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RESEARCH ARTICLE

Interaction of the external and internal genitalia during copulation in *Eupithecia abbreviata* Stephens, 1831 (Lepidoptera: Geometridae: Larentiinae)

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Abstract

During light trapping in SW Germany, a pair of the geometrid moth *Eupithecia abbreviata* Stephens, 1831, in copula, the male no longer possessing its head, was collected to study the interaction of the genitalia in this species. The pair were in a tail-to-tail position but were not in the same horizontal plane, with the male slightly rotated to one side. Externally, the valvae of the male's genitalia capsule, as well as the 8th sternite, seemed to exert pressure on the female's abdomen. In addition, the cornuti of the male vesica were found to coincide with membranous extensions in the female corpus bursae, suggesting a possible lock-and-key mechanism. The dorsal and ventral habitus, as well as the genital structures of both sexes, separately and in copula, are illustrated.

Key words: 8th sternite, cornuti, corpus bursae, Eupitheciini, genitalia, morphology, vesica.

Zusammenfassung

Während eines Lichtfanges in Südwestdeutschland wurde ein Pärchen des Eichen-Blütenspanners *Eupithecia abbreviata* Stephens, 1831, in Kopula gesammelt, wobei das Männchen seinen Kopf nicht mehr besaß. Dies ermöglichte es, die Interaktion männlicher und weiblicher Genitalien bei dieser Geometridenart zu untersuchen. Es wurde beobachtet, dass sich Weibchen und Männchen während der Kopulation mit ihren Abdomina nicht in derselben horizontalen Ebene befanden, sondern das Männchen leicht zur Seite gedreht war. Äußerlich schienen die Valven der männlichen Genitalkapsel sowie der achte Sternit Druck auf den Hinterleib des Weibchens auszuüben. Außerdem wurde festgestellt, dass die Cornuti der männlichen Vesica mit häutigen Fortsätzen in der weiblichen Corpus bursae übereinstimmen, was möglicherweise die Schlüssel-Schloss-Hypothese unterstützt. Der dorsale und ventrale Habitus sowie die Genitalstrukturen beider Geschlechter sind einzeln und in Kopula abgebildet.

Introduction

The endless variety of structures in the male and female genitalia of Lepidoptera, especially the sclerotized parts, provides an incredible source of information and has been extensively used in taxonomic investigations for the separation of species (MILLER 1988; SCOBLE 1992; HAUSMANN 2001; SIHVONEN 2007; WANKE & RAJAEI 2018). In moths, the interaction of these structures during copulation has been well documented in some noctuids and in the geometrid genus *Scopula* Schrank, 1802 (e.g., CALLAHAN 1960; CALLAHAN & CHAPIN 1960; SIHVONEN 2007). A perfect anatomical match of the male spermatophore and the female bursa copulatrix was described by PETERSEN (1909) for the genus *Eupithecia* Curtis, 1825. For North American *Eupithecia* species, MIKKOLA (1994) demonstrated an interaction of the male's 8th sternite with structures (Bolte's pockets) on the 7th segment of the females, functionally similar to those seen in some *Scopula* species (SIHVONEN 2007). Furthermore, MIKKOLA (1994) sug-

gested that the male ventral plate acts as a tool for opening the colliculum in the early stages of copulation. SIHVONEN (2007) proposed, for species of the genus *Scopula*, that the cerata are involved in sexual stimulation of the female.

The genus *Eupithecia* contains more than 1,300 species worldwide, making it the most species-rich genus of Geometridae (MIRONOV 2003). Of this immense number of species, 65 are present in Germany and 59 occur in the Baden-Württemberg region (GAEDIKE & HEINICKE 1999; GELBRECHT 1999; RATZEL 2003). *Eupithecia abbreviata* Stephens, 1831 is distributed in Europe from Portugal to the Balkan Peninsula and outside of Europe in North Africa and the Caucasus (MIRONOV 2003). *Eupithecia abbreviata* specimens are rather easy to recognize; although they can be confused with some relatives, they differ by the following characters (see MIRONOV 2003; RATZEL 2003): wings with a pointed apex, termen rounded, wing pattern pale and dark (Figs. 1–3); male genitalia with valva with large ventral process and aedeagus with one particularly long cornutus (the two other cornuti shorter)

(Figs. 6–8); female genitalia with an elongated patch of spinules in the ductus bursae and corpus bursae with spinules at the base and larger marginal spines (Fig. 9).

