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Index terms: *Lobiopa insularis*, crop pest, morphology, Neotropical region, oviposition.

Comportamento de cópula e dimorfismo sexual da praga do morangueiro *Lobiopa insularis* (Coleoptera: Nitidulidae)

Resumo – O objetivo deste trabalho foi descrever o dimorfismo sexual e o comportamento de cópula de *Lobiopa insularis*, para o desenvolvimento de programas de controle e manejo de pragas. As características morfológicas para separação de machos e fêmeas foram descritas com auxílio de estereomicroscópio. A sequência de cópula foi registrada por 72 horas com câmera digital. Há diferenças entre machos e fêmeas nos segmentos finais do abdômen, e os machos mantêm posição de guarda após a cópula. Os dados obtidos podem ser usados em programa de monitoramento para uma identificação precisa do sexo do inseto, além de lançarem luz sobre os traços ecológicos desta praga e de Nitidulidae.

Termos para indexação: *Lobiopa insularis*, praga agrícola, morfologia, região neotropical, oviposição.

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Resumo – O objetivo deste trabalho foi descrever o dimorfismo sexual e o comportamento de cópula de *Lobiopa insularis*, para o desenvolvimento de programas de controle e manejo de pragas. As características morfológicas para separação de machos e fêmeas foram descritas com auxílio de estereomicroscópio. A sequência de cópula foi registrada por 72 horas com câmera digital. Há diferenças entre machos e fêmeas nos segmentos finais do abdômen, e os machos mantêm posição de guarda após a cópula. Os dados obtidos podem ser usados em programa de monitoramento para uma identificação precisa do sexo do inseto, além de lançarem luz sobre os traços ecológicos desta praga e de Nitidulidae.

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Lobiopa insularis (Laporte, 1840), a beetle of order Coleoptera and family Nitidulidae, is a species found worldwide in Neotropical, Nearctic, and Palearctic regions (Parsons, 1943; Cline & Kinnee, 2012). Larvae and adults of *L. insularis* feed on crops such as melon (*Cucumis melo* L.), blueberry (*Vaccinium* sect. *Cyanococcus* A.Gray), tomato (*Solanum lycopersicum* L.), peach [*Prunus persica* (L.) Batsch], fig (*Ficus carica* L.), pineapple [*Ananas comosus* (L.) Merr.], and, especially, strawberry [*Fragaria* × *ananassa* (Weston) Duchesne ex Rozier] (Guimarães et al., 2009; Greco et al., 2017).

In Brazil, this pest is one of the most important of strawberry crops, standing out among the species with a frugivorous habit (Dorzenoni et al., 2019). In the country, an outbreak of *L. insularis* larvae and adults caused injuries in strawberry fruits produced for in natura consumption and commercialization, representing a damage of 14.3% per insect/fruit and 70% on total production (Bernardi et al., 2015; Dorzenoni et al., 2019; Souza et al., 2019). In this scenario, strategies to monitor and control this pest have been implemented based on the life cycle of the species and on pesticide use (Guimarães et al., 2009; Bernardi et al., 2015; Greco et al., 2017). However, despite the economic importance of *L. insularis*, its morphology still needs to be further detailed and its sexual behavior described.

The objective of this work was to describe the sexual dimorphism and mating behavior of *Lobiopa insularis* for the development of pest control and management programs.

For the experiment, adults of *L. insularis* were collected from strawberry crops in the municipality of São José dos Pinhais, in the state of Paraná, Brazil. The specimens were identified according to the key proposed by Parsons (1943) and to additional information from Guimarães et al. (2009) and Lasoń & Przewoźny (2009).

For an accurate sexual differentiation (N=5), male and female genitalia were analyzed following procedures modified from Cline & Kinnee (2012). First, the beetles were killed by freezing and, then, their cuticula was softened in warm and soapy water. Afterwards, the specimens were boiled in 10% KOH water solution to clean internal body tissue and facilitate its removal. Morphological features were observed at the Electronic Microscopy Center of Universidade Federal do Paraná, under the Discovery. V8 stereomicroscope (Zeiss, Oberkochen, Germany), and imagens were captured by the VEGA-3 LMU scanning electron microscope (Tescan Group, a.s., Kohoutovice, Czech Republic).

After visually observing sexual dimorphism by the analysis of the pygidium and hypopygidium, live insects (N=40) were sexed, selected, and kept under laboratory conditions, at a temperature of 25°C±2, humidity of 70%±5, and photoperiod of 12 hours of light/12 hours of darkness.

