

Biology and phylogenetic placement of a new species of *Lasiokapala* Ashmead from Argentina (Hymenoptera: Eucharitidae)

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Abstract. Within the ant-parasitic wasp family Eucharitidae (Hymenoptera), the Kapala clade is a monophyletic group attacking Ectatomminae and Ponerinae. Members often express extreme phenotypic features, especially in the morphology of the paired frenal spines. Although the means of attack and developmental history of the eucharitid wasps within the ant nest are very similar, the means by which they oviposit and optimize encounters of their active first-instar larvae with ants is highly variable. The relationships and life-history strategies of *Lasiokapala* Ashmead (Hymenoptera: Eucharitidae) and related taxa within the Kapala clade are discussed based on phylogenetic analyses of morphological and molecular data. Descriptions are provided for the adults (both sexes), eggs and planidia of *Lasiokapala spiralicornis* sp.n. from Santiago del Estero (Argentina). Females deposit their eggs on the underside of leaves of *Sida cordifolia* L. (Malvaceae) and the likely host is postulated to be the genus *Ectatomma* (Formicidae: Ectatomminae). Even within a closely related group of genera, there is extreme independent divergence in morphology of scutellar spines, antennae and other features, but the larvae and larval biology are highly conserved across a much larger group of Eucharitidae.

This published work has been registered in ZooBank, <http://zoobank.org/urn:lsid:zoobank.org:pub:3536985B-D193-4771-BFA3-25A59EFF6AB5>.

Introduction

Eucharitidae (Hymenoptera: Chalcidoidea) are parasitoids of the immature stages of ants (Heraty, 2002). Females lay their eggs inside or on plant tissues (leaf buds, floral buds, underside of leaf or into fruits), either individually or in masses (Clausen, 1940). Deposited remotely from the host, it is the active first-instar larva (planidium) that is responsible for getting into the ant nest through various associations with foraging adult ants (Torréns, 2013). Once in contact with the larval ant host, the planidium either remains as an external parasite or partially burrows into the host (Heraty & Murray, 2013). After development on the host pupa, the adults must emerge and leave the nest (Clausen, 1923). Several species of the genus *Kapala*

(Eucharitini) have been observed being carried by the ants from the nest and then deposited in the accumulation of colony waste (Pérez-Lachaud *et al.*, 2006a; Buys *et al.*, 2010). Lachaud & Pérez-Lachaud (2012) and Pérez-Lachaud *et al.* (2015, cf. Video S1) further documented how *Ectatomma* pick up and transport adults of *Kapala* (related members within Eucharitini) using the paired long spines that extend from the posterior margin of the scutellum (Figs 1A–C, 2A–C). Given the aggressive behaviour of these ants, the spines may protect the wings during specimen transport out of the nest, and might account for their independent development in different Eucharitini (Heraty *et al.*, 2015).

Four subfamilies and 56 genera are recognized that are distributed in almost every zoogeographical region of the world (Heraty, 2002; Heraty *et al.*, 2004; Torrén & Heraty, 2013). *Lasiokapala* was first proposed by Ashmead (1899) with the type species, *L. serrata*, described later by Ashmead (1904). Heraty (2002) included *Lasiokapala* within the Kapala clade,

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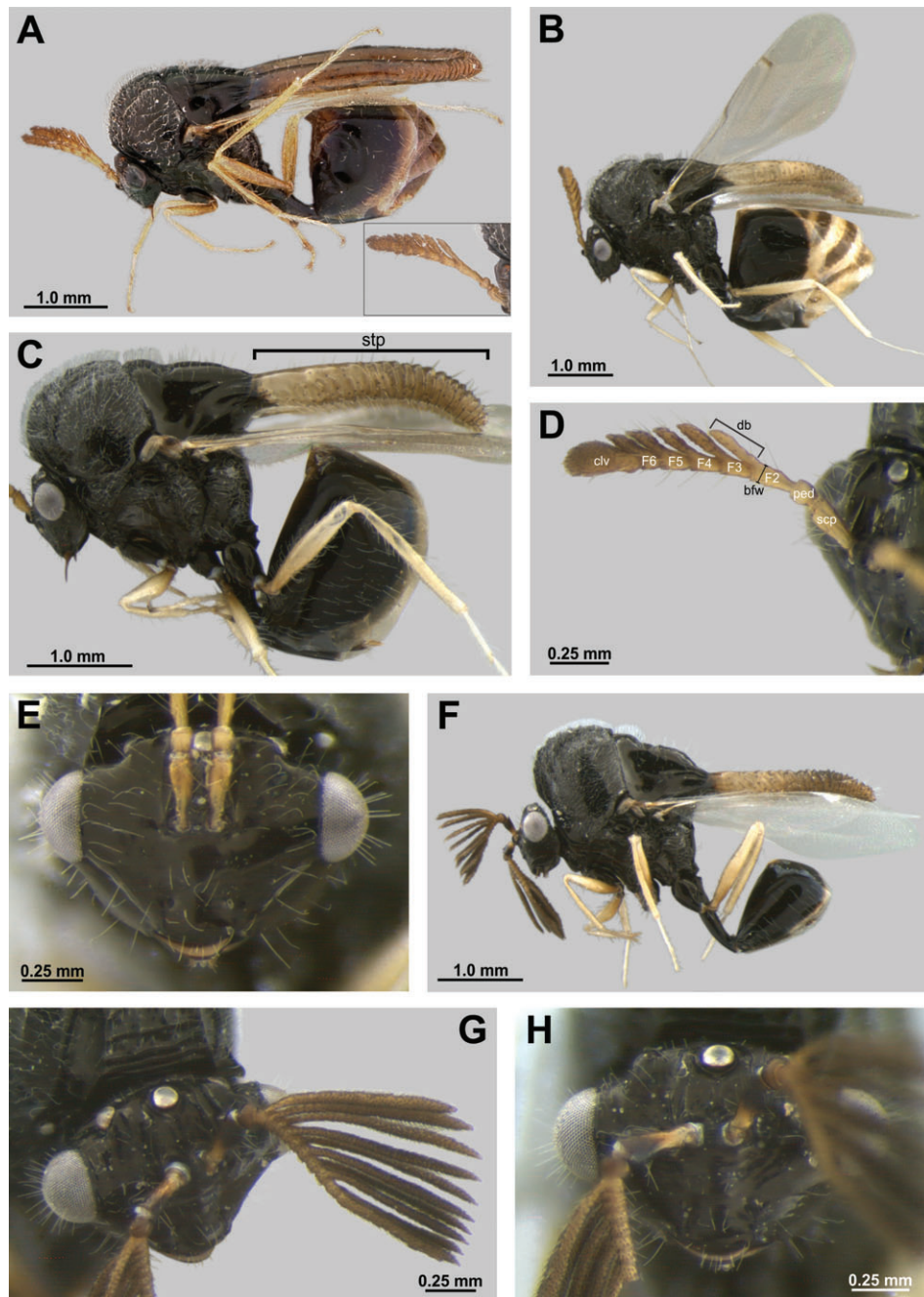


