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PENSOFT

First records of the genus *Elatosara* Malzacher, 2020 (Ephemeroptera, Caenidae) from the Lao People's Democratic Republic

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Abstract. The genus *Elatosara* was described by Malzacher, 2020 from the Mekong River in Thailand, with *E. phanomensis* Malzacher, 2020 as its type species. Here, we report *E. phanomensis* for the first time from central and southern Lao People's Democratic Republic (Lao PDR). A few morphological variations were observed in the Lao PDR population as compared to the paratypes of *E. phanomensis*. The discovery of *E. phanomensis* extends the distribution of the genus to the Lao PDR.

Keywords. Aquatic insects, Clypeocaenini, distribution, diversity, mayflies

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Introduction

The family Caenidae is mostly unexplored in the Indo-Burma Biodiversity Hotspot, which comprises Cambodia, Lao People's Democratic Republic (Lao PDR), Myanmar (formerly Burma), Thailand, and Vietnam, as well as parts of southern China and northeastern India. Nine genera and 20 species have been documented (Soldán 1991; Sites et al. 2001; Hoang and Bae 2006; Jung et al. 2008; Malzacher 2015, 2019; Malzacher and Sangpradub 2017, 2020; Martynov and Palatov 2017; Chhorn et al. 2020; Sripanya et al. 2023). Reports on caenids in this area are predominantly from Thailand: *Caenis gephyria* Malzacher, 2015, *C. guttata* Malzacher, 2015, *C. nigropunctatula* Malzacher, 2015, *C. ulmeriana* Malzacher, 2015, *C. acutostilata* Malzacher, 2020, *C. karenae* Malzacher, 2020, *C. longiforcipata* Malzacher, 2020, *C. ludovici* Malzacher, 2020, *C. nasuta* Malzacher, 2020, *C. obtusostilata* Malzacher, 2020, *C. picea* Kimmins, 1946, *Clypeocaenis oligosetosa* Soldán, 1983, *Elatosara phanomensis* Malzacher, 2020, *Kalimaenis procera* Malzacher, 2020, *Thainis kalimaenoides* Malzacher, 2020, *T. munensis* Malzacher, 2020, *Caenoculis bishopi* Soldán, 1986, *Mekongellina sangpradubae* Malzacher, 2019 and *Tigrocercus dangi* (Soldán, 1986) (Malzacher 2015; Malzacher and Sangpradub 2017, 2020; Martynov and Palatov 2017). *Clypeocaenis oligosetosa*, *Caenoculis nhahoensis* Soldán, 1986 and undescribed species of *Caenis* have been reported in Vietnam (Soldán 1991; Hoang and Bae 2006; Jung et al. 2008). Only the family level of Caenidae has been reported from Cambodia (Chhorn et al. 2020). Recently, research on aquatic insects has received more attention in Myanmar. Nevertheless, only a few species of mayfly have been discovered, but the family Caenidae have not been reported so far (Lin et al. 2018a, 2018b; Chen and Zheng 2022; Zheng and Chen 2023). Knowledge of the Caenidae in the Lao PDR is also limited, and only two genera, *Caenis* Stephens, 1835 and *Caenoculis* Soldán, 1986, have been documented (Jung et al. 2012; Mekong River Commission 2014, 2019a, 2019b). Recently, Sripanya et al. (2023) have reported five genera of Caenidae in wadable rivers and streams in the Lao PDR, including species of *Caenis*, *Caenoculis*, *Cercobrachys* Soldán, 1986, *Clypeocaenis* Soldán, 1978, and *Tigrocercus* Malzacher, 2006. In the present study, we report the

finding of the genus *Elatosara* Malzacher, 2020 in the Lao PDR for the first time. The genus *Elatosara* (subfamily Caeninae, tribe Clypeocaenini) was established by Malzacher (2020) in 2020, for only one species *E. phanomensis* Malzacher, 2020, which is the only *Elatosara* species known (Malzacher and Sangpradub 2020). The Clypeocaenini is monophyletic, defined by (1) the reduction of filaments on abdominal gill III to 8 filaments at most, and (2) head bulged in lateral view, clypeus more or less protruding (Malzacher 2013). Herein, we provide a detailed description of the larval and eggs of *E. phanomensis*.

Methods

The studied specimens were collected from Xe Nou River, Khammouane Province (Figs. 7, 8A) and Tha Hao stream, Savannakhet Province (Figs. 7, 8B) from 2019 to 2022. The Xe Nou River is located in a residential area flanked on the left bank by villages. The riparian zone consists of a forest area and a few agricultural plots. Substrates were dominated by bedrock and partly boulders, gravel, and sand. The current velocity is moderate to fast flowing in the middle of the river. Substrates at Tha Hao stream are composed of mainly boulders and bedrock, followed by mud, gravel, sand, a few patches of leaf litter, and dead wood, and the water velocity is moderate. The stream is bordered by a small canopy, farmland, and a small village. Both sampling sites appeared to be disturbed by human activities. *Elatosara* larvae were collected using a D-frame dip net (30 cm in width and with a mesh size of 450 µm). The specimens were preserved in 95% ethanol.

