



Epoicocladus flavens (Malloch, 1915) (Diptera: Chironomidae): A New Record from the Republic of North Macedonia

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Abstract: The aim of the study is to report new country records of the symphoretic midge *Epoicocladus flavens* (Malloch, 1915) on its mayfly host *Ephemera danica* Müller, 1764 (Ephemeroptera: Ephemeridae). Sampling was conducted during spring 2022, in the upper section of the watershed of the Golema Reka River, Republic of North Macedonia. The specimens were collected with a hydrobiological hand net (mesh size 500 µm), through applying the kick-and-sweep multihabitat procedure. According to the published data, the distribution of *E. flavens* is scattered mainly across the Northern Hemisphere (Europe, North America and Asia). The species is usually found in greater numbers; however, we recorded only one midge of *E. flavens* on a specimen of *E. danica*. This finding contributes to the enrichment of the entomofauna of the Republic of North Macedonia but also adds new data on the distribution of the midge *E. flavens* within the Balkan Peninsula.

Key words: Balkan Peninsula, commensalism, host, macroinvertebrates, stream, symbiotic species

Introduction

Epoicocladus Sulc & Zavrel, 1924 is one of the chironomid genera (Orthoclaadiinae) that are found living in symphoretic or commensal associations with species of Ephemeroptera (Svensson 1979, Tokeshi 1993, Jacobsen 1995). The genus has a scattered distribution, mainly across the Northern Hemisphere, including North America, Europe and the eastern part of Asia (GBIF Secretariat 2023a). At present, four species of *Epoicocladus* are recognised: *E.*

flavens (Malloch, 1915) - known from Europe and North America; *E. gynocera* (Edwards, 1937) - from Europe (Finland) and Asia; *E. itachisecunda* (Sasa & Kawai, 1987) - from Japan and *E. wangi* Yan, Liu, Cao, Zhang, & Liu, 2019 - from China (GBIF Secretariat 2023a, b). To date, the information on the distribution of the two European species is scarce, while *E. ephemerae* (Kieffer, 1924), *Camptocladus ephemerae* Kieffer, 1924 and *C. flavens* Malloch, 1915 assigned as accepted synonyms of *E. flavens* (GBIF Secretariat 2023b).

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The relationship of *E. flavens* with the mayfly hosts in earlier accounts was firstly classified as phoresy (Svensson 1979). Later, it has been more accurately termed as commensalism (Svensson 1980, Tokeshi 1986) or as symphoresy, i.e. the representatives of this midge are temporarily attached to the larger host (*Ephemera danica* Müller, 1764) for transportation; the mayfly does not gain any nutritional benefits or developmental penalties, but it is highly beneficial for the midge (Svensson 1980, Tokeshi 1993, Roque 2004, Henriques-Oliveira & Nessimian 2009, Grzybkowska et al. 2016).

Epoicocladius flavens is generally univoltine; however, the size and frequency distribution of its larvae suggest that there might be a second generation (Svensson 1980, Soldán 1988). In Europe, this midge has been found living in commensal associations on two mayfly species, *E. danica* and *E. vulgata* Linnaeus, 1758; in North America - on mayflies of genus *Hexagenia* (Svensson 1980). Soldán (1988) listed *E. flavens* as abundant across Czechia and Slovakia and speculated that the species was likely to occur within the whole European continent where the northern and southern borders of the distributional area of the midge are not yet confirmed. From the Balkan Peninsula, so far the species has been reported from Croatia (Ivković et al. 2020) and Serbia (Milošević et al. 2011). Nymphs of *E. danica* infected with *E. flavens* have been recorded from the territory of former Yugoslavia, Bulgaria and Greece (Soldán 1988), but these data have not been included in the GBIF dataset (GBIF Secretariat 2023b). The goal of this study is to present a first record of *E. flavens* in the Republic of North Macedonia based on morphological characters.

Materials and Methods

Study site: Golema Reka River is one of the permanent tributaries of the watershed of the Prespa Lake (Fig. 1). The studied stretch of the river was upstream the Izbishta Village (N 41.132344°, E 21.006513°) and has the characteristics of mountain rivers (type 1) in the Hellenic Western Balkans (Ecoregion 6). Moreover, according to Rimcheska (2022), typical for the major tributaries of Prespa Lake (including the Golema Reka River) are the high variations of their hydrological regimes with very low water discharges or even no water in the riverbed during late summer period/s.

