THE FIRST FINDING OF THE DRUSUS BOSNICUS GROUP (INSECTA, TRICHOPTERA, LIMNEPHILIDAE) IN CROATIA WITH SOME NOTES ON DIVERSITY, TAXONOMY, DISTRIBUTION AND ECOLOGY OF GENUS DRUSUS IN CROATIA AND IN DINARIC KARST OF THE BALKAN PENINSULA

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In the spring area of the Una River the caddisfly Drusus vespertinus Marinković-Gospodnetić, 1976, which belongs to the Drusus bosnicus group, was recorded for the first time in Croatia. This is the first finding of the Drusus bosnicus group species in Croatia. This paper presents the diversity, distribution and ecology of the genus Drusus in Croatia and in the Dinaric karst on the Balkan Peninsula. Additionally, in this paper we describe the female of Drusus vespertinus.

Ključne riječi: Drusus, bosnicus group, Croatia, Dinaric karst, Balkan Peninsula


Na izvorišnom području rijeke Une zabilježena je u stadiju adulta i ličinke vrsta Drusus vespertinus Marinković-Gospodnetić, 1976, koja pripada Drusus bosnicus grupi. Ovo je prvi nalaz neke od vrsta Drusus bosnicus grupe u Hrvatskoj. U radu se prikazuje raznolikost, rasprostranjenost i ekologiju roda Drusus u Hrvatskoj i u dinarskom kršu Balkanskog poluoatoka. Također, u radu se daje opis ženke vrste Drusus vespertinus.

Ključne riječi: Drusus, bosnicus grupa, Hrvatska, dinarski krš, Balkanski poluoatok

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INTRODUCTION

One of the most interesting genera within Trichoptera is the genus *Drusus* Stephens, 1837. With seven other genera (Pauls et al., 2006) the genus *Drusus* belongs to a subfamily Drusinae Banks, 1916 (Waringer & Graf, 1997), family Limnephilidae Kolenati, 1848. The subfamily Drusinae has a total of 96 described species (Oláh & Kovács, 2013; Oláh, 2010, 2011; Malicky, 2004; Marinković-Gospodnetić, 1971a, 1971b). The genus with the highest diversity is *Drusus* with 80 described species (Oláh, 2010, 2011; Malicky, 2004). Species of the genus *Drusus* usually inhabit springs and crenal sections of mountain streams and rivers characterized by low water temperature with small annual variations (Graf et al., 2008). This important life history trait determines their distribution patterns, especially the isolation of mountain populations and high species diversity (e.g. Malicky 2004, 2005; Kumanski, 1988; Marinković-Gospodnetić 1976, 1978, 1979).

One of the most interesting groups in the genus *Drusus* is the *Drusus bosnicus* group with high diversity and many endemic species. Numerous endemic species described from the Balkan Peninsula belong to the *Drusus bosnicus* group (e.g. Oláh & Kovács, 2013; Oláh 2010, 2011; Marinković-Gospodnetić 1971b; Klapálek, 1899). Therefore this group and the genus *Drusus* are very interesting for morphologic, taxonomic, phylogenetic, phylogeographic and evolutionary studies (e.g. Kučinić et al., 2011a; Previšić et al., 2009, 2014; Pauls et al., 2008; Waringer et al., 2007, 2008, 2013; Marinković-Gospodnetić, 1971a, 1971b). All species of the *Drusus bosnicus* group have some common morphological features like the shape of male genitalia, the dark coloration of adults (Schmid, 1956) and the daily activity patterns of adults.

