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Bioerosion of siliceous rocks driven by rock-boring freshwater insects

Ivan N. Bolotov^{1,2✉}, Alexander V. Kondakov^{1,2}, Grigory S. Potapov¹, Dmitry M. Palatov³, Nyein Chan⁴, Zau Lunn^{4,5}, Galina V. Bovykina^{1,2}, Yulia E. Chapurina¹, Yulia S. Kolosova¹, Elizaveta A. Spitsyna¹, Vitaly M. Spitsyn¹, Artyom A. Lyubas¹, Mikhail Y. Gofarov^{1,2}, Ilya V. Vikhrev^{1,2}, Vasily O. Yapaskurt⁶, Andrey Y. Bychkov^{6,7} and Oleg S. Pokrovsky^{8,9}

Macro-bioerosion of mineral substrates in fresh water is a little-known geological process. Two examples of rock-boring bivalve molluscs were recently described from freshwater environments. To the best of our knowledge, rock-boring freshwater insects were previously unknown. Here, we report on the discovery of insect larvae boring into submerged siltstone (aleurolite) rocks in tropical Asia. These larvae belong to a new mayfly species and perform their borings using enlarged mandibles. Their traces represent a horizontally oriented, tunnel-like macroboring with two apertures. To date, only three rock-boring animals are known to occur in fresh water globally: a mayfly, a piddock, and a shipworm. All the three species originated within primarily wood-boring clades, indicating a simplified evolutionary shift from wood to hardground substrate based on a set of morphological and anatomical preadaptations evolved in wood borers (e.g., massive larval mandibular tusks in mayflies and specific body, shell, and muscle structure in bivalves).

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INTRODUCTION

A wide array of rock-boring animals and their bioerosion traces was described from marine environments^{1–3}. These animals are common in recent and fossil sea beds⁴ and belong to multiple invertebrate taxa such as molluscs^{5,6}, crustaceans⁷, polychaetes⁸, sipunculids⁹, sea urchins^{10–12}, sponges^{13,14}, and bryozoans^{2,15}. Seawater rock borers play a significant role as ecosystem engineers that may greatly increase the complexity of hardground substrates and biodiversity of associated communities¹⁶. Furthermore, a number of marine geological processes such as sedimentation, erosion of rock surfaces, and evolution of coastal profiles and coral reefs are greatly influenced by rock-boring animals globally^{17–22}.

Conversely, rock borers are extremely rare in freshwater habitats and the relevant organisms are usually derived from marine bioeroders²³. Until recently, macro-bioerosion of rock substrates in modern freshwater environments was unknown^{23,24}. There were a few reports describing bioerosion structures in freshwater mollusc shells that are largely associated with caobangiid polychaetes (Annelida: Sabellidae)^{25,26}. These minute worms are an exclusively freshwater group that contains seven species boring into shells of gastropods and bivalves in Southeast Asia, India, and Sri Lanka^{26,27}. Microborings in subfossil and recent freshwater bivalve shells were also discovered in North America but the producers of these boreholes remain unknown^{28,29}. Other works describe microborings in freshwater mollusc shells produced by endolithic cyanobacteria in North America³⁰ and Argentina³¹.

Conversely, available paleo-ichnological data reveal that fossil macro-bioerosion structures are widespread in freshwater settings globally^{32–34}, although these settings have received little attention

compared with their marine counterparts³⁵. A variety of trace fossils is known to occur in freshwater deposits, including those associated with putative invertebrate macroborings^{3,36,37}. Most of these ichnotaxa were described in calcareous hardgrounds³.

The first modern silicate rock-boring freshwater organism was discovered in the middle reaches of the Kaladan River in Myanmar in 2018²⁴. It is a close relative of marine piddocks and belongs to the bivalve species *Lignopholas fluminalis* (Blanford, 1867) (Pholadidae). This species was known to occur exclusively in estuaries³⁸ but the Kaladan's population is adapted to live in fresh water²⁴. The clavate borings of this species in siltstone rocks are covered by a microbial biofilm, the members of which could promote bivalve bioerosion through rock weathering by dissolving Mn-rich chlorites³⁹. A year later, the second freshwater rock-boring animal was described from the Philippines⁴⁰. This unusual bivalve species, *Lithoredo abatanica* Shipway, Distel & Rosenberg, 2019, a member of the Teredinidae (commonly known as shipworms), drills into and ingests the limestone substrates in the lower reaches of a small river on the Bohol Island⁴¹.

A small body of available literature describes burrows made by recent freshwater insects in various firmgrounds such as claystones, sandstones, shales, and marls^{42–44}. These burrows were produced by larval stages of mayflies (Ephemeroptera), nonbiting midges (Diptera: Chironomidae), and caddisflies (Trichoptera). The present study extends our knowledge on bioerosion in freshwater settings via studying a previously unknown case of a siltstone (aleurolite) boring produced by mayfly nymphs from tropical Asia (Myanmar).

This contribution (1) reports on the discovery of a rock-boring freshwater insect; (2) describes this insect as a species of mayfly

¹N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk 163000, Russia. ²Northern Arctic Federal University, Arkhangelsk 163002, Russia. ³A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow 119071, Russia. ⁴Fauna & Flora International – Myanmar Programme, 34 D/9 San Yae Twin Street, Kaba Aye Pagoda Road, Bahan Township 11201 Yangon, Myanmar. ⁵Biology Department, University of New Brunswick, PO Box 5050100 Tucker Park Road, Saint John, NB E2L 4L5, Canada. ⁶Faculty of Geology, Lomonosov Moscow State University, Moscow 119991, Russia. ⁷V. I. Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Moscow 119991, Russia. ⁸Geosciences and Environment Toulouse, UMR 5563 CNRS, Toulouse 31400, France. ⁹BIO-GEO-CLIM Laboratory, Tomsk State University, Tomsk 634050, Russia. ✉email: inepras@yandex.ru

