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A Comparative Morphological Study of *Anthrenus pimpinellae pimpinellae* (Fabricius, 1775) and *Anthrenus amandae* Holloway, 2019 (Coleoptera: Dermestidae)

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Abstract

Recent research has shown that *Anthrenus pimpinellae* (Fabricius, 1775) is a complex of species numbering at least 21 in the Palearctic region. No work has been published illustrating what *A. pimpinellae pimpinellae* looks like relative to any other species in the complex, which interferes with accurate recording. Two species are considered here: *A. pimpinellae pimpinellae* and *Anthrenus amandae* Holloway, 2019. Identification of both species is confirmed by examination of the male genitalia. There are consistent differences between the species and relatively little intraspecific variation, offering routes to identification under field conditions. In addition, the validity of *A. amandae* as a distinct species is clarified.

Key Words: skin beetles, identification, phenotypic variation, ventrites, aedeagus

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Introduction

Taxonomy is vital for biodiversity research (McNeely 2002). Without taxonomy, how can we document biodiversity (Fontaine *et al.* 2012)? Following on from taxonomy, though, is research to facilitate recognition of species, perhaps in the field or maybe in the laboratory using binomial keys. Recognition of species through taxonomic and field-based techniques is often different (Quicke 1993). Taxonomists frequently deal with small details that consistently vary among species to offer accurate, but not always swift, recognition. Being able to recognize a species quickly is almost always a requirement for population studies since large numbers of specimens are involved.

Recognition of species in the field has become more important in recent years with the advent of web-based, citizen-science recording schemes (Schmeller *et al.* 2009). Field recognition involves being familiar with a range of very common species to facilitate the inclusion and differentiation of less common species. For example, familiarization of *Pterostichus madidus* (Fabricius, 1775), *Abax parallelepipedus* (Piller and Mitterpacher, 1783), and *Carabus violaceus* Linnaeus, 1758 is very useful when recording Carabidae in the UK, since these three species will account for a high proportion of the records (UK Beetle Recording, www.coleoptera.org.uk/family/carabidae). The species of Dermestidae encountered most frequently across many parts of the world is *Anthrenus verbasci* (Linnaeus, 1767); it is extremely common, almost cosmopolitan, and highly variable. Another dermestid considered to be very common with a wide global range is *Anthrenus pimpinellae pimpinellae* (Fabricius, 1775) and, as such, should be a familiar species among recorders. *Anthrenus pimpinellae pimpinellae* is subspecific with *A. pimpinellae isabellinus* Küster, 1848, but here is referred to simply as *A. pimpinellae*.

Anthrenus pimpinellae has been known for a long time for its variability, with a range of named varieties and subspecies. Kadej *et al.* (2007) conducted a study of the Palearctic *A. pimpinellae* complex, which included a detailed examination of male genitalia. Kadej *et al.* (2007) concluded that there were 17 well-defined species in the *A. pimpinellae* complex in the Palearctic, accounting for much of the variability previously attributed to *A. pimpinellae*. Kadej and Háva (2011) added a further three species, and Holloway (2019) added one more to bring the total number of described species within the complex in the

Palearctic to 21. Prior to Kadej *et al.* (2007), it is very likely that many species were being identified as *A. pimpinellae*. Judging by images that can be found on the web, confusion of species within the *A. pimpinellae* complex continues. Following on from the work to identify the true nature of the species within the complex, no effort has been made to clarify what *A. pimpinellae* truly looks like. Without this knowledge, it is difficult to establish the true distribution of *A. pimpinellae* and other closely related species. It is claimed to be almost cosmopolitan, but the first study to consider whether or not the species should be retained on the British checklist failed to find any evidence validating its inclusion (Holloway *et al.* 2018). *Anthrenus pimpinellae* is no longer considered to be a British species.

Ideally, character states used to differentiate between species should be qualitative. However, quantitative characters in keys are frequently used where they are easier to access for the reader than qualitative features. A character state might be described as longer, rounder, redder, and so on, but it is not always easy to decide what is long, round, and red without comparative material. In this study, we consider the morphological features that can be used to help recognize *A. pimpinellae* compared with a recently described species, *Anthrenus amandae* Holloway, 2019, with which *A. pimpinellae* may be confused. A comparative approach helps to appreciate quantitative habitus variation that might be used to aid identification under field conditions. A secondary objective was to establish *A. amandae* as a distinct species to counter doubts over its validity (Háva and Herrmann 2019).

