

SYMBIOCLADIUS RHITHROGENAE (ZAVREL, 1924) (DIPTERA: CHIRONOMIDAE): A NEW FINDING FOR THE ENTOMOFAUNA OF SERBIA

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Abstract

During hydrobiological research on the territory of Serbia in 2011 and 2012, the species *Symbiocladius rhithrogenae* (Zavrel, 1924) (Diptera: Chironomidae) was found in the Rača stream at three localities. Our study is the first record of this parasite in Serbia. The parasite only infected the larvae of *Rhithrogena* cf. *semicolorata* (Ephemeroptera: Heptageniidae) and was not found in any other species from the family Heptageniidae. This study contributes to the knowledge about the diversity of the family Chironomidae in Serbia.

KEY WORDS: *Symbiocladius rhithrogenae*; parasite; *Rhithrogena* cf. *semicolorata*; Rača stream

Introduction

One of the most widely represented and diverse families in aquatic ecosystems from the order Diptera (suborder Nematocera) is the family Chironomidae, which has an extensive distribution with over 4,000 described species (Ferrington 2008). In freshwaters, the family Chironomidae accounts for almost 50% of the macroinvertebrates present (Martins *et al.*, 2021). Species in this family play a fundamental role in the food chain by processing organic material and transferring energy and nutrients to invertebrates, fish, and birds that feed on them (Wonglersak *et al.*, 2021; Martins *et al.*, 2021). They have different feeding strategies: herbivores, detritivores, predators, grazers, and filter-feeders (Wonglersak *et al.*, 2021). Chironomidae species exhibit a

wide range of habitat preferences, including numerous symbiotic, commensalistic, and parasitic relationships between Chironomidae larvae and other aquatic invertebrates (Wiens *et al.*, 1975; Tokeshi, 1993; Jacobsen, 1995).

Symbiocladius rhithrogenae is a species of chironomid that can occur as an ectoparasite on mayflies when they are in their aquatic life stage, in the nymph and larval stages. The species is rheophilic, preferring fast-flowing mountain and sub-mountain streams (epirhithral to hyporhithral parts), which strongly influences the choice of its host (Schmedtje & Colling, 1996; Moller-Pillot, 2013; Antonov Dashinov & Nikolova Vidinova, 2018; Vilenica *et al.*, 2018). The first instar larva of *S. rhithrogenae* is probably the only free-living stage in search of a host (Gonser & Spies, 1997). The larvae of *S. rhithrogenae* attach to their host nymphs between or under the wing covers as ectoparasites, with the young larva crawling under the host's wing sheaths. The mouthparts of the parasite are modified, as are the parapods and anal sets. The parasites feed on their host's hemolymph and associated tissues (Schieffels, 2009). Soldan (1979), who studied this species, found that when the chironomid larva, still attached to its host, enters the pupal stage, it can cause sterility and, in some cases, death of the host. However, in a later study by Kriska *et al.* (1998), infestation with *S. rhithrogenae* was defined as a mild form of parasitism, and the parasite can separate from its host without causing host death.

S. rhithrogenae prefers hosts from the family Heptageniidae and chooses species of the genera *Ecdyonurus* Eaton, 1868; *Electrogena* Zurwerra & Tomka, 1985; *Epeorus* Eaton, 1881; *Heptagenia* Walsh, 1863 and *Rhithrogena* Eaton, 1881 (Gilka *et al.*, 2007).

The lack of faunistic and taxonomic work on the parasitic chironomid fauna of Serbia is due to the small number of experts dealing with this family Chironomidae, as well as the complex and lengthy process of identification of individuals to species level. However, research on aquatic parasites is important because aquatic parasites fulfill crucial functions in the freshwater ecosystem. Aquatic parasites are also important drivers of key evolutionary and ecological dynamics that shape, control, and regulate ecosystem function (Selbach *et al.*, 2022).

Materials and Methods

Study area

The material was collected in the Rača stream in western Serbia, and according to Illies (1978) and Paunović *et al.* (2012), it belongs to the zoogeographical region V (Dinaric Western Balkans). The ecoregional approach is a widely used basis for the spatial classification of waters according to areas that represent entities in regard to the distribution of aquatic biota. The Rača stream is 19.6 km long and originates on the northwestern slopes of the Tara Mountains at an altitude of 970 m. There are several springs, the most famous of which is the alkali-thermal karst spring Lađevac at an altitude of 498 m. The Rača spring emerges from the riverbed as a strong spring between huge rocks. Upstream, it flows through an inaccessible canyon and later behaves like an abyssal river. The total area of the Rača catchment is 59 km² (Jevtić, 1999). The catchment is typically mountainous, with steep slopes and a steep network of main and tributary streams. During heavy rains and in the period of snowmelt, the watercourse assumes a distinctly torrential character.

Sampling

The study of the Rača stream was carried out in 2011 (April, June, September, October, December) and 2012 (February and May). Sampling of macrozoobenthos was done at seven sites (RČ1, RČ2, RČ3, RČ4, RČ4', RČ5, RČ6) (Fig. 1). Site RČ1 is located 383 m above sea level, at the point where the river gorge passes

through a dense forest complex. Site RČ2 is located 10 m upstream from the water intake for the pond at 359 m a.s.l. Site RČ3 (350 m a.s.l.) is located 10 m downstream after the water from the pond is discharged into the Rača stream. Sites RČ4 and RČ4' are located at 337 and 334 m a.s.l. downstream, respectively. They pass through dense forests, and the banks are sharp and steep. Site RČ5 is located 1.5 km downstream from the pond at about 298 m a.s.l. Site RČ6 is located 4 km from the pond at about 273 m a.s.l.

