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CROP PROTECTION

Terrestrial gastropods as *Fragaria* x *ananassa* pests in southern Brazil: morphological identification

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ABSTRACT: *Considering the damage caused by terrestrial gastropods in strawberry producing areas in southern Brazil, this study aimed to i) identify the species associated with Fragaria x ananassa in rural properties in municipalities of Paraná State, Brazil; ii) describe and illustrate the diagnostic morphological and conchology features of the mollusk species; iii) construct an illustrated key based on these morphological and conchology features; iv) analyze two species collected in March 2017 to investigate the possible occurrence of parasitic nematodes of medical importance. Taxonomic determination of the mollusks was performed through morphological analysis of samples, and parasitological analysis was carried out using artificial digestion. The following gastropod species were identified: Bradybaena similaris (Bradybaenidae), Rumina decollata (Subulinidae), Deroceras leave (Agriolimacidae), Limacus flavus (Limacidae), and Meghimatium pictum (Philomycidae). Rumina decollata, L. flavus,and M. pictum were recorded for the first time as damaging strawberries in Brazil. No larvae of medical or veterinary importance, associated with samples, were reported. The main diagnostic features are described and illustrated. Besides, the potential of the gastropods as crop pests and vectors of nematodes associated with zoonotic diseases are discussed. Results can facilitate identification of gastropod species in strawberry crops and provide background information for future studies on bioecology and pest control.*
Key words: *Mollusca, Stylommatophora, pests, morphology, illustrated key, strawberry crops.*

Gastrópodes terrestres como pragas do cultivo de *Fragaria* x *ananassa* no sul do Brasil: identificação morfológica

RESUMO: *Considerando os danos causados por moluscos terrestres em regiões produtoras de morangueiro no Sul do Brasil, neste estudo tivemos como objetivos i) identificar as espécies de moluscos associadas à Fragaria x ananassa em propriedades rurais localizadas em municípios do estado do Paraná; ii) descrever e ilustrar as características morfológicas e conquiliológicas diagnósticas das espécies de moluscos encontradas; iii) construir uma chave pictórica com base em características morfológicas e conquiliológicas; iv) analisar parasitologicamente amostras de duas espécies coletadas em março de 2017, visando a identificação de possíveis nematódeos de interesse médico. A determinação taxonômica foi realizada através da análise morfológica das amostras e a parasitológica através da técnica de digestão artificial. Foram identificados os caracóis Bradybaena similaris (Bradybaenidae), Rumina decollata (Subulinidae), Deroceras leave (Agriolimacidae), Limacus flavus (Limacidae) e Meghimatium pictum (Philomycidae). Rumina decollata, L. flavus e M. pictum foram pela primeira vez registradas causando danos ao morangueiro no Brasil. Larvas de interesse médico e/ou veterinário não foram encontradas associadas às amostras analisadas. Os principais caracteres diagnósticos para cada espécie são descritos e ilustrados, assim como discutida a potencialidade das mesmas como pragas agrícolas e hospedeiras de nematódeos de importância médico-veterinária. Os resultados auxiliam a identificação destas espécies na cultura do morangueiro, servindo de base para estudos de bioecologia e controle nestas áreas.*
Palavras-chave: *Mollusca, Stylommatophora, pragas, morfologia, chave pictórica, morangueiro.*

INTRODUCTION

Gastropoda is the largest class concerning diversity within the phylum Mollusca, with over 100 thousand species described, representing about 80% of all known mollusk species (HASZPRUNAR et al., 2008). Besides acting as intermediate hosts of helminths that can affect human health, their natural and mainly anthropogenic dispersal causes some species to affect the economy, society, environment, and agriculture (GRISOTTI & ÁVILA-PIRES, 2011).

