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Two males, one female: triplet-style mating behaviour in the Darwin wasp *Xorides ater* (Gravenhorst, 1829) (Hymenoptera, Ichneumonidae, Xoridinae) in the Swiss Alps

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Abstract

The Darwin wasp *Xorides ater* is a solitary idiobiont ectoparasitoid of wood-boring beetle larvae. It occurs across the Palaearctic, and in Switzerland seems to be restricted to areas above 980 m altitude. Very little is known about its biology and behaviour. We here report three separate instances of a female *X. ater* mating simultaneously with two males, observed on the same occasion on a *Picea abies* woodpile near Lenk, Switzerland. Only two copulae that involved one male and one female were observed at the same time, which might indicate that this mating mode is rather common in *X. ater*. We illustrate our observations with photo and video material and compare it to mating behaviour known from other species of Darwin wasps.

Key Words

Altitudinal distribution, Copulation stance, Phenology

Introduction

Hymenoptera have a haplo-diploid reproductive mode (Crozier 1975) and, although thelytoky is not uncommon in the order, most species reproduce sexually through union between a haploid male and a diploid female. The latter then lays eggs that are either haploid (male-producing) or diploid (female-producing) through her ability to control access of sperm to the egg as it passes down the oviduct and past the spermatheca (Gerber and Klostermeyer 1970; Cole 1981; Strand 1989). Female hymenopterans are thus able to produce viable offspring without having to mate, although the offspring then would only be of the male sex. This mode of sex determination, known also as arrhenotokous parthenogenesis, is believed to have had a great bearing on the success of the order (Gauld and

Bolton 1988; Heimpel and De Boer 2008). It is especially through the ability of mated females to control the sex of the eggs they lay that the ensuing complex behavioural and evolutionary ecology of parasitoid Hymenoptera has been so well-studied, albeit in a very limited number of species (Godfray 1994; Wajnberg et al. 2008).

Detailed courtship studies have been made only in a minute fraction of Darwin wasp species, but it is usual for female parasitoid Hymenoptera to be receptive to mating for only a short time following their emergence (Quicke 1997). Courtship is typically rather brief but can involve elaborate cues. It is usually initiated by volatile pheromones emanating from females (Quicke 1997; McClure et al. 2007) and may then involve wing-fanning “songs” (Bredlau and Kester 2019), male-male struggles over access to females (Eggleton 1991), or the transfer of contact

pheromones from the antennae of the male to those of the female (Steiner et al. 2010).

It is by no means unusual for several males of a range of parasitoid wasps to simultaneously court a female, and aggregations of competing males are commonly observed, especially in parasitoids of wood-boring insect larvae such as Pimplinae (*Theronia maculosa* Kreiger, 1906, M. Giannota observations) and Rhyssinae (Eggleton 1991). However, to the best of our knowledge, only a single male has ever been observed to achieve copulation at any one time, even though females are sometimes receptive to repeated matings with the same or a different male for a short time afterwards (Quicke 2015; M. R. Shaw observations). With the exception of a very brief mention of *Rhyssa persuasoria* Linnaeus, 1758 being observed with two males apparently attached, though this was under conditions of disturbance whereby a large courting aggregation was being deliberately dismantled (Baker 1992), we have not encountered reports of more than one male achieving simultaneous copulation (whether ejaculation or not) with a single female in any species of Ichneumonoidea. We have never observed it ourselves during many decades of extensive rearing and field observations and, on that basis, we presume that it is highly unusual for it to occur.

It was therefore a surprise to observe three instances of a female of the xoridine ichneumonid *Xorides ater* Gravenhorst, 1829 in copula with two males, and apparently comfortably so for a prolonged time. During the same observation period, we observed only two standard pairings in the same location. In this brief report, we describe and illustrate these observations.

