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FIRST SEM MICROGRAPHS OF REPRESENTATIVES OF *PACHYLEUCTRA* DESPAX, 1929 AND *TYRRHENOLEUCTRA* CONSIGLIO, 1957 (PLECOPTERA: LEUCTRIDAE)

Bill P. Stark[1] and Charles H. Nelson[2]

[1] Department of Biology, Box 4045, Mississippi College, Clinton, Mississippi, 39058, U.S.A.
E-mail: stark@mc.edu

[2] Department of Biology, Geology and Environmental Science, 615 McCallie Avenue, The University of Tennessee-Chattanooga, Chattanooga, Tennessee, 37403, U.S.A.
E-mail: charles-nelson@utc.edu

ABSTRACT

Adult terminalia of two populations of *Pachyleuctra* Despax, 1929 and three populations of *Tyrrhenoleuctra* Consiglio, 1957 were examined with scanning electron microscopy in order to provide details of various anatomical features exhibited by species in these genera.

Keywords: Plecoptera, Leuctridae, *Pachyleuctra*, *Tyrrhenoleuctra,* scanning electron microscopy

INTRODUCTION

From a phylogenetic perspective, the small stonefly genera *Pachyleuctra* Despax, 1929 and *Tyrrhenoleuctra* Consiglio, 1957 merit interest. The proposed phylogeny of the subfamily Leuctrinae (Nelson & Hanson 1973, Shepard & Baumann 1995), hypothesizes that *Tyrrhenoleuctra* is a basal lineage and the sister group of the clade comprising the remaining included genera of the subfamily. *Pachyleuctra*, however, is a member of an apical clade and the sister group of the species-rich genus *Leuctra* Stephens, 1835. This genus is a Pyrenean group with three recognized species after the studies of Aubert (1952) and Despax (1951). *Tyrrhenoleuctra,* a stonefly genus known from the Iberian Peninsula, North Africa and several Mediterranean islands, was proposed by Consiglio (1957) as a replacement name for *Strobliella* Klapálek, 1903, after the latter generic name was discovered to be a junior homonym of *Strobliella* Kieffer, 1898. This genus currently includes five species (DeWalt et al. 2019); two of these are morphologically indistinguishable and were recently recognized on the basis of molecular data (Fochetti & Tierno de Figueroa 2009, Tierno de Figueroa & Fochetti 2014).

Beginning in the 1970's the scanning electron microscope (SEM), by providing high resolution and increased magnification while retaining great depth of field, has been a useful tool in morphological studies requiring fine structural detail of the phenotype (Wipfler et al. 2016). This present study is based on a SEM analysis of a small sample of *Pachyleuctra* and *Tyrrhenoleuctra* specimens and seeks to provide baseline information on specific surface structures of these insects and to augment previous morphology based taxonomic studies as revealed by line drawings using the light microscope.

MATERIALS AND METHODS

Pachyleuctra and *Tyrrhenoleuctra* specimens were provided by Dr. Romolo Fochetti, Universitá degli Studi della Tuscia, Viterbo, Italy (UST), Dr. M.A. Puig Collection, Girona, Spain (MAPC), and Dr. Gilles –Vinçon Collection, Grenoble, France (VIN). To optimize SEM examination of body anatomical structures, wings were removed and the torsos placed in an ultrasonic cleaner and vibrated for 15 seconds before they were dehydrated in solutions of 90%, 95% and 100% ethanol for 10 minutes in each solution. Following this treatment, the specimens were placed in hexamethyldisilizane for 1 hour, mounted on aluminum stubs with double stick copper tape, coated with a gold/palladium alloy in a Hummer coater and examined with an Amray 1810d scanning electron microscope.

RESULTS

Pachyleuctra Despax, 1929

(Figs. 1–8)

Leuctra (*Pachyleuctra*) Despax, 1929: 298, type species *Leuctra* (*Pachyleuctra*) *montana* Despax = *Pachyleuctra benllochi* (Navás, 1917)

Current species and type localities:

P. benllochi (Navás, 1917), San Juan del Erm, Lérida, Spain
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:4308

P. bertrandi Aubert, 1952, Etang de Puig, Pyrenees Mountains, France
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:4307

P. ribauti Despax, 1929, Saint-Béat, Haute-Garonne, France
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:4305

Material examined: ***P. benllochi*: FRANCE:** Ger Tributary, Pyrenees Mountains, Mente Pass, 1300m, 27 May 1993, G. Vinçon, 5♂ 4♀ (VIN). **SPAIN:** Lleida, Pyrenees Mountains, Escrita River basin spring, 1809m, 2 July 2008, M.A. Puig, 1♂ (MAPC).

