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First record of *Unionicola* (*Myanmaratax*) *savadiensis* from India, with remarks on parasite-host relationship in unionicolid mites (Acari, Unionicolidae)

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Original research

ABSTRACT

Freshwater mites of the family Unionicolidae are associated with freshwater mussels and snails in lakes and streams on all continents except Antarctica. The majority of the currently known 265 Unionicolidae species are associated with molluscs during at least one stage of their life cycle. A new host record is presented here from India: *Unionicola* (*Myanmaratax*) *savadiensis* from the host *Lamellidens corrianus*. In addition to *L. corrianus*, *U. savadiensis* was also found associated with two other mussel species, i.e., *Lamellidens marginalis* and *Corbicula cashmirensis*, but DNA evidence confirms parasite-host association between *U. (M.) savadiensis* and *L. corrianus*, indicating that its findings in the two other mussel species should be considered as 'vagrant' associations. This report is thus significant for two reasons: (i) the record of the new host and geographic associations of the mite and mussels extends our knowledge on the known mite-mussel associations, and (ii) the study emphasized the importance of including experts from acarology, malacology, and molecular biology, to ascertain the nature of mite-mussel associations.

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Keywords mussel; mite; India; vagrant associations; parasitism

Introduction

The majority of the 265 known unionicolid freshwater mites (Acari: Unionicolidae: *Unionicola*) are associated with molluscs during at least one stage of their life cycle. The mites are currently known to infest five of the six families of freshwater mussels (Bivalvia: Unionoida) (Edwards and Vidrine 2013a, b). The mollusc-associated mites are readily divided into two groups: mantle mites (the mantle and foot tissue of their hosts as sites of oviposition) and gill mites (the gill tissues of their hosts as sites of oviposition) (Edwards and Vidrine 2006, 2013a, b, 2020). In the Northern Hemisphere, it is not uncommon to find the representatives of both groups of mites in a single host individual, however, the mites apparently are highly specific as to not only as sites of oviposition but also as to their selection of host species. The water mite literature

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supporting these hypotheses was summarized in Edwards and Vidrine (2013a, b, 2021), and Ernsting *et al.* (2014), and more recently in Chapurina *et al.* (2021, 2022a, b). Discovery of species new to science as well as associations new to science are also not uncommon, however, each discovery is important and helps to qualify significant hypotheses in evolutionary ecology of the complex 200-million year history of mites and molluscs associations (Edwards and Vidrine 2020). The substantiation of this history with molecular evidence from a variety of sources, including both molluscan and acarine DNA sequences, is important.

In historical Indian collections, Karl Viets (1926) described two mites from a single host species in India. *Lamellidens marginalis* (Lamarck, 1819) was infected by *Unionicola* (*Prasadatax*) *diversipes* Viets, 1926 (a gill mite) and *U. (Imamuratax) scutigera* Viets, 1926 (a mantle mite). Also, in Majumder's collections (Majumder and Pal 1987, 1988, 1990), *U. diversipes*, *U. scutigera*, and *Unionicola* sp. (= *U. (Majumderatax) hankoi* Szalay, 1927) (a mantle mite) were found in the same host species. John and Inasu (2004) reported *U. diversipes* and *U. (Myanmaratax) brandti* Vidrine, 1985 from the same host species. It is possible that the latter record was actually a mite belonging to one of the species described in the new subgenus in Chapurina *et al.* (2022a), but lacking evidence supporting this, the record is considered doubtful. Chapurina *et al.* (2022a, b) reported a number of species from *Lamellidens* Simpson, 1900 as gill mites from Myanmar, including *U. diversipes*, *U. (Dimockatax) huangthayaensis* (Chapurina *et al.*, 2022b), *U. (Myanmaratax) generosa* Chapurina, Vidrine, Kondakov, Vikhrev & Bolotov, 2022 (in Chapurina *et al.*, 2022a) and *U. (Myanmaratax) savadiensis* Chapurina, Vidrine, Kondakov, Vikhrev & Bolotov, 2022 (in Chapurina *et al.*, 2022a).

The records of *U. (M.) savadiensis* in new host species, accompanied with molecular analyses, contribute significantly to our global picture of the co-evolution of mussels and mites. The series of co-phylogenetic hypotheses is discussed elsewhere (Edwards and Vidrine 2013a), and our focus here is on the mites of the genus *Lamellidens* (Unionidae: Parreysiinae, Lamellidentini).

