REDESCRIPTION OF *ANAPHES ATOMARIUS* (BRÈTHES) (HYMENOPTERA: MYMARIDAE) AND COMPARISON WITH SIMILAR SPECIES IN EUROPE AND NORTH AMERICA

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Abstract

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Anaphes atomarius (Brèthes) (Hymenoptera: Mymaridae) is redescribed based on the holotype and specimens reared from *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) in Brazil that are assumed tentatively to be conspecific with the type. *Anaphes archettii* Ghidini from Italy is also redescribed, a lectotype designated, and both species are compared to *A. listronoti* Huber and *A. victus* Huber from North America.

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Introduction

The Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) is native to South America. It was accidentally introduced into New Zealand where it was discovered in 1927 (Dymock 1989) and has become a major economic pest (Timlin 1964). A search for potential biological control agents was begun by staff at the Commonwealth Institute of Biological Control, South American Station, San Carlos de Bariloche, Argentina, and an egg parasitoid was found and identified as *Anaphes atomarius* (Brèthes) (Hymenoptera: Mymaridae). In 1966 and 1967, consignments of parasitized eggs were sent to New Zealand (Clausen 1977) and specimens were released at Nelson, Lincoln (Canterbury) and Waikato but the species failed to become established as a result of not being able to overwinter (Ferguson *et al*. 2007). Ahmad (1977, 1978) detailed the rearing technique for *L. bonariensis* and the egg parasitoid. Because *L. bonariensis* may occur as a contaminant in grain shipments from New Zealand or elsewhere it is listed as a quarantine pest of pasture grasses and cereals in the European Union (Ostoja-Starzewski 2011). The original 5-line Latin description and sketchy line drawings of wings and antenna are inadequate to define *Anaphes atomarius* and because of its potential for biological control the species is redescribed here, based on the holotype and several other specimens reared from *L. bonariensis* in Brazil. It is compared with similar species reared from known hosts in Europe and North America.

Methods

Non-type specimens were slide mounted in Canada balsam using the method described by Noyes (1990). Photographs of slide preparations were taken with a ProgRes C14plus digital camera attached to a Nikon Eclipse E800 compound microscope, and the resulting layers combined electronically using Auto-Montage® (Synoptics Group, Cambridge) or Zerene Stacker™ (http://zerenestacker.com) and, except for primary types, retouched as needed with Adobe® Photoshop (Adobe Systems for Windows). Measurements of morphological structures are given in micrometres (µm), following Huber (1992, 2006). Abbreviations used are: fl_x = funicle or flagellar segment, mps = multiporous plate sensillum. Specimens are deposited in the following institutions.

CNC – Canada, Ontario, Ottawa, Canadian National Collection of Insects.
DEZA – Italy, Naples, Portici, Dipartimento di Entomologia e Zoologia Agraria dell'Università degli Studi di Napoli «Frederico II».
MACN – Argentina, Buenos Aires, División Entomología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia".

Anaphes atomarius (Brèthes)

Anaphoidea atomaria Brèthes, 1913: 100 (original description).
Patasson atomarius: Ogloblin, 1964: 39 (generic transfer).
Patasson atomarius: De Santis, 1967: 109 (catalogue).
Patasson atomarius: Clausen,1977: 272 (host, biological control).
Patasson atomaria: De Santis, 1979: 371 (catalogue).
Patasson atomarius: Ahmad, 1977: 151 (host, percent parasitism).
Patasson atomarius: Ahmad, 1978: 161 (laboratory rearing, longevity).
Patasson atomarium: Dymock, 1989: 23 (biological control).
Anaphes atomarius: Huber, 1992: 72 (list, implied generic transfer).

Type material. Holotype ♀ (MACN), on slide (Fig. 2) labelled: 1. "Patasson atomarius ♀ Brethes]. Det. A. Ogloblin". 2. "A 14". 3. "Anaphoidea atomaria Br. 10545". Some illegible letters in faded ink and the number 53 in pencil are also on the labels. Type locality: the original description gives the type locality and collecting date as General Urquiza and 1.xi.1912. The locality is now in Villa Urquiza, an area in greater Buenos Aires.

Other Material Examined. BRAZIL. Rio Grande do Sul: Passo Fundo, 14.viii.1985, D.N Gassen, ex. *L. bonariensis* (1♀ and 4♂, CNC).

Diagnosis. *Anaphes atomarius* belongs to a complex of species with 2 mps on fl_2 of each antenna in females. The holotype differs from *A. archettii* (described below) and *Anaphes listronoti* Huber by the fore wing with double line of setae separating the medial space from the posterior margin of the wing (a single line in *A. listronoti*), and narrower fore wing.