Fig. 1. *Lasiokapala* spp. (A) *Lasiokapala serrata*, habitus (female, antenna magnified); (B) *L. spiralicornis* sp.n., habitus (female); (C) *L. spiralicornis*, mesosoma (female, lateral); (D) *L. spiralicornis*, antenna (female, sublateral); (E) head (female, frontal); (F) habitus (male); (G) antenna and head (male, sublateral); (H) head (male, frontal). Abbreviations: db, dorsal branch; bfw, flagellomere basal width; clv, clava; F2–6, flagellomeres; ped, pedicel; scp, scape; stp, scutellar process.

which is restricted largely to the Neotropics and southern Nearctic regions and defined largely by the long parallel mesoscutellar (frenal) spines. Species within the Kapala clade are known to attack ant genera in the Ectatomminae and Ponerinae (Heraty, 2002; Lachaud & Pérez-Lachaud, 2012), and are known to oviposit into developing flower buds or on the abaxial

surface of leaves (Clausen, 1940; Heraty & Darling, 1984; Torrén, 2013).

Lasiokapala was based only on the type species and thus far is known only from Brazil, with no information on the host ant, host plant or the male. In the morphological phylogenetic reconstructions of Heraty (2002), *Lasiokapala* was recovered

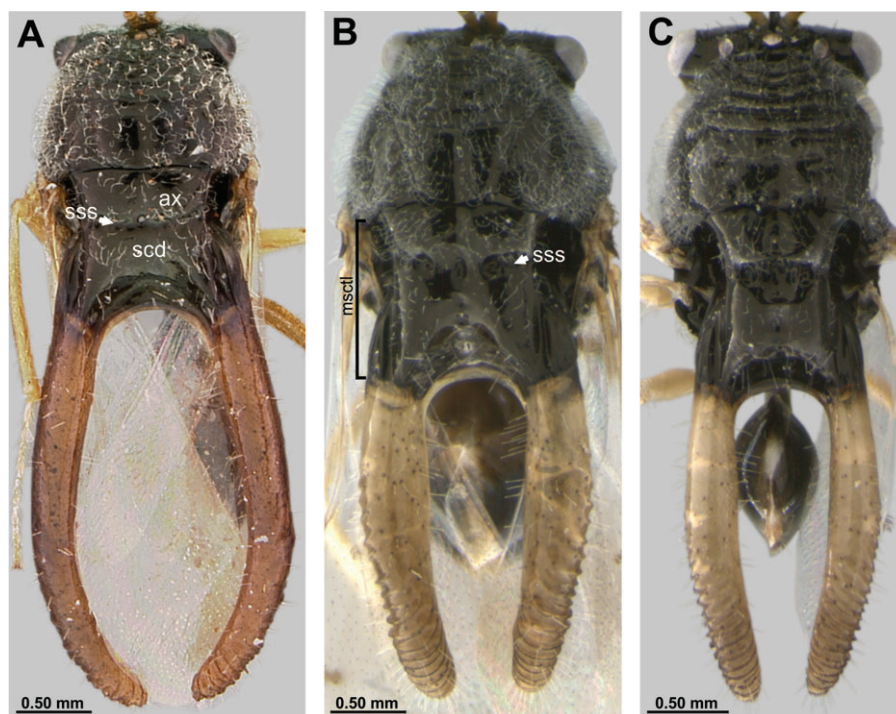


Fig. 2. *Lasiokapala* spp. (A) *L. serrata*, mesosoma (female, dorsal); (B) *L. spiralicornis*, mesosoma (female, dorsal); (C) *L. spiralicornis*, mesosoma (male, dorsal). Abbreviations: ax, axilla; msctl, mesoscutellum; scd, scutellar disc; sss, scutoscutellar sulcus.

as the sister group of *Dicoelothorax* Ashmead, sharing an enlarged gena, reduced forewing venation and reduced number of funicular segments.

Herein we describe the adults, immature stages, oviposition habits and plant associations of *Lasiokapala spiralicornis* sp.n. *Lasiokapala* express a number of extreme features especially in antenna and scutellar spine morphology. In contrast, the first-instar larvae are highly conserved in their morphology. The ant host has not yet been documented, but a proposed host is discussed. The development of some of these peculiar phenotypic features is discussed based on proposed relationships of related genera within this clade, using both morphological and molecular data.

Material and methods

Collections and terminology

Adults of *Lasiokapala spiralicornis* sp.n. were collected on *Sida cordifolia* L. (Malvaceae) while ovipositing on the underside of leaves. Leaves with eggs were placed into a 10 × 10 cm cylindrical glass container with dampened cotton until emergence of the first-instar larvae (planidia). Planidia and eggs were preserved in ethanol. Planidia were later cleared in 10% KOH and both larvae and eggs slide-mounted in Hoyer's medium.

Images of adults were obtained using GT-Vision[®] Ento-Vision software on a Leica M16 zoom lens linked to a JVC KY-F75U 3-CCD digital video camera, and Leica Application Suite

(v3.5.0) software operating on a Leica MZ12 linked to a digital video camera Leica DFC295. Images were enhanced with Corel PhotoPaint and Corel Draw (v15), or processed with CombineZP (Alan Hadley). The distribution map was constructed in Simple Mappr (Shorthouse, 2010). The biogeographical distribution and classification of ecoregions was constructed from Morrone (2001) and Morello *et al.* (2012). GPS coordinates that are reported in brackets were estimated from GoogleEarth.

Morphological terms are from Heraty (2002) and Heraty & Darling (1984), with details on sculpture from Eady (1968) and Harris (1979). The basal flagellomere is counted as F2 following Heraty (2002). The funiculars include F2 and the following unfused flagellomeres to the clava. The paired spines of the mesoscutellum arise from the frenum and we refer to these as the frenal spines (character 50; Heraty, 2002). Morphological abbreviations: ax, axilla; db, dorsal branch; bfw, flagellomere basal width; clv, clava; F2-6, flagellomeres; md, mandible; msctl, mesoscutellum; ped, pedicel; plst, pleurostomal seta or spine; scd, scutellar disc; scp, scape; stp, scutellar process; sss, scutoscutellar sulcus; tp, tergopleural line.

Specimens are deposited in the Instituto Fundaci n Miguel Lillo, Tucum n, Argentina (IFML); Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina (MACN); University of California, Riverside, CA, U.S.A. (UCRC); and National Museum of Natural History, Washington, DC, U.S.A. (USNM). All specimens received a specimen identifier (UCRCENT) barcode label, with the museum of deposition identified on each barcode.

