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A morphometric analysis of *Anthrenus munroi* Hinton, 1943, and a key for citizen scientists to the Western European species in the *Anthrenus pimpinellae* complex (Coleoptera: Dermestidae)

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ABSTRACT

Species that make up the *Anthrenus pimpinellae* complex are difficult to distinguish: a thorough examination of all the known species is required for progress in their taxonomy. Here a morphometric examination of *A. munroi* and its male genitalia is carried out to complement similar examinations of other species from the complex. A key is presented to differentiate among Western European species in the *A. pimpinellae* complex, including *A. munroi*. The key is aimed at citizen-scientist field images taken for submission to biodiversity websites; it relies entirely on external features that should be distinguishable in clear images.

Keywords: *Anthrenus*, *amandae*, *angustefasciatus*, *chikatunovi*, *delicatus*, *isabellinus*, identification

INTRODUCTION

The Palaearctic *Anthrenus pimpinellae* (Fabricius, 1775) species complex consists of some 23 species (Holloway 2021) and sits within the speciose genus *Anthrenus* Geoffroy, 1762 (Háva 2022). Most species within the complex share a colour pattern consisting of a white or cream trans-elytral band on a black background, accompanied by variable number of orange or brown scales. As a result, species with this colour pattern are considered difficult to differentiate. Indeed, throughout the 19th and most of the 20th century, many species carrying this colour pattern were considered variants or sub-species of *A. pimpinellae*. Occasionally, variation in the antennal structure was noted but even then specimens were still frequently described a variant of *A. pimpinellae*, *e.g.*, *A. pimpinellae dorsatus* Mulsant & Rey, 1868, (which is now recognized as a synonym of *A. isabellinus* Küster, 1848 (Holloway *et al*. 2020)). Sometimes specimens were noted with such distinctive antennal structures that they were declared a new species, *e.g.*, *A. munroi* Hinton, 1943. For the most part, though, it was generally accepted that any specimen with an *A. pimpinellae* type colour pattern was *A. pimpinellae*, thus reducing the incentive to study more closely and in particular to dissect specimens for species confirmation.

Beal (1998) dissected several species demonstrating considerable inter-specific variation in male genitalia structure. Kadej, Háva & Kalik (2007) took this further, dissecting all known species belonging to the Palaearctic *A. pimpinellae* complex and establishing 17 species (from approximately nine). Further work by Kadej & Háva (2011) and Holloway (2019, 2020, 2021) added six more species, bringing the number of species in the complex to 23. The similarity of *A. pimpinellae* complex species necessitates careful description of every species, but we are still far from achieving this. Of the Western European species, Holloway & Bakaloudis (2020) described *A. amandae* Holloway, 2019, and *A. pimpinellae* in detail, and Holloway *et al*. (2020) extended this level of detail to consider *A. isabellinus*. *Anthrenus munroi*, although relatively straight-forward to recognise under a microscope with a good view of antennal structure, has not yet been subject to the same morphological examination as these species. Part of the current study was to produce a more detailed examination of the morphology of *A. munroi*, particularly because Hinton, in his original description, considered *A. munroi* difficult to differentiate from *A. pimpinellae* (Hinton 1943).

In recent years, entomology has evolved with citizen scientists engaging in the study of insects through the submission of images to digital platforms, such as iNaturalist (2022) and others that feed into GBIF (2022), illustrating species distribution and phenology. For citizen science data to be useful, images must be reliably identifiable. To this end, *A. pimpinellae* complex species need to be presented differently from that way they have been to date: to facilitate identification in the field and from images. There are two identification guides to Dermestidae (Peacock 1993; Háva 2011), including *Anthrenus*, but both these publications utilize features not easily noted in images taken in the field. Very few *A. pimpinellae* complex museum specimens have been dissected, suggesting that collectors and subsequent visitors to museum collections believe they can identify specimens using external characters. External identification features are what citizen scientists require, but these identification characters infrequently make their way into the literature.

