

DNA identification and morphological description of the first confirmed larvae of Hetaeriinae (Coleoptera: Histeridae)

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Abstract. Using DNA sequences of nuclear ribosomal (18S) and mitochondrial (cytochrome oxidase I) genes (a modified DNA barcoding approach), we positively identify, for the first time, larvae of hetaeriine Histeridae. Species in this subfamily occur as obligate associates of social insect colonies, particularly those of neotropical army ants. Of several larval specimens collected from bivouacs of *Eciton burchelli*, we identify the two larval instars of *Paratropinus scalptus*, and discuss a quite different first instar larva near *Euxenister*. The larvae are described and illustrated, with attempts to homologize all chaetotaxy to other known histerid larvae. Phylogenetic trees for 18S and cytochrome oxidase I, for over twenty hetaeriine taxa, are compared with each other and with a previous hypothesis of relationships in the subfamily.

Introduction

The Hetaeriinae represent an extraordinary diversification of inquiline Histeridae. Most species of the over 100 described hetaeriine genera are obligate associates of the colonies of ants and termites (but see Kapler, 1999). The bulk of this diversity occurs in the Neotropics, primarily with army ants (Formicidae: Ecitoninae) (Helava *et al.*, 1985). Knowledge of their biology is limited largely to simple host associations (Helava *et al.*, 1985; Dégallier, 1998a,b, 2004), although some adult behaviours have been described (see Wheeler, 1908; Reichensperger, 1926; Rettenmeyer, 1961; Akre, 1968; Akre & Rettenmeyer, 1968). Major questions have centred on the larval biology of the subfamily, and the degree of association with host colonies (Akre, 1968). However, no hetaeriine larvae have ever been identified definitively. Böving & Craighead (1931) illustrated a termitophilous larva that has since been tentatively attributed to Hetaeriinae (Newton, 1991), and a few additional suspected hetaeriine larvae are known (Diniz *et al.*, 1998; A. F. Newton, pers. comm.). In this paper, we present the first definitive association of larvae

with the subfamily, based on DNA sequences, discuss their possible taxonomic identities and present detailed descriptions of their morphology. These data will facilitate the eventual resolution of histerid subfamily and tribal level phylogeny. The larval morphology of Histeridae has not received a great deal of study, but available data indicate strong potential for helping to resolve these relationships (Kovarik & Passoa, 1993; Caterino & Vogler, 2002).

This paper presents a relatively unsung application of DNA sequences. Although larval identification is commonplace in forensic science (e.g. Wells & Sperling, 2001), few studies have utilized nucleotide data to identify and associate unknown insect larvae for systematic purposes (but see Alarie & Bilton, 2005 and Miller *et al.*, 2005). Rearing, generally considered to be the only infallible method of association of larvae with their respective adults, is time intensive, and successful rearing conditions for many species are difficult to achieve. DNA sequencing, which has become increasingly straightforward, accessible and inexpensive, represents an obvious alternative. This application ultimately will require a much more representative database of identified sequences than is available currently for most insect groups to be broadly applicable, but in cases such as ours, where larvae and adults are collected together, association may be attempted by sequencing individuals from both pools of specimens to seek sequence matches.

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Materials and methods

Larval specimens

Four specimens, representing three distinct larval types, are discussed in this paper. They are referred to by the following codes throughout. MSC1: first instar larva from BELIZE: Cayo: Las Cuevas Field Station (16°44'N, 88°59'W), 24.v.2000, sifted from beneath bivouac of *Eciton burchelli* (Westwood) (determined by B. Bolton), deposited in M. S. Caterino collection (MSCC). Adult Hetaeriinae collected from this host in the area included *Daitrosister* sp. (four of 16 adult specimens), *Euclasea godmani* Lewis (one specimen), *Psalidister distinctus* Reichensperger (seven specimens), *Troglosternus ecitonis* Mann (three specimens), *Ulkeus* sp. (one specimen). AKT1, AKT2, AKT3: second instar larvae from ECUADOR: Napo, Yasuni Research Station (00°40'S, 77°24'W), between 29.vii and 6.viii.1999, from refuse deposit of *Eciton burchelli* bivouac, deposited in Louisiana State Arthropod Museum (LSAM) collection. Associated adult Hetaeriinae included *Synoditulus* sp. (13 of 82 adult specimens), *Paratropinus scalptus* Reichensperger (33 specimens), *Psalidister* spp. (three species, 20 specimens) and *Daitrosister* sp. (eight specimens). Additional first instar larvae from this sample were not sequenced, but are discussed as they differ from the second instars below.

Adult specimens

In addition to specimens of all of the adult Hetaeriinae mentioned above, we obtained sequences from numerous additional adults, collected by both authors at these and other localities. For the purposes of definite placement within Hetaeriinae, sequences of several non-hetaeriine Histeridae also were included. All specimens sequenced and their localities, identities (if known) and GenBank accession numbers are listed in Table 1. Outgroups included several Exosternini (Table 1). Although additional histerid outgroups were included in preliminary 18S analyses, it became immediately clear that our larvae were indeed Hetaeriinae, and removal of these extraneous taxa permitted a more thorough evaluation of relationships within this group.

Genes

We obtained sequences of two genes for this study. The nuclear ribosomal 18S gene has been used in a previous study of histerid phylogeny, and has demonstrated valuable information for resolving relationships among subfamilies of Histeridae (Caterino & Vogler, 2002). Although this gene might not be expected to vary much within a subfamily, it was hoped that these sequences would provide at least a conclusive subfamily level association. This gene also is

sequenced readily for poorly preserved specimens, and allowed inclusion of several additional taxa in the analysis. We obtained a relatively short segment of the gene, between primers 18Sai–18Sb0.5 (see Shull *et al.*, 2001 and our online supplementary material), spanning about 350 bases. The region contains one hypervariable region, flanked by strongly conserved regions on either side.

The more rapidly evolving cytochrome oxidase I (COI) gene also was sequenced to provide more informative variation amongst candidate species. This gene is amongst the most widely used in species level systematics of insects (Caterino *et al.*, 2000), and has been advocated as an ideal marker to underlie a DNA-based taxonomy, or 'DNA barcode' (Hebert *et al.*, 2003). We sequenced a region of about 660 base pairs (bp) at the 5' end of the gene between primers C1-J-2334 and L2-N-3014 (Pat; see Simon *et al.*, 1994 and online supplementary material).

Molecular methods

Most specimens were collected in >95% ethanol, although a few had been frozen directly at –80 °C. Genomic DNA was extracted using Qiagen's DNeasy tissue kit (Qiagen, Valencia, California). Adult specimens were broken open between the pro- and mesothorax and bathed in extraction buffer and proteinase K (both as provided with the kit) for ~2 h. Body fragments were then removed, rinsed in ethanol and mounted as vouchers (see Table 1 for voucher repositories). Subsequent treatment of the extract followed the manufacturer's protocol. Larval specimens were pierced with an insect pin in several places and similarly bathed in extraction buffers, and then removed and stored in ethanol. Polymerase chain reactions (PCRs) were carried out using a common profile (thirty-five cycles of 95 °C for 30 s, 45 °C for 30 s and 72 °C for 1 min, preceded by a 3-min initial denaturation at 95 °C and followed by a 5-min final extension at 72 °C). PCR fragments were sequenced in both directions.

Analysis

Alignment of 18S sequences was accomplished using CLUSTALX (Thompson *et al.*, 1997; fixing alignment parameters following a previous analysis of histeroid 18S; Caterino & Vogler, 2002). COI sequences were length invariant and easily aligned. Trees for each dataset were produced by similar methods. A parsimony analysis was carried out in PAUP* (PAUP 4.0b10; Swofford, 2003) with 500 random taxon addition replicates (limiting trees stored at each step to 1000), followed by tree bisection and reconnection (TBR) branch swapping. Branch support for parsimony trees was estimated using decay indices as calculated in TREEROT (version 2c; Sorenson, 1999). We also conducted maximum likelihood analyses on both datasets with models and parameters selected using the Akaike

Table 1. Taxa and specimens sequenced for this study. Collection locations for Hetaeriinae refer to one of three localities, described more precisely in the text. Yasuni is in Ecuador, La Selva in Costa Rica and Las Cuevas in Belize.

