Living on the edge: *Meoneura obscurella* in the ‘Wieliczka’ Salt Mine (southern Poland) exhibits the first case of lecithotrophic ovoviviparity in the family Carnidae (Diptera)

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Abstract

During the studies on the invertebrate fauna of the subterranean part of the “Wieliczka” Salt Mine in Wieliczka, Poland, the presence of many specimens of the dipteran species *Meoneura obscurella* (Fallén 1823) was observed. Organic remains and faeces related to the presence of mice (*Mus musculus*) were indicated as a potential food source for the insects. *M. obscurella* displays lecithotrophic viviparity (ovoviviparity), which has developed due to extremely harsh abiotic conditions and a lack of food. This is the first documented case of lecithotrophic viviparity within the fly family Carnidae. Based on the ability of this species to inhabit and reproduce in the conditions of the subterranean part of the “Wieliczka” Salt Mine, it is proposed to classify the species as a troglophile. The occurrence of *Niptus hololeucus* (Faldermann 1835), a species of beetle representing the family Ptinidae, was also confirmed in the subterranean part of the “Wieliczka” Salt Mine.

Keywords: *Meoneura obscurella*, *Niptus hololeucus*, troglophiles, biology, lecithotrophic ovoviviparity, “Wieliczka” Salt Mine, Poland

Introduction

Even though salt deposits and salt mines are widespread throughout the world (Kozary et al. 1968; Lefond 1969), information about the fauna living in such habitats is rare and fragmented (Zejszner 1843; Nitzu et al. 1998–1999; Kłys et al. 2018). Consequently, there is a widespread belief that the high salinity of this specific underground environment is unsuitable to the fauna that inhabits it (Humphreys et al. 2009), which in turn makes studies of such fauna very rare.

The salt mine in Wieliczka has been in continuous operation since the mid-13th century. The mine consists of nine levels reaching up to 327 metres deep, with a total of 26 open-top shafts and 180 shafts connecting the different levels. Since the 13th century, salt has been mined from over 2000 chambers, and over 240 km of galleries have been dug, creating a labyrinth that stretches from Level I (57 metres below the ground) to Level IX (327 metres below the ground) (Dębkowski et al. 2005).

Geologically speaking, the mine is related to the Carpathian foreland plate, which was filled 15–20 million years ago (Late Miocene) with the waters of the Baden Sea. Crystallisation of the salt dissolved in the seawater created sediments that have accrued in an area called the Carpathian Foredeep. These sediments are primarily composed of rock salt, anhydrite and gypsum, all of which are present in Wieliczka (Gawel 1962). Detailed information about the origin and geology of the Carpathian Foredeep can be found in many articles (e.g., Oszczypko 1996, 1997; Andreyeva-Grigorovich et al. 2003; Oszczypko et al. 2006).

The salt industry in the neighbourhood of Wieliczka began with the extraction of salt from natural, surface sources of brine (Jodłowski 1988). In 1964, the mining of rock salt in Wieliczka was discontinued, and on 30 June 1996, all mining ceased completely (Beiersdorf 2011). Currently, salt is produced from brine pumped out of the mine to the surface, while the mine itself and its...
picturesque surroundings have become a tourist attraction, with a museum and a health resort.

Because a study in 2018 confirmed the presence of beetles representing *Niptus hololeucus* (Faldemann 1835) (Coleoptera: Ptinidae) (Klys et al. 2018), which had already been observed over 150 years ago (Zejszner 1843) in abandoned areas of the mine, the authors of this paper decided to conduct further research on the entomofauna of the subterranean environment in the “Wieliczka” Salt Mine.

**Methods**

Research in the subterranean part of the “Wieliczka” Salt Mine was conducted between April 2018 to October 2019, in areas where the specimens of *Niptus hololeucus* (Coleoptera: Ptinidae) had previously (Klys et al. 2018) been found. The Barber traps baited with bovine liver were used to collect specimens from 14 sites (so-called “chambers”) located in the excavations on various mine levels. The traps were checked and replaced on average once per month. Between June 2019 and August 2019, individual traps were also set on Levels IV through VII.

The collected insects were placed in 70% ethyl alcohol, inappropriately labelled tubes, and identified based on specialised identification keys (Trojan 1957; Papp 1978; Borowski 1996; Brake 2011). Determination of embryonic stages and their morphological nomenclature follows Panagopoulos (2012) and Martin-Vega and Hall (2016).

Photographs of the specimens were taken using the following equipment: Olympus SZX-9 stereo microscope, CX-41 optical microscope, Olympus DP-12 cameras and Analysis software.

**Results**

Many specimens of the Diptera species *Meoneura obscurella* (Fallén 1823) representing the family Carnidae were observed during studies in the Kieratowa Moritz Chamber (Figure 1). These flies were not detected anywhere outside the chamber. They were also extremely annoying to the people who entered the chamber.

Over 60 adult specimens of *Meoneura obscurella* (Diptera: Carnidae) (Figure 2) were collected in the study area. An examination of their morphology revealed the presence of between one and several large eggs (in the abdomens of about a dozen females). The eggs were transferred onto slides and observed using the aforementioned optical microscope. The samples were immersed in paraffin oil.

The eggs were found to contain embryos at different stages of development (Figure 3) or even fully-formed larvae (Figure 4(a)). Older larvae and pupae (Figure 4(b)) were also observed outside the flies bodies.