Sampling and taxonomic identification: The specimens for this study were collected in May 2022, during a regular monitoring of the river, using a standard pond net (mesh size 500 µm). We applied the kick-and-sweep multihabitat procedure (Cheshmedjiev et al. 2011), following EN ISO 10870: 2012 for collection of benthic fauna (ISO 10870 2012). Back in the laboratory, the macroinvertebrate specimens were separated into systematic groups and preserved in 80% ethanol. For the taxonomic identification of the chironomid larva a permanent microscope slide was prepared, following a prior maceration in 10% KOH for approximately 3 h, a dehydration of the chironomid larva, a decapitation of its head capsule and then the capsule and body were mounted on a slide in Euparal. The pictures were taken with a Samsung Galaxy S10e camera and using a stereomicroscope Olympus SZ51 (WHSZ10x-H/22) and a microscope ZEISS Axiolab 5 (100x ocular: PI 10x/22, objectives 10x/0.65 for Figs 2B, 3B and 400x/0.65 for Figs 2C, 3A, C, D). The taxonomic identification followed

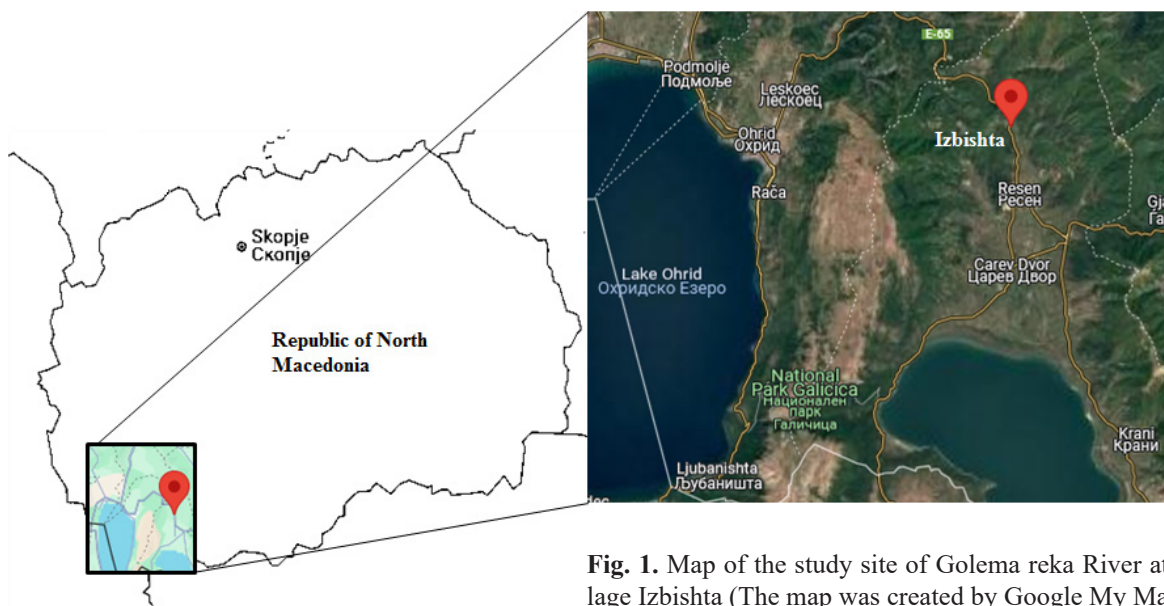


Fig. 1. Map of the study site of Golema reka River at village Izbishta (The map was created by Google My Maps).

Jacobsen (1992) for *E. flavens* and Bauernfeind & Humpesch (2001) for *E. danica*.

Results and Discussion

We collected 15 individuals of *E. danica* from the Golema Reka River. A single specimen of *E. flavens* was found attached to the ventral side of the thorax of one specimen of the mayflies (Fig. 2A). We identified the larva based on the following morphologi-

cal features: (i) length of 4th and 5th instar larvae: 5-7 mm (Fig. 2B); (ii) thoracic and abdominal segments 1-8 with >100 setae, dorsal setae spine-like; (iii) lateral view of 4th abdominal segment (Fig. 3A); (iv) long anal setae, responsible for the attachment to the host (Fig. 3B, C); (v) each posterior parapod with six dark-brown, stout claws (Fig. 3B, D); (vi) mentum with six teeth (Fig. 2C).

