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Research paper

Taxonomy and systematics of three species of the genus *Eumerus* Meigen, 1822 (Diptera: Syrphidae) new to southeastern Europe

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ABSTRACT

The genus *Eumerus* Meigen (Diptera: Syrphidae) is considered one of the most species-rich hoverfly genera. Here, we present two new species, *E. montanum* Grković, Radenković et Vujić sp. nov. (Montenegro, Greece) and *E. rubrum* Grković et Vujić sp. nov. (Greece), and one species, *E. uncipes* Rondani, 1850, recorded for the first time in southeastern Europe. The species are members of three different taxon groups, respectively *E. strigatus* sensu Speight et al. (2013), *E. tricolor* sensu Chroni et al. (2017) and *E. clavatus* as defined here. Diagnostic characters for each of the three taxon groups and descriptions of the two new species are provided. In addition, we employed morphological and molecular data for available taxa of the *E. strigatus* taxon group in order to corroborate their taxonomical status and systematic position. Finally, we discuss the diversity of these taxon groups (*E. clavatus*, *E. strigatus* and *E. tricolor*) and give a detailed overview of the differences between closely-related species.

1. Introduction

During the last glaciations, southeastern Europe, including all countries south of Alpine region and Pannonian plain, harboured a tremendous number of animal species, serving as a center of biodiversity and endemism (Džukić and Kalezić, 2004; Gaston and David, 1994; Hewitt, 1999, 2000, 2011; Poulakakis et al., 2014; Schmitt, 2007). Various projects have explored the biodiversity, ecology and biogeography of southern parts of Europe, revealing the conservation and evolutionary statuses of different species; amphibians and reptiles (Džukić and Kalezić, 2004, Marzahn et al., 2016), beetles (Drees et al., 2016), flies (Vujić et al., 2016; Ståhls et al., 2016), molluscs (Psonis et al., 2015), spiders (Sagonas et al., 2014) to name a few. One very species-diverse target group of such studies is the family of hoverflies (Speight, 2014). Thompson et al. (2010) stated that hoverflies consisted of 6100 species worldwide whereas, five years previously, only 6000 species were known (Thompson, 2005). Studies on hoverfly taxonomy and systematics have increased tremendously over recent years; an assessment of Web of Science records (keywords: hoverflies/syrphidae and taxonomy or systematics) on 07 November 2016 revealed that the number of papers produced during the last decade is almost equal to the number produced from 1970 to 2005. Besides the vast amount of papers the number of newly described species is even higher during the last decade compared with the 35 years period mentioned before. This is partly a consequence of new methods (e.g. DNA barcoding) and approaches (e.g. integrative taxonomy) that have assisted and facilitated species diagnoses and delimitations where species identifications were previously complicated (Chroni et al., 2017; Jordaens et al., 2015; Nedeljković et al., 2015; Ståhls et al., 2008; Suk and Han, 2013; Young et al., 2016).

The genus *Eumerus* Meigen, 1822 is an example of high hoverfly species diversity (Speight, 2014) and endemism (Grković et al., 2015; Ricarte et al., 2012) in southeastern Europe. To date, the European fauna of *Eumerus* comprises more than 50 described species (Speight, 2014; van Steenis, pers. comm.), of which 31 listed by Speight (2016) occur in southeastern Europe (as well as an additional three species that are not on that list, Grković et al., 2015). Notwithstanding the considerable species diversity of *Eumerus*, the genus faces taxonomic challenges posed by the lack of an up-to-date European identification key. In addition, there are still uncertainties about nomenclature (e.g. synonyms) and unclarified taxonomic statuses to be resolved before a clear view of the genus' species diversity can be perceived.

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Eumerus species exhibit considerable morphological variability; they are generally medium-sized flies, with a distinctive habitus showing strong metalegs and a narrow or broadly oval abdomen (Doczkal et al., 2016). Doczkal and Pape (2009) studied the adult morphology of the tribe Eumerini (*Eumerus* Meigen, 1822, *Azpeytia* Walker, 1865, *Lyneborgimyia* Doczkal and Pape, 2009, *Merodon* Meigen, 1803, *Platynochaetus* Weidemann, 1830) and provided strong support for Eumerini monophyly, but not for the genus *Eumerus*. Doczkal and Pape (2009) concluded that *Eumerus* is paraphyletic, but did not assign any taxon groups (hereafter called as 'group') except for the *Eumerus maculipennis* group. Following this, Speight et al. (2013) presented the "*E. strigatus* group" of morphologically similar species. Doczkal (1996) had previously mentioned the *E. tuberculatus* group, with some species that were subsequently covered by the *E. strigatus* group sensu Speight et al. (2013). Recently, DNA sequences were used to infer species delimitation within *Eumerus* (Chroni et al., 2017), and that analysis suggested the presence of two major clades and seven groups within the genus based on tree inference approaches. One clade, named as *Eumerus tricolor* group, consisted of species with red parts on the tergites to an entirely red abdomen: *E. armatus* Ricarte and Rotherey, 2012, *E. grandis* Meigen, 1822, *E. sinuatus* Loew, 1855, *E. tricolor* (Fabricius, 1798), as well as *E. niveitibia* Becker, 1921 (a species with a blue appearance), and *E. aurofinis* Grković, Vujić & Radenković, 2015 (a species endemic to eastern Mediterranean islands) (Grković et al., 2015). The remaining species formed the second clade, in which six groups were assigned: *E. basalis* Loew, 1848, *E. minotaurus* Claussen & Lucas, 1988, *E. ornatus* Meigen, 1822, *E. pulchellus* Loew, 1848, *E. strigatus* (Fallen, 1817) and *E. sulcitibius* Rondani, 1868 (Chroni et al., 2017).

In the current study, we present two new species (*E. montanum* sp. nov. and *E. rubrum* sp. nov.) and one new record (*E. uncipes* Rondani, 1850) for southeastern Europe. These species are members of three different groups: *E. strigatus* sensu Speight et al., 2013, *E. tricolor* sensu Chroni et al., 2017 and a *E. clavatus* group defined here (for more details on *Eumerus* groupings, see Chroni et al., 2017; Doczkal and Pape, 2009; Speight et al., 2013). Our objectives are to: (a) provide descriptions for the new species; (b) revise the geographic distribution of *E. uncipes*; and (c) discuss and overview the assignments of the aforementioned species to groups based on morphological and molecular data for the available taxa.

2. Material and methods

2.1. Taxon sampling

The insect material that we considered in this study was collected over the past few decades from Southeastern Europe, including the following countries: Bulgaria, Croatia, Greece, Romania, Serbia, Slovenia and FYR Macedonia. Insects were collected by researchers from the Laboratory for Biodiversity Research and Conservation of the University of Novi Sad, using hand nets. Specimens belonging to new species (*E. montanum* sp. nov. and *E. rubrum* sp. nov.) were collected from continental parts of the Balkan Peninsula (on Mt. Pindos in Greece and Mt. Durmitor in Montenegro) and in the Peloponnese (Mt. Chelmos and Mt. Taygetos, Greece), respectively. Specimens of *E. uncipes* were collected in Greece (Corfu Island). Several specimens of each assessed taxon were studied, and these have mainly been deposited in the collections of the Department of Biology and Ecology of the University of Novi Sad. We also considered the female holotype of *E. tauricus* Stackelberg, 1952, from Crimea, coll. 10.V.1905, leg. Kirichenko.

For molecular analyses, we examined DNA barcodes (mitochondrial cytochrome c oxidase subunit I, COI/cox1) for *E. montanum* sp. nov. and *E. uncipes* with 13 additional *Eumerus* species. We were unable to obtain DNA barcodes for *E. rubrum* sp. nov. The sequenced material originated mainly from Greece and Turkey, as well as from Germany, Italy, Montenegro, Russia and Serbia (for more details see Table S1). The sequence used for *E. funeralis* Meigen, 1822 (Accession No. GMGMJ702-14) was retrieved from GenBank. Outgroup taxa consisted of *Megatrigon tabanoides* Doczkal et al. (2016) (Accession No. KX083393), *Merodon erivanicus* Paramonov, 1925 (Accession No. KX083391) and *Platynochaetus setosus* (Fabricius), 1794 (Accession No. KM224512).

All specimens studied are deposited in the collections of the following institutions: FSUNS – Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia; NBC – Natural Biodiversity Centre, Leiden, The Netherlands and MAegean – The Melissotheque of the Aegean, University of the Aegean, Mytilene, Greece.

2.2. Morphological characters

The morphological characters used in the descriptions and drawings are based on the terminology established by Thompson (1999), and those related to the male genitalia by Hurkmans (1993) and Doczkal (1996). Colour characters are described from dry-mounted specimens. Male genitalia were extracted from specimens using standard methods for studying male hoverfly genitalia, as explained in detail in Grković et al., 2015. Drawings were created by using photographs of characters taken with a Leica DFC 320 (Wetzlar, Germany) camera attached to a Leica MZ16 binocular stereomicroscope and then processed in Adobe Photoshop CS3 V 10.0 software (Adobe Systems, San Jose, CA, USA).

2.3. DNA extraction and PCR amplification

Total genomic DNA was extracted using 2 to 3 legs from each specimen and following the Chen et al. (2010) protocol for DNA extractions, with slight modifications (Grković et al., 2015).

DNA barcodes were generated by amplifying the mitochondrial COI gene fragment, using the primer pair LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994). PCR amplifications and DNA sequencing were performed as described in Grković et al. (2015).

2.4. Molecular analyses

We used BioEdit 7.2.5 (Hall, 1999) to edit sequences by eye and to trim to their final length of 612 bp. Multiple sequence alignments were implemented in MAFFT version 7 by employing the L-INS-i algorithm (Katoh et al., 2005; available at http://mafft.cbrc.jp/alignment/server/index.html). We implemented K2P genetic distance analyses in MEGA6 (Tamura et al., 2013) to corroborate species delimitation for E. montanum sp. nov. (15 species, outgroups were excluded from the analyses; Table S2).

In order to resolve the phylogenetic positions of *E. montanum* sp. nov. and *E. uncipes*, we produced a dataset of 41 *Eumerus* DNA sequences plus three outgroup sequences (the 'total' dataset). All trees were rooted based on the *P. setosus* sequence. We employed Neighbor-Joining (NJ), Maximum likelihood (ML) and Maximum parsimony (MP) phylogenetic methods. The NJ analysis was performed in MEGA6 (Tamura et al., 2013) with the Tamura-Nei nucleotide substitution model with a Gamma distribution, which was the second-best nucleotide substitution model (the first one was GTR + G + I) proposed by the Akaike Information Criterion (AIC 5601.91). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are presented in units of number of base substitutions per site. All positions containing gaps and missing data were eliminated, resulting

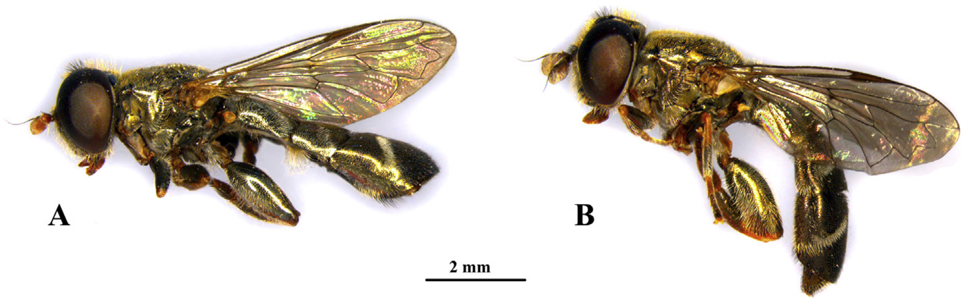

Fig. 1. *E. uncipes*: a) male; b) female.

in a total of 601 positions in the final dataset. The ML analysis was executed in RAxML 8.0.9 (Stamatakis, 2006; Stamatakis et al., 2008) in the Cipres Science Gateway (Miller et al., 2010) under the general time-reversible (GTR) evolutionary model with a gamma distribution (GTR + G) (Rodriguez et al., 1990) with 1000 bootstrap replicates. The MP analysis was performed in NONA (Goloboff, 1999), spawned in WINCLADA version 1.00.08 (Nixon, 2002). A heuristics search algorithm with 1000 random addition replicates (mult x 1000) was performed, holding 100 trees per round (hold / 100), max trees set to 100 000 and applying TBR branch swapping.

