A larval key to the Drusinae species (Trichoptera: Limnephilinae) of Austria, Germany, Switzerland and the dinaric western Balkan[1]

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A b s t r a c t : A larval key of the Drusinae of Central Europe and the dinaric western Balkan is presented. Phylogeographic relationships are discussed in the light of molecular genetics, feeding ecology and larval morphology.

K e y w o r d s : Larval stages, Drusinae, key, feeding type, phylogeny.

Introduction

Geographically, the subfamily Drusinae is restricted to Eurasian mountains from the Caucasus in the East to the Iberian Peninsula in the south-west. Three quarters are endemics limited to a single or very few mountain ranges, making the group an ideal model for studying evolutionary processes like speciation and diversification. The evolutionary interest in the Drusinae is demonstrated by a number of key papers and species grouping concepts (SCHMID 1956, MARINKOVIC-GOSPODNETIC 1976, KUMANSKI 1988, MALICKY 2005, SIPAHILER 1999, 2002). Recently, mitochondrial DNA sequence data have proved to be successful in detecting genetic differentiation within *D. discolor*, *D. romanicus*, *D. croaticus* and others (PAULS 2004, PAULS et al. 2006, 2008, LEHRIAN et al. 2008, PREVIŠIĆ et al. 2009). These studies provide various scenarios on when and how diversification and major climatic shifts correlate, generating general hypotheses on the role of Pliocene-Pleistocene climate change in aquatic insects which may have reacted very differently than terrestrial species. Most species presumably retreated to refugia during periods of unfavourable climate conditions (HEWITT 1996, 2000, BERNATCHEZ & WILSON 1998, WIDMER & LEXER 2001, SCHMITT & SEITZ 2001, HÄNFLING et al. 2002, SCHMITT & HEWITT 2004, HABEL et al. 2005, HAUBRICH & SCHMITT 2007) where isolated populations diverged genetically before they postglacially started to recolonise Central Europe (BENNET et al. 1991, HEWITT 1999). Where secondary contact between previously isolated lineages occurs, suture zones and hybridisation are often observed between lineages (ZWICK 1982b, TABERLET et al. 1998, HEWITT 2000, BARTON 2001, PFENNINGER & POSADA 2002, LEESE 2004), although this was never observed in Drusinae. Pleistocene range expansion and regression patterns and postglacial migration routes have been frequently discussed and fairly well studied in aquatic organisms (e.g. THIENEMANN 1950, ILLIES 1953, ZWICK 1982a, 1982b, MALICKY 1983, ENGLBRECHT et al. 2000, HÄNFLING et al. 2002, RIBERA & VOGLER 2004, ŠLECHTOVÁ et al. 2004). For caddisfly species inhabiting cold, turbulent running water in hard-substrate channels, MALICKY (1983) suggests that favourable habitats existed in periglacial mountain streams in the vicinity of the inland ice sheets (e.g. Koralpe,

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[1] This paper is dedicated to Prof. Dr. Hans Malicky on the occasion of his 75th birthday.

Soboth, South-Western Alps) which may have served as glacial refugia for caddisflies in general (MALICKY 1983, 2006, ENGELHARDT et al. 2008) and Drusinae in particular (GRAF et al. 2009, PAULS et al. 2006). In addition, a recent multilocus molecular phylogeny has raised the question whether shifts in feeding ecology in the Drusinae may have been a key innovation, driving most of the diversification in the subfamily (PAULS et al. 2008). Taken together, Drusinae provide ideal study objects for testing hypotheses on relationships within and between genera and the trait evolution in these organisms at a worldwide scale by fusing both phylogeographical, morphological and genetic approaches.

In addition to this phylogenetic appeal, caddisflies are considered primary indicator taxa in monitoring water quality (Barbour et al. 1999, Smith et al. 1999, BARBOUR & YODER 2000, WRIGHT et al. 2000, AQEM consortium 2002, GRAF et al. 2002, HERING et al. 2006). Unfortunately, contemporary larval keys of European Trichoptera are still incomplete. Adult Trichoptera can be identified using the excellent key by MALICKY (2004). For caddisfly larvae in Austria and its surroundings, WARINGER & GRAF (1997, 2004) condensed the present knowledge on larval taxonomy. Since then, several new descriptions of Central European Drusinae larvae were published (WARINGER et al. 2007b, 2008a, b, GRAF et al. 2005, GRAF et al. 2009) which provided additional information. In the present paper we concentrate, therefore, on this larval-taxonomic aspect of subfamily Drusinae by providing a key for all known species of Austria, Germany, Switzerland and the dinaric western Balkan. We hope that this key will be helpful in using the high bioindication potential of this aquatic insect group.

Material and methods

The key is based on numerous larval collections made in Austria, Germany, Switzerland and Italy within the last twenty years. To support conspecific association between larval and adult specimens either ripe pupae were collected, final instar larvae reared to emergence or we sequenced and analysed a 498bp long fragment of the mitochondrial cytochrome oxidase I gene (mtCOI) of larvae and adults following the methods outlined in PAULS (2004) and PAULS et al. (2006). We generated uncorrected pairwise distances between individuals using the DNADist function as implemented in BioEdit 7.0.5.3 (HALL 1999). To further support the association of specimens we estimated a phylogeny using new and previously published sequence data. The Phylogeny was estimated using a Bayesian approach. Bayesian Markov chain Monte Carlo (B/MCMC) analysis was performed using the program MrBayes 3.1.2 (HUELSENBECK et al. 2001) assuming the GTR + I + G model. Two parallel MCMC samplings were performed with 4 simultaneous chains each for 5 million generations. Trees were sampled every 1000th generation for a total of 5000 trees from each sampling run. Log likelihood scores of samples were plotted against generation time using Tracer 1.3 (http://evolve.zoo.ox.ac.uk/ software.html?id=tracer) to determine stationarity (HUELSENBECK et al. 2001). The initial 2500 trees of each run were discarded as 'burn-in'. In a subsequent morphological analysis larval standard characters that allow morphological differentiation were checked and used for constructing the key.

Drusinae of Austria Germany, Switzerland and the dinaric western Balkan

Currently, 24 Drusinae species are reported from Austria Germany and Switzerland: sixteen species of genus *Drusus*, four *Ecclisopteryx* species, two *Metanoea* species and one species each of genus *Anomalopterygella* and *Crypthothrix* (LUBINI-FERLIN & VICENTINI 2005,