Although, externally, the bodies of male and female *L. insularis* appeared quite similar, the pygidium and hypopygidium differed significantly (Figure 1). For males, the pygidium had a wider basal part, becoming narrow and triangular near its apical part, whereas the hypopygidium had an angulate apical margin. For females, the pygidium was membranous without a triangular part near the apical part, while the hypopygidium had a broad apical part.

Among Nitidulidae, a similar sex dimorphism in tergite VIII was observed in males of *Aethina tumida* Murray, 1867 and *Carpophilus dimidiatus* (Fabricius, 1792), which showed a pygidium with an angulate apical margin (Neumann et al., 2013) and a concave structure in ventrite V at the top of the pygidium (Reales et al., 2018), respectively.

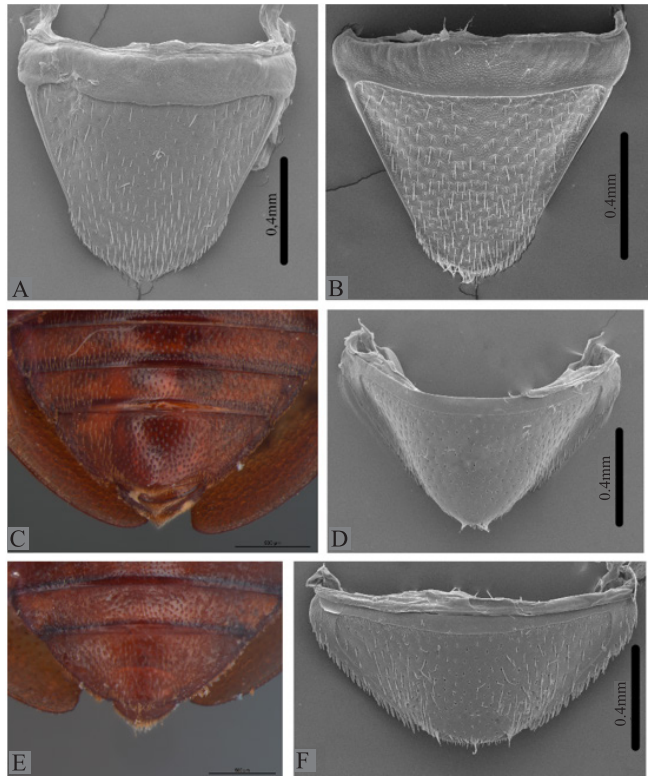

Figure 1. Images of the sexual dimorphism of *Lobiopa insularis* captured with a scanning electron microscope, showing: A, male pygidium with a wider basal part, becoming narrow and triangular near its apical part; B, membranous female pygidium without a triangular part near the apical part; C–D, male hypopygidium with an angulate apical margin; and E–F, female hypopygidium with a broad apical part.

Before the beginning of the experiment, the diet of the insects was changed every two days, and, then, only strawberry was offered for five days before reproductive behavior was observed. For this, ten insect couples were kept in five Petri dishes (5 cm height x 10 cm internal diameter), covered with a PVC film perforated (N=30) with a needle (1 mm in diameter) to allow air circulation and avoid their exit. The behavior of each couple was recorded over 72 hours using the DSC-H400 digital camera (Sony, Tokyo, Japan), directed to the arena with the Petri dishes. The behavior sequences observed during the experiment were used for reproductive description.

Disregarding oviposition, after the behavior analysis, the three main stages established for *L. insularis* were: pre-copulation, copulation, and post-copulation, according to the terminology and methods of Kamiya et al. (2015) and Mustafa et al. (2015).

The main stages of reproductive behavior were observed for *L. insularis* (Figure 2). In the pre-copulation stage under a natural diet with ripe strawberries, the males approached the females from the lateral side during feeding, starting sexual arousal by performing a brief courtship by vibrating the right and left hind legs three times. A similar courtship behavior was reported for *A. tumida*, with a similar pattern of male mating stages and female choice (Neumann et al., 2013; Mustafa et al., 2015). In this stage, the male touches the female's pronotum with its antennas, and, if accepted, climbs on the female, then touches the female's head and pronotum with its antennas. In Tenebrionidae, a family of the same infraorder (Coleoptera: Cucujiformia) as Nitidulidae (Lawrence et al., 2011), beetles also displayed leg movements during mating behavior, but associated to a possible sperm transference (Fedina & Lewis, 2015). In the present work, the leg movements of the analyzed male *L. insularis* were observed before mating, which may indicate two possibilities: a behavior of sexual arousal or to check mating viability. The taxis behavior of a male touching the body of a female is a way of identifying the correct partner through the cuticular hydrocarbons present in the female's body, as observed by Kamiya et al. (2015) for *Cyrtomon luridus* (Curculionidae) and Mustafa et al. (2015) for *A. tumida*. Based on this finding, the mating interaction of *L. insularis* could be related to a mixture of visual and chemical cues; however, to confirm this assumption, further studies are necessary.

