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Dolerus asper Zaddach, 1859 and *Dolerus brevicornis* Zaddach, 1859 (Hymenoptera: Tenthredinidae), with notes on their phylogeny

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Abstract. Concordant differences in morphology, phenology and RAMS markers, as well as in sequenced mtDNA (COI, COII, *cyt*b) and nuclear DNA (ITS2) fragments, indicate that *Dolerus asper* Zaddach, 1859 and *Dolerus brevicornis* Zaddach, 1859 are valid species. On the basis of morphology, molecular markers, and distributional records, both species are distinct from *Dolerus gibbosus* Hartig, 1837 (= *Dolerus planatus* Hartig, 1837). Taxonomy of the species is clarified and the neotypes of *Dolerus asper* Zaddach, 1859 and *Dolerus brevicornis* Zaddach, 1859 are designated. The synonymies of *Dolerus asper* Zaddach, 1859, to *Dolerus planatus* Hartig, 1837 and *Dolerus derzavini* Malaise, 1931, spec. rev. to *D. asper* Zaddach, 1859 are abandoned. *Dolerus carbonarius* Zaddach, 1859 and *Dolerus fumosus* Zaddach, 1859 are considered to be *species inquirendae*. Phylogenetic analyses of the ITS2 fragment and fragments of ITS2 + COI and ITS2 + COII yielded the topology [*D. asper*, (*D. brevicornis*, *D. gibbosus*)], while those of all other markers and their combinations resulted in the topology [*D. brevicornis*, (*D. asper*, *D. gibbosus*)]. In the latter hypothesis the clade *asper* + *gibbosus* is also supported by structural synapomorphies.

INTRODUCTION

Dolerus Panzer, 1801 [type species: *Tenthredo pedestris* Panzer, 1801 by monotypy = *Dolerus germanicus* (Fabricius, 1775)] is a diverse Holarctic sawfly genus with several taxonomically unresolved species groups. The subgenus *Poodolerus* Zhelochovtsev, 1988 with about 60 species in the western Palearctic is the most species-rich and taxonomically difficult of this genus. Detailed studies involving the concurrent analyses of morphological, ecological and molecular data can significantly contribute to the recognition of closely related species complexes and the reliable differentiation of their members.

Blank & Taeger (1992) synonymized *Dolerus asper* Zaddach, 1859 with *Dolerus planatus* Hartig, 1837, and included *Dolerus brevicornis* Zaddach, 1859 in the list of synonyms of *D. planatus* (Blank & Taeger, 1998) following the literature (S.M. Blank, pers. comm.). Recently, *D. planatus* was recognized as the male of *D. gibbosus* Hartig, 1837 (Heidemaa & Viitasaari, in press). A preliminary study of the males of *Dolerus asper* auct. from Estonia and Finland by MH and MN revealed two morphologically distinct forms based on the structure of the penis valves, form of the clypeus, form of the postocellar furrows, and length of setae on vertex, pronotum and mesepisternum of females. Because these forms are morphologically different from *D. gibbosus* (= *D. planatus*), we suggest that they are distinct species. To check this, we did a detailed taxonomic study of *Dolerus asper* auct. incorporating morphometrical, phenological and distributional data as well as molecular markers.

In addition to the taxonomic difficulties concerning *D. asper* auct. nomenclatural problems are involved because the type material of *Dolerus* species described in Zaddach (1859) is not available. Here we review the taxonomy of *Dolerus asper* Zaddach, 1859 and *Dolerus brevicornis* Zaddach, 1859 and designate neotypes. We also discuss the phylogenetic relationships between *D. asper*, *D. brevicornis* and *D. gibbosus*.

MATERIAL AND METHODS

Morphological study

The morphological study is based on material deposited in the following institutional collections:

Department of Applied Biology, University of Helsinki, Finland (DABUH);
Deutsches Entomologisches Institut, Eberswalde, Im Leibniz-Zentrum für Agrarlandschafts- und Landnutzungsforschung, Germany (DEI, ZALF);
Finnish Museum of Natural History, Helsinki, Finland (ZMH);
Institute of Biology, University of Latvia, Salaspils, Latvia (IBUL);
Institute of Plant Protection, Estonian Agricultural University, Tartu, Estonia (IPP);
Museum für Naturkunde der Humboldt-Universität zu Berlin, Bereich Zoologisches Museum, Germany (MNHUB);

Museum of Zoology, University of Tartu, Tartu, Estonia (MZUT);
The Natural History Museum, London, Great Britain (BMNH);
Naturhistorisches Museum Wien, Vienna, Austria (NHMV);
Naturhistoriska Riksmuseet, Sektionen för Entomologi, Stockholm, Sweden (NHRM);
Zoology Department of the Tromsø Museum, Tromsø, Norway (ZDTM);
Zoological Institute in St. Petersburg, Russia (ZISP);
Zoological Museum, University of Oslo, Oslo, Norway (ZMUO);
Zoologische Staatssammlung München, Munich, Germany (ZSM).

The type material listed below was examined:
Dolerus asper megapteroides Muche, 1964; paratype ♀ (MNHUB);
Dolerus derzavini Malaise, 1931; holotype ♀ (NHRM);
Dolerus docilus Benson, 1956; holotype ♂, paratypes 5♂, 4♀ (BMNH);
Dolerus harwoodi Benson, 1947; holotype ♂, 2♀ paratypes (BMNH);
Dolerus oblongus Cameron, 1882; holotype ♀(BMNH);
Dolerus planatus Hartig, 1837; lectotype ♂, 2♂ paralectotypes (ZSM).

In addition, specimens from the private collections of Stephan M. Blank, Manfred Kraus, Jean Lacourt, Jaan Luig, Jouko Nuorteva, Fausto Pesarini, Andreas Taeger, Matti Viitasaari and Veli Vikberg, and the authors MH and MN were studied. Over 1200 specimens of *D. asper* auct. were examined.

Several metrical characters of 20 female and 40 male specimens of *D. asper* auct. were measured using a Wild M8 stereomicroscope with a measuring scale. The measuring units were 0.01 mm for the penis valve and 0.02 mm for other characters. Specimens of the two different forms were not measured in groups, but were measured alternately; a specimen of one form was followed by a specimen of another form. Pairwise combination of the measured characters on x–y scatter diagrams was used to explore if discrete clusters can be found corresponding to the groups of *D. asper* auct. recognized on the basis of structural characters.

The scanning electron micrographs (SEM) in Figs 6, 8, and 12 were taken in digital format with a JEOL 840 attached to a PC with the computer program SEMAPHORE (version 1.2). The genitalia were processed in KOH (10%) prior to their dissection and study. The Figs 4, 7 and 9–11 are based on micrographs taken with an Olympus Camedia 4040Z digital camera mounted to an Olympus SZX-9 stereomicroscope. Serrulae of the *Dolerus derzavini* holotype (Fig. 5) are photographed with a Canon AV-1 camera (film Kodakchrome 25/36) using a Nikon 1.25 microscope (with object-glass: Ph3DL Eplan 40/0.65 160/0.17). Morphological terminology follows Goulet (1986) and Viitasaari (2002). The morphological study was carried out by MH and MN.

DNA isolation and sequencing

Insect samples used for DNA sequencing were collected by MH and US from different localities in Estonia and stored in 96% ethanol. Both mitochondrial and nuclear markers were analysed. 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 3 specimens of the species under study (*D. asper*, *D. brevicornis*), as well as in four specimens of *D. gibbosus* and two specimens of *D. stygius* Förster, 1860 (used as outgroup in phylogenetic study), were determined. 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' CCC 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 a 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 10 µl of PCR reaction and incubated for 27 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 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 Progene Thermal Cycler in a total volume of 10 µl. 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 carried out by US.

Multivariate techniques

In the taxonomic study, multidimensional scaling (MDS) was used for ordination of the similarity matrix representing differences between the analysed samples on the basis of sequence data. The combination of ITS2 and *cyt*b sequences was selected, as these markers showed most intraspecific variation. The similarities between the pairs (x, y) of binary coded sequences were calculated as percent of disagreement (number of $x_i \neq y_i/i$) using the cluster analysis module, and the ordination was performed using the MDS procedure available in STATISTICA (data analysis software system), version 6 (StatSoft Inc., 2001). The UPGMA dendrogram (Fig. 14) was constructed using the MEGA software package (Kumar et al., 1993).

Phylogenetic analysis

The sequenced mitochondrial (*cyt*b, COI, COII) and nuclear (ITS2) DNA markers (Table 1) as well as structural characters of the imaginal stage (Table 2) were used to estimate of the phylogenetic relationships. Sequences of the ITS2 fragment were aligned with multiple alignment procedure in Clustal W, which was run in the software package BIOEDIT, version 5.0.9 (Hall, 1999). Sequence data were analysed using the neighbour joining (NJ), unweighted maximum parsimony (MP) and minimum evolution (ME) methods available in the MEGA software package, version 2.1 (Kumar et al., 2001). Phylogenetic analysis using

morphological data was conducted using the exhaustive search MP procedure in PAUP* 4.0b8 (Swofford, 1998). Molecular and morphological datasets were analysed separately. The mtDNA and nuclear sequences were analysed separately and in all possible combinations. MP analysis was performed using branch-and-bound search with alignment gaps (present in ITS2 only) treated as missing. Support for individual branches was evaluated using the nonparametric bootstrap method with branch-and-bound search and 10,000 pseudoreplicates in all cases. All trees inferred from sequence data were rooted from midpoint. The cladogram resulting from cladistic analysis of morphological data was rooted using *D. stygius*. The phylogenetic analysis was performed independently by MH and US.