Subfamily	Tribe	Genus/species	Collection location	MSC extract	Voucher repository	GenBank 18S	GenBank COI
Histerinae	Exosternini	<i>Rhyphochares saprinoides</i>	South Africa	46	MSCC	DQ124332	DQ124304
Histerinae	Exosternini	<i>Phelister</i> nr. <i>williamsi</i>	Las Cuevas	64	MSCC	AY028354	DQ124305
Histerinae	Exosternini	<i>Scapomegas auritus</i>	Tiputini	116	MSCC	DQ124333	DQ124306
Histerinae	Exosternini	<i>Tribalister striatellus</i>	WI: Grant Co.	132	MSCC	DQ124334	DQ124327
Histerinae	Exosternini	<i>Operclipygus</i> sp.n.	Las Cuevas	66	MSCC	AY028356	DQ124307
?		AKT1	Yasuni	114	LSAM	DQ124350	DQ124320
?		AKT2	Yasuni	115	LSAM	DQ124351	–
?		AKT3	Yasuni	117	LSAM	DQ124352	DQ124321
?		MSC1	Las Cuevas	113	MSCC	DQ124348	DQ124319
Hetaeriinae		<i>Cheilister lucidulus</i>	Yasuni	111	MSCC	DQ124341	DQ124314
Hetaeriinae		Genus nr. <i>Ecclisister</i>	Yasuni	102	MSCC	DQ124344	DQ124316
Hetaeriinae		<i>Clientister</i> sp.	La Selva	106	MSCC	DQ124342	DQ124315
Hetaeriinae		<i>Anasynodites striatus costaricae</i>	Yasuni	98	MSCC	DQ124339	DQ124313
Hetaeriinae		<i>Daitrosister</i> sp.n.	Las Cuevas	90	MSCC	DQ124345	DQ124317
Hetaeriinae		<i>Paratropinus scalptus</i>	Yasuni	80	MSCC	DQ124337	DQ124310
Hetaeriinae		<i>Psalidister distinctus</i>	Las Cuevas	92	MSCC	AY028364	DQ124311
Hetaeriinae		<i>Euclasea godmani</i>	Las Cuevas	93	MSCC	DQ124336	DQ124309
Hetaeriinae		<i>Nymphister monotonus</i>	Las Cuevas	94	MSCC	DQ124338	DQ124312
Hetaeriinae		<i>Hetaerius</i> sp.	CA: Sonoma Co.	45	MSCC	DQ124346	DQ124318
Hetaeriinae		<i>Hetaerius morsus</i>	CA: Ventura Co.	129	MSCC	DQ124347	DQ124330
Hetaeriinae		<i>Euxenister caroli</i>	Panama: Colon	127	MSCC	–	DQ124328
Hetaeriinae		<i>Euxenister wheeleri</i>	Panama: Colon	128	MSCC	–	DQ124329
Hetaeriinae		<i>Euxenister wheeleri</i>	Yasuni	77	MSCC	DQ124349	–
Hetaeriinae		<i>Troglosternus</i> sp.n.	Yasuni	75	MSCC	DQ124335	–
Hetaeriinae		<i>Troglosternus ecitonis</i>	Las Cuevas	91	MSCC	–	DQ124308
Hetaeriinae		<i>Scapicoelis</i> sp.	Las Cuevas	73	MSCC	AY028366	DQ124326
Hetaeriinae		<i>Ulkeus</i> sp. ^a	Las Cuevas	87	MSCC	DQ124354	DQ124323
Hetaeriinae		<i>Ulkeus</i> (s. l.) sp.1 ^a	Las Cuevas	88	MSCC	DQ124353	DQ124322
Hetaeriinae		<i>Ulkeus</i> (s. l.) sp.2 ^a	Las Cuevas	86	MSCC	DQ124355	DQ124324
Hetaeriinae		<i>Opadosister</i> sp.n.	Las Cuevas	85	MSCC	DQ124356	DQ124325
Hetaeriinae		<i>Reninus</i> sp.	Costa Rica	130	MSCC	–	DQ124331
Hetaeriinae		<i>Synoditulus</i> sp.n.	Yasuni	72	MSCC	AY028365	–
Hetaeriinae		<i>Aphanister fungifer</i>	La Selva	97	MSCC	DQ124340	–
Hetaeriinae		<i>Pulvinister nevermanni</i>	La Selva	95	MSCC	DQ124343	–
Hetaeriinae		<i>Terapus</i> sp.n.	FL: Putnam Co.	89	MSCC	DQ124357	–
Hetaeriinae		<i>Iugulister</i> sp.n.	Yasuni	82	MSCC	DQ124358	–
Hetaeriinae		<i>Tylois</i> sp.	Yasuni	56	MSCC	DQ124359	–

COI, cytochrome oxidase I; LSAM, Louisiana State Arthropod Museum; MSCC, M. S. Caterino collection.

^aThe boundaries of the genus *Ulkeus* are particularly unclear. We place these taxa in a loosely defined *Ulkeus*, although they expand its current scope considerably. The specimen representing extract 86, in particular, is very similar to the presently monotypic *Ulkeopsis*. This latter genus, however, seems likely to have been derived from within *Ulkeus*.

information criterion as implemented in MODELTEST (version 3.6; Posada & Crandall, 1998). These were then fixed in PAUP*, and TBR branch swapping was run to completion using each gene's respective parsimony topology as a starting point.

Results

Phylogenetic results and identities of larvae

The trees obtained for both genes, and by all methods, were similar in overall topology and identical with respect to the closest relationship of the unidentified larvae to

particular reference sequences (Figs 1 and 2). Firstly, all suspected larvae examined were found to be Hetaeriinae; monophyly of the subfamily (excluding the problematic *Synoditulus*; see Caterino & Vogler, 2002), including the larval sequences, is found in all trees, with eleven and two steps decay support by COI and 18S, respectively. The alignment of the 18S variable region as reconstructed by CLUSTAL is shown in Fig. 3 (and the full datasets are available as online supplements).

The most unambiguous specific larval association is that of larva AKT3 with *Paratropinus scalptus*. Their 18S sequences are identical, whereas they differ by 3 bp in COI (<0.5%), which is well within the range of many insect species (Langor & Sperling, 1997). As *Paratropinus scalptus*