On the 17th of April 2018, in the grounds of the Entomological Society of Stuttgart in Markgröningen (Ludwigsburg district, Baden-Württemberg), I collected a pair of *E. abbreviata* in copula, at light. Surprisingly, the male was lacking its head, which might have prevented the female from breaking free, even after the pair was transferred into a cyanide jar; this allowed the specimens to be stored, dissected and studied while still in the copulatory position. Here, I document the dissected genitalia of the *E. abbreviata* pair, compare them to additional specimens of the species, and comment on the interaction of the male and female structures during copulation.

Material and methods

Examined material

1 ♂ and 1 ♀ (in copula), Germany, BW [Baden-Württemberg], Markgröningen, EVS [Entomologischer Verein Stuttgart] – Stückerle im Leudelsbachtal, 17.iv.2018, 270 m, LF [Lichtfang], leg. D. WANKE, g.prep. 0774/2020 D. WANKE; 1 ♂, [Germany], Markgröningen, Rotenacker, Li [Licht], 17.iv.1971, leg. W. SCHÄFER, g.prep. 0775/2020 D. WANKE; 1 ♀, Germania [Germany], Württemberg, Markgröningen, Rotenacker, Lichtfang, 350 m, 16.iv.1976, leg. W. SCHÄFER, g.prep. 0775/2020 D. WANKE; all in State Museum of Natural History Stuttgart (SMNS).

Morphological examination

The species was identified based on the literature and comparison with identified material in the SMNS collection. Dorsal and ventral habitus photographs of all specimens and close-up photographs of the interlocked abdomens of the copulating pair were taken using a Visionary Digital photography system (LK Imaging System, Dun. Inc.) equipped with a Canon EOS 5DSR camera and 65 mm macro lens, and stacked in Helicon Focus. Specimens were dissected following standard techniques (ROBINSON 1976), and the genitalia structures of the additional male and female were embedded in Euparal on permanent slides. The genitalia of the copulating pair were dissected and documented step by step in alcohol, using the mechanical fixation methods described by WANKE & RAJAEI (2018), WANKE et al. (2019) and WANKE et al. (2021). All genitalia images were taken using a Keyence VHX-5000 and its associated software.

Results and discussion

Comment on the mating position

As said, the pair of *E. abbreviata* was collected during mating and was in a tail-to-tail position (Fig. 3). The male and female were not in the same horizontal plane, with the male rotated relative to the female, as shown in Figs. 3–5. This tail-to-tail mating position occurs in most species in Lepidoptera (PHELAN & BAKER 1990; SIHVONEN 2007); however, there are also exceptions, such as *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Lymantriidae),

in which the male faces in the same direction as the female (CHARLTON & CARDÉ 1990).

