

First record of *Pisidium nevilleianum* Theobald, 1876 (Bivalvia: Sphaeriidae) from Myanmar

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ABSTRACT. *Pisidium nevilleianum* Theobald, 1876, a common pea clam species that widely ranged from India to Indochina, was recorded in the Indawgyi Lake, Northern Myanmar for the first time. These specimens share the same or very similar 16S rRNA haplotypes as specimens from Nepal (geographic distance of over 1,400 km), which could reveal a high dispersal rate of the species, likely via water birds. Same high dispersal possibilities were observed for many other sphaeriid species.

Introduction

Pisidium nevilleianum Theobald, 1876, a widespread subtropical pea clam species, is known from India, Bangladesh, Nepal, Thailand and Vietnam [Theobald, 1876; Brandt, 1974; Subba Rao, 1989; Nesemann *et al.*, 2001; Nesemann, Sharma, 2005; Schultheiß *et al.*, 2008; Clewing *et al.*, 2013]. Brandt [1974] assumed that it also has to be distributed in Burma, as it was found in Thailand near the Burmese border. However, no records of this species are currently available from Myanmar.

In the present communication, we describe the first record of *Pisidium nevilleianum* from Myanmar based on morphological and molecular data.

Material and methods

During the field trip in 2014, sphaeriids were collected from Lake Indawgyi, Northern Myanmar using an aquatic net sampler. Sampled specimens were immediately transferred in 96% ethanol. They are deposited in the Russian Museum of Biodiversity Hotspots (RMBH) of the Institute of Ecological Problems of the North of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Rus-

sia. Species identification was performed based on shell morphology, anatomy and molecular data, using the available taxonomic information [Brandt, 1974; Nesemann *et al.*, 2001]. Anatomical and morphological details were investigated using a stereomicroscope (Leica M165C, Leica Microsystems, Germany and Carl Zeiss Axio Lab.A1, Carl Zeiss Microscopy, Germany). All images were processed with Corel Photo-Paint version X6.

Genomic DNA was extracted from individual clams using proteinase K and phenol [Sambrook, Russel, 2001]. The standard primers 16Sar and 16Sbr [Palumbi, 1996] were used for the amplification of the 16S rRNA gene. The PCR mix contained approximately 200 ng of total cellular DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µl of PCR buffer (with 10×2 mmol MgCl₂), 0.8 units Taq DNA polymerase (SibEnzyme Ltd., Novosibirsk, Russia), and H₂O, which was added to provide a final volume of 25 µl. Thermocycling included one cycle at 95°C (4 min), followed by 38–40 cycles of 95°C (50 sec), 48°C (50 sec), and 72°C (50 sec) and a final extension at 72°C (5 min). Forward and reverse sequencing was performed on an automatic sequencer (ABI PRISM® 3730, Applied Biosystems) using the ABI PRISM® BigDye™ Terminator v. 3.1 reagent kit. Resulting sequences (502-bp long) were checked using a sequence alignment editor [BioEdit version 7.2.5, Hall, 1999]. In addition, available 16S sequences from three specimens were obtained from the GenBank sequence database of the National Center for Biotechnology Information (NCBI) (Table 1). The sequence alignment was performed using the ClustalW algorithm implemented in MEGA6 [Tamura *et al.*, 2013]. Each sequence of the aligned dataset was trimmed, leaving a 437-bp fragment. The sequence dataset

was collapsed into haplotypes using an online fasta sequence toolbox [FaBox 1.41, Villesen, 2007]. Genetic differences (*p*-distances) between analyzed haplotypes were calculated using MEGA6 [Tamura *et al.*, 2013].

Results

Pisidium nevillianum Theobald, 1876 (Fig. 1, Table 1)

Pisidium nevillianum Theobald, 1876: 188.

Material examined. Myanmar: Indawgyi Lake, near source of Indawgyi River, 25°13'56" N, 96°22'49" E, 9 specimens, Bolotov, Gofarov, Spitsyn and Vikhrev leg.