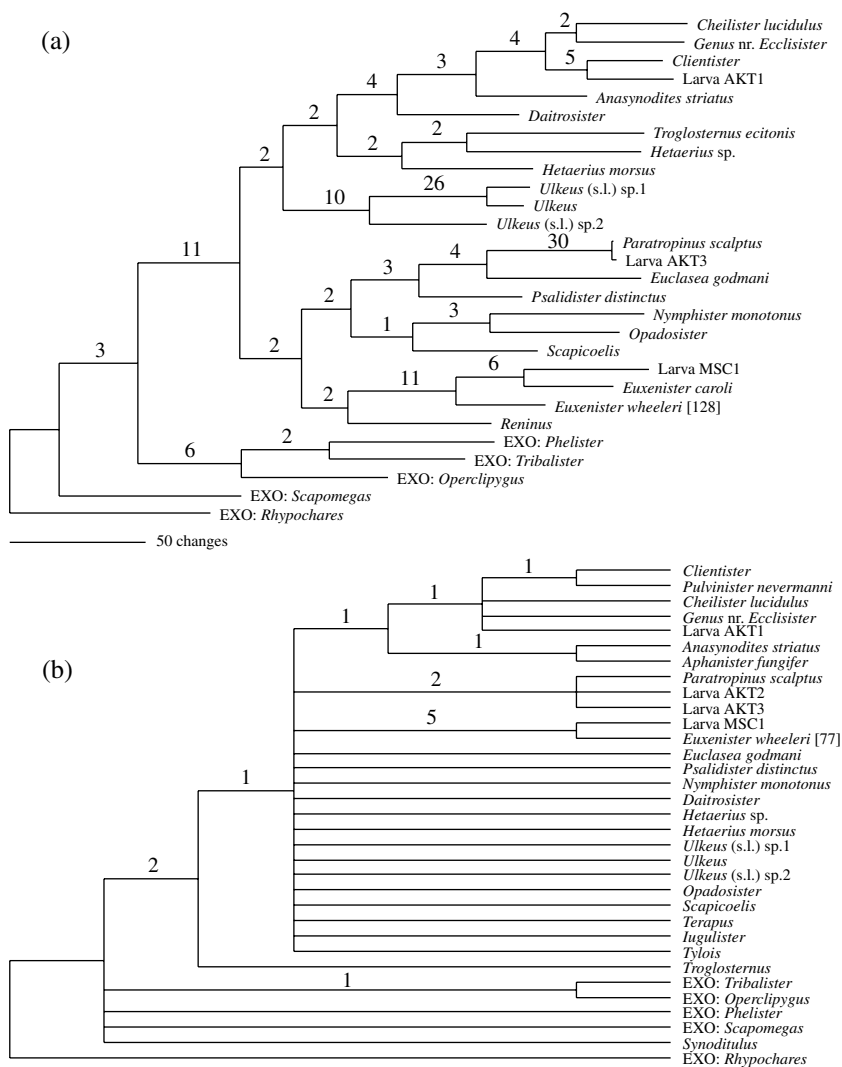


Fig. 1. (a) Single most parsimonious tree based on cytochrome oxidase I (COI) (1883 steps; CI = 0.3168; RI = 0.4144). (b) Strict consensus of 23 797 equally parsimonious trees based on 18S (127 steps; CI = 0.5748; RI = 0.7453). Decay indices are shown above the branches.

was the most abundant taxon of adult beetle associated with this particular larval collection, it seems justified to conclude a definite species association. We describe this larva fully below. Larva AKT2 was identical to AKT3 in morphology and 18S sequence, and seems likely to represent this taxon as well, but we were unsuccessful in obtaining a COI sequence from it, and therefore exclude it from further consideration.

We find a suggestive association between larva MSC1 and the genus *Euxenister*. Their close, exclusive relationship is supported by all analyses, with good decay support (eleven and five steps by COI and 18S, respectively). The larva and the one *Euxenister* adult sequenced for 18S are nearly identical and 36 bp longer in the V4 region than any other hetaeriine (although they do differ by one transition in this region). Data from COI are, however, difficult to interpret: we obtained COI sequences of two (of three) described species of *Euxenister* – the only two known from Central America – and larva MSC1 (from Belize) was very distinct from either (12.0% from *Euxenister caroli*; 14.9% from

Euxenister wheeleri). At these divergences, any particular species association is unlikely. However, whether this means that the larva represents an undescribed *Euxenister* or some other unsampled (and unsuspected) relative of *Euxenister* is impossible to say. Despite this inconclusive association, in the interest of broadening the known scope of morphological variation amongst hetaeriine larvae, we provide a description of this larva to the extent that it differs from AKT3.

The third larval type for which we obtained both COI and 18S data (larva AKT1) also cannot be conclusively identified. It is identical in 18S sequence to both *Cheilister* and an undescribed genus near *Ecclisister*, both of which have been collected at the same Yasuni site as the larva (although neither at the same time, and with a different host in the latter case). However, in COI sequence, it is distinct from both, and resembles *Clientister* (from which it differs in 18S by a single nucleotide change). All of these genera were assigned to subgroup E4 of Helava *et al.* (1985), along with a few others we have not sampled.

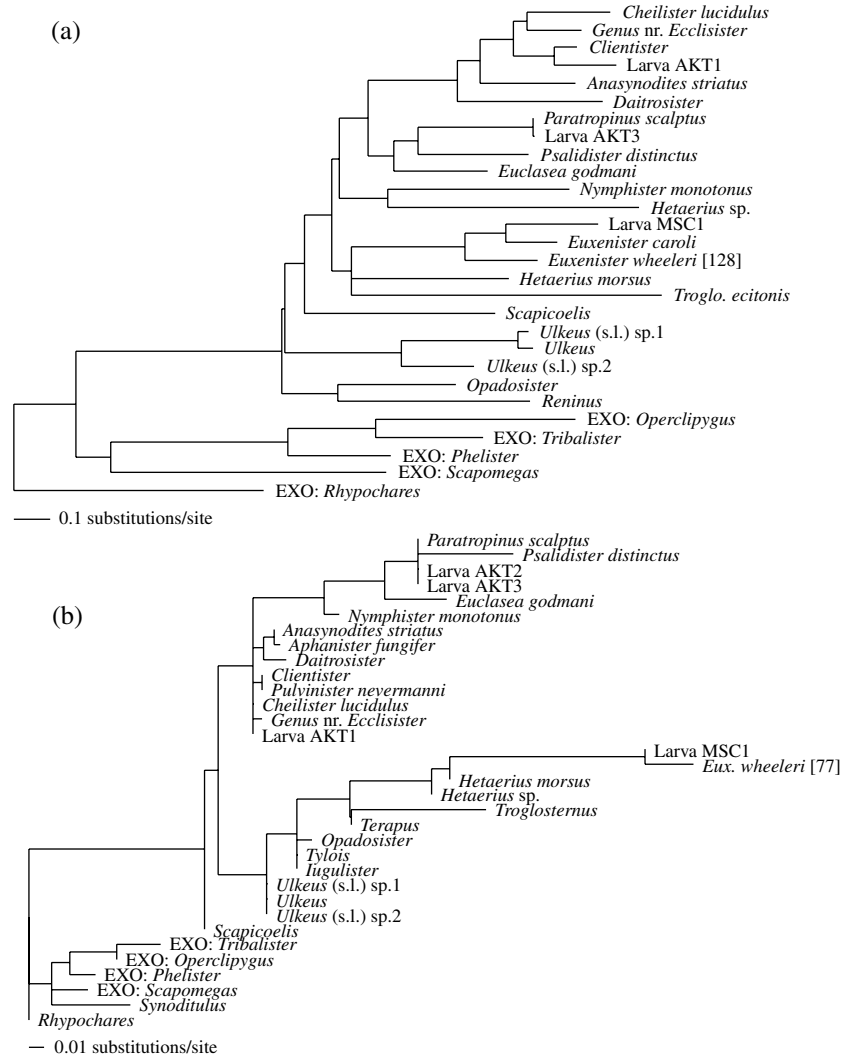


Fig. 2. (a) Maximum likelihood phylogram for cytochrome oxidase I (COI) sequences based on a GTR + I + Γ [ln L = -7701.5434; model settings (in PAUP format): Base = (0.3952 0.2079 0.0635) Nst = 6 Rmat = (0.7035 36.7178 3.3179 8.0006 45.6086) Rates = gamma Shape = 0.5120 Pinvar = 0.4547]. (b) Maximum likelihood phylogram for 18S sequences based on a GTR + I + Γ [ln L = -1116.9870; model settings (in PAUP format): Base = (0.1532 0.2810 0.3311) Nst = 6 Rmat = (0.0999 2.3590 0.1805 0.0001 7.2782) Rates = gamma Shape = 0.6233 Pinvar = 0.7522].

	1	11	21	31	41	51	61	71	81	91	101	111			
LarvaMSC1	TGGACGC	-GGTCTC	-CGGTCG	TCTCTCG	-CGTGGC	-TCGTCG	CTTC	GTCTCTCT	CCGGGGAC	G- A-	-CGGCGT	CGTCG	GGGTGG	GCGATCGG	-GGCGTCC
Euxenister wheeleri	TGGACGC	-GGTCTC	-CGGTCG	TCTCTC	-CGCGT	CGCTCG	CTCTCC	GTCTCTCT	CCGGGGAC	G- A-	-CGGCGT	CGTCG	GGGTGG	GCGATCGG	-GGCGTCC
LarvaAKT2	TGGACGC	-GGTCTG	T-T-CG	TTC	-GC	-G	-G	-G	-G	-G	-G	-G	-G	-G	-G
LarvaAKT3	TGGACGC	-GGTCTG	T-T-CG	TTC	-GC	-G	-G	-G	-G	-G	-G	-G	-G	-G	-G
Paratropinus scalptus	TGGACGC	-GGTCTG	T-T-CG	TTC	-GC	-G	-G	-G	-G	-G	-G	-G	-G	-G	-G
LarvaAKT1	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Clientister	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Genus nr. Ecclisister	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Cheilister lucidulus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Pulvinister nevermanni	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Anasynodites striatus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Aphanister fungifer	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Daitrosister	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Euclasea godmani	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Psalidister distinctus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Nymphister monotonus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Hetaerius sp.	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Hetaerius morsus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Troglosternus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Synoditulus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Ulkeus (s.l.) sp1	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Ulkeus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Ulkeus (s.l.) sp2	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Terapus	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Opadosister	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Iugulister	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Scapicoelis	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT
Tylois	TGGACGC	-GGTCT	-CG-C-GT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT	-CGT

Fig. 3. The V4 region of the 18S rDNA gene for Heteriinae only. This is the alignment of this region produced by CLUSTAL. Although obvious improvements suggest themselves, this inevitably involves an unacceptable level of subjectivity.

