A new *Contradens* from Laos (Bivalvia: Unionidae: Contradentini)

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Abstract
Here, we describe *Contradens novoselovi* sp. nov. (Bivalvia: Unionidae), a new freshwater mussel species from the Mekong Basin in Laos. Phylogenetic and morphological analyses reveal that the new taxon is closely related to *Contradens comptus* (Deshayes & Jullien, 1874) but can be distinguished from it by a smaller size, sub-ovate shell shape, tiny wrinkles near umbo, stronger pseudocardinal and lateral teeth, and fixed nucleotide substitutions. *Contradens novoselovi* sp. nov. is known only from the type locality in northwestern Laos and may represent a rare endemic species with restricted range.

Key words: Freshwater bivalve, Unionida, new species, Mekong River, Southeast Asia.

Introduction

*Contradens* Haas 1911 is a widely distributed freshwater mussel genus, whose range covers the Mekong, Chao Phraya and Mae Klong basins, rivers of the Malay Peninsula and the Greater Sunda Islands (Zieritz et al. 2016; Bolotov et al. 2017a, 2017b; Pfeiffer et al. 2018; Konopleva et al. 2019). During recent decades some researchers attempt to revise the genus *Contradens* by means of an integrative taxonomic approach (Bolotov et al. 2017b; Lopes-Lima et al. 2017; Jeraththitikul et al. 2019; Konopleva et al. 2019). Based on a growing body of integrative taxonomic research, several nominal taxa were transferred from other genera to *Contradens*, e.g. *C. eximius* (Lea, 1856), *C. misellus* (Morelet, 1865), *C. pallegoixi* (Sowerby, 1867), *C. peninsularis* (Simpson, 1900), and *C. comptus* (Deshayes & Jullien, 1874) (Pfeiffer et al. 2018; Konopleva et al. 2019). Furthermore, *Contradens rolfbrandti* Jeraththitikul & Panha, 2019 was recently described from Thailand (Jeraththitikul et al. 2019). In summary, the genus *Contradens* contains at least 13 valid species (Graf and Cummings 2019). However, there are still several taxa with an uncertain status, e.g. *C. fulvaster* (Drouet & Chaper, 1892) and *C. subcircularis* Brandt, 1974 requiring further investigations using a molecular approach.

In the present study, we describe a new species of *Contradens* from northwestern Laos based on multi-locus molecular and morphological data. Until recently, this species was treated as a separate
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phylogenetic lineage preliminary labelled as *Contradens* sp. “Vieng Phou Kha” without a valid taxonomic name (Konopleva et al. 2019).

Materials and methods

The type specimens of *Contradens novoselovi* sp. nov. (*N* = 10) were studied in the malacological collection of the Russian Museum of Biodiversity Hotspots (RMBH thereafter), Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Arkhangelsk, Russia. This sample was collected from northwestern Laos (Fig. 1). A comparative analysis of shell morphology was carried out based on shell shape, the structures of pseudo-cardinal and lateral teeth, muscle attachment scars, and umbo position (Konopleva et al. 2019). The length, height, and width of the shell (all at the maximum diameter) of each specimen were measured using dial calipers (±0.1 mm).

DNA extraction, PCR, sequencing and sequence alignment were performed as described in Bolotov et al. (2017b). New sequences of the mitochondrial *cytochrome c oxidase subunit I* (*COI*), *small ribosomal RNA* (*16S rRNA*), and the nuclear *large ribosomal RNA* (*28S rRNA*) gene fragments from three specimens of the new species were generated (Table 1).

Phylogenetic analysis was based on the combined data set of Contradentini and Rectidentini (*COI* + *16S rRNA* + *28S rRNA*) with 86 unique haplotypes (Appendix 1). Sequences of *Pseudodon* species were used as outgroup. The best-fit evolutionary models applied to each partition were calculated by ModelFinder within the IQ-Tree web-server (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017), based on the Bayesian Information Criterion (BIC). The models were selected as follows: F81 (1st codon of *COI*), GTR + G (2nd codon of *COI*), TNe + G (3rd codon of *COI*), TIM2 + G (*16S rRNA*), and TIM3 + I (*28S rRNA*). Bayesian inference analysis was performed in MrBayes v. 3.2.6 (Ronquist et al. 2012) at the San Diego Supercomputer Center through the CIPRES Science Gateway (Miller et al. 2010). Three runs, each with three heated (temperature = 0.2) and one cold Markov chain, were conducted for 15 million generations. Trees were sampled every 1000th generation. The first 25% of trees were discarded as burn-in (pre-convergence part). The effective sample size (ESS) value for each parameter sampled from the MCMC analysis was always recorded as >1100.