MALICKY 1999, 2009, ROBERT 2004, Table 1). In the dinaric western Balkan, the larvae of four species/subspecies are known (Table 2). Although Drusinae are restricted to European mountain ranges, chorological patterns within the area of Austria, Germany and Switzerland are highly variable. There are widespread species such as *Drusus annulatus*, *D. biguttatus*, *D. chrysotus*, *D. discolor*, *Ecclisopteryx guttulata* and *E. madida* which cover large areas within the framework of this key as well as species with more restricted distribution patterns, for example *Cryptothrix nebulicola* (an alpine species lacking in Germany), *Drusus improvisus*, a species of the Apennine peninsula which spreads northwards to the Swiss Alps (Table 1) or *D. croaticus* which is known only from Croatia and Slovenia (Table 2). Especially interesting are closely related species pairs which show a distinct east-west disjunct distribution pattern in the Alps (Fig. 1): according to MALICKY (2004), *D. alpinus* is restricted to the western Alps; records exist from Switzerland (LUBINI-FERLIN & VICENTINI 2005) and Italy (CIANFICCONI 2002). *Drusus franzi* is a micro-endemic species of the eastern Alps in Austria, covering a small area situated mainly in intermediate altitudes of Saualpe, Soboth and Weinebene in south-eastern Austria (Styria and Carinthia). East-west alpine disjunctions are known in *Metanoea rhaetica* (east) and *M. flavipennis* (west), *Drusus monticola* and *D. nigrescens*, *D. adustus* and *D. melanchaetes*, *D. chrysotus* and *D. muelleri* (Fig. 1), but also among alpine Limnephilinae, e.g. *Allogamus* (*uncatus* and *mendax*).
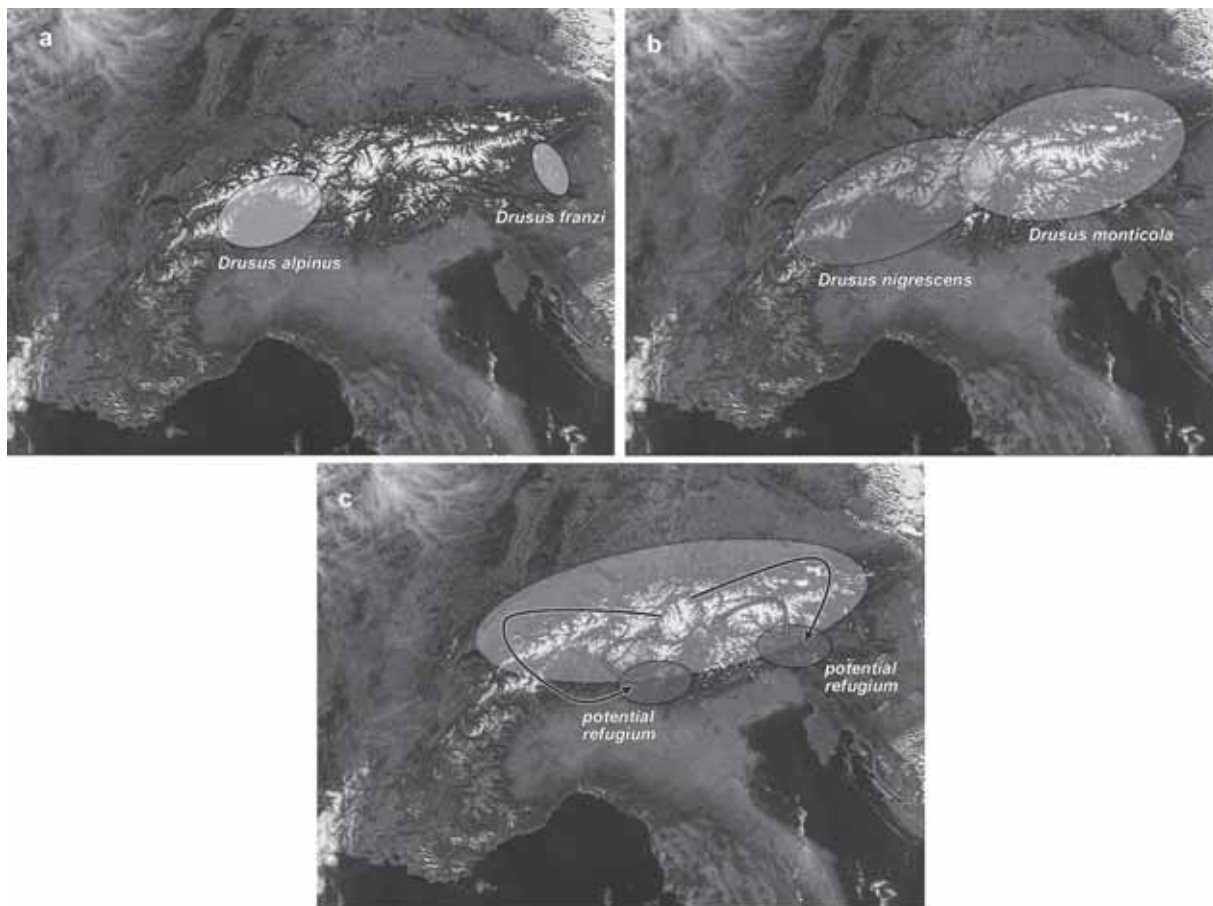
Fig. 1: Examples of alpine east-west disjunct distribution patterns of genetically identified Drusinae sister species (cf. Fig. 2): a) *Drusus franzi – Drusus alpinus*; b) *Drusus monticola – D. nigrescens*; c) Hypothetical scenario illustrating the separation of an ancestral taxon by regression into southwestern and southeastern refugia due to increasing glaciation of the central Alps (blue arrows). In the refugial areas speciation took place; after glaciation, the new species recolonized the central Alps and secondary contact zones (redrawn after GRAF et al. 2007).

These patterns imply comparable mechanisms of regression, speciation and subsequent expansion. Alpine mountain chains are aligned primarily in west–east orientation and represent massive dispersal barriers. Most probably major valley systems served as recolonisation pathways into the central Alps from southern or circum-alpine refugia. The disjunction of sister species reveals a new aspect in reconstructing the Pliocene and Pleistocene history of glacial survival and recolonisation of formerly glaciated areas in freshwater insects. The patterns may support the Dinodal concept of MALICKY (1983), which suggests preglacial and glacial species-specific areas within the Alps. Patterns of east-west lineage diversification have also been observed within species (e.g. *D. discolor*, PAULS et al. 2006, *Rhyacophila pubescens*, ENGELHARDT et al. 2008). Areas of contact between these species pairs or intraspecific lineages vary in size. For example, *D. alpinus* and *D. franzi* are restricted to small areas in the western and eastern Alps, respectively, and are separated by several hundred kilometres (Fig. 1, GRAF et al. 2009). On the other hand, many examples of species pairs with eastern and western areas greatly extend towards the central Alps, thereby representing a model group for speciation processes within glacial refugia and a subsequent expansion east- and westwards within the alpine chain. Independent of range proximity or overlap, the observed disjunctions all fit well within the east-west-separation of the Alps along the "Splügen-line" in eastern Switzerland and western Austria. Thus, while actual distribution patterns of closely-related species pairs are relatively well known and documented, the mechanisms of regression, diversification and subsequent expansion are poorly understood.

Phylogenetic analysis

The phylogenetic analysis (Fig. 2) supports monophyly of the subfamily Drusinae (clade 1). All species where more than one specimen was analysed are monophyletic, although not always significantly supported. Within the Drusinae, there is a basal clade with *Cryptothrix nebulicola*, *Drusus muelleri*, *D. romanicus*, *D. chrysotus* and *D. discolor* (clade 2, Fig. 2). Within this clade, *D. muelleri* and *D. chrysotus* are basal to *D. discolor* and *D. romanicus*. The remaining species fall into two major clades. One clade (clade 4, Fig. 2) includes *Drusus alpinus* and *D. franzi*. Clade 4 is sister to a clade comprising the remaining Drusinae (clade 5). Clade 5 falls into four supported smaller clades: clade 5a comprises two well-resolved sister groups with *Drusus balcanicus* and *D. discophorus pallidus* and *D. botosaneanui*, *Ecclisopteryx dalecarlica*, *E. guttulata* and *E. madida*, respectively. Clade 5b groups *D. trifidus* and *D. brunneus*. Sister to this clade is *Anomalopterygella chauviniana*. *Drusus annulatus* and *D. rectus* form an unsupported clade which is basal to clades 5a and 5b. Clade 5c groups *E. asterix* and *E. malickyi*. Finally, clade 5d groups the remaining *Drusus* species. The current generic concept is not supported by this analysis. The genera *Anomalopterygella*, *Cryptothrix*, *Ecclisopteryx* and *Metanoea* are nested in *Drusus*. *Ecclisopteryx* is polyphyletic and falls into two separate clades. Within Drusus three groups distinguished by adult genital morphology were supported: the *Drusus alpinus* group, *D. discophorus*-group and *D. bosnicus*-group. The remaining species groups within *Drusus* are not supported in our study.

