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Ecdyonurus submontanus Landa, 1969 and *Electrogena affinis* (Eaton, 1887) new for Belgium (Ephemeroptera: Heptageniidae)

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Abstract

Two new species of mayflies for Belgium are recorded: *Ecdyonurus submontanus* Landa, 1969 and *Electrogena affinis* (Eaton, 1887). *E. submontanus* can be confused with *Ecdyonurus dispar* (Curtis, 1834) (as adults) or with *Ecdyonurus venosus* (FABRICIUS, 1775) (as larvae). It is found in medium large rivers and brooks in the hills of the Ardennes region. It was expected to occur in Belgium based on records in the German Eifel and in Luxembourg. A single imago female of *E. affinis* was found in the downstream part of the river Lesse. A second individual, a subimago, was later photographed along the Semois, but not collected. It was not possible to find a population of larvae of this species. For both species an overview is given of the diagnostic characters according to the most recent insights.

Keywords: genetic barcoding, morphologic characters, identification, CO1, mayflies.

Samenvatting

In dit artikel worden twee nieuwe soorten eendagsvliegen gemeld voor België: *Ecdyonurus submontanus* Landa, 1969 en *Electrogena affinis* (Eaton, 1887). *E. submontanus* kan worden verward met *Ecdyonurus dispar* (als imago) en met *Ecdyonurus venosus* (als larve). *E. submontanus* is gevonden in middelgrote rivieren en bij beken in de Ardense heuvels. De soort werd verwacht op basis van de waarnemingen in de Duitse Eifel en in Luxemburg. Van *E. affinis* werd één vrouwtje imago gevonden en verzameld bij het benedenstroomse deel van de Lesse. Een tweede exemplaar, ditmaal een subimago, werd louter gefotografeerd bij de Semois. Larven werden nog niet gevonden. Voor beide soorten worden de kenmerken volgens de meest recente inzichten beschreven.

Résumé

Deux nouvelles espèces d'éphémères ont été rapportées pour la première fois de Belgique: *Ecdyonurus submontanus* Landa, 1969 et *Electrogena affinis* (Eaton, 1887). *E. submontanus* est difficile à identifier et peut être confondue avec *Ecdyonurus dispar* (mâles au stade imago) ou *Ecdyonurus venosus* (larves). L'espèce a été trouvée dans des rivières de dimension moyenne et dans des ruisseaux en Ardenne. Seule une femelle adulte de *E. affinis* a été trouvée dans l'aval de la Lesse. Un deuxième exemplaire (subimago) a été photographié près de la Semois, mais pas récolté. On n'a pas réussi à trouver une population de larves. Les caractères diagnostiques des deux espèces sont décrits en détail selon les connaissances actuelles.

Introduction

Since the latest checklist of the Belgian mayflies (LOCK & GOETHALS, 2011), only one new addition to the mayfly fauna was published, as DEMARTEAU (2015) discovered a population of *Rhithrogena germanica* (Eaton, 1885) in the river Ourthe. Over the last few years, many other interesting findings were made in Belgium, amongst them the findings leading to this publication of two species not found in Belgium before. Both species belong to the Heptageniidae family: a family that is well diversified in the Holarctic region, with many species in Europe (BAUERNFEIND & SOLDÁN, 2012). All larvae are dorsoventrally flattened and adapted to life in running waters. The family is more diversified on higher altitudes, which is also the case in the hilly regions south of the rivers Sambre and Meuse in Belgium. The taxonomy in many genera of this group is unclear (VUATAZ *et al*., 2016) and also for Belgium, the genus of *Ecdyonurus* and especially *Rhithrogena* should be thoroughly investigated morphologically and genetically, because there seems to be several inconsistencies in the morphology and preliminary DNA barcoding. In this paper, one conundrum regarding *E. submontanus* Landa, 1969 and *Ecdyonurus dispar* (Curtis, 1834) will be addressed. Both species are members of the *Ecdyonurus venosus*-group (cf. Bauernfeind & Soldán, 2012). In the past, the imagines of *E. submontanus* could have been confused with *E. dispar*, while the larvae could have been confused with *Ecdyonurus venosus* (FABRICIUS, 1775). However, to our knowledge, *E. submontanus* has never been found before in Belgium and the same applies for *Electrogena affinis* (Eaton, 1883), the other species newly recorded for the Belgian fauna.

