FIRST RECORD OF *BAEOLIDIA MOEBII* BERGH, 1888 (NUDIBRANCHIA: AEOLIDIIDAE) FROM INDIA BASED ON INTEGRATIVE TAXONOMY

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Abstract Baeolidia *is the most diverse genus under the family Aeolidiidae. The genus has a widespread distribution with records from the Indo-Pacific, central and eastern Pacific, Red Sea, and Mediterranean Sea. Until now, this genus was represented by only one species from India,* Baeolidia salaamica, *however, we report* Baeolidia moebii *as a new record to India and the second species from the subcontinent. This paper not only provides the first sequences of mitochondrial (COI and 16S) DNA for the species from the Indian Ocean but also discusses the possibilities supporting its Lessepsian migration and the relationship between* B. moebii *and* B. australis.

Key words Heterobranchia, Northern Indian Ocean, Agatti Island, Lakshadweep, new record, Lessepsian migration

INTRODUCTION

The Lakshadweep Islands, situated in the Arabian Sea off the southwest coast of India, are one of the four major coral reef ecosystems in India and the only coral atolls of the country. A total of 102 species of heterobranchs is reported from Lakshadweep Islands (Ravinesh and Biju Kumar 2015; Snehachandran *et al*. 2017) but none of them have been subjected to molecular studies.

The genus *Baeolidia* Bergh, 1888 is the largest genus under the family Aeolidiidae with fourteen accepted species (WoRMS, 2021). This genus is reported from the Indo-Pacific, including the East African coast, Mauritius, Seychelles, Japan, and the Red Sea (Carmona *et al*. 2014b). Recently, this genus was reported from the Mediterranean Sea, being represented by *Baeolidia moebii* Bergh, 1888 as an alien species (Paz-Sedano *et al*. 2019) along the coasts of Cyprus and Turkey, suggesting Lessepsian migration (i.e., species of the Indo–Pacific entering the Mediterranean through the Suez Canal).

From India, only one species, *Baeolidia salaamica* (Rudman, 1982) is reported from the western mainland, *i.e.,* Gujarat coast (Apte and Desai 2017). However, despite the presence of large coral reef regions in the country, the widely distributed species *B. moebii* has not been recorded to date, revealing gaps in aeolid distribution data of India. As far as molecular data for Indian aeolids is concerned, only four species from India are sequenced, *Anteaeolidiella fijensis* Carmona *et al.*, 2014, *Anteaeolidiella poshitra* Carmona *et al.*, 2014, *Cratena poshitraensis* Bharate *et al*. 2020 and *Cratena pawarshindeorum* Bharate *et al*. 2020.

The present work records *Baeolidia moebii* from the Agatti Atoll, Lakshadweep, which is a new record to Indian waters. Support is provided by photographic, morphological, and molecular evidence, which also provides the first CO1 and 16S sequences for this species from the Indian Ocean.

MATERIALS AND METHODS

Two specimens of *Baeolidia moebii* were collected from 10m depth in the lagoon off Agatti Atoll (coll by. S. Dixit; 10°51'58''N 72°10'57''E; 03.05.2018) by SCUBA diving (Fig. 1). The specimens were observed together under a small rock in the lagoon's sandy substratum. Live specimens were photographed *in-situ* and *ex-situ* by Sony RX100 M5 with underwater housing. One specimen (CMLRE IO/DV/GAS/00021) was preserved in 99% ethyl alcohol for molecular studies while the other specimen (CMLRE IO/DV/GAS/00022) was fixed in frozen 10% formalin buffered with seawater to maintain its morphological structures. The fixed specimens were later photographed under a stereomicroscope (LEICA

