We do not select, nor are we choosy: reproductive biology of Strepsiptera (Insecta)

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The cryptic entomophagous parasitoids in the order Strepsiptera exhibit specific adaptations to each of the 34 families that they parasitize, offering rich opportunities for the study of male–female conflict. We address the compelling question as to how the diversity of Strepsiptera (where cryptic speciation is common) arose. Studying 13 strepsipteran families, including fossil taxa, we explore the genitalic structures of males, the free-living females of the Mengenillidia (suborder), and the endoparasitic females of the Stylopidia (suborder). Inferring from similarity between aedeagi of males either between congeners, heterogeners, or between species within the same taxonomic family, the same of which is true of the cephalothoraces of females, we predict that male–female conflict and a co-evolutionary morphological arms race between sexes is not likely to exist in most species of Strepsiptera. We then review the non-genitalic structures that play a role during sexual communication, and present details of copulatory behaviour. We conclude that Strepsiptera fall within the synchronous sensory exploitation model where short-lived males take advantage of a pre-existing sensory system involving pheromone signals emitted by females. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **00**, 000–000.

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INTRODUCTION

Divergent evolution of males is one characteristic of sexual selection, which, in insects, is more apparent in the external genitalia than in any other morphological structure. That characters of the male genitalia used during sperm transfer are important became apparent when Waage (1979) reported that the damselfly penis served the dual function of sperm removal and transfer. Eberhard (1993) went so far as to state that the elaborate male genitalia in some insects are as dramatic as the plumage of birds. Although it is now known that sexual selection has an effect on insect genitalia, this concept has not been explored in parasitic insects.

The traditional model involving female mate choice (Eberhard, 1985) predicts that the morphological characters of males coming into contact with a female during sexual reproduction are subject to selection, and that they diverge more rapidly, and into more elaborate forms, than other nonsexual structures. The morphological characters of males are often species-specific, and are routinely consulted

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for species identity (West-Eberhard, 1983; Eberhard, 1985, 1996, 2004a, b, 2009; Andersson, 1994). In most species, the genitalia of males present more discerning information than any other aspect of their morphology, behaviour or physiology (Eberhard, 2009).

When males engage in an evolutionary arms race, or in antagonistic coevolution with females for the control of reproduction, selection towards rapid divergence of copulatory structures often ensues. This selection is driven by male competition and results in the co-evolution of corresponding female structures (Parker, 1979; Rowe, 1994; Alexander, Marshall & Cooley, 1997; Holland & Rice, 1998; Partridge & Hurst, 1998; Gavrilets, 2000). The 'lock-and-key' hypothesis proposed by Dufour (1844) concerns the rapid divergence of morphological traits, which prevents cross-fertilization among species, with females evolving complex genitalia to exclude heterospecific males. The concept envisaged by Dufour's hypothesis was considered to apply to most insects with internal insemination but, within the vast diversity of insects, there are exceptions to this rule (Eberhard, 1985; Shapiro & Porter, 1989).

The new arms race model predicts that females initiating an encounter with a conspecific male (e.g. via species-specific long-range pheromone) are 'protected' from unwanted attention and harassment by heterospecific males (Eberhard, 2004a). In this context, a male–female conflict and a co-evolutionary morphological arms race for control of reproduction are less likely to evolve.

Genital structures of females that do not come into contact with males during copulation may have some function other than reproduction (e.g. defence: Darwin, 1871; Eberhard, 1985; Alexander *et al.*, 1997). In these cases, the sexual antagonistic co-evolution (SAC) hypothesis predicts that males would lack species-specific characters because there would be no corresponding female characters to which they need to adapt (Eberhard, 2004b). When there has been no arms race between the sexes, a simple design results together with 'secondarily' lost or reduced structures (Eberhard, 2009).

Genitalia should also not diverge rapidly in those species that have evolved in isolation from close relatives, as on islands or in caves, or, indeed, in parasites that are isolated when they parasitize different host species (Eberhard, 2009). Yet the data do not always exhibit this trend (Eberhard, 1985) and, in some of these groups, the male genitalia have distinguishing features (Poinar & Herre, 1991). There are also groups where the morphology of the female is not species-specific but that of the male is (Eberhard, 2004a, b).

Species-specific genitalia are rare in parasitic Hymenoptera, perhaps because of monandry in females (Eberhard, 1985). Arnqvist (1998) found that male genitalia are morphologically less similar in polyandrous species than in monandrous species. Post-mating sexual conflict and sexual selection will be absent or minimal in monandrous species, and polyandrous species will tend to be more speciose (Arnqvist *et al.*, 2000). The hypothesis is that the genitalia of males in monandrous species are simple and slow evolving. Soft female genitalic structures are also less likely to be involved in co-evolutionary arms races with males, and thus select less rapid divergence in male genitalia (Eberhard, 2004a).

A related trend is towards simplicity of males and variation of the females' external morphology in the body part where sperm is deposited, as seen in cimicid bugs Eberhard, (2006). Via a sharp intromittent organ, cimicid males directly inject sperm, together with accessory fluids, into the body of the female (traumatic insemination). Females, in turn, have developed a secondary genital system, the ectospermalege, lying over an area filled with haemocytoid cells (Carayon, 1966). In cimicids, spermaleges are highly divergent and genera specific. This is because females have adapted to control fertilization rather than intromission (Eberhard, 2006). Traumatic insemination is assumed by some to occur in Strepsiptera (Tatarnic, Cassis & Siva-Jothy, 2014), although this conclusion appears to be too general to accurately reflect the diversity of mating systems in Strepsiptera (see below).

Cryptic species with identical or near-identical genitalia have evolved less rapidly and distinctly than closely-related species that have distinct genitalic structures (Eberhard, 1985). One exception comprises fireflies, where females are protected from male harassment because they must signal their location to mate-seeking males, thereby putting females in 'control'. Male genitalia are typically species-specific in this type of system (McDermott & Buck, 1959; Lloyd, 1997).

Both the traditional sexual selection model and the new arms race model predict the rapid divergence of genitalia. These two types of models differ in the genitalic functions and the ecological contexts in which morphological divergence of genitalia occurs (Eberhard, 2004a). Although taxonomic surveys of morphology have failed to show or support the SAC hypothesis (Eberhard, 2004b), it still follows that SAC may exist in physiological traits (Eberhard, 2004b).

Evolutionary trends predicted by the SAC hypothesis for insects including Strepsiptera were studied by Eberhard (2006). The study had drawbacks in that genitalia might have been used to distinguish

congeneric species, although there might have been only subtle differences among species. This would have led to 'overestimates' of the value of male genitalia in species recognition. Accordingly, Eberhard (2006) suggested reclassifying these genitalia as 'lacking differences', although, initially, they were said to differ intra-generically, especially in cimicids and strepsipterans. Furthermore, Eberhard (2006) had combined eight families of Strepsiptera into a single group, instead of studying each family separately.

Here, we examine mating systems of the Strepsiptera and give a comprehensive account of the genitalic and non-genitalic traits used during sexual contact (insemination) between males and females in 48 genera of 13 extinct, basal, and extant families. We also describe details of mating behaviour and discuss monandry in females.

Furthermore, we examine morphological traits of the aedeagi of free-living males, the birth opening of free-living females of the basal family Mengenillidae, and the cephalothorax of the endoparasitic females of the Stylopidia (with derived characteristics), comprising 13 families (both extinct and extant) and 48 genera of Strepsiptera (see Supporting information, Data S1). We also study the literature on Strepsiptera regarding the non-genitalic structures (e.g. sensory receptors) that play a role during sexual communication, mediating male attraction and mating behaviour.

GENERAL OVERVIEW OF STREPSIPTERA

Strepsiptera are a taxonomic order of the Insecta divided into two suborders: Mengenillidia and Stylopidia (Kinzelbach, 1971; Kathirithamby & Engel, 2014). As parasitoids, Strepsiptera display a variety of unusual genetic and phenotypic features (Kinzelbach, 1971, 1978; Kathirithamby, 1989, 2009; Pohl & Beutel, 2005; McMahon, Hayward & Kathirithamby, 2011). Strepsiptera (especially Stylopidia) exhibit extreme sexual dimorphism and are obligate parasitoids in the larval stages which undergo apolysis without ecdysis (Kathirithamby et al., 1984). In the basal suborder Mengenillidia, males and females emerge to pupate outside their hosts (Fig. 1A) (Parker & Smith, 1933, 1934; Silvestri, 1940, 1941a, 1943). In the derived suborder Stylopidia, males pupate when they are still partially embedded within their hosts,
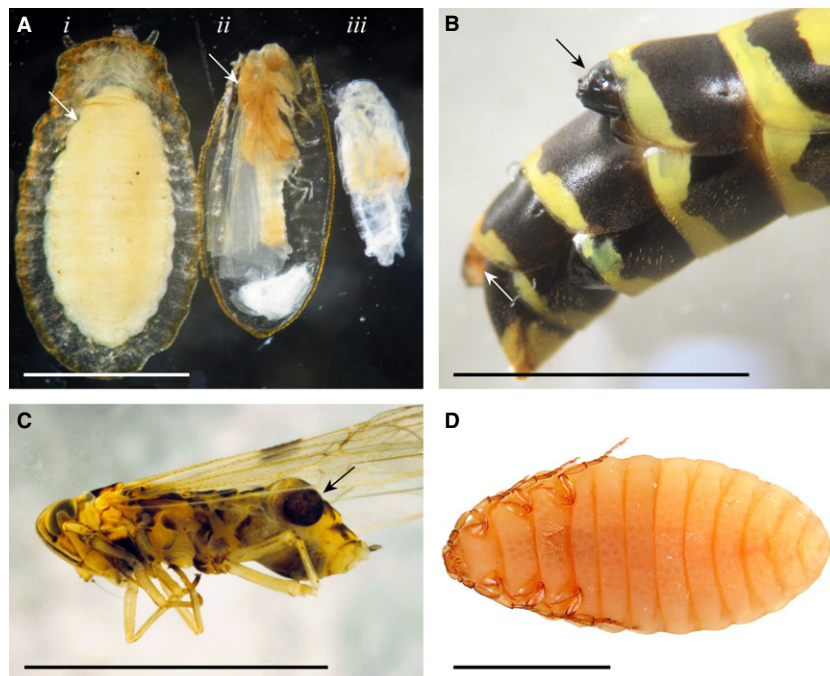

Figure 1. A, *Mengenilla* sp.: (*i*) Neotenic female (arrow) enclosed in a puparium. (*ii*) Adult male (arrow) emerging from a puparium with shed pupal and prepupal cuticles at the base of the puparium. (*iii*) Shed cuticle of male prepupa (taken out of the puparium). B, paper wasp (*Polistes dominula*) parasitized by male *Xenos vesparum*, male cephalotheca (black arrow), female cephalothorax (white arrow). C, planthopper (*Sogatella furcifera*) parasitized by *Elenchus japonicus*, male cephalotheca (black arrow). D, *Eoxenos laboulbenei* neotenic female, ventral view. Scale bars: (A, B) 5 mm; (C, D) 2 mm.

whereas females, as obligate endoparasites, are neotenic and do not have a pupal instar (Fig. 1B, C) (Kinzelbach, 1971; Kathirithamby, 1989, 2009; Erezyilmaz *et al.*, 2014). Strepsiptera parasitize a broad range of insect hosts belonging to 34 families comprising apterygotes, endopterygotes, and exopterygotes, and have coevolved with their hosts' life cycles (Kathirithamby, 1989, 2009). Cryptic species are widespread in Strepsiptera, as is now being revealed by molecular data (Kathirithamby & Johnston, 2004; Kathirithamby, 2009; Hayward, McMahon & Kathirithamby, 2011; Matsumoto *et al.*, 2011; Isaka, Ueda & Itin, 2012; Nakase & Kato, 2013; Juzová, Nakase & Straka, 2015).