Material and methods

Collection and deposition of Ephemeroptera

During 2016 – 2019 the Belgian region south of Sambre and Meuse was searched for mayflies to study their morphology. Subimagines and female imagines were caught using a large butterfly net on a telescopic stick from bridges and river banks, when specimens emerged from the water surface. Swarming male imagines were caught from open, yet sheltered areas close to rivers. Some of the mayflies that were collected, were used for DNA extraction. All collected specimens were photographed alive on a white background and stored in 95% ethanol to preserve the morphological characters for further study. Larvae were caught using a kicknet over submerged stones and pebbles, so that the current carried them into the net. Specimens were sorted in a white tray filled with a small layer of water, photographed alive and stored on ethanol. Collected specimens were either kept in the collection of the author or transferred to the collections of Naturalis Biodiversity Center in Leiden, The Netherlands. In a large scale DNA-barcoding project at Naturalis called DNA Waterscan, some of the interesting findings of mayflies were added to retrieve a CO1 mtDNA barcode. Additionally, two specimens of *E. submontanus* have been transferred to the collection of the Royal Belgian Institute of Natural Sciences (RBINS) in Brussel, Belgium, in order to have them in their country of origin as well. Of the new species for Belgium, the following collected material is available:

Ecdyonurus submontanus: Belgium: Aisne, Villers-Sainte-Gertrude 26.VIII.2017, 3 larvae, 2 in collection Naturalis, RMNH.5009124 (Fig. 8) and RMNH.509125 (Fig. 9). Lomme, Poix-Saint-Hubert 25.VIII.2017, 3 subimago, 2 reared to male imago, 2 in collection D. Drukker and 1 in collection RMNH, RMNH.5012616 (Figs 10 and 11). Aisne, Villers-Sainte-Gertrude 2.IX.2018, 3 male imago in collection D. Drukker, 2 male imago in collection RBINS, Brussels, IG 34109. 1 male imago in collection Naturalis RMNH.5086375 (Fig. 2), Rau Basseilles, Lavacherie 25.VIII.2017, 2 male imago collection D. Drukker.

Fig. 1. *Electrogena affinis* (Eaton, 1887). Habitus female imago (alive). The first specimen discovered in Belgium, river Lesse at Furfooz, 10.VIII.2016. © Daan Drukker.

Fig. 2. *Ecdyonurus submontanus* Landa, 1969. Habitus male imago (alive). Caught swarming at the river Aisne at Villers-Sainte-Gertrude, 2.IX.2018. © Daan Drukker.

Fig. 3. *Ecdyonurus dispar* (Curtis, 1834). Habitus male imago for comparison (alive). River Lesse at Furfooz, 10.VIII.2016. © Daan Drukker.

Electrogena affinis: Belgium, Hulsonniaux, river Lesse, 10.VIII.2016, 1 female imago, collection Naturalis, RMNH.5009074 (Fig. 1). Bouillon, river Semois, 25.VI.2018, 1 female subimago, photographed by Manuel Valdueza (Fig. 6), not collected.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from legs, using a NucleoMag 96 Tissue kit (MachereyNagel Gmbh & Co.) on a KingFisher Flex magnetic particle processor (Thermo Scientific). A volume of 150 µl was used for elution. A 658 bp fragment of the mtDNA cytochrome c oxidase I (COI) gene was amplified using primer combinations provided in Table 1. 25 µl of PCR reaction mix contained 5 µl of 5x Phire II Reaction Buffer (ThermoFisher), 1 µl of each primer (10 pM), 0.5 µl of Phire Hot Start II DNA Polymerase (ThermoFisher), 0.5 µl of dNTPs and 1 µl of DNA template. The amplification protocol consisted of 30 sec at 98°C followed by 40 cycles of 5 s at 98°C, 5 s at 50°C and 15 s at 72°C and a final 5 min at 72°C. Bi-directional Sanger sequencing was performed at BaseClear, Leiden, The Netherlands. Sequences were assembled, edited and checked for stop-codons, using Geneious Prime 2019.2.1 (https://www.geneious.com).

Table 1. Primer combinations used for amplification of COI.

