

First Record of *Lobothorax Typus* from Odisha Coast, India, with Molecular Characterisation and Observation of its Biphasic Moulting

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Abstract

Two distinct morphologies of Cymothoidae isopod, *Lobothorax typus* were collected from the marine water of Bay of Bengal, Goapalpur-on-Sea as the first record of this parasite from coastal water of Odisha, India. All specimens were found attached to the buccal region of different individuals of the same host fish *Trichiurus lepturus*. With the aid of COI gene sequencing and morphological analysis, the individuals were found to be conspecific. The most prominent variation among the two morphologies includes the size of 5th pereonite and pleon length to total body length ratio. These variations are as a result of the biphasic moulting process. Maximum Likelihood (ML) tree analysis based on COI gene sequences concluded the monophyletic taxonomy of different buccal attaching genera under the family Cymothoidae which is in congruence with their morphological divergence.

Introduction

The study of the representatives of the family Cymothoidae are important as they are obligate ectoparasites to many fresh, brackish and marine fish species. Most of these parasites are show a high scale of the site and host-specific (Ravichandran et al. 2019). These parasites are known to cause a wide range of damaging effects to many host fish species which includes tissues damage, varieties of wounds, growth inhibition, behavioural changes, impacting the reproductive potential, even cause death to the small fishes; thereby impacting the fisheries resources (Rameshkumar et al.2013; Kumar et al .2015; Aneesh et al. 2018, 2019; Seth et al. 2020 a, b). Indian water is known to harbour 48 valid species belonging to 16 genera under the family Cymothoidae. Most of the studies on species of the family Cymothoidae in Indian water are confined to Southern Indian coast (Ravichandran et al. 2019). The information on the isopods of the family Cymothoidae from the coastal water of Odisha, India is scanty and requires a comprehensive long-term study to come up with an updated data (Seth et al. 2020 a, b).

The representatives of genera: *Cymothoa* Fabricius, 1793, *Ceratothoa* Dana, 1852, *Cinusa* Schiödte and Meinert, 1884, *Glossobius* Schiödte and Meinert, 1883, *Lobothorax* Bleeker, 1857, and *Smenispa* Özdikem, 2009 of the family: Cymothoidae are known to cause infection in the buccal cavity of the host fishes (Aneesh et al.2018).The genus *Lobothorax* of the family: Cymothoidae is consisting of three species viz., *L. aurita* (Schiödte & Meinert, 1883), *L. laevis* Richardson, 1910, and *L. typus* Bleeker, 1857. The species *L. typus* is known to occur from the South-eastern Asia region: Java, Jakarta, Indonesia (as Batavia Bleeker 1857, Schiödte and Meinert 1883), East Malaysia (Kumar et al. 2015), and South China Sea (Yu & Bruce 2006). In India, it is documented from the South-eastern coast (Rameshkumar et al. 2013; Ravichandran et al. 2019). The species *L. aurita* and *L. laevis* are known to occur from the Philippines (Schiödte and Meinert 1883; Richardson, 1910). The species *L. typus* is known to cause infection in the buccal region of the host fish species i.e. *Trichiurus lepturus* (Ravichandran et al. 2019) and *Lepturacanthus savala* (Yu and Bruce 2006). The details taxonomic features of *L. typus* was given by Yu and Bruce (2006). The present record of the species *L. typus* from water of Bay of Bengal, Gopalpur-on-Sea is the first material evidence of this isopod parasite from the Odisha coast, India.

Study of life cycle especially moulting in isopods is very important as it affects growth, development and reproduction. The biphasic moulting is generally observed in isopods in which posterior body part moults before the anterior half and break between the two halves at the junction between the 4th and 5th pereonites. The moulting in isopods has four stages: intermoult, premoult, ecdysis and postmoult. The changes, which are occurring in different stages of moulting can be observed from the observation of their appendages. In the female, moulting takes place by the shedding of old oostegites (brood plates). One pair of oostegites are shed through the ecdysis of the posterior half; followed by the shedding of remaining three pairs of oostegites through the subsequent ecdysis (Aneesh and Kappalli 2020; Sahadevan 2019). This manuscript only provides a note on the observation on the variation among the two distinct morphologies of the isopod *L. typus* in terms of 5th pereonite and pleon to total body length ratio, which are the results of the biphasic moulting.