Phylogenetic analyses

Ten species in seven genera were scored for 100 morphological characters as proposed by Heraty (2002). The choice of taxa focussed on *Lasiokapala* and related Kapala clade members, following the results of Murray *et al.* (2013) and Murray (2014). We included the new genus *Neolirata* Torr ns & Heraty, which includes taxa removed from *Lirata* Cameron (*sensu* Heraty, 2002), with the latter now considered as distantly related to the *Neolirata*–*Lasiokapala* clade (Torr ns & Heraty, 2013; Murray, 2014). Host choice (character 101, Heraty, 2002) was not used, but would be uninformative within the clade because *Ectatomma* (Ectatommatinae) is a common host for all of the genera with known biology herein. Only morphological data were available for *Parakapala* Gemignani and *Lasiokapala serrata*.

For the molecular partition, five gene regions were utilized: three nuclear ribosomal (*18S*, *28S-D2* and *28S-D3-D5*) and two mitochondrial (*COI* and *COII*). We included sequences used and previously deposited on GenBank by Murray *et al.* (2013) and Murray (2014). GenBank numbers, DNA specimen voucher numbers and locality data are included in Table S1. DNA vouchers include *Kapala furcata* (Fabricius) (D2799), *Kapala iridicolor* (Cameron) (D2917), *Neolirata alta* (Walker) (D1101), *Neolirata daguerrei* (Gemignani) (D1067b), *Dicoelothorax platycerus* Ashmead (D3602), *Latina rugosa* (Torr ns, Heraty & Fidalgo) (D1073b), *Thoracantha striata* Perty (D1254) and *Lasiokapala spiralicornis* (D3598 and D3600). Only *L. spiralicornis* D3600 was used for the phylogenetic analyses as D3598 was identical in all five genes. All molecular taxa have data for all five gene regions except for *T. striata* which has no *COI*. The alignment follows the 2350-nucleotide dataset from Murray (2014), with any gap-only columns (due to taxon pruning) removed. There were no gaps in the alignment of *28S*, except for one uninformative three base insertion in D3602. The nexus-formatted combined morphological and molecular matrix with partition definitions is found in Figure S1.

Only the combined data were analysed. Parsimony analyses of the combined data were done with TNT v1.1 (Goloboff *et al.*, 2008) using New Technology sectorial search methods, ratchet weighting of 5% with 50 iterations, tree-drifting of 50 cycles, tree-fusing of five rounds and best hit of 10 times (alignment in File S1). Implied weights using concavity functions of $k = 3$ and 15 were applied to evaluate the shortest trees (cf. Heraty *et al.*, 2013). To verify that the resulting tree was amongst the set of most parsimonious (MP) trees, tree length was calculated after reweighting all characters to unity. Bootstrap analyses were performed using 10 000 replicates on the unweighted data. Morphological character changes were mapped using Winclada (Nixon, 1999). The MP ancestral state reconstruction of the female egg-laying behaviour was performed in Mesquite v3.0 (Maddison & Maddison, 2015). Oviposition behaviour was coded as '0' for oviposition into flower buds, '1' as oviposition at the underside of leaves and '?' for unknown (Figure S1).

MrBayes v3.2.3 (Ronquist *et al.*, 2012) was used for Bayesian inference through the CIPRES Science Gateway (Miller *et al.*, 2010). The dataset was partitioned by morphological subset

and by gene, and the *COI* and *COII* regions were further each partitioned into codon positions 1+2 and position 3. This resulted in a total of eight unlinked subsets, each with a gamma distribution to account for rate heterogeneity. Reversible jump MCMC was used so that nucleotide substitution models did not have to be designated for the molecular data. In the morphological subset, 61 characters were invariant and ignored by MrBayes, and the default Mk1 model was implemented. The analysis was run to 1 million generations, sampling every 1000. At the completion of the analysis, the average standard deviation of split frequencies was below 0.01, 25% of each run was discarded as burnin, and the 50% majority rule consensus tree was used to summarize results.

Results

Taxonomy

Lasiokapala Ashmead

<http://zoobank.org/urn:lsid:zoobank.org:act:536A33CD-2CB4-4A00-A3EB-19525B97966C>

Lasiokapala Ashmead, 1899: 240, 242. Type species (syntype, female) *Lasiokapala serrata* Ashmead (USNM); subsequently designated and described by Ashmead, 1904: 473–474. De Santis, 1980: 209 (catalogue). Heraty, 2002: 175–176 (diagnosis and redescription of genus).

Diagnosis. Both sexes with long, cylindrical, smooth frenal spines (stp) with oblique apical serrations (Fig. 2A–C). The spines are broadly separated basally and closely spaced apically. In both sexes, the mesoscutum is pilose with long, fine, hook-tipped hairs, and the scutellar disc (scd) is smooth and sloping posteriorly with a broad median depression, more marked in males. The antennal flagellum of females is dorsally pectinate, the funiculars each with progressively shorter dorsal branches, and with the number of flagellomeres (6–7) varying based on degree of fusion of the flagellomeres with the clava, and the clava comprising at least three completely fused flagellomeres (considered as one segment) (Fig. 1D); basal flagellomere (F2) 3–7 \times as long as its basal width (Fig. 1D, db:bfw). Antennal flagellum of males with 12 segments and funiculars dorsally ramose (Fig. 1G), as in all other Kapala clade members.

Synapomorphic characters (after Heraty, 2002) for the genus include posterolateral margin of gena abrupt and sharply carinate (11:1 [character: state]); labrum with five digits (17:1); female with funicular segments dorsally pectinate (27:4; 28:4); callus densely pilose (57:1); femoral groove shallow (59:0); and calcar long and slightly acuminate at the tip (72:0).

Lasiokapala serrata Ashmead

<http://zoobank.org/urn:lsid:zoobank.org:act:85C1D810-FF79-4A4D-82DE-45FE89DF4BE1>

(Figs 1A, 2A)

Lasiokapala serrata Ashmead, 1904: 473–474. Type species (syntype [one specimen] female). Deposited in USNM, type

number no. 60514 [USNMMENT 809364]. De Santis, 1980: 209 (catalog). Heraty, 2002: 176 (catalog and new localities).

Diagnosis. Female antenna with nine segments (Fig. 1A). Frenal spines reddish to dark brown, cylindrical, more than 1.2× as long as mesosoma, smooth and transversely striated apically, medially broader than mesosoma, spines with basal half straight and apical third sharply curved to medially. Scutoscuteellar sulcus (sss) narrowly impressed (Fig. 2A). Scutellar disc dorsally broader than long. Male unknown.