Beyond placing this larva with some confidence in this group, we suggest no specific identity for it, and do not describe it here.

Formal larval descriptions

Descriptive conventions

Our chaetotaxy descriptions follow the format of Kovarik & Passoa's (1993) description of the histerid *Onthophilus nodatus* LeConte with respect to presentation, terminology and numbering. We do not necessarily agree with their homology assessments (either serially amongst segments or as extended to other beetle families), but it facilitates direct comparison amongst descriptions. All setae and pores are referred to by two capital letters for each body region (MN = mandible; MX = maxilla; LA = labium; AN = antenna; SE = antennal sensorium; FR = frontale [nasale]; PA = parietal [head]; TE = thoracic and abdominal dorsum; PL = thoracic and abdominal pleuron; ST = thoracic and abdominal sternum; TR = trochanter; FE = femur; TI = tibia; UG = urogomphus; PP = pygopod [abdominal segment X]). These codes are followed by a numeral for setae and a lower case letter for pores. Where thoracic and abdominal (TE, PL, ST only) features share codes amongst segments, it is indicative of their putative serial homology. A lower case 'g' preceding a code indicates a group of setae treated collectively. A code followed by a question mark (?) indicates that our homology assessment is particularly uncertain. We invoke a few additional numbers or letters for setae and pores present in all the Hetaeriine larvae that have no possible *Onthophilus* homologue. However, many autapomorphic features lack codes. In all cases in which a number of setae or pores are given for a particular region, it refers to the number present on only one side of the body. Body measurements are abbreviated as follows: HW = maximum head width;

dHL = dorsal head length from base of epicranial emargination to tip of central nasale tooth; vHL = ventral head length from base to labial emargination; HD = maximum head depth; PnW = maximum pronotum width; PnL = pronotal length along midline.

Basis for homology assessments

Homologies of setae and pores with those present in *Onthophilus* (Kovarik & Passoa, 1993) are hypotheses, but confidence for most, at least for the *Paratropinus* larva, is relatively high. Although their arrangement varies, the numbers of chaetotaxic features present on various body regions are similar in most cases, and identical in many, to those found in *Onthophilus*. Thus, through a process of elimination, in most cases questionable setae could be homologized. Where absolute quantities are different, however, definite assignment is problematic. This results from a small number of 'missing' setae in *Paratropinus*, and from a large number of 'extra' setae in larva MSC1 (nr. *Euxenister*).

Description of Paratropinus scalptus larva (AKT3)

Second instar. Cream-coloured, largely membranous with deep convoluted folds separating segments, and shallower folds marking secondary segmentation, with head, pronotum and legs only obvious sclerotizations; body narrowed anteriorly and posteriorly, maximum width at abdominal segment V about twice head capsule width. Head capsule (Fig. 4): HW = 0.53 mm; dHL = 0.53 mm; vHL = 0.69 mm; HD = 0.37 mm; strongly sclerotized, parallel sided, lacking stemmata, deeply emarginate at dorsal base, in lateral view, narrowed anteriorly, deepest approximately one-fifth from base; ventral tentorial pits confluent at middle; nasale with broad central tooth with

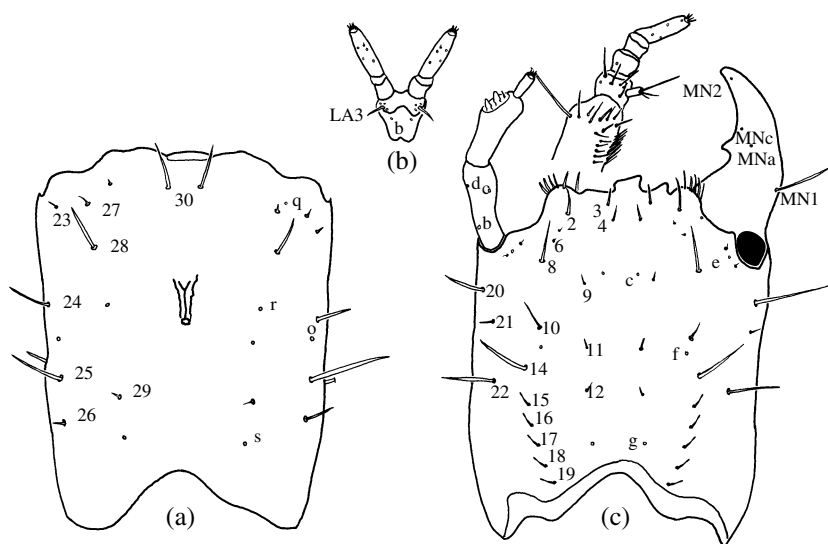


Fig. 4. Second instar larva of *Paratropinus*: a, venter of head; b, dorsal view of labium; c, dorsal view of head capsule, antenna and maxilla.

oblique apex, and 2 slightly divergent, smaller lateral teeth; central tooth with 2, and lateral teeth with one, minute sensoria, with visible conduits into epistoma; nasale anterolaterally with 5 medium length frontal marginal setae (gFR), in addition to pair of subnasal setae arising beneath nasale between lateral tooth and lateral frontal lobes; dorsum of head capsule with PA2–22 and PAb–h present (pore PAi possibly hidden by anterior pronotal margin), arranged as in Fig. 4(c); venter with short, shallow longitudinal depressions behind prementum, with PA23–26 laterally, PA27–29 midlaterally, PA30 at middle behind anterior margin, and PAn, o, q–s, PAK with seta. Antenna: scape with dorsal ANa, c near inner edge, and ANb, d near outer edge, pedicel with AN3 about one-third from base, apex with large sensoria SE1, SE2, with 4 smaller sensoria, 2 dorsad of SE2, 2 at mesal base of flagellar segment; flagellum with 5 apical sensillae. Mandible: narrowed and evenly curved from base to apex, with single, relatively small mesal tooth, and dense penicillus restricted to small membranous area near mesal base; with MN1 long, one-third from base, MN2 minute, near apex along dorsum, pore MNa near base of tooth, MNb below lateral margin two-thirds from base, MNc obscure, distal to tooth near mesal edge. Maxilla: stipes with gMX1 dense, arising from desclerotized mesal area, with few setae plumose near their apices; with MX2–6 present, gMX2 (along outer stipital margin) not evident; apical membrane of stipes with 4 non-articulated setae; galea (digitiform appendage of basal palpomere; Beutel, 1999) with 2 short (MX9 + unnumbered) and long MX8; basal palpomere with medium MX7 and MXe, its apical membrane with 3 non-articulated setae; palpomeres 2–3 lacking setae, palpomere 3 with single series of circumferential pores; apical palpomere with numerous pores, and about 5 small terminal setae. Labium: dorsum of prementum with LAb near apex of basal sclerotized portion, with MX3 in its apical membrane, with unnumbered pores anteromedially; ventral surface of prementum with LAa basal and close to medium LA2; basal labial palpomere without setae, with single dorsal and ventral pores; apical palpomeres missing from this individual.

