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An exploration of the hidden endosymbionts of *Corbicula* in the native range

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In a sense, a single individual host can be considered an ecosystem. For example, a host may be seen as an environment that can be colonized by a high diversity of entities such as different mutualists, commensals, and parasites. These entities may interact with each other, potentially competing for space, energy and resources, and ultimately being also highly influenced by the overall ecophysiological condition of the host (Rynkiewicz et al., 2015). A key point in this rationale is that when we consider a host as an ecosystem we should recognize that individual hosts are not homogenous environments in which symbionts, resources and immune components are similar (Rynkiewicz et al., 2015). This situation is highly useful to assess possible spatial and temporal differences in mutualists, commensals, and parasites dynamics using also the different host compartments (e.g., organs, tissues) as different habitats inside the ecosystem where nutrients, energy and parasites can circulate between the distinct compartments (Rynkiewicz et al., 2015).

In the last 4 years, we have been working with *Corbicula* clams in Southeast Asia, and we have been using this rationale of individual hosts as ecosystems mainly to describe their hidden diversity.

The taxonomic identification of single species of genus *Corbicula* is still difficult even using a molecular approach (for a review please refer to Pigneur et al., 2014). Conversely, research into this genus has rapidly increased in the past few decades in Europe and North America, mainly because of interest in their invasive behavior (Ilarri & Sousa, 2012). Despite the high interest in the study of this genus, particularly the ecological and economic impacts generated in the invaded range, to date there have been very few studies on endosymbionts associated with *Corbicula* clams. Currently, only some trematodes and *Unionicola* mites have been reported for *Corbicula* clams in Asia and North America (Brian & Aldridge, 2019; Taskinen et al., 2021). According to Taskinen et al. (2021) *Corbicula* spp. are completely free from parasites in European waterbodies. Therefore, this absence of enemies in the invaded range could, in part, explain their invasive success. Escape from natural enemies such as predators, competitors, and parasites (The Enemy Release Hypothesis [ERH]) was proposed as a possible mechanism explaining the success of introduced species (Torchin et al., 2003). Nevertheless, it is not clear whether the absence of enemies in the invaded range is due to a lack of enemies there, or because the invaders naturally have low enemy pressure anywhere. The loss of parasites could be highly beneficial, as parasites have been shown to depress freshwater bivalve populations (Bolotov et al., 2019; Brian et al., 2021). However, the lack of studies in the native range impairs further comparisons and the validity or not of the role of the ERH on the invasion success of *Corbicula*.

Since 2018 we have been sampling several Asian countries, including South Korea (July 2018), Thailand (March 2018), and Laos (March 2020) (Appendix S1; Appendix S1: Figure S1). Although these investigations have been mostly focused on the phylogeny and taxonomy of freshwater bivalves, we were able to collect representative material on endosymbionts of *Corbicula* clams (Figure 1).

In summary, it was discovered for the first time that mayflies, chironomids, and fishes can use the *Corbicula* clams as a host for their larval development (Figure 1; Appendix S1: Table S1). The larvae of mayflies *Symbiocloeon* spp. (Ephemeroptera: Baetidae) were found on the gills of *C. fluminea* from Thailand and Laos. In addition, the larvae of chironomids (Chironomidae) were discovered under the mantle of *C. fluminea* from Laos. Finally, the embryos of Soldatov's thicklip gudgeon *Sarcocheilichthys soldatovi* were detected in the mantle cavity of *C. leana* from South Korea (Appendix S1). It should be pointed out that we did not examine *Corbicula* for trematodes in this study.

The mayfly larvae were discovered on the gills of *C. fluminea* from the Lam Chae Stream and Mun River (Mun River basin) in Thailand and from the Sein Kaphoe River in Laos (undescribed species *Symbiocloeon* sp. 1 and *Symbiocloeon* sp. 2, respectively). In total, 211 *Corbicula* individuals from two localities were examined and among them 55 clams were infested by 56 larvae of two species of *Symbiocloeon* (Figure 1d–g; Appendix S1: Table S1).

The associations of mayflies with freshwater bivalves have been previously recorded in Southeast Asia, India, and Africa (Gillies & Elouard, 1990; Müller-Liebenau & Heard, 1979). This relationship of bivalve-associated mayflies with their hosts is usually considered as commensalism (Müller-Liebenau & Heard, 1979) or inquilinism (Gillies & Elouard, 1990). These mayfly larvae have some adaptations for completing these stages of their life cycles inside the mantle cavity of freshwater mussels (Order Unionida) (Gillies & Elouard, 1990; Müller-Liebenau & Heard, 1979) and in our particular case we found a reduction in integument chitinization of breast and abdomen segments, as well as the disappearance of stiff bristles, teeth, and spines.