Distribution. Brazil (fig. 18).

***Lasiokapala spiralicornis* sp.n.**

<http://zoobank.org/urn:lsid:zoobank.org:act:F73FF9BD-0509-4049-9F82-792246457C23>

(Figs 1B–H, 2B, C)

Diagnosis. Distinguished from *L. serrata* by the female antenna having eight segments (vs nine) (Fig. 1D); frenal spines yellowish, cylindrical, smooth basally and transversely striated in posterior half, and with striations sometimes extended to base of spine on the outer and ventral surfaces (Fig. 2B, C); in dorsal view, spines only slightly curved; spines 5.3–5.6× as long as broad, 2.4–2.6× as long as scutellum and 1.0–1.2× as long as mesosoma; sss broadly impressed and smooth (Figs 1C, 2B). Scutellar disc dorsally quadrate. Male: face smooth except for some weak transverse striations on the gena and vertical carinae lateral to the scrobe (Fig. 1H); dorsal branch of basal flagellomere equal or slightly longer than head height (Fig. 1G); and mesoscutal midlobe with irregular, transverse carinae (Fig. 2C).

Description. *Female.* Length, 4.0–4.5 mm. Head, mesosoma except frenal spines, coxae and petiole black; antenna yellowish to light brown, with apex of flagellum slightly darker; frenal spines pale yellowish and translucent; legs pale yellowish to white, except basal half of femora slightly darker (Figs 1B–D, 2B). Wings hyaline, venation light brown to yellow (Fig. 1B). *Head* 1.4–1.5× as broad as high, smooth, except for a few vertical carinae lateral to the scrobe (Fig. 1D). Face including eyes and gena with long, erect hairs. Eyes separated by 2.4–2.7× their height (Fig. 1E); malar space 0.8–1.0× eye height; ocellar triangle elevated above occiput (Fig. 1E), median ocellus in line with lateral ocelli, with sharp occipital carina posterior to ocelli. Labrum with five digits, each digit with a single terminal long and flattened setae. Clypeus smooth and slightly swollen medially; supraclypeal area weakly impressed laterally. *Antenna* with eight segments, scape smooth, not reaching median ocellus, 2.2–2.5× as long as broad and 0.2–0.3× head height; length of flagellum 1.1–1.2× head height; basal flagellomere excluding branch 0.8–1.2× length of scape, 3.8–5.0× as long as basal width and 1.3–2.0× as long as next flagellomere; branch of basal flagellomere 0.8–1.2× as long as basal length of flagellomere and 3–3.5× as long as its basal width (Fig. 1D, db:bfw);

five funiculars and apical flagellomeres fused into a single clava, funiculars pectinate, with single dorsal branches (db) decreasing in length apically (Fig. 1D). *Mesosoma.* Mesoscutum broadly rounded with a smooth surface; mid lobe anteriorly with few transverse carinae, dorsomedially with shallow linear depression (Fig. 2B); mesosoma covered with long fine hairs that are apically hooked (Figs 1C, 2B). Axillae broadly rounded dorsally, smooth and densely covered by fine setae, 0.5–0.6× as long as scutellar disc (scd); sss broadly and deeply impressed (Fig. 2B); scutellar disc and axillae with a medial longitudinal depression; mesoscutellum with pair of long frenal spines (Fig. 1C, stp), 5.3–5.6× as long as broad, 2.4–2.6× as long as mesoscutellum (Fig. 2B, msctl) and 1.0–1.2× as long as mesosoma, with scattered long erect setae; spines cylindrical and broadly separated basally, slightly bowed in dorsal view and closely spaced apically, smooth basally and transversely carinate in posterior half, with carinae sometimes extending to base of spine on outer and ventral surfaces (Figs 1C, 2B); inferior surface of frenum smooth. Propodeal disc smooth and slightly swollen with a longitudinal medial depression, and with two or three setae laterally; callus swollen, smooth and covered with fine long hooked hairs. Mesepisternum smooth and slightly concave, with a few scattered setae; upper mesepimeron smooth, acropleuron with strong carinae (Fig. 1C). Metacoxa semiglobose and smooth, 1.3–1.6× as long as broad. Metafemur with scattered erect and hook-tipped setae. Fore wing 2.5–2.7× as long as broad; stigmal vein 1.0–1.5× as long as broad; postmarginal vein not pigmented, if visible 2.4–2.6× as long as broad; wing disc with dense short setae except basal area without setae. *Metasoma.* Petiole 3.1–3.6× as long as broad and 1.5–1.8× as long as metacoxa; petiole subcylindrical and smooth. Gastral terga with scattered long setae.

Male. Length 4.3 mm. Similar to female except for following: head 1.5–1.6× as broad as high; eyes separated by 2.5–2.8× their height; malar space 0.8–1.1× as long as eye height. Antenna with 12 segments, flagellum dorsally pectinate, branches long (Fig. 1F, G); scape dark brown to black, flagellum with same colouration as female but darker; scape 1.8–2.2× as long as broad; base of basal flagellomere quadrate and very short (Fig. 1G); branch of basal flagellomere similar or slightly longer than head height. Mesosomal midlobe with strong transverse carinae (Fig. 2C); axillae 0.4–0.5× as long as scutellar disc; frenal spines 5.3–5.8× as long as broad, 2.6–2.7× as long as mesoscutellum (ax + scd) and slightly shorter than mesosoma (Figs 1F, 2C). Metacoxa 1.7× as long as broad. Petiole with irregular, weak carinae laterally, 4.6–4.8× as long as broad and 1.8–1.9× as long as metacoxa (Fig. 1F).

Eggs. Length of egg body 0.20 mm and caudal stalk 0.07 mm. Undeveloped eggs are whitish and translucent with a smooth chorion, slightly flattened dorsally and convex ventrally, with a caudal stalk less than half the length of the egg body. After 4 days the sclerotized planidium is apparent inside of the egg body (Fig. 4B). The egg is similar to other Eucharitinae as described by Heraty & Darling (1984).