The Dinaric karst is a 650 kilometres long and very diverse mountain area in the Balkan Peninsula, stretching from Slovenia (north-western border) to Albania (south-eastern border). This region harbours many endemic and relict species and subspecies of different animal groups (e.g. Bilandžija et al., 2013; Klobučar et al., 2013; Kučinić et al., 2013; Casale et al., 2012; Ikvovic et al., 2012; Muráň, 2011; Žganec et al., 2011; Lukić et al., 2010; Bedek & Taiti, 2009; Lakota et al., 2009; Mustafić et al., 2008; Mihoci et al., 2007; Bedek et al., 2006; Mihoci & Šašić, 2005; Pavlek & Ozimec, 2005; Gottstein et al., 2002; Sket et al., 2001). The central and Mediterranean part of Croatia, Bosnia and Herzegovina and Montenegro belong to the Dinaric karst region, but also some smaller parts of Slovenia on the west, and Kosovo, Serbia and Albania on the southeast. The inland lowland part of Croatia does not belong to the Dinaric karst area of the Balkan Peninsula.


MATERIAL AND METHODS

Research into caddisflies in the spring area of the Una River (Fig. 1) was conducted in the period 2005–2012. Within this period, caddisflies were sampled several times. Larvae were collected by handpicking while adults were collected with entomological nets during daytime and with UV light traps during the night. Specimens were stored in containers with 80% and 96% EtOH, for both morphological and molecular analysis. Collected specimens are deposited in the collections of M. Kučinić (Croatian Natural History Museum in Zagreb), Ana Previšić (Faculty of Science, Zagreb) and R. Ćuk (Hrvatske Vode).
Fig. 1. Spring of the Una River (photo: A. Delić).

Fig. 2. Male genitalia of *Drusus vespertinus* Marinković-Gospodnetić, 1976 collected in the spring of the Una River (photo: M. Franjević & M. Kučinić).

The female of *D. vespertinus* was drawn based on material collected on 23.06.2001 in the Bastašica spring, Drvar, Bosnia and Herzegovina (2 females, leg. M. Kučinić & A. Delić), in the Sanica River, Bosnia and Herzegovina (1 female, leg. P. Kružić & A. Previšić)
and on a specimen collected on 10.06.2010 in the spring of the Una River (2 females, leg. I. Mihoci & M. Kučinić).

Macrophotographing of larvae and adults was done at the Faculty of Forestry, University of Zagreb, using a Leica Wild MZ8 stereomicroscope and Olympus SP-500 UZ digital camera, processed with the computer program Olympus Quick Photo Camera 2.2. Photographing in the field (spring area of the Una River) was done with Olympus Photo camera EU 570. Taxonomic identification of the collected adults was conducted by Malicky (2004).

We have defined the area of the Dinaric karst according to Bilandžija et al. (2013) and the border of the Balkan Peninsula according to Reed et al. (2004). In our analyses we have included three species from the genus Drusus which live near or at the border of Dinaric karst: D. bosnicus, Klapálek, 1899, D. botosaneanui Kumanski, 1968 and D. serbicus. Marinković-Gospodnetić, 1971.

RESULTS AND DISCUSSION

Diversity and distribution of the genus Drusus in Croatia with description of the female of Drusus vespertinus Marinković-Gospodnetić, 1976

The finding of Drusus vespertinus Marinković-Gospodnetić, 1976 which belongs to the Drusus bosnicus group in the spring area of the Una River (Fig. 1) is the fifth species of the genus Drusus found in Croatia. In the spring area of the Una River (Fig. 1) adult and larval specimens of D. vespertinus were collected (leg. I. Mihoci, A. Delić, I. Vučković, M. Kučinić) while in Loskun only larval specimen were sampled (leg. R. Ćuk, K. Žganec). Analysis of morphological features of male genitalia of adult specimens collected in the spring area of the Una River confirms that they belong taxonomically to Drusus vespertinus Marinković-Gospodnetić, 1976 (Fig. 2). Before this finding in Croatia, this species was recorded only in the western part of Bosnia and Herzegovina at three localities: the spring areas of rivers Bastašica, Ribnik and Sanica (Marinković-Gospodnetić, 1979). Therefore, recent findings in Croatia represent the fourth and fifth localities where this species was found and the first finding of the Drusus bosnicus group in Croatia.

The female of Drusus vespertinus has not previously been described (Malicky, 2004; Marinković-Gospodnetić, 1976). Based on specimens collected in the Bosnia and Herzegovina and Croatian we present a description of the female of this species.