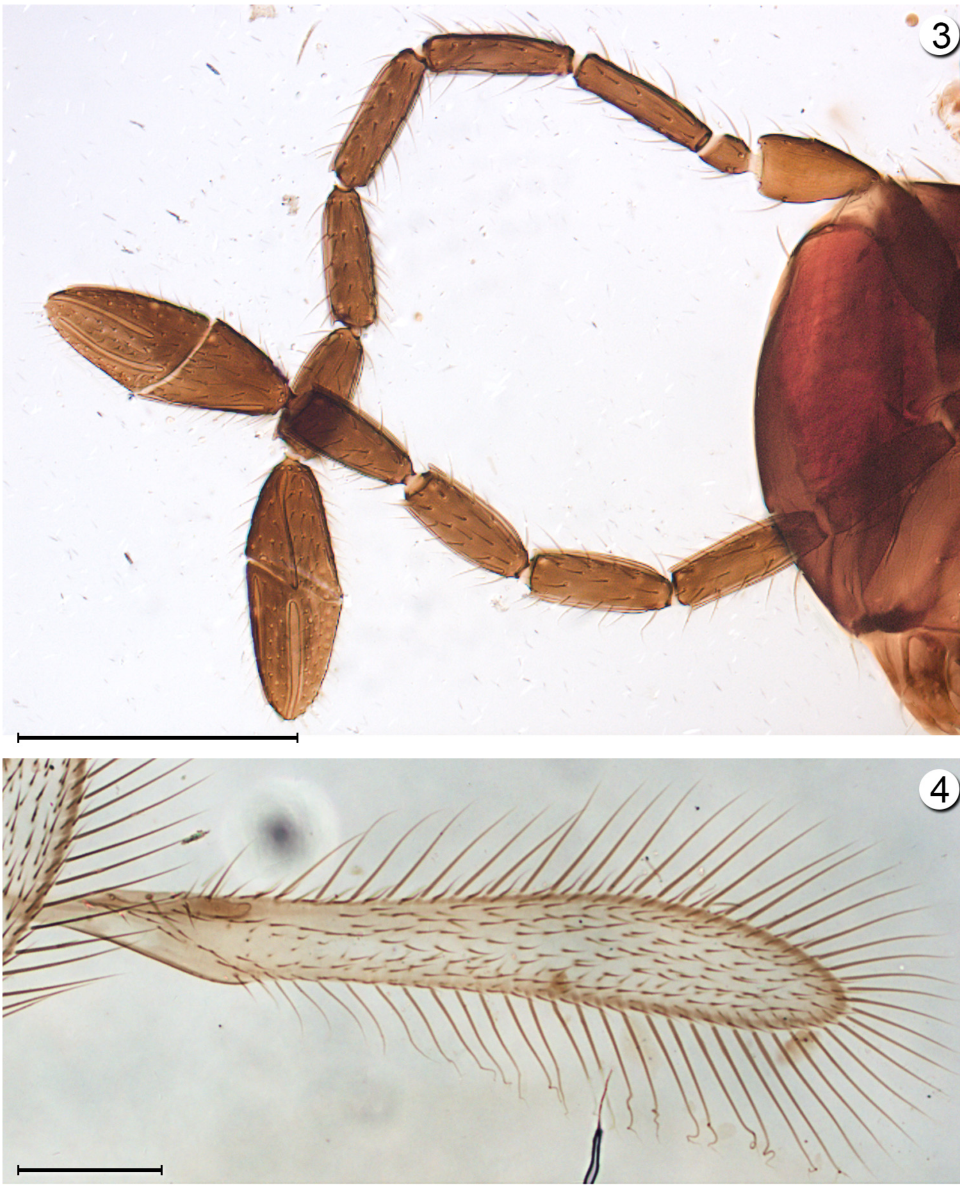
Incidentally, the line drawing of the fore wing of the holotype of *A. listronoti* (Huber *et al*. 1997, fig. 11) differs from its photograph (Huber 2006, fig. 23) in that the setal line between the medial space and posterior margin appears partly double in the former but single in the latter. I rechecked the holotype and the photograph showing a single setal line is correct. *Anaphes atomarius* differs from *A. victus* Huber by the narrower fore wing, with length to width ratio at least 8.0 (at most 6.7 in *A. victus*). The reared female from Brazil that I tentatively identify as *A. atomarius* has the fore wing with a single setal line separating medial space from hind margin and a slightly wider wing (length to width ratio of 7.37).

Description. Female. Holotype (Fig. 1) body length 445 (mesosoma + metasoma only) (total length including head = 500 in original description). **Head**. Head width 189. **Antenna**. Length to width ratio of segments: scape + radicle 79/25 (3.16), pedicel 49/29 (1.69), $f1_1$ 21/14 (1.5), fl_2 57/19 (3.00), fl_3 57/21 (2.71), fl_4 57/22 (2.59), fl_5 56/21 (2.67), fl_6 52/22 (2.36), club 100/38 (2.63); fl_2–fl_6 each with 2 mps (Fig. 3). **Wings**. Fore wing (Fig. 4) length to width ratio 620/77 (8.05); longest marginal setae about 122, marginal space length 62, with double line of setae separating marginal space from hind margin (Fig. 4). Hind wing length 394, width 23, longest marginal setae about 109. **Legs**. Metatibia length 214, metatarsomere 1–4 lengths 32, 38, 34, 31; metatasomere 1 0.84 × length of metatarsomere 2. **Metasoma**. Ovipositor sheath length 277, extending under mesosoma to about level of anterior margin of mesocoxa (Fig. 5) and slightly exserted posteriorly (Fig. 6); ovipositor length to metatibia length ratio 1.29.

