DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

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MARKO PROUS

Taxonomy and phylogeny of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae)

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LIST OF ORIGINAL PUBLICATIONS

This dissertation is a summary of the listed articles, which are referred to by the respective roman numerals:

I. Prous M, Heidemaa M, Soon V (2011) *Empria longicornis* species group: taxonomic revision with notes on phylogeny and ecology (Hymenoptera, Tenthredinidae). Zootaxa 2756: 1–39.

II. Prous M, Heidemaa M, Shinohara A, Soon V (2011) Review of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae) in Japan. ZooKeys 150: 347–380. doi: 10.3897/zookeys.150.1968

III. Prous M, Heidemaa M (2012) *Empria formosana* sp. n. from Taiwan with notes on *E. wui* species group (Hymenoptera, Tenthredinidae). Deutsche Entomologische Zeitschrift (in press).

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My personal contribution to the articles referred to in this thesis is as follows:

I – identification of most of the material, morphometric measurements and most of the analyses, microphotographing, laboratory procedures, part of the phylogenetic analyses, responsible for writing the article.

II – identification of most of the material, morphometric measurements, microphotographing, laboratory procedures, part of the phylogenetic analyses, responsible for writing the article.

III – identification of most of the material, morphometric measurements, microphotographing, laboratory procedures, phylogenetic analyses, responsible for writing the article.

INTRODUCTION

Knowing accurately species (taxonomy) and their evolutionary history (phylogenetics) is fundamental to many areas of biological research. Taxonomy and phylogenetics are the main components of biological systematics, which can be summarized as the science about delimiting, naming, classifying, and describing taxa (species, genera, and other higher taxa), as well as providing identification keys, distribution data, and reconstructing phylogenetic relationships. Knowing the species and their phylogeny is not only important to other biological disciplines (ecology, evolutionary and conservation biology etc.), but also to agriculture (e.g. pests and their biological enemies), medicine (e.g. pathogens and drug development), and even economics (Futuyma 2004; Yates et al. 2004; Springer and Murphy 2007; Domazet-Loso and Tautz 2008). Although systematists have been describing biodiversity for more than 250 years, large gaps in our knowledge still remain, because only 10–20% of eukaryotic species are estimated to have been discovered (Hamilton et al. 2010; 2011; Mora et al. 2011). At the same time, biodiversity still continues to decline (Butchart et al. 2010; Leadley et al. 2010). Fortunately, rapid developments in biodiversity informatics (Smith and Penev 2011; Thessen and Patterson 2011; Maddison et al. 2012) and molecular methods (Hebert et al. 2010; Bybee et al. 2011) promise to accelerate biodiversity research, while making the results more easily accessible to other researchers, policy makers, and the general public.

Insects form the largest proportion of known biodiversity, yet they are among the taxa with smallest proportion of estimated species described (Hamilton et al. 2010; 2011). Although sawflies (Symphyta) are globally relatively species poor (about 8600 species; Taeger and Blank 2011) compared to many other insect groups, they form a significant component in northern ecosystems and are a promising group for many ecological and evolutionary studies (Kouki et al. 1994; Viitasaari 2002; Price et al. 2005; Craig and Itami 2008; Nyman 2010). Sawflies are phylogenetically quite well studied at the level of families and superfamilies (Schulmeister 2003; Heraty et al. 2011), but at lower taxonomic levels only few groups have received attention from a molecular phylogenetic perspective (Nyman et al. 2006; Leppänen et al. 2012). Among the sawflies, tenthredinids (Tenthredinidae) are the most species rich (about 5700), but most of the species are relatively poorly known, which is the source of many taxonomic problems.

The sawfly genus *Empria* Lepeletier & Serville, in Latreille et al. 1828 with about 50 species is one of the largest in the tenthredinid subfamily Allantinae. The adults of *Empria* are about 5–7 (9) mm long and characteristic of the genus are pale paired patches on abdominal terga (Fig. 1). Most species in the genus are externally rather similar to each other, which makes species identification difficult. However, the differences in the structure of ovipositors and penis valves are often very clear even between closely related species, which usually makes species identification using sufficient magnification rather straightforward. The genus is distributed in the northern hemisphere (mostly Holarctic; Taeger et al. 2010) and the species mostly inhabit areas with a cool climate which are found towards northern latitudes and higher altitudes in the south. The genus appears to be at least 24 million years (MA) old, which is the approximate date for the Rott site of the Oligocene (Utescher et al. 2000), the type locality of the only known fossil *Empria* species, *E. oligocaenica* (Meunier, 1923) (see also Statz 1936). The species are monophagous (mostly) or oligophagous on species of Betulaceae, Rosaceae, and Salicaceae. Adults are usually on the wing for about 1–4 weeks in early spring. Eggs are laid in the fresh leaves or stems of host plants (Kontuniemi 1951; Smith 1979; **I**), from which I instar larvae hatch within 1–2 weeks (depending on the temperature). The larvae feed on the host plant for about 3–5 weeks after which they enter the prepupal stage which overwinters in a chamber prepared from plant stems or in the soil. There is only one generation a year. Apart from some information about host plants of European and Nearctic species, ecological and evolutionary information about the genus is virtually absent.

Figure 1. Habitus of *Empria* (*E. longicornis*, male).

Empria is taxonomically rather problematic because of the difficulties in species identification (genitalia must usually be dissected) and lack of any taxonomic revisions of Palaearctic species since Conde (1940). Although the 11 Nearctic and one Neotropic species (10 of them endemic to the western hemisphere) have been revised by Smith (1979; 1980), most of the *Empria* species are Palaearctic. Few poorly known species are also known from the northern parts of the Oriental (Indomalaya) region (Yan et al. 2009; Taeger et al. 2010). Of the Palaearctic region, only European species (Zhelochovtsev and Zinovjev 1988; Heidemaa and Viitasaari 1999) were reasonably well known before this study (papers **I–III** and this thesis). Even within Europe, many taxonomic problems remained, notably species delimitation in the *E. longicornis* species group.

The results presented here attempt to solve many of these problems. Taxonomic revision of *E. longicornis* group is the focus of paper **I** and papers **II–III** fill in some gaps in the knowledge on East Palaearctic and Oriental species of *Empria*. The results from papers **I–III** and subsequent new findings are summarized here in the form of a key to all known species of Palaearctic and Oriental *Empria* and brief taxonomic treatments of these species.

Prior to this work there were no attempts to reconstruct the phylogeny of *Empria*. The papers presented here are the first to use molecular sequence data to study phylogeny of the genus, although these are limited by taxonomic scope or geographic region (**I–III**). Attempts to propose morphology-based subdivisions of the genus have remained scarce. Nevertheless, *Empria* is sometimes divided into the subgenera *Parataxonus* MacGillivray, 1908 [now comprising *E. candidata* (Fallén, 1808) and *E. multicolour* (Norton, 1862); **II**] and *Empria* s. str. (all other species) (Ross 1936; Zhelochovtsev and Zinovjev 1988; 1996; Yan et al. 2009). Within *Empria* s. str., the *E. hungarica* (Konow, 1895) (see Heidemaa and Viitasaari 1999), *E. longicornis* (Thomson, 1871) (see Benson 1938; Lindqvist 1968; **I**), and *E. immersa* (Klug, 1818) (Smith 1979; Zhelochovtsev and Zinovjev 1988; **I**) species groups have been proposed. Close affinity of *E. liturata* (Gmelin, 1790), *E. ignota* (Norton, 1867), *E. nordica* Ross, 1936, *E. obscurata* (Cresson, 1880), *E. eosa* Smith, 1979, and *E. mexicana* (Cameron, 1883) could be suggested based on the similarities between these species (Ross 1936; Benson 1938; Smith 1979; **II**). Here, the molecular datasets from papers **I–III** are expanded to investigate the phylogeny of *Empria* globally (including also Nearctic and Neotropic regions).

MATERIAL AND METHODS

Collecting and studied collections

An essential part of any taxonomic revision is the examination of type material, especially in groups where there are no or only few good external diagnostic characters and species delimitation has varied between the authors. Genus *Empria* is undoubtedly one of such groups. The examination of type material is essential to link taxonomic names with biological species and to know which species have already been described, in case the original descriptions are insufficient for that matter. For the revision of Palaearctic *Empria*, a significant proportion of types have been examined, which are listed in the Results section of this thesis and in papers **I–III**. The type and non-type material have been loaned from the following institutional and personal collections:

BMNH The Natural History Museum, London, United Kingdom (G. Broad, N. Dale-Skey Papilloud, S. Ryder, N. Springate);
CEH Collection of Erik Heibo, Lierskogen, Norway;
CJL Collection of Jean Lacourt, Igé, France;
CJM Collection of Jan Macek, Prague, Czech Republic;
CGTK Collection of Guy T. Knight, Liverpool, United Kingdom;
CMH Collection of Mikk Heidemaa, Tartu, Estonia;
CMV Collection of Matti Viitasaari, Helsinki, Finland;
COL Collection of Ole Lønnve, Oslo, Norway;
CSCS Central South University of Forestry and Technology, Changsha, China (M.-C. Wei);
CTN Collection of Tommi Nyman, University of Eastern Finland, Joensuu, Finland;
DEI Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany (A. Taeger, S. M. Blank, A. D. Liston);
EIHU Hokkaido University, Sapporo, Japan (M. Suwa);
HNHM Hungarian Natural History Museum, Budapest, Hungary (G. Puskás, S. Csősz, L. Zombori);
MNHN Muséum National d'Histoire Naturelle, Paris, France (C. Villemant, A. Touret-Alby, T. Yoshida);
MZAT Museum Zoologicum Åbo Academi, Turku, Finland (A. Teräs);
MZH Finnish Museum of Natural History, Helsinki, Finland (P. Malinen; J. Paukkunen);
MZLU Lunds universitet, Lund, Sweden (R. Danielsson);
NHRS Naturhistoriska Riksmuseet, Stockholm, Sweden (H. Vårdal);
NSMT National Museum of Nature and Science, Tokyo, Japan (A. Shinohara);
RSME National Museums of Scotland, Edinburgh, United Kingdom (R. Lyszkowski);
SIZK Schmalhausen Institute of Zoology, Kiev, Ukraine (I. N. Pavlusenko);
TUZ Zoological Museum of the University of Tartu, Estonia (J. Luig);

UOPJ Osaka Prefecture University, Sakai, Japan (T. Hirowatari);
USNM National Museum of Natural History, Washington DC, USA (D. R. Smith);
UUZM Uppsala University, Museum of Evolution, Uppsala, Sweden (H. Mejlon);
YUIC Yeungnam University Insect Collections, Gyeongsan, South-Korea (J.-W. Lee);
ZIN Russian Academy of Sciences, Zoological Institute, St. Petersburg, Russia (S. Belokobylskij, A. Zinovjev);
ZMHB Museum für Naturkunde der Humboldt-Universität, Berlin, Germany (F. Koch);
ZMUC University of Copenhagen, Zoological Museum, Copenhagen, Denmark (L. Vilhelmsen).

Occurrence data of the studied specimens (collection locality and date, identifications etc.) have been digitized in most cases. The data of papers **I–III** is available at:
http://empriini.myspecies.info/files/imports/long_group_spec.txt (**I**);
http://dx.doi.org/10.5061/dryad.fs262s48 or
http://ipt.pensoft.net/ipt/resource.do?r=japanese_empria (Prous et al. 2011; **II**);
http://dx.doi.org/10.5061/dryad.g6v8v5kq (Prous and Heidemaa 2012; **III**).

Most of the occurrence data of the material collected by the author (using sweeping nets, Malaise traps or reared *ex ovo* or *ex larva*) have also been deposited in the database of Estonian animal collections (Abarenkov et al. 2010; http://elurikkus.ut.ee/collections.php?lang=eng).

Rearing of larvae and imagines

To study larval morphology and determine the host plants of *Empria*, and to confirm species identity of males in *E. longicornis* group (**I**), rearing of larvae was carried out indoors in 2004–2012. Host plants were planted in plastic flower pots filled with soil. To ensure that the hosts were without previously-laid sawfly eggs, they were planted before the first adults appeared or, if planted later, they were carefully inspected. Newly hatched first instar larvae were moved to Petri dishes, each containing 1–4 larvae. Larvae were fed by fresh leaves (replaced after every 1–2 days). Shed cuticles of all larval instars were preserved. If possible, at least one specimen of all instars was preserved in ethanol (70–80% or 96%). When the larvae ceased feeding and moulted to prepupal stage, they were moved to glass jars or plastic vials, which contained sterilized moist sand, sphagnum moss, and pieces of raspberry and other plant stems. The jars and vials with prepupae were taken in a basement for overwintering (minimum temperature about 3°C). During February-March, prepupae were left outside on a balcony (where temperature fell below freezing, but not below –10°C) for a few days and then taken step by step to room temperature (the first step of applying freezing temperatures was not always implemented).

Morphological analyses

For morphological analyses, male penis valves, female lancets (valvula 1), and external characters of the adults were studied. To dissect the penis valves, genital capsules were separated from the specimens and macerated in KOH or NaOH (10–15%) for 6–12 hours at room temperature, or treated with proteinase K using High Pure PCR Template Preparation Kit (Roche, Mannheim) and following manufacturer's protocol.

Ovipositors and penis valves were photographed using a digital camera attached to a microscope. Set of images taken along the z axis, where each separate image had only some parts in focus, were combined to create single composite digital image with all parts in focus using the program CombineZM (Alan Hadley; http://www.hadleyweb.pwp.blueyonder.co.uk/index.htm). In some cases the study object was photographed in 2–4 partially overlapping parts and assembled into a single image using the plugin MosaicJ (Thévenaz and Unser 2007) implemented in ImageJ version 1.39u (Wayne Rasband; http://rsb.info.nih.gov/ij/). All images made for papers **I–III** were deposited in the Morphbank database (http://www.morphbank.net/?id=583165, 592670, 716084).

Morphological terminology follows (Viitasaari 2002). To differentiate between species, some distances were measured on the head capsule (**I–III**): maximal lengths and breadths of flagellomeres, length of head (Fig. 1A in **II**), head breadth behind the eyes (Fig. 1B in **II**), length between lateral margins of lateral ocelli ("breadth of postocellar area"; Fig. 1C in **II**), length of the postocellar area (Fig. 1D in **II**), length of head behind the eye in dorsal view (Fig. 1E in **II**; head positioned with posterior margins of lateral ocelli and eyes aligned), length and height of the eye (Figs 1F in **II**; 1C in **I**), antero-posterior diameter of lateral ocellus, length between toruli (antennal sockets; Fig. 2A in **II**), maximal and minimal length of the temple (http://www.morphbank.net/?id=781392), and the length of malar space (Fig. 2B in **II**; from here on referred to as "malar space").

Images of penis valves and the valvulae 1 of ovipositors were used to measure some linear distances (Figs 9–10 in **I**) and to carry out geometric morphometric (GM) analyses (**I**). Linear distances and angles were measured using the program ImageJ version 1.39u. GM analyses were used to study shape differences between the penis valves of the males in the taxonomically difficult *E. longicornis* group, because discrete qualitative differences were lacking (**I**). Although serrulae of female ovipositors are in most cases sufficiently distinct in different species of the *E. longicornis*-group, GM analyses were used also in this case to reveal additional differences for species discrimination. For GM analyses, landmarks were digitized using tpsDig 2.12 (Rohlf 2008a). Some of the landmarks could not be precisely located (Fig. 9 in **I**) and these were defined as semilandmarks (sliders) using tpsUtil 1.4 (Rohlf 2008b). Digitized landmark data were analysed using tpsRelw 1.45 (Rohlf 2007).

The angle between dorsal and basal margins of valviceps was measured in the following way: the tip of the dorsal line starts at the spine (if present) of valviceps and moves basally so that it touches about the middle part of dorsal margin of valviceps; and the tip of basal line starts at dorsal joint between valvura and valviceps and moves dorsally so that it touches the basal-most part of valviceps (Fig. 30).

Molecular analyses

Molecular data can verify or falsify taxonomic hypotheses based on morphological data and reveal additional insights regarding evolution and phylogeny of a taxon under study. Papers presented here (**I–III**) are the first to use molecular methods to study *Empria*. To obtain additional data for solving taxonomic problems in the *E. longicornis* group (**I**) and to investigate phylogeny of *Empria* in general (**II–III**, this study) molecular sequence data were used.

DNA was extracted and purified with High Pure PCR Template Preparation Kit (Roche, Mannheim) according to the manufacturer's protocol and stored at –20°C for later use. In the case of larvae, whole trunk or thorax (and often also head) was used. In the case of imagines, thorax muscles, a leg, or, as suggested by Knölke et al. (2005), genitalia were used. One mitochondrial and one nuclear region were amplified using primers listed in Table 1. From mitochondrial DNA (mtDNA), a fragment containing tRNA-Cys, tRNA-Tyr, cytochrome c oxidase I (COI), and tRNA-Leu was sequenced. This region was amplified in 1–4 parts (Table 2). From the nuclear DNA, internal transcribed spacers 1 and 2 (ITS1 and ITS2) within the rRNA locus were sequenced. For most of the specimens, ITS1 (in 1–2 fragments) and 2 were amplified separately, but in some cases ITS1 and 2 were amplified in a single fragment obtaining also the full 5.8S rRNA gene between them (Table 2). For some older air-dried museum specimens, it was possible to obtain the mitochondrial and nuclear sequences only partially (Table 2).

PCR reactions were carried out in a total volume of 20 µl containing, 5 pmol of primers, 0.2 mM dNTP mixture (Fermentas, Vilnius), 1X of Advantage 2 PCR buffer, 1U of Advantage 2 Polymerase mix (BD Biosciences, San Jose), and purified genomic DNA. The PCR programme consisted of an initial denaturing step at 95°C for 1 min, followed by 35–45 cycles of 20 s at 95°C, 30 s at 46–65°C depending on a primer set used (Tabel 1) and 30–120 s (depending on the amplicon size) at 68°C; the last cycle was followed by a final 7 min extension step at 68°C. To improve amplification success, 2.5 µl of combinatorial enhancer solution (CES) (Ralser et al. 2006) was added to PCR reactions when ITS1 and 2 were amplified together in a single fragment. PCR products were visualised on a 1.6% agarose gel and 10 µl of each product was purified with FastAP and exonuclease I. 1U of both enzymes (Fermentas, Vilnius) were added and incubated for 10 min at 37°C, followed by 15 min at 80°C. The purified PCR product was directly used for sequencing.