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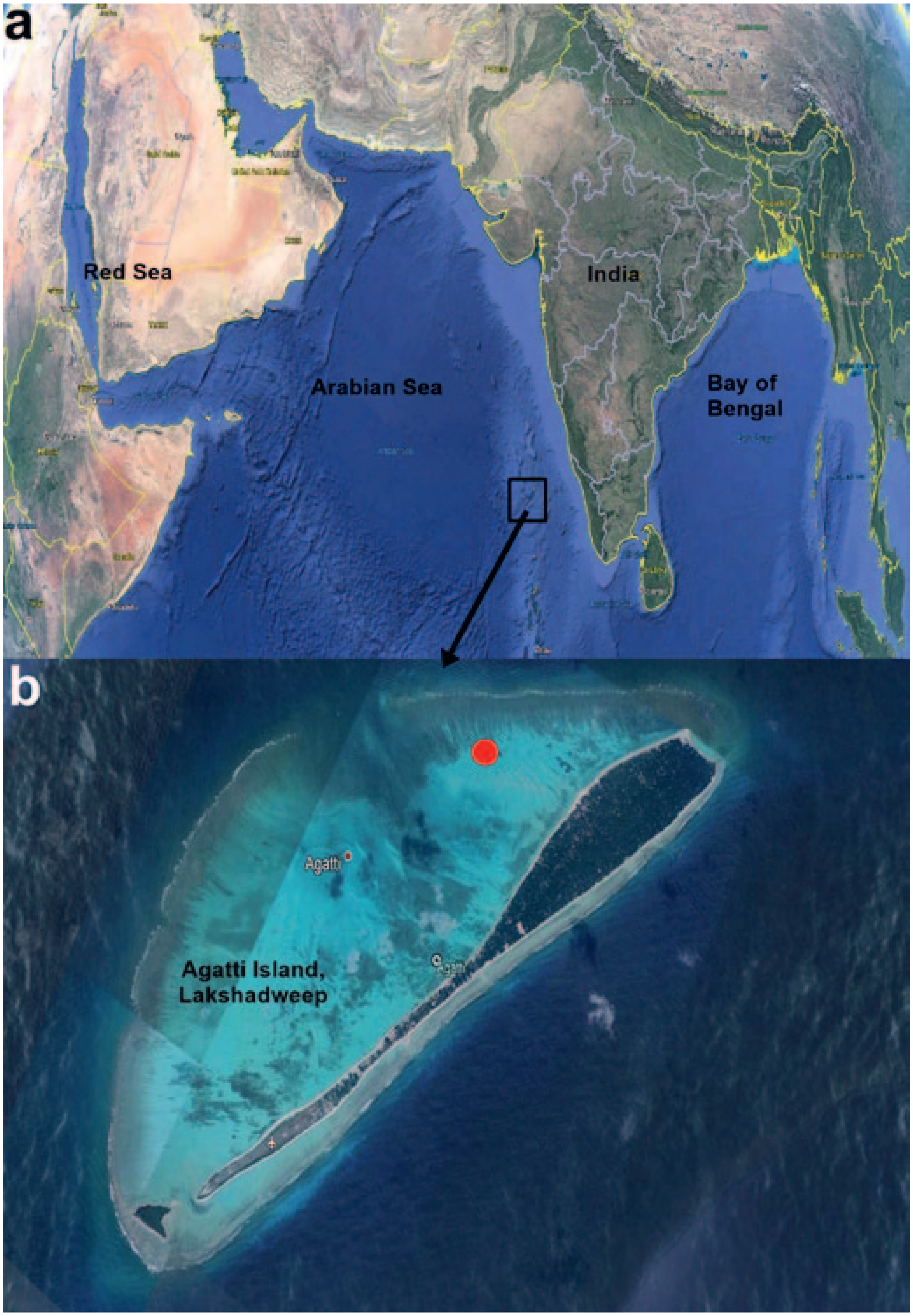

Figure 1 (a) Lakshadweep group of Islands (black rectangle) off west coast of southern India; (b) Agatti Island; Collection site of *Baeolidia moebii* – Red dot. (Source: Google Earth)

M80) and measurements (in mm) of the specimens (total length and width) were made. All identified materials are deposited in the Referral Centre at Centre for Marine Living Resources and Ecology (CMLRE), Kochi, India, which is the regional node of the Ocean Biodiversity Information System (OBIS) for the Indian Ocean. The occurrence data associated with these specimens is available at the OBIS portal (https://obis.org/).

Table 1 Species used for molecular analyses, including voucher no., locality and GenBank accession numbers for both CO1 and 16S Abbreviations used: CASIZ — California Academy of Science (California, USA); MNCN and MNCN/ADN — Museo Nacional de Ciencias Naturales (Madrid, Spain); SZN — Stazione Zoologica Anton Dohrn (Naples, Italy). Specimen used for present study is in **bold**.