Despite intensive hydrobiological monitoring on the territory of the Republic of North Macedo-

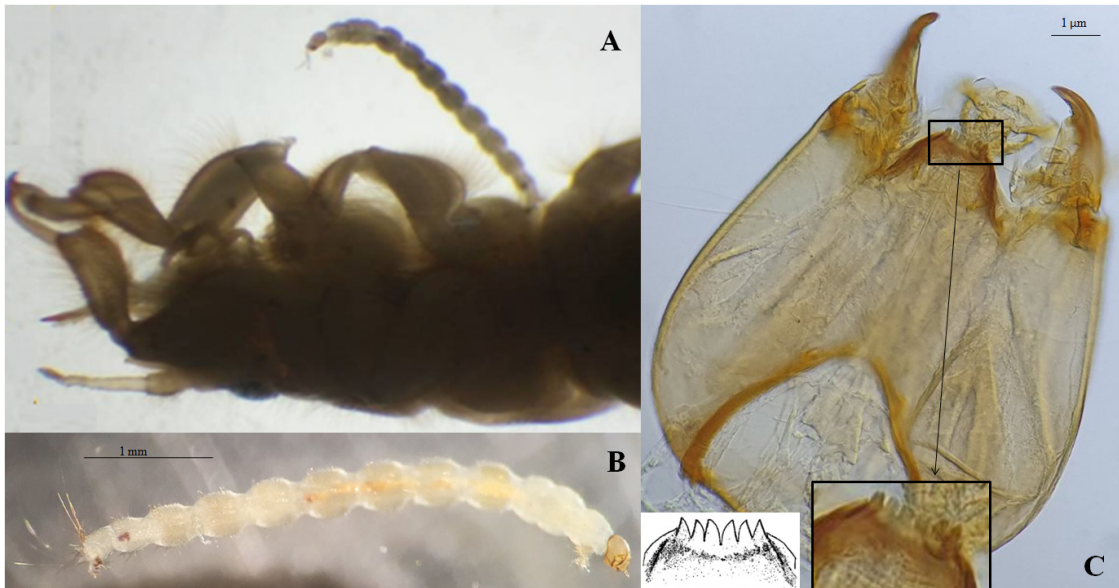


Fig. 2. A: *Epoicocladius flavens* attached to the ventral part of the thorax of the mayfly *Ephemera danica*; and B: Mentum with first lateral teeth (drawing by Jacobsen1992); B: The larval stage of the collected specimen *Epoicocladius flavens* (ocular: P1 10x/22, objective: 10x/0.65); and C: Head capsule (ocular: P1 10x/22, objective: 40x/0.65) and mentum with first lateral teeth (drawing by Jacobsen1992).

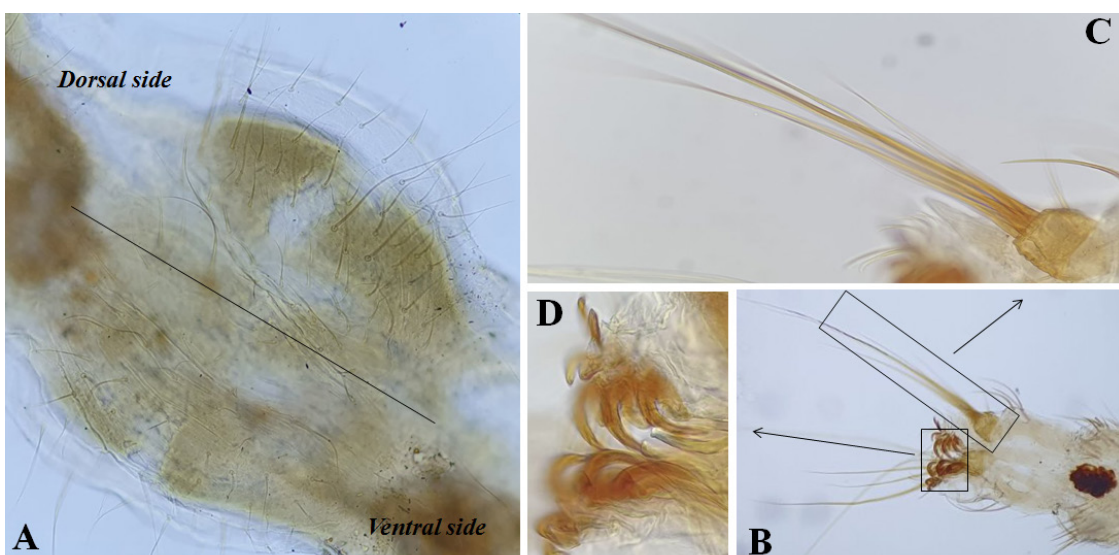


Fig. 3. *Epoicocladius flavens*: A) 4th abdominal segment side view; B) The last body segment with anal setae and parapods; C) Anal setae; D) Each posterior parapod with 6 dark-brown, stout claws). For the photos the magnifications for ocular (P1 10x/22) and objective (40x/0.65) were used.

nia, this is the only known country record of *E. flavens* (temporarily attached to the specialised host *E. danica*). All recent data include the available papers/contributions on the synonymised *E. ephemerae* (Grzybkowska et al. 2016, GBIF Secretariat 2023b).