Adult habitus. Slightly brachypterous to macropterous in available specimens. General color of body and wings dark brown to black without distinctive contrasting pigment.

Male genitalia (n = 3). Epiproct strongly reduced (Vinçon & Ravizza 2001). A moderately slender pair of long, closely appressed paraprocts (specilla) form a recurved probe that extends from below the cerci to above the apex of the abdomen (Fig. 3); apex of paraprocts recurved over abdomen, tips flattened and bearing a small circular orifice near the inner subapical margins (Figs. 2–4). Apical half of paraprocts bearing long slender setae along caudal surface and lateral surfaces of the apex (Figs. 3–4). Abdominal terga 5, 7–9 bear a median pair of spine-like lobes on the anterior segmental margins. Cerci somewhat conical in shape and bearing a small apical nipple-like process (Figs. 1–2). Sternum 9 bearing a circular, densely hairy vesicle (Figs. 5–6).

Female genitalia (n = 2). Subgenital plate on sternum 8 consists primarily of a pair of ear-like lobes that extend from the basoposterior margin of sternum 8 across most of sternum 9 (Fig. 7). Lobes and much of sternum 8 clothed with long, slender setae.

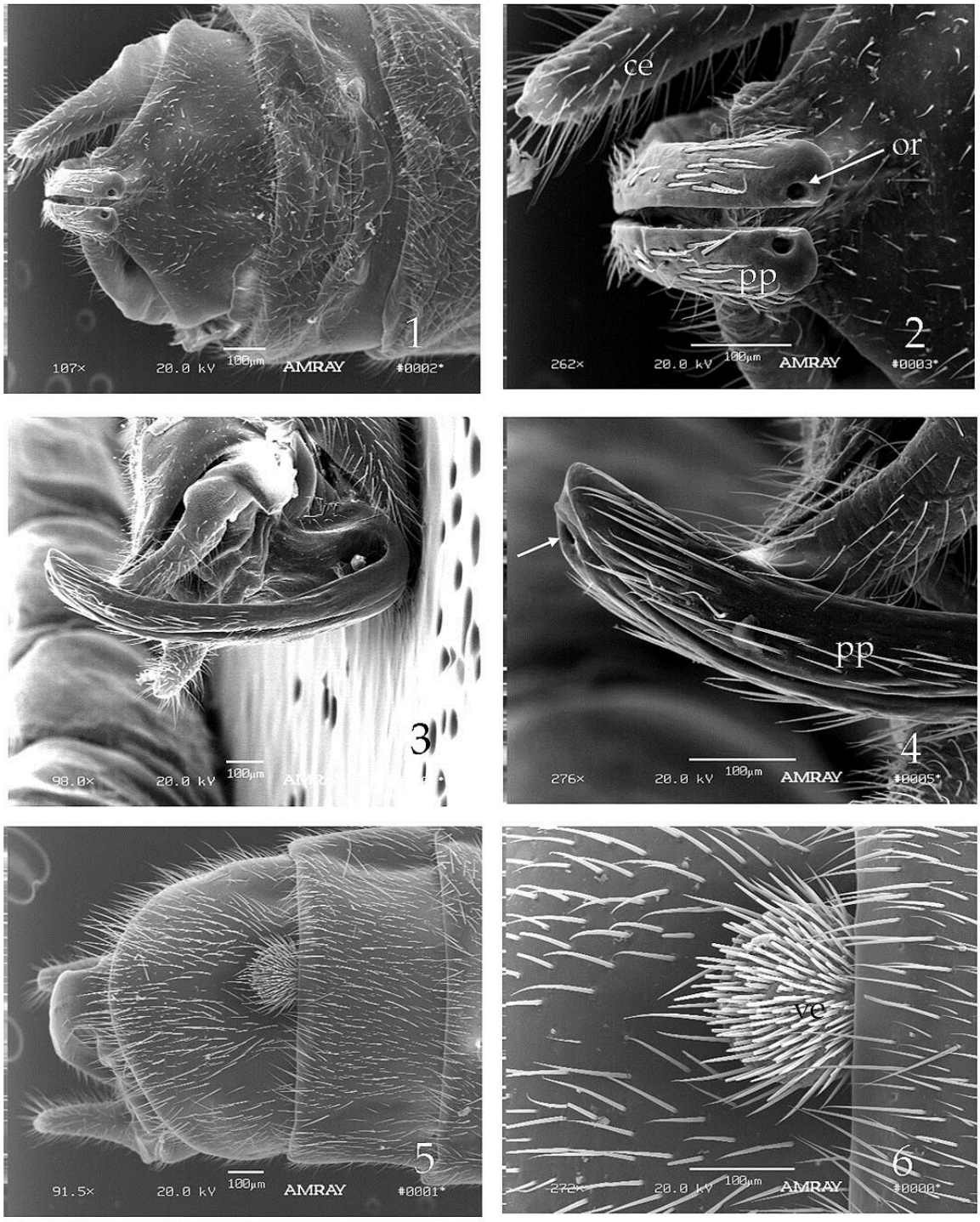
Mesosternum (n = 1). Mesosternal furcasternum circular in outline (Fig. 8); mesosternal basisternum bearing a narrow, dark, hairless median line that extends across at least half of the basisternum surface (Fig. 8). Mesosternal spinasternum relatively long, slender and distinctly separated from mesosternal postfurcasterna. Metasternal prefurcasterna united (Fig. 8).