Material and methods

The unionid freshwater mussels were collected by the second author (AAAKK) from two sites in Jammu and Kashmir of India in September-October 2022: 1/. Gou Manhasan, a tributary of River Chenab (32.7232°N; 74.7334 °E), 20 km from Jammu town and Gajansoo Marh, and 2/. a tributary of River Chenab (32.7651°N, 74.7150°E), 12 km from Jammu town - about 2 m from the bank of the river at a depth of 5 cm in the sediment in Jammu and Kashmir of India (Fig. 1B-C). Mites were collected from gills of *Corbicula cashmiriensis* Deshayes, 1854, *Lamellidens corrianus* (Lea, 1834), and *L. marginalis* by manual dissection in a closed chamber using autoclaved surgical blade, forceps and needles (Fig. 1D-E). Mites were taken from the upper layer of gills of *C. cashmiriensis* and *L. marginalis*, and from a deeper layer of gills of *L. corrianus*. The mite samples were preserved in 96% ethanol immediately after collecting. For morphological research, permanent slides of mite specimens were prepared.

After DNA extraction, the specimen vouchers were morphologically examined. Some of these vouchers were dissected and slide mounted in Faure's medium, while the rest was transferred to Koenike-fluid and stored in the private collection of Vladimir Pešić. DNA sequences prepared in the course of this study were deposited in BOLD (The Barcode of Life Data System) and GenBank (see Tab. 1). The DNA extracts were archived at -80 °C at the Centre for Biodiversity Genomics in Guelph, Canada (CBG; biodiversitygenomics.net).

Molecular analyses were conducted at the Canadian Centre for DNA Barcoding, Guelph, Ontario, Canada (CCDB; <http://ccdb.ca/>). The specimens were sequenced for the barcode region of COI using standard invertebrate DNA extraction (Ivanova *et al.* 2007), amplification (Ivanova and Grainger 2007a) and sequencing protocols (Ivanova and Grainger 2007b).

The final dataset included 28 COI sequences of the genus *Unionicola*: *U. (M.) savadiensis* (n=8), *U. generosa* (n=4), *U. trapezidens* (n=4), *U. robacki* (n=9), *U. sella* (n=2) and *U.*