DNA cycle sequencing was performed by using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City) with the addition of CES (Ralser et al. 2006) in some cases (amplicons of ITS1 and ITS1+2). 33 cycles (15 s at 95°C, 15 s at 45–55°C and 60 s at 60°C) were performed in a total volume of 10 µl. To obtain unequivocal sequences, both sense and antisense strands were sequenced, using the primers listed in Table 1. Sequences were resolved on 3130xl automated DNA sequencers (Applied Biosystems, Foster City). Based on chromatograms from both DNA strands, consensus sequences were created with Consed (Gordon et al. 1998) and double-checked by eye with BioEdit (Hall 1999). Few ITS sequences of females and one male were polymorphic for length and in those cases insertions/deletions (indels) were reconstructed using the program In*d*elligent v.1.2 (Dmitriev and Rakitov 2008), available at http://ctap.inhs.uiuc.edu/dmitriev/indel.asp. Ambiguous positions (i.e. double peaks in chromatograms of both strands) due to heterozygosity or intragenomic variation were coded using IUPAC symbols.

Sequences reported in papers **I–III** have been deposited in the GenBank (NCBI) database (accession numbers HM177266–HM177415, HQ412768–HQ412770, JN029842–JN029898, and JN788309–JN788331).

COI and tRNA sequences were aligned manually. Delimitation and alignment of tRNA sequences was aided by secondary structure, which was detected using tRNAscan-SE 1.21 (Lowe and Eddy 1997). Regions of tRNA sequences, which remained ambiguously aligned were removed prior to analyses. Among COI sequences, *Monsoma pulveratum* (Retzius, 1783) contained insertion of three base pairs, which was located by translating nucleotides into amino acids (using invertebrate mitochondrial genetic code). *Empria quadrimaculata* and *E. rubicola* had a three base-pair insertion before the stop codon at the 3′ end, but because this could not be unambiguously located, the last three amino acid coding codons of these species were treated as missing data. Boundaries of ITS2 sequences were identified with ITS2-Annotation tool (which uses HMMer; Eddy 1998) available through the ITS2 Database (Selig et al. 2008).

There are three main methods to reconstruct phylogeny from molecular sequences: parsimony, likelihood, and distance methods. Parsimony and likelihood (maximum likelihood, Bayesian inference) methods use a matrix of discrete characters to evaluate candidate phylogenetic trees according to an explicit optimality criterion. Under parsimony, a phylogenetic tree is sought which minimizes the amount of character change required to explain the data. In contrast to parsimony, explicit model of character evolution (e.g. character state frequencies, evolutionary rate variation across characters) is assumed in likelihood methods. Under maximum likelihood, the tree (topology and branch lengths) and parameters of the evolutionary model are sought that maximize the probability of observing the data. Disadvantage of using a model of character evolution is that the true model is not known. A solution is to evaluate different models and select the one having significantly higher likelihood among the models compared. Application of Bayesian inference requires, in addition to a model of character evolution, the prior probability distributions (priors) for parameters to be estimated. These priors can be uniform, in which case all possible values of parameters are assigned equal probability. Given the model, the priors, and the data, trees and model parameters with the highest posterior probability are estimated using Markov chain Monte Carlo (MCMC) sampling (see e.g. Lemey et al. 2009). Distance methods use a character matrix to calculate pairwise distances between taxa, from which a tree is inferred. A disadvantage of the distance methods is that converting characters into single distance values between taxa leads to information loss, which might also affect phylogeny reconstruction. However, some clustering algorithms perform rather well (e.g. neighbour-joining, which does not assume a constant rate of evolution across taxa) when appropriate substitution models are used to correct distances. An advantage of distance methods is that large datasets (e.g. thousands of taxa with millions of characters) can be analysed within reasonable amount of time, while for parsimony and especially for likelihood methods this can be prohibitive. When datasets are not prohibitively large, character-based phylogenetic methods should be preferred. Among character-based methods, likelihood methods should generally be preferred, because parsimony can significantly underestimate the amount of sequence evolution and lead to incorrect phylogeny. Lower topological accuracy of parsimony compared to likelihood methods has been shown theoretically and empirically (Felsenstein 1978; Swofford et al. 2001; Liu and Warnow 2012). For these reasons, maximum likelihood and Bayesian inference methods are here used for phylogeny reconstruction.

Sequence data was analysed using the maximum likelihood (ML, with PhyML 3.0.1 at http://www.atgc-montpellier.fr/phyml/; Guindon and Gascuel 2003), and Bayesian methods (MrBayes 3.1.2, Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; and BAli-Phy 2.1.1, Suchard and Redelings 2006; Redelings and Suchard 2007). In PhyML nearest neighbor interchanges (NNI) and subtree pruning and regrafting (SPR) were always used to estimate tree topologies (i.e. using the extensive tree search option). Robustness of reconstructed trees was estimated with 1000 bootstrap replicates. In case of MrBayes, 10 million MCMC generations were run, with trees and lnLs sampled at intervals of 1000 generations. We used a threshold of 0.01 for the average standard deviation of split frequencies between the two runs as a diagnostic for convergence. This threshold was achieved well before 2.5 million generations, which were discarded as “burn-in”.

For the datasets analysed with MrBayes and PhyML, the best fitting DNA substitution model was selected using jModelTest 0.1.1 (Posada 2008), which uses PhyML (Guindon and Gascuel 2003) for likelihood calculations. Model selection was done on the basis of the Akaike Information Criterion (AIC). When the model selected was not implemented in MrBayes or online version of PhyML (http://www.atgc-montpellier.fr/phyml), the next best model according to AIC score was used.

Because of numerous indel events (insertions/deletions) several regions of ITS genes proved to be difficult to align, therefore BAli-Phy was used to include information from these regions and also indel events. This program has implementations to handle difficult-to-align sequences and to include indels in phylogenetic analyses but it is computationally intensive. In order to enhance the speed of calculation, ITS1 and ITS2 sequences were aligned separately for detecting and fixing the conserved positions prior to analyses with Bali-Phy. For aligning, we used MAFFT 6.5 online version (http://align.bmr.kyushu-u.ac.jp/mafft/software/) with the alignment strategy Q-INS-i, which considers RNA secondary structure (Katoh and Toh 2008). Two to four independent runs consisting of 100680–322660 generations with burnin 10–50% were analysed with BAli-Phy. Tracer 1.4 (http://beast.bio.ed.ac.uk/Tracer) was used to ensure the stationary of output values.

Table 1. Properties of primers used in this study including annealing temperatures for DNA cycle sequencing (CS); f/r, forward or reverse primer.

Primer	f/r	Primer sequence (5′ to 3′)	Primer location	CS (°C)	Reference
TW-J1301	f	GTTAAWTAAACTAATARCCTTCAAA	tRNA-Trp	48	Simon *et al.* (2006)
TY-J1460	f	TTTACAATTTATCGCCTAAAMTTCAGCC	tRNA-Tyr	47	**I**
C1-J1718	f	GGAGGATTTGGAAAYTGAYTAGTWCC	COI	49	Nyman *et al.* (2006)
C1-J1718a	f	GGAGGATTTGGAAATTGAYTARTHCC	COI	49	This study
C1-J1751 (Ron)	f	GGATCACCTGATATAGCATTCCC	COI	46	Simon *et al.* (1994)
C1-J1751a	f	GGAGCNCCTGATATAGCATTYCC	COI	46	This study
C1-N1760	r	GGTARAAATCARAATCTTATATTAT	COI	48	**I**
C1-J2186	f	CAACAYYTATTTTGATTTTTTGGWCA	COI	48	**I**
C1-N2191	r	CCCGGTAAAATTAAAATATAAACTTC	COI	46	Simon et al. (1994)
C1-N2191a	r	GCTGGAATAATTARAATATAWACTTC	COI	46	This study
C1-J2435	f	ACAGGAATTAAAATTTTTAGRTG	COI	48	**I**
A2590	r	GCTCCTATTGATARWACATARTGRAAATG	COI	49	Normark *et al.* (1999)
A2590a	r	GCYCCTATTGATARAACATARTGAAAATG	COI	49	This study
TL2-N3018 (1)	r	CCATTGCATTTTTCTGCCAT	tRNA-Leu	47	**I**
C2-N3083	r	TAAAARTTWGYTCATGTTGTCAT	COII	48	**I**
CAS18sF1	f	TACACACCGCCCGTCGCTACTA	18S rRNA	60	Ji *et al.* (2003)
ITS1F1 (2)	f	ACTGGTACGCGGCAATGTCTCGGCATT	18S rRNA	60	This study
CAS5p8sB1d	r	ATGTGCGTTCRAAATGTCGATGTTCA	5.8S rRNA	60	Ji *et al.* (2003)
EmpITS1F	f	GAACGWCGTAACGGCCGGTRT	ITS1	60	**I**
EmpITS1F1	f	TCGAACGWCGTAACGGCCGG	ITS1	60	This study
EmpITS1R	r	TCGTGCAGAGCGCCGGGTCGGA	ITS1	60	**I**
CAS5p8sFc	f	TGAACATCGACATTTYGAACGCACAT	5.8S rRNA	60	Ji *et al.* (2003)
CAS28sB1d	r	TTCTTTTCCTCCGCTTATTRATATGCTTAA	28S rRNA	60	Ji *et al.* (2003), **I**
AM1	f	TGTGAACTGCAGGACACATGA	5.8S rRNA	60	Heidemaa et al. (2004)
AM2	r	ATGCTTAAATTTAGGGGGTAGTC	28S rRNA	60	Marinucci et al. (1999)

(1), reverse complement of TL2-J3037 (Simon *et al.* 1994).

(2), when ITS1 and 2 were amplified in a single fragment, this primer was used in cycle sequencing reactions instead of CAS18sF1, which did not work properly with ITS1+2 amplicon.

Table 2. Primer pairs used in amplification. Ta, annealing temperature for the primers.

Primer pair	Amplified regions	Ta (°C)
TW-J1301 + C1-N1760	partial tRNA-Trp, tRNA-Cys, tRNA-Tyr, partial COI	48
TW-J1301 + C1-N2191a	partial tRNA-Trp, tRNA-Cys, tRNA-Tyr, partial COI	47
TY-J1460 + C1-N1760	partial tRNA-Tyr, partial COI	47
C1-J1718/C1-J1718a + A2590/A2590a	partial COI	49
C1-J1718/C1-J1718a + C1-N2191/C1-N2191a	partial COI	46
C1-J1751a + C2-N3083	partial COI, tRNA-Leu	48
C1-J2186 + A2590/A2590a	partial COI	47
C1-J2435 + A2590	partial COI	48
C1-J2435 + C2-N3083	partial COI, tRNA-Leu	48
CAS18sF1 + CAS5p8sB1d	partial 18S rRNA, ITS1, partial 5.8S rRNA	65
CAS18sF1 + EmpITS1R	partial 18S rRNA, partial ITS1	60
EmpITS1F/EmpITS1F1 + EmpITS1R	partial ITS1	60
CAS18sF1 + CAS28sB1d	partial 18S rRNA, ITS1, 5.8S rRNA, partial 28S rRNA	62
EmpITS1F/EmpITS1F1 + CAS5p8sB1d	partial ITS1, partial 5.8S rRNA	60
CAS5p8sFc + CAS28sB1d	partial 5.8S rRNA, ITS2, partial 28S rRNA	62
AM1 + AM2	partial 5.8S rRNA, ITS2, partial 28S rRNA	60

RESULTS AND DISCUSSION

Taxonomic results

Empria longicornis species group

This species group is one of the most problematic taxonomically in the Palaearctic region (**I**) with 10 species currently recognized (**I, II**): *E. alector, E. alpina, E. basalis, E. japonica, E. loktini, E. longicornis, E. minuta, E. mongolica, E. tridens*, and *E. sp11*. In most cases, reliable identification of species in this group cannot be done without studying ovipositors or penis valves. It is especially difficult to identify males, because there are no clear qualitative differences even between penis valves of different species. For this reason, geometric morphometric analyses were used to find if there are differences in overall shape of the penis valves (**I**). To ensure that males in *E. longicornis* group were correctly associated with conspecific females, *ex ovo* rearings and DNA sequencing were carried out (**I**). Results of geometric morphometric analyses of penis valves in conjunction with some external characters (particularly flagellomere length) showed that males can be identified in most cases as reliably as females (**I**). Species delimited by morphological characters where in accordance with nuclear ITS sequence data (**I**, this study), although *E. tridens* was not monophyletic in ITS tree, but nevertheless did not share any haplotypes with other species. Existence of different species was also supported by differences in host plant use (**I**), although host plants are unknown for several species (*E. japonica, E. loktini, E. minuta, E. sp11*) and *E. longicornis* and *E. tridens* share the same host plant (*Rubus idaeus*). Results of mitochondrial DNA analyses, on the other hand, were inconsistent with morphological and nuclear data, as the specimens of different species did not form monophyletic groups and were intermingled (Fig. 54; **I**). Because it is known that mitochondrial phylogeny can be significantly incongruent with morphology and nuclear phylogeny, possibly because of mitochondrial introgression (Chan and Levin 2005; Linnen and Farrell 2007; Wahlberg et al. 2009; Near et al. 2011), the results from mtDNA do not disprove the existence of species delimited by other means.

Japanese and Taiwanese *Empria*

To fill in the gaps in the knowledge regarding *Empria* in the East Palaearctic and Oriental region, material from Japan and Taiwan was studied (**I–III**). Previously, only two species had been identified from Japan (Takeuchi 1952a; b; Abe and Togashi 1989), and one of them actually turned out to be species of *Monsoma* MacGillivray, 1908, *M. pallipes* (Matsumura, 1912) (**II**). No species of *Empria* were known from Taiwan. Results revealed that there are at least 13 species in Japan (*E. candidata, E. japonica, E. liturata, E. loktini, E. plana,* *E. quadrimaculata, E. rubicola, E. tridens, E. tridentis, E. honshuana, E. takeuchii*, *E. sp11*, and *E. sp8*; **I–III**) and one in Taiwan (*E. formosana*; **III**). Four species were described as new (*E. japonica, E. honshuana, E. takeuchii*, and *E. formosana*), but there were two more probably new species from Japan (*E. sp11*, *E. sp8*), which were not described as new due to insufficient material (**II, III**).

Key to Palaearctic and Oriental *Empria* and *Blennallantus*

The only previous key to Palaearctic *Empria* (Conde 1940) is clearly outdated and included only 17 species, most of them European. The key presented here includes all known 50 Palaearctic and Oriental *Empria* species. Because of small amount of external morphological variation and extensive overlap between the species, the following key is mainly based on ovipositors and penis valves. Additional characters are provided whenever possible, but often there is certain degree of overlap in character variation between the couplets. If the external character states of a specimen to be identified are in the overlap region, ovipositors or penis valves should be studied. Still, in many cases it should be possible to identify specimens (especially females) without the study of ovipositors or penis valves. In some species groups (*longicornis*-group, *immersa*-group), however, studying ovipositors and penis valves is the primary means of species identification. Included in the key and the taxonomic section is also *Blennallantus compressicornis* Wei, 1998, which might belong to *Empria* (see paper **III**). Although *Blennallantus* Wei, 1998 would fit the diagnosis of *Empria* given here (see also paper **III**), we did not yet synonymise it, because of lack of sufficient material (females currently unknown and no molecular data available).

1. Clypeus flat without median keel; at least facial orbits dorsally and part of temples pale; on hind wing cross-vein m-cu absent; claws simple or with minute subbasal tooth; number of serrulae 18–21 (Fig. 14 in **II**); posterior margin of sternum 9 in male notched, penis valve as in Fig. 25 in **II**; Holarctic .... ***E. candidata***

– Clypeus with median keel (distinct mostly in anterior part of clypeus only); facial orbits and temples black; on hind wing cross-vein m-cu present or absent; number of serrulae 13–18(19) (Figs 2–29; 36–46 in **I**; 15–24 in **II**; 8 in **III**); claws variable; posterior margin of sternum 9 in male round 2

2. Serrulae triangular (Figs 2–3), penis valves as in Figs 10, 11 in **III**; on forewing vein 2A+3A incomplete (Fig. 14 in **III**); on hind wing cross-vein m-cu absent; flagellum 1.5–1.8 times longer than breadth of head in female, 1.8–2.1 times longer in male...70

– Serrulae and penis valves various (Figs 4–51; 36–58 in **I**; 15–24, 26–36 in **II**; 8, 9, 12 in **III**); on forewing vein 2A+3A complete or incomplete; on hind wing cross-vein m-cu mostly present; flagellum (1.2)1.3–2.7 times longer than breadth of head in female, 1.4–3.9 times longer in male...........3

3. On forewing vein 2A+3A incomplete and abdominal terga with 2(3) pairs of barely visible pale patches; on hind wing cross-vein m-cu present; flagellum 3.2–3.5 times longer than breadth of head in male; penis valve as in Fig. 12 in **III**; female unknown; China (Shaanxi, Sichuan) ....***Blennallantus compressicornis***

– On forewing vein 2A+3A complete and abdominal terga with 0–8 pairs of pale patches or vein 2A+3A incomplete and abdominal terga with 4–5 pairs of pale patches; on hind wing cross-vein m-cu mostly present; flagellum (1.2)1.3–2.7 times longer than breadth of head in female, 1.4–3.9 times longer in male; penis valves different (Figs 30–51; 47–58 in **I**; 26–36 in **II**; 9 in **III**); serrulae as in Figs 4–29, 36–46 in **I,** 15–24 in **II,** 8 in **III** ...4

4. Malar space 2.1–2.6 times longer than lateral ocellus diameter in female, 1.8–2.3 times longer in male, and abdominal terga with 4–7 pairs of pale patches; legs often extensively yellow; serrulae as in Figs 17–19 and 17 in **II**; valviceps with long apical spine (Figs 42–44; 28 in **II**) 51

– Malar space 0.9–2.0 times longer than lateral ocellus diameter in female, 0.9–1.6 times longer in male, and abdominal terga with 0–8 pairs of pale patches, or malar space 1.9–2.2 times longer than lateral ocellus diameter in female, 1.5–1.9 times longer in male, and abdominal terga with 2–3 (or 2–4 in male) pairs of pale patches; legs black and white or yellow; serrulae different (Figs 4–16, 20–29; 36–46 in **I**; 15, 16, 18–24 in **II**; 8 in **III)**; valviceps without long apical spine (Figs 30–41, 45–51; 47–58 in **I**; 26, 27, 29–36 in **II**; 9 in **III**)...5

5. Legs extensively yellow; flagellum 1.3–1.9 times longer than breadth of head in female, 1.4–2.5 times longer in male; serrulae as in Figs 8, 9, 16, 21, 27, 28; penis valves as in Figs 32, 34, 45, 49 65

– Legs black and white; flagellum (1.2)1.4–2.7 times longer than breadth of head in female, 1.6–3.9 times longer in male; serrulae and penis valves different (Figs 4–7, 10–15, 20, 22–26, 29–31, 33, 35–41, 46–48, 50, 51; 36–58 in **I**; 15, 16, 18–24, 26, 27, 29–36 in **II**; 8, 9 in **III**)...............................6