Examination was made under a stereomicroscope (Olympus SZX9, Japan), and photographs were taken using a stereomicroscope (Nikon SMZ25, Japan). Mouthparts, legs, and gills were dissected and studied under a scanning electron microscope. Subsequently, all parts were dehydrated through a stepwise immersion in absolute ethanol, then dried by critical-point drying (Quorum Critical Point Dyer, model K850, United Kingdom) and mounted on SEM stubs. Following that, the mounted materials were coated with a 20 nm Au layer (Cressington, Sputter Coater 108, United Kingdom) and photographed with a FEI scanning electron microscope (FEI Helios NanoLab G3 CX, FEI company, USA) and scanning electron microscope (LEO 1450vp, TZSupplies, USA).

Identification and description of the morphological characters of the new materials follows Malzacher and Sangpradub (2020). Finally, all photographs were adjusted for contrast and tonality with Adobe Photoshop 2020. The geographical distribution map was made with SimpleMappr (https://simplemappr.net). The examined specimens are deposited in the Department of Biology, Khon Kaen University in Khon Kaen, Thailand (KKU-AIC) and the Department of Biology, Faculty of Natural Sciences, National University of Laos, Lao PDR (NUoL-AIC).

Results

***Elatosara phanomensis* Malzacher, 2020**
Figures 1–6

Type locality. Thailand, Nakhon Phanom Province, Mekong River.

New records. LAO PDR – **Savannakhet Province** • Xaybury District, Tha Hao stream; 16°51′54.47″N, 104°54′3.77″E; 140 m alt.; 23.XI.2019; V. Vannachak leg. 4 ♀ and 2 ♂, NUoL-AIC – **Khammouane Province** • Xebangfay District, Xe Nou River. 17°04′05.56″N, 105°04′13.68″E; 154 m alt.; 6.I.2022; V. Vannachak leg. 13 ♀ and 5 ♂, KKU-AIC.

Identification. Larva length: body: 2.1–2.2 mm (male, n = 4), 2.5–2.8 mm (female, n = 5); cerci: 1–1.4 mm (male), 1.7–1.8 mm (female). General coloration variable among individuals: penultimate instar larva head, thorax, operculate gills, and abdominal terga brownish to yellow; legs and cerci whitish (Fig. 1A, B). Morphology: cuticle of head, pro- and mesonotum, femora, and abdominal segments with net-meshes; each mesh with a small denticle or granule; on surface of pro- and mesonotum with small setation. Operculate gill denticulate, jointed by net meshes. Head: outline of head in lateral view slightly bulged (Fig. 2A). Clypeus slightly protruding and straight, with numerous small bristles. Antenna with thin bristles, pedicel twice as long as scape, with 4 or 5 bristles (Fig. 2B). Labrum dorsal surface with moderate fine, frayed bristles (Fig. 2C). Mandibles with 3–5 relatively short, thin bristles on dorsolateral surface (Fig. 2D, E). Hypopharynx with minute, hair-like setae on apical margin; superlingua with long, simple setae on apical margin (Fig. 2F). Maxillary palp short; length to width ratio of segment 3 3.5–4.0. Segments 2 and 3 subequal in length at labial and maxillary palps; both segments together nearly equal in length at labial and maxillary palpus (Fig. 2G). Lateral margins of pronotum slightly diverging anteriorly or straight-parallel, with scattered short bristles; meso and metathorax approximately oval (Fig. 2B). Coxal processes inconspicuous, sickle-shaped, with 1–3 short, thin bristles. Forefemur with an irregular transverse row of 10–12 long or moderate thin bristles, 1/4 of femur length from apical end (Fig. 3A). Femora of mid and hind legs with clearly long, thin bristles on outer margin, length of bristles ca. 2× longer than bristles on inner margin (Fig. 3B, C). Foreclaw slender and slightly bowed, mid and hind claws basally broader and apically more or less bowed, with 4–6 small denticles (Fig. 4A–C). Abdomen: posterolateral processes of abdominal segments short, slightly longer in segments IV–VI (Fig. 4D). Tergum II with triangular, pointed posteromedian process. Hind margins of terga VII and VIII with ca. 12–14 and 5–9 long, thin bristles, respectively; terga IX and X with small denticles (Fig. 4D). Hind part of sternum IX has obtusely angled triangle with a broad, rounded tip, marginally provided with long to moderate bristles, apical one shorter and bent

Figure 1. *Elatosara phanonensis* Malzacher, 2020, habitus. **A.** Male larva; **B.** Female larva. Scale bars: 1 mm.