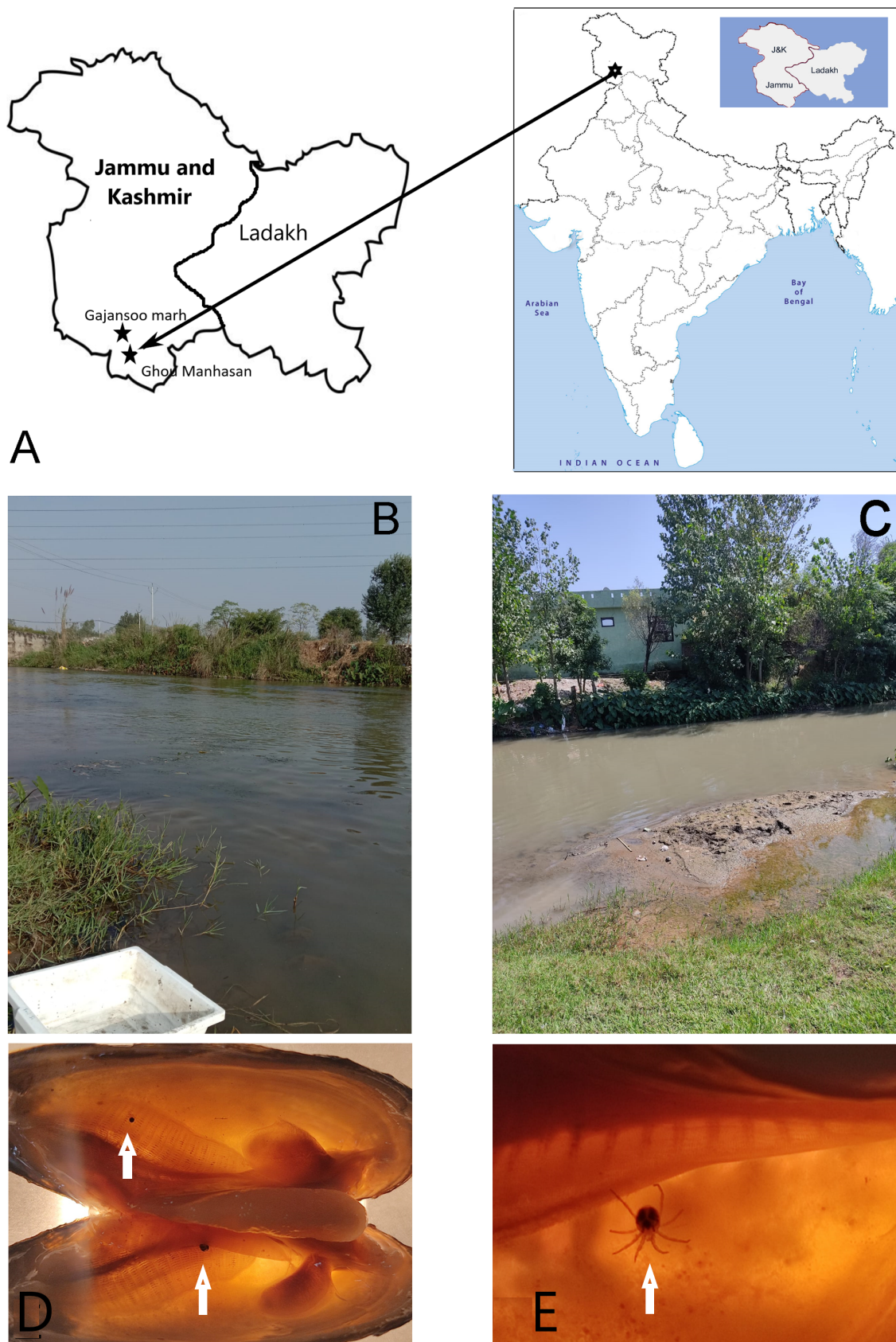


Figure 1 A – Map of studied area; B – Collection area - Gou Manhasan, a tributary of River Chenab; C – Collection area - Gajansoo Marh, a tributary of River Chenab; D–E – Mites on gills of *Lamellidens marginalis* (arrow indicating mites).

ypsilophora (n=1). COI sequence data were additionally taken from Chapurina *et al.* (2021, 2022a) and Pešić *et al.* (2021), and downloaded from the respective sequence data archives. Sequences were aligned with MUSCLE (Edgar 2004). MEGA X software (Kumar *et al.* 2018) was used to calculate Neighbor-Joining NJ trees based on the Kimura 2-parameter (K2P; Kimura 1980) distances (standard for barcoding studies) and pair wise deletion of missing data. The support for tree branches was calculated by the nonparametric bootstrap method (Felsenstein 1985) with 1000 replicates and shown next to the branches. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair.

Results

Molecular Analysis

A 639-658 bp fragments for three specimens of *Unionicola* collected from mussels were successfully amplified. Species identification of studied specimens based on BOLD and GenBank databases is shown in Table 1. Three specimens were identified to species level as they showed sequence matches of 99.53-99.54% with *U. (M.) savadiensis* sequences available in GenBank database. For another three specimens, morphologically attributed to *U. savadiensis* (two specimens collected in the gills of *C. cashmiriensis* from Gou Manhasan and one specimen collected in the gills of *L. marginalis* from Gajansoo Marh; Tab. 1), we amplified and sequenced DNA of the mussel *L. corrianus* instead of water mite DNA. These sequences showed sequence match of 99.65-99.67% with *L. corrianus* (AF339457) from India submitted by Lopes-Lima *et al.* (2021).

To further confirm the taxonomic status of the examined specimens, 28 COI *Unionicola* sequences were downloaded from the BOLD database. The Neighbor Joining (NJ) tree was constructed in MEGA X software and displayed in Figure 2. The sequences retrieved from *Unionicola* specimens from Jammu and Kashmir, formed a distinct cluster with sequences of *U. (M.) savadiensis* from Myanmar (Fig. 2).

Systematics

Family Unionicolidae Oudemans, 1909

Genus *Unionicola* Haldeman, 1842

Subgenus *Myanmaratax* Chapurina, Vidrine, Kondakov, Vikhrev & Bolotov, 2022

Unionicola (Myanmaratax) savadiensis Chapurina, Vidrine, Kondakov, Vikhrev & Bolotov, 2022

Figure 3A-D

Material examined — Jammu and Kashmir: **CC Vial 1**, Gou Manhasan, a tributary of River Chenab, 32.7232°N, 74.7334 °E, from the gills of *C. cashmiriensis*, 2♀; **CC Vial 2**,

Table 1 Details on barcoded specimens, including data and coordinates of sampling sites.