Study species

Xorides ater (Fig. 1) is a member of the putative sister group to all other Darwin wasps, the subfamily Xoridinae (Klopfstein et al. 2019; Bennett et al. 2019). By analogy with congeners *X. brachylabis* Kriechbaumer, 1889 and *X. irrigator* Fabricius, 1793 (Chrystal and Skinner 1931), and *X. corcyrensis* Kriechbaumer, 1894 (Sharifi and Javadi 1971), *X. ater* can safely be presumed to be an idiobiont parasitoid (i.e., one that kills or permanently paralyzes its host at the time of oviposition) whose solitary larva develops on the body of its beetle larva, prepupa or pupa host. The parasitoid would pass the winter in its cocoon; however, neither the host range nor the developmental biology of *X. ater* is properly understood, nor is there any report of its courtship and mating behaviour.

Materials and methods

Circumstances of observations

During a period of general collecting of Ichneumonoidea near Lenk in the Swiss Canton of Bern, we came across an exposed woodpile of cut and partly split spruce logs

(*Picea abies*), originally up to about 20 cm diameter before being split. The woodpile was located just below Läheweid, about 600 m NW of the cable car station Bettelberg, Stoos (46°26'40"N, 7°25'31"E) at about 1510 m altitude. The observations took place on 3.vii.2019 in the early afternoon from 13.00 hrs to 15.00 hrs of a humid and intermittently sunny day. The dimensions of the woodpile, which had a corrugated iron cover, was approximately 10 × 2 × 1 m and ran length-ways from West to East with one side in shade (at 23 °C) and with the majority of insect activity observed on the shaded side. From the insect species seen on the woodpile and their activity, we deduced that it had been present for at least two years.

Photographs and short video recordings were taken with an Olympus EM-5 Mark II with a 60 mm macro-Olympus lens and later viewed and processed in Adobe Photoshop 2020 and Adobe Illustrator 2020. The composite video was edited in iMovie 10.2.3.

Results

Activity and mating observations

Arriving at the woodpile, we immediately observed a very high level of Ichneumonoidea activity, at one point counting more than 40 males in flight in front of the shaded side of the woodpile. Not only males but also females were present, and closer examination revealed that most of the individuals belonged to the ichneumonid *X. ater* (Fig. 1) with fewer of the similarly sized (but koinobiont endoparasitoid) braconid *Helcon nunciator* Fabricius, 1793. At least some of the females of *X. ater* present were clearly freshly emerged, as matings were occurring, though there were also some females resting without being courted that may or may not have already mated. A few males of *X. ater* were observed at rest but more often they were flying close to the woodpile and apparently in search of females, although without evident aggregation. Although some adult parasitoids were seen on the sunny (southern) side of the woodpile, insects there were too highly active to be easily observed or collected, and most of our observations were made on the shaded (northern) side, where insects were more abundant and easier to observe.

In the braconid *H. nunciator*, we observed about three matings, each between one male and one female. In *X. ater*, on the other hand, over a period of about two hours we saw three instances of a female *X. ater* with two males attached in apparent mating mode (Fig. 2A, B; Suppl. material 1: Movie 1). Only two copulations involving a single individual of each sex by this species were observed during the same period (Fig. 2C, D; Suppl. material 1: Movie 1). Unfortunately, no courtships or commencements of copulation were seen, so whether the attachment of the two males in the triplets happens simultaneously or sequentially is unknown.

In addition to the fact that the triplet-style matings appeared to happen rather easily and possibly even



Figure 1. *Xorides ater* female. Freshly eclosed female resting on a *Picea abies* woodpile in the Swiss Canton of Bern.