Fig. 2: B/MCMC inference for 53 Drusinae taxa based on 1468 base pairs from three gene regions (mtCOI, mtLSU nuWG). Bold branch ends indicate posterior probabilities ≥ 0.95. Numbers on the nodes indicate clades referred to in the text. Shown is the 50 % majority rule consensus tree from 80002 trees samples in two parallel runs with 11 heated and 1 cold chain each. Tree space was searched for 5000000 generations. Boxes on the right indicate mandible with teeth (T), spoon-shaped mandible without teeth (S), presence of filtering bristles (B) and feeding type (F). Taken from PAULS et al. (2008).
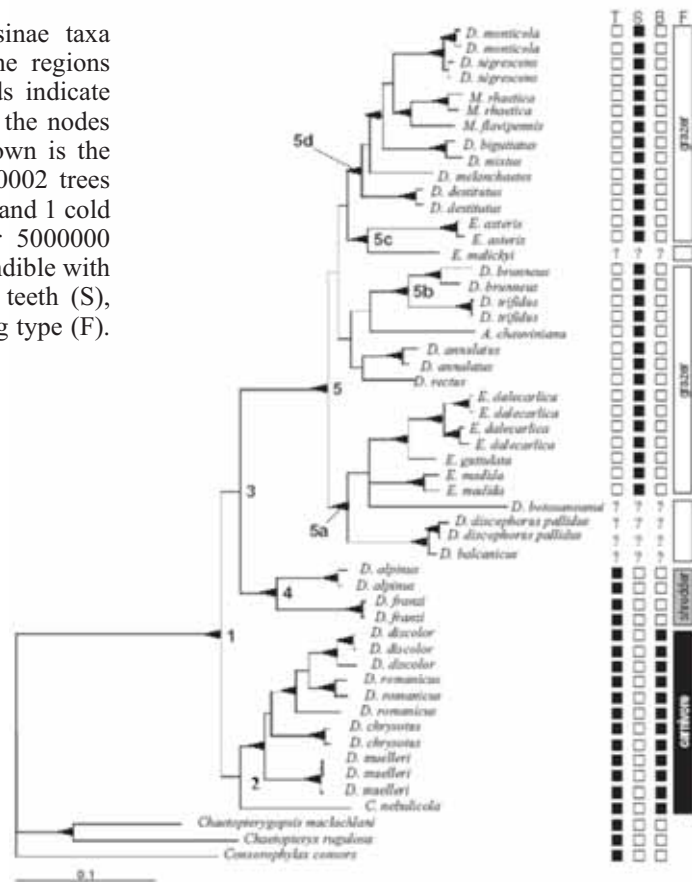
Larval key to the Drusinae larvae of Austria, Germany, Switzerland and the dinaric western Balkan

Larvae of family Limnephilidae share the following set of morphological characters:

- Sclerites present on pro-, meso- and metanotum (Fig. 3p, s, t) except Apataniinae (*Apatania* spp.), some Stenophylacini (*Parachiona picicornis*, *Leptotaulius gracilis*) and Limnephilini (*Limnephilus hirsutus*, *L. extricatus*).

- Mesonotum completely covered by one sclerite or more than one sclerite in close contact to each other (Fig. 3s).

- Metanotum incompletely sclerotized (Fig. 3t).

- Mesonotum covered by two sclerites (Fig. 3s).

- Prosternal horn present (Fig. 4, arrow) except *Drusus chrysotus* which is immediately identified by the deep median concavity (Fig. 5) of its head capsule.

- Fleshy protuberances present laterally and dorsally at first abdominal segment (Fig. 3, arrows); antenna distinctly offset from anterior eye margin (Fig. 6, arrow).

Species groups within Limnephilidae may be identified by the following key.

Key to Limnephilidae species groups

1   Larvae terrestrial; gills are lacking in all instars (Fig. 7) ....*Enoicyla* spp.

-    Larvae aquatic, final instars with gills (e.g. Fig. 3g)...2

2   Metanotum covered by five (Fig. 8t) or six (Fig. 3t) small sclerites..3

-    The anterior and sometimes also the posterior metanotal sclerites are replaced by groups of setae (e.g. Fig. 10, arrows) 4

3   Metanotum with one pair of lateral sclerites, one pair of posteromedian sclerites and a single anteromedian sclerite (Fig. 8t); first abdominal sternum with two large sclerites bearing setae (Fig. 9).. *Hydatophylax infumatus*

-    Metanotum with three pairs of sclerites (Fig. 3t) 6

4   Gills consisting of single filaments only (e.g. Fig. 3g) 5

-    Some gills consisting of two to four filaments, the remaining of single filaments (Fig. 11) ....
.... some Limnephilini

5   In the anterior third of the pronotum a transverse rim is present (Fig. 12, arrow) ....
....some Chaetopterygini and Stenophylacini

-    Without transverse rim at the anterior third of the pronotum (Fig. 13, arrow) .... *Apatania* spp.

6   Abdominal dorsal and ventral gills consisting of 8-24 filaments (Fig. 14).............. *Ironoquia dubia*

-    Abdominal dorsal and ventral gills consisting of 1-4 filaments (Figs 3g, 11) 7

7   Some gills consisting of 2-4 filaments (Fig. 11), the remaining of single filaments..........................
....Limnephilini and *Mesophylax*

-    All gills consisting of single filaments (Fig. 3g)..
....Drusinae, Chaetopterygini and Stenophylacini

Key to Drusinae **(Chaetopterygini and Stenophylacini not keyed out in detail)**

1   Cutting edge of mandibles with teeth (Fig. 15)..2

-    Mandibles without teeth along cutting edge (Fig. 16) 7

2   Long filtering bristles are present at the first abdominal sternum and at the legs (Fig. 17).............3

-    Long filtering bristles are missing at the legs; first abdominal sternum with short setae only (e.g. Fig. 18) 5

3   Head and pronotum with a dense layer of woolly hairs (Fig. 19)..............................*Drusus discolor*

-    Without dense woolly hairs at head and pronotum...4

4   Frontocylpeal suture running in a rim-like depression (Fig. 20)....................*Cryptothrix nebulicola*

-    Dorsal head surface with a deep concavity (Fig. 5) ....*Drusus chrysotus*

-    Dorsal head surface distinctly flattened and coarsely granulated; anterior section of frontoclypeus steeply sloped (Fig. 21) ....*Drusus muelleri*

5   Anterior third of pronotum without transverse rim; posterior third of pronotum with a small, rounded hump; pronotal surface granulated (Fig. 22) 6

-   Anterior third of pronotum with transverse rim; pronotal surface smooth (as in Fig. 12, arrow); most species of Chaetopterygini and Stenophylacini key out here (not included in this key)

6   At each median insertion of mid and hind coxa, only one seta is present (Fig. 23) (Eastern Alps).... ....*Drusus franzi*

-   At each median insertion of mid and hind coxa, small groups of 3-4 setae are present (Western Alps) .... *Drusus alpinus*

7   In addition to standard setation, short spines or tapering bristles are present at the head capsule (e.g. Fig. 24) 8

-   In addition to standard setation, spinules (small spines, approximately 0.03 mm long) are present at the head capsule (laterally at inner eye margins, Fig. 48) 24

-   Without additional spines or bristles at the head capsule 11

8   Pronotum dorsally rounded (Fig. 24) 9

-   Pronotum with a distinct dorsal ridge (Fig. 25) 10

9   Head and pronotum yellow, pronotal spines blackish-brown (Fig. 24) .....*Ecclisopteryx dalecarlica*

-   Head and pronotum blackish-brown, pronotal spines yellow (Fig. 26, arrows) .... *Drusus trifidus*

10 The number of frontoclypeal setae is 12 – 16; at least some head spines are yellowish in colour (Abb. 27) .... *Ecclisopteryx guttulata*

-   The number of frontoclypeal setae is >20; head spines dark (Fig. 25) ....*Ecclisopteryx madida*

11 Sternum of first abdominal segment with a large sclerotized patch (as in Fig. 23s).......................12

-   Sternum of first abdominal segment without a large sclerotized patch; small sclerites at the base of individual setae may be present (Fig. 18) 14

12 Posterolateral gills are lacking at the second and third abdominal segment .... *Metanoea rhaetica*

-   Posterolateral gills are present at the second and third abdominal segment 13

13 Pronotum with a prominent, sharp dorsal ridge (Fig. 28) (Western Alps)............*Drusus nigrescens*

-   Pronotum with high, rounded dorsal hump; in the geographical framework of this atlas, in Switzerland only (Fig. 29)..*Drusus improvisus*

-   Pronotum with rounded dorsal hump which is very low in profile (Fig. 30)... *Metanoea flavipennis*

14 Pronotum with distinct dorsal ridge (Figs 31, 32)..15

-   Pronotum without distinct dorsal ridge, +/- evenly rounded (e.g. Figs 33, 34) 16

15 Dorsal pronotal ridge high, extending anterolaterally and reaching the anterior edges of the pronotum (Fig. 31) .... *Anomalopterygella chauviniana*

-   Dorsal pronotal ridge much lower and not extending anterolaterally, ridge positioned in the posterior third of the pronotum (Fig. 32) ....*Drusus monticola*

-   Dorsal pronotal ridge much lower and not extending anterolaterally, positioned in the posterior half of the pronotum and divided into two humps by the ecdysial line (Figs 49, 50) (Dinarides; Bosnia & Herzegovina) ....*Drusus ramae*

16 Faces of mid and hind femora without setae (Fig. 35)............................. *Melampophylax mucoreus* ....*Melampophylax nepos*

In *M. mucoreus*, hind tarsal length of fifth instar larvae is ≥ 0.77 mm, there are 9-10 posterodorsal setae on the eighth abdominal dorsum and the sclerotized setal bases at the first abdominal sternum are almost never fused. In *M. nepos*, hind tarsal length of fifth instar larvae is < 0.60 mm, there are 11-14 posterodorsal setae on the eighth abdominal dorsum and there is a marked tendency of fusing of the sclerotized setal bases at the first abdominal sternum.