Tyrrhenoleuctra Consiglio, 1957

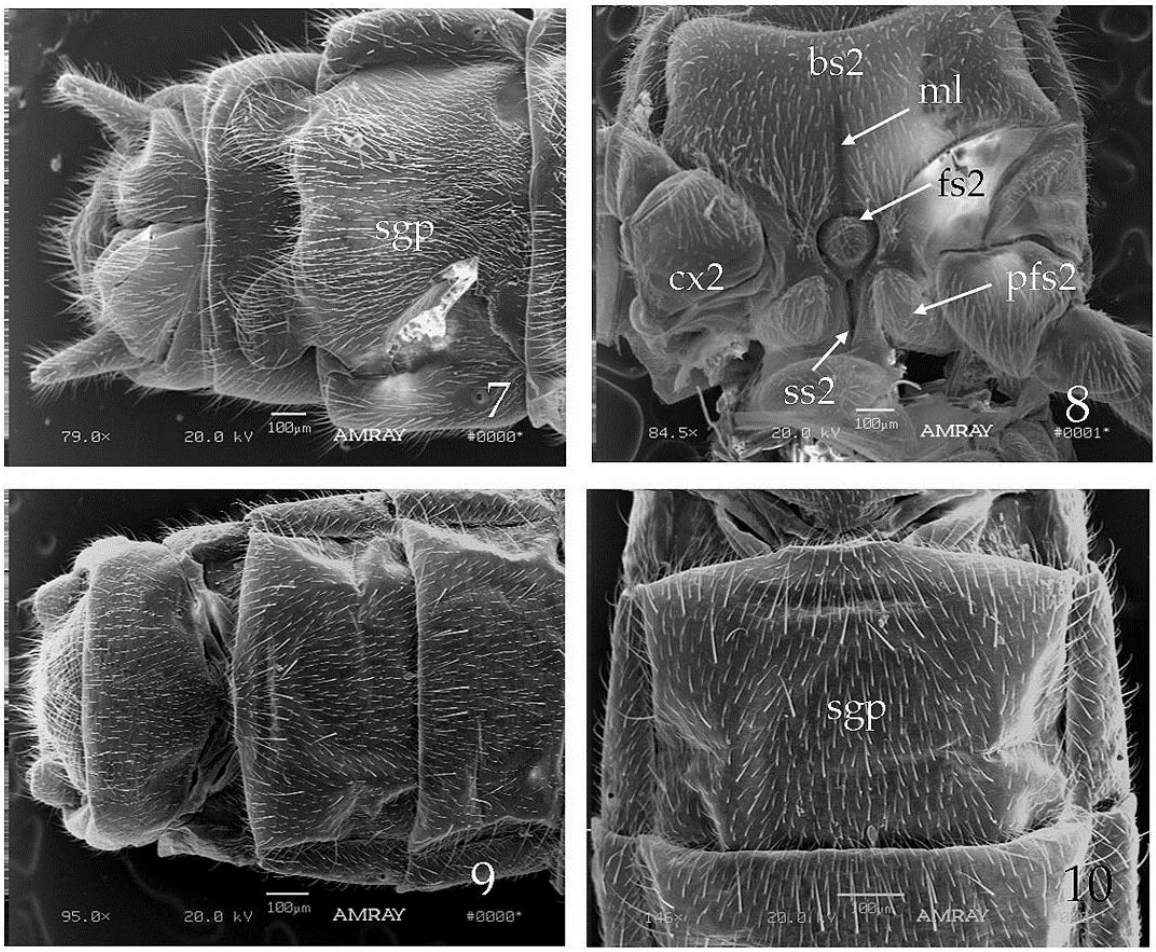
(Figs. 9-–22)