Ecology of E. flavens: The width of the sampled river stretch of the Golema Reka River was 2-5 m, the water depth did not exceed 0.40 m and the site shading was over 80%. The water was clear, oxygen-rich and cold. The bottom substrata were heterogeneous, dominated by stones (<10 cm) and gravel (60%) and including organic debris from the riverbank trees (leaves and small branches: <30%), with the remainder consisting of sand and silt (<10%). *Epoicocladus flavens* prefers well-oxygenated, small-scale fluvial habitats with water temperature being the limiting factor for its distribution: the species is not recorded in high mountain biotopes (Soldán 1988, Ivicheva et al. 2018). Its larvae feed on organic particles settled on the hairy body of the nymphs of its hosts (Svensson 1980, Grzybkowska et al. 2016). The larvae of *E. flavens* attach ventrally to the thorax of the host, which optimizes their positioning for pupation on the host's surface and improves foraging and respiration. These processes are further facilitated by the host's behaviour: the intensive gill movement of the mayfly ensures an influx of food and oxygen to the midge (Pennuto 2003, Roque et al. 2004).

Midge infestation intensity scales with host size, implying synchronised life-history traits between the ectosymbiont and the host (Soldán 1988, Tokeshi 1988, Jacobsen 1998, 1999, Pennuto 2003, Grzybkowska et al. 2016). The high-density infestation on the host would result in low emergence success for the midge (e.g. Pennuto 2000, Grzybkowska et al. 2016). Svensson (1980) observed higher densities of associated symphoretic midge larvae on nymphs of *Ephemera* inhabiting stony substrata as compared to nymphs in homogeneous soft bottom substrata. Furthermore, high organic pollution and low pH (< 6.0) were shown to limit the distribution of *E. danica* where both *E. danica* and *E. flavens* were absent from most densely inhabited part of the studied rivers and restricted to the forested central reaches (Svensson 1980, Ivicheva et al. 2018). We also collected the species from the forested part of the Golema Reka River, with less organic pollution and optimal pH values (8.15), with environmental conditions corresponding closely the defined "reference" conditions (Rimcheska 2022). Similar environment for recording of the above mentioned symphores were also observed in unpolluted or weakly polluted fluvial ecosystems across Central and Southern Europe (Soldán 1988).

Larvae of *E. flavens* tend to attach on the ventral side of the thorax of the host (Pennuto 2003). The primary factors governing this behaviour are host size and spring emergence timing, which determines that midge development must be completed prior to the pupation of the host (Pennuto 2000, Pennuto et al. 2002). Identifying life cycle divergences is vital, given that symbionts with life history durations mirroring their hosts are likely to show high developmental synchrony (Pennuto 2003). Host density is ostensibly the critical factor influencing the intensity of infestation (Svensson 1976, 1980, Tokeshi 1986, Soldán 1988, Tokeshi 1988, Grzybkowska et al. 2016); specifically, the success of *E. flavens* in exploiting *E. danica* at high host densities—well below carrying capacity—suggests that midge larvae has a limited surface to colonize sparse host populations (Svensson 1980). Bearing this in mind, some studies explored the pattern of infestation dynamics when two commensals (e. g. ciliated protozoan and chironomid) existed on one host (Svensson 1980, Tokeshi 1988, Grzybkowska et al. 2016). Additionally, Svensson (1980) showed that in the absence of *E. flavens* on the surface of large nymphs subjected to organic pollutants, the host becomes completely colonised by ciliates. Minimizing the density of commensals per host is a crucial mechanism for managing space and nutrient competition (Grzybkowska et al. 2016).

Conclusions

This study contributes new records to the entomofauna of the Republic of North Macedonia but also adds new data on the distribution of the midge *E. flavens* from the Balkan Peninsula. Additional monitoring activities could provide better understanding of the symbiotic interspecific relation of the potential phoretic and parasite representatives of Chironomidae in the Republic of North Macedonia.

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