# *PARALEPTOPHLEBIA WERNERI* ULMER, 1919 (EPHEMEROPTERA, LEPTOPHLEBIIDAE) FROM THE WEST CAUCASIAN CAVES WITH NOTES ON MAYFLIES' POTENTIAL FOR CAVE COLONIZATION[1]

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ABSTRACT: Larval and winged stages of *Paraleptophlebia werneri* Ulmer, 1919 were found in Andreevskaya Cave and Verkhnyaya Shakuranskaya Cave (W Caucasus, Abkhazia). No considerable differences in chaetotaxy, coloration and body size of larval and winged stages from these caves and epigean water bodies were noted. Mayfly potential for cave colonization is discussed.

KEY WORDS: Mayflies, West Caucasus, development in cave, colonization potential

Caves are unique subterranean natural habitats without sunlight, with constant temperature and high relative humidity; thus their colonization often requires or results in special morphological, physiological and ecological adaptations. Not all insect orders are able to settle these habitats due to complicated life cycles, unique nutritive bases which could be absent in caves, and other factors. In our opinion, there are no substantial limiting factors for underground water bodies' colonization by mayflies. However, all mayfly species developing in caves represent typical inhabitants of surface waters and do not possess any special adaptations for life in complete darkness (Kawai, 1964; Hippa et al., 1985; Mc Cafferty et al., 2010; Derka et al., 2012; etc.). Moreover, all mayfly larvae obtained from caves have been found mainly near their entrances.

We have found one more species of mayflies which can develop in caves, but has not been treated as troglobiont or troglophile – *Paraleptophlebia werneri* Ulmer, 1919. Larvae and winged stages of target species were registered in Verkhnyaya Shakuranskaya and Andreevskaya Caves (West Caucasus).

Verkhnyaya Shakuranskaya and Andreevskaya Caves are situated in Gumishhinsko-Panavsky speleological district of Southern Slope region in Crimea-Caucasus speleological country. Gumishhinsko-Panavsky district is located between the rivers Inguri and Aapsta. Their karst cavities are dated to the limestones of Upper Cretaceous and Paleogene periods. Severe erosion dismemberment is the reason for a large number of caves in valleys of rivers in the district. The largest karst cavity of Southern Slope region - the Novo-Afonskaya Cave (1900 m in length, volume - 1700000 $m^3$) - is situated at Gumishhinsko-Panavsky speleological district.

The Verkhnyaya Shakuranskaya Cave is 800 m long and has 22 000 $m^3$ in volume. There is a stream flowing out from the cave (water discharge – up to

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6 liters/sec, water temperature – up to 12°C). The Andreevskaya Cave is 220 m long. There are numerous rimstone pools (depth up to 1.5 m) in it. The current of water in this cave is almost absent at low water, but during seasonal floods a stream in the cave is formed, which has strong discharge from the entrance to the cave (Dublyanskiy et al., 1987; original data).

## MATERIALS AND METHODS

All material was collected by Dr. Vargovitsh during investigations of Abkhazian caves in 2009, 2010, 2011. Winged stages of mayflies were collected on the water surface and on the walls of caves. Larvae were collected manually, without using a net. All specimens are preserved in 85-95% ethanol. Two larvae were mounted on slides in Canada balsam.

Material: *Paraleptophlebia werneri* Ulmer, 1919: 4 mature larvae (1♂, 3♀) (one in slide № 537), 2 subimagoes (♀♀), West Caucasus, Abkhazia, Gulripsh District, Amtkel Village vicinity, Verkhnyaya Shakuranskaya Cave (transl. as "Upper Shakuran Cave"), on the walls above the underground stream, about 500 meters from the entrance, leg. Vargovitsh R., 16.08.2009; mature larva (♀) (in slide № 538) and imago (♀), ibid, leg. Vargovitsh R., 18.08.2010; 5 immature larvae (♀♀) – first instars, West Caucasus, Abkhazia, Akhalsheni Village vicinity, Andreevskaya Cave, in rimstone pools (diameter ~2 m), about 50 m from the entrance, leg. Vargovitsh R., 29.08.2011.

Material is deposited in the collection of A. V. Martynov in the National Museum of Natural History at the National Academy of Sciences of Ukraine.

## RESULTS AND DISCUSSION

*Paraleptophlebia werneri* is the West-Palaearctic species. Target species is common in Caucasus, and in its southern slope especially. *P. werneri* inhabits different types of water bodies: lotic and lentic epigean waters, zones of waterflows from epirhithral to hypopotamal ones. The most typical habitat of the species is potamal zone of streams and small rivers with low current velocity, macrophytic vegetation and a large quantity of detritus (Bauernfeind and Soldán, 2012; original data).

The stream in Verkhnyaya Shakuranskaya Cave, where the larvae of *P. werneri* live, represents a system of rimstone pools, flowing lakes and waterfalls depending on cave relief. During floods the stream discharges out of the cave through the entrance to this cave. Larvae were sampled in the stream at a distance of about 500 m from the entrance. Subimagoes and imagoes were also found here on the water surface and on the walls.