The generated NJ and ML trees were merged into a split network in order to extract a united tree topology. The split network was produced in SplitsTree4 4.14.3 (Huson and Bryant, 2006) (http://www.splitstree.org/) under SuperTree, Z-closure super-network from partial trees and heuristic analysis (number of runs: 1000).

Additional MP analyses were performed in order to further evaluate species systematic positions (tree topology) within the *E. strigatus* group with reference to species morphology. We included seven species from within that group (for which DNA sequences were available): *E. amoenus* Loew, 1848, *E. consimilis* Šimić and Vujić (1996), *E. montanum* sp. nov., *E. funeralis*, *E. pannonicus* Ricarte, Vujić & Radenković, 2016, *E. sogdianus* Stackelberg, 1952 and *E. strigatus*, and 21 additional *Eumerus* specimens (plus the three outgroup sequences). We performed MP analyses (with run settings as described above) separately and in combination for the morphological and DNA sequence datasets. The morphological matrix scored 24 male characters, related to size, head, thorax and genitalia (Table S3), which was created in Mesquite v2.73 (Maddison and Maddison, 2011).

3. Results

3.1. The E. clavatus group

Diagnosis. Medium-sized species, dark-coloured, with parallel-sided elongated abdomen having lunulate shaped oblique maculae of silver-to-white pollinosity on tergites II–IV (Fig. 4c, d). Distinctive processus on distal margin of abdominal sternite III in males (Fig. 4a, b). Male abdominal sternite IV with characteristic pliers-like shape. Antenna in females oval with more or less noticeable groove medially (Fig. 2d).

Male. *Head.* Eyes holoptic, eye contiguity 8–10 omattidia long. Eye with very scattered and short pile. Eye margins slightly broadening ventrally. Face black, covered with white pollinosity and long white or yellow pile. Frons and postocular orbit black with long yellow pile, except ocellar triangle with black pile. Ocelli forming an equilateral triangle, longer than wide (Fig. 2a). Antenna yellowish-brown. Basoflagellomere slightly longer than broad (Fig. 2c), with straight anterior margin and deep central incision (sensory pit). Long ventral pile of pedicel yellow to white.

Thorax. Thorax black with golden lustre. Mesoscutum covered with short golden-yellow pile, reclined posteriorly, medially with two vittae of silvery pollinosity. Scutellum black with short yellow pile, pile slightly longer along posterior margin. Pleurae black with metallic golden lustre and covered with golden pollinosity. Anepisternum, posterior katepisternum and anepimeron covered with long yellow pile. Wing transparent, entirely microtrichose. Calypter and halter white to yellow. Metafemur swollen, black with metallic lustre. Long white pile on hind femur ventrally, as long as three-quarters the depth of the femur (Fig. 2e). Metatibia dark-coloured, yellow in proximal part.

Abdomen. Abdomen elongated, black with short white pile, tergites II–IV with pairs of white to silver pollinose lunulate shaped oblique maculae (Fig. 4c, d). Tergite IV long, almost twice the length of tergite III. Sternite III with medial prominence, covered with long whitish pile, and a characteristic processus ventro-medial of the posterior margin (Fig. 4a, b). Sternite IV with a specific pliers-like shape.

Genitalia. Cerci oval, flattened laterally, with a row of strong setae dorsally (Fig. 3a, b). Interior accessory lobe of posterior surstylar lobe densely covered in pilosity. Posterior surstylar lobe simple, tapered, with short spines laterally and on apicoventral ridge. Hypandrium broad, curved, and simple (Fig. 3c, d). Aedeagal apodeme with dorsal processus (Fig. 3e, f).

Female. Similar to the male, except for usual sexual dimorphism (Figs. 1b, 2b, d, f) and the following characteristics: face and frons black and covered with yellow to golden pile, white and golden pollinosity on the face; frons flattened; silver pollinosity along eye margin anterior to ocellar triangle and at the eye corner posterior to ocellar triangle (Fig. 2b).

3.1.1. Species of the E. clavatus group in southeastern Europe

The following two species belonging to the *E. clavatus* group have been recorded on the Balkan Peninsula and on eastern Mediterranean islands: *E. clavatus* Becker, 1923 (Bradescu, 1991; Vujić and Šimić, 1999) and *E. uncipes* recorded during research presented here.

Taxonomic notes about *Eumerus uncipes* Rondani, 1850 (Fig. 1)

Remark. This is the first record of *E. uncipes* in southeastern Europe. To clarify the relationship between the related species *E. uncipes* and *E. clavatus*, we present here diagnoses for *E. uncipes* and molecular data for both species.

Material examined. 2♂♂, 1♀, Greece [Corfu], Strinilas [39°44’20.4“N 19°50’13.2“E], [642 m.a.s.l.] coll. 08.VIII.2014, 1♂, 2♀♀ coll. 10.VIII.2014,; 1♀, Stroggili [39°30’43.7“N 19°55’00.2“E], [153 m.a.s.l.], 10.VIII.2014. leg. Vujić A. (FSUNS).

Diagnosis. This species is closely related to *Eumerus clavatus*, from which it clearly differs in the shape of the processus on abdominal sternite III (Fig. 4a, b); quadratic in *E. uncipes* and as a triangular thorn in *E. clavatus*. In addition, *E. uncipes* has a pointed triangular extension apico-ventrally on the metatibia (Fig. 2g), which is absent in *E. clavatus*. Male genitalia of both species are very similar (Fig. 3). The posterior surstyle lobes are slightly differently shaped (Fig. 3a,

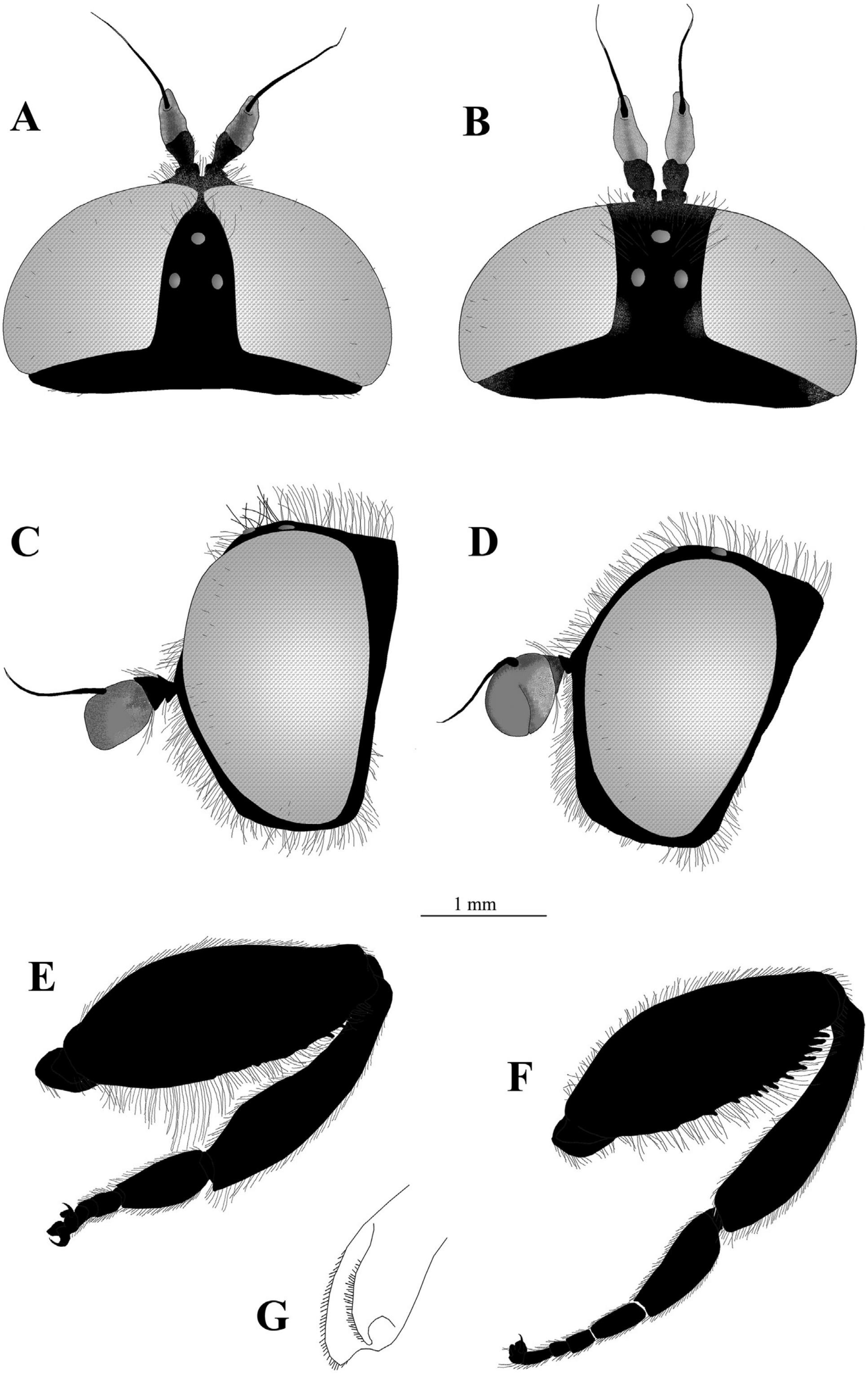

Fig. 2. *E. uncipes*, head: Dorsal view: (A) male; (B) female. Lateral view: (C) male; (D) female. Leg: (E) male; (F) female; (G) Apicoventral part of male metatibia with conspicuous notch.

b). Dorsal spines on the posterior surstyle lobe of *E. clavatus* are longer than in *E. uncipes*. A row of strong bristles is present dorsally on the cercus of *E. uncipes* (Fig. 3a); in *E. clavatus*, the pile on the cercus is not so distinctive (Fig. 3b). Aedeagal apodeme in *E. clavatus* has a spine-like process dorsally (Fig. 3f), whereas in *E. uncipes* the same process is keel-shaped (Fig. 3e).

Females are very similar, but in *E. uncipes* antennae are more reddish-brown than in *E. clavatus* (which are more yellowish-brown) and basoflagellomere with a noticeable arced groove medially.

In both sexes, the abdomen of *E. clavatus* is more robust and broad with noticeable silvery-white pollinose lunulate oblique

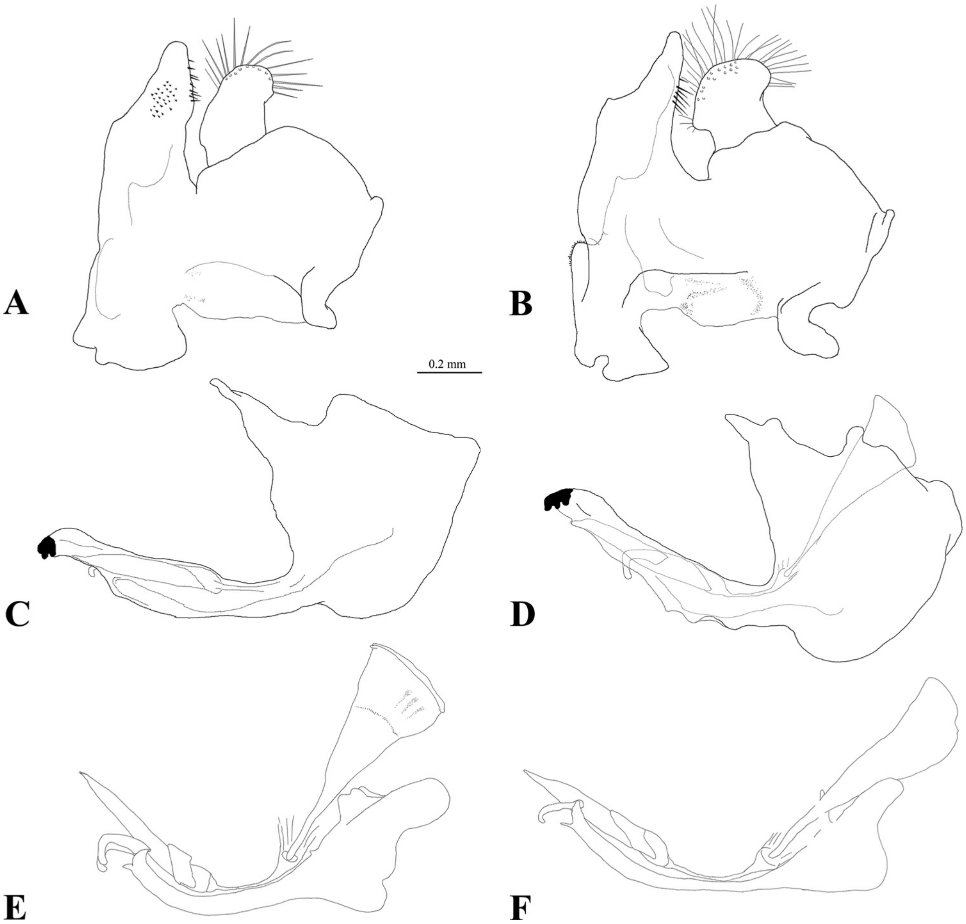

Fig. 3. Male genitalia. Epandrium: (A) *E. uncipes*; (B) *E. clavatus*. Hypandrium: (C) *E. uncipes*; (D) *E. clavatus*. Aedeagus and accessory structures: (E) *E. uncipes*; (F) *E. clavatus*.

maculae, which contrasts with *E. uncipes* that has a narrower abdomen with grey maculae on its tergites (Fig. 4c, d).