MALES

Adult males are free-flying and possess characters of a 'typical' holometabolous insect. Emergent males (Fig. 2) live for 3–6 h and, being capable of flight, locate the flightless females (Roff, 1990). Males of all Strepsiptera, including those of extinct species, have elaborate flabellate antennae that differ between families, genera, and species, with numerous chemoreceptors (Figs 2, 3A).

FEMALES

The neotenic females of the Mengenillidia are free-living at the adult stage, possessing body appendages typical of insects, except wings (Fig. 1D). The antennae lack flabellae and their mandibles, maxillae, and legs resemble those of adult males.

Females of Stylopidia are highly derived, without a distinct head, thorax or body appendages (Figs 1B, 4, 5, 6A). On stylopization, they live an endoparasitic life-style and are totally host-dependent. Females resemble a 'bag of eggs' (Figs 6B, 7A, B), mate, and, when still endoparasitic, release the planidia (first-instar) larvae (Fig. 7C, D). The females' cephalothorax is the only externally visible structure to extrude through the host cuticle (Kinzelbach, 1971; Kathirithamby, 1989, 2009) (Figs 1B, 4, 5, 6A). We propose that the total endoparasitism of Stylopidia females leads to a simplification of their body plan and represents a state of extreme neoteny (retention of juvenile characteristics in adults), as opposed to the partial neoteny of the free-living primitive females of Mengenillidia.

The specific reproductive characteristics of the Mengenillidia and Stylopidia are described below.

Mengenillidia

Males of both *Mengenilla* and *Eoxenos* fertilize eggs of the free-living females via traumatic insemination (*sensu* Lange *et al.*, 2013) (Parker & Smith, 1933, 1934; Silvestri, 1940, 1941a, 1943). Males perforate any part of the female's body (except the head), inseminating directly into her body cavity. As a result of the absence of ovarioles, eggs lie free in the haemocoel, filling the abdominal region (Parker & Smith, 1933). Embryos and planidia larvae develop within the mother's body (Parker & Smith, 1933; Silvestri, 1940, 1941a, 1943). This haemocoelous vivipary, first described by von Siebold (1843), is common to all strepsipterans. Planidia larvae emerge via a slit-like opening composed of two thick lips on the seventh abdominal segment (Fig. 1D). Internally, the opening extends as a tube to the fifth segment, where it ends abruptly and floats in the haemocoel (Parker & Smith, 1933).

Stylopidia

The cephalothorax of the endoparasitic females comprises a vestigial head fused to indistinct and reduced thoracic segments (Figs 1B, 4, 5, 6, 7A, B). It is the only part of the endoparasitic female with which the male comes into contact during insemination. In the Corioxenidae (Fig. 4), the planidia larvae emerge via the same opening through which the
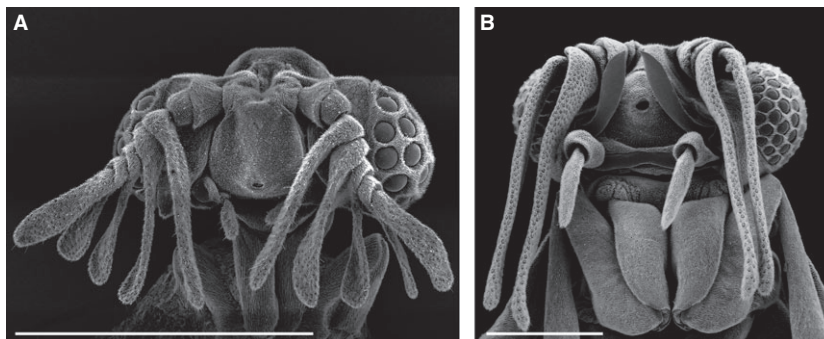

Figure 2. A, male *Halictophagus fulmeki* (frontal view) [scanning electron micrograph (SEM)] (Kathirithamby *et al.*, 2012). B, male *Xenos vesparum* (frontal view) (SEM) (Kathirithamby, 2009). Scale bars: (A) 0.5 mm; (B) 1.0 mm.
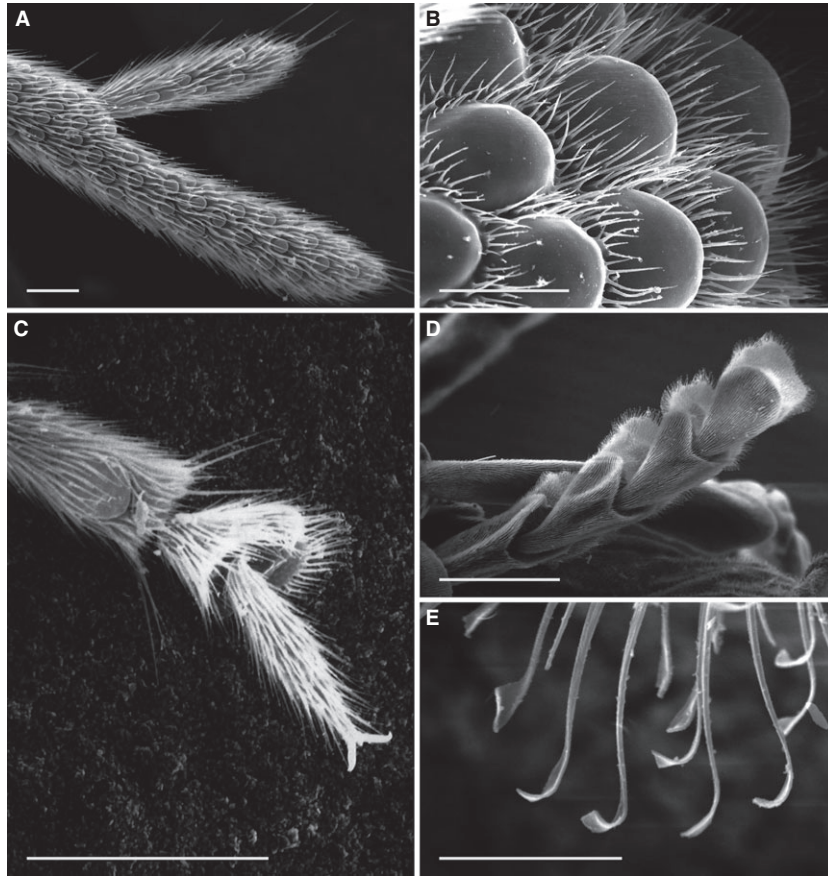
Figure 3. A, sixth and seventh antennal segments of a male of *Blissoxenos esakii* [scanning electron micrograph (SEM)]. B, eyelets of male *Blissoxenos esakii* (SEM). C, tarsi with claws of male *Triozocera* sp. (SEM). D, tarsi of male *Xenos vesparum* (SEM). E, tarsal hairs of male *Xenos vesparum* (SEM). Scale bars: (A, B) 20 μm; (C, D) 200 μm; (E) 10 μm.
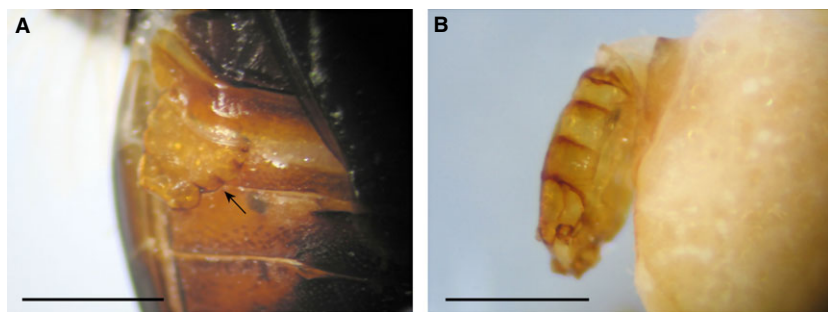

Figure 4. A, Cephalothorax of female *Triozocera* sp. (arrow) extruded through the anterior abdominal segments of host. B, cephalothorax of female *Triozocera* sp. Scale bars: (A, B) 200 μm.

male inseminates the female (Kirkpatrick, 1937). Females of Stylopiformia are inseminated through the brood canal opening (Meinert, 1896; Hughes-Schrader, 1924; Lauterbach, 1954; Linsley & Mac-Swain, 1957; Kathirithamby, 2000) (Fig. 5). The embryos develop in the body cavity of the mother (von Siebold, 1839, 1843) and active planidia larvae

emerge from their mother via the same opening (Smith & Hamm, 1914; Linsley & MacSwain, 1957; Kinzelbach, 1971; Kathirithamby, 1989, 2000, 2009) (Fig. 7D).