6. Serrulae as in Fig. 24; penis valve as in Fig. 50; abdominal terga with 0–1(2) pairs of pale patches; tegula at most anterolaterally and metatibia at most in basal 1/3 pale; East Palaearctic .... ***E. sp7***

– Serrulae and penis valves different (Figs 4–7, 10–15, 20, 22, 23, 25, 26, 29–31, 33, 35–41, 46–48, 51; 36–58 in **I**; 15, 16, 18–24, 26, 27, 29–36 in **II**; 8, 9 in **III**); abdominal terga with 2–8 pairs of pale patches; tegula black to completely pale; metatibia black to pale in basal 2/3 7

7. Serrulae as in Figs 6, 7; penis valves as in Figs 30, 31; clypeus deeply excised in front; claws with large subbasal tooth; postocellar area 1.6–1.9 times wider than long in female, 1.9–2.1 times wider in male; first abdominal tergum usually with pair of faint pale patches 54

– Serrulae and penis valves different (Figs 4, 5, 10–15, 20, 22, 23, 25, 26, 29, 33, 35–41, 46–48, 51; 36–58 in **I**; 15, 16, 18–24, 26, 27, 29–36 in **II**; 8, 9 in **III**); clypeus moderately excised in front and / or claws simple; postocellar area 1.5–2.7 times wider than long; first abdominal tergum with or without pair of pale patches 8

8. Serrulae as in Figs 10–15, penis valves as in Figs 33, 35–39; first abdominal tergum with pair of pale patches (sometimes hardly visible); claws simple or with subbasal tooth … *E. hungarica* group in part 55

– Serrulae and penis valves different (Figs 4, 5, 20, 22, 23, 25, 26, 29, 40, 41, 46–48, 51; 36–58 in **I**; 15, 16, 18–24, 26, 27, 29–36 in **II**; 8, 9 in **III**); first abdominal tergum without pair of pale patches; claws variable 9

9. Trochanters, trochantelli, and sometimes coxae at least partly pale; tegula completely pale 61

– Trochanters, trochantelli, and coxae almost completely black, only metatrochantellus sometimes clearly pale anteromedially; tegula black or pale 10

10. Serrulae as in Figs 25, 26, 29, and 15, 16 in **II**; penis valves as in Figs 47, 48, 51, and 26, 27 in **II**; postocellar area (1.9)2.0–2.6 times wider than long in female, (2.1)2.2–2.7 times wider in male; abdominal terga with 2–3(4) pairs of pale patches in female, with 2–4(5) pairs in male 41

– Serrulae and penis valves different (Figs 4, 5, 20, 22, 23, 40, 41, 46, 36–58 in **I**; 18–24, 29–36 in **II**; 8, 9 in **III**); postocellar area 1.5–2.1 times wider than long in female, 1.6–2.3 times wider in male, and abdominal terga with 2–6 pairs of pale patches, or postocellar area 1.9–2.5 times wider than long and abdominal terga with 5–6 pairs of pale patches in female, with 4–5 pairs in male...11

11. Serrulae as in Figs 4, 5; penis valve as in Fig. 41 (male of *E. sp3* unknown); claws simple; clypeus mostly deeply excised in front; postocellar area 1.5–1.7 times wider than long in female, 1.6–1.8 times wider in male; abdominal terga with 5–6 pairs of pale patches in female, with 4 pairs in male … *E. pallimacula* group 50

– Serrulae and penis valves different (Figs 20, 22, 23, 40, 46, 36–58 in **I**; 18–24, 29–36 in **II**; 8, 9 in **III**); claws mostly with subbasal tooth; clypeus mostly moderately excised in front; postocellar area 1.5–2.5 times wider than long in female, 1.6–2.3 times wider in male; abdominal terga with 2–6 pairs of pale patches 12

12. ♂ 26

– ♀ 13

13. Serrulae papilliform (Figs 36–38 in **I**; 20 in **II**; 8 in **III**)............................18

– Serrulae not papilliform (Figs 20, 22; 39–46 in **I**; 21–24 in **II**) 14

14. Serrulae as in Fig. 22; abdominal terga with 5(6) pairs of pale patches; metatibia in basal 1/3 pale; Palaearctic...***E. gelida***

– Serrulae different (Figs 20; 39–46 in **I**; 21–24 in **II**); abdominal terga with 2–6 pairs of pale patches; metatibia black to pale in basal 1/2..................15

15. Serrulae triangular (Figs 39–42, 44–46 in **I**; 22–24 in **II**); abdominal terga with 2–6 pairs of pale patches … *E. longicornis* group in part 22
– Serrulae not triangular (Figs 20; 43 in **I**; 21 in **II**); abdominal terga with 2–4(5) pairs of pale patches 16
16. Serrulae with ventral margin almost at the same level along its entire length, not protruding (Fig. 43 in **I**); West Palaearctic ....***E. alector***
– Serrulae different (Figs 20; 21 in **II**) 17
17. Serrulae as in Fig. 20; abdominal terga with 2(3) pairs of pale patches; West Palaearctic...***E. kuznetzovi***
– Serrulae as in Fig. 21 in **II**; abdominal terga with 4 pairs of pale patches; Japan (Honshu) .... ***E. honshuana***
18. Serrulae as in Figs 36–38 in **I** … *E. longicornis* group in part...................20
– Serrulae as in Figs 20 in **II** and 8 in **III** 19
19. Serrulae as in Fig. 20 in **II**; on forewing vein 2A+3A complete; claws simple or with minute subbasal tooth; abdominal terga with 5–6 pairs of pale patches; Palaearctic ....***E. liturata***
– Serrulae as in Fig. 8 in **III**; on forewing vein 2A+3A complete or incomplete; claws with conspicuous subbasal tooth; abdominal terga with 4 pairs of pale patches; Taiwan ....***E. formosana***
20. Serrulae not conspicuously papilliform (Fig. 38 in **I**); length of eye 1.6–1.7 times longer than length of head behind eyes; Palaearctic ....***E. minuta***
– Serrulae conspicuously papilliform (Figs 36, 37 in **I**); length of eye 1.3–1.7 times longer than length of head behind eyes...21
21. Serrulae as in Fig. 36 in **I**; length of eye 1.2–1.4 times longer than length of head behind eyes; posterior margin of pronotum and tegula black; Holarctic ....***E. alpina***
– Serrulae as in Fig. 37 in **I**; length of eye 1.5–1.7 times longer than length of head behind eyes; posterior margin of pronotum pale; tegula completely pale; East Palaearctic ....***E. mongolica***
22. Serrulae weakly developed and 4–5 basal-most serrulae without denticles (Fig. 44 in **I**); abdominal terga with (3)4–5 pairs of pale patches; West Palaearctic...***E. basalis***
– Serrulae more robust and 0–3 basal-most serrulae without denticles (Figs 39–42, 45, 46 in **I**; 22–24 in **II**); abdominal terga with 2–6 pairs of pale patches 23
23. Serrulae as in Fig. 39 in **I**; 24 in **II**; number of serrulae 13–14(15); abdominal terga with 2–3(4) pairs of pale patches; flagellum 1.8–1.9 times longer than breadth of head; East Palaearctic .... ***E. loktini***
– Serrulae different, more triangular in shape (Figs 40–42, 45, 46 in **I**; 22, 23 in **II**); number of serrulae (15)16–18; abdominal terga with 3–6 pairs of pale patches; flagellum 1.9–2.7 times longer than breadth of head...........24
24. Maximal length of temple 1.40–1.55 times greater than minimal length of temple (Fig. 3 in **I**); length of basal-most annulus of valvula 1 (Figs 40 in **I**;

23 in **II**) more than 0.1 mm; flagellum 2.5–2.7 times longer than breadth of head; Japan (Hokkaido) .... ***E. japonica***

– Maximal length of temple 1.20–1.35 times greater than minimal length of temple; length of basal-most annulus of valvula 1 (Figs 41, 42, 45, 46 in **I**; 22 in **II**) less than 0.1 mm; flagellum 1.9–2.7 times longer than breadth of head...25

25. Serrulae as in Figs 41, 46 in **I**; 3rd flagellomere mostly 3.5–4.5 times longer than broad; flagellum mostly 2.3–2.7 times longer than breadth of head; abdominal terga with 3 pairs of large and 1 pair of small pale patches; Palaearctic... ***E. longicornis***

– Serrulae as in Figs 42, 45 in **I**; 22 in **II**; 3rd flagellomere mostly 2.9–3.5 times longer than broad; flagellum mostly 1.9–2.3 times longer than breadth of head; abdominal terga mostly with 4 pairs of large and 1 pair of small pale patches; Palaearctic ....***E. tridens***

26. Dorsal margin of valviceps convex with minute dorso-apical tooth (often very inconspicuous) and angle between dorsal and basal margin of valviceps acute, forming a notch (rarely almost absent) between valviceps and valvura (Figs 47–58 in **I**; 33–36 in **II**); flagellum 2.1–3.8 times longer than breadth of head … *E. longicornis* group..31

– Dorsal margin of valviceps concave and / or angle between dorsal and basal margin of valviceps obtuse; valviceps often with large dorso-apical tooth or spine (Figs 40, 46; 31, 32 in **II**; 9 in **III**); flagellum 1.7–3.0 times longer than breadth of head 27

27. Valviceps very narrow, about two times broader than valvura; apically dorsal and ventral margins of valviceps parallel (Fig. 40); flagellum 1.7–2.1 times longer than breadth of head; abdominal terga with 4–5 pairs of pale patches; Mongolia... ***E. montana***

– Valviceps broad, about three or more times broader than valvura; apically dorsal and ventral margins of valviceps not parallel (Figs 46; 31, 32 in **II**; 9 in **III**); flagellum 1.8–3.0 times longer than breadth of head; abdominal terga with 2–5 pairs of pale patches 28

28. Dorsal margin of valviceps concave (Fig. 31 in **II**); claws with subbasal tooth; abdominal terga with (2)3–4 pairs of pale patches; Japan (Honshu) .... ***E. honshuana***

– Dorsal margin of valviceps convex (Fig. 46; 32 in **II**; 9 in **III**); claws simple or with subbasal tooth; abdominal terga with 2–5 pairs of pale patches 29

29. Penis valve as in Fig. 46; abdominal terga with 2–3 pairs of pale patches; West Palaearctic..***E. kuznetzovi***

– Penis valves different (Fig. 32 in **II**;. 9 in **III**); abdominal terga with 4–5 pairs of pale patches 30

30. Penis valve as in Fig. 32 in **II**; on forewing vein 2A+3A complete; abdominal terga with 5 pairs of pale patches; claws simple or with minute subbasal tooth; Palaearctic ....***E. liturata***

– Penis valve as in Fig. 9 in **III**; on forewing vein 2A+3A complete or incomplete; abdominal terga with 4(5) pairs of pale patches; claws with minute or conspicuous subbasal tooth; Taiwan .... ***E. formosana***

31. Penis valves with relatively deep notch (Figs 53, 54 in **I**; 36 in **II**) and flagellum 2.7–3.5 times longer than breadth of head 39

– Penis valves with relatively shallow notch (Figs 47–52, 55–58 in **I**; 33–35 in **II**) and / or flagellum 2.1–2.7 times longer than breadth of head 32

32. Flagellum less than 2.7 times longer than breadth of head 37

– Flagellum more than 2.7 times longer than breadth of head 33

33. Basal lobe of valviceps long, valviceps (0.57)0.60–0.75(0.80) times as long as valvura (Figs 51, 56, 58 in **I**) 36

– Basal lobe of the valviceps short, valviceps 0.45–0.63(0.69) times as long as valvura (Figs 47–50 in **I**; 34, 35 in **II**) 34

34. Maximal length of temple (1.30)1.35–1.50 times greater than its minimal length (Fig. 3 in **I**); flagellum 3.2–3.8 times longer than breadth of head; valviceps 0.58–0.62 times as long as valvura (Figs 47 in **I**; 35 in **II**) and metatibia at least in basal 1/3 and tegula anterolaterally to almost completely pale; Japan (Hokkaido) .... ***E. japonica***

– Maximal length of temple 1.15–1.35(1.38) times greater than its minimal length; flagellum 2.4–3.1(3.3) times longer than breadth of head and valviceps 0.52–0.63(0.69) times as long as valvura (Figs 49, 50 in **I**; 34 in **II**) or flagellum (3.0)3.1–3.8 times longer than breadth of head and valviceps 0.45–0.56(0.59) times as long as valvura (Fig. 48 in **I**); tegula and metatibia black or pale 35

35. Penis valve as in Fig. 48 in **I**, valviceps 0.45–0.56(0.59) times as long as valvura; flagellomere 3 mostly longer than 0.7 mm; flagellum (3.0)3.1–3.8 times longer than breadth of head; abdominal terga mostly with 3 pairs of large and 1 pair of small pale patches; Palaearctic .... ***E. longicornis***

– Penis valves as in Figs 49, 50 in **I**; 34 in **II**; valviceps 0.52–0.63(0.69) times as long as valvura; flagellomere 3 mostly shorter than 0.7 mm; flagellum 2.4–3.1(3.3) times longer than breadth of head; abdominal terga mostly with 4 pairs of large and 1 pair of small pale patches; Palaearctic .... ***E. tridens***

36. Penis valves as in Figs 51, 56 in **I**; tegula and metatibia mostly black; claws mostly with conspicuous tooth; West Palaearctic .... ***E. alector***

– Penis valve as in Fig. 58 in **I**; tegula and metatibia partly pale; claws mostly simple or with inconspicuous tooth; Palaearctic .... ***E. minuta***

37. Apical part of valvular duct extending clearly further from dorsal rim of valvura (Fig. 55 in **I**; 33 in **II**); abdominal terga mostly with 2–3 pairs of pale patches; flagellum 2.3–2.4 times longer than breadth of head; East Palaearctic .... ***E. loktini***

– Apical part of valvular duct reaching almost the dorsal rim of valvura or extending only slightly further from it (Figs 49, 50, 52 in **I**; 34 in **II**);

abdominal terga mostly with 4–5 pairs of pale patches; flagellum 2.1–3.1(3.3) times longer than breadth of head 38

38. Basal lobe of valviceps short (Figs 49, 50 in **I**; 34 in **II**), flagellum 2.4–3.1(3.3) times longer than breadth of head; Palaearctic .... ***E. tridens***

– Basal lobe of valviceps long (Fig. 52 in **I**); flagellum 2.1–2.7 times longer than breadth of head; West Palaearctic .... ***E. basalis***

39. Penis valve as in Fig. 36 in **II**, valviceps more than 0.80 times as long as valvura; Japan (Hokkaido) .... ***E. sp. 11***

– Penis valves as in Figs 53, 54 in **I**, valviceps 0.53–0.65(0.67) times as long as valvura 40

40. Penis valve as in Fig. 53 in **I**; length of eye 1.3–1.5 times longer than length of head behind eyes; posterior margin of pronotum black; Holarctic .... ***E. alpina***

– Penis valve as in Fig. 54 in **I**; length of eye 1.6–2.2 times longer than length of head behind eyes; posterior margin of pronotum pale; East Palaearctic .... ***E. mongolica***

41. ♂ 46

– ♀ 42

42. Serrulae weakly developed, rather flat (Fig. 26) and 4–5 basal-most serrulae without denticles; number of serrulae 12–14(15); flagellum 1.7–2.0 times longer than breadth of head; Palaearctic .... ***E. parvula***

– Serrulae papilliform (Figs 25, 29; 15, 16 in **II**), at most 1–2 basal-most serrulae without denticles; number of serrulae 15–19; flagellum 1.8–2.5 times longer than breadth of head 43

43. Serrulae as in Fig. 29; metatibia black; abdominal terga with 3(4) pairs of pale patches; West Palaearctic .... ***E. sexpunctata***

– Serrulae as in Figs 25 and 15, 16 in **II**; abdominal terga with 2–3 pairs of pale patches; metatibia at least in basal 1/2 pale … *E. quadrimaculata* group in part 44

44. Malar space 1.4–1.5 times longer than lateral ocellus diameter; flagellum about 2.0 times longer than breadth of head; serrulae as in Fig. 25; China .... ***E. zhangi***

– Malar space 1.7–2.0 times longer than lateral ocellus diameter; flagellum 1.9–2.5 times longer than breadth of head; serrulae as in Figs 15, 16 in **II** 45

45. Abdominal terga mostly with 2 pairs of pale patches; flagellum mostly 2.1–2.5 times longer than breadth of head; in most specimens flagellomeres 1 and 2 about equally long; number of serrulae 17–19 (Fig. 15 in **II**); cannot always be distinguished morphologically from *E. rubicola*; Japan (Honshu, Shikoku, Kyushu) .... ***E. quadrimaculata***

– Abdominal terga mostly with 3 pairs of pale patches; flagellum mostly 1.9–2.2 times longer than breadth of head; in most specimens flagellomere 1 longer than flagellomere 2; number of serrulae 16–18 (Fig. 16 in **II**);

cannot always be distinguished morphologically from *E. quadrimaculata*; East Palaearctic (Hokkaido, Sakhalin) .... ***E. rubicola***

46\. Penis valve as in Fig. 47; abdominal terga with (3)4–5 pairs of pale patches; metatibia black; West Palaearctic .... ***E. sexpunctata***

– Penis valves as in Figs 48, 51; 26, 27 in **II**; abdominal terga with 2–3(4) pairs of pale patches; metatibia black to pale in basal 2/3 47

47\. Penis valve as in Fig. 48, with large dorsoapical tooth; flagellum 2.0–2.7 times longer than breadth of head; metatibia black or basally slightly pale; Palaearctic .... ***E. parvula***

– Penis valves as in Figs 51 and 26, 27 in **II**, with small dorsoapical tooth; flagellum 2.4–3.3 times longer than breadth of head; metatibia at least in basal 1/3 pale ... *E. quadrimaculata* group in part....................................48

48\. Malar space 0.9–1.0 times longer than lateral ocellus diameter; flagellum about 2.4–2.5 times longer than breadth of head; penis valve as in Fig. 51; China...***E. zhangi***

– Malar space 1.5–1.8 times longer than lateral ocellus diameter; flagellum 2.6–3.3 times longer than breadth of head; penis valves as in Figs 26, 27 in **II** 49

49\. Valviceps with small basal lobe, ventroapical part of valviceps slightly bent towards its basal part (Fig. 26 in **II**); flagellum 2.9–3.3 times longer than breadth of head; in most specimens flagellomere 7 not distinctly shorter than length of eye in dorsal view; Japan (Honshu, Shikoku, Kyushu)..***E. quadrimaculata***

– Valviceps with large basal lobe, ventroapical part of valviceps strongly bent towards its basal part (Fig. 27 in **II**); flagellum 2.6–3.0 times longer than breadth of head; in most specimens flagellomere 7 distinctly shorter than length of eye in dorsal view; East Palaearctic (Hokkaido, Sakhalin) .... ***E. rubicola***