We also found 13 larvae of one species of chironomid under the mantle of 11 *C. fluminea* clams from Laos (among 36 observed) (Figure 1i–k; Appendix S1: Table S1). It is noteworthy to mention that one *Corbicula* contained both chironomid and mayfly larvae (Figure 1g), and two clams had two chironomid larvae under the mantle of the right and the left valves. Chironomid larvae in freshwater bivalves have been found in Europe, New Zealand, Canada, and the USA (Brian & Aldridge, 2021; Forsyth & McCallum, 1978; Funk et al., 2018; Gordon et al., 1978). Some authors have classified these associations as inquiline commensalism (Forsyth & McCallum, 1978). Funk et al. (2018) and Gordon et al. (1978) think that the larvae of chironomids are parasitic on bivalves. According to the authors, the larvae actively feed upon the gill tissue or eggs and/or glochidia of bivalves. We also found damage in the mantle of *Corbicula* clams, probably in the place for the larva's penetration (Figure 1i,k). Therefore, and in agreement with Funk et al. (2018) and Gordon et al. (1978), the association between *Corbicula* and chironomid larvae can be considered as parasitism. Funk et al. (2018) presumed that chironomid larvae spend all or nearly all of their larval life within their mussel host, starting with first instars, free-living inside the mantle cavity, and later entering the gills to complete larval development. However, we did not find chironomid larvae on the gills of *Corbicula* clams. The larvae of all instars and pupae were located under the mantle (Figure 1h,j). Therefore, the larvae do not necessarily migrate to the gills to complete their development. Available literature data show that chironomid larvae are usually found in the mantle cavity around the gills, gonads, and siphons (Brian & Aldridge, 2021; Funk et al., 2018). Forsyth and McCallum (1978) observed chironomid larvae in mussels between the outer surface of the mantle and the inner surface of the valve, and then they migrated to the margin of the valve opposite the inhalation siphon (Forsyth & McCallum, 1978). Here we present a novel observation, because the chironomid larvae were found under the mantle and burrowing through it.

Our results and a review of available published data may indicate that the clam-associated mayflies and chironomids are narrow host specialists (Müller-Liebenau & Heard, 1979; Funk et al., 2018). Probably, all larval stages of the life cycles of these mayfly and chironomid species take place in the mantle cavity of *Corbicula* clams.

Finally, in the mantle cavity of two investigated (among four observed) *C. leana* individuals from an irrigation channel near Mangyeong River in South Korea, five eggs of Soldatov's thicklip gudgeon were found (Figure 1a–c; Appendix S1: Table S1). The genus *Sarcocheilichthys* (Cyprinidae) has an unusual mating system, in which the females lay eggs inside the mantle cavity of freshwater mussels and *Corbicula* clams (Nishino, 2012). The