At the end of the fourth instar, the strepsipteran female extrudes the anterior region through the host cuticle and becomes sexually mature, while retaining
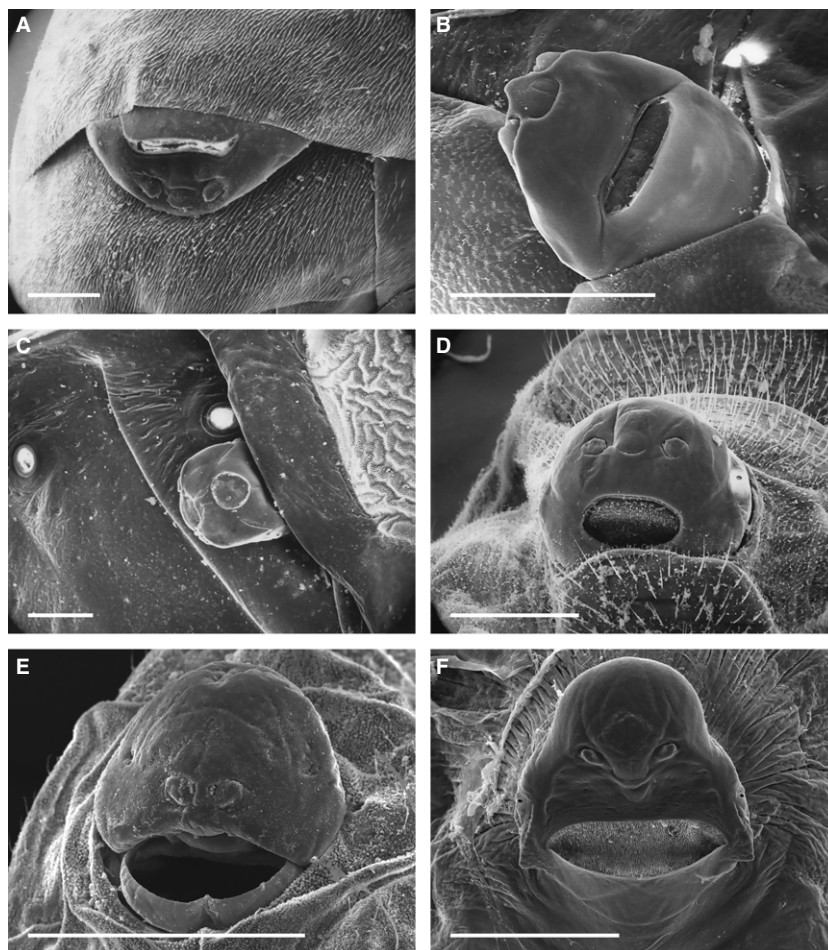
Figure 5. Cephalothorax of female [scanning electron micrograph (SEM)]. A, *Xenos vesparum*. B, *Halictophagus australiensis*. C, *Coriophagus rieki*. D, *Dipterophagus daci*. E, *Elenchus varleyi*. F, *Caenocholax* sp. Scale bars: (A, C) 1200 μm; (B, D–F) 250 μm.
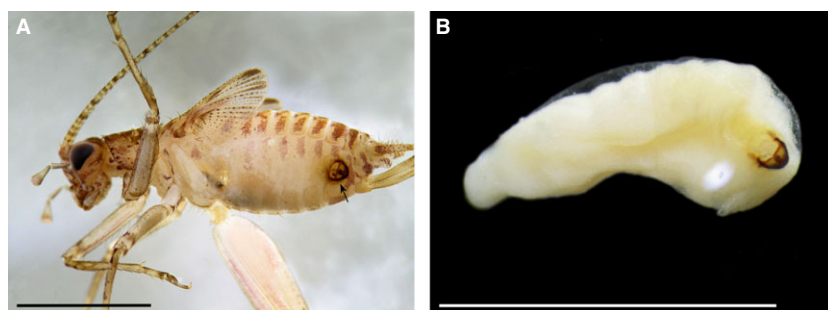

Figure 6. A, endoparasitic female of *Caenocholax* sp. (arrow) in a host cricket. B, neotenic female of *Caenocholax* sp. excised from host (Kathirithamby, 2009). Scale bars: (A, B) 4 mm.

larval characters (Fig. 6B). On extrusion, the anterior region comprising the head and prothoracic region is fused to form the cephalothorax, with a brood canal opening, which leads into a brood canal (Fig. 7A, B). The Nassonov (pheromone) glands open into the brood canal (Dallai *et al.*, 2004). The brood canal opening is covered by the brood canal membrane, which is reported to be broken by the male before he transfers sperm (Hughes-Schrader, 1924; Kirkpatrick, 1937; Silvestri, 1941b; Lauterbach, 1954;
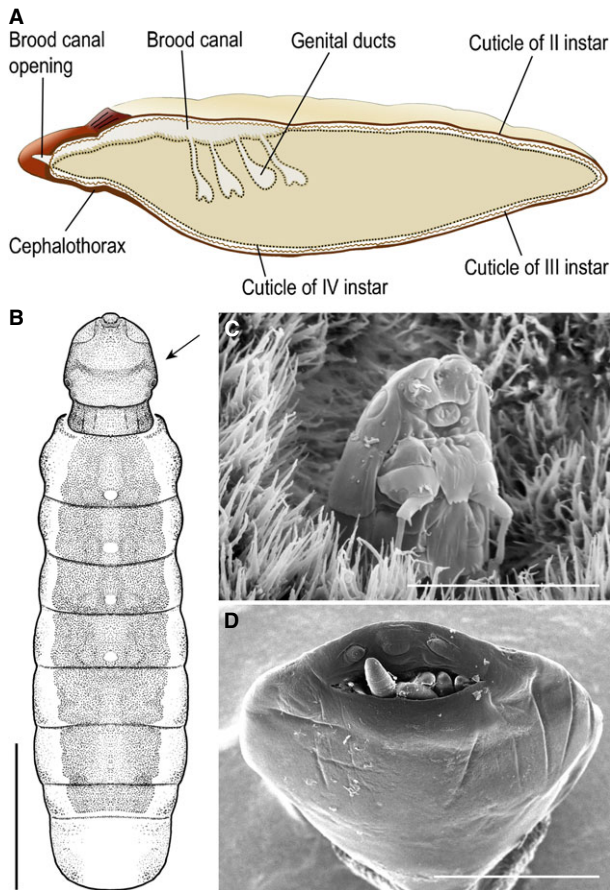
Figure 7. A, diagrammatic representation of neotenic female with brood canal and genital ducts (lateral view). B, neotenic female *Xenos vesparum*, cephalothorax (arrow) (ventral view). C, planidia larva emerging from genital duct of paternal female *Stichotrema dallatorreanum* [scanning electron micrograph (SEM)]. D, cephalothorax of female *Xenos vesparum* (SEM) with first-instar planidia larvae emerging from brood canal opening. Scale bars: (A, B) 1 mm; (C) 10 µm; (D) 400 µm.

Linsley & MacSwain, 1957): however, this is not likely because a closed brood canal membrane may impede pheromone dissemination. We hypothesize that the brood canal membrane is ruptured during the super-extrusion of the female's cephalothorax, thereby facilitating the release of pheromone out of the brood canal during mate signaling (see below).

The brood canal typically leads into one to five invaginations (genital ducts/birth openings) per segment (Fig. 7A, B) (Kinzelbach, 1971; Kathirithamby, 2000). Some species, such as the large *Stichotrema dallatorreanum* Hofeneder, have 25–35 genital ducts per segment, which allow easy exit for the hundreds of thousands of planidia larvae (Kathirithamby, 2000). The genital ducts end blindly in the haemo-

coel of the female (von Siebold, 1839, 1843; Nassonov, 1892; Meinert, 1896; Smith & Hamm, 1914; Hughes-Schrader, 1924; Lauterbach, 1954; Kinzelbach, 1971; Kathirithamby, 2000). Sperm passes via the brood canal opening into the brood canal and via the genital ducts into the haemocoel of the female containing the oocytes (Hughes-Schrader, 1924). Planidia larvae emerge from their mother's haemocoel via the genital ducts (Fig. 7C) into the brood canal, and out of the body of the mother via the brood canal opening (Fig. 7D).

The brood canal and the associated structures of endoparasitic females are also referred to as the ventral canal (Lauterbach, 1954), an extra-genital duct system (Lange *et al.*, 2013), or 'apron' (Kathirithamby, 2000), which lies ventral to the brood canal opening and is a unique adaptation in the endoparasitic, extremely neotenic, female Stylopidia.

GENITAL STRUCTURES USED FOR SPERM DELIVERY AND RECEPTION

The microstructure of aedeagi of the free-living males and that of the cephalothoraces of the endoparasitic females (when known) of 13 families of Strepsiptera is described in the Supporting information (see Data S1). The phylogeny of Strepsiptera is based on the study by McMahon *et al.* (2011).

Male aedeagus

The aedeagus of Strepsiptera is a simple structure. In the extinct fossil species *Cretostylops engeli* Grimaldi & Kathirithamby (approximately 100 Mya) and *Mengea tertiaria* Kulicka (approximately 39–50 Mya), the basal extant species *Bahiaxenos relictus* Bravo *et al.*, and in the Mengenillidae, and the derived Corioxenidae (Stylopidia), the aedeagi are tube-like and slightly bent anteriorly, ending in a sharp acumen (Fig. 8A, C). The aedeagi of corioxenid males are particularly long (Fig. 8B, C) and can thus reach the females' cephalothorax, which is bent at 180° and extrudes at the anterior abdominal host segments, just under the metathoracic wings (Fig. 4). Aedeagi of derived Stylopiformia males are hook-shaped dorsally, with or without an anterior dorsal spur or prolongation (*spina dorsalis*), and with a sharp acumen (Fig. 8D, F, G). However, the aedeagi of *Caenocholax* males, unlike those of other strepsipterans, are highly varied and, dorsally, have an elaborate anchor-shaped structure with either lateral spines (*spinae lateralis*), a *spina dorsalis*, and a sharp acumen (Fig. 9A, B, C, D), or a *spina dorsalis* and a sharp acumen without *spinae lateralis* (Fig. 9E) There is often no substantial variation in the
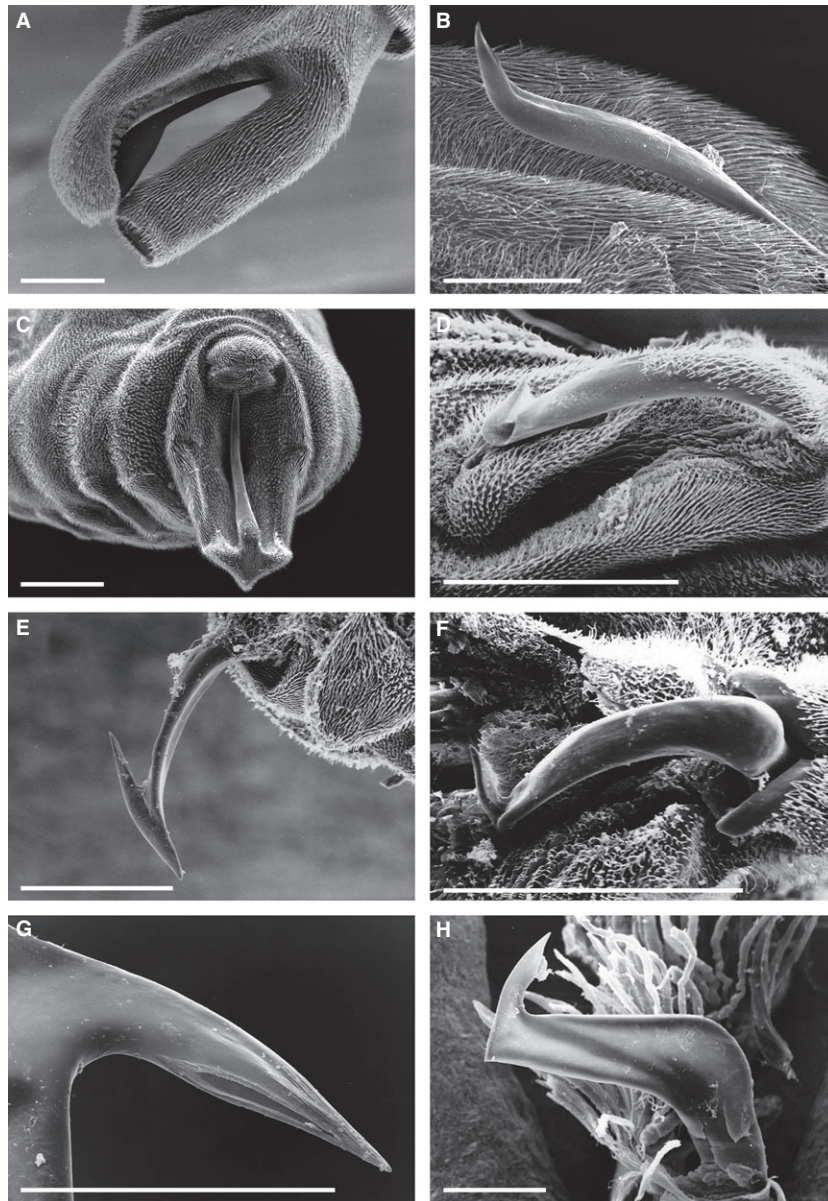
Figure 8. A, ninth and tenth abdominal segments of male *Mengenilla* sp. (lateral view) [scanning electron micrograph (SEM)]. B, aedeagus of *Triozocera* sp. (lateral view) (SEM). C, tenth abdominal segment of male *Blissoxenos esakii* (frontal view) (SEM). D, aedeagus of *Stichotrema robertsoni* (lateral view) (SEM). E, aedeagus of *Myrmecolax* sp. (lateral view) (SEM). F, aedeagus of *Xenos vesparum* (lateral view) (SEM). G, sharp acumen of *Xenos vesparum* (SEM). H, aedeagus of *Halictophagus moorookensis* (lateral view) (SEM). Scale bars: (A–H) 100 μm.

