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Short Communication

First record of *Atherigona reversura* Villeneuve (Diptera: Muscidae) feeding on Bermudagrass (*Cynodon dactylon* cv. Jiggs, Poaceae) in Brazil: morphological and molecular tools for identification



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ABSTRACT

Bermudagrass (*Cynodon dactylon* cv. Jiggs) is an important food source for dairy cattle in the semi-intensive milk production systems most often used in southern Brazil. Although many insect pests are associated with feed grasses, we report here the first occurrence of the fly *Atherigona* (*Atherigona*) *reversura* Villeneuve, 1936 (Diptera: Muscidae) feeding on bermudagrass in Brazil. This potential pest was observed in April 2015 in three localities (Abelardo Luz, Palmitos, and Videira) in western Santa Catarina, in southern Brazil. The infested plants had senescent and necrotic terminal leaves that reduced plant growth. New growth had to sprout new tillers from basal nodes, which resulted in a reduced plant growth rate. We also provide a morphological identification key (with figures) for *A. (Atherigona) reversura* and *A. (Acritochaeta) orientalis* Schiner, 1868. A molecular identification based on COI is also provided to better differentiate species.

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Dairy farming has increased recently in Brazil due to changing market demands (Muniz et al., 2013). Southern Brazil is among the most important milk producing regions and has the greatest increase in production (Síntese Anual da Agricultura de Santa Catarina 2014–2015). Although cattle diets are often supplemented (especially with corn silage and hay), most farmers use pastures as the main source of feed. Thus, management of cultivated pasture is important to ensure constant forage supply and to maintain high levels of productivity while keeping costs low.

In tropical and subtropical regions, *Cynodon* grasses (bermudagrass) are very productive (over 20 t ha<sup>-1</sup>), adaptable to regional soil conditions, pest and disease tolerant, and therefore of great forage potential (Oliveira et al., 2000; Rodrigues et al., 2006b). During the last 20 years, bermudagrass became the most important pasture grass for milk production in southern Brazil, used in more than 80% of milk farms (Fernandes, 2012). To date, few insect pests, including spittlebugs (Hemiptera: Cercopidae) (Lohmann et al.,

2010; Chiaradia et al., 2013), caterpillars (Lepidoptera: Noctuidae) (Assunção-Albuquerque et al., 2010), and grass bug (Hemiptera: Miridae) (Chiaradia and Poletto, 2012) were problems for perennial grasses in southern Brazil.

We report, for the first time in Brazil, herbivory and damage by *Atherigona* (*Atherigona*) *reversura* Villeneuve, 1936 (Insecta, Diptera: Muscidae) on bermudagrass *Cynodon dactylon* cv. Jiggs. This is also the first record of the species in South America. *Atherigona* (*Atherigona*) *reversura* larvae were found infesting bermudagrass in April 2015 in three localities: Abelardo Luz (26°39'22" S; 52°12'17" W), Palmitos (27°7'56" S; 53°6'9" W), and Videira (27°1'23" S; 51°11'53" W) in the western region of the state of Santa Catarina in southern Brazil. These larvae caused the death of apical leaves of infected tillers by feeding apically starting at the terminal node and which damages vascular tissue (Fig. 1). Once larvae begin feeding, senescence and necrosis of the upper part of tillers occurs, mostly due to the death of the two newest leaves. Feeding this way causes a reduction in plant growth because regrowth must begin through new tillers originating at basal nodes, or the apical node prior to the damage. Thus, feeding by the fly larvae causes a reduction in establishment into new areas as well as biomass production in areas already established.

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**Fig. 1.** (A) *Cynodon dactylon* cv. Jiggs showing plant damage. *Atherigona* (*Atherigona*) *reversura*: (B) larvae; (C) puparium; (D) adult. Scale bars = 1 mm.