Description: general appearance: dark brown; sclerites and tergites brown; cephalic and thoracic setal areas brown, abdominal setal areas light brown; body setation light brown; legs brown; haustellum and intersegmental teguments pale, whitish. Spur formula: 133 (N=3). Forewing length: 12.00 – 13.69 mm (N=3).

Female terminalia (Fig. 3 A-D): Lateral lobe of segment IX membraneous, triangular from lateral with a dorsal sclerotized setose part, the latter distinct and elongated from lateral and evenly rounded from dorsal and ventral. Segment X proximally wider than distally, from dorsal with 2 lateral lobes and a deep and round median excision leaving 2 distinct median protrusions in lateral, ventral and dorsal view; (Fig. 3 A-B, D); tips of lateral lobes sharp and distinct in dorsal and ventral view, approximately as long as supragenital plate; ventrally unsclerotized. Supragenital plate quadrangular in lateral, ventral and caudal view (Fig. 2 A-C). Vulvar scale with 2 lateral lobes in ventral view, lobes roundly oval with converging tips; median lobe reduced to a small triangular prominence (Fig. 3 D). The presented description enables identification of D. vespertinus
Fig. 3. A-D. Morphological features of female genitalia of *Drusus vespertinus*: A) lateral view; B) dorsal view; C) caudal view; D) ventral view.

females. So far, among the *D. bosnicus* group species occurring in the Dinaric karst area only females of *D. bosnicus*, *D. plicatus* and *D. krusniki* are described (Radovanović, 1942; Klapálek, 1899) and depicted in Malicky (2004).

However, in order to separate females of various species of the *D. bosnicus* group, a detailed revision and comparative analysis of morphological features of females of all *D. bosnicus* group species is necessary.

In the previous studies four species of the genus *Drusus* were recorded in Croatia: *D. croaticus* Marinković-Gospodnetić, 1971, *D. chrysotus* (Rambur, 1842), *D. discolor* (Rambur, 1842) and *D. schmidi* Botosaneanui, 1960 (e.g. Cerjanec, 2012; Previšić et al., 2012, 2013; Habdija, 1979; Marinković-Gospodnetić, 1971a, 1979) (Fig. 4, Tab. 1). *Drusus croaticus* is the most common species of the genus *Drusus* in Croatia, recorded in approximately 20 springs and spring areas (Cerjanec, 2012; Previšić & Popijač 2010; Previšić et
According to literature data *D. croaticus* and *D. krusniki* are among the most frequent species of this genus in the Dinaric karst of the Balkan Peninsula (e.g., Ibrahim, 2011; Oláh, 2010; Previšić et al., 2009, 2014; Marinković-Gospodnetić, 1979; M. Kučinić, A. Previšić unpublished data). *Drusus croaticus* was also found in the Dinaric karst in Slovenia (Urbanić, 2004), an area that does not belong to the Balkan Peninsula.

The second species, *D. discolor* was found in Croatia for the first time, in the larval stage in one locality in the Mržnica River, by Habdija (1979). In further studies *D. discolor* was recorded in adult stages at two localities in Croatia, in the springs of the Čabranka River and the Dobra River (Cerjanec, 2012; Previšić & Popijač, 2010) (Fig. 4). *Drusus discolor* is a widely distributed European species, ranging from Central Europe, the Pyrenees, Alps and Carpathians to the Balkan Peninsula (Pauls et al., 2006).