Thorax (Figs 5, 6). Virtually all non-sclerotized regions densely microspinose (appearing finely granulate at lower magnification); segments separated by distinct intersegmental constrictions. Prothorax: PW = 0.66 mm; PL = 0.48 mm; pronotal shield well sclerotized, with thin median ecdysial line, with setae TE1–5, 7, 9–13, 17; TE21 inserted in front of anterolateral corner of pronotal shield, prominent; lateral propleural membrane with TE23, 24 and 27 on underside near anterior corner, and TE19 above insertion of procoxa, prominent; presternal sclerites lightly sclerotized; median presternal sclerite with PR7, 8; lateral presternal sclerite with PR4, 6; ST40 present on small sclerite immediately above coxa; prosternite with ST30, 44, 46; posterolateral sternite absent. Mesothorax: 5 major sclerites present above level of spiracle; median mesotergite strongly transverse, weakly desclerotized along thin ecdysial line,

with TE7–11, TEf–h; dorsolateral sclerite with TE3–5 and TEe, i; lateral sclerite with TE15–21 and TEj; anterior pleurite with one seta, PL24?; small posterior pleurite with PL27, 28; mesepisternum not seen; mesepimeron immediately posterodorsad of mesocoxa, with PL40; mesosternum with large median sclerite bearing ST30, 44, 46, lateral sternite absent; small anterolateral sclerite (precoxite of Kovarik & Passoa, 1993) with ST31, 32; ST36 borne separately on minute sclerite posterad and facing procoxa. Metathorax: 7 major sclerites present above level of spiracle; mesonotum with broadly ovoid median tergite, with fine median ecdysial line, bearing TE9–11 and TEf, g; TE1, 2 present, on separate very small sclerites near anterior segmental boundary (TE1 within intersegmental constriction); lateral portions of metatergite separated from median tergite (relative to mesotergite), bearing TE7, 8, and TEh adjoined to socket of TE8; dorsolateral tergite bearing TE3–5 and TEe, i; anterior pleurite with two setae (PL24? and 25?); posterior pleurite with PL27, 28; metepisternum not present as a sclerite, but minute PL34, 35 apparently present; metepimeron present, bearing PL40; metasternum with anterior portion (bearing ST30) partly separated from main portion (complete separation seen on abdominal sterna); main portion of metasternite bearing ST44, 46; minute anteromedian sclerites bearing ST29; anterolateral precoxite bearing ST31, 32; ST36 present on minute sclerite behind and facing mesocoxa.

Legs (Fig. 5a). All legs similar in form and chaetotaxy; coxa with approximately 8 medium setae around apical margin, with several minute setae around basal margin; trochanter with 2 pores TRc?, g?, and 2 medium setae, TR3?, 5?; femur with seta FE2 at middle of mesal surface, and FE3–8 at apex, FEb present dorsally between FE5 and FE6; tibia with irregular circumferential series of setae TI1–6 less than one-half from base, and 8–10 unhomologized apical setae; tarsungulus not divided into basal ring and apical claw (as shown by Kovarik & Passoa, 1993 for *Onthophilus*), with 2 basal setae.

Abdomen (Figs 5–7). Lacking organized transverse asperities; non-sclerotized integument densely microspinose; segments separated by strong intersegmental constriction; each segment further subdivided all the way around the body by weaker constriction into major anterior (bearing all sclerites) and minor posterior subsegments. Segment I (Fig. 5): 8 major sclerites present on each side above level of spiracle; additional paired minute sclerites bearing TE1 at anterior segmental margin; anterior series of 4 sclerites: median with single seta TE2; anterolateral tergite with TE3 and intimately adjoined pore TEe; minute sclerite with TE13? in membrane anterolaterad of TE3; lateral tergite with TE16; supraspiracular tergite with TE19–21 and pore TEj; posterior series of sclerites: median with TE9–11, TE9 with adjoined TEg, and TE11 with adjoined TEf; posterolateral tergite with TE6, 7, TE6 with adjoined TEi; mediolateral tergite with TE4; lateral tergite with TE5. Pleuron with 3 lateral sclerites; postspiracular sclerite with single

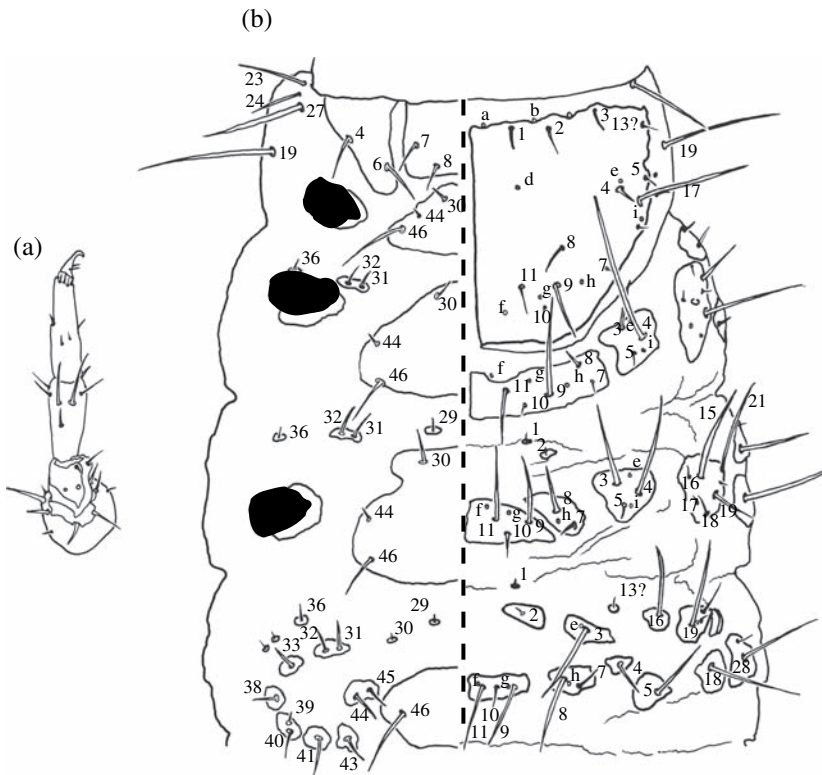


Fig. 5. a, ventral view of mesothoracic leg of *Paratropinus*; b, thorax and first abdominal segment of *Paratropinus*. Left, ventral view; right, dorsal view.

seta TE18; subpostspiracular pleurite with PL27?, 28 and large pore PLk (not recognized in Kovarik & Passoa, 1993); anterior pleurite with 4 setae, PL23–26?. Sternum with weakly developed proleg; sternum with large median sclerite, smaller paired anteromedian sclerites, and 7 sclerites surrounding each proleg; median sclerite with ST46; anteromedian sclerite with ST30; minute ST29 present anterior to ST30 in intersegmental constriction; first posterolateral sternite with ST44, 45, second with ST43, third with ST41; posterolateral sternite with ST40 and minute ST39; lateral sternite with ST38; first anterolateral sternite with ST31, 32, second with ST33; ST34–36 borne separately on minute sclerites anterolaterad of proleg. Segments II–VIII (Fig. 6): chaetotaxy identical to segment I; prolegs well developed. Segment IX (Figs 6 and 7): prolegs absent; lacking several sclerites found on *Onthophilus*; homologies particularly difficult to assign confidently; dorsally with anterolateral unisetose sclerite borne anterior to transverse fold, TE16?; major posterior subsegment with 3 major sclerites on each side; median tergite with TE9, mediolateral tergites with TE5, lateral tergites with TE19, 21?; 2 minute sclerites anteriorly on subsegment near transverse fold, each with seta, TE1 (anteriormost) and TE3? just posterolaterad; pleural region with 2 sclerites, larger posteriormost with PL26, 27, and a small seta of unassignable homology; small anterior pleurite with PL28; sternal region not clearly distinct from pygopodial region, with 4 small sclerites on each side anterior to pygopod, the anteromedial-most minute sclerite with ST30, immediately behind these a slightly larger mediosternite with ST46; posterolateral to

mediosternite a similar sized sclerite is present with PP6?; posterolateral to this is slightly larger sclerite with ST38, 40. Urogomphi (Figs 6 and 7): basally urogomphal sclerites fused more or less indistinguishably with basal urogomphal segment, with UG5?, 6? posterobasally, UG3?, 4?, 7? in circumferential series about one-third from base, and UG8–10 and UGc, d at apex of basal segment; apical segment with UG12, 13, and UGf (previously unrecognized, or possibly apically displaced UGe, which was not otherwise observed) at apex. Pygopod (Figs 6 and 7): dorsolateral sclerite with PP2, 3, and PPb basad of PP3 (possibly PP1 with seta lost); lateral sclerite with PP4; apparently single ventral sclerite with, on each side, PPa proximally and PP7 distally; sclerite with putative PP6 displaced anteriorly among sternites of segment IX.