- Setae are present at the faces of mid and hind femora (e.g. Fig. 36) 17

17 Anteromedian metanotal tergites large and ovoid (Fig. 37)...18

- Anteromedian metanotal tergites small and narrow (Fig. 38)......................... *Micropterna testacea*

18 A row of long setae along the anterior pronotal margin extends almost up to center (Fig. 39, arrows)...19

- At center of the anterior pronotal margin long setae are lacking 23

19 Dorsal-edge setae of mid and hind tibia are restricted to the distal third of the tibia (Fig. 40 b)....21

- Dorsal-edge setae of mid and hind tibia are present along the whole length of the tibia (Fig. 40 a).. 20

20 Lateral profile of frons straight, with a small step at eye level (Fig. 41); pronotum with ventrolateral bulge (Fig. 42, arrow); 2-4 dorsal setae are present at the eighth abdominal segment (Western Alps) .... *Drusus melanchaetes*

- Lateral profile of frons rounded (Fig. 43); a ventrolateral bulge is lacking at the pronotum; 4-8 dorsal setae are present at the eighth abdominal segment (Eastern Alps)...................*Drusus adustus*

21 Lateral fringe starting at last third of third abdominal segment; dorsal profile of pronotum, in lateral view, is angled (Fig. 44) (Southern Alps) .... *Ecclisopteryx asterix*

- Lateral fringe starting at anterior border of third abdominal segment 22

22 Dorsal profile of pronotum distinctly angled (Fig. 45) .... *Drusus annulatus*

- Dorsal profile of pronotum evenly rounded (Fig. 46)... *Drusus biguttatus*

23 Pronotum bearing numerous light spines (Fig. 47) .... *Drusus trifidus*

- Pronotum without numerous light spines; distribution: Northern Switzerland............*Drusus mixtus*

- Pronotum without numerous light spines; distribution: Croatia, Slovenia .... *Drusus croaticus* (Fig. 51)

24 Anterodorsal part of pronotum with numerous white, recumbent setae (Fig. 52) (Dinarides; Bosnia & Herzegovina)... *D. medianus*