3.2. The E. strigatus group

Diagnosis. The *E. strigatus* group was named by Speight et al. (2013) in assigning a group of species related to *E. strigatus*. Chroni et al. (2017) presented the same group in their studies based on molecular data. The *E. strigatus* group comprises relatively small, inconspicuous species with usually a bronze shine and without coloured markings on their tergites; basoflagellomere usually rectangular and from reddish to dark-brown or black coloured; sternites simple, without distinct apomorphic structures (as in the *E. clavatus* group – Fig. 4); legs connections, apex of femora and basal thirds of tibiae bright yellow; abdominal sternite IV in males differently shaped but always with a v-shaped notch on the posterior margin (Fig. 8); cerci elongated (Fig. 7a, d–h). The main diagnostic character is the shape of the male genitalia: epandrium in all examined species has an elongated, posterior surstyle lobe with species-specific shape (Fig. 7a, d–h).

Male. *Head*. Eyes holoptic, eye contiguity 7–9 ommatidia long. Eye with 2–4 ommatidia-long scattered pile. Distance from anterior ocellus to posterior ocellus approximately equal to the distance from posterior ocellus to eye corner. Distance between posterior ocellus and eye margin is equal to the diameter of ocellus or less. Face with white to yellow pile and silver to grey pollinosity (in some species, ranging to golden). Vertex black with metallic shine (in some species with pollinose maculae from posterior ocellus to upper eye corner along eye margin and sometimes in front of anterior ocellus). Basoflagellomere slightly longer than wide, oval to rectangular with ventral margin pointed, reddish to dark brown coloured. Depth of pedicel is about four-fifths the depth of the basoflagellomere medially. Longest pile on pedicel as long as the depth of pedicel.

Thorax. Black, weakly punctuated, with bronze to golden shine. Pile of thorax from white to yellow, ranging to golden, slightly longer on scutellum than pile of mesonotum. Mesonotum with two vittae of silvery-white pollinosity, which extend almost its entire length or are clearly visible only on the anterior part of mesonotum. Pleurae black with bluish or bronze to golden metallic shine and pale pile, longer than pile on scutum. Metafemur enlarged, 1.5 to 2 times wider than metatibia, black with yellow apex. Metatibia black, yellow to yellowish-brown in basal quarter to third. Metatrochanter simple, metafemur with anterolateral row of 6–7 spines and posterolateral row of 10–12 spines. Pilosity on legs from white to yellow, ranging to golden.

Abdomen. Tergites entirely shiny black with bluish or bronze tinge, weakly punctuated. Pairs of white to grey lunules of pollinosity on tergites II–IV, usually more grey-coloured in the middle of the tergite and white to silver laterally. Posterior margin of sternite IV with a sharp to shallow v-shaped notch and sometimes with two spatulate or sharp projections laterally (Fig. 8).

Genitalia. Hypandrium simple, with apically-situated ctenidion (Fig. 7b). Cerci simple, more or less curved posteriorly (Fig. 7a, d–h). Posterior surstyle lobe more or less elongated and always spatulate in some way (Fig. 7a, d–f, h), with the exception of species related to *E. bactrianus* Stackelberg, 1952 that has a specifically-shaped surstylus (bifurcated apically, Fig. 7g). Anterior lobe of surstylus is well-developed (Figs. 7a, d–h, 11i).

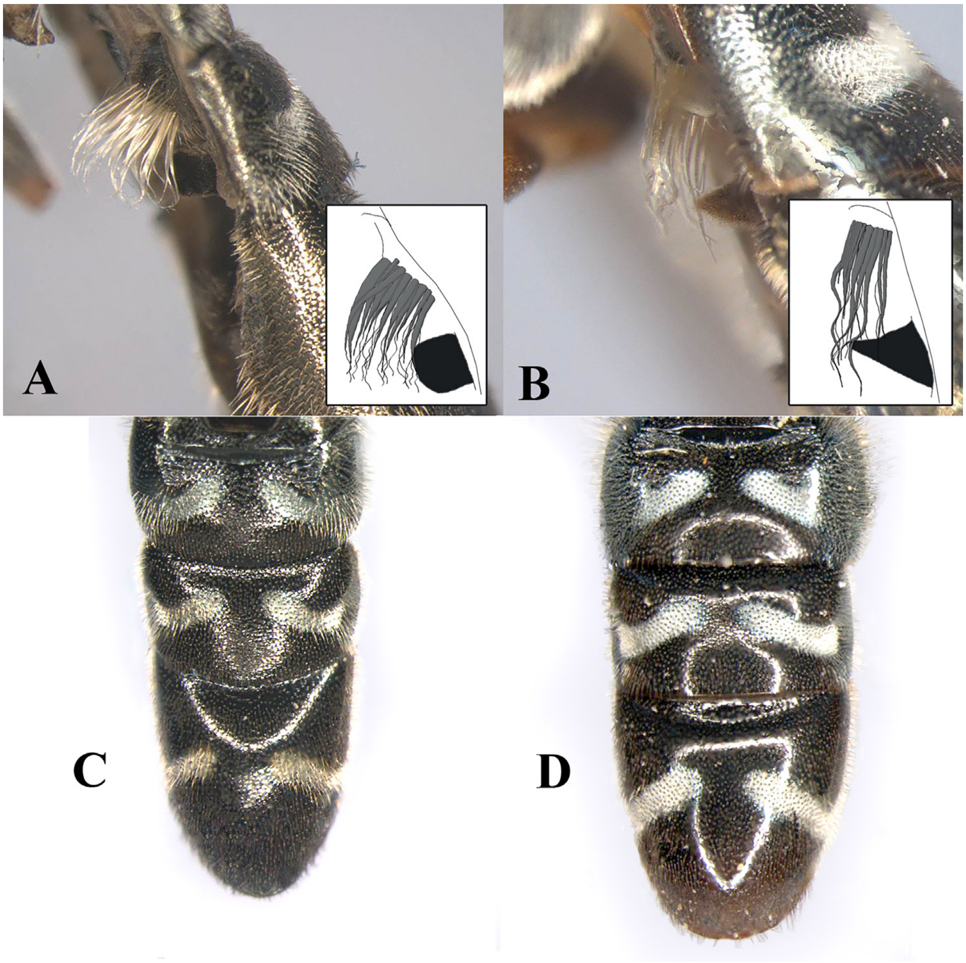

Fig. 4. Third abdominal sternite in male: (A) *E. uncipes*; (B) *E. clavatus*. Male tergites: (C) *E. uncipes*; (D) *E. clavatus*.

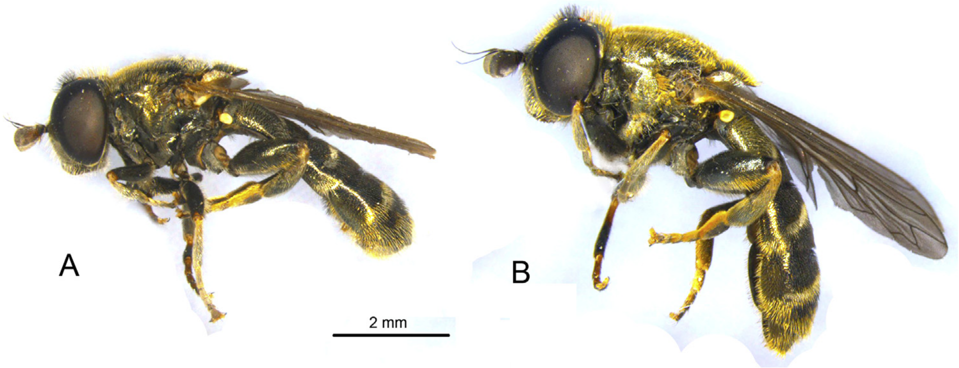

Fig. 5. *E. montanum* sp. nov.: (A) male; (B) female.

Female. *Head*. Pile on face and frons white-yellow to golden, black in region of ocellar triangle, this contrast in pile colour is more distinctive than in males (Fig. 5b). Face covered with silver-grey to bronze pollinosity (Fig. 6d). Frons with dense vitta of bronze-green pollinosity from face anteriorly to anterior ocellus, along eye margin. Some species have a triangular macula of pollinosity adjacent to the dorsal eye corner and posterior to the posterior ocellus, usually with pollinosity along the dorsal eye margin.

Thorax. Black with metallic bronze to golden shine. Two vittae of grey pollinosity on mesoscutum, extending from half to almost the entire length of the mesoscutum. Scutellum black with bronze to golden reflection. Metafemur black with yellow apex. Metatibia black, yellow in basal quarter to a third. Pile of legs yellow to golden.

Abdomen. Tergites with two or three pairs of white lunulate shaped oblique maculae of pollinosity. Tergite IV posteriorly with slightly longer white to yellow pilosity.

3.2.1. *Species from the E. strigatus group in southeastern Europe*

The species belonging to the *E. strigatus* group that have been recorded from the Balkan Peninsula and eastern Mediterranean islands are: *E. amoenus*, *E. consimilis* (Figs. 7d, 8c), *E. funeralis* (Figs. 6h, 7h, 8d), *E. montanum* sp. nov. (species described below; Figs. 5, 6a–g, 7a–c, 8a), *E. strigatus* (Figs. 7f, 8b), *E. sogdianus* (Figs. 7e, 8e,

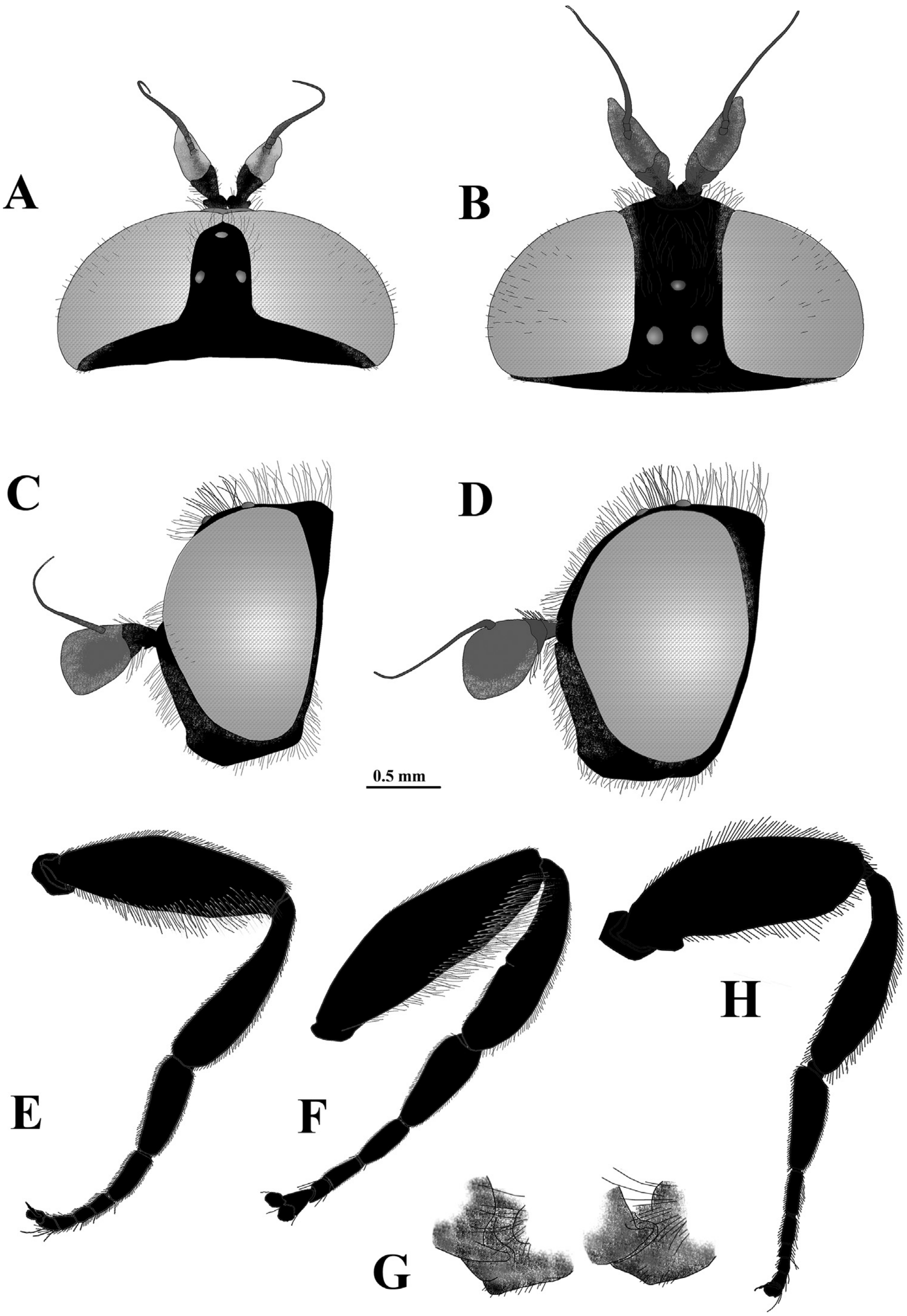

Fig. 6. *E. montanum* sp. nov., head. Dorsal view: (A) male; (B) female. Lateral view: (C) male; (D) female. Leg: (E) male; (F) female; (G) Metatrochanter, lateral view, in this order: male, female; (H) Leg of *E. funeralis*.

