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A Study of Trichoptera of the Blinajë Hunting Reserve Including the First Records of *Ironoqua dubia* (Stephens, 1837) (Limnephilidae) from the Hellenic Western Balkans

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ABSTRACT

The results of investigation of Trichoptera of the Blinajë Hunting Reserve in Kosovo are presented. Two different sampling techniques for collecting adult caddisflies are compared. The most significant faunistic records are of *Ironoquia dubia* (Stephens, 1837) (Limnephilidae). This species has not been previously recorded from Ecoregion 6, the Hellenic Western Balkans. The Kosovo records are put in the context.

Key words: Trichoptera, Balkan Peninsula, *Ironoquia dubia*, Ecoregion 6, Kosovo, sampling techniques.

INTRODUCTION

The territory of the Blinajë hunting area designated as a special reserve zone is located in central part of Kosovo, 15 km on the western side of Lipjan town. The total surface of Blinajë Hunting Reserve is 5 500 ha and stretches into the territory of three municipalities: Lipjan, Shtime and Drenas. The altitude within this territory ranges from 670 to 860 m above sea level. There are 33 artificial lakes present inside Blinajë Hunting Reserve. This part of the Balkan Peninsula has been poorly investigated for caddisflies.

It has been known for a long time (e.g. Crichton, 1965; Svensson, 1974; Usseglio-Polatera, 1986) that different sampling techniques capture a different range of species. This is especially important in biodiversity studies where the number of species, and especially including those with low densities, is increased by implying more sampling techniques.

The life cycle of *Ironoquia* Banks, 1916 species that have been studied is most unusual. The fully grown larvae leave the water and burrow into the bank or piles of

leaf litter by its side. They remain very moist but are effectively terrestrial. They stay as resting larvae until they pupate in the autumn. Larvae of *I. dubia* inhabit woodland streams, ditches and ponds in which the bottom is covered with dead leaves. They are found in a wide saprobic variety of freshwater habitats from totally unpolluted streams up to very organically polluted rivers and thus the species has no saprobic value (Graf *et al*., 2002; Ćuk, Vučković, 2010). *I. dubia* has no legal protection in annexes of EU directives but is listed as rare, threatened, presumably vulnerable or critically endangered in Denmark, Carinthia (Austria), some cantons of Germany and Hungary and it is also a priority species within the United Kingdom Biodiversity Action Plan (e.g. Nogradi, Uherkovich, 1999; Shirt, 1987). It is given the IUCN category of Critically Endangered in the United Kingdom in Wallace (2016). Wallace (2011) summarised information on the species.

The goal of this paper is to give an overview of the composition of the caddisfly fauna of the Blinajë Hunting Reserve and also to compare species composition by using two different sampling techniques for adult caddisflies: ultra-violet light trap and hand searching and netting. The composition of the macrozoobethos during the spring and winter period has also been investigated. Another goal of this paper is to contribute to the knowledge on biogeographic distribution, ecological features and population characteristics of *I. dubia* in South-eastern Europe.

MATERIAL AND METHODS

Sample collection and processing

Adult caddisfly specimens were collected with entomological net, sweeping net, handpicking and ultraviolet light trap. The sampling was carried out during the period May - December 2013. Collected samples were preserved in 80% ethanol. The specimens were identified under a stereomicroscope with determination keys from Malicky (2004) and Kumanski (1985; 1988). The collection is deposited at the Laboratory of Zoology of the Faculty of Natural and Mathematical Sciences, University of Prishtina, Kosovo.

In order to verify the presence of larvae of *I. dubia*, macrozoobenthos specimens were collected with standard Surber net and D-net from the bottom of the stream during 2013 twice, during March and December, from various microhabitats in the investigated area. Macrozoobenthos specimens were not conserved but were released back to their habitat.

Study site

The sampling site is located at the spring area of the only stream inside the Blinajë Hunting Reserve, adjacent to the biggest lake (Table 1). This first order stream is surrounded by a forested area, is in average about 1.2 m wide and 15 cm deep. A significant portion of the water from the spring goes directly through a pipe for use in the Administration Office of the Blinajë Hunting Reserve. The remaining water flows beside the lake and discharges near Lipjan town into Sitnicë River. The substrate is made by gravel, cobles and boulders surrounded by fine particles and is shaded

throughout the year by nearby vegetation. The amount of the water during the summer significantly decreases and according to the information from the staff working in this hunting reserve, during some years the amount of flowing water is very low or absent at all giving the stream the characteristics of a temporary stream.

