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SYSTEMATIC STUDIES ON SAWFLIES OF THE GENERA *DOLERUS*, *EMPRIA*, AND *CALIROA* (HYMENOPTERA: TENTHREDINIDAE)

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PRESS

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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 Heidemaa, M. & Viitasaari, M. Taxonomy of the *Dolerus gibbosus* species group, with descriptions of *Dolerus zhelochovtsevi*, sp. nov., and males of *D. blanki* Liston, 1995, and *D. quadrinotatus* Biro, 1884, and redescription of the male of *D. gibbosus* Hartig, 1837 (Hymenoptera: Tenthredinidae) (submitted manuscript).

II Heidemaa, M., Nuorteva, M., Hantula, J. & Saarma, U., 2004. *Dolerus asper* Zaddach, 1859 and *D. brevicornis* Zaddach, 1859 (Hymenoptera: Tenthredinidae), with notes on their phylogeny. *European Journal of Entomology* (in press).

III Heidemaa, M. & Saarma, U. Phylogenetic relationships in *Dolerus gibbosus* species group and *Dolerus varispinus* complex (Hymenoptera: Tenthredinidae) as inferred from molecular and morphological data, with revisory notes on *D. varispinus* complex (manuscript).

IV Heidemaa, M. & Zinovjev, A., 2004. *Dolerus anatolii*, n. sp., the first Palaearctic member of the subgenus *Neodolerus* (Hymenoptera: Tenthredinidae). *Proceedings Of The Entomological Society Of Washington* 106, 1: 159–165.

V Heidemaa, M. &Viitasaari, M., 1999. Taxonomy of the *Empria hungarica* species-group (Hymenoptera, Tenthredinidae) in Northern Europe. *Entomologica Fennica* 10, 2: 95–101.

VI Heidemaa, M. & Prous, M., 2004. The larvae of *Empria pumila* (Konow, 1896) and *E. pumiloides* Lindqvist, 1968 (Hymenoptera: Tenthredinidae). *In*: Schmidt, S., Taeger, A. & Blank, S. M. (Eds.) *Recent Sawfly Research: Synthesis and Prospects* (in press).

VII Heidemaa, M., 1999. A new sawfly species *Caliroa crypta* sp. nov. from Northern Europe (Hymenoptera, Tenthredinidae). *Entomologica Fennica* 10, 3: 183–186.

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The author of the present dissertation claims that his personal contribution to the articles included is as follows: VII – 100%, I, III–VI – 75%, II – 50%.

INTRODUCTION

Importance of sympatric speciation in animals has been reassessed during the last decades. It is suggested that a striking terrestrial biodiversity pattern, the extraordinary richness of insect species, may be a result of sympatric speciation and that the insect phytophags and parasitoids are immensely diverse because sympatric speciation plays a particularly significant role (*e.g.* Bush & Smith, 1997; Berlocher & Feder, 2002). Mallet (2001) predicted that the years 1990–2010 will prove to be revolutionary in the study of speciation. Contemporary advances in molecular biology and bioinformatics have raised systematics to a new level, and the first results employing a genome-scale approach in phylogenetic studies have already been published (*e.g.* Rokas *et al*., 2003). Concurrently, a new excitement in taxonomy, driven partly by advances in technology and partly by newly perceived needs stemming from the biodiversity crisis, is evident (Mallet & Willmott, 2003). These developments undoubtedly promote systematic studies and will hopefully lead to a new renaissance in systematics.

Well-founded phylogenetic hypotheses are based on the basis of experimentally testable predictions about the evolution of many biological systems, from biochemical and physiological systems, to behavioural studies (McLennan *et al*., 1988). Success of the approach depends significantly on recognition of the monophyletic groups and successful differentiation of their members, as well as on confidently resolved phylogenetic relationships between them. The many levels of hypothesis testing in systematics, from characters to species to clades are essential for all evolutionary biology (Lipscomb *et al*., 2003).

Sawflies (Symphyta) are economically important insects that include major forest and horticultural pests worldwide; they also illustrate some of the major themes in basic insect ecology (Raffa & Wagner, 1993). Although being relatively poor in species on a global scale, with 8 000 species (Abe & Smith, 1991), sawflies display amazing diversity of life histories and a remarkably high proportion of monophagous species (Viitasaari, 2002). More than one-half of the known species are associated with woody plants (Benson, 1950; Lorenz & Kraus, 1957; Smith, 1979), but the proportion of the species that occurs on herbaceous plants would probably be in the majority if all their hosts were be known (Smith, 1993).

Symphyta serve as a model group in a wide variety of laboratory and field experiments carried out in ecological and evolutionary studies. The chemical defence of plants against herbivorous insects (Hanhimäki, 1993; Niemelä & Tuomi, 1993), adaptive responses of herbivores to the chemical barriers of hosts (Géri *et al*., 1993; McCullough & Wagner, 1993), effects of seasonal environment on the genetic architecture of insect herbivores (Kause *et al*., 2001; Kause & Morin, 2001), and the ecology and evolution of host use in gall forming insects (Price *et al*., 1987, 1997, 1998; Nyman, 2000) are only a few examples.

Sawflies display an unusual diversity pattern compared with most other insect groups, a reversed latitudinal gradient of species richness. Unlike most other insects, sawflies have their greatest species diversity in northern latitudes (Kouki *et al*., 1994). Their biomass is significant as a part of the food chain in

northern ecosystems; under subarctic conditions sawfly larvae consume an average of 0,5...1,1% of the leaf biomass (Bogacheva, 1977). It has been proposed that sawflies may be essentially different from lepidopterans and coleopterans in their moulting physiology. Their ability to moult quickly at low temperatures may contribute to the remarkable ecological success of the group in high-latitude environments (Matsuki & MacLean, 1990; Matsuki *et al*., 1994).

The larvae of sawflies have been identified as an important food source in farmland habitats for the chicks of several bird species known to be in decline (Barker *et al*., 1999). Potts (1970) found a significant correlation between chick survival in the Grey Partridge (*Perdix perdix*) in barley and the number of sawfly larvae. To explore these patterns in greater detail, long-term studies of dolerine sawflies in relation to farmland bird numbers are being carried out by the Game Conservancy Trust in Great Britain (Aebischer, 1991).

Despite the remarkable importance and lower species richness of sawflies in general, the taxonomy of this group is clearly less well developed, than that of most other herbivorous insects. There are many taxonomically unresolved species groups in Symphyta, and their phylogenetic relationships on the levels of species and genera are almost entirely unknown. Tenthredinidae comprise the largest family of symphytans, with over 5000 known species in about 350 genera (Viitasaari, 2002).

This dissertation is a summary of articles dealing with the systematics of some tenthredinid species groups (Hymenoptera, Tenthredindae) from the genera *Dolerus*, *Empria* and *Caliroa*. The main objectives of the dissertation are to define the *Dolerus gibbosus* species group (*s. str.*), revise its taxonomy (**I, II**), and the taxonomy of the *D. varispinus* complex, as well as to reconstruct the phylogenetic relationships between their members on the basis of cladistic analyses of molecular and morphological data (**II**, **III**). In addition, it includes descriptions of three new tenthredinid species, *Dolerus* (*Poodolerus*) *zhelochovtsevi* Heidemaa & Viitasaari (**I**), *Dolerus* (*Neodolerus*) *anatolii* Heidemaa & Zinovjev, 2004 (**IV**), and *Caliroa crypta* Heidemaa, 1999 (**VII**), as well as the descriptions of previously unknown larvae of *Empria pumila*, *E. pumiloides* (**VI**), and *Dolerus zhelochovtsevi*, and the overlooked males of *D. blanki* Liston, 1995, *D. gibbosus* Hartig, 1837, and *D. quadrinotatus* Biró, 1884 (**I**). It also renders an improved identification key for the North-European species of the *Empria hungarica* species group and provides diagnostic characters for differentiation of the imaginal and larval stages of *Empria pumila* (Konow, 1896) and *E. pumiloides* Lindqvist, 1968 (**V**, **VI**).

OVERVIEW OF THE PROBLEMS

Detailed analyses of covariation patterns between different character sets (morphological, ecological and molecular) significantly contribute to taxonomic studies of difficult species groups and sibling species, and enhance reliability of their differentiation. Precise measurements sometimes reveal bimodal characteristics, and the two modes can be correlated with additional characters (Mayr & Ashlock, 1991), indicating that separate species might be involved. Failures to segregate externally very similar species may introduce hidden variables into a study system and must be avoided in all kinds of experimental and comparative biological studies. Morphologically inseparable or only partially separable sibling species and recently diverged species (neospecies) are most problematic in this regard. According to Mayr (1963), sibling species are common in most animal groups. Insect phytophages and parasitoids include numerous abundant and widespread taxa whose status and phylogenetic relationships are unresolved, and, in this regard, sawflies are not an exception.