Description. The maximal shell length is 3.6 mm. The shell has a triangular shape, moderately convex, yellowish or beige colored, subtransparent (Figs. 1A and 1B). Ventral margin is slightly curved, without angles, smoothly following to the anterior margin. The beaks are prominent, relatively narrow and markedly shifted posteriorly. Hinge plate relatively broad (Figs. 1C and 1D). The shell surface sculpture is pronounced, represented by regularly spaced ribs (Fig. 1F). Internal pores are well marked, dense (Fig. 1G). The cardinal tooth of the right valve is curved and split on the posterior end, its branches diverge (Fig. 1H). The inner cardinal tooth of the left valve is curved, longer than outer cardinal tooth, which is thin and straight (Fig. 1I). Only one (exhalant) siphon present (Fig. 1K). The nephridia belong to a closed type (Fig. 1L).

Remarks. *P. nevillianum* is closely related to *P. clarkeanum* G. et H. Nevill, 1871, differing in its much heavier triangular shell, regular sculpture and broader hinge plate. In several cases of sympatry with *P. clarkeanum*, significantly differed also in having less pores density. Bundles of inner radial mantle muscles in *P. nevillianum* are definitely shorter, but stronger. Shell somewhat smaller than in *P. clarkeanum*. Siphons, siphonal muscles, gills and nephridia as in *P. clarkeanum* [Nesemann *et al.*, 2001].

Molecular data. Three sequenced specimens share two 16S rRNA haplotypes with *p*-distance 0.2% (Table 1).

Habitat. Specimens were collected on a grey clay ground with macrophyte roots from a shallow eutrophic area in the lake (Fig. 2).

Discussion

Our record is the first evidence to the species distribution in Myanmar. The Indawgyi specimens share same morphological and anatomical characters as those from Nepal [Nesemann *et al.*, 2001]. This species is common across a wide range of streams, small rivers, agricultural irrigation channels, and is dominant in larger lakes in the mid-hills [Nesemann *et al.*, 2001; Nesemann, Sharma, 2005]. Our data is in accordance with the known habitat preference of the species. Pea clam species in the Indawgyi Lake were not yet studied, excluding a record of *P. clarkeanum* [Prashad, 1930]. Thus, *P. nevillianum* is the second documented species, which is usually found together with *P. clarkeanum* [Nesemann *et al.*, 2001]. According to some authors, the Indawgyi Lake might have a pre-Pleistocene origin [Dumont, Green, 2005]. The lake fauna is poorly studied but several local endemic taxa were described from this area [Rao, 1929; Prashad, 1930; Hee, 1999; Dumont, Green 2005].

Two of the sequenced specimens of *P. nevillianum* from Indawgyi Lake have the same 16S rRNA haplotype as specimens from Nepal [Schultheiß *et al.*, 2008; Table 1]. These data indicate the appropriate possibility for the species to overcome the geographic distance of over 1,400 km. Similar results were obtained for *P. stewarti* Preston, 1909 from the Tibetan Plateau where specimens from geographically very distant locations (max. ca. 1,400 km) share the same haplotype [Clewing *et al.*, 2013]. However, a *P. nevillianum* specimen from Vietnam [Clewing *et al.*, 2013; Table 1] has a divergent haplotype with *p*-distances 0.9–1.1% of

Table 1. List of 16S rRNA sequences of *Pisidium nevillianum* Theobald, 1876

Haplotype	NCBI's GenBank acc. no.	Specimen Voucher	Locality	Latitude (N)	Longitude (E)	Reference
NEV-1	KM514316	INEP-875	Myanmar, Indawgyi Lake	25.23222	96.38027	Our data
NEV-2	KM514317	INEP-877	Myanmar, Indawgyi Lake	25.23222	96.38027	Our data
NEV-1	KM514318	INEP-878	Myanmar, Indawgyi Lake	25.23222	96.38027	Our data
NEV-1	EU559132	3510	Nepal, Lamahi	27.83527	82.53972	Schultheiß <i>et al.</i> 2008
NEV-1	EU559133	3511	Nepal, Lamahi	27.83527	82.53972	Schultheiß <i>et al.</i> 2008
NEV-3	KF483322	UGSB 10986	Vietnam, Ninh Binh	20.23361	105.72194	Clewing <i>et al.</i> 2013