In Brazil, terrestrial mollusks have been recorded as pests of numerous crops including soybeans, beans, tobacco, coffee, citrus, and grapes, as well as home and vegetable gardens (BRUSCHI-FIGUEIRÓ & VEITENHEIMER-MENDES, 2002; OHLWEILER et al., 2010, GRISOTTI & ÁVILA-PIRES, 2011). Strawberry (*Fragaria* x *ananassa*

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Duch) crops are one of the most affected by pests, where one of the main problems has been the diversity of species that can affect production. In recent years, an increase in mollusk infestation has been observed in strawberries from crops in southern Brazil (ZAWADNEAK et al., 2014). Use of *mulching* techniques and drip irrigation create favorable conditions for the survival of the mollusks since these organisms prefer dark and humid environments, in addition to the constant food availability. Mollusks can attack not only leaves, which compromises plant development but also fruits, by opening galleries that can serve as a doorway to other pests and diseases (ZAWADNEAK et al., 2014; SOUZA & ZAWADNEAK, 2018). Another critical damage; although indirect, is caused by the presence of slime (mucus), which depreciates the fruits by affecting market value. Consequently, income generation for strawberry farmers in the Paraná State and other regions infested by mollusks has been jeopardized (SOUZA & ZAWADNEAK, 2018).

Mollusks presence in crops may also raise public health issues since several species can be naturally infected with helminths causing human diseases, such as *Angiostrongylus costaricensis* (Moreira & Céspedes, 1971), which causes abdominal angiostrongyliasis, and *Angiostrongylus cantonensis* (Chen, 1935), which is the etiological agent of eosinophilic meningitis (OHLWEILER et al., 2010). Human beings are accidental hosts in the life cycle of these helminths, and contamination usually occurs due to ingestion of raw mollusks or unwashed foods contaminated with third-stage larvae, which are present in the mollusk slime (BONETTI & GRAEFF-TEIXEIRA, 1998).

In this context, this study aimed to i) identify the species associated with *Fragaria* x *ananassa* in rural properties in municipalities of Paraná State, Brazil; ii) describe and illustrate the diagnostic morphological and conchology features of mollusk species; iii) construct an illustrated key based on these morphological and conchological features; and iv) analyze two species collected in March 2017 to investigate the possible occurrence of parasitic nematodes of medical importance.

MATERIALS AND METHODS

Mollusks analyzed in this study were obtained from the collection of the Laboratório de Entomologia Prof. Costa Lima, Curitiba, Brazil. The collection was created to receive specimens of qualitative sampling of the "Produção Integrada do Morango PR" project, from commercial strawberry crops in rural properties located in the municipalities of Pinhalão, São José dos Pinhais, and Quatro Barras in Paraná State, southern Brazil. Specimens were collected during three years (2015-2017).

Fixation of specimens followed the distension technique described by THOMÉ (1975). Individuals were submerged in water in a closed container and kept refrigerated at 6°C for 24 hours and subsequently transferred to 70% ethanol. Species identification was based on dissection of five specimens per each morphotype found. They were dissected under a stereomicroscope for analyzing the external morphological features and genitalia, which were further compared with specialized literature (GODAN, 1983; ARAÚJO, 1989; THOMÉ et al., 1996; BARKER, 1999; SCHILEYKO, 1999; OHLWEILER et al., 2010). The family names follow BOUCHET et al. (2005).

Twelve specimens were collected in March 2017 in the strawberry crops. They were analyzed for parasites using the artificial digestion method modified from WALLACE & ROSEN (1969), with six specimens of *L. flavus* from Quatro Barras (n=3) and São José dos Pinhais (n=3), and six specimens of *M. pictum* from São José dos Pinhais. Artificial digestion was carried out at the Laboratório de Referência Nacional para Esquistossomose-Malacologia (LRNEM), Instituto Oswaldo Cruz (IOC), Brazil.

RESULTS AND DISCUSSION

Five species of terrestrial mollusks were identified in the strawberry crops of the sampled rural properties, *Meghimatium pictum* (Stoliczka, 1873), *Bradybaena similaris* (Férussac, 1821), *Rumina decollata* (Linnaeus, 1758), *Deroceras laeve* (Muller, 1774), and *Limacus flavus* (Linnaeus, 1758) (Table 1, Figure 1, 2). All of them are exotic and classified into Stylommatophora, which includes monoic gastropods with invaginable (rather than contractile) ommatophores and a respiratory pore (THOMÉ et al., 2006). The snail *B. similaris* and the slug *D. laeve* were previously reported as associated with strawberries (ZAWADNEAK et al., 2014). Both species have been reported as natural hosts of *A. cantonensis* (OHLWEILER et al., 2010). Conversely, *L. flavus*, *M. pictum*, and *R. decollata* are here reported for the first time as associated with strawberry crops.

