

The discovery after 94 years of the elusive female of a myrmecolacid (Strepsiptera), and the cryptic species of *Caenocholax fenyesei* Pierce sensu lato

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Due to its extreme sexual dimorphism and disparate hosts, no female myrmecolacid has been matched to its conspecific male to date. Here, for the first time to our knowledge, a morphological description is given of the matched female and male myrmecolacid, *Caenocholax fenyesei waloffi* ssp. nov. from Veracruz, Mexico: the female parasitic in a cricket and the male parasitic in an ant. For examined segments of DNA, the male and female are identical. Male *C. fenyesei* Pierce sensu lato was described 94 years ago from Veracruz. The male from Texas USA, which, for the same DNA segments, shows 15% divergence from the morphologically identical male from Veracruz, is given subspecies status, and is named *Caenocholax fenyesei texensis* ssp. nov. The discovery of the female finally enables many interesting studies to be pursued, such as speciation in morphologically cryptic taxa, the sexes of which parasitize disparate hosts. *Caenocholax fenyesei* sensu lato may also be evaluated for biocontrol of the red imported fire ant, *Solenopsis invicta* Buren, which is a pest in the USA and Australia.

Keywords: Strepsiptera; Myrmecolacidae; *Caenocholax fenyesei* sensu lato; Mexico; Texas

1. INTRODUCTION

The discovery of a female myrmecolacid parasitic in an orthopteran comes 94 years after the free-living male *Caenocholax fenyesei* sensu lato was described from the same area in Mexico (Pierce 1909), and is a major breakthrough in the study of Strepsiptera. The family Myrmecolacidae exhibits an extreme form of heterotrophic heteronomy (Kathirithamby 1991), where males develop in ants, and the females in grasshoppers, crickets and mantids (Ogloblin 1939; Kathirithamby 1989; Kathirithamby & Hamilton 1992). This feature, together with the fact that the sexes exhibit extreme dimorphism in Strepsiptera, means that it is impossible to match females and males

morphologically. The males and females of only two species of this family have been described so far, and even these matches might be conjectural. Among the 108 Myrmecolacidae described, the ant hosts of only eight species of males are known (see tables 1 and 2 in electronic Appendix A, available on The Royal Society's Publications Web site). The rest of the species have been described from free-living males that have been caught in traps.

Previous records of female myrmecolacids were mostly of single specimens, except for two instances from Papua New Guinea: 15 specimens of *Stichotrema jeyasothiae* Kathirithamby (Kathirithamby *et al.* 2001) and large numbers of female *S. dallatorreanum* Hofeneder, a parasite of an oil palm pest (Solulu *et al.* 1998). Females parasitic in *Hapithus agitator* Uhler (Gryllidae) were originally identified as *C. fenyesei* (Cook *et al.* 1996). However, molecular analysis confirmed that this identification was incorrect (Halbert *et al.* 2001). Kathirithamby & Hamilton (1992) discussed the intriguing phenomenon of the scarcity of female Myrmecolacidae.

Male *C. fenyesei*, described by Pierce in 1909, is the type species by monotypy and we here propose two nominotypical subspecies of the Fenyesei group. Kifune (1979) stated that, except for records dating from 1935 of a dead male in a banana thrash boat from Panama and Argentina (Bohart 1941), no distributional data are available. However, males of morphologically identical *C. fenyesei* sensu lato have since been found in large numbers in traps from the neotropics and southern USA (Kathirithamby 1992; Kathirithamby & Hughes 2002), and are parasitic in several species of ants throughout the neotropics and southern USA (see table 1 in electronic Appendix A).

We present, to our knowledge, the first morphological description of a female myrmecolacid, parasitic in a cricket *Macroanaxipha mecilenta* (Saussure) and that was confirmed by molecular characterization as a 100% match to a sexually dimorphic male parasitic in an ant *Dolichoderus bispinosus* Olivier (J. Kathirithamby, L. D. Ross, G. Moya-Raygoza, D. P. Hughes and J. S. Johnston, unpublished data). It has hitherto been impossible to match males and females of Myrmecolacidae because of their heterotrophic heteronomous biology and their extreme sexual dimorphism. Now, however, with the help of molecular tools, one such match has been established.

During the present study it was also found that the male *C. fenyesei* sensu lato from Texas parasitic in *Solenopsis invicta* Buren is 15% divergent from the morphologically identical male from Mexico, parasitic in *D. bispinosus* (J. Kathirithamby, L. D. Ross, G. Moya-Raygoza, D. P. Hughes and J. S. Johnston, unpublished data). Hence, *C. fenyesei* sensu lato from Texas is given subspecies status. It is not clear whether these are (hitherto unrecognized) reproductive isolated sibling species that parasitize disparate hosts, or if the sexes exhibit distinct (disparate) host preferences in the absence of any barriers to gene flow between races.