At present, at least 189 species of the Holarctic sawfly genus *Dolerus* Panzer are described. Of those, 120 species occur in the Palaearctic region and 75 in the Nearctic, while only 6 are recorded in both regions. The most diverse lineage of this genus in the Palaearctic region is the subgenus *Poodolerus* Zhelochovtsev, 1988 (= *nitens*-group of Goulet, 1986) with at least 55 species. The larvae of *Poodolerus* species feed on Poaceae and Cyperaceae, but the larvae of several species and their host plants are still unknown. The taxonomy of some species groups of this subgenus is controversial and phylogenetic relationships between the species are mostly unknown. The *Dolerus gibbosus* species group and the *D. varispinus* complex include some relatively common forms whose taxonomic status has remained unresolved. Several other tenthredinid genera, including *Empria* and *Caliroa*, comprise species that pose taxonomic difficulties. The problems recognized and discussed in the present dissertation are briefly introduced in the following paragraphs of this chapter. Some of them led to revisory studies of some species groups of the genus *Dolerus*; these attempt to resolve the taxonomic status and nomenclature of the members of those groups, as well as to delineate the groups in cladistic terms and to reconstruct the phylogenetic relationships between the species recognised. Others resulted in the descriptions of new tenthredinid species or previously unknown larvae or males of already known species.

1. Hartig (1837) and the following authors (*e.g.* Thomson, 1871; Konow, 1884a; Enslin, 1909, 1913; Malaise, 1932 and Berland, 1947) treated *Dolerus gibbosus* and *D. blanki* as distinct species until Zhelochovtsev (1935) proposed that they are conspecific. Afterwards, probably following Zhelochovtsev's concept, Benson (1947, 1954), Hellén (1955) and Muche (1970) regarded them also as conspecific; however, Lindqvist (1969) and Ermolenko (1975) treated *D. gibbosus* and *D. blanki* as separate species. Blank & Taeger (1992) emphasized the need for a revison of *D. planatus*, *D. gibbosus*, *D. blanki*, and *D. mega-pterus*, suggesting that *Dolerus gibbosus* sensu Zhelochovtsev (= *D. gibbosus* auct., nec Hartig) and *D. gibbosus* Hartig are separate species. In addition, they regarded

D. asper Zaddach, 1859 as a junior subjective synonym of *D. planatus* Hartig, 1837 and later included also *D. brevicornis* Zaddach, 1859 in the synonymy of *D. planatus* Hartig (Blank & Taeger, 1998). Viitasaari *et al*. (1998) also referred to taxonomic problems concerning these species groups because the authors M. Heidemaa and M. Nuorteva suggested that two differentiable morphs can be recognized among *Dolerus asper* auct., and MH noticed some morphological differences between the type material of *D. planatus* and the specimens of *D. asper* auct. Inconsistencies with determination and association of the females and males of *D. blanki*, *D. gibbosus* and *D. gibbosus* auct. by the aforementioned authors were revealed when attempts to match the species definitions proposed by different authors were made or the definitions were applied to real specimens. The male of *D. quadrinotatus* Biró has not been recognised and the only known specimen of *D. stygius* Förster, 1860 was its lectotype. The larvae and host plants of the species were mostly found to be unknown.

2. Taxonomy of the *Dolerus varispinus* complex has also remained contradictory and the forms with different colour pattern on their legs and thorax have been regarded as separate species (Kiaer, 1898; Haris, 2000), separate subspecies (Zhelochovtsev, 1988; Lacourt 1999), or have been treated as conspecific (Benson, 1956). Preliminary study revealed two morphologically differentiable forms among females and males, which have been treated as *D. liogaster* Thomson by previous authors.

3. The females of a *Dolerus* species that did not fit any species of this genus described from the Palaearctic region (Zhelochovtsev, 1928; Zhelochovtsev, 1935; Malaise, 1931; Muche, 1965; Haris, 1996; Wei, 1997; Wei & Nie 1997; Haris 2000, 2001), and is not one of the Nearctic species treated by Goulet (1986), were discovered by A. G. Zinovjev.

4. The Holarctic tenthredinid genus *Empria* with 45 species worldwide and 27 species in the Western Palaearctic region also poses taxonomic difficulties and the host plants and larvae of many species are still unknown. A nomenclatural problem was recognized when it was found that the syntypes series of *Empria pumila* (Konow) is heterogeneous, and the only specimen, labelled by Konow as *E. pumila*, was determined as *E. pumiloides* Lindqvist. Also, there was no key for determination of the North European species of the *Empria hungarica* group based on the easily accessible imaginal characters, and the larvae of *E. pumila* and *E. pumiloides* have remained unknown.

5. An interesting *Caliroa* female was collected by the author from Northern Estonia on the 18th of July 1993. Attempts to determine this specimen using available identification keys to this genus were unsuccessful. Later, one male collected in Southern Finland was found from a sawfly collection in Finland. Its penis valve structure did not match with any known *Caliroa* species and the male was considered as conspecific to the above mentioned female because they shared some unique characteristics that clearly differentiated them from all previously described *Caliroa* species.

MATERIAL AND METHODS

Collecting and studied collections

Sawfly imagos were collected mainly by sweeping net and light entomological net. Some specimens were captured using Malaise traps and yellow funnel traps.

The pinned sawfly samples of the studied taxa are from the following institutional collections:

- Department of Applied Biology (formerly Dept. of Applied Zoology), University of Helsinki, Finland;
- Deutsches Entomologisches Institut, Eberswalde, Im Leibniz-Zentrum für Agrarlandschafts- und Landnutzungsforschung, Germany;
- Département d'Entomologie, Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium;
- Finnish Museum of Natural History, Helsinki, Finland;
- Institute of Plant Protection, Estonian Agricultural University, Tartu, Estonia;
- Institute of Zoology and Botany, Estonian Agricultural University, Tartu, Estonia;
- Museum für Naturkunde der Humboldt-Universität zu Berlin, Bereich Zoologisches Museum, Berlin, Germany;
- Museum of Zoology, University of Tartu, Tartu, Estonia;
- Museum of Zoology and Entomology, Lund University, Lund, Sweden;
- Naturhistorisches Museum Wien, Vienna, Austria;
- Naturhistoriska Riksmuseet, Sektionen för Entomologi, Stockholm, Sweden;
- The Natural History Museum, London, Great Britain;
- Zoology Department of the Tromsø Museum, Norway;
- Zoological Institute in St. Petersburg, Russia;
- Zoological Museum, University of Oslo, Norway;
- Zoologische Staatssammlung München, Munich, Germany.

Specimens from the private collections of Kaupo Elberg (Tartu, Estonia), Stephan Blank (DEI), Manfred Kraus (Nürnberg, Germany), Jean Lacourt (Le Pâty, Igé, France), Jaan Luig (Tartu, Estonia), Jouko Nuorteva (Helsinki, Finland), Matti Nuorteva (Helsinki, Finland), Carsten Ritzau (Oldenburg, Germany), Andreas Taeger (DEI), Matti Viitasaari (DABUH), Veli Vikberg (Turenki, Finland), and the author were also studied.

The type material listed below was examined:

Dolerus asper megapteroides Muche, 1964 – paratype ♀ (holotype unavailable);
Dolerus carinatus Konow, 1884 – syntypes: 2 ♂♂;
Dolerus derzavini Malaise, 1931 – holotype ♀;
Dolerus docilus Benson, 1956 – holotype ♂, paratypes: 5 ♂♂ 4 ♀♀;
Dolerus gibbosus Hartig, 1837 – lectotype ♀;
Dolerus harwoodi Benson, 1947 – holotype ♂, paratypes: 2 ♀♀;
Dolerus liogaster Thomson, 1871 – syntypes: 2 ♀♀ 2 ♂♂;
Dolerus megapterus Cameron, 1881 – holotype ♀;
Dolerus oblongus Cameron, 1882 – holotype ♀;

Dolerus planatus Hartig, 1837 – lectotype ♂, paralectotype ♂;
Dolerus rugosus Konow, 1884 – syntype ♂;
Dolerus soniensis Dubois, 1920 – holotype ♀;
Dolerus stygius Förster, 1860 – lectotype ♀;
Dolerus schmidti Konow, 1884 – holotype ♀;
Dolerus schneideri Kiaer, 1898 – syntypes: 3 ♀♀;
Dolerus thoracicus (Fallén, 1808) – syntype ♀;
Dolerus thoracicus Klug, 1818 – syntypes: 4 ♀♀;
Dolerus varispinus Hartig, 1837 – lectotype ♂, paralectotypes: 2 ♀♀;
Empria pumila (Konow, 1896) – syntypes: 1 ♀ 2 ♂♂;
Empria pumiloides Lindqvist, 1968 – holotype ♀, paratype ♂;
Empria tricornis Lindqvist, 1968 – holotype ♂, paratype ♀.

