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# Field Observations on the Mating Behavior of *Aclees* sp. cf. *foveatus* Voss (Coleoptera: Curculionidae), an Exotic Pest Noxious to Fig Orchards

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## Abstract

*Aclees* sp. cf. *foveatus* Voss is a new and exotic pest recently reported as responsible for severe damages to Italian fig orchards and nurseries. Investigating its mating behavioral sequence could help to unravel mate assessment and choice dynamics of this pest, adding useful information to build-up behavior-based control strategies. In this study, we observed *A. sp. cf. foveatus* mating behavior under field conditions. The mating behavior is divided into precopulatory, copulatory and postcopulatory phases. Simultaneous antennal tapping and rostral rubbing acts performed by the male on female's body raises male's chances to copulate, while males that performed only antennal tapping on the partner's body did not achieved mating success. Copulation attempt phase lasted  $37.80 \pm 7.65$  s. Copulation lasted  $1116.00 \pm 229.73$  s and was followed by a motionless postcopulatory phase ( $8.40 \pm 1.21$  s). Same-sex sexual interactions were observed among *A. sp. cf. foveatus* males. Male-male sexual interactions lasted about  $140.17 \pm 19.45$  s and were composed of antennal tapping and rostral rubbing followed by one or more mounting attempts. Further research is ongoing to investigate the exact nature of male- and female-borne olfactory and tactile cues routing mate recognition in *A. sp. cf. foveatus*.

## Keywords

Alien pest fig weevil *Ficus carica* mating cues male-male sexual interactions Molytinae

## Introduction

Fig, *Ficus carica* L., is an important worldwide crop, which probably originated in the Middle East. Wild forms of *F. carica* are found in Mediterranean countries and Arabia, Iran, Asia Minor as well as in Central Asia and Transcaucasia. Hundreds of cultivars are listed in the literature and almost all grown are the result of old selection and they are maintained by vegetative propagation. Just in case of Italy, there are 2,409 ha of fig orchards, with a total production of 12,171 t, and the number of involved farms continues to grow (FAOSTAT [2011](#)).

A new and exotic pest belonging to the genus *Aclees* (Coleoptera: Curculionidae), initially mistakenly identified as *A. cribratus* Gyllenhal 1835, was recently reported as responsible of severe damage in many Italian fig nurseries and orchards, particularly in the Tuscan provinces of Pistoia and Lucca (Ciampolini et al. [2005](#)).

The genus *Aclees* Schoenherr 1835 is composed of about 35 species distributed in tropical Africa, Madagascar, Zanzibar and East Asia, including New Guinea. The taxonomy of *Aclees* is still extremely confused and no revisions have ever been published. The only available key was provided by Morimoto (1982), but it included only four species. Most of the species described from East Asia are morphologically very similar and this has often led to confusion in identification of specimens preserved in private and museum collections. This was the cause of the misidentification of the first record for Italy (Ciampolini et al. 2005) that was based on comparison with specimens erroneously labeled as *A. cribratus* in the collections of the Natural History Museum in Paris (Perrin H, personal communication and Meregalli M, personal observation). Soon after, one of the authors (M.M.), after the study of the types of about 20 taxa of *Aclees*, noticed that the species introduced to Italy could not be referred to *A. cribratus*. In the absence of any monograph or complete key, a precise identification of the Italian species proved to be quite complex. A close similarity in morphology was seen with *A. foveatus* Voss 1932. This species was described based on specimens from northwestern China [Yunnan, Soling-ho Valley = (Longchuan Jiang, 25°55'N, 101°54'E), (Voss 1932)], a region with a humid subtropical climate. This name also applies to specimens found at the end of the 19th century and at the beginning of the 20th century in western China, which have hitherto been unidentified or identified erroneously (e.g. specimens at the Museum of Natural History, Paris, identified as *A. cribratus*; or specimens seen at the Senckenberg Natural History Collections, Dresden, labeled *A. poricollis* Faust, a name *in litteris*, Meregalli M, personal observation). For the present time, and until a complete study is carried out, it seems justified to identify the introduced species as *A. sp. cf. foveatus* Voss 1932.

