<https://doi.org/10.11646/zootaxa.4975.1.1>

<http://zoobank.org/urn:lsid:zoobank.org:pub:01E9B1B4-A465-4DEC-A522-83AB67B376B5>

The *Rhyacophila fasciata* Group in Europe: *Rhyacophila fasciata* Hagen 1859 and formerly synonymized species (Trichoptera: Rhyacophilidae), with new description of *Rhyacophila fasciata* and *Rhyacophila septentrionis* McLachlan 1865 (stat. prom.).

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Abstract

The presence and distribution of *Rhyacophila fasciata* Hagen 1859 in Europe were revised, based on bibliographic study, collection specimens, and new material collected in different countries. The status of formerly synonymized species, *Rhyacophila ferruginea* (Scopoli 1763) and *Rhyacophila septentrionis* McLachlan 1865 was also assessed. The type of *R. ferruginea* is missing, the taxon is still unidentified, and thus we propose *Rhyacophila ferruginea* as a *nomen dubium*. Morphological features and genetic evidence revealed that *R. septentrionis* differs from *R. fasciata*, so we propose to change its status to *status resurrectus*. We therefore include new descriptions of the different stages (larva, pupa, male, and female) of *R. fasciata* and of *R. septentrionis*, together with a molecular analysis based on mitochondrial cytochrome oxidase I (mtCOI) and ecological notes. The species *R. coppai* Oláh 2020 **NEW SYNONYM** and *R. soreda* Coppa & Oláh 2020 **NEW SYNONYM** are synonyms of *R. sociata* Navás 1916; the species *R. kopasa* Oláh & Coppa 2020 **NEW SYNONYM** and *R. rova* Oláh & Coppa 2020 **NEW SYNONYM** are synonyms of *R. denticulata* McLachlan 1879; the species *R. matrensis* Oláh & Szczęsny 2020 is probably a synonym of *R. fasciata*, so more study of this species is needed in order to confirm or deny that it is a valid species.

Key words: Taxonomic revision, morphology, larva, pupa, imago, mitochondrial COI, ecology, distribution

Resumen

Se ha revisado la presencia y distribución de *Rhyacophila fasciata* Hagen 1859 en Europa, basada en el estudio de la bibliografía, especímenes de colecciones y en nuevo material recolectado en diferentes países. Se evaluó asimismo el estado de las especies anteriormente sinonimizadas *Rhyacophila ferruginea* (Scopoli 1763) y *Rhyacophila septentrionis* McLachlan, 1865. El ejemplar tipo de *R. ferruginea* se ha perdido y el taxón permanece todavía sin identificar, por lo que proponemos *Rhyacophila ferruginea* como un *nomen dubium*. Las características morfológicas y las evidencias genéticas indican que *R. septentrionis* difiere de *R. fasciata*, por lo que proponemos un cambio de estatus a *status resurrectus*. Se incluyen nuevas descripciones de las distintas etapas (larva, pupa, machos y hembras) de *R. fasciata* y de *R. septentrionis*, junto con el análisis molecular del gen mitocondrial Citocromo Oxidasa subunidad I (COI_{mt}), y notas ecológicas. Las especies *R. coppai* Oláh 2020 **NUEVO SINÓNIMO** y *R. soreda* Coppa & Oláh 2020 **NUEVO SINÓNIMO** son sinónimos de *R. sociata* Navás 1916; las especies *R. kopasa* Oláh & Coppa 2020 **NUEVO SINÓNIMO** y *R. rova* Oláh & Coppa 2020 **NUEVO SINÓNIMO** son sinónimos de *R. denticulata* McLachlan 1879; la especie *R. matrensis* Oláh & Szczęsny 2020 es probablemente un sinónimo de *R. fasciata*, por lo que se necesita un estudio más detallado para confirmarla o rechazarla como especie válida.

Palabras clave: Revisión taxonómica, morfología, larva, pupa, adulto, COI mitocondrial, ecología, distribución

Introduction

In the introduction of his monograph on genus *Rhyacophila* Pictet 1834 and family Rhyacophilidae Stephens 1836, Schmid (1970) concluded that *Rhyacophila* is one of the most primitive and important genera of caddisflies, because it is present in almost all lotic environments in the Holarctic and Oriental Regions, and because it included 465 species at that time, a number that has increased to 792 valid species up to date (and 30 valid subspecies) (GBIF 2020; Morse 2020). Genus *Rhyacophila* shows an evolutionary stage essential for inferring the phylogeny of Trichoptera, mainly due to the male genitalia, where high variability in details is mixed with a high stability in general architecture. Furthermore, in their geographic distribution, many of the species are restricted to small areas, sometimes to only a single mountain, providing the opportunity for phylogenetic and zoogeographical inferences (Schmid 1970).

Pictet (1834) described *Rhyacophila vulgaris* from brooks in the Lemán Lake basin and noticed a great variability in the specimens, predicting the future description of new species. Hagen (1859), studying Stephens', Curtis', and Pictet's private collections, classified these specimens in five different species: *R. vulgaris* Pictet 1834, *R. dorsalis* (Curtis 1834), *R. paupera* Hagen 1859, *R. ferruginea* Scopoli 1763, and *R. fasciata* Hagen 1859. The characters that defined *R. fasciata* were similar to those of *R. ferruginea*: dorsal lobe circular, covering the preanal appendages almost totally, but the specimens are bigger in size and wings show three dark transverse bands.

In his study of the British Trichoptera, McLachlan (1865) considered *R. ferruginea* similar to *R. fasciata* in the form of inferior appendages and to *R. septentrionis*, described in the 1865 publication from Scottish specimens and

from which *R. ferruginea* differed only in size. In his publication of 1868, McLachlan considered three species, *R. fasciata* (specimens from Carinthia), *R. septentrionis*, and *R. ferruginea*, although he doubted the validity of the last one. Finally, McLachlan (1879), studying several specimens of *R. fasciata* from different countries of Europe, concluded that those from Central Europe could be *R. septentrionis* while those from the Pyrenees could be a new species [this species was described by Navás in 1916 as *R. sociata* (Valladolid *et al.* 2018)].

Ulmer (1909), in his publication about Trichoptera, included descriptions of *R. fasciata* and *R. septentrionis* males, the first species from Elberfeld, while the second one with a wider distribution in Germany (Hamburg, Holstein, Harz, Thuringia, Black Forest, Sachsen, Hesse, Odenwald) and Poland (Szczecin and Silesia).

Décamps (1967a) published a paper on Trichoptera species from the Pyrenees, based on bibliographic references, collections, and specimens captured and reared to adults. He proposed that the synonymy *R. fasciata* = *R. septentrionis* was wrong and it would be necessary to examine Hagen's type material (different from the Pyrenees' specimens) as well as the variability of the species in Central Europe. On the other hand, Schmid (1970) kept this synonymy in his publication about the Rhyacophilidae.

To resolve the question whether there is one or two different species, we studied their distributions, morphology, and DNA. We conclude that these are two species and redescribe them (males, females, pupae, larvae) as well as their geographic distribution and ecology.

Material and methods

In this list we include all the specimens, confirmed previously as *R. fasciata* through morphological and genetic characters studied.

Specimens studied for this project are deposited in: the Collection of Trichoptera NIP, Croatian Natural History Museum, Zagreb, Croatia (CNHM); the Trichoptera Collection of the Museo Civico di Scienze Naturali "E. Caffi," Bergamo, Italy (MCSN); the Macedonian National Collection of Invertebrates, Department for Invertebrates at the Institute of Biology (Faculty of Natural Sciences and Mathematics), Skopje, Republic of North Macedonia (MNCI); the Collection of Entomology, Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); the M. Valladolid Collection, Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales, Madrid, Spain (MVC); the Collection of Entomology, Natural History Museum, London, United Kingdom (NHMUK, previously BMNH); the Collection of Entomology, National Museum, Praga, Czech Republic (NMP), the Oláh Private Collection, Debrecen, Hungary (OPC) and the World Museum of Liverpool, Liverpool, United Kingdom (WML).

Material examined

Adults.

Bosnia and Herzegovina: *R. fasciata*: 1 ♂ + 3 ♀, river Bosna, Zenica-Doboj, Maglaj, 44.550N, 18.105E, 170 m a.s.l., 29–31/vii/13, leg. J. Louda [ID Data number of Access Catalog 28055 (MCSN)].

Croatia: *R. fasciata*: 1 ♂ + 1 ♀ (K1–K2), river Kutjevačka, upper course, 45.476N, 17.866E, 470 m a.s.l., 16/v/05, leg. R. Čuk [nos. 5400–5401 (CNHM)]. 3 ♂, river Veličanka, upper course, near Požega, 45.491N, 17.649E, 430 m a.s.l., 03/v/15, leg. R. Čuk [nos. 5402–5404 (CNHM)]. 4 ♂, river Voćinska, upper course, Papuk Mt., 45.619N, 17.544E, 215 m a.s.l., 19/v/15, leg. P. Crnčan [nos. 5405–5408 (CNHM)]. 3 ♂ + 1 ♀, 03/viii/15; 3 ♂, 28/x/15, river Rijeka, Bastaji, 45.636N, 17.316E, 150 m a.s.l., leg. R. Čuk [nos. 5409–5415 (CNHM)]. 1 ♀, 15/v/15; 2 ♂ + 7 ♀, 05/viii/15, river Pakra, Kusonje, 45.457N, 17.226E, 180 m a.s.l., leg. A. Čukušić & A. Delić [nos. 5416–5425 (CNHM)].

Czech Republic: *R. fasciata*: 4 ♂ + 1 ♀, Jizerské mountains, brook Ješkrabec, Rejdice (N Bohemia), 50.733N, 15.365E, 650–750 m a.s.l., 02/vi/08 [3 ♂ + 1 ♀ (NMP); 1 ♂, MNCN_Ent 269386 (MNCN)]. 2 ♂ + 2 ♀, Bohemian Switzerland National Park, brook Bílý (N Bohemia), 50.925N, 14.401E, 330 m a.s.l., 22/vi/10 [1 ♂ + 1 ♀ (NMP); 1 ♂, MNCN_Ent 269384, 1 ♀, MNCN_Ent 269385 (MNCN)]. 5 ♂ + 2 ♀, Bílé Karpaty mountains, stream Drietomice (E Moravia), 48.952N, 17.875E, 390 m a.s.l., 08/vii/10 [4 ♂ + 1 ♀ (NMP); 1 ♂, MNCN_Ent 269390, 1 ♀, MNCN_Ent 269391 (MNCN)]. 3 ♂, brook near Nové Strašecí (C Bohemia), 50.145N, 13.827E, 430 m a.s.l., 31/v/11 [2

♂ (NMP); 1 ♂, MNCN_Ent 269389 (MNCN)]. 1 ♂ + 1 ♀, Krušné hory mountains, stream Hluboký, near Dolní Nivy (W Bohemia), 50.236N, 12.604E, 540 m a.s.l., 31/viii/15 [1 ♂, MNCN_Ent 269387, 1 ♀, MNCN_Ent 269388 (MNCN)], leg. P. Chvojka.

Kosovo: *R. fasciata*: 4 ♂, Shushtë, 42.281N, 21.359E, 570 m a.s.l., 15/xi/19 [MNCN_Ent 269410, MNCN_Ent 269411, MNCN_Ent 269412, MNCN_Ent 269413 (MNCN)]. 1 ♂, Lugu i Kopilaqës, 42.246N, 21.431E, 1175 m a.s.l., 3/xi/19 [MNCN_Ent 269414 (MNCN)], leg. H. Ibrahim.

Finland: *R. fasciata*: 7 ♂ + 2 ♀, 3–25/vi/16 [2 ♀, MNCN_Ent 269376, MNCN_Ent 269377; 2 ♂, MNCN_Ent 269378, MNCN_Ent 269379 (MNCN); 2 ♂ (MCSN), ID Data number of Access Catalog 39740; 3 ♂ (MVC)]; 5 ♂ + 5 ♀, 18/7–19/viii/16 [2 ♀ MNCN_Ent 269380, MNCN_Ent 269381; 2 ♂ MNCN_Ent 269382, MNCN_Ent 269383 (MNCN); 2 ♀ (MCSN), ID Data number of Access Catalog 39741; 3 ♂ + 1 ♀ (MVC)], Tampere, brook Tiikonoja (Pirkanmaa), 61.720N, 24.031E, 120 m a.s.l., leg. I. Rasimus. 1 ♂ + 1 ♀, Kuhmo, Viiksimonjoki, 64.311N, 30.299E, 195 m a.s.l., 10/viii/19 [1 ♂, MNCN_Ent 269403, 1 ♀, MNCN_Ent 269404 (MNCN)], leg. E. Viitanen. 1 ♂ + 1 ♀, Kuusamo, brook Putaanoja, 66.380N, 29.424E, 225 m a.s.l., 12/ix/02 [1 ♂, MNCN_Ent 269804, 1 ♀, MNCN_Ent 269416 (MNCN)], leg. J. Salokannel.

Poland: *R. fasciata*: 4 ♂, river Pasłęka (NE Poland), 53.618N, 20.326E, 180 m a.s.l., 11/v/85 (MVC). 1 ♀, river Łyna (NE Poland), 53.441N, 20.413E, 190 m a.s.l., 11/v/91 (MVC), leg. S. Czachorowski.

Republic of North Macedonia: *R. fasciata*: 4 ♂, river Trnovska, Osogovo Mountain, 42.258N, 22.361E, 820 m a.s.l., 12/viii/18 [MNCI_RfT10J01, MNCI_RfT10J02, MNCI_RfT10J03, MNCI_RfT10J04 (MNCI)]. 3 ♂, river Kriva before Kriva Palanka, Osogovo Mountain, 42.231N, 22.363E, 710 m a.s.l., 12/viii/18 [MNCI_RfT8J01, MNCI_RfT8J02, MNCI_RfT8J03 (MNCI)], leg. J. Hinić & V. Slavevska Stamenković.

Serbia: *R. fasciata*: 1 ♂, river Kamenica, 44.048N, 20.045E, 540 m a.s.l., 17/xi/19 [MNCN_Ent 269451 (MNCN)], leg. D. Stojanov.

Slovakia: *R. fasciata*: 2 ♂ + 3 ♀, High Tatras, stream Biely Váh (N Slovakia), 49.133N, 20.021E, 1400 m a.s.l., 7/x/90 [1 ♂ + 2 ♀ (NMP); 1 ♂, MNCN_Ent 269392, 1 ♀, MNCN_Ent 269393 (MNCN)]. 8 ♂ + 3 ♀, Oravské Beskydy Mountains, left tributary of stream Bystrá below Babia hora mountain (N Slovakia), 49.560N, 19.510E, 1200 m a.s.l., 15/x/91 [6 ♂ + 1 ♀ (NMP); 1 ♂, MNCN_Ent 269394, 1 ♀, MNCN_Ent 269395 (MNCN); 1 ♂ + 1 ♀ (OPC)]. 1 ♂, Strážov mountains, stream Sučiansky, NW Nitrianske Sučany (W Slovakia), 48.745N, 18.450E, 450 m a.s.l., 24/vi/15 [MNCN_Ent 269415 (MNCN)], leg. P. Chvojka.

United Kingdom. *R. fasciata* (= *R. septentrionis*): 1 ♂ (paratype), near Longniddry & Aberlady, 18/vi/1865 [NHMUK 013803988 (NHMUK)]. 1 ♀ (cleared preparation), Lanark, Carlisle, 1882 [NHMUK 010115586 (NHMUK)]. 1 ♂, Afon Marslandwr, 53.290N, 3.241W, 110 m a.s.l., 16/v/99. 1 ♀, stream, Hendre, 53.199N, 3.211W, 160 m a.s.l., 25/iv/99 (WML), leg. B. & I.D. Wallace.

Pupae

Finland. *R. fasciata*: 1 immature pupa, Kuusamo, brook Oulanka, 66.326N, 29.537E, 190 m a.s.l., 14/vi/19 [MNCN_Ent 269407 (MNCN)], leg. P. Majuri.

