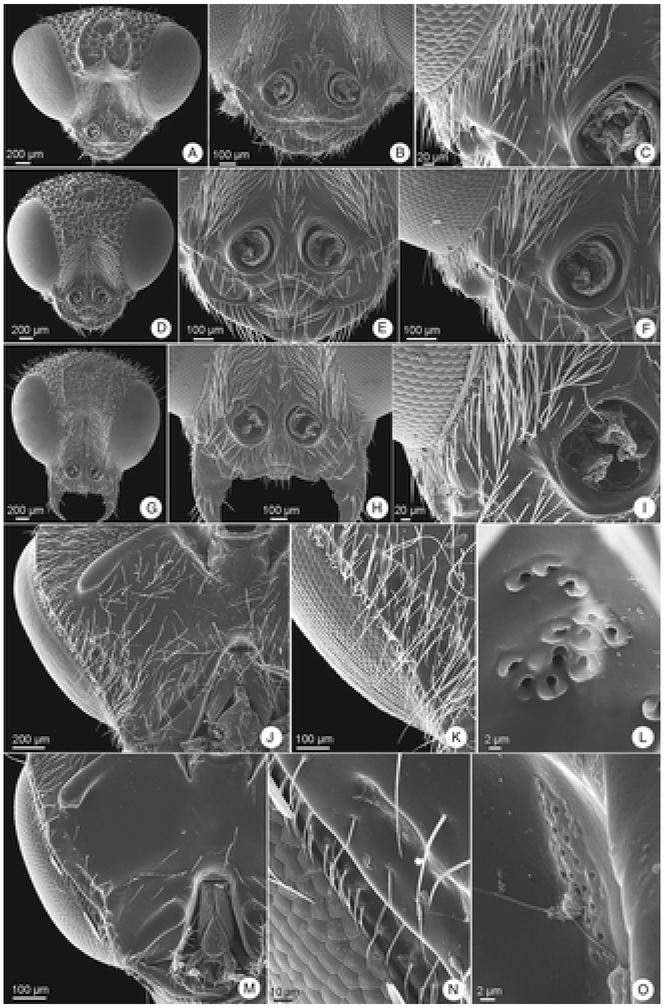

Figure 3

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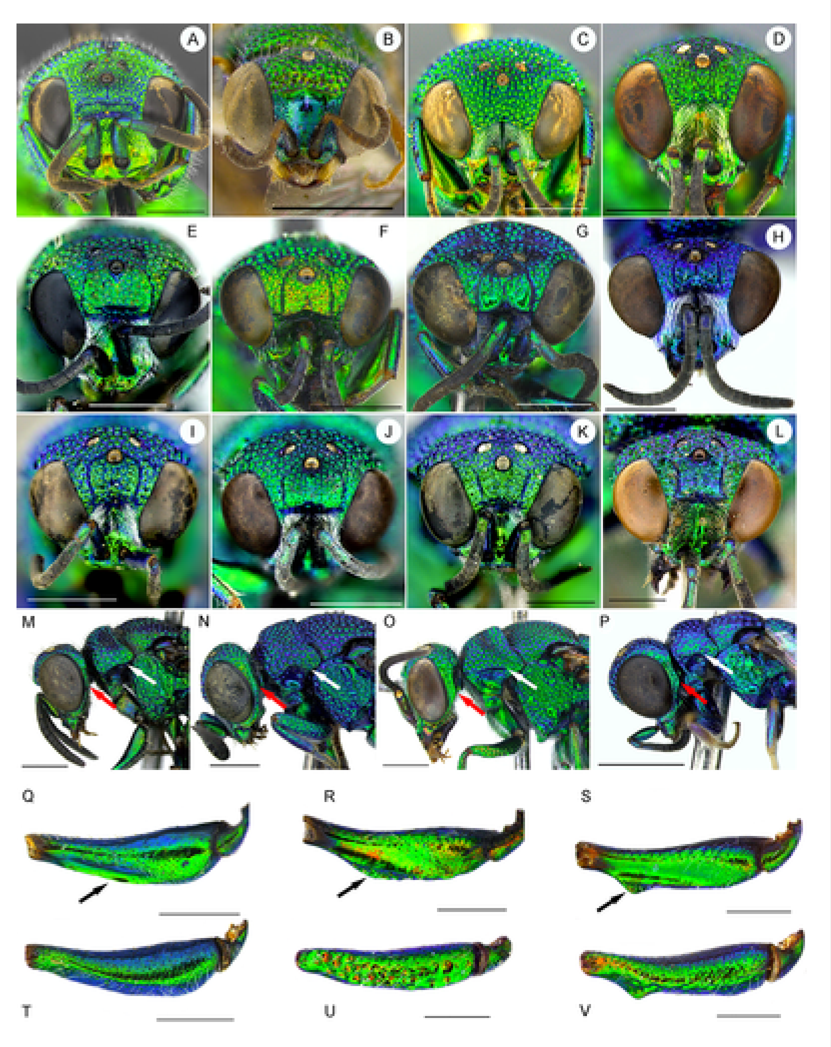