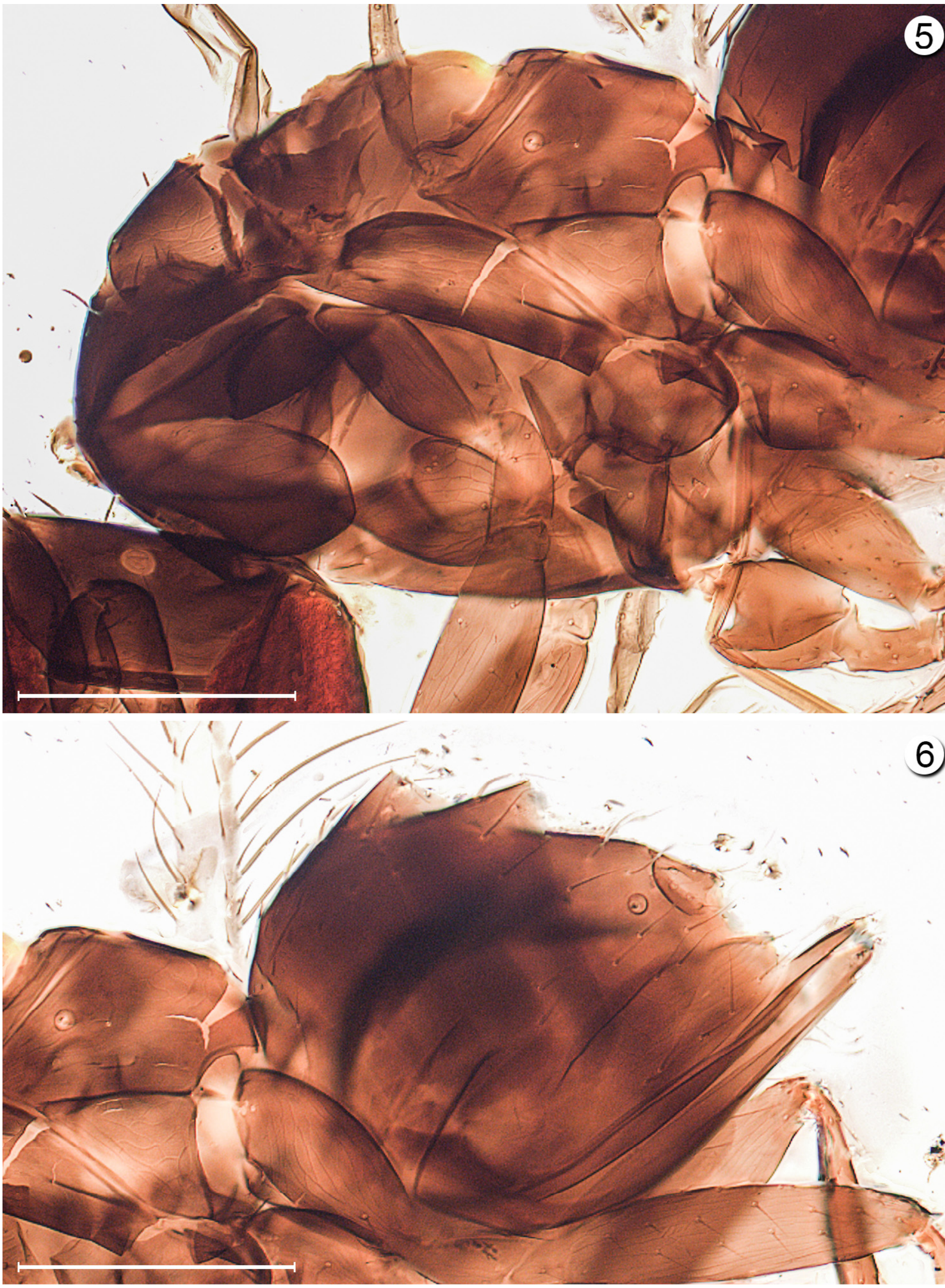
Reared female specimen from Brazil. Body length 490 (mesosoma + metasoma only). **Head**. Head (Fig. 7) width 193. **Antenna**. Scape with faint oblique striations on inner surface (Figs 7, 8). Length to width ratio of antennal articles: scape + radicle 107/24 (4.46), pedicel 49/28 (1.75), $f1_1$ 26/16 (1.63), fl_2 64/17 (3.76), fl_3 64/17 (3.76), fl_4 62/16 (3.88), fl_5 62/18 (3.44), fl_6 58/20 (2.90), club 104/37 (2.81); fl_2–fl_6 each with 2 mps (Fig. 8). **Mesosoma**. Scutellum (Fig. 9) with campaniform sensilla separated by 3.2 × their diameter. **Wings**. Fore wing length to width ratio 656/89 (7.37); longest marginal setae about 127, marginal space length 101, with single line of setae separating marginal space from hind margin. Hind wing length to width ratio (for a male specimen) 642/29. **Legs**. Metatibia length 208, metatarsomere 1–4 lengths 33, 39, 40, 35; metatasomere 1 0.85 × length of metatarsomere 2. **Metasoma**. Gaster (Fig. 10) about 0.9 × as long as mesosoma. Ovipositor length 294, extending under mesosoma to about level of anterior margin of mesocoxa (Fig. 11); ovipositor length to metatibia length ratio 1.41.

Reared male specimens from Brazil. Body length (n=1, on slide) 645. **Head** as in Figs 12 and 13. **Antenna**. Length of segments (n=3) (Fig. 14): scape + radicle 91–97, pedicel 48, fl_1 4–5, fl_2 76–81, fl_3 85–86, fl_4 81–83, fl_5 80–82, fl_6 78–79, fl_7 76–82, fl_8 76–78, fl_9 80–84, fl_{10} 76–80, fl_{11} 77–82. Length/width of fl_5 3.75–4.04. Total flagellum length 797–815. **Mesosoma**. As in Fig 16. **Wings**. As in Fig 15. **Metasoma**. Gaster (Fig. 17) sligthly longer than high. Genitalia as in Fig. 18 (and see comments in Discussion). The four males are assumed to be conspecific with the reared female based on being obtained from the same rearing event.