Planidium. As described for other Eucharitinae by Heraty & Darling (1984), but distinguished as follows: pleurostomal spine

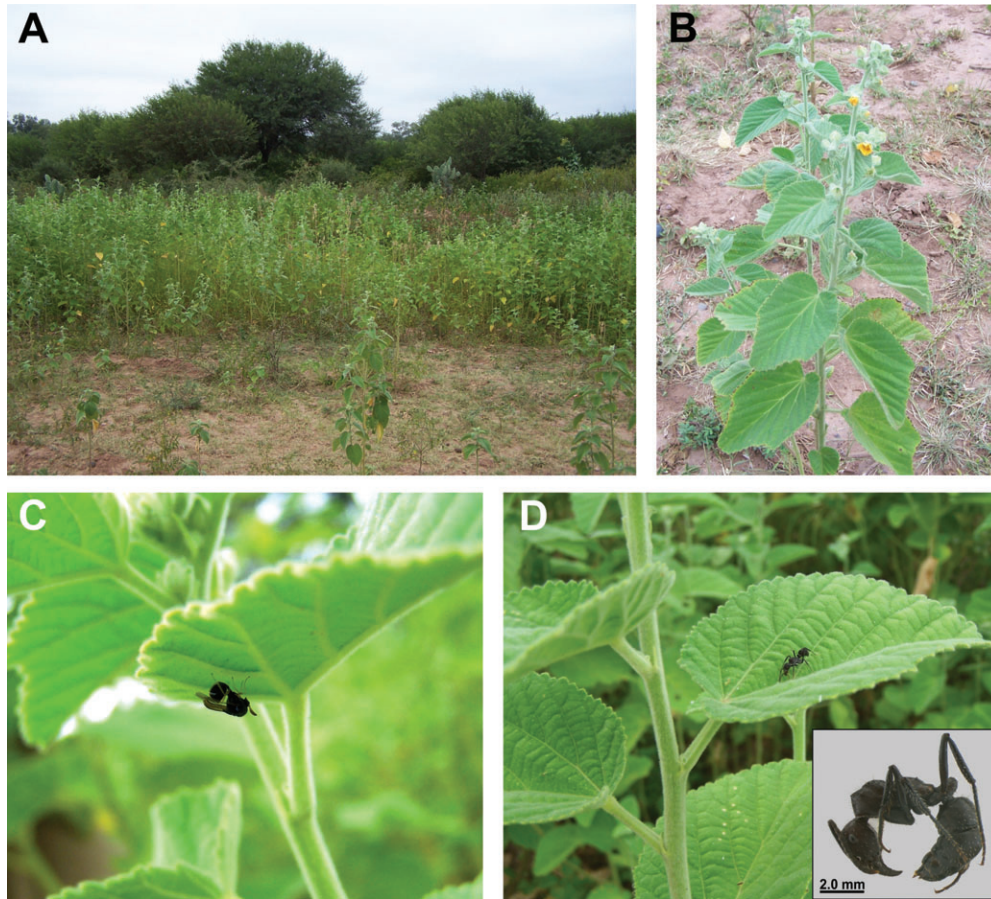


Fig. 3. Biology of *Lasiokapala spiralicornis* sp.n. (A) habitat; (B) *Sida cordifolia* (host plant); (C) female of *L. spiralicornis* ovipositing on underside of leaf of *S. cordifolia*; (D) ant visiting *S. cordifolia* and (inset) habitus of *Ectatomma brunneum* (ant worker).

(plst) enlarged and blunt (Fig. 4C); tergopleural line (tp) present; ventral spine present on TIII; TV–VIII with elongate posteriorly projecting spine on ventral margin and subtriangular spine along posterior margin lateral to tp; TVI with long spine-like setae lateral to tp that reaches to apex of TIX; posteroventral margin of tergite IX with long apically spatulate process reaching to apex of TXII; posterior margin of TXII broadly rounded; apical cerci stiff and as long as TXI–XII combined.

Etymology. From Latin *spiralis*, meaning spiral and *cornus* for horn, referring to the sculpture of the frenal spines. Gender is feminine.

Material examined. *Holotype* ♀. ARGENTINA: Santiago del Estero, La Unión (Colonia Negrito), 26°16'51"S, 62°50'7"W, 20.iii.2012, J. Torrén & P. Fidalgo, T12-009, UCRCENT 00357439. Deposited in IFML. *Paratypes*. Same data, UCRCENT 0033654, 00333656 (1♀, IFML, sequence voucher D32598; 1♂, IFML, sequence voucher D3600); same data, UCRCENT 00357440, 00242265-8 (1♀, IFML; 1♂ and 3♀ MACN); same location, 13.iii.2013, J. Torrén & P. Fidalgo, T13007, UCRCENT 00412539-43 (6♀, UCRC); same location, 29.iii.2013, J. Torrén, T13009, UCRCENT 00439055-58,

00412524-26 (4♀, IFML; 3♀, MACN); same location, 4.iv.2014, J. Torrén, UCRCENT00412531 (1♀, UCRC).

Geographical area and study site

The geographical area corresponds to a typical habitat of Chaco *sensu* Morrone (2001) or Chaco Seco *sensu* Morello *et al.* (2012) (Fig. 5). This ecoregion is characterized by a semi-arid environment with a marked dry season and few rain events during the year, and with summer temperatures exceeding 40°C, with 47.3°C recorded at Campo Gallo (Morello *et al.*, 2012). It is dominated by xeric forest and shrub vegetation, consisting of spiny ligneous vegetation forming an irregular mosaic. This type of environment developed through forest fires and human management (Morello *et al.*, 2012).

The collection site was located 35 km north of Campo Gallo, Santiago del Estero, (26°16'51"S 62°50'7"W) (Fig. 3A); the total area of collection was approximately 3000 m², on both sides of Provincial Route 5. The predominant vegetation is composed of *Aspidosperma quebracho-blanco* Schltr. (Quebracho blanco) (Apocynaceae), *Schinopsis lorentzii* (Griseb.) Engl. (Quebracho colorado santiagueño) (Anacardiaceae), *Prosopis*

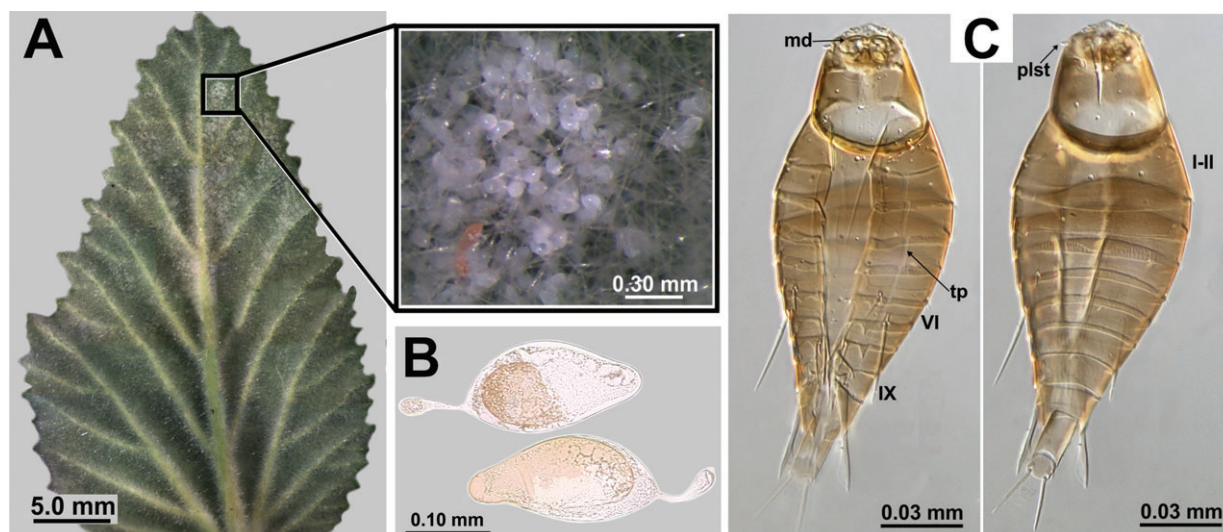


Fig. 4. Biology of *Lasiokapala spiralicornis* sp.n. (A) underside of leaf of *Sida cordifolia* with eggs (magnified area with eggs); (B) eggs after 4 days; (C) planidium (ventral and dorsal). Abbreviations: md, mandible; plst, pleurostomal seta or spine; tp, tergopleural line.