Besides, the conducted study confirmed the presence of many specimens of the beetle *Niptus hololeucus* in the analysed excavations within the mine.

**Discussion**

The research to date has been unable to identify the anatomical, morphological and physiological traits that allow a species inhabiting such extreme environments...
as the “Wieliczka” Salt Mine to survive and reproduce. This subterranean habitat is characterised by a lack of light, a stable temperature (about 11.3°C throughout the year), high relative humidity (75%) and high salinity (Kłys et al. 2018). Furthermore, the mine’s only connection to the surface environment is through a series of corridors (Szlązak et al. 2006).

All sampled specimens of *Meoneura obscurella* (Diptera: Carnidae) inhabited only the Kieratowa Mortiz Chamber. To date, this species has been observed only in epigeal environments within its entire Holarctic range (Brake 2011; Stuke & Bächli 2015).

The presence of *M. obscurella*, a species rarely observed in Europe (Stuke & Bächli 2015), has only been recorded in Poland twice (Brake 2011), one of which is a mention in a catalogue of Polish fauna (Nowakowski 1991). Karl (1936) is the only author to indicate a particular site inhabited by this species in Poland; namely, Słupsk (*Stolp-Waldkatze*, currently Zalesiczki).

Specimens of *M. obscurella* primarily inhabit the nests of birds, such as the blackbird, thrush, finch and sand martin; however, they have also been frequently observed on dead vertebrates and in vertebrate faeces (Smith 1989). Consequently, it can be speculated that thanks to such a specific diet, the species can easily make use of all, even the smallest, of the organic remains available in the mine.

Also, an interesting adaptation of *M. obscurella* never previously recorded in this species was observed during the study, which may aid in its survival in the harsh environment of a salt mine, namely, the development of eggs within the bodies of the females. The eggs collected from the

Figure 2. Adult male of *Meoneura obscurella*. Scale bar = 1 mm.
abdomen of females contained embryos at different stages of growth or even fully-formed larvae (Figures 3–4).

According to the subject literature, lecithotrophic viviparity in Diptera has evolved independently at least 61 times (Meier et al. 1999) due to a harsh environment and a lack of food. Using the small amounts of resources provided by a quickly-decaying food supply (faeces and dead animals) enforces a shortening of the development time for larvae outside the body of the mother and a decrease in the number of larvae. Lecithotrophic viviparity naturally reduces the feeding time of the larvae and decreases their number (Sulikowska-Drozd 2012).

It is worth mentioning that to date, *M. obscurella* has never been indicated as a species with lecithotrophic viviparity; however, Engel (1931) suggested such a possibility. He observed some larvae in powdered tobacco (snuff) and decided to conduct an experiment in which he attempted to breed them in this environment. He described both the larvae and the pupae, and noted that no egg-laying took place, nor were any eggs present in the powdered tobacco. Instead, fully-formed larvae appeared in the tobacco.

The presence of a previously observed beetle, *Niptus hololeucus* (Zejszner 1843; Klys et al. 2018) in the “Wieliczka” Salt Mine is unsurprising, as this species has inhabited the mine for many years and is

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Figure 3. (a) A stage 10 follicle. FC – follicle cells. NC – nurse cells. OC – oocyte. VM – vitellin membrane. (b) An egg at the stage of about 40% of its total embryonic development. SI – stomodeal invagination. (c) An egg at the stage of 50% of its total embryonic development. CF – cephalic furrow. CL – clypeolabrum. Scale bar = 0.1 mm.
a permanent part of its fauna. *N. hololeucus* originates from East Asia, and was introduced to North and South America and to Europe, where it inhabits human settlements, reaching in Poland even the northern-most range of the continent (Burakowski et al. 1986). The species has also been observed in European caves (Jeannel 1909) and Kan-Gohar Cave in the Fars Province of Iran (Dashan et al. 2014). Because *N. hololeucus* has also been observed in rodent burrows (Borowski 1996), it may be speculated that its habitat in the “Wieliczka” Salt Mine is in the nests of mice, which are abundant there, and that the organic remains and faeces of the mice are an excellent material for the cocoons to house the beetle larvae (Borowski 1996; Kłys et al. 2018).

Engel’s study was completely omitted in a monograph by Meier et al. (1999) on viviparity and lecithotrophic viviparity in Diptera. The only Carnidae species mentioned in this monograph that may display lecithotrophic viviparity was *Carnus hemapterus* (Nitzsch, 1818). However, detailed research has not confirmed this possibility (Meier et al. 1999). Consequently, it should be concluded that *M. obscurella* (Fallén, 1923) is the first recorded representative of the family Carnidae to display lecithotrophic viviparity, which allows it to inhabit and reproduce in the extremely harsh conditions of a salt mine or even in powdered tobacco.

Both species observed in the “Wieliczka” Salt Mine, *N. hololeucus* and *M. obscurella*, seem to be more troglophilic than was previously thought. Their specimens were collected throughout the year, which means that they can complete their life cycles in both epigean and subterranean environments. The original populations of these two species likely allowed for migration and gene flow between both environments. Currently, the populations present in the mine are most likely completely isolated. However, only genetic research can verify this hypothesis.

The small number of insect species inhabiting the “Wieliczka” Salt Mine is related to the specific abiotic conditions in the mine and limited contact with the surface (Halse et al. 2014).

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Disclosure statement

No potential conflict of interest is reported by the authors.

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