



Argonauta hians Lightfoot, 1786 (Cephalopoda: Argonautidae) in the gut of *Alepisaurus ferox* from Indian waters and its phylogenetics

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Abstract

The Paper Nautilus, *Argonauta hians* Lightfoot 1786, belongs to the superfamily Argonautoidea and is widely distributed along the tropical and subtropical oceans. The present study describes the record of *A. hians* recorded from the gut of a long-snouted lancetfish, *Alepisaurus ferox* (133cm TL and 2 kg weight). *A. ferox* was hooked during the regular multifilament tuna longlining onboard MFV Yellow Fin on 20th October 2021 off Goa (Lat. 15° 19.44 N; Long. 70° 50.20 E) at 2652 m depth along the Central west coast of India. A detailed note on the distribution of *A. hians* in Indian waters is provided along with morphological characterization of the specimen and validation with molecular marker.

Keywords: Octopus, paper nautilus, winged argonaut, Arabian Sea

Introduction

The pelagic octopuses belonging to the family Argonautidae, currently have four valid species within the genus *Argonauta* Linnaeus, 1758 (Finn, 2014) viz. *Argonauta argo* Linnaeus, 1758, *Argonauta hians* Lightfoot, 1786, *Argonauta nodosus* Lightfoot, 1786 and *Argonauta nouryi* Lorois, 1852 (Mollusca Base eds., 2023). Adding on to the four current valid species, studies from New Zealand had placed on record another species *Argonauta oweri* from the early Pliocene period, which is considered to be extinct (Saul and Stadum, 2005; Martill and Barker, 2006). Argonaut octopuses are widely distributed in tropical and temperate waters and exhibit sexual dimorphism (Beesley *et al.*, 1998; Finn, 2013; Vaitheeswaran *et al.*, 2014). Females of this genus are capable of secreting a paper-thin, fragile chamber made up of a calcareous structure similar to nautilus, hence popularly known as "Paper Nautilus". Further, the female also has two specialized arms called

hectocotyli, which are used for reproduction. Males lack this distinctive shell and are significantly smaller than females (Sukhsangchana *et al.*, 2009; Finn, 2014). Previous studies have recorded two species of argonaut octopuses from Indian waters viz. *A. argo* Linnaeus, 1758 and *A. hians* (Roper *et al.*, 1984; Ravinesh *et al.*, 2017; Mollusca Base eds., 2023). *A. hians* is widely distributed in Indian waters and they have been recorded from Andaman and Nicobar Waters (Ramakrishna, 2010; Tripathy, 2013; Devi, 2015), Mumbai–Northwest coast of India (Sundaram and Sushant, 2015); Kerala–Southwest coast of India (Ravinesh *et al.*, 2017); Tamil Nadu–Southeast coast of India (Crichton, 1940; Vaitheeswaran *et al.*, 2014); Odisha–Northeast coast of India (Mohanty *et al.*, 2021) and also from the Lakshadweep Sea (Silas, 1968). *Argonauta boettgeri* Maltzan, 1881 is reported to be synonymised with *A. hians* (Finn, 2014; Mollusca Base eds., 2023). The present work focuses on the record of *A. hians* off Goa and its taxonomic characterization based on morphological and molecular methods.

Material and methods

A single specimen of *A. hians* was collected from the buccal cavity of long-snouted Lancetfish, *Alepisaurus ferox* Lowe, 1833 (Total length 133cm and weight 2 Kg) hooked from the area Lat. 15° 19.44N; Long. 70° 50.20 E at 2652 m depth contour during the tuna long line survey conducted onboard MFV Yellow Fin on 20th October 2021 (Fig. 1). The specimen of *A. hians* was intact and would have been freshly consumed or accidentally ingested by *A. ferox* during hauling of the longline gear. Morphometric measurements were taken using a digital calliper (Baker DDC series, 0-150mm to 0-300mm range) and photographs were taken with a Canon SLR (EOS 550 D, 18.7 megapixel)

camera. The specimen was deposited in the museum of Mormugao Zonal Base of Fishery Survey India (MFSI), Goa with registration number MFSI/01/2023.

Taxonomic delineation using molecular markers was carried out by analyzing the mitochondrial Cytochrome oxidase subunit I (COI) gene sequence. The tissue sample of *A. hians* was collected aseptically and preserved in 99.5% ethanol at ambient temperature until further analysis. Genomic DNA was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. The quality of the extracted DNA was checked by agarose gel electrophoresis. The gels were visualized in a UV transilluminator (Genei, India) and the image was captured using a Gel documentation system (Bio-Rad, CA, USA).