kuntzei Harms (It n) (Leguminosae), *Prosopis ruscifolia* Griseb. (Vinal) (Fabaceae), *Prosopis* sp. (Algarrobo) (Fabaceae) and *Acacia precox* Griseb. (Garabato) (Fabaceae). Periodic disturbance due to weeding along roadsides favours the growth of grasses and other plants such as *Sida cordifolia* L. (Malvaceae), *Sphaeralcea bonariensis* (Cav.) Griseb. (Malvaceae) and *Pseud-abutilon* sp. (Malvaceae).

Host plant and oviposition habit

Sida cordifolia L. (Malvaceae) is a perennial shrub, widely distributed in tropical and subtropical climates (www.GBIF.org). The long and slender stems are yellow-green and pubescent, and the leaves are oblong-ovate and pubescent (Fig. 3B). This plant also was recorded as a host plant for *Dicoelothorax platycerus* Ashmead (Torr ns & Fidalgo, 2013) and *Galearia latreillei* (Gu rin-M neville) (Torr ns, 2013) in Campo Gallo (Santiago del Estero).

Females deposit about 500 eggs amongst the leaf trichomes on the underside of leaves of *S. cordifolia* (Figs 3C, 4A). More than one female could be observed ovipositing on the same leaf, and on one occasion four females were observed concurrently ovipositing on the same leaf (Video S1). Observations were made in mid-afternoon (~2 PM), and three of these females were observed on the same leaves until 19.00 hours, but in the last few hours no oviposition was observed.

Eggs hatched within 7 days; the first instars (planidia) are very mobile and have a propensity to jump.

Potential host ants: presence and activity

We could not confirm the host ant for *L. spiralicornis*; however, *Ectatomma brunneum* Smith (Ectatomminae) [identified

with the key of Kugler & Brown (1982)] was common in the study area (Fig. 3A, D). This species has been cited as a host for *Dicoelothorax platycerus*, *Galearia latreillei* (Gu rin-M neville) and *Kapala* sp. (Lachaud *et al.*, 2012; Torr ns & Heraty, 2012; Torr ns, 2013; Torr ns & Fidalgo, 2013). Some *Ectatomma* are attacked by more than one genus of eucharitid; for example, *Ectatomma tuberculatum* (Olivier) is attacked by *Dilocantha*, *Isomeralla* and *Kapala* (P rez-Lachaud *et al.*, 2006b).

Ectatomma brunneum is common in South America, being recorded in Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Panama, Paraguay, Peru, Suriname and Venezuela (Kusnezov, 1978; Lachaud *et al.*, 2012; The Global Biodiversity Information Facility, 2013; Ward, 2013). It is a mesophilic species found in cleared or savannah areas, often adjacent to forested areas (Kusnezov, 1978; Kugler & Brown, 1982). In the study area, the ants were most active during the morning (09.00–13.00 hours) and near sunset (18.00–20.00 hours). At other times, they were barely seen outside the nest, and were not observed foraging on very hot days.

A total of 20 nests of *E. brunneum* in close proximity to the adult collection and oviposition sites were excavated, but only 10 included immature ant stages. Two nests contained ant larvae parasitized by planidia (one of 39 ant larvae parasitized; two of two ant larvae parasitized), and one nest had a pupa of *Kapala* sp. The association of the planidia found parasitizing the ant larva is difficult because in the same site we collected *Dicoelothorax platycerus*, *Galearia latreillei* and *Kapala* sp., which are all potential parasitoids of *E. brunneum* (Torr ns & Heraty, 2012; Torr ns, 2013; Torr ns & Fidalgo, 2013). The only means of confirming an exact association would be by discovery of a developed eucharitid pupa within the host cocoon.

Excavation was difficult because of the depth of the nests that may in part be caused by the periodic maintenance of the roadside: this involves removal of all vegetation thus forcing the

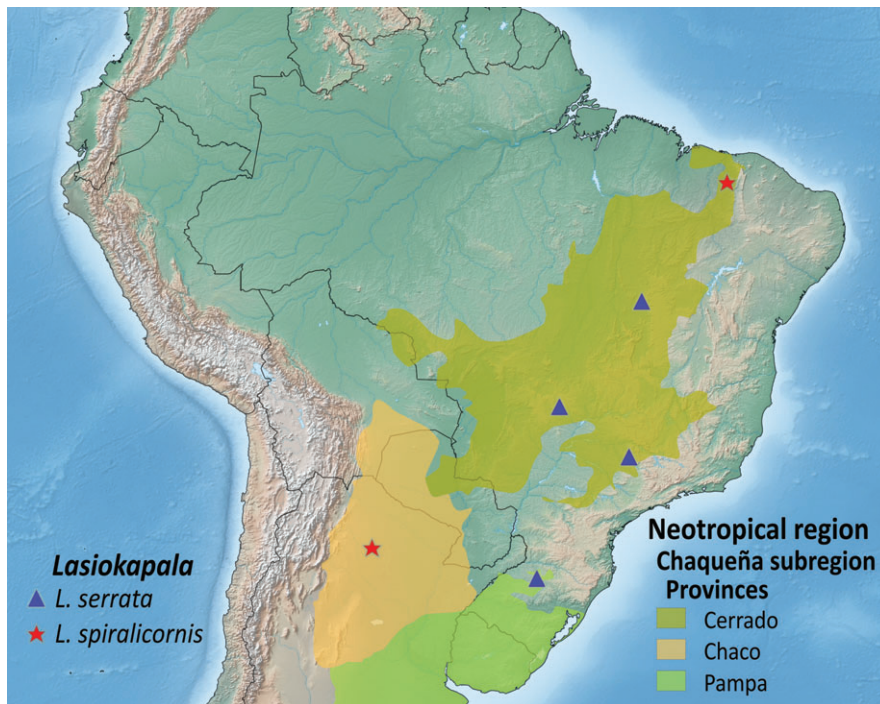


Fig. 5. Distribution of *Lasiokapala* spp.

ants to dig deeper to protect their brood. This behaviour also was observed by Lapola *et al.* (2003) for *Ectatomma brunneum*.

