
Johann Waringera*, Wolfram Grafb, Steffen Paulsc and Fernanda Cianficconid

*aDepartment of Freshwater Ecology, University of Vienna, Vienna, Austria; bInstitute of Hydrobiology and Aquatic Ecology Management, University of Natural Resources and Applied Life Sciences, Vienna, Austria; cUniversity of Minnesota. Department of Entomology, Saint Paul, MN, USA; dDipartimento di Biologia Animale ed Ecologia, Facoltà di Scienze MM.FF.NN, Perugia, Italy*

(Received 30 January 2008; final version received 8 July 2008)

The larvae of *Drusus improvisus* McLachlan, 1884, *Drusus camerinus* Moretti, 1981 and *Drusus aprutiensis* Moretti, 1981 are re-described and discussed in the context of contemporary keys of European Drusinae species. In addition, phylogenetic, zoogeographical and ecological notes are included.

**Keywords:** Trichoptera; *Drusus improvisus*; *Drusus camerinus*; *Drusus aprutiensis*; description; habitat; ecology

**Introduction**

In his famous key on the Italian caddisfly larvae, G.P. Moretti (1983) figured and briefly described the larvae of *Drusus improvisus* and *Drusus camerinus*. In addition, he stated that the larva of *D. aprutiensis* would be morphologically close to *D. camerinus*, although he did not include the former species in his Drusinae key. Unfortunately, the data provided did not allow inclusion of the three species into contemporary larval keys devoted to the hitherto known Central European Drusinae species. We therefore attempted to collect or obtain larval material from these species and recently succeeded. During collection trips made in 2007 to the *lozi typici* (springs and spring brooks in the Alpi Apuani, Italy), W. Graf managed to collect larvae of an unknown Drusinae species whose pupae and adults from the larval habitats clearly identified as *Drusus improvisus*. In addition, F. Cianficconi generously provided larvae of *D. improvisus*, *D. camerinus* and *D. aprutiensis*, which were also checked and identified by G.P. Moretti himself. This material enabled us to provide additional morphological details usable for the separation from other Central European Drusinae and to include *Drusus improvisus*, *D. camerinus* and *D. aprutiensis* in contemporary larval keys. We further used the fresh material of *D. improvisus* to test a recently published hypothesis on the phylogenetic relevance of feeding ecology in the Drusinae (Pauls, Graf, Haase, Lumbsch and Waringer 2008).

*Corresponding author. Email: johann.waringer@univie.ac.at*
Description of the fifth instar larvae of *Drusus improvisus*, *D. camerinus* and *D. aprutiensis*


The body length of final instar larvae ranges from 6.2 to 9.1 mm (fourth instar: 5.8 mm) in *D. improvisus*, from 6.3 to 8.6 mm in *D. camerinus* and from 7.7 to 8.3 mm in *D. aprutiensis*. The head width, in the same sequence of species, ranges from 1.10 to 1.32 (fourth instar: 0.84 mm), from 1.20 to 1.30 mm and from 1.33 to 1.34 mm, respectively. Larval case length ranges from 6.7 to 8.9 mm (fourth instar: 5.5 mm), 6.2 to 8.7 mm and 8.0 to 9.3, anterior case width from 2.2 to 2.6 mm (fourth instar: 1.5 mm), 2.2 to 1.8 mm and 2.3 to 2.4 mm; finally, posterior case width ranges from 1.2 to 2.0 mm (fourth instar: 1.0 mm), 1.3 to 1.5 mm and 1.2 to 1.3 mm. The cases of all three species are slightly curved, tapering at posterior end and consist completely of mineral particles with grain sizes increasing distinctly in anterior direction (Figure 19).

**Head**

Head capsules and all body sclerites are dark brown to black brown. The head capsules (Figures 1–4) lack the additional setae or spines known from other Drusinae larvae (e.g. *Ecclisopteryx* spp., *Drusus trifidus*). The mandibles lack terminal teeth along edges as well as ridges in the central concavity (Figure 2, arrow).