medially, and at top a couple of short, bifurcated bristle. Operculate gill square, with rounded posterolateral corner; Y-shaped ridges well developed, broad and strongly bulged, with conspicuously granulated, inner ridge basally with 4 strong bristles of moderate length (Fig. 5A, B); lateral margin with a row of long, thin bristles at basal end and decreasing in length towards posterolateral corner, on posteromedian corner with a row of 6–8 long, thin bristles; ventral side of operculate gill with small microtrichia forming irregular row situated away from lateral and hind margins (Fig. 5C), shape of microtrichia on ventral side of the gill square or more or less rounded (Fig. 5D). Gill I about 1/3 as long as gill II. Gill III with about 6–9 filaments with 3 branches and about 14–22 filaments with 1 or 2 branches (Fig. 4D). Eggs were taken from a last instar female larva: egg elongate, length ca. 2× as long as width, chorion rough, with 2 cap-shaped epithermata (Fig. 6A), with a large number of single threads, each with a terminal tassel-shaped knob, running from polar region and forming cap-shaped epithemata (Fig. 6B) and a micropyle of moderate length with a circular sperm guide (Fig. 6C). The shape and appearance of the egg from the Lao PDR are similar to those of the paratype specimen from Thailand (Fig. 6D, E).

Distribution (Fig. 7). Thailand: Nakhon Phanom Province, Mekong River (Malzacher and Sangpradub 2020), Lao PDR: Khammouane Province, Xebangfay District, Xe Nou River and Savannakhet Province, Xaybury District, Tha Hao stream (first records herein).