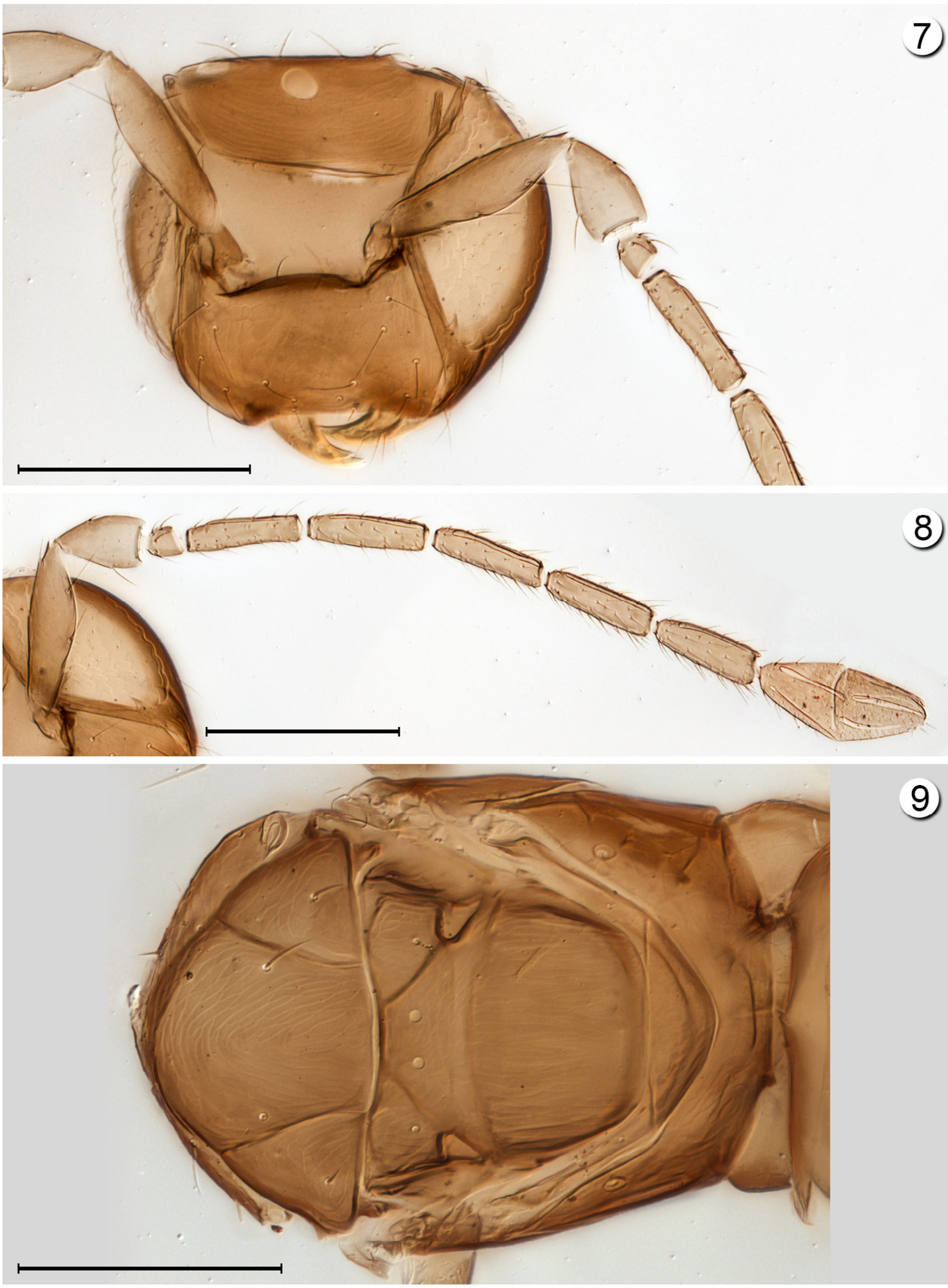
FIGURES 1–2. *Anaphoidea atomaria*, holotype. 1, habitus; 2, type slide. Scale bar = 500 µm.



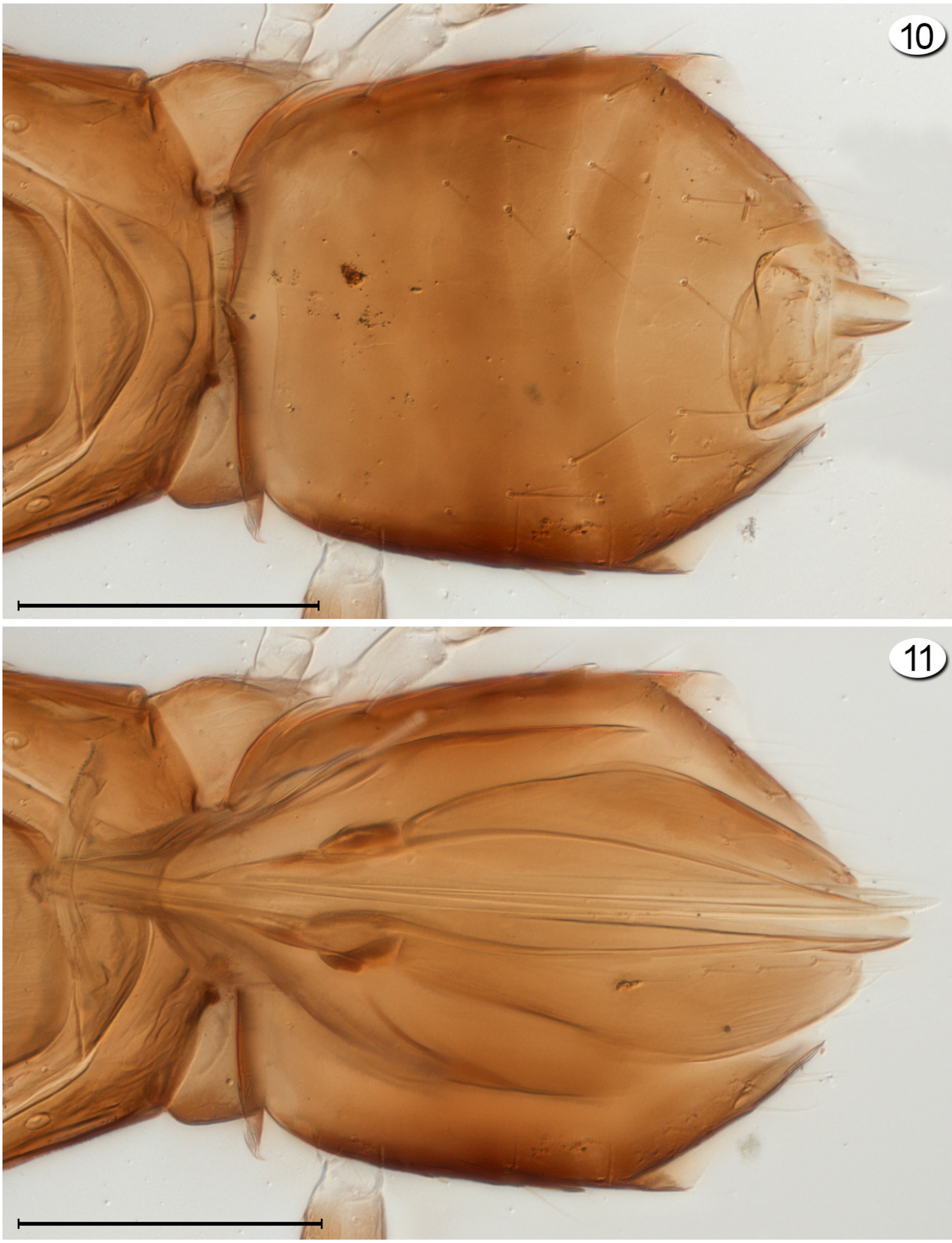
FIGURES 3–4. *Anaphoidea atomaria*, holotype. 3, antennae; 4, fore wing. Scale bars = 100 µm.