Material and Methods

Beetles were collected from sites in and around Thessaloniki (Fig. 1), Greece, 6–8 May 2019. A variety of Dermestidae were gathered together, including several individuals of *A. pimpinellae*. All specimens were preserved in 2% acetic acid prior to examination. The *A. amandae* individuals examined were F_1 beetles derived from adults collected from Mallorca during May 2018 (Holloway 2019). The F_1 of *A. amandae* was reared on bird feathers. Rearing them ensured that all specimens belonged, without question, to the same species.

Nineteen (nine males, 10 females) specimens of *A. pimpinellae* and 40 (21 males, 19 females) specimens of *A. amandae* were dissected. Dissection involved detaching the abdomen from the rest of the beetle by using two entomological micropins. The soft tergites were then peeled from the harder ventrites to expose the genitalia. Using pins, structures associated with the genitalia were carefully removed, and the aedeagus was extruded between the terminal tergite and terminal sternite using a No. 2 entomological pin.

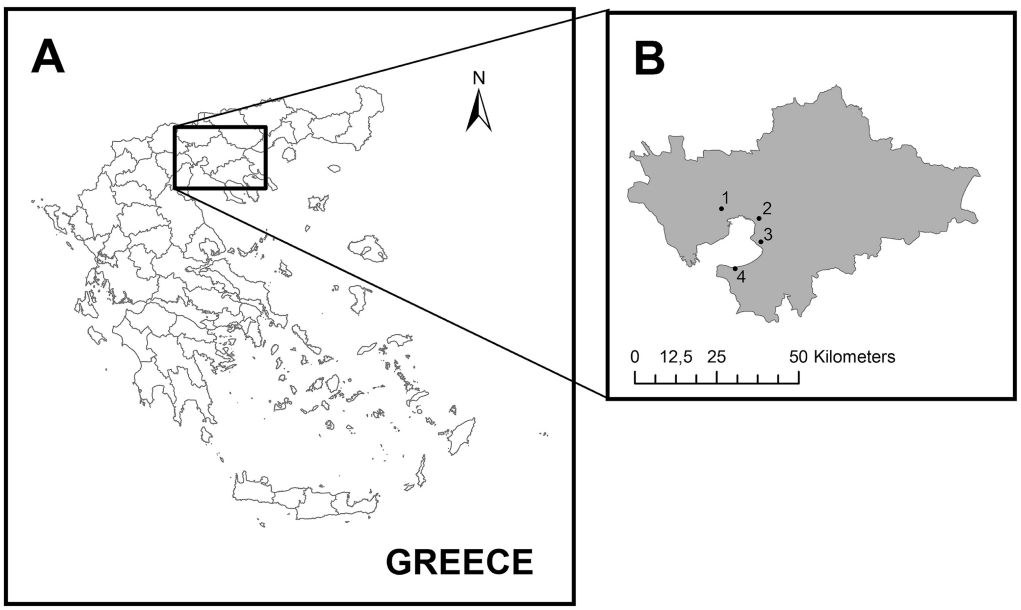

Fig. 1. The Prefecture of Thessaloniki (B) and its location in Greece (A), indicating the collection sites around Thessaloniki. The sites were 1: Sindos (40°39′55″ N, 22°48′33″ E), 12 individuals; 2: Kedrinos Lofos, Thessaloniki (40°38′16.26″ N, 22°57′54.67″ E), one individual; 3: School of Forestry & Natural Environment, Thessaloniki (40°34′03.06″ N, 22°58′14.94″ E), one individual; 4: Perea (40°29′56.01″ N, 22°53′14.25″ E), five individuals.

The aedeagus was carefully detached from the ring sclerite retaining the capsule over the anterior end of the aedeagus. In addition to the aedeagus, sternite IX was also very carefully detached from the ring sclerite. Images of the aedeagus and sternite IX were captured at 100× magnification with a camera mounted on a Brunel monocular SP28 microscope. After dissection, all body parts were mounted on a card. The antennae were teased out and images were taken at 63× magnification through the stereo microscope. All images were fed through Helicon Focus 7-Pro focus-stacking software. Morphometric analysis was carried out using DsCap.Ink Software version 3.90. Identification of *A. pimpinellae* was confirmed using images provided by Herrmann (2019) and Kadej *et al* (2007), and *A. amandae* was confirmed following Holloway (2019).