simple aedeagi either between congeners, heterogeners, or most importantly, between species, within the same taxonomic family, except in the genus *Caenocholax*, where aedeagi significantly differ between species.

There are no parameres or claspers in any male strepsipterans, including *Caenocholax* (Fig. 8A, B, C, D). In some families of the Stylopiformia, aedeagi bear (sensory) hairs on the frontal side (Kinzelbach,

1971) (Figs 8D, E, 9). The aedeagi of mengenillid and corioxenid males and those of males in other stylopid families are devoid of hairs (Fig. 8A, B, C). When the aedeagus is in an upright position, just before and during copulation, its hairs come into contact with the female's cephalothorax. If these hairs have a sensory function, they may perhaps guide the aedeagus towards the brood canal opening.
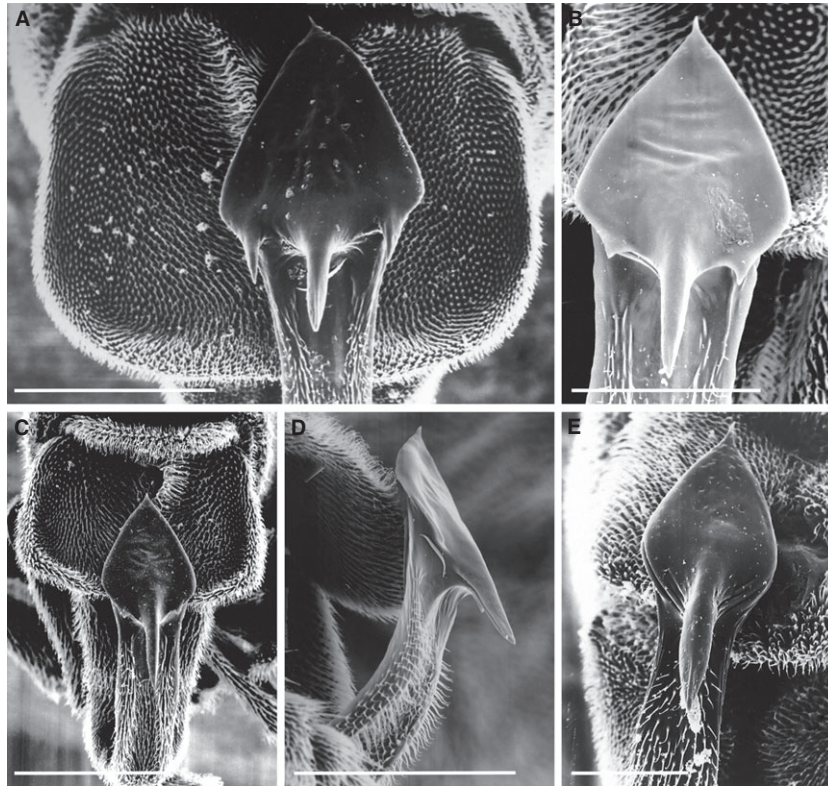
Figure 9. A–E, varied shield-shaped aedeagus of *Caenocholax* sp. [scanning electron micrographs (SEM)] from: Los Tuxtlas, Mexico (A); Los Tarrales Reserve, Guatemala (B); Tapachula, Mexico (C); College Station, TX, USA (D); and Barro Colorado Island, Panama (E). Scale bars: (A–E) 100 μm.

FEMALE CEPHALOTHORAX

The novel extra-genital duct system is linked to the evolution of endoparasitism in females, and is present in all Stylopidia (McMahon *et al.*, 2011) (which is absent in the basal extant Mengenillidia). It facilitates the fertilization of the endoparasitic female and the emergence of her planidia offspring (Fig. 7A). Mengenillid males engage in traumatic insemination, and planidia larvae emerge from their mother's abdomen via a simple birth opening (Parker & Smith, 1933; Silvestri, 1940, 1941a, 1943). By contrast, Stylopidia females (except corioxenid females) are inseminated via the brood canal opening, which planidia larvae later use for emergence. We hypothesize that, in the Corioxenidae, the evolution of an opening at the anterior-most region of the cephalothorax facilitates the exit of planidia larvae because the cephalothorax extrudes through the anterior abdominal segments, below the wings of the host (Fig. 4A). It appears that the extra-genital ductal system of the Corioxenidae is transitional between the absence of a duct system in the Mengenillidae and the elaborate duct system of the Stylopiformia.

Females of the primitive extinct families are not known, and only a few are known in the basal genera *Mengenilla* and *Eoxenos*; therefore, no definitive conclusion is possible regarding the birth-opening in females of the basal families. However, cephalothoraces of recent Stylopiformia families often do not exhibit structural variation either between congeners, heterogeners, or species within the same family, not even in the genus *Caenocholax*.

Cephalothoraces in Stylopiformia may: (1) appear curved up 180° towards the body, with an anterior opening but with no brood canal opening (Fig. 4); (2) exhibit an extended oblong/triangular sclerotized structure, with a large brood canal opening in the middle or on the anterior third (Fig. 5A, B, C, D); (3) display thickened sclerotized dorsal and ventral lobes, with a brood canal opening between the lobes (Fig. 5E); (4) appear round and highly sclerotized, with further sclerotization inside the host, as in the largest (approximately 30 mm) female strepsipteran, *S. dallatorreanum* or; (5) exhibit a dorsal weakly sclerotized lobe, with a brood canal opening below but lacking a ventral lobe or any other lobe (Figs 5F, 6), being mutually similar among all cryptic species

of *Caenocholax* examined be thus far (J. Kathirithamby, pers. observ.).

OTHER RECEPTOR SYSTEMS AND SIGNALS THAT MAY PLAY A ROLE DURING SEXUAL COMMUNICATION

Contact-chemo, tactile, and olfactory receptor systems may play a role in mate finding and mate assessment. These receptor systems and communication signals include the tarsi, antennae, maxillary palps, aedeagus, and pheromones.

TARSI

Males of the Stylopiformia and some Corioxenidae possess elaborate hairs on their tarsi, allowing them to cling to a female during sperm transfer (Fig. 3D, E). A chemosensory function of these hairs is also conceivable but has not yet been confirmed. By contrast, males of the primitive (extinct) Protoxenidae, Cretostylopidae and Mengeidae, the basal (extant) taxa Bahiaxenidae and Mengenillidae, and males of some subfamilies in the Corioxenidae, have tarsi with claws (Fig. 3C). Corioxenidae is again seen as a transitory family, where males of some subfamilies (e.g. Triozocerinae, Uniclavinae) have tarsal claws, the function of which can be determined only when conspecific females have been found and the structure of their cephalothorax studied. The complete endoparasitism of stylopid females may have led to strong selection on the part of the free-living males to develop specialized tarsi with adhesive hairs (microtrichia) (Kinzelbach, 1971) (Fig. 3D, E). Tarsi of strepsipteran males are not species-specific, nor are the tarsal claws of free-living mengenillid males.