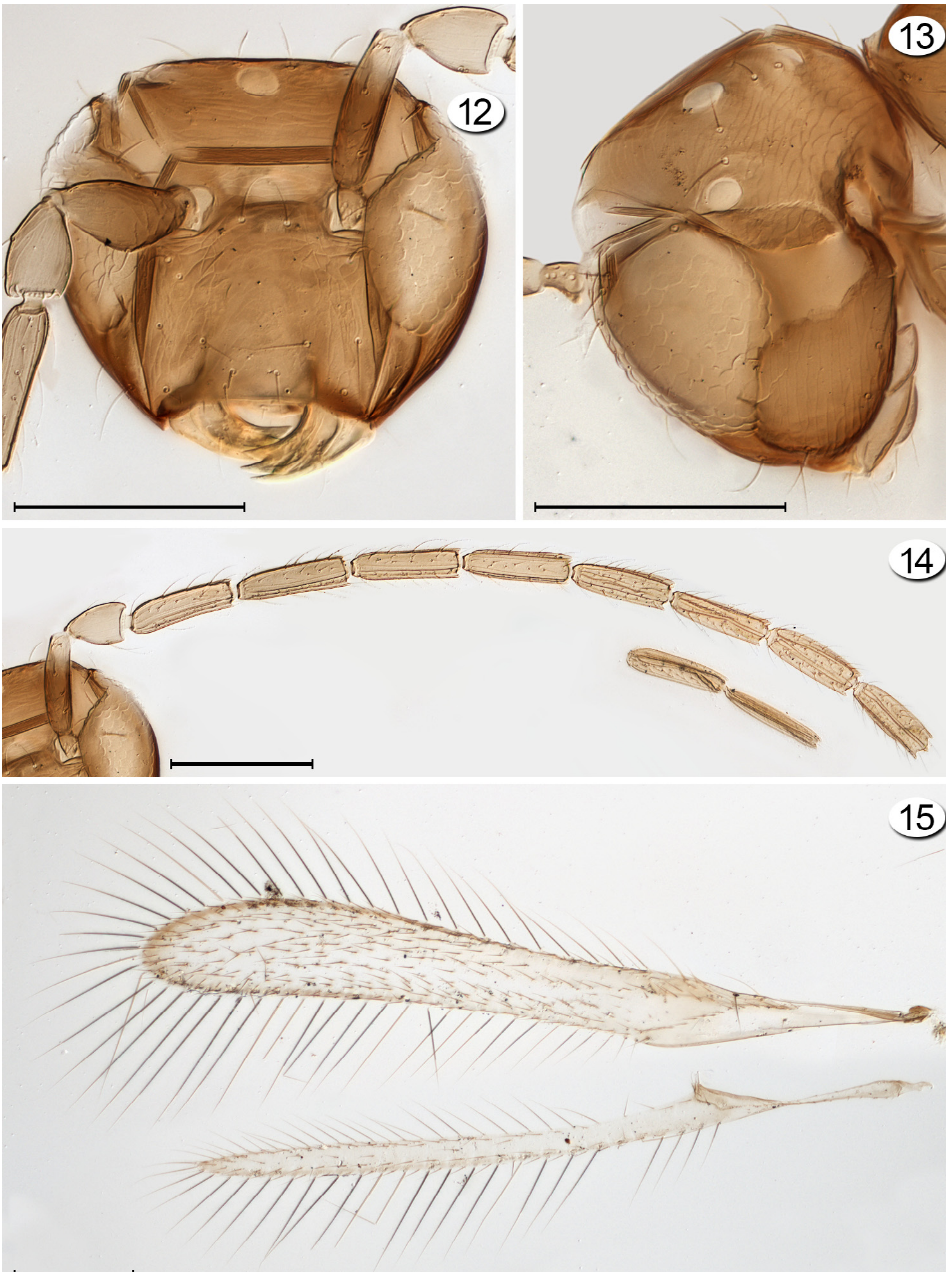
FIGURES 5–6. *Anaphoidea atomaria*, holotype. 5, mesosoma, lateral; 6, metasoma, lateral. Scale bars = 100 µm.