11i) and *E. pannonicus* (Fig. 7g) (Bradescu, 1991; Šimić and Vujić, 1996; Vujić and Šimić, 1999; Ricarte et al., 2012: Markov et al., 2016).

3.2.2. Description of Eumerus montanum Grković, Radenković & Vujić sp. nov

Material examined. **Holotype**. 1♂, Montenegro [Durmitor Mountain], Komarnica, beside Pošćensko Lake [42°58’39.2“N 19°04’15.9“E], [1045 m.a.s.l.], coll. 16.VIII.2015, leg. Vujić A. (FSUNS). **Paratypes.** 1♂, Greece [Epirus], Pindos, Smolikas Mountain, Paleoselli [1365 m.a.s.l.], coll. 04.VIII.1994, leg. Renema W. (NBC); 4♂♂, 2♀♀, Montenegro [Durmitor Mountain]: Komarnica, coll. 23.VII.2014, 1♂, 1♀, Pošćensko Lake, [42°58’39.2“N 19°04’15.9“E], [1045 m.a.s.l.], coll. 16.VIII.2015, leg. Vujić A., 5♂♂, 3♀♀, coll. 1–04.VI.2016, leg. Vujić A. et al., 1♂, coll. 08.VII.2016, leg. Grković A., 21♂♂, 5♀♀, coll. 30.VII.2016, leg. Vujić A., 23♂♂, coll. 31.VII.2016, leg. Vujić A.; Canyon Sušica, Skakala, 1♂, coll. 13.VIII.2015, legs. Vujić A. and Veličković N.; 6♂♂, 3♀♀, coll. 06.VII.2017, legs. Vujić A. et al. (all in FSUNS).

Diagnosis. It differs from other species of the *E. strigatus* group in the shape of sternite IV in the male: posterior margin of sternite IV with v-shaped, shallow notch with two spatulate projections laterally with tufts of golden pile (Fig. 8a). The new species has distinctive golden pilosity on the posterior ventral part of its

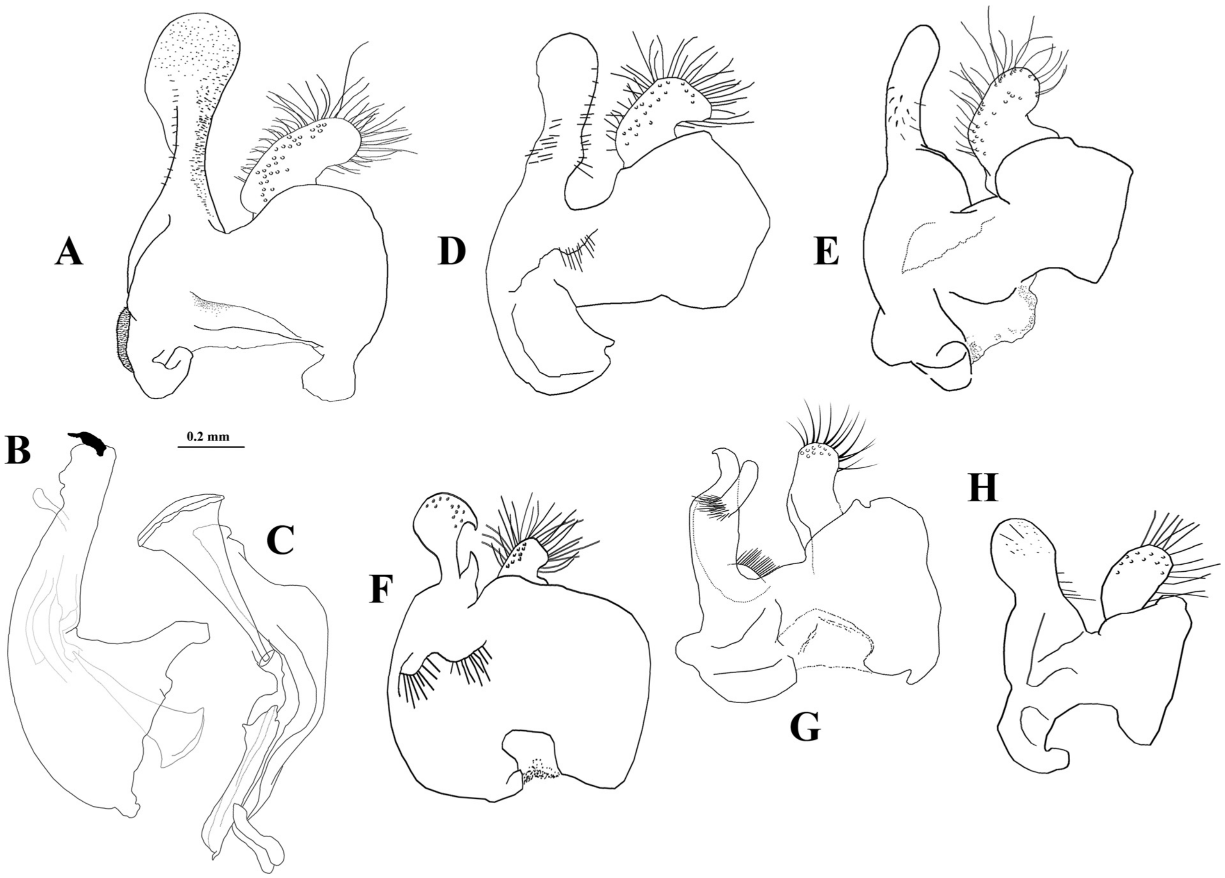

Fig. 7. Male genitalia, *strigatus* group. *Eumerus montanum* sp.n.: (A) epandrium, (B) hypandrium, (C) aedeagus and accessory structures; epandrium: (D) *E. consimilis*, (E) *E. sogdianus*, (F) *E. strigatus*, (G) *E. panonnicus*, (H) *E. funeralis*.

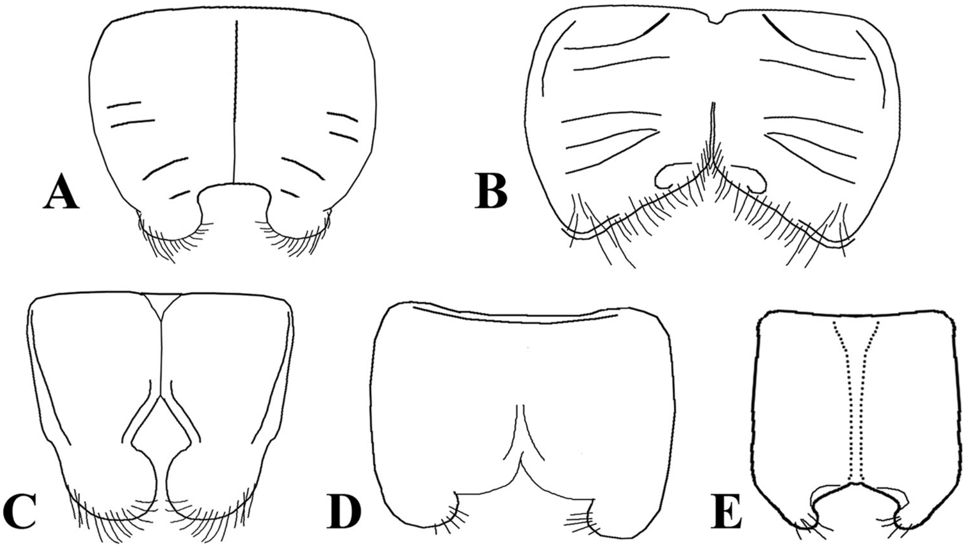

Fig. 8. Males 4th abdominal sternite: (A) *E. montanum* sp. nov., (B) *E. strigatus*, (C) *E. consimilis*, (D) *E. funeralis*, (E) *E. sogdianus*.

metafemur (Figs. 5, 6e, f). Despite these differences, *E. montanum* sp. nov. can easily be confused with similar species, so the most valid identification character is that of the male genitalia; the epandrium has cerci curved backwards and an elongated posterior surstylar lobe (Fig. 7a). Females of *E. montanum* sp. nov. are extremely similar to females of other species of the group, differing by a darker appearance and golden pile particularly noticeable on the posteroventral side of the metafemur and the posterior half of tergite IV. Further, females of this species have similar antenna to the males, i.e., slightly elongated and dark (Fig. 6c–d). More detailed differences between females of this group are given below in the taxonomic notes.

General description.

Male. *Head.* Eyes holoptic, eye contiguity 9–11 omattidia long (Fig. 6a). Face, frons and postocular orbit black with long yellow pile except on ocellar triangle where the pile is black. Face with white pollinosity anterior to antennal socket, golden pollinosity posteriorly. Ocelli making an isosceles triangle, slightly longer than wide (Fig. 6a). Distance from anterior ocellus to posterior ocellus equal to distance from posterior ocellus to eye corner. Face margin slightly protruding (Fig. 6c). Antenna dark; basoflagellomere brown to brownish-red, slightly elongated, covered with white velvet pollinosity. Arista dark-brown, inserted dorsomedially of basoflagellomere, thickened basally. Ventral pile of pedicel yellow on median side, dark on lateral side, and as long as depth of pedicel. Dorsal pile of pedicel short, yellow and dark.

Thorax. Black, weakly punctuated, with golden metallic lustre, entirely covered with yellow pile and with two vittae of white pollinosity, sometimes barely visible. Anepisternum and anepimeron yellow pilose, katepisternum posteriorly with white pile. Scutellum simple, black, with yellow pile. Pleurae with metallic golden shine. Wing with brownish tinge, entirely trichose; halters white. Calypter white to bright yellow. Legs dark, with connections, basal thirds of tibiae and basal tarsi brown. Metatrochanter angular, in distinctive characteristic shape (Fig. 6g), metafemur black with reddish to black tip, covered with long white to yellow pile, longer at inner side, posteriorly with distinctive golden pile (Figs. 5a, 6e). Metatibia black, brown in basal quarter to third. Tarsi with golden pile ventrally.

Abdomen. Black, white pilose with golden lustre. Tergites II–IV with pairs of lunulate shaped oblique maculae of pollinosity. Tergite IV apically with slightly longer grey to golden pile (Fig. 5a). Abdominal sternite IV on posterior margin with two spatulate projections (Fig. 8a). Distal margin of sternite IV with longer erect golden pile laterally.

Genitalia. Cerci oval, elongated backwards (Fig. 7a). Interior accessory lobe of posterior surstyle lobe covered with dense pile. Posterior surstylar lobe very long, expanded like a paddle, with dense pile, particularly in the lateromedial part (Fig. 7a). Hypandrium simple, broad, curved (Fig. 7b). Ctenidion situated apically. Lateral sclerite of aedeagus slightly enlarged (Fig. 7c). Aedeagal apodeme without dorsal processus.