Fig. 1 Study area and sampling localities. **a** Map of Myanmar with location of the study area. **b** Map of the Bago River basin (light red filling). The red stars indicate freshwater macrobioerosion sites. The maps were created using ESRI ArcGIS 10 software (<https://www.esri.com/arcgis>). The topographic base of the maps was created with Natural Earth Free Vector and Raster Map Data (<https://www.naturalearthdata.com>), Global Self-consistent Hierarchical High-resolution Geography, GSHHG v2.3.7 (<https://www.soest.hawaii.edu/wessel/gshhg>), HydroSHEDS (<https://www.hydrosheds.org>)⁹⁷, The General Bathymetric Chart of the Oceans, GEBCO (<https://www.gebco.net>), and Vector Map (VMap) Level 0 (<http://gis-lab.info/qa/vmap0-eng.html>). (Maps: Mikhail Y. Gofarov).

new to science using morphological, ethological, and DNA-based evidence; (3) provides a description of recent and sub-recent bioerosion traces produced by mayfly nymphs in siliceous substrate; (4) briefly discusses ichnotaxonomic issues regarding macroborings produced by freshwater invertebrates; and (5) proposes a hypothesis on the evolutionary origin of rock-boring freshwater invertebrates.

RESULTS

Discovery of rock-boring freshwater insects

Siliceous rock outcrops with bioerosion traces were recorded at two sites of the Bago (historically Pegu) River, Myanmar (Figs. 1a, b, 2a–f, 3a–e, 4a–c, and 5a–e). The first site is located at the middle reaches of the river (17.6797°N, 96.2318°E, altitude 40 m), while the second site is situated at the upstream section (18.0791°N, 96.0449°E, altitude 195 m) (Fig. 1). Both sites are freshwater and are well above the upper limit of tidal influence.

At the first site, the river crosses a continuous outcrop of Miocene siltstone (aleurolite) rocks of approximately 300 m long (Fig. 2a, b). The siltstones are exposed at the right shore (Fig. 2b) and throughout the river bottom (Fig. 2a, b). Multiple insect borings densely penetrate the upper layer (up to 20–30 mm deep) of the submerged rocks. The typical boring represents an elongate tunnel-like structure, which is oriented horizontally, and shares two circular end openings (apertures) at the outer surface (Fig. 3a–c). Numerous living mayfly nymphs were recorded inside the borings (Fig. 3d, e), whereas a number of borings were abandoned and, in some cases, filled with clay. A substance with a silky texture covers the inner surface of borings inhabited by nymphs (Fig. 3e)

and, rarely, of abandoned borings (Fig. 5b). There are also elongate grooves of various depth at the rock surface, representing eroded borings having a more or less concave bottom, with the oldest ones being shallower (Fig. 3b, c). Siltstone rocks with sub-recent traces of the same morphology were also recorded at the surface of the youngest river terrace, above the level of the present floodplain (Supplementary Fig. 1).

At the upstream section, the river valley shares a characteristic V-shaped profile and is deeply incised into Miocene clay sediments⁴⁵ with numerous outcrops of siltstone rocks (Fig. 2c). The upper parts of siltstone rocks at this site were exposed, because the water level in the upstream was dropped drastically due to the lack of rainfalls at the end of the dry season (24 March 2020) (Fig. 2d). The surface of rocks was completely covered by a dense network of grooves and borings, which were morphologically similar to those recorded in the middle reaches of the river (Fig. 2e, f). At this site, all the borings were recently abandoned due to a drop in the water level, although a few fragments of dead insect larvae were collected inside some tunnels. The rock surface with eroded borings often bears a thin encrustation of an unidentified freshwater sponge (order Spongillida) (Fig. 2e). In many cases, the bioerosion surface and borings were filled with clay originated from the deposition of river suspended matter (Fig. 2f), while some smaller bioerosion beds were almost completely overlapped by alluvial clay sediments. These features indicate rather a sub-recent than recent origin, although the exact age of these traces was not estimated.

Based on morphological and genetic analyses, the rock-boring nymphs from the middle reaches of the river were found to be representatives of a mayfly species from the genus *Languidipes*

Fig. 2 Examples of macrobioerosion in the Bago River, Myanmar. **a** Right shore with continuous siltstone outcrop at the middle reaches of the river. The red arrows show the location of submerged rocks with bioerosion traces. **b** Submerged part of the siltstone rock outcrop (indicated by the red arrows) at the middle reaches of the river. **c** Emerged siltstone rocks with bioerosion traces (indicated by the red arrows) at the upper reaches of the river. **d** Close-up view of siltstone outcrop with multiple bioerosion traces at the upstream section of the river. **e** Siltstone fragment densely covered by sub-recent bioerosion traces from the upper reaches of the river (plan view). The yellow arrows show crusts of a freshwater sponge species. **f** Siltstone surface with a dense network of sub-recent bioerosion traces from the upper reaches of the river (plan view). The blue arrows show the macroborings filled with clay. The green arrow shows a pea clam in a bioerosion groove (*Bivalvia*: *Sphaeriidae*). Scale bars = 10 mm (**e**, **f**). (Photos: Mikhail Y. Gofarov [**a–b**] and Ilya V. Vikhrev [**c–f**]).

Hubbard, 1984 (Ephemeroptera: Polymitarcyidae). This species is new to science and is described here. Though we were unable to identify the larvae from the upstream section with certainty because of the very poor condition of available body fragments, these borings were likely produced by the same species as those in the middle reaches of the Bago River. The sub-recent bioerosion beds in the river valley should also be attributed to the dwelling activity of this mayfly species because these traces are morphologically similar (if not identical) to the borings made by modern mayfly nymphs.