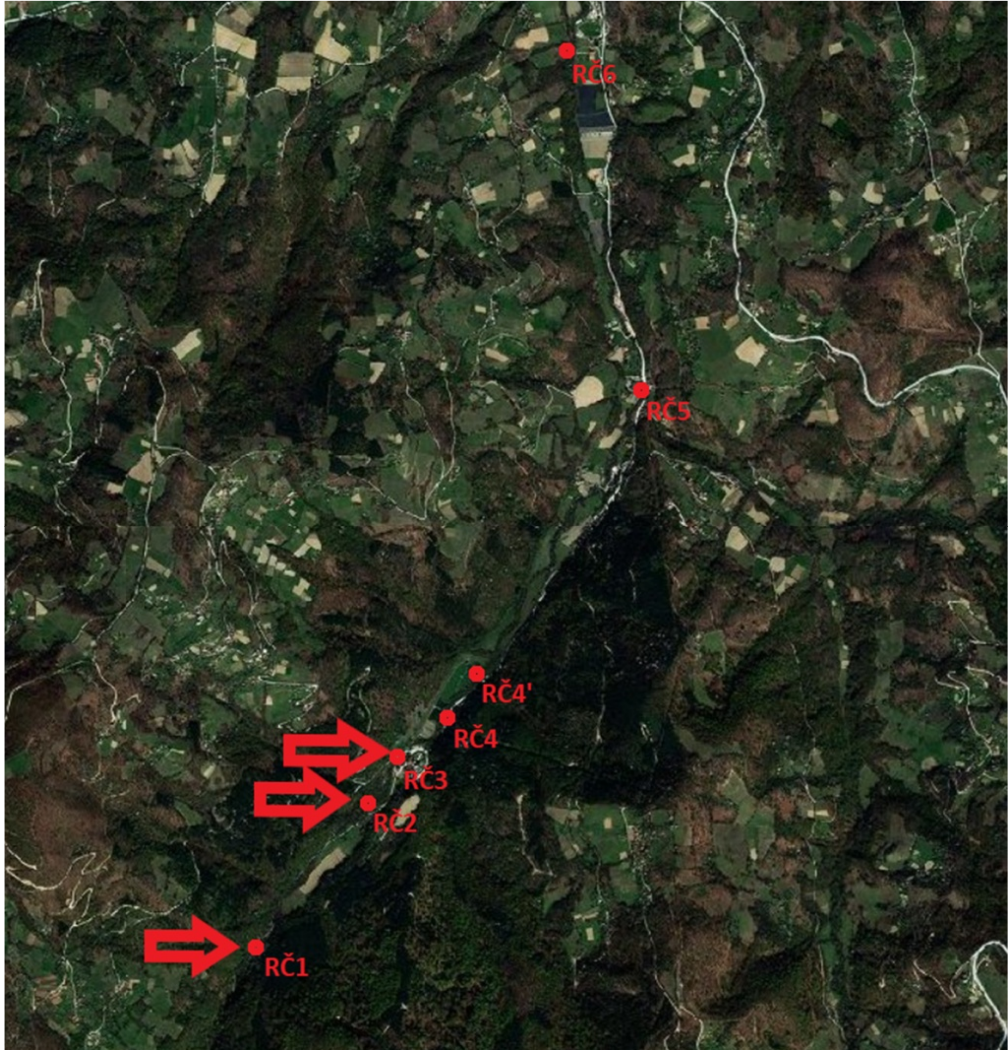


Figure 1. Sampling sites in the Rača stream (marked with a red arrow where *Symbiocladius rhithrogenae* was found).

The material was sampled using the quantitative method with a Surber net with an area of 300 cm² and a mesh diameter of 250 µm. Three subsamples were taken at each studied site in all study periods, mainly along the transect on both sides of the coast. Sampling was standardized so that the collection of organisms in a

horizontal collapsible frame aligned against the water current took 10 min for each sampling. After thoroughly washing the net, the organisms left in the collection bag at the end of the net were placed in plastic bottles and fixed with 96% alcohol. After separating the organisms from mud, gravel, and detritus, they were identified to the lowest possible taxonomic categories, species (species group), or genera, except in the case of Hydracarina, which were not identified in this study. The material was separated and identified using a binocular magnifier (ZEISS Discovery V8 stereomicroscope) at the Institute of Zoology, Faculty of Biology, Belgrade, where the material is kept. For organism identification, the relevant literature was consulted (Langton, 1991, Schmid, 1993, Nilsson, 1996, Bauernfeind & Soldan, 2012). Photographs of the pupa and larvae of *S. rhithrogenae* were taken in the laboratory using a Leica DFC295 camera (Leica Microsystems, Germany).



Figure 2. Sampling sites in the Rača stream where *Symbiocladius rhithrogenae* was found A – RČ1 locality; B – RČ2 locality; C – RČ3 locality (photo: Katarina Stojanović).

Physical and chemical parameters

During the study, measurements of the physical and chemical parameters were taken at each site in all sampling periods, and the mean value was calculated based on the values obtained. Measurements of water temperature (t_w , °C), dissolved oxygen content (DO, mg/l), pH, and electrical conductivity (EC, $\mu\text{S}/\text{cm}$) were made on-site with equipment from PCE-PHD (Germany). Elevation, longitude, and latitude were also recorded using GPS equipment (Geko 201, Garmin). Stream depth (m) and width (m) were measured with a meter, flow velocity (V , m/s) was determined with a velocity meter (GEOPACKS Stream Flowmeter, UK), and water flow rate (Q , m^3/s) was calculated according to Stojanović (2017). The analysis of the substrate type was performed by visual assessment of the substrate, according to the method described by Paunović (2007). The composition of the substrate is divided into five classes: Fine substrates – silt (<0.125 mm), sand (0.125-2 mm), gravel (2-64 mm), stone (64-256 mm) and rock (>256 mm) (Table II).

Results

Qualitative and quantitative composition of the macrozoobenthos community

The part of the Rača stream studied is characterized by a diverse community of macroinvertebrates, with 177 determined taxa from five taxonomic categories. The great diversity of these communities is mainly due to the numerous representatives of the family Chironomidae, of which 41 species were determined (Table I). The species *Symbiocladius rhithrogenae* was found only in three localities (RĈ1 (June 2011.), RĈ2 (May 2012.), and RĈ3 (May 2012.)) (Fig. 2) and represents the first record of this parasite in Serbia (Fig. 3).



Figure 3. A – Pupa of *S. rhithrogenae*, dorsal view (red circle); B – Larva of *S. rhithrogenae*, dorsal view (red circle); C – Larva of *S. rhithrogenae*, lateral view (red circle) (photos: Milenka Božanić and Katarina Stojanović).