50\. Serrulae as in Fig. 4; penis valve as in Fig. 41; Palaearctic... ***E. pallimacula***

– Serrulae as in Fig. 5; male unknown; Russia (Primorsky Krai)........... ***E. sp3***

51\. Serrulae papilliform (Figs 18, 19; 17 in **II**); penis valves as in Figs 42, 43; 28 in **II**; claws bifid; apical terga and sterna in female black or extensively pale...52

– Serrulae more or less triangular (Fig. 17); penis valve as in Fig. 44; claws with subbasal tooth; apical terga and sterna in female extensively pale; Palaearctic... ***E. fletcheri***

52\. Basal papilliform part of serrulae small (Fig. 18); angle between dorsal and apical margins of valviceps clearly more than 90° (Fig. 42); at least coxae, trochanters, and trochantelli usually black in most part; clypeus completely and metafemora in most part often black; apical terga and sterna in female black; males cannot always be distinguished morphologically from *E. plana*; East Palaearctic .... ***E. camtschatica***

– Basal papilliform part of serrulae large (Figs 19 and 17 in **II**); angle between dorsal and apical margins of valviceps about 90° or more (Figs 43 and 28 in **II**); clypeus, tegulae, coxae, trochanters, trochantelli, and metafemora pale or black; apical terga and sterna in female black or extensively pale 53

53. Serrulae as in Fig. 19; angle between dorsal and apical margins of valviceps about 90° or slightly greater (Fig. 43); clypeus, coxae, trochanters, and trochantelli black; tegulae and metafemora black to pale in most part; apical terga and sterna in female black; West Palaearctic.. ***E. immersa***

– Serrulae as in Fig. 17 in **II**; angle between dorsal and apical margins of valviceps clearly more than 90° (Fig. 28 in **II**); clypeus, tegulae, coxae, trochanters, trochantelli, and metafemora pale in most part in most of the specimens from mainland Asia; apical terga and sterna in female black or extensively pale; males cannot always be distinguished morphologically from *E. camtschatica*; East Palaearctic .... ***E. plana***

54. Serrulae as in Fig. 6; penis valve as in Fig. 30, angle between dorsal and basal margin of valviceps 54–61°; West Palaearctic .... ***E. excisa***

– Serrulae as in Fig. 7; penis valve as in Fig. 31, angle between dorsal and basal margin of valviceps 65–74°; West Palaearctic (North-Africa).. ***E. africana***

55. Serrulae as in Fig. 15; penis valve as in Fig. 39; postocellar area 1.5–1.7 times wider than long in female, 1.5–1.9 times wider in male; flagellum 1.5–1.6 times longer than breadth of head in female, 1.8–2.0 times longer in male; claws simple; West Palaearctic.................................. ***E. pumiloides***

– Serrulae as in Figs 10–14; penis valves as in Figs 33, 35–38; postocellar area (1.7)1.8–2.2 times wider than long in female, (1.8)1.9–2.4 times wider in male; flagellum 1.6–2.1 times longer than breadth of head in female, 2.1–3.9 times longer in male; claws simple or with subbasal tooth............56

56. Serrulae as in Fig. 10; penis valve as in Fig. 33; claws with subbasal tooth; flagellum about 3.3 times longer than breadth of head in male (no female with completely intact flagella was available for study); West Palaearctic.. ***E. ushinskii***

57. Serrulae and penis valves different (Figs 11–14, 35–38); claws simple or with minute subbasal tooth; flagellum 1.6–2.1 times longer than breadth of head in female, 2.1–3.9 times longer in male 58

58. Serrulae as in Fig. 11; penis valve as in Fig. 35; stigma white in female; claws simple; flagellum 2.0–2.1 times longer than breadth of head in female, 3.6–3.9 times longer in male; West Palaearctic .... ***E. hungarica***

– Serrulae and penis valves different (Figs 12–14, 36–38); claws simple or with subbasal tooth; flagellum 1.6–1.7 times longer than breadth of head in female, 2.1–2.7 times longer in male..59

59. Serrulae as in Fig. 12; penis valve as in Fig. 36; claws simple; abdominal terga with 8 pairs of pale patches in female, with 5–6 pairs in male; West Palaearctic.. ***E. pumila***

– Serrulae and penis valves different (Figs 13, 14, 37, 38); claws with conspicuous or minute subbasal tooth (in males sometimes absent); abdominal terga with 5–6 pairs of pale patches 60

60. Serrulae as in Fig. 13; penis valve as in Fig. 37; tegula black in male; wings infuscate; West Palaearctic...***E. persephone***

– Serrulae as in Fig. 14; penis valve as in Fig. 38; tegula laterally pale in male; wings hyaline; West Palaearctic ....***E. granatensis***

61. Serrulae as in Fig. 23; claws simple; trochanters and trochantelli completely, and coxae at least partly pale; flagellum 1.4–1.6 times longer than breadth of head in female; male unknown; East Palaearctic .... ***E. sp12***

– Serrulae and penis valves different (Figs 22; 37, 54 in **I**; 18, 19, 29, 30 in **II)** (male of *E. gelida* unknown); claws with subbasal tooth; trochanters and trochantelli black to completely pale; coxae black or partly pale; flagellum (1.5)1.6–2.4 times longer than breadth of head in female, 2.2–3.7 times longer in male..62

62. Serrulae as in Fig. 18 in **II**; valviceps with large dorsobasally pointing spine at dorsoapical part (Fig. 29 in **II**); flagellum 2.2–2.4 times longer than breadth of head in female, 2.6–3.7 times longer in male; abdominal terga with 3 pairs of pale patches in female, with 3–4 pairs in male; East Palaearctic... ***E. tridentis***

– Serrulae and penis valves different (Figs 22; 37, 54 in **I**; 19, 30 in **II** (male of *E. gelida* unknown); flagellum (1.5)1.6–2.1 times longer than breadth of head in female, 2.2–3.4 times longer in male; abdominal terga with 3–6 pairs of pale patches in female, with (2)3–4 pairs in male 63

63. Serrulae as in Fig. 19 in **II**; penis valve as in Fig. 30 in **II**; flagellum 1.8–2.0 times longer than breadth of head in female, 2.2–2.7 times longer in male; abdominal terga with 3–4 pairs of pale patches in female, with (2)3 pairs in male; Japan (Hokkaido, Honshu)..................................***E. takeuchii***

– Serrulae and penis valves different (Figs 22; 37, 54 in **I)** (male of *E. gelida* unknown); flagellum (1.5)1.6–2.1 times longer than breadth of head in female, 2.7–3.4 times longer in male; abdominal terga with 5–6 pairs of pale patches in female; with 4–5 pairs in male..64

64. Serrulae as in Fig. 22; apical terga and sterna in female black; male unknown; Palaearctic...***E. gelida***

– Serrulae as in Fig. 37 in **I**; penis valve as in Fig. 54 in **I**; apical terga and sterna in female often pale; East Palaearctic ....***E. mongolica***

65. Serrulae as in Fig. 16; penis valve as in Fig. 34; claws simple; clypeus moderately excised in front; postocellar area 1.4–1.6 times wider than long in female, 1.5–1.7 times wider in male; flagellum 1.3–1.4 times longer than breadth of head in female, 1.4–1.5 times longer in male; first abdominal tergum with or without (in specimens from East Palaearctic) pair of pale patches; Palaearctic.. ***E. testaceipes***

– Serrulae and penis valves different (Figs 8, 9, 21, 27, 28, 32, 45, 49); claws with subbasal tooth; clypeus deeply or moderately excised in front; postocellar area 1.7–2.1 times wider than long in female, 1.9–2.3 times wider in male; flagellum 1.3–1.9 times longer than breadth of head in female, 1.7–2.5 times longer in male; first abdominal tergum without pair of pale patches 66

66. Serrulae as in Figs 21, 27, 28; penis valves as in Figs 45, 49 (male of *E. pravei* unknown); clypeus moderately excised in front; postocellar area 1.8–2.1 times wider than long in female; 2.0–2.3 times wider in male; flagellum 1.7–1.9 times longer than breadth of head in female, 2.4–2.5 times longer in male 68

– Serrulae and penis valves different (Figs 8, 9, 32) (male of *E. sp4* unknown); clypeus deeply excised in front; postocellar area 1.7–1.9 times wider than long in female; 1.8–2.0 times wider in male; flagellum 1.3–1.5 times longer than breadth of head in female, 1.7–1.8 times longer in male 67

67. Serrulae as in Fig. 8; penis valve as in Fig. 32; West Palaearctic ....***E. archangelskii***

– Serrulae as in Fig. 9; male unknown; Greece (Crete)......................... ***E. sp4***

68. Serrulae as in Fig. 21; penis valve as in Fig. 45; tegula completely pale; China (Hunan, Zhejiang) ....***E. sulcata***

– Serrulae and penis valves different (Figs 27, 28, 49) (male of *E. pravei* unknown); tegula centrally black 69

69. Serrulae as in Fig. 27; length of head 2.7 times greater than length of head behind eyes in female; Russia (Stavropol Krai); male unknown.....***E. pravei***

– Serrulae as in Fig. 28; penis valve as in Fig. 49; length of head 3.2–3.3 times greater than length of head behind eyes in female, 3.3–3.7 times greater in male; Iran... ***E. sp2***

70. ♀ 71

– ♂ 72

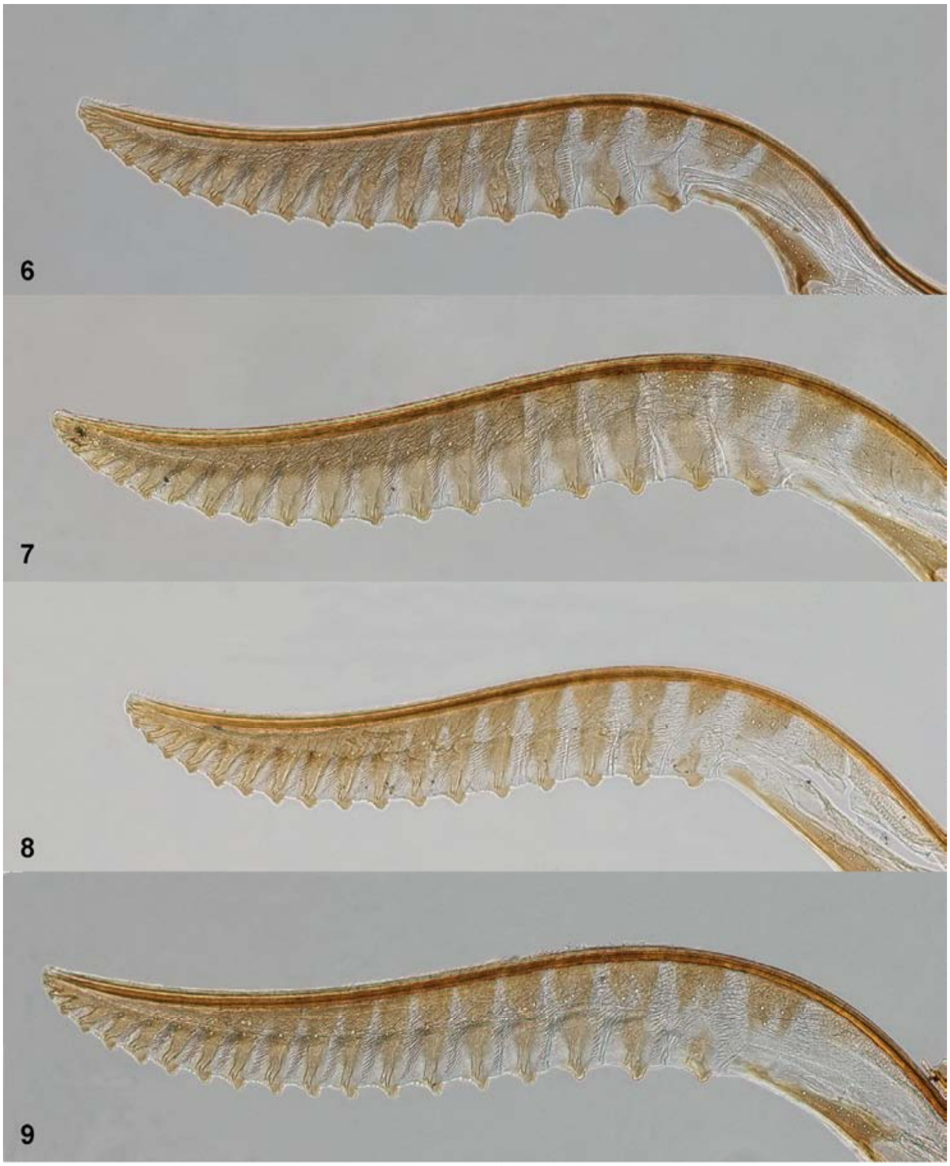
71. Serrulae as in Fig. 2; China...***E. wui***

– Serrulae as in Fig. 3; China.. ***E. sp9***

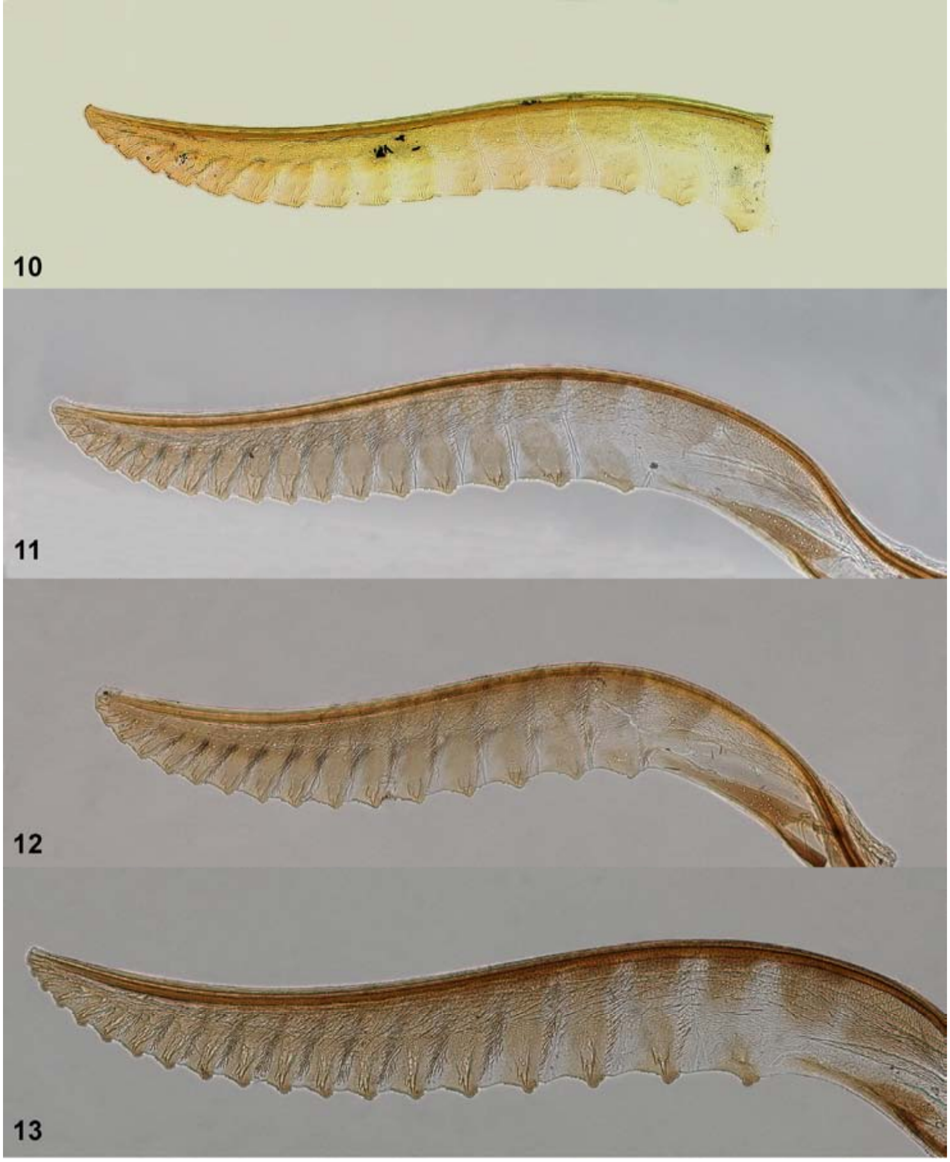
72. Spine of valviceps small, subapically placed (Fig. 11 in **III**); conspecificity with *E. wui* cannot be excluded; China.. ***E. sp13***

– Spine of valviceps large, apically placed (Fig. 10 in **III**); Japan .... ***E. sp8***

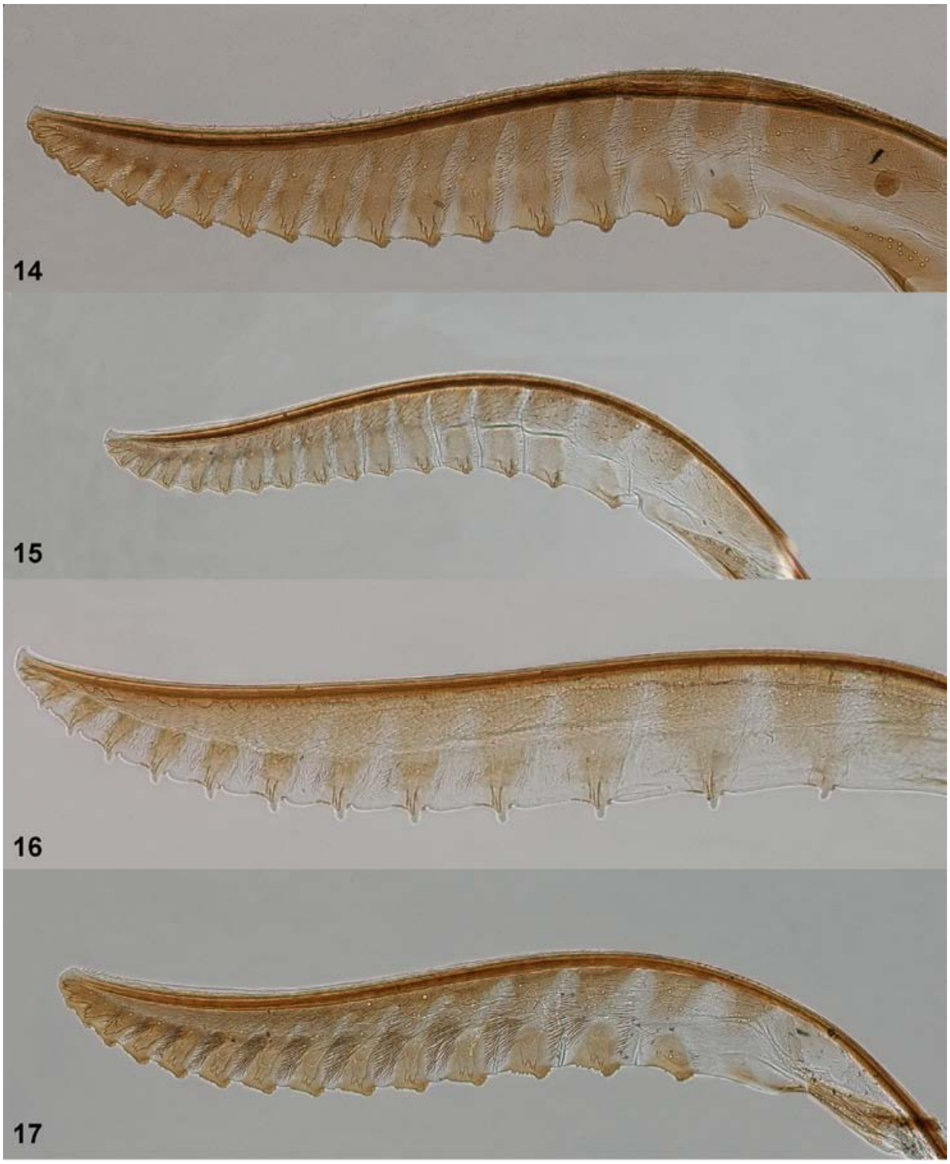
Figures 2–5. Lancets (valvulae 1) of *Empria*. **2** *E. wui* (holotype) **3** *E. sp9* **4** *E. pallimacula* **5** *E. sp3*.