Meghimatium pictum (Figure 1A) – body up to 6cm long and 1.5cm wide (GOMES et al., 2011). Mantle whole, body tapering posteriorly; keel absent. Shell sac present but shell absent. In

Table 1 - List of gastropod species (Mollusca: Gastropoda: Stylommatophora) reported in the survey of strawberry crops, in rural municipalities of Paraná State, Brazil (2015-2017).

FAMILY	SPECIES	MUNICIPALITIES
Philomycidae	*Meghimatium pictum* (Stoliczka, 1873)	São José dos Pinhais; Quatro Barras
Bradybaenidae	*Bradybaena similaris* (Férussac, 1821)	São José dos Pinhais; Quatro Barras
Subulinidae	*Rumina decollata* (Linnaeus, 1758)	Pinhalão
Agriolimacidae	*Deroceras leave* (Muller, 1774)	São José dos Pinhais; Quatro Barras
Limacidae	*Limacus flavus* (Linnaeus, 1758)	Quatro Barras

dorsal view, body with a brown, medial longitudinal stripe and two stripes along the dorsum, on a light beige background. Small, oval gray spots present dorsally between stripes and laterally on the body. The respiratory pore is a short slit near anterior right mantle edge. Pale-colored foot covers the entire length of the body. Upper tentacles grayish.

Meghimatium pictum has been reported as causing damage to vines (Vitis labrusca) in southern Brazil (BARONIO et al., 2014) The species has also recently been associated with the transmission of abdominal angiostrongyliasis in humans in the Rio Grande do Sul State (RODRIGUEZ et al., 2018). These authors also associated the risk of infection with grape consumption in areas where the *M. pictum* is a pest, showing the importance of its appropriate management in the field to reduce the contamination risk of strawberry farmers. According to BARONIO et al. (2014), this species is not able to perforate the surface of grapes but takes advantage of existing damage to reach and feed on grape pulp. This is not true for strawberries since there is no protective exocarp skin; and therefore, it is easier for the mollusks to feed on them.

Bradybaena similaris (Figure 1B) – small snail, the shell up to 1.5cm in diameter. Body light to dark beige. Tentacles darkened. Dorsal surface of upper tentacles usually with a dark stripe that extends through the head. Heliciform shell with five to six whorls and, opened and not deep umbilicus, with a semilunar aperture (ARAÚJO, 1989), partially covered by the extremity of the columella. Color varying from yellowish to brown, sometimes with a reddish-brown band along sutures and the last whorl through the aperture. The reproductive system in this species present a single dart sac with two multifidus glands connected to it, an oval bursa copulatrix with a very long and narrow duct, and a cylindric, muscular and smooth phallus involved by a muscular sheath with an epiphallus (THOMÉ et al., 1996).

Bradybaena similaris has been found in several fruit crops including vines, citrus, banana, and strawberry, besides coffee, maize, wheat, and horticultural products like spinach, broccoli, mustard, lettuce, chicory, and green onions (BRUSCHI-FIGUEIRÓ & VEITENHEIMER-MENDES, 2002; BARKER, 2002; ARAÚJO, 1989; ZAWADNEAK et al., 2014; ZAWADNEAK et al., 2017). The species has also been reported to be a natural host for *A. costaricensis* and *A. cantonensis* (CALDEIRA et al., 2007; OHLWEILER et al., 2010). It has also been associated with the fluke *Eurytrema coelomaticum* (Giardet Billet), a parasite of bovine cattle, goats, and pigs, in Porto Alegre, Rio Grande do Sul State, Brazil. Finally, the species has been associated with *Postharmostomum gallinum* Witenberg, a cecal fluke found in chickens (OHLWEILER et al., 2010).

Rumina decollata (Figure 1C) – has a shell is turriculated, longitudinally striated with irregular elevated lines and wrinkles. It is a decollated shell, which is a shell with a broken apex. Commonly with four whorls and three cm in height, although variations are also known (BATTS, 1957; PRÉVOT et al., 2015). Penis elongate, with characteristic internal coiling folds. It is also possible to notice the slit-like opening of the vas deferens, which is delimited by denser tissue. Opposite to this opening, there is a conspicuous glandular area. Vagina wide and short. Bursa copulatrix with short, narrow duct and well-developed sac (SCHILEYKO, 1999).