In the recent decades, molecular identification based on cytochrome c oxidase subunit 1 (CO1) of mitochondrial genome has been successfully used in taxonomic studies, description of various species and diversity assessments of Crustaceans (Raupach et al. 2015). Due to intra and inter specific variations, species of the family Cymothoidae are known to exhibit high degree of polymorphism (Bruce 1981; Smit et al. 2014). In this juncture, molecular characterisation based on conserved region like COI gene is very useful in species level delineation and helpful in understating the phylogeny of the species of the family Cymothoidae. Till date there is no such report on the molecular characterisation of the COI gene of the species *L. typus*; the present report on the molecular characterisation of this species will be very useful to supplement its morphological data and intra-specific variation found in the species. The present manuscript also describes the phylogenetic relationship among the buccal attaching representing belonging to the family: Cymothoidae through Kimura 2 Parameter (K2P) distance and ML tree analysis based on COI genes of this parasite and other buccal attaching parasites obtained from the NCBI database.

Materials And Methods

The representatives of the host *T. lepturus* were collected during gill net operation in December 2018 from the water of Bay of Bengal, Gopalpur-on-Sea (19°15' 43.22"N, 84°54' 50.01" E), Odisha coast, India. The host fish species was identified as per the standard keys and description provided in Fish Base (Forese and Pauly 2018). The isopods of the family Cymothoidae were gently removed from their respective attachment sites of the host fish species and kept in 70% alcohol. The isopod parasites were identified after following the features as described by Yu and Bruce (2006) and Ravichandran et al. (2019). All the parasites and hosts are deposited and registered in repository of the Estuarine Biological Research Centre (EBRC), Zoological Survey of India (ZSI), Gopalpur-on-Sea, Odisha, India. Molecular identification and its characterization of these isopod parasites were carried out based on the COI gene sequence. DNA isolation from the isopod tissue samples was done by using the EX pure Tissue DNA isolation kit developed by Bogar Bio Bee stores Pvt Ltd. PCR amplification and next generation sequencing using Oxford Nanopore sequencing platform were carried out as per the description of Seth et al. (2019) and Cuscó et al. (2019). Especially 650–700 base pair of the full COI gene was targeted for amplification and sequencing purpose.

For both PCR amplification and sequencing of the COI gene, the universal LCO 1490: 5'-ggcaacaaatcataaagatattgg-3' and HCO 2198: 5'-taaacttcagggtgaccaaataatca-3' primers set were used. The PCR reaction mixture (25 µL) for COI gene was composed of isolated DNA (5 µL), forward primer (1.5 µL) and reverse primer (1.5 µL of), molecular grade deionised water (5 µL), and Taq Master Mix (12 µL). The PCR thermal regime was done by setting an initial denaturation step for the duration of 4 minutes at 95°C, followed by 34 cycles for the duration of 45 second at 95°C, 30 second at 40°C annealing temperature, 50 second at 72°C for the extension step, and for 10 minutes at 72°C for the final step, followed by indefinite hold at 4°C. The sequences were assembled by using Macvector 16.0.10. The obtained sequences were checked in the NCBI blast tool for similarity checking. The final sequences were submitted to NCBI to get the accession numbers. The accession number obtained from the NCBI for the submitted COI gene sequence of the parasites with 5th pereonite smaller (gravid female) and bigger (intermoult female) are MK672880 (number of base pair: 657) and MK672881 (number of base pair: 681) respectively.

For multiple sequences alignment, pair wise K2P distance and ML using Kimura-2-parameter model (Kimura 1980) for COI genes between the species *L. typus* and other species of the buccal attaching genera under the family Cymothoidae were carried through MEGA 10 (Kumar et al. 2018). We obtained available COI gene sequences from different database for the genera: *Glossobius* and *Cinusa*, but unable to retrieve any sequence for the genus: *Smenipisa*. Bootstrap analysis (Felsenstein 1985) was carried out to verify the robustness of the internal nodes in the evolutionary tree using 1000 replications.