Species	Voucher no.	Location	(CO1)	. (16S)
Baeolidia australis (Rudman, 1982)	ZFMK Wägele 208	Albany, Australia	-	MK100935
Baeolidia moebii (Bergh, 1888)	SZN-MOL0020	Cyprus	MK922511	-
Baeolidia moebii (Bergh, 1888)	MNCN 15.05/54987	Hawaii	HQ616771	-
Baeolidia moebii (Bergh, 1888)	CASIZ 180327	Hawaii	JQ997060	JQ996857
Baeolidia moebii (Bergh, 1888)	CASIZ177602	Philippines	HQ616770	HQ616733
Baeolidia moebii (Bergh, 1888)	MNCN/ADN: 51948	Marshall Island	-	JX087481
Baeolidia moebii (Bergh, 1888)	CASIZ 186211	Philippines	-	JQ996858
***Baeolidia moebii* (Bergh, 1888)**	**IO/DV/GAS/00021**	**Lakshadweep, India**	**MT884061**	**MT592801**
Baeolidia variabilis (Carmona, Pola, Gosliner & Cervera, 2014)	CASIZ 177716	Philippines	JQ997051	JQ996847
Baeolidia variabilis (Carmona, Pola, Gosliner & Cervera, 2014)	CASIZ 186210	Philippines	JQ997054	JQ996852
Baeolidia rieae (Carmona, Pola, Gosliner & Cervera, 2014)	CASIZ 184525	Japan	JQ997046	JQ996842
Baeolidia scottjohnsoni (Carmona, Pola, Gosliner & Cervera, 2014)	CASIZ 184503	Marshall Islands	JQ997045	JQ996840
Baeolidia japonica (Baba, 1933)	CASIZ 184520	Japan	JQ997058	JQ996855
Baeolidia japonica (Baba, 1933)	CASIZ 186795	Marshall Island	JQ997059	JQ996856
Baeolidia nodosa (Haefelfinger & Stamm, 1958)	CASIZ 186792	Madeira Is. (Portugal)	JQ997081	
Baeolidia nodosa (Haefelfinger & Stamm, 1958)	MNCN/ADN 24.923	Balearic Is. (Spain)	HQ616768	
Baeolidia nodosa (Haefelfinger & Stamm, 1958)	CASIZ 184521	Bahamas	-	JX087527
Baeolidia nodosa (Haefelfinger & Stamm, 1958)	MNCN/ADN51959	France	-	JX087494
Chelidonura hirundinina (Quoy & Gaimard, 1833)	ZMBN95971	Lizard Is. Australia	MF036524	-
Chelidonura hirundinina (Quoy & Gaimard, 1833)	ZMBN95973	Lizard Is. Australia	-	MF036333

MOLECULAR ANALYSES

DNA isolation and PCR

The genomic DNA was extracted from the foot tissue by using the ORIGIN marine animal kit following the manufacturer's protocol. Fragments of the mitochondrial cytochrome oxidase subunit I (COI) and 16SrRNA genes were amplified using universal primers LCO1490/HCO2198 and 16Sar/16Sbr (Palumbi, 1996) (Folmer *et al*. 1994). PCR reactions were performed in 25 µl, containing 50 ng of DNA, 2.5 mM MgCl2, 0.3 mM of each primer. Amplification reaction consisted of an initial denaturation at 94ºC for 4 min, followed by 35 cycles of denaturation at 94ºC for 30 s; annealing for 40 s at 52ºC for 16S and 50ºC for COI and extension at 72ºC for 1 min. The final extension was at 72ºC for 5 min. The PCR products were electrophoresed and visualised on 1.2% agarose gel containing ethidium bromide. The raw DNA sequences were edited using BioEdit sequence alignment editor version 5.0.9 (Hall, 1999). The COI and 16S sequences from the closely related species were obtained from the GenBank, NCBI database (Table 1). Maximum Likelihood Phylogenetic analyses were performed using MEGA version 7 (Kumar *et al*. 2016).

MOLECULAR RESULTS

Seven *Baeolidia* species found in GenBank were used for the comparison with the present studied specimen (Table 1). Analyses of the partial mitochondrial COI and 16S rRNA sequences from various locations revealed a well-differentiated clade for *B. moebii*. The Indian specimen analysed here is not only clearly conspecific with all the Indo-Pacific *B. moebii* (Figs 3, 4) sequenced but also with the recently reported specimen (Paz-Sedano *et al*. 2019) from Cyprus in the Mediterranean. The intra-species K2P genetic difference among the *B. moebii* specimens from different geographical locations range from 0 to 0.7% for COI (Table 2) and 0–0.3% for 16SrRNA (Table 3).