Figure 4

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Heads (frontal view), lateral habitus, and inner and outer surfaces of fore femora. (A) *Chrysis intricata*; (B) *Gaullea argentina*; (C) *Neochrysis lecointei*; (D) *Neochrysis carina*; (E) *Pleurochrysis jenseni*; (F) *Pleurochrysis leucophris*; (G) *Exochrysis imperforata*; (H) *Exochrysis leucostigma*; (I) *Ipsiura oaxacae*; (J) *Ipsiura obidensis*; (K) *Ipsiura bohartiana*; (L) *Ipsiura marginalis*; (M) *Exochrysis leucostigma*; (N) *Neochrysis lecointei*; (O) *Ipsiura lata*; (P) *Pleurochrysis allotria*; (Q) *Ipsiura lilloi*, inner; (R) *I. lata*, inner; (S) *Ipsiura neolateralis*, inner; (T) *I. lilloi*, outer; (U) *I. lata*, outer; (V) *I. neolateralis*, outer. (A–P) scale bars 1 mm. (Q–V) scale bars: 0.5 mm. [Colour figure can be viewed at wileyonlinelibrary.com.]

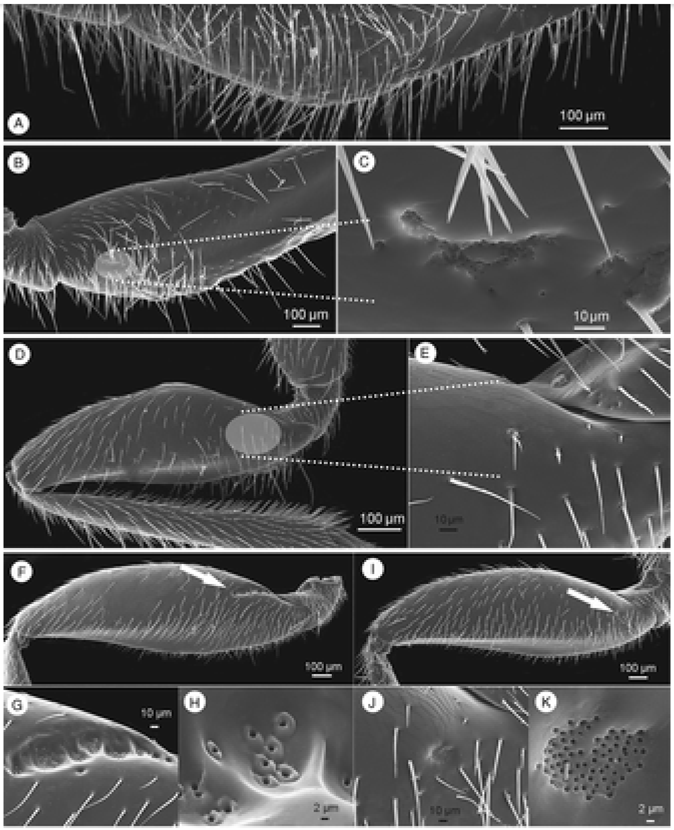

Figure 5

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Legs (scanning electron microscopy images). (A) *Exochrysis leucostigma*, fore femur, ventral view; (B, C) *Ipsiura lata*, fore femur, ventral view, enlarged; (D, E) *Pleurochrysis allotria*, hind femur, ventral view, enlarged; (F–H) *Exochrysis leucostigma*, hind femur, ventral view; arrow indicates enlarged areas; (I–K) *Ipsiura myops*, hind femur, ventral view, arrow indicates areas in higher magnification.