**Thorax**

In profile, the dorsal line of the pronotum shows a distinct and evenly rounded hump within the posterior third in *D. improvisus* and *D. camerinus* (Figures 11 and 20b), whereas this hump is distinctly weaker, lower in profile and much more flattened in *D. aprutiensis* (Figure 20d). There are also differences in the arrangement of long setae along the anterior border of the pronotum: in *D. improvisus*, this row extends close to the dorsal midline (Figure 17), whereas in *D. camerinus* and *D. aprutiensis* there is a large median gap in this row of long setae (Figure 18). The black brown pronotal surface of all three species is covered by dark brown setae of unequal length: the longest ones are situated at the anterior border and in the central area (Figure 11). The prosternites are inconspicuous and a prosternal horn is present in all three species. The mesonotum is completely covered by two chestnut-brown sclerites (Figure 5). The metanotum is partially covered by three pairs of sclerites with the anterior metanotal sclerites being large and oval; their median separation is distinctly smaller than their maximum extension along the body axis (Figure 5). The setal bases at the central section of the first abdominal sternum differ greatly within the three species: in *D. improvisus*, the bases of many central setae are fused, creating one or two large, multi-lobed sclerotised patches with an irregular outline (Figures 8 and 14c). In *D. camerinus*, these patches are smaller and distinctly separated (Figures 9 and 14d). Finally, in *D. aprutiensis*, only isolated sclerotised setal bases are present: two large setal bases of the median setae and about ten tiny sclerotised bases of much smaller setae.
Figures 1–6. Fifth instar larvae of *Drusus*. (1) *D. improvisus*, head, frontal view; (2) *D. improvisus*, head, right lateral view, arrow: spoon-shaped mandible; (3) *D. camerinus*, head, frontal view; (4) *D. aprutiensis*, head in frontal view; (5) *D. improvisus*, thorax and first abdominal segment, dorsal view; m: metanotum; (6) *D. improvisus*, first to third abdominal segment, right lateral view, arrow = beginning of lateral fringe. Scale bars: 1 mm.
Figures 7–14. (7–10) First abdominal sternum of fifth instar larvae of (7) *Metanoea flavipennis*, (8) *Drusus improvisus*, (9) *D. camerinus*, (10) *D. aprutiensis*. (11–13) Pronotum of fifth instar larvae of: (a) *Metanoea rhaetica* and *M. flavipennis*, (b) *Drusus nigrescens*, (c) *D. improvisus*, (d) *D. camerinus*, (e) *D. aprutiensis*; only sclerotised patches and setal bases are shown. Scale bars: 1 mm.
Erratum

Please replace the figure captions at page 272 by the following:

Figs. 7 - 10: First abdominal sternum of fifth instar larvae of 7: *Metanoea flavipennis*; 8: *Drusus improvisus*; 9: *D. camerinus*; 10: *D. aprutiensis*. Figs. 11 – 13: Pronotum of fifth instar larvae, right lateral view, of: 11: *D. improvisus*, 12: *M. flavipennis*, 13: *D. nigrescens*. Fig. 14: Central sections of first abdominal sternum of fifth instar larvae of: a: *Metanoea rhaetica* and *M. flavipennis*; b: *Drusus nigrescens*; c: *D. improvisus*; d: *D. camerinus*; e: *D. aprutiensis*; only sclerotized patches and setal bases are shown. Scale bars: 1mm.
(Figures 10 and 14e), a situation often observed in other Drusinae species. At the eighth abdominal dorsum, two long posterodorsal setae are present in all three species.

**Abdomen**

All gills consist of single filaments only. Dorsal gills are present from the second (presegmental position) to the sixth (presegmental position) in *D. aprutiensis*, to the sixth (postsegmental) in *D. improvisus* and to the seventh segment (presegmental position) in *D. camerinus*. Ventral gills range from second (presegmental) to seventh segment (pre- or postsegmental) in all three species. Lateral gills are present on the second and third segment (postsegmental position), in *D. aprutiensis* also on the fourth segment (postsegmental). The lateral fringe is uniformly present from the last third of the second (Figure 6) to the beginning of the eighth abdominal segment. Setae are present at the anterior and posterior faces of all femora. The row of dorsal setae at the middle and hind leg tibiae are restricted to the distal third of the segment (Figure 15). A synopsis for the identification of *D. improvisus*, *D. camerinus* and *D. aprutiensis* is given in Table 1.

**Morphological separation of Drusus improvisus, D. camerinus and D. aprutiensis from other European Trichoptera**

A summary of morphological features for the identification of Limnephilidae and Drusinae larvae is given in Waringer (1985). Within the framework of the Limnephilidae key by Waringer and Graf (1997, 2004), *Drusus improvisus* and *D. camerinus* are separated from other species by the following features:

- Metanotum covered by three pairs of small sclerites (Figure 5m);
- Head and pronotum without a thick layer of woolly hairs (Figures 1–4);
- Head capsule without groups of additional spines, without central concavity and rims surrounding the frontoclypeus (Figures 1–4);
- Mandibles lacking terminal teeth along edges as well as ridges in the central concavity (Figure 2, arrow);
- First abdominal sternum with a large median sclerotised patch or distinct concentrations of fused setal bases, thereby creating multi-lobed sclerotised patterns (Figures 7–9, 14a–d).