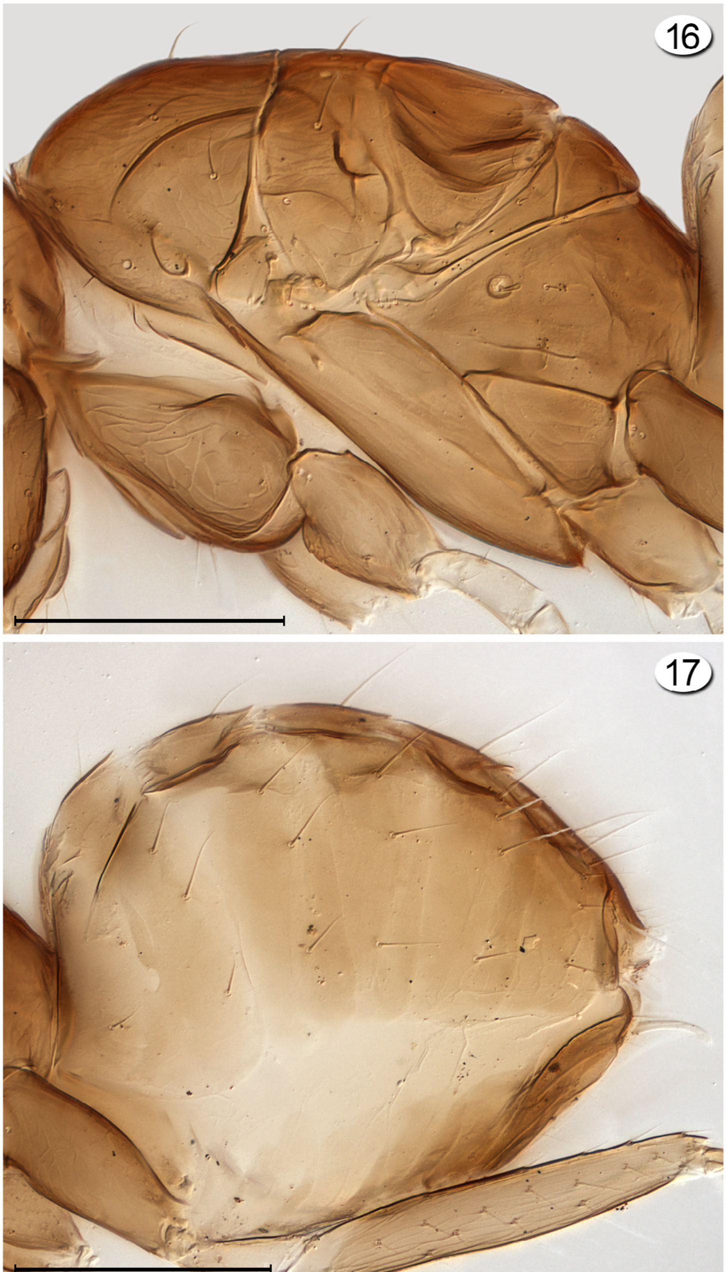
FIGURES 7–9. *Anaphes ?atomarius*, reared female from Brazil. 7, head, anterior; 8, antenna; 9, mesosoma, dorsal. Scale bars = 100 µm.



FIGURES 10–11. *Anaphes* ?*atomarius*, reared female from Brazil, apex of mesosoma + metasoma; 10, dorsal surface; 11, ovipositor, dorsal view (as seen through metasoma). Scale bars = 100 µm.



FIGURES 12–15. *Anaphes ?atomarius*, reared male from Brazil. 12, head, anterior; 13, head, dorsolateral; 14, antenna; 15, wings. Scale bars = 100 μm.



FIGURES 16–17. *Anaphes* ?*atomarius*, reared male from Brazil, lateral. 16, mesosoma; 17, metasoma. Scale bars = 100 µm.

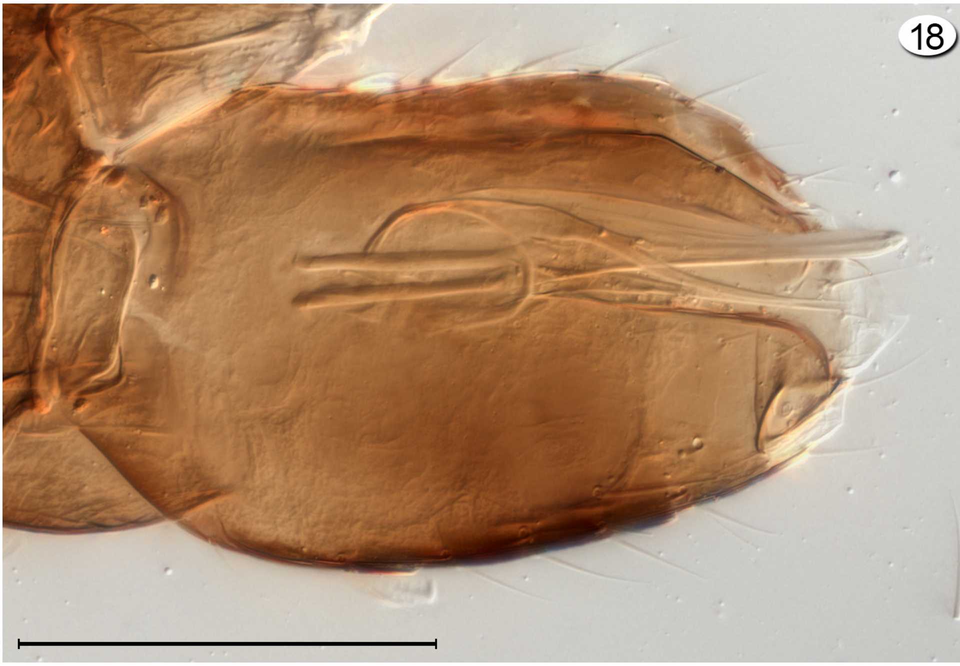


FIGURE 18. *Anaphes ?atomarius*, reared male from Brazil, dorsal, genitalia. Scale bar = 100 µm.

Anaphes archettii Ghidini

Anaphes archettii Ghidini, 1945: 39 (original description).
Anaphes archettii: Viggiani and Jesu, 1988: 1020 (host cited).
Anaphes archettii: Huber, 1992: 72 (list).
Anaphes archettii: Viggiani, 1994: 472 (male genitalia).
Anaphes archettii: Pagliano and Navone, 1995: 36 (list).
Anaphes archettii: Jesu, 2002: 111 (host cited).
Anaphes archettii: Pintureau, 2012: 33 (list).