Phylogenetic analyses

Our combined morphological and molecular analyses focused on the relationship between *Lasiokapala* and related genera in the Kapala clade, using *Kapala iridicolor* and *Kapala furcata* as the outgroup taxa following the analyses of Murray (2014).

We report only the results of the implied weights parsimony analysis, which resulted in a topology matching one of the two unweighted parsimony trees (263 steps) (Fig. 6; thin branches collapse in the consensus tree). The relationship of *Thoracantha*, *Dicoelothorax* and *Lasiokapala* were the same in both parsimony and Bayesian results. *Latina*, *Neolirata* and *Parakapala* were unresolved on the strict consensus of three MP trees, and in the Bayesian results *Parakapala* was sister group to the *Thoracantha*–*Lasiokapala* clade (Figure S1). The lack of resolution was likely due to the absence of molecular data for *Parakapala*. Our results were similar to those obtained by Heraty (2002, cf. fig. 445) and Murray (2014, cf. fig. 3), with placement of *Parakapala* varying across the three studies. Based on morphological data only (Heraty, 2002), *Parakapala* were sister to the rest of the ingroup clade, with *Neolirata* (identified as *Lirata alta* in Heraty, 2002) as sister to *Dicoelothorax* + *Lasiokapala*.

All *Lasiokapala* share the following synapomorphies: postero-lateral margin of gena abrupt, with a sharp carinate posterior margin (11:1), funicular segments beyond basal flagellomere

pectinate in female (27:4; at most serrate in the other taxa), and femoral groove present and shallow (59:0). *Lasiokapala* characters homoplastic with other members of the clade include four or five labral digits (17:1; shared with *Latina*), callus densely pilose (57:1; shared with *Kapala* and *Latina*), propodeal disc flat, evenly sculptured and with a pronounced carina bordering the disc (53:2; shared with *Kapala* and *Thoracantha*) and calcar long, curved and bifid (72:0 shared with *Kapala* and *Latina* in part). Dorsally pectinate antennal segments in females are only rarely encountered in some distantly related genera within Eucharitidae (Heraty *et al.*, 2009, 2015).

The relationship between *Lasiokapala* and *Dicoelothorax* (DL, Fig. 6) is supported by the enlarged gena (12:1), reduction of funicular segments (25:5 in part, see below), basal funicular segment very long (26:2, shared with *Latina*), shallowly impressed notauli (42:1), and venation faint but with the stigma still apparent (77:1) (shared with *Parakapala*, in part); the last three characters are also shared with *Neolirata*. The funiculars of *Dicoelothorax* females were scored as dorsally serrate, but these are pronounced and approaching the form in *Lasiokapala* (cf. Fig. 6), and thus may be another character supporting monophyly of these two genera. Heraty (2002) cited the absence of a postmarginal vein (78:2) as also supporting a relationship between *Lasiokapala* and *Dicoelothorax* (shared with *Kapala* in part). In our study, we scored this feature as 2.4–2.6× as long as broad in *Lasiokapala*, and in some specimens is not discernible thus we change the state 2 as ‘postmarginal vein absent or less than 3.0× as long as broad’.

There is an apparent reduction and fusion of funicular segments in females across the clade. The *Kapala furcata* and

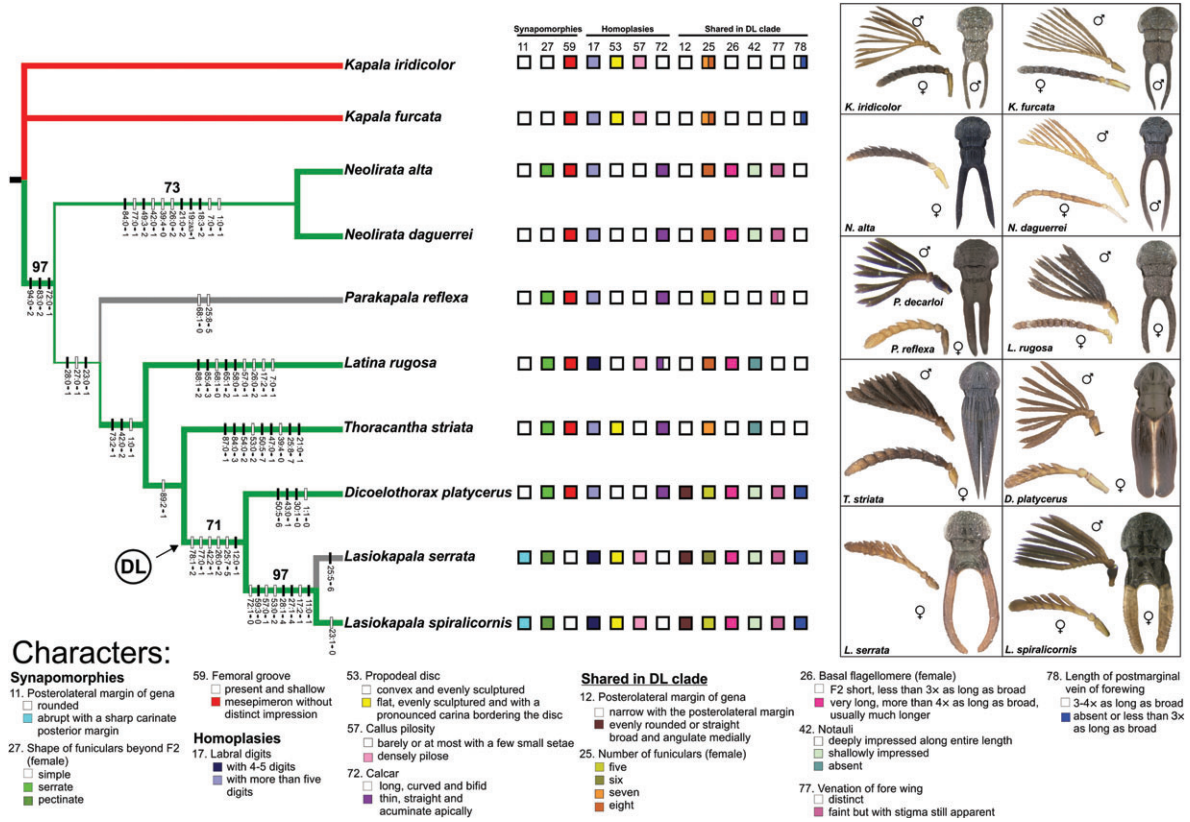


Fig. 6. Phylogenetic relationships of *Kapala* clade members related to *Lasiokapala*. Single most parsimonious tree (implied weights) representing combined morphological and molecular analyses (bootstrap support values above branches); thin branches represent collapse in strict consensus of the three best unweighted trees. Columns to right of tree show the distribution of informative characters (see text); across bottom are the character and state descriptions. Antenna and mesosoma in dorsal view shown. Branches are coloured according to ancestral state reconstruction results. Red branches indicate deposition of eggs into flower buds; green branches indicate laying eggs on underside of leaves; grey branch represents taxa with unknown laying behaviour and no molecular data. Character vertical bars shading: black = unique derivation within clade; white = homoplasy (reversal or convergence). DL, *Dicoelothorax* + *Lasiokapala* clade. [Colour figure available on the online version on Wiley Online Library.]