Differences in putative instar I. Head with epicranial suture, stem present in basal one-third, lyriform lateral arms diverging from stem between PA11 and PA12, recurved posterad of Paf, extending anterolaterally to antennal foramen. Head and thoracic chaetotaxy identical to instar II. Egg bursters present on abdominal segments I and II. Seta TE2 apparently absent from these segments. Abdominal chaetotaxy otherwise apparently identical to that of instar II.

Comparative description of larva MSC1

The first instar larva MSC1 (near *Euxenister*) is quite distinct from that described above. Although we hesitate

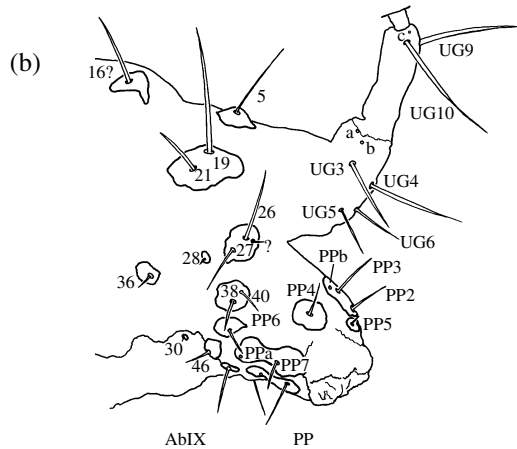
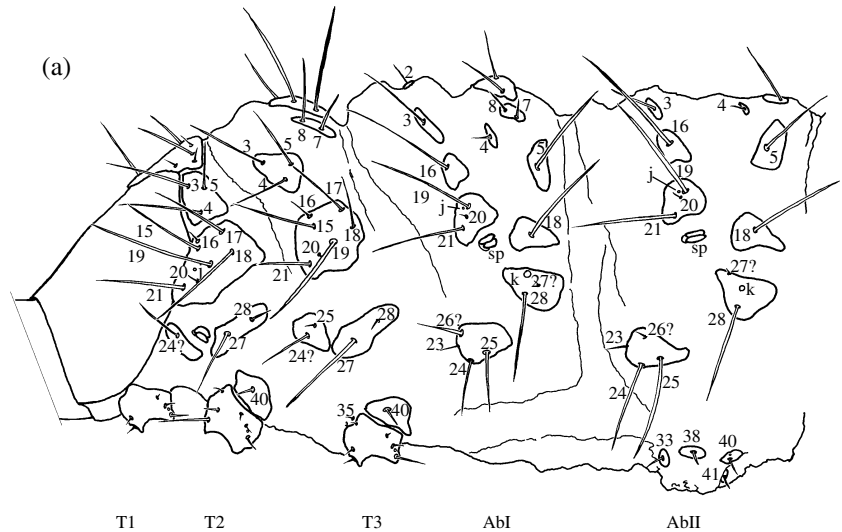


Fig. 6. Second instar larva of *Paratropinus* (legs from trochanter distad removed): a, lateral view of thorax and abdominal segments I, II; b, lateral view of abdominal segment IX, pygopod and base of urogomphus (see Fig. 7 for whole urogomphus).

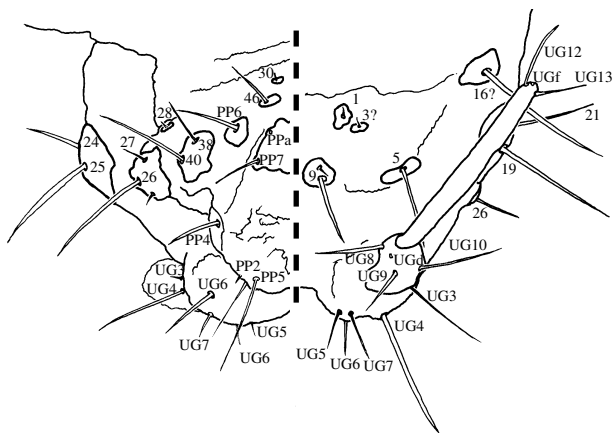


Fig. 7. Abdominal segment IX, pygopod and urogomphus of second instar larva of *Paratropinus*. Left, ventral view; right, dorsal view.

to present a full description based on a larva with such an incomplete identification, its morphology extends the known range of variation for hetaeriine larvae considerably, and this information will help in recognizing and placing additional larvae as they become available. In particular, the head and thorax possess numerous setae not present in the *Paratropinus* larva. This renders most setae of these regions difficult to homologize. In some cases, it appears that groundplan pores bear setae. For example, its median mesonotal sclerite bears 7 distinct setae, and only one pore, whereas that of *Paratropinus* bears 5 setae and 3 pores, all of which have obvious homologues in *Onthophilus*. In others, setae appear to have been lost, whereas pores remain (although with only a single specimen, it is impossible to discount the possibility that setae have simply broken off both sides). The clearest example here is on the frons, where PA11 is absent but a distinct pore is present at the site. This larva displays less than complete bilateral symmetry in chaetotaxy, with numerous nonmirrored arrangements of apparently homologous setae, as well as several cases of setae on one side of the

body without counterparts on the other. An obvious implication is that conspecifics may differ somewhat from the description presented here.

First instar. Cream-coloured, largely membranous, with head, pronotum and true legs only obvious sclerotizations; body slender, head and pronotum equally wide, body tapering evenly posterad (larva probably recently eclosed, and not yet distended). *Head capsule* (Fig. 8): HW = 0.53 mm; dHL = 0.42 mm; vHL = 0.47 mm; HD = 0.31 mm; head capsule slightly narrowed to base (in dorsal view), of relatively even depth from base to apex, texture very finely, deeply reticulate; nasale narrow, symmetrical, tridentate, with median tooth slightly elevated above the others, with a minute seta on each side between teeth; all major setae of head capsule distinctly blunt tipped; dorsal parietal setae PA2–5, 7–10, 12, 14–19 present (PA6, 11 absent); PA3, 4 more widely separated than in *Paratropinus*; PA10 inserted more anterad, not closely associated with PAF; head with 11 dorsolateral setae where only 4 (PA13, 20–22) are found above; pore present behind epicranial suture, posterolateral to pore PAF where none is present above (although may correspond to strongly displaced PAh, which is otherwise not seen); seta present posteriorly near epicranial stem, corresponding in position and possibly homologous to pore PAg; side of head with oblique series of 3 pores, the anteriormost possibly PAo; venter of head with setae PA23, 27, 28, 30 identifiable in similar positions to above; gular region with 3 setae in more or less longitudinal series on either side of tentorial bridge; posteriorly and laterally with numerous setae, including

PA24–26, 29, plus about 15 not corresponding to any observed in *Paratropinus* or *Onthophilus*. Most of these unique setae display imperfect bilateral symmetry. Mandible with distal inner edge finely serrulate from tooth to tip; maxilla, labium and antennae all short, segments relatively compressed, maxillary palpomeres 1–3 much broader than long; scape about as long as apical width; chaetotaxy of cephalic appendages as for *Paratropinus*.