Stylops males (Stylopidae) strike the cephalothorax of females during copulation (Grabert, 1953; Linsley & MacSwain, 1957), a behaviour that might be considered as copulatory courtship. Males of *Halictophagus silwoodensis* Waloff (Halictophagidae) search first with their front tarsi for the endoparasitic female in the host *Ulopa reticulata* (F.), and then bend their abdomen for copulation (Henderickx, 2008). Males of *Xenos peckii* Kirby approach host wasps in response to the 'calling female', which assumes a particular calling posture (see below; Hrabar *et al*., 2014) (Fig. 10) (see Supporting information, Video Clip S1). After a male *X. peckii* has been attracted to a female, he typically lands on the anterior dorsal section of the host wasp's abdomen and then steps backwards until he makes contact with the female's cephalothorax (see Supporting information, Video Clip S2).
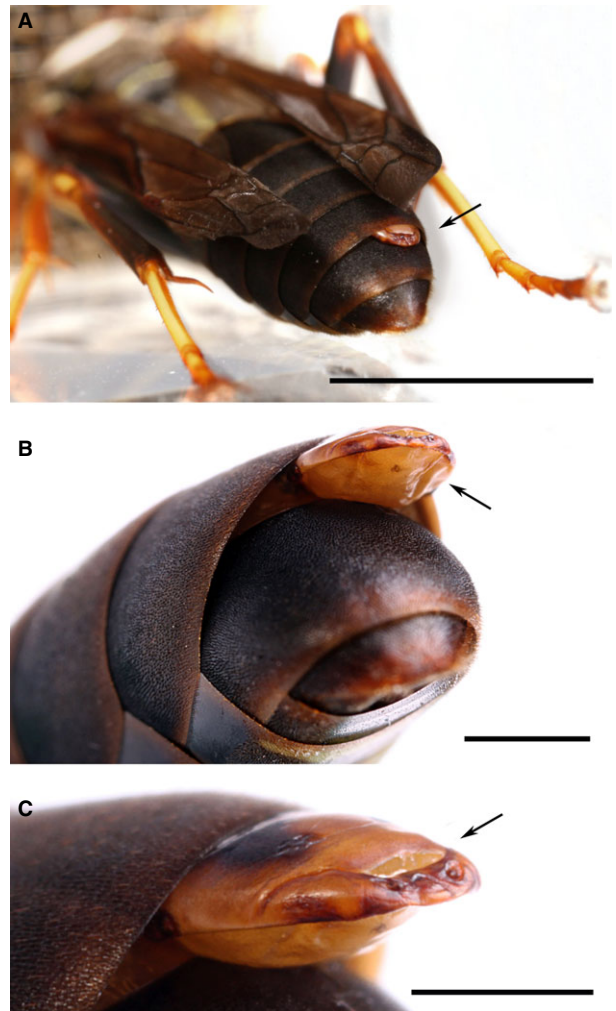

Figure 10. Cephalothorax of female *Xenos peckii* extruded from host paper wasp *Polistes fuscatus*. A, extruded female (arrow) prior to calling. B, C, calling super-extruded female during calling, with inflated cephalothorax (arrow). B, dorsal view. C, lateral view. Scale bars: (A) 10 mm; (B, C) 1 mm.

A sensory patch on his metathoracic legs, as described on legs of other strepsipterans (Dubitzky, 2001; Pohl & Beutel, 2004; Henderickx, 2008), appears to mediate sensory recognition of the female: as soon as the mesothoracic legs touch the female's cephalothorax, they grasp it tightly, and the male initiates copulation through the opening of the female's brood canal (Hrabar *et al*., 2014) (see Supporting information, Video Clip S2).

ANTENNAE AND MAXILLARY PALPS

The branched antennae of males have four to eight antennomeres, some with several (six to eight) flabellae (Fig. 2). These characters are family- and

sometimes genera-specific. At the species level, the antennae can be obviously or subtly species-specific. The flabellate antennae have numerous chemoreceptors that are presumably capable of detecting the sex pheromone emitted by conspecific females (Figs 2, 3A), as has been demonstrated in *Stylops melittae* Kirby (Tolasch, Kehl & Dötterl, 2012) and *X. peckii* (M. Hrabar, A. Danci, S. McCann, P.W. Schaefer, & G. Gries, unpubl. data).

FEMALE SEX ATTRACTANT PHEROMONE

Strepsipteran females emit a sex pheromone capable of attracting males. That mated females are no longer attractive to males was noted more than 60 years ago (Silvestri, 1941b; Linsley & MacSwain, 1957). Males of *Corioxenos antestiae* Blair, a parasite of pentatomid bugs, are first attracted to the host by sight, and then respond to female by touch (Kirkpatrick, 1937). *Stylops* males are even attracted to the female cephalothorax *sans* body (Grabert, 1953). On emergence, males of *Eoxenos laboulbenei* De Peyerimhoff recognize a female but, if she has eggs 'at an advanced stage of development', the males show no interest (Silvestri, 1941b). Males of *Stylops pacificus* Bohart are attracted to females by a 'scent mechanism' involving the male antennae (Linsley & MacSwain, 1957). Males of *Xenos vesparum* Rossi are attracted to caged receptive females (Hughes *et al.*, 2003; E. F. Riek, unpubl. data; R. K. Kinzelbach, pers. comm.). Similarly, virgin *X. vesparum* females are attractive to newly emerged males (Dallai *et al.*, 2004).

Studying *S. melittae*, Nassonov (1910) found glandular structures in the cephalothorax and speculated that they produce semiochemicals attracting males. Further examination of the Nassonov gland in *Stylops* females (Lauterbach, 1954) and *X. vesparum* females (Dallai *et al.*, 2004) supported the conclusion that the gland is modified to produce pheromones. The Nassonov gland is a secretory structure comparable to the epidermal gland cells of class 3 (Noirot & Quennedey, 1974, 1991). Gap junctions are laterally placed to help coordinate pheromone production and emission by different clusters of cells. The degeneration of pheromone gland cytoplasm in mated females explains why males were found not to be attracted to mated females (Dallai *et al.*, 2004).

The sex pheromone of female *S. melittae*, $(3R,5R,9R)$-3,5,9-trimethyldodecanal (Tolasch *et al.*, 2012), is the first pheromone to be identified in Strepsiptera. It elicits strong responses from male antennae in electrophysiological recordings and is strongly attractive to males in behavioural bioassays (Tolasch *et al.*, 2012). Males approach and walk on pheromone impregnated filter paper with their aedeagus in an upright position (Cvačka *et al.*, 2012; Tolasch *et al.*, 2012). Female *S. melittae* continue to release pheromones until they mate, upon which they immediately and drastically decrease pheromone emission (Tolasch *et al.*, 2012). Mated females have only trace amounts of pheromone in their cephalothorax and are not attractive to males (Lagoutte *et al.*, 2013).

MATING

The small size, rapid mating schedule, and cryptic nature of Strepsiptera make definitive observations on mating difficult. Thus, the paucity of records has led to inconsistent terminology and generalizations that appear unjustified because they are based on very few observations of mating events.

Upon emergence from the puparium, male strepsipterans are generally active for approximately 1–2 h, after which they are incapable of sustained flight (Kirkpatrick, 1937; Silvestri, 1941a, 1943). They copulate with a female for a few seconds to a minute, and sometimes re-copulate with the same female before they search for new mates (Hofeneder, 1923; Hughes-Schrader, 1924; Kirkpatrick, 1937; Silvestri, 1940, 1943; Grabert, 1953; Linsley & MacSwain, 1957; Waloff, 1981). Males were observed to copulate with as many as 12 females, and also with two females that extruded from the same host (Kirkpatrick, 1937).

Generally, males are attracted to females 2–3 days after they extrude their cephalothorax. They do not copulate with mated females whose brood canal membrane has been perforated by an aedeagus, or with females that have started egg development (Hughes-Schrader, 1924; Silvestri, 1940, 1941a, b, 1943; Grabert, 1953; Lauterbach, 1954; Linsley & MacSwain, 1957; Dallai *et al.*, 2004; Tolasch *et al.*, 2012). For example, if several *S. pacificus* males arrive at a receptive female, all leave when the first male begins to copulate (Linsley & MacSwain, 1957). *Xenos peckii*, *S. pacificus*, *S. melittae*, and *X. vesparum* have all been shown to be monandrous (Hughes-Schrader, 1924; Grabert, 1953; Linsley & MacSwain, 1957; Dallai *et al.*, 2004).

The unusual pre-copulatory behaviour of Strepsiptera was first studied in detail in *X. peckii* (Hrabar *et al.*, 2014). Free-living males emerge shortly after females have reached sexual maturity. Two or three days after extrusion (Fig. 10A), females initiate 'calling' (pheromone emission) by assuming a particular calling position (Fig. 10B, C). During 'calling', female *X. peckii* inflate and super-extrude their cephalothorax from the host abdomen beyond its normal position. In this super-extruded position, the cephalothorax is inflated and tilted (Fig. 10B, C) (see

Supporting information, Video Clip S1). The inflation occurs in daily cycles and proceeds gradually over a period of approximately 30 min to several hours, and is accompanied by a somewhat more rapid tilting motion whereby the cephalothorax protrudes away from the host's abdomen. In *X. peckii*, this 'super-extrusion' of the female gradually subsides during the course of the evening, and resumes the following day. The pheromone titre is greatest when females are in a calling posture (M. Hrabar, A. Danci, S. McCann, P.W. Schaefer & G. Gries, unpubl. data).

This 'calling' posture, as Hrabar *et al.* (2014) suggest, might help disseminate pheromones and provide visual cues for mate-seeking males. It may also facilitate contact chemo-recognition through aligment with sensory patches on the males' tarsi. Similarly, when males of *H. silwoodensis* approach a female, her cephalothorax 'heaves up' (Waloff, 1981). Whether these observations on *X. peckii* and *H. silwoodensis* apply to other strepsipteran taxa will become apparent as more studies are conducted. Interestingly, there is a distinct diel periodicity of the females 'calling' behaviour, which coincides closely with the diel periodicity of the males' emergence from their puparia (Hrabar *et al.*, 2014). This remarkable synchronization of the females' and the males' reproductive cycles may help ensure that the short-lived males (3–6 h) have the best possible chance of locating the cryptic, endoparasitic females (Fig. 11). Within minutes after a *X. peckii* male has mated and detached himself, the female retreats, leaving only the very tip of her cephalothorax extruded from the host (Hrabar *et al.*, 2014) (Fig. 10A). The mated female remains in this retreated position, neither posturing, nor pheromone-signaling again.

In *Mengenilla* species, which exhibit traumatic insemination, Silvestri (1943) observed that a male hurled himself several times onto the body of the same free-living female, or various other females. Parker & Smith (1934) observed that copulating *E. laboulbenei* males pierced the ventral 'skin of the female's abdomen' rather than her birth opening, although the true nature of traumatic insemination was reported in mengenillids after Silvestri's (1940, 1941a, 1943) acute observations on *Mengenilla* sp. and *E. laboulbenei*.

Studying *Halictophagus tettigometrae* Silvestri: (Halictophagidae), Silvestri (1941b) noted that the aedeagus was introduced into the brood canal opening, piercing the 'cephalo-prothoracic membrane'; however, he found no sperm in the 'ventral chamber or tubes' (i.e. the brood canal and genital ducts) 30 min after copulation. Sperm movement is rapid and sperm absence in the brood canal 30 min after insemination (as observed by Silvestri, 1941b) is not unexpected. Nevertheless, Silvestri (1941b) concluded that the sperm must pass directly into the thoracic cavity, instead of entering the brood canal. After his study of *H. tettigometrae* (and his earlier study of mengenillids) (Silvestri, 1940, 1941a), Silvestri (1941a, b) concluded that fertilization is 'extra-vulvar' (both in the Mengenillidae and the Stylopidia).