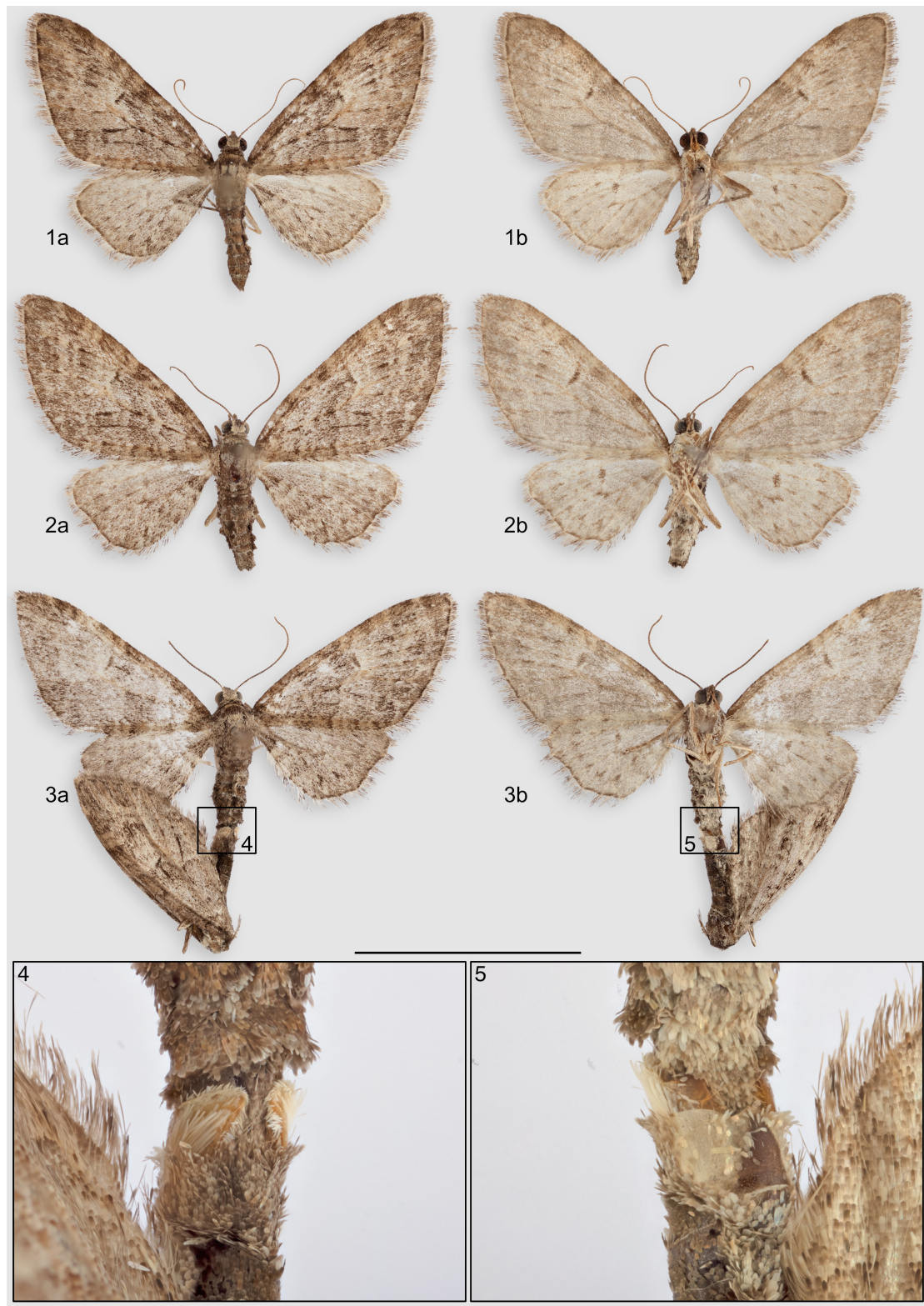
Interaction of the external genitalia

Prior to dissection, the male valvae and 8th sternite (dark brown, triangular in Fig. 5) were the only characters visible externally (Figs. 4–5), and the valvae seemed to be in a position such as to exert lateral pressure on the female's abdomen. The uncus has been shown to be inserted between the papillae anales of female Notodontidae and Noctuidae during copulation, where it performs an additional clasping function (MILLER 1988; LÖDL 2000); although not observed in this study, a similar function of the uncus in *E. abbreviata* cannot be ruled out. After maceration, the valvae and the uncus became detached from the female abdomen and a possible connection of the uncus with the papillae anales could no longer be verified (Fig. 10). During the maceration process, I was also able to observe that the membranous and setose labides of the male were in contact with the female's papillae anales, but their potential function during copulation remains unclear.