The second objective of the current study was to produce a key to the identification of species from the *A. pimpinellae* complex that might be encountered in Western Europe (*i.e*., the area west of a north-south line that includes Italy), using specimens from the Natural History Museum (NHM), London, and others recently collected in the field. Images are shown in conjunction with the key to produce a guide that citizen scientists might find useful, and to encourage more people to submit *Anthrenus* records to aid better our understanding of these insects' distribution and phenology.

MATERIAL AND METHODS

Anthrenus munroi Hinton, 1943, specimens were collected from Pollensa, Mallorca (39.853814N, 3.0571317E) in 2019, and Bunyola, Mallorca (39.696857N, 2.700120E) in May 2021. The insects, including the NHM specimens, had been stored dry before preparation when they were dropped into a solution of 2% acetic acid for five days softening before dissection. Dissection was carried out under a Brunel BMSL zoom stereo LED microscope. Dissection involved detaching the abdomen from the rest of the insect using two entomological pins. The soft tergites were then peeled off the harder ventrites to expose the genitalia. For males, the aedeagus was detached from the ring sclerite, and sternite IX was detached from the ring sclerite and the aedeagus. Images of male and female habitus, upper side and underside, were captured at ×20 magnification using a Canon EOS 1300D Camera mounted on the BMSL microscope. Images of aedeagi and sternite IX were captured at ×100 magnification using the EOS 1300D Camera mounted on a Brunel monocular SP28 microscope. After dissection, all body parts were mounted on card. The antennae were teased out and images were taken at ×63 magnification through the BMSL microscope. All images were fed through Helicon Focus Pro version 7.7.4 focus-stacking software. Morphometric measurements were made using DsCap.Ink Software version 3.90.

Measurements taken

Body length (BL): distance from anterior margin of pronotum to the posterior of the elytra; Body width (BW): maximum distance across the elytra; Antennal club length (AL): length of the last three antennomeres; Antennal club width (AW): maximum width across the terminal antennomere; Paramere length (PL): distance from the anterior end of the parameres to the apex of the parameres; Sternite IX length (SL): distance from the tip of one anterior horn to the tip of the posterior margin.

Statistical analysis: Analyses were carried out using Minitab version 20.3. Differences among groups were tested using Kruskal Wallis test and t-tests.

RESULTS

Examples of the *Anthrenus munroi* habitus (dorsal and ventral aspect), antennal club, aedeagus, and sternite IX are shown in Fig. 1.

Morphometrics

24 specimens were collected from Mallorca (n = 10♂♂, n = 14♀♀). There was no difference in the size of specimens from the two collection localities (H = 0.75, df [degrees of freedom] = 1, ns [not significant]) so the two data sets were combined for further analysis. Data were checked by sex for normality and homoscedasticity.

Males were significantly smaller (♂ mean BL = 2.66 ± 0.12 sd [standard deviation] mm) than females (♀ mean body length (BL) = 2.96 ± 0.24mm) (t_{22} = 3.65, $p < 0.001$). 95% of ♂ BL would be expected to fall between 2.39–2.93mm (study sample minimum/maximum = 2.50mm and 2.79mm, respectively). 95% of ♀ BL would be expected to fall between 2.44 and 3.48mm (study sample minimum/maximum = 2.68mm and 3.35mm, respectively).

Body width/body length (BW/BL) was calculated as a measure of body shape. ♂ mean BW/BL = 0.690 ± 0.016; ♀ mean BW/BL = 0.691 ± 0.016. Male BW/BL did not differ significantly from ♀ BW/BL (t_{22} = 0.13, ns [not significant]). Combining the two data sets (mean = 0.691 ± 0.016), 95% of all values would be expected to fall between 0.66–0.72 (study sample minimum/maximum = 0.67 and 0.73, respectively).

The antennal club is large and smoothly rounded. Male antennal clubs (♂ mean antennal length (AL) = 204 ± 12µm; antennal width (AW) = 163 ± 9µm) were significantly smaller than female antennal clubs (♀ mean AL = 229 ± 20µm; AW = 177 ± 14µm) (AL t_{19} = 2.89, p = 0.009, AW t_{19} = 2.53, p = 0.021). The antennal club is broad with ♂ and ♀ (antennal length/antennal width (AL/AW) = 1.27 ± 0.11 and 1.3 ± 0.06, respectively. There was no difference in AL/AW between the sexes (t_{19} = 0.68, ns). Female body length (BL) is 11% larger than ♂ BL whilst ♀ antennal length (AL) is 12% larger than ♂ AL. There is no evidence that ♀♀ have proportionately larger antennal clubs than ♂♂, nor does the shape of the antennal club vary between the sexes.