Description of the rock substrate

The rock substrate was classified as a siltstone (aleurolite) (primary grain size of 2–62 μm). Samples from the middle reaches and the upstream section of the Bago River share minor differences (Figs. 2, 3). In particular, the X-ray diffraction (XRD) analyses indicate that the rock from the middle reaches of the river consists of quartz (40 wt.%), clay minerals (17 wt.%), feldspars (14 wt.%), mica (14 wt.%), chlorite (11 wt.%), and pyrite (4 wt.%). Backscattering Electron (BSE) images show a thin-grain rock with organic matter inclusions (black) and pyrite concretions (Supplementary Fig. 2a). The siltstone contains large (ca. 100 μm) grains of mica and chlorite. The microindentation hardness (Vickers test) ranged from 0.42 to

0.50 GPa. Based on the XRD analyses, the siltstone rock from the river's upstream consisted of quartz (59 wt.%), feldspars (19 wt.%), chlorite (10 wt.%), mica (8 wt.%), small amounts of clay minerals (3 wt.%), and calcite (1 wt.%). The BSE images show that this rock is uniformly grained, and also contains organic matter inclusions (Supplementary Fig. 2b). The upstream siltstone contains carbonate-clay cement, which makes it more resistant to swelling. A higher content of quartz and feldspars gives elevated microhardness values (Vickers test) within a range of 0.60–0.75 GPa. The siltstone (aleurolite) studied in this work is not subjected to wetting and preserves its shape under pressure. The siltstone is cemented and can be considered as hard substrate given its hardness, cementation, and capacity to preserve its shape.

Description of the recent and sub-recent mayfly borings

The mayfly dwelling traces are described here on the basis of siltstone specimens collected from submerged rock outcrop (Figs. 4a–c and 5a–c). The upper surface of rock specimens contains apertures of the untreated borings produced by the new mayfly species, as well as a network of elongated grooves associated with its eroded borings. A typical trace has a total length of 20.1 mm and a maximum width (diameter) of 4.4 mm (Fig. 4c). It represents

Fig. 3 Siltstone rock substrate with borings and living nymphs of the rock-boring mayfly species *Languidipes lithophagus* sp. nov. from the middle reaches of the Bago River, Myanmar. **a** Rock fragment with borings (lateral view). **b** Rock fragment showing apertures (circular openings) and partly eroded borings (plan view). **c** Rock surface with apertures and partly eroded abandoned borings. **d** Living nymphs in their borings. **e** Living nymph in its boring. The white arrow shows a fragment of a silky substance covering the inner side of the boring. Scale bars = 10 mm (**a**, **c**), 20 mm (**b**), and 5 mm (**d**, **e**). (Photos: Ilya V. Vikhrev).

a horizontally oriented, banana-shaped, tunnel-like macroboring, circular in cross-section; its bottom outline is curved, while the upper outline is straight (Figs. 4c, 5c–e). It is connected with the outer surface of the rock by two circular apertures (end openings) (Fig. 5a). In some cases, one of the two apertures is not fully developed, representing a narrow, ovate or irregular hole (Fig. 5a). The inner side of the boring is rather smooth, without showing clear scratches in the examined traces (Fig. 5b). The macroboring is separated from the outer surface by a very thin and fragile rock layer. This layer could usually be traced by a few minute fractures at the rock surface (Fig. 5a). In sub-recent borings, the upper layer is often lost due to ongoing erosion processes. In such case, the partly eroded boring represents a narrow, elongated groove with

parallel (sometimes slightly convex or concave) sides and concave bottom in plan view (Fig. 5b), and looks like a member of the calcareous ichnogenus *Petroxestes* Wilson & Palmer, 1988 (Rogerellidae)^{3,46}.

Phylogenetic affinities of the rock-boring mayfly species

The cytochrome *c oxidase subunit I* (*COI*) gene sequences were obtained from five nymphs of the rock-boring mayfly (Supplementary Table 1). These sequences returned two unique and rather divergent *COI* haplotypes with a mean uncorrected *p*-distance of 2.48% (Supplementary Table 2). In some cases, such a genetic distance between *COI* haplotypes could indicate interspecific differences but our PTP species delimitation analysis

Fig. 4 Traces of the rock-boring mayfly species *Languidipes lithophagus* sp. nov. in siltstone rock specimens from the middle section of the Bago River, Myanmar. **a** Siltstone rock specimen RMBH BA-0076 with mayfly traces (plan view). The red line reveals the longitudinal section through the boring, illustrated in Fig. 4c. The white arrows show apertures of this boring. **b** Siltstone rock specimen RMBH BA-0077 with mayfly traces (plan view). **c** Longitudinal section through a boring (lateral view) in the specimen RMBH BA-0076. The yellow arrows show the apertures. The red arrows show the concave bottom outline. Scale bars = 10 mm. (Photos: Artyom A. Lyubas).

suggested that the two haplotypes most likely belong to a single rock-boring species. The average genetic distances between the new species and two already known *Languidipes* taxa range from 7.83 to 8.68% that is well above an intraspecific level. In its turn, the mean *COI* *p*-distance between *Languidipes corporaali* (Lestage, 1922) and *L. taprobanes* (Hubbard, 1984) is a bit lower, 5.97%. Our phylogenetic analyses revealed that the genus *Languidipes* represents a well-supported clade (Supplementary Fig. 3). The rock-boring species was recovered as sister to the wood-boring *L. corporaali* and *L. taprobanes*.

Taxonomic description of the rock-boring mayfly species

Order Ephemeroptera Hyatt & Arms, 1891

Family Polymitarcyidae Banks, 1900

Subfamily Asthenopodinae Edmunds & Traver, 1954

Genus *Languidipes* Hubbard, 1984

Type species: *Asthenopus corporaali* Lestage, 1922 (by original designation).

Taxonomic richness: This genus contains three valid species as follows: *L. corporaali* (Lestage, 1922) [wood-boring nymphs; Indonesia: Java, Sumatra, and Simeulue, Malaysia, and Thailand]^{47,48}, *L. taprobanes* (Hubbard, 1984) [wood-boring nymphs; Sri Lanka and India]^{47,49}, and *L. lithophagus* sp. nov. [rock-boring nymphs; Myanmar: Bago River]. The latter species is described here. An undescribed species was recorded from Assam, India but it is known from a single century-old female specimen^{47,50,51}. The Barcode of Life Data System (BOLD; <http://www.boldsystems.org>)⁵² contains four *COI* sequences of one more undescribed species from the Bago River, Myanmar.