SYSTEMATICS

Class Gastropoda Cuvier, 1795
Sub Class Heterobranchia Burmeister, 1837
Order Nudibranchia Cuvier, 1817
Family Aeolidiidae Gray, 1827
Genus *Baeolidia* (Bergh, 1888)

Baeolidia moebii (Bergh, 1888)

Description

Description is based on one specimen, CMLRE IO/DV/GAS/00022; 6×3mm. When alive, the body was elongated and narrow towards the posterior end (Fig. 2a, b). Background colour brown with numerous white coloured spots on dorsum. Oral tentacles and lamellated rhinophores are speckled with irregularly spaced white spots. Oral tentacles white-tipped. A brown patch bordered with white on the head region between oral tentacles (Fig. 2b). Cerata arranged in 7–9 arches with 5 or 6 cerata on either side. The middle portion of the body is devoid of any cerata. Cerata flat and pointed at the apex with translucent tips followed by broad white and then faint yellow band. Numerous small, white spots on the entire cerata. Branches of the digestive glands in cerata (Fig. 2c) and rhinophores studded with rounded knobs (Fig. 2d) are clearly visible in the preserved specimen.

Remarks

Based on characters described in Carmona *et al*. (2014b) such as "broad body tapering towards the end of the foot; dorsum with bright white patches; whitish ring on the head continuing towards oral tentacles; oral tentacles white tipped; rhinophores studded with minute knobs; cerata dorso-ventrally flattened and leaf-like, translucent white at apices followed by a yellow band, broad at the base and narrowing towards the apices", and molecular results, the specimens studied in the present study are identified as *B. moebii.* The intraspecific genetic distance between different specimens from different localities based on K2P analyses, from 0 to 0.7% including both the genes further support the identification of the present specimen as *B. moebii.* Our specimen, based on interspecies genetic analysis of 16S rRNA sequence formed a distinct clade (Fig. 4) from *B. australis* (Rudman, 1982) collected from Albany, Australia (Goodheart *et al*. 2018). The K2P genetic distance range is from 7.2 to 7.4% (Table 3) which is more than the threshold genetic distances generally considered as interspecific distances used for species delimitation and validation in molluscs.

DISCUSSION

The genus *Baeolidia* is entirely Indo-Pacific in distribution except for the Atlantic species *Baeolidia cryoporos* Bouchet, 1977. Only *B. moebii* Bergh, 1888 and *B. salaamica* (Rudman, 1982) are recorded from both the Indian and the Pacific oceans (Carmona *et al*. 2014). Recently, the genus *Baeolidia* represented by *B. moebii* was reported from Cyprus and Turkey as an alien species (Paz-Sedano *et al*. 2019) suggesting its Lessepsian migration. With this study, the record of *B. moebii* from the Lakshadweep Islands further support the theory of the transportation of this species from the Indo-Pacific to the Mediterranean Sea because of the presence of these islands in the middle of one the busiest shipping routes (Fig. 5) of the world (shipping route from Malacca Strait to Suez Canal via India). Although the type locality of *B. moebii* is in Mauritius and the species is also reported from the East African coast such as Tanzania and Mozambique, these areas are not well connected to the Mediterranean via major shipping routes (Fig. 5). The genetic variation based on K2P analyses is 0.5% between the present specimen and the specimen sequenced from Cyprus show a high level of genetic similarity. However, genetic data from the specimen from the type locality or nearby east African coast is required to compare the genetic variation between specimen from Mediterranean and India.

Table 2 Pair wise genetic distance (K2P) based on COI

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	***Baeolidia moebii* *MT884061***														
2	*Baeolidia moebii* HQ616770.1	0.002													
3	*Baeolidia moebii* JQ997060.1	0.002	0												
4	*Baeolidia moebii* JX087550.1	0.003	0.002	0.002											
5	*Baeolidia moebii* HQ616770.1	0.002	0	0	0.002										
6	*Baeolidia moebii* HQ616771.1	0.005	0.003	0.003	0.005	0.003									
7	*Baeolidia moebii* MK922511.1	0.005	0.003	0.003	0.005	0.003	0.007								
8	*Baeolidia nodosa* JQ997081.1	0.25	0.247	0.247	0.25	0.247	0.247	0.25							
9	*Baeolidia japonica* JQ997059.1	0.278	0.276	0.276	0.278	0.276	0.281	0.271	0.257						
10	*Baeolidia japonica* JQ997058.1	0.283	0.281	0.281	0.283	0.281	0.286	0.276	0.26	0.009					
11	*Baeolidia nodosa* HQ616768.1	0.252	0.25	0.25	0.252	0.25	0.25	0.253	0.014	0.248	0.25				
12	*Baeolidia rieae* JQ997046.1	0.281	0.281	0.281	0.278	0.281	0.276	0.281	0.256	0.18	0.177	0.252			
13	*Baeolidia scottjohnsoni* JQ997045.1	0.26	0.258	0.258	0.26	0.258	0.263	0.258	0.265	0.139	0.145	0.268	0.193		
14	*Baeolidia variabilis* JQ997051.1	0.235	0.235	0.235	0.233	0.235	0.23	0.24	0.243	0.241	0.246	0.24	0.241	0.228	
15	*Baeolidia variabilis* JQ997054.1	0.233	0.233	0.233	0.23	0.233	0.228	0.237	0.24	0.238	0.243	0.238	0.241	0.233	0.003