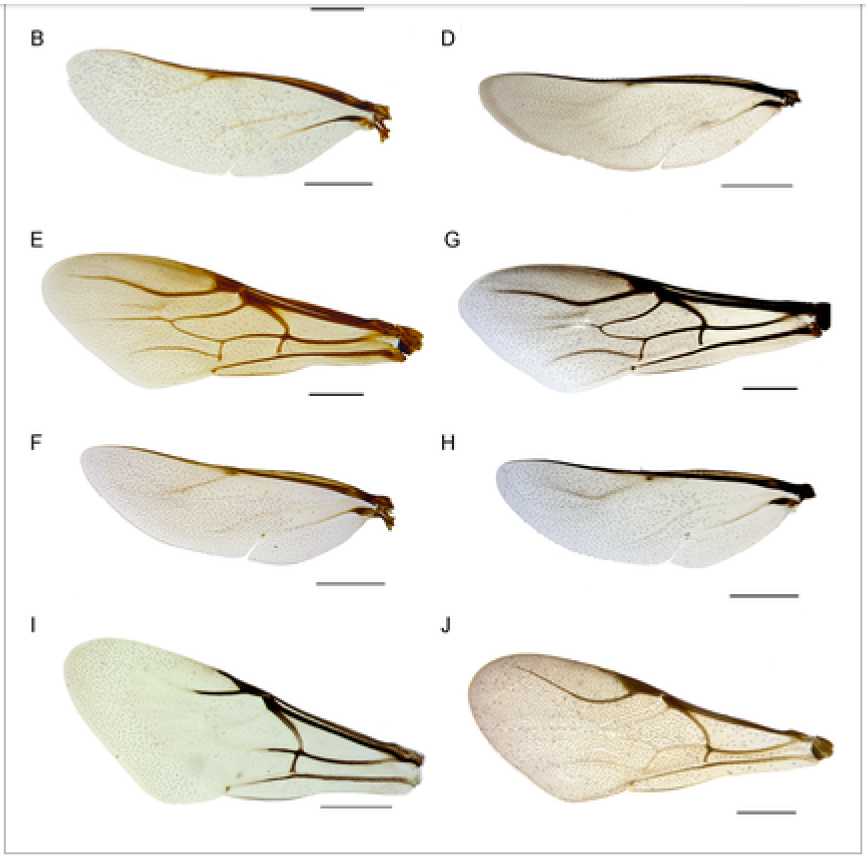

Figure 6

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Fore- and hindwings. (A, B) *Chrysis excavata*; (C, D) *Exochrysis leucostigma*; (E, F) *Ipsiura marginalis*; (G, H) *Neochrysis carina*; (I) *Gaullea argentina*, forewing; (J) *Pleurochrysis allotria*, forewing. Scale bars: 0.5 mm. [Colour figure can be viewed at wileyonlinelibrary.com.]