Thorax (Figs 9, 10). PW = 0.53 mm; PL = 0.28 mm; pronotal shield with numerous setae unmatched, and unhomologizable with any in *Paratropinus*, most displaying imperfect symmetry; transverse series of about 10 setae along anterior margin, including, although none specifically identifiable as, TE1–3, 12, 13; lateral pronotal margin with 8 setae; central portion of disc with about 4 setae behind anterior margin, a pair of setae about midway to posterior margin (the anterolateral of which is probably TE8), and transverse cluster of 7 setae near base, including TE7, 9–11, and 3 lacking counterparts in *Paratropinus*; lateral propleural membrane with TE19, 23, 24, 27 recognizable just anterad of midpoint; median presternite weakly sclerotized, with PR7, 8; lateral presternite more distinctly sclerotized, with 3 setae, possibly PR4–6 (PR5 not seen in *Paratropinus*); median prosternite with PR30, 44, 46, as above; propleuron with PR40. *Mesothorax*: median mesotergite with 7 setae, TE7–11 in similar positions to above, plus 2 possibly inserted in pores TEf, h; one pore present; dorsolateral mesotergite with 4 setae, TE3–5 plus one not present in *Paratropinus*, possibly corresponding to either

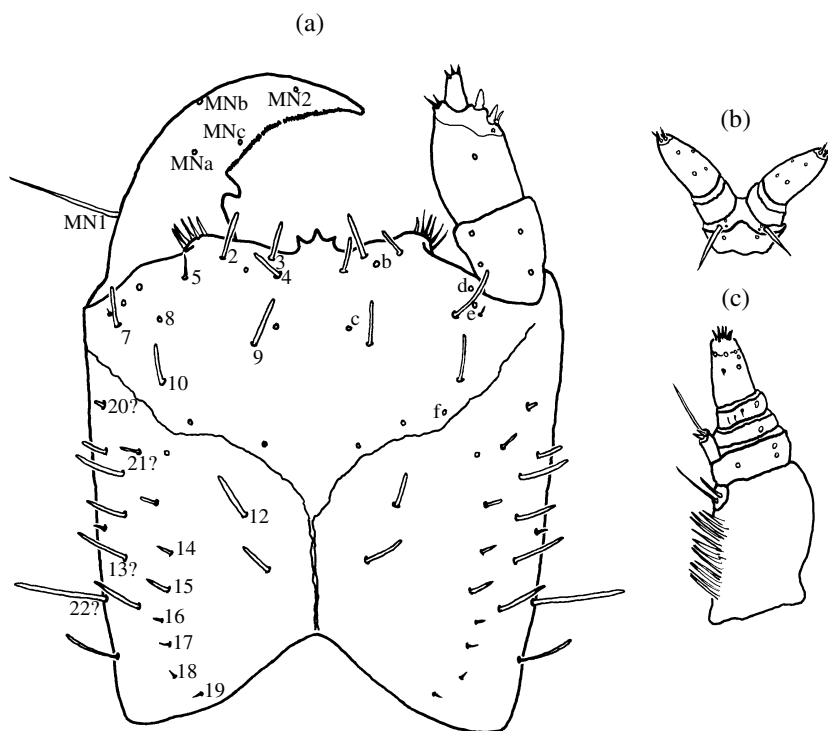


Fig. 8. Putative first instar larva of *Euxenister*: a, dorsal view of head capsule, left mandible and right antenna; b, dorsal view of labium; c, dorsal view of right maxilla.

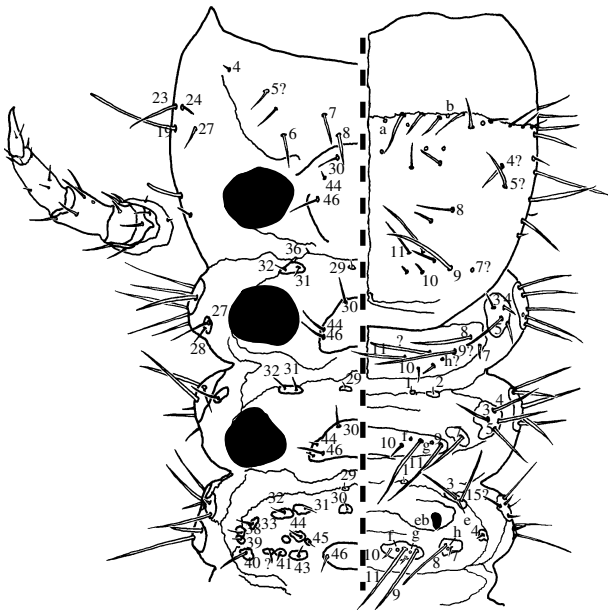


Fig. 9. Thorax and first abdominal segment of first instar larva near *Euxenister*. Left, ventral view; prothoracic leg at side; right, dorsal view.

TEe or TEi; lateral mesotergite with 6 setae, TE16–21 and one pore TEj, corresponding exactly to those of *Paratropinus* (although lacking TE15); small prespiracular pleurite with one seta PL23 or PL24; postspiracular pleurite with PL27, 28 (as above); mesepisternum not evident; metepipleuron slightly removed posterodorsad from coxa with PL40; mesosternum as above, although with ST36 situated more mesally, immediately anterad of ST31. *Metathorax*: median metatergite with 3 setae on each side, probably TE9–11, although arranged differently from above, with small TE10 displaced mesad, closest to ecdysial line, with 2 pores TEg, f, as above; minute anterior TE1, 2 as above; lateral metatergite with TE7, 8, lacking pore; dorsolateral and lateral tergites as on mesothorax; PL24 and PL25 present on separate minute sclerites anterodorsad of

metacoxa; PL27, 28 together on small sclerite as on mesothorax; metasternal chaetotaxy exactly as preceding segment.

Legs (Fig. 9). Well developed; despite lack of larval distension, relatively larger compared to body than in *Paratropinus*; chaetotaxy without obvious differences from *Paratropinus*.

Abdomen (Figs 9, 10). Egg burster (eb) present only on segment I, as a prominent posteriorly directed tooth on each side at the posterior margin of a large sclerite. *Segment I*: minute sclerite bearing TE1 in intersegmental constriction anteromesad of egg burster; median tergite bearing 2 long (TE9, 11?) and 2 short setae, one of the latter probably TE10, the other without homologue in *Paratropinus*, and 2 pores (TEf, g) free, not adjoining setal sockets as above; dorsolateral tergite with 2 setae (TE7, 8) and TEh; small dorsolateral tergite bearing TE4?; small sclerite anterolaterad of egg burster with 2 long setae, one corresponding to TE3, the other either TE4? or TE15?, with pore TEe between and adjoining socket of TE3; lateral (supraspiracular) tergite with TE16–19, 21 and TEj; postspiracular pleurite as on mesothorax; subspiracular pleurite with PL24, 25 together on one sclerite (separated on metathorax); sternum with prolegs weakly developed; sternal chaetotaxy as for AI of *Paratropinus* except ST31, 32 borne on separate sclerites, ST44, 45 borne on separate sclerites, and ST42 present at inner corner of proleg (apparently on one side only, and not on subsequent segments); ST39, 40 borne on separate sclerites on left side, but together on right. *Segments II–VIII*: identical to segment I except small sclerite in place of egg burster, with one pore (corresponding exactly in position to seta TE2 on abdomen of *Paratropinus*); small sclerite bearing TE13 anterad of TE3; 2 small sclerites also present posterolaterad of TE4?, each with minute seta, possibly corresponding to TE5? and TE18?; prolegs well developed. *Segment IX*: chaetotaxy as for *Paratropinus* except lacking PL28, PL36 (and their associated sclerites). *Urogomphi* (no figure): basal sclerotized elements more or less fused

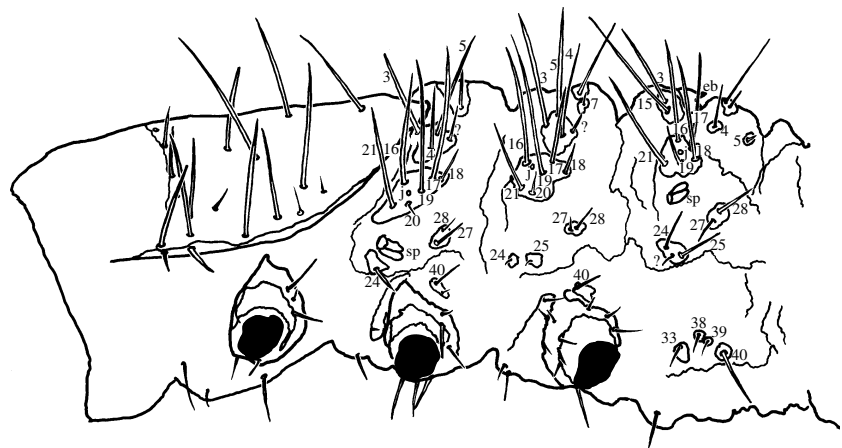


Fig. 10. Lateral view of pro-, meso- and metathorax, and abdominal segment I of first instar larva near *Euxenister* (legs removed).

to basal urogomphal segment as above, with UG5–7 forming transverse basal series from the posterior to the inner surface, and UG3, 4, and 2 additional unique setae forming slightly more distal (although still within basal half) transverse series, extending around the posterior aspect from the lateral to the mesal surface; UG8–10 and UGc, d at apex of basal segment as above; apical urogomphal segment with 2 long (UG12, 13) and one minute (unique) apical setae. *Pygopod*: as for *Paratropinus* except PP5 and PP2 (distal-most dorsal setae) borne together on small sclerite.