Random amplified microsatellite analysis

Because some females of *D. asper* auct. (under 10%) could not be differentiated with confidence using morphological characters, the random amplified microsatellite (RAMS) technique was used to search for suitable markers for their discrimination. The technique is based on primers composed of microsatellite DNA motives and an anchor sequence (Zietkiewicz et al., 1994, Hantula et al., 1996). In RAMS analysis, the DNA between the distal ends of two closely located microsatellites is amplified and the resulting PCR products are separated electrophoretically (Zietkiewicz et al., 1994).

The insect samples for RAMS analysis were collected from Finland (5♀ of *D. brevicornis* and 1♀ *D. asper*) and Norway (4♀ of *D. asper*), and stored in 96% ethanol. The geographical origin of the samples are: ASP 1 (Nilsiä, Finland); ASP 2, ASP 4 (Boensaeter, Norway); ASP 3 (Vestvatnet, Norway); ASP 5 (Solli, Norway); BRE 1, BRE 3–5 (Nilsiä, Finland); BRE 2 (Hattula, Finland). DNA was isolated by a modification of the method described by Vainio et al. (1997). Tissue from the abdomen of each specimen was transferred to a microcentrifuge tube and disrupted using quartz sand in lysis buffer. Three phenol-chloroform (1 : 1) and one chloroform: isoamyl alcohol (24 : 1) extractions were carried out, and the DNA was precipitated using polyethylene glycol 6000 and NaCl. The pellet was washed with 70% ethanol, and after drying under a vacuum, the DNA was resuspended into 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA.

The PCR-reactions were carried out in reaction conditions suggested by the manufacturer of Dynazyme II DNA-polymerase (Finnzymes Ltd., Finland), except that 2 µm of the primer 5′DHB$(CGA)_5$ (where D = A/G/T, H = A/C/T, and B = C/G/T) was used. The samples were denatured by 10 min incubation at 95°C after which 37 cycles of amplification were carried out (30 s denaturation at 95°, 45 s annealing at 61°C, 2 min primer extension at 72°). After the cycles, the reaction was terminated with a 7 min extension at 72°.

Amplification products were separated by electrophoresis on agarose gels containing 1.0% SynerGel (Diversified Biotech) and 1.0% agarose (FMC BioProducts). The electrophoresis was run in TAE-buffer (40 mM Tris-Acetate pH 8.0, 1 mM EDTA), and the amplification products were visualised using ethidium bromide under UV-light. The lengths of the amplification products were estimated by comparing them to 100 bp DNA ladder (Gibco BRL). Distance matrix was calculated from RAMS markers using Band Sharing Indices calculated according to Lynch (1990). The RAMS analysis was conducted and interpreted by JH.

RESULTS AND DISCUSSION

Taxonomy and nomenclature of *D. asper* auct.

If the metrical characters of the specimens of *D. asper* auct., segregated in two morphological groups by structural characters, were represented graphically on x–y scatter diagrams, the character combinations resulting in two distinct clusters were found for both sexes (Fig. 1A–B). Two discrete clusters, consisting of ASP 2, ASP 3, ASP 4 and BRE 1, BRE 3, BRE 9, corresponding to the morphologically distinct groups formed if the similarity matrix representing differences between the samples on the basis of their nucleotide sequences was ordinated using nonmetric multidimensional scaling (MDS). To achieve maximal spread of the samples on a scatterplot, the number of calculated dimensions was set on maximum (9). The resulting ordination of the samples in first 2 dimensions is given in Fig. 2. It can be seen that three males (GIB 31, GIB 36, and GIB 37) of the third species, morphologically identical to the lectotype of *D. planatus*, fall in the same cluster with a female (GIB 33) (Fig. 2) that morphologically matches the lectotype of *D. gibbosus*. This confirms that *D. planatus* is the male of *D. gibbosus* as was suggested earlier on the basis of shared structural characters (Heidemaa & Viitasaari, in press).

In the two morphological groups detected in *D. asper* auct. the association of females and males, initially based on their shared structural characters, is also supported by molecular markers and corresponding phenological patterns of the sexes. The flight period of both sexes in one group (*D. brevicornis*) begins and ends earlier than in the other (*D. asper*) (Fig. 3). Accordance of the variation patterns observed in the different character sets shows that *D. asper* auct. includes two distinct species. Application of the name *D. asper* Zaddach, 1859 has to be fixed by selecting its neotype because the original type material is lost and the description or any other reference information would not ensure unambiguous usage of this name in the present taxonomic situation. Zaddach (1859) emphasized the similarity of the males of *D. asper* and *D. brevicornis*: "Diese Art ist dem *D. brevicornis* sehr ähnlich ...".

All facts suggest that Zaddach's sawfly collection was destroyed during Word War II and none of the recent sawfly workers have studied the type material of *Dolerus* species described in Zaddach (1859). According to Horn et al. (1990), the Hymenoptera collection of Gustav Zaddach was partially destroyed during World War II. Preliminary information indicating that the collection was in the Zoological Institute in St. Petersburg (Blank & Taeger 1998) was later rejected (A. Zinovjev, pers. comm., A. Taeger, pers. comm.). All our efforts to trace the Zaddach's *Dolerus* types as well as requests sent to several zoological institutions in Lithuania (Vilnius), Germany (Berlin), Poland (Krakow), Russia (Kaliningrad = Königsberg) and Ukraine (Kharkov), were unsuccessful.

Only the female is mentioned in the original description of *D. carbonarius* Zaddach, 1859. Unfortunately, the additional specimens with the type material referred to in Zaddach (1859) as "Das mir aus dem Berliner entomolo-

