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***Anthrenus dorsatus* new to the United States and a comparison with *Anthrenus pimpinellae* ssp. *pimpinellae* (Coleoptera: Dermestidae)**

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ABSTRACT: *Anthrenus dorsatus* Mulsant et Rey 1868 is recorded from the United States for the first time. Species confirmation is achieved through dissection and genitalia examination. Images of *A. p. pimpinellae* Fabricius 1775 from the United States are examined and compared with confirmed images of *A. p. pimpinellae* from Europe. It is suggested that specimens from the United States identified as *A. p. pimpinellae* more likely belong to *A. dorsatus*. Habitus shapes (body width/body length) of images of United States specimens are too broad for *A. p. pimpinellae*. In addition, the elytral and ventrite patterns of United States specimens are not consistent with *A. p. pimpinellae*. To establish which species from the *A. pimpinellae* complex occur across the United States, dissection and genitalia examination is required

KEYWORDS: Identification, phenotypic variation, pattern, ventrites, aedeagus

Hoebeke *et al.* (1985) was the first published record of *Anthrenus pimpinellae* *pimpinellae* Fabricius 1775 in the United States. Hoebeke *et al.* (1985) described *A. p. pimpinellae* from Delaware and Pennsylvania. Later, Hoebeke and Wheeler (1990) noted *A. p. isabellinus* Küster 1848 from Virginia. Beal (1998) comments on the distribution of *A. p. pimpinellae* in the United States but does not indicate that it had dispersed further than the eastern seaboard, even though it is known to have been in the United States from at least 1906 (Hoebeke *et al.* 1985). Given that it has been in the United States for in excess of 100 years, it is very likely that it is more widely spread but has simply gone unnoticed.

Both, Hoebeke *et al.* (1985) and Beal (1998) noted the high level of phenotypic variation associated with *A. p. pimpinellae* but both also commented that it was very likely that many of these forms were different species. It was not until Kadej *et al.* (2007), followed by Kadej and Háva (2011) and Holloway (2019), that the various forms of *A. p. pimpinellae* in the Palaearctic were confirmed as separate species, largely through the examination of male genitalia. Currently, 21 species are now known to belong to the Palaearctic *A. pimpinellae* complex. Hoebeke *et al.* (1985), Beal (1998) and Kadej (2011) produced binomial keys facilitating the separation of all or some Nearctic *Anthrenus* species, but in all cases several species from the *A. pimpinellae* complex would key out as *A. p. pimpinellae* s.str. Hoebeke *et al.* (1985), Beal (1998) and Kadej (2011) do not show line drawings or illustrations of *A. pimpinellae* aedeagus from the United States, so it would be interesting to know which species from the *A. pimpinellae* complex was originally

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noted by Hoebeke *et al.* (1985) and which species from the *A. pimpinellae* complex inhabits the United States now.

Holloway and Bakaloudis (2020) carried out a morphological analysis of *A. p. pimpinellae*. They found, contrary to general agreement, that *A. p. pimpinellae* does not display conspicuous phenotypic variation. The data produced by Holloway and Bakaloudis (2020) provide the means to examine images of specimens identified as *A. p. pimpinellae* from the United States and consider whether they belong to that species. In addition, a small number of '*pimpinellae*' individuals were collected from Maryland, United States, providing an opportunity to examine which species exists in the field.