The following variables were parameterized (all in mm):

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: Aedeagal 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

Analyses of the data were carried out using Minitab (version 18).

Images of the dorsal and ventral habitus were captured by using a Canon EOS 1300D camera mounted on a Brunel BMSL zoom stereo LED microscope. Habitus images were taken at 20× magnification.

Results

Anthrenus pimpinellae (Fabricius)

Typical examples of male and female dorsal habitus types are shown in Fig. 2A. For neither *A. pimpinellae* nor *A. amandae* was there evidence of sexual dimorphism in color pattern. The elytra of both species carried a mixture of white or cream, orange to brown, and black scales. *Anthrenus pimpinellae* had denser regions of orange scales spread around the apex of the elytra, from the apex along the elytral suture, and from the apex along the outer elytral margins. There were also orange scales on the pronotum. The white band crossing the elytra was relatively narrow (compare with *A. amandae*). A significant feature was that the scales, particularly evident on the white band, were not overlapping, thus giving the pattern a 'scruffy' appearance.

Anthrenus pimpinellae is not large. Female BL (mean = 2.600±0.078 mm) is marginally greater than male BL (mean = 2.538±0.077 mm), but not significantly ($F_{1,17}$ = 0.31). Body length did not deviate significantly from normality, so 95% of the individuals in the population would lie between 2.073 mm and 3.075 mm. None of the 19 individuals examined fell outside this range. Mean BW/BL (0.688±0.029 mm) did not differ significantly between the sexes ($F_{1,17}$ = 0.58) and indicated that *A. pimpinellae* is relatively slim. The ratio of body width to body length is a highly conserved character with a coefficient of variation of 1.86%.

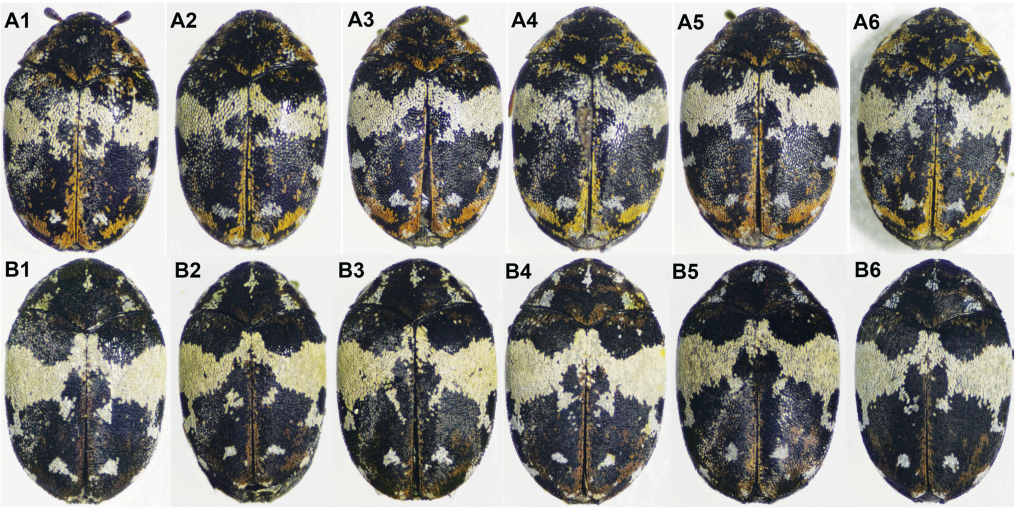

Fig. 2. Habitus types of *Anthrenus pimpinellae* (A) and *Anthrenus amandae* (B). 1–3 = males; 4–6 = females.

Typical examples of male and female ventrites are displayed in Fig. 3A. The scales on the ventrites of *A. pimpinellae* were grayish and brown. As with the scales on the elytral band, the scales did not generally overlap to produce a scruffy appearance. The black spots on the outer edges of each ventrite were composed of black scales. These patches were large on each ventrite but largest on the first ventrite. The gray scales extending inwards across the first ventrite were sparsely distributed.

A typical example of the antenna of *A. pimpinellae* is shown in Fig. 4A. There was no difference between the sexes in the mean AL ($F_{1,17}$ = 1.08) and mean AW ($F_{1,17}$ = 1.76). Mean AL was 0.179±0.003 mm and mean AW was 0.143±0.002 mm. Mean AL/AW was 1.254±0.009.