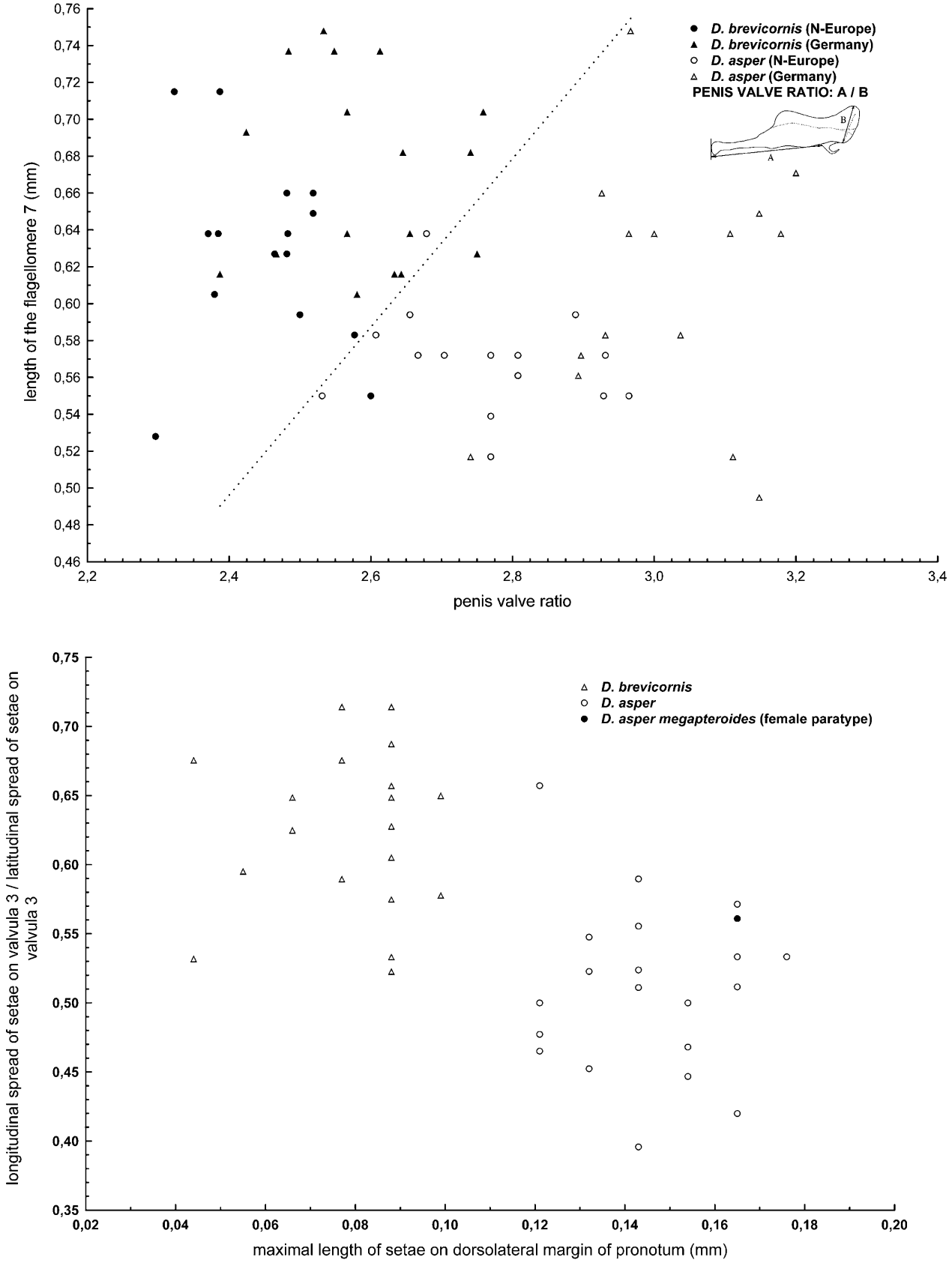


Fig. 1. Relationships of some metrical characters of adults of *D. asper* Zaddach, spec. rev. and *D. brevicornis* Zaddach, spec. rev. A – males: penis valve ratio (see inset figure) (x) vs length of the flagellomere 7 (y). Dotted line designates the best linear discrimination (misclassification minimised); B – females: maximum length of setae on dorsolateral margin of pronotum (x) vs longitudinal spread of setae on valvulae 3 / latitudinal spread of setae on valvulae 3 (y).

gischen Museum zur Ansicht geschickte Weibchen des *Dolerus anthracinus* gehörte zu dieser Art.", which might help resolve the taxonomic identity of *D. carbonarius*, are not in the Berlin Museum (S.M. Blank, pers. comm.). This species, traditionally listed in the synonymy of *D. asper* Zaddach, 1859, has to be regarded as "species inquirenda". Also, *Dolerus fumosus* Zaddach, 1859 (preoccupied by *Dolerus fumosus* Stephens, 1835 = *Dolerus zaddachi* Kirby, 1882, new name proposed for *D. fumosus* Zaddach) is considered a "species inquirenda" because its type is not available and there is no other information for resolving the identity of this species in present taxonomic context.

For the sake of nomenclatural stability we do not propose any new names but recognize *D. asper* Zaddach, 1859 and *D. brevicornis* Zaddach, 1859 as distinct species, earlier synonymised to *D. planatus* Hartig, 1837 (= *D. gibbosus* Hartig, 1837), and fix the usage of these names by selecting neotypes. This solution is available because the species recognized in *D. asper* auct. match the original descriptions of *D. asper* Zaddach, 1859 and *D. brevicornis* Zaddach, 1859. In Zaddach's key (Zaddach, 1859, p. 15), the specimens with clearly defined postocellar furrows run to "*fissus* [valid name: *Dolerus nigratus* (O. F. Müller, 1776)], *carbonarius* and *brevicornis*" while the specimens with obsolete postocellar furrows go to "*fumosus* and *asper*". Other characters given in Zaddach's descriptions, for example the sculpture on tergum I and the relative length of the basal flagellomeres, have no taxonomic value. Because the male of *D. asper* and *D. brevicornis* are described in Zaddach (1859) and the males of the species recognized in *D. asper* auct. can be determined on the basis of their penis valves, the neotypes selected are males. The short antennae of *D. brevicornis*, according to Zaddach's description, are in the female 4.3 mm and in the male 5 mm. This is not contradictory because the length of the antennae is rather variable and the measurements are not precise.

Morphotaxonomy

The males of *D. asper* and *D. brevicornis* are most reliably differentiated from each other and from *D. docilus* Benson, 1956 and *D. gibbosus* by penis valve structure. *D. docilus* is not conspecific to *D. gibbosus* as proposed by Lacourt (1999) but a separate species (Heidemaa & Viitasaari, in press). Its larva is unknown but the adult resembles *D. asper*. The outline of the penis valve of *D. gibbosus* in lateral view resembles that of *D. asper* and *D. brevicornis*, but the hooklike process at its ventroapical corner is less developed. It is easy to destroy this structure during dissection or if the valves are processed for too long in KOH. In dorsal view, the sharply broadening apical part of the penis in *D. gibbosus* (Fig.

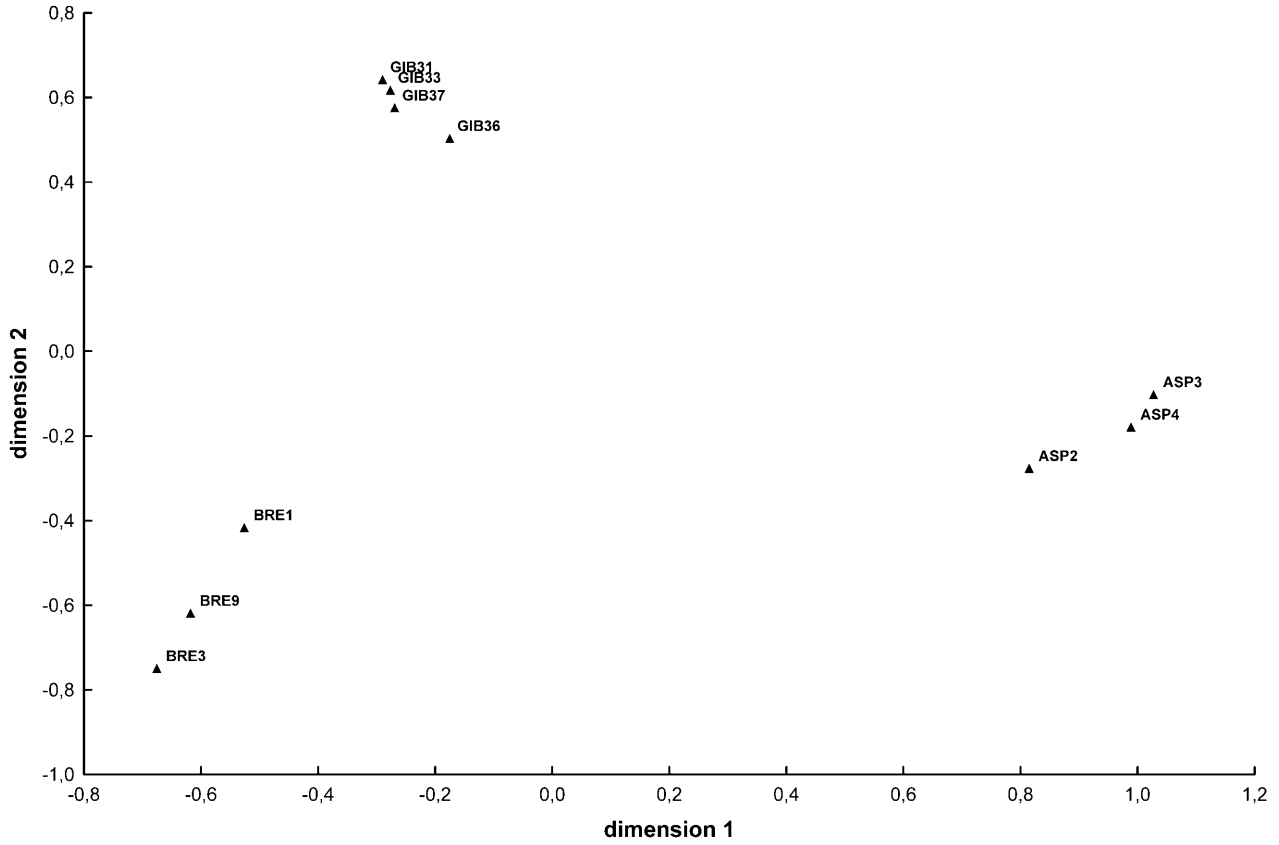


Fig. 2. Nonmetric multidimensional scaling ordination of the similarity matrix representing the differences (percent of disagreement) between the samples of *D. asper* Zaddach, spec. rev., *D. brevicornis* Zaddach, spec. rev., and *D. gibbosus* Hartig based on the combined binary coded *cyt*b and ITS2 fragments' nucleotide sequences. The first two of the 9 calculated dimensions were used for the ordination of the samples (Final stress = 1.6×10^{-5}).