At this position *Drusus improvisus* and *D. camerinus* key out together with *Metanoea rhaetica*, *M. flavipennis* and *D. nigrescens*. The three *Drusus* species are separated from the

<p>| Table 1. Synoptic key for the identification of <em>D. improvisus</em>, <em>D. camerinus</em> and <em>D. aprutiensis</em>. |
|--------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Species/character</th>
<th>Long median setae at the anterior border of the pronotum</th>
<th>1st abdominal sternum</th>
<th>Dorsal profile of pronotum in lateral view</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. aprutiensis</em></td>
<td>– (Figure 18)</td>
<td>Two large, isolated, sclerotised setal bases only (Figures 10, 14e)</td>
<td>Flat (Figure 20d)</td>
</tr>
<tr>
<td><em>D. camerinus</em></td>
<td>– (Figure 18)</td>
<td>Fused sclerotised patches (Figures 9, 14d)</td>
<td>Rounded (Figure 20b)</td>
</tr>
<tr>
<td><em>D. improvisus</em></td>
<td>+ (Figure 17)</td>
<td>Fused sclerotised patches (Figures 8, 14c)</td>
<td>Rounded (Figure 20b)</td>
</tr>
</tbody>
</table>
two *Metanoea* species by the multi-lobed and highly fragmented sclerotised structure of this patch (Figures 8, 9, 14c, d), whereas in the two *Metanoea* species this patch is heavily concentrated, mostly unfragmented and oval in shape (Figures 7 and 14a). In addition, the evenly-rounded dorsal line of the pronotum has a very low profile in *Metanoea* (Figures...
I12, 20c), whereas the rounded hump is distinctly higher in *D. improvisus* and *D. cameronus* (Figures 11, 20b and 21b); in *D. nigrescens*, the dorsal profile line of the pronotum is completely different, bearing a distinct, serrated ridge in its posterior third (Figure 13; Waringer, Graf, Pauls and Lubini 2007). *D. improvisus* and *D. cameronus* may be easily separated by the presence of long median setae at the anterior border of the pronotum in *D. improvisus* (Figure 17) which are lacking in *D. cameronus* (Figure 18). Within *Metamoea*, species may be separated by the presence of lateral gills in *M. flavipennis* that are completely lacking in *M. rhaetica* (cf. Waringer, Graf and Maier 2000).

In *Drusus aprutiensis*, a large median sclerotised patch or distinct concentrations of fused setal bases as figured in Figures 7–9 and 14 a–d are lacking; in this species, only two large, isolated, sclerotised setal bases are present at the centre of the first abdominal sternum (Figures 10 and 14c), as it is frequently observed in other Drusinae species. In the Limnephilidae key by Waringer and Graf (1997, 2004), this leads to the following characters:

- Pronotum without ridge; in profile, dorsal outline evenly rounded in its posterior third, thereby creating a very low dorsal hump (Figure 20d);
- Middle and hind leg femora faces with additional setae (e.g. Figures 15 and 16, arrows);
- Anteromedian metanotal sclerites large, oval, their median separation being distinctly smaller than their maximum extension along the body axis (Figure 5);
- Without long median setae at the anterior border of the pronotum (as in Figure 18).

At this position *D. aprutiensis* keys out together with *D. mixtus*; both species may be easily separated from each other by the arrangement of the dorsal edge setae at the mid and hind tibiae: in *D. mixtus*, they extend over almost the whole length of the tibia (e.g. Figure 16) whereas in *D. aprutiensis* these setae are restricted to the distal third of the segment (e.g. Figure 15).

In case the sclerotised patches of the first abdominal sternum of *D. improvisus* and *D. cameronus* have been interpreted as agglomeration of isolated sclerotised setal bases, *D. improvisus* keys out together with *D. biguttatus*, *D. annulatus*, *D. carpathicus* and *Eclissopteryx asterix* whereas *D. cameronus* keys out together with *D. mixtus* and *D. aprutiensis*. In the first group, *E. asterix* can be readily identified by the fact that the lateral fringe starts at the last third of the third abdominal segment (cf. Urbanic, Waringer and Graf 2003) whereas in the other four species it starts immediately at the border of the second/third abdominal segment. *D. carpathicus* is easily identified by its lack of dorsal abdominal gills, which are present in the other species keyed out here. Furthermore, in
D. improvisus and D. camerinus the dorsal pronotal profile is distinctly higher than in D. biguttatus and D. annulatus (Figures 20a, b, 21a). The most difficult separation is between D. annulatus and D. biguttatus (cf. Figure 41 in the Limnephilidae key by Waringer and Graf 1997); in the latter species, the dorsal line of the pronotal hump, in lateral view, is evenly rounded whereas in D. annulatus the dorsal line is angled.