The third species of the genus *Drusus* recorded in Croatia is *D. chrysotus* founded only in the spring of the Dobra River near the town of Skrad, in the mountain region Gorski kotar (Cerjanec, 2012; Previšić et al., 2012) (Fig. 4). Previously, *D. chrysotus* was found only in the Alps; therefore, the finding in Croatia indicates a disjunct population of this species and the easternmost point in the distribution area of *D. chrysotus*, distributed now also in the Dinaric karst of the Balkan Peninsula (Previšić et al., 2012).
The last species from the genus *Drusus* recorded in Croatia is *D. schmidi* (Previšić et al., 2013) and this is very interesting information. This species was found in the Dubočanka Stream and Jankovac Spring on Mt Papuk in eastern Croatia, the Slavonia region (Previšić et al., 2013) (Fig. 4). This is the first finding of this species in the area lying outside of the Balkan Peninsula and the Dinaric karst. *Drusus schmidi* was also found in several locations in Bosnia and Herzegovina and Montenegro and in two locations on Kosovo (Previšić et al., 2014; Ibrahim, 2011; Stanić-Koštroman, 2009; Marinković-Gospodnetić, 1979). The population on Mt Papuk is the northernmost point in the distribution area of this species. Some additional species from the genus *Drusus* could possibly be found in the future in Croatia, like the widely distributed European species *D. biguttatus* (Pictet, 1834) or some species from the *D. bosnicus* group distributed in Bosnia and Herzegovina.

Diversity and distribution of the genus *Drusus* in Dinaric karst of the Balkan Peninsula

In the area of the Balkan Peninsula 40 species from genus *Drusus* are recorded (e.g. Oláh & Kovács, 2013; Oláh, 2010, 2011; Malicky, 2004, 2005; Krušnik, 1997; Kumanski, 1988; Marinković-Gospodnetić, 1971c, 1976, 1978, 1979). This area is the centre of diversity of this genus with 33 endemic species. Seven species are distributed also in other areas: *D. discolor* and *D. biguttatus* have wider European ranges, *D. botoananiu* is also recorded in Turkey, *D. tenellus* (Klapálek, 1898) in the Carpathians (Kumanski, 1988; Malicky, 2004), *D. schmidi* in continental lowland part of Croatia, *D. chrysotus* in the Alps and *D. croaticus* in Slovenia (Previšić et al., 2013) (Tab. 1).

Most of the endemic *Drusus* species in the Balkan Peninsula belong to the *D. bosnicus* Group. *Drusus bosnicus* group is also distributed in some other area e.g. the Alps

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
<th><em>D. bosnicus</em> group</th>
<th>Larva</th>
<th>Diet pattern</th>
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</thead>
<tbody>
<tr>
<td><em>Drusus biguttatus</em></td>
<td>European species</td>
<td>no</td>
<td>described</td>
<td>grazers</td>
</tr>
<tr>
<td><em>Drusus bosnicus</em></td>
<td>B &amp; H</td>
<td>yes</td>
<td>described</td>
<td>grazers</td>
</tr>
<tr>
<td><em>Drusus botoananiu</em></td>
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<td>undescribed</td>
<td>carnivorous filterers</td>
</tr>
<tr>
<td><em>Drusus chrysotus</em></td>
<td>Croatia, Alps</td>
<td>no</td>
<td>described</td>
<td>grazers</td>
</tr>
<tr>
<td><em>Drusus croaticus</em></td>
<td>Croatia, Slovenia</td>
<td>no</td>
<td>described</td>
<td>carnivorous filterers</td>
</tr>
<tr>
<td><em>Drusus discolor</em></td>
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<td>no</td>
<td>described</td>
<td>?</td>
</tr>
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<td><em>Drusus gombos</em></td>
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<td>yes</td>
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<td>?</td>
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<td>undescribed</td>
<td>?</td>
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<tr>
<td><em>Drusus klapaleki</em></td>
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<td>yes</td>
<td>described</td>
<td>grazers</td>
</tr>
<tr>
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<td>yes</td>
<td>described</td>
<td>grazers</td>
</tr>
<tr>
<td><em>Drusus radovanovici</em></td>
<td>B &amp; H</td>
<td>yes</td>
<td>described</td>
<td>grazers</td>
</tr>
<tr>
<td><em>Drusus ramae</em></td>
<td>B &amp; H</td>
<td>yes</td>
<td>described</td>
<td>grazers</td>
</tr>
<tr>
<td><em>Drusus schmidt</em></td>
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<td>no</td>
<td>undescribed</td>
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<tr>
<td><em>Drusus serbicusa</em></td>
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<td>grazers</td>
</tr>
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<td>no</td>
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</tr>
<tr>
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<td>?</td>
</tr>
<tr>
<td><em>Drusus tenellus</em></td>
<td>B &amp; H, Macedonia, Romania</td>
<td>no</td>
<td>undescribed</td>
<td>?</td>
</tr>
</tbody>
</table>