Primer name	**Direction**	**Sequence (5' to 3')**	**Reference**
ODO_LCO1490d	F	TTTCTACWAACCAYAAAGATATTGG	DIJKSTRA *et al.*, 2014
ODO_HCO2198d	R	TAAACTTCWGGRTGTCCAAARAATCA	DIJKSTRA *et al.*, 2014
LepFolF	F	RKTCAACMAATCATAAAGATATTGG	HEBERT *et al.*, 2004
LepFolR	R	TAAACTTCWGGRTGWCCAAAAAATCA	HEBERT *et al.*, 2004

Molecular phylogenetic analysis

Multiple sequence alignments were performed using MAFFT 7 (KATOH *et al*., 2013) under default parameters. ML analyses were run with RAX-ML 8 (STAMATAKIS, 2014) using the GTRCAT model. The best ML tree was calculated using the –D parameter. A multi-parametric bootstrap search was performed, which automatically stopped based on the extended majority rule criterion. The Bayesian inference was performed with ExaBayes 1.5 (ABERER *et al*., 2014) using the GTR substitution model. Four independent runs were run for 10,000,000 generations. Bootstrap supports and posterior probabilities were depicted on the branches of the best ML tree using P4 (FOSTER, 2004). The resulting tree was visualized in FigTree 1.4.3 (RAMBOUT, 2016).

Results

Ecdyonurus submontanus Landa, 1969

Location and period

Specimens of this species were found in August and September on several locations in the Ardennes region. Subimagines were collected emerging from the river Lomme south of Poix-Saint-Hubert (province of Luxembourg) on the 25th of October 2017. Swarming male imagines were found and collected on the same date near the brook of Basseilles, near Laneuville-au-Bois and on the 2nd of September 2018 near the river Aisne at Villers-Sainte-Gertrude (both in the province of Luxembourg as well). From the last location, two larvae were identified as *E. submontanus* and confirmed by mtDNA barcoding. These specimens were collected on the 26th of August 2017, which was also the reason to return to this location in 2018, when the imagines were caught. The rivers Lomme and Aisne are relatively small rivers, between 5 and 15 meters wide at the stations where the specimens were collected.

Table 2. Characters of male imagines of *Ecdyonurus submontanus* Landa, 1969 and *Ecdyonurus dispar* (Curtis, 1834) based on SOWA (1971) and LANDA (1970) in accordance with the Belgian material in collection D. Drukker.

Character	***E. submontanus***	***E. dispar***
Pterostigma	Dark grey, well defined (Fig. 2), fades to light brown in ethanol	Transparent, milky to light brown, not conspicuously different from rest of wing (Fig. 3)
First longitudinal veins in fore wing	Dark to pitch brown, same colour as other veins (Fig. 2)	Pale to yellowish brown, lighter than other veins (Fig. 3)
Abdominal sterna	Two white divergent lines and two spots on each sternum	Pattern of light lines and stripes inconspicuous
Styliger projections	Symmetrical, straight out	Asymmetrical, usually arched inward
Abdominal terga	Reddish brown without obvious diagonal markings (Fig. 2)	Yellowish brown background with obvious diagonal markings, especially in the lateral parts of the terga (Fig. 3)
Penis lobes	S-shaped basal sclerite. Lobes more quadrangular, caused by a more massive lateral sclerite (Fig. 7 A, B, C, D)	Convex basal sclerite. Lobes more triangular caused by less massive lateral sclerite (Fig. 7 E, F, G, H)

Distribution and ecology

According to LANDA (1970), the species is moderately abundant in the highlands of the former Czechoslovakia, but absent from the mountains and lowlands. BAUERNFEIND & SOLDÁN (2012) mention a distribution from the Rhodopes and Carpathians Westward to Poland, Austria and Germany. Additionally, the species is also found in the Grand Duchy of Luxembourg (det. Arne Haybach: DOLISY, 2000; HAYBACH, 2006), which was the most western finding until now. In the German Eifel, the species turned out to be less rare than expected in smaller brooks and rivers and a distribution extending to Belgium was already predicted by HAYBACH (2006).