In the second group, D. mixtus is again easily separated by the arrangement of the dorsal tibial setae which extend over almost the whole length of the tibia (e.g. Figure 16); D. camerinus and D. aprutiensis may be separated by the dorsal shape of the pronotum (lateral view): in D. camerinus the dorsal hump is very distinct whereas in D. aprutiensis the lateral profile of this structure is very low (Figure 20b, d).

Mouthpart anatomy, feeding ecology and phylogenetic placement

With respect to mouthpart anatomy, three distinct species groupings exist in Central European Drusinae (Waringer et al. 2007; Graf, Waringer and Pauls, accepted). In D. improvisus, D. camerinus and D. aprutiensis, a spoon-shaped mandible without teeth – as known from most European Drusinae species – identifies them as scrapers that feed mainly on epilithic algae. Filtering carnivores are characterised by teeth on the mandible edges and filtering spines on the first abdominal sternum and the legs. Shredders also have mandibles with teeth but lack filtering spines. Pauls et al. (2008) examined the evolution of feeding ecology in the group and concluded that the subfamily Drusinae comprises three main evolutionary units, which correspond to the three feeding types. Of the possible scenarios for the evolution of feeding ecology, the progression from ancestral omnivorous shredders (e.g. Drusus franzii, D. alpinus) to both filtering carnivores (e.g. D. muelleri, D. chrysotus) and epilithic grazers (most Drusinae species) seems most likely based on the fact that almost all other Limnephilidae are known to be shredders. This progression could not be resolved with the data available to Pauls et al. (2008). However, mandibles with teeth appear to be the ancestral state; the spoon-shaped grazer mandible seems to be derived, having reduced or lost the teeth on the mandible edge (Pauls et al. 2008). Adding sequence data from other species provides the opportunity to test the validity of the feeding ecology based grouping observed by Pauls et al. (2008). With the material at hand, we can only test if feeding ecology corresponds to phylogenetic placement for D. improvisus, where we have fresh material collected in 2007.

With the same data and analyses we can also test previous phylogenetic hypotheses on the relationships within the genus Drusus, with regard to D. improvisus. Schmid (1956) placed D. improvisus next to D. spelaeus in the mixtus-group, in which he also included D. biguttatus, D. bolivari, D. brumius, D. mixtus, and D. tridus. Pauls et al. (2008) included four of these species and rejected the monophyly of the mixtus-group as circumscribed by Schmid (1956). The addition of D. improvisus provides the opportunity to further test the validity of the mixtus-group.

To test both the validity of the mixtus-group and the feeding ecology based grouping, we generated sequence data for three gene loci (mitochondrial cytochrome oxidase I, mitochondrial ribosomal 16S, nuclear wingless) following the methods described in Pauls et al. (2008). We predict that including D. improvisus in our analyses will not alter our previous observations and that monophyly of the mixtus-group remains questionable. We also predict that D. improvisus falls within the “grazer” clade. We obtained sequence data from one male and larvae collected in the Val di Luce (Abetone), Italy, by W. Graf on 18.07.2007, and one male collected in Equi Terme at the Alpi Apuane, Italy, by W. Graf on 17.07.2007 (Gen Bank Accessions EU999187-EU999195). We added these data to the
matrix previously published (GenBank Accessions AY954395, AY954398, AY954400, AY954401, EF464556, EF464560, EF464562, EF464565, EU215079-EU215218) and ran a Bayesian Markov-Chain Monte Carlo phylogenetic analysis for the complete data set including 29 species and 56 specimens using MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The analysis was run with four chains for $2 \times 10^6$ generations assuming the GTR+I+G model of nucleotide substitution. We discarded the first $1.75 \times 10^5$ generations as burn-in and sampled the tree with the best likelihood score every 100th generation. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that stationarity was achieved after the first $1.75 \times 10^6$ generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium plateau. From the remaining 5000 trees of both runs we generated a majority-rule Consensus tree with average branch lengths using the sumt option of MrBayes. Posterior probabilities (pp) were obtained for each clade.