K. iridicolor sister groups have 7–8 (25:7/8). *Thoracantha* has seven distinct funiculars in females (25:7), which is considered plesiomorphic. *Lasiokapala serrata* has six funiculars. A reduction to five funiculars occurs in *L. spiralicornis*, *Dicoelothorax* and *Parakapala* (Fig. 6). Our tree supports at least two independent reductions, but we lack molecular data for *Parakapala* to establish a more certain relationship.

Discussion

Within Eucharitidae, scutellar processes or spines are known only in Akapalinae (host unknown) and Eucharitini, with members of the PEM clade (Ponerinae, Ectatomminae and Myrmeciinae parasitoids) displaying the greatest variety of form (Heraty *et al.*, 2015). Within this PEM clade, the *Kapala* clade consists of 13 genera (Heraty, 2002; Torr ns & Heraty, 2013), all of which have a pair of cylindrical frenal (scutellar) processes. The spines are sexually dimorphic in *Dicoelothorax*, *Dilocantha*, *Galearia* and *Parakapala* (Heraty, 1998, 2002; Torr ns, 2013; Torr ns & Heraty, 2013). This dimorphism

is absent in *Kapala*, *Neolirata*, *Latina*, *Thoracantha* and *Lasiokapala*. Our results suggest that dimorphism in spine morphology is independently derived in the different genera. Spines that form an arching carapace over the metasoma (50:7; Heraty, 2002) occur in females of *Parakapala*, *Thoracantha* and *Dicoelothorax*, as well as *Galearia* and *Dilocantha*. Again our results suggest multiple independent derivations of these forms. *Lasiokapala* has a unique spine morphology unrelated in form to any other Eucharitinae, other than the reduction or loss of parallel carinae on the spines of *Dicoelothorax* and *Lasiokapala*.

Oviposition behaviour is relatively well known across Eucharitidae. Within the *Kapala* clade, oviposition can be into flower buds or on the underside of leaves. Within the genus *Kapala*, only one species, *Kapala terminalis* Ashmead, is known to oviposit on the underside of leaves (Clausen, 1940), whereas the other known species oviposit into flower buds (Clausen, 1940; Heraty & Darling, 1984; Heraty & Woolley, 1993; Torr ns, 2013) (102:0&1). In the *Kapala* species included in our analyses, oviposition takes place in floral buds (102:1, red line in Fig. 6); whereas in the rest of the genera, except for *Parakapala*

whose oviposition strategy is unknown, females lay their eggs on the undersides of leaves (Heraty, 2002; Torrén *et al.*, 2007; Torrén & Heraty, 2012, 2013) (102:0, green line in Fig. 6). In eucharitids, oviposition *onto* the leaf surface is rare outside of the Kapala clade. *Gollumiella* embed the tip of the egg into the leaf tissue, and Oraseminae and *Schizaspidia antennata* Gahan oviposit fully into the leaf tissue (Clausen, 1940; Heraty, 2000; Heraty *et al.*, 2004). Most of the Old World members of the PEM clade (*sensu* Murray *et al.*, 2013) oviposit into leaf or flower buds (Clausen, 1940). Laying eggs on the underside of leaves between trichomes would seem to occur almost exclusively within the Kapala clade; however, one species in the Schizaspidia clade, *Ancyлотropus montanus* (Girault), deposits eggs that resemble white powder on the underside of the leaves (Ishii, 1932). Leaf oviposition may be a more common trait across the entire PEM clade as we accumulate more biological data.

According to Clausen (1940), the selection of a plant suitable for oviposition by eucharitids appears to be governed by structural characteristics of host plants, with little focus on taxonomic placement. The leaves of *Sida cordifolia* are used by three species: *Dicoelothorax platycerus* (Torrén & Fidalgo, 2013), *Galearia latreillei* (Torrén, 2013) and *Lasiokapala spiralicornis*. The abundance of trichomes on the leaves surrounding the eggs offer protection from predators and mechanical damage, and may also conserve humidity near the epidermis of the leaves and therefore the eggs.

Extremes in the eucharitid morphology of the frenal spines and other features are most pronounced within the Schizaspidia and Kapala Clades, which are together monophyletic in the PEM clade (Murray *et al.*, 2013; Heraty *et al.*, 2015). Projecting frenal spines are derived independently, often multiple times within both clades, but not within the sister Chalcura Clade (Heraty *et al.*, 2015). Despite morphological divergence in adults, the larvae are very highly conserved in both morphology and behaviour. All members of the Chalcura, Schizaspidia, and Kapala clades have highly active, minute planidia larvae, with similar morphology that includes a tergopleural line, distinctive posterior spines and terminal processes on tergites VI and IX. These traits are associated both with rapid movement and a propensity to jump over long distances, which we assume offers a greater chance of encountering a foraging host ant for transport back to the nest. The conserved morphology of the planidium is likely tied to the constraints surrounding host access via an ant vector.

We observed the proposed host, *Ectatomma brunneum*, on both the plant host and soil surrounding the plant. Recent studies by Schwitzke *et al.* (2015) in the genus *Chalcura* (Old World Chalcura clade) suggest that planidia may attack and kill adult ants visiting the host plant. They sampled a variety of ants on the plant, but none were the likely ponerine host. In their case, it is possible that the host ants forage below the host plant and remove dead ants infested with planidia as food for their brood. In our case, with *Ectatomma* found on the host plant, the interaction is less clear. More information is needed on both adult and immature behaviours across the group to get a better understanding of interactions between planidia, their hosts and the extreme morphological diversification of the adults.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12176

Table S1. GenBank accession numbers and collection locality data.

File S1. Combined morphological and molecular data.

File S2. Bayesian tree and nexus files. Bayesian phylogeny figure and input nexus sequence file along with the Mesquite nexus file for ancestral state reconstruction.

Video S1. Adult *Lasiokapala spiralicornis* ovipositing on *Sida*.

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