Type material. Lectotype ♀, here designated (DEZA), on slide (Fig. 20) labelled 1. Littoria, 13.v.1943 ex Lixus junci coll. F.M Ghidini". 2. "Lectotype ♀ des. Huber 2014". 3. "Anaphes archettii Ghidini 2♀". 4. "Paralectotype ♀ Anaphes archettii'. Type locality: Italy, Lazio, Agro Pontino [a plain in Latina Province south and southeast of the provincial capital, Latina). The former name of Latina was Littoria (used in the original description).

Paralectotypes. 1♀ and 1♂ (DEZA), with same data as lectotype. The female paralectotype is on the same slide as the lectotype, the male on another slide; both were examined. Three other specimens (DEZA) remain from the original series but are in poor condition; they were not examined. All other original specimens are lost (G. Viggiani, personal communication).

Diagnosis. *Anaphes archettii* belongs to the same species complex as *A. atomarius*. It differs from *A. atomarius* by four features: 1) longer body length (at least 770 long vs 500 in *A. atomarius* holotype), 2) fore wing with a single line of setae separating the medial space from the posterior margin (double line in *atomarius* holotype), 3) fore wing length to width ratio 5.39 (8.05 in *atomarius* holotype) and 4) ovipositor to metatibia length ratio 1.77 (1.29 in *A. atomarius*). The body length of *A. archettii* is at least 770, based on Ghidini (1945) compared to at most 693 in *A. victus* and 723 in *A. listronoti*.

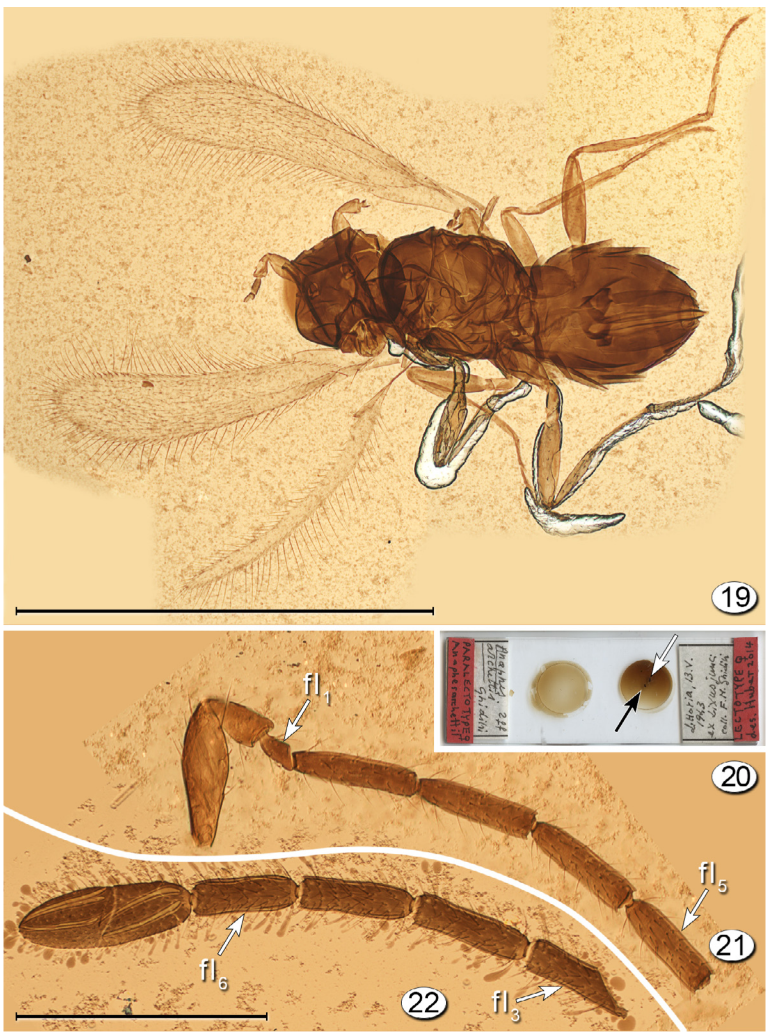
Description. Female. Lectotype (Fig. 19) body length (mesosoma + metasoma only) 792 (total length including head = 770–850 in original description). **Head**. Head width 314. **Antenna**. Length to width ratio of segments (scape–fl_5 from paralectotype): scape + radicle 155/47, pedicel 65/38, fl_1 36/19, fl_2 101/26, fl_3 101/27, fl_4 99/30, fl_5 91/29, fl_6 90/30, clava 145/45; fl_2–fl_6 each with 2 mps (Figs 21, 22). **Wings**. Fore wing (Fig. 26 [male]) length to width ratio 1013/188 (5.39); longest marginal setae about 150, marginal space length 146, with single line of setae separating marginal space from hind margin. Hind wing length 904, width 58, longest marginal setae about 130. **Legs**. Metatibia length (paralectotype) 334, metatarsomere 1–4 lengths 61, 66, 58, 35; metatarsomere 1 0.92 × length of metatarsomere 2. **Metasoma**. Ovipositor sheath length 592, extending under mesosoma to about level of anterior margin of mesocoxa (Fig. 23); ovipositor length to metatibia length ratio 1.77.

Male. Body length (from original description) 0.65–0.70 mm. **Antenna**. Length of segments (Fig. 24) (paralectotype): scape + radicle 128/40, pedicel 51/39, fl_1 9, fl_2 125, fl_3 119, fl_4 118, fl_5 119/23, fl_6 116, fl_7 116, fl_8 115, fl_9 111, fl_{10} 109, fl_{11} 114. Length to width ratio of fl_5 5.04. Total flagellum length 1171. Fore wing as in Fig. 26. Genitalia as in Fig. 25 (and see comments in Discussion).