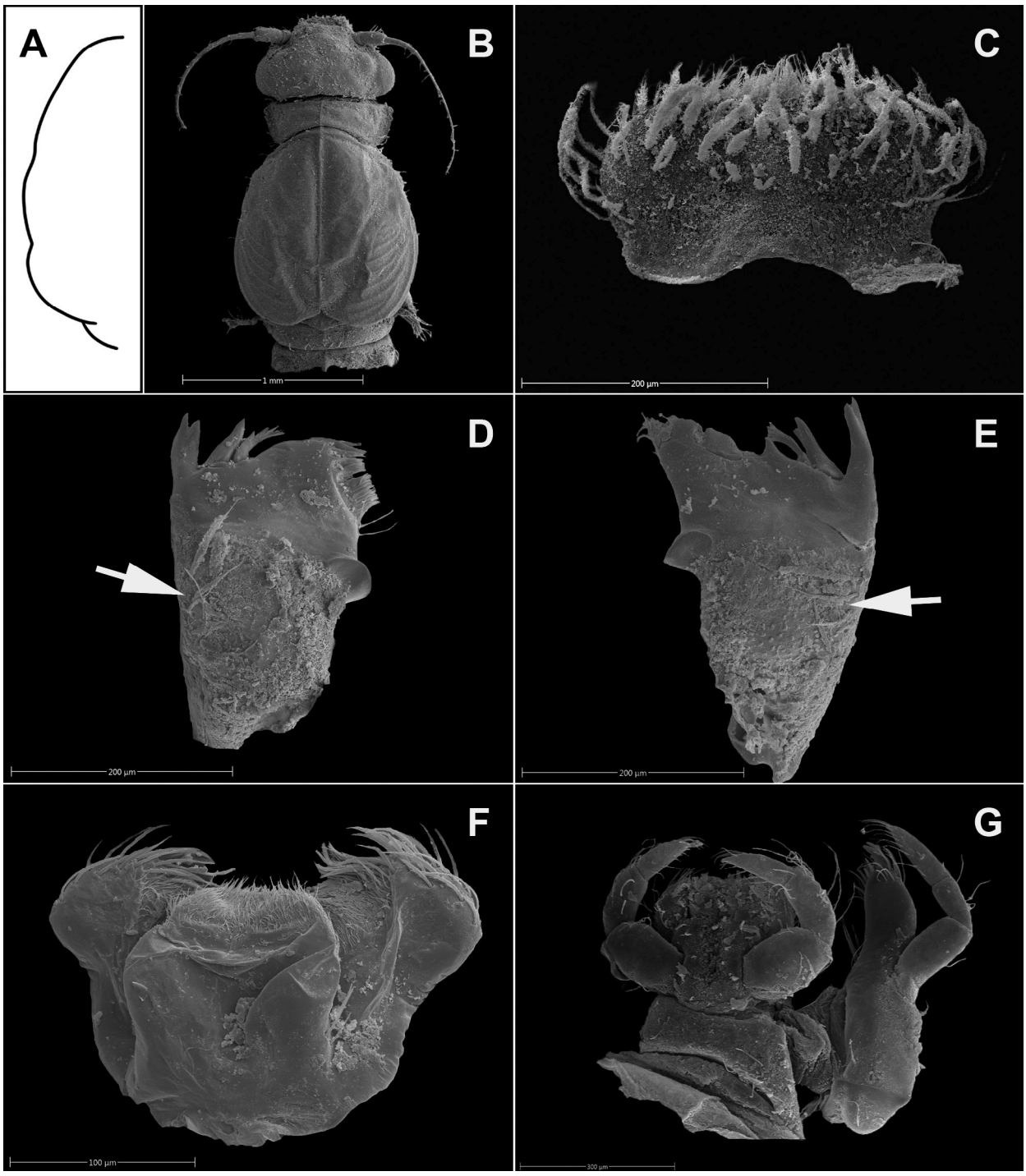

Figure 2. *Elatosara phanomensis* Malzacher, 2020, larval morphology. **A.** Outline of head in lateral view. **B.** SEM view of head and thorax, dorsal view. **C.** SEM view of labrum, dorsal view. **D.** SEM view of left mandible (arrow point the setation). **E.** SEM view of right mandible (arrow point the setation). **F.** SEM view of hypopharynx. **G.** SEM view of labium (left) and maxilla (right). Scale bars: B = 1 mm; C–E = 200 µm; F = 100 µm; G = 300 µm.

Discussion

According to original description by Malzacher and Sangpradub (2020), our Lao PDR specimens of *Elatosara* are classified to *E. phanomensis*. The Lao specimens largely agree with the type specimens from Thailand in the partly diagnostic characters, which are the outline of the head, mouth parts, length of bristles on forefemora, gills, and abdomen. However, some characters differ: i) the scale-shaped microtrichia are very small and slightly broader with a single irregular row of band arrangement, whereas the individuals from Thailand have 2 or 3 irregular rows of band arrangement (compare Fig. 5D here with Malzacher and Sangpradub 2020: fig. 19); ii) the inner marginal bristles on the mid femur are present on both the distal and proximal halves, whereas in the Thai specimens bristles are present on the distal half (Malzacher and Sangpradub 2020: fig. 17e) or present on both the distal and proximal halves; iii) claws with 4–6 small bristles,