- Anterodorsal part of pronotum without numerous white, recumbent setae (Fig. 53) (Dinarides; Bosnia & Herzegovina)...*D. radovanovici septentrionis*
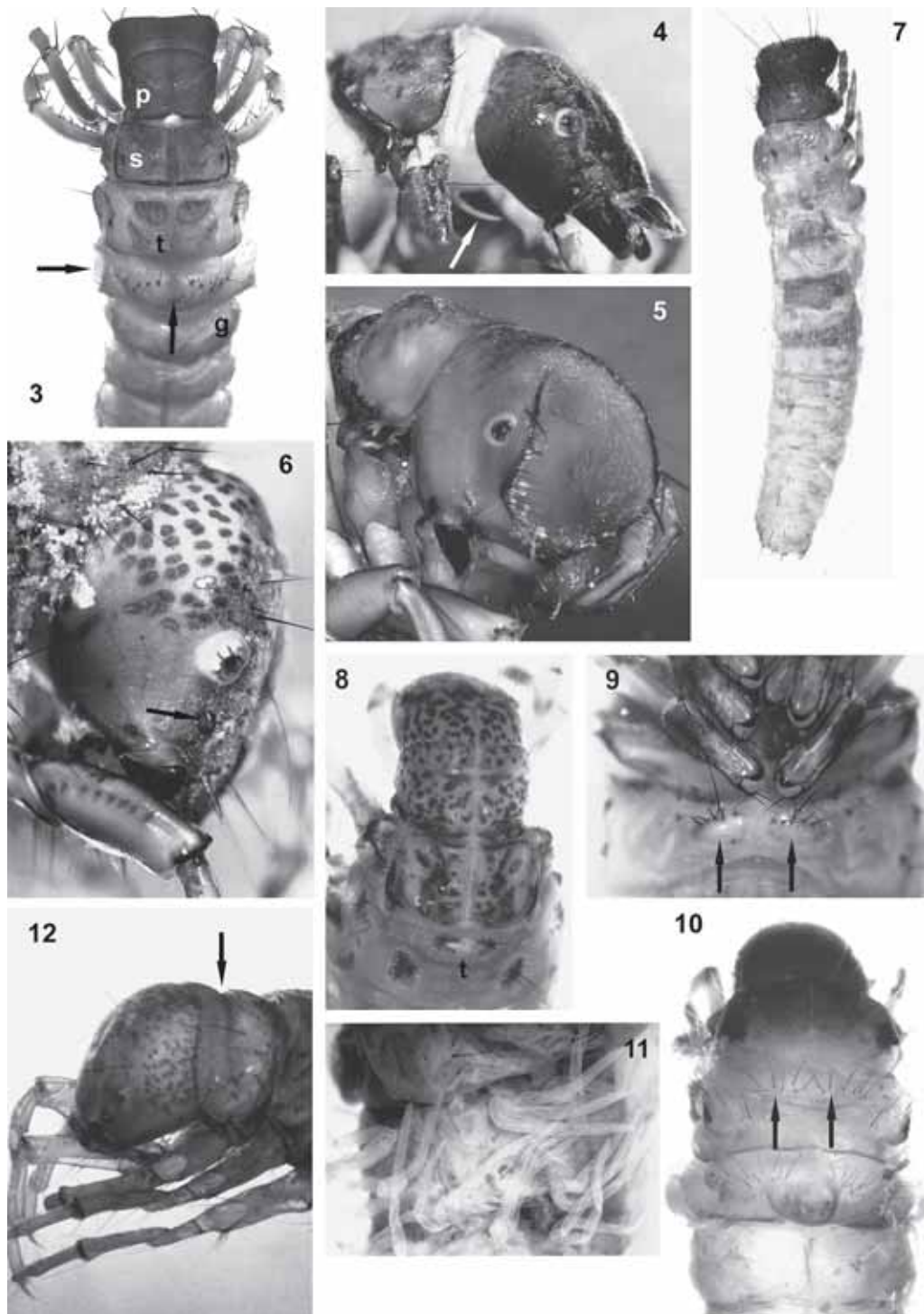
Figs 3-12: (**3**) *Drusus chrysotus*, dorsal view of fifth instar larva; (**4**) *Annitella apfelbecki*, fifth instar larva, head and pronotum, right lateral view; (**5**) *Drusus chrysotus*, fifth instar larva, head and pronotum, right fronto-lateral view; (**6**) *Halesus* sp., fifth instar larva, head, right lateral view; (**7**) *Enoiclya reichenbachi*, fifth instar larva, dorsal view; (**8**) *Hydatophylax infumatus*, fifth instar larva, dorsal view; (**9**) *Hydatophylax infumatus*, fifth instar larva, first abdominal sternum, ventral view; (**10**) *Apatania* sp., fifth instar larva, dorsal view; (**11**) *Grammotaulius nigropunctatus*, fifth instar larva, gills of second abdominal segment; left lateral view; (**12**) *Grammotaulius nigropunctatus*, fifth instar larva, head and pronotum, left lateral view.
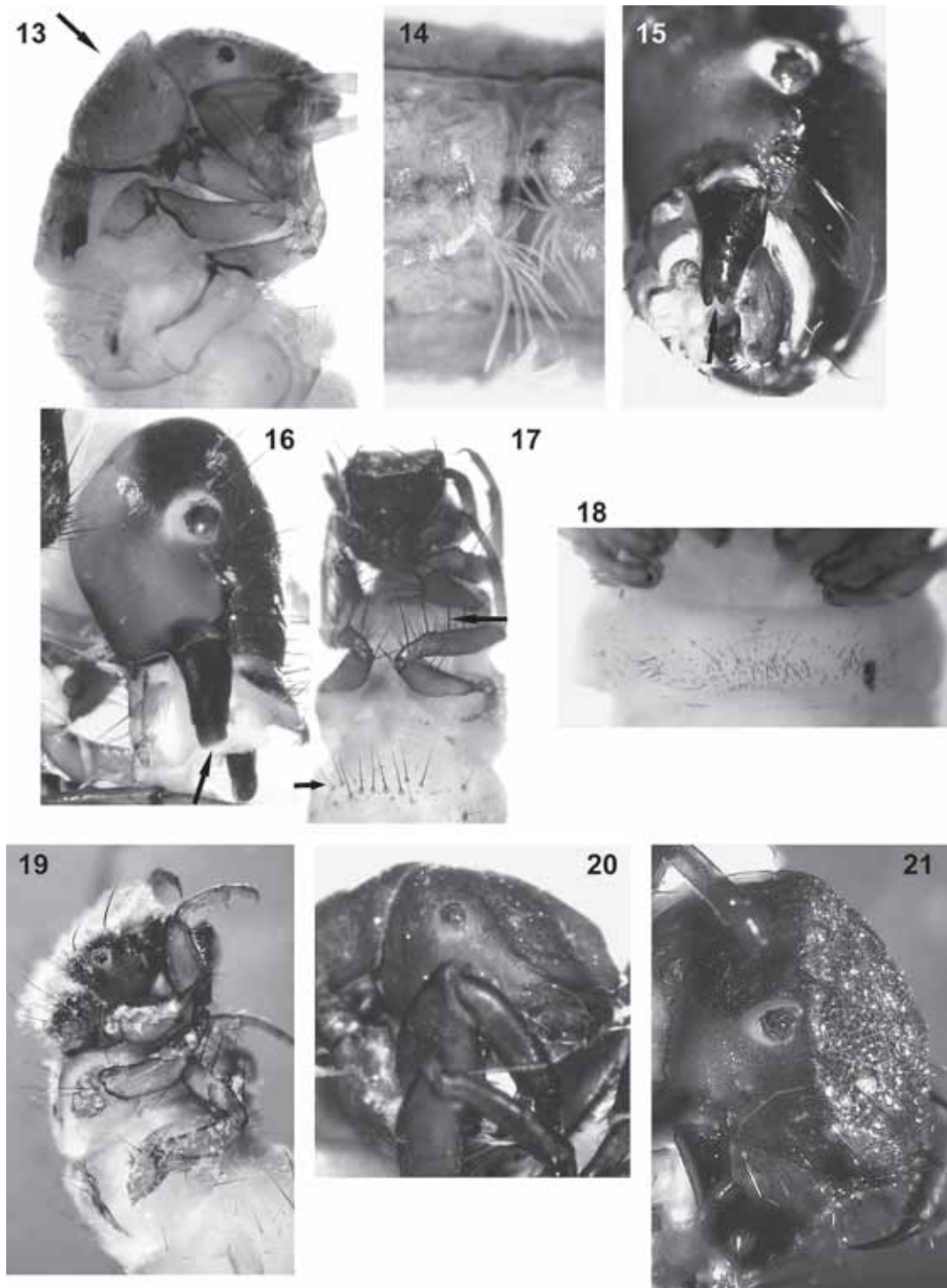
Figs 13-21: (**13**) *Apatania* sp., fifth instar larva, head and thorax, right lateral view; (**14**) *Ironoquia dubia*, fifth instar larva, gills; (**15**) *Drusus franzi*, fifth instar larva, mandible; (**16**) *Drusus improvisus*, fifth instar larva, mandible; (**17**) *Drusus muelleri*, fifth instar larva, ventral view; (**18**) *Drusus monticola*, fifth instar larva, first abdominal sternum; (**19**) *Drusus discolor*, fifth instar larva, head and thorax, right lateral view; (**20**) *Cryptothrix nebulicol*a, fifth instar larva, head; (**21**) *Drusus muelleri*, fifth instar larva, head.
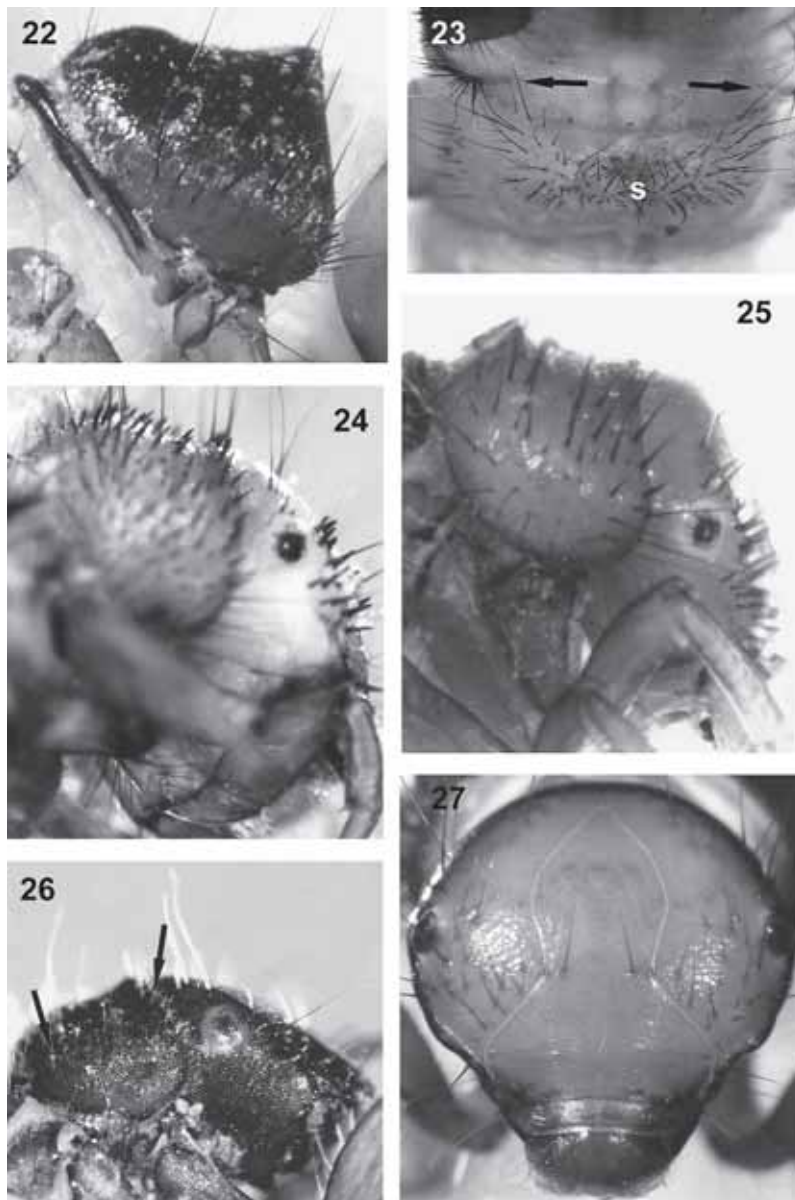
Figs 22-27: (**22**) *Drusus improvisus*, fifth instar larva, pronotum from right; (**23**) *Drusus franzi*, fifth instar larva, metathorax and first abdominal segment, ventral view; (**24**) *Ecclisopteryx dalecarlica*, fifth instar larva, head and pronotum from right; (**25**) *Ecclisopteryx madida*, fifth instar larva, head and pronotum from right; (**26**) *Drusus trifidus*, fifth instar larva, head and pronotum from right; (**27**) *Ecclisopteryx guttulata*, fifth instar larva, head.
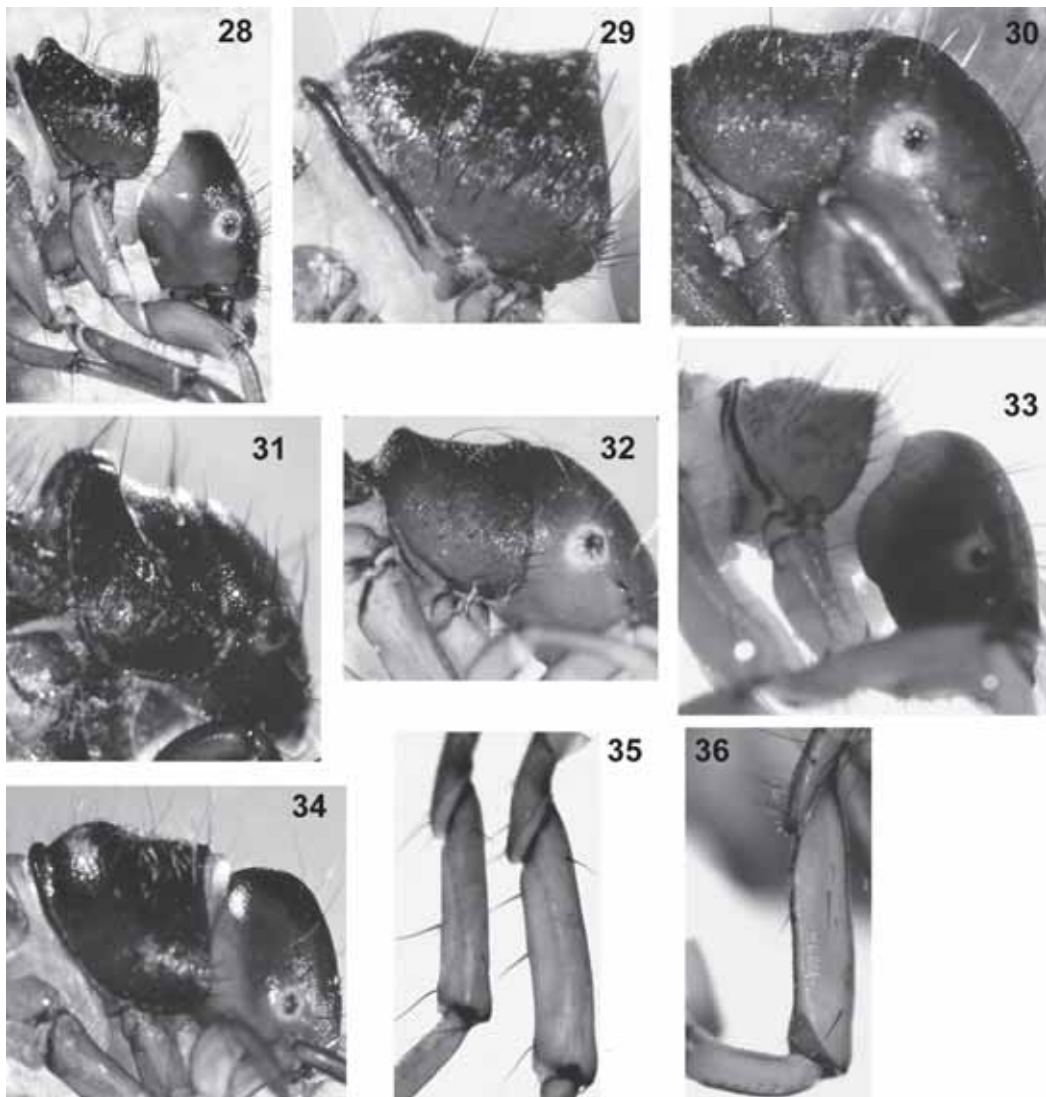
