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THE FIRST RECORD OF *Dixella aestivalis* (Meigen, 1818) (DIPTERA: DIXIDAE) IN SERBIA

Predrag M. Simović[1], Vladica M. Simić[1], Ana S. Petrović[1]*

[1]*University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia*
*****Corresponding author; E-mail: ana.petrovic@pmf.kg.ac.rs

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ABSTRACT. The meniscus midges *Dixella aestivallis* (Meigen, 1818) was recorded for the first time on the territory of Serbia. This species was found in the Karamejdan Lake on the territory of the protected nature reserve Pešter plateau, the highest karst plateau of the Balkan Peninsula. The larva of *D. aestivalis* was detected in a locality with well-developed aquatic vegetation and high value of oxygen concentration. *Dixella aestivalis* is a eurytopic species, which prefers to live in different types of small stagnant waters. This study is a result of the ongoing monitoring of freshwater ecosystems. This finding is the starting point for further studies of these species in Serbia.

Keywords: Dixidae, *Dixella aestivalis*, Pešter plateau, Karamejdan Lake, Serbia.

INTRODUCTION

Dixidae, also known as meniscus midges, represent one of the smallest families within the order Diptera (SAVARY, 1992). There is poor knowledge about this family worldwide and the total number of recorded species in different zoogeographical areas varies considerably (CORRÊA and GIL-AZEVEDO, 2018). Moreover, WAGNER *et al*. (2007) stated that the mentioned number of species represents only about 20% of the world biodiversity of the Dixidae family. Dixidae is reminiscent of related Culicidae, with which they belong to the superfamily Culicoidea, within the suborder Nematocera (KOÇ *et al.,* 2006; KANG *et al.,* 2014). They are distributed in inland waters of all continents except Antarctica, with the greatest diversity recorded in the Palearctic and Nearctic regions (WAGNER *et al*., 2007; OBOŇA *et al*., 2015). The global diversity of the Dixidae family includes 197 known species, classified into nine genera. Species from *Dixa* and *Dixella* genera inhabit European waters (GREENWALT and MOULTON, 2016; MITRA *et al*., 2016; ADLER and COURTNEY, 2019). Twenty species of the genus *Dixella* are reported for Europe (WAGNER, 2014).

Adults dixids are poor fliers concealed in overhanging vegetation near the water. They do not feed (PETERS and ADAMSKI, 1982; WAGNER, 2004; IVANKOVIĆ *et al*., 2019). Among European dixids, larvae of the genus *Dixa* usually live in small to medium-sized streams with clear water and abundant coastal vegetation, with shade (IVKOVIĆ and IVANKOVIĆ, 2019). On the other hand, larvae of the genus *Dixella* occur on the edges of different types of small stagnant waters such as ponds, wetlands, lakeshores and bog pools (SAVARY, 1992; SALMELA

et al., 2014; BYLAK, 2016). *Dixella aestivalis* larvae occur in the meniscus that forms the interface between the water and leaves of macrophytes or can be trapped in the surface film itself (LOCK *et al*., 2014). In general, such a way of life is characteristic of most species of the genus *Dixella* (KREBS, 1982; WAGNER *et al*., 2007).

Most of the species of the genus *Dixella* have two generations each year, the first in the spring and the second in the autumn (HÅLAND, 2009; BYLAK, 2016). The larvae of the genus *Dixella* are filter feeders and live in or just above the aquatic meniscus, on rocks, fallen branches, and leaves, where they take a characteristic U-shaped position (GOLDIE-SMITH and THORPE, 1991; EBJER, 2000; KOÇ *et al*., 2006; BRISCOE *et al*., 2015). They swim with fast zig-zag movements on the water's surface, using a pair of rear “oars” with floating hairs (BRACKENBURY, 1999). As uniquely adapted to life in surface films, they are sensitive to pollution by surfactants and oils (BULÁNKOVÁ and HALGOŠ, 1999; ADLER and COURTNEY, 2019).