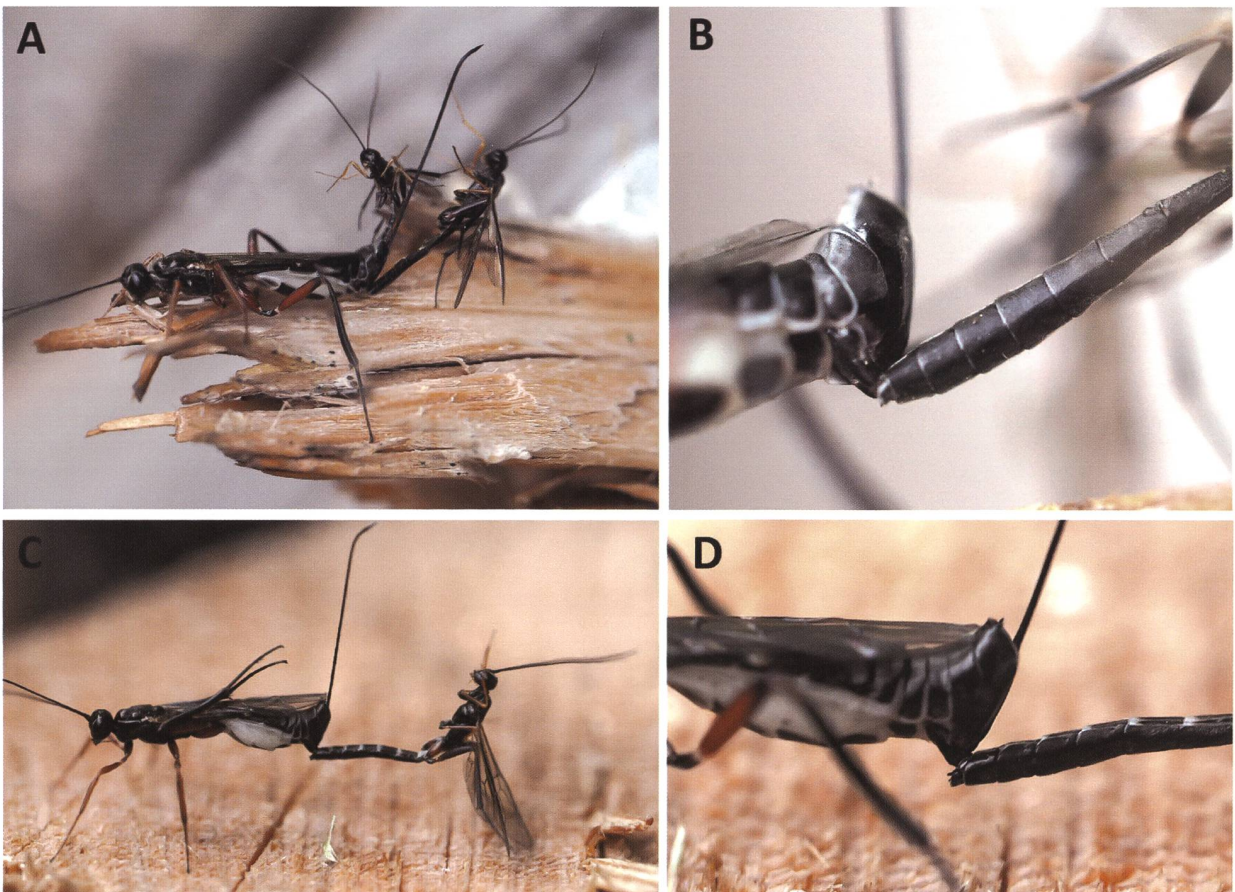


Figure 2. *Xorides ater* mating behaviour **A** Adult female (left) in copula with two attached males (right). [For greater clarity, figure 2A has been edited to create a stacked composite image of two less-focused images. The original raw files are available in Suppl. material 2: S.Fig. 1A and S.Fig. 1B]; **B** Close up of genitalic attachment points – left paramere of male (right) attached to the sixth sternite of female (left). Parameres of second male (background) are obscured from view; **C** Adult female (left) in copula with single male (right). Only the hind legs of the male are in contact with the wood; **D** Close up of genitalic attachment points – left paramere of male (right) attached to the sixth sternite of female (left).

regularly, several things struck us. In the first place, the union seemed to be satisfying all three participants as, overall, the assemblages were as quiescent as the two normal copulae seen at the same time and appeared to persist for an equivalent period (at least 5–10 minutes, with separation occurring only as we collected them). Second, there was no evident jostling or attempt by one male to dislodge the other; indeed, they seemed extremely relaxed, with legs twitching and often held outstretched and clear of everything (usually at least one hind leg touched the substrate, but sometimes all legs were completely free). The females, too, seemed unperturbed and made no attempt to dislodge either male. Third, the positioning of the genitalia appeared to be normal, although the precise mechanism of attachment of males to the female's sixth sternite (hypopygium) in the triplets could not be investigated in any detail. However, the single paramere (= harpe, sensu Schulmeister 2001) that was visible appeared to be normally deployed (Fig. 2B) and was presumably attached to the female's sixth sternite by means of the usual pincer action of the cuspis and digitus of the volsella. The other side of the male external genitalia was not visible, and how it was deployed or indeed whether insemination would be possible, could not be deduced.