In general, there are no information on ecology and mating behavior for the majority of *Aclees* species. However, in Japan, Taiwan and China, larvae of *Aclees hirayamai* Kôno feed on *Ficus erecta* Thunberg and *F. elastica* Roxb., creating serious damage in nurseries of these ornamental plants (Morimoto 1982). Not all species of the genus are associated with figs, since an as-yet unidentified species was recorded as a pest of Cedar (*Cedrela odorata* L., Meliaceae) in Vietnam (Thu et al. 2010), the larvae boring in the inner bark and sapwood of young trees, leading to the death of the trees. In Italy, the life cycle of *A. sp. cf. foveatus* is closely related to the fig phenology, from which this pest depends for nutrition and reproduction (Ciampolini et al. 2005, 2007). In plant nurseries, the pest completes two annual cycles, in June and September. The mated female digs a series of holes in the wood using the rostrum and then places an egg in the bottom of each hole. Larvae come out after an incubation period ranging from 10 to 20 days, depending on environmental conditions. Larvae live as xylophagous both in roots and tree branches, causing the complete destruction of the wood (Ciampolini et al. 2007). All varieties of *F. carica* grown in the nursery are damaged (Ciampolini et al. 2005). Before the complete decay of wood by feeding larvae, the aboveground portion of the plant accuses dieback, reduced vigor, alteration and yellowing of leaves followed by leaf drop. Mortality of *F. carica* trees is caused by the damage caused to the underground portion of the trunk from repeated attacks of larvae born from the females of different annual cycles (Ciampolini et al. 2007, 2008). Furthermore, adults feed on fruits and young buds. Overall, the larvae of *A. sp. cf. foveatus* are the main cause of *F. carica* decline and application of systemic insecticides has not been successful in controlling this pest. Furthermore, these approaches are highly expensive, polluting and inconclusive and do not allow to avoid persistent re-infection risk caused by the continuous flow of adults from the wild (Ciampolini et al. 2005, 2008). Moderate success has been achieved recently against adult stages with applications of contact insecticides (e.g. pyrethroids) on tree canopy and soil in close proximity of the trunk (Ciampolini et al. 2008). However, research on the use of both biotechnological and biocontrol tools are needed to reduce the damage caused by this weevil, in order to build-up eco-friendly and low-cost control strategies. In this regard, research on the mating behavior of insects is the first step toward a full understanding of their evolutionary behavioral ecology (Kirkendall 1983). Furthermore, investigating mating behavioral sequences could help to unravel mate

assessment and mate choice dynamics of pests, adding useful information for behavior-based control strategies (Jiménez-Pérez et al. [2013](#)).

Based on our knowledge, little research has been done on the mating strategies in the superfamily Curculionoidea (Condrashoff [1968](#); Wojcik [1969](#); Fockler and Borden [1972](#); Schmitz [1972](#); Walgenbach and Burkholder [1987](#); Alcock [1994](#); Vanderbilt et al. [1998](#); Campbell [2005](#); Sato and Kohama [2007](#); Mutis et al. [2009](#); Silva et al. [2012](#)) and no information are available about the reproductive ethology of *A. sp. cf. foveatus*. In this study, we carried out field observations aimed to produce an ethogram describing the *A. sp. cf. foveatus* mating behavior. Furthermore, same-sex sexual behaviors can absolve key adaptive functions, thus shaping the evolution of mating systems (Bailey and Zuk [2009](#)). Particularly, male-male sexual interactions have been noted in other Curculionoidea (Condrashoff [1968](#); Vanderbilt et al. [1998](#)). However, little is known on their occurrence from a quantitative point of view. On this basis, in this research we quantified the magnitude of same-sex sexual interactions among *A. sp. cf. foveatus* males, highlighting the possible mechanisms evoking same-sex behaviors in this species.