METHODS

The nomenclature and zoogeography follow Háva (2015; 2020), and the conventional nomenclature, including of the taxa under study, is used. Insects were collected from Williamsport, Maryland from hemlock, *Conium maculatum* L. 1753, by knocking the insects into a small tray and subsequently tipping into a 75x25mm glass tube. Of 53 individuals of *Anthrenus* Geoffroy 1762 gathered, two were '*pimpinellae*' type, a male and a female. Both specimens were preserved in 2% acetic acid prior to examination. Images of the habitus, dorsal and ventral side, were captured using a Canon EOS 1300D camera mounted on a Brunel BMSL zoom stereo LED microscope. Habitus images were taken at x20 magnification. Dissection involved detaching the abdomen from the rest of the insect using two entomological micropins. The soft tergites were then peeled off the harder ventrites to expose the genitalia. Using pins, structures associated with the genitalia were carefully removed and the genitalia were extruded between the terminal tergite and sternite using a No 2 entomological pin. The male aedeagus was detached from the ring sclerite retaining the capsule over the anterior end of the aedeagus. In addition to the aedeagus, sternite IX was also detached from the ring sclerite. The soft female bursa copulatrix was left attached to the terminal tergite and sternite to avoid damage. Images of the aedeagus, sternite IX, and bursa copulatrix were captured at x100 magnification using the camera mounted on a Brunel monocular SP28 microscope. After dissection, all body parts were mounted on card. The antennae were exposed, and images were taken at x63 magnification through the stereo microscope. All images were stacked using the Helicon Focus 7-Pro focus-stacking software. Morphometric analysis was carried out using DsCap.Ink Software version 3.90.

The following metrics were taken:

BL: Body length from the front edge of the pronotum to the tip of the elytra (accounting for any expansion that might have occurred between the thorax and elytra during storage)

BW: Body width across the widest part of the elytra

AE: Aedeagus length from the tip of the paramere to the tip of the anterior cap

AL: Length of the antennal club

AW: Width of the antennal club

Online images of specimens identified as *A. p. pimpinellae* and *A. p. isabellinus* were obtained from Hoebeke *et al.* (1985), Hoebeke and Wheeler (1990), Kadej (2011), Benisch

(2020), Herrmann (2020), and Tykarski *et al.* (2020). These images were downloaded into DS Cap to enable habitus ratios to be derived. The habitus ratios were obtained from pixel counts.

Analyses of the data were carried out using Minitab (version 19).

RESULTS

Two specimens collected from the field (Williamsport, Maryland: Lat 39.594523, Long -77.827292) were dissected: a male and a female. Both proved to be *Anthrenus dorsatus* Mul-sant et Rey 1868. Both specimens (labelled *Anthrenus Isabellinus*, see Holloway *et al.*, 2020) are deposited in the Montana Entomology Collection, Montana State University, Bozeman, MT.

Habitus

Figure 1A shows the habitus of the female specimen, Fig. 1B the male specimen, and Fig. 1C a typical example of *A. pimpinellae* ssp. *pimpinellae* from Holloway and Bakaloudis (2020). The overall impression is that Figs. 1A and 1B are much broader individuals with more rounded elytral margins than Fig. 1C. The BL were 3.232 mm and 2.803 mm for Figs. 1A and 1B, respectively. The average *A. p. pimpinellae* length is 2.569 ± 0.233 (SD)mm (Holloway and Bakaloudis, 2020). The BL for Fig. 1A lies above the expected BL range for *A. p. pimpinellae* while the BL for Fig. 1B lies towards the top end of the range (Holloway and Bakaloudis, 2020). The BW/BL ratio for Fig. 1A and Fig. 1B was 0.731 and 0.737, respectively, reflecting the broad, rounded appearance of the habitus. Holloway and Bakaloudis (2020) found the BW/BL ratio to be highly conserved in *A. p. pimpinellae* with a coefficient of variation of just 1.8% making a good character to use to aid with the separation of some species of *Anthrenus*. The average BW/BL ratio in *A. p. pimpinellae* is 0.688 ± 0.029 , indicating that *A. p. pimpinellae* is a much narrower species than the specimens of *A. dorsatus* illustrated in Figs. 1A and 1B.