The phylogenetic inference placed *D. improvisus* in a highly supported clade (pp = 1.0) with *D. adustus*, *D. melanchaetes*, *D. mixtus*, *D. biguttatus*, *D. monticola*, *D. nigrescens*, *Metanaoea rhaetica* and *M. flavipennis* (hereafter referred to as clade A). Beyond clade A, the topology of the inference is identical with the one obtained in Pauls et al. (2008, cf. Figure 2). Clade A falls within the grazer clade, which in turn is highly supported (pp = 1.0). Thus *D. improvisus* falls within the grazers, as we predicted. The other feeding types form monophyletic groups (shredders, pp = 1.0; filtering carnivores, pp = 0.94). Filtering carnivores is not significantly supported. This result was also observed in Pauls et al. (2008). However, the other two feeding groups are significantly supported (pp > 0.95). Filtering carnivores is thus supported by species exclusion from other feeding type clades.

All species represented by more than one specimen are monophyletic (pp > 0.98) except *D. romanicus*. Within clade A several significantly supported species pairs are observed: *D. monticola* and *D. nigrescens* (pp = 1.0), *Metanaoea rhaetica* and *M. flavipennis* (pp = 1.0), *D. biguttatus* and *D. mixtus* (pp = 1.0). However, the relationships within clade A remain largely unresolved. These results were also observed and discussed in detail in Pauls et al. (2008). Three members of the putative *mixtus*-group (*D. improvisus*, *D. biguttatus*, *D. mixtus*) occur in clade A, but do not form a monophyletic group. The other two putative members, *D. brumaeus* and *D. trifidus*, fall outside clade A, thus rejecting monophyly of the *mixtus*-group as proposed by Schmid (1956). From our results it appears that *D. improvisus* is closely related to members of clade A, but its position within this clade cannot be resolved with the data at hand.

Our results confirm that feeding ecology corresponds to our molecular inference of the evolution of the Drusinae. More studies are warranted and necessary to examine the full breadth of diversity of larval morphology in the group. Including more taxa in the molecular phylogeny will help to fully resolve and understand the evolution of this highly diverse group of caddisflies.

**Phenology, habitat and distribution**

Last instar larvae of *D. improvisus* were collected on 17 July 2007 at Equi Terme (338 m a.s.l.) at the Alpi Apuane and on 18 April 1966 at the Sorgente Camporio, Monteluco (1050 m a.s.l.). The species is endemic to Italy and known from the central Apennine (Emilia Romagna, Tuscany, Umbria, Marche, Lazio and Abruzzo regions; Moretti and Cianficconi, 1974, 2002; Moretti 1981; Malicky 2004). However, one record also exists from the central alpine region of Switzerland (Lubini and Vicentini 2005). According to Moretti (1983),
adults can be collected in winter and summer. *D. improvisus* is a typical crenal species (Cianficconi et al. 1998) inhabiting springs and spring brooks from 200 to 1300 m a.s.l. At Equi Terme, *D. improvisus* could only be found at a small groundwater-fed stretch of a streamlet. At this location, *D. improvisus* was sympatric with *Rhyacophila tristis, Catagapetus nigrans, Tinodes dives* and *Odontocerum albicorne*. At Abetone the following caddisfly species could be collected together with *D. improvisus*: *Rhyacophila inaequalis, Glossosoma conformis, Wormaldla occipitalis, Philopotamus ludificatus, Hydropsyche tenus, Drusus discolor, Sericostoma personatum* and *Odontocerum albicorne*; At Vinca, *Catagapetus nigrans, Ptilocoleptus granulatus, Wormaldla copiosa, Botosaneanu*, *Wormaldla occipitalis, Philopotamus ludificatus, Diplecmenta magna, Micropterus wageneri, Crumoeia irrorata* and *Adicella filicornis* were sympatric with *D. improvisus*.

*Drusus cameratus* and *D. aprutensis* are also endemics of the Apennine peninsula with the former being recorded from the Umbria, Marche and Lazio regions and the latter species known from the Lazio and Abruzzo regions (Cianficconi 2002). Both species inhabit small springs and spring brooks from 400 to 1200 m a.s.l. (Moretti 1983). Males and egg-laying females can be observed in deep winter (December and January) with fifth instar larvae being collected as early as 31 April.

Acknowledgements
We wish to thank Dr W. Lechthaler for providing the two photographs (Figures 13 and 16). This paper is part of the outcomes of a project dealing with larval taxonomy of Central European Drusinæ (project number P18073-B03, PI: J. Waringer) funded by the Austrian Science Fund (FWF).

References


