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FIRST REPORT OF VIVIPARITY OF THE STONEFLIES

CAPNIA KHINGANA (PLECOPTERA: CAPNIIDAE)

IN THE LOW AMUR RIVER BASIN

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Summary. For the first time on the south of the Russian Far East, in small cool spring-like streams of the Low Amur River Basin, viviparity or live-bearing reproduction of stoneflies *Capnia khingana* (Plecoptera: Capniidae) was recorded. Apparently, the development of nymphs in eggs takes more than two months. Mature embryos were found throughout the abdomen, thorax, and head of *C. khingana* females, their number varied from 178 to 124. The first-instar nymphs hatched inside female body and exiting under the genital plate had head capsule with apparent eyespots, thorax, abdomen, legs, cerci and antennae. The lengths of the first-instar nymphs varied from 910.33 to 920.35 µm, and the width of the head capsule, from 161.85 to 171.13 µm.

Key words: stoneflies, viviparity, oviparity, first instar nymphs, streams, Russian Far East.

В. А. Тесленко, Н. М. Яворская. Первое сообщение о живорождении у веснянки *Capnia khingana* (Plecoptera: Capniidae) в бассейне Нижнего Амура // Дальневосточный энтомолог. 2020. N 417. С. 17-24.

Резюме. Впервые на юге Дальнего Востока России в малых холодноводных родниковых ручьях бассейна Нижнего Амура отмечено живорождение или живородящее размножение веснянки *Capnia khingana* (Plecoptera: Capniidae). По-видимому, эмбриональное развитие личинок продолжалось более двух месяцев. Зрелые эмбрионы были обнаружены в брюшной полости, грудной клетке и голове самок *C. khingana*, их количество варьировало от 178 до 124. Личинки первой стадии, сформировавшиеся внутри тела самки и выходящие из-под генитальной пластинки, имели головную капсулу с видимыми пятнами пигмента глаз, грудь, брюшко, ноги, церки и усики. Длина личинок первой стадии варьировала от 910,33 до 920,35 мкм, а ширина головной капсулы – от 161,85 до 171,13 мкм.

INTRODUCTION

Capnia khingana Teslenko, 2019 is a small brachypterous winter stonefly found in streams on the spurs of the Lesser Khingan Mountains (Khinganskii Reserve, Amurskaya oblast), south

of the Russian Far East. Stoneflies inhabit the upper reaches of small, cool spring-like streams that are tributaries to the Mutnaya River in the Middle Amur River Basin. *C. khingana* adults are found crawling on ice and snow within 100 m of unfrozen sections of streams in mid-March (Teslenko, 2019). Any additional information concerning the life cycle, biology, and ecology of this species remains elusive. Here we describe the unique reproductive behavior of *C. khingana* that was discovered as a result of stonefly fauna research in the Komsomolskii State Nature Reserve.

MATERIAL AND METHODS

Komsomolskii State Nature Reserve encompasses the confluence of the Gorin River and Amur River in the Khabarovskii Krai on the South of the Russian Far East. The Gorin River is a left tributary of the Amur River (Low Amur River Basin); it is approximately 390 km long. Samples were collected from the right tributaries of the Gorin River in small, cold, foothill streams. The second author collected stonefly specimens in the Khankuka stream (length = 13 km, altitude of the source = 360 m above sea level) and Kamenskaya stream; stonefly specimens were also collected in a few unnamed streams from May 9–14, 2020. The small foothill streams differ from the Amur River in several characteristics such as water transparency, current flow, and the thermal regime (Levanidov, 1969). The difference in the water temperature between the Amur River and small foothill streams ranges from 5°C to 10°C and, in some cases, 15°C in summer. The Amur River bottom is sandy, but the bottoms of the tributaries contain pebble-rock substrate. The insular permafrost in the mountainous upper parts of the Gorin River Basin significantly decreases the temperature of the ground and surface stream waters since the frozen ground layers lie at a shallow depth. The stream water temperatures varied within the range of 5–7°C.

A modified drift net (hole diameter = 0.28 m, net length = 0.8 m) was sometimes used to collect stoneflies. Adult female stoneflies were hand-collected with entomological forceps primarily from the surface film of water and foam that formed in calm areas of river folds. The specimens were also collected through the sweeping of coastal vegetation using an entomological net. Samples were fixed with 75% ethanol.