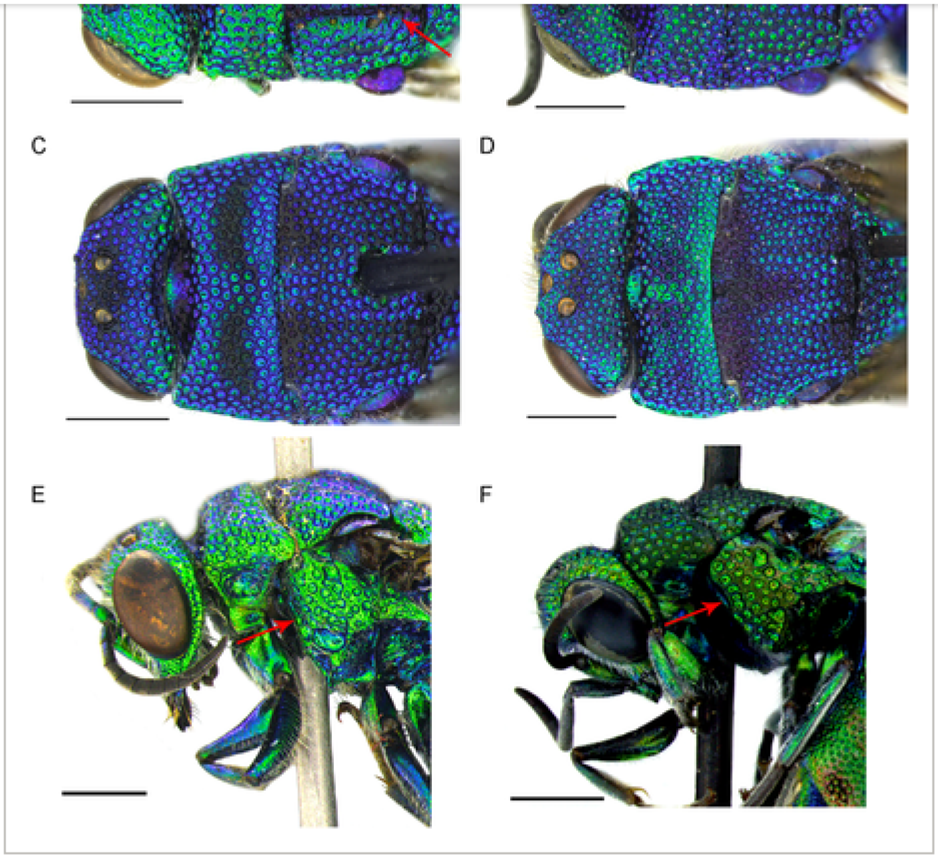

Figure 7

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Dorsal view and lateral habitus. (A) *Pleurochrysis cavifrons*; arrow indicates the marked mesoscutum notauli; (B) *Neochrysis lecointei*; (C) *Ipsiura lata*; (D) *Ipsiura leucobasis*; (E) *Chrysis excavata*; arrow indicates the scrobal sulcus; (F) *P. jenseni*; arrow indicates the scrobal sulcus. Scale bars: 1 mm. [Colour figure can be viewed at wileyonlinelibrary.com.]