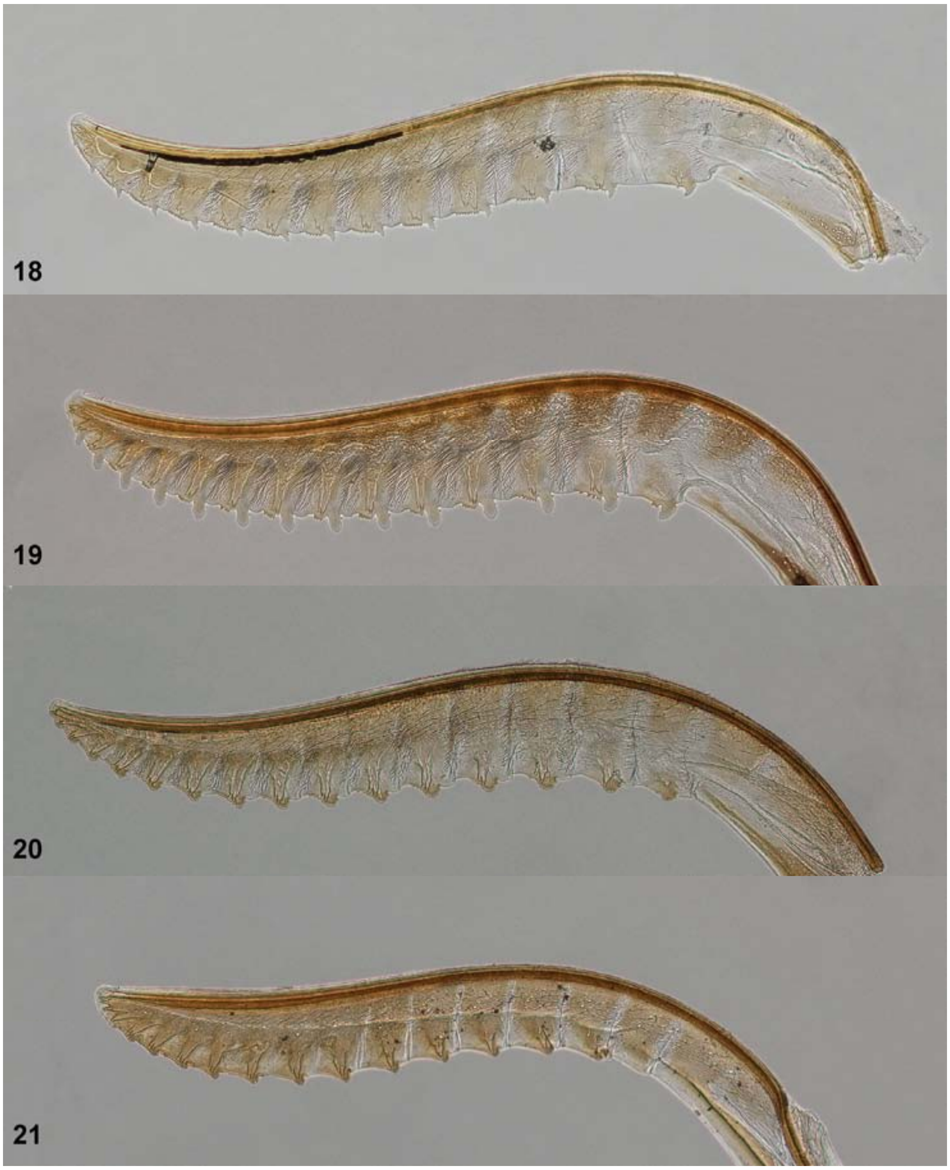
Figures 6–9. Lancets (valvulae 1) of *Empria*. **6** *E. excisa* **7** *E. africana* (paratype) **8** *E. archangelskii* **9** *E. sp4*.



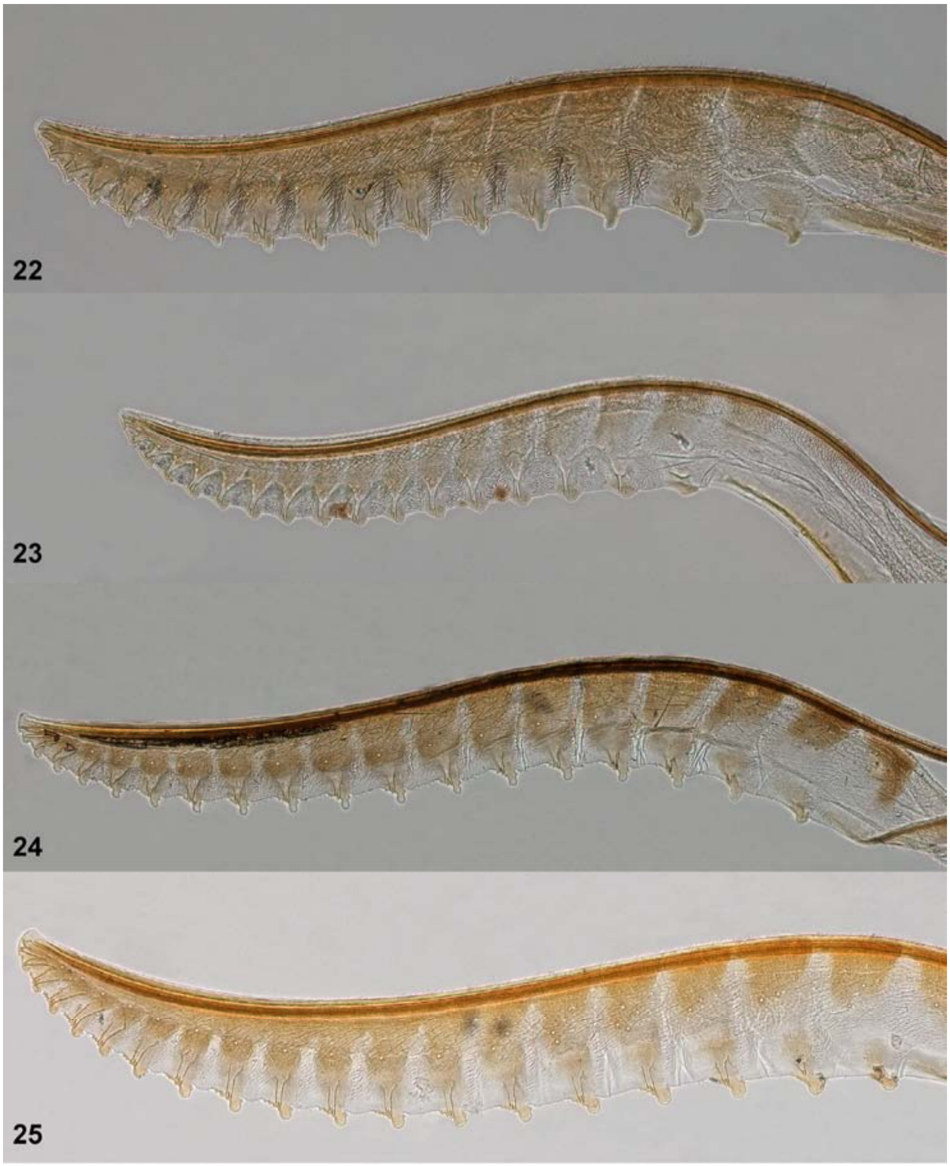
Figures 10–13. Lancets (valvulae 1) of *Empria*. **10** *E. ushinskii* **11** *E. hungarica* **12** *E. pumila* **13** *E. persephone*.



Figures 14–17. Lancets (valvulae 1) of *Empria*. **14** *E. granatensis* (holotype) **15** *E. pumiloides* **16** *E. testaceipes* **17** *E. fletcheri*.

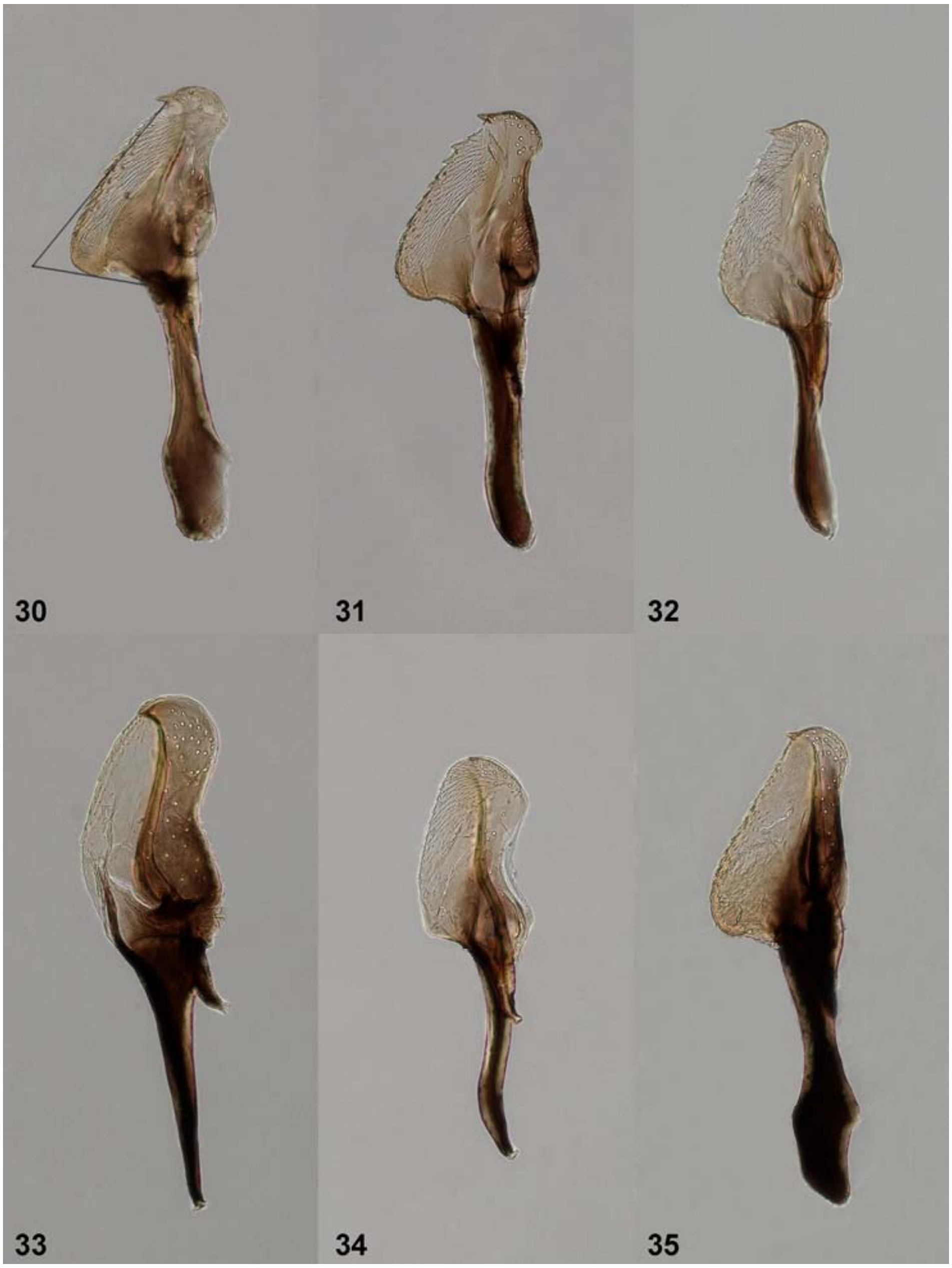


Figures 18–21. Lancets (valvulae 1) of *Empria*. **18** *E. camtschatica* (holotype) **19** *E. immersa* **20** *E. kuznetzovi* **21** *E. sulcata*.



Figures 22–25. Lancets (valvulae 1) of *Empria*. **22** *E. gelida* **23** *E. sp12* **24** *E. sp7* **25** *E. zhangi* (paratype).

Figures 26–29. Lancets (valvulae 1) of *Empria*. **26** *E. parvula* **27** *E. pravei* (syntype) **28** *E. sp2* **29** *E. sexpunctata*.



Figures 30–35. Penis valves of *Empria*. **30** *E. excisa* **31** *E. africana* **32** *E. archangelskii* **33** *E. ushinskii* **34** *E. testaceipes* **35** *E. hungarica*. Angle between the dorsal and basal margins of valviceps were measured between the lines indicated on Fig. 30.

Figures 36–41. Penis valves of *Empria*. **36** *E. pumila* **37** *E. persephone* **38** *E. granatensis* **39** *E. pumiloides* **40** *E. montana* **41** *E. pallimacula*.

Figures 42–47. Penis valves of *Empria*. **42** *E. camtschatica* **43** *E. immersa* **44** *E. fletcheri* **45** *E. sulcata* **46** *E. kuznetzovi* **47** *E. sexpunctata*.

Figures 48–51. Penis valves of *Empria*. **48** *E. parvula* **49** *E. sp2* (image courtesy of Stephan M. Blank, DEI) **50** *E.* sp7 **51** *E. zhangi*.

Taxonomy of Palaearctic and Oriental *Empria* and *Blennallantus*

All the Palaearctic and the Oriental species covered in this thesis are treated below. Nomenclatural information (synonyms, type localities) is given only for the species absent in the papers (**I–III**).

Empria Lepeletier & Serville, 1828

Empria Lepeletier & Serville, 1828: 571. Type species: *Dolerus pallimacula* Serville, 1823 [= *Empria pallimacula* (Serville, 1823)].
See Taeger et al. (2010) for the complete list of synonyms.

Diagnosis. Abdominal terga and sterna posteriorly pale, terga with pale paired patches (in one species can be completely missing). Clypeus bi- or tridentate, with or without a median keel; pit in the central part of the frontal field present; malar space about equal to, shorter, or longer than distance between antennal sockets; malar space 0.9–2.6 times longer than lateral ocellus diameter; maximal length of temple 1.1–1.6 times greater than minimal length of temple; postocellar area 1.4–3.1 times of lateral ocellus diameter; postocellar area 1.3–2.7 times wider than long; length of head 2.2–4.0 times greater than length of head behind eyes; flagellum 1.2–2.7 times longer than breadth of head in female, 1.4–3.9 in male; length of pedicel less than or equal to breadth; antennae with 7 (very rarely 8) flagellomeres; first flagellomere 0.4–0.8 times as long as flagellomeres 2–3 combined; propleura meeting narrowly or not meeting in front; metapostnotum narrow and carinated in middle; tarsal claws simple, with subbasal tooth, or bifid. Posterior margin of sternum 9 in male notched or round. Wing venation (modified from Smith 1979): in forewing, veins M and 1m-cu parallel, vein 2A+3A complete or incomplete, separated from 1A by oblique anal crossvein, veins M and Rs+M meeting Sc + R at same point; first free sector of vein Rs present or absent, therefore either four or three cubital cells, respectively; hind wing with cell Rs absent, cell M present or absent, anal cell petiolate
(http://www.morphbank.net/?id=579063;
http://www.morphbank.net/?id=716093;
http://www.morphbank.net/?id=786402).

Blennallantus compressicornis Wei, 1998

Blennallantus compressicornis Wei, 1998: 149–150. Holotype ♂, CSCS [examined]. Type locality: China, Shaanxi, Huoditang.

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from China (Shaanxi, Sichuan).

Notes. Female unknown.

Empria africana Forsius, 1918

Empria africana Forsius, 1918: 3–4. Holotype ♀, paratype ♀, MZAT [examined]. Type locality: Algeria, Algiers, Husseyn-Dey.

Host plants. Unknown, but could be *Rubus ulmifolius* (in Morocco) or other closely related species (see Lacourt 1986; 1999 under the name *E. excisa*).

Distribution. West Palaearctic. Specimens studied are from Algeria and Morocco.

Notes. Although the serrulae of valvula 1 of female specimens from Algeria (including the paratype) are quite distinct (are more protruding) from *E. excisa* (cf. Figs 6, 7), conspecificity with *E. excisa* cannot be entirely excluded, because serrulae of females studied from Morocco seem somewhat intermediate between *E. africana* and *E. excisa*. Because there appear to be also small differences between male penis valves from Africa (only specimens from Morocco were available for study) and Europe, we tentatively treat specimens from Africa as distinct species.

Empria alector Benson, 1938 (I)

Host plants. *Filipendula ulmaria* (Kontuniemi 1955; **I**).

Distribution. West Palaearctic. Specimens studied are from Denmark, Estonia, Finland, France, Germany, Russia (Leningrad Oblast), Sweden, Switzerland, and United Kingdom.

Empria alpina Benson, 1938 (I)

Host plants. *Dryas octopetala* (see paper **I**).

Distribution. Holarctic. Specimens studied are from Finland, France, Germany, Italy, Switzerland, and United Kingdom.

Empria archangelskii Dovnar-Zapolskij, 1929

Empria (Empria) archangelskii Dovnar-Zapolskij, 1929: 38, 44. Syntype ♀, ZIN [examined]. Type locality: Russia, Krasnodar Krai, Tuapse.

Host plants. Unknown.

Distribution. West Palaearctic. Specimens studied are from Cyprus, Israel, Lebanon, Russia (Krasnodar Krai), Syria, and Ukraine.

Empria basalis Lindqvist, 1968 (I)

Host plants. *Geum rivale* and *G. urbanum* (**I**; this study). Possibly also *Alchemilla* sp. (see Notes).