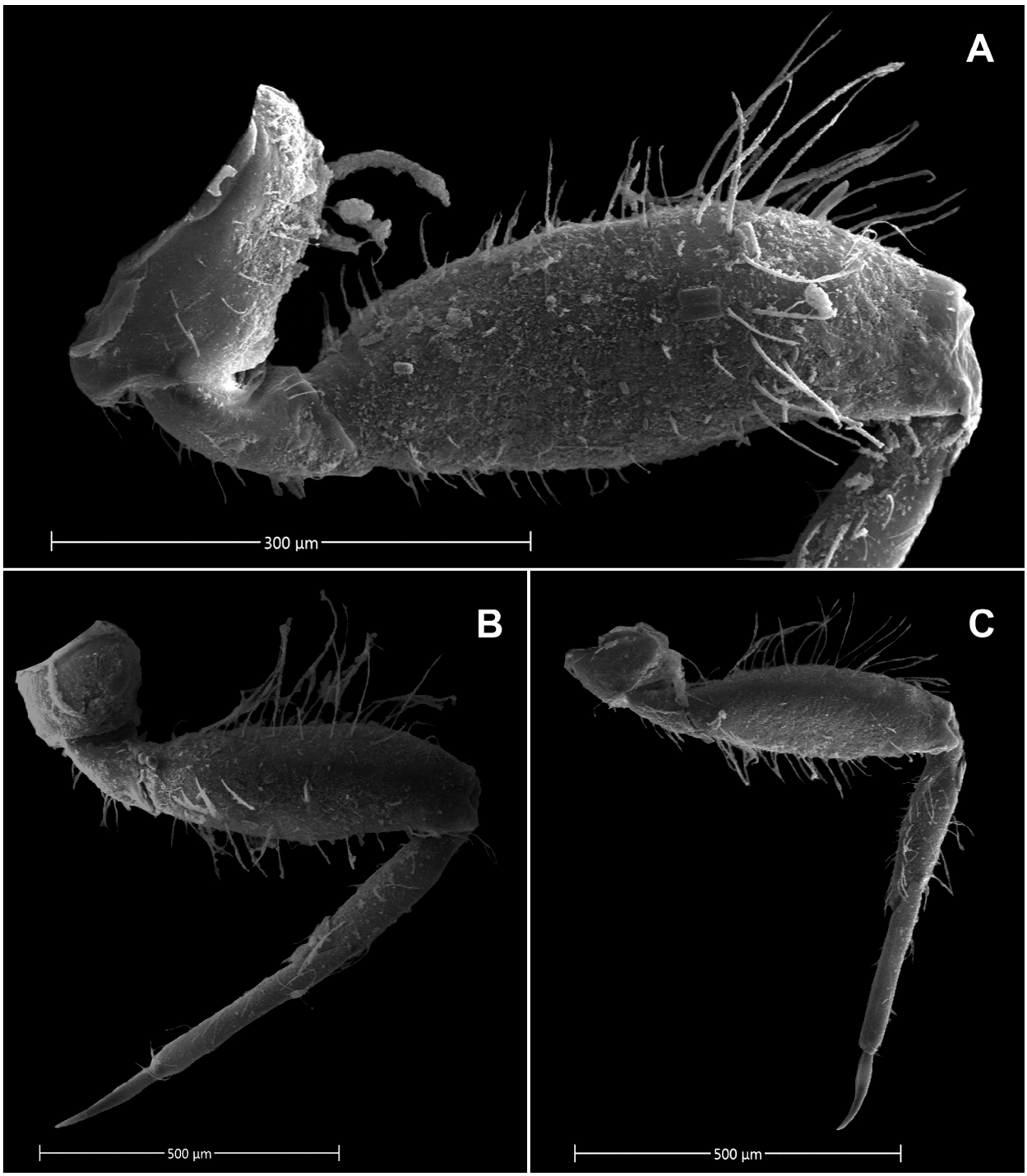

Figure 3. *Elatosara phanomensis* Malzacher, 2020, larval morphology. **A.** SEM view of forefemur. **B.** SEM view of mid leg. **C.** SEM view of hind leg. Scale bars: A = 300 µm; B–C = 500 µm.

whereas in Thai specimens claws have 5–7 small denticles. These differences may be intraspecific variation. The type locality is on the border between Thailand and Lao PDR at an altitude of 133 m, and the site is surrounded by small villages and the riparian zone consists of a few agricultural plots, floating houses, a shoreline, some trees on the bank, and small-scale fish farms. The sampling site seems to be moderately disturbed due to rubbish disposal, agricultural runoff, fish farming, and bank erosion (Mekong River Commission 2019a). In the Xe Nou River and Tha Hao stream in southern Lao PDR (Fig. 7), the altitude ranges from 140 to 154 m, and both sites are in residential areas and have similar environments and microhabitats. At Xe Nou River, the left bank is surrounded by villages, another riverbank riparian zone consists of a forest area and a few agriculture plots; substrates were dominantly bedrocks and partly boulders, gravel and sand, moderate to high flow velocity at the middle of the river (Fig. 8A). However, the Tha Hao stream has different substrates, made up of boulders and bedrock, as well as by mud, gravel, sand, and a few patches of leaf litter and dead wood. Water velocity is moderate (Fig. 8B), and the stream banks are covered by a small canopy, farmland, and