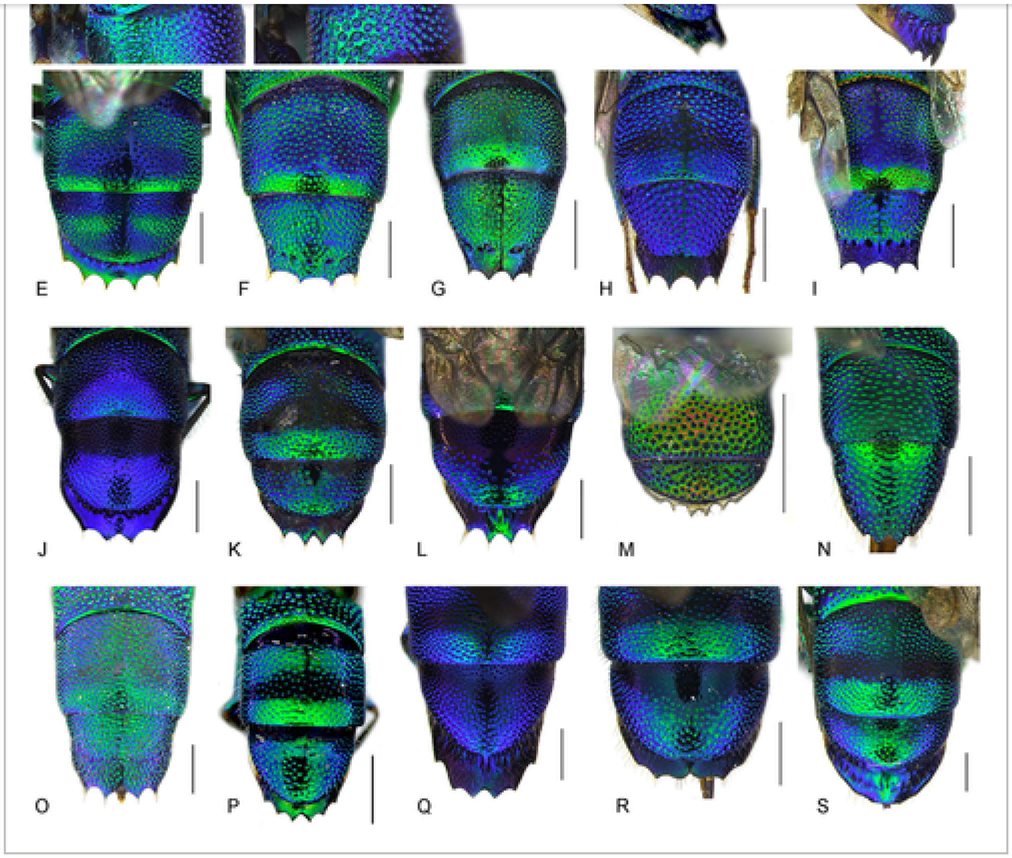

Figure 8

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Lateral views of T1–T3, and postero-dorsal views of T3. (A) *Neochrysis tysis*, T1; (B) *Exochrysis leucostigma*, T1; (C) *Pleurochrysis postica*, T2; (D) *Ipsiura neolateralis*, T2; (E) *Chrysis intricata*, T3; (F) *Pleurochrysis morosa*, T3; (G) *Pleurochrysis leucophris*, T3; (H) *Pleurochrysis postica*, T3; (I) *Pleurochrysis cavifrons*, T3; (J) *Exochrysis panamensis*, T3; (K) *Exochrysis imperforata*, T3; (L) *Exochrysis leucostigma*, T3; (M) *Gaullea argentina*, T3; (N) *Neochrysis inseriata*, T3; (O) *Neochrysis lecointei*, T3; (P) *Ipsiura obidana*, T3; (Q) *Ipsiura klugi*, T3; (R) *Ipsiura leucobasis*, T3; (S) *Ipsiura marginalis*, T3. Scale bars 1 mm. [Colour figure can be viewed at wileyonlinelibrary.com].