Rearing of larvae

Ex ovo rearing was used for obtaining the previously unknown larvae of known sawfly species because it enables comparisons of different larval stages of the studied species and determination of their host plant spectrum. Rearing of *Dolerus spp*. (**I**, **II**) was carried out indoors in 2001, 2002 and of *Empria pumila* and *E. pumiloides* (**VI**) in 2003 and 2004. Species' host plants with more or less intact roots were taken from the collecting sites of the imagos and planted in plastic flowerpots filled with soil. To ensure that the plants used in *ex ovo* rearing were without previously laid *Dolerus* eggs, some sods of sprouting *Carex* plants were isolated with wooden frames covered with plastic net before the first *Dolerus* adults appeared. *Ex ovo* rearing was carried out as suggested by Finnish sawfly researchers (*e.g.* Kontuniemi, 1951; Viitasaari, 2002; Vikberg, pers. comm.). The planted host was enclosed in a transparent plastic bag that was attached bottom up to the upper margin of the flowerpot using a rubber band. Numerous small holes about 2 mm in diameter were punched in the bag for ventilation. One upper corner of the cover bag was removed, folded, and fastened with a paper clip, enabling release and feeding of the sawfly females without having to remove the bag. The cover bag maintained the required illumination and humidity level, prevented escape of the specimens, and enabled monitoring of the ovipositing females. The plants with sawflies were kept inside the bags and placed at a north-facing window on a balcony (to avoid direct sunlight) at a temperature 2–3 degrees higher than outdoors. The sawflies were fed in separate Petri dishes using a piece of cotton saturated with a light sugar solution. Newly hatched first stage larvae were soon put in Petri dishes together with pieces of the host plant leaves or were transferred there using a soft watercolour brush. Small bundles of the host leaves, wrapped in a piece of moist tissue at their petioles, were used as food for the larvae and were replaced daily. All larval skins and some samples of fully-grown larvae were stored in ethanol. When the larvae stopped feeding and started to crawl around actively, a layer of clean sterilized moist sand was placed in the bottom of dishes. The dishes with larvae were taken to a basement for overwintering and in late February the pupae were taken step by step up to room temperature and kept in stable conditions until the imagos emerged.

Morphological study

Morphological terminology follows Wong (1963), Goulet (1986), and Viitasaari (2002). The genitalia were processed in KOH (10%) prior to their dissection and study. Ovipositors and penis valves were afterwards mounted in Canada balsam or euparal between rectangular cover slips, or glued on pieces of paper and pinned with the corresponding specimens.

The dissected larvae (**I**, **VI**) were decapitated, their trunks were cut lengthwise along the ventral side (between the legs) with a small scalpel and the intestines removed. The cuticles and heads of the larvae were treated in hot KOH during 3–4 minutes and carefully washed in distilled water. Afterwards, the cuticles were spread, mounted in glycerol or euparal, covered with cover slips, and studied under a microscope using a magnification of 100–400 times. Mandibles, maxillae, labrum, and labium of the dissected larvae were separated from the head capsule. The mandibles were glued on pieces of paper and other parts were mounted in euparal. The frons was separated from the larval skin, and its setae were counted. Glandubae and setae on the annulets of each thoracic and abdominal segment were counted and their form and arrangement were also studied.

Digital micrographs of the microscopic slides were taken with an Olympus BX50 System Microscope and Olympus DP11 camera while those photographed on film (Kodakchrome 25/36) were produced with a Canon AV-1 camera using a Nikon 1.25 microscope (with object-glass: Ph3DL Eplan 40/0.65 160/0.17). Micrographs of larger (3-dimensional) objects were taken with an Olympus stereomicroscope SZX9 and a digital camera C-4040ZOOM. Some line drawings were made from printed micrographs (**IV**), prepared from digital images applying special graphics filters (**I**), or using a drawing device attached to a microscope (**V**).

Illustrations of the heads of larvae in frontal view (**VI**) were prepared using the extended focal imaging (EFI) technique (called also extended depth of focus imaging – EDFI): from a set of several images taken along the z axis, where each separate image had only some parts in focus, a single composite digital image with all parts in focus was created using the freeware program CombineZ (version 4.1) compiled by Alan Hadley.

For SEM study (**I**, **II**) the heads and mandibles of the larvae and male genitalia of the species were treated with KOH, washed in distilled water, mounted on double-carbon tape on a metal block and, after drying, were covered with a thin film of gold. The scanning electron micrographs were taken in digital format with a JEOL 840 attached to a PC with the computer programme SEMAPHORE (version 1.2).

The metrical characters of the adults were measured with a measuring scale using a Wild M8 stereomicroscope (**II**, **V**) or an Olympus SZX-9 stereomicroscope (**I**, **IV**). The measuring units were 0.01 mm for the penis valve and 0.015 or 0.02 mm for other characters. To exclude the possibility that differences found between the compred groups were due to systematic measurement error, the specimens of the compared groups were not measured in groups, one group fol-

lowing after another, but were measured alternately; a specimen of one group was followed by a specimen of another group.

Molecular study

mtDNA (COI, COII, and *cyt*b) and nuclear (ITS2) sequence characters were analysed in order to resolve the taxonomy of the *Dolerus gibbosus* species group and the *D. varispinus* complex and to reconstruct the phylogenetic relationships between their members (**II**, **III**). The random amplified microsatellite (RAMS) analysis for discrimination between *Dolerus asper* and *D. brevicornis* (**II**) was conducted and interpreted by Jarkko Hantula (Finnish Forest Research Institute, Vantaa Research Center, Vantaa, Finland).

DNA for sequencing was isolated mainly from freshly frozen or ethanol preserved samples, but in some cases, air-dried specimens were used. Sequences of the mitochondrial gene fragments of cytochrome *b* (*cyt*b), cytochrome oxidase subunit I (COI), cytochrome oxidase subunit II (COII) and of the DNA internal transcribed spacer (ITS2) fragments in 2–5 specimens of 12 studied *Dolerus* species were determined for taxonomic and phylogenetic study. If possible, the sequences of different genomic markers were obtained from the same specimen of a species but in some cases different markers from different specimens of the same species were used.

DNA was extracted and purified with the QIAamp DNA Mini kit (Qiagen) according to the manufacturers protocol and stored at –20°C until required. The mitochondrial gene fragments were: *cyt*b, amplified with the primers CB-J-10933 (5′ TAT GTA CTA CCA TGA GGA CAA ATA TC 3′) and TS1-N-11683 (5′ TAT TTC TTT ATT ATG TTT TCA AAA C 3′); COI, amplified with the primers C1-J-1751 (5′ GGA TCA CCT GAT ATA GCA TTC CC 3′) and C1-N-2191 (5′ CCA GGT AAA ATT AAA ATA TAA ACT TC 3′); COII, amplified the primers TL2-J-3037 (5′ATG GCA GAA AAA TGC AAT GG 3′) and C2-N-3661 with 3′-end base omitted (5′ CCA CAA ATT TCT GAA CAT TGA CC 3′) (Simon *et al*., 1994). Of the nuclear DNA, the sequence of a ITS2 fragment was amplified using the modified AM1 primer with 2 bases of its 3′ end omitted (5′ TGT GAA CTG CAG GAC ACA TGA 3′), and AM2 (5′ ATG CTT AAA TTT AGG GGG TAG TC 3′) (Marinucci *et al*., 1999).

PCR reactions were carried out in a total volume of 20 µl containing 4...100 ng of genomic DNA, 4 pmol of primers, 1.5 mM $MgCl_2$, 0.2 mM dNTP mixture, PCR buffer and 1U of Platinum Taq DNA polymerase (Life Technologies). PCR was performed on Progene Thermal Cycler (Techne), cycling parameters were 5 min denaturing step at 94°C, followed by 35–41 cycles of 1 min at 94°C, 1 min at 46...50°C depending on the primer set used and 1 min at 72°C. PCR product was purified with shrimp alkaline phosphatase/exonuclease I treatment. 1U of both enzymes (USB) were added to 10µl of PCR reaction and incubated for 30 min at 37°C, followed by inactivation of enzymes 15 min at 80°C. The purified PCR product was used directly for sequencing. DNA cycle sequencing was performed

by using DYEnamic ET Terminator Cycle Sequencing kit (Amersham Biosciences). 33 cycles (15 sec at 95°C, 15 sec at 50°C and 60 sec at 60°C) were performed on a Progene Thermal Cycler in a total volume of 10µl. To obtain unequivocal sequences, both sense and antisense strands were sequenced using the same primers as for PCR amplification. Sequences were resolved on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems). Based on the sequences of both strands, a consensus sequence of each marker was created for every specimen. DNA isolation and sequencing were performed by Urmas Saarma (Institute of Zoology and Hydrobiology, University of Tartu, Estonia).