Discussion

Only four species of *Anaphes* have been described from South America: three in *A.* (*Yungaburra*) and one, *A. atomarius*, in *A.* (*Anaphes*) (Huber 1992). *Anaphes atomarius* belongs to the *crassicornis* species group, in which the clava is 2-segmented. Among species described from the Western Hemisphere *A. atomarius* would key to couplet 12 in Huber (2006), which leads to *A. listronoti*, *A. sordidatus* (Girault) and *A. victus*. *Anaphes victus* and some specimens of *A. listronotus* Huber were reared from *Listronotus oregonensis* (LeConte) (Coleoptera: Curculionidae) among other species, and *A. sordidatus* was reared from *Tyloderma foveolatum* (LeConte) (Coleoptera: Curculionidae). Specimens of all three species sometimes or always have 2 mps on fl_2 of the female antenna, in contrast to other *Anaphes* species that have at most 1 or, usually, 0 mps on fl_2. Several Old World (European) species also have 2 mps on fl_2, but only one of them, *A. archettii*, is treated here for comparison with *A. atomarius* because the types were reared from a known host.

The specimens from Passo Fundo, about 900 km from the type locality of *A. atomarius*, match the holotype fairly well but not perfectly. I tentatively treat the differences as intraspecific variation until shown otherwise by further rearing and morphological study of additional specimens reared from *L. bonariensis*, preferably from nearer the type locality. Because of the slight morphological differences, the species name *atomarius* may not be correctly applied to the reared specimens I examined. Regarding specimens introduced into New Zealand, it is not known who made the species identification, whether voucher specimens from the releases or studies were kept or, if so, where they are deposited. Therefore their identity cannot be checked. Because no voucher specimens were located from previous



FIGURES 19–22. *Anaphes archettii*, lectotype. 19, habitus; 20, type slide, 21, paralectotype antenna, from scape (radicle missing) to fl_5; 22, lectotype antenna, from fl_3 (part) to clava. Scale bars: 19 = 1000 µm, 21 and 22 = 200 µm.

FIGURES 23–26. *Anaphes archettii*, types. 23, female paralectotype, body dorsal; 24, male paralectotype, head + antenna; 25, male genitalia, lateral; 26, fore wing. Scale bars = 200 µm.

publications that use the name *A. atomarius* I cannot be sure whether the species name was correctly applied in those publications either. Like most species of *Anaphes*, the holotype of *A. atomarius* was not reared so its host is unknown. It would be expedient to assume that the name *A. atomarius* was correctly applied to all specimens reared from *L. bonariensis* because then the name would be associated with specimens reared from a known host that happens also to be a pest of economic importance. But this cannot be done until more evidence of conspecificity is obtained. That may be impossible because the holotype is slide mounted so other lines of evidence such as DNA barcoding or biological information cannot be obtained from it for comparison with freshly reared specimens from known hosts.

The possibility exists that a complex of similar *Anaphes* species in South America uses *L. bonariensis* as a host, just as a complex of species exists on *L. oregonensis* in North America. Species in the latter complex differ in biology, e.g., in the number of individuals reared from a single host egg of *L. oregonensis*—*A. listronotus* is gregarious and *A. victus* is solitary (Huber *et al*. 1997). Unfortunately, publications on the biology of *A. atomarius* do not state how many adults emerge from a single host egg and this information was not recorded in the five reared specimens in this study. Another possibility is that *A. atomarius* is the same as one of the North American species. The fact that one species was described from Brazil and the others from Canada or the United States of America is not a problem because various species of Mymaridae in the Western Hemisphere are known to have wide distributions that extend from Canada, or at least somewhere north of Mexico, to Argentina. Additional rearing is needed of *A*. '*atomarius*' from *Listronotus* spp. in South America for detailed morphological study and, if colonies can be established, laboratory crossing experiments with the North American species, preferably with the addition of molecular evidence to see if species are the same or different.

Ghidini (1945) reared numerous specimens of *A. archettii* from *Lixus junci* Boheman (Coleoptera: Curculionidae) on sugar beet (*Beta vulgaris* Linnaeus) (Chenopodiaceae) in Italy but did not state how many emerged from a single weevil egg. Apart from the specimens discussed above, the original material is lost (Viggiani, personal communication). Viggiani (1994) illustrated the male genitalia (photographed in Fig. 25) and showed that various *Anaphes* species could be distinguished by measurements of various genitalic parts. The problem is that association of males with females is only certain for the few *Anaphes* species reared from economically important hosts, whereas descriptions of most *Anaphes* species are based on females only, the corresponding males being unknown or not certainly associated. Because the genitalia of only three males of *A. atomarius* from Brazil and one of *A. archettii* are available for study little can be said about variation. In any case, there appears to be no difference in measurements.

Body length in the four *Anaphes* species discussed above may be correlated with host egg size and number of individuals developing in a single egg. The gregarious or solitary nature of *A. atomarius* and *A. archettii* must first be determined, however. A host for each of the four species is known if one expediently, but perhaps incorrectly, assumes that specimens reared from *L. bonariensis* are indeed *A. atomarius*. If eggs of *L. junci* are larger than those of any of the *Listronotus* species that may account for the larger body size of *A. archettii* compared to the other *Anaphes* species. It would be interesting to obtain living *A. archettii* from *L. junci* and try to rear them on *L. oregonensis* in order to determine whether the host range can be extended and, if so, see if specimens reared from *L. oregonensis* are smaller than when reared on *L. junci*. If they are, then the body length difference proposed above to separate *A. archettii* from *A. listronoti* or *A. victus* does not distinguish these species and other differences need to be found. Ultimately, molecular evidence and cross breeding may be needed to distinguish correctly these (and other) *Anaphes* species. It may show that at least two of them are conspecific.

Acknowledgements

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