(a) *Myrmecolacidae* Saunders

Myrmecolacides Saunders (1872).

Myrmecolacidae Pierce (1908).

Strichotrematoidae Hofeneder (1910).

Stichotrematidae Hofeneder (1910).

(b) *Caenocholax Pierce**Caenocholax* Pierce (1909).

Type species: male, *Caenocholax fenyesei* Pierce, Córdoba, Veracruz, Mexico USNM type 10081, originally part of the Fenyese collection at the California Academy of Sciences, San Francisco.

Aedeagus: shield-shaped with a pair of lateral spines and a ventral projection.

Host: unknown.

(c) *Caenocholax fenyesei* Pierce *sensu lato*

Caenocholax fenyesei Pierce (1909, 1911, 1918); Bohart (1941); Kifune & Brailovsky (1987, 1988, 1997); Kathirithamby & Johnston (1992); Maes & Kathirithamby (1993); Kathirithamby & Peck (1994); Kathirithamby & Hughes (2002).

(d) *Synonymy*

Caenocholax brasiliensis Oliveira & Kogan (1959), Tesón & de Remes Lenicov (1979) (sym. nov.) is synonymized with *C. fenyesei* *sensu lato* based on the unique structure of the aedeagus and the ninth abdominal segment.

2. DESCRIPTION**(a) *Caenocholax fenyesei waloffi* ssp. nov****(i) *Female***

The extruded cephalothorax lies flat on the host abdomen (figure 1a–c), light brown with rounded anterior region; brood canal opening broad. The cephalothorax always extrudes through the abdominal pleurites of the host and is cryptic (figure 1a). Numerous spines on inner surface of mandibles (figure 1c).

Genital apertures/invasions in the apron: one on each of the second and third abdominal segments.

Cephalothorax length of 0.55 mm; width of 0.43 mm.

Brood canal opening length of 0.18 mm; width of 0.33 mm.

Total length of female of 1.91 mm.

First-instar larva: total length of 0.89 mm.

(ii) *Host*

Macroanaxipha macilenta (Saussure) (Orthoptera: Gryllidae: Trigonidiinae) (figure 1a) is most abundant during the evening after 18.00.

Distribution: neotropical.

(iii) *Male*

Morphologically identical males have been described from Texas (Kathirithamby & Johnston 1992), the Bahamas (Kathirithamby & Peck 1994), Belize (Kifune & Brailovsky 1997), Nicaragua (Maes & Kathirithamby 1993), Argentina, Brazil, Costa Rica, Guatemala, Mexico, Panama, Trinidad and Venezuela (Kathirithamby & Hughes 2002). The aedeagus is unique for Strepsiptera. All other species examined have a variation of a simple hook-shaped structure. In *C. fenyesei* *sensu lato*, the distal end is shield-shaped with two shorter lateral spines and one longer central spine (Kathirithamby & Johnston 1992).

(iv) *Host*

Dolichoderus bispinosus Olivier (Formicidae: Dolichoderinae) (described as Strepsiptera: Myrmecolacidae;

Hughes *et al.* 2003) from Veracruz, Mexico (J. Kathirithamby, L. D. Ross, G. Moya-Raygoza, D. P. Hughes and J. S. Johnston, unpublished data) (figure 1d).

Distribution: tropical species—neotropical.

(v) *DNA analysis*

The female parasitic in the cricket *M. macilenta* is 100% identical to the male parasitic in the ant *D. bispinosus* (J. Kathirithamby, L. D. Ross, G. Moya-Raygoza, D. P. Hughes and J. S. Johnston, unpublished data).

(vi) *Collection of taxa*

Estación de Biología Tropical 'Los Tuxtles', Instituto de Biología UNAM, 30 km Carretera Catemaco, Montepio Apdo, San Andrés, Tuxtla, Veracruz, Mexico, (18°35' N 95° 5' W), between 20 and 24 June 2002.

(vii) *Etymology*

C. fenyesei waloffi ssp. nov. is named after the late Nadia Waloff.

(viii) *Voucher specimens*

Two neotenic female endoparasitic in *Macroanaxipha macilenta* (Saussure) (Orthoptera), Mexico: Estación de Biología Tropical 'Los Tuxtles', Instituto de Biología UNAM, 30 km Carretera Catemaco, Montepio Apdo, San Andrés, Tuxtla, Veracruz, Mexico, (18°35'N, 95°5'W), June 2002 (G. Moya-Raygoza) (Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, in alcohol).

Two neotenic female endoparasitic in *M. macilenta*, Mexico: same data as above (Oxford University Museum of Natural History, Oxford, UK, in alcohol).