Anthrenus amandae Holloway

Typical male and female habitus types are shown in Fig. 2B. The overall impression is of a narrow and very dark species, darker than *A. pimpinellae*. The orange scales displayed by *A. pimpinellae* were a dark brown in *A. amandae*, and it is not immediately obvious that there were any colored scales mixed in with the black. The brown scales on *A. amandae* showed a similar distribution to the orange scales of *A. pimpinellae*, both on the elytra and the pronotum. The patches of white scales (other than the band) were more obvious and extensive in *A. amandae* and differed from *A. pimpinellae*, particularly on the pronotum. The white band in *A. amandae* was broader and consisted of tighter packed, more overlapping scales. A consistent difference between the two species was that *A. amandae* showed a well-developed, white finger of scales extending backwards from the lower edge of the white band close to the outer elytral margin. This white finger was absent in *A. pimpinellae* or rudimentary when present.

There is a size difference between the sexes. Males are significantly shorter (BL mean = 2.578±0.05 mm) than females (BL mean = 2.814±0.03 mm) ($F_{1,38}$ = 16.82, $p < 0.001$). The BL of neither males nor females deviates significantly from normality, so 95% of male body lengths would lie between 2.137 mm and 3.019 mm, and 95% of female body lengths would lie between 2.553 mm and 3.072 mm. Only one male lay outside of the range (2.125 mm), but none of the females. The predicted body length range for *A. amandae* is almost identical to the predicted body length range for *A. pimpinellae*. Body shape does not differ significantly between the sexes (mean BW/BL = 0.683±0.022, $F_{1,38}$ = 1.59) and is almost identical to the BW/BL value for *A. pimpinellae* (mean = 0.688±0.029).

Typical examples of male and female ventrites are displayed in Fig. 3B. The color difference between the ventrites of *A. amandae* and *A. pimpinellae* was striking. While the scales on *A. pimpinellae* were dirty gray/brown and relatively sparse, the scales on *A. amandae* were bright white, dense, and overlapping. The spots of black scales along the outer edges of the ventrites were as long but narrower than those on *A. pimpinellae*. In particular, the black spot on the outer edge of the first ventrite, when present, was very small. The white scales extending inwards from the lateral margin of the first ventrite were more extensive than those on *A. pimpinellae* and more densely packed together.

A typical antenna of *A. amandae* is shown in Fig. 4B. There was a significant difference between the sexes in mean AL ($F_{1,38}$ = 4.44, p = 0.042) and mean AW ($F_{1,38}$ = 4.53, p = 0.040). Mean male AL and AW were 0.170±0.002 mm and 0.132±0.002 mm, respectively, and mean female AL and AW were 0.176±0.002 mm and 0.137±0.002 mm, respectively. Antennal club AL/AW also differed between the sexes ($F_{1,38}$ = 5.03, p = 0.031); female antennal clubs (mean AL/AW = 1.228±0.008) were larger than male antennal clubs (mean AL/AW = 1.252±0.008). The AL/AW values were very similar to those of *A. pimpinellae*, although the shapes of the antennal clubs appear to be different. Antennomere 8 is shorter and wider in *A. amandae* than in *A. pimpinellae* (Figs. 4A and B).

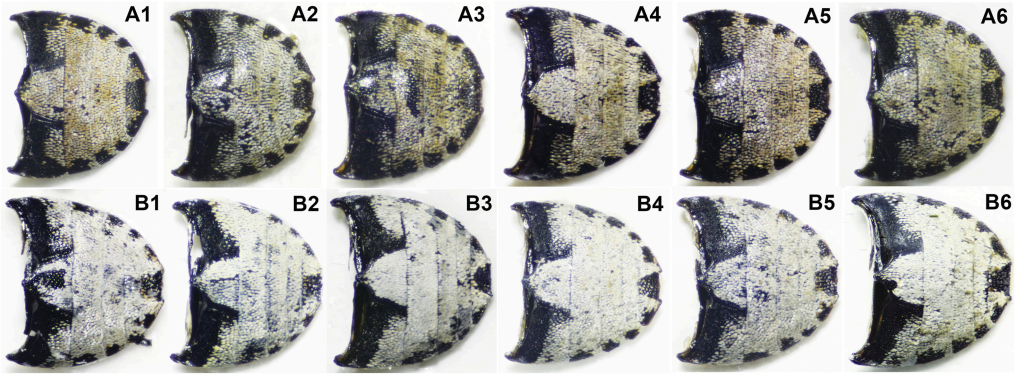

Fig. 3. Ventrites of *Anthrenus pimpinellae* (A) and *Anthrenus amandae* (B). 1–3 = males; 4–6 = females.