Strobliella Klapálek, 1903: 13, Preoccupied (Kieffer, 1898)

Tyrrhenoleuctra Consiglio, 1957: 1, novum nomen, type species, *Strobliella minuta* Klapálek = *Tyrrhenoleuctra minuta* (Klapálek)



Figs. 1–6. SEM micrographs of *Pachyleuctra benllochi* male structures (Escrita River basin, Pyrenees Mountains, Spain). 1. Terminalia, dorsal. 2. Details of paraprocts and cerci, dorsal. 3. Terminalia, caudolateral. 4. Paraproct apex caudolateral (arrow indicates orifice). 5. Terminalia, ventral. 6. Details of vesicle. (ce = cerci; or = orifice; pp = paraproct; ve = vesicle)



Figs. 7–10. SEM micrographs of *Pachyleuctra benllochi* (Escrita River basin, Pyrenees Mountains, Spain) and *Tyrrhenoleuctra zavattarii* (Rio Pirastzu, Sardinia, Italy) adult structures. 7. *P. benllochi* female terminalia, ventral. 8. *P. benllochi*, adult mesosternum, ventral. 9. *T. zavattarii*, female terminalia, ventral. 10. *T. zavattarii*, female subgenital plate. (bs2 = mesobasisternum; cx2 = mesocoxa; fs2 = mesofurcasternum; ml = median line; pfs2 = mesopostfurcasternum; sgp = subgenital plate; ss2 = mesospinasternum)

Current species and type localities:

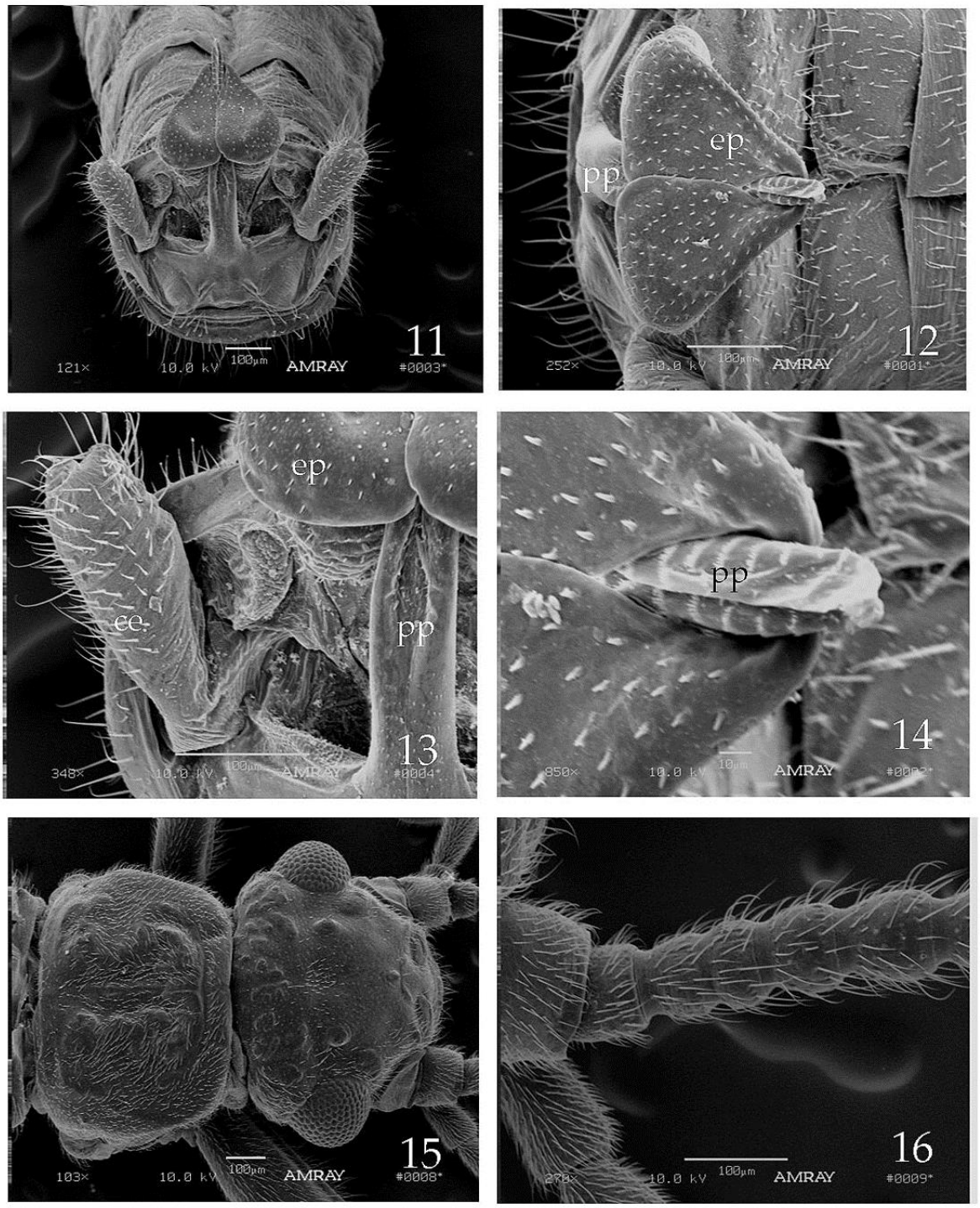
T. antoninoi Fochetti & Tierno de Figueroa, 2009, Mallorca Island, Spain
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:4121