Analysis of taxonomic characters

Variation of most morphological (structural) characters was assessed visually, without digital image analysis, because this was usually sufficient for segregation of the discrete groups of specimens and their unambiguous association with the type specimens (**I**, **V**). In some cases the covariation patterns between structural and metrical characters (**II**, **V**), structural and molecular (**II**, **III**) as well as between structural characters and phenology (**II**, **V**) were explored in order to reveal concordant variation patterns that could help to recognise and delineate possible sibling species, to classify the type specimens, or to verify if the females and males of some externally very similar species are correctly associated with. Scatter diagrams (x–y) were used to visualize variation of the samples on the character plane and also the results of ordination. Ordination of the analysed samples was carried out using nonmetric multidimensional scaling and employing a similarity matrix computed from DNA sequence data (**II**, **III**). The computations were performed and the results visualised in STATISTICA, version 6 (StatSoft Inc., 2001).

Phylogenetic analysis

If possible, the molecular sequences of a species were obtained from at least 2–3 specimens originating from geographically isolated localities. Mitochondrial as well as nuclear gene fragments were sequenced and used for phylogeny reconstruction.

Morphological (structural) characters of the imaginal stage (Table 2, **II**; Table 3, **III**) and the sequences of mitochondrial (*cyt*b, COI, COII) and nuclear (ITS2) DNA regions (variable and/or parsimony informative sites in Table 1, **II**; Table 1–2, **III**) were used for phylogenetic inference (**II**, **III**). The sequences of the ITS2 fragment were aligned with multiple alignment procedure in CLUSTAL W, version 1.4 (Thompson *et al*., 1994) that was run in the software package BIOEDIT, version 5.0.9 (Hall, 1999), used for sequence editing.

A chi-squared test of homogeneity of base frequencies (for confirmation that there were no significant differences among studied taxa) was performed using

PAUP* 4.0. The homogeneity of the phylogenetic signal from different data partitions was assessed by conducting a series of incongruence length difference tests (Farris *et al*., 1994) using 500 replicates for each analysis as implemented in PAUP*, with invariant characters excluded (Cunningham, 1997). To detect if the base composition differs significantly in different parts of trees, the nucleotide data were analysed in the PC programme STATIO (Rzhetsky & Nei, 1995) (**III**).

The extent of substitutional saturation for different markers was evaluated using an entropy-based index of substitution saturation (Xia *et al*., 2003) and the saturation diagrams, both implemented in the PC programme DAMBE, version 4.2.13 (Xia & Xie, 2001). For testing hypotheses of the molecular clock and checking for the proportionality of the relative rates of nucleotide substitution for different markers, the hierarchical likelihood ratio (hLRT) tests were carried out in the PC version of HYPHY (.96beta) (Pond, 2001) (**III**).

Sequence data were analysed using unweighted maximum parsimony (MP) and minimum evolution (ME) methods as implemented in the MEGA software package, version 2.1 (Kumar *et al*., 2001) (**II**). Phylogenetic analyses of sequence data under the maximum likelihood criterion (ML) and of morphological data were conducted using different methods in PAUP*, PC versions 4.0 β8 / 4.0 β10 (Swofford, 2002). The combined analyses of morphological and all available molecular data (**III**) were performed in MRBAYES, version 3.0 β4 (Ronquist & Huelsenbeck, 2003), which implements a Merropolis Coupled Markov Chain Monte Carlo method for sampling trees according to their probability, given the data, priors, and the substitution model (Huelsenbeck & Ronquist, 2001). The programme allows the specification of different substitution models for separate data partitions and the unlinking of the model parameters across the partitions, if necessary. For each analysis, five separate sessions starting from different randomly chosen topologies were run to ensure that stationarity had been reached and that the chains provided valid samples from the posterior probability distribution of the estimated parameters (Huelsenbeck *et al*., 2002). Phylogenetic genetic inference based on molecular data was carried out using the following phylogenetic reconstruction methods: the best-fit model under maximum likelihood, equally weighted (unweighted) parsimony, and the minimum evolution (ME). The sequences of the different gene fragments (data partitions) were analysed separately (using ME, ML, and MP analyses), as well as in combination (using ML, Bayesian analysis) but considering the results of the incongruence length difference (ILD) test (partition-homogeneity test) before combining the data for MP analysis (significantly incongruent partitions were not usually analysed using the MP). The Akaike information criterion (AIC) implemented in software programmes MODELTEST, version 3.06 (includes 56 different nucleotide substitution models) (Posada & Crandall, 1998; Posada, 2001, 2003) and HYPHY (enables evaluation of all possible 203 substitution models) were used to identify the simplest (best-fit) models of sequence evolution for phylogenetic reconstruction to which the addition of parameters does not result in significant improvement. If the best-fit sequence evolution models found for separate partitions were different, the different models were specified for the corresponding partitions, and the pylogenetic analyses were conducted using MRBAYES (**III**).

The cladograms were viewed and manipulated in MEGA 2.1 and TREEVIEW, version 1.6 (Page, 1996). Support for nodes on the likelihood and parsimony trees were evaluated by nonparametric bootstrapping (Felsenstein, 1985) with 500 (ML) or 1000 / 10 000 (MP) pseudoreplicates and NNI branch swapping (ML) or TBR swapping (MP) (**II**, **III**). Bremer support indices (Bremer, 1988) for the datasets analysed by parsimony were calculated in AUTODECAY, version 5.0 (Eriksson, 2001) using 100 random addition replicates per constraint and TBR branch swapping (**III**).

Monophyly testing was performed using trees from the Bayesian and parsimony analyses. Constrained tree for the monophyly of the proposed species group, having only one resolved internode branch (that between the group and the other taxa included) was created and the proportion of post-stationarity trees from Bayesian analyses, compatible with the corresponding constrained tree, was calculated (Lewis, 2001). This number is the posterior probablity of the hypothesis represented by the constrained tree. Length difference between most parsimonious constrained and unconstrained trees was evaluated using the Wilcoxon signed-rank tests.

RESULTS AND DISCUSSION

Taxonomic and nomenclatural results

Descriptions of new species and previously unknown forms. — Descriptions of three new tenthredinid species, as well as two previously unknown males and larvae of some species are given. The three new species described are: *Caliroa cryptа* Heidemaa (**VII**), *Dolerus* (*Neodolerus*) *anatolii* Heidemaa & Zinovjev (**IV**), and *Dolerus zhelochovtsevi* Heidemaa & Viitasaari (**I**). The males of *Dolerus blanki* Liston and *D. quadrinotatus* are described for the first time, and the male of *Dolerus gibbosus* Hartig is re-described (**I**). The previously unknown larvae of *Dolerus zhelochovtsevi* (**I**), *Empria pumila* and *E. pumiloides* (**VI**), obtained by *ex ovo* rearing, are also described and illustrated.

Designated neotypes and lectotypes. — In revisory studies the neotypes of *Dolerus asper* Zaddach, 1859 and *D. brevicornis* Zaddach, 1859 were designated (**II**). The lectotypes of *Dolerus carinatus* Konow, 1884, *D. liogaster* Thomson, 1871, *D. rugosus* Konow, 1884 (Konow, 1884b), *D. schneideri* Kiaer, 1898, *D. thoracicus* Fallén, 1808, *D. thoracicus* Klug, 1818, and *Empria pumila* (Konow, 1896) were also designated (**I**, **V**).

Dolerus gibbosus species group (**I**, **II**). — *Dolerus gibbosus* species group (*s. str*) is defined on the basis of the synapomorphic ventroapical hooklike process of the penis valve in its members. At present, 7 species of this species group are known: *Dolerus asper* Zaddach, *D. brevicornis* Zaddach, *D. blanki* Liston, *D. docilus* Benson, *D. gibbosus* Hartig, *D. harwoodi* Benson, and *D. stygius* Förster.

Concordant differences in morphology, phenology, and RAMS markers, as well as in sequenced mtDNA (COI, COII, *cyt*b) and nuclear DNA (ITS2) fragments, indicated that *Dolerus asper* Zaddach, 1859 and *Dolerus brevicornis* Zaddach, 1859 are valid species. Because the nomenclatural types of these species are lost and their original descriptions or any other reference information do not ensure unambiguous usage of these names in the present taxonomic situation, the neotypes of *Dolerus asper* Zaddach, 1859 and *Dolerus brevicornis* Zaddach, 1859 are designated for the sake of nomenclatural stability. Because the basal serrulae of the lancet and the outline of the mesepisternum of *Dolerus derzavini* Malaise holotype in frontal view fall outside the range of variation of these characters in *Dolerus asper* and *D. brevicornis*, *D. derzavini* is regarded as valid species (*spec. rev.*) (**II**).