The adult females were dissected under a compound microscope in transmitted light and the embryos and their position in the abdomen was studied in detail and documented by digital cameras (Nikon Coolpix 995 and Toups View 3.7) and with the stereomicroscope Olympus SZX1 6 and digital camera Olympus DP74, and stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software. The material is deposited in the collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia [FCBV].

RESULT AND DISCUSSION

Approximately 568 females of *Capnia khingana* and the majority of swimming first-instar nymphs were collected. We dissected 60 *C. khingana* gravid females with body lengths of 4.3–5.3 mm. *C. khingana* females, like many *Capnia* representatives, do not possess a seminal receptacle (Zhiltzova, 2003). Sperm accumulate in the oviducts and remain in them until the eggs mature. Due to this feature, egg fertilization is performed in the ovaries or oviducts; thus, embryo development occurs inside the female body (Zhiltzova, 2003) (Fig. 1). The embryos in most *C. khingana* female specimens were at immature developmental stages (Fig. 1), and only seven females contained nymphal embryos as well-developed first-instar nymphs with apparent eyespots (Figs 2, 3). The average number of embryos varied from 178 to 124. Unhatched nymphal embryos were enveloped in coverings and curled within their eggs,

Fig. 1. Female reproductive system of *Capnia khingana*. Abbreviations: Ov – developing oocytes, Ovd – oviducts, C – gut, Vg – vagina.

Fig. 2. Female of *Capnia khingana* with developing nymphal embryos throughout abdomen, thorax, and head.

which were oval-shaped and white; body segmentation was also distinguishable within the eggs (Figs 2, 3). The eggs were distributed throughout the abdomen, thorax, and occasionally the head of female specimens (Fig. 2).

We fixed first-instar hatched nymphs exiting under the genital plate that were hooked to each other by claws, legs, or cercal segments (Figs 3, 5). The discovery of these nymphs within two females from our samples confirmed viviparity or live-bearing reproduction in *C. khingana* females as they retained developing eggs inside their reproductive tracts and gave birth to their young (Blackburn, 2015). Here, we follow the terminology of Blackburn and define viviparity as live-bearing reproduction and oviparity as egg-laying reproduction. We avoid the use of the term ovoviviparity, which has been given several different definitions and sometimes implies knowledge of an embryo's nutritional source (Kohler *et al.*, 2004; Trumbo, 2012; Blackburn, 2015).

The hatched nymphal embryos inside of the body have distinguishable eyespots as well as legs, cerci, antennae that are also easily distinguished (Fig. 4A). No membranous chorion was detected on the surface of the hatched embryos (Fig. 4A). The body of the hatched nymphal embryos was covered with setae, the length of the curled embryo did not exceed 500 μm , and the formed mouthparts were very visible (Fig. 4B). Hatched first-instar nymphs exiting abdomen possessed three cercal segments and nine antennal segments (Fig. 4A). Their body lengths varied from 910.33 to 920.35 μm , and their head capsule width ranged from 161.85 to 171.13 μm .

Viviparity among Plecoptera occurs. Only Claassen (1924) has observed nymphs hatched within the body of *Allocapnia vivipara* (Claassen, 1924), but Frison (1929) could not confirm the live birth in *A. vivipara* with his experimental work. Embryogenesis within adult females and the live birth of nymphs were detected in the second cohort of a unique *Capnia lacustra* Jewett 1965 population inhabiting Lake Tahoe in the Sierra Nevada mountain range, USA (Caires, *et al.*, 2016). *C. lacustra* has two asynchronous reproductive cohorts that develop simultaneously, and each has a life-cycle duration of one year. Females from the autumn cohort were viviparous, whereas the spring cohort was oviparous. To the best of our knowledge, this paper is the first time facultative viviparity has been described in Plecoptera (Caires *et al.*, 2016). Eggs were found throughout the abdomen, thorax, and head of *C. lacustra* females. Egg-bearing females lived for up to three months in the laboratory following field collection but died shortly after depositing eggs or nymphs. The mass emergence of *C. khingana* near unfrozen sections of streams was recorded in the Khinganskii Reserve in the Middle Amur River Basin in mid-March. The number of males slightly exceeded the number of females (Teslenko, 2019). According to our research in the streams of the Komsomolskii Reserve (Low Amur River Basin), the mass hatching of the new generation first-instar nymphs occurred in mid-May. Gravid *C. khingana* females dominated samples, while mature males were not found, presumably due to the protandry. Most males appeared to have mated with females and died by the time collections occurred in May. By taking the time of *C. khingana* emerging from the streams of Khinganskii Reserve into account, with a certain degree of confidence, we can assume that the embryonic development of *C. khingana* eggs takes more than two months.