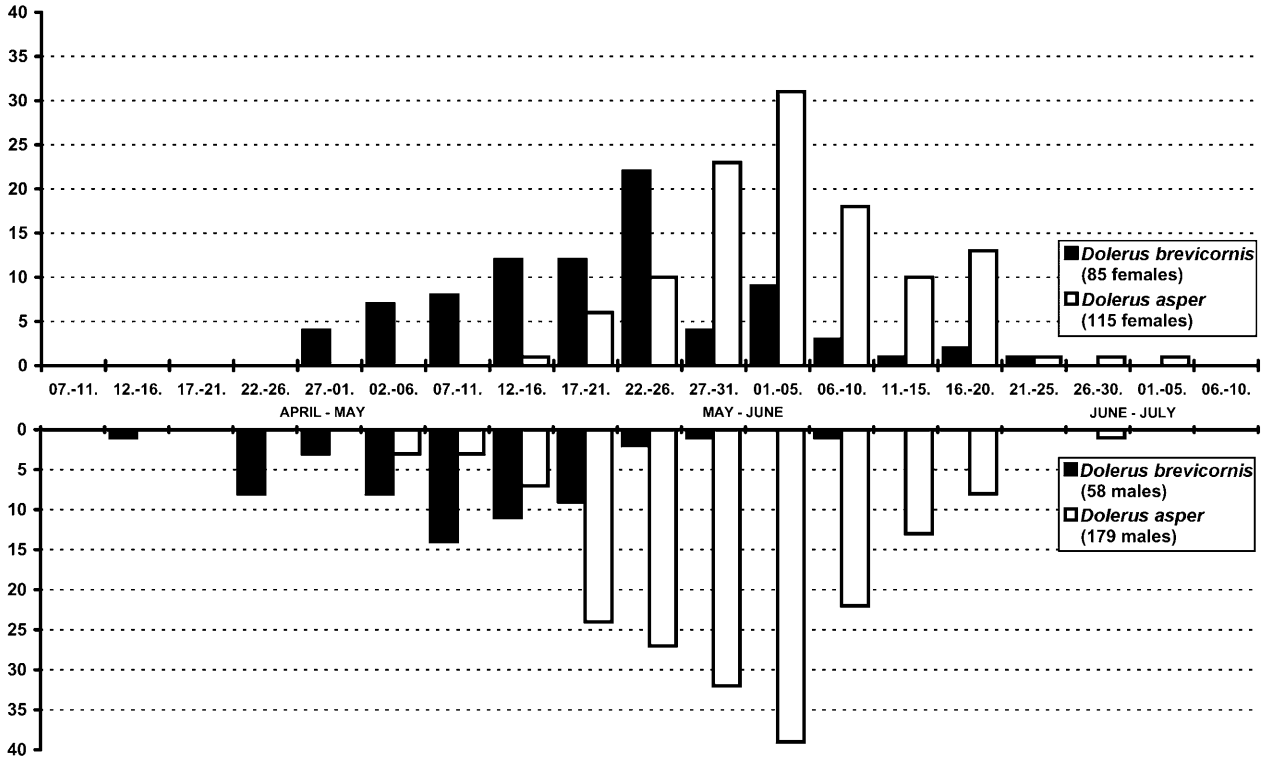


Fig. 3. Phenology diagrams for the imaginal stages of *D. brevicornis* Zaddach, spec. rev. and *D. asper* Zaddach, spec. rev. based on the numbers of adults collected in Vuohiniemi (Southern Finland) on different dates in April–July, from 1946 to 1997.

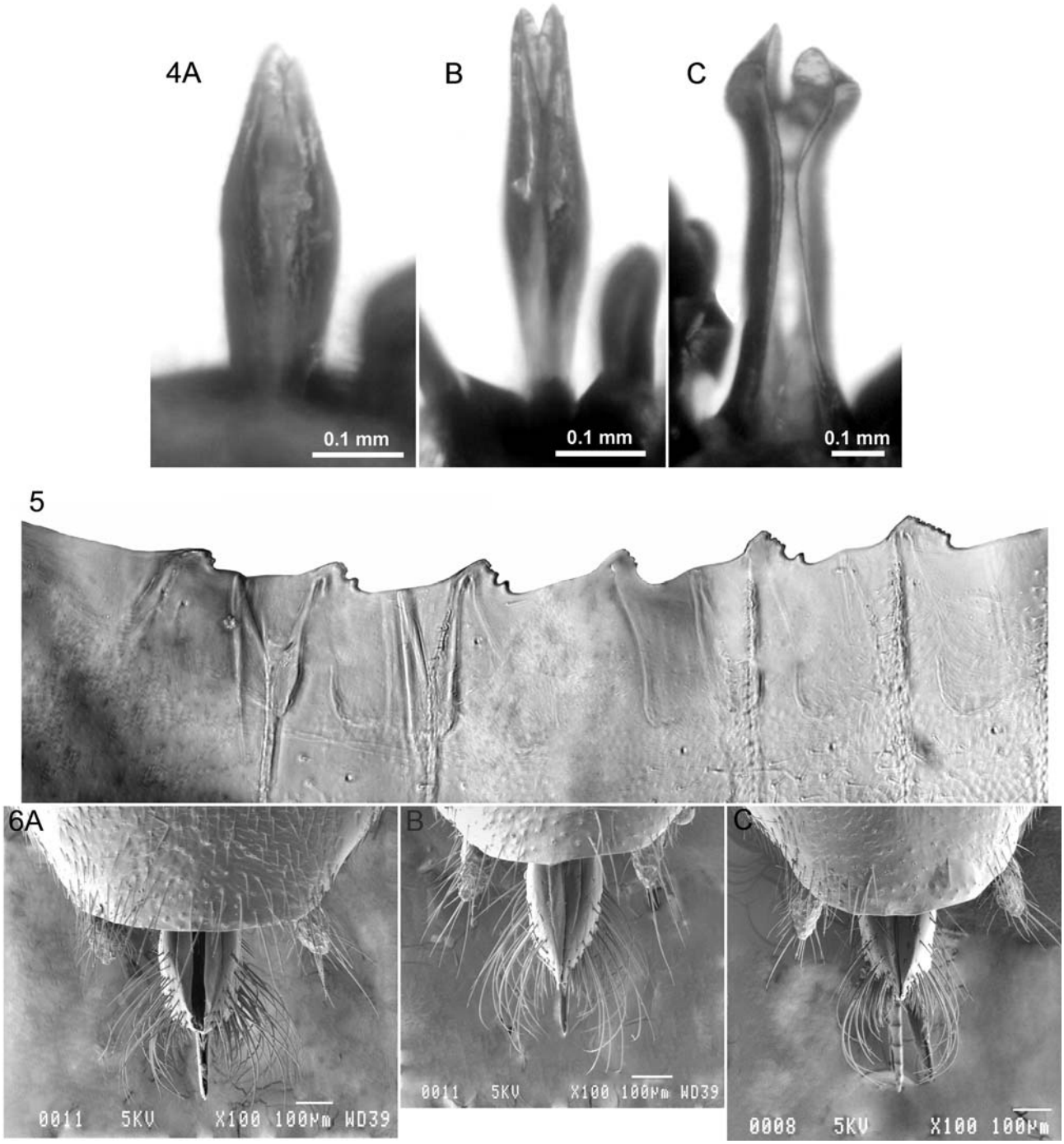
4C) differs from *D. asper* (Fig. 4A) and *D. brevicornis* (Fig. 4B), but resembles *D. harwoodi*. Examination of the 2♀ paratypes of *Dolerus harwoodi* Benson, 1947 revealed that a specimen collected from Dalby (Sweden) and identified by Benson as *D. harwoodi* is actually *D. asper*. *Dolerus derzavini* Malaise, 1931, described from the Russian Far East from one female (male is unknown), was later regarded as a synonym of *D. asper* by Zhelochovtsev (1935). However, the basal serrulae of lancet (Fig. 5) and the outline of mesepisternum of *D. derzavini* holotype in frontal view fall outside the range in variation of these characters in *D. asper* and *D. brevicornis*. Therefore, the synonymy of *D. derzavini* to *D. asper* is abandoned.

Lacourt (1999) raised *Dolerus asper megapteroides* Muche, 1964 to species rank. Its holotype was not found and the sex of the type specimen not mentioned in Muches's description (Muche, 1964). According to Muche (1964) the type was deposited in his collection. So far, only a female paratype in the Berlin Museum is known (S.M. Blank and J. Lacourt, pers. comm.). According to structural characters, it is conspecific to *D. asper*. Also, the metrical characters indicate that the paratype female of *D. asper megapteroides* falls in the same cluster as specimens of *D. asper* (Fig. 1B). Additional material from the type locality of *D. asper megapteroides* (Bolu-daglari [= Bolu mountains], Anatolia) consists of 3♀ (coll. M. Kraus), all of which belong to *D. asper*.

Comparison of the penis valves of *D. asper* auct. illustrated in the literature by different authors also reveals two distinct types. We suggest that the illustrations given in Goulet (1986: Fig. 170) and Benson (1952: Fig. 235, 1956: Fig. 15) for *D. asper* are indeed the penis valves of *D. asper* Zaddach, spec. rev. while those given in Zhelochovtsev (1935: Fig. 4) and Muche (1964: Fig 5) for *D. asper* are those of *D. brevicornis* Zaddach, spec. rev. The true identity of the *Dolerus asper megapteroides* penis valve given in Muche (1964: Fig. 4) remains ambiguous as long as the males collected from the Bolu mountains in Anatolia by Muche are not traced. Benson (1968) suggested that it is *D. megapterus* Cameron, 1881; however, it seems more likely that the penis valve figure of *D. asper megapteroides* is really that of *D. asper*, as indicated also by other characters given by Muche (1964). Of course, it cannot be excluded that the material regarded by Muche (1964) as *D. asper megapteroides* includes more than one species or the penis valve drawing by him (Muche, 1964: Fig 4) is imprecise and misleading. Identity of the two rather rough sketches of the penis valves drawn by Muche (1969: Figs 67–68) for *D. asper* also remains uncertain. They look very different from *D. asper* and may be *D. gibbosus* and *D. blanki* Liston, 1995, respectively. Because the setae on the mesepisternum of a female of *D. asper* illustrated in Benson (1952: Fig. 210) are very short, we regard it as *D. brevicornis* while the penis valve he illustrated for *D. asper* was most probably based on that of a male of *D. asper*.