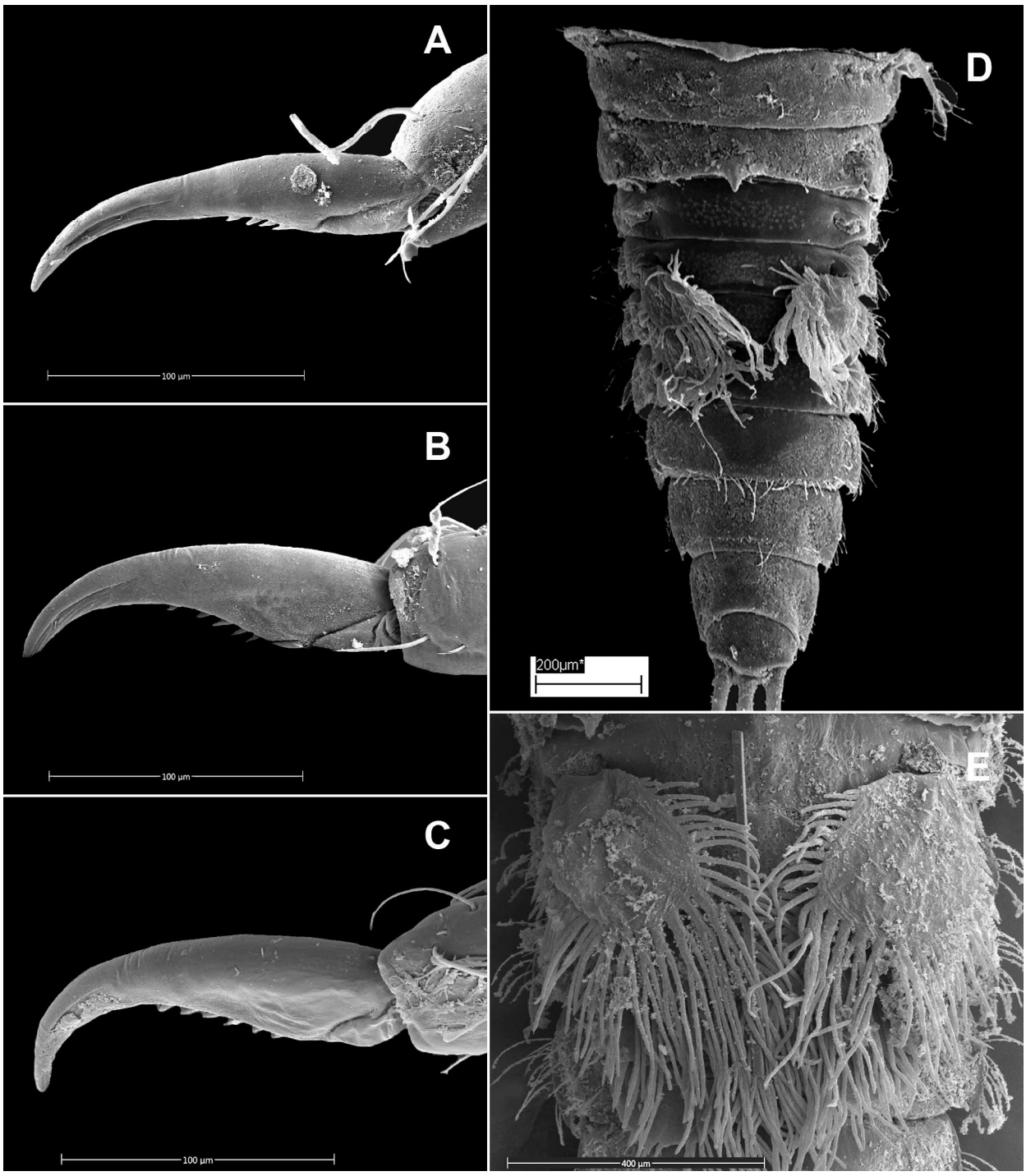

Figure 4. *Elatosara phanomensis* Malzacher, 2020, larval morphology. **A.** SEM view of foreclaw. **B.** SEM view of mid claw. **C.** SEM view of hind claw. **D.** SEM view of abdomen. **E.** SEM view of gill III, dorsal view. Scale bars: A–C = 100 µm; D = 200 µm; E = 400 µm.

small villages. Therefore, these sampling sites appear to be disturbed by human activities. Thus, the ecological preferences of *E. phanomensis* in similar in both Thailand and Lao PDR.

Our study increases the known genera of caenid mayflies in Lao PDR to six and extends the distribution of *E. phanomensis* to the Lao PDR. We expect that there are many more unreported caenid species waiting to be discovered in other parts of the Lao PDR. With the paucity of caenid data, more work needs to be carried out in the country.

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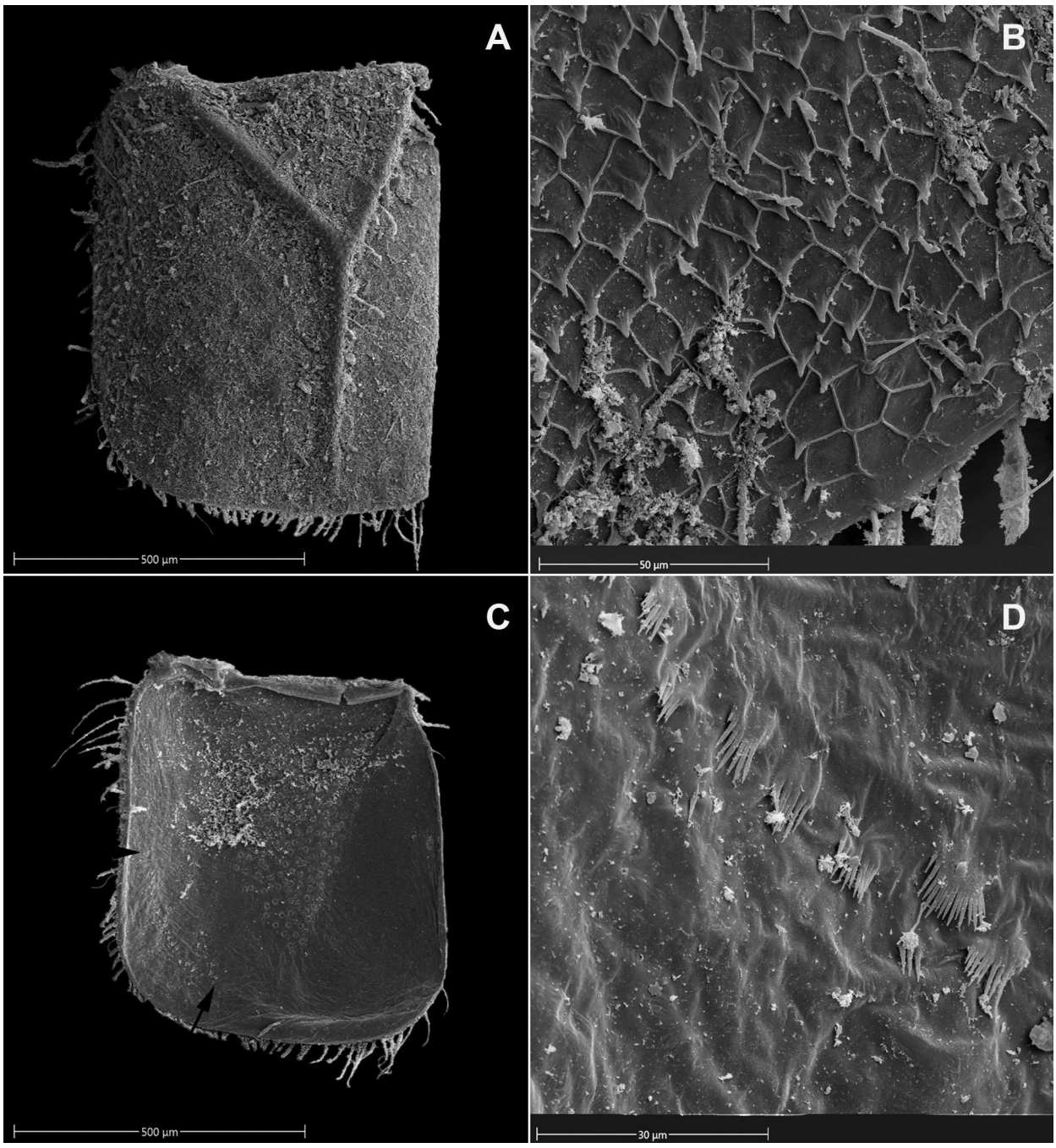

