



Note

First record of the leech, *Glossiphonia complanata* (Linnaeus, 1758) infection in freshwater pearl mussel *Lamellidens marginalis* (Lamarck, 1819)

A. PAUL, S. PATTANAYAK, S. PRADHAN, S. SAURABH AND P. K. SAHOO

ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar - 751 002, Odisha, India

e-mail: pksahoo1@hotmail.com

ABSTRACT

Demand and production of freshwater pearl mussel *Lamellidens marginalis* (Lamarck, 1819) have increased many folds in the last few decades. There are only very scanty reports of infective agents and their pathogenicity affecting this particular mussel species. Heavy infestation and mortality of *L. marginalis* were observed in the ponds of Freshwater Pearl Mussel Unit of ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar during April, 2017. All (100%) of the pond collected pearl mussels were infected with leeches both on outside as well as internal parts of the shell at 5-20 numbers per mussel. The infected mussels showed low surgical success rate for pearl production. Mortality to the tune of 30% was recorded in the tanks of 35 l capacity stocked with 10 numbers of mussels. The infectious leech was identified as *Glossiphonia complanata* by 28S rDNA PCR followed by sequencing and phylogenetic analysis. The present communication is the first report on *G. complanata* causing mortality in *L. marginalis* farming.

Keywords: *Glossiphonia complanata*, Infection, *Lamellidens marginalis*, Mortality, Mussel

Freshwater mussels are having high economic significance in aquaculture owing to pearl production as well as food value. Mussels being filter feeders help to improve water quality in aquaculture systems by filtering of water and therefore mussel culture is environment friendly (Saurabh *et al.*, 2016). Different types of culture systems *viz.*, ponds, rivers, lakes and reservoirs are utilised for production of freshwater pearls. The pearl mussel *Lamellidens marginalis* (Lamarck, 1819) rules over the total freshwater pearl industry in the country. India has more than 52 species of mussels distributed in various freshwater resources. Among these, *L. marginalis*, *L. corrianus* and *Parreysia corrugate* produce good quality pearls (Janakiram, 2003). Generally, pH neutral to slightly alkaline waters are conducive for mussel production. Management of good quality of natural food sources in the culture environment is essential in mussel cultivation. Dissolved oxygen (DO), pH and other water quality parameters are also important. However, disease outbreak in the mode of parasitic infection is one of the limiting factors in this species. So far, viral disease evidence was reported from the mussel *Hyriopsis cumingi*, which is commonly found in China (Zhang *et al.*, 1986; Shao *et al.*, 1993). Parasites and infectious agents generally cause lesions that include inflammation and regressive phenomenon. The other reported pathogens in freshwater mussels and other freshwater bivalve molluscs include bacteria and different groups of parasites but their roles in diseases are yet to be established (Grizzle and Brunner,

2009). Leeches occur worldwide in all ecosystems except in Antarctica (Govedich and Bain, 2006). *Glossiphonia complanata* (Linnaeus, 1758), is having ubiquitous ecological distribution (Elliot and Mann, 1979) which primarily feeds from different gastropods (Harding, 1910; Wrona *et al.*, 1979). The present study intended for molecular identification and characterisation of a leech infection causing large scale mortality in cultured *L. marginalis*.

Heavy infestation and mortality of freshwater pearl mussel (*L. marginalis*) were observed in the Freshwater Pearl Mussel Unit of ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar during April, 2017. Samples of live mussels were collected and observed for disease causing agent. All (100%) of the mussels collected from the pond ecosystem were found to be infested with leech, at various densities of infection (5-20 no. mussel⁻¹). The infested mussels were stocked at 10 no. per 35 l water for further investigations. Visible leeches were observed on the outside of shell as well as on internal body parts of infected mussels (Fig. 1a,b). Cumulative mortality of 30% was observed in the cultured tanks within 2-3 days after collection from the pond. The infected mussels also showed low surgical success rate as compared to uninfected mussels. Individual parasites were collected with the help of sterile forceps, examined and preserved in 100% ethanol (EMSURE Merck) for molecular identification.