Figs 28-36: (**28**) *Drusus nigrescens*, fifth instar larva, head and pronotum from right; (**29**) *Drusus improvisus*, fifth instar larva, pronotum from right; (**30**) *Metanoea flavipennis*, fifth instar larva, head and pronotum from right; (**31**) *Anomalopterygella chauviniana*, fifth instar larva, head and pronotum from right; (**32**) *Drusus monticola*, fifth instar larva, head and pronotum from right; (**33**) *Consorophylax* sp., fifth instar larva, head and pronotum from right; (**34**) *Drusus adustus*, fifth instar larva, head and pronotum from right; (**35**) *Melampophylax mucoreus*, fifth instar larva, faces of mid and hind femur; (**36**) *Stenophylax permistus*, fifth instar larva, face of mid femur.
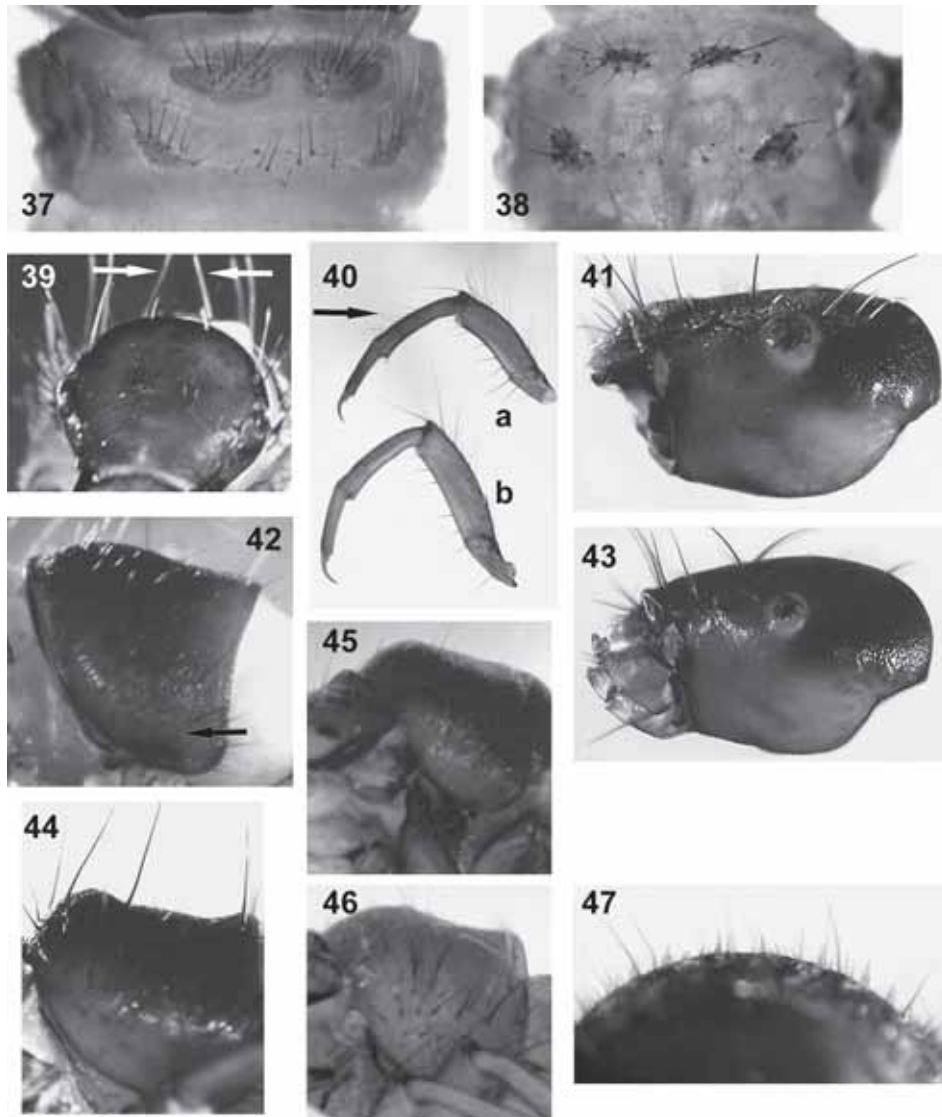
Figs 37-47: (**37**) *Drusus adustus*, fifth instar larva, metanotum, dorsal view; (**38**) *Micropterna testacea*, fifth instar larva, metanotum, dorsal view; (**39**) *Drusus melanchaetes*, head and pronotum, anterior view; (**40**) mid tibia, fifth instar larvae; (**a**) *Drusus adustus*; (**b**) *Drusus biguttatus*; (**41**) *Drusus melanchaetes*, fifth instar larva, head from left (Photo: W. Lechthaler); (**42**) *Drusus melanchaetes*, fifth instar larva, pronotum from right; (**43**) *Drusus adustus*, fifth instar larva, head from left (Photo: W. Lechthaler); (**44**) *Ecclisopteryx asterix*, fifth instar larva, pronotum from right; (**45**) *Drusus annulatus*, fifth instar larva, pronotum from right; (**46**) *Drusus biguttatus*, fifth instar larva, pronotum from right; (**47**) *Drusus trifidus*, fifth instar larva, anterior view of pronotum.
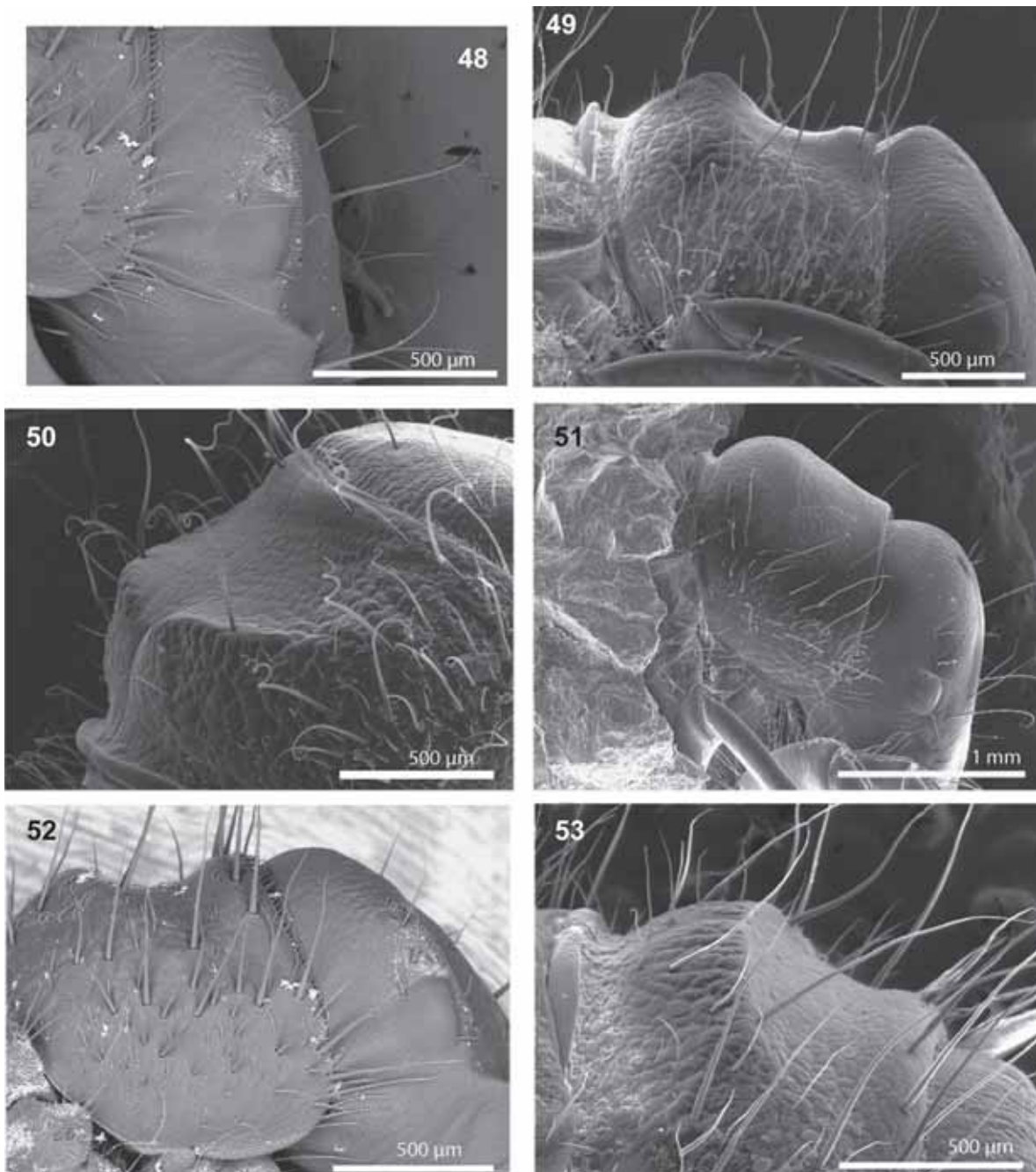
Figs 48-53: (**48**) *Drusus medianus*, fifth instar larva, head with spinules, right lateral view; (**49**) *Drusus ramae*, fifth instar larva, head and pronotum, right lateral view; (**50**) *Drusus ramae*, fifth instar larva, dorsolateral view of pronotum; (**51**) *Drusus croaticus*, fifth instar larva, head and pronotum, right lateral view; (**52**) *Drusus medianus*, fifth instar larva, head and pronotum, right lateral view; (**53**) *Drusus radovanovici septentrionis*, fifth instar larva, anterolateral view of pronotum.

