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The Larvae of *Drusus improvisus* McLachlan, 1884, *Drusus camerinus* and *Drusus aprutiensis* (Trichoptera: Limnephilidae: Drusinae)

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Abstract

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 to include 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 *loci 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 et al., 2008).

Description of the fifth instar larvae of *Drusus improvisus*, *D. camerinus* and *D. aprutiensis*

Material examined: *D. improvisus*: 34 fifth instar and 1 fourth instar larvae from Equi Terme (338 m a.s.l.), Tuscany, Italy, leg. W. Graf 17 July 2007; one third instar larva from Vinca (945 m a.s.l.), leg. W. Graf 17 July 2007, 6 fifth instar larvae from Abetone, Val di Luce (1417m.a.s.l.), Emilia Romagna, leg. W. Graf 18 July 2007, two fifth instar larvae from Sorgente Camporio, Monteluco, (1050 m a.s.l.), Umbria, Italy, leg. Venturi 18 April 1966, det. G.P. Moretti. *D. camerinus*: 2 fifth instar larvae from Fonti di Brescia (680 m a.s.l.), Marche, Italy, leg. Bellini 31 April 1950, det. G.P. Moretti. *D. aprutiensis*: 2 fifth instar larvae from Rio Arno, Abruzzo (1200 m a.s.l.), Italy, leg. Tricinci 25 June 1977, det. G.P. Moretti.

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 – 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 (Fig. 19).

Head

Head capsules and all body sclerites are dark brown to black brown. The head capsules (Figs. 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 (Fig. 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* (Figs. 11, 20 b), whereas this hump is distinctly weaker, lower in profile and much more flattened in *D. aprutiensis* (Fig. 20 d). 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 (Fig. 17), whereas in *D. camerinus* and *D. aprutiensis* there is a large median gap in this row of long setae (Fig.

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 (Fig. 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 (Fig. 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 (Fig. 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 sclerotized patches with an irregular outline (Figs. 8, 14 c). In *D. camerinus*, these patches are smaller and distinctly separated (Figs. 9, 14 d). Finally, in *D. aprutiensis*, only isolated sclerotized setal bases are present: two large setal bases of the median setae and about ten tiny sclerotized bases of much smaller setae (Figs. 10, 14 e), 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) 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 (Fig. 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 (Fig. 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 & 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 (Fig. 5 m);
- Head and pronotum without a thick layer of woolly hairs (Figs. 1 - 4);
- Head capsule without groups of additional spines, without central concavity and rims surrounding the frontoclypeus (Figs. 1 - 4);
- Mandibles lacking terminal teeth along edges as well as ridges in the central concavity (Fig. 2, arrow);

- First abdominal sternum with a large median sclerotized patch or distinct concentrations of fused setal bases, thereby creating multi-lobed sclerotized patterns (Figs. 7 – 9, 14 a - 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 two *Metanoea* species by the multi-lobed and highly fragmented sclerotized structure of this patch (Figs. 8, 9, 14 c, d), whereas in the two *Metanoea* species this patch is heavily concentrated, mostly unfragmented and oval in shape (Figs. 7, 14 a). In addition, the evenly-rounded dorsal line of the pronotum has a very low profile in *Metanoea* (Figs. 12, 20 c,) whereas the rounded hump is distinctly higher in *D. improvisus* and *D. camerinus* (Figs. 11, 20 b, 21 b); in *D. nigrescens*, the dorsal profile line of the pronotum is completely different, bearing a distinct, serrated ridge in its posterior third (Fig. 13; Waringer, Graf, Pauls & Lubini, 2007). *D. improvisus* and *D. camerinus* may be easily separated by the presence of long median setae at the anterior border of the pronotum in *D. improvisus* (Fig. 17) which lack in *D. camerinus* (Fig. 18). Within *Metanoea*, species may be separated by the presence of lateral gills in *M. flavipennis* that are completely lacking in *M. rhaetica* (cf. Waringer, Graf & Maier, 2000).

In *Drusus aprutiensis*, a large median sclerotized patch or distinct concentrations of fused setal bases as figured in Figs. 7 – 9 and 14 a - d are lacking; in this species, only two large, isolated, sclerotized setal bases are present at the centre of the first abdominal sternum (Figs. 10, 14 e), as it is frequently observed in other Drusinae species. In the Limnephilidae key by Waringer & 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 (Fig. 20 d);
- Middle and hind leg femora faces with additional setae (e.g. Figs. 15, 16, arrows);
- Anteromedian metanotal sclerites large, oval, their median separation being distinctly smaller than their maximum extension along the body axis (Fig. 5);
- Without long median setae at the anterior border of the pronotum (as in Fig. 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. Fig. 16) whereas in *D. aprutiensis* these setae are restricted to the distal third of the segment (e.g. Fig. 15).