Identification key

Dolerus gibbosus species group was defined on the basis of synapomorphic hooklike process of the penis valves in its members by Heidemaa & Viitasaari (in

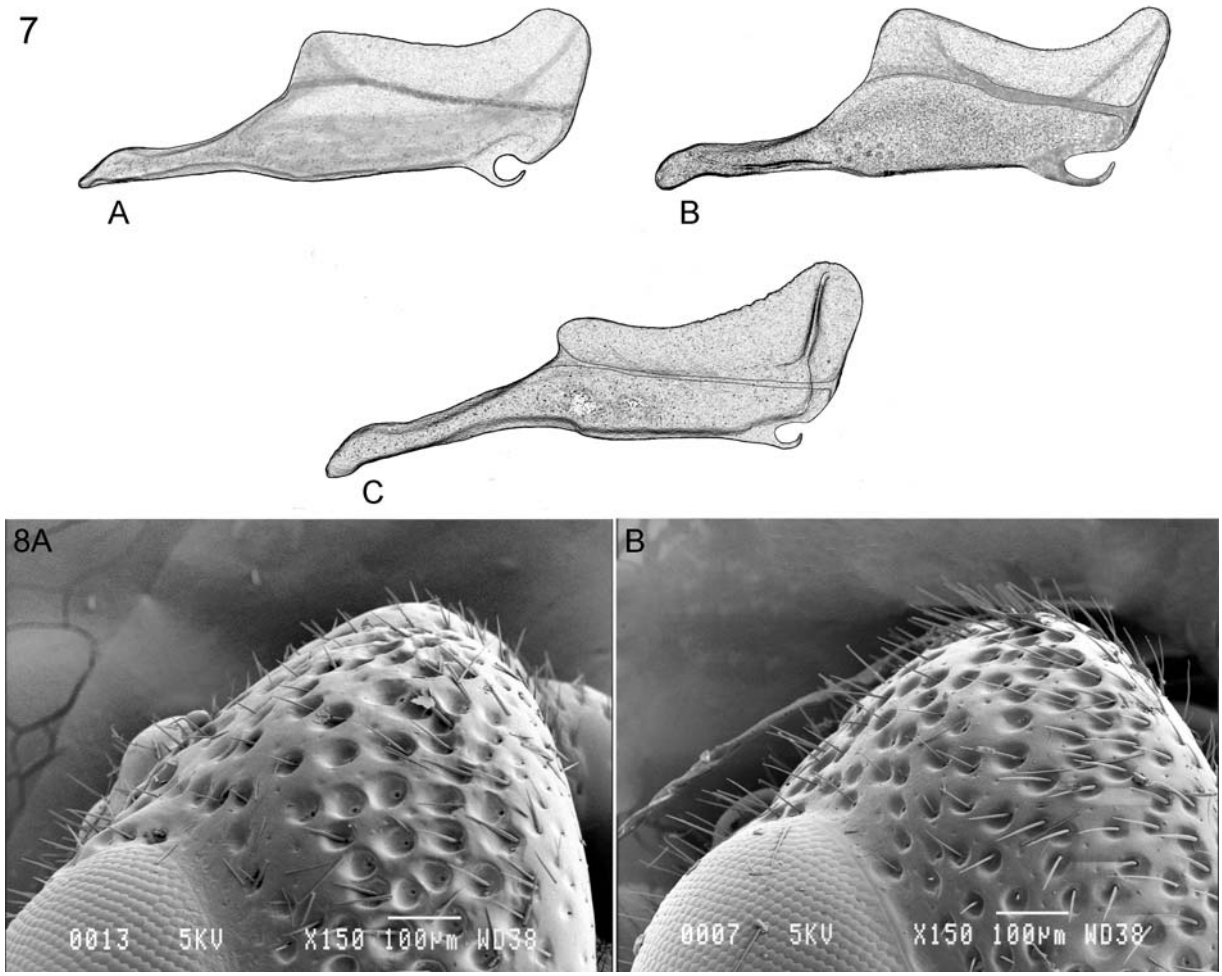


Figs 4–6. 4 – Penis valves in dorsal view. A – *D. asper* Zaddach, spec. rev.; B – *D. brevicornis* Zaddach, spec. rev.; C – *D. gibbosus* Hartig. 5 – Holotype of *Dolerus derzavini* Malaise, 1931, basal serrulae. 6 – Valvulae 3 (apical sheath) in dorsal view (SEM). A – *D. asper* Zaddach, spec. rev.; B – *D. brevicornis* Zaddach, spec. rev.; C – *D. harwoodi* Benson.

press). An identification key in Heidemaa & Viitasaari (in press) also includes similar species that probably do not belong to this species group, although the females *D. asper* and *D. brevicornis* are not distinguished (they run to *D. asper* auct.). The following key is for the differentiation of *D. asper* Zaddach, 1859 and *D. brevicornis* Zaddach, 1859 from each other, and from the female of *D. harwoodi* and the male of *D. gibbosus*, which have often been confused with the respective sexes of the aforementioned species.

Key to the imagos of *D. asper*, *D. brevicornis*, *D. harwoodi* (l), and *D. gibbosus* (k)

1 Female. Body black; longest setae of valvulae 3 (apical sheath) in dorsal view curved from their base and in contour forming a longitudinal oval or circle. (Fig. 6A–C). 2
– Male. Body black; ventroapical corner of the penis valve bears hooklike process (Fig. 7A–C). 6
2 Longest setae on upper part of head shorter (Fig. 8A) or at most only slightly longer than ocellus diameter (Fig. 8B); body length typically under 9.5 mm. 3



Figs 7–8. 7 – Penis valves in lateral view. A – *D. asper* Zaddach, spec. rev.; B – *D. brevicornis* Zaddach, spec. rev.; C – *D. gibbosus* Hartig (schematic). 8 – Setae on upper part of head of females (SEM). A – *D. brevicornis* Zaddach, spec. rev.; B – *D. harwoodi* Benson.

– Longest setae on upper part of head at least 1.5 × longer than ocellus diameter; large species with body length typically over 9.5 mm. . . . . other species (a key by Heidemaa & Viitasaari, in press)

3 Upper halves of the lateral portions of mesepisternum in frontal view more or less parallel or slightly converging (Fig. 9A–B) with setae either uniform and shorter than ocellus diameter (Fig. 9B) or of variable length, some about as long as ocellus diameter or even longer, and some distinctly shorter (Fig. 9A); longest setae on valvulae 3 in dorsal view directed more or less backwards and form a longitudinal oval contour (Fig. 6A–B). 4

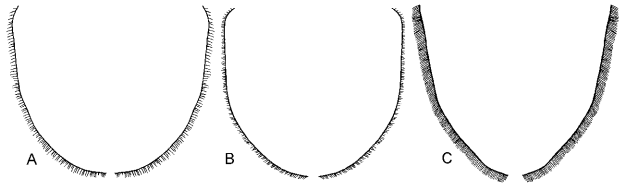
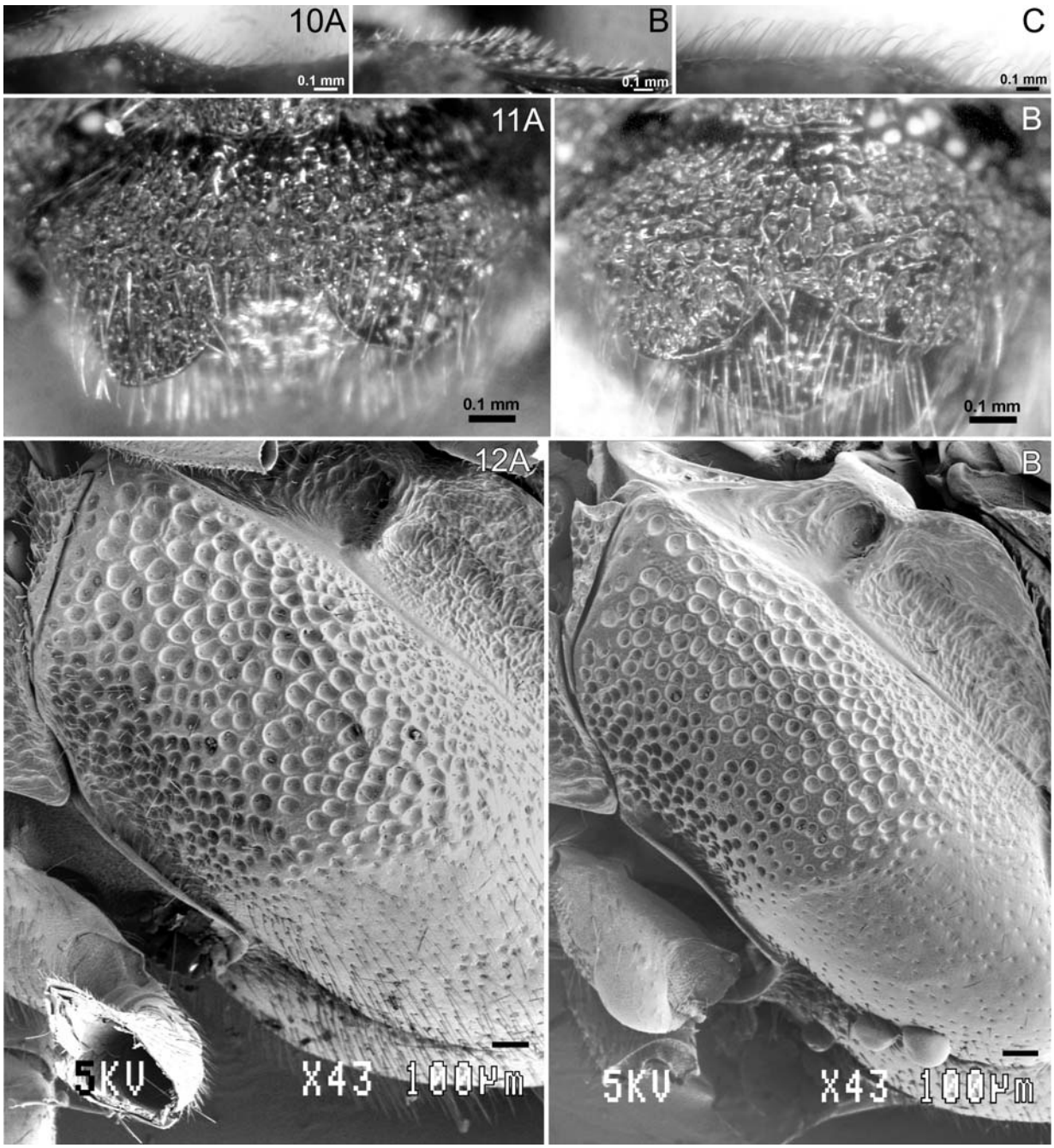