Distribution. West Palaearctic. Specimens studied are from Austria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Norway, Russia (Leningrad Oblast and Ulyanovsk Oblast), Slovakia, Sweden, Ukraine, United Kingdom.

Notes. The studied specimens (2 ♀♀, 1 ♂) from Austria (DEI-GISHym14920–DEI-GISHym14922) were reared from *Alchemilla* sp. by Ewald Altenhofer (personal communication). Nuclear ITS sequences from one of the males suggest that it is not *E. basalis*, but morphology of the ovipositors and the penis valves suggest otherwise (http://www.morphbank.net/?id=799225–799227).

Empria camtschatica Forsius, 1928

Empria camtschatica Forsius, 1928: 46–47. Holotype ♀, ZMH [examined]. Type locality: Russia, Kamchatka Krai, Bolsheretsk [Bolscheretsk].

Empria asiatica Muche, 1965: 121–124, **syn. n.** Holotype ♀, paratypes 3 ♀♀ & 1 ♂, ZMHB [examined]. Type locality: Mongolia, 80 km NW of Ulan Bator.

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Mongolia and Russia (Kamtschatka Krai, Magadan Oblast).

Empria candidata (Fallén, 1808) (II)

Host plants. *Betula pendula, B. pubescens* (Verzhutskii 1966; original *ex ovo* rearings; Jan Macek, personal communication).

Distribution. Holarctic. Specimens studied are from China (Heilongjiang), Estonia, Finland, Japan (Hokkaido), Russia (Kamchatka Krai, Khabarovsk Krai, Leningrad Oblast, Primorsky Krai), South-Korea, Sweden, Switzerland, United Kingdom, USA (Maine).

Empria excisa (Thomson, 1871)

Poecilosoma excisa Thomson, 1871: 233. Syntype ♀, MZLU [not examined]. Type locality: Southern Sweden, Ringsjön lake [Ringsjön i Skåne].

Host plants. *Filipendula vulgaris* (original *ex ovo* rearings).

Distribution. West Palaearctic. Specimens studied are from Estonia, Finland, France, Germany, Spain (Mallorca), Switzerland, and United Kingdom.

Empria fletcheri (Cameron, 1878)

Poecilosoma fletcheri Cameron, 1878: 20. Syntype ♀, BMNH [not examined]. Type locality: United Kingdom, Scotland, Rannoch or Braemar.

Host plants. *Betula humilis*, *B. nana*, and *B. pubescens*. Based on original *ex ovo* rearings from *B. humilis* (larvae and adults), *B. nana* (few larvae), and young *B. pubescens* (I instar larvae). Larvae have also been reared *ex ovo* from *B. nana* by Veli Vikberg (personal communication) in Finland.

Distribution. Palaearctic. Specimens studied are from Estonia, Finland, Mongolia, Russia (Yamalo-Nenets Autonomous Okrug), Sweden, United Kingdom (Scotland).

Empria formasana (III)

Host plants. Unknown.

Distribution. Oriental. Specimens studied are from Taiwan.

Empria gelida (Erichson, 1851)

Tenthredo (*Poecilostoma*) *gelida* Erichson in Ménétriés In: Middendorf, 1851: 61–62. Holotype ♀, ZIN [examined]. Type locality: Russia, Khabarovsk Krai, Udskoe [Udskoj Ostrog].

Poecilosoma alboscutellata Konow, 1894: 132, **syn. n.** Holotype ♀, DEI [examined]. Type locality: Czech Republic, Moravia.

Host plants. Unknown.

Distribution. Palaearctic. Specimens studied are from Czech Republic and Russia (Kamchatka Krai, Khabarovsk Krai, Primorsky Kray).

Notes. Male unknown.

Empria granatensis Lacourt, 1988

Empria granatensis Lacourt, 1988: 309–312. Holotype ♀, paratype ♂, JL [examined]. Type locality: Spain, Granada, Padul.

Host plants. Unknown.

Distribution. West Palaearctic. Specimens studied are from France and Spain.

Empria honshuana Prous & Heidemaa, 2011 (II)

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Honshu).

Empria hungarica (Konow, 1895)

Poecilosoma hungarica Konow, 1895: 51–52. Holotype ♀, HNHM [destroyed, see Liston 2006]. Type locality: Romania, Mehádia (previously Hungary).

Empria (*Triempria*) *zacharovi* Dovnar-Zapolskij, 1929: 41. Syntype ♀, ZIN [examined]. Type locality: Russia, Kuban-Gebiet, Mirskaja.

Host plants. *Filipendula vulgaris* (Liston 2006; original *ex ovo* rearings).

Distribution. West Palaearctic. Specimens studied are from Estonia, Germany, Russia (West Ciscaucasia), Sweden, Ukraine.

Empria immersa (Klug, 1818)

Tenthredo (Emphytus) immersa Klug, 1818: 284. Holotype ♂, ZMHB [examined]. Type locality: Germany.

Synonymy in Taeger et al. (2010).

Host plants. *Salix alba, Salix caprea, Salix aurita* (Brischke 1883 under the name *Poecilosoma pallimacula* Le Peletier, 1823, misident.; Kontuniemi 1960; Jan Macek, personal communication).

Distribution. West Palaearctic. Specimens studied are from Denmark, Estonia, Finland, France, Germany, Italy, Russia (Leningrad Oblast), Sweden, United Kingdom.

Empria japonica Heidemaa & Prous, 2011 (I)

Host plants. Perhaps *Rubus idaeus* ssp. *melanolasius* (see paper **I**).

Distribution. East Palaearctic. Specimens studied are from Japan (Hokkaido).

Empria kuznetzovi Dovnar-Zapolskij, 1929

Empria (*Triempria*) *kuznetzovi* Dovnar-Zapolskij, 1929: 40. No type specimens were found in ZIN. Type locality: Russia, Volgograd oblast, Sarepta.

Host plants. *Rubus caesius* (Jan Macek, personal communication).

Distribution. West Palaearctic. Specimens studied are from France, Germany, Greece, Hungary, Russia (Ulyanovsk Oblast). Additional confirmed country record is Czech Republic (Jan Macek, personal communication).

Notes. The binomen was synonymized with *Empria parvula* (Konow, 1892) by Conde (1940). However, there is clearly a distinct species (cf. Figs 20, 26, 46, 48), which could easily be confused with *E. parvula* by its external morphology. The only more or less clear external difference is that the postocellar area in *E. kuznetzovi* is 1.8–2.1 times wider than long, while in *E. parvula* it is 2.1–2.6 times wider. This difference can easily be overlooked and the overlap between the species cannot be excluded. Because the original description of *E. kuznetzovi* by Dovnar-Zapolskij (1929) generally fits with the specimens of "*E. kuznetzovi*" studied here, no new name is proposed, although we were not able to study any types of *Empria kuznetzovi* Dovnar-Zapolskij, 1929 [hence the synonymy with *Empria parvula* (Konow, 1892) is still a possibility].

Empria liturata (Gmelin, 1790) (II)

Host plants. *Filipendula ulmaria, Geum rivale* (original *ex ovo* rearings), *Geum urbanum,* and *Filipendula vulgaris* (Jan Macek, personal communication). *Fragaria vesca* has also been suggested (Enslin 1914), but this requires confirmation.

Distribution. Palaearctic. Specimens studied are from Belgium, Croatia, Czech Republic, Denmark, Estonia, France, Germany, Hungary, Italy, Japan (Hokkaido), Russia (Leningrad Oblast), Sweden, Switzerland, United Kingdom.

Empria loktini Ermolenko, 1971 (I)

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Hokkaido) and Russia (Sakhalin Oblast).

Empria longicornis (Thomson, 1871) (I)

Host plants. *Rubus idaeus* (Kontuniemi 1951; **I**).

Distribution. Palaearctic. Specimens studied are from Austria, Denmark, Estonia, Finland, France, Germany, Hungary, Romania, Slovakia, Sweden, and Switzerland.

Notes. The actual distribution might be restricted only to West Palaearctic, as the East Palaearctic records of *E. longicornis* by Zhelochovtsev & Zinovjev (1988; 1996) might be incorrect due to possible misidentifications of *E. japonica* or *E. tridentis*. The record from Mongolia (Zombori 1972) is certainly incorrect due to misidentification of *E. mongolica* males.

Empria minuta Lindqvist, 1968 (I)

Empria minuta Lindqvist, 1968: 30. Holotype ♀, ZMH [examined]. Type locality: Finland, vicinity of Helsinki.

Host plants. Unknown.

Distribution. Palaearctic. Specimens studied are from Estonia, Finland, Mongolia, Russia (Leningrad Oblast, Yamalo-Nenets Autonomous Okrug), and Sweden.

Empria mongolica (Konow, 1895) (I)

Host plants. *Dasiphora fruticosa* (see Verzhutskii 1981).

Distribution. East Palaearctic. Specimens studied are from Mongolia, Russia (Kamchatka Krai).

Empria montana Koch, 1984

Empria montana Koch, 1984: 19–20. Holotype ♂, ZMHB [examined]. Type locality: Mongolia, Uvs, Harhiraa [Charchira], 30 km SW Ulaangom [Ulangom], alt. 1710 m.

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Mongolia.

Notes. Female unknown.

Empria pallimacula (Serville, 1823)

Dolerus pallimacula Serville, 1823: 55. Lectotype ♀, MNHN [examined]. Type locality: France, Picardy, Aisne, Soissons [Du Soissonnais].

Dolerus (*Emphytus*) *pallimacula* Lepeletier, 1823: 117–118. Primary homonym of *Dolerus pallimacula* Serville, 1823 [= *Empria pallimacula* (Serville, 1823)].

Empria baltica Conde, 1937: 106. Syntype ♀ & ♂, DEI [not examined]. Type locality: Latvia.

Host plants. *Filipendula ulmaria* (Kontuniemi 1951; original *ex ovo* rearings).

Distribution. Palaearctic. Specimens studied are from Belgium, Denmark, Estonia, Finland, France, Germany, Norway, Russia (Irkutsk Oblast, Leningrad Oblast), Sweden, United Kingdom.

Empria parvula (Konow, 1892)

Poecilosoma parvula Konow, 1892: 215. Syntypes 2 ♀♀ & 4 ♂♂, DEI [not examined]. Type locality: Germany.

Empria (*Empria*) *pseudo-klugi* Dovnar-Zapolskij, 1929: 39. Syntypes 2 ♀♀ & 1 ♂, ZIN [examined]. Type locality: Russia, Volgograd oblast, Sarepta.

Host plants. *Geum urbanum* (preferably) and *G. rivale* (original *ex ovo* rearings; Jan Macek, personal communication).

Distribution. Palaearctic. Specimens studied are from Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Russia (Khabarovsk Krai, Primorsky Krai), Switzerland.

Notes. The specimens of *Empria pseudoklugi* Dovnar-Zapolskij, 1929 studied bear the identification labels “Empria klugiformis paratypus 1928. m. D. Dovnar det.” (♀, ♂) and “Empria klugiformis typus 1928. m. D. Dovnar det.” (♀). Because the locality labels and characters of the specimens match with the original description of *E. pseudoklugi* and Dovnar-Zapolskij never published the name *E. klugiformis*, these specimens are considered as syntypes of *Empria (Empria) pseudoklugi* Dovnar-Zapolskij, 1929.

Empria persephone Benson, 1954

Empria persephone Benson, 1954: 279–280. Holotype ♂, BMNH [examined]. Type locality: France, Var, Les Arcs.

Host plants. *Fragaria viridis* and *Sanguisorba minor* (Jan Macek, personal communication).

Distribution. West Palaearctic. Specimens studied are from Estonia, France, Czech Republic

Empria plana (Jakovlev, 1891) (II)

Host plants. Possibly *Salix* sp. See Verzhutskii (1966; 1981) under the name *E. immersa*.

Distribution. East Palaearctic. Specimens studied are from Japan (Hokkaido, Honshu), Mongolia, Russia (Amur Oblast, Irkutsk Oblast, Kamtschatka Krai, Khabarovsk Krai, Primorsky Kray, Sakha Republic).

Empria pravei Dovnar-Zapolskij, 1925

Empria pravei Dovnar-Zapolskij, 1925: 35, 37–38. Syntype ♀, ZIN [examined]. Type locality: Russia, Stavropol Krai, Stavropol.

Host plants. Unknown.

Distribution. West Palaearctic. Only one syntype female studied from Russia (Stavropol Krai).

Notes. Male unknown.

Empria pumila (Konow, 1896)

Poecilosoma (*Poecilosoma*) *pumila* Konow, 1896: 54, 58–59. Lectotype ♂, paralectotypes ♀ & ♂ (*E. pumila* and *E. pumiloides*), DEI [see Heidemaa and Viitasaari 1999]. Type locality: Poland, Carvin/Pommern near Debica.

Host plants. *Filipendula ulmaria* (see Heidemaa and Prous 2006).

Distribution. West Palaearctic. Specimens studied are from Belgium, Denmark, Estonia, Finland, France, Germany, Russia (Leningrad Oblast), Sweden, United Kingdom. Confirmed country record is also Poland (see Heidemaa and Viitasaari 1999).

Empria pumiloides Lindqvist, 1968

Empria pumiloides Lindqvist, 1968: 31–32. Holotype ♀, paratype ♂, MZH [see Heidemaa and Viitasaari 1999]. Type locality: Finland, Uusimaa, near Helsinki.

Empria tricornis Lindqvist, 1968: 32–33. Holotype ♂, paratype ♀, MZH [see Heidemaa and Viitasaari 1999]. Type locality: Finland, Uusimaa, Helsinki.

Host plants. *Filipendula ulmaria* (see Heidemaa and Prous 2006).

Distribution. West Palaearctic. Specimens studied are from Belgium, Estonia, France, Germany, Russia (Leningrad Oblast), Ukraine. Confirmed country record is also Finland (see Heidemaa and Viitasaari 1999).

Empria quadrimaculata Takeuchi, 1952 (II)

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Honshu, Kyushu, Shikoku).

Notes. The record from Sakhalin Oblast (Russia) by Zhelochovtsev & Zinovjev (1996) might be incorrect due to possible misidentification of *E. rubicola* or *E. tridentis*.

Empria rubicola Ermolenko, 1971 (II)

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Hokkaido) and Russia (Sakhalin Oblast).

Empria sexpunctata (Serville, 1823)

Tenthredo sexpunctata Serville, 1823: 43–44. No type specimens were found in MNHN (see also Lacourt 2000). Type locality: France, Paris.

Tenthredo sex-punctata [sic!] Lepeletier, 1823: 102. Primary homonym of *Tenthredo sexpunctata* Serville, 1823 [= *Empria sexpunctata* (Serville, 1823)].

See Taeger et al. (2010) for full list of synonyms.

Host plants. *Geum rivale* (Conde 1937; original *ex ovo* rearings) and possibly *G. urbanum* (Lacourt 1999).

Distribution. West Palaearctic. Specimens studied are from Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Russia (Leningrad Oblast), Slovakia, Switzerland, United Kingdom.

Empria sulcata Wei & Nie, 1998

Empria sulcata Wei & Nie, 1998: 364, 386. Holotype ♂, CSCS [examined]. Type locality: China, Zhejiang, Longwangshan Nature Reserve.

Host plants. Unknown.

Distribution. Oriental. Specimens studied are from China (Hunan, Zhejiang).

Empria takeuchii Prous & Heidemaa, 2011 (II)

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Hokkaido, Honshu).

Empria testaceipes (Konow, 1896)

Poecilosoma (*Poecilosoma*) *testaceipes* Konow, 1896: 54, 57–58. Holotype ♂, DEI [examined]. Type locality: Czech Republic, Moravia.

Host plants. *Sanguisorba officinalis* (see Liston et al. 2007).

Distribution. Palaearctic. Specimens studied are from Czech Republic, Mongolia, Russia (Amur Oblast), Slovakia, Ukraine.

Empria tridens (Konow, 1896) (I)

Host plants. *Rubus idaeus* (Miles 1936; Lorenz and Kraus 1957; **I**) and possibly *Rubus fruticosus* complex (**I**).

Distribution. Palaearctic. Specimens studied are from Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Japan (Hokkaido), Mongolia, Norway, Russia (Amur Oblast, Kamchatka Krai, Kostroma Oblast, Leningrad Oblast, Primorsky Kray, Sakhalin Oblast, Volgograd Oblast), Sweden, Switzerland, Turkey, Ukraine, and United Kingdom.

Empria tridentis Lee & Ryu, 1996 (II)

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Hokkaido, Honshu), Russia (Khabarovsk Krai, Primorsky Kray), South-Korea.

Empria ushinskii Dovnar-Zapolskij, 1929

Empria (Triempria) ushinskii Dovnar-Zapolskij, 1929: 41–42. Syntypes 2 ♀♀, 3 ♂♂, ZIN [examined]. Type locality: Ukraine, Kiev.

Host plants. Unknown.

Distribution. West Palaearctic. Specimens studied are from Czech Republic.

Notes. The three specimens studied by the author (one female, two males) clearly have a pair of pale patches on the first abdominal tergum, as well as two additional males studied by Jan Macek (personal communication). The original description of *E. ushinskii* states that these pale patches on the first abdominal tergum can be present or absent (Dovnar-Zapolskij 1929). All of the studied syntypes of *E. ushinskii* (2 ♀♀, 3 ♂♂) actually belong to *E. tridens* (for that reason the lectotype was not designated), which do not have pale patches on the first abdominal tergum. It is possible that there exist additional syntype specimens by Dovnar-Zapolskij with pale patches on the first abdominal tergum, which might belong to *E. ushinskii* as currently understood (see Zhelochovtsev and Zinovjev 1988).

Empria wui Wei & Nie, 1998

Empria wui Wei & Nie, 1998: 363–364, 385–386. Holotype ♀, CSCS [examined]. Type locality: China, Zhejiang, Longwangshan Nature Reserve.

Host plants. Unknown.

Distribution. Oriental. Only one female specimen (holotype) studied from China (Zhejiang).

Notes. Male unknown.

Empria zhangi Wei & Yan, 2009

Empria zhangi Wei & Yan in Yan, Wei & He, 2009: 248–250. Holotype ♀, paratypes 1 ♀ & 2 ♂♂, CSCS [examined]. Type locality: China, Hunan, Mt. Heng, Nantianmen, 27°14′N 112°51′E.

Host plants. Unknown.

Distribution. East Palaearctic, Oriental. Specimens studied are from China (Hunan).

Empria sp2

Host plants. Unknown.

Distribution. West Palaearctic. Specimens studied are from Iran.

Empria sp3

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Russia (Primorsky Krai).

Notes. Male unknown.

***Empria* sp4**

Host plants. Unknown.

Distribution. West Palaearctic. Specimens studied are from Greece (Crete).

Notes. Male unknown.

***Empria* sp7**

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Russia (Primorsky Krai) and South-Korea.