Table 3 Pair wise genetic distance (K2P) based on 16S

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	***Baeolidia moebii*** MT592801													
2	*Baeolidia moebii* JX087481.1	0.003												
3	*Baeolidia moebii* JQ996857.1	0.003	0											
4	*Baeolidia_moebii* HQ616733.1	0.003	0	0										
5	*Baeolidia moebii* JQ996858.1	0.003	0	0	0									
6	*Baeolidia japonica* JQ996856.1	0.071	0.069	0.069	0.069	0.069								
7	*Baeolidia japonica* JQ996855.1	0.071	0.069	0.069	0.069	0.069	0							
8	*Baeolidia australis* MK100935.1	0.074	0.072	0.072	0.072	0.072	0.086	0.086						
9	*Baeolidia variabilis* JQ996852.1	0.1	0.097	0.097	0.097	0.097	0.075	0.075	0.074					
10	*Baeolidia variabilis* JQ996847.1	0.094	0.091	0.091	0.091	0.091	0.069	0.069	0.069	0.005				
11	*Baeolidia rieae* JQ996842.1	0.091	0.088	0.088	0.088	0.088	0.044	0.044	0.094	0.08	0.075			
12	*Baeolidia scottjohnsoni* JQ996840.1	0.077	0.074	0.074	0.074	0.074	0.034	0.034	0.077	0.072	0.066	0.044		
13	*Baeolidia nodosa* JX087527.1	0.135	0.132	0.132	0.132	0.132	0.138	0.138	0.154	0.151	0.151	0.154	0.141	
14	*Baeolidia nodosa* JX087494.1	0.138	0.135	0.135	0.135	0.135	0.141	0.141	0.157	0.154	0.154	0.151	0.144	0.003