Comparison of the morphological characters of the lectotypes of *Dolerus planatus* Hartig, 1837 and *D. gibbosus* Hartig, 1837 revealed that they represent different sexes of the same species. For taxonomic reasons, *Dolerus gibbosus* Hartig is establised as the valid name of this species while *D. planatus* Hartig is regarded as its junior subjective synonym (**I**). The association of the females and males of *D. gibbosus* and their distinctness from the resembling species is also supported by comparative analysis of the sequenced gene fragments (**II**).

The following new subjective synonyms are established: *Dolerus planatus* Hartig, 1837, *syn. nov.* of *Dolerus gibbosus* Hartig, 1837; *Dolerus megapterus* Cameron, 1881, *Dolerus carinatus* Konow, 1884, and *Dolerus crassus* Konow, 1884 are all *syn. nov.* of *Dolerus stygius* Förster, 1860. The synonymies of *Dolerus gibbosus* auct., nec Hartig, 1837 to *D. stygius* Förster, 1860, *D. docilus* Benson, 1956 to *D. gibbosus* Hartig, *Dolerus asper* Zaddach, 1859 and *D. brevicornis* Zaddach, 1859 to *Dolerus planatus* Hartig, 1837, and *Dolerus derzavini* Malaise, 1931 to *D. asper* Zaddach, 1859 are abandoned. *Dolerus carbonarius* Zaddach, 1859 and *Dolerus fumosus* Zaddach, 1859, usually treated as conspecific to *Dolerus asper* Zaddach, are considered as *species inquirendae* because their type material is not available and the original descriptions do not allow the determination of their status in the present taxonomic situation.

Dolerus varispinus complex (**III**). — Concordant variation patterns between structural and metrical characteristics, sequenced DNA markers, and phenology (flight periods) of imagos provide evidence that *D. varispinus* complex includes three separate species: *Dolerus liogaster* Thomson, *D. schmidti* Konow, and *D. varispinus* Hartig. Thus, *Dolerus schmidti* Konow, 1884 is removed from the synonymy of *D. liogaster* Thomson, 1871 and regarded as a valid species (*spec. rev.*) Analyses of morphological and sequence data revealed no basis for differentiation of *D. schneideri* from *D. liogaster* and the synonymy already proposed by Lindqvist (1943) is sustained. The following new synonyms are established: *Dolerus rugosus* Konow, 1884 and *Dolerus rugosulus* Dalla Torre, 1894 are junior synonyms (*syn. nov.*) of *Dolerus varispinus* Hartig, 1837 and *Dolerus soniensis* Dubois, 1920, is a new synonym (*syn. nov.*) of *Dolerus schmidti* Konow, 1884. The lectotypes of *Dolerus liogaster* Thomson, 1871, *Dolerus rugosus* Konow, 1884 and *Dolerus schneideri* Kiaer, 1898 are designated. As long as the type specimen of *Dolerus annulatus* R. R. von Stein, 1896 cannot be found, this species has to be considered as *species inquirenda* because the characteristics given in its original description do not enable the determination of its taxonomic identity in present situation.

Dolerus (*Neodolerus*) *anatolii* Heidemaa & Zinovjev (**IV**). — This is the first species of the subgenus *Neodolerus* Goulet recorded from outside the Nearctic region. Within the subgenus *Neodolerus*, the species is closest to *D. parasericeus* MacGillivray, 1908; however, it differs by the mostly separated punctures behind the compound eyes, with only some in the upper third (just behind the eyes) fused, the mesoscutellar appendage transversely concave, outer surface of the metacoxa with numerous punctures, and the lamnium with 16 segments. It is noteworthy that characteristic features of *Neodolerus* occur in some species of the mostly Palaearctic subgenus *Poodolerus*, for example, in *Dolerus vulneratus* Mocsáry, 1878. This species has two characters in common with *Neodolerus* species: the distinctly outlined furrow on the outer surface of the metatibia and the large flat triangular surface at the posterior angle of the median lobe. On the other hand, the penis valve of *D. vulneratus* is very different from all *Neodolerus* species and resembles species of the *Dolerus alpinus* and *D. affinis* groups.

Further study incorporating DNA data should shed more light on the phylogenetic relationships between *Neodolerus* and *Poodolerus*.

Empria hungarica species group (**V**, **VI**). — A study of the syntypes of *Empria pumila* (Konow) revealed that two species were involved and the only specimen, labelled by Konow as *E. pumila,* actually belonged to *E. pumiloides* Lindqvist. The earlier proposed synonymy of *E. tricornis* Lindqvist and *E. pumiloides* by Viitasaari (1981) was supported. Because the head of the female was not the same as in typical *E. pumila*, the specimen was not selected as lectotype, but a male with penis valve structure and other characteristics matching well with the interpretation of *E. pumila* by later authors (*e.g.* Lindqvist, 1968; Lacourt, 1988; Zhelochovtsev, 1988) was designated. A study of the larvae of *E. pumila* and *E. pumiloides* revealed that they are differentiable from each other by several morphological features, of which some are visible even to the naked eye. It was found also that the imagos of those species could be determined using external characteristics, and an identification key for their differentiation was prepared. The *Empria* species with a paired whitish patch on tergum 1 were regarded tentatively as a species group termed the *Empria hungarica* group, but a further phylogenetic study is needed to validate its monophyly.

Caliroa crypta Heidemaa (**VII**). — Original description of this species was based on holotype female collected from Estonia and a paratype male found from Finland. Compared with other European species of *Caliroa*, the species exhibits some resemblance with *C. tremulae* Chevin, but it is smaller, has a petiolate anal cell in the hind wing, less shining temples with rough punctures, and a distinct sculptured groove behind the compound eye. The mesopleuron is smooth and shining, with sparse but distinct punctures on its surface, resembling *C. cerasi* (L.) in this respect. The punctures are robust on the pronotum, mesonotum, and frontal area.

Lacourt (2002) has studied the holotype of *Caliroa crypta* and regarded it as a valid species in his taxonomic revision of Western Palaearctic *Caliroa*; however, no additional records of the species have been found so far and its larva and host plant are still unknown.

Phylogenetic results

Dolerus gibbosus species group (**II, III**). — Fragments of the mtDNA genes (*cyt*b, COI, and COII) and of nuclear DNA (ITS2) from 2–3 specimens of each species were PCR-amplified and both strands were sequenced. The sequences were aligned, trimmed, and the resulting sequences with 517 bp of *cyt*b, 272 bp of COI, 356 bp of COII and 479 bp of ITS2 were used for phylogenetic reconstruction. The number of variable and parsimony informative sites (in parentheses) of the analysed gene fragments was the following: 17 (15) for *cyt*b, 7 (6) for COI, 7 (7) for COII, and 45 (40) for ITS2. Altogether 76 variable and 68 parsimony

informative sites were found. The consensus trees from the unweighted MP analyses of ITS2 alone and the combinations of COI + ITS2 and COII + ITS2 have the ingroup topology (*D. asper*, (*D. brevicornis*, *D. gibbosus*)). The consensus tree of the 12 most parsimonious trees with a length of 61 is given in Fig. 15A (**II**). The same ingroup topology was produced if the ME criterion was used and both transitional and transversional substitutions were included. However, the ingroup topology (*D. brevicornis*, (*D. asper*, *D. gibbosus*)) emerged from the ME analysis of COI + ITS2 and COII + ITS2 if only the transitions were considered. Furthermore, all consensus trees inferred from the unweighted MP and NJ analyses of all other possible combinations of the sequenced markers resulted in the topology (*D. brevicornis*, (*D. asper*, *D. gibbosus*)). The MP analysis, based on all four markers combined (1624 bp), yielded three most parsimonious trees with a length of 91. The resulting consensus tree, with the 75% cut-off value and the bootstrap supports indicated, is given in Fig. 15B (**II**). Briefly, the phylogenetic analyses most of the markers and their combinations resulted in the ingroup topology (*D. brevicornis*, (*D. asper*, *D. gibbosus*)), with high bootstrap support for the clade *asper* + *gibbosus*, while only a few resulted in the topology (*D. asper*, (*D. brevicornis*, *D. gibbosus*)). In the latter, the clade *brevicornis* + *gibbosus* had a low bootstrap support value (Fig. 15A, **II**).