Comments: This taxon was described as a subgenus of *Povilla* Navás, 1912⁴⁷. Later, it was elevated to the full generic rank based on a set of morphological evidences⁴⁸. Our results support this

Fig. 5 Morphological features and neoichnological model of the rock-boring mayfly species *Languidipes lithophagus* sp. nov. traces from the middle section of the Bago River, Myanmar. **a** Close-up view of the apertures (plan view). The white arrows show minute fractures tracing the upper layer of the borings. The yellow arrows show larger fractures corresponding to the initial stage of rock erosion leading to the origin of grooves (i.e., borings, the upper layer of whose was lost). **b** Plan view of a groove with concave bottom originated via partial erosion of the boring. The groove bears fragments of a silky substance produced by the tracemaker (shown by white arrows). **c** Transverse cross-section of the borings showing their circular tunnel-like shape. Neoichnological model of the boring: lateral view of the longitudinal section (**d**) and plan view of the two apertures at the rock surface (**e**). Orange filling indicates the boring. Scale bars = 10 mm (**a**) and 5 mm (**b–e**). (Photos: Artyom A. Lyubas; Graphic Art: Ivan N. Bolotov).

decision, as the African wood-boring mayfly *Povilla adusta* Navás, 1912 (the type species of the genus *Povilla*) is found to be distant phylogenetically from *Languidipes* taxa, including *L. corporaali* (the type species of the latter genus).

Languidipes lithophagus Bolotov, Kondakov, Potapov, Palatov & Spitsyn sp. nov.

Figure 6a–d, Supplementary Figs. 4a–h, 5a–f, and 6a–j

LSID: <http://zoobank.org/urn:lsid:zoobank.org:act:25ABEA26-A694-482D-A704-2BE1F09A5866>

Type material (ethanol-preserved mature nymphs): Holotype RMBH N54_8 [preserved in 96% ethanol] (Fig. 6a, b); paratypes: 16 specimens [preserved in 96% ethanol: voucher numbers RMBH 54; RMBH N54_1; N54_2; N54_3; N54_4; N54_5; N54_6; N54_7; and N54_9; and mounted on a permanent slide: voucher number ZMMU Eph-0001]. The *COI* reference sequence accession numbers for the holotype and paratypes are given in Supplementary Table 1. The type series is deposited in the Russian Museum of Biodiversity Hotspots [RMBH], N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia (the holotype and 15 paratypes) and in the collection of Zoological Museum of Moscow State University [ZMMU], Moscow, Russia (one paratype).

Fig. 6 Nymph of the rock-boring mayfly species *Languidipes lithophagus* sp. nov. from Bago River, Myanmar. **a** Holotype RMBH N54_8 (dorsal view); **b** holotype (lateral view); **c** left and **d** right mandible (dorsal view). Scale bars = 5 mm (**a**, **b**) and 1 mm (**c**, **d**). (Photos: Elizaveta A. Spitsyna).

Type locality: MYANMAR: middle reaches of the Bago River, 17.6797°N, 96.2318°E, altitude 40 m, 22 March 2020, Bolotov, Gofarov, Kondakov, and Vikhrev leg.

Differential diagnosis (mature nymph): The new species is morphologically similar to the congeneric *L. taprobanes* and *L. corporaali* but could be distinguished from these taxa by the following combination of characters: anteromedian projection of the head semicircular, subapical mandibular tubercle of both tusks small. *L. taprobanes* also shares a semicircular anteromedian projection but differs from the new species by having a left mandibular tusk with four teeth. In its turn, *L. corporaali* could be distinguished from the new species by having a triangular anteromedian projection of the head and much larger, rounded subapical mandibular tubercle. The apical teeth of the rock-boring species are much more massive and rounded than those of the two wood-boring taxa.

DNA-based diagnosis: The new species differs from two congeners by 31 fixed nucleotide substitutions in the *COI* gene barcode fragment (658 bp) as follows: 16 T, 38 A, 61 C, 67 T, 85 T, 88 A, 95 G, 133 T, 205 T, 217 G, 220 C, 238 T, 274 T, 284 T, 298 A, 337 C, 346 A, 355 T, 364 C, 373 T, 379 A, 391 A, 475 T, 478 A, 536 T, 538 G, 556 G, 562 T, 586 T, 628 T, 634 C.

Description: *Mature nymph*. Length (mm): body 14.3, cerci 3.2, paracercus 4.4 (holotype); body 11.3–17.9, cerci 2.0–3.2, paracercus 3.3–4.7 (paratypes; $N = 15$). Head is semicircular in dorsal view, with narrow setose areas, yellowish with two dark brown, irregular stripes laterally. Frontal process of the head is small and semicircular (Supplementary Fig. 6a). Antenna is white, its total length is nearly equal to that of head, scape bare is long, pedicel is slightly shorter and with a single long seta (Supplementary Fig. 6b), flagellum bears multiple annuli, decreasing in length proximally (Supplementary Fig. 4c). Labrum with rounded anterior margin and numerous bristles (Supplementary Fig. 4a). Hypopharyngeal lingua is nearly triangular, rounded apically, superlinguae are subrectangular with convex margins (Supplementary Fig. 4b). Glossae are slightly asymmetric, shorter by 1/3 than paraglossae (Supplementary Fig. 4d). Segment I of labial palps is oval, with a group of 25–30 strong bristles situated on dorsal surface. Segment II is asymmetrical, spoon-shaped, 2.5 times longer than broad, with slightly concave inner margin (Supplementary Fig. 4c). Maxillae are asymmetric with a single long and narrow, acutely pointed chitinous spine (Supplementary Fig. 4h) and two groups of long bristles: ventral and dorsal (Supplementary Fig. 4g). Segment I of maxillary palps is oblong-shaped with a single group of short setae on outer distal corner; segment II is oval, asymmetric, about three times longer than a wide and strongly haired on inner margin (Supplementary Fig. 4g). Mandibles are black apically, covered by long yellowish setae basally, without tooth on outer border (Supplementary Fig. 4e, f). Mandibular incisors are reduced. Mandibular tusks are massive, robust, and stout (Fig. 6c, d). Left tusk with three (in holotype) or four (in paratypes) apical teeth, median teeth smallest, apex of all teeth rounded (Supplementary Fig. 4e). Right tusk with two teeth of nearly triangular shape, with rounded apex, the inner tooth is smaller than the outer tooth (Supplementary Fig. 4f). Inner margin of both tusks with a small subapical tubercle. Dorsal surface of tusks bears numerous setae and a prominent, ovate dorsal tubercle. Thorax: anterior ring of pronotum with dark gray shading and with a line of yellow setae along posterior margin; posterior ring of pronotum with a large ovate patch densely covered by yellow setae (Supplementary Fig. 6d); mesonotum and metanotum are white; pleura and sternum are whitish. Wingbuds are white with dark brown markings pattern. Legs are white, with dense yellowish setae distally (Supplementary Fig. 5). Leg I: femora broad and well developed, with a double ventro-basal row of long filtering setae; tibio-tarsus (fused) with 3 rows of filtering setae (one on anterior face dorsally, one on outer margin, and one on inner margin) (Supplementary Fig. 5a); tarsal claw with a row of