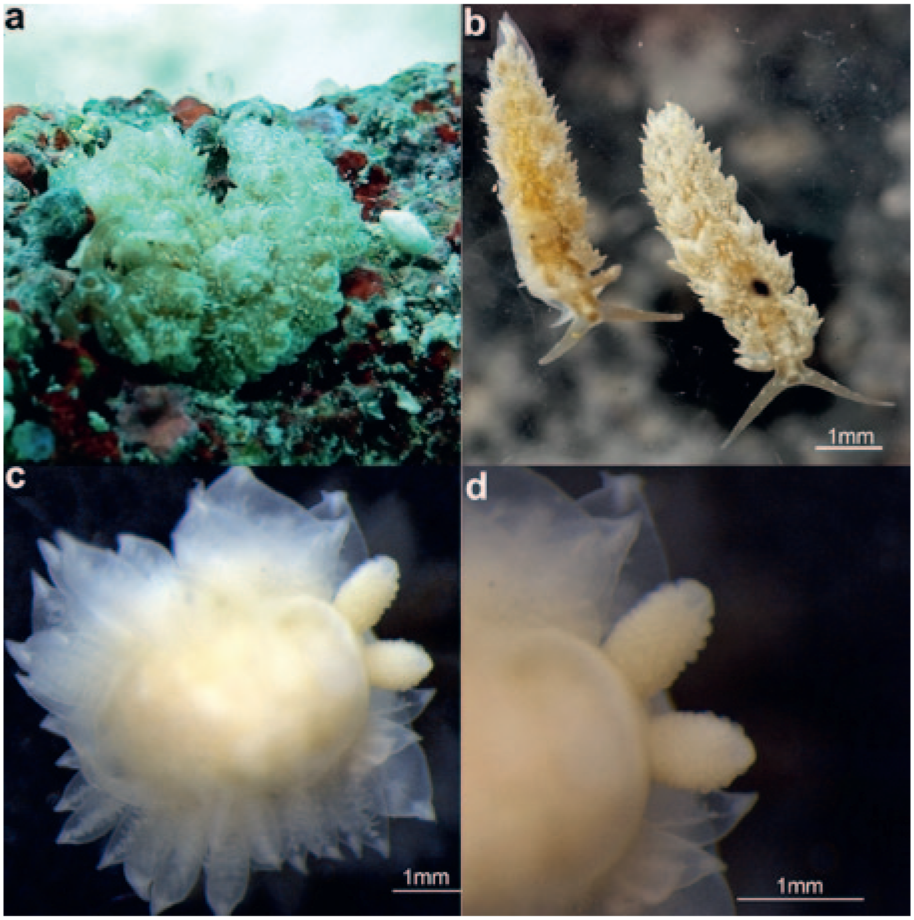

Figure 2 *Baeolidia moebii* (a) *in-situ*; (b) *ex-situ*; (c) preserved specimen; (d) rhinophores in preserved specimen

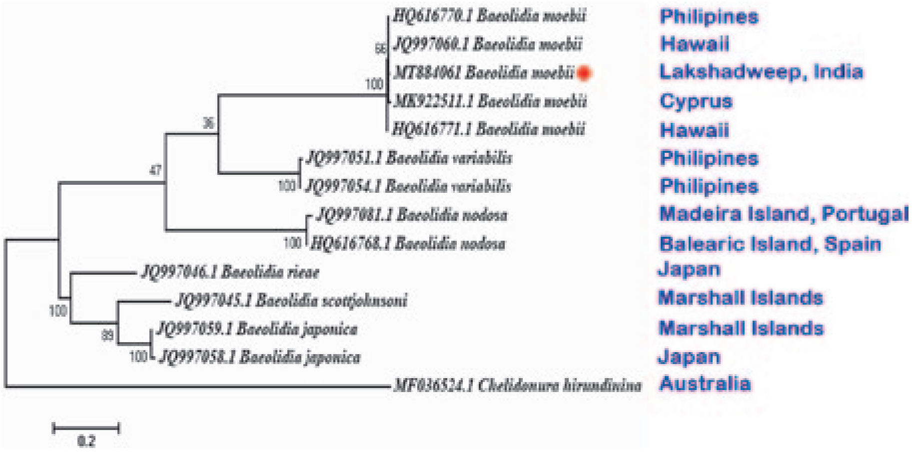

Figure 3 Maximum likelihood phylogenetic analysis based on mitochondrial COI gene. Red dot – specimen in present study. Numbers near nodes are bootstrap values.

Fig. 6 illustrates the widespread distribution of *B. moebii* and emphasises the need for further genetic data. Only five localities in three oceans have been sampled to date, but to reliably record its presence and further spread, more genetic data is required. More data from additional localities would assist in plotting this species spread from the Indian Ocean to the Pacific and the Mediterranean.

The present specimens also form a separate clade from *B. australis* (Rudman, 1982) based on the 16S gene sequence. The validity of *B. australis*

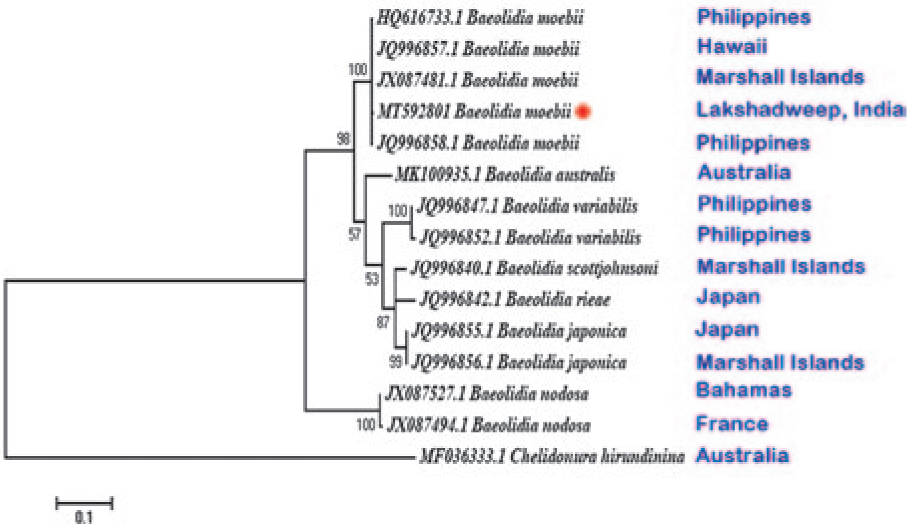

Figure 4 Maximum likelihood phylogenetic analysis based on mitochondrial 16SrRNA gene. Red dot – specimen in present study. Numbers near nodes are bootstrap values.

