

of MO. The seeded cells were cultured at 38.5°C under 5% CO<sub>2</sub> in the air for 24 h and monitored cell attachment rate and proliferation. The morphological changes after 24 h of culture were captured using an inverted microscope (Nikon, Tokyo, Japan) at 200× magnifications. We observed that seeded somatic cells were not attached to culture surface and cells have bleb membrane similar to embryos (Figure 2 a). Somatic cells were attached and proliferated when MO was not overlaid in the culture dishes. To reconfirm MO toxicity, above-mentioned cell assay was performed with a new lot of MO, and it was observed that seeded cells were attached and proliferated. This indicates that an old lot of MO was toxic to embryos. Hereafter, we have regularly been testing each lot of procured MO using the described somatic cell assay to avoid MO toxicity to embryos.

The present study describes a cheap and simple somatic cell assay to test the quality of MO. This assay can easily be performed by embryologists who are working in human IVF and farm animal

laboratories. In addition to somatic cell assay, further analysis can be done to determine the actual level of peroxidation, alkenes and aldehydes, and residual Triton X-100 in each lot of MO. Since the human IVF embryos are not available in a large number and they are mainly used to produce babies, we suggest using the embryos of farm animals to screen the quality of MO and other compounds such as serum and antibiotics.

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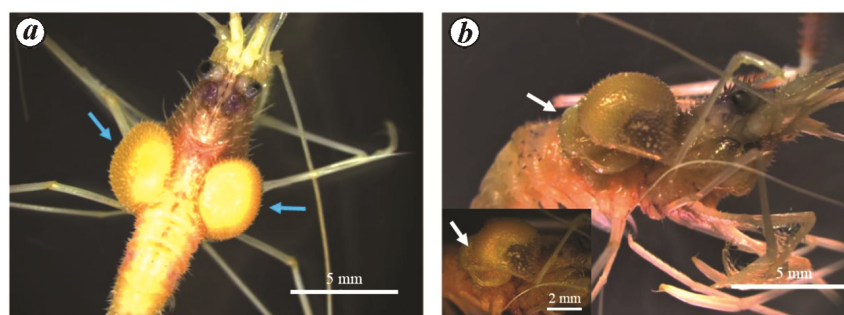
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## Infestation of bopyrid isopod parasite (Bopyridae) on ‘coral banded boxing’ shrimp *Stenopus hispidus* Olivier, 1811 (Stenopodidae) in the Lakshadweep archipelago

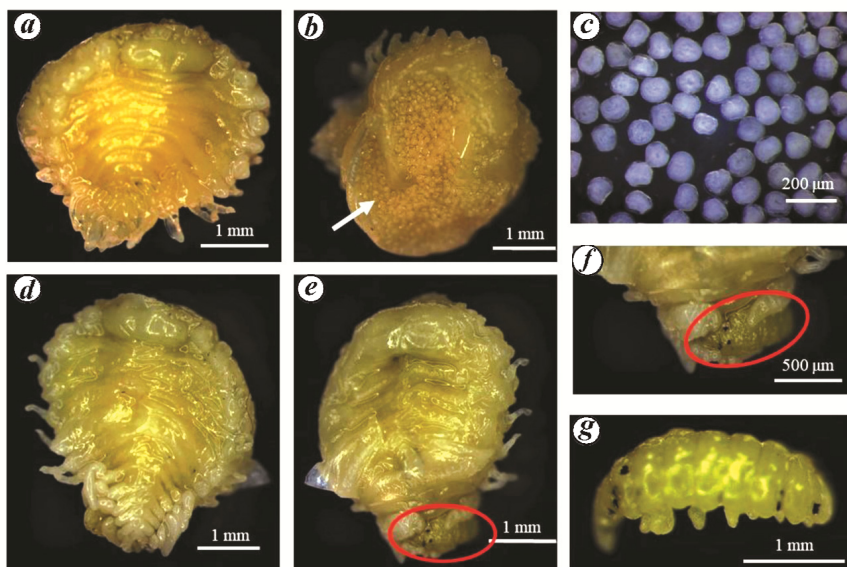
Bopyrid parasitic isopods (family Bopyridae Rafinesque) are unique and well known to utilize marine crustaceans as both intermediate and definitive host during their life cycle<sup>1,2</sup>. The family Bopyridae is the largest and highly diverse group with 605 species under 8 subfamilies<sup>2,3</sup>. The males are smaller than females (exhibiting reverse sexual dimorphism) and are attached to the ventral posterior region of the female abdomen<sup>4</sup>. Bopyrids used to infest either branchially or abdominally on a variety of crustaceans including brachyurans, anomurans, penaeids and carideans<sup>1,2,5–7</sup>. Infestation of parasites has deleterious effects on host such as reduction in growth, energy and mainly resulting in reproductive failures<sup>8–11</sup>. It can also affect the escape response of host to predators<sup>12</sup>. Extensive studies have revealed bopyrid parasite infestation on caridean shrimps<sup>3</sup>. However, the infestation of

parasite on Stenopodean shrimps has been rarely documented<sup>10,13–15</sup>. The present contribution is one such rare observation on the infestation of bopyrid isopod parasite on the marine ornamental shrimp *Stenopus hispidus* from Lakshadweep.