Known elsewhere as the bermudagrass stem maggot (BSM), *A. (Atherigona) reversura* was first discovered in the New World in southern USA state of Georgia in July 2010 where it was infesting bermudagrass hayfields, pastures and turf and is now found throughout the southeastern United States (Grzywacz et al., 2013). Although the extent of damage varies among cultivars (Ikeda et al., 1991), BSM causes an average decrease of ~8% in total dry biomass of bermudagrass cultivars in the USA (Baxter et al., 2014). Thus, due to this potential problem, *A. (Atherigona) reversura* should be monitored to assess its establishment and behavior as a potential pest of pastures in Brazil. Additional study is also necessary to quantify damage by the fly larvae to bermudagrass quality and yield, to develop integrated management.

In the laboratory, under controlled conditions ( $26 \pm 2$  °C, RH:  $70 \pm 10\%$  and photoperiod: 14L:10D hours), larvae were reared and the resulting adults were identified as *A. (Atherigona) reversura* using the revision of Pont and Magpayo (1995). Adrian C. Pont (Oxford University Museum of Natural History – UMO) also confirmed the species identification by figures and photographs we sent to him. Voucher specimens were deposited at the Coleção Entomológica da Epagri/Cepaf, in Chapecó, and in the Coleção Entomológica Pe. Jesus Santiago Moure, in the Department of Zoology of the Universidade Federal do Paraná, Curitiba (DZUP).

We also used molecular data to confirm identity and to provide another tool to aid in the identification of this pest. Thus, we provide a partial COI sequence of *A. (A.) reversura* and compared it

to *Atherigona* sequences available at GenBank (Table 1). Our objective in comparing the sequences was to use additional evidence that the species we were dealing was not any other with known sequences. We also intended to verify if COI data was enough to differentiate *Atherigona (Atherigona) reversura* from other species of the genus, especially *Atherigona (Acritochaeta) orientalis*. We highlight this analysis is not an inference of the phylogenetic relationships of the genus, which is very speciose. We also examined the *Atherigona (Acritochaeta) orientalis* voucher that provided the sequence KP161673 to verify its identity. We amplified the COI gene using Folmer et al. (1994) primers (LCO-1490f and HCO-2198r), the region chosen by DNA Barcoding project as the standard

**Table 1**

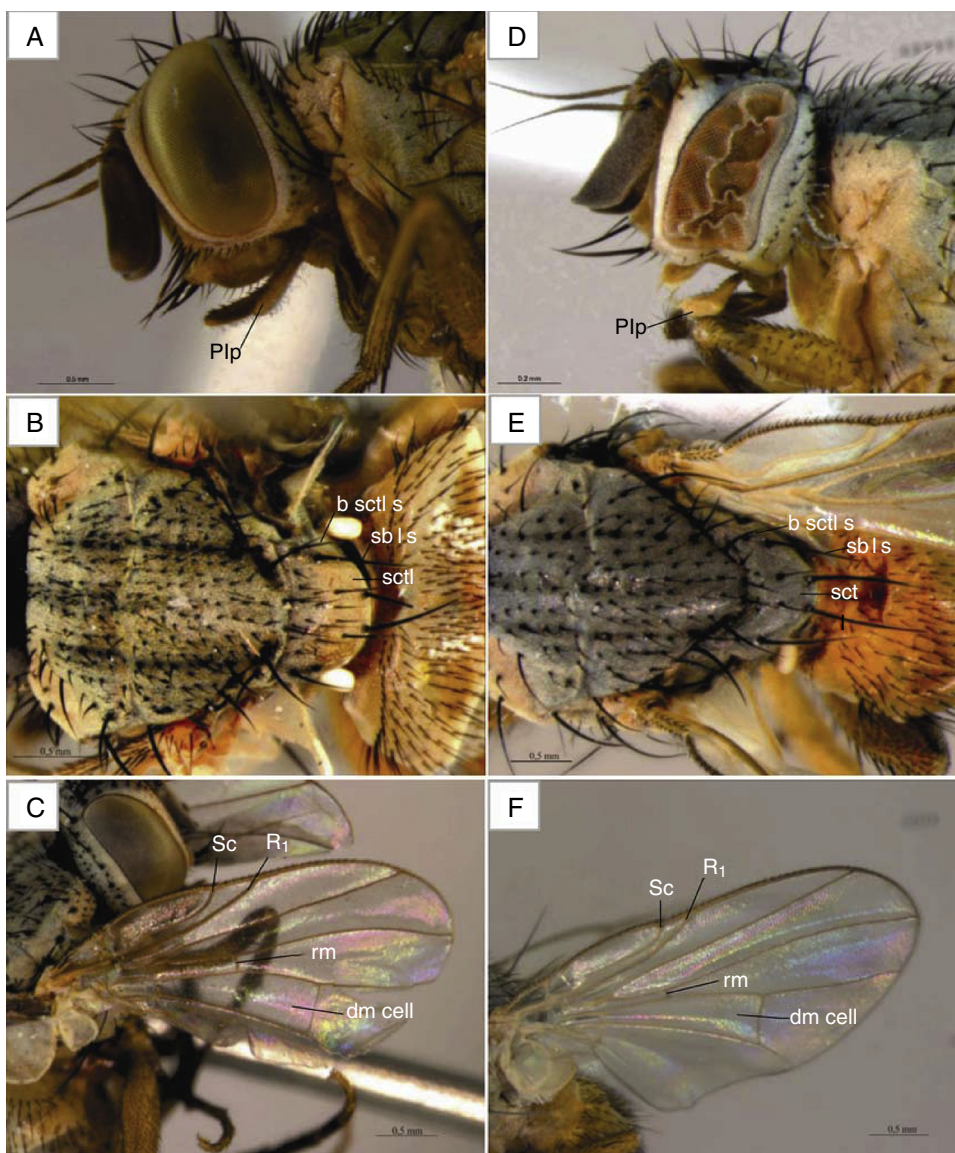
List of *Atherigona* species used in this study with GenBank number.