Figure 5. *Elatosara phanomensis* Malzacher, 2020, larval morphology. **A.** SEM view of operculate gill, dorsal view. **B.** SEM view of operculate gill, dorsal view higher magnification. **C.** SEM view of operculate gill, ventral view (arrows show end point of microtrichia band). **D.** SEM view of microtrichia from operculate gill. Scale bars: A, C = 500 µm; B = 50 µm; D = 30 µm.

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Author Contributions

Conceptualization: NS, VV. Investigation: VV, NS. Methodology: VV, NS. Resources: VV, NS, PM. Visualization: VV, NS. Writing – original draft: VV, NS, PM. Writing – review and editing: NS, VV, PM.

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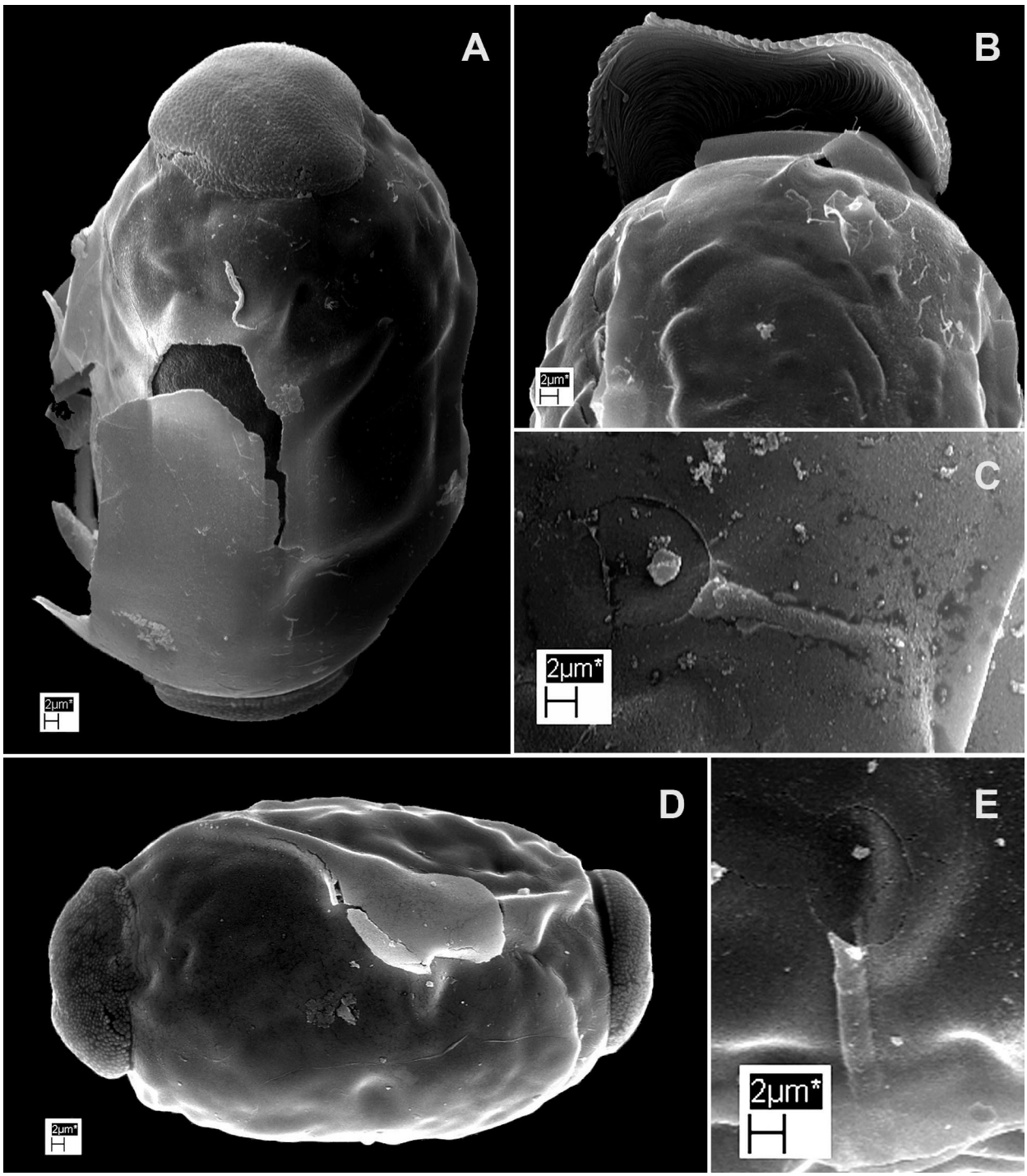