The *A. munroi* aedeagus is shown in Figs 1C and 1D, and sternite IX is shown in Figs 1E and 1F. Mean paramere length (PL) = 372 ± 11µm. Mean paramere length/body length (PL/BL) = 0.14 ± 0.005. Mean sternite length (SL) = 445 ± 21µm. SL is on average nearly 20% longer than PL.

Identification key for Western European species in the *Anthrenus pimpinellae* complex

As noted above, specimens of species compared for inclusion in the following key were derived from the NHM collection and field collections from Mallorca and Greece. Some specimens collected east of Italy were also included, but these were of species whose range extends into Western Europe (*e.g.*, *A. isabellinus*). For each species, a good number of specimens (see Table 1) were dissected to confirm identification and to provide opportunity to consider intra- and inter-specific pattern variation allowing us to focus on characteristics that varied consistently between species.

TABLE 1. — NUMBERS OF EACH WESTERN EUROPEAN *ANTHRENUS PIMPINELLAE* COMPLEX SPECIES DISSECTED TO CONFIRM IDENTIFICATION AND TO FACILITATE CONSIDERATION OF INTRA- AND INTER-SPECIFIC PATTERN VARIATION. THE ORIGIN OF THE SPECIMENS EXAMINED IS PROVIDED

Species	**No. dissected**	**Origin**
A. amandae	52	Mallorca
A. angustefasciatus	32	Western Europe
A. chikatunovi	3	Pyrenees
A. delicatus	48	Western and Eastern Mediterranean
A. isabellinus	113	Western and Eastern Mediterranean
A. munroi	24	Mallorca
A. pimpinellae	64	Western Europe, Hungary and Greece

Fig. 1. — *Anthrenus munroi* Hinton, 1943: A, habitus dorsal aspect (scale bar = 1mm); B, antenna (scale bar = 0.1mm); C, aedeagus (dorsal surface) under high transmitted light (scale bar = 0.1mm); D, aedeagus (ventral surface) under low transmitted light (scale bar = 0.1mm); E, Sternite IX under high transmitted light (scale bar = 0.1mm); F, Sternite IX under low transmitted light (scale bar = 0.1mm).

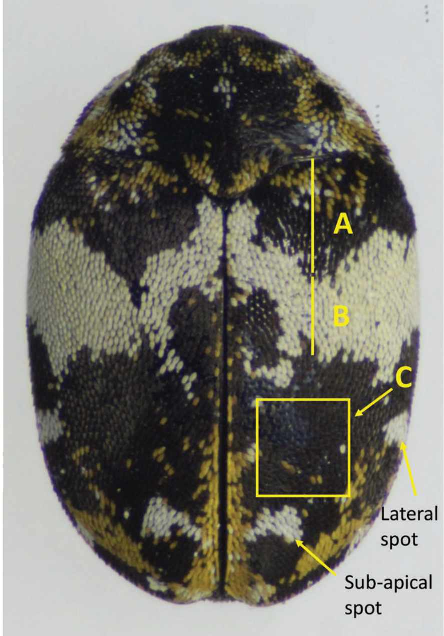

Fig. 2. — Characters used in identification key for Western European species in the *Anthrenus pimpinellae* complex.

The key uses habitus colour pattern to separate species belonging to the *Anthrenus pimpinellae* complex. All seven species confirmed from Western Europe are included. Fig. 2 indicates the features used in the key. Line B lies across the white band from the point where the band is furthest from the basal margin of the elytron. Line A is a continuation from line B, from beyond the white band to meet the basal margin of the elytron. The position of Area C is indicated in Fig. 2.