Fig. 9. Outline of the mesepisternum with setae of females (frontal view). A – *D. asper* Zaddach, spec. rev.; B – *D. brevicornis* Zaddach, spec. rev.; C – *D. harwoodi* Benson (schematic).

– Upper halves of the lateral portions of mesepisternum in frontal view distinctly converging with setae dense and almost uniform, mostly longer than ocellus diameter (Fig. 9C); longest setae on valvulae 3 in dorsal view directed inwards and form a more or less circular contour (Fig. 6C). . *Dolerus harwoodi* Benson, 1947

4 Setae on dorsolateral edge of pronotum (half way towards tegula) about as long as ocellus diameter or longer (Fig. 10A); clypeus usually asymmetrical with emargination about 0.5 × as deep as its median length (Fig. 11A); interspaces between the punctures on central part of mesepisternum more or less uniform, typically narrower than diameter of larger punctures on mesepisternum and without distinct sculpture (Fig. 12A); setae on mesepisternum of variable length, longest about as long as ocellus diameter or slightly longer and shortest about half its diameter; postocellar furrows typically obsolete; some of the longest setae of valvulae 3 in dorsal view usually directed more backwards than inwards, and form a slightly longitudinal oval contour (Fig. 6A). . . . . *Dolerus asper* Zaddach, 1859, spec. rev.

– Setae on dorsolateral edge of pronotum (halfway towards tegula) distinctly shorter than ocellus diameter (Fig. 10B); clypeus more or less symmetrical with emargination about 0.35 × as deep as its median length (Fig. 11B); interspaces between punctures on central part of mesepisternum usually



Figs 10–12. 10 – Setae on dorsolateral edge of pronotum. A – *D. asper* Zaddach, spec. rev.; B – *D. brevicornis* Zaddach, spec. rev.; C – *D. gibbosus* Hartig. 11 – Clypeus. A – *D. asper* Zaddach, spec. rev.; B – *D. brevicornis* Zaddach, spec. rev. 12 – Surface of the mesepisternum of females (SEM). A – *D. asper* Zaddach, spec. rev.; B – *D. brevicornis* Zaddach, spec. rev.

not uniform, at least some occupy an area comparable with larger punctures on mesepisternum, and display distinct sculpture (Fig. 12B); setae on mesepisternum sparse, usually shorter than ocellus diameter; postocellar furrows typically distinct; longest setae of valvulae 3 in dorsal view point more or less uniformly inwards and form a distinctly longitudinal oval contour (Fig. 6B). . . . . *Dolerus brevicornis* Zaddach, 1859, spec. rev.

6 Penis in dorsal view gradually broadening and then narrowing (Fig. 4A–B); maximum height of apical part of the valviceps in lateral view less or little greater than its maximum basal height (Fig. 7A–B); ventroapical hooklike process well developed; tergum I more or less matt and with several punctures; clypeus emargination variable (0.35–0.5 × its median length). 7

– Penis valves in dorsal view sharply broadening and then narrowing (Fig. 4C); maximum height of apical part of the valviceps in lateral view clearly exceeds its maximum basal height (Fig. 7C); ventroapical hooklike process weakly developed; tergum I glossy, without or with few punctures; clypeus emargination about 0.5 × as deep as clypeal median length; penis valves in lateral view as in Fig 7C. . . . . *Dolerus gibbosus* Hartig, 1837

7 Clypeal emargination deeper, about 0.5 × as deep as clypeal median length; penis valves in lateral view as in Fig 7A, tip of ventroapical hooklike process usually not reaching the

level of distal margin of valviceps. . . . . *Dolerus asper* Zaddach, 1859, spec. rev.

– Clypeal emargination shallower, about 0.35 × as deep as clypeal median length; penis valves in lateral view as in Fig. 7B, tip of ventroapical hooklike process usually reaches the level of distal margin of valviceps. . . . . *Dolerus brevicornis* Zaddach, 1859, spec. rev.

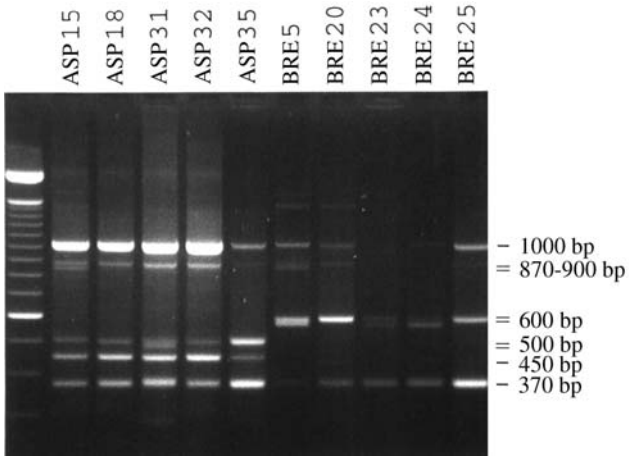


Fig. 13. RAMS patterns in specimens of *D. asper* Zaddach, spec. rev. (labelled ASP 15–ASP 35) and *D. brevicornis* Zaddach, spec. rev. (labelled BRE 5 – BRE 25). The RAMS markers discussed in the text are shown on the right.

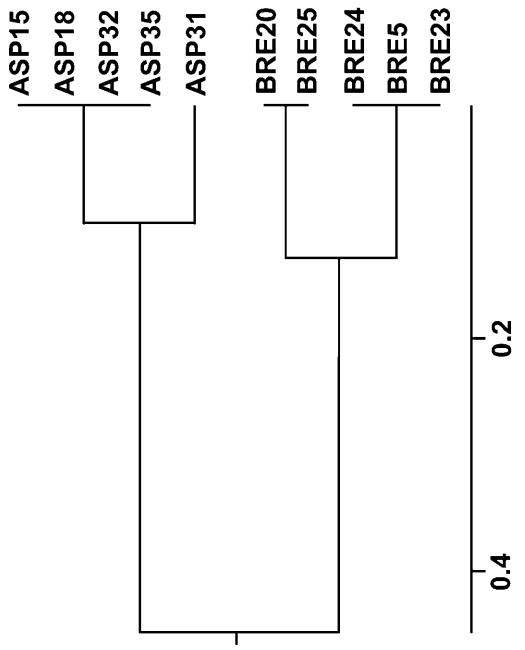


Fig. 14. Dendrogram of UPGMA clustering of RAMS markers in *D. asper* Zaddach, spec. rev. and *D. brevicornis* Zaddach, spec. rev.

Dolerus asper Zaddach, 1859, spec. rev.

Dolerus asper Zaddach, 1859: 21

Dolerus asper auct. partim.: Benson (1952: Fig. 235, 1956: Fig. 15), Goulet (1986: Fig. 170)

Dolerus oblongus Cameron, 1882: 177

Dolerus asper megapteroides Muche, 1964: 33–34; synonymy proposed by Benson (1968), (♀ *nec* ♂)

Neotype. Deposited in DEI, labelled: "ESTONIA 2003, Ta: Leetsi, ME77 12.05., NL 58:24:49 EL 26:31:20, M. Heidemaa leg." [white printed label]; "Water flood of the River Emajõgi, catkins of *Salix* sp., sweep net" [white printed label]; "NEOTYPUS ♄, *Dolerus asper*, ZADDACH, 1859, M. Heidemaa design." [red printed label]; "*Dolerus asper*, Zaddach 1859, M. Heidemaa det." [white printed label].