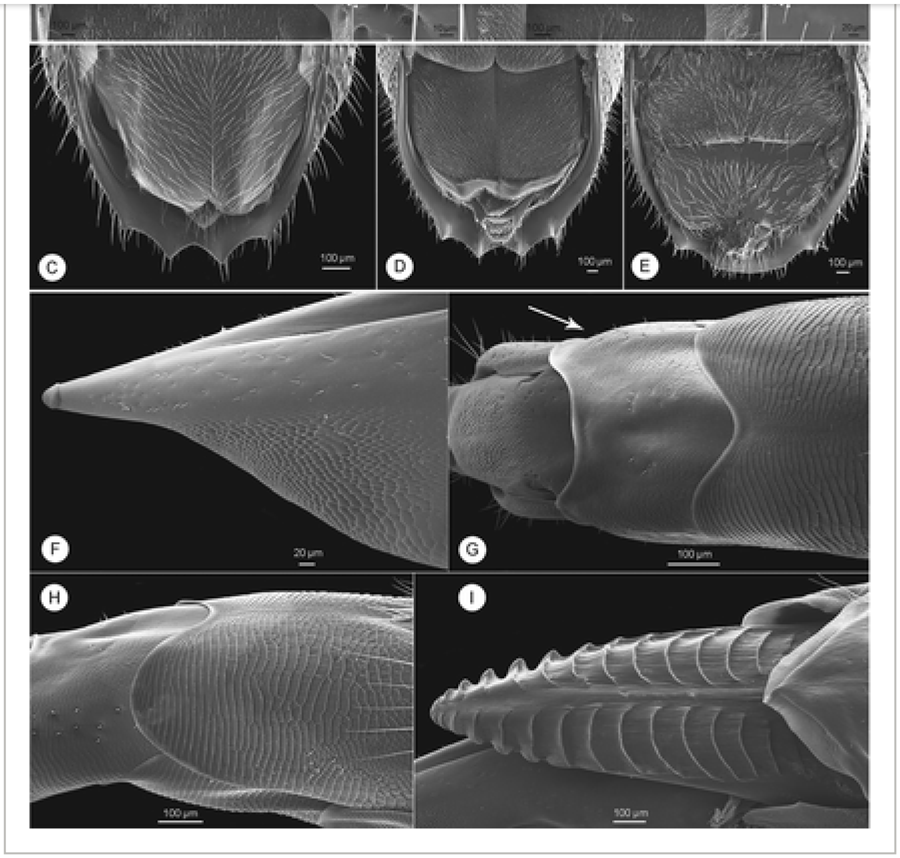

Figure 9

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Female S1, male S3–S4 and female T5–T6 (SEM images). (A) *Ipsiura lata*, female S1 foveae, ventral view, amplified on right; (B) *Pleurochrysis cavifrons*, female S1,ventral view; amplified view is shown on the right; (C) *Pleurochrysis allotria*, male S3, ventral view; (D) *Exochrysis leucostigma*, male S3–S4, ventral view; (E) *Ipsiura myops*, male S3–S4, ventral view; (F) *Ipsiura marginalis*, female T6, lateral view; (G) *I. lata*, female T6, dorsal view, indicated by the arrow; (H) *I. lata*, female T5, lateral view; (I) *I. marginalis*, female T5, lateral view.