1 B usually longer than A . . . . **A. isabellinus* Küster, 1848 (Figs 3A, 4A)
[Additional features: lateral spots broadly connected to band, apical spots round to broadly elongated, many orange scales in area C, ventrite 1 black spot not meeting lateral margin. For aedeagus structure see Holloway *et al.* (2020)]
– B usually shorter than A . . . . (2)

2 Lateral spot not (or occasionally very weakly) connected to band . . . . (3)
– Lateral spot clearly connected to band . . . . (5)

3 Area C with few orange scales, and those that are present do not line up . . . *A. pimpinellae* Fabricius, 1775 (Figs 3B, 4B)
[Additional features: ventrite 1 black spot meets lateral margin, underside scruffy, off-white. For aedeagus structure, see Holloway & Bakaloudis 2020]
– Area C with many orange scales often forming anterior posterior lines . . . . (4)

4 Inner half of band very narrow, and often with a small break across the band . . . . *A. angustefasciatus* Ganglbauer, 1904 (Figs 3C, 4C)
[Additional features: lateral spot often very small or vague and distant from band, ventrite 1 black spot meets lateral margin, broad across shoulders. For aedeagus structure see Kadej, Háva & Kalik 2007]
– Band broader and never broken . . . . ***A. delicatus* Kiesenwetter, 1851 (Figs 3D, 4D)
[Additional features: lateral and sub-apical spots large, usually roundish, and obvious, ventrite 1 black spot not meeting lateral margin, flat-topped and vase-shaped antennal club. For aedeagus structure, see Holloway 2020]

5 Very few orange scales in Area C .... *A. amandae* Holloway, 2019 (Figs 3E, 4E)
[Additional features: ventrite 1 black spot not meeting lateral margin, few orange scales on pronotum so white spots on pronotum very obvious, only recorded from Mallorca to date. For aedeagus structure, see Holloway 2019 or Holloway & Bakaloudis 2020]
– Orange scales in Area C .... (6)

6 Apical spots usually reduced to very narrow, sometimes vague, lines often forming anterior pointing chevrons .... *A. munroi* (Figs 3F, 4F)
[Additional features: more orange scales in Area C often tending to form lines, lateral spot strongly connected with band, ventrites 1 black spot not meeting lateral margin, antennal clubs large and rounded. For aedeagus structure, see Figs 1C, 1D in current study]
– Apical spots round or slightly elongated but not very narrow .... *A. chikatunovi* Holloway, 2020 (Figs 3G, 4G)
[Additional features: ventrite 1 black spot not meeting lateral margin, only recorded from Pyrenees and extreme NE part of Spain to date. *NB* right hand lateral spot in Fig. 3G damaged by pin hole. For aedeagus structure, see Holloway 2020]

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* *Anthrenus isabellinus*'s dorsal colour pattern is highly plastic (Holloway, Bakaloudis & Cocks 2022). The majority of individuals resemble the one shown in Fig. 3A. In about 20% of individuals, the white band is broader with white scales bleeding down towards the elytral apices producing in some cases an almost completely white individual. This form of plasticity is only known from *A. isabellinus* so all individuals displaying this type of colour pattern are easy to identify.

** *Anthrenus delicatus* has two colour variants, the commonest of which is shown here (Fig. 3D). A second variant, referred to as *A. delicatus armstrongi* Háva, 2007, has no or very few black scales and is almost wholly covered in orange and white scales. It is possible that *A. delicatus armstrongi* is more common in eastern Europe so not covered in this key.

DISCUSSION

Many of the original descriptions of species belonging to the Palaearctic *Anthrenus pimpinellae* complex species do not adequately differentiate among the various species. Beal (1998) noticed that the genitalia, particular of males, varied considerably among species, suggesting that studying the genitalia would be a better way to proceed towards understanding the taxonomy of the complex. Kadej, Háva & Kalik (2007) and Kadej & Háva (2011) took this further and described six new species. These studies provided a good start to a process that could unravel the taxonomy of the complex and address misconceptions were they to be taken further. Holloway (2019, 2020, 2021) did this and found more species from field and museum collections. Additional work highlighted taxonomic errors that had been perpetuated for over 150 years (Holloway *et al*. 2020) resulting in a lack of understanding of global distribution (Holloway, Bakaloudis & Foster 2021). All work on this complex since, and including Beal (1998), has demonstrated the absolute necessity to have good descriptions of the structure and morphometrics of genitalia, supplemented with habitus comparisons.