Smith & Hamm (1914) argue that it would be difficult for sperm to reach the body cavity of the female (where oocytes reside) and conclude that eggs perhaps develop by parthenogenesis. The presence of pigment scars in the invagination of the brood canal in *S. melittae* was evidence for Lauterbach (1954) that these scars were caused by traumatic insemination. Studying *X. vesparum*, Beani *et al.* (2005) present no conclusive evidence for traumatic insemination. They document insertion of sperm into the brood canal as previously observed (von Siebold, 1839, 1843) but go on to speculate that the 'aedeagus could perforate the cuticle and the underlying epithelium near
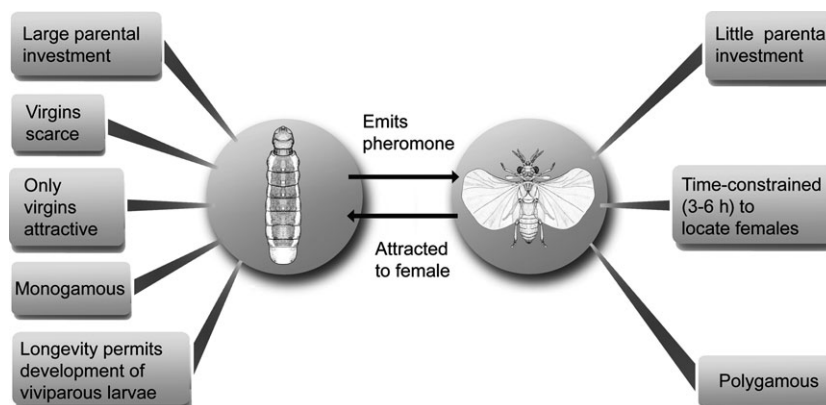

Figure 11. Diagrammatic representation of life-history traits, sexual communication, and reproductive synchrony in Strepsiptera.

the opening … (hypodermic insemination) into the female haemocoel'. Beani *et al.* (2005) then argue that 'hypodermic insemination' is a possible mode of insemination in *X. vesparum*, although they could not determine the location where the aedeagus might have been inserted into the female. They proceed to suggest that traumatic insemination is a recent development in Strepsiptera 'to bias paternity'. This suggestion does not appear to be supported by the evidence currently available (see below).

In their review of traumatic insemination in terrestrial arthropods, Tatarnic *et al.* (2014) citing studies by Smith & Hamm (1914), Silvestri (1940), and Beani *et al.* (2005), conclude (with reference to Strepsiptera): 'It is widely accepted that mating occurs by traumatic insemination whereby a male seeks out a parasitized host insect and, using his needle-like aedeagus, stabs and inseminates the female through the cephalothorax, ejaculating into the haemocoel'. However, their conclusion contradicts the very references they cite in support of the claim: (1) Silvestri (1940) only witnessed traumatic insemination in Mengenillidae, where males seek out free-living rather than endoparasitic females as mates, inseminating them through the abdomen; (2) Smith & Hamm (1914) suggest parthenogenetic egg development, dismissing traumatic insemination as a means of reproduction in the Stylopidae; (3) Silvestri (1940, 1941a, b) refers to fertilization as 'extra-vulvar', which differs from traumatic insemination; and (4) Beani *et al.* (2005) did not find conclusive evidence for traumatic insemination, stating that it is not known whether mating in strepsipterans occurs via hypodermic injection directly into the hemocoel, or through the brood canal opening, or via both routes.

Citing Meinert (1896) and Hughes-Schrader (1924), Tatarnic *et al.* (2014) proceed to state that 'males fertilize the female though the brood canal', and that 'the female incurs no damage'. Tatarnic *et al.* (2014) base their generic conclusion of traumatic insemination in Strepsiptera mainly on the study by Beani *et al.* (2005), who admit their evidence is inconclusive (see above).

Traumatic insemination does occur in the basal extant family Mengenillidae, where males pierce any body part (except the head) of free-living females (Silvestri, 1940, 1941a, 1943). However, traumatic insemination has never been conclusively demonstrated in the Stylopidia. Indeed, the evidence suggests that traumatic insemination represents a basal mode of reproduction in the free living forms of Strepsiptera (Mengenillidia). We suggest that mating via the brood canal is a recent adaptation, necessitated by the total endoparasitism of the neotenic females that extrude only their cephalothorax through the host. Mating directly through the abdomen is prevented by shielding of the host's sclerotized integument. Males insert sperm into the an opening (in Corioxenidae), or via the brood canal opening in Stylopiformia (Meinert, 1896; Hughes-Schrader, 1924; Kirkpatrick, 1937; Grabert, 1953; Linsley & MacSwain, 1957; Hrabar *et al.*, 2014). We therefore conclude that non-traumatic insemination ('brood canal mating') is the mode of sperm transfer in Stylopidia. In this case, sperm is delivered into the brood canal (an extra-genital duct system), representing a distinct adaptation to the females' extreme neoteny and endoparasitic lifestyle. We do not concur with the conclusion of Tatarnic *et al.* (2014) that traumatic insemination is a 'by-product of female neoteny and endoparasitism'. We argue that the currently available information supports the concept that traumatic insemination has disappeared phylogenetically and has been 'replaced' by brood canal mating as an adaptation to endoparasitism in female Stylopidia (McMahon *et al.*, 2011).

DISCUSSION

On the basis of the 48 genera of Strepsiptera that we examined, there is no substantial divergence in the general morphology of the male's aedeagus between congeners, heterogeners, or even between species within the same taxonomic family. However, the genus *Caenocholax* is an exception to this rule because aedeagi differ between species. Furthermore, parameres or claspers, or any other genitalic structures used during sexual contact and copulation, are absent (Fig. 8).

Similarly, often there is uniform morphology of the female's cephalothorax between congeners, heterogeners or even between species of the families of the suborder Stylopidia, including the genus *Caenocholax* (Figs 5F, 6). The widespread cryptic species in the Strepsiptera might be 'partitioned' by their hosts, with genitalia not diverging rapidly in taxa that are isolated by parasitizing different hosts. An exception is the genus *Caenocholax*, where males are generalist parasitoids of ants and have distinctly different aedeagi between species (Fig. 9). By contrast, *Caenocholax* females parasitize specific cricket hosts and have almost identical cephalothoraces between species (Hayward *et al.*, 2011; J. Kathirithamby, unpub. data; Figs 5, 6).

In endoparasitic females of the suborder Stylopidia, the cephalothorax and associated structures on the 'apron' (*sensu* Kathirithamby, 2000), also referred to as extra-genital duct system (*sensu* Lange *et al.*, 2013), have two functions: (1) receiving sperm (Meinert, 1896; Hughes-Schrader, 1924; Lauterbach, 1954; Linsley & MacSwain, 1957; Kathirithamby,

1989, 2009; Hrabar *et al.*, 2014) and (2) providing a passage for the emergence of the planidia larvae from their endoparasitic mother (Smith & Hamm, 1914; Linsley & MacSwain, 1957; Kinzelbach, 1971; Kathirithamby, 1989, 2000, 2009; Hrabar *et al.*, 2014). Although the cephalothorax, as well as the associated brood canal and genital ducts, function in sperm reception, it is not a genitalic structure but rather a contact organ for the male. For this reason, fertilization has been referred to as extra-vulvar (Silvestri, 1941b), a process that has been mistaken for traumatic insemination. In basal Mengenillidae, where fertilization does occur by traumatic insemination, there is a birth opening that serves only for the emergence of the live planidia larvae (Parker & Smith, 1933, 1934; Silvestri, 1941a, 1943), but not for sperm reception.

Tatarnic *et al.* (2014), citing studies by Smith & Hamm (1914), Silvestri (1940) and Beani *et al.* (2005), conclude that traumatic insemination is the widely utilized mode of sperm transfer in Strepsiptera. This generalization, however, does not appear to be justified in light of the studies they cite. Particularly, Beani *et al.* (2005) clearly refrain from making a conclusive statement, writing that sperm is transferred either via the brood canal opening or tramautically.

The aedeagi of males of the basal family Mengenillidae (*Mengenilla* and *Eoxenos* spp.) and of the early branching Corioxenidae (Stylopidia) are smooth and devoid of hairs, whereas hairs are present on parts of the frontal surfaces of the aedeagi in other stylopid families (Figs 8D, 9). When the aedeagus is in an upright position, just before and during copulation, this surface comes in contact with the female. We suggest that the smooth, simple, slightly curved aedeagi of Mengenillidia males are ideally suited for traumatic insemination, whereas the more elaborate and sometimes hair-bearing aedeagi of Stylopidia males are adapted for insemination via the brood canal opening. The hairs could serve to guide the aedeagus to the brood canal opening of the cephalothorax.

Sexually antagonistic coevolution theory predicts that males lack species-specific characteristics when conspecific females have no specific contact organs to which males need to adjust (Eberhard, 2004b). The cephalothorax of strepsipteran females is one such nonspecific contact organ that may serve in a context other than defense against copulation by heterospecific males (Alexander *et al.*, 1997). The endoparasitic females of Stylopidia are largely soft-bodied, except for their extruded cephalothorax, which has (weakly) sclerotized swellings (lips) or an extended structure. The cephalothorax and its extra-genital duct system (brood canal opening, brood canal and genital ducts)

have presumably evolved as adaptations to reduce trauma and wounding during sperm transfer. If so, morphological characteristics of the cephalothorax may not enable the female to defend against copulations, thus exerting no selection pressure on strepsipteran males to evolve species-specific genitalic characteristics.

The genus *Caenocholax* is an exception to the above rule because the males' aedeagi are varied and species-specific between species (J. Kathirithamby, unpubl. data), even though the cephalothorax of females is uniform, simple, and comprises only a dorsal chitinized swelling, with the brood canal opening below. Ventral to the brood canal opening is nonchitinized, exposed cuticle. Heterospecific *Caenocholax* females are reproductively isolated by their cricket hosts, and host switches (males parasitize ants) are uncoupled between sexes (Hayward *et al.*, 2011). The simple cephalothorax in *Caenocholax* is extruded latero-ventrally from the cricket host, contrasting with dorsal or ventral extrusions of strepsipterans from other hosts. Latero-ventral extrusion of the cephalothorax from the host makes copulation a little more precarious for males. Furthermore, males copulate quickly to reduce the likelihood of being brushed off by the living host. This phenomenon has been observed in planthopper hosts that constantly rub their hind leg against their abdomen, apparently in an attempt to rid themselves of the strepsipteran male (J. Kathirithamby, pers. observ.). The elaborate, anchor-shaped aedeagi of *Caenocholax* males exhibit a *spina dorsalis*, a sharp acume of various lengths, and often possess a pair of *spinae lateralis* of various lengths (Fig. 9). These structures may have evolved to give the copulating male added support (in addition to support via tarsi) to enable him to cling to the dorsal lobe of the females' simple cephalothorax, and to the host. This extra support provided by the spines may also help prevent damage to the nonchitinized area below the brood canal opening. Structures of the aedeagi, as seen in *Caenocholax*, enabling males to improve their mating success, have been documented by Arnqvist & Rowe (1995).