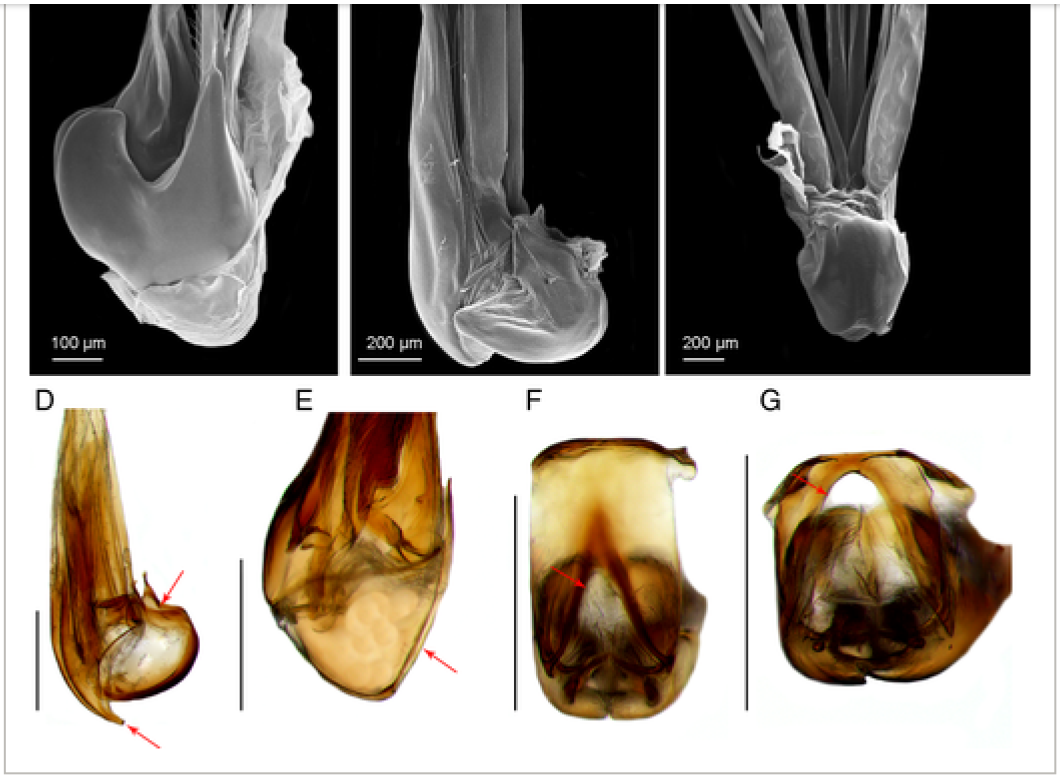

Figure 10

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Male genital capsules. (A) *Caenochrysis nigropolita*, lateral view; asterisk indicates the ventral surface [scanning electron microscope (SEM)]; (B) *Ipsiura frieseana*, lateral view, asterisk indicates the ventral surface (SEM); (C) *Neochrysis carina*, ventral view (SEM); (D) *Exochrysis leucostigma*, lateral view; arrows indicate the ventral process of paramere (below) and the relative position of foramen genitale (above); (E) *Chrysis excavata*, lateral view; arrow indicate the relative position of foramen genitale; (F) *Pleurochrysis postica*, cupula, ventral view; arrow indicates the medial furrow; (G) *Exochrysis imperforata*, cupula, ventral view; arrow indicates the medial furrow. (D–G) Scale bars: 0.5 mm. [Colour figure can be viewed at wileyonlinelibrary.com.]

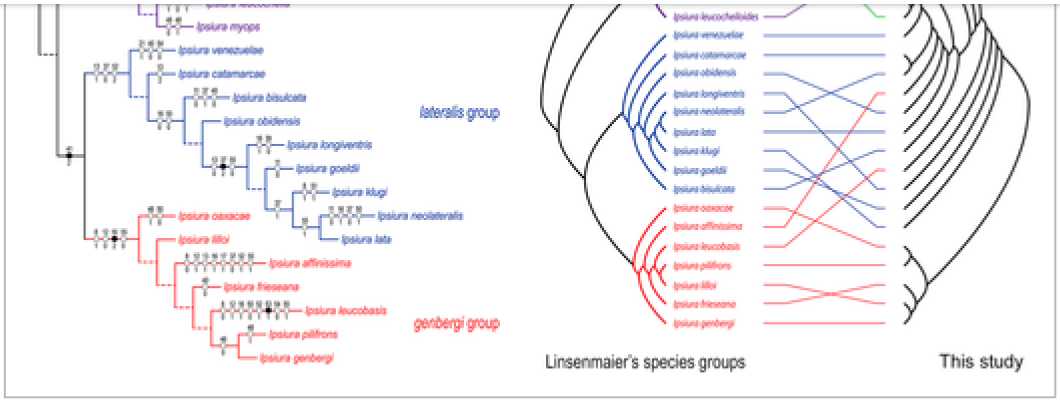

Figure 11

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Tree reconciliation. (A) Morphological characters optimized onto a hypothetical tree representing Linsenmaier's views on species and species groups affinities within *Ipsiura* [L = 108, consistency index (CI) = 27, retention index (RI) = 42]. Character state transformations are depicted as solid (unequivocal changes) and empty (reversed or multiple changes) circles; only unambiguous transformations are shown. Unsupported nodes are depicted as dashed lines. (B) Tanglegram comparison between Linsenmaier's species groups (L = 108, CI = 27, RI = 42) and the results found in this study (L = 69, CI = 43, RI = 71). Taxonomic sampling was adjusted in both trees to allow for a match between them. [Colour figure can be viewed at wileyonlinelibrary.com.]