Anthrenus munroi is a small species with mean body length (BL) of males just exceeding 2.5mm and in females falling below 3.0mm. Only two other species considered here are as small: *A. amandae* and *A. pimpinellae* (Holloway & Bakaloudis 2020). Differentiating *A. munroi* from *A. amandae* and *A. pimpinellae* is straightforward with the use of a stereo microscope since the antennal club of *A. munroi* is broad and rounded (Fig. 1B), whereas the antennal clubs of the other two species are cubic in shape (see Holloway & Bakaloudis 2020). If the antennae are missing, inter-specific variation in aedeagal structure makes species separation a reliable and simple task.

The genitalia of *A. munroi* have been illustrated elsewhere: Herrmann (2022) provides an image of the aedeagus, but not sternite IX; Kadej, Háva & Kalik (2007) also show images and illustrations of *A. munroi* genitalia, but not clearly. Furthermore, the illustration of sternite IX shown by Kadej, Háva & Kalik (2007) is incorrect and does not show the flaps on the inside of the two anterior pointed horns (see Figs 1E and 1F in the current paper).

When constructing keys, taxonomists understandably prefer to use qualitative features that consistently and reliably differentiate among species. Sometimes characteristics used are small or not simple to access (such as aedeagus structure in *Anthrenus*). Quantitative characters, such as colour or pattern, are often avoided because of the intra-specific variability of such features, although Hermand & Holloway (2021) found that the dorsal colour pattern in the case of *Attagenus rufiventris* Pic, 1927 (Dermestidae) was not very variable.

However, the landscape of entomology is changing with the advent of digital photography and cameras (and smartphones) that provide opportunities to take excellent images of insects under field conditions. Whilst some of the people taking images of insects in the field are established entomologists, most photographers do not fall into this category. This new breed of entomologist is not necessarily interested in the internal structures used to differentiate definitively among species, but requires the means to establish identification using features that can be seen and assessed from images. An army of photographers patrolling the field can provide valuable information about distribution and phenology of species (*e.g.*, see iNaturalist, 2022), and produce large data sets that a relatively small number of specialist entomologists would find difficult to generate. It is in the interest of every entomologist to explore ways to identify species using external features such as colour and pattern.

Holloway & Bakaloudis (2020) displayed images of *A. amandae* and *A. pimpinellae* and showed that they could be quite easily differentiated on the basis of their colour patterns. The work of Holloway & Bakaloudis (2020) provided the impetus to study in greater detail the colour patterns of more species from the *A. pimpinellae* complex and to construct the key (and images) provided here. Kadej, Háva & Kalik (2007) prepared a key based on internal features, but the current study is the first attempt to produce a key based purely on components of the colour patterns from images. Kadej (2005) produced a key to some *Anthrenus* species using illustrations, although his work pre-dated much of the research to elucidate *A. pimpinellae* complex taxonomy. Sometimes the inter-specific differences in colour patterns are subtle, but should be visible on clear images. Quantitative features are by their very nature variable, so it is possible that some specimens cannot be identified with total confidence, especially if some of the elytral scales are missing, but it should be possible to attach identity to all but a small number of specimens.

There is one further species from the *A. pimpinellae* complex claimed from Europe: *A. goliath* Saulcy in Mulsant & Rey, 1868. Kadej, Háva & Kalik (2007) examined *A. goliath* but the line drawing of the aedeagus differs considerably from the black and white image provided in the same publication for *A. goliath*. The line drawing resembles *A. corona* Holloway, 2021. There remains confusion over what *A. goliath* looks like and, consequently, where it is found. Kadej, Háva & Kalik (2007) studied 60 putative *A. goliath* specimens from the Mediterranean but did not specify exact locations. Holloway (2021) described *A. corona* from a specimen in the NHM found in Turkey, so it is possible that the specimens studied by Kadej,