An oscillation in the total abundance of macroinvertebrate taxa was observed in the Rača stream. From the first to the second locality, the total abundance decreased, increasing significantly in abundance at the third locality, compared to the second, fourth, and sixth localities, where again a decrease in the total abundance of macroinvertebrates was observed. The value of $12,786 \pm 3,180$ ind/m² was the highest abundance recorded, at the third locality.

Table I. Qualitative composition of macrozoobenthos communities in the Rača stream

| Taxa | RĈ1 | RĈ2 | RĈ3 | RĈ4 | RĈ4' | RĈ5 | RĈ6 |
|---|-----|-----|-----|-----|------|-----|-----|
| Platyhelminthes | | | | | | | |
| <i>Dugesia lugubris</i> (Schmidt, 1861) | + | + | + | | | + | + |
| Mollusca | | | | | | | |
| <i>Ancylus fluviatilis</i> Müller, 1774 | + | + | + | + | + | + | + |
| <i>Bithynia tentaculata</i> (Linnaeus, 1758) | + | + | + | + | | | |
| <i>Planorbarius corneus</i> (Linnaeus, 1758) | | + | | + | + | | |
| <i>Radix auricularia</i> (Linnaeus, 1758) | + | + | + | + | + | | + |
| <i>Pisidium</i> sp. | | + | + | + | | | + |
| Annelida | | | | | | | |
| <i>Eiseniella tetraedra</i> (Savigny, 1826) | + | + | | | + | + | + |
| <i>Fridericia</i> sp. | | | | | + | | |
| <i>Limnodrilus hoffmeisteri</i> Claparede, 1862 | | | + | | | | |
| <i>Nais barbata</i> Müller, 1774 | | | | + | | | |
| <i>Nais bretsheri</i> Michaelsen, 1899 | | | + | + | | | |
| <i>Nais</i> sp. | | | | + | | | |
| <i>Stylodrilus heringianus</i> Claparede, 1862 | | | + | | | + | + |
| <i>Tubifex tubifex</i> (Müller, 1774) | | | + | + | | | + |

Table I – continued

| | | | | | | | |
|--|---|---|---|---|---|---|---|
| <i>Erpobdella testacea</i> (Savigny, 1820) | + | + | + | + | + | + | + |
| Chelicerata | | | | | | | |
| Hydracarina | + | + | + | + | + | + | + |
| Crustacea | | | | | | | |
| <i>Gammarus balcanicus</i> Schaferna, 1922 | | | + | + | + | + | + |
| <i>Gammarus fossarum</i> Koch, 1835 | | + | + | + | | | |
| <i>Gammarus</i> sp. | + | | | | | + | |
| Insecta – Ephemeroptera | | | | | | | |
| <i>Baetis (Baetis) alpinus</i> (Pictet, 1843) | + | + | + | + | + | + | + |
| <i>Baetis (Baetis) alpinus</i> gr. | | | + | + | | | |
| <i>Baetis (Baetis) lutheri</i> Müller-Liebenau, 1967 | | + | + | + | + | + | + |
| <i>Baetis (Baetis) melanonyx</i> (Pictet, 1843) | + | + | + | + | + | + | |
| <i>Baetis (Nigrobaetis) muticus</i> (Linnaeus, 1758) | + | | + | + | + | + | + |
| <i>Baetis (Rhodobaetis) rhodani</i> (Pictet, 1843) | + | + | + | + | + | + | + |
| <i>Baetis</i> sp. | + | + | + | + | + | + | + |
| <i>Ecdyonurus (Ecdyonurus) cf. venosus</i> (Fabricius, 1775) | | | + | + | | | |
| <i>Ecdyonurus (Helvetoraeticus) cf.</i> | + | | | | | + | |
| <i>Ecdyonurus (Helvetoraeticus) sp.</i> | + | | + | + | + | + | + |
| <i>Ecdyonurus</i> sp. | + | + | + | + | + | + | + |
| <i>Epeorus (Epeorus) assimilis</i> Eaton, 1885 | + | + | + | + | + | + | + |
| <i>Ephemera (Ephemera) danica</i> Müller, 1764 | | | | + | + | + | + |
| <i>Ephemera</i> sp. | + | + | + | + | + | + | + |
| <i>Ephemerella (Serraltella) ignita</i> (Poda, 1761) | + | + | + | + | + | + | + |
| <i>Ephemerella</i> sp. | + | + | + | + | + | + | + |
| <i>Habroleptoides confusa</i> Sartori & Jacob, 1986 | + | + | | + | + | + | + |
| <i>Habroleptoides</i> sp. | | | + | + | + | + | + |
| <i>Rhithrogena cf. braaschi</i> Jacob, 1974 | | | | | + | | |
| <i>Rhithrogena hybrida</i> gr. | | | | + | | | |
| <i>Rhithrogena semicolorata</i> (Curtis, 1834) | + | | + | | | | + |
| <i>Rhithrogena semicolorata</i> gr. | + | + | + | + | + | + | + |
| <i>Rhithrogena</i> sp. | + | + | + | + | + | + | + |
| <i>Torleya major</i> (Klapalek, 1905) | | | | + | | | |
| Insecta – Plecoptera | | | | | | | |
| <i>Amphinemura sulcicollis</i> (Stephens, 1836) | | | | | + | | |
| <i>Brachyptera risi</i> (Morton, 1896) | | | + | + | | + | + |
| <i>Dinocras</i> sp. | + | + | | + | + | + | + |
| <i>Isoperla grammatica</i> (Poda, 1761) | | | + | | | | |
| <i>Isoperla</i> sp. | | | | | | + | |
| <i>Leuctra fusca</i> gr. | | | | | + | | |
| <i>Leuctra hippopus</i> Kempny, 1899 | + | + | | + | + | + | + |
| <i>Leuctra inermis</i> Kempny, 1899 | | | | + | | | + |
| <i>Leuctra nigra</i> (Olivier, 1811) | | | | | | | + |
| <i>Leuctra prima-hippopus-inermis</i> gr. | | | | | | + | + |
| <i>Leuctra</i> sp. | + | + | | + | + | + | + |
| <i>Nemoura</i> sp. | + | + | | | | | + |
| <i>Perla marginata</i> (Panzer, 1799) | + | + | + | + | + | + | + |
| <i>Perla pallida</i> Guerin, 1838 | | | | | | | + |
| <i>Perla</i> sp. | | + | | | | + | + |
| <i>Protonemura praecox</i> (Morton, 1894) | + | + | + | | + | | |
| <i>Protonemura</i> sp. | | + | + | + | | | |
| Insecta – Coleoptera | | | | | | | |
| <i>Elmis aenea</i> (Müller, 1806) | + | + | + | + | + | + | + |
| <i>Hydraena gracilis</i> Germar, 1824 | + | + | + | + | + | + | + |
| <i>Hydrocyphon</i> sp. | + | + | + | + | + | + | + |