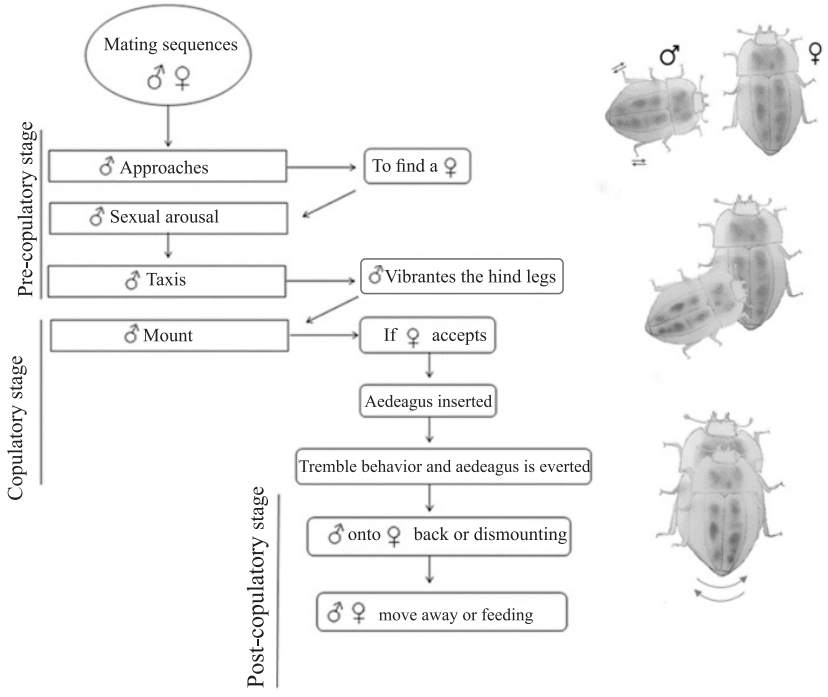

Figure 2. *Lobiopa insularis* mating sequence scheme recorded over 72 hours using a digital camera.

After pre-copulation, the copulatory stage began, when the male started to tremble with the aedeagus everted and inserted in the female genitalia (Figure 2); the tremble behavior occurred from right to left for ±5 s.

The post-copulatory stage was initiated with the everted aedeagus, when males stayed over the female's body for a very short period, no longer than 1 min, which could suggest a possible mate guarding behavior. In fact, mate guarding plays an important role as it aims to protect and ensure paternity after mating as observed by Eberhard (1997) for other arthropods.

In the present study, it was also noted that the females only laid their eggs when the males were present. This reproductive behavior was also recorded by Greco et al. (2017) for *L. insularis*, meaning it could be another mechanism for paternity protection. Therefore, other researches are necessary to verify if the presence of more males can interfere in the reproductive behavior of courtship, mating, and oviposition since only couples were evaluated in the present study.

This is the first known work to confirm and describe the sex dimorphism of *L. insularis* based on high-resolution images, as well as some aspects of the species mating behavior. These data could contribute to a precise sex identification for monitoring program purposes, also shedding light on the ecological features of this insect pest and Nitidulidae.

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References

BERNARDI, D.; BOTTON, M.; NAVA, D.E.; ZAWADNEAK, M.A.C. **Guia para a identificação e monitoramento de pragas e seus inimigos naturais em morangueiro**. Brasília: Embrapa, 2015.

CLINE, A.R.; KINNEE, S.A. A new species of sap beetle (Coleoptera: Nitidulidae) from Baja California Sur, Mexico, with a review of the genus *Lobiopa* Erichson. **Pan-Pacific Entomologist**, v.88, p.202-211, 2012. DOI: https://doi.org/10.3956/2012-25.1.

DORZENONI, R.R.; FORNAZIER, M.J.; ZANUNCIO JUNIOR, J.S.; MARTINS, D. dos S.; FORNAZIER, D.L.; FORNAZIER, M.L.; BOTACIN, L.A.; COSTA, A.F. da. Outbreaks of *Lobiopa insularis* (Laporte, 1840) (Coleoptera: Nitidulidae, Nitidulinae) in the state of Espírito Santo, Brazil. **Revista Científica Intelletto**, v.4, p.43-54, 2019.