Rumina decollata has an omnivorous feeding habit (plants, eggs, worms) (REYNA & GORDILLO, 2018) and may be cannibalistic or prey on other mollusks (CARDILLO et al., 2016). This predatory habit led to its introduction to California (USA) as a biological control agent against *Cornu aspersum* (Müller, 1774) (FISHER, 1966; COWIE, 2001; CARDILLO, et al., 2014) and later to Argentina, in 1988, with the same purpose (DE FRANCESCO & LAGIGLIA, 2007; CARDILLO et

al., 2016). Recent studies in Argentina indicated that *R. decollata* is host to several parasitic nematodes like Toxocara cati Schrank, 1788, which may parasitize human beings (CARDILLO et al., 2016), as well as *Aelurostrongylus abstrusus* (Railliet, 1898), which affects the respiratory system of cats (CARDILLO et al., 2014). Besides, this species is considered pest of crops and horticulture (REYNA & GORDILLO, 2018). In Brazil, *R. decollata* has been recorded on states of São Paulo, Santa Catarina, Rio Grande do Sul and recently also on the Minas Gerais (OLIVEIRA & ABREU, 2013). This is the first report of *R. decollata* damaging strawberry plants on the Paraná state. Considering its ability to rapidly spread and deleteriously impact the native biota, commerce, and health of pets and humans, monitoring its distribution and implementing early control efforts is warranted (REYNA & GORDILLO, 2018).

Deroceras laeve (Figure 1D) – small slug, body length about 2.5cm, anterior part of the mantle delimiting the area where the vestigial internal shell is located. This area reaches approximately the medial part of the body. The specimens observed were medium brown, with the foot pale brown; although, color can vary to light brown or grayish. Short keel in the dorsal posterior portion of the body. Mucus secretion transparent. Pneumostome in the post-medial region. Penis long, sinuous, with an appendage, proximal area with several small papillae more or less differentiated, the phallus glands, distally with a small internal stimulator which can be hemispheric or conical (BARKER, 1999; FORSYTH, 2004). Bursa copulatrix oval to globular, with a duct slightly longer than the bursa itself.

Deroceras laeve has been recorded in vegetable gardens, home gardens, and crops, and is often reported on horticultural products like spinach, beet, broccoli, kale, cabbage, mustard, arugula, and lettuce (MAURER et al., 2002; BRUSCHI-FIGUEIRÓ & VEITENHEUIMER-MENDES, 2002). An epidemiologic study in Nova Itaberaba, Santa Catarina State, Brazil, reported *D. leave* for the first time as host of *A. costaricensis* (MAURER et al., 2002). Those authors mentioned that this small slug is frequently reported on folds of leafy vegetables and thus could transmit the parasite to human beings if ingested accidentally.

Limacus flavus (Figure 1E) – slug up to 12cm in length (BARKER, 1999), with yellowish body with green spots. Spots can be sparse or cover most part of the body, giving a more uniform

Figure 1 - Terrestrial gastropods found damaging *Fragaria* x *ananassa* in rural properties in Paraná State, Brazil. (A) *Meghimatium pictum*; (B) *Bradybaena similaris*; (C) *Rumina decollata*; (D) *Deroceras laeve*; (E) *Limacus flavus*.

greenish color to the body. The first third is marked by the mantle shield, which delimits the area where the reduced internal nail-shaped shell. A white medial line starting on the second third of the body is present. Head pale, without spots, upper tentacles grayish blue. Foot is yellowish white. Mucus secretion yellowish. Bursa copulatrix constituted by a short duct and a small and oval sac. Atrium and vagina are very short. Penis is long, narrow, cylindrical, variably coiled or folded. Vas deferens is short and penetrates the phallus adjacent to phallus retractor muscle. Prostatic gland is very slender and not connected to oviduct at its begining.

Limacus flavus has been recorded as a host for *A. costaricensis* in the municipalities of Coronel Vivida (Paraná State, Brazil) and Santa Rosa (the Rio Grande do Sul State, Brazil) (GRAEFF-TEIXEIRA et al., 1993; OHLWEILER et al., 2010). Two other species of the family *Limacidae* are reported in Brazil, *Limax maximus* Linnaeus, 1758 e *Lehmannia valentiana* (Férussac, 1822) (THOMÉ & GOMES, 2011).