The partial sequence of the mitochondrial COI gene was amplified using the universal primers (Folmer *et al.*, 1994), LCO 1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO 2498 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') in 10 µl reaction volume of Phire Tissue Direct PCR Master Mix (Thermo Scientific, USA). PCR reactions were carried out in Gene Amp PCR System 9700, (Applied Biosystems, USA) following an initial preheating at 98 °C for 30 sec., followed by 10 cycles of denaturation at 98 °C for 5 sec., annealing at 45 °C for 10 sec., extension at 72 °C for 15 sec., and further 30 cycles of denaturation at 98 °C for 5 sec., annealing at 50 °C for 10 sec., extension at 72 °C for 15 sec and a final extension for 1 min at 72 °C. The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. The gel was visualized on a Gel documentation system (Bio-Rad, USA). Removal of unused primers and dNTPs from the PCR product mixture was done using Exo SAP-IT™ PCR product

clean-up reagent (GE Healthcare CA, USA). The purified PCR products were sequenced using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following the manufacturer protocol.

Taxonomic annotation was done using NCBI-nt database. Homologous sequences were obtained from the GenBank database of NCBI and the phylogenetic tree was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980) with the aid of MEGA XI (Tamura *et al.*, 2021). The bootstrap consensus tree was inferred from 1000 replicates (Felsenstein, 1985) to represent the evolutionary history of the taxa analyzed. The genetic distance matrix was also inferred using MEGA XI (Tamura *et al.*, 2021).

Results

Order : Octopoda Leach, 1818
 Superfamily : Argonautoidea Cantraine, 1841
 Family : Argonautidae Cantraine, 1841
 Genus : Argonauta Linnaeus, 1758
Argonauta hians Lightfoot, 1786

Synonyms

Argonauta boettgeri Maltzan, 1881; *Argonauta crassicosta* Blainville, 1826; *Argonauta hians f. aurita* Martens, 1867; *Argonauta hians f. obtusangula* Martens, 1867; *Argonauta kochianus* Dunker, 1852; *Argonauta nitidus* Lamarck, 1822; *Argonauta owenii* A. Adams & Reeve, 1848; *Argonauta politus* Conrad, 1854; *Argonauta raricosta* Blainville, 1826 and *Ocythoe cranchii* Leach, 1817.

Material examined

One female specimen of *A. hians* (MFSI/01/2023) with a total length of 33 mm and maximum shell breadth of 19 mm, was recovered from the buccal cavity of *A. ferox* (Fig. 2 A & B).

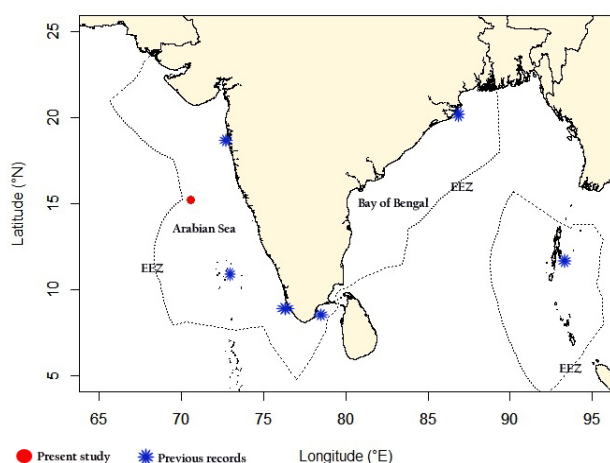


Fig. 1. Map showing the recorded distribution of *A. hians* Lightfoot, 1786 along the Indian Exclusive Economic Zone

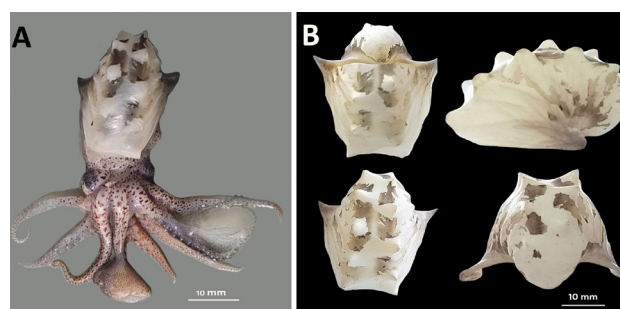


Fig. 2. A) *A. hians* with shell (MFSI/01/2023), B) Different views of the shell

Table 1. Morphometric and meristic details of *A. hians* in comparison with previous records. Weight in grams (g) and measurements in millimetres (mm)