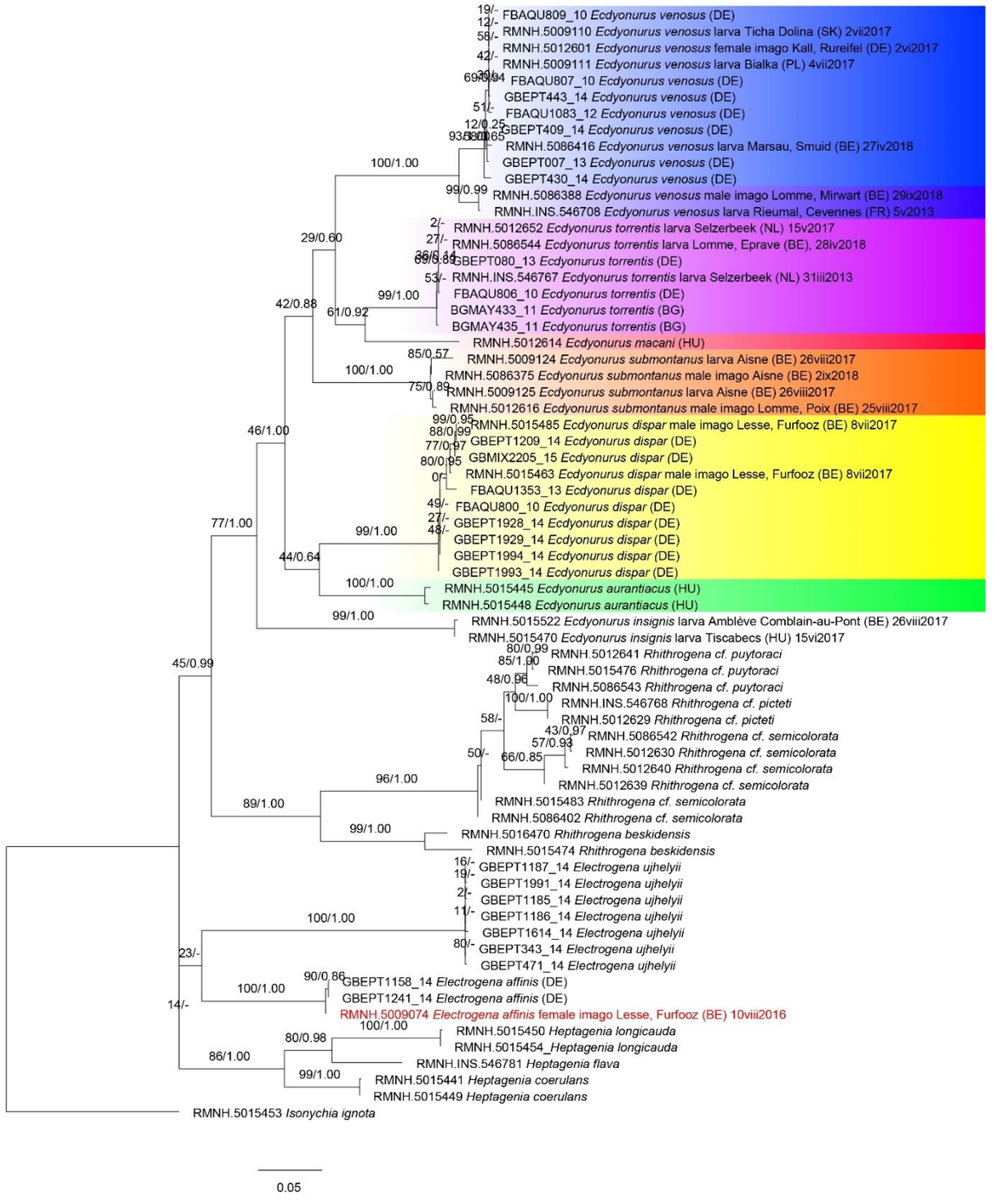

Fig. 4. Maximum likelihood best tree of 17 taxa of Ephemeroptera, based on COI. Branch support values (ML bootstrap percentage/Bayesian Inference posterior probability) are depicted on branches only if below 100%. The highlighted clades represent the northwest European taxa of *Ecdyonurus* Eaton, 1868 (of the nominate subgenus), showing the new species for Belgium *Ecdyonurus submontanus* Landa, 1969 being well-separated from the other species that are known from Belgium and surrounding countries. The specimen of *Electrogena affinis* (Eaton, 1887) marked in red characters represents the female imago described in this article, first for Belgium. *Isonychia ignota* (Walker, 1853) is taken as outgroup. The specimens of *Heptagenia* Walsh, 1863 and *Rhithrogena* Eaton, 1881 are used as a check within the Heptageniidae family.

Description and identification

Up till now, only one species of *Ecdyonurus* with a relatively narrow penis was known in Belgium: *E. dispar*. A large amount of morphological variation was noted in the specimens caught by the first author that were identified as *E. dispar* in Belgium and the identification of larvae was particularly difficult. Some larvae that were caught exhibited narrow first gills and other characters pointing towards *E. submontanus*. When the imagines I caught on the same location also had *E. submontanus* characteristics, more literature was consulted than the standard identification work of BAUERNFEIND & HUMPESCH (2001), where the main focus lies on the male genitalia. The shape of the penis lobes of the Belgian *E. submontanus* is difficult to separate from the Belgian *E. dispar*, however, the shape of the projections on the styliger do seem different and in accordance with BAUERNFEIND & HUMPESCH (2001). The shape of the penis lobes is more like the description given by LANDA (1970) in his description of the species and in more detail by SOWA (1971) (Fig. 7). This and the other differences between the Belgian specimens of male imagines of this group are given in Table 2.