Tab. 1. Species of the genus *Drusus* in the Dinaric karst of the Balkan Peninsula with: distributional data, belonging or not to the *Drusus bosnicus* group, described or undescribed larvae and diet pattern (B & H = Bosnia and Herzegovina; MNG = Montenegro).
Kučinić, M. et al.: The first finding of the Drusus bosnicus group in Croatia with some notes ...

(Waringer et al., 2007). In the Dinaric karst there are 19 species of the genus Drusus, out of which 10 species of the D. bosnicus group were recorded: seven in Bosnia and Herzegovina (Oláh, 2013) in Montenegro, Drusus krusnik Malicky, 1981 in Kosovo, Montenegro and Albania and Drusus kerek Oláh, 2011 in Albania (Oláh & Kovács, 2013; Oláh, 2010, 2011; Kučinić et al., 2008, 2010, 2011a, 2011b; Malicky, 1981; Marinković-Gospodnetić, 1979) (Tab. 1). As adults the Drusus bosnicus group species have limited dispersal abilities. They are active diurnally and are mostly restricted to spring areas and small zones of nearby streams/rivers resulting in disconnected populations and allopatric distribution. These biological features are the most important reasons for the high diversity and endemism of this group, as well as some geological and hydrological features (e.g. Previšić et al., 2014; Kučinić et al., 2011). Some other, nocturnal species from this genus with higher dispersal abilities of adults have larger distribution areas, e.g. species D. schmidi. Within the distribution area of D. schmidi (Bosnia and Herzegovina and Kosovo) eight endemic species of the Drusus bosnicus group have been recognized, but with smaller distribution areas and higher diversity. On the east, the Drusus bosnicus group is distributed in the areas of Albania and Macedonia and possibly in Bulgaria and Greece that are not a part of Dinaric karst. The western distribution border of the Drusus bosnicus group in the Balkan Peninsula is the area of Bosnia and Herzegovina, reaching Croatian territory only at a small and isolated part, in the spring area of the Una River. In other parts of Dinaric karst, in the central Croatia the Drusus bosnicus group was not found.

The spring of the Una River (Fig. 1) is situated at the eastern side of Mt Lička Plješivica. The border is bounded by the mountains among which the biggest are Lička Plješivica and Dinara which lie northwest to southeast, 130 km in length. These two mountains represent a natural border that prevents the dispersal of the Drusus bosnicus group further to the west. At the west side of these mountains Drusus croaticus, which does not belong to the Drusus bosnicus group, is distributed. Species of the Drusus bosnicus group and D. croaticus have never been recorded as co-occurring species in one spring area, and neither have two different species from the Drusus bosnicus group. This pattern was observed for some other Drusus species; e.g. D. discolor – D. septentrionis, D. chrysotus – D. discolor, D. croaticus – D. discolor, D. botosaneanui – D. plicatus, (Čerjanec, 2012; Previšić & Popijač, 2010; Marinković-Gospodnetić, 1979). An explanation for the Drusus bosnicus group can be found in the possibility that the taxa of this group were separated in different periods during the Pleistocene (due to allopathic fragmentation), probably from a single, common ancestor with a large area of distribution in the Balkan Peninsula, which resulted in high diversity with numerous different, endemic taxa with small distribution areas (Kučinić et al., 2011a; Marinković-Gospodnetić 1976, 1978). Recent phylogeographic study showed that two different Drusus species of the Drusus bosnicus group inhabit the spring of the Bosna River (Previšić et al., 2014). This is possibly an indication of a more complex distribution pattern and evolutionary history of the Drusus bosnicus group than previously recognised (e.g. Kučinić et al., 2011a). Thus, full understanding of the latter remains a challenge for the future. Very likely D. croaticus had a different process of speciation in the separate area than the Drusus bosnicus group. In the future, some new Drusus species might be found in the Dinaric karst and the Balkan Peninsula; recently seven new species were described from Albania, one from Greece and one from Montenegro (Oláh & Kovács, 2013; Oláh, 2010, 2011). Possibly, some of them belong to the Drusus bosnicus group according to morphology of adults and larvae, and ethology (daily activity). For these species, belonging to the Drusus bosnicus group will be defined in the future.
Ecological notes on the genus *Drusus* in Dinaric karst of the Balkan Peninsula