Ecological observations

In addition to copulation behaviour, some information on the ecology of *Xorides ater* can be given here. First, we were interested in the altitudinal distribution of the species in Switzerland and thus obtained data from museum collections (Table 1). All 71 specimens found were collected

above 980 m altitude, and the narrow flight time from late June to early August indicates that *X. ater* is univoltine. Furthermore, three out of the 26 collection events included at least a dozen males, while females were always only collected one or two at a time, adding support to our observation of high male abundance (Table 1).

The few and mostly unconfirmed host records that can be found for *X. ater* in the literature all include cerambycid beetles in conifers (Hilszczański 2003; Sheng and Wen 2008). The only suitably sized adult beetles seen at our site were the three species of *Callidium* (Coleoptera: Cerambycidae) that occur in Switzerland: *Callidium aeneum* De Geer, 1775, *C. coriaceum* Paykull, 1800, and *C. violaceum* Linnaeus, 1758. However, there was no direct evidence that any of these was serving as host.

While the females of *X. ater* seen were rather uniform in size, males were substantially more variable in that respect, suggesting that hosts of different sizes had been accepted, but whether of the same or different host species is unknown.

Discussion

Copulatory position in *X. ater* and other Hymenoptera

In the majority of Hymenoptera, the male genitalia are orthandrous, resulting in a mating stance whereby the male is mounted on the top of the female, the same way up and with head-to-head orientation and the apex of his metasoma curled below that of the female (Schulmeister 2001). The major exception is the Tenthredinoidea and

Table 1. Collection data from 71 individuals of *Xorides ater* collected in Switzerland.

Canton.	Locality	Date	N, E	Altitude	Males	Fem.	Legator	Collection ¹
BE	Adelboden	13 Aug.1922	46°29.54, 7°33.35	1340	0	1	T. Steck	NMB
GR	National park	22 Jul.1923		>1400	1	0	A. Barbey	MZL
GR	National park	26 Jul.1926		>1400	3	1	A. Barbey	MZL
GR	Somvix, Val Sumvitg	25 Jul.1891	46°40.80, 8°58.08	1200	0	1		NMBE
GR	Versam	9 Jul.1898	46°47.50, 9°19.49	908	1	0		NMBE
GR	Zernez, Il Fuorn	21 Jul.1949	46°39.85, 10°12.6	1794	0	1	J. de Beaumont	MZL
GR	Zernez, Val dal Spöl, Praspöl	5 Jul.1922	46°39.47, 10°9.40	1800	2	0	C. Ferrière	BNM
GR	Zernez, Val dal Spöl, Praspöl	5 Jul.1922	46°39.47, 10°9.40	1800	12	2	C. Ferrière	BNM
SG	Pfäfers, Vättis	12 Jul.1912	46°54.40, 9°26.03	980	0	1	T. Steck	NMBE
VS	Agettes, Les, Les Mayens-de-Sion	Aug.1946	46°12.05, 7°22.67		0	1	J.L. Nicod	MZL
VS	Ayer, Zinal	26 Jul.1926	46°7.94, 7°37.19	1675	0	1		NMBE
VS	Champéry, Col de Bretolet	18 Jul.1964	46°8.60, 6°47.80	1920	0	1		MZL
VS	Evolène	7 Aug.1933	46°6.33, 7°29.42	1371	0	1	T. Steck	NMB
VS	Evolène	8 Jul.1911	46°6.33, 7°29.42	1371	1	0	T. Steck	NMBE
VS	Evolène	7 Jul.1911	46°6.33, 7°29.42	1371	12	1	T. Steck	NMBE
VS	Evolène	12 Jul.1911	46°6.33, 7°29.42	1371	13	1	T. Steck	NMBE
VS	Evolène, Les Haudères	27 Jun.1915	46°4.71, 7°30.20	1550	0	1	T. Steck	NMB
VS	Evolène, Les Haudères	1 Jul.1925	46°4.71, 7°30.20	1550	1	0	T. Steck	NMB
VS	Grimentz	16 Jul-5 Aug 1942	46°10.81, 7°34.56	1600	2	1	J. de Beaumont	MZL
VS	Grimentz	16 Jul-5 Aug 1942	46°10.81, 7°34.56	1600		1	J.F. Aubert	MZL
VS	Lötschental	16 Jun.1919	46°25.18, 7°49.73	1700	1	0	T. Steck	NMB
VS	Saas	Jul.1938			1	0	J. de Beaumont	MZL
VS	Saas Fee	Aug.1912	46°6.27, 7°55.03	1798	1	1		NMB
VS	Vissoie	22 Jun.1921	46°12.80, 7°34.87	1200	0	1	T. Steck	NMB
VS	Vissoie	24 Jun.1921	46°12.80, 7°34.87	1200	1	0	T. Steck	NMB
VS	Vissoie	23 Jun.1921	46°12.80, 7°34.87	1200	1	0	T. Steck	NMB