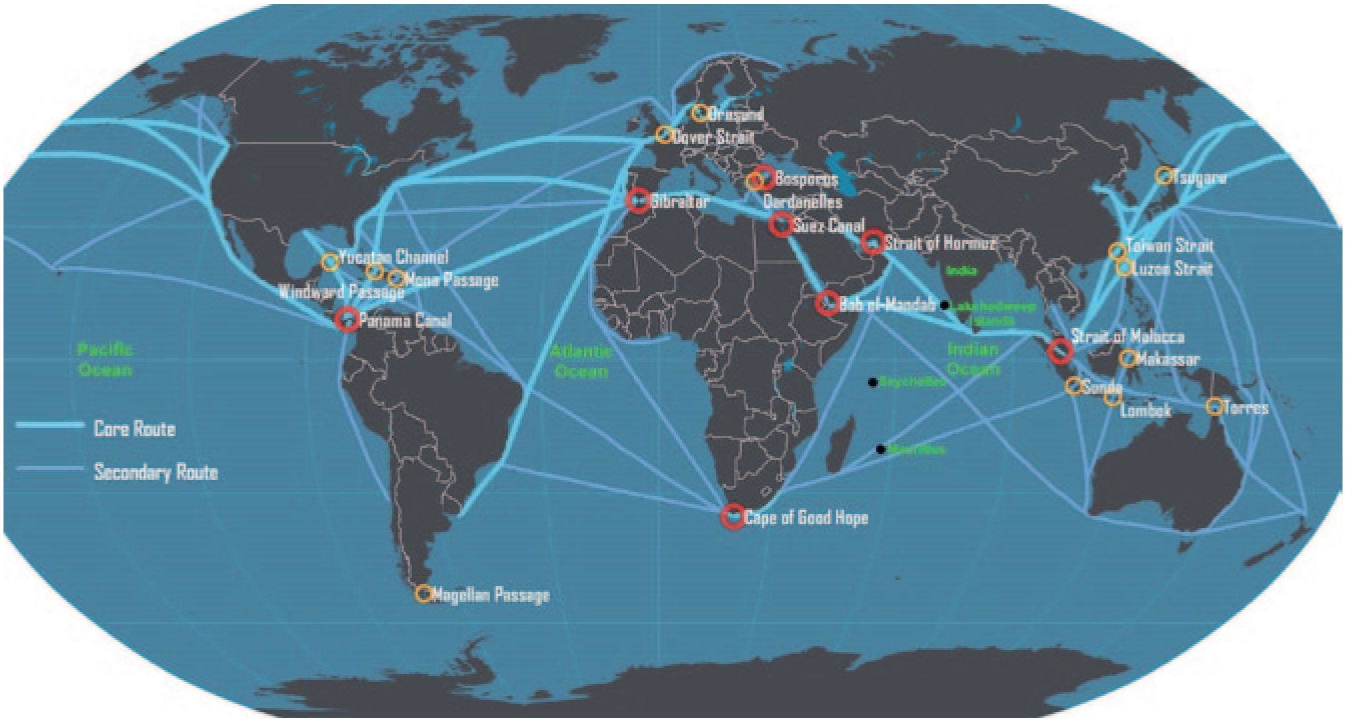

Figure 5 Major maritime shipping routes and strategic passages (adapted from Rodrigue J.P. 2017).

as a distinct species has always been in question. According to Carmona *et al*. (2014b), Rudman (2007) questioned the validity of *B. australis* and the authors then specified the need to examine specimens of *B. moebii* and *B. australis* from both morphological and molecular points of view. The morphological differences between the two species were elaborated in Carmona *et al*. (2014b) and four years later molecular evidence was provided in Goodheart *et al*. (2018), wherein *B. australis* formed a separate clade from the specimens of *B. moebii*, both collected from Australia (Goodheart *et al*. 2018: Fig. 5). However, the authors did not comment on taxonomy of both the species in the paper. As of now, there is only one mitochondrial gene dataset available for *B. australis* in the GenBank (16S gene) and if we believe the taxonomic identification of the specimen (ZFMK Wägele 208) used for 16S as correct, then the molecular analyses in the present study