Fig. 5. Ventral view of the abdomen of *Electrogena affinis* (Eaton, 1887) female imago in ethanol. Same individual as Fig. 1. © Daan Drukker.

Fig. 6. *Electrogena affinis* (Eaton, 1887). Male subimago (alive). Specimen not collected, but identifiable by the combination of the markings on the femora (especially the fore femur), abdomen pattern and annulated tails. River Semois near Bouillon. © Manuel Valdueza.

The *E. dispar*-group can be distinguished in the adult stages by the following characters: penis lobes rounded and compact (extended laterally in *Ecdyonurus torrentis* Kimmins, 1942 and *E. venosus*), projections on styliger present (absent in *E. venosus*, but present in *E. torrentis*). The full description of *E. submontanus* was given by LANDA (1970) and SOWA (1971). The imago male is overall quite dark, with dark wing venation, a well-defined dark pterostigmatic area, pitch brown thorax, brownish abdomen without well-defined oblique stripes on the terga. The pattern on the sterna consists of two paler diverging lines and two white dots. Male imagos of *E. dispar* differ from *E. submontanus* mainly in colouration of the pterostigma (dark and well-defined in *E. submontanus* (Fig. 11), while it is unremarkable in *E. dispar* (Fig. 3)). The wing venation differs slightly as well (first three longitudinal veins yellow-brown in *E. dispar* (Fig. 3), while these are dark to pitch brown in *E. submontanus* (Fig. 11)) and there are some minute differences on the penis lobes, such as an S-shaped basal sclerite and more quadrangular lobes in *E. submontanus* (LANDA, 1970; SOWA, 1971, red lines in Fig. 7D and 7H). The subimagines of *E. submontanus* have dull grey wings, similar to *E. dispar*. The specimens that were caught near the river Lomme showed a very vague light zigzag pattern (Fig. 10). *E. dispar* should not have any pattern and the imago pattern of the terga is often already visible in subimagines. Confusion with the second generation of *E. venosus* is therefore more likely, because this species has a more striking pattern on the wings in the subimago stage.