Discussion

Ecology of Drusinae and global warming effects

Drusinae species are mostly stenotopic, cold-adapted inhabitants of low-order mountain brooks (except *Anomalopterygella chauviniana*) and, therefore, typically confined to higher altitudes in the mountains (BOTOSANEANU & MALICKY 1978, GRAF et al. 2008, PITSCH 1993). They are therefore very exposed to global warming effects, which have their most severe implications in Alpine ecosystems, which, in turn, makes them ideal "target species" in terms of global change (BOHLE 1983, 1987, GOWER 1973, KUMANSKI 1973, LAVANDIER 1992). As the water temperature of springs is closely correlated with mean annual air temperature of the location and Drusinae species oviposit underwater (MALICKY 1973) and spend their embryonic, larval and pupal stages there, warming of headwaters strongly affects their persistence. This assumption is backed up by different climate scenarios for interglacial, glacial and postglacial periods which demonstrate that the areal dynamics of Drusinae changed dramatically (MALICKY 1983, 2000, PAULS 2004). There is also strong evidence for vertical migrations according to optimal temperature conditions. However, such adaptive strategies might perhaps be of little use for micro-endemics of the Eastern Alps (e.g. *Drusus franzi*, *Drusus noricus)* which inhabit the peaks of rather isolated mountain areas (Klugveitl [Stainz], Kaltenbachalm, Saualpe) where vertical and/or horizontal migrations to colder climate zones are not possible, a situation possibly leading to their extinction.

Phylogenetic relationships in Central European Drusinae

Recent results of phylogenetic studies based on molecular genetic data (Fig. 2; PAULS et al. 2008) supports monophyly of the subfamily when compared with outgroups from subfamily Limnephilinae. However, at the level of species groups, there are differences with respect to species grouping concepts based on adult morphology (e.g. SCHMID (1956): whereas genus *Metanoea* is monophyletic, *Drusus* is polyphyletic with *Anomalopterygella*, *Ecclisopteryx* and *Metanoea* nested within. In addition, two contemporary genera, *Drusus* and *Ecclisopteryx*, are not monophyletic. In the same analysis *Ecclisopteryx* falls into two well-supported clades within Drusus: the first clade comprises *E. asterix* and *E. malickyi*, the second clade groups *E. dalecarlica*, *E. guttulata* and *E. madida* with *Drusus balcanicus*, *D. botosaneanui* and *D. discophorus pallidus* (PAULS et al. 2008). SCHMID (1956) stated that the morphological differences between *Ecclisopteryx* and *Drusus* are limited to the genital armature, but consistent and stable enough to support two genera. In contrast, the study of PAULS et al. (2008) suggests that *Ecclisopteryx* and *Drusus* are not true evolutionary units.

Of special interest is the phylogenetic situation of vicariant species pairs. *D. alpinus* and *D. franzi* are endemics of the western-central and south-eastern Alps, respectively; *D. nigrescens* is a local endemic of the western-central Alps, while *D. monticola* mainly occurs further east. PAULS et al. (2008) propose that both species pairs may be examples of refugial lineage divergence of a common ancestor which was forced to retreat to south-eastern and south-western refugia during the early or middle Pleistocene (HEWITT 2004, PAULS et al. 2006).

Functional feeding ecology and feeding type evolution in Central European Drusinae

With respect to mouthpart anatomy, three distinct species groupings exist in Central European Drusinae (WARINGER et al. 2007a): in *Cryptothrix nebulicola*, *Drusus chrysotus*, *D. discolor* and *D. muelleri*, mandibles with teeth around edges are present; this, together with additional setae on the legs, head-capsule modifications, and long filtering bristles on the first abdominal sternum, identifies this group as carnivorous filterers (Figs 5, 17, 19-21). In order to switch into filtering mode, the ventral section of the anterior case opening is most commonly attached to the substrate by silk. Then the legs are spread out, much as in Brachycentridae, and the filtering bristles exposed to the current as described by BOHLE (1983) for *Drusus discolor*. The filtering bristles on the first abdominal sternum probably assist in catching prey drifting close to the stream bottom. According to gut analyses by BOHLE (1983, 1987), aquatic insect prey in *Cryptothrix nebulicola*, *Drusus chrysotus* and *D. discolor* comprised larval Ephemeroptera, Plecoptera, Trichoptera and Diptera (Chironomidae and Simuliidae), as well as small fragments of Spermatophyta and Bryophyta. Only early instars of aquatic insects were consumed in toto, larger prey items were cut into pieces using the teeth on the mandible cutting edges.