Species	GenBank number
<i>Atherigona (Atherigona) nigritibiella</i> Fan & Liu, 1982	EU627708
<i>Atherigona (Acritochaeta) orientalis</i> Schiner, 1868	EU627707
<i>Atherigona (Acritochaeta) orientalis</i> Schiner, 1868	KP161673
<i>Atherigona (Atherigona) oryzae</i> Malloch, 1925	KP161674
<i>Atherigona (Atherigona) reversura</i> Villeneuve, 1936	KT906365
<i>Atherigona (Atherigona) seticauda</i> Malloch, 1926	KJ510607
<i>Atherigona (Atherigona) theodori</i> Hennig, 1963	KJ510608
<i>Atherigona (Atherigona) varia</i> (Meigen, 1826)	KJ510609
<i>Cyrtoneurosis veniseta</i> (Stein, 1904)	KJ510614
<i>Cyrtoneurosis veniseta</i> (Stein, 1904)	KP161656

barcode for most animal groups. DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen, Valencia, United States) following the protocol provided by the manufacturer, with the following modifications: the specimen was incubated in proteinase K and buffer for 48 h, with a final addition of 60  $\mu$ l of buffer to obtain concentrated genomic DNA. The PCR reaction used Platinum Taq DNA Polymerase (Invitrogen, Whaltham, United States) with the following: 14.3  $\mu$ l of water, 2.5  $\mu$ l of each MgCl<sub>2</sub>, dNTP and PCR buffer (all at 25 mM), 1  $\mu$ l of each primer (10 pmol/ $\mu$ l), 0.2  $\mu$ l of taq and 1  $\mu$ l of genomic DNA, under the following conditions: first denaturation step at 95 °C (5 min), 33 cycles of denaturation at 93 °C (20 s each), annealing at 50 °C (40 s), first elongation step at 72 °C (2 min), second elongation step at 72 °C (5 min) following Lessard et al. (2013). The PCR product was purified using QIAquick PCR Purification Kit (Qiagen, Valencia, United States) following the protocol provided by the manufacturer, and then sent to the Centro de Pesquisa sobre o Genoma Humano e Células-Tronco (Universidade de São Paulo) for sequencing in both directions. The voucher (DZUP342114) was also deposited at DZUP.