Poland. *R. fasciata*: 3 immature pupae (IP) + 1 ♂P + 1 ♀P, Rurka, river Rurzyca (W Poland), 52.999N, 14.481E, 130 m a.s.l., 07/xii/96; 6 IP, 17/ix/97 (MVC). 1 ♂P + 1 ♀P, river Walsza (NE Poland), 54.236N, 20.128E, 70 m a.s.l., 19/iv/99 (MVC). 6 IP + 1 ♂P + 1 ♀P, Gaznoty, river Gizela (NE Poland), 53.534N, 19.901E, 190 m a.s.l., 9/vi/99 (MVC). 6 IP + 1 ♂P + 1 ♀P, Zajączki, river Gizela (NE Poland), 53.560N, 19.882E, 170 m a.s.l., 9/vi/99 (MVC); 9 IP + 2 ♀P, 17/x/00 (MVC). 5 IP + 2 ♂P + 3 ♀P, river Mała Wkra (NE Poland), 53.552N, 19.964E, 140 m a.s.l., 17/iv/00 (MVC). 10 IP + 4 ♂P + 6 ♀P, Elbląg, river Kumiela (N Poland), 54.158N, 19.414E, 15 m a.s.l., 10/vii/03 (MVC). 4 IP + 1 ♂P + 2 ♀P, brook Srebrny Potok (NE Poland), 54.162N, 19.491E, 135 m a.s.l., 11/vii/03 (MVC), leg. S. Czachorowski.

Russia. *R. fasciata*: 1 ♂P + 1 ♀P + 1 IP, Moscow, Tuchkovo, spring brook near river Moskva, 55.627N, 36.386E, 170 m a.s.l., 21/iv/19 [1 ♂P, MNCN_Ent 269400, 1 ♀P, MNCN_Ent 269401 (MNCN), 1 IP (MVC)], leg. M. Chertoprud.

United Kingdom. *R. fasciata* (= *R. septentrionis*): 1 ♂P + 1 ♀P, stream, Ysceifiog, 53.237N, 3.275W, 140 m a.s.l., 20/ix/20 [1 ♂P, MNCN_Ent 269447, 1 ♀P, MNCN_Ent 269446 (MNCN)], leg. B. & I.D. Wallace.

Larvae

Finland. *R. fasciata*: 1 L, Tampere, brook Keltolampi (Pirkanmaa), 61.609N, 23.997E, 110 m a.s.l., 22/v/18

(MVC), leg. J. Salokannel. 2 L, Obb Simo, brook Kuivasoja, 66.005N, 25.459E, 110 m a.s.l., 17/x/17 [MNCN_Ent 269405, MNCN_Ent 269406 (MNCN)]. 1 L, Kuusamo, brook Oulanka, 66.326N, 29.5376E, 190 m a.s.l., 14/06/19 [MNCN_Ent 269407 (MNCN)], leg. P. Majuri. 1 L, Kuusamo, brook Uopajanpuro, 66.339N, 29.520 E, 180 m a.s.l., 14/vi/19 [MNCN_Ent 269409 (MNCN)], leg. P. Majuri.

Poland. *R. fasciata*: 2 L, Góry Stołowe, brook Kudowski Potok (SW Poland), 50.482N, 16.337E, 840 m a.s.l., 19/ix/94 (MVC). 1 L, Gaznoty, river Gizela (NE Poland), 53.534N, 19.901E, 190 m a.s.l., 9/vi/99 (MVC); 1 L, 27/v/09 (MVC). 6 L, Zajączki, river Gizela (NE Poland), 53.560N, 19.882E, 170 m a.s.l. 17/x/00 (MVC). 1 L, river Mała Wkra (NE Poland), 53.552N, 19.964E, 140 m a.s.l., 17/iv/00 (MVC). 5 L, Elbląg, river Kumiela (N Poland), 54.158N, 19.414E, 15 m a.s.l., 10/vii/03 (MVC), leg. S. Czachorowski.

Russia. *R. fasciata*: 3 L, Moscow, Lesodolgorukovo forest, Ryjiy Ovrage, stream Bruchanicha, 56.036N, 36.440E, 270 m a.s.l., 01/i/14 [2 L, MNCN_Ent 269396, MNCN_Ent 269397 (MNCN); 1 L (MVC)]. 3 L, Moscow, Tuchkovo, spring brook near river Moskva, 55.627N, 36.386E, 170 m a.s.l., 21/iv/19 [1 L, MNCN_Ent 269402 (MNCN); 2 L (MVC)]. 1 L, Murmansk, Luvenga, stream Medvejiy, 67.136N, 32.702E, 150 m a.s.l., 25/vi/19 [MNCN_Ent 269399 (MNCN)]. 5 L, Moscow, Paramonovo, stream near river Volgusha, 56.242N, 37.405E, 150 m a.s.l., 14/ix/19, [1 L, MNCN_Ent 269398 (MNCN); 4 L (MVC)], leg. M. Chertoprud.

United Kingdom. *R. fasciata* (= *R. septentrionis*): 1 L, stream, Ysceifiog, 53.237N, 3.275W, 140 m a.s.l., 20/ix/20 [MNCN_Ent 269448 (MNCN)]. 1 L, stream, Maes-maenan, 53.239N, 3.321W, 95 m a.s.l., 20/ix/20 [MNCN_Ent 269449 (MNCN)], leg. B. & I.D. Wallace.

Methods

Morphology

Morphological characters of adults, pupae, and larvae were examined and the last instars of larvae also measured with a binocular microscope. Selected parts of genitalia from adult males (parameres) were photographed using a light microscope and multiple stacked images were integrated with focal stacking software (Auto-Montage Essentials, Syncroscopy©). The following descriptions of *R. fasciata* and synonymized species (*R. ferruginea*, *R. septentrionis*) were consulted: Bertrand (1954), Buholzer (1978), Edington & Hildrew (1981), Eidel (1974), Fotius-Jaboulet (1964), Hagen (1859), Hickin (1954, 1967), Higler (2005), Klapálek (1893), Kumanski (1973, 1985), Lepneva (1964), Mackereth (1954), Malicky (2004), Malicky & Sipahiler (1993), McLachlan (1865, 1868, 1879), Nielsen (1942), Nývák (1963), Pitsch (1993), Rinne & Wiberg-Larsen (2017), Rousseau (1921), Schmid (1970), Scopoli (1763), Sedlak (1985), Silfvenius (1905), Tobias & Tobias (2010), Ulmer (1909), and Waringer & Graf (1997, 2011).

Terminology for larval structures, including chaetae and sensorial pits, follow Williams & Wiggins (1981) and Friedrich *et al.* (2015); for adult genitalia according to Schmid (1970); and for wing venation according to Ulmer (1909) and Holzenthal *et al.* (2007).

DNA Extraction, Amplification, and Sequencing

Muscle tissue of legs and thorax from adults, larvae, and pupae was dissected. Samples were fully immersed in 0.5 ml digestion buffer (Gilbert *et al.* 2007) and incubated overnight at 55°C with gentle agitation. The buffer consisted of 5 mM CaCl₂, 2% sodium dodecyl sulphate (SDS), 40 mM dithiothreitol (DTT), 250 mg/ml proteinase K, 10 mM Tris buffer pH 8.0, 2.5 mM EDTA (Ethylene-Diamine-Tetra-Acetic acid) pH 8.0, and 10 mM NaCl₂ (final concentrations). After incubating, nucleic acids were extracted from the digestion buffer using a Qiaquick PCR purification kit (QIAGEN, Alda *et al.* 2007). A 508 bp region of the mitochondrial COI gene (Cytochrome Oxidase I) was amplified with the primers C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer *et al.* 1994; Simon *et al.* 1994). Three microlitres of the DNA solution were used as a template. Other components of the 25 µl PCR reaction were: 1x of the corresponding buffer [75 mM Tris HCl, pH 9.0; 50 mM KCl and 20 mM (NH₄)₂SO₄], 2 mM MgCl₂, 10 mM dNTPs mix, 0.1 µM of both primers, 0.02% BSA, and 0.125 units AmpliTaq Gold® DNA Polymerase (Applied Biosystems). Five microlitres of PCR products were electrophoresed through a 1.5% agarose gel and visualized with SYBR Safe™ DNA Gel Stain (Invitrogen) under ultraviolet light. PCR products were purified by treatment with ExoSAP-IT (USB Amersham, Buckinghamshire, UK) and incubated at 37 °C for 45 min, followed by 80 °C for 15 min to inactivate

the enzyme. Purified PCR product was then used to sequence in both directions using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc., Foster City, USA) in a 10 µL volume, containing 15–20 ng of purified product and 3 pmol of primer. To verify that the sequences obtained came from a *Rhyacophila*, they were compared with sequences from GenBank using Blast (Altschul *et al.* 1997). The alignment of all *Rhyacophila* COI gene sequences generated in our lab was performed and edited manually using MEGA X (Kumar *et al.* 2018). Fine adjustments were made by eye, as the COI does not present any gaps.

A total of 162 DNA sequences were obtained and used for the study: 118 were prepared in laboratory, 42 were downloaded from GenBank (2020) (accession numbers in Table 1) and BOLD (2020) and 2 sequences were reported directly by Valentina Stamenković and Jelena Hinić.

Phylogenetic analyses were accomplished using maximum likelihood (ML) and Bayesian inference (BI) methods. The CIPRES Science Gateway v.3.3 online server (Miller *et al.* 2010) was used to run jModeltest v.2.1.9 (Darriba *et al.* 2012; Guindon & Gascuel 2003) which was used to calculate the nucleotide substitution model and the ML with RAxML-HPG BlackBox (Stamatakis 2014), using 10,000 bootstrap replicates to assess node support. BI analyses were run in MrBayes-3.2.7 (Ronquist *et al.* 2012). We used the substitution model space with the option lset nst=mixed rates=invgamma, for 1.5×10^6 generations, discarding the first 25% generations as burn-in and synthesizing the resulting cladogram. Distances among and within species were calculated using the maximum composite likelihood and p-distance models with MEGA X. The trees with summarized information were made with MEGA X. Nucleotide sequences were deposited in GenBank (2021) (accession numbers and MNCN: ADN collection numbers in Table 1).

Geographical distribution

The analysis of the species distribution was based on the localities of the studied samples, the Global Biodiversity Information Facility page (GBIF 2020) and references from literature as follows: **Europe:** Botoşăneanu (1967), Malicky & Sipahiler (1993), Neu *et al.* (2018), Oláh *et al.* (2020). **Austria:** Adlmannseider (1965, 1973), Graf *et al.* (2006), Komzák & Chvojka (2018), Kownacka & Margreiter (1978), Malicky & Sipahiler (1993), Satori (1942), Schultz *et al.* (2006). **Belgium:** Botoşăneanu & Schmid (1973), McLachlan (1879), Smissaert (1959), Stroot (1984a, 1984b). **Bosnia and Herzegovina:** Botoşăneanu (1960, 1961), Stanić-Koštroman (2009), Stanić-Koštroman *et al.* (2012, 2015). **Bulgaria:** Botoşăneanu (1956), Kownacki & Kownacka (1973), Kumanski (1969, 1971, 1997), Malicky & Sipahiler (1993), Russev (1961), Uherkovich & Nógrádi (2002). **Croatia:** Cerjanec (2012), Graf *et al.* (2008), Habdija *et al.* (2004), Kučinić (2002), Kučinić *et al.* (2008, 2010, 2011, 2017), Malicky (2009), Marinković-Gospodnetić (1979), Oláh (2010), Previšić *et al.* (2007, 2010, 2013), Previšić & Popijač (2010), Vučković (2011). **Czech Republic:** Adámek (1977), Chvojka (2008), Chvojka & Komzák (2006, 2008), Chvojka & Novák (2001), Klapálek (1890, 1891, 1897), Komzák & Chvojka (2012, 2018), Macek *et al.* (2019), McLachlan (1879), Novák (1962, 1971, 1996), Obr (1984), Sedlák (1967, 2001), Skála *et al.* (2019), Soldán *et al.* (2001), Sukop (2008). **Denmark:** Gørtz (1998), Lindegaard & Thorup (1975), Moth Iversen *et al.* (1978), Thorup & Lindegaard (1977), Wiberg-Larsen *et al.* (2000). **Estonia:** Timm, personal communication, Viidalepp *et al.* (2011). **Finland:** McLachlan (1879), Salokannel & Mattila (2018). **France:** Berland & Mosely (1937), Cayrou *et al.* (2000), Décamps (1967a, 1967b), Faessel *et al.* (1993), Fotius-Jaboulet (1964), Guerold *et al.* (1991), McLachlan (1879), Mosely (1932), Statzner (2011), Verneux (1968). **Germany:** Burmeister & Burmeister (1974), Caspers (1972), Eidel (1974), Fey & Mertschenk (1977), Franz (1980), Görtz (1988), Hackbarth *et al.* (2005), Hagen (1859), Harthum (1999), Hohmann (2010), Illies (1953), Marten *et al.* (1996), Mey (2019), Richarz (1983), Röser (1979), Schröder (1986), Thienemann (1912), Ulmer (1909), Werner & Werner (1968). **Greece:** Malicky (2005). **Hungary:** Andrikovics & Kéri (1991), Csabai *et al.* (2005), Ficsór & Szabó (2011), Kiss (2008), Kiss *et al.* (2006), Móra & Csabai (2002), Móra *et al.* (2006, 2007, 2011), Nándor *et al.* (2005), Nógrádi (1984, 1989), Nógrádi & Uherkovich (1988, 1989, 1990a, 1990b, 1994, 2002), Nógrádi *et al.* (1984, 1991, 1996, 1999), Novák (1962), Nowinszky *et al.* (2014), Schmera (2001), Schmera & Eros (2006), Uherkovich & Nógrádi (1988, 1991, 1994, 1999, 2006). **Italy:** Cianficconi & Moretti (1987), Lodovici & Valle (2020a, 2020b), Valle (2001), Wolf *et al.* (2012). **Kosovo:** Ibrahimović & Sejdiu (2018); Ibrahimović *et al.* (2012a, 2012b, 2014, 2016, 2018, 2019a, 2019b). **Lithuania:** Plūraitė & Kesminas (2004), Višinskienė (2009). **Luxembourg:** Gerecke *et al.* (2005), Schrankel *et al.* (2002). **Netherlands:** Fischer (1934), Smissaert (1959). **Norway:** Andersen & Hagenlund (2012), Andersen *et al.* (1990, 1993a, 1993b). **Poland:** Buczyńska (2008), Buczyński *et al.* (2003, 2012), Czachorowski (1988), Czachorowski & Veith (2006), Czachorowski *et al.* (1993, 1998), Górecki (2016), Kownacka (1971), Majecki (2004), Małek (2001),

TABLE 1. Data for specimens analyzed by DNA. Species numbers refer to sequences included in the study. Sex: A= adult (sex not specified), M= male, F= female, MP= male pupa, FP = female pupa, L= larvaN, E = latitudinal and longitudinal geographical coordinates, respectively. Alt = altitude (m a.s.l.). Accession numbers from GenBank and Tissues and DNA Collection of Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas (MNCN, CSIC).