Table 1. Locality and habitat assessment data after Plafkin *et al*., (1989) and Barbour *et al*., 1999) for the sampling station in Blinajë Hunting Reserve.

Parameters	Sampling stacion characteristics
Latitude ˚N	42.5185˚N
Longitude ˚E	20.9788˚E
Altitude m	721
Distance from source (m)	5
Stream width (m)	1.2
Stream depth (cm)	15
Epifaunal substrate/ Available cover	More than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential.
Embedeness	Gravel, cobble and boulder particles are 20-50% surrounded by fine sediment.
Pool Substrate Characterization	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.
Sediment deposition	Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition.
Flow status	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.
Bank stability	Banks stable; evidence of erosion absent or minimal; <5% of bank affected.
Bank vegetation protection	More than 90% of the streambank surfaces and immediate riparian zones covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally
Riparian vegetative zone width	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.

RESULTS

Two sampling methods for collecting adult caddisflies were applied in parallel: a) with entomological net, sweeping net and handpicking, and b) with UV light trap. Two hundred and thirty specimens (Table 2) belonging to thirteen caddisfly species were found with the first method. Fourteen caddisfly species with one hundred and eighty two specimens (Table 2) were sampled with the second method. Species collected only during the daylight with the first method are: *Hydropsyche angustipennis* (Curtis, 1834)*, Athripsodes billineatus* (Linnaeus, 1758)*, Mystacides azureus* (Linnaeus, 1761)*, Mystacides niger* (Linnaeus, 1758) and *Helicopsyche bacescui* Orghidan, Botosaneanu, 1953 (Table 2). Seven species were found only with UV light trap: *Rhyacophila fasciata* Hagen, 1859, *Grammotaulius nigropunctatus* (Retzius, 1783)*, Halesus digitatus* (von Paula Shrank, 1781)*, Limnephilus vittatus* (Fabricius, 1798)*, Micropterna sequax* McLachlan, 1875, *I. dubia* and *Potamophylax pallidus* (Klapalek, 1899) (Table 2). The last specimen of *I. dubia* was found on 25th of November 2013.

Table 2. The list of species collected with entomological net, sweeping net and handpicking (D) and ultraviolet light trap (N) in Blinajë Hunting Reserve during the period May - December 2013.

Months	May		June		July		August		September		October		November		December	
Species	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Rhyacophila fasciata Hagen, 1859	4 N	2 N		1 N		2 N		3 N			1 N					
Wormaldia occipitalis (Pictet, 1834)	5 D	12 D 13 N	12 D 6 N	4 D	4 D 6 N	8 D 3 N										
Hydropsyche saxonica McLachlan, 1884		1N		3 N												
Hydropsyche angustipenis (Curtis, 1834)		2 D		4 D												
Hydropsyche spp.	11 N		16 D 13 N		7 N		6 D		2 N							
Cyrnus trimaculatus (Curtis, 1834)	4 D	5 D	14 D	7 D	5 D	3 D										
Polycentropus flavomaculatus (Pictet, 1834)				1 D				2N								
Tinodes janssensi Jacquemart, 1957	2 D	5 D	3 D 2 N				2 N									
Chaetopteryx bosniaca Marinkovic-Gospodnetic, 1959											5 D 11 N	2 D 2 N		2 D 8 N	3 D	6 D 1 N
Grammotaulius nigropunctatus (Retzius, 1783)								3 N								
Halesus digitatus (von Paula Shrank, 1781)								4 N			8 N	7 N		3 N		
Ironoquia dubia (Stephen, 1738)								1 N						1 N		
Limnephilus vittatus (Fabricius, 1798)		3 D 1N														
Micropterna nycterobia McLachlan, 1875					5N	7N	1 D	8N			7N	2N				
Micropterna sequax McLachlan, 1875						5 N	2 N									
Potamophylax pallidus (Klapalek, 1899)							2 N	11 N	5 N	7 N	1 N	1 N	2 N	5 N	1 N	
Athripsodes bilineatus (Linnaeus, 1758)			3 D	11 D												
Mystacides azureus (Linnaeus, 1761)	12 D	21 D	11 D	12 D	4 D	6 D										
Mystacides niger (Linnaeus, 1758)	6 D	12 D														
Helicopsyche bacescui Orghidan & Botosaneanu, 1953		1 D														

In total, specimens of *I. dubia* represent only 0.48% of the total caddisfly specimens collected with both methods during the whole sampling period of 2013 year. Specimens of *I.dubia* represent 1.09% of the total caddisfly specimens collected with UV light trap during the sampling period.