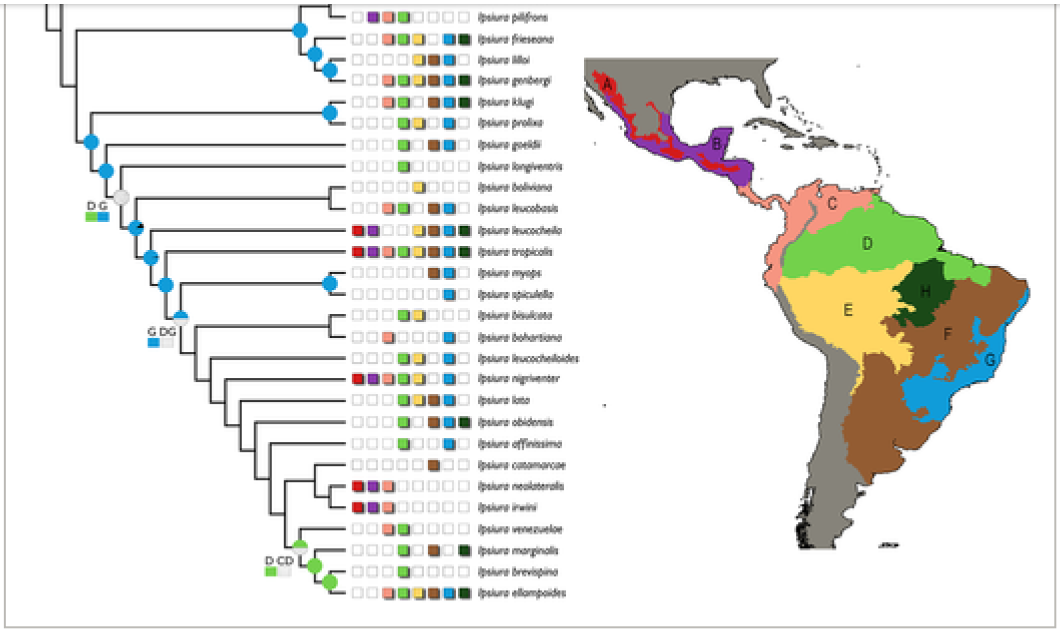

Figure 12

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Majority consensus calculated for the *Ipsiura* and distribution of species in biogeographic divisions of the Neotropical region. The right charts on the tree are colour-coded to the areas illustrated in the map and imply the occurrence of species in each area. Dispersal-vicariance analysis returned multiple biogeographical reconstructions for most nodes in the tree, but in 15 cases where one or two reconstructions resulted as the most parsimonious, charts depict them. Black slices represent a fraction of the reconstructions with high ambiguity; stippled charts denote hybrid area reconstructions. [Colour figure can be viewed at wileyonlinelibrary.com.]

Historical biogeography

The currently known distribution of *Ipsiura* species indicates how complicated their history must have been from a biogeographical viewpoint. Among the 32 *Ipsiura* considered, as many as 29 occur in more than one of the units considered for the Neotropical region (Fig. 12). In addition to the widespread species, redundant distributions contribute to making the biogeographical interpretation challenging. It is not surprising, given this scenario, that the reconstruction of the majority of the nodes was too ambiguous, and that the hypothesis yielded by DIVA drastically departs from a well-behaved hierarchical history. Despite that, DIVA indicated that at least part of the diversification of *Ipsiura* took place in association with areas presently covered with forested environments: the domains of Atlantic and Amazonian forests.

Exochrysis, *Neochrysis* and *Pleurochrysis* have similar distributional patterns (Kimsey & Bohart, 1991: 503, 514–515, 524–528).

Recently, Lucena *et al*. (2016) had provided new records for 26 of the 32 species of *Ipsiura*. Sampling biases could, nonetheless, at least partly explain the distributional configuration of taxa through history, because knowledge about other biological attributes (i.e. hosts, environmental tolerances, preferences for nest sites) is unavailable or limited for all *Ipsiura* species. An interesting indication of this bias is exemplified by areas D, E and G, which were those that were more extensively sampled by Adolpho Ducke in the early 1900s: 28 species of *Ipsiura* are recorded at least in one of these areas (Fig. 12).

Conclusions

Despite the plasticity found in many of the characters coded for the phylogenetic analyses, the results were mostly congruent using a wide scope of optimality criteria. This study dissolves some artificial species groups in the genus, and we clarified some aspects on the morphological evolution of the *Ipsiura* clade. Therefore, it became clear that the interpretation of the morphological characters, as well as the diversification of *Ipsiura* in the Neotropical region are more complex than initially thought. Our results also provide a phylogenetic basis for future taxonomic revisions with some still problematic taxa of the Neotropical Chrysidini (e.g. *Pleurochrysis*), and it will useful for investigations aiming for a better understanding of the evolution of the cleptoparasitic biology.

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References

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