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
<i>fasciata</i> 1	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX293777	
<i>fasciata</i> 2		Trøgstad, Indre Østfold (Norway)	59.746	11.272	115	KX293282	
<i>fasciata</i> 3	F	Finmark, Alta, Gargiaveien (Norway)	69.822	23.480	95	KX293192	
<i>fasciata</i> 4	L	Kuusamo, Purkupuutaanoja (Finland)	66.380	29.424	225	KX292544	
<i>fasciata</i> 5		Eidsberg, Indre Østfold (Norway)	59.603	11.286	130	KX295452	
<i>fasciata</i> 6		Flateby (Norway)	59.827	11.174	124	KX291568	
<i>fasciata</i> 7	A	Bavaria, Ueberacker (Germany)	48.234	11.298	505	KX291079	
<i>fasciata</i> 8	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX294646	
<i>fasciata</i> 9	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX294688	
<i>fasciata</i> 10	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX292344	
<i>fasciata</i> 11	F?	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX296294	
<i>fasciata</i> 12		Limburg, Veurs, Voeren (Belgium)	50.749	5.814	130	KX142884	
<i>fasciata</i> 13	M	Modrava, (Czech Republic)	48.979	13.431	1200	HQ959079	
<i>fasciata</i> 25	F	Trondheim, Nidelva (Norway)	63.339	10.442	70	KX294414	
<i>fasciata</i> 26	M	Trondheim, Nidelva (Norway)	63.339	10.442	70	KX291512	
<i>fasciata</i> 27	L	Paltamo, Vaarainjoki (Finland)	64.472	27.630	175	KX141034	
<i>fasciata</i> 28	L	Paltamo, Vaarainjoki (Finland)	64.472	27.630	175	KX143671	
<i>fasciata</i> 29	L	Mustjala, Moeldrioja (Estonia)	58.490	22.303	5	KX294243	
<i>fasciata</i> 30		Södermanland (Sweden)	59.152	17.599	20	KM225308	
<i>fasciata</i> 31		Vlaams-Brabant, Kappitel, (Belgium)	50.733	4.295	50	KX142866	
<i>fasciata</i> 32		Vlaams-Brabant, Kappitel, (Belgium)	50.733	4.295	50	KX142423	
<i>fasciata</i> 33		Limburg, Veurs, Voeren (Belgium)	50.749	5.814	130	KX142016	
<i>fasciata</i> 34		Limburg, Veurs, Voeren (Belgium)	50.749	5.814	130	KX142000	
<i>fasciata</i> 35	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816342	MNCN:ADN 92790
<i>fasciata</i> 36	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816343	MNCN:ADN 92791
<i>fasciata</i> 37	M	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816344	MNCN:ADN 92792
<i>fasciata</i> 38	M	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816345	MNCN:ADN 92793
<i>fasciata</i> 39	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816346	MNCN:ADN 92794
<i>fasciata</i> 40	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816347	MNCN:ADN 92795

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
<i>fasciata</i> 41	M	Tampere, Tikononoja (Finland)	61.720	24.031	120	MT816348	MNCN:ADN 92796
<i>fasciata</i> 42	M	Tampere, Tikononoja (Finland)	61.720	24.031	120	MT816349	MNCN:ADN 92797
<i>fasciata</i> 46	A	Hesse, Breitenbach (Germany)	50.659	9.629	240	KY262289	
<i>fasciata</i> 47	A	Hesse, Breitenbach (Germany)	50.659	9.629	240	KY262252	
<i>fasciata</i> 48	A	Hesse, Breitenbach (Germany)	50.662	9.624	280	KY262207	
<i>fasciata</i> 50	A	Hesse, Breitenbach (Germany)	50.659	9.629	240	KY261630	
<i>fasciata</i> 51	A	Hesse, Breitenbach (Germany)	50.662	9.624	280	KY261529	
<i>fasciata</i> 52	A	Hesse, Breitenbach (Germany)	50.662	9.624	280	KY261518	
<i>fasciata</i> 53	A	Hesse, Breitenbach (Germany)	50.662	9.624	280	KY261298	
<i>fasciata</i> 54	A	Hesse, Breitenbach (Germany)	50.662	9.624	280	KY261164	
<i>fasciata</i> 57	MP	River Kumiela, Elblag (Poland)	54.158	19.414	15	MT816350	MNCN:ADN 94323
<i>fasciata</i> 58	FP	River Kumiela, Elblag (Poland)	54.158	19.414	15	MT816351	MNCN:ADN 94324
<i>fasciata</i> 59	MP	River Mala Wkra (Poland)	53.552	19.964	140	MT816352	MNCN:ADN 94332
<i>fasciata</i> 60	FP	River Mala Wkra (Poland)	53.552	19.964	140	MT816353	MNCN:ADN 94333
<i>fasciata</i> 61	MP	River Walsza (Poland)	54.236	20.128	70	MT816354	MNCN:ADN 94334
<i>fasciata</i> 62	FP	River Walsza (Poland)	54.236	20.128	70	MT816355	MNCN:ADN 94335
<i>fasciata</i> 63	MP	River Gizela, Gaznoty (Poland)	53.534	19.901	190	MT816356	MNCN:ADN 94340
<i>fasciata</i> 64	FP	River Gizela, Gaznoty (Poland)	53.534	19.901	190	MT816357	MNCN:ADN 94341
<i>fasciata</i> 65	FP	River Gizela, Zajączki (Poland)	53.560	19.882	170	MT816358	MNCN:ADN 94342
<i>fasciata</i> 66	FP	River Gizela, Zajączki (Poland)	53.560	19.882	170	MT816359	MNCN:ADN 94343
<i>fasciata</i> 67	MP	Srebrny Potok (Poland)	54.162	19.491	135	MT816360	MNCN:ADN 94344
<i>fasciata</i> 68	FP	Srebrny Potok (Poland)	54.162	19.491	135	MT816361	MNCN:ADN 94345
<i>fasciata</i> 69	M	Bílý potok, Bohemia (Czech Republic)	50.925	14.401	330	MT816362	MNCN:ADN 94391
<i>fasciata</i> 70	F	Bílý potok, Bohemia (Czech Republic)	50.925	14.401	330	MT816363	MNCN:ADN 94392
<i>fasciata</i> 71	M	Ješkrabec, Rejdice, Bohemia (Czech Republic)	50.733	15.365	700	MT816364	MNCN:ADN 94393
<i>fasciata</i> 72	M	Hluboký potok, Bohemia (Czech Republic)	50.236	12.604	540	MT816365	MNCN:ADN 94394
<i>fasciata</i> 73	F	Hluboký potok, Bohemia (Czech Republic)	50.236	12.604	540	MT816366	MNCN:ADN 94395
<i>fasciata</i> 74	M	Brook W Nové Strašceci (Czech Republic)	50.145	13.827	430	MT816367	MNCN:ADN 94396
<i>fasciata</i> 75	M	Drietomice, Moravia (Czech Republic)	48.952	17.875	390	MT816368	MNCN:ADN 94398
<i>fasciata</i> 76	F	Drietomice, Moravia (Czech Republic)	48.952	17.875	390	MT816369	MNCN:ADN 94399
<i>fasciata</i> 77	F	Tatras, Biely Váh (Slovakia)	49.133	20.021	1400	MT816370	MNCN:ADN 94401

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
<i>fasciata</i> 78	F	Tributary of Bystrá (Slovakia)	49.560	19.510	1200	MT816371	MNCN:ADN 94404
<i>fasciata</i> 80	M	River Kutjevačka (Croatia)	45.476	17.866	470	MT559339	MNCN:ADN 96487
<i>fasciata</i> 81	F	River Kutjevačka (Croatia)	45.476	17.866	470	MT559340	MNCN:ADN 96488
<i>fasciata</i> 82	M	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559341	MNCN:ADN 109896
<i>fasciata</i> 83	F	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559342	MNCN:ADN 109897
<i>fasciata</i> 84	F	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559343	MNCN:ADN 109898
<i>fasciata</i> 85	F	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559344	MNCN:ADN 109899
<i>fasciata</i> 86	L	Bruchanicha str., Ryjijy Ovrage, (Russia)	56.036	36.440	270	MT816372	MNCN:ADN 109948
<i>fasciata</i> 87	L	Bruchanicha str., Ryjijy Ovrage, (Russia)	56.036	36.440	270	MT816373	MNCN:ADN 109949
<i>fasciata</i> 88	L	Stream near Volgusha river (Russia)	56.242	37.405	150	MT816374	MNCN:ADN 109952
<i>fasciata</i> 89	L	Stream Medvejy, Murmansk (Russia)	67.136	32.702	150	MT816375	MNCN:ADN 109954
<i>fasciata</i> 90	MP	Spring brook near Moskva river (Russia)	55.627	36.386	170	MT816376	MNCN:ADN 109955
<i>fasciata</i> 91	FP	Spring brook near Moskva river (Russia)	55.627	36.386	170	MT816377	MNCN:ADN 109956
<i>fasciata</i> 92	L	Spring brook near Moskva river (Russia)	55.627	36.386	170	MT816378	MNCN:ADN 109957
<i>fasciata</i> 93	M	Kuhmo, Viiksimonjoki (Finland)	64.311	30.299	195	MT816379	MNCN:ADN 110209
<i>fasciata</i> 94	F	Kuhmo, Viiksimonjoki (Finland)	64.311	30.299	195	MT816380	MNCN:ADN 110210
<i>fasciata</i> 95	L	Obb Simo, Kuivasoja brook (Finland)	66.005	25.459	110	MT816381	MNCN:ADN 110211
<i>fasciata</i> 96	L	Obb Simo, Kuivasoja brook (Finland)	66.005	25.459	110	MT816382	MNCN:ADN 110212
<i>fasciata</i> 97	L	Kuusamo, Oulanka brook (Finland)	66.326	29.537	190	MT816383	MNCN:ADN 110213
<i>fasciata</i> 98	M	Kuusamo, Putaanoja brook (Finland)	66.380	29.424	225	MT816384	MNCN:ADN 110214
<i>fasciata</i> 99	L	Kuusamo, Uopajampuro brook (Finland)	66.339	29.520	180	MT816385	MNCN:ADN 110216
<i>fasciata</i> 100	M	Shushtë (Kosovo)	42.281	21.359	570	MT816386	MNCN:ADN 110218
<i>fasciata</i> 101	M	Shushtë (Kosovo)	42.281	21.359	570	MT816387	MNCN:ADN 110219
<i>fasciata</i> 102	M	Shushtë (Kosovo)	42.281	21.359	570	MT816388	MNCN:ADN 110220
<i>fasciata</i> 103	M	Shushtë (Kosovo)	42.281	21.359	570	MT816389	MNCN:ADN 110221
<i>fasciata</i> 104	M	Lugu i Kopilaqës (Kosovo)	42.246	21.431	1175	MT816390	MNCN:ADN 110225
<i>fasciata</i> 105	M	River Trnovska (Republic of North Macedonia)	42.258	22.361	820	MW703665	
<i>fasciata</i> 106	M	River Kriva (Republic of North Macedonia)	42.231	22.363	710	MW703664	
<i>fasciata</i> 107	M	River Kamenica (Serbia)	44.048	20.045	540	MW703663	MNCN:ADN 110312
<i>septrionis</i> 1		Nothumberland National Park (United Kingdom)	55.317	-2.137	240	KY225476	
<i>septrionis</i> 2	M	Afon Marslandwr (United Kingdom)	53.290	-3.241	110	MW703657	MNCN:ADN 110304

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
<i>septrionis</i> 3	FP	Ysceifog, stream (United Kingdom)	53.237	-3.275	140	MW703658	MNCN:ADN 110305
<i>septrionis</i> 4	MP	Ysceifog, stream (United Kingdom)	53.237	-3.275	140	MW703659	MNCN:ADN 110306
<i>septrionis</i> 5	L	Ysceifog, stream (United Kingdom)	53.237	-3.275	140	MW703660	MNCN:ADN 110307
<i>septrionis</i> 6	L	Maes-maenan, stream (United Kingdom)	53.239	-3.321	95	MW703661	MNCN:ADN 110308
<i>f. delici</i> G1	M	River Gacka, Otočac (Croatia)	44.787	15.368	475	MT559345	MNCN:ADN 96483
<i>f. delici</i> G2	M	River Gacka, Otočac (Croatia)	44.787	15.368	475	MT559346	MNCN:ADN 96484
<i>f. delici</i> V1	M	Vukovića spring, river Cetina (Croatia)	43.965	16.413	375	MT559347	MNCN:ADN 96485
<i>f. delici</i> V2	F	Vukovića spring, river Cetina (Croatia)	43.965	16.413	375	MT559348	MNCN:ADN 96486
<i>f. delici</i> D1	M	River Dretulja, spring (Croatia)	45.074	15.343	390	MT559349	MNCN:ADN 96489
<i>f. delici</i> D2	F	River Dretulja, spring (Croatia)	45.074	15.343	390	MT559350	MNCN:ADN 96490
<i>f. delici</i> C1	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559351	MNCN:ADN 96491
<i>f. delici</i> C2	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559352	MNCN:ADN 96492
<i>f. delici</i> C3	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559353	MNCN:ADN 103406
<i>f. delici</i> C4	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559354	MNCN:ADN 103407
<i>f. delici</i> B11	M	River Bijela, NP Plitvice Lakes (Croatia)	44.833	15.557	750	MT559355	MNCN:ADN 103408
<i>f. delici</i> R1	M	Roški slap (waterfall), National Park Krka (Croatia)	43.906	15.975	80	MT559356	MNCN:ADN 103409
<i>f. delici</i> R2	F	Roški slap (waterfall), National Park Krka (Croatia)	43.906	15.975	80	MT559357	MNCN:ADN 103410
<i>f. delici</i> BR1	M	Brkljača channel, Udovičić (Croatia)	43.656	16.732	290	MT559358	MNCN:ADN 103411
<i>f. delici</i> CT1	F	River Cetina, tributary, Civljani (Croatia)	43.948	16.401	375	MT559359	MNCN:ADN 103412
<i>f. delici</i> BT1	M	River Butišnica, Golubić (Croatia)	44.111	16.227	350	MT559360	MNCN:ADN 103413
<i>f. delici</i> BU1	F	Brušane (Gospić) (Croatia)	44.505	15.236	620	MT559361	MNCN:ADN 109891
<i>f. delici</i> B1	L	River Bosna, Vrelo Bosne (Bosnia and Herzegovina)	43.816	18.273	560	MT772015	
<i>f. delici</i> B2	L	River Bosna, Vrelo Bosne (Bosnia and Herzegovina)	43.816	18.273	560	MT772017	
<i>f. delici</i> M1	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT765286	
<i>f. delici</i> M2	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT765287	
<i>f. delici</i> M3	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT765290	
<i>f. delici</i> M4	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT772020	
<i>f. delici</i> M5	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT772022	
<i>f. delici</i> M6	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT772028	
<i>f. delici</i> M7	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT772029	
<i>f. delici</i> RK	M	River Raska (Serbia)	43.133	20.435	560	MW703662	MNCN:ADN 110309

.....continued on the next page

TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
<i>denticulata</i> 1	M	River Balameth, Bethmale (France)	42.865	1.092	1000	MF347380	MNCN:ADN 49487
<i>denticulata</i> 2	M	River Balameth, Bethmale (France)	42.865	1.092	1000	MF347381	MNCN:ADN 49488
<i>denticulata</i> 3	F	River Balameth, Bethmale (France)	42.865	1.092	1000	MF683825	MNCN:ADN 96497
<i>kykladica</i> 1	M	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422501	MNCN:ADN 94016
<i>kykladica</i> 2	M	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422502	MNCN:ADN 94017
<i>kykladica</i> 3	M	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422503	MNCN:ADN 94018
<i>kykladica</i> 4	L	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422504	MNCN:ADN 94424
<i>kykladica</i> 5	L	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422505	MNCN:ADN 94425
<i>kykladica</i> 6	MP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422506	MNCN:ADN 94426
<i>kykladica</i> 7	FP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422507	MNCN:ADN 94427
<i>kykladica</i> 8	FP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422508	MNCN:ADN 94428
<i>kykladica</i> 9	MP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422509	MNCN:ADN 94429
<i>sociata</i> 1	M	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347382	MNCN:ADN 57431
<i>sociata</i> 2	M	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347383	MNCN:ADN 57433
<i>sociata</i> 3	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347384	MNCN:ADN 57436
<i>sociata</i> 4	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347385	MNCN:ADN 57440
<i>sociata</i> 5	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347386	MNCN:ADN 57441
<i>sociata</i> 6	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347387	MNCN:ADN 57442
<i>sociata</i> 7	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347388	MNCN:ADN 57443
<i>sociata</i> 8	M	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347389	MNCN:ADN 57444
<i>sociata</i> 9	M	River Oja, La Rioja (Spain)	42.953	-2.915	490	MF347390	MNCN:ADN 57445
<i>sociata</i> 10	M	River Oja, La Rioja (Spain)	42.953	-2.915	490	MF347391	MNCN:ADN 57434