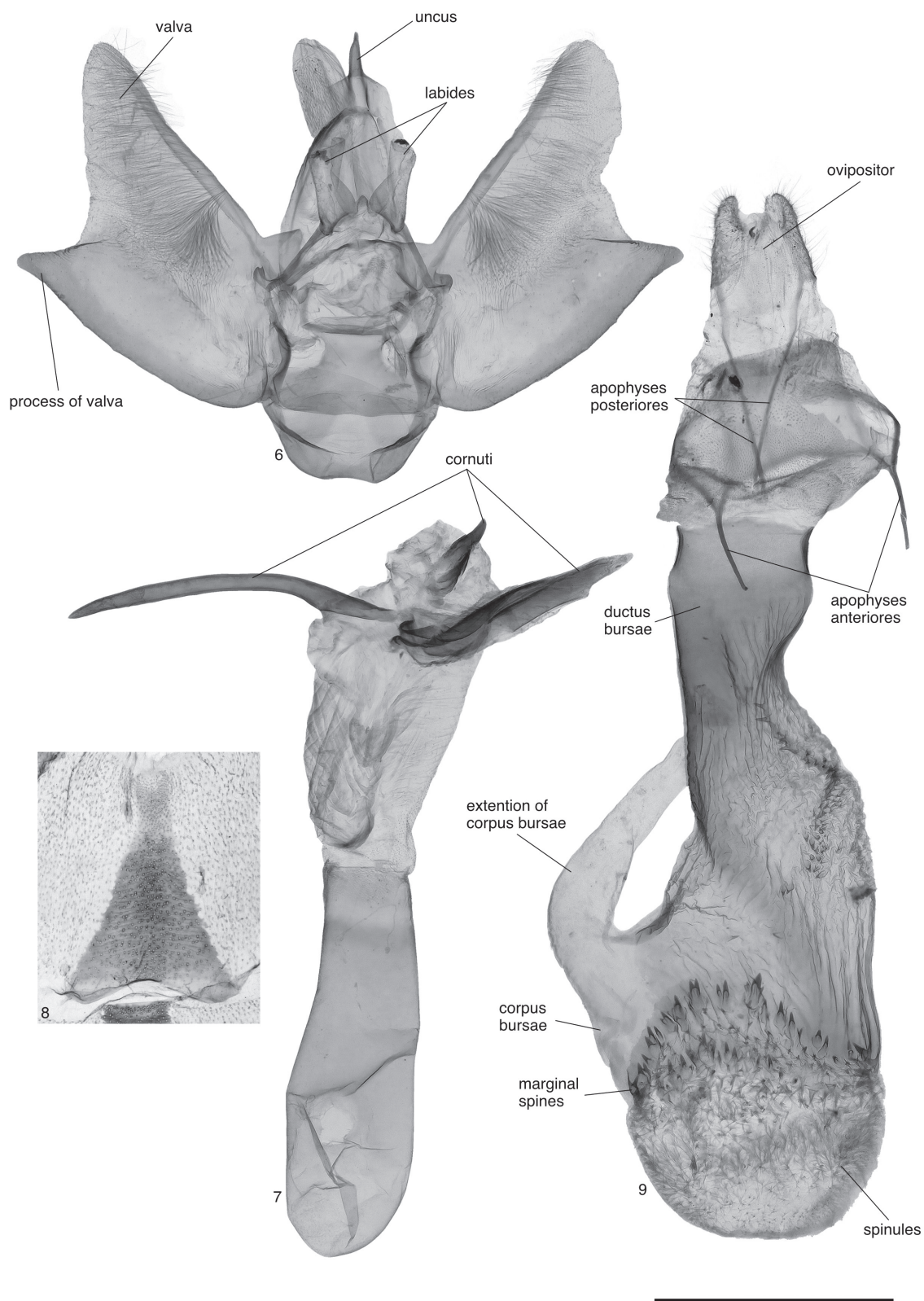
Regarding the male 8th sternite, it seemed to cause pressure on the female abdomen in its natural position prior to maceration (Fig. 5). Unfortunately, after maceration, the male 8th sternite could not be studied in more detail. Although it has been assumed that the 8th sternite has a mechanical function during copula (HAUSMANN 1999) and a forceps-like function in the North American *Eupithecia palpata* group, by means of long prolongations (rods) of the 8th sternite (MIKKOLA 1994), this sternite is a very simple triangular plate in *E. abbreviata*, and its potential function during copulation remains unclear; nonetheless, it can be assumed that the function is different from that suggested by MIKKOLA (1994) and SIHVONEN (2007), due to the different morphology of the 8th sternite in these two groups of species.