Table I – continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| <i>Orectochilus villosus</i> (Müller, 1776) | | | | | + | + | + |
| <i>Stenelmis</i> sp. | + | + | + | + | + | + | + |
| Insecta – Diptera | | | | | | | |
| <i>Antocha (Antocha) vitripennis</i> (Meigen, 1830) | | | | + | | | |
| <i>Atherix ibis</i> (Fabricius, 1798) | + | + | + | + | + | + | + |
| <i>Atherix marginata</i> (Fabricius, 1781) | + | + | + | + | + | + | + |
| <i>Bezzia</i> sp. | | | + | + | + | + | + |
| <i>Blepharicera fasciata</i> (Westwood, 1842) | + | | | + | + | + | + |
| <i>Chrysopilus auratus</i> (Fabricius, 1805) | | | | | + | | |
| <i>Clinocera nigra</i> Meigen, 1804 | | | + | + | + | + | |
| <i>Clinocera</i> sp. | | | | | | | + |
| <i>Dicranota (Dicranota) bimaculata</i> | | | | | + | + | |
| <i>Hemerodromia</i> sp. | | | | | + | | |
| <i>Hemerodromia unilineata</i> Zetterstedt, 1842 | | + | + | + | + | + | |
| <i>Hexatoma (Hexatoma) bicolor</i> (Meigen, 1818) | | | | | + | | |
| <i>Lianculus</i> sp. | | + | | | | | |
| <i>Limnophila</i> sp. | + | | | + | + | | |
| <i>Limnophora riparia</i> (Fallen, 1824) | | | + | | | | |
| <i>Nemotelus</i> sp. | | | | | | + | |
| <i>Odontomyia</i> sp. | + | + | + | + | + | | |
| <i>Tipula (Acutipula) fulvipennis</i> De Geer, 1776 | | + | | | + | | |
| <i>Tipula</i> sp. | + | + | + | | | + | + |
| Insecta – Simuliidae | | | | | | | |
| <i>Simulium (Eusimulium) sp.</i> | + | | + | + | | | |
| <i>Simulium (Simulium) maximum</i> (Knoz, 1961) | | | + | | | | |
| <i>Simulium (Simulium) ornatum</i> Meigen, 1818 | | | + | | | | |
| <i>Simulium (Simulium) sp.</i> | + | + | + | + | + | + | + |
| <i>Simulium (Simulium) variegatum</i> | | | + | | | | |
| <i>Simulium</i> sp. | | | + | | + | + | |
| Insecta – Chironomidae | | | | | | | |
| <i>Brillia bifida</i> (Kieffer, 1909) | + | | | + | + | + | + |
| <i>Brillia flavifrons</i> (Johannsen, 1905) | + | | | + | + | + | |
| <i>Chetocladius piger</i> gr. | + | | | | | + | + |
| <i>Conchapelopia melanops</i> (Meigen, 1818) | + | | | | | + | + |
| <i>Corynoneura coronata</i> Edwards, 1924 | + | | | | | | |
| <i>Corynoneura scutellata</i> grp. | + | | | + | + | + | |
| <i>Cricotopus (Cricotopus) bicinctus</i> | | | + | | | | |
| <i>Cricotopus (Cricotopus) trianulatus</i> agg. | | + | + | | | + | + |
| <i>Cricotopus (Cricotopus) trifascia</i> | | | + | | | | + |
| <i>Demicryptochironomus</i> sp. | | | | | | | + |
| <i>Diamesa insignipes</i> Kieffer, 1908 | + | + | | | | | |
| <i>Diamesa zerny</i> gr. | + | + | | | | | |
| <i>Epoicocladius flavens</i> (Malloch, 1915) | | + | | | | | + |
| <i>Eukiefferiella brevicar</i> agg. | + | | + | + | + | + | + |
| <i>Eukiefferiella cf. devonica</i> (Edwards, 1929) | + | + | + | + | + | + | + |
| <i>Eukiefferiella clypeata</i> (Thienemann, 1919) | + | + | + | | + | + | + |
| <i>Eukiefferiella minor/fittakuy</i> | | + | | | | | + |
| <i>Heleniella ornatocollis</i> (Edwards, 1929) | + | | | | + | | |
| <i>Macropelopia adauca</i> Kieffer, 1916 | | | | | + | | |
| <i>Metriocnemus hirticollis</i> agg. | + | | | | + | | |
| <i>Micropsectra bidentata</i> (Goetghebuer, 1921) | + | | + | | | | |
| <i>Micropsectra</i> sp. | + | + | + | + | + | + | + |
| <i>Microtendipes pedellus</i> (De Geer, 1776) | | | + | | | | |
| <i>Nanocladius bicolor</i> agg. | | | | | + | | + |