Female. Setae on dorsolateral edge of pronotum (halfway towards tegula) about as long as ocellus diameter or longer (Fig. 10A). Clypeus usually asymmetrical with emargination about 0.5 × as deep as its median length (Fig. 11A). Interspaces between punctures on central part of mesepisternum more or less uniform, usually distinctly narrower than diameter of larger punctures on mesepisternum and without distinct sculpture (Fig. 12A). Setae on mesepisternum of variable length: longest about as long as ocellus diameter or slightly longer and shortest about half of it. Postocellar furrows typically obsolete. Some of the longest setae of valvulae 3 in dorsal view usually point more backwards than inwards, and form a slightly longitudinal oval contour (Fig. 6A).

Male. Penis in dorsal view as in Fig. 4A; tip of the ventroapical hooklike process usually not reaching level of distal margin of valviceps (Fig. 7A). Lateral postocellar furrows obsolete in most cases. Clypeus usually asymmetrical with emargination about 0.5 × as deep as its median length (Fig. 11A).

Host. *Carex acuta* Bell., *C. cespitosa* L., *C. nigra* (L.) and most probably other *Carex* spp. Feeding success on Poaceae needs to be studied. The females also oviposited on *Poa pratensis* L. but the larvae died. Barker (in press) has reared larvae of this species on *Carex panicea* L. and *Eriophorum* sp. However, according to her observations many wide-leaved *Carex* spp. were rejected by larvae.

Distribution. Holarctic. The verified records for countries in the Palearctic are: Austria, Switzerland, Germany, Estonia, Finland, France, Latvia, Norway, Russia (European and Far East) and Sweden. Altogether over 600 specimens were examined. The northernmost records in Palearctic are from Utsjoki (69°48'N) in Finland and from Parasdalen (69°05'N) in Norway.

Dolerus brevicornis Zaddach, 1859, spec. rev.

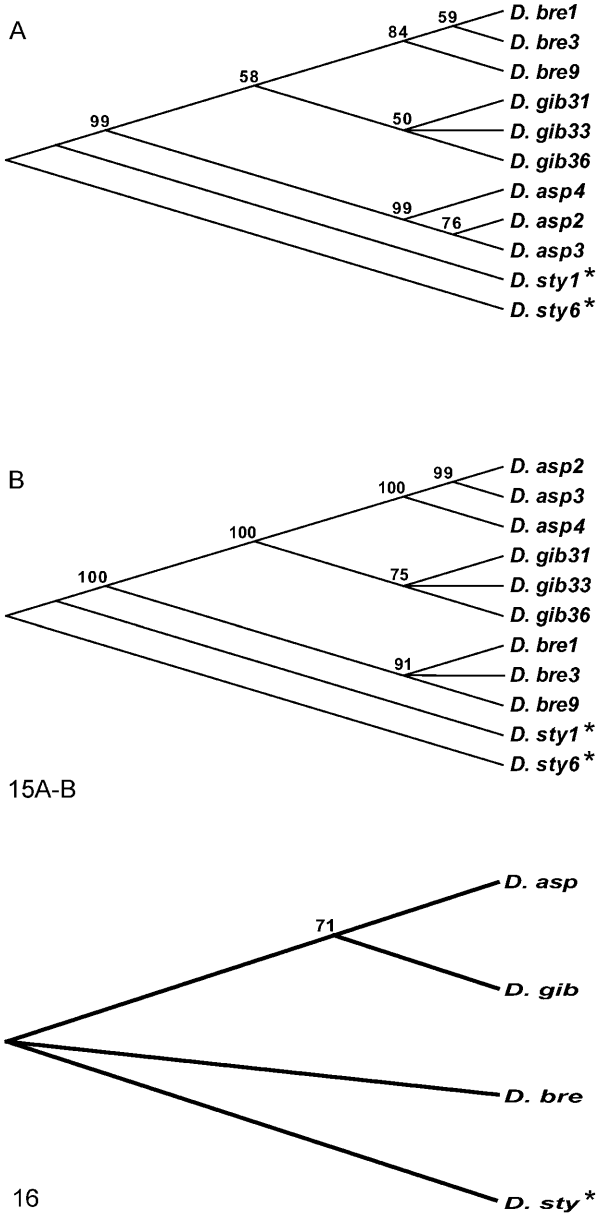
Dolerus brevicornis Zaddach, 1859: 25

Dolerus asper auct. partim.: Benson (1952: Fig. 210), Muche (1964: Fig. 5), Zhelochovtsev (1935: Fig. 4)

? *Dolerus tectus* MacGillivray, 1914: 104 (holotype female, not examined).

Neotype. Deposited in DEI, labelled: "ESTONIA 2003, Ta: Laane, ME86 12.05.03, NL 58:19:14 EL 26:40:48, M. Heidemaa leg." [white printed label]; "drain, *Carex* spp. sweep net" [white printed label]; "NEOTYPUS ♄, *Dolerus brevicornis*, ZADDACH, 1859, M. Heidemaa design." [red printed label]; "*Dolerus brevicornis*, Zaddach 1859, M. Heidemaa det." [white printed label].

Female. Setae on dorsolateral edge of pronotum (halfway towards tegula) distinctly shorter than ocellus diameter (Fig. 10B); clypeus more or less symmetrical with emargination about 0.35 × as deep as its median length (Fig. 11B); interspaces between punctures on cen-



Figs 15–16. 15 – Tree topologies for *D. asper* Zaddach, spec. rev. (*D. asp*), *D. brevicornis* Zaddach, spec. rev. (*D. bre*) and *D. gibbosus* Hartig (*D. gib*) estimated from unweighted parsimony analysis conducted in PAUP* using exhaustive search. Numbers above the branches indicate bootstrap support from 10,000 pseudoreplicates. A – consensus (cut-off value = 50%) based on the 8 most parsimonious cladograms resulting from the combined sequences of COII (356 bp) and ITS2 (479 bp) fragments. B – consensus tree, with 75% cut-off value, based on the 3 most parsimonious cladograms resulting from the combination of all analysed markers: *cyt*b (517 bp) + COI (272 bp) + COII (356 bp) + ITS2 (479 bp). 16 – Single most parsimonious cladogram resulting from an unweighted parsimony analysis of 3 imaginal characters of *D. asper* Zaddach, spec. rev. (*D. asp*), *D. brevicornis* Zaddach, spec. rev. (*D. bre*) and *D. gibbosus* Hartig (*D. gib*), conducted in PAUP* using exhaustive search. *D. stygius* Förster was used as an outgroup. Number between branches indicates bootstrap support from 10,000 pseudoreplicates, * – outgroup.

tral part of mesepisternum usually not uniform, at least some occupy an area comparable to the larger punctures on mesepisternum, and have a distinct surface sculpture (Fig. 12B); typically most setae shorter than ocellus diameter. Postocellar furrows typically distinct. Longest setae of valvulae 3 in dorsal view point more or less uniformly inwards forming a distinctly longitudinal oval contour (Fig. 6B).

Male. Penis in dorsal view as in Fig. 4B; tip of ventroapical hooklike process usually reaches level of distal margin of valviceps (Fig. 7B). Lateral postocellar furrows distinct in most cases. Clypeal emargination about 0.35 × as deep as clypeal median length (Fig. 11B).

Host. *Carex cespitosa* and some other *Carex* spp., e.g. *C. nigra* (T. Kontuniemi, unpubl.).

Distribution. Probably Holarctic, as the Nearctic records are unverified. The verified records for countries in the Palearctic are: Austria, Germany, Estonia, Finland, France, Norway, Russia (European and Far East) and Sweden. Altogether about 600 specimens were examined. The northernmost records are from Ivalo (68°38'N) and Kittilä (KemL) in Finland, and Petsamo in Russian Karelia.

Differentiation of *D. asper* and *D. brevicornis* using RAMS markers

Since some females of *D. asper* and *D. brevicornis* could not be differentiated morphologically, RAMS markers were used for discrimination. Using the primer DHB$(CGA)_5$ 4–6 amplification products were obtained from *D. asper* and 4–8 from *D. brevicornis* (Fig. 13). A marker with a size of 450 bp was present in all specimens of *D. asper* but not in those of *D. brevicornis*. In addition, in the specimens of *D. asper* but not *D. brevicornis*, one or two markers with a molecular weight of about 500 bp were present. Markers with a molecular weight of about 600 bp were observed only in *D. brevicornis*. The occurrence of one or two markers within these weight ranges could be due to the length polymorphism of the markers, although this was not confirmed. The markers of 370 bp and 1000 bp are monomorphic, and shared by both species. The markers in the molecular weight range of 870–900 bp did not differentiate the species, although some variation was observed.