T. lusohispanica Tierno de Figueroa & Fochetti, 2014, Algarve, Portugal
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:464237

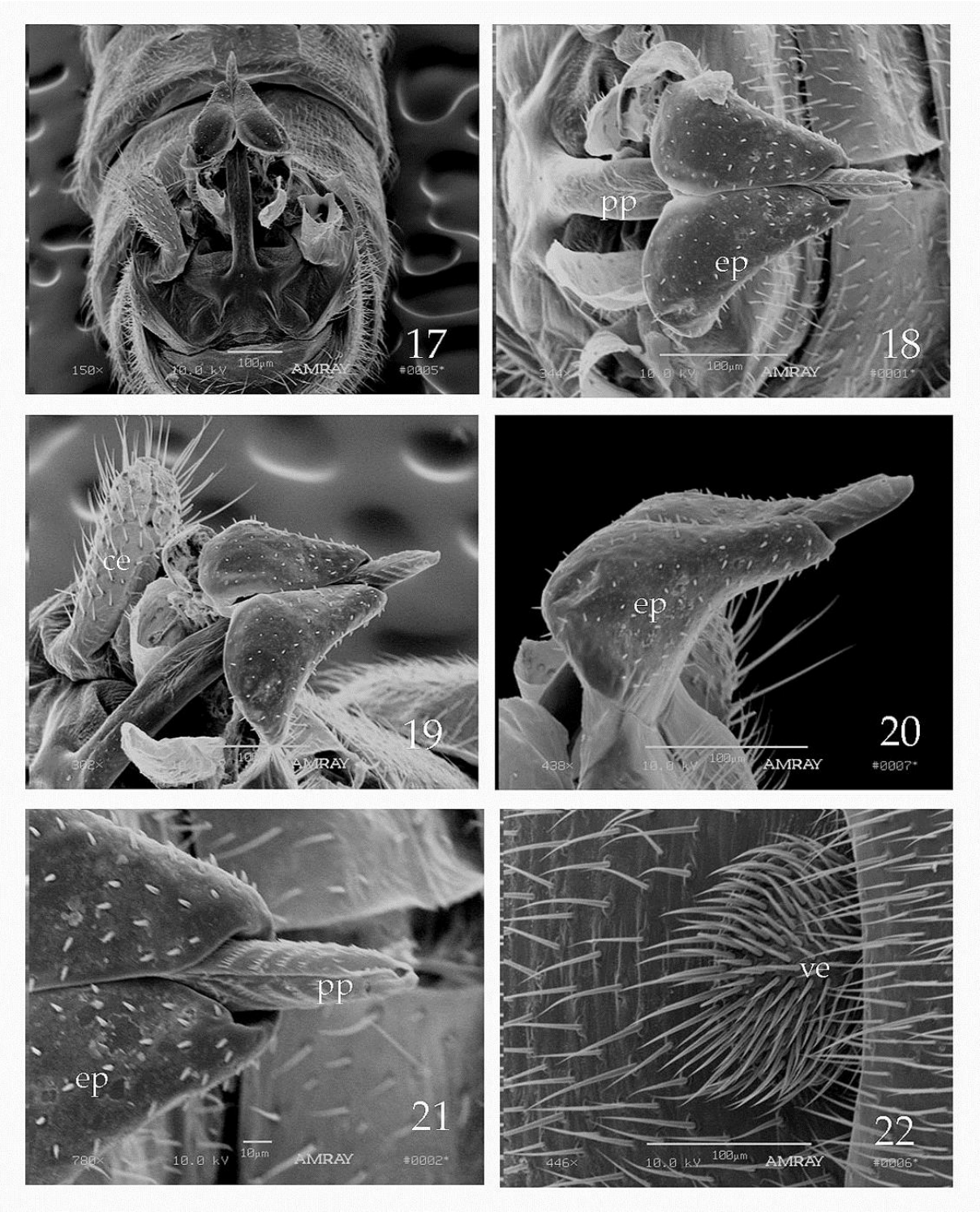
T. minuta (Klapálek, 1903), Sierra Morena, Spain
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:4127