Mean uncorrected *COI* p-distance to the sister species was calculated using MEGA 7 (Kumar et al., 2016). The molecular diagnosis of the new species was designed as described in Bolotov et al. (2017b, 2019).

Description of the new species

Family Unionidae Rafinesque, 1820
Subfamily Rectidentinae Modell, 1942
Tribe Contradentini Modell, 1942

Type genus: *Contradens* Haas, 1911 (by original designation)
Genus *Contradens* Haas, 1911

Type species: *Unio contradens* Lea, 1838 (by original designation)

*Contradens novoselovi* sp. nov.

*Contradens* sp. “Vieng Phou Kha” Konopleva et al. (2019): 2.
Figs. 1-3, Table 1

**Type material.** Holotype RMBH biv 203_4: LAOS: Mekong River basin, a tributary of Nam Fa River near Vieng Phou Kha village, 20.682°N, 101.0794°E, 24.v.2016, Spitsyn and local villagers leg.; paratypes: same locality and date, 9 specimens [RMBH biv 202_2 and biv 203_5 are sequenced; biv 202_3, biv 202_4, biv 202_5, biv 202_6, biv 203_1, biv 203_2, biv 203_3]. Spitsyn and local villagers leg.

**Etymology.** This new species is dedicated to Dr. Alexander P. Novoselov, a prominent Russian ichthyologist and our colleague.
Figure 1. Type locality of *Contradens novoselovi* sp. nov.: A) map of the type locality; B) general view of the type locality, a tributary of the Nam Fa River near Vieng Phou Kha village, Mekong Basin, Laos. (Map: M. Yu. Gofarov; photo: Vitaly M. Spitsyn).
**Differential diagnosis.** The new species is morphologically similar to *C. comptus* but can be distinguished from it by a smaller size, sub-ovate shell shape (vs. trapezoidal), broadly rounded anterior margin (vs. narrower and more elongated anterior margin), tiny wrinkles only near umbo (vs. along all dorsal margin and posterior slope), and strong lamellar pseudocardinal and lateral teeth.

**Molecular diagnosis.** Our multi-locus phylogeny (3 codons of COI + 16S rRNA + 28S rRNA) reveals that *Contradenos novoselovi* sp. nov. forms a highly supported clade within the genus *Contradens* (BPP = 1.00; Fig. 3). Phylogenetically, the new species is most closely related to *Contradens comptus* (mean uncorrected COI p-distance = 3.6±0.7%) but can be distinguished from it by six fixed substitutions in the COI gene fragment as follows: 122 T, 164 C, 185 C, 257 A, 338 C, 434 A.

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**Figure 2.** Shell and soft body morphology of *Contradenos novoselovi* sp. nov.: A) lateral view of the shell: inner side of the left valve and outer side of the right valve [holotype RMBH biv 203_4]; B) lateral view of the shell: vice versa; C) dorsal view of the shell; D) soft body morphology (right valve and corresponding mantle tissue were removed) [paratype RMBH biv 202_2]. Scale bars = 10 mm. (Photos: Ekaterina S. Konopleva and Ilya V. Vikhrev).

**Description.** Small mussel: shell length 23.0–41.1 mm, shell height 13.1–25.1 mm, shell width 8.1–16.1 mm (Table 1). Shell sub-ovate, moderately thick and inflated, anterior margin broadly rounded, ventral margin straight or slightly curved, dorsal margin elevated posteriorly. Posterior margin covered by small wrinkles elongated from the umbo. Posterior slope gradual. Periostracum smooth, brown with darker areas. Nacre bluish-white. Umbo small, slightly elevated above hinge line, eroded. Pseudocardinal teeth strong, two on each valve. On the right valve, anterior tooth thin and lamellar, posterior tooth strong, indented. On the left valve, two strong lamellar indented teeth. Lateral teeth pronounced, thin, slightly curved, two teeth on the left valve, one tooth on the right valve. Anterior adductor muscle scars drop-like or oval-shaped, contiguous with pedal retractor scars. Posterior adductor muscle scars rounded and shallow. Mantle and its edges creamy. Gills creamy or light-yellow, elongated and slightly ribbed. Anterior margin of outer gills slightly longer and wider than that of inner gills. Foot medium-sized, light-brown. Small epithelial fold divides exhalant and inhalant siphons. Papillae of the inhalant siphon represent monodactylous outgrowths,
decreasing in height in the direction of the ventral margin. Ascending lamella of the inner demibranchs attached to the visceral mass only anteriorly.