## Materials and Methods

Field observations were carried out in a small fig orchard located in “La Sughera” farm, Spianate, Lucca, Italy (43°49'00"N, 10°43'00"E) from 11:30 to 18:00 h in May and June 2013. *Aclees sp. cf. foveatus* sexual interactions were observed in close proximity of the trunk and on the branches of 30 years old *F. carica* trees highly infested by all the developmental stages of the pest. Up to five adults of *A. sp. cf. foveatus* were contemporarily observed walking on the base of *F. carica* trunks or on the branches and feeding on young buds and/or visiting crevices and holes of the bark. Behavioral observations were carried out over several days to account for daily variability.

To describe the *Aclees sp. cf. foveatus* mating behavior, when a male was located, it was directly tracked by an observer for 40 min (or until the end of the sexual interaction, if occurred). The following parameters were registered: (i) time spent by the male performing antennal tapping or antennal tapping plus rostral rubbing on the female's body; (ii) mean duration of copulation attempt (i.e. time spent by the male trying to mount the female and maintain the balance on her by grasping his legs); (iii) mean duration of copulation (i.e. from the male's insertion of the aedeagus into the female's genital chamber until genital disengagement); (iv) the duration of motionless postcopulatory phase (i.e. after dismounting both sexes remains close and motionless for short time, before moving away from each other) and (v) the duration of whole mating sequence (i.e. from the start of the male's walking towards the mate to the complete separation of partners after copulation). Nineteen pairs were observed. After the end of each sexual interaction, the weevils were collected, stored for 30 min at - 20 °C and dissected under a stereomicroscope (Leica ES2, Germany) to verify their sexual identity via the presence of gonads. The males and females that did not engage in any courtship approach or did not move for more than 30 min were discarded.

Data concerning the duration of a male's antennal tapping and rostral rubbing on a female's body were not normally distributed and were processed by JMP 7® ([www.jmp.com](http://www.jmp.com)) using a weighted generalized linear model with one fixed factor:  $y = X\beta + \epsilon$ , where  $y$  is the vector of the observations (e.g. duration of tapping and rubbing),  $X$  is the incidence matrix,  $\beta$  is the vector of fixed effects (i.e. the kind of tapping and rubbing behavior performed by the male towards the female) and  $\epsilon$  is the vector of the random residual effects.

To evaluate the magnitude of male-male sexual interactions in field conditions, encounters between pairs of *A. sp. cf. foveatus* males were directly tracked by an observer for 30 min or until the end of the sexual interactions. The number of pairs in which male-male mating attempts (i.e. tapping with

antennae and rubbing with rostrum on a conspecific male followed by one or more copulation attempts) occurred was recorded. Fourteen male pairs were observed. A chi-square test with Yates correction was used to compare the number of pairs that showed male-male sexual interactions with the number that did not. In male pairs that showed sexual approaches, the mean duration of the interaction was also registered.

## Results

The mating behavior of *A. sp. cf. foveatus* is depicted in Fig. 1. After the location of a female, the male walked towards her and approached her posterior, lateral or frontal side. When the male has reached the female, he engaged mate recognition through antennal tapping and rostral rubbing on the partner's body (Fig. 2). The male tapped and rubbed the female's elytra in case of his posterior approach as well as her head and thorax in case of his frontal and lateral approaches. Significant differences were found in the duration of tapping and rubbing behaviors performed by the male during mate recognition ( $\chi^2 = 49.09$ ,  $d.f. = 3$ ;  $P < 0.001$ ); regardless of the approach's direction, antennal tapping plus rostral rubbing behavior lasted longer than antennal tapping alone (Fig. 3). Then, the female avoided the male and walked off or remained still and the male attempted copulation. Males performing only antennal tapping on the female's body did not achieve mating success. If *Aclees sp. cf. foveatus* male performed copulation attempts [ $37.80 \pm 7.65$  s (mean  $\pm$  standard error), from a minimum of 1 to a maximum of 5 copulation attempts] from any direction and if the mount was frontal or lateral, he immediately tried to align his body with the longitudinal axis of her body. Contemporarily, the male maintained his balance during his copulation attempt through periodical leg grasping on the female's thorax and abdomen.