¹Collection abbreviations are as follows: BNM – Bündner Naturmuseum, Chur; MZL – Musée de Zoologie, Lausanne; NMB – Natural History Museum Basel; NMBE – Natural History Museum Bern.

Xyelinae, in which the male genitalia are strophandrous (twisted through 180°), resulting in a coplanar end-to-end stance with the two sexes looking in opposite directions (or positioned co-ventrally), as is typically seen in Lepidoptera. The database Taxapad (Yu et al. 2016) gives references to some 333 papers in which courtship and/or mating in Ichneumonoidea is at least mentioned, but it is beyond the scope of this work to attempt a detailed review of these, and we restrict ourselves to an outline of the most usual copulatory stances seen, and a more detailed comparison between species of *Xorides*.

In most Ichneumonoidea, especially when the sexes are of comparable size so that the heads can be more or less adjacent, the male is usually mounted on top of the female and holds her with his legs (Fig. 3A). In this position, some degree of antennal contact during mating is usual. Indeed, the antennae of males are furnished with tyloids in species of approximately ten subfamilies of Darwin wasps (Quicke 2015), which appear to play a role in antennal communication during courtship and mating (e.g., Bin et al. 1999; Bordera and Hernandez-Rodriguez 2003; Steiner et al. 2010); an extreme example of this is seen in the diplazontine *Syrphoctonus tarsatorius* Panzer, 1809 (Fig. 3B; Steiner et al. 2010). In other cases, the male may slip back, foregoing antennal contact (Fig. 3C; also see Benelli et al. 2019 for the campoplegine *Campoplex capitator* Aubert, 1960). When the sexes are dissimilar in size, the male is usually the smaller and, because he is necessarily further back, antennal contact is often literally out of reach during copulation itself. In such cases, the male might start by climbing onto the female's back to perform antennal courtship, then sliding back as the female signals receptiveness, as we have seen in the rhyssine *Rhyssa per suasoria* (Fig. 3D; S. Klopstein observations).

Once genital attachment is achieved, it seems not unusual for males, particularly in species with a large sexual size difference, to fall further back and flip through 180° so that the orientation changes to one with the heads looking in opposite directions, and the male on his back with respect to the female (Fig. 2C). In such cases, there is usually little advantage in the male using many of his legs to hold onto the substrate, and they are sometimes all left free, with only his genitalia attached to anything (Suppl. material 1: Movie 1). This orientation of the male's body was seen in both of the normal copulae (Fig. 2C) and all three triplets (Fig. 2A). It has been reported also in the tryphonine subgenus *Netelia* (*Netelia*) Gray, 1860 (Vance 1927) although, in another species of the same subgenus, Shaw (2001) recorded a more normal male-on-top stance, suggesting some degree of plasticity or variation in the group. Recently, we have seen a similar stance to that encountered in *X. ater* in an unidentified species of the ophionine genus *Enicospilus* in Australia (Fig. 3E), though in this case the male did twist round to grasp the substrate rather than hang free.