Table I – continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| <i>Nanocladius rectinervis</i> (Kieffer, 1911) | + | | + | + | | + | + |
| <i>Nilotanypus dubius</i> (Meigen, 1804) | | | | | | | + |
| <i>Orthocladus (Eudactilocladus) sp.</i> | + | + | + | | | + | |
| <i>Orthocladus (Euorthocladus) rivulorum</i> | | | + | | | + | |
| <i>Orthocladus (Euorthocladus) sp.</i> | + | + | + | + | + | + | + |
| <i>Orthocladus (Orthocladus) frigidus</i> | + | | + | | | + | |
| <i>Orthocladus (Orthocladus) sp.</i> | + | + | + | + | + | + | + |
| <i>Paracladopelma nigrilulum</i> | | | | | | | + |
| <i>Paracricotopus sp.</i> | | | + | | + | | |
| <i>Parametricnemus stylatus</i> (Spärck, 1923) | + | + | + | + | + | + | + |
| <i>Paratendipes albimanus</i> (Meigen, 1818) | | | + | | + | | + |
| <i>Paratendipes nudisquama</i> (Edwards, 1929) | | + | | | | | |
| <i>Paratrachocladus rufiventris</i> (Meigen, 1830) | | + | + | + | + | | |
| <i>Paratrissocladius excerptus</i> (Walker, 1856) | | | | + | | | |
| <i>Polypedilum (Pentapedilum) cf. tritum</i> (Walker, 1856) | | | + | | | | |
| <i>Polypedilum (Pentapedilum) uncinatum</i> | | | + | + | + | | |
| <i>Polypedilum (Polypedilum) albicorne</i> | | | + | | + | | + |
| <i>Polypedilum (Polypedilum) convictum</i> | + | + | + | + | + | + | + |
| <i>Polypedilum (Polypedilum) laetum</i> | | | + | | | | |
| <i>Pothastia gaedi</i> (Meigen, 1838) | | | | | | + | |
| <i>Pothastia longimana</i> Kieffer, 1922 | + | + | + | + | + | + | + |
| <i>Prodiamesa olivacea</i> (Meigen, 1818) | | | + | | | | |
| <i>Psectrocladius platypus</i> (Edwards, 1929) | | | | + | | | |
| <i>Rheocricotopus (Rheocricotopus) efusus</i> | + | + | + | | + | | |
| <i>Rheocricotopus (Rheocricotopus)</i> | | | + | | + | | + |
| <i>Rheotanytarsus spp</i> | | | + | | | | |
| <i>Symbiocladus rhithrogenae</i> (Zavrel, 1924) | + | + | + | | | | |
| <i>Synorthocladus semivirens</i> (Kieffer, 1909) | + | | + | | + | | |
| <i>Tanitarsus sp.</i> | | | + | | | | |
| <i>Thienemanniella acuticornis</i> (Kieffer, 1912) | | | + | | | | |
| <i>Thienemanniella clavicornis</i> agg. | + | | | + | | | |
| <i>Tvetenia calvescens</i> (Edwards, 1929) | + | + | + | + | + | + | + |
| <i>Tvetenia discoloripes</i> (Goetghebuer & | + | + | + | + | + | + | + |
| Insecta – Trichoptera | | | | | | | |
| <i>Agapetus sp.</i> | | | | + | + | | |
| <i>Anabolia nervosa</i> (Curtis, 1834) | | + | | | | | + |
| <i>Cheumatopsyche lepida</i> (Pictet, 1834) | | | | | | | + |
| <i>Glossosoma conforme</i> Neboiss, 1963 | | + | | + | | | |
| <i>Glossosoma intermedium</i> (Klapalek, 1892) | + | | + | + | + | + | + |
| <i>Glossosoma sp.</i> | | | | | + | | |
| <i>Goera pilosa</i> (Fabricius, 1775) | + | + | + | + | | + | + |
| <i>Goera sp.</i> | | | + | | | | + |
| <i>Hydropsyche angustipennis</i> (Curtis, 1834) | + | + | + | + | + | + | + |
| <i>Hydropsyche contubernalis</i> McLachlan, 1865 | | | | + | | | |
| <i>Hydropsyche pellucidula</i> (Curtis, 1834) | + | + | + | + | + | + | + |
| <i>Hydropsyche sp.</i> | + | + | + | + | + | + | + |
| <i>Hydroptila cf. sparsa</i> Curtis, 1834 | | | | | | + | |
| <i>Lepidostoma hirtum</i> (Fabricius, 1775) | | | + | | | | |
| <i>Leptocerus sp.</i> | | | | + | | | |
| <i>Leptocerus tineiformis</i> Curtis, 1834 | + | + | | + | + | + | + |
| <i>Lithax niger</i> (Hagen, 1859) | | | + | + | | + | + |
| <i>Micrasema longulum</i> McLachlan, 1876 | | | | | | + | |
| <i>Philopotamus montanus</i> (Donovan, 1813) | | | | | + | + | |
| <i>Potamophylax sp.</i> | | | | | | | + |

Table I – continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| <i>Rhyacophila fasciata</i> Hagen, 1859 | | | | + | | | |
| <i>Rhyacophila nubila</i> Zetterstedt, 1840 | + | | + | + | + | + | + |
| <i>Rhyacophila obliterata</i> McLachlan, 1863 | + | + | + | + | + | + | + |
| <i>Rhyacophila</i> sp. | | + | + | + | + | + | + |
| <i>Sericostoma personatum</i> (Kirby & Silo <i>pallipes</i> (Fabricius, 1781) | + | + | + | + | + | + | + |
| <hr/> | | | | | | | |
| Insecta – Hymenoptera | | | | | | | |
| <i>Agriotypus armatus</i> Curtis, 1832 | + | | | + | | + | + |

In our surveys of sites where *S. rhithrogenae* was found, as potential hosts we found representatives of *Ecdyonurus* cf. *venosus*, *Ecdyonurus* sp., *Epeorus assimilis*, and *Rhithrogena* cf. *semicolorata*. However, the parasites were found only in *R.* cf. *semicolorata* and in no other species of the family Heptageniidae. *R.* cf. *semicolorata* was most abundant in RČ3 (60 individuals), while the abundance in RČ2 was 56 and in RČ1 22 individuals. The abundance of *S. rhithrogenae* was the same at all three sites (11 individuals in each of the studied localities). Potential hosts for this species were also found at other sites surveyed in our study, but *Symbiocladius rhithrogenae* was not found at any site other than the first three.

Analysis of chemical and hydrological parameters

Analysis of the mean values of abiotic factors at the studied sites of the Rača stream (Table II) shows that the chemical parameters are more or less uniform. The pH values indicate that the water at the studied sites of the Rača stream is alkaline. The Rača stream is characterized by relatively cold water with an average temperature of 12°C. Oxygen concentration was uniform at all sites and ranged from 10.01 to 10.88 mg/l, and electrical conductivity, which was also uniform at the studied sites, ranged from 313 to 347 µS/cm (Table II).