Locality	Coordinates	Host	Voucher Code	BOLD/GenBank id	% Match	Match Process id	Match Species
Gou Manhasan	32.7232° N, 74.7334° E	<i>Corbicula cashmiriensis</i>	CCDB-44301-A04	HYDAL004-23	99.65	GBMNF57322-22	<i>Lamellidens corrianus</i>
Gou Manhasan	32.7232° N, 74.7334° E	<i>Corbicula cashmiriensis</i>	CCDB-44301-A05	HYDAL005-23	99.67	GBMNF57322-22	<i>Lamellidens corrianus</i>
Gou Manhasan	32.7232° N, 74.7334° E	<i>Lamellidens marginalis</i>	CCDB-44301-A07	HYDAL007-23/ OQ801389	99.54	GBMND37031- 21	<i>Unionicola savadiensis</i>
Gajansoo Marh	32.7651° N, 74.7150° E	<i>Lamellidens marginalis</i>	CCDB-44301- A08	HYDAL008- 23	99.67	GBMNF57322-22	<i>Lamellidens corrianus</i>
Gajansoo Marh	32.7651° N, 74.7150° E	<i>Lamellidens marginalis</i>	CCDB-44301- A10	HYDAL010-23/ OQ801388	99.53	GBMND37031- 21	<i>Unionicola savadiensis</i>
Gou Manhasan	32.7232° N, 74.7334° E	<i>Lamellidens corrianus</i>	CCDB-44301- B02	HYDAL014-23/ OQ801387	99.54	GBMND37031- 21	<i>Unionicola savadiensis</i>

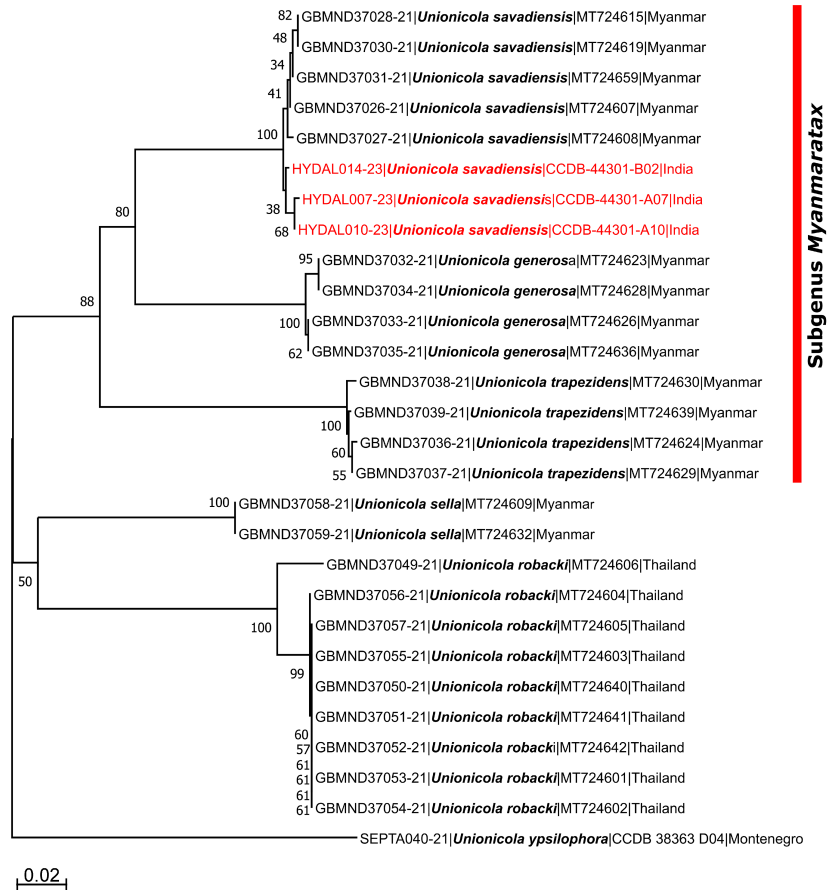


Figure 2 Neighbor-Joining tree of the selected data set of *Unionicola* spp. obtained from 28 nucleotide COI sequences.

ibid., from the gills of *C. cashmiriensis*, 1♂, dissected and slide mounted (RMNH); **CC Vial 3**, ibid., from the gills of *C. cashmiriensis*, 2♀; **LMM Vial 1**, Gou Manhasan, a tributary of River Chenab, 32.7232°N, 74.7334°E, from the gills of *L. marginalis*, 2♂ (1♂ sequenced, Tab. 1); **LMM Vial 2**, Gajansoo Marh, a tributary of River Chenab, 32.7651°N, 74.7150°E, from the gills of *L. marginalis*, 4♂, 1♀ (1♂ sequenced, Tab. 1), 1♂ dissected and slide mounted (RMNH); **LCM Vial 1**: Gajansoo Marh, a tributary of River Chenab, 32.7651°N, 74.7150°E, from the gills of *L. corrianus*, 1♂, 3♀; **LCM Vial 2**, Gou Manhasan, a tributary of River Chenab, 32.7232°N, 74.7334°E, from the gills of *L. corrianus*, 2♂, 2♀ (1♀ sequenced, Tab. 1).