Discussion

The larva of *Paratropinus scalptus* (AKT3) contrasts greatly with the distinctive MSC1 (near *Euxenister*) larva. This latter exhibits numerous unique setae relative to all other known histerid larvae, as well as blunt-tipped parietal setae, serrulate inner mandibular margins, small symmetrical nasale and short cephalic appendages. This distinctness is reflected in both the nucleotide divergence (of both genes examined here) and, to a certain degree, in the adult morphology of its nearest identifiable relative, *Euxenister*, whose phylogenetic position is very uncertain. Although this larva is the most distinctive of those examined here, that is not to say that the others lack distinctive features. The first instar we attribute tentatively to *Paratropinus* possesses a highly distinctive second pair of egg bursters (on the second abdominal dorsum) unknown in any other histerids (and not present in the near *Euxenister* larva).

Only preliminary suggestions can be made as to possible diagnostic characters of hetaeriine larvae based on these data. All specimens are separated minimally from non-Histerinae by the combination of three maxillary and two labial palpomeres, the dorsal surface of the prementum sclerotized and lacking small teeth at its base, and a lack of lateral teeth on the prementum. Separation from Histerinae is likely to prove more difficult. Although Hetaeriinae still is recognized formally as a subfamily, it has become increasingly clear that it is derived from within Histerinae (Caterino & Vogler, 2002). Consistently distinguishing hetaeriine larvae from those of other histerine tribes may well prove impossible, particularly considering the vast diversity of neotropical Exosternini, virtually none of whose larvae are known. Nonetheless, we suggest that the uniform covering of microtrichiae, the presence of five supraspiracular mesonotal sclerites and a lack of well-defined transverse asperities may distinguish many Hetaeriinae. In addition, the larvae we have seen all possess a more or less tridentate nasale, with the median tooth either broad (*Paratropinus*) or acute (nr. *Euxenister*), and the lateral teeth less prominent, as opposed to the quadridentate nasale of most Histerinae. Although these characters hold at least against known Histerini, Exosternini and Platysomatini, much descriptive larval taxonomy remains for all these groups.

It is worth briefly contrasting the larvae we have identified with termitophilous forms previously considered likely

to be Hetaeriinae by other workers (A. F. Newton, pers. comm., and unpublished tentative identifications). Those larvae have only weak thoracic and abdominal sclerotization (from T2 posterad), completely lack segmented urogomphi and bear fleshy lateral protuberances on T2 and T3 and abdominal segments 1–8. In an unpublished key, A. F. Newton further noted that these specimens lacked mesal mandibular teeth. One of these collections was associated with adult *Homalopygus latipes* Boheman. At least the moderate physogastry, as well as perhaps the reduced dorsal sclerotization, might have been considered morphological adaptations to the myrmecophilous lifestyle. That the larvae identified herein lack any such obvious specializations is somewhat unexpected. Although we have no particular doubt about these previous tentative associations, they must be reconsidered as divergent, rather than typical, examples.

The circumstances in which the larvae described here were collected shed a glimmer of light on their habits. When discussing coleopteran myrmecophily, virtually all authors have concentrated on behavioural and morphological adaptations of the adult beetles. The niche of the larvae has rarely received attention. Considering the highly mobile colonies of army ants, the predominant hetaeriine hosts in the Neotropics, we know that some adult Hetaeriinae can follow ant trails (Akre & Rettenmeyer, 1968), may run along or ride on hosts in emigration columns (Reichensperger, 1926; Akre, 1968) and may fly to find colonies. Of these, larvae are capable only of riding or being carried. An association with the starchy phase of the army ant colony would seem more likely (Akre, 1968). Indeed, Torgerson & Akre (1970) found the ovaries of *Euxenister* adults enlarged and apparently ready to oviposit near the end of the nomadic cycles of their respective hosts. Both samples that produced hetaeriine larvae for this study were collected in association with *Eciton* colonies, although in varied phases of the ant cycle. The larvae of *Paratropinus* indeed were collected in 'refuse deposits' of a starchy phase *E. burchelli* bivouac. The 'nr. *Euxenister*' larva, however, was sifted from litter beneath a large, temporary bivouac of nomadic *Eciton burchelli*. A few hours later these ants had moved on. Whether the larva would have moved on too, or been carried along, cannot be known.

Finding *Paratropinus* larvae in association with a starchy phase bivouac supports Akre's (1968) idea that, in some Hetaeriinae, there might be considerable synchrony in host–guest life cycles. However, the contrasting data for MSC1 indicate that, as with known adult behaviour, there is significant variation in larval habits. The starchy phase of *Eciton* colonies corresponds to their pupal phase (Schneirla & Topoff, 1971), and would represent the only time in the colony life cycle when limited mobility of the beetle larvae would not pose a problem. Furthermore, this is the period in the ant life cycle when accumulations of refuse occur. *Eciton burchelli* are noted for producing nutrient-rich refuse deposits (Gottwald, 1982). Given the brevity of this phase (less than 3 weeks), the general brevity of the histerid larval stage is notable. Histerids are unusual amongst beetles in

passing rapidly through only two larval instars before pupating (Lawrence, 1991; Newton, 1991; Kovarik & Passoa, 1993). This might represent an important preadaptation to myrmecophily, particularly to associations with army ants, and might help explain histerids' prevalence and diversity in this unusual niche.

Finally, although the trees presented here were not intended to provide great insight into hetaeriine phylogeny, we discuss some details. These trees show largely congruent relationships and, at less inclusive levels, considerable agreement with Helava *et al.*'s (1985) hypothesis. Our sampling for their 'group E' is particularly dense, and most of these genera are resolved in our trees as closely related (*Cheilister*, *Clientister*, *Paratropinus*, *Psalidister*, *Nymphister*, *Pulvinister*, *Aphanister*, *Daitrosister* and *Anasynodites*). Both genes resolve *Euclasea* as close to these, whereas Helava *et al.* (1985) include this in a group ('D') with *Troglosternus* and *Reninus*. Our 18S tree finds much of Helava *et al.*'s 'group C' intact as well, including in our sampling *Iugulister*, *Ulkeus* and related forms, and *Opadosister*. This clade also, however, includes *Tylois*, considered part of a separate lineage potentially containing *Euxenister* and *Terapus* ('subgroup B2') by Helava *et al.* Our sampling of their 'subgroup B1' included *Scapicoelis* and *Synoditulus*, which did not resolve together here, nor near any other 'group B' taxa. Interestingly, *Synoditulus* (for which we have only an 18S sequence) did not resolve within Hetaeriinae at all, in keeping with previous ambivalence about its inclusion (Helava *et al.*, 1985; Caterino & Vogler, 2002).

This study advances our understanding of hetaeriine biology along several fronts. In addition to eventually identifying and describing larvae of Hetaeriinae for the first time, we have identified some potentially useful variation in larval characters to aid in resolving relationships within Hetaeriinae, as well as for their placement relative to other lineages of Histerinae. Although hetaeriine larvae may not be easy to collect, targeting stately bivouacs of *Eciton* throughout the Neotropics (particularly those that appear to be used repeatedly, accumulating large refuse deposits) is likely to produce additional specimens and taxa. We also highlight the potential of nucleotide sequences for associating unknown larvae, whereas, in the past, most dead, unassociated larval collections have been considered dead ends. Proper preservation, however, in high-proof ethanol, and preferably isolated as individual specimens, must be emphasized, as soft-bodied larvae seem quite likely to leak body fluids and easily cross-contaminate each other. Ultimately, Hetaeriinae still require a tremendous amount of work, including the basic description of large numbers of species and genera, but the study of this fascinating group is becoming increasingly tractable.

Supplementary material

Supplementary material is available at www.blackwell-synergy.com under DOI reference doi: 10.1111/j.1365-3113.2006.00338.x.

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