Table II. Mean values of abiotic factors at the studied sites of the Rača stream

| Locality | RČ1 | RČ2 | RČ3 | RČ4 | RČ4' | RČ5 | RČ6 |
|--------------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| tw (°C) | 10.66 | 12.14 | 12.1 | 12.43 | 12.41 | 12.53 | 12.37 |
| DO (mg.L ⁻¹) | 10.88 | 10.34 | 10.01 | 10.33 | 10.13 | 10.17 | 10.26 |
| pH | 8.20 | 8.34 | 8.46 | 8.34 | 8.44 | 8.40 | 8.40 |
| EC(µS.cm ⁻¹) | 347 | 331 | 327 | 330 | 321 | 313 | 321 |
| Stream depth(m) | 0.12 | 0.11 | 0,16 | 0.19 | 0.19 | 0.18 | 0.15 |
| Stream width (m) | 3.13 | 2.71 | 2,35 | 3.49 | 3.2 | 3.59 | 4.19 |
| V (m.s ⁻¹) | 0.25 | 0.56 | 0.40 | 0.48 | 0.41 | 0.51 | 0.43 |
| Q (m ³ .s ⁻¹) | 0.16 | 0.12 | 0.11 | 0.31 | 0.27 | 0.33 | 0.34 |
| Silt (%) | 3.57 | 8.57 | 15.0 | 0 | 0.71 | 2.86 | 1.43 |
| Gravel (%) | 16.43 | 44.3 | 37.14 | 24.29 | 10.71 | 21.43 | 31.43 |
| Sand (%) | 6.43 | 14.29 | 17.86 | 3.57 | 7.14 | 7.86 | 1.43 |
| Stones (%) | 62.14 | 32.86 | 30.0 | 48.57 | 67.86 | 50.0 | 57.14 |
| Rocks (%) | 11.43 | 0 | 0 | 23.57 | 13.57 | 17.86 | 8.57 |
| Altitude (m) | 386 | 359 | 350 | 337 | 334 | 298 | 273 |
| Longitude and latitude | 43°55.537 19°32.392 | 43°55.802 19°32.360 | 43°55.877 19°32.455 | 43°55.969 19°32.561 | 43°56.046 19°32.561 | 43°56.580 19°33.051 | 43°57.221 19°32.861 |

Concerning the chemical parameters, the hydrological parameters and substrate composition were somewhat more variable at the studied sites. At the RČ3 site, the silt content increased compared to the upstream and

downstream sites. However, silt content was significantly higher at the first three sites than at all sites downstream of RČ3 (Table II). Regarding other elements of the substrate, sites RČ2 and RČ3 were the most similar, characterized by a greater occurrence of sand and gravel and fewer stones and rocks compared to most of the other sites. In the other localities, the dominant form of substrate was rocks and stones. In the other hydrological parameters measured, depth downstream of RČ2 and flow downstream of RČ3 were found to increase significantly, while most sites are characterized by moderate flow velocity, except for RČ2, where it is faster (0.56 m/s), and RČ1, where it is slow (0.25 m/s) (Table II).

Discussion

Symbiocladius rhithrogenae is the only species (out of six known *Symbiocladius* species) living in the Palaearctic region (Gilka *et al.*, 2007; Vilenica *et al.*, 2018). This species has been recorded in Austria, Czech Republic, France, Germany, Hungary, Romania, Bulgaria, Croatia, Poland, Spain, Slovakia, Switzerland, Ukraine, northern and central European parts of Russia, the Near East, and the eastern Palaearctic region (Soldán, 1978; Kriska *et al.*, 1998; Kovács, 2007; Gilka *et al.*, 2007; Kovács & Godunko, 2008; Hordós & Muranyi, 2008; Antonov Dashinov & Nikolova Vidinova, 2018; Vilenica *et al.*, 2018).

The results of this study indicate the dominance of aquatic insect larvae in macroinvertebrate communities of rivers in the mountainous region of Serbia. Macroinvertebrate samples were dominated by representatives of the family Chironomidae, among which the parasitic species *Symbiocladius rhithrogenae* was found. Such a large number of recorded chironomid taxa should not be surprising, as it is the group with the highest diversity and very often the highest density of communities (Marziali *et al.*, 2010).

Despite the long tradition of studying chironomid fauna in Serbia and the great diversity and occurrence of species in mountain rivers (Janković, 1978; 1980; 1981; 1987; 1998; Živić, 2005; Milošević *et al.*, 2012; Milošević, 2013; Savić, 2012; Petrović, 2014; Stojanović, 2017; Čerba *et al.*, 2022), this is the first finding of *S. rhithrogenae* for the entomofauna of Serbia. Vilenica *et al.* (2018) also reported the rare occurrence of this species in Croatia, citing the demand for habitats with good ecological conditions as a possible reason. Antonov Dashinov & Nikolova Vidinova (2018) noted that host preferences also determine the environmental preferences of this parasite, namely pristine waters close to reference conditions. However, Živić (2020), in his research on mountain streams to define reference localities in Serbia, does not mention the presence of this parasite in any of the studied areas. This observation underscores the importance of conducting thorough research on chironomids in high mountain springs. Given that this species is found in neighboring countries, it is reasonable to anticipate its presence in the pristine mountain streams of Serbia.

In our study, the values of the abiotic parameters at the sites where this parasite was found differ slightly from those reported by Gilka *et al.* (2007), who studied the biology of this parasite. They state that the species prefers an environment with a pH of around 7, while the values at our sites are higher, reaching up to 8.46, similar to what Vilenica *et al.* (2018) found in their study. Gilka *et al.* (2007) also found this species at higher flow rates than our study. Regarding elevation, we found this species at elevations of 350-386 m (Table II), while Soldán (1979) and Matěna & Soldán (1982) indicated elevations of 250-800 m and Moubayed (1991) elevations of 900-1,100 m in the Pyrenees. Gilka *et al.* (2007) recorded this species at a much higher elevation (1,400-1,500 m), the highest elevation at which this species has been found. Because the species occurs at elevations from 250 to 1500 m, there are many potential habitats for it in our country; however, this is the only finding at Rača stream. Moreover, it was not found at all the studied sites, but only at the first three. At all studied sites, the temperature, pH, electrical conductivity, and oxygen concentration values are more or less uniform (Table II). However, the composition of the substrate shows that in the first three sites where the species

occurred, there is a significant percentage of silt, gravel, and sand compared to the sites where it is absent and where the percentage is lower, with a higher percentage of rocks and stones.