Males use their tarsi to strike or cling to the female's cephalothorax before and during mating. Tarsal strikes, in the new arms race model, are considered to be a type of stimulation, where females reject a male that is less effective (Alexander *et al.*, 1997; Rice & Holland, 1999; Gavrilets, Arnqvist & Friberg, 2001; Eberhard, 2004a). There is no indication for such screening in Strepsiptera.

There is emerging evidence to suggest that receptive female Strepsiptera attract the short-lived males by means of a long-range sex pheromone (Cvačka *et al.*, 2012; Tolasch *et al.*, 2012). Males sense airborne

and contact pheromones of a prospective mate through chemoreceptors on their antennae and maxillary palps, and through sensory hairs on their tarsi and aedeagi. Once fertilized by a male, females become unattractive to other males (Linsley & MacSwain, 1957; Dallai *et al.*, 2004; Tolasch *et al.*, 2012). Females have greater parental investment than their mate because larvae develop by haemocoelous vivipary, with embryos obtaining nutrients from their mother. Until the planidia larvae have emerged, their mother and her host must stay alive.

Sexual antagonistic coevolution and male–female conflict over mating are less likely to evolve in a system where females emit long-range sex pheromones that attract males (Eberhard, 2004a, 2009). In Strepsiptera, this 'invoked' the 'design' of simple external genitalia often with limited or little variation among congeners, or heterogeners, except for the genus *Caenocholax*. Judging from the morphology of the males' antennae and their olfactory receptors, all strepsipteran males, irrespective of their taxonomic placement, presumably seek conspecific females in response to the pheromones they emit.

To locate a receptive female quickly is of paramount importance for the short-lived strepsipteran male. Immediately upon emergence, he is ready to fly and must find a conspecific female that is often scarce. Thus, one would predict that he cannot afford to be choosy, even though he may prefer females that produce a lot of pheromone, as shown in or implied for *S. melittae* (Tolasch *et al.*, 2012). If males are numerous, it might be advantageous for a male to deliver multiple ejaculates, as has been reported previously (Silvestri, 1943); however, male combat over mating has not been observed in Strepsiptera (Linsley & MacSwain, 1957). Even when a female *S. melittae* has attracted several males, only one will copulate with her, perhaps indicating that there could be subtle male–male combat (Tolasch *et al.*, 2012). That mated females lose attractiveness (Silvestri, 1941b; Linsley & MacSwain, 1957) helps males save time and focus their search on receptive virgin females.

There is no direct empirical evidence, although various studies suggest that females mate with a single male (Hughes-Schrader, 1924; Grabert, 1953; Linsley & MacSwain, 1957; Dallai *et al.*, 2004). This concept of monandry is supported by recent observations that female *X. peckii*, immediately after mating, retreat partially back into the abdomen of their host wasp (Fig. 10A), and never again posture or attract other males (Hrabar *et al.*, 2014). Furthermore, pheromone production in *S. melittae* decreases immediately after mating (Tolasch *et al.*, 2012). Postmating cessation of pheromone emission 'protects' (*sensu* Eberhard, 2004a) the female from harassment by unwanted males, and reduces the likelihood of forced copulations and sexual conflict. 'Protected females' are also less likely to invoke rapid divergence of male genitalia, which are often not species-specific (Eberhard, 2004a), as in Strepsiptera.

CONCLUSIONS

Unusual life-history traits of Strepsiptera, such as the extreme neoteny and flightlessness of free-living or endoparasitic females, and the ephemeral life expectation of males, have driven the evolution of reproductive synchrony. The rate of mate encounter is likely to be low. Females increase their chance of mating by exhibiting virtually no degree of choosiness. They attract males (by pheromone) when the reproductive cycles of females and males are in synchrony; not the synchrony of breeding (*sensu* Ims, 1990) but the synchrony of 'calling' and 'answering'.

Based on, and extrapolated from, currently available data, this synchrony entails several factors: (1) only receptive females call; (2) females emit pheromones that are highly attractive to males; (3) the diel periodicity of calling behaviour by females coincides with the diel emergence period of males, as shown in *X. pecki* (Hrabar *et al.*, 2014); (4) neither males, nor females reject potential mates; and (5) mated females are no longer attractive to males (Fig. 11).

Morphological traits of females and males facilitating sperm transfer often exhibit little or no substantial variation either between congeners, heterogeners, or even between species, within the same taxonomic family (except for the genus *Caenocholax*). This might be because sexual antagonistic coevolution and male–female conflict are less likely to occur when conspecific females have no specific contact organs to which males need to adjust, and when females emit long-range species-specific sex pheromones that attract males.

Current data support the conclusion that Strepsiptera fall under the synchronous sensory exploitation model of sexual selection.

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REFERENCES

Alexander RD, Marshall MC, Cooley JR. 1997. Evolutionary perspectives on insect mating. In: Choe JC, Crespi BJ, eds. *The evolution of mating systems in insects and arachnids*. Cambridge: Cambridge University Press, 4–31.

Andersson M. 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.

Arnqvist G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393:** 784–786.

Arnqvist G, Rowe L. 1995. Sexual conflict and arms races between the sexes: a morphological adaptation for control of mating in a female insect. *Proceedings of the Royal Society of London Series B, Biological Sciences* **261:** 123–127.

Arnqvist G, Edvardsson M, Friberg U, Nilsson T. 2000. Sexual conflict promotes speciation in insects. *Proceedings of the National Academy of Sciences of the United States of America* **97:** 10460–10464.

Beani L, Giusti F, Mercati D, Lupetti P, Paccagnini E, Turillazzi S, Dallai R. 2005. Mating in *Xenos vesparum* (Rossi) (Strepsiptera, Insecta) revisited. *Journal of Morphology* **265:** 291–303.

Carayon J. 1966. Traumatic insemination and paragenital system. In: Usinger RL, ed. *Monograph of the Cimicidae*. College Park, MD: Thomas Say Foundation, Entomological Society of America, 81–166.

Cvačka J, Jiroš P, Kalinova B, Straka J, Černá K, Šebesta P, Tomčala A, Vašíčková S, Jahn U, Šobotník J. 2012. Stylopsal: the first identified female-produced sex pheromone of Strepsiptera. *Journal of Chemical Ecology* **38:** 1483–1491.

Dallai R, Lupetti P, Giusti F, Mercati D, Paccagnini E, Turillazzi S, Beani L, Kathirithamby J. 2004. Fine structure of the Nassonow's gland in neotenic endoparasitic female *Xenos vesparum* (Rossi) (Strepsiptera, Insecta). *Tissue and Cell* **36:** 211–220.

Darwin C. 1871. *The descent of man and selection in relation to sex, 2nd edn.* New York, NY: Hurst & Co.

Dubitzky A. 2001. Die äußere Morphologie von *Xenos vesparum* im REM (Strepsiptera: Stylopidia). *Mitteilungen der Münchner Entomologischen Gesellschaft* **91:** 71–76.

Dufour L. 1844. Anatomie générale des dipterés. *Annales des Sciences Naturelles* **1:** 244–264.

Eberhard WG. 1985. *Sexual selection and animal genitalia*. Cambridge, MA: Harvard University Press.

Eberhard WG. 1993. Evaluation models of sexual selection: genitalia as a test case. *American Naturalist* **142:** 564571.

Eberhard WG. 1996. *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Prenceton University Press.

Eberhard WG. 2004a. Male–female conflict and genitalia: failure to confirm predictions in insects and spiders. *Biological Reviews* **79:** 121–186.

Eberhard WG. 2004b. Rapid divergent evolution of sexual morphology: comparative tests of antagonistic coevolution and traditional female choice. *Evolution* **58:** 1947–1970.

Eberhard WG. 2006. Sexually antagonistic coevolution in insects is associated with only limited morphological diversity. *Journal of Evolutionary Biology* **19:** 657–681.

Eberhard WG. 2009. Postcopulatory sexual selection: Darwin's omission and its consequences. *Proceedings of the National Academy of Sciences of the United States of America* **106:** 10025–10032.

Erezyilmaz DF, Hayward A, Yan H, Paps J, Acs Z, Delgado JA, Collantes F, Kathirithamby J. 2014. Expression of the pupal determinant *broad* during metamorphic and neotenic development of the strepsipteran *Xenos vesparum* Rossi. *PLoS ONE* **9:** e93614.

Grabert B. 1953. *Bau der Geschlechtsorgane und Kopulation beim Stylopsmännchen (Insecta, Strepsiptera)*. Berlin: Diplomarbeit, Zoologisches Institut der Freien Univ.

Gavrilets S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403:** 886–888.

Gavrilets S, Arnqvist G, Friberg U. 2001. The evolution of female choice by sexual conflict. *Proceedings of the Royal Society of London Series B, Biological Sciences* **268:** 531–536.

Hayward A, McMahon DP, Kathirithamby J. 2011. Cryptic diversity and female host specificity in a parasitoid where the sexes utilize hosts from separate orders. *Molecular Ecology* **20:** 1508–1528.

Henderickx H. 2008. Faunistische Bemerkingen over Strepsiptera met onderzoek van een populatie *Halictophagus silwoodensis* (Halictophagidae) in het Nationaal Park Hoge Kempen (Maasmechelen). *Phegea* **36:** 103–107.

Hofeneder K. 1923. *Stylops* in copula. *Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien* **73:** 128–134.

Holland B, Rice WR. 1998. Chase-away selection: antagonistic seduction vs. resistence. *Evolution* **52:** 1–7.

Hrabar M, Danci A, McCann S, Schaefer P, Gries G. 2014. New findings on life history traits of *Xenos peckii* (Strepsiptera: Xenidae). *Canadian Entomologist* **146:** 514–527.

Hughes DP, Beani L, Turillazzi S, Kathirithamby J. 2003. Prevalence of the parasite Strepsiptera in *Polistes* as detected by dissection of immatures. *Insectes Sociaux* **50:** 62–68.

Hughes-Schrader S. 1924. Reproduction in *Acroschismus wheeleri* Pierce. *Journal of Morphology* **39:** 157–205.

Ims RA. 1990. The ecology and evolution of reproductive synchrony. *Trends in Ecology and Evolution* **5:** 135–141.

Isaka Y, Ueda S, Itin T. 2012. Molecular phylogenetic affinities of Japanese Xenid and stylopid strepsipterans (Strepsiptera: Xenidae, Stylopidae) parasitizing wasps andbees. *New Entomology* **61:** 15–20.

Juzová K, Nakase Y, Straka J. 2015. Host specialization and species diversity in the genus *Stylops* (Strepsiptera: Stylopidae), revealed by molecular phylogenetic analysis.