Female. Similar to the male (Figs. 5b, 6b, d, f–g). Antenna dark, basoflagellomere oval, slightly darker apically. Arced groove extends transversally to the apical fourth of basoflagellomere. Face metallic bronze with white pollinosity (Fig. 6d). Frons with dense white to yellow pollinosity along eye margin (Fig. 6b). Ocelli making an isosceles triangle, longer than wide (Fig. 6b). Distance from anterior ocellus to posterior ocellus twice as long as distance from posterior ocellus to upper eye corner. Thorax with golden lustre and golden pile, anepisternum posteriorly with a fringe of longer golden pile. Metatrochanter not rounded, angular. Metafemur ventrally and postero-laterally with golden pile, as in males but more distinctive (Fig. 6f). Tergite IV with bronze shine and golden pile in the posterior one third.

Taxonomic notes. Since females of *E. montanum* sp. nov. are very similar to females of related species, we summarize in Table 1 the differences between females of *E. amoenus*, *E. consimilis*, *E. strigatus*, *E. montanum* sp. nov., *E. sogdianus* and *E. funeralis*. Females of *E. montanum* sp. nov. have a more robust abdomen, a distinctive bronze-golden shine on the posterior third of tergite IV (the anterior margin of which forms a reversed v and has distinctive golden-yellow pile), and narrow pollinose markings on tergites. In *E. montanum* sp. nov., the ocellar triangle is clearly isosceles and pollinosity behind the posterior ocelli is absent or almost invisible in this species (Fig. 6b). What is also characteristic of the species is the rectangular shape of the metatrochanter, but this feature is not always as clearly visible as it is in males (Fig. 6g). Females of *E. amoenus* can be quite easily distinguished from *E. montanum* sp. nov. and the other species by the lunules of tergite IV that are clearly visible but short, tear-shaped, mid-positioned, and about half the length of the lunules on tergite III. Both males and females of *E. amoenus* have a pair of shallow depressions on tergite IV, in an area of white spots, which are more conspicuous than in other species where this feature is present. Also, females of *E. pannonicus* are easy to distinguish by the absence of pollinose markings on T4, the elongated basoflagellomere and the small ocellar triangle. In contrast to *E. montanum* sp. nov., females of *E. sogdianus* have moderately narrow pollinose markings on the tergites, an almost equilateral ocellar triangle, and pollinose patches behind the posterior ocelli that extend behind the eye line. Females of *E. sogdianus* have characteristic antenna, similar to those in males, which are slightly peaked ventrally. Females of *E. strigatus* have a characteristic ocellar triangle that is always equilateral or even wider than long. Pollinosity near and behind the posterior ocelli in this species is usually limited to the eye line and the pollinose markings on the tergites are quite broad.

More problematic is to distinguish females of *E. montanum* sp. nov. and *E. consimilis* by morphological characters. Females of *E. consimilis* have broad lunulate-shaped oblique maculae on tergites II–IV, and the width of the crescent-shaped spots on tergite III are approximately a quarter of the length of the tergite and wider than the distance between two lunules of the same tergite. However, this feature is quite variable, with some females of *E. consimilis* having narrower markings; although, in that case, the distance between two markings of the same tergite is not larger than the width of the markings. In *E. montanum* sp. nov., the maculae on the tergites are more linear, and the width of the spots on tergite III are about 5–6 times shorter than the length of the tergite and they are shorter than the distance between lunules of the same tergite.

Although the morphology is very similar between the species, molecular data clearly defined all analyzed taxa.

Etymology. The name reflects that the species is found in mountain habitat.

3.3. The *E. tricolor* group

Diagnosis. Chroni et al. (2017) defined the *E. tricolor* group based on DNA sequencing and named the group after *E. tricolor*. Species belonging to the group are large-sized, predominantly black and usually with red markings on the tergites, but sometimes with a metallic blue shine. The basoflagellomere is characteristic by: square shaped with more or less marked wrinkles; sometimes it is very small and oval, but in some females oval, extremely enlarged. Eyes dichoptic or only slightly holoptic. The main synapomorphic character connecting all analyzed species is the poorly developed anterior surstyle lobe of the male genitalia (Fig. 11e–h) and the usually large wing-shaped interior accessory lobe of the posterior surstylar lobe which is densely pilose (Fig. 11e–h). The less pronounced differentiation of the male genitalia is characteristic of the group.

Male. *Head*. Eyes dichoptic to holoptic. If holoptic usually slightly dichoptic with a space of 1–4 ommatidia in breadth between eye margins and an eye contiguity of 3–7 ommatidia long. Eyes with long dense pile or almost bare. Ocelli in an equilateral or isosceles triangle. Antenna usually dark brown to black, but yellow in some species. Basoflagellomere more or less square distally with a flattened ellipsoidal area and more or less wrinkled from the base to the top. Face, frons and postocular orbit black, but with a blue shine in some species.

Thorax. Black, sometimes with a golden or metallic blue shine. Legs usually dark-coloured. Metafemur moderately swollen, postero-ventrally with two rows of 7–9 distinctive sharp spines.

Abdomen. Black, tergites II–IV usually red-coloured to differing degrees (Fig. 10), but entirely black or with metallic blue shine in some species.

Genitalia. Posterior surstylar lobe of epandrium simple, oval to square with strong bristles dorsally. Genitalia do not differ much

Table 1
Characters to distinguish adult females of *E. amoenus* Loew, 1848, *E. consimilis* Šimić and Vujić (1996), *E. strigatus* (Fallen, 1817), *E. montanum* sp. n., *E. sogdianus* Stackelberg, 1952 and *E. funeralis* Meigen, 1822.

E. amoenus	*E.consimilis*	*E. strigatus*	*E. montanum sp. nov*	*E. sogdianus*	*E. funeralis*
Tergite 4 sharply tapering to the top	Tergite 4 slightly tapering to the top	Tergite 4 sharply tapering to the top	Tergite 4 slightly tapering to the top	Tergite 4 slightly tapering to the top	Tergite 4 sharply tapering to the top
Tergite 4 black with short white pile	Tergite 4 with bronze shine in posterior half, below lunules, golden-yellow pile slightly longer posteriorly	Tergite 4 with barely visible bronze shine and golden-yellow pile slightly longer in posterior half	Tergite 4 in posterior 1/3 with bronze-gold shine which anterior margin forms reversed v-shape and distinctive golden-yellow pile in that part, other parts with dark pile mixed with yellow	Tergite 4 sharp pointed with bronze shine in posterior half, below lunules, golden-yellow pile slightly longer posteriorly	Tergite 4 black with short white pile
Tergite 5 black with pale pilosity	Tergite 5 bronze with yellow pilosity	Tergite 5 bronze with pale pilosity	Tergite 5 bronze with yellow to gold pilosity	Tergite 5 bronze with pale pilosity	Tergite 5 black with pale pilosity
Abdomen black, sometimes with a blue tinge and with a bronze shine on lateral margins, short white pile	Abdomen black with bronze shine and blurry blue tinge, pale to yellow pile	Abdomen black with bronze shine and blurry blue tinge, pale to yellow pile	Abdomen black with bronze-gold shine, particulary on lateral parts of tergites with pale to yellow ranged to gold pile	Abdomen black with bronze shine and blurry blue tinge, pale to yellow pile	Abdomen black with blurred blue tinge and unobtrusive bronze shine, pale short sparse pile
Scutum with bronze-shine and pale short pilosity	Scutum with gold shine and yellow to gold pilosity	Scutum with gold shine and yellow to gold pilosity	Scutum with gold to bronze shine and dense gold pilosity	Scutum with gold shine and pale to yellow pile	Scutum with bronze shine and pale to yellow pilosity
Pleural pile pale to white	Pleural pile from pale to gold	Pleural pile from pale to gold	Pleural pile from white to very gold	Pleural pile from white to yellow	Pleural pile pale to yellow
Ventral pile of hind femur white, long about 1/4 of depth of the femur	Ventral pile of hind femur yellow, long about 1/3 of depth of the femur	Ventral pile of hind femur yellow, long about 1/3 of depth of the femur	Ventral pile of hind femur yellow to gold, long about half of depth of the femur	Ventral pile of hind femur pale, long about 1/3 of depth of the femur	Ventral pile of hind femur pale, long more then half of depth of the femur
Ocelli making isoscale triangle, longer then wide	Ocelli making almost equilateral triangle	Ocelli making equilateral triangle	Ocelli making isoscale triangle, longer then wide	Ocelli making almost equilateral triangle	Ocelli making equilateral triangle
Space between posterior ocellus and eye margin is about 2 ocelli wide	Space between posterior ocellus and eye margin is about 1 ocellus wide	Space between posterior ocellus and eye margin is about 1 ocellus wide	Space between posterior ocellus and eye margin is about 1 ocellus wide	Space between posterior ocellus and eye margin is about 1 ocellus wide	Space between posterior ocellus and eye margin is about 1 ocellus wide

between species, but they remain the best diagnostic character for species identification (Fig. 11a–h).

Female. Similar to the male, except for normal sexual dimorphism. In some species, basoflagellomere strongly enlarged (for instance in *E. armatus*).

3.3.1. *Species of the E. tricolor group in southeastern Europe*

Eleven species belonging to the *E.tricolor* group have been recorded from the Balkan Peninsula and eastern Mediterranean islands: *E. armatus* (Figs. 10d, 11c, g), *E. aurofinis*, *E grandis*, *E. richteri* Stackelberg, 1960, *E. niveitibia*, *E. sinuatus*, *E. ovatus* Loew, 1848, *E. tarsalis* Loew, 1848 (Figs. 10b, 11b, f), *E. tauricus*, *E. tricolor* (Figs. 10c, 11d, h) and *Eumerus rubrum* sp. nov., described below (Vujić and Šimić, 1999; Ricarte et al., 2012; Grković et al., 2015).

3.3.2. *Description of Eumerus rubrum Grković & Vujić sp. nov*

Material examined. **Holotype**. 1♂, Greece [Peloponnese], Achaia, from Chelmos Mountain above Kalavryta [38°00’30.9“N 22°07’08.3“E], [1700 m.a.s.l.], coll. 16.V.2007; legs. Dils, Faes and Langemark (NBC). **Paratype.** Laconia: 1♀ from Varvara, shelter of Taygetos, [37°02’12.4“N 22°23’00.8“E], [843 m.a.s.l.], 02.VI.1993; leg. Den Hollander G. (NBC).

Diagnosis. Belongs to the *E. tricolor* group. *E. rubrum* sp. nov. is clearly different from all other European species of the *E. tricolor* group by having an almost entirely reddish-yellow abdomen (more extensively red coloured and brighter than in other similar species)(Fig. 10), with a small, yellow basoflagellomere (Fig. 9b), and by the long pile of abdominal sternite IV in males that are longer than half the length of the metabasitarsus and the long yellow ventral pile of the metafemur. Male genitalia are different in all similar species (Fig. 11a–h), except in *E. tauricus*, the genitalia of which are identical to those of a new species (see Barkalov, 1990, as *E. carasukensis* sp. n.). Excluding *E. tauricus*, the genitalia of *E. rubrum* sp. nov. and *E. tarsalis* are the most similar, which suggests that these too are closely related species (Fig. 11a, b, e, f). In contrast to *E. tarsalis* (Fig. 11b), the setae of the posterior lobe of the surstylus in *E. rubrum* sp. nov. are very strong, more dense and almost transparent, and the posterior lobe is entirely covered with alveolae (Fig. 11a). Since the male genitalia of *E. rubrum* sp. nov. and *E. tauricus* are practically identical, the features that distinguish these two species are the following: pile on the ocellar triangle are yellow in *E. rubrum* sp. nov. and black in *E. tauricus*; ventral pile on the metafemur in *E. tauricus* are denser and longer compared to the new species; and, most distinctively, tergite I in *E. rubrum* sp. nov. is broadly yellow at the posterior margin (Figs. 10a, 12d), whereas in *E. tauricus* tergite I is black or only narrowly orange-red in the posterolateral corner. This last feature is also present in females. In addition, females of these two species can be distinguished by the wide ocellar triangle in *E. rubrum* sp. nov. (wider than long and with the anterior angle greater than 90°) (Fig. 9d, 12a), whereas in females of *E. tauricus* the anterior angle of the ocellar triangle is closer to 80° (Fig. 12b), and by the conspicuous transverse suture in *E. rubrum* that envelops the whole metafemur anteriorly (Figs. 9f, 12c).

General description.

Medium-sized species (10–11 mm) with a characteristic reddish-yellow abdomen.