1A



1B



1C



Fig. 1. Images of habitus of A: female and B: male *Anthrenus dorsatus* found in Maryland, United States compared with C: *A. p. pimpinellae* (Holloway and Bakaloudis, 2020)

The color patterns also vary between Figs 1A and 1C (Fig. 1B is a color variant and not comparable). *Anthrenus dorsatus* (Fig. 1A) has a broader pale band across the elytra than *A. p. pimpinellae* (Fig. 1C). The white markings on the pronotum are more evident in *A. dorsatus* than *A. p. pimpinellae*. In addition, from above, *A. dorsatus* has two white extensions of scales extending in a posterior direction from the trailing edge of the band of white scales crossing the elytra close to the elytral margins. This feature was absent (or on occasions poorly developed) in all specimens of *A. p. pimpinellae* examined by Holloway and Bakaloudis (2020). The pattern features mentioned here were also present on *A. dorsatus* specimens described by Holloway *et al.* (2019) and Holloway and Bakaloudis (2019).

Figure 2 shows the ventrites: Fig. 2A and 2B are the female and male *A. dorsatus* specimens, respectively, and Fig. 2C is an *A. p. pimpinellae* specimen from Holloway and Bakaloudis (2020). *Anthrenus dorsatus* ventrites are white, *A. p. pimpinellae* ventrites are brownish. The spots of black scales on the lateral edges of the sternites are large in *A. p. pimpinellae* (Fig. 2C) compared with the smaller black spots shown by *A. dorsatus* (Figs. 2A and 2B). The extent of pale scales extending inwards from the lateral margin of the first sternite of *A. p. pimpinellae* (Fig. 2C) is much reduced compared with the equivalent patches of scales on *A. dorsatus* (Figs. 2A and 2B).

The antennae of female and male *A. dorsatus* are shown in Fig. 3A and 3B, respectively. All antennal segments, including the antennal club, are slimmer than *A. p. pimpinellae* antennae

2A

2B

2C



Fig. 2. Images of ventrites from A: female and B: male *Anthrenus dorsatus* collected from Maryland, United States compared with C: *A. p. pimpinellae* (Holloway and Bakaloudis, 2020)

(**Fig. 3C:** Holloway and Bakaloudis, 2020). The AL are 0.213 mm and 0.204 mm for the individuals shown in Figs. 3A and 3B, respectively. Mean antennal club length of *A. p. pimpinellae* is 0.179 ± 0.012 mm. AL/AW ratios for the antennae shown in Figs. 3A and 3B are 1.392 and 1.388, respectively. Mean AL/AW for *A. p. pimpinellae* is 1.297 ± 0.039 . Antennal clubs of the studied specimens are longer but comparatively narrower than *A. p. pimpinellae* antennal club.