The ‘barber pole’ or ‘coral banded boxing’ shrimp *Stenopus hispidus* Olivier (Infraorder Stenopodidea) is recognized for its remarkable coloration and circum-tropical distribution<sup>16,17</sup>. It is the only species among the family Stenopodidae to successfully pass through the major



**Figure 1.** Bopyrid parasite infested ‘coral banded boxing’ shrimp *Stenopus hispidus* Olivier. *a*, Dorsal view of carapace region showing the bulbous structure (blue arrows) on the branchial region of cephalothorax; *b*, Lateral view of carapace (right side) showing the presence of bopyrid parasite (female) (white arrow), inset: the adult female carrying eggs (white arrow).



**Figure 2.** Bopyrid parasite *Argeiopsis inhacae* Kensley. *a*, Ovigerous female, TL 4.60 mm, dorsal view; *b*, same, ventral view carrying eggs in the abdomen region (white arrow); *c*, developing eggs; *d*, non-ovigerous female, TL 4.72 mm, dorsal view; *e*, same, ventral view, showing male attached to the posterior region of abdomen (red circle); *f*, same, magnified view; *g*, male, lateral view, TL 2.0 mm.

biogeographical barriers<sup>18,19</sup> and inhabits the tropical coral reefs of the Indo-Pacific, including Red Sea and the Atlantic Oceans<sup>17,20</sup>. Several studies have used *S. hispidus* as a model organism to study the reproduction and mating<sup>21–23</sup>, population dynamics<sup>24</sup>, phylogeny and phylogeography<sup>20,25</sup>. It is also widely collected from the coral reef areas of Indo-Pacific and supplied to meet the demand of global marine aquarium trade<sup>26</sup>. In India, *S. hispidus* has been recorded from the major coral reef regions of India such as Lakshadweep<sup>27</sup>, Andaman and Nicobar Islands<sup>28</sup> and Gulf of Mannar<sup>29</sup>.

Surprisingly, during the recent marine biodiversity survey conducted at Lakshadweep to collect ornamental shrimps for captive propagation, we obtained a group of six individuals of *S. hispidus* (2 males and 4 females including 2 ovigerous) using scoop nets from the south eastern tip of the Agatti island by adopting snorkeling and skin-diving methods. All the individuals looked good and healthy except for one with a bulbous structure on both sides of the branchial regions (Figure 1 *a*). Careful examination of the individual indicates the infestation of bopyrid isopod parasites on both sides of the branchial chamber (Figure 1 *b*). Subsequently, the infested parasite was identified as *Argeiopsis inhacae* Kensley<sup>13,14</sup> which is a specific parasite infesting *S. hispidus*. Isopod

parasite infestation has been extensively documented from the penaeid and caridean shrimps<sup>7</sup> and food fishes of Indian waters<sup>30</sup>. Nevertheless, the knowledge of parasite infestation on stenopodidean shrimps is still missing.

In the laboratory, the preserved specimen of *S. hispidus* (carapace length, CL, 7.35 mm; total length, TL, 17.2 mm) was measured under stereo zoom microscope (Leica SMZ165FC fixed with DFC310X camera, Germany) with an accuracy of 0.01 mm. The sex of the shrimp was determined as female, but no visible gonadal or embryonic development was observed. In total, three individuals of parasites (two females including one ovigerous and one male) were retrieved from both the sides of the branchial chamber. The TL of females was 4.60 mm (ovigerous) (Figure 2 *a–c*) and 4.72 mm (non-ovigerous) (Figure 2 *d–f*) and the TL of male was 2.0 mm (Figure 2 *e–g*). The ovigerous female contains almost 2560 eggs with the presence of distinct blastoderm (Figure 2 *b* and *c*). The parasite exhibits clear reverse sexual dimorphism with larger females and smaller male, indicating socially monogamous pairs as reported for other parasitic bopyrids<sup>31</sup>. Interestingly, the presence of both ovigerous and nonovigerous female on either side of the host suggests that the male may frequently roam between the females for copulation

indicating promiscuous mating behaviour. Experimental studies on individual-level reproductive behaviour will provide insights into the evolution of mating strategies in the bopyrid parasites.

Infestation of *A. inhacae* on *S. hispidus* has been observed from South eastern Africa<sup>13</sup>, the Philippines<sup>10,14</sup>, Indonesia and Hawaii (JW Goy, pers. obs.) and in India (present study). Lastly, the observation presented here does not reveal any information on the natural history of the parasite. Therefore, we argue in favor of additional exploration in the major coral reef areas of India to understand the diversity of bopyrid isopod parasites infesting marine crustaceans. Further, knowledge on parasite–host interaction and the impact of parasite on host reproductive strategies will help to develop successful protocols for the captive propagation of marine ornamental shrimps for sustainable aquarium trade in India<sup>32</sup>.

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