Male. *Head.* Eyes bare, slightly dichoptic, spaced two ommatidia apart, eye contiguity 3–4 ommatidia long, almost bare; ocelli form an isosceles triangle, slightly wider than long (Fig. 9a). Face and frons black with long pale pile. Eye margins broadening ventrally. Scape and pedicel brownish-yellow, bristles of pedicel pale; basoflagellomere small, yellow, oval with rough margin, slightly extended ventrally (Fig. 9b). Arista dark, basally thickened.

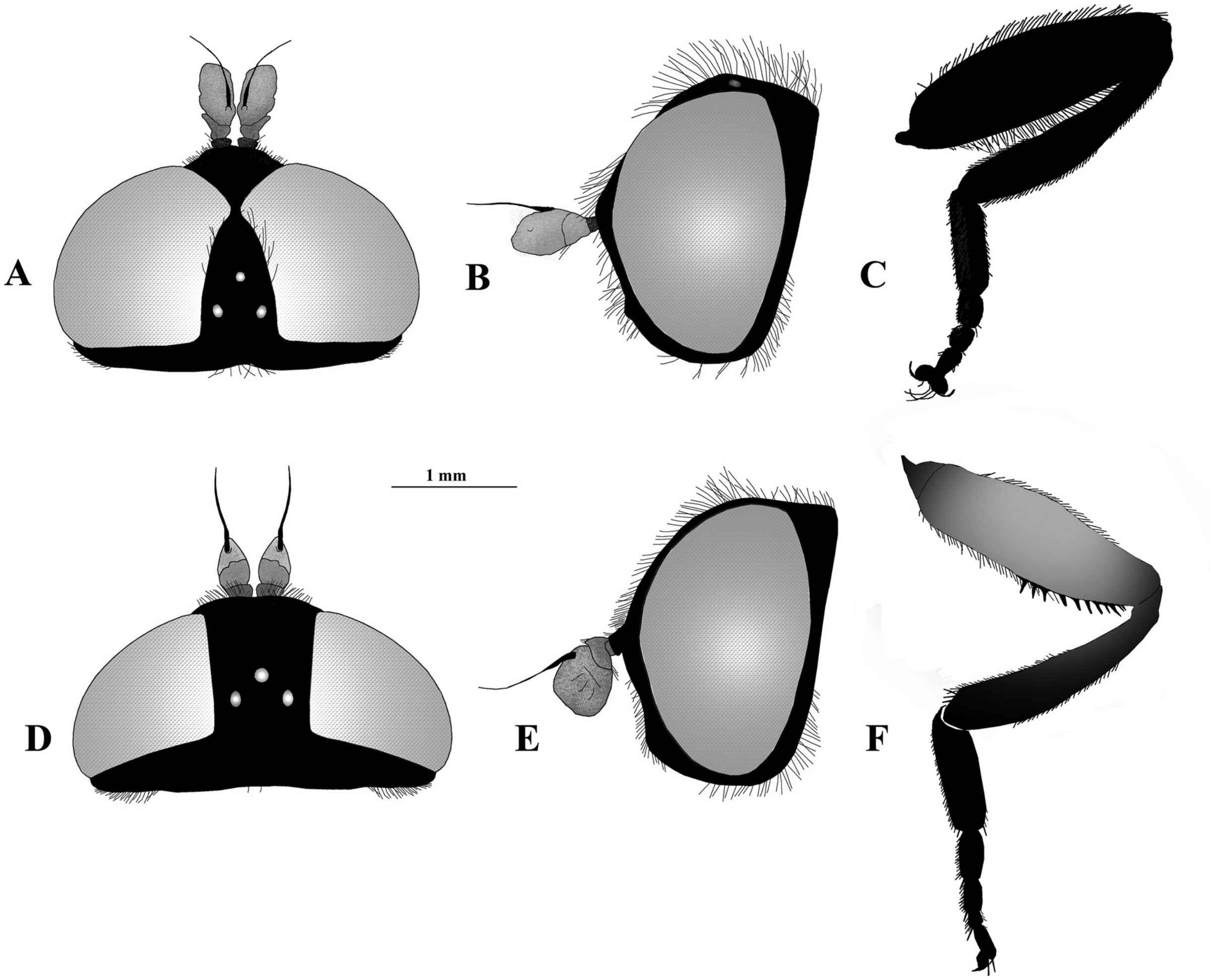

Fig. 9. *E. rubrum* sp. nov., male: head: (A) dorsal view, (B) lateral view. Leg: (C); female: head: (D) dorsal view, (E) lateral view. Leg: (F).

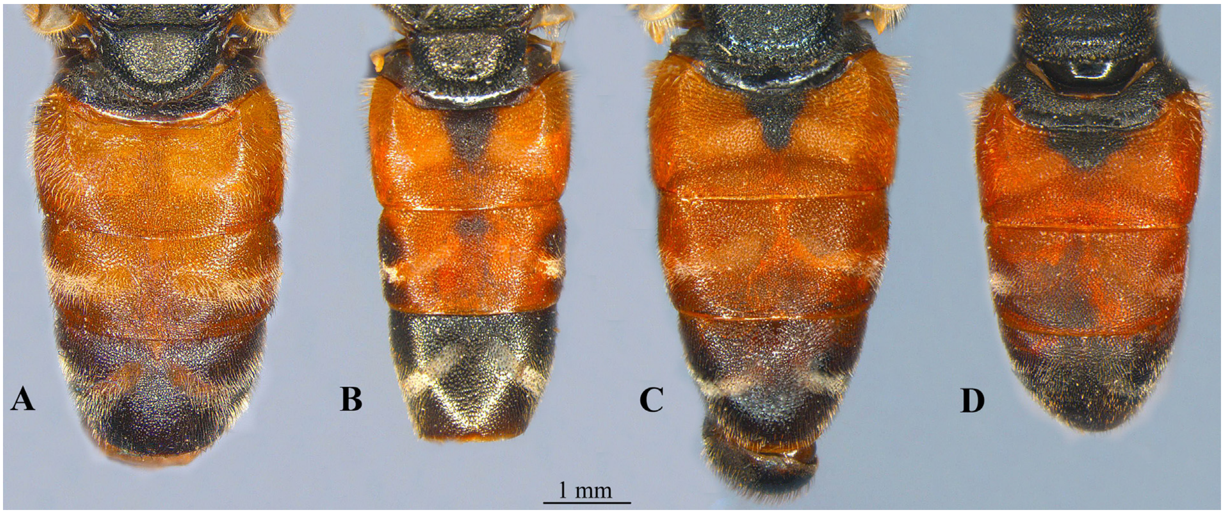

Fig. 10. Male tergites of the *E. tricolor* group: (A) *E. rubrum* sp. nov.; (B) *E. tarsalis*; (C) *E. tricolor*; (D) *E. armatus*.

Thorax. Scutum, scutellum and pleurae entirely black with barely visible golden shine, densely and roughly punctuated. Scutum and scutellum with dense yellowish pile. Pleurae with longer yellowish pile. Legs dark with bases of tibiae and basal tarsi of front and mid legs paler. Metafemur slightly swollen (Fig. 9c), simple with long white to gold pile, ventrally as long as the depth of femur. Antero-laterally with barely visible transverse suture. Metafemur apically with a row of 9–10 sharp black spines on anterior ridge and a row of 7–8 spines on the posterior ridge. Tarsi with golden pile ventrally. A row of dense black spinulae on mesonotum above the wing. Wing entirely microtrichose, transparent with gentle shading apically. Calypter white to gold. Halter whitish, almost transparent.

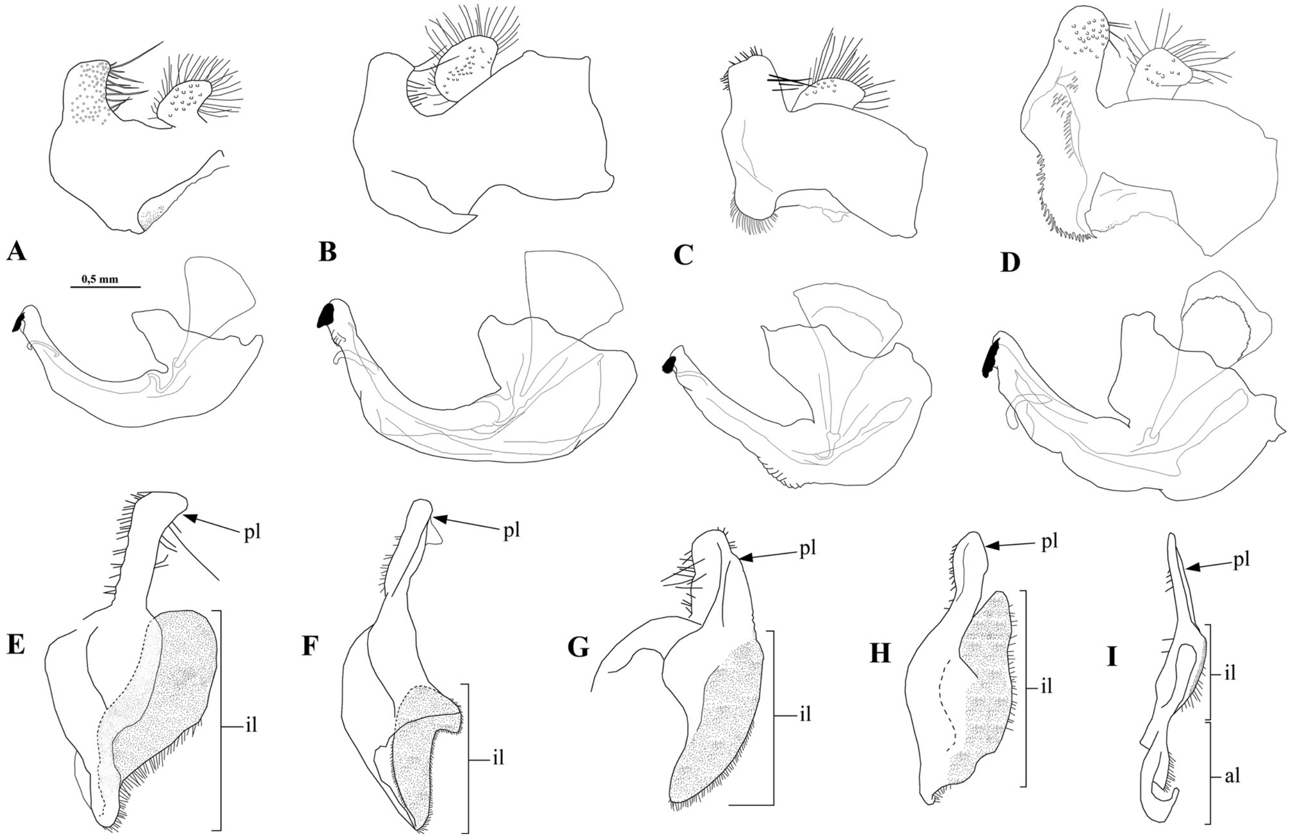

Fig. 11. Male genitalia of the *E. tricolor* group, lateral view: upper- epandrium, lower-hypandrium: (A) *E. rubrum* sp. nov.; (B) *E. tarsalis*; (C) *E. armatus*; (D) *E. tricolor*; ventral view of epandrium: (E) *E. rubrum* sp. nov., (F) *E. tarsalis*, (G) *E. armatus*, (H) *E. tricolor*; (I) *E. sogdianus*. Abbreviations: pl, posterior lobe of surstylus; il, interior accessory lobe of surstylus; al, anterior lobe of surstylus.

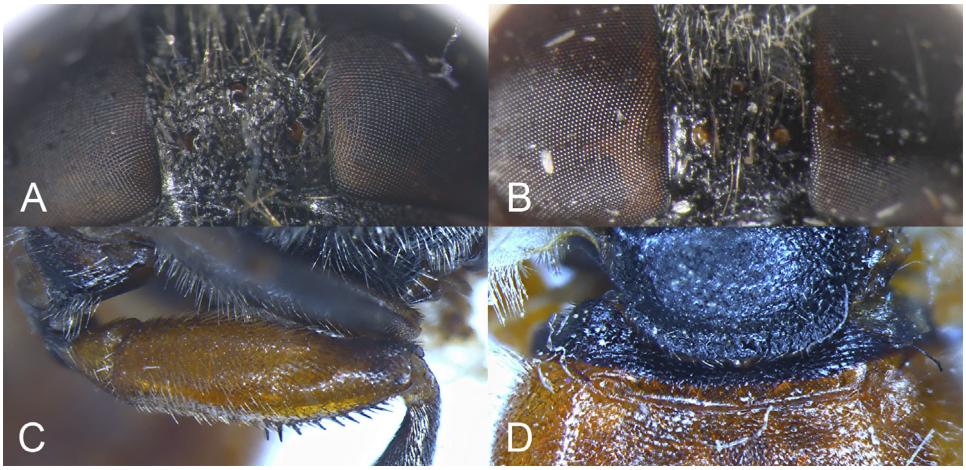

Fig. 12. Some diagnostic characters of females: ocellar triangle: (A) *E. rubrum*, (B) *E. tauricus*; (C) metafemur, lateral view: *E. rubrum*; (D) tergite I: *E. rubrum*.