Illustrated key based on external morphological characters

1. Shell present 2
Shell absent..... *Meghimatium pictum*

2. Shell external.....3
Shell internal, vestigial, under the mantle of anterior dorsal portion.....4

3. Shell up to 2cm in diameter, spire with five or six whorls and open umbilicus.....*Bradybaena similaris*
Shell up to 3cm long, fusiform, elongated, castaneous .....*Rumina decollata*

4. Body length 1 to 2.5cm; color from grayish to medium brown; short keel in the posterior portion of the body, mucus secretion transparent ....*Deroceras laeve*

5. Body length up to 10cm; color yellowish gray or greenish yellow, rugosities yellowish, mantle with greenish spots; keel absent; mucus secretion yellowish.....*Limacus flavus*

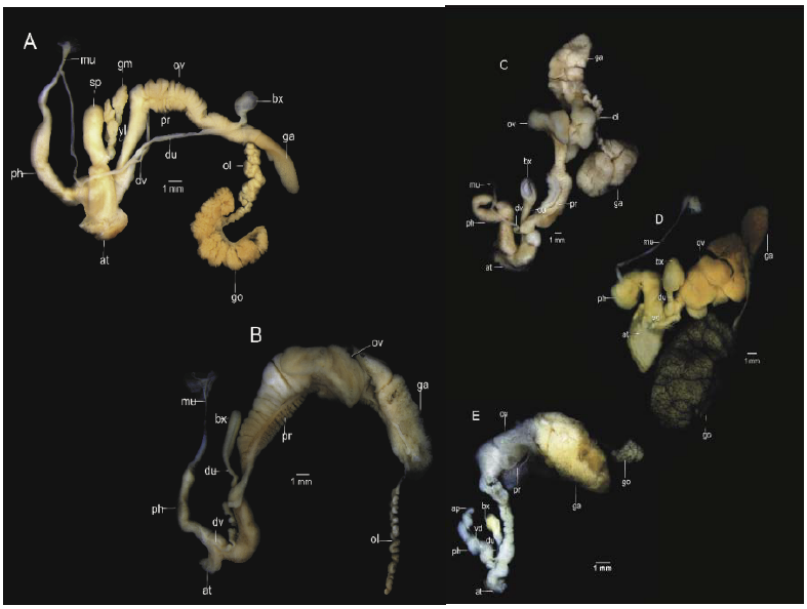

Figure 2 - Reproductive system of the five gastropod species obtained in the sampled localities in rural properties in Paraná State, Brazil: (A) *Bradybaena similaris* (CMIOC 10.006); (B) *Rumina decollate* (CMIOC 11.103) - For *R. decollata*, the gonad does not appear in the picture as it could not be dissociated from the digestive gland; (C) *Limacus flavus* (CMIOC 10.785); (D) *Meghimatium pictum* (CMIOC 10.784); (E) *Deroceras leave* (CMIOC 148). Acronyms: at, atrium; du, bursa copulatrix duct; dv, vas deferens; ga, albumen gland; go, gonad; mu, penis retractor muscle; ov, oviduct; ol, hermaphrodite duct (duct which conducts gametes from the gonad); ph, penis; pr, prostate.

All specimens tested of *L. flavus* and *M. pictum* were negative for *A. cantonensis* and for *A. costaricensis*. Specimens-vouchers were deposited in the malacological collection of the Instituto Oswaldo Cruz (CMIOC), Rio de Janeiro, Brazil. *Bradybaena similaris* (CMIOC 10.006); *Rumina decollata* (CMIOC 11.103), *Limacus flavus* (CMIOC 10.785); *Meghimatium pictum* (CMIOC 10.784); *Deroceras laeve* (CMIOC 148).

The information about mollusk species associated with crops and terrestrial mollusks, in general, is scarce in Brazil (SANTOS et al., 2009). We identified here five species associated with strawberry crops. Further studies on morphology, biology, and physiology are necessary to develop adequate management methods for controlling these pests (THOMÉ et al., 2006).

CONCLUSION

Terrestrial gastropods *M. pictum*, *L. flavus* and *R. decolata* were recorded for the first time damaging *Fragaria* x *ananassa* in rural properties in Paraná State, Brazil. Species identification can be performed using morphological and conchology features. Although, no larvae of medical or veterinary importance were detected in the analyzed samples, the consumption of fresh strawberries *in natura* and the presence of these gastropods in crops may increase the transmission risk of zoonoses.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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