Mating behaviour has been described for two other species of *Xorides*, in both cases from captive material in the laboratory. Chrystal and Skinner (1931) observed several pairings of *X. brachylabis*, noting that in courtship the male stroked the female's antennae with his own

and, having approached slowly, mounted the female and grasped her with all six legs while stroking her thorax with his antennae throughout the copulation, which lasted from 15 to 40 minutes. This head-to-head alignment with the male mounted on top corresponds to our Fig. 3A. The mating behaviour of a single pair of *Xorides corcyrensis* described by Sharifi and Javadi (1971) differs in several respects but the male, although perhaps a little further back, was also essentially mounted head-to-head on the female. During copulation the mounted male placed his fore and mid legs on the female's mesoscutum and scutellum, with the fore legs sometimes suspended in the air.

Although unfortunately we did not witness the initiation of copulation, the position finally arrived at by *X. ater* in the copulations we observed, whether involving one or two males, differed markedly from these literature descriptions of union in other *Xorides* species. As in the case of *Netelia* mentioned above, this indicates that uniformity within a genus should not necessarily be expected. It is possible that the copulatory position of *X. ater* may be one factor facilitating matings involving two males, as more space around the female's external genitalia is exposed than in a male-on-top stance. However, additional mating observations under natural circumstances are needed to assess whether triplet-style matings are common in the genus or whether they are restricted to *X. ater*.

Are triplet-style matings of adaptive significance or just a side-effect of scramble competition?

Although males certainly predominated, we were able to find several females of *X. ater* on the woodpile that were not mating and that did not seem to attract much attention at all from the numerous males. Although they were not necessarily receptive, some females did appear freshly emerged, judging from the distended membranous separation of metasomal tergites from sternites and from particles of wood dust on them not yet cleaned away. There is thus no reason to believe that the triplet-style mating was a unique result of particularly high male density and a shortage of females, and we did not observe anything resembling scramble competition among the many males present.

Whether or not insemination was taking place in the triplets and, if so, whether it was from one or both of the males, are key questions that unfortunately we are not able to resolve at present. There are, however, several interesting considerations pertaining to this that can be briefly summarised here. If sperm transfer from both males takes place, is it a fitness strategy of the female to permit or even encourage simultaneous mating by two males, perhaps thereby ensuring a higher genetic mix for her progeny, or offsetting her risk of mating with an infertile male? By decreasing mating time while simultaneously maximising overall sperm transfer, a triplet-style mating strategy may lower the cost (for example the increased risk of predation, or simply the