The macrozoobenthos is composed by the following groups: Trichoptera with six families, Plecoptera with three families, Ephemeroptera with three families, while Odonata, Diptera, Amphipoda and Gastropoda are represented each with one family only (Table 3). Only one larvae of *I. dubia* has been found during the sampling period of macrozoobenthos.

DISCUSSION

Out of twenty taxa found during this investigation most are common and widespread species in the Balkan Peninsula and Europe. Most of the found species belong to the family Limnephilidae due to the characteristics of the stream: slow flow, low water level throughout the year and large quantities of food available for larvae of these species. It was noted the absence of species of the Rhyacophilidae family with the exception of *R. fasciata* which is a generalist occurring in different habitats. Most of the species of this family usually require fast flowing streams with high water level. Three species found during this investigation are notorious in terms of their scarce distribution in

the Balkan Peninsula: *Tinodes janssensi* Jacquemart, 1957, *Chaetopteryx bosniaca* Marinkovic-Gospodnetic, 1959 and *Ironoquia dubia*. The endemic species of the Balkan Peninsula, *T. janssensi* beside Blinajë Hunting Reserve is only found in few localities in Albania, Greece and Kosovo (e.g. Ibrahimi *et al*., 2016a). The autumn limnephilid species *C. bosniaca* found during the autumn-winter period has been rarely sampled in Kosovo but also elsewhere in the Balkan Peninsula as well (e.g. Ibrahimi *et al*., 2015). However the most important faunistic finding during this investigation was the record of *I. dubia.*

Table 3. The composition of the macrozoobenthos in the Blinajë Hunting Reserve during March and December 2013.

Taxa / Sampling month	March	December
Trichoptera		
Rhyacophilidae	5	12
Philopotamidae	2	3
Hydropsychidae	19	8
Psychomyiidae		8
Leptoceridae	18	3
Limnephilidae	31	19
Ironoquia dubia	1	
Plecoptera		
Perlidae	3	14
Nemouridae		12
Chloroperlidae	15	2

Taxa / Sampling month	March	December
Ephemeroptera		
Baetidae	13	21
Ephemeridae	12	8
Leptophlebidae	8	5
Odonata		
Gomphidae		5
Diptera		
Tipulidae	2	1
Amphipoda		
Gammaridae	11	13
Gastropoda		
Lymnaeidae	2	

The known distribution range of *I. dubia* in Europe (Fig. 1) extends over the United Kingdom, central and northern Europe up to Caucasus (Graf *et al*., 2015). It has been suggested that this species is absent from South-eastern Europe (e.g. Reding, 2006) but this seems to be incorrect proven by recent Croatian records (Čuk, Vučković, 2010) and current investigation. Our finding of this species is the first genus and species record for the Republic of Kosovo. It is reported for the first time from Ecoregion 6, Hellenic Western Balkans. *I. dubia* has been reported for the first time from several European countries and regions only during the last decade (e.g. Reding, 2006; Roos, Semmler-Elpers, 2008). Despite intensive caddisfly investigations in more than 100 sampling sites all over Kosovo during 2010 - 2016 including streams, brooks, rivers, standing waters, temporary waters and lakes *I. dubia* has not been found anywhere else up to now (e.g. Ibrahimi *et al*., 2007; 2013; 2014; 2015; 2016b; 2016c; Ibrahimi, Gashi, 2008). This suggests this species is extremely rare in Kosovo. In total, during the whole sampling period with entomological net, sweeping net, handpicking and UV light trap only two males have been found. However, this is usually a very secretive species and difficult to collect as an adult. At Millbarn Pond over several years only a single adult specimen was sampled among thousands of caddisflies (e.g. Crichton, 1960; 1965). Hiley (1970) reports larvae from the stream only a few metres from the