Although potential hosts were present, *S. rhithrogenae* was not found at all sites studied. Also, in our study, the parasite was only detected on *Rhithrogena* cf. *semicolorata* nymphs, despite the presence of other potential hosts from the family Heptageniidae at these sites. As in our study, Antonov Dashinov & Nikolova Vidinov (2018) found the parasite only on nymphs of the genus *Rhithrogena* (i.e., species from the *semicolorata* group). In our study, the reason could be that the number of individuals per square meter was much lower on the other hosts than on *Rhithrogena* cf. *semicolorata*. However, Antonov Dashinov & Nikolova Vidinova (2018) state that although the genus *Ecdyonurus* was the most dominant, followed by the genus *Epeorus*, *S. rhithrogenae* infested only *Rhithrogena* at the sites studied, while Gilka *et al.* (2007) state that it parasitized *Ecdyonurus*.

Conclusion

Based on the above, and in particular the limited number of parasites found in this study, we cannot draw any conclusions about the host choice preferences of this species. Further studies on *Symbiocladius rhithrogenae* are needed for a better understanding of its biology and host preferences.

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References

- Antonov Dashinov, D., & Nikolova Vidinova, Y. (2018). First records of the parasite *Symbiocladius rhithrogenae* (Zavrel, 1924) (Diptera: Chironomidae) in several streams of Rila Mountain, Bulgaria. *Aquatic Insects*, 38(4), 255-259.
- Bauernfeind, E., & Soldán, T. (2012). *The mayflies of Europe (Ephemeroptera)*. Apollo, Ollerup, 781 pp.
- Čerba, D., Koh, M., Vlaičević, B., Turković Čakalić, I., Milošević, D., & Stojković Piperac, M. (2022). Diversity of periphytic Chironomidae on different substrate types in a floodplain aquatic ecosystem. *Diversity*, 14(4), 264.
- Ferrington, L. C. (2008). Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. *Freshwater animal diversity assessment*, 447-455.
- Gilka, W., Klonowska-Olejnik, M., & Godunko, R. J. (2007). On the biology of *Symbiocladius rhithrogenae* (Zavrel, 1924) (Diptera: Chironomidae) from the Chornohora Mts., Ukraine. *Polish Journal of Entomology*, 4(76).
- Gonser, T., & Spies, M. (1997). Southern Hemisphere *Symbiocladius* (Diptera, Chironomidae) and their mayfly hosts (Ephemeroptera, Leptophlebiidae). *Ephemeroptera & Plecoptera: Biology-Ecology-Systematics. MTL. Fribourg*, 455-466.
- Hordós, N., & Murányi, D. (2008). Contribution to the Ephemeroptera Fauna of Maramures, Romania. *Studia Universitatis "Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series)*, 18, 221.
- Illies, J. (1978). *Limnofauna Europaea*. Stuttgart and New York: Gustav Fischer Verlag, 532 pp.
- Jacobsen, R. E. (1995). Symbiotic associations between Chironomidae (Diptera) and Ephemeroptera. In: Corkum D. L. & Ciborowski I. I. H. (Ed.): *Current directions in research on Ephemeroptera*. Toronto: Canadian Scholars' Press Inc., 317-332.

- Janković, J. M. (1987). Nove vrste Chironomidae (Diptera) u fauni Jugoslavije. *Biosistematika*, 13(1), 87-90.
- Janković, M. (1978). Fauna Chironomidae (Diptera, Nematocera) Jugoslovenskog dela Dunava i njegovog plavnog područja i karakteristike zajednica koje njihove larve obrazuju na različitim staništima, *Zbornik radova o entomofauni SR Srbije*, SANU 2, 29-89.
- Janković, M. (1980). Fauna Chironomidae sliva Blatave, *Zbornik radova o fauni SR Srbije*, SANU 1, 17-29.
- Janković, M. (1981). Sastav i struktura faune Chironomidae (Diptera) donjeg toka Save, *Glas CCCXXIX*, knjiga 48, 111-120.
- Janković, M. (1998). Fauna Chironomidae Velike Morave, *Zbornik radova o fauni SR Srbije*, SANU 5, 106-139.
- Jevtić, Lj. (1999). *Glavni projekt pastrmskog ribnjaka Manastira Rača*. Valjevo: dr Ljubiša Jevtić
- Kovács, T. (2007). Data to the distribution of three species of *Electrogena* in Hungary, based on larvae (Ephemeroptera: Heptageniidae). *Folia Historico-naturalia Musei Matraensis*, 31, 133-137.
- Kovács, T., & Godunko, R. J. (2008). Faunistical records of larvae of Ephemeroptera, Odonata and Plecoptera from the Zakarpats'ka Region, Ukraine. *Folia historico-naturalia Musei matraensis*, 32, 87-91.
- Kriska, G., Andrikovics, S., & Sztitó, A. (1998). Phenological Data on a Parasitic Relationship between *Electrogena lateralis* (Curtis, 1834) (Ephemeroptera) and *Symbiocladius rhithrogenae* (Zavrel, 1924) (Chironomidae). *Opuscula Zoologica Budapest*, 31, 79-84.
- Langton, P. H. (1991). *A key to pupal exuviae of West Palaearctic Chironomidae*. Privately published by the author. 386 pp.
- Martins, I., Castro, D. M., Macedo, D. R., Hughes, R. M., & Callisto, M. (2021). Anthropogenic impacts influence the functional traits of Chironomidae (Diptera) assemblages in a neotropical savanna river basin. *Aquatic Ecology*, 55, 1081-1095.
- Marziali, L., Armanini, D. G., Cazzola, M., Erba, S., Toppi, E., Buffagni, A., & Rossaro, B. (2010). Responses of Chironomid larvae (Insecta, Diptera) to ecological quality in Mediterranean river mesohabitats (South Italy). *River Research and Applications*, 26(8), 1036-1051.
- Matěna, J., & Soldán, T. (1982). Taxonomy and ecology of the subgenus *Symbiocladius* s. str. (Diptera, Chironomidae). *Folia Facultatis Scientiarum Naturalium Universitatis Purkynianae Brunensis*, 23(7), 83-86.
- Milošević, Đ. (2013). Larve familije Chironomidae (Diptera, Insecta) sliva Južne Morave i njihova primena u proceni ekološkog statusa tekućih vodenih ekosistema. Doktorska disertacija. Kragujevac: Prirodno-matematički fakultet Univerziteta u Kragujevcu.
- Milošević, D., Simić, V., Stojković, M., & Živić, I. (2012). Chironomid faunal composition represented by taxonomic distinctness index reveals environmental change in a lotic system over three decades. *Hydrobiologia*, 683, 69-82.
- Moller Pillot, H. K. (2013). *Chironomidae Larvae of the Netherlands and Adjacent Lowlands Biology and Ecology of the Aquatic Orthoclaadiinae*. Zeist: KNNV Publishing. 314 pp.
- Moubayed, J. (1991). *Symbiocladius rhithrogenae* Kieffer & Zavrel (Dipt. Chironomidae) redescription of the female imago and pupar exuviae. *Bulletin de la Société d'histoire naturelle de Toulouse*, 127, 81-84.
- Nilsson, A. (Ed.). (1997). *Aquatic insects of North Europe: A taxonomic handbook* (Vol. 1). Apollo books, Stenstrup, Denmark, 274 pp.
- Paunović, M. (2007). Struktura zajednica makroinvertebrata kao indikator tipova tekućih voda Srbije. Doktorska disertacija. Beograd, Srbija: Biološki fakultet, Univerzitet u Beogradu.
- Paunović, M., Tubić, B., Kračun-Kolarević, M., Marković, V., Simić, V., Zorić, K., & Atanacković, A. (2012). Ecoregions delineation for the territory of Serbia. *Water Research and Management*, 2(1), 65-74.
- Petrović, A. (2014). Mogućnosti korišćenja baze podataka u strategiji konzervacije biodiverziteta makroseskičmenjaka kopnenih voda na nacionalnom nivou. Doktorska disertacija, Prirodno-matematički fakultet, Univerzitet u Kragujevcu, Srbija, 1-237.