The most important ecological features of aquatic insects include the feeding ecology of larvae, emergence patterns of adults and characters of life cycle.

Information on larval morphology (e.g. mandible, head, legs) enables knowledge of feeding ecology. All described and recorded larvae of the *Drusus bosnicus* group in Dinaric karst of the Balkan Peninsula belong to a group of grazers (Kučinić et al. 2008, 2010, 2011a, 2011b) (Tab. 1). Preliminary data indicate the same diet pattern for *Drusus vespertinus* larva (Previšić et al., 2009; unpublished data A. Previšić, M. Kučinić). Because phylogenetically related species within the genus *Drusus* have the same feeding ecology (Pauls et al., 2008), we believe that all species from the *D. bosnicus* Group belong to the group of grazers. Larval diet is known for three out of four Drusus species occurring in Croatia: the larvae of *D. croaticus* are grazers, while the larvae of *D. chrysotus* and *D. discolor* belong to the group of carnivorous filterers (Previšić et al., 2012; Kučinić et al., 2008; Pauls et al., 2008). Diet pattern is very important for evolutionary processes and for diversity of the genus *Drusus* in general (Pauls et al. 2008).

In general, species from the genus *Drusus* usually inhabit rheocrene and limnocrene-type karst springs and crenal sections of mountain streams. In the Balkan Peninsula, species from the genus *Drusus* emerge in different periods, from spring to autumn (e.g. Previšić et al., 2007; Kučinić, 2002). Some species, like *D. tenellus* or *D. botosaneanui* emerge in summer and autumn (Ibrahimi et al., 2012; Stanić-Koštroman, 2009; unpublished data, M. Kučinić), while many other species, especially from the *Drusus bosnicus* group emerge in spring and then in the late summer and autumn. According to the literature data and our investigation, in the Dinaric karst of the Balkan Peninsula, only one species from the *Drusus bosnicus* group, *D. ramae*, emerges in spring alone and has a typical univoltine life cycle (Kučinić et al., 2010; Stanić-Koštroman, 2009). All other species emerge from spring to autumn (bivoltine life cycle). Investigation of *D. septentrionis* and *D. croaticus* showed the possibility that both generations, the vernal and the autummal, have a one year life cycle (Kučinić et al., 2008). This type of a life cycle is also possible for some other species from the *Drusus bosnicus* group. Adults of different species from the *Drusus bosnicus* group are on the wing from April to November (e.g., Ibrahimi, 2011; Oláh, 2010; Previšić et al. 2007; Marinković-Gospodnetić, 1971c).

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Prvi nalaz Drusus bosnicus grupe (Insecta, Trichoptera) u Hrvatskoj s osvrtom na raznolikost, taksonomiju, rasprostranjenost i ekologiju roda Drusus u Hrvatskoj i dinaridskom kršu Balkanskog poluotoka

M. Kučinić, A. Previšić, I. Mihoci, R. Ćuk, A. Delić, K. Žganec, D. Cerjanec & I. Vučković

Na području Balkanskog poluotoka zabilježeno je 40 vrsta iz roda Drusus, od toga 19 na području Dinarskog krša. Vrste roda Drusus u pravilu naseljavaju izvore i izvorišna područja planinskih tekuća što uvjetuje alopatrijski tip rasprostranjenosti, procese specijacije i nastanak većeg broja vrsta, najčešće s malim područjem rasprostranjenosti. Zbog velike raznolikosti i intenzivnih procesa specijacije jedna od najzanimljivijih skupina unutar roda...