Zoological Journal of the Linnean Society. doi:10.1111/zoj/72233.

Kathirithamby J. 1989. Review of the order Strepsiptera. *Systematic Entomology* **14:** 41–91.

Kathirithamby J. 2000. Morphology of the female Myrmecolacidae (Strepsiptera) including the *apron*, and an associated structure analogous to the peritrophic matrix. *Zoological Journal of the Linnean Society* **128:** 269–287.

Kathirithamby J. 2009. Host-parasitoid associations in Strepsiptera. *Annual Review of Entomology* **54:** 227–249.

Kathirithamby J, Engel MS. 2014. A revised key to the living and fossil families of Strepsiptera with the description of a new family Cretostylopidae. *Journal of the Kansas Entomological Society* **87:** 385–388.

Kathirithamby J, Johnston JS. 2004. The discovery after 94 years of the elusive female of a myrmecolacid (Strepsiptera), and the cryptic species of *Caenocholax fenyesi* Pierce sensu lato. *Proceedings of the Royal Society of London Series B, Biological Sciences* **271**(Suppl. 3)**:** S5–S8.

Kathirithamby J, McMahon DP, Anober-Lantican GM, Ocampo VR. 2012. An unusual occurrence of multiparasitism by two genera of Strepsiptera (Insecta) in a mano leafhopper Idioscopus clypealis (Lethierry) (Hemiptera: Cicadellidae) in the Philippines). *Zootaxa* **3268:** 16–28.

Kathirithamby J, Spencer Smith D, Lomas MB, Luke BM. 1984. Apolysis without ecdysis in larval development of a strepsipteran, *Elenchus tenicornis* (Kirby). *Zoological Journal of the Linnean Society* **82:** 335–343.

Kinzelbach RK. 1971. Morphologische Befunde an Fächerflüglern und ihre phylogenetische Bedeutung (Strepsiptera: Insecta). *Zoologica* **41:** 256.

Kinzelbach RK. 1978. Strepsiptera. *Die Tiewelt Deutschlands* **65:** 166.

Kirkpatrick TW. 1937. Studies of the ecology of the coffee plantations in East Africa. II. The autecology of *Antestia* spp. (Pentatomidae) with a particular account of a strepsipterous parasite. *Transactions of the Royal Entomological Society* **86:** 247–343.

Lagoutte R, Šebesta P, Jiroš P, Kalinova B, Jirošova A, Straka J, Černa K, Šobotnik J, Cvčka J, Jahn U. 2013. Total synthesis, proof of absolute configuration, and biosynthetic origin of stylopsal, the first isolated sex pheromone of Strepsiptera. *European Journal of Chemistry* **19:** 8513–8524.

Lange R, Reihnardt K, Michiels NK, Anthes N. 2013. Functions, diversity, and evolution of traumatic mating. *Biological Reviews* **88:** 585–601.

Lauterbach G. 1954. Begattung und Larvengeburt bei den Strepsipteren. Zugleich ein Beitrag zur Anatomie der *Stylops*-Weibchen. *Zeitschrift für Parasitenkunde* **16:** 255–287.

Linsley EG, MacSwain JW. 1957. Observations on the habits of *Stylops pacifica* Bohart. *University of California Publications in Entomology* **11:** 395–423.

Lloyd J. 1997. Firefly mating ecology, selection and evolution. In: Choe JC, Crespi BJ, eds. *The evolution of mating systems in insects and arachnids*. Cambridge: Cambridge University Press, 184–192.

Matsumoto Y, Matsumura M, Hoshizki S, Sato Y, Noda H. 2011. The Strepsipteran parasite *Elenchus japonicus* (Strepsiptera, Elenchidae) of planthoppers consists of three genotypes. *Applied Entomology and Zoology* **46:** 435–442.

McDermott FA, Buck JB. 1959. The lampyrid fireflies of Jamaica. *Transactions of the American Entomological Society* **85:** 1–112.

McMahon DP, Hayward A, Kathirithamby J. 2011. First molecular phylogeny of Strepsiptera (Insecta) reveals an early burst of molecular evolution correlated with the transition to endoparasitism. *Public Library of Science One* **6:** e21206.

Meinert FR. 1896. Contribution à l'histoire des Strepsiptères. *Oversigt over det Kongelige Danske Videnskabernes Selskabs Forhandlinger* **1896:** 67–76.

Nakase Y, Kato M. 2013. Cryptic diversity and host specificity in the giant *Xenos* strepsipterans parasitic in large *vespa* hornets. *Zoological Science* **30:** 331–336.

Nassonov NV. 1892. Position des Strepsiptères dans le system selon donnés du développement postembryonal et de l'anatomie. *C. R. Congress of Internal Zoology* **2:** 174–184.

Nassonov NV. 1910. Untersuchungen zur Naturgeschichte der Strepsipteren Aus dem Russischen übersetzt von Alexander v. Sipiagen. Mit Anmerkungen und einem kritischen Anhang über einige Ansichten Meinerts betreffs der Anatomie des Weibchens. Herausgegeben von Karl Hofeneder S. J. *Berichte des Naturwissenschaftlich-medizinischen Vereins in Innsbruck* **33:** 1–206.

Noirot C, Quennedey A. 1974. Fine structure of insect epidermis glands. *Annual Review of Entomology* **19:** 61–80.

Noirot C, Quennedey A. 1991. Glands, gland cells, glandular units: some comments on terminology and classification. *Annales de la Société Entomologique de France* **27:** 123–128.

Parker GA. 1979. Sexual selection and sexual conflict. In: Blum MS, Blum N, eds. *Sexual selection and reproductive competition in insects*. New York, NY: Academic Press, 123–166.

Parker HL, Smith HD. 1933. Additional notes on the strepsipteran *Eoxenos* laboulbenei Peyerimhoff. *Annals of the Entomological Society of America* **26:** 217–231.

Parker HL, Smith HD. 1934. Further notes on *Eoxenos laboulbenei* Peyerimhoff with description of the male. *Annals of the Entomological Society of America* **27:** 466–477.

Partridge L, Hurst LD. 1998. Sex and conflict. *Science* **281:** 2003–2008.

Pohl H, Beutel RG. 2004. Fine structure of adhesive devices of Strepsiptera (Insecta). *Arthropod Structure and Development* **33:** 31–43.

Pohl H, Beutel RG. 2005. The phylogeny of Strepsiptera (Hexapoda). *Cladistics* **21:** 328–374.

Poinar GO, Herre EA. 1991. Speciation and adaptive radiation in the fig wasp nematode *Parasitodiplogaster* (Diplogasteridae: Rhabditidae) in Panama. *Révue de Nematologie* **14:** 361–364.

Roff DA. 1990. The evolution of flightlessness in insects. *Ecological Monographs* **60:** 389–421.

Rowe L. 1994. The costs of mating and mate choice in water striders. *Animal Behaviour* **48:** 1049–1056.

Rice W, Holland B. 1999. Reply to comments on the chase away model of sexual selection. *Evolution* **53:** 302–306.

Shapiro AM, Porter AH. 1989. The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *Annual Review of Entomology* **34:** 231–245.

von Siebold CT. 1839. Beiträge zur Naturgeschichte der Wirbellosen Thiere – V. Über Xenos sphecidarum und dessen Schmarotzer. *Schriften der Naturforschenden Gesellschaft in Danzig* **3:** 72–87.

von Siebold CT. 1843. Über Strepsiptera. *Archiv für Naturgeschichte* **9:** 137–162.

Silvestri F. 1940. Descrizione preliminare di una specie nuova di '*Mengenilla*' (*M. spinulosa*, Insecta, Strepsiptera) della Sicilia e notizie sul suo ciclo e sul particolare modo di fecondazione. *Atti dell'Accademia d'Italia Rendiconti delle Classe di Scienze Fisiche, Matematiche e Naturali* **18:** 614–617.

Silvestri F. 1941a. Studi sugli 'Strepsiptera' (Insecta) I. Redescrizione e ciclo dell' *Eoxenos laboulbenei* De Peyerimhoff. *Bollettino del Laboratorio di Zoologia generale e della agraria della Facoltà Agraria in Portici* **31:** 311–341.

Silvestri F. 1941b. Sulla maniera di fecondazione della femmina negli 'Stylopidae' (Insecta, Strepsiptera). *Atti dell' Accademia d'Italia Rendiconti delle Classe di Scienze Fisiche, Matematiche e Naturali* **19:** 550–558.

Silvestri F. 1943. Studi sugli 'Strepsiptera' (Insecta) III. Descrizione e biologie di 6 specie italiane di *Mengenilla*. *Bollettino del Laboratorio di Zoologia generale e della Facoltà Agraria in Portici* **31:** 197–281.

Smith G, Hamm AH. 1914. Studies in the experimental analysis of sex. Part II. On *Stylops* and stylopisation. *Quarterly Journal of Microscopical Science* **60:** 435–461.

Tatarnic NJ, Cassis G, Siva-Jothy MT. 2014. Traumatic insemination in terrestrial arthropods. *Annual Review of Entomology* **59:** 245–261.

Tolasch T, Kehl S, Dötterl S. 2012. First sex pheromone of the order Strepsiptera: (3R,5R,9R)-3,5,9-Trimethyldodecanal in *Stylops melittae* Kirby, 1802. *Journal of Chemical Ecology* **38:** 1493–1503.

Waage JK. 1979. Dual function of the damselfly penis: sperm removal and transfer. *Science* **203:** 916–918.

Waloff N. 1981. The life history and description of *Halictophagus silwoodensis* n. sp. (Strepsiptera) and its host *Ulopa reticulata* (Cicadellidae) in Britain. *Systematic Entomology* **6:** 103–113.

West-Eberhard MJ. 1983. Sexual selection, social competition, and speciation. *Quarterly Review of Biology* **58:** 155–183.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Data S1. List of families and genera of Strepsiptera.

Video Clip S1. Super-extrusion of the cephalothorax of a *Xenos peckii* female during 'calling'. The process of super-extrusion by female *Xenos peckii* was video recorded at 1920 × 1080 pixel resolution, 60 frames/s (fps), interlaced, using a Sony HDR-XR550 camera (Sony of Canada Ltd) (Hrabar *et al.*, 2014). Video replay is at 7.4 fps (4× real-time speed).

Video Clip S2. Fight approach and mating sequence: *Xenos peckii* male approaching the cephalothorax of a female for mating. We filmed the flight approach of a male *X. peckii* and the mating sequence using the Fastec imaging camera IN1000M2GB equipped with Fastec Imaging software, version 3.0.4 (Fastec Imaging). We obtained footage at 300 frames/s (fps), 640 × 480 pixel resolution (Hrabar *et al.*, 2014). Video replay is at 30 fps (10% real-time speed).