Results

Cymothoidae parasites collected from the buccal region of different individuals of the same host fish *T. lepturus*, belong to same species *L. typus*; and are having brood pouch, so they belongs to same sex i.e. female. The parasites along with the host are shown in the figure 1. These parasites are in different stages of moulting and can be distinguished into two morphologies based on the size of the 5th pereonite (in terms of length of 5th pereonite to total body length percentage). The length of the 5th pereonite to total body length is 4.5-4.76 % in the four individuals having smaller 5th pereonite (gravid female) vs 7.7-7.81% in the four other individuals having bigger 5th pereonite (intermoult female). There is also difference in pleon to total body length comparison between these two morphologies. Pleon to body length percentage is comparatively larger in gravid female (28.18-30.43% vs 23.49-26.01%). The diagnostic features of the parasites are as per the description of Yu and Bruce (2006) and Rabichandran et al. (2019). The morphometric measurements of these parasites are provided in table 1. Taxonomic account of the isopod parasites are given below:

Order: Isopoda Latreille, 1817

Family: Cymothoidae, 1818

Genus: *Lobothorax* Bleeker, 1857

Type species and type locality: *Lobothorax typus* Bleeker, 1857; by monotypy, collected from Jakarta, Java, Indonesia (Rabichandran et al. 2019).

Diagnosis: The detailed identifying features and description of this isopod was provided by Yu and Bruce (2006) and Rabichandran et al. (2019).

Description: Number of materials examined: 08. Registration number: EBRC/ZSI/Cr-110911 A-D (04 examples, 5th pereonite smaller, gravid female) and Registration number: EBRC/ZSI/Cr-11090 A-D (04 examples, 5th pereonite bigger, intermoult female). Body of the parasite is straight. Pereon is having mid-dorsal longitudinal ridge, cephalon is triangular, and eyes are distinct and moderate in size. Pereonite 1st is the longest one and having expanded anterolateral process. Pereonites 2-4 are almost equal in length and distinctly shorter than the 1st one. Pereonites 5-7 are distinctly shorter than 1-4. Pereonite 5th is overlapped partly by the 4th pereonite. All pleonites are visible and sub-equal in length. The Pleonite 1 is narrow and partly overlapped by the pereonite 7. Pleonites 2-5 are gradually wider. The Pleotelson is subrectangular in shape and its posterior margin tapering to caudomedial point. The antennule is slightly shorter than antenna. The antennule is robust and consisting of 8 articles, the antenna is slender and consisting of 9 articles. The colour of body is whitish and there is presence of black and green chromatophores on the pleon region (Fig. 1).

Gravid female: It is about 2.54-2.69 times as long as wide (at 5th pereonite). 5th pereonite is distinctly smaller in terms of length as compared to the 6th and 7th pereonite. The length of the 5th pereonite is about 4.5-4.76 % percentage of total body length. Pleon is short; it is about 28.18-31.28 % of the total body length. The pleotelson is about 1.50-2.25 times as long as the wide. (Table 1; Fig. 1: b1 and b2)

Intermoult Female: It is about 2.15-2.98 times as long as wide (at 5th pereonite). 5th pereonite is distinctly bigger in terms of length as compared to the 6th and 7th pereonite. The length of the 5th pereonite is about 7.7-7.81 percentage of total body length. Pleon is short; it is about 23.49-27.07 percentage of the total body length. Pleotelson is about 1.6-2.01 times as long as the wide. (Table 1; Fig. 1: c1 and c2).

The COI gene of the *L. typus* is rich with AT nucleotides. The AT nucleotides percentage is about 63.51%. There is no difference in the K2P distance between the COI gene sequences of *L. typus*. The K2P distance between *L. typus* and species of the genus *Cymothoa* is 24-28%, between *L. typus* and species of the genus *Ceratothoa* is 30-34%, between *L. typus* and *Glossobius auritus* is 30-31% and between *L. typus* and *Cinusa tetrodontis* is 29-30%. The K2P distance between the genus *Cymothoa* and *Ceratothoa* is 26-33%, between *Cymothoa* and *G. auritus* is 28-30% and between *Cymothoa* and *C. tetrodontis* is 28-30%. The K2P distance between genus *Ceratothoa* and *G. auritus* is 26-30% and between *Ceratothoa* and *C. tetrodontis* is 26%. The K2P distance between *G. auritus* and *C. tetrodontis* is 27% (Table 2).

In the ML tree analysis: all the three COI gene sequences of *L. typus* belong to the same clade with a strong bootstrap value of 100%. All the buccal attaching genera: *Ceratothoa*, *Cinusa*, *Cymothoa*, and *Lobothorax* are monophyletic with bootstrap support of moderate to strong. Representative considered under the genus *Cymothoa* belongs to same clade with a strong bootstrap support of 71%, representatives of the genus

Ceratothoa belongs to same clade with a strong bootstrap support of 76%, single sequence of genera *Cinusa* and *Glossobius* are separated with moderate bootstrap value of 58% and 36% respectively (Fig. 2).