### Table 1. Shell measurements and reference DNA sequences for the type series of *Contradens novoselovi* sp. nov.

| Status of specimen | Specimen Voucher No.* | Shell length, mm | Shell height, mm | Shell width, mm | NCBI’s GenBank acc. nos. |
|--------------------|-----------------------|------------------|------------------|----------------|-------------------------|
| Holotype           | RMBH biv 203_4        | 40.8             | 25.1             | 15.8           | KY561632 KY561647 KY561664 |
| Paratype           | RMBH biv 202_2        | 37.0             | 21.9             | 14.7           | KY561630 KY561645 KY561662 |
| Paratype           | RMBH biv 203_5        | 32.6             | 20.5             | 13.7           | KY561631 KY561646 KY561663 |
| Paratype           | RMBH biv 202_5        | 35.3             | 20.5             | 14.1           | n/a n/a n/a |
| Paratype           | RMBH biv 202_3        | 29.2             | 16.6             | 10.2           | n/a n/a n/a |
| Paratype           | RMBH biv 202_4        | 26.5             | 15.2             | 9.2            | n/a n/a n/a |
| Paratype           | RMBH biv 202_6        | 23.0             | 13.1             | 8.1            | n/a n/a n/a |
| Paratype           | RMBH biv 203_1        | 35.7             | 21.6             | 13.8           | n/a n/a n/a |
| Paratype           | RMBH biv 203_2        | 39.7             | 24.7             | 15.3           | n/a n/a n/a |
| Paratype           | RMBH biv 203_3        | 41.1             | 25.0             | 16.1           | n/a n/a n/a |

*RMBH – Russian Museum of Biodiversity Hotspots, Federal Center for Integrated Arctic Research, Russian Academy of Sciences, Arkhangelsk, Russia. n/a – not available.

**Distribution.** This species is known only from its type locality in northwestern Laos (Fig. 1).

**Habitat.** The new species was recorded in a mixed substrate of tree roots, silty and clay, near the banks on the slow flowing sections of the stream. *Bineurus mouhotii* (Lea, 1863) (Bivalvia: Unionidae: Pseudodontini) was found in sympathy with the new species.

**Discussion**

Although Southeast Asian fauna of freshwater bivalves has been subject of intense research efforts during the last five years (e.g. Zieritz et al. 2016; Bolotov et al. 2017a, 2017b, 2019; Lopes-Lima et al. 2017; Jeratthitikul et al. 2019; Konopleva et al. 2019), the fauna of Laos is still poorly known (Zieritz et al. 2018). For example, modern information on the population status and ecology is only available for a single species, *Gibbosula laosensis* (Lea, 1863) (Bivalvia: Margaritiferidae) (Bolotov et al. 2014). Nevertheless, molecular data on freshwater mussels from Laos is presented in a few works (Pfeiffer and Graf 2015; Bolotov et al. 2017a, 2017b; Lopes-Lima et al. 2017) but the taxonomic placement of those sequences is still uncertain. Here, a freshwater mussel sample from Laos was recovered as the new species, *Contradens novoselovi* sp. nov. Phylogenetic analyses, diagnostic substitutions and the level of genetic divergence clearly demonstrate that *Contradens novoselovi* sp. nov. represents a separate lineage of this genus which is sister to *C. comptus*.

The type series of *Contradens novoselovi* sp. nov. was collected from a single locality in northwestern Laos, what may point to a rather restricted distribution of this species. However, this species requires further surveys for the evaluation of its distribution and conservation status. Unfortunately, Northern Laos is under high anthropogenic pressure leading to habitat degradation, river pollution and damming, fish and mussel overharvesting, and intense soil erosion (Bolotov et al. 2014). Therefore, *Contradens novoselovi* sp. nov. may be under severe threat as a prospective local endemic species with restricted range and need a long-term population monitoring and conservation efforts.

The Upper Mekong Basin in northern Laos is a well-known biodiversity hotspot, especially for birds, butterflies and fishes (Pierre 2004; Tordoff et al. 2011). However, the levels of species richness and endemism of freshwater mussels in northern Laos are virtually unknown due to the absence of reliable data (Zieritz et al. 2018). The Annamite Range harboring numerous endemic animal and plant species (Tordoff et al. 2011) seems to be a potential high-priority area for future field surveys of freshwater mussel diversity in Laos.

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Figure 3. Phylogenetic tree recovered from Bayesian inference analysis of the combined data set of mitochondrial and nuclear sequences of the Rectidentini (collapsed) and Contradentini (five partitions: three codons of COI + 16S rRNA + 28S rRNA). *Pseudodon species* were used as outgroup (not shown). Scale bar indicates the branch lengths. Black numbers near nodes are Bayesian Posterior Probabilities (BPP); asterisk (*) indicates high BPP values ≥ 0.95. *Contradens novoselovi* sp. nov. is colored red, and its sister species, *C. comptus* is colored blue. (Photos: Ekaterina S. Konopleva).

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