Authentication of *Nassodonta insignis* H. Adams, 1867 (Gastropoda: Nassariidae) from the Kodungallur-Azhikode backwater, southwest coast of India using mitochondrial COI marker


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Present study identifies *Nassodonta insignis* from brackish water environments of Kodungallur – Azhikode backwater and confirms the occurrence of the species in these localities with molecular data. Mitochondrial DNA COI sequences (KT985460, KT985461, KT985462, KT985463, KT985464) with 663 base pair length were developed for *N. insignis*. GenBank BLAST analysis shows 89% similarity with *Nassarius (Varicinassa) variciferus* and 44% bootstrap value in the Maximum Likelihood tree analysis.

**Key words:** Gastropoda; Nassariidae; Nassodonta; Cochin (Kochi) – Kodungallur – Azhikode backwaters; India

**Introduction**

Species of the family Nassariidae mainly live in sandy or muddy sediments and are widely distributed from the intertidal to the neritic zone and even to deeper waters\(^1\)\(^-\)\(^7\). Exceptionally they live in brackish water; among them are three valid species in the genus *Nassodonta* (H. Adams, 1867): *Nassodonta annesleyi* Benson, 1861, *Nassodonta insignis* H. Adams, 1867 and *Nassodonta dorri* Wattebled, 1886. Present diversity of the family Nassariidae stands at 420 valid species, of which 85% are in the subfamily Nassariinae\(^5\)\(^-\)\(^10\). The type locality of *N. insignis* is “Peiho river, China”. Smith\(^11\), Preston\(^12\) and Cernohorsky\(^13\) confirmed the occurrence of *N. insignis* in Indian brackish waters. Cernohorsky\(^13\) synonymised *Nassodonta gravelyi* as *N. insignis*\(^14\) and recently Strong et al. again transferred and synonymised them as *Nassodonta annesleyi*\(^15\). It was originally described by Benson in 1861 as *Clea annesleyi*, collected from “a tank between the sea and the canal which communicates with Cochin to the north of Quilon” in Kerala\(^16\). *Nassodonta dorri* from Vietnam, is the another valid species in this genus. It was originally described in the genus *Canidia* as *Canidia dorri* by Wattebled in 1886; Kantor and Kilburn\(^17\) allocated it to the family Nassariidae in the genus *Nassodonta* based on its shell morphology and radula. The type locality of *N. dorri* is Kao-hai Lagoon (near Hue, Vietnam). Lack of molecular data makes it impossible for proper validation of the species in this genus. Present study confirms the occurrence of *N. insignis* in Cochin (Kochi) – Kodungallur – Azhikode backwaters by
morphological and molecular methods and also provides the molecular data on *N. insignis*.

**Materials and Methods**

Cochin (Kochi) – Kodungallur – Azhikode backwaters (CBW) (9°30′ to 10°20′ N and 76°13′ to 76°50′ E) lies at the northern arm of Vembanad backwater system and parallel to the coast between the Arabian Sea to the west and the Western Ghats to the east in Peninsular India. It has two openings to Arabian Sea, one at Munambam (Kodungallur-Azhikode estuary) and another at Cochin (Cochin [Kochi] estuary). Kodungallur – Azhikode backwater (10°11′–10°12′N and 76°10′–76°13′E) receives the tributaries of Periyar river (that is Chalakkudy and Karuvannur river)18–20. Forty specimens were collected from Anapuzha – Kottapuram region (Fig.1.) of Kodungallur – Azhikode backwater monthly sampling for a period of two years (July 2009 - June 2011), for monthly study using a van-Veen grab sampling from the research vessel ’R.V. King Fisher’.