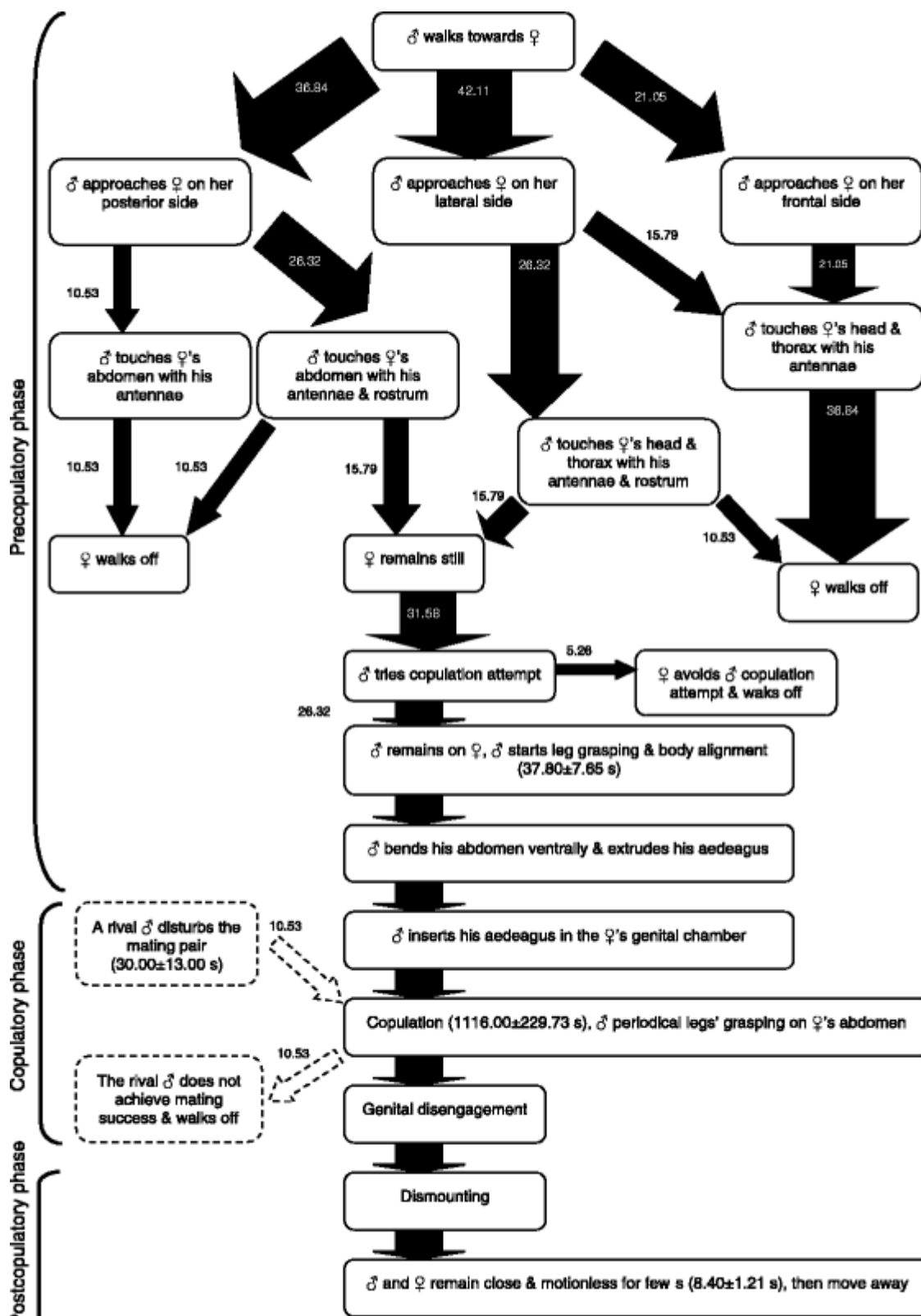


Fig. 1

Ethogram of the mating behavior of *Aclees sp. cf. foveatus*. The thickness of the arrow and the number near it indicate the proportion of individuals displaying different behavioral phases. The values in brackets indicate the mean duration (s) and the relative standard error. The dashed lines evidenced how the mating behavior sequence can be affected by other males. Nineteen mating pairs were observed