- Savić, A. V. (2012). Ekološka analiza zajednice makrozoobentosa reke Nišave. Doktorska disertacija. Beograd: Biološki fakultet, Univerzitet u Beogradu.
- Schiffels, S. (2009). Commensal and parasitic Chironomidae. *Lauterbornia*, 68, 9-33.
- Schmid, P. E. (1993). *Key to the Larval Chironomidae and their Instars from Austrian Danube Region Streams and Rivers – Part I. Diamesinae, Prodiamesinae, Orthoclaadiinae*, Wien: Federal Institute of Water Quality of Ministry of Agriculture and Forestry. 513 p.
- Schmedtje, U. & Colling, M. (1996). Ökologische Typisierung der aquatischen Makrofauna. Bayrisches Landesamt für Wasserwirtschaft. *München, Germany*, 4, 543.
- Selbach, C., Mouritsen, K. N., Poulin, R., Sures, B., & Smit, N. J. (2022). Bridging the gap: aquatic parasites in the One Health concept. *Trends in Parasitology*, 38(2), 109-111.
- Soldán, T. (1978). Die Wirtsspezifität und Verbreitung von *Symbiocladius rhithrogenae* (Diptera, Chironomidae). *Acta entomologica bohemoslovaca*, 75, 194-200.
- Soldán, T. (1979). The effect of *Symbiocladius rhithrogenae* (Diptera, Chironomidae) on the development of reproductive organs of *Ecdyonurus lateralis* (Ephemeroptera, Heptageniidae). *Folia Parasitologica*, 26, 45–50.
- Stojanović, K. Z. (2017). Uticaj pastrmskih ribnjaka na zajednice makrozoobentosa tekućica sa posebnim osvrtom na larve roda *Baetis* (Ephemeroptera, Insecta). Doktorska disertacija Biološki fakultet, Univerzitet u Beogradu.
- Tokeshi, M. (1993). On the evolution of commensalism in the Chironomidae. *Freshwater Biology*, 29(3), 481-489.
- Vilenica, M., Ergović, V., & Mihaljević, Z. (2018). Mayfly (Ephemeroptera) assemblages of a Pannonian lowland mountain, with first records of the parasite *Symbiocladius rhithrogenae* (Zavrel, 1924) (Diptera: Chironomidae). In *Annales de Limnologie-International Journal of Limnology*, 54, 31.
- Wiens, A. P., Rosenberg, D. M., & Evans, K. W. (1975). *Symbiocladius equitans* (Diptera: Chironomidae), an ectoparasite of Ephemeroptera in the Martin River, Northwest Territories, Canada. *Entomologica Germanica*, 113-120.
- Wonglersak, R., Fenberg, P. B., Langdon, P. G., Brooks, S. J., & Price, B. W. (2021). Insect body size changes under future warming projections: a case study of Chironomidae (Insecta: Diptera). *Hydrobiologia*, 848(11), 2785-2796.
- Živić I. (2020). Istraživački monitoring bioloških, fizičko-hemijskih i hidromorfoloških parametara u cilju definisanja referentnih lokaliteta na području Republike Srbije, *Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije. Republička direkcija za vode*.
- Živić, I. (2005). Faunistička i ekološka studija makrozoobentosa tekućica sliva Južne Morave sa posebnim osvrtom na taksonomiju larvi Trichoptera (Insecta). Doktorska disertacija. Beograd: Biološki fakultet.

SYMBIOCLADIUS RHITHROGENAE (ZAVREL, 1924) (DIPTERA: CHIRONOMIDAE): НОВИ НАЛАЗ ЗА ЕНТОМОФАУНУ СРБИЈЕ

МИЛЕНКА БОЖАНИЋ, КАТАРИНА СТОЈАНОВИЋ, ЂУРАЋ МИЛОШЕВИЋ,
ВАЊА МАРКОВИЋ И ИВАНА ЖИВИЋ

Извод

Током хидробиолошких истраживања на територији Србије током 2011. и 2012. године врста *Symbiocladius rhithrogenae* (Zavrel, 1924) (Diptera: Chironomidae) пронађена је у реци Рачи на три локалитета и представља први налаз овог паразита у Србији. Ова врста је инфицирала само ларве *Rhithrogena* cf. *semicolorata* (Ephemeroptera: Heptageniidae) и није пронађена ни на једној другој врсти из породице Heptageniidae. Неопходне су даље студије о биологији ове паразитске врсте да би се боље разумео њен избор домаћина.

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