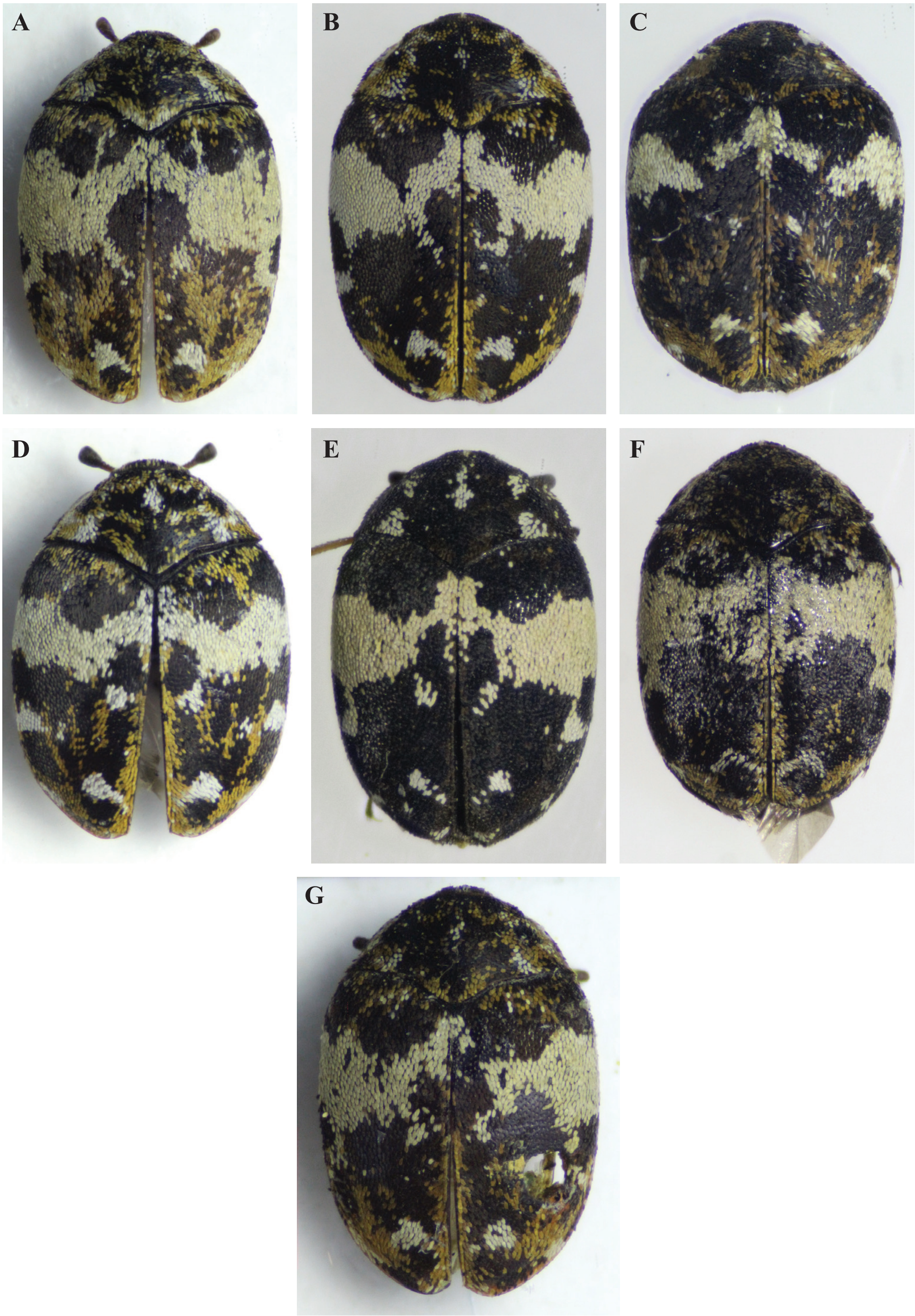

Fig. 3. — Dorsal surface of A, *Anthrenus isabellinus*; B, *A. pimpinellae*; C, *A. angustefasciatus*; D, *A. delicatus*; E, *A. amandae*; F, *A. munroi*; G, *A. chikatunovi.*