Abdomen. Tergite I black with broadly reddish-yellow posterior margin; tergites II and III entirely reddish-yellow, tergite IV dark brown with reddish anterior and lateral part (Fig. 10a). Tergite II and III with pairs of barely visible lunulate shaped oblique maculae of white pollinosity, covered with white pile. The rest of the tergites covered with very short black pile, except the postero-lateral part of tergite II which is covered with long white pile. Abdominal sternites reddish-yellow covered with pale pile. Sternite IV darker, covered with long white pile, particularly on lateral margins.

*Genitalia.*Posterior lobe of surstylus slightly pointed posteriorly and with strong long, almost transparent setae, almost entirely covered in alveolae (Fig. 11a). Inner accessory lobe of surstylus with short dense pile (Fig. 11e). Hypandrium simple and thin (Fig. 11a).

Female. *Head.* Eyes bare (Fig. 9d–e). Frons moderately wide, almost two times wider than the width of basoflagellomere. Ocelli arranged in an isosceles triangle, wider than long (Fig. 9d). Anterior angle of ocellar triangle in female paratype is about 95° (Fig. 12a). Face, frons and postocular orbit shiny black with shorter yellow pile. Pronounced longitudinal notch from antennal socket to anterior ocellus. Scape and pedicel brownish-yellow. Basoflagellomere moderately small, oval, yellow (Fig. 9e). Arista dark, basally thickened.

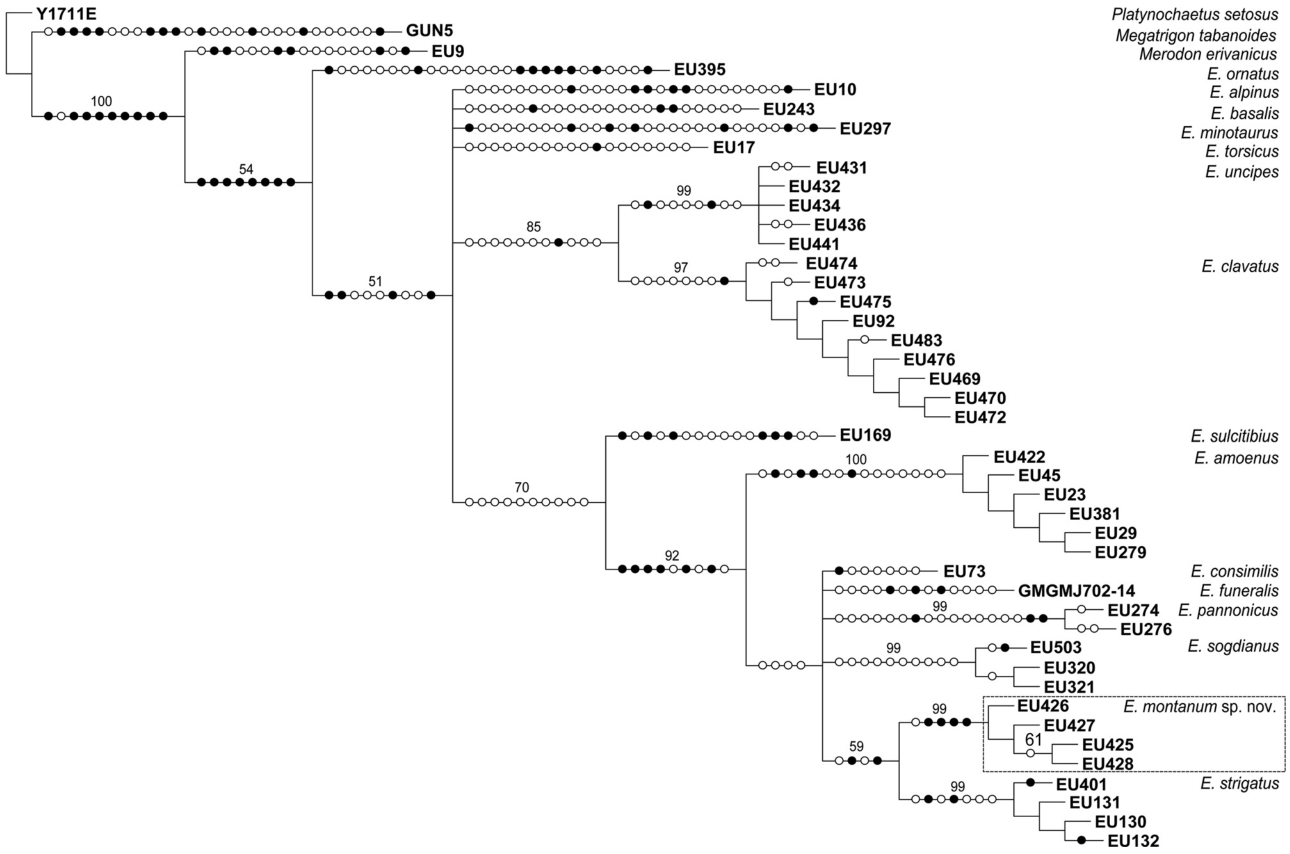

Fig. 13. Maximum parsimony analysis for the 'total' dataset (total length 612 bp); 27 equally parsimonious trees were produced from which the consensus tree is illustrated here. Length 442 steps, Consistency index (CI) = 48, Retention index (RI) = 76; filled circles denote unique changes, open circles non-unique. Bootstrap support values are illustrated above the branches.

Thorax. Mesonotum and pleurae black, densely punctuated, with short, yellow pile. Mesonotum anteriorly with rudiments of three vittae of white pollinosity. A row of dense black spinulae on mesonotum above the wing, more pronounced than in males. Scutellum black, striated transversely. Legs predominantly brown with yellowish coxae, yellow to translucent metafemur (Figs. 9f, 12c), tips of tibiae and ventral side of tarsi yellowish. Metafemur anteriorly with conspicuous transversal suture that envelops the whole femur (Figs. 9f, 12c). Metafemur apico-ventrally with rows of sharp black spines, 9–10 on anterior row and 7–8 on posterior row. Pile on legs short and yellow, only slightly longer ventrally on metafemur.

Abdomen. Tergite I black with broadly reddish-yellow posterior margin (Fig. 12d), as in males. Tergites II–III completely and tergite IV anteriorly reddish-yellow. Tergites with short yellow pile with intermingled brown pile and three pairs of crescent-shaped lunules of pollinosity on tergites II–IV. Tergite V dark with brown pile. Sternites I–IV reddish with short sparse yellow pile. Sternite V dark with brown pile.

Etymology. The Latin adjective "*rubrum*" indicates that it is a species with a ruby appearance.

3.4. Molecular analyses

The ML alignment of the 'total' dataset contained 0.06% gaps and completely undetermined characters, and 164 distinct alignment patterns. The NJ, ML and MP tree topologies can be viewed in the Supplementary material (Figs. S1 and s3) and in Fig. 13, respectively. The genetic distance (K2P) computed for the different species of *Eumerus* in the 'total' dataset was found to be 0.023-0.107 (Table S2).

In all phylogenetic trees, *E. uncipes* was clustered with *E. clavatus* (*E. clavatus* group) and together these taxa were highly differentiated, with bootstrap support values of 87 (ML) and 85 (MP). *E. montanum* sp. nov. was clustered within the *E. strigatus* group and close to *E. strigatus*, with bootstrap support values of 75 (ML) and 59 (MP). The split network analysis identified the *E. strigatus* and *E. clavatus* groups, indicating the phylogenetic relationships between the species constituting these groups (Fig. 14). Regarding the *E. strigatus* group, the MP tree topologies showed a clear clustering of the seven studied species (Figs. 15–17), with separate and combined DNA/morphological analyses supporting the affinity between the species *E. montanum* sp. nov. and *E. strigatus*.

4. Discussion

To date *Eumerus* has been considered as encompassing 34 species in southeastern Europe. Here, we introduce and provide descriptions of two new species (*E. montanum* sp. nov. and *E. rubrum* sp. nov.). We revise the geographic distribution of *E. uncipes*, thereby increasing the total number of *Eumerus* species to 37 in southeastern Europe. In addition, the assignment of *Eumerus* species into groups is discussed and, where feasible, based on an integrative approach.

Based on morphology, we found that *E. montanum* sp. nov. belongs to the group defined as *E. strigatus* which consists of species "closely similar morphologically in the adult stage to *E. strigatus*", sensu Speight et al. (2013). Based on the study of Speight et al. (2013), in Europe, the *E. strigatus* group includes five species: *E. consimilis*, *E. funeralis*, *E. narcissi* Smith, 1928, *E. sogdianus* and *E. strigatus*. According to the same study, the similarity between species of the *E. strigatus* group was based on morphology not on a phyloge-

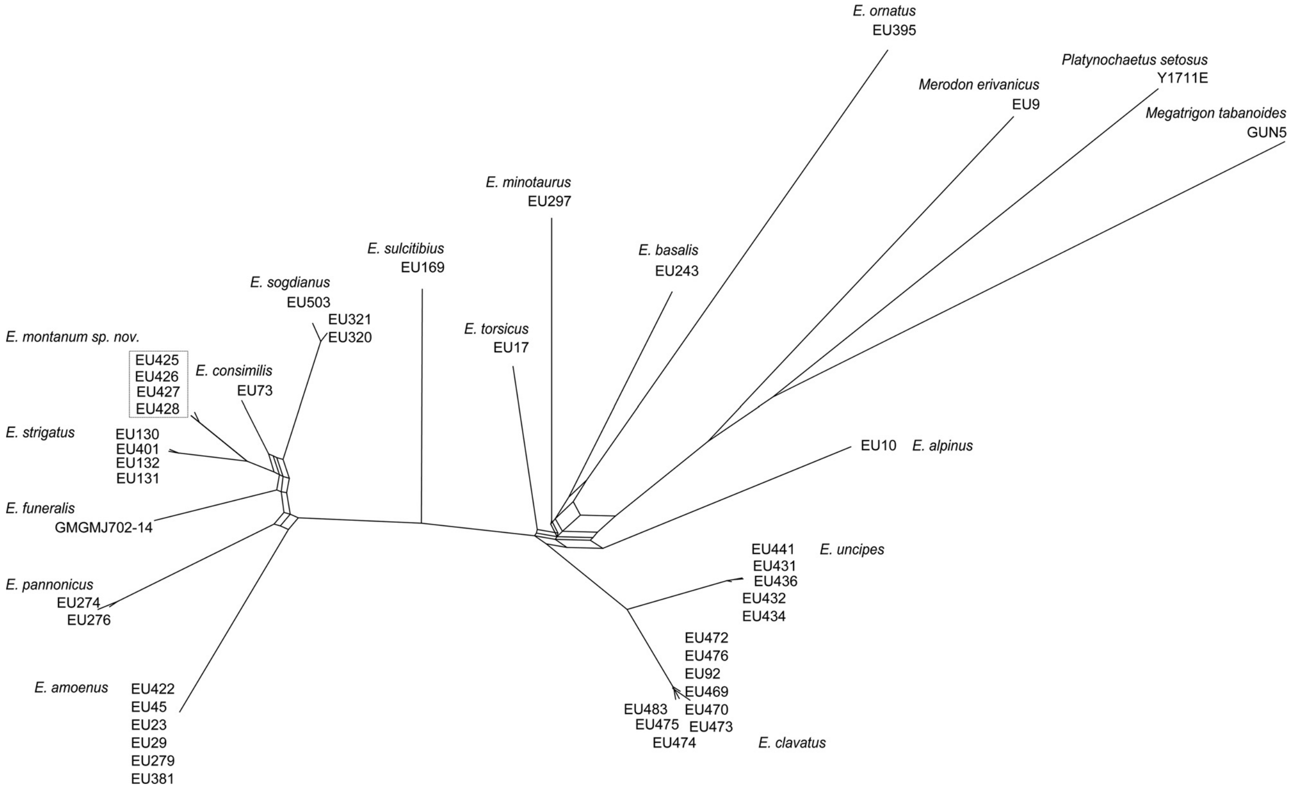

Fig. 14. A phylogenetic network from NJ and ML tree results of the 'total' dataset.