Concordance of complementary strands was checked. Alignment was carried out using the standard configuration in the program MUSCLE (Edgar, 2004), in MEGA 6.05 (Tamura et al., 2013). GTR+I was the nucleotide substitution model used (chosen by JModel Test 2.1.4, Posada, 2008). We analyzed the relationships between seven *Atherigona* species with COI (Table 1) using MrBayes 3.2.2 (Bayesian Posterior Probabilities, Ronquist et al., 2012) using standard configurations. We carried out two simultaneous runs with eight chains of 1 million generations, saving a tree every 1000 generations. We used *Cyrtoneuropsis veniseta* (Stein, 1904) to root the tree, due to the close phylogenetic relationship of *Cyrtoneuropsis* Malloch, 1925 and *Atherigona* Rondani, 1856 (Haseyama et al., 2015), and FigTree 1.4 (Rambaut and Drummond, 2012) to create the tree figure.

*Atherigona* genus (includes more than 220 species) can be distinguished from other genera by the following characters (Pont and Magpayo, 1995; de Carvalho and Couri, 2002): angular head, with long very sunken face and antennal flagellomere, almost reaching lower facial margin in lateral view; arista bare; thoracic setae very reduced in size, prealar absent, acrostichal setae 0+1;



**Fig. 2.** *Atherigona (Acritochaeta) orientalis*: (A) Palpus male, (B) Mesonotum male, dorsal view, (C) Wing. *Atherigona (Atherigona) reversura*: (D) Palpus male, (E) Mesonotum male, dorsal view, (F) Wing. Abbreviations: b sct l s, basal scutellar seta; dm cell, discal median cell; plp, palpus; R1, radial 1 vein; r-m, radial-medial crossvein; sb l s, subbasal lateral seta; Sc, subcostal; sct l, scutellum.

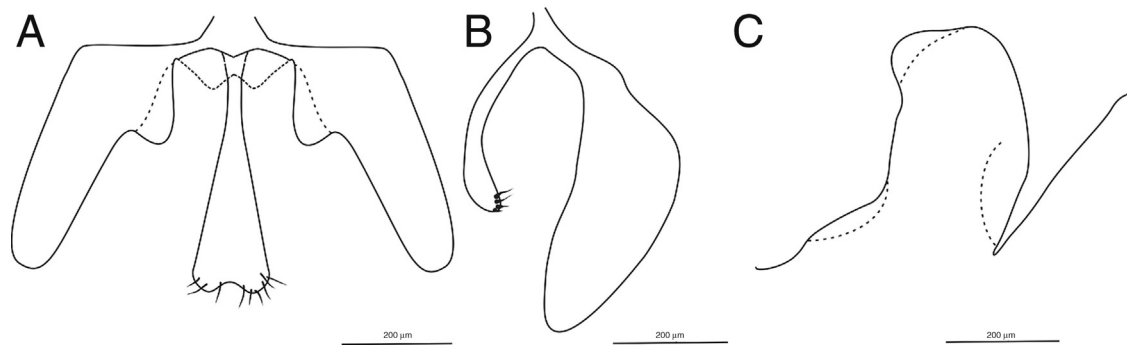


Fig. 3. *Atherigona (Atherigona) reversura*: (A) trifoliate process, dorsal view; (B) trifoliate process, lateral view; (C) hypopygial prominence, lateral view.

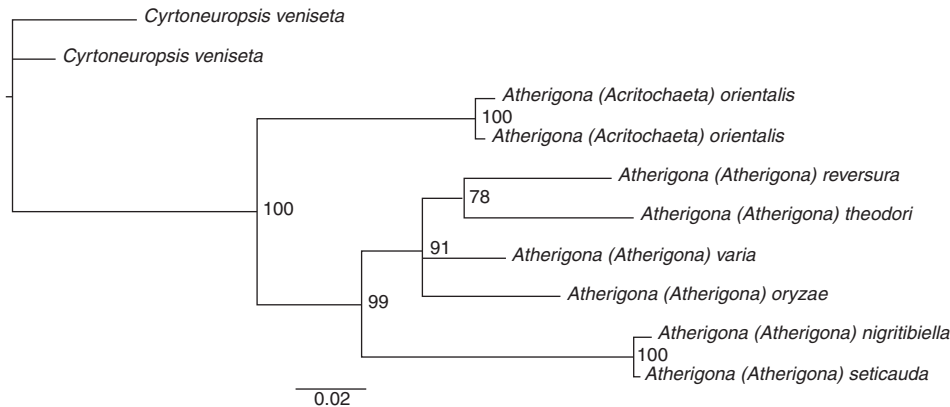


Fig. 4. Analysis using Bayesian posterior probabilities (values shown) using 10 COI sequences for seven species of *Atherigona* and *Cyrtoneuropsis veniseta*.

katapisternals setae 1 + 2, lower one weaker, equidistant from the upper two; wing veins, except costa, bare; hind tibia without posterodorsal seta (calcar) in apical half. *Atherigona* species are uniform in general appearance, small (body length: 5.0–7.0 mm), yellowish-gray in general coloration.