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
<i>sociata</i> 11	M	River Lez, Audressein (France)	42.930	1.023	500	MF347392	MNCN:ADN 49486
<i>sociata</i> 12	M	River Aude, Languedoc-Roussillon (France)	42.954	2.449	500	KX141525	
<i>sociata</i> 13	M	River Vernal, Aragón (Spain)	42.811	-0.833	970	HM395984	
<i>sociata</i> 14	M	River Cares, Asturias (Spain)	43.193	-4.908	570	MF347393	MNCN:ADN 92806
<i>sociata</i> 15	M	River Cares, Asturias (Spain)	43.193	-4.908	570	MF347394	MNCN:ADN 92807
<i>sociata</i> 16	M	River Correc de la Sogueta, Sorède (France)	42.500	2.958	390	MF683826	MNCN:ADN 96499
<i>sociata</i> 17	F	River Correc de la Sogueta, Sorède (France)	42.500	2.958	390	MF683827	MNCN:ADN 96500
<i>f. viteceki</i> N1	M	River Neretva, Bačevići (Bosnia and Herzegovina)	43.254	17.825	35	MT559365	MNCN:ADN 96493
<i>f. viteceki</i> N2	M	River Neretva, Bačevići (Bosnia and Herzegovina)	43.254	17.825	35	MT559366	MNCN:ADN 96494
<i>f. viteceki</i> N3	M	River Neretva, Bačevići (Bosnia and Herzegovina)	43.254	17.825	35	MT559367	MNCN:ADN 96495
<i>f. viteceki</i> B1	M	River Bunica (Bosnia and Herzegovina)	43.243	17.855	30	MT559368	MNCN:ADN 96496
<i>f. viteceki</i> S1	M	River Sturba, Livno (Bosnia and Herzegovina)	43.762	17.030	755	MT559362	MNCN:ADN 94430
<i>f. viteceki</i> S2	M	River Sturba, Livno (Bosnia and Herzegovina)	43.762	17.030	755	MT559363	MNCN:ADN 94431
<i>f. viteceki</i> S3	F	River Sturba, Livno (Bosnia and Herzegovina)	43.762	17.030	755	MT559364	MNCN:ADN 94432
<i>cf. obliterated</i> 1	FP	Kudowski Potok stream (Poland)	50.482	16.337	840	MT816391	MNCN:ADN 94336
<i>cf. obliterated</i> 2	FP	Stream near Volgusha river (Russia)	56.242	37.405	150	MT816392	MNCN:ADN 109950
<i>cf. obliterated</i> 3	FP	Stream near Volgusha river (Russia)	56.242	37.405	150	MT816393	MNCN:ADN 109951
<i>cf. nubila</i> 1	L	Stream Medvejij, Murmansk (Russia)	67.136	32.702	150	MT816394	MNCN:ADN 109953

Mikulski (1931), Raczyńska *et al.* (2000), Riedel (1961, 1966), Rychla & Buczyńska (2013), Serafin (2004), Sowa (1961, 1975), Szczęsny (1974, 1986, 1987, 1990, 1998, 2000, 2002, 2003, 2005), Ulmer (1909), Wargacka (2004), Zacwilichowska (1968). **Republic of North Macedonia:** Kovachev *et al.* (1999), Kumanski (1997), Slavevska Stamenković & Hinić (unpublished data). **Romania:** Botoșăneanu (1961), Botoșăneanu & Tabacaru (1963), Ciubuc (1993, 2009, 2017), Cupșa *et al.* (2009), Curtean-Bănăduc (2008), Curtean-Bănăduc & Radu (2010), Curtean-Bănăduc *et al.* (2006), Dumbravă-Dodoacă *et al.* (2010), Sători (1944), Surugiu & Cristea (2008), Tatole (1999), Ujvárosi (1997, 2002), Ujvárosi & Chisu (1999), Ujvárosi *et al.* (1995). **Russia:** Chertoprud (2011), Chertoprud & Palatov (2013), Ivanov (2011), McLachlan (1879), Silfvenius (1906), Vuori *et al.* (1999), Yakovlev (2005). **Serbia:** Djikanović *et al.* (2008), Filipović (1954, 1969), Ibrahimi *et al.* (2017), Marinković-Gospodnetić (1975), Marković *et al.* (1997, 1998, 1999), Paunović *et al.* (2006), Petković *et al.* (2015), Stojanović (2017), Vulić *et al.* (2014), Živić (2005), Živić *et al.* (2003, 2004, 2005). **Slovakia:** Beracko *et al.* (2012), Bulánková *et al.* (2001), Chvojka (1992, 1993, 2002), Chvojka & Novák (2001), Derka *et al.* (2001), Dudich *et al.* (1943), Elexová *et al.* (2010), Hradil (1965), Husárová-Dudiková (1960), Jusko *et al.* (2009), Krno *et al.* (1994), Nógrádi (1988), Nógrádi & Uherkovich (1987), Novikmec (2005), Novikmec & Hamerlik (2000), Novikmec *et al.* (2007), Obr (1984), Obrdlík (1968), Pastuchová (2006), Rúfusová *et al.* (2014). **Slovenia:** Hrovat (2007), Hrovat & Urbanič (2012), Malicky & Sipahiler (1993), Tavzes *et al.* (2006), Urbanič (2002), Urbanič *et al.* (2005). **Spain:** Bonada *et al.* (2004, 2008), López-Llaneza (1984), Navás (1907, 1917). **Sweden:** Illies (1956), Ulfstram *et al.* (1974). **Switzerland:** Buholzer (1978), Felber (1908), Malicky & Sipahiler (1993), Ris (1889). **Turkey:** Kalyoncu & Zeybek (2011), Kumanski & Sipahiler (2002), Malicky & Sipahiler (1993), Sipahiler (2007, 2012), Sipahiler & Malicky (1987), Uherkovich & Nógrádi (2002). **Ukraine:** Szczęsny & Chvojka (2008), Szczęsny & Godunko (2008a, 2008b). **United Kingdom:** Hickin (1954), Mackereth (1954), McLachlan (1879).

The distribution map was drawn using ArcGIS 10.3 (ESRI 2011)

Results

Systematic part

Rhyacophila ferruginea (Scopoli), nomen dubium

Phryganea ferruginea Scopoli, 1763: 266, fig. 691.

Rhyacophila ferruginea (Scopoli): Hagen, 1859: 153.

The original description of this species, as *Phryganea ferruginea* (Figs 1a, 1b) was:

“*Phryganea ferruginea*. Length of 6 $\frac{1}{3}$ lines [supposedly French lines, that gives approximately 14.287 mm of length. M.A. Alonso Zarazaga, personal communication].

FIGURE 1. Original description of *Rhyacophila ferruginea* (Scopoli 1763) in *Entomologia Carniolica*, downloaded from Biodiversity Heritage Library (BHL). 1a, drawing and label. 1b, description (Scopoli 1763).

Diagnosis. All rust colored; forewings reticulated dark; black eyes. In forests by the waters; month of May. Abdomen with lateral black dots, as many as segments of it. Antennae 6 lines long, rusty. The other sex with a round white common spot, and white spots (5–6) on the outer margin of the forewings.”

Hagen (1854), in his study of the species of *Phryganea* included in Scopoli’s *Entomologia Carniolica* stated that the description of *P. ferruginea* does not match the drawing, even with other *Phryganea*. Following the “reticulate wings” and “white spots” of wings, he proposed three options that could be one of the following: *Chaetopteryx villosa* (Fabricius, 1798) (based on body contour and reticulation of wings), one of two *Hydropsyche* Pictet 1834, and one *Rhyacophila* (reticulation of wings); the color (all rust) only matched with the mentioned *Rhyacophila* (maybe *R. nebulosa* Stephen), and, as he had only the female, he couldn’t check if males also had the white spots on the wings.

In 1859 Hagen cited this species and *R. fasciata*, together with *R. vulgaris*, *R. dorsalis* and *R. paupera*: “4. *R. ferruginea* Scop. Anal appendage rounded, almost completely covering the lateral appendages. The smallest species. Carniola, Austria. Maybe dark colored females of my collection from Zurich belong to it.”

“5. *R. fasciata* Hag. The appendages are very similar to the previous species, but the size of the animal is much more significant and the upper wings have three dark cross-ties. A single male from Elberfeld”.

McLachlan (1865), at the end of his description of *Rhyacophila septentrionis* wrote: “I have some doubt in announcing this as a new species, since the form of the appendices agrees very well with the figure of those of *R. ferruginea*, sent to me by Dr. Hagen. I submitted an example to that gentleman, and he says that it is larger and darker than his continental types of that species and may be distinct. *R. ferruginea* is described as ‘die kleinste Art’ of the group (the true genus *Rhyacophila*) to which it belongs”. In his publication of 1868, McLachlan said: “*R. ferruginea*, (Scopoli) Hagen. I have not seen this insect, which, according to Hagen (*in litt.*) is smaller than *septentrionis*, but with similar lobes and appendices. Scopoli’s name cannot be said to apply to this species with certainty.” Finally, in his publication of 1879, McLachlan concluded at the end of the paragraph about *Rhyacophila septentrionis*: “I do not feel justified in adopting Scopoli’s name *ferruginea* (as was done by Hagen for the example from Carniola). Even supposing that Scopoli had a *Rhyacophila* before him (which is just possible), the particular species must remain uncertain.” We agree with this statement, due to the difficulty of identification from the original species described, so we propose to change the status of *Rhyacophila ferruginea* (Scopoli 1763) to *nomen dubium*.

***Rhyacophila fasciata* Hagen**

Rhyacophila fasciata Hagen, 1859: 153.

Lectotype: 1 male. Entomology 11067, Entomology Collection of the Museum of Comparative Zoology, Harvard University.

Type locality: Elberfeld (Germany).

Other information: (in label) 57; E.-illegible; Hagen; 107; ML; type 11067; (vial); Lectotype *Rhyacophila fasciata* Hagen. (MCZBase, 2020). (Figs 2a, 2b, 30a, 30b)

Ross (1952) included the species in his publication about the lectotypes of Hagen in the collection of the Museum of Comparative Zoology at Harvard University, Cambridge, Massachusetts.

Description. This species has been described by several authors over many years. The first publication where this species is cited is that of Hagen (1859), where he provided a very short description: “A. Lower appendages undivided at the top.

1. The tongue-like extension of the last abdominal segment with an enlarged end.

5. *R. fasciata* Hag. The app. are very similar to the previous species (*R. ferruginea* Scop.), but the size of the animal is much more significant, and the upper wings have three dark cross bands. A single male from Elberfeld” (Fig 30a).”

McLachlan (1868) described it as follows: “*R. fasciata* Hagen, may be distinguished almost with certainty by the dark fasciae on the anterior wings. The form of the dorsal lobe is very distinct from the preceding (*R. paupera*), being broad and rounded, and nearly concealing the app. sup. The second joint of the app. inf. is obliquely truncate (Pl. XIV. fig. 16). I have three ♂ from Carinthia from Zeller.”

FIGURE 2. Lectotype of *Rhyacophila fasciata* Hagen 1859, in the Entomology Collection of the Museum of Comparative Zoology of Harvard University (Cambridge, MA, USA; MCZ). 2a, labels and male genitalia in cork-stoppered microvial. 2b, genitalia of *Rhyacophila fasciata* lectotype (male). Reproduced with permission of MCZ.

In 1879, McLachan described *R. fasciata* and *R. septentrionis*. In the description of *R. fasciata* he included the original specimen from Elberfeld, his specimens from Carinthia, saying that they should be referred to *septentrionis*, and 7 ♂ and 1 ♀ from the Pyrenees (France), writing “it may be that these should form a distinct species”. These specimens from the Pyrenees were described in 1916 by Navás as *Rhyacophila sociata* (Valladolid *et al.* 2018). On the other hand, in the description of *R. septentrionis* McLachlan included his specimens from Scotland, as well as others from different countries of North and Central Europe. Based on results of our studies of morphology and genetics, we can say that most of the specimens cited belong to *R. fasciata*, keeping only those from Scotland as *R. septentrionis*.

Klapálek (1893) described the larva and the pupa of *R. septentrionis* in Bohem, but the area matches with those of *R. fasciata*, so it should be this species. The same situation pertains to Silfvenius (1905), who described larvae and pupae from South Finland as *R. septentrionis* that should be *R. fasciata*.

Ulmer (1909), based on previous authors, described the imagos and larvae of *R. fasciata* and *R. septentrionis*, first one from Elberfeld and the second one from different places in Germany. We consider that these specimens all belong to *R. fasciata*.

There are more recent authors that have described *R. fasciata* and *R. septentrionis* specimens: Nielsen (1942) as *R. septentrionis* from Denmark; Nývák (1963) as *R. fasciata* from Czechoslovakia; Hickin (1954) and Mackereth (1954) as *R. septentrionis* from United Kingdom; Fotius-Jaboulet (1964) as *R. septentrionis* from France; Lepneva (1964) as *R. septentrionis* from northwest, west and southwest Russia; Eidel (1974) as *R. fasciata* from Austria; Buholzer (1978) as *R. fasciata* from Switzerland; Sedlak (1985) as *R. fasciata* from Central Europe; Pitsch (1993) as *R. fasciata* from Central Europe. Of all these descriptions, only those from the United Kingdom could belong to *R. septentrionis*; the rest, from continental Europe, should be *R. fasciata* or some related species.

Finally, Malicky & Sipahiler (1993) illustrated the differences among male specimens of *R. fasciata* from Europe, Turkey, and Lebanon that they considered as subspecies of the *R. fasciata* Group. Some of them have been promoted to species (*R. denticulata* McLachlan 1879, by Valladolid *et al.* 2018; *R. kykladica* Malicky & Sipahiler 1993, by Valladolid *et al.* 2019), one species has been resurrected (*R. sociata* Navás 1916, previously a synonym of *R. denticulata*, by Valladolid *et al.* 2018), and others could be considered also valid species (Valladolid, unpublished data).

***Rhyacophila septentrionis* McLachlan, status resurrectus**

Rhyacophila septentrionis McLachlan, 1865: 157

Rhyacophila septentrionis McLachlan: McLachlan 1868: 305, 1879: 444; Klapálek 1893: 126–128; Silfvenius 1905: 146–

148; Ulmer 1909: 17, 220; Nielsen 1942: 337–358; Hickin 1954: 59–61; Mackereth 1954: 150–151; Ross 1956: 118; Fischer 1960: 131–136, 1971: 120–122 (and other references herein); Fotius-Jaboulet 1964: 1–13; Lepneva 1964: 220–227; Botosaneanu 1967: 291 (as synonym of *R. fasciata* Hagen); Schmid 1970: 120 (as synonym of *R. fasciata* Hagen); Tobias & Tobias 1981: 38–39 (as synonym of *R. fasciata* Hagen).

Lectotype: 1 male NHMUK 012504718 (Figs 3, 31a).

Type locality: Near Longniddry & Aberlady, Scotland.

Other information: Captured 18/vi/1865, at a little stream running into the sea on the coast of the Firth of Forth, near Longniddry.

FIGURE 3. Lectotype of *Rhyacophila septentrionis* McLachlan 1865, in Entomology Collection of the Natural History Museum, London, United Kingdom (NHMUK, previously BMNH). Photo: Dr. Ben Price, Curator.

Description. According to McLachlan (1865), “Antennae testaceous, with indistinct paler annulations. Head, palpi and mesothorax pale brown; the former with scattered golden hairs. Anterior wings broad, the apex nearly elliptical, golden-grey, thickly reticulated with dark grey; dark greyish-fuscous spots round the apical margin, one at the termination of each apical sector; the pale dorsal blotch is ill defined, and is indicated by an irregular pale space, scarcely reticulated; a similar but still more indistinct paler space occupies the whole of the lower two-thirds of the apical portion of the wing; neuration fuscous. Posterior wings pale greyish, subhyaline; apical veins darker grey, sometimes margined with grey at their points of insertion in the apical margin. Legs testaceous; the tips of the anterior and intermediate tibiae externally fuscous; tibial spurs dark brown. Abdomen fuscous, ochreous beneath, testaceous at the apex above, and with testaceous appendices. In the male the superior lobe is short, rather attenuated at the base, but soon greatly dilated, the apical portion being nearly orbicular (McLachlan, 1879, pl. XLVI, *Rh. septentrionis*, fig 1) (herein Fig 31b); app. sup. nearly concealed under the lobe, the external edge rounded, slightly

excised in front (herein Fig 31b); app. inf. rather short, the basal joint broad, with the sides parallel, the apical joint scarcely shorter than the basal and of equal breadth, the apex very obliquely truncated (McLachlan, 1879, pl. XLVI, *Rh. septentrionis* fig 2); penis furnished above with a short broad dark chesnut-brown superior cover; lower sheaths bent slightly downwards (their direction varies in dead specimens) (Fig 31c), dark shining chestnut-brown, clear testaceous at the extreme base (McLachlan, 1879, pl. XLVI, *Rh. septentrionis* fig 3). Expanse of fore-wings 12–13 lines”.