***Empria* sp8**

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Honshu).

Notes. Female unknown.

***Empria* sp9**

Host plants. Unknown.

Distribution. East Palaearctic. Only one female specimen studied from China (Shanxi).

Notes. Male unknown.

***Empria* sp11 (II)**

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Japan (Hokkaido).

Notes. Female unknown.

***Empria* sp12**

Host plants. Unknown.

Distribution. East Palaearctic. Specimens studied are from Russia (Primorsky Krai) and South-Korea.

Notes. Male unknown.

***Empria* sp13**

Host plants. Unknown.

Distribution. East Palaearctic, Oriental. Only one male specimen studied from China (Hubei).

Notes. Female unknown.

Phylogenetic results and discussion

First phylogenetic results on *Empria* were presented in papers **I–III**, which, taken together, covered 29 species. Here, these results are updated by including 46 species in the phylogenetic analyses. In addition we updated the phylogeny of *E. longicornis* group by including more specimens to test if the different phylogenetic patterns found based on mitochondrial and nuclear sequences will hold.

Amplified ITS1 was 956–1153, ITS2 485–602, ITS1 and 2 combined 1574–1749 bp, and mitochondrial fragment 1724–1814 bp long. In comparison to all other species amplified, tRNA-Cys and tRNA-Tyr are apparently missing between tRNA-Trp and cytochrome c oxidase I (COI) in *Empria testaceipes*' mtDNA. Instead there is an unidentified 79 bp sequence, which was excluded from the analyses. Although the length is suggestive for the presence of one tRNA gene, tRNAscan-SE 1.21 (Lowe and Eddy 1997) did not find any.

For some specimens, the full 5.8S rRNA gene was also sequenced (when ITS1 and 2 were amplified together), but it was identical in almost every specimen sequenced (only a specimen of *E. obscurata* had an autapomorphic substitution in the middle of the gene) and therefore was excluded from the phylogenetic analyses.

Three kinds of phylogenetic analyses were done: ITS1, ITS2, and mtDNA were all analysed separately, ITS1 and ITS2 were combined, and then all sequence data were combined. It is generally advisable to combine different gene regions into single data matrix in order to minimize stochastic error and to amplify any phylogenetic signal which might be present (Gatesy and Baker 2005; Philippe et al. 2005). However, if the phylogeny differs between the sampled genes and individual gene phylogenies are not entirely congruent with the species phylogeny (due to incomplete lineage sorting, hybridization, or horizontal gene transfer), analyses of combined data matrix might yield misleading results. Therefore, the three different gene regions (ITS1, ITS2, and mtDNA) were also analysed separately to examine if there were any significant conflicts. “Significant” here means that alternative phylogenetic relationships are supported by posterior probabilities (PP) more than 0.95 and by maximum likelihood bootstrap proportions (BP) more than 70%.

In case of ITS sequences, most of the analyses were done by excluding *Monsoma* and *Parataxonus* (*E. candidata* and *E. multicolor*), because these were too different from *Empria* s.str., making alignment problematic. Although BAli-Phy is designed to handle difficult-to-align sequences, *Monsoma* and *Parataxonus* were excluded to save computation time (by excluding these taxa, more unambiguously aligned regions were available to define as constraints in the analyses) and avoid serious alignment errors that might be caused by large degree of divergence between *Monsoma*-*Parataxonus* and *Empria* s.str. The analyses including *Monsoma*-*Parataxonus* unambiguously showed that *Empria* s.str. is monophyletic (not shown; **III**). The following presented analyses including ITS sequences are without *Monsoma* and *Parataxonus*.

Although rDNA, which includes ITS1 and ITS2, is tandemly repeated usually hundreds or thousands of times in the genomes of eukaryotes, it can be considered as a single gene because of highly efficient concerted evolution, which usually keeps the tandemly repeated copies essentially identical (Ganley and Kobayashi 2007). Because of the proximity of ITS1 and ITS2 in the genome (only about 150 bp of 5.8S rRNA separates those gene regions), these would be generally expected to be linked markers and therefore to be phylogenetically congruent. However, the large number of rDNA tandem-repeats increases the chance of recombination, which might also lead to phylogenetic incongruency between different parts of the rDNA repeat array. This appears to be a case, because there were two significantly (PP>0.95) supported differences in the separate analyses of ITS1 and ITS2 sequences using Bali-Phy: 1) *E. parvula* is sister to *E. sexpunctata* in the analysis of ITS1 sequences (PP=1.0) (not shown), but forms a well supported clade with *E. kuznetzovi* and species of the *E. hungarica* group (PP=1.0) in the analysis of ITS2 sequences (not shown); 2) *E. pumila* forms a well supported clade (PP=0.98) with *E. persephone* and *E. hungarica* in the analysis of ITS1 sequences and there are at least 30 substitution and indel events distinguishing *E. pumila* and *E. pumiloides* (when we take into account that ITS1 is nearly twice as long as ITS2, at least 15 differences would still be expected), but based on ITS2 sequences, *E. pumila* and *E. pumiloides* are indistinguishable (based on two specimens studied from both species there are only 0–2 differences). Results of phylogenetic analyses of mitochondrial sequences are more similar to ITS2 phylogeny regarding the position of *E. parvula*, but more similar to ITS1 phylogeny regarding the position of *E. pumila* (not shown). However, it should be mentioned that in both cases there are important additional differences in the phylogeny of mitochondrial sequences compared to nuclear sequences.

When we removed *E. parvula* and *E. pumila* from the combined analyses of ITS1 and ITS2 sequences, the following significant differences (disregarding internal phylogeny of the *E. longicornis* and *E. immersa* groups, which are discussed below) are apparent between nuclear and mitochondrial DNA analyses: *E. takeuchii* is sister to the *E. immersa* group in the ITS tree, but falls outside a clade composed of the *E. immersa* and *E. longicornis* groups and *E. tridentis* in the mtDNA tree; *E. ushinskii* is sister (actually nearly identical) to *E. pumiloides* in the ITS tree, but it is part of a clade composed of the

E. pallimacula group and *E. parvula* in the mtDNA tree; *E. persephone* is sister to *E. hungarica* in the ITS tree, but sister to the *E. hungarica* group minus *E. ushinskii* in the mtDNA tree; and the *E. hungarica* group is sister to *E. kuznetzovi* in the ITS tree, but it is (without *E. ushinskii*) sister to a clade composed of the *E. pallimacula* group, *E. parvula*, and *E. ushinskii*. The most puzzling of these conflicts is the position of *E. ushinskii*. Based on morphological data, this species was expected to be closely related to *E. hungarica* (males of both species have the longest flagellum in the *E. hungarica* group, more than 3 times the breadth of head) or *E. testaceipes* (similar penis valves; Figs 33, 34). The ITS phylogeny agrees with the morphological data in that *E. ushinskii* falls within the *E. hungarica* group, but the sister group relationship with *E. pumiloides* was completely unexpected (see the identification key). That *E. ushinskii* is more closely related to the *E. pallimacula* group and *E. parvula* than to the *E. hungarica* group in the mitochondrial phylogeny is quite puzzling, even though *E. ushinskii* together with the *E. pallimacula* group and *E. parvula* are sister to the *E. hungarica* group. Longer mitochondrial sequences are needed to resolve this phylogenetic anomaly.

The other differences (except within the *E. longicornis* and *E. immersa* groups) are not significantly supported primarily due to low resolution of the mitochondrial tree (only few clades are well supported) in contrast to nuclear ITS tree (when excluding *E. parvula* and *E. pumila*) reconstructed with Bali-Phy, in which most of the clades are supported with posterior probabilities 0.99–1.00. Resolution of the mitochondrial phylogeny can be improved with longer sequences, which might reveal additional significantly supported differences with nuclear ITS tree.

Combined nuclear ITS and mitochondrial phylogeny revealed the following well supported groups within *Empria* s.str. (Fig. 52): the *E. immersa, E. longicornis, E. quadrimaculata, E. hungarica, E. pallimacula, E. liturata, E. wui* species groups; *E. immersa* group + *E. longicornis* group + *E. takeuchii* + *E. tridentis*; and *E. liturata* group + *E. maculata*.

For some species it was possible to obtain sequences only partially, namely *E. granatensis*, *E. eosa* Smith 1979, *E. ignota* (Norton, 1867), and *E. evansi* Smith, 1980. Their phylogenetic position, based on analysis of partial mitochondrial and ITS sequences (not shown), is shown on the tree based on combined analysis of full COI and ITS sequences (Fig. 52). As expected from morphology, *E. granatensis* falls within the *E. hungarica* group (Lacourt 1988; Heidemaa and Viitasaari 1999), and *E. eosa* and *E. ignota* within the *E. liturata* group (Smith 1979; **II**) (Fig. 52). *Empria evansi* was found to be a sister species of *E. maculata* (Fig. 52), consistent with the similarities between these species as noted by Smith (1980).

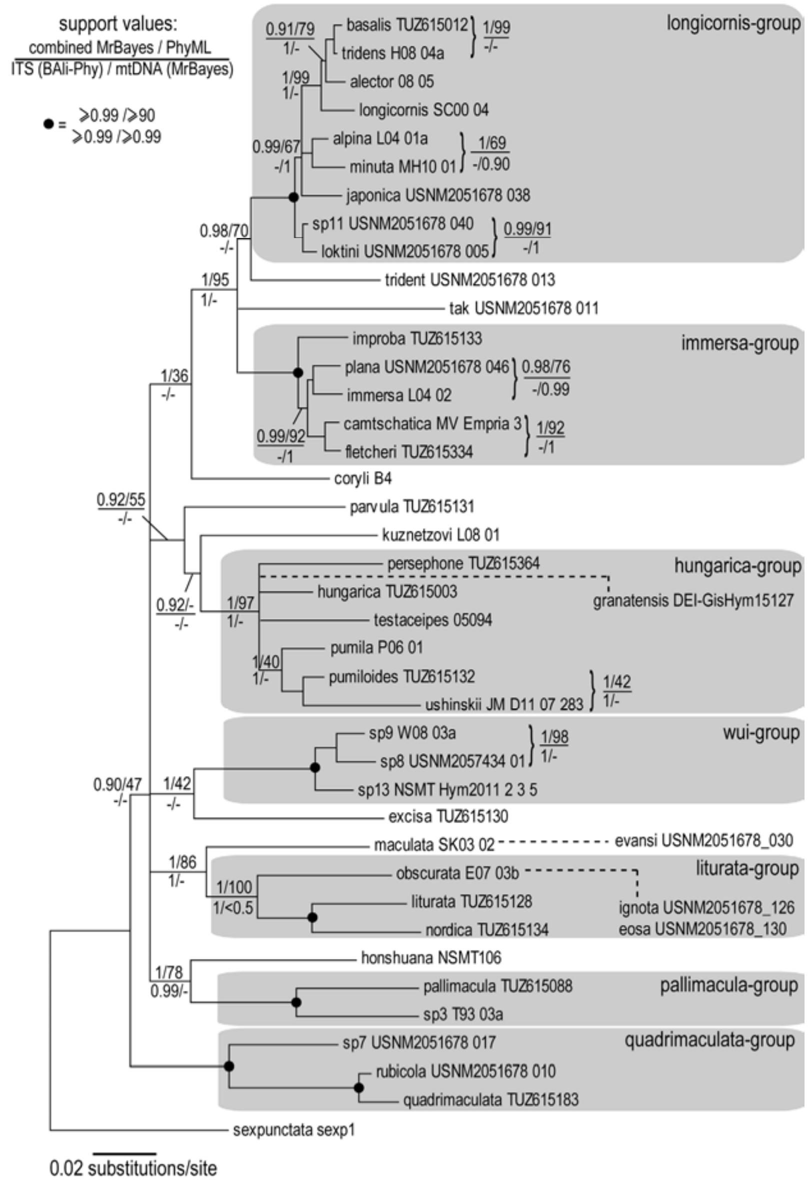
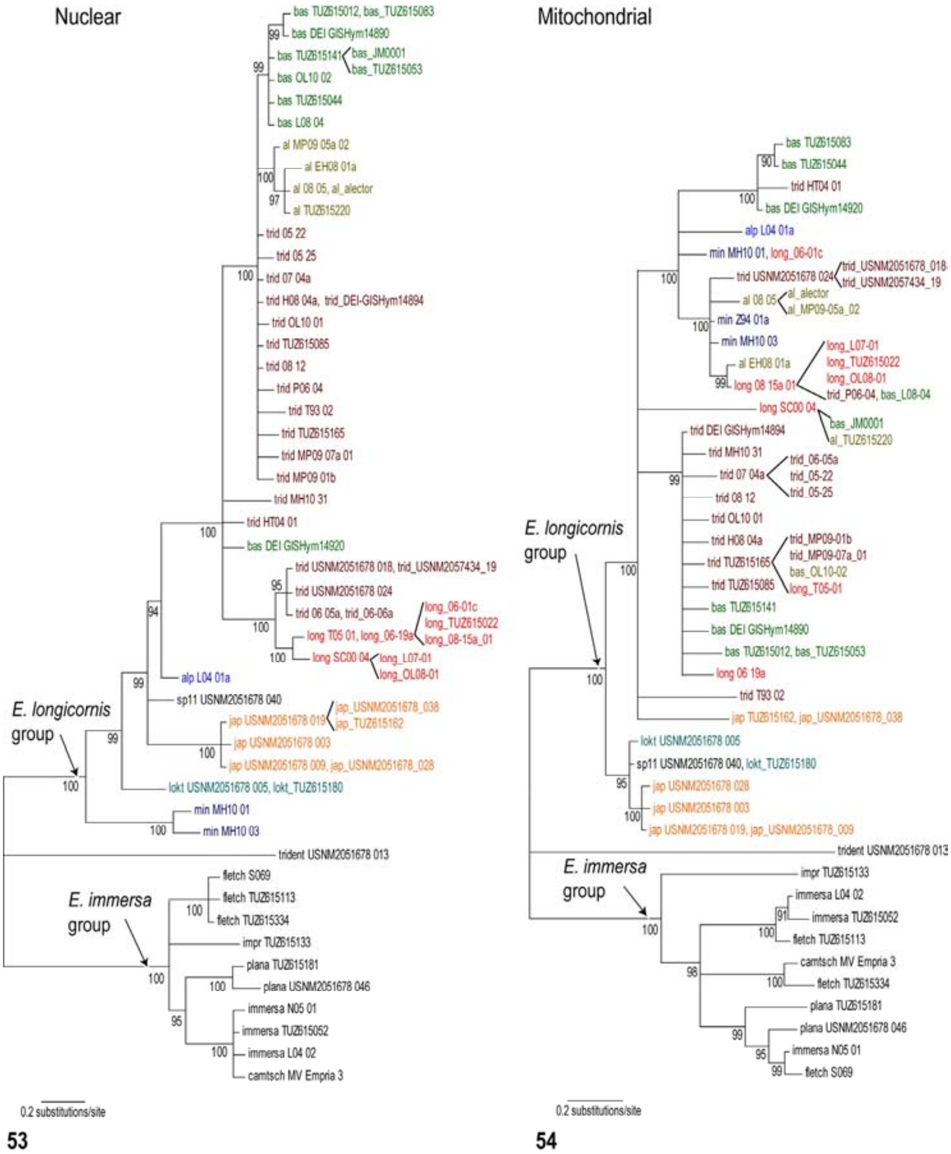


Figure 52. Phylogeny of *Empria* s.str. based on combined analysis of ITS and mitochondrial sequences using MrBayes (GTR + I + G model). The ITS alignment included in the combined analysis was estimated using maximum posterior decoding based on four independent runs (150050–152640 generations, "burn-in" 40000) with Bali-Phy. Although the four runs did not converge (the average standard deviation of split frequencies was 0.084) and the effective sample size of several output values was below 100 (preferably should be more than 200), the consensus trees computed separately for the four independent runs were topologically nearly identical to each other and to the consensus tree computed simultaneously from all four runs. Clades with posterior probabilities (PP) less than 0.9 were collapsed. Support values (PP or BP) for the clades are shown, from left to right: combined analysis (MrBayes, PP / PhyML, BP), nuclear ITS only (BAli-Phy, PP), mitochondrion only (MrBayes, PP). Filled circles indicate clades receiving maximum or nearly maximum support (PP≥0.99, BP≥90%) from all the analyses and "–" indicates a clade not recovered in the respective analysis. Dashed lines indicate the phylogentic positions of *E. granatensis* (209 bp of ITS1 and 1292 bp of mtDNA), *E. evansi* (594 bp of ITS1, 563 bp of ITS2, and 1290 bp of mtDNA), *E. ignota* (237 bp of ITS1 and 1286 bp of mtDNA), and *E. eosa* (587 bp of ITS1, 568 bp of ITS2, and 1287 bp of mtDNA) based on separate analyses using shorter nuclear and mitochondrial sequences. The tree was rooted between *E. sexpunctata* and all other species as suggested by nuclear ITS analysis including *Parataxonus* and *Monsoma pulveratum*.

Only approximate phylogenetic positions can be suggested for some species based on morphology and in some cases also based on short ITS1 (>300 bp) fragment: *E. mongolica* (also ITS1) within the *E. longicornis* group (**I**); *E. archangelskii*, *E. africana*, and *E. sp4* (ITS1) close to *E. excisa* (this study); *E. zhangi* within the *E. quadrimaculata* group (**II**); *E. mexicana* (Cameron, 1883) close to *E. obscurata* (Cresson, 1880) (Smith 1979) (i.e. within the *E. liturata* group); *E. sulcata* close to *E. honshuana* (**II**); *E. wui* and *E. formosana* close to *E. sp8*, *E. sp9*, *E. sp13* (**III**); *E. pravei* and *E. sp2* close to *E. parvula* (this study). In case of the remaining known species of *Empria* (*E. gelida*, *E. montana, E. sp12*), it can only be said that most probably these species fall within *Empria* s.str., but more exact phylogenetic position remains unclear.