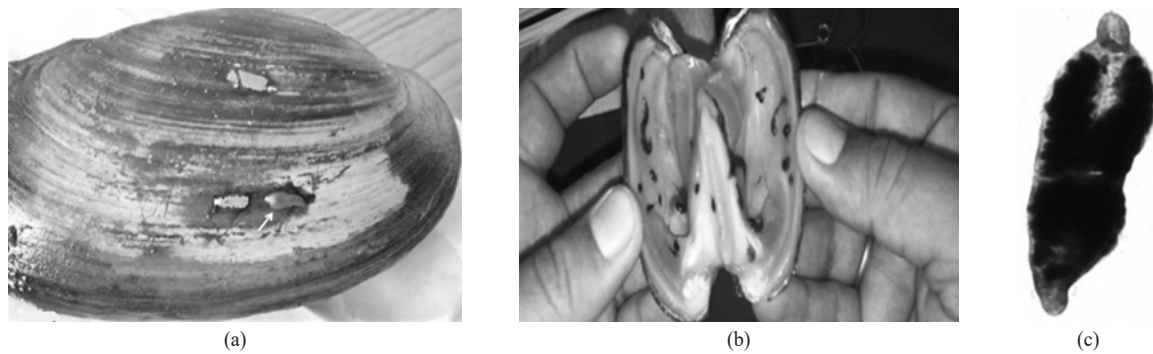


Fig. 1. (a) Outer shell of *L. marginalis* infected with *G. complanata* and its egg clutches; (b) Body cavity of *L. marginalis* infected with *G. complanata* and (c) Ventral view of the isolated leech

DNA extraction was performed following phenol-chloroform extraction method of Sambrook and Russell (2001) from the individual parasite. DNA obtained were subjected to 28S rDNA PCR followed by sequencing. Already published 28s DG primers (forward primer: 5'ACCCGCTGA ATTTAAGCAT 3' and reverse primer: 5' CTCTTCAGAGTACTTTTCAAC 3', IDT, Mumbai, India) described by Randrianambinintsoa *et al.* (2014) were used in this study. PCR reaction mixture (25 μ l) comprised nuclease free water (19.50 μ l), assay buffer (2.50 μ l, 10X), dNTPs (0.50 μ l), forward primer (0.50 μ l) and reverse primer (0.50 μ l), *Taq* DNA polymerase (0.25 μ l) and leech DNA (1.25 μ l of 50-100 ng μ l⁻¹). The PCR conditions were followed as mentioned by Randrianambinintsoa *et al.* (2014) and PCR was performed in an automatic programmable thermal cycler (Applied Biosystem). PCR amplification was followed by electrophoresis with an expected product size of 400 bp. The gel image was captured using gel documentation (Bio Rad, Germany). Targeted PCR product obtained was sequenced at the sequencing facility of M/s AgriGenome Lab, Kochi, India.

NCBI-BLAST program was used to compare the obtained sequence of 400 bp with the available sequences in GenBank. The obtained consensus sequence of the isolate was deposited in NCBI-GenBank. Phylogenetic analysis was carried out using the Maximum Likelihood method of MEGA software (version 4.1) and phylogenetic tree obtained was displayed with Tree View software (version 1.6.6). Previously published 28s rDNA sequences of *G. complanata* obtained from GenBank were used to draw relationships between the present leech isolate and other leech species. Bootstrapping was performed to calculate the confidence values of clusters formed.

The parasites were identified as leeches on morphological examination (Fig. 1c). Eggs were laid in clutches and each clutch carried 23 to 45 numbers of

eggs. PCR using 28s rDNA primers produced an amplicon size of 400 bp (Fig. 2). The partial sequence (400 bp) of 16S rDNA of *G. complanata* isolate was deposited in the GenBank database (Accession no. MF326542). The sequence obtained showed 99% homology with that of *G. complanata* (GenBank Accession no. JQ821578) having only three bases of difference between two sequences (Fig. 3) and the leech isolate was confirmed. The leech isolate in the present study upon sequencing and phylogenetic analysis clustered with *G. complanata* isolates earlier reported from elsewhere (Fig. 4).

In benthic ecosystem of ponds and other aquatic bodies, role of freshwater mussels is very much noteworthy. Many people belonging to the hilly parts of India use this species (*L. marginalis*) as food and it is a species of choice for small scale aquaculture. *L. marginalis* has medicinal value besides its use for pearl culture (Dey, 2008; Prabhakar and Roy, 2009; Chakraborty *et al.*, 2012; Suryawanshi and Kulkarni, 2014). The genus *Lamellidens* is represented by nine species and two

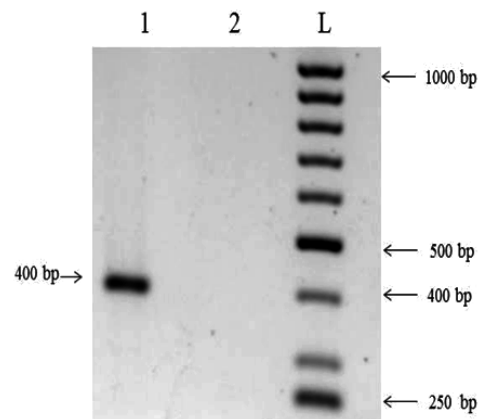


Fig. 2. Gel image of the products (400 bp) amplified with the primer DGR45 F/R. Lane 1 - Sample amplified with DGR45 F/R, Lane 2 - Negative control; L - 50 bp ladder