Our observations document the viviparity of fully developed nymphs, which is not the typical oviparity well known to stonefly species of the Capniidae, Leuctridae, and Gripopterygidae families. Egg development within adult females is known to occur in capniid stoneflies from Lake Baikal, wingless *Baikaloperla elongata* Zapekina-Dulkeit *et Zhiltzova*, 1973, and *Baikaloperla kozhovi* Zapekina-Dulkeit *et Zhiltzova*, 1973 (Zapekina-Dulkeit & Zhiltzova, 1973). The embryonic development of eggs may continue for approximately three months from

Fig. 3. Female of *Capnia khingana* with developing eggs, non hatched and hatched nymphal embryos inside of body, hatched nymphs exiting abdomen.

Fig. 4. Female of *Capnia khingana* hatched embryos: A – first instar nymph exiting of body; B – curled inside of body.

April to June. In June, there were eggs with fully formed embryos inside females; within these embryos, one could distinguish features such as a head with dark eyespots, thorax, abdomen, and legs. Additionally, the egg mass could reach the back of the female head. Nymphs of both *Baikaloperla* species hatch soon after oviposition (Zhiltzova, 2003). According to Zhiltzova (2003), the reproductive capacity of two East Palaearctic capniid species (*Capnia tshukotica* Zhiltzova et Levanidova, 1978 and *Capniella nodosa* Klapalek, 1920) is also characterized by oviparity. *Zwicknia bifrons* (Newman, 1838) (reported as *Capnia bifrons*) females are also oviparous with egg-laying reproduction, and eggs hatch soon after oviposition into the water (Hynes, 1941; Brinck, 1949; Khoo, 1964; Elliott, 1986; Lillehammer *et al.*, 1989; Bo *et al.*, 2013). Oviparity has been observed for the New Zealand gripopterygid stonefly species *Zelandoperla decorata* Tillyard, 1923. A single female at the water edge was ovipositing an eggs with developed eyespots (Smith & Storey, 2018).

Fig. 5. Female of *Capnia khingana* giving birth to first instar nymph.

C. khingana females emerge with immature ovarioles and are capable of surviving several weeks during which they have to feed to get the energy required for egg development. According to generalized data on the trophic ecology of stoneflies (Tierno de Figueroa & López-Rodríguez, 2019), the food composition varies among adults of Capniidae species. However, lichens, Cyanoprokaryota, fungi, and pollen are the main components of the Capniidae diet in the riparian zone. The specific feeding behavior of *C. khingana* adults has not been observed. Some gravid *C. khingana* females have been collected with food items in their guts (Fig. 1), which suggested that food availability is essential for egg development and maturation.

C. khingana females were found in springs and small spring-like foothill streams, where water temperatures did not exceed 6–7°C in May. Species in the family Capniidae commonly require cool temperatures for development. The present findings suggest a trend of viviparity as a reproductive life-history strategy associated with avoiding high temperatures. As a result, *C. khingana* adults emerge in the spring, and nymphal growth is delayed or undergoes diapause in summer until the water cools in late fall and winter; at that time, they feed and rapidly grow until maturity.

This study clearly documented the viviparity of *C. khingana*, but whether viviparity is obligate or facultative in this species remains to be determined.

Females of *C. khingana* were collected with female of *Capnia* sp., *Capniella nodosa* Klapálek, 1920, females and males of *Paraleuctra* sp., larvae of *Nemoura* sp. and exuvia of *Arcynopteryx* sp. mature nymphs.

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