32–35 marginal denticles (Fig. 5b). Leg II: smaller, femora thinner, with a row of short pointed bristles on dorsal margin; tibia and tarsi with row of long setae on outer (dorsal) margin, ventrally with dense shorter setae subapically and with a brush of thick setae distally; tarsus bears a row of long setae on outer margin and a group of narrow stout setae distally (Supplementary Fig. 5c); tarsal claw without denticles (Supplementary Fig. 5d). Leg III: similar to leg II but larger, femora bears dense, short setae along inner margin, dorsal surface with a field of short, thick spines proximally; tibia bears a row of long setae on outer margin, a brush of short, thick setae on inner margin, and a distal brush of short, thick setae (Supplementary Fig. 5e); tarsus and tarsal claw as those in Leg II (Supplementary Fig. 5f). Abdomen is whitish, terga II–IX with a median row of long hair-like setae (Supplementary Fig. 6e), tergum X is shaded with dark gray and bears a few hair-like setae on the surface. Gill I is uniramous (Supplementary Fig. 6f); gills II–VII are biramous, white with dark gray median line (Supplementary Fig. 6g). Paraprocts are covered with sparse pointed setae, each lobe with a distinct distal projection (Supplementary Fig. 6j). Cerci with rich marginal fringe (Supplementary Fig. 6h). Paracercus is longer by about 1/4 without marginal fringe (Supplementary Fig. 6i). Cerci and paracercus are covered with pointed scales, especially numerous on basal segments. *Male and female imago and subimago*. Unknown.

Etymology: The name of the new species is derived from two words: *lithos* ('stone' in Greek) and *phagus* ('eater of' in Latin), as a reference to its rock-boring habit.

Biology: Nymphs of the new species are filter feeders, boring into siliceous (siltstone) rocks in riverine environment.

Distribution: Possible endemic to the Bago River basin, Myanmar.

Conservation: At first glance, the new species could be treated as endangered, because it shares a restricted range and inhabits a very specific freshwater environment. Currently, the Bago River basin is impacted by a variety of anthropogenic loads arising from agricultural and urban land use, deforestation, sewage inputs, garbage littering, and general habitat degradation⁵³. These loads may negatively affect the rock-boring mayfly population.

DISCUSSION

Macrobioerosion in fresh water and the origin of freshwater rock borers

To the best of our knowledge^{23,54}, the present discovery of the new mayfly species *Languidipes lithophagus* sp. nov. from Myanmar represents the first example documenting macrobioerosion of hardgrounds by modern freshwater insects globally. Earlier, freshwater insect burrows were recorded in bedrock sediments of the Conecuh River, Alabama but these substrates represented firmgrounds and were subjected to wetting⁴⁴. Furthermore, the new mayfly species appears to be the first rock-boring animal of non-marine ancestry ever reported from freshwater environments²³. Currently, two more freshwater invertebrate species are known to produce macroborings in rocks, i.e., the piddock *Lignopholas fluminalis*^{24,39} and the shipworm *Lithoredo abatanica*^{40,41}. Both mollusc species are secondary freshwater members of primarily marine bivalve clades^{24,40}.

Interestingly, the three rock-boring freshwater taxa of invertebrates outlined above were originated within primarily wood-boring clades. *Languidipes lithophagus* sp. nov. belongs to a group of wood-boring mayflies and sisters to other taxa, the nymphs of which burrow in wood and bamboo⁴⁷. Similarly, the piddock genus *Lignopholas* Turner, 1955 contains estuarine and freshwater bivalves, most of which bore into wood, with *Lignopholas fluminalis* being the only exception³⁸. A putative Mesozoic stem lineage of this group, *Palaeolignopholas kachinensis* Bolotov et al.⁵⁵ from the mid-Cretaceous Burmese amber, also bored into wood

and hardening resin of coniferous trees⁵⁵. Finally, the shipworm *Lithoredo abatanica* is a unique rock-boring representative among a large monophyletic group of xylophagous bivalves, the Teredinidae^{40,56–58}, although a few species in this group are considered as seagrass borers⁵⁹.

Based on these data, we can assume that the evolutionary shift from wood to hardground substrate in fresh water is easier than that from free-living to rock-boring life style due to a set of specific preadaptations already available in wood borers. In particular, wood-boring mayfly nymphs have massive mandibular tusks⁴⁷ that could be useful to bore into lithic substrates such as siltstone. The larval mandibular tusks of *Languidipes lithophagus* sp. nov. are similar to those in wood-boring taxa⁴⁷ but have thicker and more rounded apical teeth (see Fig. 6c, d and Supplementary Fig. 4e, f for detail). The wood-boring bivalves (Pholadidae and Teredinidae) also actively use mechanical pathways to make cavities and tunnels in lignic substrates, and their morphological and anatomical preadaptations (specific structure of body, shell, and muscles)^{38,39,58} might facilitate an evolutionary shift to sedimentary rocks that was discovered in *Lignopholas fluminalis*^{24,39} and *Lithoredo abatanica*^{40,41}.