So far, the diversity and distribution of Dixidae have not been adequately studied in Serbia with only two species of the genus *Dixa* mentioned in the dissertation by PETROVIĆ (2014). However, it is believed that several other species have been found, but identification was made only to the genus level (PETROVIĆ, 2014), because species recognition at the larval stage is extremely difficult.

Here, we present the first record of the genus *Dixella* and the species *D. aestivalis* (Meigen, 1818) on the territory of Serbia.

MATERIALS AND METHODS

Study area

A study of the macroinvertebrate community in Karamejdan Lake (43°04'59.4"N 20°06'49.6"E) (Figure 1A), in the area of the Pešter plateau (alternatively Pešterska visoravan), the highest karst plateau of the Balkan Peninsula, was performed in June 2021.

In the geological past (neogene), the central part of the Pešter plateau was a big lake. The area gradually drained through karst ruptures, the lakes remnants left in the certain parts, and one of them being Karamejdan Lake. It is located at an altitude of 1159 m, between the villages of Karajukića Bunari and Braćak. Due to long and harsh winters, with temperatures low to -40°C, short cool summers, strong and constant winds, the research area, as well as the whole Pešter plateau, is also known as Balkan (Serbian) Siberia (PAPP *et al*., 2014; ČAVLOVIĆ *et al*., 2017). The lake represents a peat habitat anthropogenically changed by the formation of an artificial canal for water collecting.

The aquatic macrophyte communities in the lake are very rich and diverse. The coastal zone is dominated by meadows and pastures vegetation, and near the lake, there are several ephemeral and permanent water ponds.

Pešter plateau is included in the "EMERALD ecological network in Serbia", as well as the Ramsar list of wetlands of international importance, "IPA - International Important Plant Areas", "IBA - Important Birds Area in Serbia", and "PBA - Selected Areas for Butterfly in Serbia" (LAZAREVIĆ, 2014).

Sampling methodology

Samples were collected using the benthological hand net (25x25 cm, mesh size diameter 500 μm), according to the EUROPEAN STANDARD EN 10870 (2012). Samples were conserved in 4% formaldehyde and deposited in the Institute of Biology and Ecology collection, Faculty of Science, University of Kragujevac, Serbia.

The macroinvertebrates were identified under NIKON SMZ 800 stereomicroscope with a MOTIC camera and Nikon Eclipse E100 microscope, based on morphological characteristics given in serial identification keys: CONCI and NILSEN, 1956; ROZKOŠNÝ, 1980; SPAČKOVA and KNOZ; 1980; and ELLIOTT *et al*. 1988.

Simultaneously with gathering benthic invertebrates samples in the field, the following physical and chemical parameters were measured: water temperature (°C), oxygen concentration (mgL), saturation (%), pH, conductivity (μs/cm), and water hardness ($CaCO_3$ mg/L) according to EUROPEAN STANDARD EN 5667-3:2018 (2018).

RESULTS AND DISCUSSION

A detailed analysis of the collected material (one specimen) and a review of the relevant literature showed that the Karamejdan Lake is the first site in Serbia where the genus *Dixella* and species *D. aestivalis* were found. The record of *D. aestivalis* was identified according to SPAČKOVA and KNOZ (1980).

The physical and chemical parameters of the investigated locality are summarized in Table 1. *Dixella aestivalis* was recorded in water with a high value of oxygen concentration (9.16 mg/L) and relatively low temperature (10.8°C). The species was collected in the Karamejdan Lake (Figure 1: A and B) with dense vegetation of hydrophile macrophytes, in full sunlight. The most common macrophytes species were: *Potamogeton natans* L., *Phragmites australis* (Cav.) Trin. ex. Steud., *Ranunculus aquatilis* L., *Typha* sp., *Nymphaea alba* L., *Utricularia australis* R. Br., *Alisma plantago-aquatica* L., *Glyceria* sp., *Equisetum* sp. and *Carex* sp.