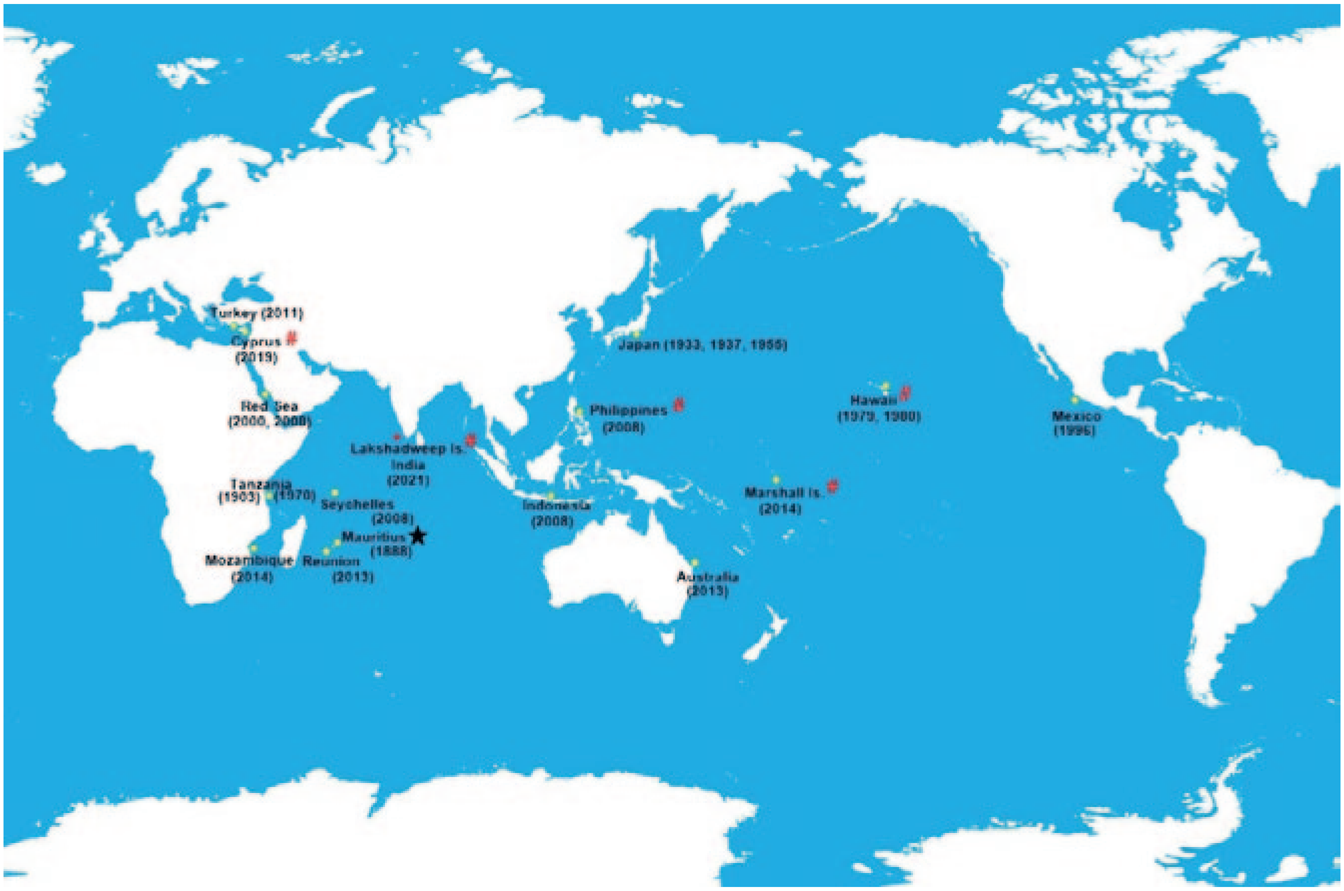

Figure 6 Distribution of *Baeolidia moebii* in world's oceans depicting the year of record. * Type locality, # places from where *B. moebii* is sequenced for molecular data (refer to Carmona *et. al*. 2014b for distributional references)

provide additional evidence to retain *B. australis* as a distinct species, at least until any topotypical material is sequenced.

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