An extended data set, based on all known members of this species group, was obtained later and analysed in a separate study (**III**). The fragments of the mtDNA genes (cytb, COI and COII) from 2–3 specimens of each species were used. The resulting sequences with 518 bp of *cyt*b, 272 bp of COI, 356 bp of COII, and 487 bp of ITS2 were used. The number of variable and parsimony informative sites (in parentheses) of the analysed DNA fragments was the following: 74 (22) for *cyt*b, 25 (9) for COI, 24 (8) for COII, and 96 (51) for ITS2.

Because the amplification of the *cyt*b fragment from *D. docilus* specimens was not successful, phylogenetic reconstruction was carried out using two separate datasets. The first included the sequences from the COI, COII, and the *cyt*b fragments of the mtDNA, and from the ITS2 region for 2–3 specimens of each species, however, with *Dolerus docilus* excluded. The second data set contained only the sequences of COI and COII (one set per species), but included all known members of the *gibbosus*–group (including *D. docilus*) and also a data set of imaginal morphology.

Although significant incongruence between the gene partitions was revealed by the partition homogeneity test (ILD test), the results are disputable, as it has been demonstrated that the test can yield significant values indicating incompatibility due to a length difference between the analysed sequences (Dowton & Austin, 2002), or due to their different substitution rates, even if they are not actually in conflict (Dolphin *et al*., 2000). Still, the sequence data were preferably combined for MP analysis only if they passed the ILD test. Hence, only *cyt*b and COII were used for combined analysis under the MP criterion while the COI and ITS2 fragments were initially excluded from analysis. The MP analysis of COII + *cyt*b yielded in the single most parsimonious tree of a length 108 (CI=0.94, RI=0.84) but the clade *brevicornis* + *harwoodi* + *stygius* remained unresolved. A 50% majority rule consensus tree from 1000 bootstrap preudoreplicates, using the

tree weights resulted in the same topology (Fig. 7, **III**). The ML analysis with the best-fit model (TIM+I), selected for all mtDNA combined, resulted in a compatible topology (lnL= -2247.27) but with the clade *brevicornis* + *harwoodi* + *stygius* resolved: ((*D. brevicornis*, *D. harwoodi*), *D. stygius*). If the model selection was carried out separately for each marker, it appeared that three of the four selected best-fit models were of different types (TrN+I for COI and COII, K81uf+Γ for *cyt*b, and TVM+Γ for ITS2). Because this result also indicated a significant heterogeneity between different markers, the Bayesian analysis, employing the corresponding best-fit models (MODELTEST, AIC), was performed in MRBAYES, with the shared model parameters for the partitions (*e.g.* "shape", "revmat", "pinvar") unlinked. The resulting topology (Fig. 9, **III**) was identical to that estimated from the combined mdDNA (under the TIM+I model), and all posterior probability values for the clades were between 0.90–1.00. A combined MP analysis of the morphological data (with *D. docilus* included) and the parsimony informative sites of the COII fragment resulted in three equally parsimonious MP trees with a length of 116 steps (CI=0.84, RI=0.61). A 50% majority rule consensus tree from 1000 pseudoreplicates, generated by nonparametric bootstrapping and using the tree weights, yielded the topology (*D. asper*, (*D. gibbosus*, *D. docilus*) (Fig. 8, **III**). At first COI was excluded because of a significant incongruence revealed in the ILD test ($P < 0.02$). The hypotheses of the molecular clock were rejected ($P<0.001$) for all four markers ("global", "local", and "allroots" tests, implemented in HYPHY were used). Finally, all four markers were analysed in combination under the MP criterion. One most parsimonious tree (L=313, CI=0.75, RI=0.67) was identical to the ML and Bayesian trees (Fig. 9, **III**).

Because the *D. gibbosus* species group (*s. str.*) can be defined in cladistic terms, on the basis of a synapomorphic hooklike process in the ventroapical part of the penis valve in its members (**I**), its monophyly was tested using the Bayesian as well as the MP analysis, with all available DNA data combined. The results from the post-stationarity trees, obtained from 5 independent runs (altogether 40 000 trees), resulted in the mean value of 0.999, which means that the monopyhyly of the *D. gibbosus* species is supported with 99.9% probability. Also, under the MP criterion, the constrained tree (with ingroup non-monophy) was significantly longer (305 steps) than the unconstrained tree (296) ($P < 0.049$, Wilcoxon signed-rank test). Because of significant data heterogeneity between the partitions, the results of the Bayesian analysis should be more consistent than those of the MP analysis. However, as *Dolerus docilus* was not included in the analyses of the complete data set (*cyt*b and ITS2 sequences for this species are yet unavailable), the results should still be considered preliminary.

The phylogenetic reconstructions, based on the suboptimal (too simplistic) models of sequence evolution (*e.g.* JC69), placed the clades containing *D. blanki* and *D. stygius* at the root of the tree. The possible reason for this could be the long-branch attraction that probably affects the placement of the short internal node containing *D. blanki*, *D. brevicornis*, *D. harwoodi*, and *D. stygius*. According to the cladistic analysis of the DNA sequence data and morphological characters, *D. asper* and *D. brevicornis* are not sister species, although they are externally most

similar; however sister species are *D. asper* and *D. gibbosus*. Thus, the similarity of *D. asper* and *D. brevicornis* might be secondary, caused by convergent evolution.

Dolerus varispinus complex (**III**). — Of the 307 bp for COI, 80 (26%) were variable and 18 (6%) were parsimony informative, for COII, 36 (8%) of the 462 bp were variable and 16 (3.5%) were parsimony informative, and for ITS2, 45 (12%) of the 384 bp were variable and 8 (2%) were parsimony informative. Partition homogeneity test for the combination of the COII and ITS2 sequences indicated insignificant incongruence (P=0.3124) between the two partitions. However, if one or both of these markers were combined with COI, the ILD test produced a significant values ($P < 0.001$) rejecting the congruence. The parsimony analysis of the COII sequences resulted in the single most parsimonious tree of 38 steps (CI=0.88, RI=0.87). The bootstrap support values obtained from 1000 pseudoreplicates were 100% for the clade *varispinus* + *schmidti* + *liogaster* and 92% for the clade (*schmidti* + *liogaster*). The same topology resulted also from the MP analysis of COI. The MP analysis of ITS2 region resulted in 5 most parsimonious trees (L=131, CI=0.93, RI=0.91) of different ingroup topology: ((*D. varispinus*, *D. schmidti*), *D. liogaster*), but with much lower bootstrap support values for the clade *varispinus* + *schmidti* (71%), whereas the bootstrap value for the clade *varispinus* + *schmidti* + *liogaster* remained still high (97%). Under the minimum evolution (ME) criterion, the same topologies with slightly different bootstrap support values were obtained. Two-gene combined data sets under the MP and ME criteria mostly supported the clade *schmidti* + *liogaster* with the bootstrap support values ranging from 61% to 95%. The combination of CO1 with ITS2 did not resolve the clade *liogaster* + *schmidti* + *varispinus*, while under ME it yielded the ingroup topology ((*D. varispinus*, *D. schmidti*), *D. liogaster*); however, the bootstrap value for the clade *schmidti* + *varispinus* was rather low (50%).

The combined analyses of all three genes in combination, with the use of different optimality criteria, produced trees supporting the clade *liogaster* + *schmidti*, with the bootstrap values ranging from 79% (ME) to 92% (MP and ML). The models of sequence evolution, used in the maximum likelihood (ML) analyses were GTR+I+Γ (a general time reversible with a proportion of invariable sites and with gamma-distributed rates) and TrN+I (with the estimated proportion of invariable sites I=0.85; selected in MODELTEST on the basis of the AIC criterion). Because the ILD test indicated a significant incongruence ($P<0.001$), when COI was combined with *cyt*b and/or ITS2, the combined analysis of these markers was also carried out in MRBAYES under the best-fit models for the each partition. The analysis resulted in the tree with identical ingroup topology, with the posterior probability for the clade *varispinus* + *schmidti* + *liogaster* 1.00 and for the clade *schmidti* + *liogaster*, 0.96. When the model selection for all three markers was performed separately, the TVM model was selected for COI, and the TrN+I model was selected for both COII and ITS2. Because the fragments of COII and ITS2 were successfully sequenced for 2–3 specimens, the ILD test revealed no significant incongruence between the two gene regions (P=0.537), and the same best-fit model (TrN+I) was selected for both MP and

ML, analyses of the combined data were also carried out. However, as the relative rates test rejected the null hypothesis assuming that the rates for one data set were proportional to the rates for another (constrained model lnL= - 2146.705 vs unconstrained model lnL= - 2077.21, - 2(likelihood ratio)=138.995; constrained parameters: 27, P<0.0001), the Bayesian analysis, with the model parameters unlinked (estimated independently for separate partitions), was also performed. In all cases the specimens of *D. puncticollis* (samples *pun1* and *pun2*) formed the outgroup. The MP analysis resulted in one most parsimonious tree with a length of 182 steps (Fig. 6A, **III**), however, the nonparametric bootstrap and Bremer support values for the clade *liogaster* + *schmidti* were relatively low (60% and 2, respectively). The Bayesian analysis (TrN+I model with parameters unlinked) resulted in a similar ingroup topology (Fig. 6B, **III**), with high posterior probablity for the clade *liogaster* + *schmidti* (0.96). For all tree markers, the hypotheses of the molecular clock were rejected (P<0.001).