3A

3B

3C

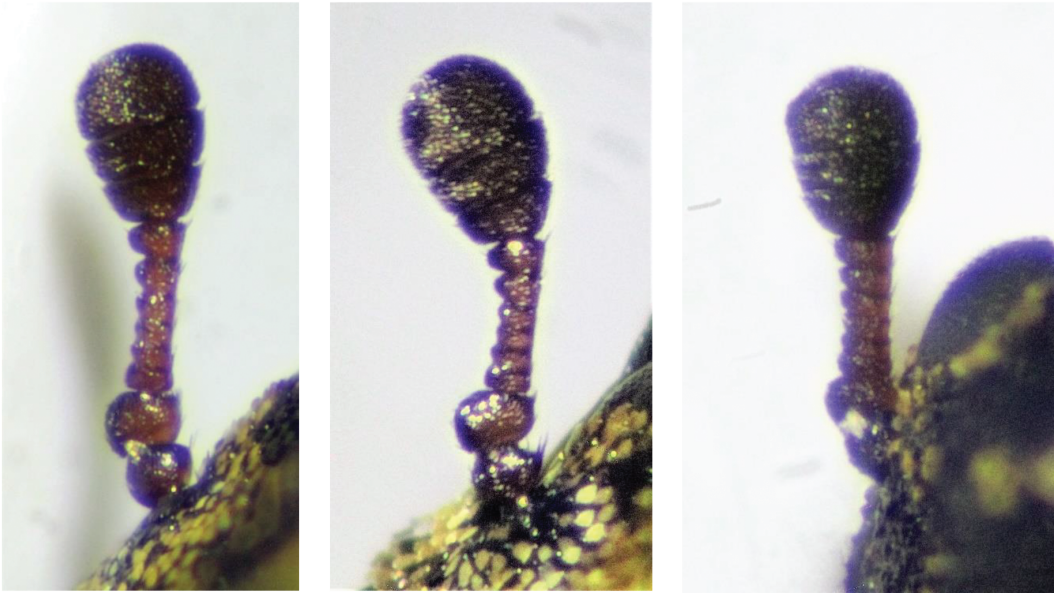


Fig. 3. Images of antennae from A: female and B: male *Anthrenus dorsatus* found in Maryland, United States, compared with C: antenna of *A. p. pimpinellae* (Holloway and Bakaloudis, 2020)

Genitalia

Bursa copulatrix

Figure 4A shows the ventral surfaces of the bursa copulatrix of the female of *A. dorsatus* collected in the United States. There is little information in the literature to compare this image with, except the bursa copulatrix from European *A. dorsatus* (Fig. 4B: Holloway and Bakaloudis, 2019). Fig. 4C shows the bursa copulatrix from a female *A. p. pimpinellae* (Holloway and Bakaloudis, 2020). Very little work has been carried out on the taxonomic value of the bursa copulatrix as a useful character to identify *Anthrenus* species (cf Lackner and Tarasov, 2019). However, a superficial inspection of the bursa copulatrices in Figs. 4A and 4B reveals that they are similar in form and pattern. Conversely, the bursa copulatrix shown in Fig. 4C differs in form and pattern from the bursa copulatrices shown in Figs. 4A and 4B. This provides more evidence that the images in Figs. 4A and 4B belong to the same species and that the bursa copulatrix shown in Fig. 4C is derived from a different species.

4A



4B



4C



Fig. 4. Bursa copulatrix from A: female *Anthrenus dorsatus* found in Maryland, United States, B: bursa copulatrix from Greek *A. dorsatus* (Holloway and Bakaloudis, 2019), and C: bursa copulatrix from *A. p. pimpinellae* (Holloway and Bakaloudis, 2020)

Aedeagus

Figure 5A shows the aedeagus of the male specimen captured in the United States. Figure 5B shows an aedeagus of a European male of *A. dorsatus* (Holloway and Bakaloudis, 2019). The aedeagi shown in Figs. 5A and 5B are very similar. Figure 5C shows a typical aedeagus from a male *A. p. pimpinellae* (Holloway and Bakaloudis, 2020). It is evident that there are differences between Fig. 5C and Figs. 5A and 5B. The *A. p. pimpinellae* parameres are heavily hooked and splay out so that the posterior end is wider than the anterior end; this is not the case in *A. dorsatus*. The tips of the *A. p. pimpinellae* parameres show evident clear windows that the *A. dorsatus* parameres do not have. The median lobe of the *A. p. pimpinellae* aedeagus is very broad at its base, tapering to a thick tip. The median lobe of the *A. dorsatus* aedeagus is narrower at the base and tapers to a very slim tip. AE of the specimen of *A. dorsatus* collected in the United States (Fig. 1A) was 0.213mm, equating to 22.8% of BL. Mean AE for *A. p. pimpinellae* ($0.440 \pm 0.0266\text{mm}$) is considerably shorter than *A. dorsatus*, equating to $16.9 \pm 0.1\%$ of BL (Holloway and Bakaloudis, 2020).