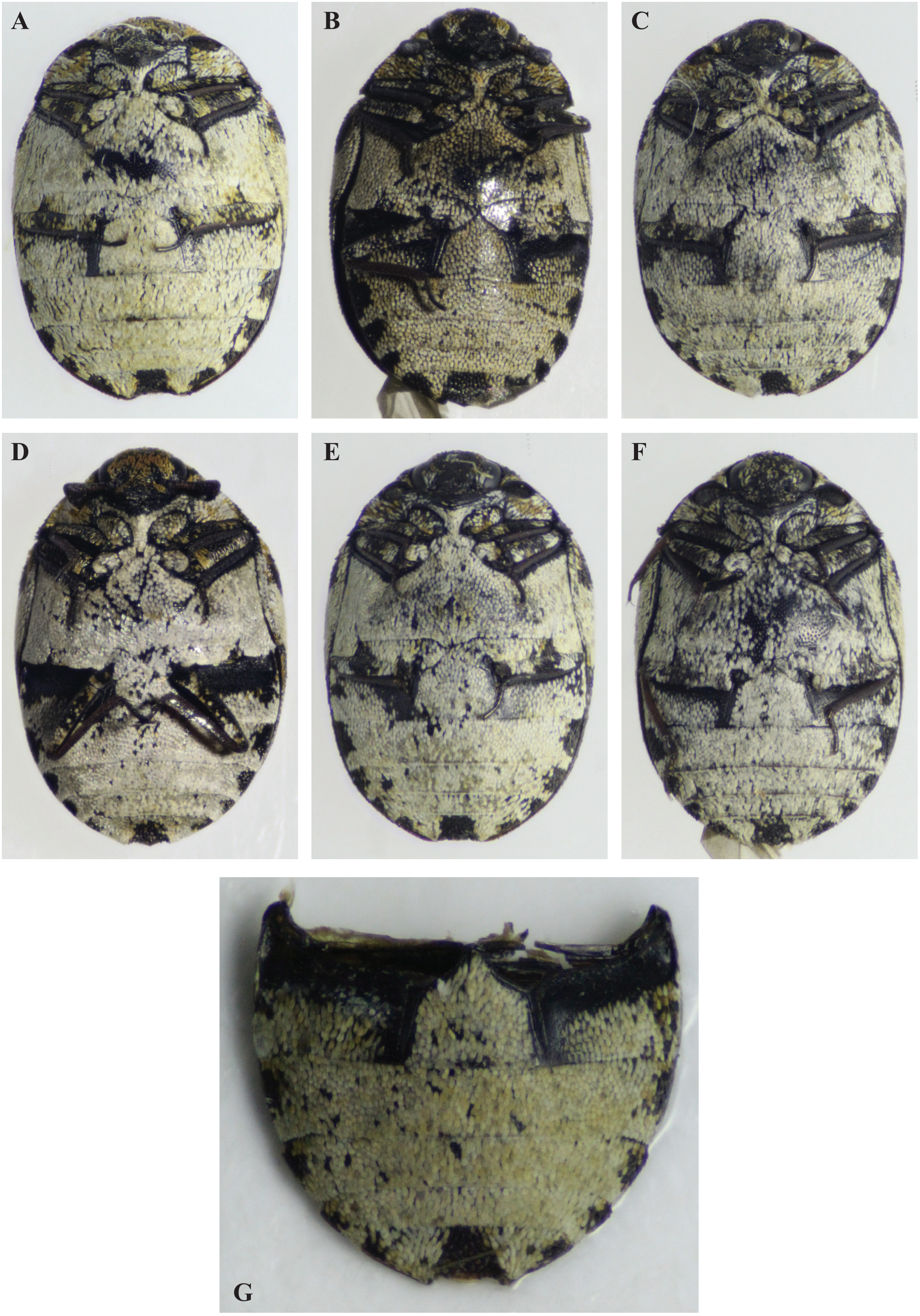

Fig. 4. — Ventral surface of A, *Anthrenus isabellinus*; B, *A. pimpinellae*; C, *A. angustefasciatus*; D, *A. delicatus*; E, *A. amandae*; F, *A. munroi*; G, *A. chikatunovi.*

Háva & Kalik (2007) were from the eastern Mediterranean. Háva (2022) claims that *A. goliath* is distributed across southern Europe. This requires confirmation, and as a result *A. goliath* has not been included in the current study.

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