light trap site, but running in a deep channel below it. Crichton, Baker (1959) cite communications with Swedish colleagues that suggest *I. dubia* adults never leave the stream bed during flight. Vick (1992) collected many adults in a Malaise Trap set close to the stream bed. However this situation is totally different in a locality in south east Norway, where this species was collected in large numbers as a dominant species (Andersen *et al*., 1990). Čuk, Vučković, (2010) report *I. dubia* larvae from 30% of the streams they investigated indicating thus that it is easier to collect it in larval stadium. However during our investigation even the standard methods in collecting macrozoobenthos did not produce a significant number of larvae of this species. Only one larvae of this species was found during March around the leaf package close to the stream shore. This indicates that either the species is relatively rare in this site or more efforts are needed in collecting larger number of specimens. It has been reported that *I. dubia* has a short flight period from the middle of September to the first week in October (Higler, 2008). The last specimen of *Ironoquia dubia* during our investigation was found during the third week of November. This implies that at least in Kosovo, *I. dubia* has longer flying period than previously thought. This may also depend on the weather conditions from year to year since it was observed in other species that during the years with higher temperatures during the late autumn months, the flying period of adult caddisflies is extended.

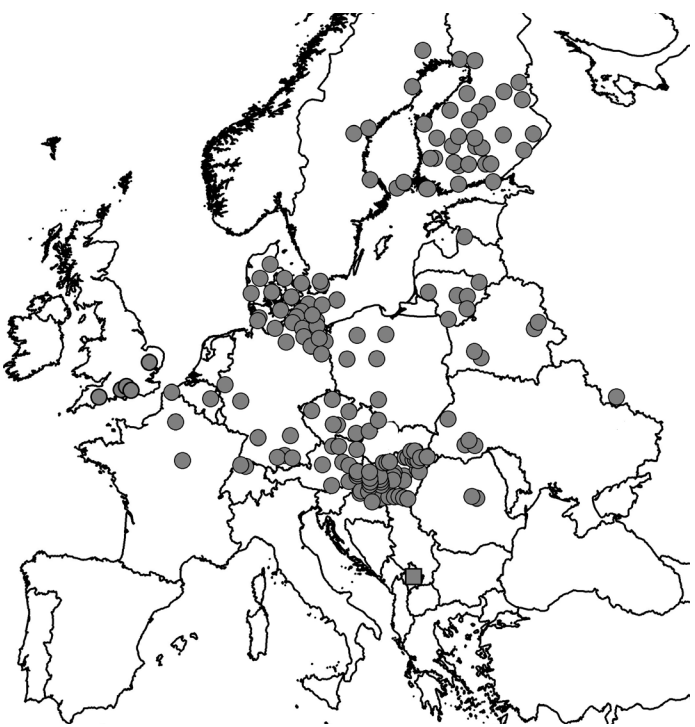

Fig. 1. Distribution of *Ironoquia dubia* in Europe (full circles-data from DAET 2014, Čuk, Vučković 2012, Urbanić, 2001; 2006), square - distribution in Kosovo (current investigation).

By implying two different methods in collecting adult caddisflies the number of found species has been significantly increased. There is a significant difference in qualitative composition of caddisfly samples collected by applying two mentioned methods. The coefficient of similarity is only 40%. It is known that adult caddisflies of different species express their activity during the different parts of the day and thus many species often remain unexplored because only one particular method is used in sampling. Our investigation proves the importance of using different sampling techniques for adult caddisflies in alpha diversity studies. Finding of a rare species such as *I. dubia* during this investigation is a result of a combination of different sampling techniques. This was noted earlier as well, for example in Brezne Lake in Kosovo, where by implying different sampling techniques a rare species in the Balkan Peninsula, *Triaenodes bicolor* (Curtis, 1834) has been found (Ibrahimi *et al*., 2017). According to the predictions of Chao1 diversity index (Chao, 1984) when first samples contain several species with singletons, there are high chances that in these sites are hidden other species with low population densities. This was the case during this investigation and consequently, increased sampling efforts and especially different sampling techniques were proved to be a successful tool in sampling these species with small populations.

This research shows that there are still poorly investigated areas in the Balkan Peninsula which contain rare or less known species of caddisflies. In addition to this, this investigation proves that application of different sampling techniques results in discovery of more species and especially those with small populations which are usually overlooked when only one particular method in collecting caddisflies is implied. This investigation highlights the Blinajë Hunting Reserve as an important area in regards to the caddisfly species. Some of them found during this investigation, or those reported earlier (Ibrahimi *et al*., 2016a or b) are rarely sampled in Kosovo or in the Balkan Peninsula in general.

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