Boring and burrowing taxa among mayflies and other freshwater invertebrates

Among freshwater invertebrate taxa, species making boreholes in submerged wood appear to be more widespread than those boring into rocks and burrowing into firmgrounds. Examples of freshwater wood borers were reported in mayflies^{47,60,61}, beetles (Lutrochidae, Elmidae, and Oedemeridae; Coleoptera)^{62,63}, flies (Tipulidae and Axymyiidae; Diptera)^{62,64}, and bivalves (*Lignopholas rivicola* (Sowerby, 1849); Pholadidae)^{24,38}. A few firmground burrowers were discovered recently among chironomids and caddisflies⁴⁴. Finally, the wood-boring shipworm *Nausitora* sp. (Teredinidae) co-occurs with the rock-boring species *Lithoredo abatanica* in the same freshwater setting of the Abatan River, Bohol Island, Philippines⁴¹.

There is a large, monophyletic group of the so-called 'tusked burrowing mayflies', belonging to the infraorder Scaphodonta and characterized by having well-developed larval mandibular tusks^{65,66}. Most of wood-boring mayflies belong to the family Polymitarcyidae (subfamily Asthenopodinae)^{61,67,68}, as does the rock-boring *Languidipes lithophagus* sp. nov. Examples of wood-boring behavior of Asthenopodinae nymphs were described from Southeast Asia^{47,48,69}, Sri Lanka and India^{47,49}, Sub-Saharan Africa^{47,60}, and South America⁶¹ (Supplementary Table 3). The majority of available works are dedicated to wood-boring nymphs of the African mayfly *Povilla adusta*^{60,70–72}. In some cases, these nymphs were found to burrow into other kinds of substrate such as freshwater sponges, macrophytes, and sedge (e.g., *Cyperus*) stems^{73,74}, as well as lithic firmgrounds⁷⁵. Nymphs of *Tortopsis* sp. (Polymitarcyidae: Campsurinae) produce U-shaped burrows in hard clay deposits of the Caquetá River, Columbia⁷⁶. *Palingenia fuliginosa* (Georgi, 1802) (Palingeniidae) seems to be the only non-polymitarcyid mayfly species, the nymphs of which are known to bore into wood occasionally^{67,77}. Recent pouch-like burrows recorded from firm mud in rivers of Eastern Europe were attributed to *Palingenia* and *Polymitarcis* (*Ephoron*) taxa⁴³. In summary, wood borers appear to be more common in mayfly nymphs than firmground burrowers, whereas *Languidipes lithophagus* sp. nov. represents the only example of rock borers ever reported in freshwater insects. It was shown that mayflies play a number of ecosystem services such as bioturbation, bioirrigation, decomposition, nutrition for many animals, and nutrient cycling in freshwater environments⁷². Our discovery of a rock-boring mayfly species adds bioerosion of hard substrates to this extensive list. Interestingly, *Languidipes lithophagus* sp. nov. covers its tunnels by silky substance (see Fig. 5b for detail), as does the wood-boring

species *Povilla adusta* from Africa^{60,70}. It is unknown what is the function of this silk and whether other wood-boring mayflies produce such a substance. Borings of *Lithoredo abatanica* are lined with calcite tubes⁴¹ but it is unclear whether these structures and mayfly silky tubes could be attributable to convergent evolution.

Ichnotaxonomic issues related to bioerosion in fresh water

Here, we show that mayfly nymphs may produce a specific 'banana-like' macroboring in siliceous rocks. There is a strong recommendation to avoid ichnotaxonomic descriptions of modern traces, the producers of which were recorded directly in the field⁷⁸. Here, we follow these requirements and do not introduce an ichnotaxonomic name for the mayfly macroboring discovered at the Bago River, although some rock surfaces with sub-recent bioerosion beds, which are largely overlapped by alluvial clay sediments, were recorded at the youngest terrace in the midstream section of the Bago River. Though the tracemaker of massive bioerosion beds recorded at the upstream section of the river, as well as of those above the present floodplain levels in the midstream site, is unknown, these beds were almost certainly produced by the same rock-boring mayfly species. This conclusion is based on the morphological similarity of sub-recent macroborings and those produced recently by modern mayfly nymphs.

The macroboring produced by the modern mayfly nymphs is quite different morphologically from ichnogenera and ichnospecies that were discovered in hard substrates so far³. Moreover, this trace is morphologically distinct from recent mayfly burrows that were recorded in various soft and xylitic substrates^{43,79}. Recent and sub-recent dwelling activity of mayfly nymphs in firmgrounds, mud, and other soft sediments typically results in characteristic U-shaped burrows with long branches^{42,43,54,76,80}, while *Languidipes lithophagus* sp. nov. produces a horizontally oriented tunnel with convex bottom and two apertures, but without clear branches. Wood-boring mayflies also produce U-shaped burrows in wood, bamboo, and other kinds of xylitic substrate^{47,60,70,79}.