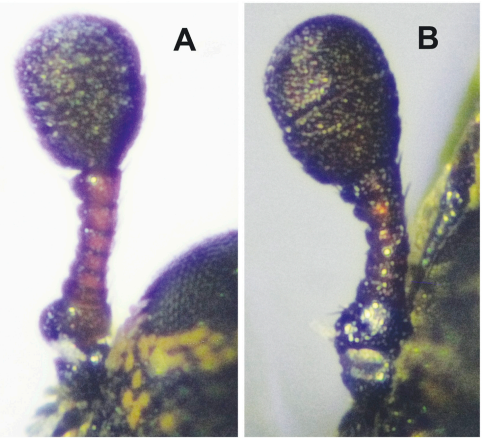

Fig. 4. Antennae of *Anthrenus pimpinellae* (A) and *Anthrenus amandae* (B).

Confirmatory Characters. The male aedeagi of *A. pimpinellae* and *A. amandae* are compared in Fig. 5. The aedeagus of *A. pimpinellae* (Fig. 5A) had a distinctive shape. The parameres were broad and hooked at the posterior apices. The dorsal surface of the paramere was covered in long, shaggy, black setae. The aedeagus widened considerably from the anterior cap towards the posterior end. There was a small membranous 'window' at the posterior end of each paramere. The cap was evenly curved to the pointed anterior end. The median lobe was very broad at the anterior end and tapered evenly to a blunt, relatively broad tip falling well short of the ends of the parameres. Mean AE was 0.428±0.13 mm, which equated to 16.9±0.3% of body length.

The aedeagus of *A. amandae* (Fig. 5B) differed from that of *A. pimpinellae* in almost every respect. The overall shape was approximately rectangular, but the width at the posterior and anterior ends were similar. The parameres were broad and hooked but were much less setose than in *A. pimpinellae*. The membranous windows in the parameres were much longer, extending from the posterior tip and down the inner edge of each paramere. The cap was obviously shouldered towards a blunt, square-ended anterior tip. The median lobe was narrower than in *A. pimpinellae* at the anterior end and did not narrow evenly towards the blunt, relatively broad tip. From the anterior end, the lobe expanded before tapering towards the posterior tip that fell short of the ends of the parameres. Mean AE was 0.469±0.006 mm, which was significantly longer than the mean AE of *A. pimpinellae* ($F_{1,27}$ = 10.97, p = 0.003). The aedeagus was 18.3±0.3% of the body length, which was a significantly greater proportion of the body length than in *A. pimpinellae* ($F_{1,27}$ = 8.94, p = 0.006).

Examples of sternite IX for both species are shown in Fig 6. Sternite IX lies underneath the aedeagus *in situ*, ensuring smooth movement of the aedeagus. There was a great deal of variation between the species in many structural components of sternite IX. The two species differed notably in the width relative to length of the posterior stem, the shape of the apex of the posterior stem, and the size and distribution of setae along the margins and apex of the stem.

Discussion

The ease with which species within the *A. pimpinellae* complex can be identified varies depending on the species. Some are more straightforward than others, such as *Anthrenus angustefasciatus* Ganglbauer, 1904 and *Anthrenus mroczkowskii* Kalik, 1954, because of breaches in the white elytral band (Herrmann 2019; Kadej 2005; Kadej *et al.* 2007). Indeed, *A. angustefasciatus* has been added to the British list by virtue of field-based features (Foster and Holloway 2015). Characters other than genitalia that can be used to recognize other species from the complex remain less well studied.