T. tangerina (Navás, 1922), Fondak, Morocco
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:4125

T. zavattarii (Consiglio, 1956), Monte Limbara, Sardinia, Italy
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:4123



Figs. 11–16. SEM micrographs of *Tyrrhenoleuctra zavattarii* (Rio Pirastzu, Sardinia, Italy) and *Tyrrhenoleuctra* sp. (Rio Andila, Estrema Dura, Spain). 11. *T. zavattarii*, male terminalia, dorsal. 12. *T. zavattarii*, details male epiproct, dorsal. 13. *T. zavattarii*, male cerci and base of paraprocts, caudodorsal. 14. *T. zavattarii*, apex of epiproct and paraprocts, dorsal. 15. *T.* sp., head and pronotum, dorsal. 16. *T.* sp. basal antennal segments and palpus. (ce = cercus; ep = epiproct; pp = paraprocts)



Figs. 17–22. SEM micrographs of *Tyrrhenoleuctra* sp. (Rio Andila, Estrema Dura, Spain) male structures. 17. Male terminalia, caudodorsal. 18. Male epiproct and paraprocts dorsal. 19. Male epiproct and paraprocts, dorsolateral. 20. Epiproct, lateral. 21. Epiproct and paraprocts, dorsoapical. 22. Vesicle, ventral. (ce = cercus; ep = epiproct; pp = paraprocts; ve = vesicle)

Material examined: ***T. c.f. minuta*: SPAIN:** Sevilla, Arroyo del Moro, Sierra Morena, 38° 02′ 23″ N, 5° 56′ 10″ W, 347m, 18 February 2015, J.M. Luzón-Ortega, 2♂ 2♀ 1 larva, specimens damaged (UST). ***T. zavattarii*: ITALY:** Sardinia, Sardegus, Rio Pirastzu, Strada, S. Teresa- Costelsordo Ponte, 100m, 6 February 2000, 10♂ 3♀ (UST). ***Tyrrhenoleuctra* sp.: SPAIN:** Estrema Dura, Calera De Leon, Rio Andila, 650m, 24 February 2001, 5♂ 2♀ (UST).

Adult habitus. Brachypterous or macropterous among male specimens examined (3–6 mm), forewings usually reaching midlength of abdomen; females fully winged, forewing length (7.5–8.0 mm). General color of wings and body pale brown, without distinctive contrasting pigment pattern; head with numerous (~30) occipital rugosities (Fig. 15), at least among specimens of *Tyrrhenoleuctra* sp. listed above. Pronotum with a small number of rugosities (~10). Antennae and palpi hairy (Fig. 16).