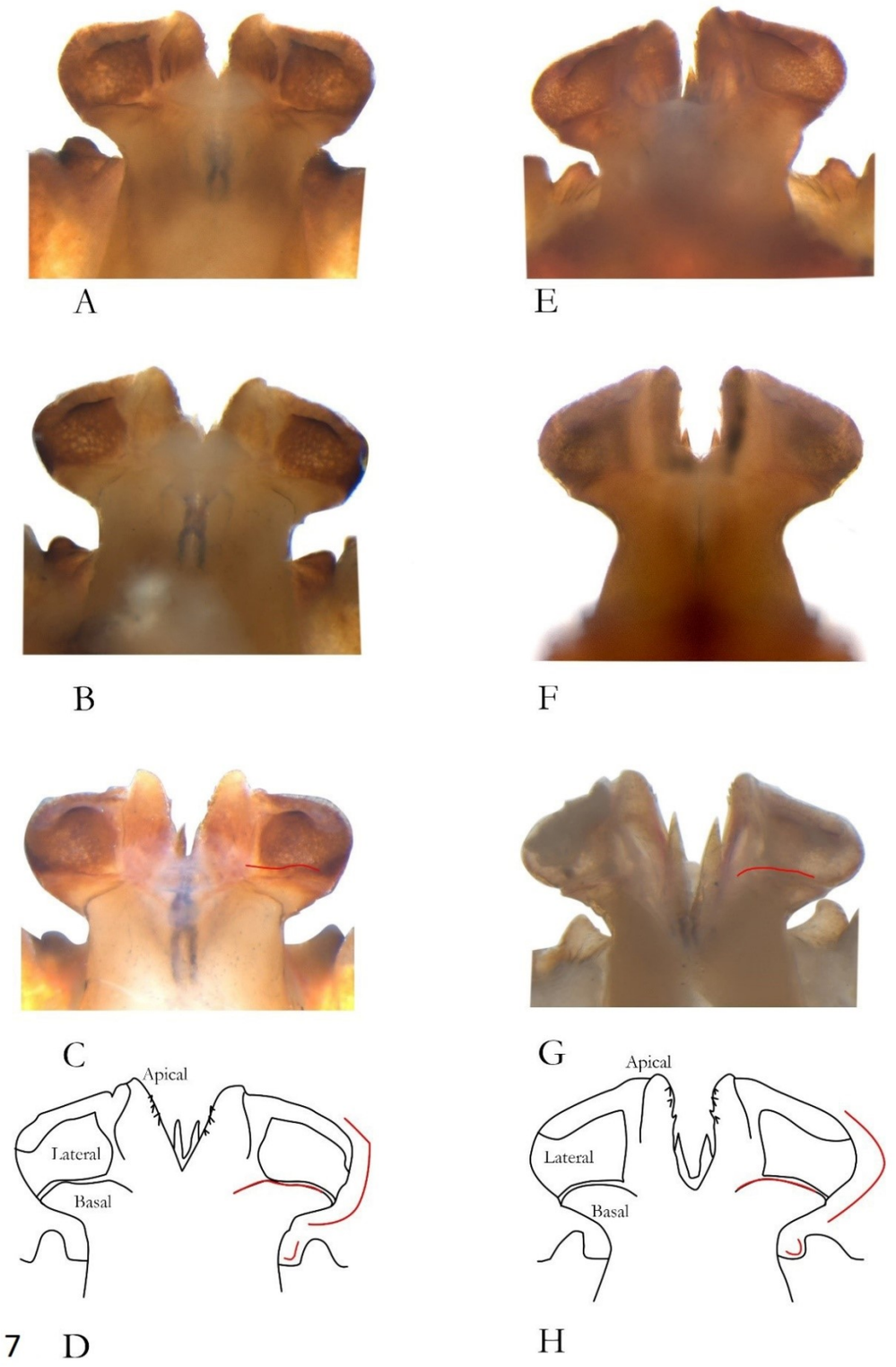

Fig. 7. Comparison of the genitalia of *Ecdyonurus submontanus* Landa, 1969 (left) and *Ecdyonurus dispar* (Curtis, 1834) (right) in dorsal view. Main differences lie in the shape of the protuberances of the styliger and the shape of the basal sclerite (red line). A. *E. submontanus*, Lomme, Poix-Saint-Hubert (RMNH.5012616), B. *E. submontanus*, Basseilles, Tenneville (not sequenced), C. *E. submontanus*, Aisne, Villers-Sainte-Gertrude (RMNH.5086375), D. Drawing based on 7B. E. *E. dispar*, Lesse, Furfooz (not sequenced), F. *E. dispar*, Lesse. Furfooz (RMNH.5015485), G. *E. dispar*, Lomme, Mirwart (not sequenced), H. Drawing based on 7E and F.

The larvae of *Ecdyonurus* in Central Europe were studied by BAUERNFEIND (1997), HAYBACH (1999) and EISELER (2005), the last author providing the most user-friendly key. *E. submontanus* has a narrow first gill, in contrast to *E. dispar*. This makes confusion with *E. venosus* more likely. The main differences are given in Table 3. Several collected specimens were used for CO1 mtDNA barcoding (two male imago *E. dispar*, two larvae and one male imago of *E. submontanus*). Those that were identified as *E. submontanus* formed a cluster well apart from those that were identified as *E. dispar* and *E. venosus* (Fig. 4).

***Electrogena affinis* (Eaton, 1887)**

Location and period

The first record for Belgium was a female imago depositing eggs in the river Lesse between Furfooz and Hulsonniaux (province of Namur) collected around sunset on August 10th, 2016. Here, situated in the lower range of the calcareous region, the river Lesse is large and flows through steep rocky outcrops and forested valleys (Fig. 12). A second individual, a subimago, was photographed by Manuel Valdueza on the 25th of June, 2018, near the river Semois, downstream from the city of Bouillon. An *Electrogena* larva photographed along the river Semois near Alle (province of Namur) could also concern *E. affinis* based on the habitat (observation Bob Lens on Observations.be). The specimen was not collected (Bob Lens pers. comm), so it was not possible to identify it with certainty.

Fig. 8. *Ecdyonurus submontanus* Landa, 1969. Habitus of larva (alive) and its left mandible (in ethanol) (RMNH.5009124). Aisne, Villers-Sainte-Gertrude. © Daan Drukker.

Fig. 9. *Ecdyonurus submontanus* Landa, 1969. Habitus of larva (alive) and its left mandible (in ethanol) (RMNH.5009125). Aisne, Villers-Sainte-Gertrude. © Daan Drukker.