It can be concluded that the sequences of the mitochondrial genes (*cyt*b, COI, and COII) and of the nuclear ITS2 region, especially the combination of *cyt*b and COII, contain a significant phylogenetic signal for reconstruction of the phylogenetic relationships between closely related species in some species groups of the genus *Dolerus*, which can also resolve the taxonomic problems (**II**, **III**).

Systematists generally agree that multiple specimens should be included at the level below the taxonomic rank of interest, and this should also apply to the species level (Funk & Omland, 2003). Mitochondrial genes are viewed as advantageous for pylogenetic analysis because they are generally easier to amplify than nuclear genes, they lack non-coding regions (introns), and are clonally inherited (through the maternal lineage) and non-recombining, rendering recombination, paralogy, and heterozygosity less problematic for phylogenetic analysis. Also, mitochondrial genes are generally thought to evolve at higher rates than nuclear protein-coding genes, which is especially useful in studies of recently diverged and closely related taxa (Lin & Danforth, 2004).

New host plants and distribution data

According to field observations and *ex ovo* rearing, the previously unknown host plants of *Dolerus blanki*, *D. brevicornis*, *D. gibbosus,* and *D. zhelochovtsevi*, and *Empria pumiloides* are recorded (**I**, **II**, **VI**).

Dolerus asper and *D. brevicornis* are common species in the Palearctic region. The current distribution of the less common *D. gibbosus* seems incomplete, but its distribution is different from that of the two former species, as it occurs in Estonia but is absent from Finland (Viitasaari *et al*., 1998). Based on the studied collections, it seems that *D. asper* is more common in Lapland than *D. brevicornis*. The Nearctic material of *D. asper* and the holotype female of *Dolerus tectus* MacGillivray, 1914 were not studied, but the illustration of the penis valve of *D. asper* in Goulet (1986: fig. 170a) indicates that *D. asper* occurs

in the Nearctic. Study of the North American specimens is required to confirm the occurrence of *D. brevicornis* in the Nearctic.

Until now, the subgenus *Neodolerus* was considered endemic to North America. *Dolerus anatolii* is the first species of the subgenus *Neodolerus* Goulet recorded from outside of the Nearctic, in the Palaearctic region (Russian Far East and South Korea). Thus, the range of this subgenus is not restricted to the Nearctic region, as was regarded earlier, but is Holarctic (**IV**).

Empria pumiloides is reported as a new species for the fauna of Germany, Russia, and the Baltic region (**V**, **VI**). The specimens of *E. hungarica*, collected from Saaremaa (Kaugatuma, Viidumägi), are probably the northernmost records of this species (**V**); the species displays patchy distribution, occurring mostly in hilly and mountainous regions.

Dolerus schmidti Konow (*spec. rev.*) is reported as new for the fauna of Finland, Belgium, Norway, Sweden, and Russia (**III**).

CONCLUSIONS

1. The *Dolerus gibbosus* species group (*s. str.*), currently consisting of *Dolerus asper* Zaddach, *D. blanki* Liston, *D. brevicornis* Zaddach, *D. docilus* Benson, *D. gibbosus* Hartig, *D. harwoodi* Benson, and *D. stygius* Förster, can be defined on the basis of a synapomorphic hooklike process in the ventroapical part of the penis valve. Using the cladistic analysis of the sequenced DNA markers (COI, COII, cytb, and ITS2), this group can be preliminarily regarded as monophyletic.

2. Based on the cladistic analysis of the currently available morphological and molecular data under different optimality criteria, the topologies ((*D. docilus*, (*D. asper*, *D. gibbosus*)), (*D. blanki*, (*D. stygius*, (*D. brevicornis*, *D. harwoodi*))) for the *Dolerus gibbosus* species group and (((*D. liogaster, D. schmidti*), *D. varispinus*), *D. gonager*) for the *D. varispinus* complex can be suggested as the most likely.

3. The sequences of the studied mitochondrial genes (*cyt*b, COI, and COII, especially the combination of *cyt*b and COII) contain a sufficient phylogenetic signal (although not completely homogeneous) for reconstruction of the phylogenetic relationships between closely related species in some species groups of the genus *Dolerus* and can contribute to the resolving of taxonomic problems.

4. *Dolerus asper* auct. includes two separate species, *D. asper* Zaddach and *D. brevicornis* Zaddach, which are both distinct from *D. gibbosus* Hartig. Although these two species are rather similar, they are not sister species according to the cladistic analysis of the DNA sequence data and the morphological characters; they also display differences in their phenology and distribution.

5. The lectotype male of *Dolerus planatus* Hartig and the lectotype female of *D. gibbosus* Hartig represent different sexes of the same species; hence, *Dolerus planatus* Hartig, 1837 and *Dolerus gibbosus* Hartig, 1937 are synonyms; however, it is less confusing if *D. gibbosus* Hartig is retained as the valid name of the species and *D. planatus* Hartig is treated as its synonym.

6. *Dolerus planatus* Hartig, 1837 is a new synonym (*syn. nov.*) of *D. gibbosus* Hartig, 1837; *Dolerus megapterus* Cameron, 1881, *Dolerus carinatus* Konow, 1884, and *Dolerus crassus* Konow, 1884 are *syn. nov.* of *Dolerus stygius* Förster, 1860; *Dolerus rugosus* Konow, 1884 and *Dolerus rugosulus* Dalla Torre, 1894 are *syn. nov.* of *Dolerus varispinus* Hartig, 1837, and *Dolerus soniensis* Dubois, 1920 is *syn. nov.* of *Dolerus schmidti* Konow, 1884.

7. *Dolerus carbonarius* Zaddach, 1859 and *Dolerus fumosus* Zaddach, 1859, previously regarded as conspecific to *D. asper* Zaddach, and *D. annulatus* Stein, 1896, treated earlier as conspecific to *D. liogaster* Thomson, should be

considered *species inquirendae* because as their type material is not available, and the original descriptions do not enable determination of their status in the present taxonomic situation.

8. The synonymies of *Dolerus asper* Zaddach, 1859 and *D. brevicornis* Zaddach, 1859 to *D. planatus* Hartig, 1837, of *Dolerus docilus* Benson, 1956 to *D. gibbosus* Hartig, 1837, of *Dolerus derzavini* Malaise, 1931 to *D. asper* Zaddach, and of *Dolerus schmidti* Konow, 1884 to *D. liogaster* Thomson, 1871 are abandoned.

9. *Dolerus zhelochovtsevi* Heidemaa & Viitasaari (=*Dolerus gibbosus* auct., nec Hartig) is a bisexual species, its females have been misidentified as *D. gibbosus* Hartig, 1837, while males have been incorrectly ascribed to *D. blanki* Liston by previous authors. The proper males of *Dolerus blanki* and *D. gibbosus* have been misidentified and the male of another bisexual species, *D. quadrinotatus* Biró, has been overlooked earlier.

10. *Dolerus liogaster* Thomson, *D. schmidti* Konow (*spec. rev.*) and *D. varispinus* Hartig are separate bisexual species that display some differences in their phenology and distribution; *D. derzavini* Malaise is also a valid species (*spec. rev.*), the taxonomic identity of its male and it phylogenetic placement remain unknown.

11. In most cases, imagos of the *Dolerus gibbosus* species group and the *D. varispinus* complex can be determined using the external morphological characters alone; however, examination of the structure of the penis valve is strongly recommended for reliable differentiation of their males.

12. According to field observations and *ex ovo* rearing, *Carex acuta* L. is the confirmed host plant of *Dolerus blanki* and *D. gibbosus*, while *C. cespitosa* L. is the confirmed host of *D. brevicornis* and *D. zhelochovtsevi*, however, the species can probably feed on some other sedges as well.

13. *Dolerus anatolii* Heidemaa & Zinovjev is the first species of the subgenus *Neodolerus* Goulet recorded from the Palaearctic region; the range of this subgenus is not restricted to the Nearctic, as regarded earlier, but is Holarctic.

14. Adults and larvae of *Empria pumila* (Konow, 1896) and *E. pumiloides* Lindqvist, 1968 can be easily differentiated on a morphological basis. The host plant of *E. pumiloides* is *Filipendula ulmaria* L.