5A



5B



5C

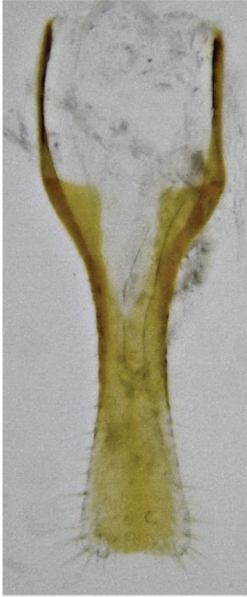


Fig. 5. Images of aedeagi from A: *Anthrenus dorsatus* found collected in Maryland, United States B: Greek *A. dorsatus* (Holloway and Bakaloudis, 2019), and C: *A. pimpinellae* (Holloway and Bakaloudis, 2020)

Sternite IX

Fig. 6A shows the sternite IX of the *A. dorsatus* specimen collected in the United States. By way of comparison and confirmation that the sternite IX is typical of *A. dorsatus*, Fig. 6B shows an image of the sternite IX from a European male *A. dorsatus* (Holloway and Bakaloudis, 2019). The two sternites are very similar; in particular, both show the diagnostic flaps between the two anterior horns (Kadej *et al.* 2007). By contrast, Fig. 6C shows the sternite IX of *A. p. pimpinellae* (Holloway and Bakaloudis, 2020). The sternite IX of *A. p. pimpinellae* does not possess the flaps between the horns, has a much thicker posterior stem, and has much thicker and longer setae than the sternite IX of *A. dorsatus*.

6A



6B



6C



Fig. 6. Images of sternite IX from A: male *Anthrenus dorsatus* collected in Maryland, United States, B: Greek *A. dorsatus* (Holloway and Bakaloudis, 2019), and C: *A. pimpinellae* (Holloway and Bakaloudis, 2020).

Analysis of Published Images

Holloway and Bakaloudis (2020) found that the BW/BL ratio was highly conserved and is therefore a useful way to separate *A. dorsatus* from *A. p. pimpinellae*. Several images of United States specimens were obtained from the literature that were labelled as *A. p. pimpinellae* including Hoebeke *et al.* (1985) [*A. p. pimpinellae*], Hoebeke and Wheeler (1990) [*A. p. pimpinellae* and *A. p. isabellinus*], and Kadej (2011) [*A. p. pimpinellae* and *A. p. isabellinus*]. The BW/BL ratios for the *A. p. pimpinellae* images were 0.734, 0.747, and 0.711, respectively. The BW/BL ratios for the *A. p. isabellinus* images were 0.723 and 0.742, respectively. The mean BW/BL ratio for *A. p. pimpinellae* is 0.688 ± 0.029 , whereas the BW/BL ratios for the specimen collected in Maryland, United States were 0.731 and 0.737. To ensure that these measurements were not simply artifacts of downloading web-based images, images of *A. p. pimpinellae* from reliable sources were also downloaded and measured. Three images were found: Herrmann (2020), the Polish Biodiversity Information Network (Tykarski *et al.*, 2020) and Benisch (2020). Herrmann (2020) also shows an image of an *A. p. pimpinellae* aedeagus confirming identification. The BW/BL ratios for the *A. p. pimpinellae* images displayed on these sites were 0.682, 0.677, and 0.696, respectively, with an average of 0.685, almost identical to the mean value found by Holloway and Bakaloudis (2020).

The dimensions of published images of United States records are not consistent with *A. pimpinellae*.