Descriptive part

Rhyacophila fasciata Hagen 1859

Description of the larva (Figs 4–9, 21).

The description of the specimens examined will be complemented with descriptions of previous authors, adding or confirming features.

Biometrics of last instar larva and prepupa: Length: 14.25–19.00 mm ($\bar{x}$ = 16.09, n = 13). Maximum width: 2.98–3.90 mm ($\bar{x}$ = 3.28, n = 13) (Lepneva 1964, 5th instar larval length: 23.00–23.40 mm; Nielsen 1942, 5th instar larval length: 12.00–23.40 mm; Ulmer 1909, larval length: 22.00–24.00 mm, larval width: 3.00–3.50 mm). Maximum width of larva at metathorax.

Colour: Live specimen greenish, dorsally with dark areas and light stripes, pale ventrally. Specimens preserved in ethanol reddish brown, dorsum dark, ventrally pale; on abdomen, semicircular pale anterior dorsolateral areas, dorsocentral area of segments with irregular pale spots, connected with anterior dorsolateral areas. Sclerotized areas of head and thorax brown with pale areas and stripes, black in some places (Fig 21a).

Head (Figs 4, 21b). Length: 1.49–2.20 mm ($\bar{x}$ = 1.90, n = 13). Width: 1.14–1.50 mm ($\bar{x}$ = 1.30, n = 13). Maximum width in posterior third of head, lateral margins almost parallel. Cephalic capsule (Figs 4a, 4b), dorsally and dorsolaterally light brown in anterior third, areas adjacent to frontoclypeal and anterior coronal sutures and anterior dorsolateral areas paler; ventral side variable (Silfvenius 1905), pale (Pitsch 1993), or with dark spots in middle central area (Sedlák 1985, p. 111, table 3, fig 10); anterior edge and submentum reddish brown, posterior edge black (Nielsen 1942); oval brown area with dark spots extending laterally from anterior edge of occipital foramen to anterior third of head (Fig 4b) (Klapálek 1893; Lepneva 1964, p. 223, figs 181, 182B). Frontoclypeal apotome (Fig 4c) pale; 2 oval dark brown muscle attachment spots in central anterior third, between setae #4; dark pigmentation in posterior third heart-shaped, including pits #4 but not setae #6, with 4–6 oval brown muscle attachment spots with dark borders (Klapálek 1893; Silfvenius 1905; Nielsen 1942; Lepneva 1964; Pitsch 1993). Two-thirds of posterior dorsal area of head light brown, marked with darker muscle attachment spots; centrally one pair of dark brown patches, wing-shaped, outside posterior edge of frontal suture; light circular area on juncture of frontal and coronal sutures; three pale spots around setae #15, #16, and #19–21; dorsal head pits with dark border, central posterior occipital foramen black.

Mandibles (Fig 5) asymmetrical, as in other rhyacophilids; left one bigger than right one. Inner blade of left mandible irregular, convex, right mandible with small tooth in middle (Nielsen 1942, pl. 2, fig 13; Lepneva 1964, p. 223, fig 184). Labrum and maxillolabium as in other *Rhyacophila* species (Nielsen 1942, pl. 2, figs 12A, 12B, 14A, 14B; Lepneva 1964, p. 223, figs 183A, 183B, 185). Mentum rectangular, its anterior half slightly sclerotized. Labrum with small transparent area in central anterior edge.

Thorax (Figs 6, 21c): Anterior half of pronotum pale or very light coloured. Sinuous posterior margin bordered by dark band, brown in middle of each half, black posteromesally and posterolaterally, light brown submesally; posterior half of pronotum (Fig 6a) with three dark areas separated by light zones, central dark region tapered posteriorly and reaching posterior edge of sclerite, anteriorly truncate, wider than posteriorly, two lines of dark brown spots inside central dark region and reaching posterior edge, several dark brown spots along posterior edge; each pronotal plate with lateral dark area almost triangular, from anterior submesal region to posterolateral edge of sclerite, posterior area with two–three rows of brown spots with dark borders distributed in circular-oval shape and parallel to posterior border. Four–six brown spots around setae #5; curved row of dark dots from setae #5 toward anterior submesal corner, curving around circular spot on anterior half (Fig 6a); pale posterior and median areas connected (Fig 6b); with narrow black band in anterolateral angles, extending posterad laterally to posterolateral

margins, inner to margins (Fig 6b; Lepneva 1964); anterior margin light brown, with black spot near anterolateral angle; central area of posterior marginal band of each sclerite dark brown or black (Fig 6b) (Nielsen 1942, pl.3, fig 17A; Lepneva 1964, p. 223, fig 188).

Legs (Fig 9): Similar to those other *Rhyacophila* species. Colour yellowish, proximal regions of coxa, femur and distal region of trochanter of fore- and midlegs black or dark brown. Narrow pale reddish-brown band in proximal dorsolateral area of tibia and distal dorsolateral area of femur of each pair of legs, each inner and outer face of each femur with band of circular spots. Forelegs each ventrally with spicules, extending from distal half of trochanter, all of femur, and distal two-thirds of tibia and tarsus. Setae black or dark brown, some transparent in ventral area.

Abdomen (Figs 7, 8, 21d, 21e): Similar to other *Rhyacophila* species. Colour as for meso- and metathoracic segments, greenish with dark dorsal areas and light stripes in living larvae, in preserved specimens semicircular pale anterior dorsolateral areas, dorsocentral area of segments with irregular pale spots, connected with anterior dorsolateral areas, pale yellow ventrally. Widths of anterior abdominal segments similar, narrower in posterior segments. Lateral abdominal gills in tufts of 25–30 filaments (20–30 filaments, Lepneva 1964). Dorsal sclerite of segment IX (Figs 7, 21d) brown, with continuous black band on anterior edge and two small and widely separated black bands on posterior edge, under setae #2 (Lepneva 1964); band of anterior edge narrower in center, with pair of narrow extensions backward beyond and surrounding pit #1; with some brown spots submesally near posterior margin; in darker larvae, extensions of anterior edge and spots of posterocentral area connected by dark stripes, creating X-shape figure; three pale areas along posterior margin, on either side of two black bands and central area. Anal prolegs typical of group, each with long sword process (Figs 8a, 8b, 21e). Anal claws each with three short teeth on ventral edge (Figs 8a, 8c, 21e), basoventral hook black basally and reddish brown apically (Figs 8a, 8b).

Description of the pupa

Biometrics of pupa and cocoon. Pupal length (total): 9.70–15.70 mm ($\bar{x}$ = 12.20, n = 28); male pupa: 10.07–12.28 mm ($\bar{x}$ = 11.06, n = 11) (length: 11.20–12.20, Lepneva 1964; Nielsen 1942); female pupa: 9.70–15.70 mm ($\bar{x}$ = 12.94, n = 17) (length: 12.40–15.50, Lepneva 1964; Nielsen 1942; up to 16.00 mm, Silfvenius 1905). Cocoon length (total): 12.37–18.35 mm ($\bar{x}$ = 15.11, n = 68) (length: 12.50–18.00 mm, Nielsen 1942); male cocoon length: 12.39–16.27 mm ($\bar{x}$ = 14.43, n = 10); female cocoon length: 13.43–17.73 mm ($\bar{x}$ = 15.99, n = 16).

Head: Distribution of dorsal setae: four before, two between and two in middle of antennal pads, three on posterior edge of each cephalic tubercle. Mandibles brown, left one with two large teeth and right one with three, with numerous fine teeth on inner blade (Klapálek 1893; Nielsen 1942; Ulmer 1909). Antennae of variable length, reaching from middle of abdominal segment IV to posterior edge of segment VI (to anterior edge of segment IV in females and posterior edge of segment VI in males, Klapálek 1893; to abdominal segment VIII in males and V in females, Lepneva 1964; Nielsen 1942).

Thorax: Tubercles of prothorax each with 5 setae, directed backwards, two central setae directed forwards. Hind wing pads variously reaching from anterior edge of abdominal segment III to posterior edge of segment IV (forewing pads reaching anterior edge of abdominal segment IV, Klapálek 1893; forewing pads reaching abdominal segment V in males and IV in females, Lepneva 1964; hind wing pads reaching end of segment IV in females, Silfvenius 1905).

Abdomen: Paired anterior hook plates pedunculated, straight basally and dilated posteriorly, palmate, and present on abdominal terga IV to VII (Fig 10) (Lepneva 1964; Ulmer 1909), slightly bigger on segments V and VI, each with 15–20 spines; also, pair of inconspicuous small flat plates without spines on tergum III. Paired posterior hook plates sessile on terga III–V, slightly oblong transversally (length: width = 1:1.3) (Klapálek 1893; paired anterior hook plates present on abdominal terga III to VIII, paired posterior hook plates on terga III to V; Silfvenius 1905: paired anterior hook plates present on abdominal terga IV to VII, sometimes without plates on segment III, paired posterior hook plates present on terga III to V), each with variable number of spines, more than 25.

Description of the imago

Male body length: 7.04–10.46 mm ($\bar{x}$ = 9.23, n = 36) each forewing: 10.16–13.54 mm ($\bar{x}$ = 11.67, n = 36), each hind wing: 8.52–11.99 mm ($\bar{x}$ = 10.25, n = 36); females, length: 8.73–12.19 mm ($\bar{x}$ = 10.46, n = 19), each forewing: 10.05–13.90 mm ($\bar{x}$ = 12.14, n = 19), each hind wing: 8.24–12.03 mm ($\bar{x}$ = 10.68, n = 19) [Ulmer 1909: male length (*R. fasciata*) = 10.00 mm, wingspan = 27.00 mm; male length (*R. septentrionis*) = 6.50–9.00 mm, wingspan = 22.50–29.00 mm; Malicky 2004: male forewing length = 10.00–14.00 mm, female forewing length = 11.00–14.00 mm].

FIGURES 4–8. Larva of *Rhyacophila fasciata* Hagen 1859. 4a–4c, head: 4a, dorsal; 4b, right lateral; 4c, frontoclypeus, dorsal. 5D, 5V, mandibles from last instar larval exuviae, left (*l*) and right (*r*): 5D, dorsal; 5V, ventral. 6a, 6b, prothorax: 6a, dorsal; 6b, left hemisclerite, left lateral. 7, dorsal sclerite of abdominal tergum IX. 8a–8c, details of anal prolegs: 8a, right anal claw, right lateral; 8b, detail of basolateral plate with sword process and basoventral hook, right lateral; 8c, detail of claw, right lateral. Scale bar: unlabelled = 1 mm; • = 0.5 mm.

FIGURE 9. *Rhyacophila fasciata* Hagen 1859, right legs. 9A, anterior views; 9P, posterior views. I–III: pro-, meso-, and metathoracic legs, respectively. Scale bar: 1 mm.

Colour: Specimens preserved in ethanol reddish brown dorsally, yellowish ventrally, spurs reddish, setae golden brown, females generally darker than males; small black or dark brown spots in dorsal area; in lateral view, some specimens with large black spots marking border between dorsal and ventral areas. Forewings dark with small pale spots, three transversal dark bands (fascias) in dry specimens (Fig 30a), pale in preserved specimens, darker in females, hind wings pale: each with dark area on leading edge between subcostal (Sc) and second radial (R_2) veins in fore- and hind wings; each forewing hyaline in irregular zone connecting medial (M) and medio-cubital ($m-cu$) veins and in distal area of second cubital vein (Cu_2) (Holzenthal *et al.* 2007); in each hind wing proximal segment of medial veins and connection area (M_{1-4}) not conspicuous, second cubital (Cu_2) and first anal (A_1) veins not conspicuous either totally or partially, proximal portion of cubital vein (Cu) and distal portions of first cubital vein (Cu_{1a-1b}) thicker.

Male genitalia (Figs 2b, 12, 23, 30b): Apical segment of each inferior appendage (Figs 2b, 12A, 23a) with basal and distal edges diverging, dorsal and ventral edges diverging, posterior edge of 2nd segment of each inferior appendage slightly concave dorsally and slightly convex ventrally, ventral edge slightly concave (Figs 12A, 23a). Apicodorsal vertex slightly angular, apicoventral angle rounded, projecting as thick lobe narrowing progressively to apex.

Parameres in ventral view curved posteromesad in apical half (Figs 12BV p, 23c). In lateral view (Fig 12BL) each constricted at short distance beyond base, dilated at middle, with almost parallel dorsal and ventral margins in central area (S-shape), two files of long and thick spines on midventral margin, pointing outward; midlateral surface covered by sparse thin spicules or setae, from middle posteroventral to posterodorsal edge and covering most of apex, absents in middle anteroventral and dorsal edges.

FIGURES 10, 11. *Rhyacophila fasciata* Hagen 1859 and *Rhyacophila septentrionis* McLachlan 1865, paired abdominal hook plates of male pupa. 10*l* and 10*r*, *Rhyacophila fasciata* left and right hook plates, respectively. 11*l* and 11*r*, *Rhyacophila septentrionis* left and right hook plates, respectively. A = anterior hook plates, dorsal, P = posterior hook plates, dorsal; III–VII = abdominal terga III through VII, dorsal. Scale bar: 50 µm

FIGURES 12, 13. Male and female genitalia of *Rhyacophila fasciata* Hagen 1859. 12, male: 12A, 2nd segment of left inferior appendage, left lateral; 12BV, 12BL, parameres: 12BV, parameres (p) and ventral lobe of aedeagus (phallicata) (vl), ventral; 12CL, 12CD, aedeagus (phallicata) and lateroventral lobes: 12CL, aedeagus and ventral lobe, left lateral; 12CD, aedeagus and lateroventral lobes (lvl), dorsal. 12DD, apicodorsal lobe of segment IX (al) and preanal appendages (pa); 12DV, segment X, ventral, ab= apical band, as = anal sclerites, va = non-sclerotized ventral area. 13, female segments VIII–XI: 13La, left lateral; 13Lb, left lateral, posterolateral edge of segment VIII valves variations: 1 = two specimens from Finland, 2 = three specimens from Czech Republic and Slovakia, 3 = one specimen from Croatia; 13Da, dorsal; 13Db, posterodorsal edge of segment VIII valves variations; 13V, ventral. Scale bars: unlabelled = 1 mm; • = 0.5 mm; * = 200 μ m.

Aedeagus (phallicata) in lateral view (Fig 12CL) with dorsal margin concave and posterior corner of concavity hooked anterad, posterior edge of aedeagus straight, projected posteroventrad, subapically rounded ventrally (Fig 12CL); ventral lobe of aedeagus triangular (Fig 12BV vl), lateral edges slightly rounded; lateroventral lobes of phallus straight, posterior edges strongly convex, apicolateral margins rounded (Fig 12CD lvl).

Apicodorsal lobe of segment IX slightly dilated subapicolaterally, almost round in shape (Figs 12DD al, 23b), with small apicomesal excision in some specimens; preanal appendages (Fig 12DD pa) round, shorter than apicodorsal lobe, almost covered by it; in ventral view, apical band V-shaped (Fig 12DV ab) nearly as wide as long, non-sclerotized inside area with posteromesal excision, posterior edges rounded (Fig 12DV va), anal sclerites triangular (Fig 12DV as).

Female genitalia (Figs 13, 25): In lateral view (Figs 13La, 13Lb, 25L), posterodorsal margin of segment VIII valves on each side irregular posteriorly, convex on ventral edge, with dorsolateral projection, in some specimens slightly indented posteriorly, posterolateral margin irregular, with 1–2 posterior projections, ventral projection more conspicuous (Fig 13Lb 1–3).