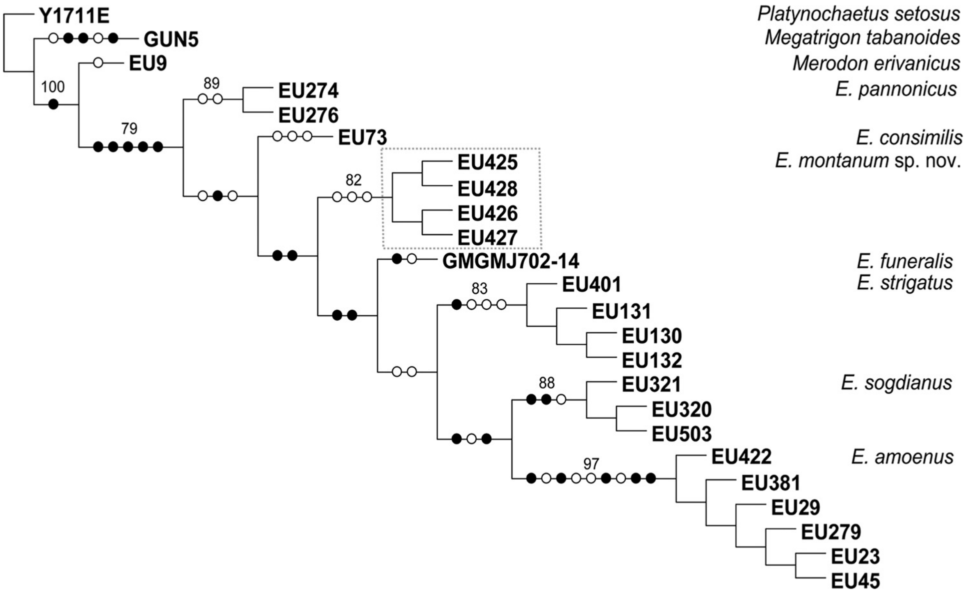

Fig. 15. Maximum parsimony analysis for the dataset of 21 *Eumerus* plus the 3 outgroups; 24 morphological characters were included (characters numbered 0 to 23). One tree was produced and is illustrated here. Length 79 steps, Consistency index (CI) = 68, Retention index (RI) = 86; filled circles denote unique changes, open circles non-unique. Bootstrap support values are illustrated above the branches.

netic hypotheses. Here, we found that the aforementioned species belong to one monophyletic group which also encompasses *E. montanum* sp. nov. Besides our species description of *E. montanum* sp. nov., we attempted to morphologically define the *E. strigatus* group after corroborating through DNA barcoding that all species considered by Speight et al. (2013), with the addition of *E. amoenus* (Chroni et al., 2017) and *E. pannonicus*, really belong to one monophyletic group. We have included neither *E. narcissi* nor *E. vanderbergei* Doczkal, 1996 in our molecular analyses due to lack of available DNA barcodes, but we have considered them part of the *E. strigatus* group based on morphological similarity. *E. narcissi* was initially described from North America (Santa Cruz, California). Speight et al. (2013) recorded *E. narcissi* for the first time in Europe (France) and redescribed the species, both males and females. We have used the detailed description of Speight et al. (2013) to compare *E. narcissi* with other species of the group. We have also assigned *E. vanderberghei* to the same group based on Doczkal (1996), who described

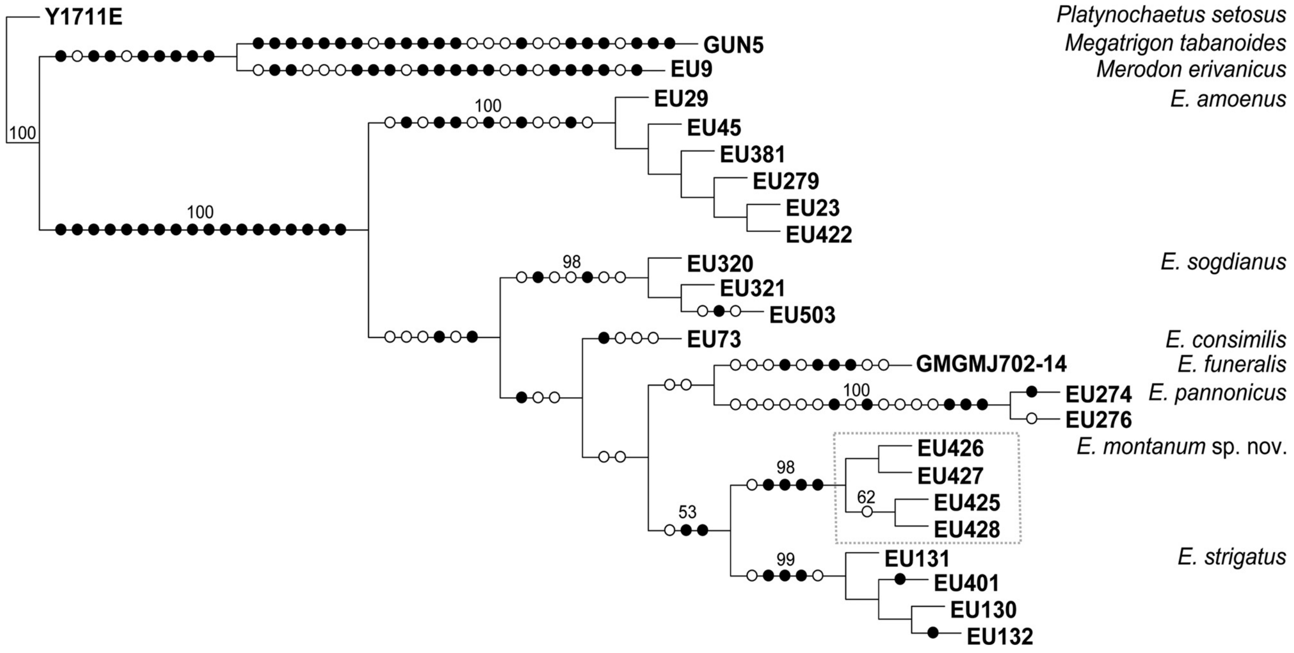

Fig. 16. Maximum parsimony analysis for the dataset of 21 *Eumerus* plus the 3 outgroups (total length 612 bp). One tree was produced and is illustrated here. Length 233 steps, Consistency index (CI) = 71, Retention index (RI) = 81; filled circles denote unique changes, open circles non-unique. Bootstrap support values are illustrated above the branches.

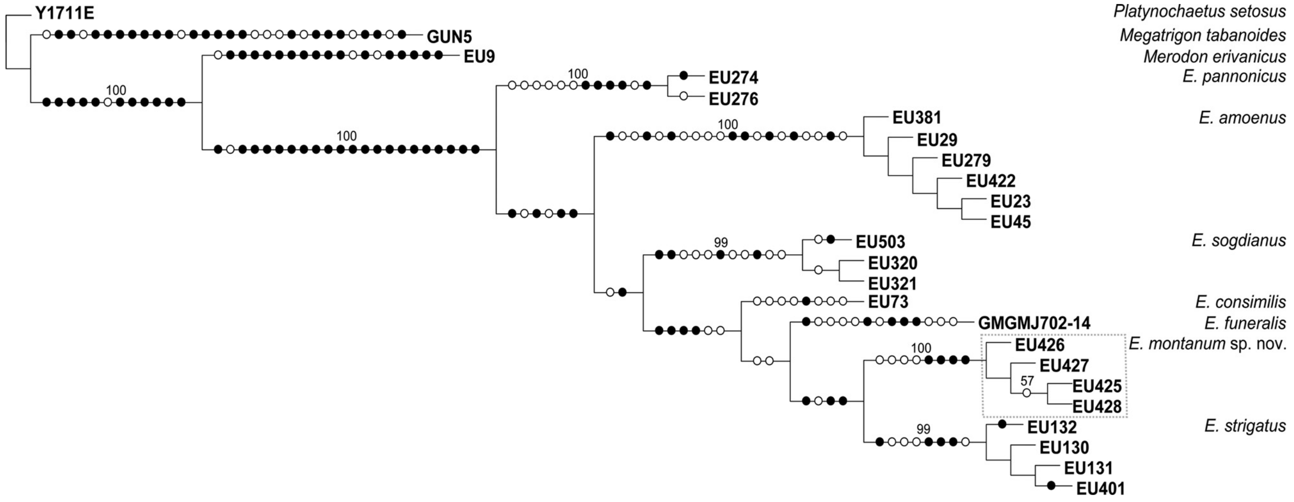

Fig. 17. Maximum parsimony analysis for the dataset of 21 *Eumerus* plus the 3 outgroups; 24 morphological (character states 1-24) and 612 molecular characters (character states 25-636) were included. One tree was produced and is illustrated here. Length 317 steps; filled circles denote unique changes, open circles non-unique. Bootstrap support values are illustrated above the branches.

E. vandenberghei as an endemic species to Corsica and Sardinia and mentioned its affinity to *E. funeralis*.

In order to conclude phylogenetic positioning and clustering, as well as to confirm species assignments within the *E. strigatus* group, we conducted various analyses (separately and combined for morphological characters and DNA sequences). Analyses with morphological characters alone did not cluster *E. montanum* sp. nov. with *E. strigatus*, probably due to the insufficient number of morphological features considered. However, DNA sequences alone and the combined analyses did reveal the strong similarity between *E. montanum* sp. nov. and *E. strigatus*. After examining more thoroughly the morphology of adults of these two species, we found considerable similarity in the shapes of the fourth abdominal sternite in males (Fig. 8a,b). Our analyses showed *E. amoenus* and *E. pannonicus* to be the most distinct from the other species of the *E. strigatus* group, which is indeed reflected in the morphology of the males (specific shapes of sternite IV and distinctive morphology of the genitalia). According to Speight et al. (2013), *E. narcissi* can be distinguished from other species of the group by the arrangement of the ocelli that form an equilateral triangle, whereas in other species the ocelli form an isosceles triangle. We found that this feature is also present in *E. strigatus*, but in this species it has a tendency to be wider than long. As mentioned previously, species related to *E. bactrianus* (such as *E. pannonicus*) also belong to the *E. strigatus* group, but since the shape of the surstylar lobe of the epandrium of those species is so distinct, we suspect that they probably form a separate subgroup (Fig. 7g).

Regarding the other newly-described species, *E. rubrum* sp. nov., we have assigned it to the *E. tricolor* group. Species from the *E. tricolor* group are predominantly black, but with a characteristic reddish abdomen (Fig. 10). Some species in the group present a metallic blue lustre on the head and thorax (*E. ovatus*) or are entirely blue (*E. niveitibia*). However, *E. aurofinis*, which also belongs to this group, is completely black in appearance. The common morphological character for most species within the *E. tricolor* group are the narrowly-spaced eyes, which is more strongly pronounced in *E. aurofinis* and *E. sinuatus*. Another unifying character is the striated basoflagellomere that is present in almost all species of the *E. tricolor* group.

We present a new record for the species *E. uncipes* occurring in Greece. *E. uncipes* is related and morphologically similar to *E. clavatus*; a species with a relatively wide range in Europe (northeast France south to Spain, Germany, Danube floodplain of Romania, the former Yugoslavia, Ukraine and the Caucasus; Speight, 2014). According to Speight (2014), the range of *E. clavatus* overlaps with that of *E. uncipes* (whose range is imperfectly known: from the Rhine valley south to the Mediterranean in France, Germany, northern and central Italy, Romania), but also extends to North Africa. We found that Corfu Island (Greece) probably represents the southeasternmost point of the distribution of *E. uncipes*. Our phylogenetic analyses clustered these two species together, highlighting that they form one monophyletic group that we named *E. clavatus*. From a morphological point of view, this group contains species with a distinctive projection on the posterior margin of abdominal sternite III of males.

In summary, our study contributes to systematic studies of the genus *Eumerus* in Europe by introducing two new species and one new record for a species in southeastern Europe, thereby increasing the total number of *Eumerus* species in this region to 37. We make further conclusions regarding genus clustering and species assignments, with the existence of a new group (*E. clavatus*, defined here) and two other groups being discussed based on morphological and molecular features. An overview of the morphological characters that cluster species into specific groups is provided.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jcz.2017.10.007.

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