Fig. 2

A pivotal phase of *Aclees* sp. cf. *foveatus* mating behavior: a male engaging mate recognition through antennal tapping and rostral rubbing on the partner's head and thorax

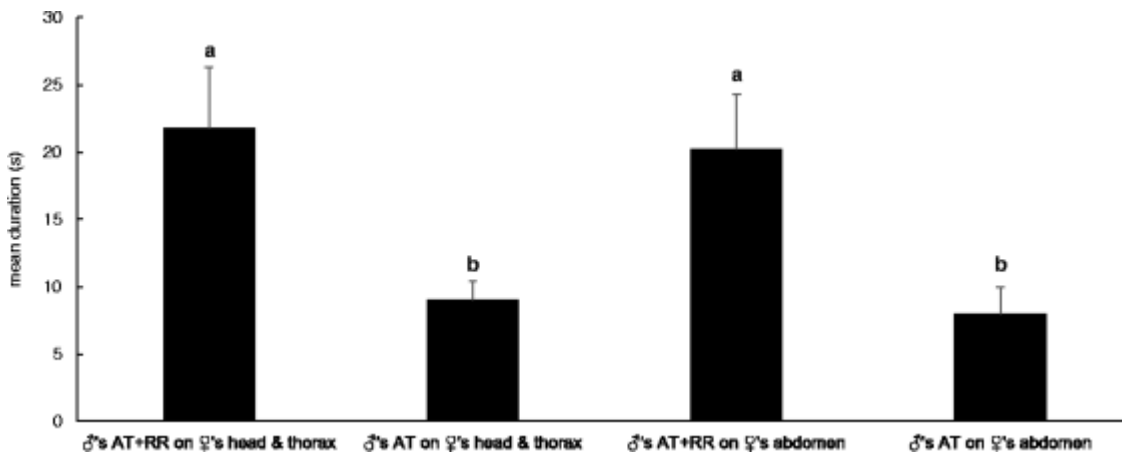


Fig. 3

Mean duration of mate recognition performed by *Aclees* sp. cf. *foveatus* males. AT = antennal tapping on the female's head and thorax ( $n = 7$ ) or abdomen ( $n = 2$ ); AT + RR = antennal tapping plus rostral rubbing on the female's head and thorax ( $n = 5$ ) or abdomen ( $n = 5$ ). T-bars indicate standard errors. Different letters indicate significant differences (Generalized Linear Model;  $P < 0.05$ )

If the mounting attempt was successful, the male bended his abdomen ventrally and extruded his aedeagus and inserted it into the female's genital chamber. During copulation ( $1116.00 \pm 229.73$  s), both sexes remained motionless ( $8.40 \pm 1.21$  s), with the exception of periodical short walking activities carried out by the female. Occasionally, copulation was briefly disturbed by a conspecific male that performed mounting attempts on the mating pair, without success. Copulation ended with genital disengagement. Then, the male dismounted slowly, the partners remained close and motionless for a few seconds then move away from each other. The duration of the whole mating behavior was  $1595.40 \pm 290.61$  s.

Male-male sexual interactions were observed in a similar number of pairs to pairs that did not exhibit sexual approaches (6 vs. 8 male-male encounters, respectively) ( $\chi^2 = 0.643$ ,  $d.f. = 1$ ;  $P = 0.422$ ). The mean duration of male-male sexual interactions was  $140.17 \pm 19.45$  s (mean  $\pm$  standard error;  $n = 6$ ). In the majority of same-sex interactions, at least two copulation



attempts were performed by the approaching male towards a conspecific ( $n = 4$ ). However, also one ( $n = 1$ ) or three copulation attempts ( $n = 1$ ) for each male-male sexual approach were occasionally registered.