In dorsal view (Figs 13Da, 13Db, 25D), with indentation between segment VIII valves, with rounded apical and subapical corners, delimiting oval space, small projection in apical corner in some specimens (Fig 13Db).

In ventral view (Fig 13V, 25V) segment VIII valves forming two elongate sclerites, basal 1/4 of mesal edges parallel, distal 3/4 of mesal edges irregular, slightly concave, diverging; intersegmental membrane with pair of dark, ovoid or slightly elongate sclerites.

***Rhyacophila septentrionis* McLachlan 1865**

Description of the larva

Colour: Live specimen golden-olive (Hickin 1954), pale ventrally. Specimens preserved in ethanol reddish brown, dorsum dark, ventrally pale; on abdomen, semicircular-triangular pale anterior dorsolateral areas, dorsocentral area of segments with darker circular pale spots, connected caudally with anterior dorsolateral areas through pale lines, forming W-shape. Sclerotized areas of head and thorax dark brown with pale areas, black in some places (Fig 22a).

Head (Figs 14, 22b). Maximum width in posterior third of head, lateral margins almost parallel (tapering slightly towards anterior end, but not constricted at level of eyes, Mackereth 1954, fig 1S; Hickin 1967, fig 40; tapering anteriorly, Edington & Hildrew 1981). Cephalic capsule (Figs 14a, 14b), dorsally and dorsolaterally dark brown, anterior third and areas adjacent to frontoclypeal and anterior coronal sutures and anterior dorsolateral areas paler (dorsal posterior region of head with conspicuous pattern of dark spots on lighter background, Edington & Hildrew 1981, Fig 33, arrow a); ventral side pale, with central and slightly darker U-shape area from middle to posterior end of protogula covered with pale spots (Fig 14c), aboral margins of genae just before union with small black fold (Hickin 1954, fig 4B, 1967, fig 54); in lateral view anterior edge and submentum reddish brown, posterior edge black; oval brown area with dark spots extending laterally from anterior edge of occipital foramen to anterior third of head (Fig 14b). Frontoclypeal apotome (Fig 14d) pale; 2 oval dark brown submesal muscle attachment spots in anterior third between setae #4; dark brown pigmentation in posterior third heart-shaped (crescent shaped, Mackereth 1954, fig 1S; Hickin 1967, fig 40), including pits #4 but not setae #6, with pair of oval lateral and 2–3 circular mesal paler brown muscle attachment spots with dark borders, anterocentral area pale with two oval pale brown spots in anteromesal position (Figs 14a, 22a, 22b). Two-thirds of posterior dorsal area of head brown, marked with darker and paler muscle attachment spots; submesally one pair of dark brown triangular patches with paler spots with dark borders outside posterior edges of frontal sutures; light triangular area on juncture of frontal and coronal sutures, small pale area around pit #9; three pale spots around setae #15, #16, and #19–21, last one connected with posterior pale areas; dark brown dots in posterior area of genae (Mackereth 1954, fig 1S; Hickin 1967, fig 40); dorsal head pits with dark brown borders, posteromesal edge of occipital foramen black.

Mandibles (Fig 15) asymmetrical, as in other rhyacophilids; left one bigger than right one. Subapicomesal blade of left mandible slightly concave, right mandible with small tooth in middle, both with darker apices (Figs 15, 22a). Labrum and maxillolabium as in other *Rhyacophila* species. Mentum rectangular, its anterior half slightly sclerotized. Labrum with small transparent area in central anterior edge.

FIGURES 14–18. Larva of *Rhyacophila septentrionis* McLachlan 1865. 14a–14d, head: 14a, dorsal; 14b, right lateral; 14c, ventral; 14d, frontoclypeus, dorsal. 15D, 15V, mandibles from last instar larval exuviae, left (*l*) and right (*r*): 15D, dorsal; 15V, ventral. 16a, 16b, prothorax: 16a, dorsal, numbers: setae #2, #3, #5 and #6; 16b, right hemisclerite, right lateral. 17, dorsal sclerite of abdominal tergum IX. 18a–18c, details of anal prolegs: 18a, right anal claw, right lateral; 18b, detail of basolateral plate with sword process and basoventral hook, right lateral; 18c, detail of claw, right lateral. Scale bar: unlabelled = 1 mm; ● = 0.5 mm

FIGURES 19, 20. Male and female genitalia of *Rhyacophila septentrionis* McLachlan 1865. 19, male: 19A, 2nd segment of left inferior appendage, left lateral; 19BV, 19BL, parameres: 19BV, parameres (p) and ventral lobe of aedeagus (phallicata) (vl), ventral; 19BL, left paramere, left lateral. 19CL, 19CD, aedeagus (phallicata) and lateroventral lobes: 19CL, aedeagus and ventral lobe, left lateral; 19CD, aedeagus and lateroventral lobes (lvl), dorsal. 19DD, apicodorsal lobe of segment IX (al) and preanal appendages (pa); 19DV, segment X, ventral, ab= apical band, as= anal sclerites, va= non-sclerotized ventral area. 20, female segments VIII–XI: 20L, left lateral; 20D, dorsal; 20V, ventral. Scale bars: unlabelled = 1 mm; • = 0.5 mm; * = 200 μ m.

FIGURES 21, 22. Larvae of *Rhyacophila fasciata* Hagen 1859 and *Rhyacophila septentrionis* McLachlan 1865. 21, *Rhyacophila fasciata*: 21a, head and prothorax, dorsal; 21b, detail of head, dorsal; 21c, detail of prothorax, dorsal; 21d, dorsal sclerite of abdominal tergum IX, dorsal; 21e, left anal proleg, left lateral. 22, *Rhyacophila septentrionis*: 22a, head and prothorax, dorsal; 22b, detail of head, dorsal; 22c, detail of prothorax, dorsal; 22d, dorsal sclerite of abdominal tergum IX, dorsal; 22e, left anal proleg, left lateral. Scale bar: 1mm.

FIGURES 23–26. Males and females of *Rhyacophila fasciata* Hagen 1859 and *Rhyacophila septentrionis* McLachlan 1865. 23, male of *Rhyacophila fasciata*: 23a, 2nd segment of left inferior appendage, left lateral; 23b, apicodorsal lobe of segment IX and preanal appendages, dorsal; 23c, parameres and ventral lobe of aedeagus (phallicata), ventral; 23d, left paramere, left lateral. 24, male of *Rhyacophila septentrionis*: 24a, 2nd segment of left inferior appendage, left lateral; 24b, apicodorsal lobe of segment IX and preanal appendages, dorsal; 24c, parameres and ventral lobe of aedeagus (phallicata), ventral; 24d, left paramere, left lateral. 25, female segments VIII–XI of *Rhyacophila fasciata*: 25L, left lateral; 25D, dorsal; 25V, ventral. 26, female segments VIII–XI of *Rhyacophila septentrionis*: 26L, left lateral; 26D, dorsal; 26V, ventral. Scale bars: Figures 23a–c, 24a–c = 0.5 mm, Figures 23d, 24d = 200 μ m, Figures 25, 26 = 1 mm.

Thorax (Figs 16, 22c): Anterior half of pronotum pale or very light coloured, posterior half with three darker areas, one median and two lateral (Edington & Hildrew 1981, fig 35; Hickin 1954, fig 5B, 1967 fig 56), in darker specimens posterior half brown, with mesal area darker. Sinuous posterior margin bordered by black band, posteriorly and posterolaterally light brown submesally; posterior half of pronotum (Fig 16a) dark, with narrow semicircular area extending anterolaterally and small area laterally, lateral area pale in each pronotal plate; central dark region triangular, tapered posteriorly and reaching posterior edge of sclerite, anteriorly truncate and wider than posteriorly, pale brown spots inside triangular region reaching posterior edge; each side of pronotal plate with two or three lines of pale brown spots with dark border of different shapes and parallel to posterior border; pale areas around setae 2–3#, 5# and 6#, four–six brown spots around setae #5; curved row of two or three dark dots extending from setae #5 toward anterolateral corner, two pale brown spots with dark borders anterolateral and posterolateral of seta 6#, circular and elongate respectively (Figs 16b, 22c); with narrow black band in anterolateral angles, extending posterad laterally above lateral margins to posterolateral corners (Fig 16b); anterior margin light brown, with a line of black spots near anterior angle; mesal area of posterior marginal band black (Fig 16b, 22c).

Legs: Similar to those of other *Rhyacophila* species. Colour pale yellowish, each with tarsus and claw darker, proximal parts of coxa, femur and distal parts of trochanters of fore- and midlegs black or dark brown. Narrow pale dark brown band in proximal dorsolateral area of tibia and distal dorsolateral area of femur of each pair of legs, each inner and outer face of each femur with band of dark brown circular spots. Forelegs each ventrally with spicules, extending from distal half of trochanter, all of femur, tibia and tarsus. Setae black or dark brown, some transparent in ventral area.

Abdomen (Figs 17, 18, 22d, 22e): Similar to other *Rhyacophila* species. Colour as for meso- and metathoracic segments, in preserved larvae semicircular-triangular pale anterior dorsolateral areas, dorsocentral area of each segment with darker circular pale spots, connected caudally with anterior dorsolateral areas through pale lines, forming W-shape, pale yellow ventrally. Widths of anterior abdominal segments similar, narrower in posterior segments. Lateral abdominal gills in tufts of 25–30 filaments. Dorsal sclerite of segment IX (Figs 17, 22d) light coloured, darker in central area, with continuous black band on anterior edge and two discontinuous black bands on posterior edge, posterior to setae #2; band of anterior edge narrower in center, surrounding anteriorly pit #1; pale spots inside central area, two pale areas laterally. Anal prolegs typical of group, each with long sword process (Figs 18a, 18b, 22e). Anal claws each with three short teeth on ventral edge (Figs 18a, 18c, 22e), basoventral hook black basally and reddish brown apically (Figs 18a, 18b).

Description of the pupa

Biometrics of pupa and cocoon. Male pupa: 10.00 mm (1 specimen); female pupa: 11.60 mm (1 specimen). Cocoon length: 14.40 mm (1 specimen).

Head: Distribution of setae on dorsal head similar to *R. fasciata*. Mandibles dark brown, left one with two large teeth and right one with three, anterior tooth bigger, with numerous fine teeth on inner blade. Antennae of variable length, reaching posterior edge of segment VII and posterior edge of segment IV in male and female studied, respectively.

Thorax: Tubercles of prothorax each with 6 setae on male and 4 setae on female studied. Hind wing pads reaching posterior edge of abdominal segment III of male and posterior edge of segment IV of female studied.

Abdomen: Paired anterior hook plates pedunculated, almost circular and present from abdominal terga IV to VII (Fig 11), more elongated on segment VII, each with more than 30 spines; also tergum III with pair of small flat hook plates each with 10–15 spines. Paired posterior hook plates sessile on terga III–V, almost circular, each with more than 30 spines.

Description of the imago

Male body length: 7.70 mm (1 specimen) each forewing: 12.20 mm, each hind wing: 10.60 mm; female, length: 8 mm (1 specimen), each forewing: 11.50 mm, each hind wing: 10.30 mm.

Colour: Specimens preserved in ethanol dark brown dorsally, yellowish ventrally, spurs yellowish brown, setae brown, females generally darker than males; small black or dark brown spots dorsally. Forewings dark with small pale spots, pale in preserved specimens, darker in females, hind wings pale; each with dark area on leading edge between subcostal (Sc) and middle space between first radial (R_1) and second radial (R_2) veins in fore- and hind wings, with some darker stripes; each forewing hyaline in circular-oval zone, connecting medial (M) and

medio-cubital (*m-cu*) veins and in distal area of second cubital vein (Cu_2) (Holzenthal *et al.* 2007); in each hind wing proximal segment of medial veins and connection area (M_{1+4}), not conspicuous, second cubital (Cu_2) vein not conspicuous either totally or partially.

Male genitalia (Figs 19, 24, 31b, 31c, 34): Apical segment of each inferior appendage (Figs 19A, 24a, 34c) with basal and distal edges diverging, dorsal and ventral edges slightly diverging, posterior edge of 2nd segment of each inferior appendage slightly concave, ventral edge slightly convex, 2.5 times longer than dorsal edge (Figs 19A, 24a). Apicodorsal vertex angular, apicoventral angle rounded, projecting as thick lobe narrowing progressively to apex. Parameres in ventral view curved posteromesad in apical half (Figs 19BV p, 24c, 34d). In lateral view (Fig 19BL) each constricted at short distance beyond base, dilated at middle, with rounded dorsal margin, without spines in midventral margin, few long and thick spines on lateroventral area; midlateral surface covered by fine spicules or setae from middle to ventral edge, absent in middle anteroventral and posterodorsal edges.

Aedeagus (phallicata) in lateral view (Fig 19CL) with dorsal margin concave and posterior corner of concavity hooked anterad, posterior edge of aedeagus straight, projected posteroventrad in small ventral apex (Fig 19CL); ventral lobe of aedeagus semicircular (Fig 19BV vl). Lateroventral lobes of phallus straight, posterior edges strongly convex, apicolateral margins semicircular (Fig 19CD lvi).

Apicodorsal lobe of segment IX dilated subapicolaterally (Figs 19DD al, 24b, 31b, 34b), preanal appendages (Fig 19DD pa, 24b, 31b, 34b) round, almost as long as apicodorsal lobe, covered partially by it, mainly posteriorly; in ventral view apical band (Fig 19DV ab, 31c) V-shaped, longer than wide, posterior edges rounded, almost parallel, non-sclerotized inside area (Fig 19DV va) straight, anal sclerites triangular (Fig 19DV as).

Female genitalia (Figs 20, 26, 32a, 35): In lateral view (Figs 20L, 26L, 32a, 35c), posterodorsal margin of segment VIII valves on each side round, posterolateral margin with small excision in middle, ventral edge convex, dorsal edge with dorsolateral triangular projection (Fig 20L).

In dorsal view (Figs 20D, 26D, 35b), with indentation between segment VIII valves, projections triangular with rounded corners, delimiting oval space (Fig 20D).

In ventral view (Fig 20V, 26V, 35d) segment VIII valves forming two elongate sclerites, distal 2/3 of proximal edges diverging, distal edges straight or slightly concave; intersegmental membrane with pair of deep-dark, slightly elongate sclerites.

Morphological characters diagnosing larvae of *R. fasciata* and *R. septentrionis*

Head: In *R. fasciata* ventral side of head pale, in some specimens one or two rows of low conspicuous pale spots from posterior edge to halfway up the head. In *R. septentrionis* central area with slightly darker U-shape area from middle to posterior end of protogula, covered with pale spots.

In *R. fasciata* the pair of dark brown patches outside posterior edge of frontal suture in the shape of open wings, with dark spots inside. In *R. septentrionis* the pair of dark brown patches more nearly triangular, with paler spots with dark borders inside.

In *R. fasciata* mandibles dark reddish brown, uniformly coloured, left mandible with inner blade slightly convex. In *R. septentrionis* mandibles reddish brown, with darker apices, left mandible with inner blade slightly concave.

Thorax: In *R. fasciata* on posterior half three dark areas, one median and two lateral, two lines of dark spots inside median area, circular spot on anterior half; posterior area with two to three rows of brown spots with dark border in circular-oval shape and parallel to posterior border. In *R. septentrionis* on posterior half three dark areas (in Hickin 1954) or dark base with darker mesal stripe with two lines of pale spots inside, two pale brown spots with dark borders anterolateral and posteriolaterally to seta 6#, circular and elongate respectively; posterior area with two-three lines of darkly-bordered pale brown spots of different shapes and parallel to posterior border. In *R. fasciata* anterior margin with black spot near anterolateral angle; mesal area of posterior marginal band of sclerite dark brown or black posteriorly. In *R. septentrionis* anterior margin with a line of black spots near anterolateral angle, light brown in middle; mesal area of posterior marginal band of each sclerite black posteriorly.

Abdomen: In *R. fasciata* dorsal sclerite of segment IX uniformly coloured, brown spots in posteromesal area, band of anterior edge with two extensions surrounding pit #1, connected with dark stripes in darker larvae. In *R. septentrionis* sclerite of segment IX light coloured, mesal area slightly darker, with paler spots inside.