Remarks — *Unionicola* (*M.*) *savadiensis* was originally described from the Indaw Lake in Myanmar. The holotype was collected from the gills of the freshwater mussel, *L. savadiensis* (Nevill, 1877), and the paratypes from the gills of the type host species and of *L. generosus* (Gould, 1847). Chapurina *et al.* (2022a) assigned *U. savadiensis* to the newly described subgenus *Myanmaratax* Chapurina *et al.* 2022, which, in addition to *U. (M.) savadiensis*, includes three more mussel-associated gill mites, i.e., *U. (M.) brandti* Vidrine, 1985, *U. (M.) generosa*, and *U. (M.) trapezidens* Chapurina, Vidrine, Kondakov, Vikhrev & Bolotov, 2022. Members of this subgenus use freshwater mussel (Bivalvia: Unionidae) species of the genera *Lamellidens* and *Trapezidens* in Western Indochina as well as *Contradens* and *Ensidens* in the Sundal and Subregion (Chapurina *et al.* 2022a) as the primary hosts.

The collected specimens from Jammu and Kashmir match the original description of *U. (M.) savadiensis* in regard to shapes of coxal plates and genital fields (Fig. 3A, C), palps (Fig. 3B) and terminal segments of the fourth legs (Fig. 3D).

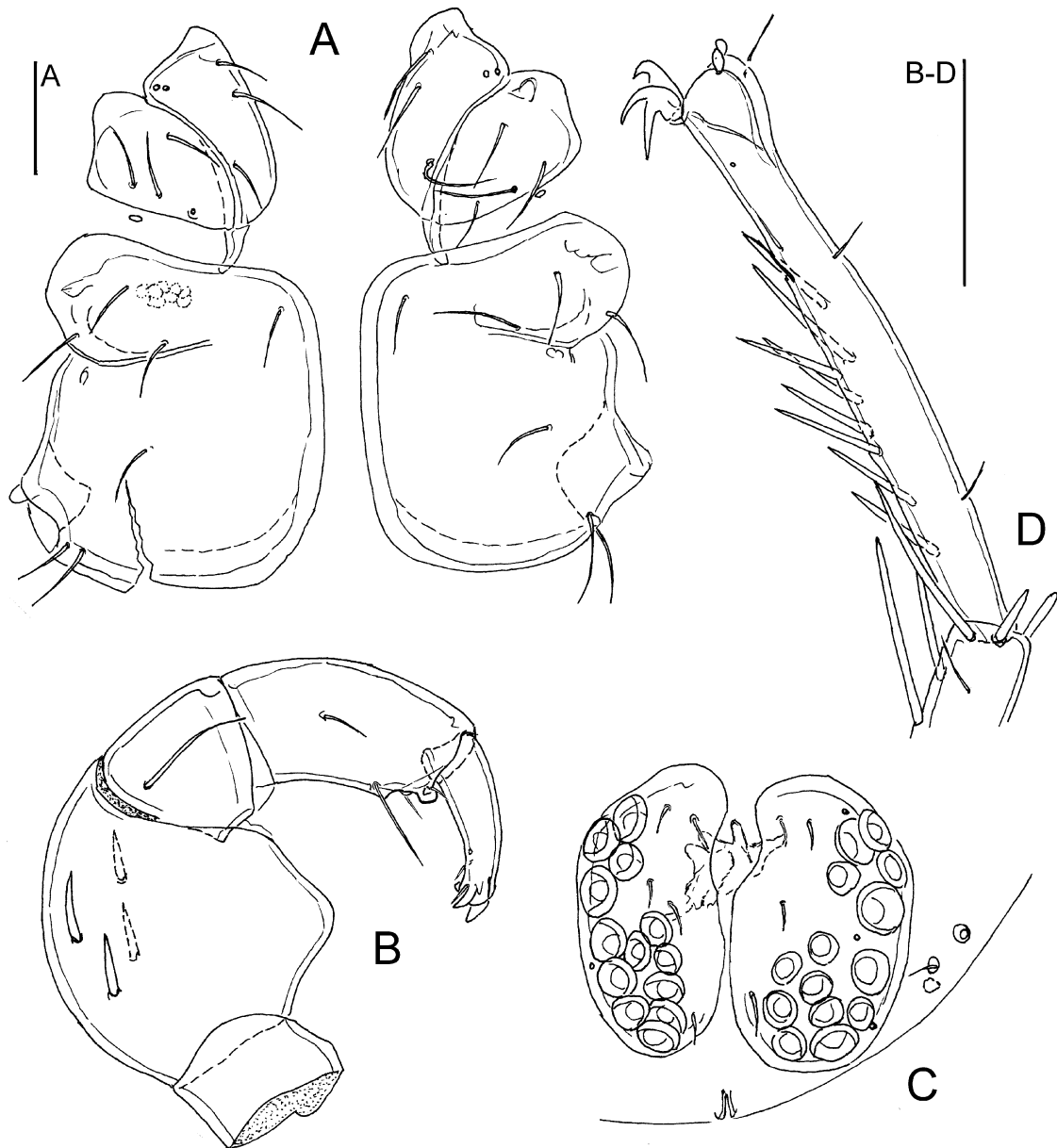


Figure 3 *Unionicola savadiensis* ♂ (A, C – CC Vial 2 Gou Manhasan; B, D – LMM Vial 1, Gou Manhasan). A – coxal field; B – palp; C – genital field; D – terminal segment of the fourth leg. Scale bars = 100 µm

Discussion

In this study, *U. (M.) savadiensis* was found to be associated with three species of molluscs: *L. marginalis*, *L. corrianus* and *Corbicula cashmirensis*. Our data reveal a much wider range extension of *U. (M.) savadiensis* and its mussel host, and fit the hypotheses of coevolution and the rather tight host-parasite association within Asian mussels, a phenomenon that has also been proven for gill mites and their unionid mussel hosts in North America (Edwards and Vidrine 2006, 2013b, 2020; Edwards *et al.* 2010; Ernsting *et al.* 2014).

We tend to refute the discovery of the genus *Corbicula* serving as a host for *U. (M.) savadiensis*. *Unionicola* mites have not been verified from the family Cyrenidae Gray, 1840, which includes the genus *Corbicula*. Spurious records however exist, including records from