## Discussion

The mating behavior of *A. sp. cf. foveatus* is divided into precopulatory, copulatory and postcopulatory phases. The sequence of events leading to copulation is comparable with those reported for other Curculionoidea, including *Hypothenemus hampei* (Ferrari) (Silva et al. [2012](#)), *Rhynchophorus cruentatus* (Fabricius) (Vanderbilt et al. [1998](#)), *Ips pini* (Say) (Schmitz [1972](#)), *Trypodendron lineatum* (Oliver) (Fockler and Borden [1972](#)) and *Sitophilus granarius* (L.) (Wojcik [1969](#)).

Interestingly, simultaneous antennal tapping and rostral rubbing acts performed by *A. sp. cf. foveatus* male on female's body raised the male's chances to copulate, while males that performed only antennal tapping on the partner's body did not achieve mating success. Antennal tapping and rostral rubbing are commonly displayed by many Coleoptera species to recognize their potential partners at close range (Wojcik [1969](#); Vanderbilt et al. [1998](#); Silva et al. [2012](#)). Indeed, peculiar female-borne cuticular hydrocarbons could act as contact pheromones thus mediating mate recognition, as demonstrated in *Aegorhinus superciliosus* (Guérin) (Coleoptera: Curculionidae) (Mutis et al. [2009](#)). On the other hand, we cannot exclude that tapping and rubbing behaviors performed by *A. sp. cf. foveatus* males towards females could absolve other functions, working as placating behaviors before the copulation attempt starts, as in bark beetles (Birch [1978](#)).

Copula duration in *A. sp. cf. foveatus* is comparable to other Curculionoidea species, such as *S. granarius* (i.e. from 30 to 72 min) (Wojcik [1969](#)), while copulation lasted longer in other species [e.g. *Sitophilus zeamais* (Motschulsky), 4.8 h] (Walgenbach and Burkholder [1987](#)).

As recently proved for *H. hampei* (Silva et al. [2012](#)), two evidences show that both postcopulatory mate guarding (Alcock [1994](#); Sato and Kohama [2007](#)) and "passive phase", in which the male maintains the copulation position on the female without genital contact (Parker [1974](#)), are not present in *A. sp. cf. foveatus*: (i) after copulation attempts, the male quickly tried to insert his aedeagus in the genital chamber of the female; (ii) the male left the female immediately after aedeagus retraction. Unfortunately, our experiments do not allow us to know the exact age and mating status of the tested insects. However, previous research conducted on Curculionoidea indicates that multiple mating is widely present in this family (Wojcik [1969](#); Campbell [2005](#); Silva et al. [2012](#)).

Furthermore, same-sex sexual interactions are common in *A. sp. cf. foveatus* males. These interactions are composed of antennal tapping and rostral rubbing on conspecific males followed by one or more copulation attempts. Male-male sexual interactions are noted also in other Curculionoidea (Condrashoff [1968](#); Vanderbilt et al. [1998](#)). We hypothesize that *A. sp. cf. foveatus* males could be perceived as females by other males and this could be due to their displaying of female olfactory traits, as already highlighted in other insect species (Benelli et al. [2013a](#)). In several insects, same-sex sexual interactions absolve key functions (Bailey and Zuk [2009](#); Benelli and Canale [2012](#)), such as allowing individuals to assert dominance over rivals or serving as practice for heterosexual encounters (Benelli and Canale [2013](#)). However, the chance that *A. sp. cf. foveatus* same-sex sexual interaction arises as a by-product of selection on a separate trait, such as high sexual responsiveness, cannot be excluded (Bailey and Zuk [2009](#) and references therein). Moreover, benefits and costs of these homosexual interactions can be extremely variable among

different mating systems (Benelli et al. [2013b](#)) and further research is needed to shed light on this issue in *A. sp. cf. foveatus*.

Overall, this study increases our understanding of the reproductive biology of *A. sp. cf. foveatus*, highlighting that female-borne olfactory and tactile cues perceived by the male through antennal tapping and rostral rubbing during mate recognition play a pivotal role in routing the following mate choice dynamics. Once the specific identity of this pest will be definitely confirmed, further research will be required to clarify the exact nature of the chemicals involved.

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