In the rimstone pools of Andreevskaya Cave at a distance of 50–60 m from its entrance the *P. werneri* larvae of first instars were collected. These certainly hatched directly in the cave.

All larvae and winged stages from Verkhnyaya Shakuranskaya Cave were normally pigmented (Figs. 1A, 1C), while young larvae from Andreevskaya Cave were lighter, but had normally developed eyes (Fig. 1B). The young instars of mayflies from epigean water bodies are commonly lighter than the last instars too. No considerable differences in chaetotaxy, coloration and body size of larval and winged stages of *P. werneri* were noted when mayflies from the caves were compared with those from epigean water bodies.

Fig. 1. *Paraleptophlebia werneri* Ulmer, 1919 from the Abkhazian caves: A – female imago from Verkhnyaya Shakuranskaya Cave; B – immature larva from Andreevskaya Cave; C – mature larva from Verkhnyaya Shakuranskaya Cave.

Mayflies' larvae occurrence in pools or streams close to the entrances of caves (up to ca 50 m) of the West Caucasian caves is not unique; we have observed such penetrations in several cases.

Most unusual is the repeated occurrence of both larval and imaginal stages of *P. werneri* in Verkhnyaya Shakuranskaya Cave far removed (about 500 m) from the entrance for two consecutive years. It is interesting that no specimens were seen in the closer and intermediate parts of the cave. Thus their penetration through the natural entrance against the stream current is rather doubtful. At the same time some detritus (dead leaves, seeds, etc.) was observed at the distant area of the cave. It means that unknown hidden water communication of this cave site with the surface through small cracks is quite possible and some mayfly larvae could penetrate into the cave through this way. Then, obviously they continued their developmental cycle inside the cave and possibly even reproduced there.

Life cycle of *P. werneri* in European epigean water bodies is univoltine (Us). The species overwinters as the egg stage, though Landa (1968) suggested that *P. werneri* from epigean water bodies may have Uw life cycle (univoltine, overwintering as the larval stage). Imagoes fly from April to June in Europe (Bauernfeind and Soldán, 2012), while in Eastern Ukraine they are registered from the middle of May to middle of July (original data).

In Verkhnyaya Shakuranskaya Cave mature larvae and winged stages of *P. werneri* were registered in August, while in Andreevskaya Cave only larvae of first instars were found at this time. We can only suggest that in both cases the species has a univoltine life cycle with slow development of larvae due to low temperature of water or even nonseasonal life cycle (winged stages can emerge even in winter) due to constant temperature conditions during a year. Similarly the nonseasonal life cycle was noted for cavernicolous stonefly species – *Protonemura gevi* Tierno de Figueroa and López-Rodríguez, 2010 (López-Rodríguez and Tierno de Figueroa, 2012). Thus, the life cycle of *P. werneri* in caves needs further investigation.

It is rather strange that despite a large potential for cave colonization, mayflies rarely occur underground and have no troglobitic forms. The general potential of order Ephemeroptera for underground water bodies' colonization is theoretically possible due to several advantages:

1) Many species are able to propagate parthenogenetically, which excludes the necessity of both sexes meeting. This is particularly important for populations with low density (usually troglobites do not show high population density). This also excludes the need for flight in complete darkness: the oviposition may take place at the same location where the larvae become winged. Moreover, according to some authors (Degrange, 1960; Harker, 1997; Funk et al., 2010) virginal reproduction in bisexual species is periodically observed alternating between sexual reproduction and fertilization. Hitherto, obligate parthenogenesis was known only for a few mayfly species from North America, South America and Africa (Froehlich, 1969; Gibbs, 1977; Bergman and Hilsenhoff, 1978; Gillies and Knowles, 1990; etc.).

2) There is no need in a prolonged lifetime for the winged stages (imago and subimago). Mayflies are aphagous at imaginal and subimaginal stages. Their life cycles are short and eggs are fully developed by the end of the larval stages, which offers the opportunity to oviposit immediately after becoming winged. Moreover, unoviposited eggs (inside the body of dead imago, subimago and even mature larvae sunken in the water) of *Caenis knowlesi* Gillies & Knowles, 1990 are able to develop (Gillies and Knowles, 1990).

It was also supposed, that if the development of *Cloeon simile* Eaton, 1870 finishes in winter, when water bodies are covered with ice, its new generation can rise from the eggs of subimagoes, which emerged and died under the ice (Harker, 1997). Such biological flexibility within the order considerably increases the ability of mayflies to colonize subterranean waters.

3) Also, many mayfly species feed on detritus (Soldán and Zahrádková, 2000), which is often present in subterranean waters. It incomes from the surface and, in some cases, from chemosynthetic bacteria (Culver, 1985).

Summarizing what is presented above, we can conclude that despite possessing some important biological advantages for cave environment colonization, epigean mayfly species penetrating or even developing in caves have not evolved to troglobiont forms.

The causes which hamper the evolution of specialized cave forms of mayflies remain unknown to us. However, it is not excluded that the discovery of troglobitic mayflies is only a matter of time and thorough search. Similarly species of Plecoptera with cavernicolous adaptations were discovered only recently (Derka et al., 2010; López-Rodríguez and Tierno de Figueroa, 2012).

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