The clearly interpretable markers (1000 bp, 600 bp, 580 bp, 500 bp, 480 bp, 450 bp and 370 bp) were used to construct an UPGMA dendrogram (Fig. 14). Segregation of the specimens of *D. asper* and *D. brevicornis* into two clusters was clear, and both major clusters were formed from two subclusters. In this connection it should be noted that the *D. asper* individuals originated from Finland and Norway, but their origin was not reflected in the UPGMA clustering as the Finnish individual was identical to the four specimens collected from Norway. Thus, the RAMS markers with molecular weights of 450, 500 and 600 bp can be used to differentiate the females of *D. asper* and *D. brevicornis*, which are morphologically inseparable. This suggests that *Dolerus asper* auct. includes two distinct species. Furthermore, some of the RAMS markers could be used to study the intraspecific variation in these species.

TABLE 1. Variable sites in the gene fragments of *D. brevicornis* Zaddach (D.bre), *D. asper* Zaddach (D.asp), *D. gibbosus* Hartig (D.gib) and *D. stygius* Förster (D.sty). In brackets after a marker the number of variable and parsimony informative sites (in total 76/68, respectively) is indicated. POS – nucleotide position in the dataset.

	MARKER	*cyt*b (17/15)	COI (7/6)	COII (7/7)	ITS (45/40)
	P			111	111111111111111111111111111111111111111111111111
	O	111122334444	5667777	8889001	122222344444444444444444444445555555555556666
	S:	2490155733782599	2040128	1993230	711278301233333556667777788990002334456680122
		56021328013773267	5680113	5673326	167420667634789583580123445281493582486815713
Species sample:	D.bre1	AGACTATTAGTTAGTTC	CAATACT	TGACCTA	CACCGGGCGGTCGCGCAAGGACGTCCCGTCAGTCAACCTTCTGGA
	D.bre3	..T.....	.....	.....	.....T.....A.....
	D.bre9	..T.....	.....	.....	.....T.....A.....C.....
	D.asp2	.ATT.TC.T.A.GAACT	TT...TC	..CTTCG	..A.A.A.AA..A...GG.....A.....G.C.TACTCAAG
	D.asp3	TATTCT.CTA..GAACT	T.GG.T.	..CTTCG	..A...A.AA..A...GG.....AT..T..G.C.TACTCAAG
	D.asp4	TATT.T.CTA..GAACT	T..G.TC	..CTTCG	..A...A.AA..A...GG.....AT..T..G.C.TACTCAAG
	D.gib31	TATT.TC.T.A.GAACT	TT...TC	.ACTTCG	.....A.....C.....
	D.gib33	TATT.TC.T.A.GAACT	TT...TC	.ACTTCG	.....T.....C.....
	D.gib36	TATT.TC.T.A.GAACT	TT...TC	.ACTTCG	...A.....T.....C.....
	D.sty1	..T.....A.A....T	....T..	G..T...	AT...AA..AGT.T.G..CACGTCAT.T...AC.GCA.....A.
	D.sty6	..T.....A.A....T	....T..	G..T...	A....AA..AGT.TAG..CACGTCAT.T.G.AC.GCA..C...A.

Phylogenetic analysis

Phylogenetic relationships between the species were estimated from the sequences of mitochondrial and nuclear DNA fragments (Fig. 15A–B) as well as from the structural characters of the adults (Fig. 16). *Dolerus docilus*, probably a very closely related species to *D. asper*, was excluded from the study because suitable material for molecular analysis was unavailable.

Molecular data

Fragments of the mtDNA genes (*cyt*b, COI and COII) and of nuclear DNA (ITS2) from 3 specimens of each species were PCR-amplified and both strands sequenced. 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 making phylogenetic inferences. The number of variable and parsimony informative sites of the analysed gene fragments is as follows: 17 and 15 for *cyt*b, 7 and 6 for COI, 7 and 7 for COII, and 45 and 40 for ITS2, respectively (Table 1). Altogether 76 variable and 68 parsimony informative sites were found. The consensus trees from unweighted MP analyses of ITS2 alone and combinations of COI + ITS2 and COII + ITS2 have the topology [*D. asper*, (*D. brevicornis*, *D. gibbosus*)]. The consensus tree of the 12 most parsimonious trees with length 61 is given in Fig 15A. The same topology resulted if the ME criterion was used and both transitions and transversions were included. However, the topology [*D. brevicornis*, (*D. asper*, *D. gibbosus*)] emerged from the ME analysis of COI + ITS2 and COII + ITS2 if only transitions were considered. Furthermore, all consensus trees inferred from unweighted MP and NJ analyses of all other possible combinations of the sequenced markers had topology [*D. brevicornis*, (*D. asper*, *D. gibbosus*)]. The MP analysis based on all four markers combined (1624 bp) yielded three most parsimonious trees with length 91. The resulting consensus tree, with 75% cut-off value and bootstrap support values indicated, is given in Fig. 15B. Briefly, the phylogenetic analyses using most markers and their combinations resulted in the topology [*D. brevicornis*, (*D. asper*, *D. gibbosus*)], with very high bootstrap support for the clade *D. asper* + *D. gibbosus* (see Fig. 15B), while only a few resulted in the topology [*D. asper*, (*D. brevicornis*, *D. gibbosus*)]. In the latter, the clade *brevicornis* + *gibbosus* had low bootstrap support (see Fig. 15A).

TABLE 2. Data matrix of 6 morphological characters imaginal scored for the four species. Characters 1, 2 and 4 are parsimony informative while 3, 5 and 6 are not. All characters except 5 (Wagner character) are treated as unordered. * – outgroup.

Species / Characters	1. 2. 3. 4. 5. 6.
D. asper (D.asp)	1 1 0 1 1 1
D. brevicornis (D.bre)	0 0 1 1 1 0
D. gibbosus (D.gib)	1 1 0 0 2 0
D. stygius (D.sty)	0 0 0 0 0 0

1. Clypeus outline: symmetric = 0; asymmetric = 1.
2. Clypeal emargination compared to median length of clypeus: shallow (about 0.35) = 0; deep (about 0.5) = 1.
3. Setae on upper part of head: long = 0; short = 1.
4. Ventroapical hooklike process of penis valve: small = 0; large = 1.
5. Apex of penis in dorsal view: uniform = 0; gradually broadening = 1; sharply broadening = 2.
6. Postocellar furrows in males: distinct = 0; obsolete = 1.

Morphological data

In this study, only imagos were used as their larval stages are insufficiently known. Of the 6 variable structural characters, three were parsimony informative and three uninformative (Table 2). An unweighted MP analysis of the morphological dataset gave a single most parsimonious tree of 8 steps (Fig. 16). The clade *asper* + *gibbosus* with bootstrap support 71 is supported by two synapomorphies: asymmetric clypeus and clypeal emargination about 0.5 × as deep as its median height. We found no morphological synapomorphies for the clade *asper* + *brevicornis* + *gibbosus*, which gained very high bootstrap support (99–100) from the phylogenetic analyses of molecular data (see Fig. 15A–B).

Notes on phenology, host plant and distribution

The phenology diagrams (Fig. 3) for the adults of *D. asper* and *D. brevicornis* are based on 200 female and 237 male specimens captured by MN and J. Nuorteva in Vuohiniemi, Southern Finland (60°57'N, 24°04'E) from April to July during 1946–1997. In comparison with *D. asper* the flight of *D. brevicornis* begins and ends earlier.

For both species (*D. asper* and *D. brevicornis*) one male was reared from a larva collected on *Carex nigra* (L.) by T. Kontuniemi in 1949 and included in his material of *D. asper* (currently in the coll. M. Viitasaari) but not reported in Kontuniemi (1960). The ex ovo rearing of the species was successfully carried out on *Carex cespitosa* and *C. nigra* by MH in 2002–03. O. Conde described and reared the larvae of *D. carbonarius* Zaddach on *Deschampsia*, *Poa* and *Carex stricta* or *C. cespitosa*, and *D. oblongus* Cameron on *Carex* sp. (Conde, 1933). We have not studied the material reared by Conde, and its taxonomic composition is unknown to us.

Dolerus asper and *D. brevicornis* are common in the Palearctic Region. The current distribution of the less common *D. gibbosus* seems incomplete, but differs in its distribution compared with the two former species, as it occurs in Estonia and is absent from Finland (Viitasaari et al., 1998). Based on collections, it seems that *D. asper* is more common in Lapland than *D. brevicornis*. There are numerous records of *D. asper* from Finland north of the Polar Circle (the biological provinces: KemL, EnL and InL) and similarly for Norway and Russia, but for *D. brevicornis* there are three such records. We have not studied the Nearctic material of *D. asper* and the holotype female of *Dolerus tectus* MacGillivray, 1914, but the illustration of the penis valve of *D. asper* in Goulet (1986: Fig. 170a) indicates that *D. asper* occurs in the Nearctic. Also, the outline of the mesepisternum with short setae in Goulet (1986: Fig. 30) suggests that *D. brevicornis* should be present in the Nearctic. A study of the North American specimens is required to confirm the occurrence of *D. brevicornis* in the Nearctic Region.

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