Parameters	Present study	Sujit and Sushant (2015)	Mohanty <i>et al.</i> (2021)	Ravinesh <i>et al.</i> (2017)	Devi (2015)	Vaitheeswaran <i>et al.</i> (2014)	Finn (2014)
Weight in gm	54	105	—	—	—	20	—
TL in mm	33	61	64	38	35	63	106
Shell breadth	19	—	39	23	23	37	—
Nodules over the keel	17	15-23	20	—	15-20	15-23	—
Suckers on arm							
First arm	56	—	60	—	—	—	140-160
Second arm	52	—	58	—	—	—	80-110
Third arm	38	—	40	—	—	—	70-110
Fourth arm	48	—	52	—	—	—	30-50

Description

The body is muscular and the mantle is dome-shaped. Head narrow embedded within mantle with two large and protruded eyes on both sides. The body bears 4 slender arms on each side. Each arm with two rows of suckers; the first arm with 56 suckers. The second, third and fourth arms are having 52, 38 and 48 suckers respectively. Shell moderate, laterally flattened with minor lateral ribs and prominent tubercles across the keel. Shell milky in colour with brownish-black speckles over the tubercles. The lateral sides have 11 prominent rounded paired nodules present over the keel. The inside of the shell is completely white.

The amplification of the COI generated a 708 base pair long amplicon, which on NCBI Blastn analysis showed 99.84% identity to *Argonauta hians* with 100% query coverage, hence the sequence was submitted to NCBI under the accession number OR234732. The evolutionary history was inferred by constructing the phylogenetic tree (Fig. 3) and the distance matrix was obtained through MEGA XI (Tamura *et al.*, 2021).

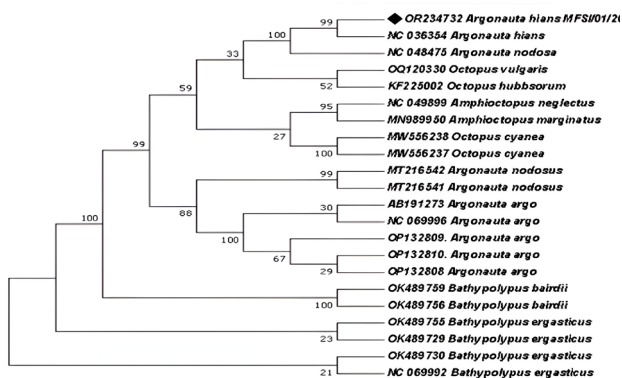


Table 2. Distance matrix of *A. hians* Lightfoot, 1786 by maximum likelihood method