15. *Caliroa crypta* Heidemaa is a rare bisexual tenthredinid species the imagos of which resemble those of *C. tremulae*; it is so far known only by its type material collected from Estonia and Finland.

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SUMMARY IN ESTONIAN (KOKKUVÕTE)

Süstemaatikaalaseid uurimusi perekondade *Dolerus*, *Empria* ja *Caliroa* lehevaablastest (Hymenoptera, Tenthredinidae)

Käesolev dissertatsioon on kokkuvõte lehevaablaste perekondade *Dolerus*, *Empria* ja *Caliroa* mõnede liigirühmade süstemaatikat käsitlevatest uurimustest. Mitme seni problemaatiliseks jäänud taksoni staatus ja piiritlemine liigirühmades *Dolerus gibbosus* ja *D. varispinus* lahendati valmikute morfoloogia, fenoloogia ja genoomse DNA fragmentide nukleotiidide järjestuste võrdleval analüüsil. Leiti sobivad tunnused vastavate liikide usaldusväärseks eristamiseks üksteisest, vajadusel kontrolliti problemaatiliste lähisliikide eri sugupoolte liigilist kuuluvust molekulaarsete tunnuste põhjal. Kladistiliste analüüside tulemusena püstitati hüpoteesid nende rühmade koosseisu kuuluvate liikide põlvnemissuhete kohta. Peenise ventroapikaalosas paikneva sünapomorfse konksja väljakasvu alusel piirtleti *Dolerus gibbosus* liigirühm kitsamas mõttes (*s. str.*) Liigirühma monofüleetilisus sai mitokondriaalse DNA geenifragmentide nukleotiidide järjestuste kladistiliste analüüsidel esialgse kinnituse. Suurimad toetused liigirühmas *D. gibbosus* pälvis topoloogia ((*D. docilus*, (*D. asper*, *D. gibbosus*)), (*D. blanki*, (*D. stygius*, (*D. brevicornis*, *D. harwoodi*))). *Dolerus varispinus*-kompleksi liikide molekulaarsete tunnuste kladistilisel analüüsil sai parimad toetused topoloogia (((*Dolerus liogaster*, *D. schmidti*), *D. varispinus*), *D. gonager*).

Selgus, et uuritud mitokondriaalsete geenifragmentide (*cyt*b, COI ja COII) ning ITS2 regiooni nukleotiidsed järjestused sisaldavad piisavalt fülogeneetilist informatsiooni, et aidata lahendada mõnede perekonna *Dolerus* liigirühmade problemaatiliste vormide taksonoomiline staatus ja rekonstrueerida põlvnemissuhted nende liigirühmade sees.

Kirjeldatud on kolm teadusele uut lehevaablaseliiki: *Caliroa crypta* Heidemaa, 1999, *Dolerus* (*Neodolerus*) *anatolii* Heidemaa & Zinovjev, 2004 ja *Dolerus zhelochovtsevi* Heidemaa & Viitasaari. Liik *Dolerus anatolii* on esimene Palearktisest kindlaks tehtud alamperekonna *Neodolerus* esindaja. Selle alamperekonna kõik eelnevalt teada olnud liigid on levinud Nearktises; seega on antud taksoni levila holarktiline, mitte nearktiline nagu senini arvati. Esmakordselt on kirjeldatud liikide *Dolerus zhelochovtsevi*, *Empria pumiloides* ja *Empria pumila* vastsestaadiumid. Kirjeldatud on ka liikide *Dolerus blanki* Liston, 1995 ja *D. quadrinotatus* Biró, 1884 isased, kes olid seni jäänud märkamatuks (*D. quadrinotatus*) või osutunud valemääranguteks (*D. blanki*). Vastsete kasvatamisega eelnevalt määratud emasloomade poolt munetud munadest (*ex ovo*) tehti esmakodselt kindlaks, et liigi *Empria pumiloides* Lindqvist toidutaimeks on angervaks (*Filipendula ulmaria*), liikide *Dolerus zhelochovtsevi* Heidemaa & Viitasaari ja *D. brevicornis* Zaddach toidutaimeks on mätastarn (*Carex cespitosa* L.) ning lii-kide *Dolerus blanki* Liston ja *D. gibbosus* Hartig toidutaim on saletarn (*C. acuta* L.). Kolme viimasena mainitud liigi vastsed võivad tõenäoliselt edukalt areneda ka teistel tarnaliikidel.

Dolerus asper auct. (autorite käsitluses) valmikute varieeruvuse analüüsi tulemusena selgus, et see takson sisaldab kahte eri liiki, millest üks on kindlasti *Dolerus asper* Zaddach. Kuna eelmainitud liigi ja sellele väga sarnase liigi *D. brevicornis* Zaddach tüüpeksemplarid pole säilinud ja liigikirjelduste või teiste olemasolevate andmete põhjal ei olnud põhimõtteliselt võimalik üheselt kindlaks teha kumma liigi puhul kasutas need kirjeldanud autor binoomenit *Dolerus asper*, siis tähistati zooloogilise nomenklatuuri stabiilsuse tagamiseks liikide *Dolerus asper* Zaddach, 1859 ja *D. brevicornis* Zaddach, 1859 neotüübid.

Binoomeneile *D. gibbosus* Hartig 1837 ja *D. planatus* Hartig, 1837 vastavate lektotüüpide üksikasjalik võrdlemine ja nendega morfoloogiliselt identsete isendite DNA analüüs näitas, et need on tegelikult ühe ja sama liigi vastassugupooled. Kuna binoomeneid *Dolerus asper* ja *D. planatus* käsitleti mõnede süstemaatikute poolt varem ekslikult subjektiivsete sünonüümidena, siis võeti selle liigi valiidse nimena kasutusele *Dolerus gibbosus* Hartig (mitte *D. planatus* Hartig). Taksoni *Dolerus gibbosus* auct. emasisendite võrdlemine liigi *D. gibbosus* Hartig lektotüübiga näitas, et üht osa selle taksoni (liigi *D. gibbosus* auct., nec Hartig) emasisendeid oli eelnevalt ekslikult käsitletud kui liigi *D. gibbosus* Hartig emaseid ja neile vastavaid isasisendeid ebaõigesti hoopis liigi *D. blanki* Liston isastena. Liigi *Dolerus gibbosus* auct., nec Hartig isendite võrdlemisel kõigi teadaolevate varem kirjeldatud sarnaste taksonite tüüpeksemplaridega selgus, et hilisemad uurijad on neid käsitlenud liigi *D. gibbosus* Hartig isendeina, tegelikult kuuluvad need aga varem kirjeldamata liiki (*Dolerus zhelochovtsevi*).

Binoomenitele *Dolerus carinatus* Konow, 1884, *D. crassus* Konow, 1884, *D. megapterus* Cameron, 1881 ja *D. stygius* Förster, 1860 vastavate tüüpeksemplaride võrdlev analüüs näitas, et need kõik kuuluvad ühte ja samasse liiki, mistõttu tuleb antud liigi praegu kehtiv nimi *D. megapterus* Cameron ja selle nooremad subjektiivsed sünonüümid *D. carinatus* Konow ja *D. crassus* Konow prioriteediprintsiibi alusel arvata binoomeni *Dolerus stygius* Förster, 1860 nooremate subjektiivsete sünonüümide hulka. Seega, selle liigi kehtiva teadusliku nimena tuleb kasututada binoomenit *Dolerus stygius* Förster, 1860.

Samuti selgus, et mitmed nooremate subjektiivsete sünonüümidena käsitletud binoomenid on tegelikult valiidsed, sest nende kandjateks olevad tüüpeksemplarid ei kuulu teistesse juba varem kirjeldatud liikidesse, nagu senini on ekslikult arvatud. Need valiidsed binoomenid on järgmised: *Dolerus asper* Zaddach, 1859 ja *D. brevicornis* Zaddach, 1859 (pole binoomeni *D. planatus* Hartig, 1837 nooremad subjektiivsed sünonüümid), *Dolerus docilus* Benson, 1956 (ei ole binoomeni *D. gibbosus* Hartig, 1837 noorem subjektiivne sünonüüm), *Dolerus derzavini* Malaise, 1931 (pole binoomeni *D. asper* Zaddach noorem subjektiivne sünonüüm) ja *Dolerus schmidti* Konow, 1884 (ei ole binoomeni *D. liogaster* Thomson, 1871 noorem subjektiivne sünonüüm).

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1989–1992	University of Tartu, diploma in biology (zoology)
1997	University of Tartu, *magister scientiarum* in systematic zoology, *MSc* thesis: "Taxonomic and faunistic studies on sawflies (Hymenoptera, Symphyta)"
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Scientific work

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