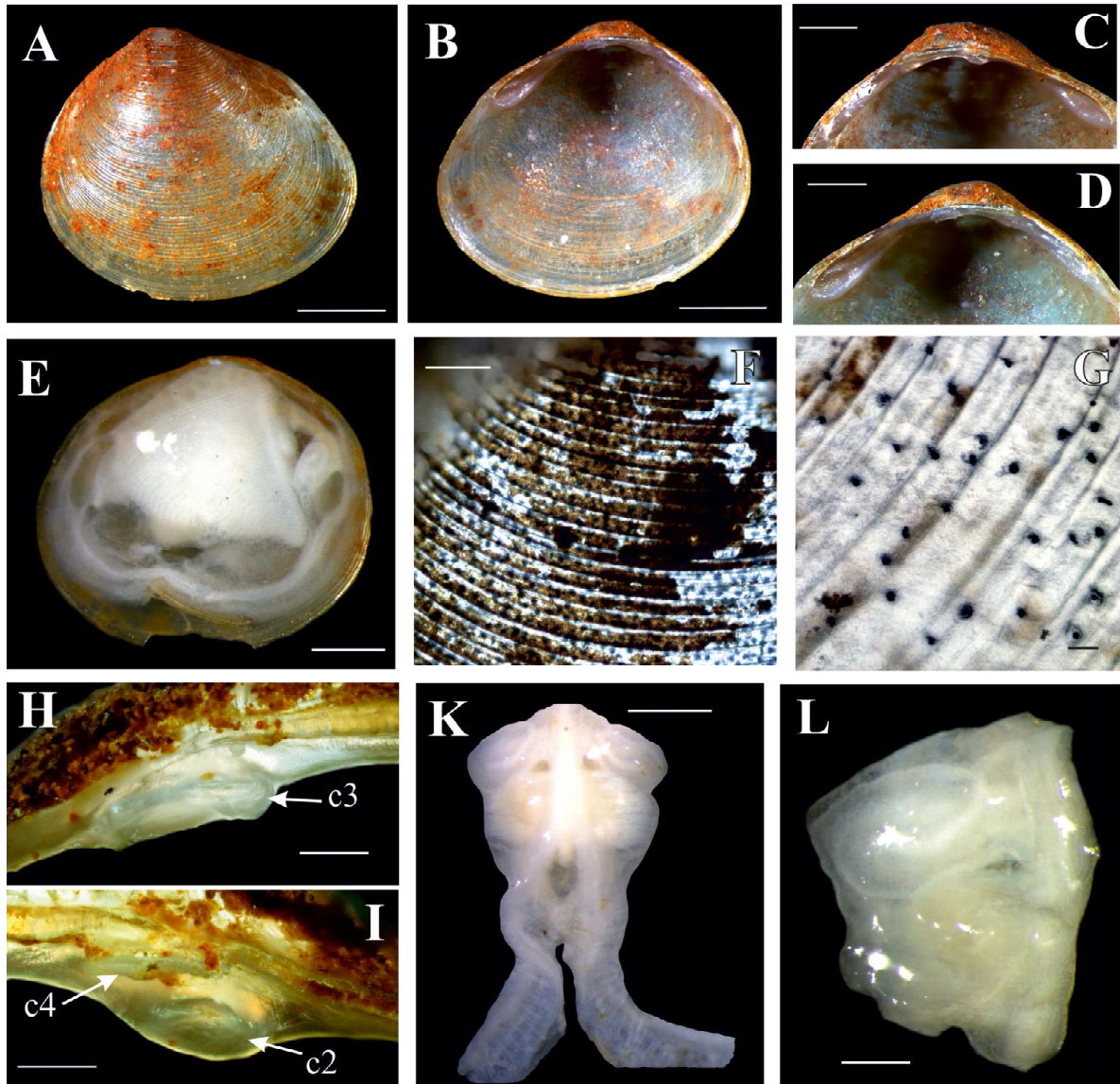


FIG. 1. Morphology of *Pisidium nevillianum* Theobald, 1876 from Indawgyi Lake, Myanmar. **A.** Exterior view of the right valve (scale bar: 1 mm). **B.** Interior view of the right valve (scale bar: 1 mm). **C.** The beak and hinge of the left valve (scale bar: 0.5 mm). **D.** The beak and hinge of the right valve (scale bar: 0.5 mm). **E.** Gross anatomy (scale bar: 0.5 mm). **F.** The shell surface sculpture (scale bar: 200 μ m). **G.** The internal pores on the surface of the left valve (scale bar: 50 μ m). **H.** The cardinal tooth (c3) of the right valve (scale bar: 100 μ m). **I.** The inner (c2) and outer (c4) cardinal teeth of the left valve (scale bar: 100 μ m). **K.** The siphon and mantle edge with musculature (scale bar: 0.5 mm). **L.** The nephridia (scale bar: 0.2 mm) (Photos: O.V. Aksenova).

РИС. 1. Морфология *Pisidium nevillianum* Theobald, 1876 из оз. Индавжи, Мьянма. **A.** Правая створка, вид с наружной стороны (масшт. линейка: 1 мм). **B.** Правая створка, вид с внутренней стороны (масшт. линейка 1 мм). **C.** Макушка и замочная площадка левой створки (масшт. линейка: 0.5 мм). **D.** Макушка и замочная площадка правой створки (масшт. линейка: 0.5 мм). **E.** Внутреннее строение (масшт. линейка: 0.5 мм). **F.** Скульптура поверхности раковины (масшт. линейка: 200 мкм). **G.** Внутренние поры на поверхности левой створки (масшт. линейка: 50 мкм). **H.** Кардинальный зуб (c3) правой створки (масшт. линейка: 100 мкм). **I.** Внутренний (c2) и внешний (c4) кардинальные зубы левой створки (масшт. линейка: 100 мкм). **K.** Сифон и край мантии с мускулатурой (масшт. линейка: 0.5 мм). **L.** Нейфридии (масшт. линейка: 0.2 мм) (фото: О.В. Аксенова).