Kadej *et al.* (2007) demonstrated that the *A. pimpinellae* complex consists of several species, many of which can be comfortably identified with access to the male genitalia. Male genitalia were used in our study to definitively confirm species identification with reference to Kadej *et al.* (2007), Herrmann (2019), and Holloway (2019). Our study demonstrated that there are considerable phenotypic differences between *A. pimpinellae* and *A. amandae*. Species from the *A. pimpinellae* complex regularly appear as images on the web. A very high proportion of them are labelled as *A. pimpinellae*. Comparison of web-based images with the images presented here suggests that many of them are incorrectly identified. It is vital that every effort is made to ensure correct identification as much of these data feed into recording schemes (*e.g.*, Mapa Bioróżnorodności Baza Danych, baza.biomap.pl; Fauna Europaea, fauna-eu.org; iRecord, www.brc.ac.uk/irecord; Kerbtier.de, www.kerbtier.de/cgi-bin/enXSearch.cgi). It is from these schemes that distribution maps and checklists are generated, and fundamental data on species of conservation concern are derived. Without confidence that species are being identified correctly, recording schemes can lose credibility and value. *Anthrenus pimpinellae* is thought to be a common and widespread species (Háva 2015; Herrmann 2019; Kadej *et al.* 2007). It is active during the daytime, easy to find, attractive, and easy to photograph. It is believed to be a common and widespread species and, as such, entomologists should ensure that it can be recognized with confidence. Modern keys generally display one typical example of a species (*e.g.*, Háva 2011) or, as is the case with Peacock (1993), it is text-based supported by line drawings. This inevitably draws questions

Fig. 5. Aedeagi (ventral and dorsal views) of *Anthrenus pimpinellae* (A) and *Anthrenus amandae* (B).

about quantitative identification features, something that one of us (GJH) hears regularly from students of entomology. Producing series of images lying against each other, as done here, is a very powerful way of appreciating variation within and between species, offering substance to quantitative variation used for identification.

Our study had two objectives. One objective was to investigate characters that might be useful in the field to identify *A. pimpinellae* but also to help students of Dermestidae to decide what is not *A. pimpinellae*. A surprising element of the study is that substantial variation between *A. pimpinellae* and *A. amandae* in

Fig. 6. Males sternites IX of *Anthrenus pimpinellae* (A) and *Anthrenus amandae* (B).

appearance exists, but also little intraspecific variation is noted. Texts dealing with Dermestidae frequently comment on the variability of *A. pimpinellae*; it is possible that this reflects a lack of research into interspecific variation across the *A. pimpinellae* complex or is a throwback to a time predating the separation of the *A. pimpinellae* complex into a series of species. In addition to the elytral color differences, the most useful distinguishing feature was scale color and character on the ventrites. The scales of *A. pimpinellae* are a mixture of gray and brown contrasting with the clean white appearance of the scales on the ventrites of *A. amandae*. Used in conjunction with further interspecific variation we described, such as antennal details, it should be a relatively straightforward task to separate these two species under field conditions by using an eyepiece, providing the scales are still present.

The other objective was to establish if *A. amandae* is a distinct species that can be differentiated comfortably from *A. pimpinellae*, the most likely confused species. The genitalia demonstrate without doubt that *A. amandae* is not *A. pimpinellae*, and, indeed, they do not resemble any other species illustrated by Kadej *et al.* (2007) and Kadej and Háva (2011). The two species are very similar in size, but beyond that they are quite distinctive. To date, *A. amandae* has only been recorded on Mallorca, Spain (Holloway 2019). This is a relatively well-studied part of Europe (Holloway 2019; Holloway *et al.* 2019), and the similarity in size could be the principal reason why *A. amandae* has not been noticed before. During two

visits to Mallorca during 5–12 May 2018 and 20–27 April 2019, no *A. pimpinellae* were found, even though these time periods coincide well with adult phenology (Kerbtier.de, www.kerbtier.de/cgi-bin/enXSearch.cgi). This raises an interesting question: Is *A. pimpinellae* currently found on Mallorca or have *A. pimpinellae* records to date (or at least recent records) been *A. amandae*? *Anthrenus pimpinellae* has recently been removed from the British faunal list. In Poland, only one record of *A. pimpinellae* has been submitted to the national recording scheme since the year 2000 (Mapa Bioróżnorodności Baza Danych, baza.biomap.pl), which might suggest that it is infrequently encountered. In Germany, *A. pimpinellae* appears to be recorded more frequently (Kerbtier.de, www.kerbtier.de/cgi-bin/enXSearch.cgi). More work needs to be carried out to establish the true contemporary distributions of species within the *A. pimpinellae* complex (*e.g.*, Holloway and Bakaloudis 2019; Holloway *et al.* 2019). To achieve this, identification characters useful under field conditions need to be determined for a range of species. It is hoped that the current study will stimulate further comparative morphological work to differentiate among species to encourage greater interest in Dermestidae and to feed into citizen science programs.

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