Distribution and ecology

Only known from Europe. Found from Greece and Bulgaria in the southeast to southern Sweden in the north. western border in England (rare) and western France (BELFIORE *et al.*, 1999; MACADAM & BENNETT, 2010; BRULIN, 2011). Type locality is Arnhem, The Netherlands. Larvae prefer large and medium large lowland rivers, mainly under 500 m altitude. The larvae are mainly found under dead wood and HAYBACH (2006) suggests that dead wood in rivers is essential for the species. The habitat where the Belgian specimens were caught, is this typical habitat, although dead wood is scarce in this stretch of the river Lesse and unknown for that part of the Semois (discussion).

Fig. 10. *Ecdyonurus submontanus* Landa, 1969. Habitus of male subimago (same individual as Fig. 11 and 7A, RMNH.5012616). Lomme, Poix-Saint-Hubert. © Daan Drukker.

Fig. 11. *Ecdyonurus submontanus* Landa, 1969. Habitus of male imago (same individual as Fig. 10 and 7A, RMNH.5012616). Lomme, Poix-Saint-Hubert. © Daan Drukker.

Description and identification

The Belgian specimens could be identified thanks to the redescription by BELFIORE *et al*. (1999). Characteristic features of all winged stages are the pattern on the abdominal segments and the fore femur, which is light with a dark median band. In other West-European *Electrogena* species, i.e. *Electrogena ujhelyii* (Sowa, 1981) and *Electrogena lateralis* (Curtis, 1834), only the middle and hind legs have a dark band, while the fore femur is light at the base and dark in the distal part. The cerci of *Electrogena affinis* are light with red annulation (Fig. 1, 6), while the other two *Electrogena* species have unringed tails (BAUERNFEIND & HUMPESCH, 2001). The abdominal terga have a light background with a broad dark reddish oblique band and a black spot on both sides of the lower distal corner (Fig. 1). The abdominal tergite pattern of *Electrogena lateralis* (Curtis, 1834) consist only of the dark spot in the lower distal corner, while the rest of the tergite is plain orange-brown, The differences on the tergites between *E. affinis* and *E. ujhelyii* are more subtle. The abdominal sterna are light with a pair of U-shaped red markings. This shape is in the Belgian female best visible on the anterior sternites (Fig. 5). Spots are absent in *E. lateralis* and black in *E. ujhelyii* (BAUERNFEIND & HUMPESCH, 2001). Subanal plate of female imagines without longitudinal median rib (Fig. 5). This rib is present in *E. ujhelyii* (SOWA, 1981). The eyes of females have more obvious lateral stripes than European congeners (Fig. 1). Thorax yellowish brown with darker markings. The wings of the subimago are grey without an obvious pattern, similar to other *Electrogena* species. The patterns on abdomen, tails and legs of the subimago are comparable to the pattern of the imago stage. For description of larvae, see BELFIORE *et al*. (1999).

Table 3. Characters of larvae of *Ecdyonurus submontanus* Landa, 1969, *Ecdyonurus dispar* (Curtis, 1834), *Ecdyonurus venosus* (FABRICIUS, 1775) and *Ecdyonurus torrentis* Kimmins, 1942 based on Eiseler (2005), Haybach (1999) and the Belgian material in collection D. Drukker.

Character	***E. submontanus***	***E. dispar***	***E. venosus***	***E. torrentis***
First gill width	Narrow	Broad	Narrow	Narrow
Upper margin first segment maxillary palp	+/- 25 hairs, only on first third of segment	>30 hairs extending to half of the segment	>35 hairs extending to two thirds of the segment	>35 hairs extending to two thirds of the segment
Bottom margin of maxillary palp	Few long hairs or hairs absent	Some long hairs, growing shorter and stronger distally	Many long hairs	Many long hairs
Tarsus claw pattern	Dark brown, darkest at the top	Dark brown at base and top, light yellow in middle part	Light brown in first half, dark brown at top	Dark brown at base and top, light yellow in middle part

The collected specimen was used for mtDNA barcoding. It resulted in a solid match with the three other barcoded specimens on BOLD, which were provided by SNSB, Zoologische Staatssammlung München, including photos of the used larva material. It was clearly different from *E. ujhelyii* (Fig. 4).