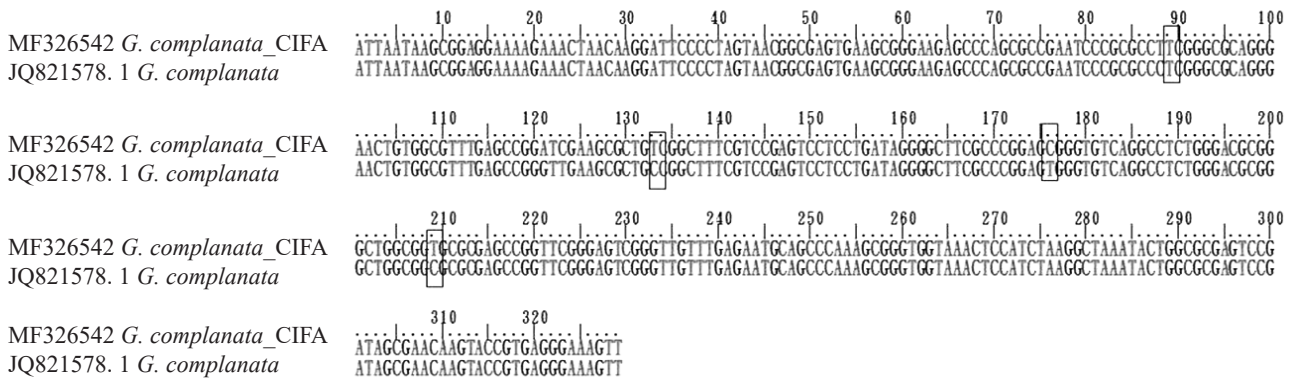


Fig. 3. 28s rDNA sequence distinctiveness of CIFA leech isolate (MF326542) with *G. complanata* reported earlier (JQ821578.1)

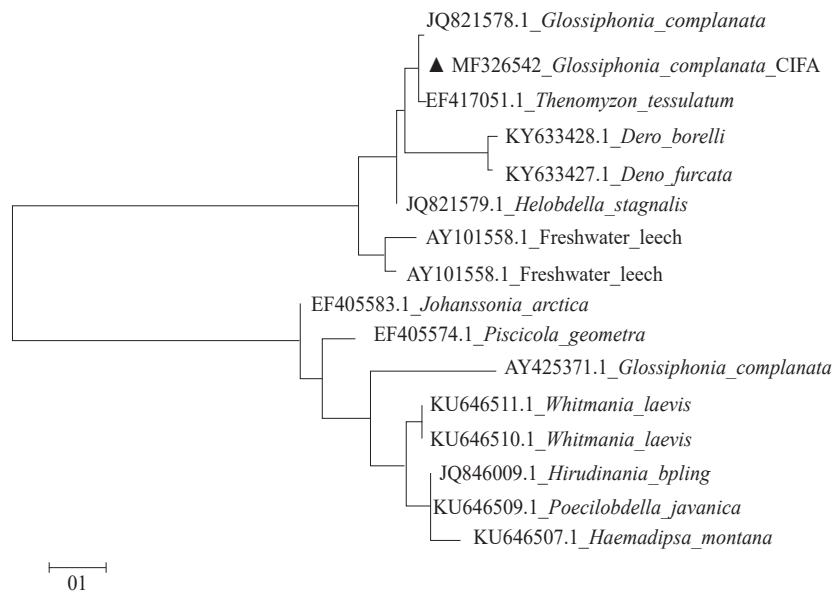


Fig. 4. Phylogenetic analysis of *G. complanata* CIFA isolate using 28s rDNA sequences already reported for other related species. Scale bar indicates genetic distance

sub-species. Generally, *Lamellidens* are distributed in slow or no flowing habitats like ponds, lakes and reservoirs (Misra and Mukhapadhyay, 2008). However, report on diseases of this particular important pearl mussel species is very scanty. In this study we used 28s rDNA PCR for molecular identification of leech infesting *L. marginalis*, which was causing high morbidity and mortality in the mussel. The parasite was observed both inside and outside parts of the mussel body. Similarly, the leech species *Placobdella parasitica* has been recorded to infest the mantle cavity of unionids (Fuller, 1974). Earlier researchers have reported on incidence of leech infestation in different snail species and the infectivity of the leeches differs with the life stages, snail species and the food availability (Bronmark and Malmqvist, 1986). It was reported that many leeches under

the family Glossiphoniidae are predators of many snail species (Bennike, 1943; Moore, 1964). Infection with *G. complanata* causing mortality of freshwater pearl mussels has not been observed earlier, although there are reports of prey-predator relationship between this particular leech species and mussels. Hence, *G. complanata* could be considered as a pathogenic organism posing threat to farming of this particular mussel species. Further studies for asseing the pathogenicity of *G. complanata* and its impact on mussel health need to be undertaken.