Two male pupa endoparasitic in *Dolichoderus bispinosus* Olivier (Formicidae), Mexico: same data as above, June 2002 (D. P. Hughes) (Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, in alcohol).

Two male pupa endoparasitic in *D. bispinosus*, Mexico: same data as above (University Museum of Natural History, Oxford, UK, in alcohol).

(b) *Caenocholax fenyesei texensis* ssp. nov**(i) *Male***

The morphologically identical males from Texas, USA, collected from Bee Creek and Lick Creek, are 16.5% and 14.3% divergent, respectively, based upon D2 and V2 nuclear gene alignments from male *C. fenyesei waloffi* ssp. nov. from Veracruz (J. Kathirithamby, L. D. Ross, G. Moya-Raygoza, D. P. Hughes and J. S. Johnston, unpublished data), and is given subspecies status.

(ii) *Host*

The red imported fire ant, *Solenopsis invicta* Buren (Formicidae: Myrmicinae) (Kathirithamby & Johnston 1992). Distribution: north Argentina, southeast Brazil, Caribbean Islands, USA (Arkansas, Florida, Texas, south California, Mississippi) and Australia.

(iii) *Female*

Unknown.



Figure 1. Female cephalothorax and male pupa of *Caenocholax fenyesei waloffi* spp. nov. (a) *Macroanaxipha macilenta* (Saussure) (Gryllidae) with extruded cephalothorax of female *Caenocholax fenyesei waloffi* spp. nov. (arrow). Magnification $\times 8$. (b) Scanning electron micrograph (SEM) of cephalothorax of *Caenocholax fenyesei waloffi* spp. nov. The arrow indicates a mandible. Scale bar, 0.1 mm. (c) SEM of spines on the mandible on the cephalothorax of *Caenocholax fenyesei waloffi* spp. nov. Scale bar, 10 μm . (d) *Dolichoderus bispinosus* Olivier (Dolichoderinae) with male pupa of *Caenocholax fenyesei waloffi* spp. nov. (arrow). Magnification $\times 7.5$.

(iv) *Etymology*

C. fenyesei texensis ssp. nov. is named after the state of Texas where this particular subspecies was first found parasitizing the red imported fire ant, *S. invicta* (Kathirithamby & Johnston 1992).

(v) *Voucher specimens*

Four males with cephalothecae, USA, Texas, Bee Creek Park, College Station, Brazos Co., March 1988 (J. S. Johnston) (University Museum of Natural History, Oxford, UK) (ref. Kathirithamby & Johnston 1992).

3. DISCUSSION

This is the first morphological description of a sexually dimorphic male and female myrmecolacid that are 100% match (J. Kathirithamby, L. D. Ross, G. Moya-Raygoza, D. P. Hughes and J. S. Johnston, unpublished data): female *C. fenyesei waloffi* ssp. nov., parasitic in an orthopteran, *M. macilenta*, and the male parasitic in an ant, *D. bispinosus*, from Veracruz, Mexico. The male *C. fenyesei waloffi* ssp. nov. differs by 16.5% and 14.3% divergence, respectively, based upon D2 and V4 nuclear gene align-

ments, from the morphologically identical male, *C. fenyesei texensis* ssp. nov., from Bee Creek and Lick Creek, TX, USA (parasitic in *S. invicta*). This divergence might be due to host speciation in the Fenyesei group.

Host speciation takes place when the gene flow between populations parasitizing different hosts is drastically reduced, so that morphologically identical parasites and/or parasitoids develop host races and thus sibling species and subspecies status. At the present time, five ant species have been recorded as hosts (see table 1 in electronic Appendix A), but only three—*S. invicta*, *Camponotus planatus* Roger (Kathirithamby & Hughes 2002) and *D. bispinosus*—can be confirmed as definite hosts. We record a new model of genetic change associated with host shifts, where the male might shift to a different host ant species with or without a concomitant shift of the sexually dimorphic female to an orthopteran species. Unlike in other host shifts reported, the first-instar free-living larva seeks and parasitizes the disparate hosts. Phenotypically, the first-instar larva is not sexually dimorphic. Given the disparate hosts of Myrmecolacidae, the question of sex determination arises. The wide distribution of *C. fenyesei*

sensu lato, and the host shifts observed and inferred mean that the host races and perhaps morphologically cryptic species of the Fenyesi group exist.

With the discovery of the female *C. fenyesi waloffi* ssp. nov. many studies can finally be carried out, of which two of the most important and urgent are the sex-determining mechanism and the potential of *C. fenyesi* sensu lato as a biocontrol agent for the red imported fire ant, *S. invicta*, in the USA and Australia.

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