Additionally, in the Karamejdan Lake, a few other species of macroinvertebrates were also recorded: *Coenagrion puella* Linnaeus, 1758., *Limnodrilus hoffmeisteri* Claparède, 1862, *Sigara nigrolineata* (Fieber, 1848), *Mesovelia furcata* Mulsant & Rey, 1852, *Lymnaea auricularia* (Linnaeus, 1758), *Tanypus* sp., *Cloeon dipterum* (Linnaeus, 1761), *Athripsodes bilineatus* (Linnaeus, 1758), *Caenis loctuosa* (Burmeister, 1839), and *Limnephilus* sp.

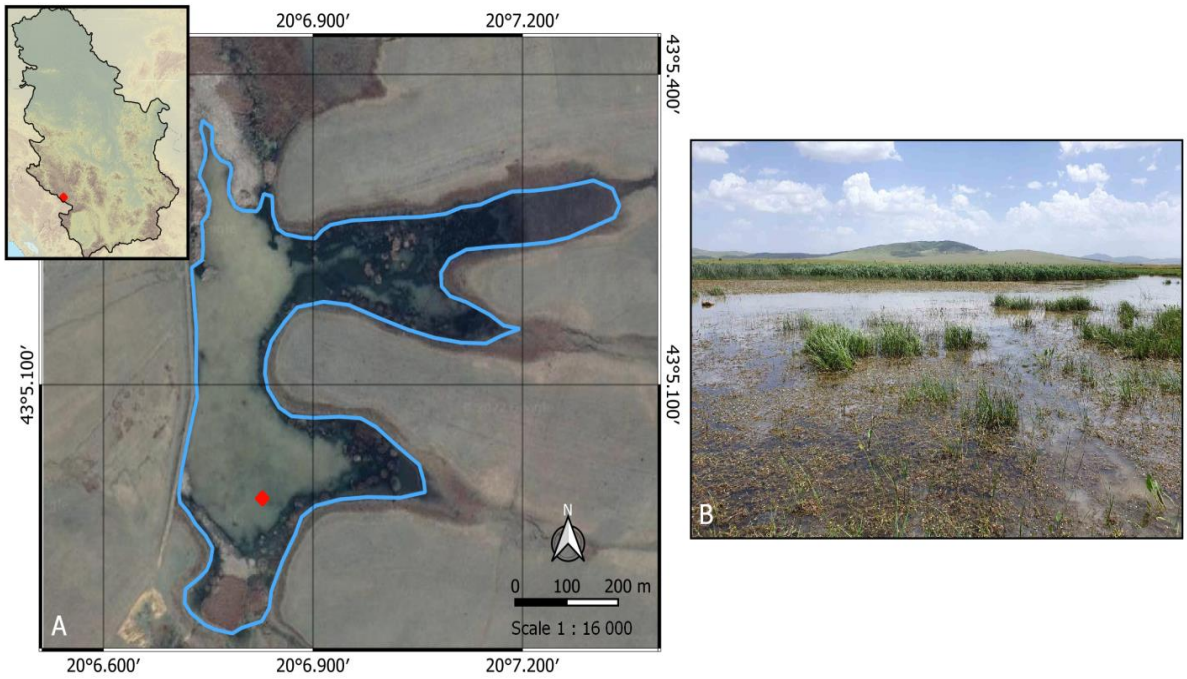

Figure 1. A. The position of Karameidan Lake in Serbia.
B. Locality where *Dixella aestivalis* was found in the Karamejdan Lake.

Table 1. Physicochemical parameters of the investigated locality in Karamejdan Lake.