OR234732_Argonauta_hians_MFSU/01/2023		0.00	13.39	13.39	13.39	13.39	13.39	13.39	13.39	13.39	0.52	12.43	12.43	12.43	13.79	13.79	13.79	13.79	13.79	13.81	13.81	12.55	15.15	13.46	12.03	14.00
NC_036354_Argonauta_hians	0.00		13.39	13.39	13.39	13.39	13.39	13.39	13.39	13.39	0.52	12.43	12.43	12.43	13.79	13.79	13.79	13.79	13.79	13.81	13.81	12.55	15.15	13.46	12.03	14.00
AB191273_Argonauta_argo	4.70	4.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.94	19.96	19.96	19.96	1.67	1.67	1.67	1.67	1.67	1.67	2.28	2.11	2.28	2.15	2.06	2.06
OP132810_Argonauta_argo	4.70	4.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.94	0.79	0.79	0.79	1.67	1.67	1.67	1.67	1.67	1.67	2.28	2.11	2.28	2.16	2.06	2.06
OP132809_Argonauta_argo	4.70	4.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.94	0.79	0.79	0.79	1.67	1.67	1.67	1.67	1.67	1.67	2.28	2.11	2.28	2.16	2.06	2.06
OP132808_Argonauta_argo	4.70	4.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.94	0.79	0.79	0.79	1.67	1.67	1.67	1.67	1.67	1.67	2.28	2.11	2.28	2.16	2.06	2.06
NC_069996_Argonauta_argo	4.70	4.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.94	19.96	19.96	19.96	1.67	1.67	1.67	1.67	1.67	1.67	2.28	2.11	2.28	2.16	2.06	2.06
NC_048475_Argonauta_nodosa	0.12	0.12	4.41	4.41	4.41	4.41	4.41	4.41	4.41	4.41		12.05	12.05	12.05	12.67	12.67	12.67	12.67	12.63	12.63	10.99	13.24	12.82	11.56	12.61	12.61
MT126542_Argonauta_nodosus	4.41	4.41	0.12	0.13	0.13	0.13	0.13	0.13	0.13	0.13	4.35	0.00	0.00	0.00	1.76	1.76	1.76	1.76	1.91	1.91	1.99	2.04	2.37	2.23	2.32	2.32
MT126541_Argonauta_nodosus	4.41	4.41	0.12	0.13	0.13	0.13	0.13	0.13	0.13	0.13	4.35	0.00	0.00	0.00	1.76	1.76	1.76	1.76	1.91	1.91	1.99	2.04	2.37	2.23	2.32	2.32
OK489755_Bathypolypus_ergasticus	4.99	4.99	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	4.81	0.19	0.19	0.19	0.00	0.00	0.00	0.00	0.32	0.32	1.97	1.93	2.30	2.39	1.82	1.82
OK489730_Bathypolypus_ergasticus	4.99	4.99	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	4.81	0.19	0.19	0.19	0.00	0.00	0.00	0.00	0.32	0.32	1.97	1.93	2.30	2.39	1.82	1.82
OK489729_Bathypolypus_ergasticus	4.99	4.99	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	4.81	0.19	0.19	0.19	0.00	0.00	0.00	0.00	0.32	0.32	1.97	1.93	2.30	2.39	1.82	1.82
NC_069992_Bathypolypus_ergasticus	4.99	4.99	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	4.81	0.19	0.19	0.19	0.00	0.00	0.00	0.00	0.32	0.32	1.97	1.93	2.30	2.39	1.82	1.82
OK489759_Bathypolypus_bairdii	5.02	5.02	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	4.83	0.21	0.21	0.21	0.08	0.08	0.08	0.08	0.00	0.00	2.23	2.38	2.04	2.08	1.72	1.72
OK489756_Bathypolypus_bairdii	5.02	5.02	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	4.83	0.21	0.21	0.21	0.08	0.08	0.08	0.08	0.00	0.00	2.23	2.38	2.04	2.08	1.72	1.72
OQ120330_Octopus_vulgaris	4.38	4.38	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	3.99	0.23	0.23	0.23	0.22	0.22	0.22	0.22	0.25	0.25	0.25	1.53	2.18	2.15	2.21	2.21
KF225002_Octopus_hubborsorum	5.14	5.14	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	4.69	0.24	0.24	0.24	0.21	0.21	0.21	0.21	0.26	0.26	0.16	0.16	2.70	2.07	1.91	1.91
NC_049899-Amphioctopus_neglectus	4.49	4.49	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	4.54	0.26	0.26	0.26	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.30	1.26	2.17	2.17	2.17
MN989950-Amphioctopus_marginatus	4.09	4.09	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.24	4.05	0.25	0.25	0.25	0.26	0.26	0.26	0.26	0.24	0.24	0.23	0.22	0.14	1.93	1.93	1.93
MW556238_Octopus_cyanea	5.04	5.04	0.22	0.23	0.23	0.23	0.23	0.23	0.23	0.22	4.72	0.24	0.24	0.24	0.19	0.19	0.19	0.19	0.19	0.19	0.23	0.19	0.23	0.20	0.00	0.00
MW556237_Octopus_cyanea	5.04	5.04	0.22	0.23	0.23	0.23	0.23	0.23	0.23	0.22	4.72	0.24	0.24	0.24	0.19	0.19	0.19	0.19	0.19	0.19	0.23	0.19	0.23	0.20	0.00	0.00

conclusion

This was the first attempt at the phylogenetic analysis of *A. hians* using a mitochondrial COI gene marker from the Indian EEZ. The present study strongly confirms the obtained specimen as *A. hians* based on morphological and molecular studies. Genetic distances between *A. hians* and other species (*eg.*, *Argonauta argo*, *Argonauta nodosa*, *Bathypolypus* spp. and *Amphioctopus* spp.) revealed varying levels of genetic divergence. Patterns of genetic divergence suggested potential evolutionary relationships within the *Argonauta* genus and highlighted the position of *Argonauta hians* in the broader context of cephalopod evolution. The genetic distance between congeners, *A. hians* and *A. argo* was found to be 4.700 substitutions per site. This value indicated a moderate level of genetic divergence between these two species, reflecting their status as distinct species within the same genus. In contrast, the intraspecific distance within *A. hians* genotypes were calculated to be 0.000 substitutions per site. This result suggests a high level of genetic similarity among *A. hians* specimens, indicating a relatively homogenous population with little genetic variation. Further, investigations considering additional molecular markers will enhance our comprehension of the evolutionary relationships within this fascinating group of these marine organisms.

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