Figure 6. *Elatosara phanomensis* Malzacher, 2020, eggs morphology. **A.** SEM view of egg. **B.** SEM view of epithema.**C.** SEM view of micropyle. **D.** SEM view of egg (from Thai specimen, paratype). **E.** SEM view of micropyle (Thai specimen, paratype). Scale bars: 2 µm.

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Figure 7. Distribution map of *Elatosara phanomensis* Malzacher, 2020. The blue dot shows the type locality in the Mekong River, Thailand and the red dots show the extended distribution in Lao PDR.

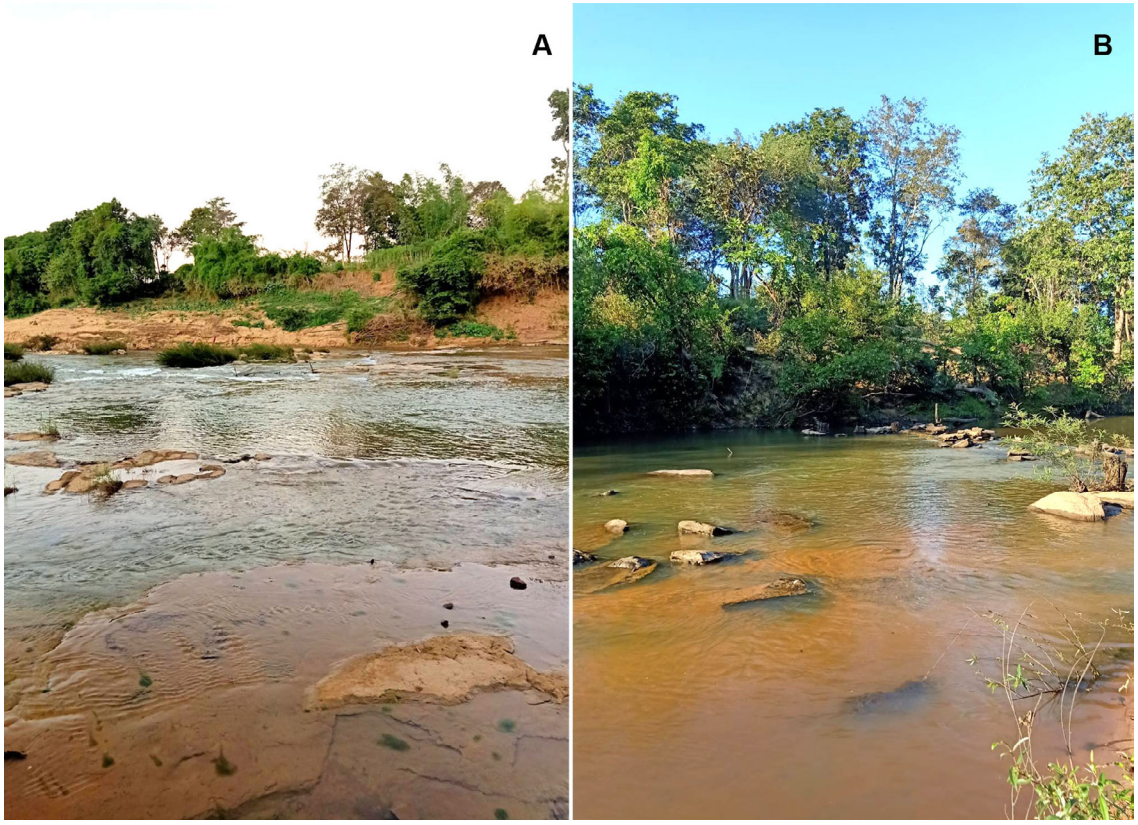

Figure 8. The sampling sites and habitats of *Elatosara phanomensis* Malzacher, 2020. **A.** Xe Nou River, Xebangfay District, Khammouane Province. **B.** Tha Hao Stream, Xaybury District, Savannakhet Province.

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