EBERHARD, W.G. Sexual selection by cryptic female choice in insects and arachnids. In: CHOE, J.C.; CRESPI, B.J. (Ed.). **The evolution of mating systems in insects and arachnids**. Cambridge: Cambridge University Press, 1997. p.32-57. DOI: https://doi.org/10.1017/CBO9780511721946.003.

FEDINA, T.Y.; LEWIS, S.M. Evaluating cryptic female choice in highly promiscuous *Tribolium* beetles. In: PERETTI, A.V.; AISENBERG, A. (Ed.). **Cryptic female choice in arthropods**: patterns, mechanisms, and prospects. Cham: Springer, 2015. p.431-459. DOI: https://doi.org/10.1007/978-3-319-17894-3_16.

GRECO, N.; CLUIGT, N.; CLINE, A.; LILJESTHRÖM, G. Life history traits and life table analysis of *Lobiopa insularis* (Coleoptera: Nitidulidae) fed on strawberry. **PLoS ONE**, v.12, e0180093, 2017. DOI: https://doi.org/10.1371/journal.pone.0180093.

GUIMARÃES, J.A.; MICHEREFF FILHO, M.; RIBEIRO, M.G.P. de M.; LIZ, R.S. de; GUEDES, Í.M.R. **Ocorrência e manejo da broca-do-morangueiro no Distrito Federal**. Brasília: Embrapa Hortaliças, 2009. (Embrapa Hortaliças. Comunicado técnico, 74).

KAMIYA, A.C.; SILVA, W.D.; LEITE, M.O.G.; TIRONI, P.; WADT, L.; BENTO, J.M.S. Mating behavior and evidence for male-produced aggregation pheromone in *Cyrtomon luridus* (Boheman) (Coleoptera: Curculionidae: Entiminae). **Journal of Insect Behavior**, v.28, p.55-66, 2015. DOI: https://doi.org/10.1007/s10905-014-9481-1.

LASOŃ, A.; PRZEWOŹNY, M. *Lobiopa insularis* (Castelnau, 1840) (Coleoptera: Nitidulidae: Nitidulinae) – an introduced beetle species new for the Palaearctic fauna. **Polish Journal of Entomology**, v.78, p.347-350, 2009.

LAWRENCE, J.F.; ŚLIPIŃSKI, A.; SEAGO, A.E.; THAYER, M.K.; NEWTON, A.F.; MARVALDI, A.E. Phylogeny of the Coleoptera based on morphological characters of adults and larvae. **Annales Zoologici**, v.61, p.1-217, 2011. DOI: https://doi.org/10.3161/000345411X576725.

MUSTAFA, S.G.; SPOONER-HART, R.; DUNCAN, M.; PETTIS, J.S.; STEIDLE, J.L.M.; ROSENKRANZ, P. Age and aggregation trigger mating behaviour in the small hive beetle, *Aethina tumida* (Nitidulidae). **The Science of Nature**, v.102, art.49, 2015. DOI: https://doi.org/10.1007/s00114-015-1300-9.

NEUMANN, P.; EVANS, J.D.; PETTIS, J.S.; PIRK, C.W.W.; SCHÄFER, M.O.; TANNER, G.; ELLIS, J.D. Standard methods for small hive beetle research. **Journal of Apicultural Research**, v.52, p.1-32, 2013. DOI: https://doi.org/10.3896/IBRA.1.52.4.19.

PARSONS, C.T. A revision of the Nearctic Nitidulidae (Coleoptera). **Bulletin of the Museum of Comparative Zoology at Harvard College**, v.92, p.119-278, 1943.

REALES, N.; ROCAMUNDI, N.; MARVALDI, A.E.; FERNÁNDEZ-GÓRGOLAS, M. del C.; STADLER, T. Morphological and molecular identification of *Carpophilus dimidiatus* (Coleoptera: Nitidulidae) associated with stored walnut in Northwestern Argentina. **Journal of Stored Products Research**, v.76, p.37-42, 2018. DOI: https://doi.org/10.1016/j.jspr.2017.12.002.

SOUZA, M.T. de; SOUZA, M.T. de; RIZZATO, F.B.; ZAWADNEAK, M.A.C.; CUQUEL, F.L. Feeding of *Lobiopa insularis* (Coleoptera: Nitidulidae) on strawberries. **Crop Protection**, v.119, p.180-184, 2019. DOI: https://doi.org/10.1016/j.cropro.2019.01.025.