Physicochemical parameters	Value
Water temperature (°C)	10.8
Concentration O_2 (mg/L)	9.16
Saturation O_2 (%)	92.9
pH (1-14)	7.81
Conductivity (μs/cm)	370
Hardness (mg/L $CaCO_3$)	180

The detected *D. aestivalis* larva is elongated, cylindrical, and somewhat flattened, with 7.8 mm in length (Figure 2). The larva is eucephalic, with a heavily sclerotized head with long antennae approximately equal length as head. The thoracic segment is simple without a leg-like structure. On the dorsal side of the body, there is not a segments of crowns of setae (different from the genus *Dixa*). The terminal complex of segments consists of a pair of lateral "paddles" with long chitinous hairs and a single narrowed caudal appendage with six long setae (float structure). The tip of the lateral paddles is clearly shorter than the caudal appendage. The ventral side of the abdominal larval segments bear ambulatory combs divided into two symmetrical halves. A small, sclerotized field named medial scleritis is developed between them, and on the investigated larva it has an isosceles triangle shape. It has taxonomic importance.

Figure 2. *Dixella aestivalis* (Meigen, 1818) dorsal view (photo by P. Simović, 2021).

When it comes to the genus *Dixella*, in regard to Serbia, the European countries count a much larger number of species. However, the biology of most *Dixella* species is unknown (BRISCOE *et al*., 2015). *Dixella aestivalis* is a rather rare species in Europe, but it is widespread throughout its territory (BYLAK, 2016). Based on literature data *D. aestivalis* has been reported in Great Britain (BRISCOE *et al*., 2015), Denmark (NIELSEN and ANDREASEN, 1998), Norway (HÅLAND, 2013), Sweden (OBOŇA *et al*., 2015), Finland (SALMELA *et al*., 2014), Belgium (LOCK *et al*., 2014), the Netherlands (VERDONSCHOT, 1992), Germany (KOCH, 1997), Czech Republic (SPAČKOVA and KNOZ, 1980), Slovakia (STARÝ, 2007; OBOŇA *et al*., 2015; BARANOVÁ *et al*., 2018), Poland (BYLAK, 2016), Lithuania (PAKALNIŠKIS *et al*., 2000), Russia, Spain, France, Switzerland, Italy, Austria, Hungary, Greece (WAGNER, 2014;

OBOŇA *et al*., 2015; BYLAK, 2016). In the mentioned studies, the findings of this species were relatively rare and were mentioned only within the studies of macroinvertebrate communities, or checklists within the order Diptera. In the territory of the Balkan Peninsula, except Greece, it has also been recorded in Croatia, but the finding dates from 1987. This finding has not been confirmed in recent research by IVKOVIĆ *et al*. (2020). Untill now published data about *D. aestivalis* only a few specimens were registered on Balkan Peninsula, this finding has great value.

Dixella aestivalis is a particularly eurytopic species (CUPENN, 1980). Literature data have shown that the species is most often observed in vegetation in the small lakes, wetlands, and ponds, most of the moderately eutrophic type. Rarely it can be found in occasional watercourses which dry out during summer, overgrown pools (cattle drinking pools), fishponds, and stream backwaters (KOCH, 1997; ANDERSEN *et al*., 2013; OBOŇA *et al*., 2015; BYLAK, 2016). In Europe, it has been observed in a wide range of altitudes, from 90 m (ANDERSEN *et al*., 2013) to 913 m (OBOŇA *et al*., 2015). Like our discovery, BARANOVÁ *et al*. (2018) recorded *D. aestivalis* at an altitude of about 800 m, in waters surrounded by forests and meadows, featured by rich aquatic macrophytes communities well adapted to the fluctuating water levels. In Poland BYLAK (2016) found *D. aestivalis* in ponds with well-developed cattail vegetation.

The region where we found *D. aestivalis* is climatically and biogeographically very diverse, and many authors have emphasized the particular importance of this geological region since, for some macroinvertebrates, Pešter waters are the only habitat in Serbia or beyond (FALNIOWSKI *et al*., 2011).

This report of the first occurrence of genus *Dixella* is a result of the ongoing monitoring of freshwater ecosystems in Serbia conducted by Hydrobiological laboratory of Faculty of Science, Kragujevac. Given the rare occurrence of certain species of the Dixidae family, this study represents an important contribution to the knowledge of the distribution of *D. aestivalis* as well as its ecological preferences.

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