In the fossil record, mayflies were thought to be producers of some traces belonging to the ichnogenera *Rhizocorallium* Zenker, 1836 [= *Glossifungites* Łomnicki, 1886³⁶], *Fuersichnus* Bromley & Asgaard, 1979, and *Asthenopodichnium* Thenius, 1979^{35–37,43}. The first ichnogenus combines U-shaped burrows, with *Rhizocorallium jenense* Zenker, 1836 being its type ichnospecies, which could be produced by burrowing mayflies in soft grounds, as it often occurs in fluvial settings^{36,37}. Furthermore, *Rhizocorallium gingrasi* (King et al.³⁵) probably represents dwelling traces of burrowing mayflies in sandstones of the Turonian age³⁵. The trace fossils belonging to *Fuersichnus* burrows look somewhat similar to *Languidipes lithophagus* sp. nov. borings in general outline (i.e., a similar 'banana-like' shape)⁸¹. These traces were described from Triassic lacustrine beds of Greenland. Members of the ichnogenus *Asthenopodichnium* such as *A. xylobiontum* Thenius, 1979 and *A. lithuanicum* Uchman et al.⁷⁹ were ascribed to possible mayfly traces in fossilized xylitic substrates^{79,82}. Conversely, *Asthenopodichnium lignorum* Genise et al.⁸³ burrows in the Miocene fossil wood from New Caledonia were probably produced by wood rotting fungi because boring mayflies are unknown from this remote island⁸³. A comparison of fossil traces with present-day burrows revealed that the link between dwelling activity of recent mayflies and *Rhizocorallium*, *Fuersichnus*, and *Asthenopodichnium* traces is rather problematic^{43,65}. Conversely, *Rhizocorallium gingrasi* shares bioglyphs that are similar to the transverse microsculpture recorded in modern U- to tongue-shaped burrows of freshwater insect larvae such as mayflies and caddisflies³⁵.

Narrow, elongated grooves of variable depth originated via destruction of the thin upper layer covering *Languidipes lithophagus* sp. nov. traces are morphologically similar to members of the ichnogenus *Petroxestes* (Rogerellidae) that was described from marine calcareous rocks^{3,46}. Furthermore, the destructed

mayfly traces strongly resemble groove-like structures that were described from the Middle Triassic Muschelkalk Group of Germany as *Sulcolithos variabilis* Knaust, 2020⁸⁴. The latter ichnospecies, however, was attributed to the combined burrowing and boring activity of marine polychaetes based on records of fossil remains of these animals in the traces⁸⁴. Our new discovery reveals that the morphology of original traces in hardground substrates could be transformed due to subsequent taphonomic processes, leading to the elimination of fragile but ichnologically important details. It was shown that taphonomic overprinting may significantly influence ichnological and even paleontological taxonomy⁸⁵. A special term, taphotaxon, was introduced to refer to taxa, the diagnostic features of which were originated taphonomically⁸⁶.

In summary, *Languidipes lithophagus* sp. nov. could be considered a regional but remarkable tracemaker of sub-recent to recent ichnofabric in siliceous successions. Occurrences of similar traces may be used as an indicator of dwelling activity of rock-boring filter-feeding insect larvae in fossil bioerosion beds preserved in fluvial settings.

METHODS

Data sampling

The siltstone rock samples with macroborings were collected using a tommy-bar from two sites of the Bago River on March 22–24, 2020. The first site is situated at the middle reaches of the river (17.6797°N, 96.2318°E, altitude 40 m), while the second site is located at the upstream section (18.0791°N, 96.0449°E, altitude 195 m) (Figs. 1, 2). Living mayfly nymphs were collected directly from macroborings at the first site, while only indistinguishable larval fragments were found in some borings at the second site. The insect samples were placed into 96% ethanol immediately after collecting. The rock fragments were air-dried at room temperature. The samples are deposited in the Russian Museum of Biodiversity Hotspots [RMBH], N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia. One paratype of the new mayfly species (on a permanent slide) is deposited in the collection of Zoological Museum of Moscow State University [ZMMU], Moscow, Russia.

Mineralogical analyses of the rock substrate

The Bago Basin is situated within an area with marine and non-marine Miocene Molasse sequences⁴⁵. Hence, the siltstone rock deposits in the river valley were considered of the Miocene age. Scanning electron microscopy (SEM) characterization of the siltstone specimens was conducted with a SEM JSM-6480LV (JEOL Ltd., Tokyo, Japan). Polished cut samples were coated with 35 nm of carbon. The backscattered electron (BSE) images were obtained at 20 kV accelerating voltage and 0.7 nA probe current. XRD analysis of siltstone powder preparations was carried out using an ULTIMA-IV X-ray diffractometer (Rigaku, Tokyo, Japan). The equipment settings were as follows: operating mode 40 kV–15 mA; copper radiation; nickel filter; measurement range 3–65° 2 θ ; and scanning angle step 0.05° 2 θ . A fixed system of focusing slits was applied. Both SEM and XRD analyses were performed at the Moscow State University, Russia. The microindentation hardness (Vickers test) of the siltstone specimens was estimated using a PMT-3M Vickers Microhardness Tester (LOMO, Russia) with 100 g load. The tester was calibrated using NaCl crystal with 10 g load. A few fragments of the siltstone were placed into briquette, were fixed with epoxy glue, and their surface was polished. Five indentations were performed on each rock fragment, and both diagonals of indentation mark were measured. The mean value was calculated on the basis of 20 measurements for each siltstone fragment.

Morphological study of the rock-boring mayfly nymphs

For the morphological study of mayfly nymphs, we applied a standard approach and terminology described by earlier authors^{47,49,68,76,87}. A permanent slide with a paratype specimen (ZMMU Eph-0001) was prepared with Faure–Berlese's mounting medium. Images of the holotype of the new mayfly species were taken using a Canon EOS 7D camera with a Sigma AF 24–70 mm f/2.8 IF EX DG Aspherical HSM Canon EF lens (Canon Inc., Tokyo, Japan). The mouthparts were photographed using a stereomicroscope AXIO Zoom.V16 (Carl Zeiss, Germany). The photographs

of morphological features were taken from a paratype slide (ZMMU Eph-0001) using a digital camera ToupCam 9.0 MP (Hangzhou ToupTek Photonics Co., Ltd., Hangzhou, China) attached to a light microscope Olympus CX21 (Olympus Corporation, Tokyo, Japan). Measurements of the type series were made with a stereomicroscope Leica EZ4D (Leica Microsystems GmbH, Germany).