Two subgenera comprise the genus: *Acritochaeta* Grimshaw, 1901 and *Atherigona sensu stricto*, whose larvae have different feeding strategies (Pont and Magpayo, 1995). Larvae of *Acritochaeta* are scavengers or predators in various types of decaying organic matter, including carrion. Larvae of *Atherigona s.s.* are phytophagous and primary pests of various species of Poaceae, often causing economic loss in agriculture in the tropics and subtropics of the Old World (Pont and Magpayo, 1995; Grzywacz et al., 2013). Today, only two species of *Atherigona* occur in Brazil, *Atherigona (Acritochaeta) orientalis* Schiner, 1868 and *A. (Atherigona) reversura*, which can be identified by the following key (Pont and Magpayo, 1995, modified):

1. Wing with cross vein r-m beyond middle dm cell, as well as beyond intersection of subcostal vein in costal vein (Fig. 2C). Male: Palpus elongate (Fig. 2A). Basal lateral setula of scutellum almost half as long as subbasal lateral seta (Fig. 2B); fore tarsus with only normal vestiture of short setulae; genitalia with trifoliate process absent . . . *Atherigona (Acritochaeta) orientalis*
- 1'. Wing with cross vein r-m always in basal half of cell dm and prior to intersection of subcostal vein in costal vein (Fig. 2F). Male: Palpus with enlarged tip (Fig. 2D). Basal lateral setula of scutellum at most 1/3 as long as subbasal lateral seta (Fig. 2E); fore tarsus with moderately long anteroventral and posteroventral setulae on tarsomere 1; genitalia with trifoliate process present (Fig. 3) . . . *Atherigona (Atherigona) reversura*

*Atherigona (Acritochaeta) orientalis* has both sanitary (Oliveira et al., 2002) and forensic (Barbosa et al., 2009) importance and

is widespread in tropical and subtropical areas in all biogeographic regions (Grzywacz and Pape, 2014). Although this fly has been collected in urban areas and forests in Brazil (Campos and Barros, 1995; Marchiori et al., 2000; Rodrigues et al., 2006a), *A. (Acritochaeta) orientalis* has been found in association with agriculture (Cahill, 1992), including peppers and tomatoes in Nigeria (Iheagwam and Nwankiti, 1980; Ogbalu, 1999; Ogbalu et al., 2005), melons in Pakistan (Chughtai et al., 1985), and corn (Panwar and Sarup, 1985) and wheat in India (Singh, 1975).

We had access to specimens from one population (from Abelardo Luz), and no COI sequence for *A. reversura* was available at GenBank. Therefore, we were unable to distinguish single nucleotide polymorphisms for this species. The result of analysis using Bayesian posterior probabilities (Fig. 4) clearly splits *Atherigona* species into two clades, one of which includes *A. (Acritochaeta) orientalis* (the only species in the subgenus *Acritochaeta* in this analysis), and the other with all of the remaining species (all in *Atherigona s.s.*). This indicates that COI sequences successfully separate *A. (Acritochaeta) orientalis* and *A. (Atherigona) reversura*.

Therefore, with the two species of *Atherigona* in Brazil, correct identification of *A. (Atherigona) reversura* is important because it is quite similar in appearance to *A. (Acritochaeta) orientalis*, and misidentification may often occur. To resolve this potential issue, we provide both morphological and molecular tools that allow correct identification of these two species. Thus, the tools we provide here can be applied to pasture management of the bermudagrass stem maggot in Brazil and elsewhere.

#### Conflicts of interest

The authors declare no conflicts of interest.

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