Discussion

Identification of male imagines, subimagines and last instar larvae of the *Ecdyonurus venosus*-group has proven to be difficult, even within a small country as Belgium, as some questions regarding the morphology still remain. Regarding the adult males of *E. submontanus*, the shape

of the penis lobes do not seem to correspond with what is given in BAUERNFEIND & HUMPESCH (2001) and BAUERNFEIND (1997). LANDA (1970) does not mention the shape of the lobes as a diagnostic difference between *E. submontanus* and *E. dispar*. Instead, he focuses on several other characters, namely the shape of the posterior edge of the basal sclerite on the dorsal side of the penis. This should be convex in *E. dispar* and sinuous in *E. submontanus*, which it indeed appears to be in the Belgian specimens (Fig. 7A, B, C). The shape of the penis of the Belgian individuals is very similar to the drawings in the description of LANDA (1970) and to the more detailed drawing provided by SOWA (1971). Characters given by LANDA (1970) regarding the length ratio of the tibia and tarsus on the foreleg were considered variable by SOWA (1971) are not used in later publications (e.g. BAUERNFEIND & HUMPESCH, 2001) and for the two previously collected Belgian individuals, no ratio corresponding to the findings of LANDA (1970) was found. Additionally, SOWA (1971), who studied the type specimens as well as 141 other specimens of all stages, altered the description of certain other features too. He considers the shape of the styliger lobes very important, mentions that the lateral sclerite of the penis in dorsal view is more massive and causes a more quadrangular shape of the penis lobe, in contrast with the more triangular shape of *E. dispar*, see also Fig. 7. There is no mention of the colour of the veins in SOWA (1971) and he mentions that although the majority of the specimens lack the oblique bands, 12 out of 103 imago males showed oblique bands to some extent.

Fig. 12. The river Lesse at Furfooz. The first specimen of *Electrogena affinis* (Eaton, 1887) for Belgium was found here, as well as many other potamal mayfly species. © Daan Drukker.

As can be seen in Fig. 4, the clade of *E. venosus* splits in two. Most specimens, including one from Belgium and one from the German Eifel belong to the clade with quite 'typical' specimens, while two specimens, one from Belgium (in autumn) and one from France (a larva in May) are genetically distinct. These two are marked with a darker shade of blue. It is possible

that they belong to a different – perhaps undescribed – species, as some characters of the imago (which was reared from subimago) are slightly different from what one would expect of *E. venosus*. This issue will be addressed in detail in the future.

Even though the two species treated by this paper both belong to the same rheophile family of Heptageniidae, their habitat preferences and conservation concerns are highly different. *E. submontanus* might occur on other locations in the Ardennes region and with expanding knowledge of its identification, more records are expected. It is recommended that all *Ecdyonurus* larvae found from July to October and all *Ecdyonurus* imagines found in August to October should be reidentified using the identification tables in this paper, in order to obtain a better understanding of the ecological preferences and distribution of this species in Belgium. *E. affinis* has suffered greatly from changes in river systems all over Europe. In the Netherlands, where the species was first described, there are only a handful of records from the 19th century, all coming from areas close to the river Rhine and the river Meuse (MOL, 1985). There have not been any observations since then. There are observations from the 1990's about 70 km east of the type locality in the Wienbach in Germany (BELFIORE *et al*., 1999). Besides that location, the river Lesse at 208 km is the second closest distance to the type locality. There are not many populations of *E. affinis* known close to the Belgian findings. The origin of the Belgian female is unclear. It could either be from the river Lesse itself, or from the France département Ardennes (BRULIN, 2011). The river quality and morphology of the Lesse should be suitable for a population of this species, as many other rare potamal species also occur on this location, such as *Rhithrogena beskidensis* Alba-Tercedor & Sowa, 1987, *Oligoneuriella rhenana* (Imhoff, 1852), *Baetis buceratus* Eaton, 1870, *Caenis macrura* Stephens, 1836 and *Potamanthus luteus* (Linnaeus, 1767). It can be considered as a refugium for potamal mayfly species that often have disappeared from other stretches of larger rivers that have suffered from anthropogenic pressures. However, this stretch of river is a popular spot for canoeing and therefore, large pieces of dead wood are actively removed from the river (Bertrand Bourtembourg (Service Public Wallonie), pers. comm. VI.2018). A lot of potential habitat is therefore lost, as the species seems to depend on dead wood in larger rivers (HAYBACH, 2006). It is likely that this species is under threat of extinction in Belgium. A short fieldtrip dedicated to detect the larvae of this species in 2018 has not yielded any records for the Lesse, but its occurrence cannot be excluded as a large stretch of river has not yet been investigated. It is recommended that future inventories focus on dead wood in the downstream part of the river Lesse and in the river Semois near Alle-sur-Semois and Bouillon. Hopefully this will result in the discovery of a population in the future.

Acknowledgments

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