In *D. franzi* and *D. alpinus*, mandibles with teeth around edges are present, but additional filtering bristles on legs and the first abdominal sternum are lacking, which is typical for omnivorous generalists feeding on a wide spectrum of aquatic invertebrates, water mosses and aquatic plants (Fig. 15). In the remaining 15 species, a spoon-shaped mandible without teeth is present and additional setae and bristles are lacking, identifying this species group as scrapers feeding mainly on epilithic algae (e.g. Fig. 16). Generally, epilithic algal growth is much higher at lotic stream sections and midstream than in lenitic sections or near the banks (GESSNER 1955). This is why scraping Drusinae species are forced to expose themselves much more during feeding than omnivorous generalists feeding near the banks. In addition, in order to feed effectively, scraping Drusinae species do not fix their cases at the substrate as the filter-feeding Drusinae do. This results in a significant over-represention of scraper Drusinae species in the drift (up to 60 % of drifting caddis larvae) when compared with their relative abundance on the stream bed (40 %; BACHER & WARINGER 1996, KÖNIG & WARINGER 2008).

The groupings observed in our genetic analyses reflect mouthpart morphology and feeding ecology. Groupings were significant for 1) epilithic grazers with smooth mandible edges, 2) carnivorous filterers with serrated mandible edges and filtering setae and bristles, and 3) omnivorous generalists with teeth on mandible edges. We conclude that in the Drusinae, larval morphology may be phylogenetically most important and highly relevant for understanding the evolution of the group.

With respect to feeding type evolution, two alternative scenarios are possible (PAULS et al. 2008): (1) progression from ancestral omnivorous shredders to both filtering carnivores and epilithic grazers or (2) evolution from filtering carnivores to omnivorous shredders and epilithic grazers. The first alternative is more likely, based on the fact that all other Limnephilids are known to be shredders, but the latter alternative cannot be ruled out with the data at hand. The mandible in the shredders *D. alpinus* and *D. franzi* is of the ancestral type with teeth along the edges (GRAF et al. 2009). Based on our ancestral character state reconstructions the mandible with teeth appears to be the ancestral state, which is maintained in the carnivorous filterers and omnivore generalist shredders. The spoon-shaped grazer mandible seems to be derived, having reduced or lost the teeth on the mandible edge. The acquisition of filtering bristles seems to be a derived character. With few exceptions, all Limnephilidae are shredders (GRAF et al. 2002). Other feeding types are only found in the

Drusinae and sporadically among other genera (*Melampophylax* and *Micropterna*). *Melampophylax mucoreus*, *M. nepos* and *Micropterna testacea*, for example, are Limnephilinae grazers with spoon-shaped mandibles (WARINGER et al. 2009). Whether the feeding type evolved only once or independently several times within Limnephilidae requires further phylogenetic analysis with a larger sampling of Limnephlidae taxa. The evolution of feeding types in the Drusinae follows the ontogeny of individuals. NIELSEN (1942) studied the larval development of *Ecclisopteryx guttulata* and observed that in first instar larvae both mandibles have a ventral tooth. Additionally, two or three dorsal teeth are present on the left and right mandible, respectively. From the second instar larvae onward, the mandibles are spoon-shaped without any teeth on the mandible edges. Most of the extant Drusinae species whose larvae are known are grazers or carnivorous filterers. WEAVER & MORSE (1986) hypothesised generally for caddisflies that feeding specialisation may have opened opportunities to colonise new ecological niches and could have promoted diversification in these organisms significantly. Considering the high number of derived grazers, such changes in feeding ecology may be responsible for much of the diversification within Drusinae (PAULS et al. 2008).

Acknowledgments

Zusammenfassung

Köcherfliegen werden in weiten Teilen der Welt als Bioindikatoren eingesetzt, wobei Vertreter der Unterfamilie der Drusinae als charakteristische Reinwasserformen gelten. Um die Gruppe für das praxisorientierte Gewässermonitoring aufzubereiten, wird ein zusammenfassender Larvenschlüssel für alle bisher bekannten Arten Zentraleuropas und des Dinarischen Westbalkans vorgestellt. Die unterschiedliche Ausbildung larvalmorphologischer Merkmale wie z.B. die Mundwerkzeuge sowie die Ernährungsweise scheinen zur Diversifikation der Gruppe wesentlich beigetragen zu haben. Phylogeographische, morphologische und genetische Aspekte werden dargestellt und diskutiert.

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Tab. 1: Drusinae species inventory of Austria, Germany and Switzerland (LUBINI-FERLIN & VICENTINI 2005; MALICKY 1999, 2009; ROBERT 2004). N= Lower Austria, O= Upper Austria, St= Styria, B= Burgenland, K= Carinthia, S= Salzburg, T= Tyrol, V= Vorarlberg; BY= Bavaria, BW= Baden-Württemberg, SL= Saar, RP= Rhineland-Palatinate, NW= North Rhine-Westphalia, HE= Hesse, NI= Lower Saxony, TH= Thuringia, ST= Saxony-Anhalt, SN= Saxony; J= Jura, M= Swiss Midlands, AN= Northern flanks of the Alps, AZ= Central Alps, AS= Southern flanks of the Alps.

Species	Austria								Germany										Switzerland				
	N	O	St	B	K	S	T	V	BY	BW	SL	RP	NW	HE	NI	TH	ST	SN	J	M	AN	AZ	AS
Anomalopterygella chauviniana (STEIN 1874)	•	•							•				•	•						•			
Crypthothrix nebulicola McL 1867			•		•	•	•	•													•	•	•
Drusus adustus (McL 1867)			•		•	•																•	
Drusus alpinus (MEYER-DÜR 1875)					•																	•	
Drusus annulatus (STEPHENS 1837)	•	•	•			•	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•
Drusus biguttatus (PICTET 1834)	•	•	•		•	•	•	•	•	•		•		•				•	•	•	•	•	•
Drusus chapmani McL 1901																					•	•	•
Drusus chrysotus (RAMBUR 1842)	•	•	•	•	•	•	•	•	•	•						•		•			•	•	•
Drusus discolor (RAMBUR 1842)	•	•	•		•	•	•	•	•	•			•			•		•	•		•	•	•
Drusus franzi SCHMID 1956			•		•																		
Drusus improvisus McL 1884																						•	
Drusus melanchaetes McL 1876					•	•	•	•													•	•	•
Drusus mixtus (PICTET 1834)																			•				
Drusus monticola McL 1876	•				•	•	•	•	•	•			•								•	•	•
Drusus muelleri (McL 1868)						•	•	•		•		•	•								•	•	•
Drusus nigrescens MEYER-DÜR 1875							•															•	
Drusus noricus MALICKY 1981					•																		
Drusus trifidus McL 1868	•	•	•		•	•	•	•	•	•		•	•	•		•			•	•	•	•	•
Ecclisopteryx asterix MALICKY 1979			•		•																		
Ecclisopteryx dalecarlica KOLENATI 1848	•	•	•			•			•	•		•	•	•	•	•	•	•		•	•	•	
Ecclisopteryx guttulata (PICTET 1834)	•	•	•		•	•	•	•	•	•		•	•	•		•	•	•	•	•	•	•	
Ecclisopteryx madida (McL 1867)	•	•	•		•	•	•	•	•	•		•	•			•	•	•		•	•		
Metanoea flavipennis (PICTET 1834)	•	•			•	•	•	•	•											•	•	•	
Metanoea rhaetica SCHMID 1955	•	•			•	•	•	•	•												•	•	

Tab. 2: Endemic Drusinae species of the dinaric western Balkan included in the key (KRUŠNIK 1987; KUČINIĆ et al. 2006, 2008, submitted; MARINKOVIĆ-GOSPODNETIĆ 1979).

Species	Croatia	Slovenia	Bosnia & Herzegowina
Drusus croaticus MARINKOVIĆ-GOSPODNETIĆ 1971	●	●	
Drusus medianus MARINKOVIĆ-GOSPODNETIĆ 1976			●
Drusus radovanovici septentrionis MARINKOVIĆ-GOSPODNETIĆ 1976			●
Drusus ramae MARINKOVIĆ-GOSPODNETIĆ 1970			●