DISCUSSION

The first report of *A. p. pimpinellae* occurring in the United States was Hoebeke *et al.* (1985). In 1990, *A. p. isabellinus* was also claimed to be present in the United States (Hoebeke and Wheeler, 1990). Beal (1998) carried out an examination of the *Anthrenus* species in the United States and, commenting on the great variation in the species across the Palaearctic, stated that many of the variants were probably separate species. Beal (1998) went further to say that recognition of these variants as species would most likely require examination of the genitalia. The work proposed by Beal (1998) was subsequently carried out by Kadej *et al.* (2007), and later by Kadej and Háva (2011) and Holloway (2019). These combined studies have so far identified 21 *Anthrenus* species, all belonging to the *A. pimpinellae* complex in the Palaearctic. The findings of Hoebeke *et al.* (1985) and Hoebeke and Wheeler (1990) pre-dated Kadej *et al.* (2007) and no genitalia examination was carried out. Beal (1998) also accepted that *A. p. pimpinellae* occurred in the United States, but neither Beal (1998), Hoebeke *et al.* (1985) nor Hoebeke and Wheeler (1990) carried out an examination of the genitalia of *A. p. pimpinellae* to confirm identification. Consequently, it is not known which species was being described and accepted as *A. p. pimpinellae*. Kadej (2011) studied the Nearctic species of *Anthrenus*, and quite reasonably at that time, accepted that *A. p. pimpinellae* occurred in the United States. It was considered to be a very widespread species and one that had been accepted as occurring in the United States for over 25 years prior to the study. Beal (1998) examined a range of *Anthrenus* genitalia from the Nearctic producing beautiful illustrations of the species considered. However, he did not dissect and illustrate any *A. p. pimpinellae* aedeagi. It is possible that the original (and subsequent) records of *A. p. pimpinellae* in the United States did not refer to that species at all but rather to another species from the *A. pimpinellae* complex. This assertion is strongly supported by the BW/BL ratios of published images. For *A. p. pimpinellae*, the BW/BL ratio averages under 0.69; for the images labelled as *A. p. pimpinellae* from the United States, the BW/BL ratios were mostly over 0.73. Examination of the images submitted by the public to Bugguide (<https://bugguide.net/node/view/15740>) show insects from the *A. pimpinellae* complex with rounded later margins, slender antennae, white ventral scales and a dorsal pattern inconsistent with *A. p. pimpinellae*. The authors found no images of United States insects consistent with *A. p. pimpinellae*, either from decades ago or more current.

Anthrenus dorsatus has recently been recorded for the first time in Mallorca (Holloway *et al.*, 2019) and Greece (Holloway and Bakaloudis, 2019) and along with Háva (2020), evidence is emerging that *A. dorsatus* is distributed in Europe to the north and south of the western Mediterranean. Here, we extend that distribution to include the United States. It is possible that *A. dorsatus* is experiencing a natural range expansion in Europe as a result of global climate change (Araújo and New, 2006; Araújo and Rahbek, 2006). Another possibility is that, since splitting the *A. pimpinellae* complex (Kadej *et al.*, 2007), little work has been carried out to establish the true species delimitation of the numerous newly described species. It is possible that several of these species have wider ranges than previously thought (see also Foster and Holloway, 2015). Consequently, it is likely that the true geographical range of *A. p. pimpinellae* following the split is much smaller than previously thought. This point was speculated by Beal (1998) who commented that *A. p. pimpinellae* could represent several species. However, apart from Beal (1998), we are not aware of other studies where this issue has been considered more thoroughly or where the appearance of *A. p. pimpinellae* has been examined in detail (cf. Holloway and Bakaloudis, 2020) to facilitate a consideration of the true range of *A. p. pimpinellae*. It is hoped that

the current study, coupled with Holloway and Bakaloudis (2020), will facilitate a better understanding of the geographical range of *A. p. pimpinellae* and will also enable the correct identification of *A. p. pimpinellae* in citizen science projects.

The purpose of this study was to illustrate that, most likely, *A. p. pimpinellae* has not been recorded in the United States and to conclusively demonstrate that *A. dorsatus* does exist in the wild in the United States. We also demonstrate that the images of insects found in the United States and labelled as *A. p. pimpinellae* are misidentifications. To establish which species from the *A. pimpinellae* complex exist in the wild in the United States, more specimens need to be dissected to allow genitalia examination. The authors would like to communicate with members of the public in the United States interested in examining this issue. The authors also appreciate that this study has also raised some taxonomic issues; these are dealt with in detail by Holloway *et al.* (2020).

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