In case the sclerotized patches of the first abdominal sternum of *D. improvisus* and *D. camerinus* have been interpreted as agglomeration of isolated sclerotized setal bases, *D. improvisus* keys out together with *D. biguttatus*, *D. annulatus*, *D. carpathicus* and *Ecclisopteryx asterix* whereas *D. camerinus* 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 & 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* (Figs. 20a, b, 21 a). The most difficult separation is between *D. annulatus* and *D. biguttatus* (cf. Fig. 41 in the Limnephilidae key by Waringer & 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. Fig. 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 (Fig. 20 b, d).

Mouthpart anatomy, feeding ecology and phylogenetic placement

With respect to mouthpart anatomy, three distinct species groupings exist in Central European Drusinae (Waringer, Graf & Pauls, 2007, Graf, Waringer & Pauls, submitted). 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 franzi*, *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. brunneus*, *D. mixtus*, and *D. trifidus*. 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 (Gen Bank Accession numbers will be provided upon acceptance of the manuscript) and one male collected in Equi Terme at the Alpi Apuane, Italy, by W. Graf on 17.07.2007 (Gen Bank Accession numbers will be provided upon acceptance of the manuscript). 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 & Ronquist 2001, Ronquist & Huelsenbeck 2003). The Analysis was run with four chains for 2×10^6 generations assuming the GTR+I+G model of nucleotide substitution. We discarded the first 1.75×10^6 generations as burn-in and sampled the tree with the best likelihood score every 100^{th} 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 1.75×10^6 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*, *Metanoea 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), *Metanoea 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. brunneus* 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, Montelupo (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 & Cianficconi, 1974, Moretti, 1981, Cianficconi, 2002; Malicky, 2004). However, one record also exists from the central alpine region of Switzerland (Lubini & 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 caddis-fly species could be collected together with *D. improvisus*: *Rhyacophila laevis*, *Catagapetus nigrans*, *Glossosoma conformis*, *Wormaldia occipitalis*, *Philopotamus ludificatus*, *Hydropsyche tenuis*, *Drusus discolor*, *Sericostoma personatum* and *Odontocerum albicorne*. At Vinca, *Catagapetus nigrans*, *Ptilocolepus granulatus*, *Wormaldia copiosa botosaneanui*, *Wormaldia occipitalis*, *Philopotamus ludificatus*, *Diplectrone magna*, *Micropterna wagneri*, *Crunoecia irrorata* and *Adicella filicornis* were sympatric with *D. improvisus*.

Drusus camerinus and *D. aprutiensis* 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.

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Figs. 1 - 6.

Figs. 1 – 2: *Drusus improvisus*, fifth instar larva; 1: Head, frontal view; 2: Head, right lateral view; arrow = spoon-shaped mandible; Figs. 3 - 4: heads, frontal view, of: 3: *D. camerinus*, 4: *D. aprutiensis*; Fig. 5: *D. improvisus*, thorax and first abdominal segment, dorsal view; m = metanotum; Fig. 6: *D. improvisus*, first to third abdominal segment, right lateral view; arrow = beginning of lateral fringe. Scale bars: 1mm.

Figs. 7 -- 14.

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.

Figs. 15--21.

Fig. 15: *D. improvisus*, right hind leg, posterior view; d= disto-dorsal setae on tibia; arrow=additional setae on face of femur; Fig. 16: *D. melanchaetes*, left hind leg, anterior view; arrow= additional setae on face of femur; bracket: dorsal setae along the whole length of the tibia. Fig. 17 - 18: Fifth instar larva, head and anterior rim of pronotum, frontal view, of: 17: *D. improvisus*, 18: *D. camerinus*; arrows: median setae of anterior setal row on pronotum; bi-headed arrow: gap at anterior pronotal border lacking long setae. Fig.19: Case of fifth instar larva of *D. improvisus*, right lateral view. Fig. 20: Right lateral profile of

pronotum of a: *D. biguttatus*; b: *D. improvisus* / *D. camerinus*; c: *M. flavipennis* d: *D. aprutiensis*. The outlines (original length: *D. biguttatus*: 1.00 mm, *D. improvisus*: 0.86 mm, *M. flavipennis*: 0.93 mm, *D. aprutiensis*: 1.00 mm) have been brought to equal length. Fig. 21: Right lateral profile of pronotum of a: *D. biguttatus*; b: *M. flavipennis* with added profile of *D. improvisus* / *D. camerinus* (in black); original length as in Fig. 20. Scale bars: 1 mm.

Table 1

Synoptic key for the identification of *D. improvisus*, *D. camerinus* and *D. aprutiensis*.

Species/character	Long median setae at the anterior border of the pronotum	1 st abdominal sternum	Dorsal profile of pronotum in lateral view
<i>D. aprutiensis</i>	– (Fig. 18)	Two large, isolated, sclerotized setal bases only (Figs. 10, 14e)	Flat (Fig. 20d)
<i>D. camerinus</i>	– (Fig. 18)	Fused sclerotized patches (Figs. 9, 14d)	Rounded (Fig. 20b)
<i>D. improvisus</i>	+ (Fig. 17)	Fused sclerotized patches (Figs. 8, 14c)	Rounded (Fig. 20b)