those from Myanmar and Nepal. Thus, we provide new evidence for long-distance dispersal of this subtropical pea clam species. Likely, its high expansion rate is associated with passive dispersal via

birds [Rees, 1965; Clewing *et al.*, 2013]. Indawgyi Lake is known as a harbor of abundant water bird populations, including many migratory duck species [Tordoff *et al.*, 2007].



FIG. 2. The habitat of *Pisidium nevillianum* Theobald, 1876: a shallow eutrophic area in the lake (Photo: V.M. Spitsyn).

РИС. 2. Местообитание *Pisidium nevillianum* Theobald, 1876: мелководный эвтрофный участок озера (фото: В.М. Спицын).

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Первая находка *Pisidium nevillianum* Theobald, 1876 (Bivalvia: Sphaeriidae) в Мьянме

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РЕЗЮМЕ. Субтропический вид *Pisidium nevillianum* Theobald, 1876, широко распространенный от Индии до Индокитая, был впервые обнаружен в озере Индавжи на территории Северной Мьянмы. Собранные особи принадлежат к двум 16S рРНК гаплотипам, один из которых идентичен гаплотипу, обнаруженному в Непале (расстояние между пунктами сбора моллюсков свыше 1400 км). Это свидетельствует об очень высоких способностях вида к расселению, вероятно, при помощи водных птиц, что характерно и для многих других представителей семейства Sphaeriidae.