Egypt (Abdel-Garber *et al.* 2018)—these were apparently compromised as mussels, including *Corbicula* and infected unionoid mussels, were held in the same container for transport and probably resulted in vagrancy among these mites. Vagrancy is common when mussels of different species hosting different mites are held together under stress (Edwards and Vidrine 2013a). We suspect that this also accounts for our current observation in India. We showed that all DNA sequences of mussels, which were amplified instead of mite DNA, belong to *L. corrianus*, also from Gou Manhasan (where *C. cashmiriensis* is listed as a “host”) and from the Gajansoo Marh (where *L. marginalis* is listed as a “host”). Therefore, we can speculate that the members of *U. savadiensis* in all three localities parasitize *L. corrianus*, but occasionally can occur also on two other species (without feeding on them), and their presence on the latter species should be treated as vagrant associations. This is confirmed by the observation that mites were found on the surface of gill in *L. marginalis* and *C. cashmiriensis*, while mites were found in deeper layers of the gill in *L. corrianus*.

Secondly, in addition to discussion of coevolution, the adaptations to new hosts, i.e. ‘host-switching’ (Cichy *et al.* 2016), must be taken into account. Such a situation may occur in the case of *Unionicola ypsilophora* (Bonz, 1783), which inhabits invasive *Sinanodonta woodiana* (Lea, 1834) in Poland (Cichy *et al.* 2016). Unlike ‘vagrancy’ — where mites move from one species of mussel to another species, ‘host-switching’ (also known as ‘Association by Colonization’ in studies of coevolution and cophylogeny) is an uncommon phenomenon, at least in the literature. However, some mite interactions can only be explained by incidences of ‘host-switching,’ with the best examples being the occurrences of unionicolid mites in freshwater snails. These snail mites are apparently derived from mussel mites, and this is evident in the morphological and molecular studies by Wu *et al.* (2009, 2012) in Chinese mussel and snail mites (see also Edwards and Vidrine 2013a). In this study, we do not suggest that ‘host-switching’ has or may be occurring among our Indian findings, but we do want to note that it was considered.

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