Interaction of the internal genitalia

Apparently, in *E. abbreviata*, penetration by the male genitalia is mainly performed by the vesica and the cornuti of the vesica, whereas the aedeagus only reaches the ductus bursae (Figs. 10–11). SIHVONEN (2007) found a similar pattern in *Scopula immorata* (Linnaeus, 1758), in which the sclerotized teeth at the apex of the aedeagus and the single cornutus rest on the inner wall of the ductus bursae, which is blocked by the fully everted vesica. The vesica is everted by aerostatic pressure and muscle action (CALLAHAN 1958). The vesica of *Eupithecia abbreviata* has three noticeable cornuti, of different lengths (Fig. 7). In the copulating pair documented herein, the long cornutus was directed towards the anterior end of the corpus bursae, the medium-sized cornutus was inserted within the lateral extension of the corpus bursae, and the short



Figs. 1–5. Habitus of *Eupithecia abbreviata*. – 1. Male ([Germany], Markgröningen, Rotenacker, g.prep. 0775/2020 D. WANKE). 2. Female ([Germany], Markgröningen, Rotenacker, g.prep. 0775/2020 D. WANKE). 3. Male and female in copula (Germany, Markgröningen, Leudelsbachtal, g.prep. 0774/2020 D. WANKE). 4. Same; close-up of copula, dorsal view. 5. Same; close-up of copula, ventral view. Abbreviations: a = upperside; b = underside. Scale bar: 1 cm.



Figs. 6–9. Genitalia structures of *Eupithecia abbreviata*. **6–8.** Male ([Germany], Markgröningen, Rotenacker, g.prep. 0775/2020 D. WANKE). – **6.** Genital capsule. **7.** Aedeagus. **8.** 8th sternite). **9.** Female ([Germany], Markgröningen, Rotenacker, g.prep. 0775/2020 D. WANKE). Scale bar: 1 mm.

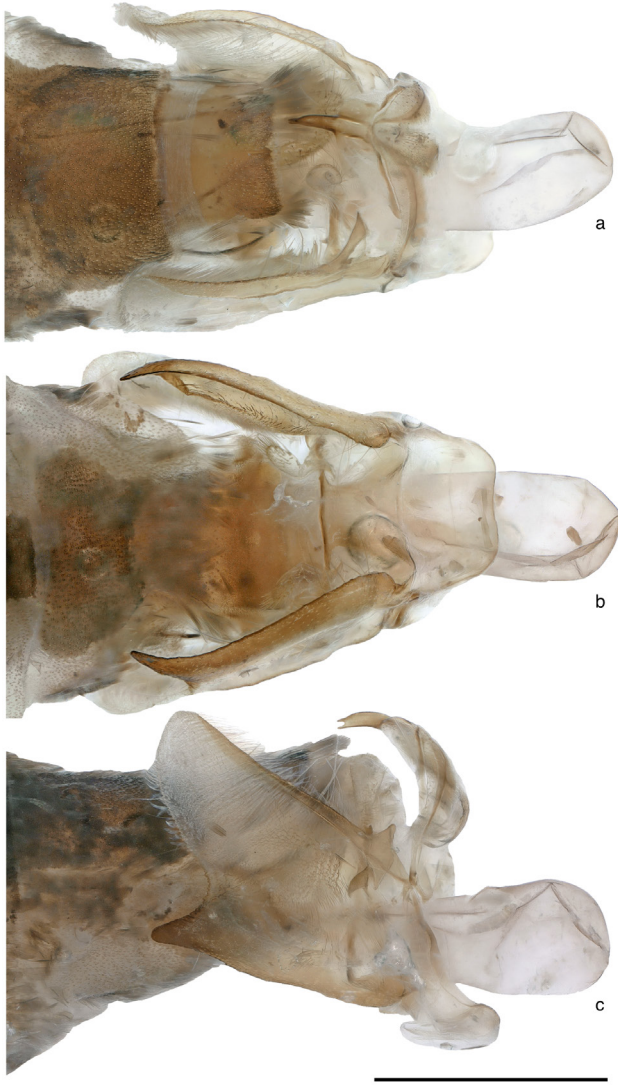


Fig. 10. Macerated male and female genitalia structures of *Eupithecia abbreviata* in copula, with the female genitalia still in the abdomen. Photos taken in water using the Fixator (WANKE et al. 2019). Abbreviations: a = dorsal view; b = ventral view; c = lateral view. Scale bar: 1 mm.