Morphological characters diagnosing pupae of *R. fasciata* and *R. septentrionis*

Abdomen: In *R. fasciata* paired anterior hook plates palmate, pair of small flat anterior plates without spines on tergum III; paired posterior hook plates slightly oblong transversally. In *R. septentrionis* paired anterior hook plates almost circular, pair of small flat anterior plates on tergum III with 10–15 spines; paired posterior hook plates almost circular.

Morphological characters diagnosing males of *R. fasciata* and *R. septentrionis*

Genitalia: In *R. fasciata*, posterior edge of 2nd segment of each inferior appendage slightly concave dorsally and convex ventrally, ventral edge slightly concave and 2.0 times longer than dorsal edge, apicodorsal vertex slightly angular. In *R. septentrionis*, posterior edge of 2nd segment of each inferior appendage completely concave, ventral edge slightly convex and 2.5 times longer than dorsal edge, apicodorsal vertex more conspicuously angular.

In *R. fasciata* parameres in lateral view each slender near base, dilated in middle, with almost parallel dorsal and ventral margins in central area (S-shape), with two rows of long and thick spines on midventral margin, pointing outward; midlateral surface with sparse fine spicules or setae, from middle posteroventral to posterodorsal edge, covering most of apex, absent on middle anteroventral and dorsal edges. In *R. septentrionis*, parameres each slender near base, dilated in middle, with rounded dorsal margin, without spines in midventral margin, few long and thick spines on lateroventral area; midlateral surface covered by fine spicules or setae, from middle to ventral edge, absent on middle anteroventral and posterodorsal edges.

In *R. fasciata* posterior edge of aedeagus straight, projected posteroventrad and rounded at posteroventral apex; ventral lobe of aedeagus triangular, lateral edges nearly straight; lateroventral lobes of phallus straight, posterior edges convex, apicolateral margins rounded. In *R. septentrionis* posterior edge of aedeagus straight, projected posteroventrad in small posteroventral protrusion; ventral lobe of aedeagus semicircular; lateroventral lobes of phallus straight, posterior edges convex, apicolateral margins semicircular.

In *R. fasciata* apicodorsal lobe of segment IX slightly dilated subapicolaterally, almost round, with small apicomeral excision in some specimens; preanal appendages round, shorter than apicodorsal lobe, almost covered by it; in ventral view, apical band V-shaped and 1.8 times as long as wide, posterior arms widely separated, non-sclerotized area between arms of apical band heart-shaped and with posteromesal excision and posterior edges rounded. In *R. septentrionis* apicodorsal lobe of segment IX strongly dilated subapicolaterally; preanal appendages round, almost as long as apicodorsal lobe, covered partially by it, mainly posteriorly; in ventral view, apical band V-shaped and 1.2 times as long as wide, posterior arms close together and almost parallel, non-sclerotized area between arms of apical band straight and narrow and without posteromesal excision.

Morphological characters diagnosing females of *R. fasciata* and *R. septentrionis*

Genitalia: In *R. fasciata* in lateral view with dorsal margin of segment VIII 3/4 as long as venter and with prominent dorsolateral projection, posterolateral margin of valve on each side irregular, with 1 or 2 posterior projections, posteroventral projection more conspicuous, ventral margin slightly concave near base and straight or convex distally, in some specimens slightly indented posteriorly. In *R. septentrionis* in lateral view, with dorsal margin of segment VIII half as long as venter and with prominent dorsolateral projection, posterolateral margin of valve on each side round and with small excision in middle, ventral margin convex.

In *R. fasciata* in dorsal view with indentation between segment VIII valves nearly enclosed by pair of thick posterolateral projections, with rounded apical and subapical corners, small projection on apical corner of some specimens. In *R. septentrionis* with indentation between segment VIII valves nearly enclosed by pair of triangular projections, with more-nearly acute apical corners.

In *R. fasciata* in ventral view segment VIII valves forming two elongate sclerites, basal third of proximal edges parallel, distal edges irregular, straight, diverging; intersegmental membrane with pair of dark ovoid or slightly elongate sclerites. In *R. septentrionis* in ventral view segment VIII valves forming two elongate sclerites, basal 1/3 of proximal edges converging, distal edges straight or slightly concave, diverging; intersegmental membrane with pair of deep-dark slightly elongate sclerites.

FIGURE 27. Neighbour-joining tree of *Rhyacophila fasciata* Hagen 1859 and *Rhyacophila septentrionis* McLachlan 1865: *Rhyacophila fasciata* (Rfas) (27a), *Rhyacophila septentrionis* (Rsep) (27b) together with *Rhyacophila cf. nubila* Zetterstedt 1840 (Rnub) (27c) and *Rhyacophila cf. oblitterata* McLachlan 1863 (Robl) (27d) as outgroups, based on mitochondrial COI sequence data. Bootstrap values of main branches are shown; data for specimens are summarized in Table 1. RfasBEL = *Rhyacophila fasciata* specimens from Belgium, RfasBAH = *Rhyacophila fasciata* specimens from Bosnia and Herzegovina, RfasCRO = *Rhyacophila fasciata* specimens from Croatia, RfasCZE = *Rhyacophila fasciata* specimens from Czech Republic, RfasEST = *Rhyacophila fasciata* specimens from Estonia, RfasFIN = *Rhyacophila fasciata* specimen from Finland, RfasGER = *Rhyacophila fasciata* specimens from Germany, RfasKOS = *Rhyacophila fasciata* specimens from Kosovo, RfasMAC = *Rhyacophila fasciata* specimens from North Macedonia, RfasNOR = *Rhyacophila fasciata* specimens from Norway, RfasPOL = *Rhyacophila fasciata* specimens from Poland, RfasRUS = *Rhyacophila fasciata* specimens from Russia, RfasSER = *Rhyacophila fasciata* specimens from Serbia, RfasSLO = *Rhyacophila fasciata* specimens from Slovakia, RfasSWE = *Rhyacophila fasciata* specimens from Sweden, RsepUK = *Rhyacophila septentrionis* specimens from United Kingdom, RnubRUS = *Rhyacophila cf. nubila* specimens from Russia, RoblPOL = *Rhyacophila cf. oblitterata* specimens from Poland, RoblRUS = *Rhyacophila cf. oblitterata* specimens from Russia. Scale bar: 1% divergence (modified tree).

FIGURE 28. Neighbour-joining tree of species of the *Rhyacophila fasciata* Group: *Rhyacophila fasciata* Hagen 1859 (Rfas) (28a), *Rhyacophila fasciata viteceki* Valladolid & Kućinić 2020 (in Valladolid *et al.* 2020) (Rfvit) (28b), *Rhyacophila fasciata delici* Kućinić & Valladolid (in Valladolid *et al.* 2020) (Rfdel) (28c), *Rhyacophila septentrionis* McLachlan 1865 (Rsep) (28d), *Rhyacophila denticulata* McLachlan 1879 (Rden) (28e), *Rhyacophila sociata* Navás 1916 (Rsoc) (28f), *Rhyacophila kykladica* Malicky & Sipahiler 1993 (Rkyk) (28g), together with *Rhyacophila cf. nubila* Zetterstedt 1840 (Rnub) (28h) and *Rhyacophila cf. oblitterata* McLachlan 1863 (Robl) (28i) as outgroup, based on mitochondrial COI sequence data. Bootstrap values of main branches are shown; data for specimens are summarized in Table 1. RfasBEL = *Rhyacophila fasciata* specimens from Belgium, RfasBAH = *Rhyacophila fasciata* specimens from Bosnia and Herzegovina, RfasCRO = *Rhyacophila fasciata* specimens from Croatia, RfasCZE = *Rhyacophila fasciata* specimens from Czech Republic, RfasEST = *Rhyacophila fasciata* specimens from Estonia, RfasFIN = *Rhyacophila fasciata* specimen from Finland, RfasGER = *Rhyacophila fasciata* specimens from Germany, RfasKOS = *Rhyacophila fasciata* specimens from Kosovo, RfasMAC = *Rhyacophila fasciata* specimens from North Macedonia, RfasNOR = *Rhyacophila fasciata* specimens from Norway, RfasPOL = *Rhyacophila fasciata* specimens from Poland, RfasRUS = *Rhyacophila fasciata* specimens from Russia, RfasSER = *Rhyacophila fasciata* specimens from Serbia, RfasSLO = *Rhyacophila fasciata* specimens from Slovakia, RfasSWE = *Rhyacophila fasciata* specimens from Sweden, RfvitBAH = *Rhyacophila fasciata viteceki* specimens from Bosnia and Herzegovina, RfdelCRO = *Rhyacophila fasciata delici* specimens from Croatia, RdelBAH = *Rhyacophila fasciata delici* specimens from Bosnia and Herzegovina, RfdelSER = *Rhyacophila fasciata delici* specimens from Serbia, RdenFRA = *Rhyacophila denticulata* specimens from France, RsocSPA = *Rhyacophila sociata* specimens from Spain, RsocFRA = *Rhyacophila sociata* specimens from France, RkykGre = *Rhyacophila kykladica* specimens from Greece, RnubRUS = *Rhyacophila cf. nubila* specimens from Russia, RoblPOL = *Rhyacophila cf. oblitterata* specimens from Poland, RoblRUS = *Rhyacophila cf. oblitterata* specimens from Russia. Scale bar: 1% divergence.

Genetic analysis

Rhyacophila fasciata has a wide area of distribution in northern and central Europe and there is no GeneBank entry from the type locality (Elberfeld, Germany), so we have included some sequences from localities around it (Belgium and Germany). In the first analysis, we used these sequences, together with others from localities that previously have been checked as *R. fasciata* from Bosnia and Herzegovina, Belgium, Croatia, Czech Republic, Estonia, Finland, Germany, Kosovo, Norway, Poland, Republic of Macedonia, Russia, Slovakia and Sweden, together with one sequence of *R. cf. nubila* from Russia and three of *R. cf. oblitterata* from Poland and Russia.

In the second analysis, to build the tree, we selected a total of 52 sequences: a maximum of two sequences of *R. fasciata* per country (25 in total), as well as 3 sequences of *R. septentrionis*, 3 sequences of *R. denticulata*, 4 sequences of *R. sociata* from Spain and France (promoted to species by Valladolid *et al.* 2018), 3 sequences of *R. kykladica* (promoted to species by Valladolid *et al.* 2019), 7 sequences of *R. fasciata delici* Kučinić & Valladolid (in Valladolid *et al.* 2020) and 3 sequences of *R. fasciata viteceki* Valladolid & Kučinić 2020 (in Valladolid *et al.* 2020), 1 sequence of *R. cf. nubila* from Russia and 3 of *R. cf. oblitterata* from Poland and Russia (see Table 1 for references). The intra- and interspecific distances were calculated with all the sequences of each species (162 in total), as well as the bootstrap values of main branches in the second tree. The second tree (as a graphic) includes only some selected specimens and the bootstrap values cited.

The first tree (Fig 27) shows that, despite the wide distribution of the specimens, the group is quite homogeneous genetically, with three well-supported branches, one for the *R. fasciata* specimens (bootstrap value of 98) (Fig 27a), a second for *R. septentrionis* specimens (bootstrap value of 99) (Fig 27b), and the last for the *cf. oblitterata* specimens (b.v. of 99) (Fig 27d). Intraspecific distances (maximum composite likelihood model) are lower than 1% (0.0041 in the *R. fasciata* Group, 0.0014 in the *R. septentrionis* Group and 0.0053 in the *R. cf. oblitterata* Group), while interspecific distances are higher than 1% (*R. fasciata*-*R. septentrionis* = 0.0290, *R. fasciata*-*R. cf. oblitterata* = 0.1040, *R. fasciata*-*R. cf. nubila* = 0.0787, *R. septentrionis*-*R. cf. oblitterata* = 0.0867, *R. septentrionis*-*R. cf. nubila* = 0.0618).

The second tree (Fig 28) shows clear differences between *R. fasciata* and the other species included, that previously were considered as *R. fasciata* or subspecies of *R. fasciata*. Based on the divergence sequences, there are seven well-supported branches, corresponding to *R. fasciata* (bootstrap value of 99) (Fig 28a), *R. f. delici* (b.v. of 98) (Fig 28c), *R. septentrionis* (b.v. of 90) (Fig 28d), *R. denticulata* (b.v. of 99) (Fig 28e), *R. sociata* (b.v. of 97) (Fig 28f), *R. kykladica* (b.v. of 100) (Fig 28g), all clearly different from *R. cf. nubila* (Fig 28h) and *R. cf. oblitterata* (b.v. of 100) (Fig 28i) branches. *Rhyacophila f. viteceki* (Fig 28b) appears as an intermediate group between *R. f. delici* and *R. fasciata* groups. Intraspecific distances (maximum composite likelihood model) are lower than 1% (Table 2a), being the lowest in *R. septentrionis*, *R. f. viteceki*, and *R. kykladica* (0.0014, 0.0017, and 0.0019, respectively) while interspecific distances are higher than 1% (Table 2b) among all of these species. The lower distances are between *R. septentrionis* and *R. f. viteceki* (0.0101) and between *R. f. delici* and *R. f. viteceki* (0.0203).

TABLE 2. Genetic distances (maximum composite likelihood model). 2a: intraspecific distances. *R. cf. nubila* = not computed (1 specimen only). 2b: interspecific distances. In **bold**: distances between *Rhyacophila fasciata* Hagen 1859 and the species of the *Rhyacophila fasciata* Group and between *Rhyacophila septentrionis* McLachlan 1865 and the species of the *Rhyacophila fasciata* Group.

TABLE 2a

Intraspecific	
<i>Rhyacophila fasciata delici</i>	0.0086
<i>Rhyacophila denticulata</i>	0.0053
<i>Rhyacophila fasciata</i>	0.0040
<i>Rhyacophila kykladica</i>	0.0019
<i>Rhyacophila septentrionis</i>	0.0014
<i>Rhyacophila sociata</i>	0.0062
<i>Rhyacophila fasciata viteceki</i>	0.0017
<i>Rhyacophila cf. oblitterata</i>	0.0053
<i>Rhyacophila cf. nubila</i>	n/c

TABLE 2b

Interspecific	<i>R. f. del.</i>	<i>R. dent.</i>	<i>R. fas.</i>	<i>R. kyk.</i>	<i>R sep.</i>	<i>R. soc.</i>	<i>R. f. vit.</i>	<i>R. cf. nub.</i>
<i>R. denticulata</i>	0.0576							
<i>R. fasciata</i>	0.0437	0.0620						
<i>R. kykladica</i>	0.0545	0.0669	0.0655					
<i>R. septentrionis</i>	0.0263	0.0519	0.0291	0.0433				
<i>R. sociata</i>	0.0613	0.0430	0.0678	0.0773	0.0546			
<i>R. f. viteceki</i>	0.0203	0.0470	0.0240	0.0506	0.0101	0.0474		
<i>R. cf. nubila</i>	0.0806	0.0911	0.0796	0.0742	0.0624	0.0904	0.0670	
<i>R. cf. oblitterata</i>	0.1087	0.1075	0.1055	0.0973	0.0877	0.1193	0.0902	0.0995

Discussion

The morphological characteristics that have been traditionally used to determine the males of these species refer mostly to weakly sclerotized (“soft”) parts of the animal, such as inferior appendages or dorsal lobes, together with strongly sclerotized (“hard”) structures as parameres or aedeagus. Soft parts, although possibly useful for the discrimination, show also more variability, not only in morphology but also in position after fixation, for example, the dorsal lobe can be more or less flat or subsequently folded after death. Hard structures are small in size and sometimes, such as the parameres, can be rotated according to their natural position, so it is necessary to check the orientation of the piece and use a compound microscope with high magnification in order to see microstructures as small as spines (only the biggest can be seen with a dissecting microscope) or setae. In females, the shape of the hard valves of segment VIII (dorsal, ventral, and lateral views) usually is a consistent character for discriminating species. Wings have also been used historically for determination, although we have observed that variability within a population can be as high as between two different species [Mosely (1939) noted variability in wing venation as a feature of *R. septentrionis* but not in other British *Rhyacophila* species]. Therefore, we include only a few venational traits in the description.