Male genitalia (n = 6). Epiproct pronounced; in dorsal or caudal view divided by a median groove (Figs. 11, 12 & 17, 18). *Tyrrhenoleuctra zavattarii* epiproct dorsally or caudally appears triangular (Fig. 11); in profile posteriorly hemispherically-shaped, abruptly becoming flattened to apex. *Tyrrhenoleuctra* sp. epiproct dorsally or caudally appears caret-shaped (Fig. 17); in profile posteriorly hemispherically-shaped sloping gradually to apex (Fig. 20). Epiproct surface of both species covered by uniformly distributed short peg-like sensilla bearing approximately 80–160 sensilla per epiproct (Figs. 12 & 18). Epiproct sensilla of *T. zavattarii* in dorsal view (Fig. 12) appear slightly more closely packed and of a more uniform length than those comprising the sparse patch of *Tyrrhenoleuctra* sp. (Fig. 18). In both *T. zavattarii* and *Tyrrhenoleuctra* sp. a single pair of long slender paraprocts (specilla) extend from a ventrobasal plate on the abdominal apex (Fig. 11); paraprocts are closely fused and extend through the median groove of the epiproct as a spear-like structure (Figs. 11–12 & 17–19). Apex of paraprocts emerge from subapical notch in epiproct and the emergent section of the paraprocts bear 5–6 partial, circular annuli formed from densely packed single rows of short setae (Figs. 12, 14 & 18–21). Paraproct tips of *T. zavattarii* broadly rounded (Figs. 12, 14), those of *Tyrrhenoleuctra* sp. bluntly pointed (Figs. 20, 21). Cerci oriented vertically to the body axis, and shaped somewhat cylindrically with truncate apices (Figs. 11, 13 & 17, 19). Vesicle small, circular and covered with long hairs throughout (Fig. 22).

Female genitalia (n = 2). Abdominal sternum 7 larger than other segments, overlapping and covering much of sternum 8; sternum 8 with extensive membranous areas on the posteromedian region of the segment (Fig. 9). Median section of posterior margin of segment 7 truncate for a short section of the segment (Fig. 10).

DISCUSSION

Based on the postulated apical systematic position of *Pachyleuctra* in Leuctrinae phylogeny several features of the genitalia examined in this study are likely apomorphies: male epiproct reduced; male paraprocts separated but closely appressed; and female subgenital plate with lobes extending from the basoposterior margin. Recently, as part of a study on Pyrenees Leuctridae, Vinçon & Ravizza (2001) reviewed the species belonging to this genus and observed that *P. benllochi* closely resembles *P. bertrandi.* Owing to this close similarity these researchers depicted the lateral view of the male terminalia of both species with one illustration (their fig. 14e, p. 113). Added fine details of the male and female genitalia of *P. benllochi* are provided by SEM micrographs of this study, but specimens of *P. bertrandi* were not available for examination. Vinçon & Ravizza (2001), however, determined that these two species can be differentiated by the shape of the tergal process found on male abdominal segments 5, 7–9.

In distinguishing between the three traditional species of *Tyrrhenoleuctra*, Aubert (1963) considered several characters: body size, wing length, leg color, male epiproct length, male cercal length, color of female sternites 8 and 9 and

shape of the female subgenital plate posterior border. Puig et al. (1990), however, described morphological variability in *T. minuta* adult male and female wing length and the female tenth sternite. Fochetti & Tierno de Figueroa (2009) and Tierno de Figueroa & Fochetti (2014) concluded that "…morphological characters cannot be confidently and consistently used for identification of species of *Tyrrhenoleuctra"*. Additionally, they suggested that new species descriptions based entirely on morphology are not reliable because of variation. Instead they relied on differences in the mitochondrial genes cytochrome oxidase subunit-1 (COI) and 12s ribosomal gene to recognize two additional cryptic species belonging to *Tyrrhenoleuctra*. Nonetheless, Fochetti et al. (2009) did observe that *T. zavattari* is both genetically as well as morphologically homogenous. SEM images in this study indicate that some ultrastructural and structural features of the epiproct and paraprocts of this species while similar to those of *Tyrrhenoleuctra* sp. are distinct. Various researchers [e.g. Wiens (2004), Okiwelu & Noutcha (2014), Wanninger (2015)], have suggested that morphology is important and that an integrated or combined approach incorporating both morphological and molecular data is necessary in systematic studies.

In summary, this initial SEM analysis of populations of *Pachyleuctra* and *Tyrrhenoleuctra* has identified a few morphological features, especially those involving male and female genitalia that might potentially be useful in subsequent taxonomic investigations. This result, however, is based on limited material and the systematic utility of these characters will need to be corroborated by additional SEM studies of the species of both genera over their known geographic range.

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