Acknowledgements

The authors wish to thank the Director, ICAR-CIFA, Kausalyaganga for providing necessary facilities during this study. Funding support from National Fisheries Development Board (NFDB), Hyderabad under the

National Surveillance Programme for Aquatic Animal Diseases (NASPAD) is duly acknowledged.

References

- Bennike, S. A. B. 1943. Contributions to the ecology and biology of the Danish freshwater leeches (Hirudinea) *Folia Limnol. Scand.*, 2: 1-109.
- Bronmark, C. and Malmqvist, B. 1986. Interactions between the leech *Glossiphonia complanata* and its gastropod prey. *Oecologia*, 69(2): 268-276. doi: 10.1007/BF00377633.
- Chakraborty, M., Bhattacharya, S., Mishra, R., Mukherjee, D. and Mishra, R. 2012. Antioxidant content and activity of the Indian freshwater pearl mussel in the prevention of arthritis in an experimental animal model. *Br. J. Nutr.*, 108: 1346-1350. doi.org/10.1017/S0007114511006805
- Dey, A. 2008. Commercial and medicinal importance of Sundarbans. In: *Record of the Zoological Survey of India. Occasional paper 289*, Zoological Survey of India, Kolkata, p. 1-54.
- Elliot, J. M. and Mann, K. H. 1979. A key to the British freshwater leeches. *Freshwater Biological Association (FBA), Scientific Publication*, 40 pp.
- Fuller, S. L. 1974. Clams and mussels (Mollusca: Bivalvia). *Pollution ecology of freshwater invertebrates*. Academic Press, New York, p. 215-273.
- Govedich, F. R. and Bain, B. A. 2006. *All about the leeches of Montezuma Well*. National Park Publication. <http://www.invertebrate.us/leech/info/leech.pdf>.
- Grizzle, J. M. and Brunner, C. J. 2009. Infectious diseases of freshwater mussels and other freshwater bivalve mollusks. *Rev. Fish. Sci.*, 17(4): 425-467. doi.org/10.1080/10641260902879000.
- Harding, W. A. 1910. A revision of the British leeches *Parasitology*, 3: 130-201. doi.org/10.1017/S003118200001992.
- Janakiram, K. 2003. Freshwater pearl culture technology development in India. *J. Appl. Aquac.*, 13: 341-349. doi.org/10.1300/J028v13n03_07.
- Misra, G. and Mukhapadhyay, P. K. 2008. Mussel farming: alternate water monitoring practice. *Aquac. Asia*, 13: 32-34.
- Moore, J. E. 1964. Notes on the leeches (Hirudinea) of Alberta. *Nat. Hist. Pap. Nat. Mus. Can.*, 27: 1-15.
- Prabhakar, A. K. and Roy, S. P. 2009. Ethno-medicinal uses of some shell fishes by people of Kosi River basin of North-Bihar, India. *Ethno-Med.*, 3: 1-4.
- Randrianambintsoa, F. J., Leger, N., Robert, V. and Depaquit, J. 2014. Paraphyly of the subgenus *Sintonius* (Diptera, Psychodidae, Sergentomyia): status of the Malagasy species. Creation of a new subgenus and description of a new species. *PloS One*, 9(6): e98065.
- Sambrook, J. and Russell, R. W. 2001. *Molecular cloning: A laboratory manual*, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Saurabh, S., Mohanty, U. L. and Jayasankar, P. 2016. Production technology of designer pearl in freshwater bivalve mussel to add farm income. *Sustainable aquaculture management*. Discovery Publishing House Pvt. Ltd., New Delhi, p. 1-7.
- Shao, J., Xiang, L., Mao, S. and Shen, Z. 1993. Fine structure, genome and polypeptides of the *Hyriopsis cumingii* plague virus. *Chin. J. Virol.*, 9: 160-166.
- Suryawanshi, A. V. and Kulkarni, A. N. 2014. Comparative study of length-weight relationship of *Parreysia corrugate* (Muller), and *Lamellidens marginalis* (Lamarck), from Nanded region, Maharashtra (India). *Indian J. L. Sci.*, 2: 77-80.
- Wrona, F. J., Davies, R. W. and Linton, L. 1979. Analysis of the food niche of *Glossiphonia complanata* (Hirudinoidea: Glossiphoniidae). *Can J. Zool.*, 57: 2136-2142. doi.org/10.1139/z79-281.
- Zhang, Z., Din, S., Xu, Y. and Wang, J. 1986. Studies on the mussel *Hyriopsis cumingii* plague. I. A new viral infectious disease. *Acta Microbiol. Sin.*, 26: 308-312.