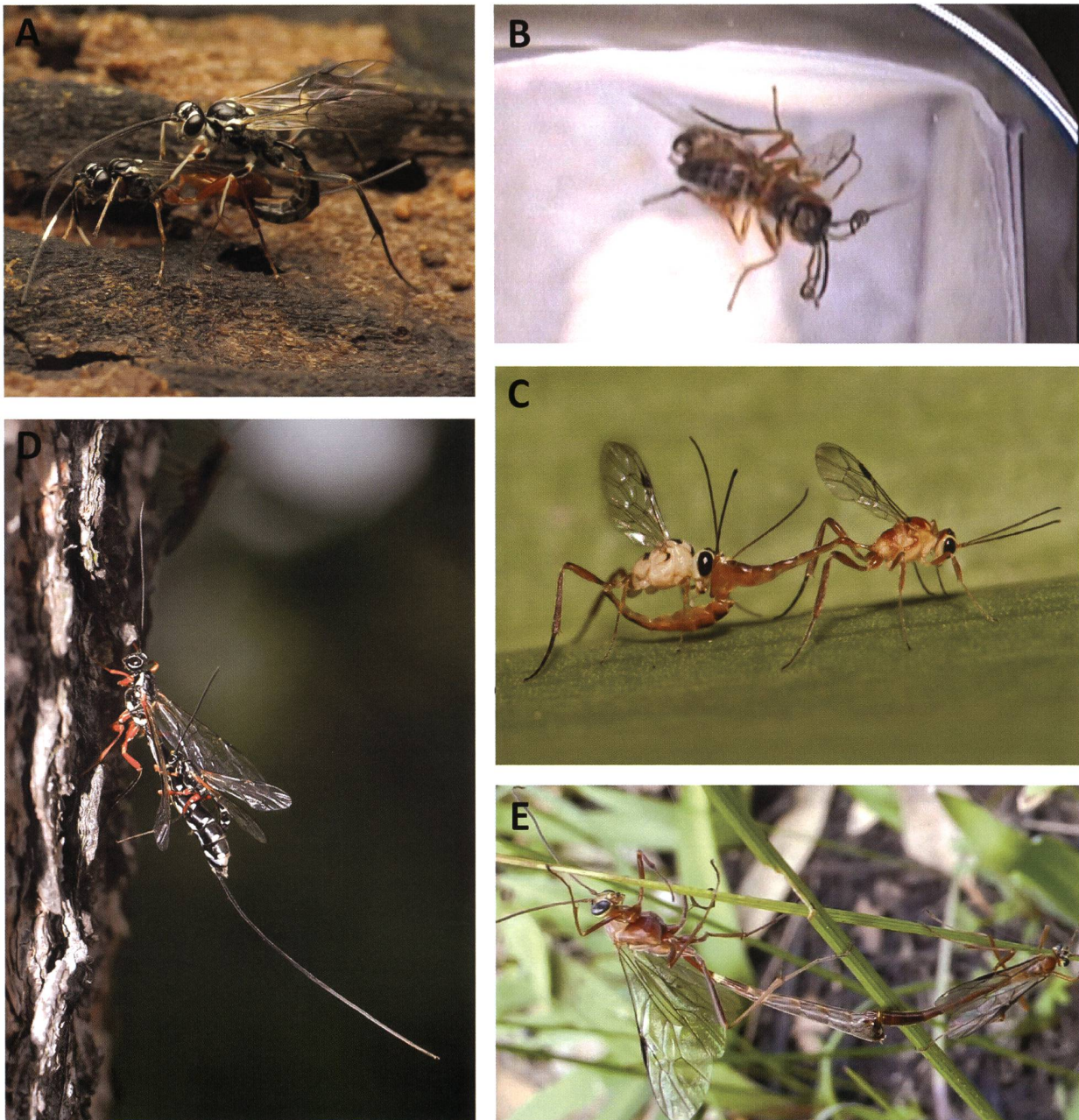


Figure 3. Various mating positions, and orthandrous attachment, of the Ichneumonidae **A** Cryptinae sp. exhibiting a common mating stance. Female – left, male – right. Image Credit: Katja Schulz; **B** Copulation in *Syrphoctonus tarsatorius* (Diplazontinae), with the male antennae coiled round those of the female (ventral view, taken from video sequence in Steiner et al. 2010); **C** Anomaloniinae sp. exhibiting a common mating stance. Male – left, female – right. Image Credit: Muhammad Mahdi Kahrin; **D** *Rhyssa persuasoria* (Rhyssinae) mating pair. The significantly smaller male (right) is seen clinging to female’s metasoma (left). The pair is not yet engaged in copulation. Image Credit: Reto Burri; **E** *Enicospilus* sp. (Ophioninae) in copula. Male – right, female – left. Image credit: Len van der Waag.

time investment) that would be associated with mating successively with more than one male. From the male side, is it just an unintended consequence of the scramble competition between males frequently observed in parasitoids of wood-inhabiting insects (e.g. Eggleton 1991), or is there a trade-off in avoiding conflict? Is there more than just passive sperm competition? Or, if insemination does not occur (in spite of the female’s presumed belief and subsequent behaviour), and if triplet-style sterile matings are common, does it constitute

a fitness impediment carried by *X. ater* by producing a higher proportion of males than would be optimal?

It would be interesting to know of additional observations of similar behaviour, whether in Xoridinae or in other ichneumonoid or hymenopteran groups. Further work is needed to elucidate whether or not sperm transfer from one or both males was successfully achieved in the triplets that evidently occur in *X. ater* at least on occasion, and possibly regularly, and any morphological modifications that might facilitate it.

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Supplementary material 1

Movie 1

Authors: Mark R. Shaw, Madalene Giannotta, Andrés F. Herrera-Flórez, Seraina Klopstein

Data type: multimedia

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Supplementary material 2

Figure S1

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Data type: Raw Images

Explanation note: Two raw images used to generate the stacked composite image, Fig. 2A, in the manuscript.

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