![Map showing the location of the study area and site (in blue spot) where *Nassodonta insignis* was collected for molecular study](image)

Specimens for molecular examination were put in very hot water for a few minutes and then preserved in 100 % ethyl alcohol and specimens for morphological studies were preserved in 4 % formaldehyde. Specimens were photographed as found then cleaned in a solution of detergent and photographed again (Fig.2A-D). Some specimens were sent for species level confirmation of *Nassodonta insignis* to Henk Dekker and Hugo Kool (both Naturalis Biodiversity Center, Leiden, The Netherlands). Type specimen was photographed by Kevin Webb, NHMUK Photographic Unit. Preservative of the alcohol samples was changed after 24 hours. The specimens are incorporated into the gastropod collection of Department of Marine Biology Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology (catalogue entry numbers MBM/SBN/JCPR/1-5/2016).

**Results and Discussion**

The samples are identified to species level according to Cernohorsky (1984). DNA extraction from tissue of individual specimens was done using the DNeasy Blood and Tissue Kit (Qiagen) following the spin column protocol for purification of total DNA from animal tissues. DNA isolates were amplified using the protocol of Takara Clontech Emerald Amp® GT PCR Master Mix (Takara Bio, Otsu, Shiga Prefecture, Japan). The primer pair LCO–1490 (5′–GGTCAACAAATCATAAAGATATTGG–3′) and HCO–2198 (5′–TAAACTTCAGGGTGACCAAAAATCA–3′) was used for amplifying mtCOI gene sequences21. Reaction mixture was composed of 25 μL PCR Master Mix, 1 μL LCO1490 (forward) primer, 1 μL HCO2198 (reverse) primer, 8 μL template DNA, and 15 μL dH2O. Thermal regime comprised an initial denaturation for 5 minutes at 94 °C, followed by 40 cycles of 1 min each at 94 °C for denaturation, 2 min at 37 °C for annealing, and 3 min at 72 °C for extension, with a final extension at 72°C for 10 min. PCR products were visualized on 1.2 % agarose gel electrophoresis. Amplified products exhibiting intense bands after agarose gel electrophoresis were purified and sequenced at SciGenom Labs (SciGenom Labs Pvt, Ltd., Kochi, Kerala, India). Sequences were compiled using BioEdit 7.0.9 and alignment was performed using ClustalX22,23. Phylogenetic analyses with a maximum likelihood tree and intraspecific pairwise sequence distance were evaluated using Kimura 2-parameter model of MEGA524. Bootstrap analysis was performed using 1000 pseudo replications and aligned sequences were submitted to National Center for Biotechnology Information (NCBI) with accession numbers.
Systematics.
Family Nassariidae Iredale, 1916
Subfamily Anentominae Strong, Galindo & Kantor, 2016
Genus Nassodonta H. Adams, 1867
Nassodonta insignis H. Adams, 1867

Nassodonta insignis H. Adams, 1867; 445
Nassodonta insignis Cernohorsky 1984: 199; text figs. 156, 157;
Nassarius insignis Kaicher1983, card 3491

Type specimen. The lectotype of N. insignis (H. Adams, 1867) is in the British Museum (Natural History), London, No. 1878.1.28.428, length 10.0 mm, width 6.6 mm.

Type locality. Peiho river, China [error]


Habitat. The species has been collected in muddysand and coarse sandy bottom at 0.5-4 m depth in brackish water environment. Salinity of occurrence during the present study was 5-10 ppt.