Separate analyses were conducted also for the *E. longicornis* and *E. immersa* groups to study their internal phylogeny more thoroughly. After the publication of the paper **I**, many more specimens belonging to the *E. longicornis* and *E. immersa* groups were sequenced (**II**, this study). Updated mitochondrial and nuclear phylogenies of the *E. longicornis* group are shown in Figs 53, 54. The results confirm and reinforce the pattern found in paper **I** that species are monophyletic according to nuclear (Fig. 53), but not according to mitochondrial sequences (Fig. 54) (non-monophyly of mitochondrial sequences does not immediately translate here to non-monophyly of the species as a whole, because species and their gene trees are not necessarily identical). Improved sampling of specimens suggests that none of the species in the *E. longicornis* or *E. immersa* groups might be monophyletic (Fig. 54) in the mitochondrial phylogeny (based on smaller sampling in paper **I** it appeared that *E. japonica* might be). Sampling of multiple specimens from *E. alpina*, *E. mongolica*, *Empria sp11*, *E. camtschatica*, and *E. improba* (Cresson, 1880) are needed to confirm this. However, non-monophyly of *E. improba* seems less likely, because it is geographically (Nearctic) as well as phylogenetically (based on mtDNA) isolated from other species of the *E. immersa* group (which are Palaearctic and form a well supported monophyletic group in the mtDNA tree). In contrast, in the nuclear phylogeny species remain monophyletic after improved sampling, except *E. tridens* and *E. immersa* (Fig. 54). Non-monophyly of *E. tridens* was expected from previous analyses (**I**) and the paraphyly of *E. immersa* is because ITS of *E. camtschatica* is nearly identical to ITS of *E. immersa* (there is only a singly apomorphy in ITS2 distinguishing *E. camtschatica* from *E. immersa*). Still, there is one interesting specimen of *E. basalis*, which has an unexpected phylogenetic position (Fig. 53). The specimen DEI-GISHym14920 lacks the synapomorphic character (and differs in many other positions of ITS1 and 2) present in ITS1 (Fig. 62 in **I**) sequences of other *E. basalis* specimens. Based solely on ITS sequences it could be identified as *E. tridens* (Fig. 54), but according to penis valves from the sequenced specimen and the ovipositors of two females collected together with this male (Austria, Tappenkarsee), it



Figures 53–54. Phylogeny of *Empria longicornis* and *E. immersa* groups based on nuclear ITS (53) and mitochondrial (54) sequences. The trees were reconstructed with MrBayes (HKY + G model for nuclear, and HKY + I + G model for mitochondrial dataset). Identical sequences were removed prior to analyses and are shown after the specimen to which these were identical to. Clades with posterior probabilities (PP) less than 0.90 were collapsed. *Empria tridentis* was used to root the tree. Note also the non-monophyly of *E. immersa* group species in the mitochondrial tree (54). **al**, *E. alector*; **alp**, *E. alpina*; **bas**, *E. basalis*; **camtsch**, *E. camtschatica*; **fletch**, *E. fletcheri*; **immersa**, *E. immersa*; **imp**, *E. improba*; **jap**, *E. japonica*; **lokt**, *E. loktini*; **long**, *E. longicornis*; **min**, *E. minuta*; **plana**, *E. plana*; **trid**, *E. tridens*; **trident**, *E. tridentis*.

is clearly *E. basalis*. Interestingly, these specimens, which can morphologically be identified as *E. basalis*, were reared *ex larva* collected from *Alchemilla* sp. (Ewald Altenhofer, personal communication), which might be novel host plant for *E. basalis* (currently known to be *Geum*) or these specimens might represent a different host race. To confirm this, *ex ovo* rearing experiments with *Alchemilla* should be done using *E. basalis* from different localities. Also, additional nuclear genes need to be sequenced to resolve the question of *E. tridens* non-monophyly and the relationship of the *E. basalis* with *Alchemilla* to other *longicornis*-group species.

In summary, based on nuclear ITS and mitochondrial COI sequences, several clades found in the analyses can be regarded as well supported, but most of the phylogenetic relationships within *Empria* s.str. remain unresolved due to insufficient amount of data (too few genes) and probably because of phylogenetic conflict between the gene regions sampled. The overall low resolution of the tree can be attributed to rapid divergence of lineages (too little time to accumulate phylogenetic signal within gene sequences), incomplete lineage sorting (Degnan and Rosenberg 2009), hybridizations (e.g. Linnen and Farrell 2007), or all of these factors combined (quite likely). In this sense, *Empria* is a good group to study different processes in speciation and rapid evolutionary radiations, because of large number of closely related species and monophagous host plant associations. Phylogenetic analysis of multiple unlinked nuclear encoding genes using newly emerging methods taking into account discordance between gene trees (Heled and Drummond 2010; Jacobsen and Omland 2011; Wu 2012) could (and arguably should) be the next interesting scientific endeavour to disentangle different evolutionary processes behind the origin of *Empria* species.

SUMMARY

Taxonomic study revealed that there are at least 50 Palaearctic and Oriental *Empria* species (excluding *Blennallantus compressicornis*, which might belong to *Empria*; **III**), which raises the number of known species to 60. Four of the species have been described as new (*E. japonica*, *E. honshuana, E. takeuchii*, *E. formosana*; **I–III**) and among the species treated here, there are 9 more which are probably new to science (unfortunately in most of these cases only females or males represented by single or few individuals are known per species). In addition to the species treated here, many more species (perhaps 10–20) are still expected globally, especially from Central Asia and China, which are currently the most poorly studied regions.

Based on the taxonomic results of this work, the following nomenclatural changes were proposed.

New synonyms:
Empria asiatica Muche, 1965 **syn. n.** (this study), synonym of *E. camtschatica* Forsius, 1928;
Empria gussakovskii Dovnar-Zapolskij, 1929, synonym of *Poecilosoma tridens* Konow, 1896 [=*E. tridens* (Konow, 1896)] (**I**);
Empria konowi Dovnar-Zapolskij, 1929, synonym of *P. tridens* Konow, 1896 [=*E. tridens* (Konow, 1896)] (**I**);
Empria itelmena Malaise, 1931, synonym of *P. plana* Jakowlew, 1891 [=*E. plana* (Jakowlew, 1891)] (**II**);
Empria waldstaetterense Liston, 1980, synonym of *E. alector* Benson, 1938 (**I**);
Poecilosoma alboscutellata Konow, 1894 **syn. n.** (this study), synonym of *Tenthredo (Poecilostoma) gelida* Erichson, 1851 [(=*E. gelida* (Erichson, 1851)].

Names resurrected from synonymy:
Empria africana Forsius, 1918) from synonymy with *E. excisa* (Thomson, 1871) (this study);
Empria kuznetzovi Dovnar-Zapolskij, 1929 from synonymy with *E. parvula* (Konow, 1892) (this study);
Empria minuta Lindqvist, 1968 from synonymy with *E. alpina* Benson, 1938 (**I**).

New combination:
Monsoma pallipes (Matsumura, 1912), transferred from *Empria* (originally in *Poecilosoma*) to *Monsoma* (**II**).

Based on *ex ovo* rearings by the author and the original results by Jan Macek (hereafter JM; National Museum, Prague), Veli Vikberg (hereafter VV; Turenki, Finland), and Ewald Altenhofer (hereafter EA; Groß Gerungs, Austria), the following new host plant records have been discovered: *E. parvula – Geum urbanum* and *G. rivale* (this study; by JM); *E. kuznetzovi – Rubus caesius* (by JM); *E. liturata – G. urbanum* and *Filipendula vulgaris* (by JM); *E. excisa – F. vulgaris* (this study); *E. hungarica – F. vulgaris* (this study); *E. persephone – Fragaria viridis* and *Sanguisorba minor* (by JM); *E. fletcheri – Betula humilis, B. nana*, and *B. pubescens* (this study; by VV); *E. alpina – Dryas octopetala* (by VV); *E. tridens – Rubus fruticosus* complex (**I**; by JM); *E. basalis – G. rivale, G. urbanum* (**I**; this study) and possibly *Alchemilla* sp. (by EA).

Molecular phylogenetic analyses based on the mitochondrial (COI) and nuclear (ITS1 and ITS2) sequences revealed several well supported clades, most of them consistent with morphological data. Of the 60 known *Empria* species (described and undescribed, excluding *Blennallantus compressicornis*), 46 (77%) were analysed. The following groups were well supported: *Parataxonus* (*E. candidata* and *E. multicolor*); *Empria* s.str. (all other species); the *E. immersa, E. longicornis, E. quadrimaculata, E. hungarica, E. pallimacula, E. liturata, E. wui* species groups; *E. immersa* group + *E. longicornis* group + *E. takeuchii* + *E. tridentis*; and *E. liturata* group + *E. maculata*. For most of the remaining species, for which molecular data (or sufficient amount of it) was not available, close relatives of the analysed species can be suggested quite confidently: *E. mongolica* within the *E. longicornis* group (**I**); *E. archangelskii*, *E. africana*, and *E. sp4* close to *E. excisa*; *E. zhangi* within the *E. quadrimaculata* group (**II**); *E. mexicana* close to *E. obscurata*; *E. sulcata* close to *E. honshuana* (**II**); *E. wui* and *E. formosana* close to *E. sp8*, *E. sp9*, *E. sp13* (**III**); *E. pravei* and *E. sp2* close to *E. parvula*. Only three species within *Empria* s.str. (*E. gelida, E. montana*, and *E. sp12*) remain unplaced phylogenetically. Because most of the relationships between or within the reconstructed clades remain largely unresolved due to insufficient amount of data (too few genes) and apparently also due to certain degree of conflict (especially the *E. longicornis, E. immersa*, and *E. hungarica* species groups) between the analysed gene regions, additional analyses using many unlinked nuclear loci are needed to tackle the remaining phylogenetic questions.

SUMMARY IN ESTONIAN

Lehevaablaste perekonna *Empria* (Hymenoptera, Tenthredinidae) taksonoomia ja fülogenees

Alusteadmised liikidest (taksonoomia) ja nende evolutsioonilisest ajaloost (fülogeneetika) on paljude bioloogiliste uurimissuundade aluseks. Taksonoomia ja fülogeneetika on peamised alustalad biosüstemaatikale, mis tegeleb liikide (ka perekondade ja muude kõrgemate taksonite) piiritlemise, nimetamise, rühmitamise ja kirjeldamisega, samuti määramistabelite koostamise, levikuandmete kogumise ja liikide põlvnemisseoste väljaselgitamisega. Teadmised liikidest ja nende fülogeneesist pole olulised mitte ainult bioloogiale, vaid ka põllumajandusele (nt. kahjurid ja nende biotõrje), meditsiinile (nt. patogeenid, ravimite väljatöötamine) ja isegi majandusele. Kuigi süstemaatikud on kirjeldanud elurikkust juba üle 250 aasta, on meie teadmised puudulikud, ainuüksi seetõttu, et kirjeldatud on hinnanguliselt ainult 10–20% maakeral elavatest liikidest. Suurema osa tuntud ja tundmata hulkraksete elurikkusest moodustavad putukad.

Kiletiivaliste hulka kuuluva lehevaablaste perekonna *Empria* liigid on põhjapoolsemates niiskemates biotoopides kevadeti üsna arvukad, kuid meie teadmised neist on üllatavalt kasinad. Perekond on peamiselt holarktilise levikuga, kus oli seni teada umbes 50 liiki (umbes 40 neist Palearktises). Liigid on perekonnas üsna raskesti määratavad, kuna usaldusväärseks määramiseks tuleb üldjuhul prepareerida isastel peenisvalvid ja emastel munetid.

Töö eesmärgiks oli piiritleda liigid perekonnas *Empria*, välja selgitada nende arv maailmas, välja töötada juhised liikide määramiseks morfologiliste tunnuste põhjal, tuvastada nende peremeestaimed, kirjeldada liikide levikut ning rekonstrueerida rühma fülogenees.

Vajalik materjal koguti autori poolt või kolleegide abiga ning laenutati erinevatest muuseumidest ja erakollektsioonidest üle maailma. Eestis esinevate liikide peremeestaimede kindlakstegemiseks viidi läbi vastsete ja valmikute kasvatuskatsed. Taksonoomiliselt keeruka *Empria longicornis* liigirühma puhul kasutati liikide piiritlemisel ja eristamisel geomeetrilise morfomeetria meetodeid ja geenijärjestusi (ITS1, ITS2, COI). Fülogeneesi rekonstrueerimiseks sekveneeriti üks rakutuuma (ITS1-ITS2; 1574–1749 aluspaari) ja üks mitokondri (COI; 1724–1814 aluspaari) genoomi piirkond. Töös kasutati paljusid võimalusi algandmete üldkättesaadavaks tegemisel: uuritud isendite etiketiandmed (leiukoht, aeg, määrang jne), nendest tehtud digipildid (peamiselt peenisvalvid ja osa munetitest) ja sekveneeritud geenijärjestused talletati rahvusvahelistes andmebaasides (GBIF, Dryad, Morphbank, GenBank).

Kollektsioonide läbitöötamisel selgus, et Palearktises ja Indo-Malai regioonis leidub vähemalt 50 *Empria* liiki (välja arvatud *Blennallantus compressicornis*, kes võib samuti *Empria* perekonda kuuluda; **III**); see teeb koos Nearktise ja Neotroopise liikidega liikide üldarvuks maailmas vähemalt 60. Kirjeldati neli uut liiki (*E. japonica, E. honshuana, E. takeuchii*, *E. formosana*;

I–III), millele lisandub veel vähemalt 9 arvatavasti kirjeldamata liiki (kahjuks on enamik neist teada ainult üksikute emaste või isasisendite põhjal). Avastamist ja kirjeldamist ootavad veel paljud selle perekonna liigid (oletatavasti 10–20), eriti Kesk-Aasiast ja Hiinast, mis on seni nähtavasti kõige vähem uuritud piirkonnad.

Taksonoomiliste tulemuste põhjal pakuti välja järgnevad nomenklatuursed parandused.

Uued sünonüümid:
Empria asiatica Muche, 1965 **syn. n.** (käesolev töö), *E. camtschatica* Forsius, 1928 sünonüüm;
Empria gussakovskii Dovnar-Zapolskij, 1929, *Poecilosoma tridens* Konow, 1896 [=*E. tridens* (Konow, 1896)] sünonüüm (**I**);
Empria konowi Dovnar-Zapolskij, 1929, *P. tridens* Konow, 1896 [=*E. tridens* (Konow, 1896)] sünonüüm (**I**);
Empria itelmena Malaise, 1931, *P. plana* Jakowlew, 1891 [=*E. plana* (Jakowlew, 1891)] sünonüüm (**II**);
Empria waldstaetterense Liston, 1980, *E. alector* Benson, 1938 sünonüüm (**I**);
Poecilosoma alboscutellata Konow, 1894 **syn. n.** (käesolev töö), *Tenthredo (Poecilostoma) gelida* Erichson, 1851 [(=*E. gelida* (Erichson, 1851)] sünonüüm.

Teadulikud nimed, mille kehtivus taastati:
Empria africana Forsius, 1918) pole sünonüümne binoomeniga *E. excisa* (Thomson, 1871) (käesolev töö);
Empria kuznetzovi Dovnar-Zapolskij, 1929 pole sünonüümne binoomeniga *E. parvula* (Konow, 1892) (käesolev töö);
Empria minuta Lindqvist, 1968 pole sünonüümne binoomeniga *E. alpina* Benson, 1938 (**I**).

Uus kombinatsioon:
Monsoma pallipes (Matsumura, 1912), tõstetud perekonnast *Empria* (algselt perekonnas *Poecilosoma*) üle perekonda *Monsoma* (**II**).

Autori *ex ovo* kasvatuskatsetele ning kolleegide Jan Macek (edaspidi JM; National Museum, Prague), Veli Vikberg (edaspidi VV; Turenki, Finland) ja Ewald Altenhofer (edaspidi EA; Groß Gerungs, Austria) tulemustele tuginedes selgitati välja perekond *Empria* liikide mitmed uued seosed peremeestaimedega: *E. parvula* – *Geum urbanum* (maamõõl) ja *G. rivale* (ojamõõl) (käesolev töö; JM tulemused); *E. kuznetzovi* – *Rubus caesius* (põldmurakas) (JM tulemused); *E. liturata* – *G. urbanum* ja *Filipendula vulgaris* (angerpist) (JM tulemused); *E. excisa* – *F. vulgaris* (käesolev töö); *E. hungarica* – *F. vulgaris* (käesolev töö); *E. persephone* – *Fragaria viridis* (muulukas) ja *Sanguisorba* *minor* (harilik mustnupp) (JM tulemused); *E. fletcheri – Betula humilis* (madal kask)*, B. nana* (vaevakask) ja *B. pubescens* (sookask) (käesolev töö; VV tulemused); *E. alpina – Dryas octopetala* (VV tulemused); *E. tridens – Rubus fruticosus* liigikompleks (**I**; JM tulemused); *E. basalis – G. rivale, G. urbanum* (**I**; käesolev töö) ja võib-olla ka *Alchemilla* sp. (kortsleht) (EA tulemused).

Molekulaarfülogeneetilistes analüüsides mitokondriaalse (COI) ja rakutuuma (ITS1 ja ITS2) järjestuste põhjal pälvisid hea toetuse mitmed klaadid, mis reeglina olid ka heas kooskõlas morfoloogiliste andmetega. Kuuekümnest teadaolevast *Empria* liigist (sh veel kirjeldamata liigid, väljaarvatud *Blennallantus compressicornis*) analüüsiti 46 (77%). Arvestatava toetuse said järgmised rühmad: *Parataxonus* (*E. candidata* ja *E. multicolor*); *Empria* kitsas mõttes (kõik teised liigid); *E. immersa, E. longicornis, E. quadrimaculata, E. hungarica, E. pallimacula, E. liturata, E. wui* liigirühmad; *E. immersa* liigirühm + *E. longicornis* liigirühm + *E. takeuchii* + *E. tridentis*; ja *E. liturata* liigirühm + *E. maculata*. Enamikul ülejäänud liikidel, kelle kohta piisavalt (või üldse mitte) molekulaarseid andmeid ei õnnestunud saada, võib üsna kindlalt välja tuua nende lähisugulased: *E. mongolica* kuulub *E. longicornis* liigirühma (**I**); *E. archangelskii*, *E. africana*, ja *E. sp4* on fülogeneetiliselt lähedased liigile *E. excisa*; *E. zhangi* kuulub *E. quadrimaculata* liigirühma (**II**); *E. mexicana* on lähedane liigile *E. obscurata*; *E. sulcata* on lähedane liigile *E. honshuana* (**II**); *E. wui* ja *E. formosana* on lähedased liikidele *E. sp8*, *E. sp9*, *E. sp13* (**III**); *E. pravei* ja *E. sp2* on fülogeneetiliselt lähedased liigile *E. parvula*. Ainult kolme *Empria* (kitsas mõttes) liigi (*E. gelida, E. montana*, and *E. sp12*) fülogeneetiline asetus jäi selgusetuks. Suurem osa fülogeneetilistest seostest hea toetuse pälvinud klaadide vahel või sees jäid käesolevas töös paraku lahendamata andmete vähesuse (liiga vähe erinevaid geene) ja nähtavasti ka analüüsitud eri geenipiirkondades sisalduva fülogeneetilise informatsiooni vastuolulisuse tõttu (iseäranis *E. longicornis*, *E. immersa* ja *E. hungarica* liigirühmades). Ülejäänud fülogeneetiliste küsimuste lahendamiseks perekonnas *Empria* tuleb teha täiendavaid analüüse kasutades mitmeid omavahel aheldumata rakutuuma geene.

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Publikatsioonid

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