Morphological study of the recent and sub-recent borings

First, we examined a series of modern borings that were evidently produced by mayfly nymphs in the midstream section of the Bago River. These borings were used to produce a neoichnological model for the sub-recent traces. Second, siltstone specimens with similar traces collected from the sub-recent bioerosion beds were studied. The sub-recent traces were compared with those produced by recent mayflies. Siltstone specimens, recent macroborings, and sub-recent traces were photographed using a Canon EOS 7D camera with a Sigma AF 24–70 mm f/2.8 IF EX DG Aspherical HSM Canon EF lens (Canon Inc., Tokyo, Japan). Longitudinal and transverse sections of the modern borings and sub-recent traces were made for morphological investigation. The morphological descriptions of borings were based on a standard approach^{3,78}.

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from the ethanol-preserved tissue of insect larvae using the DNeasy Blood & Tissue Kit (Qiagen, Germany), following the manufacturer's protocol. A barcode fragment of the mitochondrial *cytochrome c oxidase subunit I* (COI) gene was amplified and sequenced using primers LCO1490 and HCO 2198⁸⁸. The PCR mix contained approximately 100 ng of total cellular DNA, 10 pmol of each primer, 200 μ mol of each dNTP, 2.5 μ l of PCR buffer (with 20 mmol MgCl₂), 0.8 units of Taq DNA polymerase (SibEnzyme Ltd., Russia), and H₂O, which was added up to a final volume of 25 μ l. Thermocycling included one cycle at 95 °C (4 min), followed by 29–31 cycles of 95 °C (45 s), 50 °C (40 s), and 72 °C (50 s), with a final extension at 72 °C (5 min). Forward and reverse sequence reactions were performed directly on purified PCR products using the ABI PRISM® BigDye™ Terminator v. 3.1 reagents kit and run on an ABI PRISM® 3730 DNA (Thermo Fisher Scientific Inc., Waltham, MA, USA). The resulting sequences were checked using a sequence alignment editor, BioEdit 7.2.5⁸⁹.

Sequence alignment, phylogenetic analyses, and DNA-based taxonomic diagnostics

To reconstruct the phylogeny of boring and burrowing mayflies (Ephemeroptera), we used 23 available COI sequences. Altogether 11 sequences were generated in this study, i.e., five sequences of *Languidipes lithophagus* sp. nov., five sequences of *Povilla adusta*, and one sequence of *Symbiocloeon* sp. (outgroup taxon; Baetidae) (Supplementary Table 1). The other 12 sequences were obtained from NCBI's GenBank. The COI sequence dataset was aligned using the MUSCLE algorithm of MEGA7⁹⁰ and was collapsed to unique haplotypes with an online FASTA sequence toolbox, FaBox 1.5⁹¹. The server for IQ-TREE (W-IQ-TREE) was used to run a maximum likelihood phylogenetic analysis under TIM2+F+I+G4 evolutionary model⁹² and ultrafast bootstrap algorithm (UFBoot) with 1000 replicates⁹³. The Bayesian phylogeny was reconstructed with MrBayes v. 3.2.6⁹⁴ at the San Diego Supercomputer Center through the CIPRES Science Gateway⁹⁵. Four runs, each with three heated (temperature = 0.1) and one cold Markov chain, were conducted for 30 million generations. Trees were sampled every 1000th generation. We applied a 15%-burn-in to the final tree set and reconstructed a consensus phylogeny from the remaining trees.

The DNA-based taxonomic diagnostics of the new species were as follows. First, we applied the Poisson Tree Process (PTP) algorithm using the PTP web-service (<http://mptp.h-its.org>)⁹⁶ to delimit the Molecular Operational Taxonomic Units (MOTUs). As an input tree, we used the IQ-TREE COI phylogeny (see above). An outgroup taxon was excluded from the input tree using a corresponding option of the PTP server. Second, we calculated uncorrected COI *p*-distances between *Languidipes* taxa with MEGA7⁹⁰. Third, we registered fixed nucleotide substitutions in the COI gene that are characteristic for the new species in comparison with *Languidipes corporaali* and *L. taprobanes*. These substitutions were recorded with a Toggle Conserved Sites tool of MEGA7 at 50% level⁹⁰.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature (ICZN), and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains were registered in ZooBank (<http://zoobank.org>), the online registration system for the ICZN. The LSID for this publication is <http://zoobank.org/urn:lsid:zoobank.org:pub:F3A2785B-4B5F-4F4C-823D-7DC0E4961421>. The electronic edition of this paper was published in a journal with an ISSN and has been archived and is available from PubMed Central.

DATA AVAILABILITY

Supplementary material is available online. The DNA sequences generated in this study are deposited in NCBI's GenBank (see Supplementary Table 1 for accession codes). The type series of the new mayfly species (holotype and 15 paratypes) is available in the RMBH—Russian Museum of Biodiversity Hotspots, N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia. One paratype is also available in the ZMMU—Zoological Museum of Moscow State University, Moscow, Russia. Other relevant data are available from the corresponding author of this paper upon a reasonable request.

CODE AVAILABILITY

No new code was developed in this study.

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AUTHOR CONTRIBUTIONS

I.N.B. developed the concept of the study. I.N.B., I.V.V., A.V.K., M.Y.G., Z.L., and N.C. collected samples. A.V.K. and G.V.B. designed and processed molecular analyses. G.S.P., D.M.P., V.M.S., E.A.S., and Y.S.K. performed morphological research on mayflies. I.V.V. and A.A.L. made images of the rocks and borings. G.S.P., V.M.S., and E.A.S. photographed the mayfly nymphs. Y.E.C. made graphical drawings. O.S.P., A.Y.B., and V.O.Y. analyzed rock substrate characteristics. M.Y.G. created the map. I.N.B. wrote the paper, with input from O.S.P., A.Y.B., A.V.K., M.Y.G., I.V.V., Z.L., N.C., A.A.L., G.S.P., D.M.P., and Y.S.K. All the co-authors discussed the final version of the paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Ivan N. Bolotov.

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