Distribution. Apparently confined to the southern coasts of India in shallow, brackish water. Reported by Preston from Cochin (Kochi) backwaters near Ernakulam. Smith (1895) pointed out that the original locality indication of Nassodonta insignis was “Peiho river, China”, and stated that on the label accompanying the type is written “Peihoi, fresh water, Calvert, with genus Velorita”. Smith further pointed out that the genus Velorita (Villorita cyprinoides, Gray, 1825) occurs only on the west side of the Indian Peninsula, in the neighbourhood of Cochin, and in the estuary of the river Kundapur, (where it is largely eaten by natives), and that it has never been found in China, and that “Peihoi” could be some small village on the Malabar coast. The present study suggests that “Peiho river” may be “Periyar river” the largest river in the south west coast of India that feeds Cochin (Kochi) backwater. The occurrence of N. insignis in India has been subsequently confirmed through the collection of specimens from Cochin (Kochi), and the description of N. gravelyi from Cochin and Madras. The present study also confirms occurrence of N. insignis in Kodungallur-Azhikode backwaters, brackish water aquaculture ponds of Vyppen islands, Ezhupunna, South Paravoor and adjoining brackish water environments of Cochin (Kochi) - Kodungallur-Azhikode backwater in company with black clam, Villorita cyprinoides. The occurrence of N. insignis in China or the intervening areas between India and China, has never been established after Smith’s statement.25

Description. Shell up to 12.0mm in length, ovate and soild, some specimens broader than others, teleonch of only 3-3 ½ convex whorls, protoconch of 1 ½ embryonic whorls, partly macroscopically axially striate; shell smooth except for very fine axial growth lines and a heavy, flattish spiral cord at base of body whorl. Aperture moderately narrow, outerlip thickened but not strongly variced, interior with 4-5 denticles decreasing in size anteriorly. Columella concave and with a moderately broad and thick callus, a strong fold at the anterior end and occasionally with another weak plication; siphonal notch deep and broad, parietal denticle swollen, anal canal distinct. Color fawn to brown, last whorl decorated with a moderately broad, interrupted brown subsutural band and a broad dark brown band on the posterior half. Aperture white but occasionally stained with brown. Smith (1895) correctly transferred the species from the Buccinidae to the Nassariidae, based on the presence of multicuspidate rachidian tooth of radula. Operculum thick, roundish, exceptional for the family Nassariidae, two vertical teeth.
Molecular identification. A 663 base pair length of mitochondrial DNA COI sequences (n=5) were developed in respect of Nassodonta insignis and were devoid of any InDels and stop codons. The developed mtCOI sequences were submitted to the NCBI database with following accession numbers KT985460, KT985461, KT985462, KT985463 and KT985464. Five other mtCOI sequences of Family Nassariidae were obtained for constructing phylogenetic tree (Maximum Likelihood). Maximum Likelihood tree developed in the present study forms two major clades. Nassodonta insignis individuals were arranged in a single clade with high bootstrap value of 100%. Nassarius (Varicinassa) variciferus (A. Adams, 1852) formed an adjacent clade. As expected, the selected out group Villorita cyprinoides exhibited a divergent array. In order to justify the results of the phylogenetic tree, genetic distances persisting within the selected individuals were analysed. The level of inter- and intraspecific divergence persisting within the Family Nassariidae was evident from the distance matrix data (Table 1). Specifically, Nassodonta insignis possessed an intra-specific sequence divergence of 0% (Table 1) which reflects and justifies the results inferred Nassodonta insignis [KT985460], Nassodonta insignis [KT985461], Nassodonta insignis [KT985462], Nassodonta insignis [KT985463], Nassodonta insignis [KT985464], Nassarius (Varicinassa) variciferus [GU393386], Nassarius martinezi [KC970046], Nassarius radians [KC970058], Nassarius velvetosus [KC970063], Clea (Anentome) helena [KT985465] and Villorita cyprinoides [KP099608] from the ML tree. This value was adequate to establish the genetic identity of N. insignis sequences. In addition, the level of interspecific divergence among other species supported its cladistic differentiation in the Maximum Likelihood (ML) tree. GenBank BLAST analysis shows 89% similarity with Nassarius variciferus and 44% bootstrap value in the ML tree analysis. The molecular data on the other species in the genus, N. dorri and N. annesleyi is very much required for comparative study of this genus and its phylogeny.

![Fig.3. Maximum likelihood tree for Nassodonta insignis based on 1000 bootstrap pseudoreplicas](image)

Table 1. Distance matrix for inter and intraspecific percentage divergence of selected species

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References