cornutus was pointed towards the marginal spines of the corpus bursae (Fig. 11a). In contrast to *Scopula immorata*, the cornuti in *E. abbreviata* reach all the way to the inner surface of the corpus bursae (Fig. 11). In Noctuidae, the cornuti serve different functions, such as that of dragging the collum of the spermatophore into the ductus bursae or orienting the spermatophore within the corpus bursae (CALLAHAN 1958; CALLAHAN & CHAPIN 1960). Furthermore, it has been noted that cornuti sometimes break off and remain within the fertilized females; these are named deciduous cornuti, as opposed to non-deciduous cornuti (CORDERO 2010; RAJAEI et al. 2021). It is hypothesized, e.g.,

that the function of deciduous cornuti is to help the female break open the spermatophore or to gain an advantage in sperm competition (CORDERO 2010).

In conclusion, the internal genitalia of the male and female genitalia in *E. abbreviata* show anatomical correspondences, the most notable of which are the medium-sized cornutus and the long cornutus of the male vesica, which match the membranous elongations in the corpus bursae of the female. This may be in agreement with a lock-and-key hypothesis, which suggests that only the right size and shape of genitalia structures allow successful copulation, sperm transfer and fertilization (EBERHARD 1985; SHAPIRO & PORTER 1989; MUTANEN & KAITALA 2006). However, this hypothesis should be further investigated in *E. abbreviata*, as the cornuti fit within the soft, flexible corpus bursae and variation in these soft parts as well as in the length of the cornuti cannot be excluded. In addi-

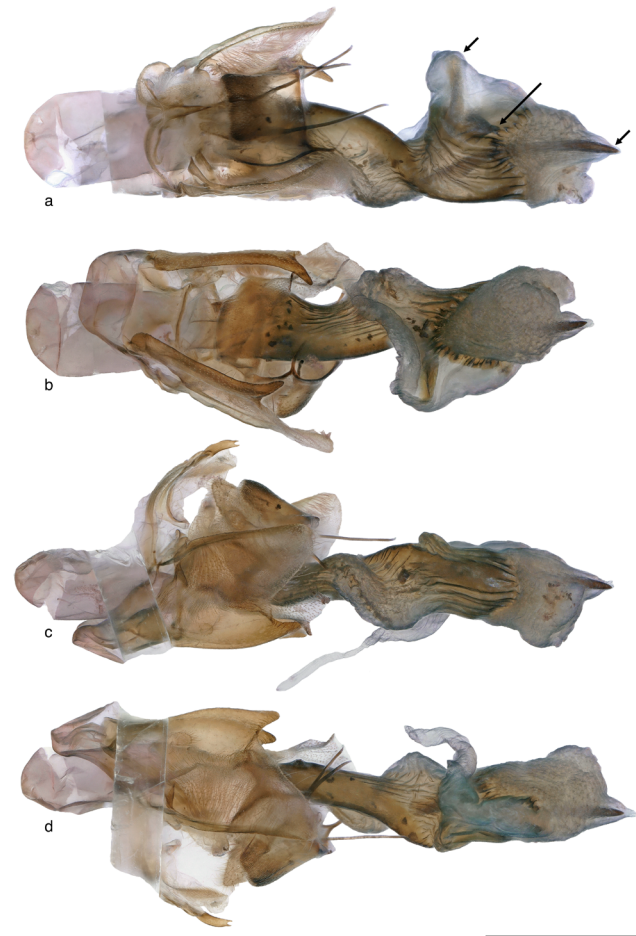


Fig. 11. Macerated male and female genitalia structures of *Eupithecia abbreviata* in copula. Photos taken in 70% ethanol using the Fixator (WANKE et al. 2019). Abbreviations: a = dorsal view (arrows indicate the three cornuti); b = ventral view; c = lateral view; d = lateral view, opposite site. Scale bar: 1 mm.

tion, it would be interesting to investigate the interaction of the genitalia in species closely related to *E. abbreviata* to support this hypothesis.

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