Mitochondrial DNA (mtDNA) sequences can be useful as a first indicator in species delineation, and, combined with careful morphological, behavioural, and ecological analysis, can help to establish accurate species boundaries (Bickford *et al.* 2007; Dasmahapatra & Mallet 2006; Galtier *et al.* 2009). Most recent studies show the importance of mtDNA in the creation of new species through mitochondrial-nuclear (mitonuclear) coevolution: Some changes in mitochondrial DNA are selected and lead to cyto-nuclear incompatibility between populations that become new species (Burton *et al.* 2013; Chou & Leu 2015; Galtier *et al.* 2009; Healy & Burton 2020; Hill 2016, 2017). Hill (2016) proposed a new definition for bisexual species: “A species is a population that is reproductively isolated from other populations by incompatibilities in uniquely coadapted mt and N-mt genes. Species boundaries are the boundaries of coadapted mitochondrial and nuclear genes, which will be boundaries already deduced from the mtCOI barcode gap.”

Among the mitochondrial DNA, the nucleotide sequence of the gene cytochrome c oxidase subunit 1 has been established as a highly effective DNA barcode for diagnosing the species boundaries of animals (Bucklin *et al.* 2011; Dasmahapatra & Mallet 2006; Hebert *et al.* 2003b; Hill 2016). In a majority of metazoan populations, there is a correspondence between the species designated by mtCOI and species previously described morphologically (Tavares & Baker 2008; Tavares *et al.* 2011) and discovery of barcode gaps often leads to the recognition of new species upon further study (Bickford *et al.* 2007; Hebert *et al.* 2004; Hill 2016).

The differences observed at genetic and morphological levels corroborate the hypothesis that *Rhyacophila fasciata* and *Rhyacophila septentrionis* are two different species: The intraspecific distances were always lower than 1% divergence, and interspecific distances were usually higher than 1% (Table 2). This result is similar to what we found in specimens of *R. denticulata* and *R. sociata* (Valladolid *et al.* 2018), *R. kykladica* (Valladolid *et al.* 2019), or *R. f. delici* and *R. f. viteceki* (Valladolid *et al.* 2020). Other authors that have analysed the divergence of mtCOI sequences between different species of several taxonomic groups found that more than 98% of the species pairs show divergences greater than 2% (e.g., Hebert *et al.* 2003b), and that the intraspecific divergences usually are less than 1% (Avice 2000). We also agree with the premise of Hebert *et al.* (2003a) that we can find new species by their

genetic divergences from known species assemblages. Therefore, it is advisable to continue with the detailed study of populations of this species in different countries to detect possible new species.

Following with the last point, in a recent publication about *Rhyacophila fasciata* in Europe (Oláh *et al.* 2020), the authors described several new species of this group, based on only a few (often only one) specimens, mainly males. For example, they include six species from northern Spain and southern France (Pyrenees Mountains), four of them new species. The authors used for their descriptions the general morphology of specimens (colour), mostly preserved in ethanol for a long time, and some characteristics of males (shapes of parameres and presence of microtrichial bands) that are difficult to see if you observe them with only a dissecting microscope instead of with a compound microscope. We think it is necessary to base new descriptions of species on more characters, more specimens, and DNA information. Due to the small number of specimens used for some of their descriptions and the intraspecific variability in species (observed, for example, in soft structures), we do not agree with the results of their paper. We conclude that *Rhyacophila gemella* Navás 1923 is a synonym of *R. sociata* Navás 1916 (Valladolid *et al.* 2018), the species *R. coppai* Oláh 2020 **NEW SYNONYM** and *R. soreda* Coppa & Oláh 2020 **NEW SYNONYM** are synonyms of *R. sociata*, and the species *R. kopasa* Oláh & Coppa 2020 **NEW SYNONYM** and *R. rova* Oláh & Coppa 2020 **NEW SYNONYM** are synonyms of *R. denticulata* (see descriptions of *R. sociata* and *R. denticulata* by Valladolid *et al.* 2018).

Another example is the description of *R. matrensis* Oláh & Szczęsny 2020 from several countries and with much morphological variation. Some of the localities cited, such as in the Czech Republic and Slovakia, are included in our study as *R. fasciata*, checked by morphology and also by genetic characters (see specimens of *R. fasciata* #71 and #72 from the Czech Republic and *R. fasciata* #78 from Slovakia), so it is probably a new synonym of *R. fasciata*. Therefore, more study of *R. matrensis* Oláh & Szczęsny 2020 is needed to confirm or deny that it is a valid species, especially considering that its distribution coincides with that of *R. fasciata*.

As we have commented in the Introduction, the identification of the species included in the *Rhyacophila fasciata* Group has been difficult since their first descriptions. The small size of specimens and the difficulties in observing structures such as male genitalia generated descriptions that were very similar for several species. We have also to consider that the first species of Trichoptera were described in the 18th and 19th centuries, and together with the technical limitations when studying the specimens, the delays in publishing results, and possibly the reduced accessibility to texts and other colleagues working on the same subject increased the number of species, although many of them had been previously described.

Rhyacophila fasciata was described from one male, but with very few details. We have searched for previous descriptions in Hagen's publications, but we have not found them. McLachlan (1868) described the species from three males from Carinthia (Austria), but in a later publication (1879), he concluded that these specimens should be referred to as *septrionis*, after comparison probably with the original specimen of Hagen. Finally, Ulmer (1909) described the two species, *R. fasciata*, from Elberfeld, and *R. septrionis*, from some regions of Germany, which coincide with the current distribution of *R. fasciata*. Several subsequent authors used the name *R. fasciata* or *R. septrionis* in their descriptions, showing the difficulties resulting from poor descriptions of both species. Having the images from the lectotype of *R. fasciata*, kindly provided by the staff from the Museum of Comparative Zoology of Harvard University (Cambridge, MA, USA), we confirm that the features that can be observed (inferior appendages and lateral parameres) match with those of the specimens we studied, so we conclude with confidence that they belong to the same species, *Rhyacophila fasciata*.

Rhyacophila septrionis was considered a synonym of *R. fasciata*. However, the original description, the images of the lectotype, and the study of one paratype, both kindly provided by Ben Price (The Natural History Museum, London), together with our study of the morphology and DNA sequences obtained from three life stages (adults, pupae, and larvae) from the United Kingdom (provided by Ian Wallace), show that *Rhyacophila septrionis* is a different species from *R. fasciata*, so we propose to change its status to *status resurrectus*.

Distribution and Ecology

Figure 29 shows the geographical distribution of *R. fasciata* and *R. septrionis*, with dates from samples studied (morphology and genetic), databases (GBIF, 2020) and literature (see *Geographical distribution* in Material and Methods). We can confirm the presence of *R. fasciata* in Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Estonia, Finland, Germany, Kosovo, Norway, Poland, Republic of North Macedonia, Russia, and Sweden. Records of these species in other countries present in databases or the literature should be checked, especially

records from countries in geographical proximity to those above, including Belarus, Denmark, Hungary, Latvia, Lithuania, and the Netherlands. The status of these taxa in other countries such as Austria, France, Greece, or Italy, will be evaluated morphologically and genetically and included in some follow-up papers. Other countries that should be studied are Romania, Slovenia, Switzerland and Ukraine.

FIGURE 29. Spatial distribution of *Rhyacophila fasciata* Hagen 1859 and *Rhyacophila septentrionis* McLachlan 1865 in Europe. In pale colour: Austria, France, Greece, Italy, Slovenia and Switzerland, countries with records of *Rhyacophila fasciata* whose material needs more research to confirm the presence of this species.

In addition to the specimens examined for this publication, adults and larvae have been identified as either *R. septentrionis* or *R. fasciata* from many sites in the United Kingdom in England, Wales, Scotland, and the Channel Islands, just off the coast of northern France. They have not been recorded from Ireland or the Isle of Man. The 200

records mapped in this paper are considered reliably this taxon. There are 700 more larval records, but these have not been included, as they include records of *R. dorsalis* (Curtis 1834) which the current UK keys can misidentify as *R. fasciata* / *septrionis*, notably those larvae with a banded pronotum, illustrated by Pitsch (1993). Further work is required to check specimens against the characters described in this publication to see if all UK adult material can be referred to *R. septrionis* and to improve the key to larvae to enable the same procedure to be adopted for larvae. All the records of this species in continental Europe should be *R. fasciata* or related species. Particularly intriguing are the few isolated records for *R. fasciata* for northwestern France, mapped by Neu *et al.* (2018) that have not yet been studied. These are geographically close to the UK.

In European Russia, *R. fasciata* inhabits cold (summer temperatures up to 10–12°C), oligosaprobic spring streams, narrow (0.7–2.0 m wide), with moderate flow (0.2–0.5 m/s), in forest ravines or in slopes of river valleys. It is found in streams throughout the year. In the Arctic zone (Murmansk Region), it is found in streams of mixed origin.

In Finland, *R. fasciata* is distributed over most of the country, but its suitable habitats (small, stony, cool brooks) are often restricted to the wooded, hilly headwaters of the river systems. Moreover, there is association with the unevenly distributed groundwater areas. *Rhyacophila fasciata* occurs seldom together with *R. nubila* Zetterstedt 1834, which is the most common species of the genus in Finland and typically present in larger and warmer running waters. Larval development is asynchronous and adults are found from June to October (Rinne & Wiberg-Larsen 2017; Salokannel & Mattila 2018).

In the Czech Republic and Slovakia *R. fasciata* inhabits almost the entire territory. It prefers cold (shaded) brooklets and small streams at an altitude of 200–1100 m a.s.l. (up to 1650 m a.s.l. in the Tatra Mts. in Slovakia). The flight period generally lasts from the beginning of May to the end of October.

In Croatia, *R. fasciata* can be found in the northern part of the country, where the distribution area is mostly covered by forests, and harbours a large network of streams and a variety of different freshwater areas.

In Bosnia and Herzegovina, *R. fasciata* can be found also in the northern part of the country. Most of the rivers belong to a mountainous region of Bosnia and Herzegovina, characterized by moderate continental climate with strong influences of mountain and subalpine climates, with harsh snowy winters and moderately warm summers (Stanić-Koštroman 2009; Stanić-Koštroman *et al.* 2015).

In the Republic of North Macedonia populations of *R. fasciata* seem to be restricted to the parts of rivers within the mountainous region. Kumanski (1997) noted the presence of this species in river Bregalnica on the southern part of Osogovo Mountain near the village Istibanja. Recent comprehensive investigation of the caddisfly fauna of the northern part of Osogovo Mountain (Hinić 2020) showed the presence of adult males in the cold waters of Trnovska and Kriva River and of larvae in the upper part of Kamenicka River (Slavevska Stamenković and Hinić, unpublished data) so we can conclude that the species could be spread over the whole Macedonian part of Osogovo Mountain. Additionally, this species lives in the western part of the country, being reported from lower elevations of the river Pena, below Tetovo (Kovachev *et al.* 1999).

In the United Kingdom larvae of *R. septrionis* are found in permanently flowing, moderate-to-fast, stony streams and rivers. It may be the only *Rhyacophila* species in stream with calcareous deposits and may be common there, sometimes together with *R. obliterata* and *R. dorsalis*. It can also be found in small numbers among other species in general (slightly alkaline) sites. It has not been recorded as adults or larvae from the chalk rivers of southern England, the Rivers Test, Itchen, or their tributaries (Wallace & O'Connor, personal communication). Adult records (Hickin 1967) indicate a flight period from June to September (Edington & Hildrew 1981). Other investigators (Wallace & O'Connor, personal communication) indicate that most adults have been taken between late April and October. Larvae that have reached full size will make pupal cocoons: if they do not complete development by late autumn, they will remain in the pupal cocoon as a resting larva until the following spring, when they will pupate and emerge. Wallace has also found developing pupae in early winter but is not sure if these would try to emerge (probably unsuccessfully) that season or in spring. Whenever it is sought at its depositing sites, it is unusual not to find final instar larvae (Wallace & O'Connor, personal communication).

FIGURES 30–33. 30. Lectotype of *Rhyacophila fasciata* Hagen 1859 (male). 30a, thorax and right forewing (pinned); 30b, abdomen and male genitalia in cork-stoppered microvial (ethanol), detail. Photos: Museum of Comparative Zoology of Harvard University, Cambridge, MA, USA (MCZH). 31–33. *Rhyacophila septentrionis* MacLachlan 1865. 31, lectotype (male): 31a, specimen (pinned); 31b, apicodorsal lobe of segment IX and preanal appendages, dorsal view; 31c, detail of genitalia, posterior view. 32, female: 32a, cleared and mounted genitalia; 32b, original mounting preparation. 33, original mounting preparation of *R. septentrionis* male genitalia. Photos: Ben Price, Natural History Museum of United Kingdom (NHMUK).

FIGURES 34–35. *Rhyacophila septentrionis* MacLachlan 1865. 34, male: 34a, label; 34b, genitalia, dorsal, 34c, genitalia, left lateral; 34d, genitalia, ventral. 35, female: 35a, label; 35b, genitalia, dorsal, 35c, genitalia, left lateral; 35d, genitalia, ventral. Photos: National Museums of Scotland (NMS).

Acknowledgements

We wish to thank the following researchers that provided us some of the specimens studied: Hans Malicky, Wolfram Graf and Johann Waringer (Austria), who also helped with the revision of manuscript; Esko Viitanen and Pekka Majuri (Finland); Gennaro Coppa (France); and Fusun Sipahiler (Turkey). Ben Price, curator of the Entomological Collection of the Natural History Museum of United Kingdom (NHMUK, previously BMNH) kindly sent photos of lectotype and other specimens of *Rhyacophila septentrionis*, as well as one paratype. Crystal Maier and Whit Farnum, of Entomology Department, and Melissa Lynne Aja, Museum Projects Coordinator & Interim Managing Editor of the Museum of Comparative Zoology of Harvard University, Cambridge, MA, USA (MCZ) kindly sent photos and permission for reproduction respectively of the lectotype of *Rhyacophila fasciata*. Lee Robertson and Craig Macadam, Conservation Director of the Buglife-The Invertebrate Conservation Trust, provided the contacts for obtaining the samples of *R. septentrionis*. Ashleigh Whiffin, Assistant Curator of Entomology, and Vladimir Blagoderov, Principal Curator of the Invertebrates Collection of the National Museums of Scotland (NMS)–National Museums Collection Center, Edinburgh, United Kingdom (NMS) kindly sent photos of *R. septentrionis* specimens in the collection.

Thanks also to Tor Erik Eriksen (Norway), K.A. Johanson (Sweden), Belma Kalamujić (Bosnia and Herzegovina) and Iliana Bysta (United Kingdom) for completing the information about some sequences obtained from GenBank, and to Henn Timm for providing distribution data for *R. fasciata* in Estonia. We thank Manuela Gallardo and Arabia Sánchez for their help with photography, and Adrián Casado for processing the samples for DNA study. Mercedes Paris, curator of the Collection of Entomology (MNCN-CSIC) helped in several steps. This research is a part of scientific project “DNA barcoding of Croatian faunal biodiversity” (IP-2016-06-9988) funded by the Croatian Science Foundation. The work of P. Chvojka was supported by the Ministry of Culture of the Czech Republic (DKRVO 2019-2023/5.I.b, National Museum, 00023272). We thank also the Editor and two anonymous reviewers for improving the final manuscript.

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Erratum

In our previous paper (Valladolid *et al.* 2020) we detected one error. In Material Examined, adults: **Croatia:** *R. f. fasciata*: 1 ♂ + 1 ♀ (K1–K2), river Kutjevačka, upper course, 16/v/05. Col. & Leg.: R. Čuk (RCC). 3 ♂, river Voćinska, upper course, near Požega, 03/v/15. Leg.: R. Čuk CNHM). 4 ♂, river Voćinska, upper course, Papuk Mt., 19/v/15.

The collection data should be as follows: **Croatia:** *R. f. fasciata*: 1 ♂ + 1 ♀ (K1–K2), river Kutjevačka, upper course, 16/v/05, col. & leg.: R. Čuk (RCC). 3 ♂, river Veličanka, upper course, near Požega, 03/v/15, leg.: R. Čuk (CNHM). 4 ♂, river Voćinska, upper course, Papuk Mt., 19/v/15.