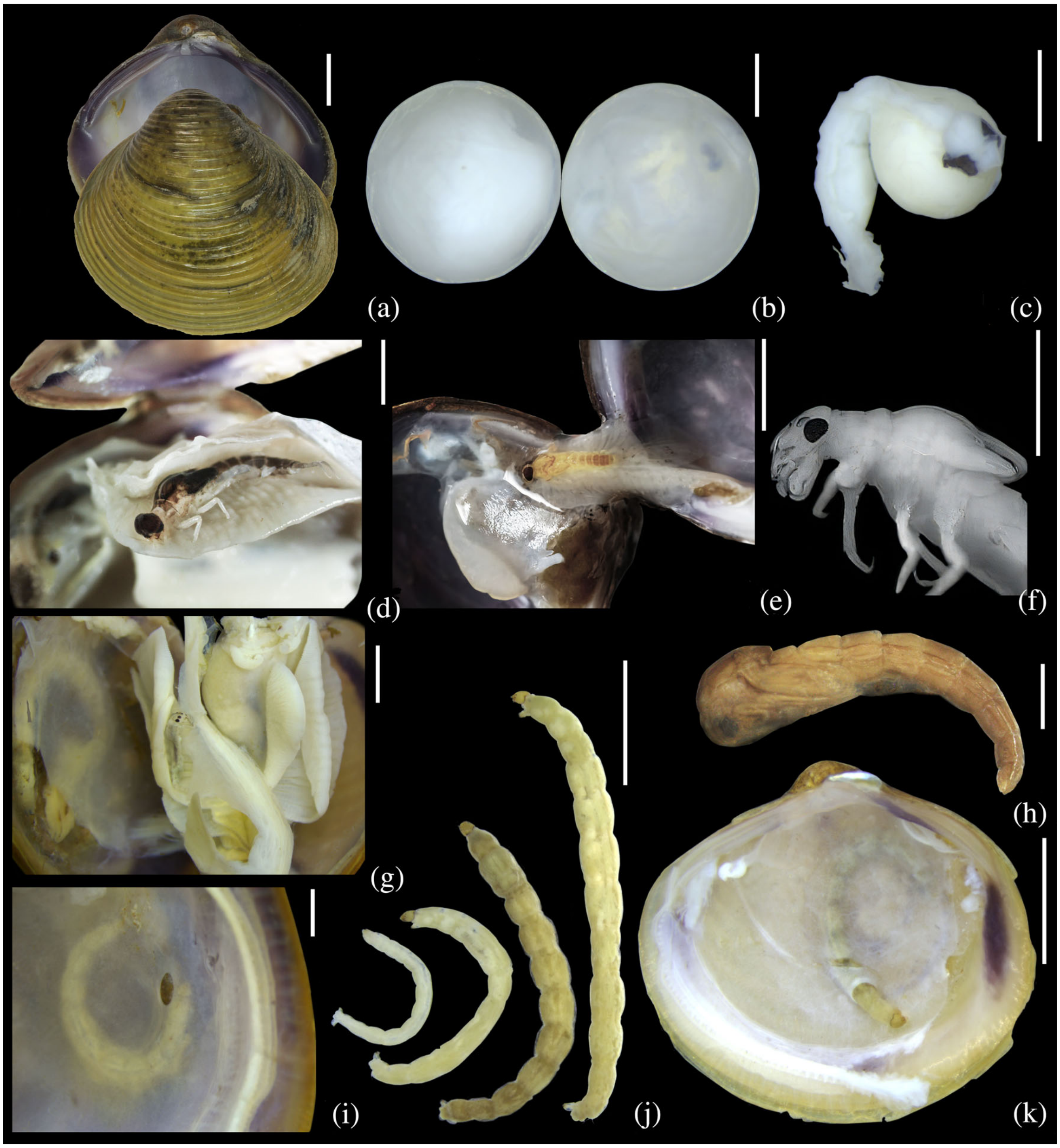

FIGURE 1 *Corbicula* clams and their endosymbionts. (a) Morphology of *Corbicula leana* from irrigation channel near Mangyeong River, South Korea, 35.9143N, 126.7077E, 11 July 2018. (b, c) Embryos of gudgeon *Sarcocheilichthys soldatovi* from the mantle cavity of *C. leana*, irrigation channel near Mangyeong River, South Korea, 35.9143N, 126.7077E, 11 July 2018. (d) Larva of *Symbiocloeon* sp. (Ephemeroptera) in the mantle cavity of *C. fluminea*, Sein Kaphoe River, Laos, 14.7710N, 106.1704E, 3 March 2020. (e, f) Larvae of *Symbiocloeon* sp. (Ephemeroptera) in the mantle cavity of *C. fluminea*, Lam Chae stream, 14.4403N, 102.2766E, Thailand, 13 March 2018. (g) Chironomid and mayfly (*Symbiocloeon*) larvae in the cavity of *C. fluminea*, Sein Kaphoe River, 14.7710N, 106.1704E, Laos, 3 March 2020. (h) Chironomid pupae from the cavity of *C. fluminea*, Sein Kaphoe River, Laos, 14.7710N, 106.1704E, 3 March 2020. (i–k) Differently sized chironomid larvae from the cavity of *C. fluminea*, Sein Kaphoe River, Laos, 14.7710N, 106.1704E, 3 March 2020.

spawning host selection was investigated earlier for *Corbicula* clams and several other *Sarcocheilichthys* species (Nishino, 2012). In the present study, it was found that *S. soldatovi* also lay eggs in the mantle cavity of *Corbicula*. A classic example of spawning relationships between fish and freshwater mussels is bitterling: unionid mussel associations (Reichard et al., 2007). Additionally, several cyprinid fishes in the genus *Barbus* and the clupeid species *Alosa sapidissima* place eggs into the gills of freshwater mussels (Wisniewski et al., 2013). Our study has added one more fish species to the list describing the complex relationship between fish and freshwater bivalves.

The taxonomic richness of insects with clam-associated larvae in Asia has been found to be largely underestimated.

This situation is especially important in a context of co-extinctions (i.e., the loss of one species as a result of the extinction of a species on which it depends; Dunn et al., 2009). Although *Corbicula* populations in the native range are still abundant and widespread, it is possible that local extinctions of *Corbicula* may result in the concurrent loss of specific mutualists and/or parasites. In addition, and outside the native range, invasive freshwater bivalves such as *Corbicula* can experience less pressure from parasites or diseases, thereby increasing their competitiveness against native species (Taskinen et al., 2021). According to these authors, *Corbicula* clams are completely free of parasites and endosymbionts in European water bodies, which may be a great ecological advantage at least in the first steps of the invasion process. Understanding the factors affecting the success of freshwater bivalve invasions, such as parasitism, can aid invasion control and conservation of local, native (endangered) bivalves (Brian & Aldridge, 2019). Future research should be aimed at studying the nature of the relationships between *Corbicula* clams (and many other bivalve species) and their endosymbionts, the possible positive and negative effects of these associations, and the life cycles of the endosymbionts. Further work is required to fully characterize host–parasite interactions in this and other similar systems. Our study is just a small example of the hidden relationships between freshwater bivalves and other aquatic animals that are still to be described in Asian (and other continents) freshwater ecosystems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The specimens are deposited (under registration numbers Fbir9, N12, N13, N67, N68) in the Russian Museum of the Biodiversity Hotspots (RMBH) of N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia). Data (Bespalaya et al., 2022) are available in Figshare at https://doi.org/10.6084/m9.figshare.19878763.

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