Discussion And Conclusion

The specimens of *L. typus* well matched with the description provided by Yu and Bruce (2006) and Rabichandran et al. (2019). The present finding of the isopod parasites *L. typus* from the buccal region of the host fish species *T. lepturus* from coastal Goaplpur-on-Sea extends its geographical range and is the first material evidence for the state of Odisha, India. The differences observed in the 5th pereonite in terms of its length and pleon to body length percentage of the two morphologies of the parasites; which are not showing any difference in other morphological features, in the K2P distance, COI gene sequence and belonging to same cluster in ML tree analysis; confirms the biphasic moulting in cymothoid isopod *L. typus*. This observation is the first material evidence of the biphasic moulting in this parasite. A long term study on the life cycle of this parasite will provide better understanding of its biology.

The Systematic study of the species of the family cymothoidae is tricky when only the data from the morphological features are available because sometimes intra-specific variation is adequately larger than the inter-specific variation. As a result of which phylogeny which is only based on morphological data may be confusing and misleading. Therefore, molecular marker like 16 s rRNA and COI gene sequences can be very useful in resolving the phylogeny of the species of the family cymothoidae (Hata et al. 2017). The present molecular characterization of COI gene of *L. typus*, will be useful for resolving the intra and inter specific variation encounter during the further study of the life cycle of this parasite. The present report of the monophyletic phylogeny of the buccal attaching isopods of the family cymothoidae is in congruence with their morphological divergence (Martin 2025; Hata et al., 2017). However, for a better understanding of the molecular systematics and phylogeny of the buccal attaching cymothoidae; more molecular data especially of the COI genes of the genera: *Cinusa*, *Glossobius* and *Smenipsa* need to be carried out.

Declarations

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Conflict of interest statements

Authors declare that they have no conflict of interest.

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Tables

Table 1: Morphometric measurements of *L. typus*

Sl. No.		Total Length of Body in mm (Head to Pletelson)	Width of Body in mm (At 5 th Pereonite)	Length of Pleotelson in mm	Width of Pleotelson in mm	Length of Pleon in mm (Pleonites to Pleotelson)	Pleon length as Percentage length of the body
1	Gravid Female	22.51	8.34	3.54	5.32	6.85	30.43
2		18.31	7.08	2.79	5.60	5.16	28.18
3		18.09	6.71	2.41	5.43	5.19	28.68
4		16.59	6.51	3.00	4.75	5.19	31.28
5	Inter-moult female	16.22	7.52	2.97	5.45	4.22	26.01
6		15.96	5.78	2.12	4.27	3.75	23.49
7		16.62	6.00	2.23	4.46	4.50	27.07
8		20.48	6.87	2.79	4.88	5.24	25.58

Table 2: Kimura 2 parameter distance (K2P) matrix in percentage

	Sp. 1	Sp. 2	Sp. 3	Sp. 4	Sp. 5	Sp. 6	Sp. 7	Sp. 8	Sp. 9	Sp. 10	Sp. 11	Sp. 12	Sp. 13	Sp. 14	Sp. 15
Sp. 1															
Sp. 2	0														
Sp. 3	0	0													
Sp. 4	28	27	27												
Sp. 5	28	27	27	0											
Sp. 6	24	24	24	20	20										
Sp. 7	25	25	25	20	20	0.5									
Sp. 8	27	26	25	0.3	0	19	20								
Sp. 9	28	28	28	0.3	0	19	20	0							
Sp. 10	27	27	26	5	5	20	20	5	5						
Sp. 11	34	34	34	28	27	33	33	27	27	29					
Sp. 12	34	34	34	28	27	33	33	27	27	29	0				
Sp. 13	31	30	30	28	27	29	29	26	27	27	22	22			
Sp. 14	31	31	30	30	30	33	33	28	30	29	30	30	26		
Sp. 15	30	29	29	30	29	32	32	28	30	29	26	26	26	27	

Note: Sp. 1-2: *Lobothorax typus*, Sp. 3: *Lobothorax sp.*, Sp. 4-5: *Cymothoa eremite*, Sp. 6-7: *Cymothoa excise*, Sp. 8-9: *Cymothoa pulchrum*, Sp. 10: *Cymothoa indica*, Sp. 11-12: *Ceratothoa oestroides*, Sp. 13: *Ceratothoa carinata*, Sp. 14: *Glossobius auritus*, Sp. 15: *Cinusa tetrodontis*

Figures

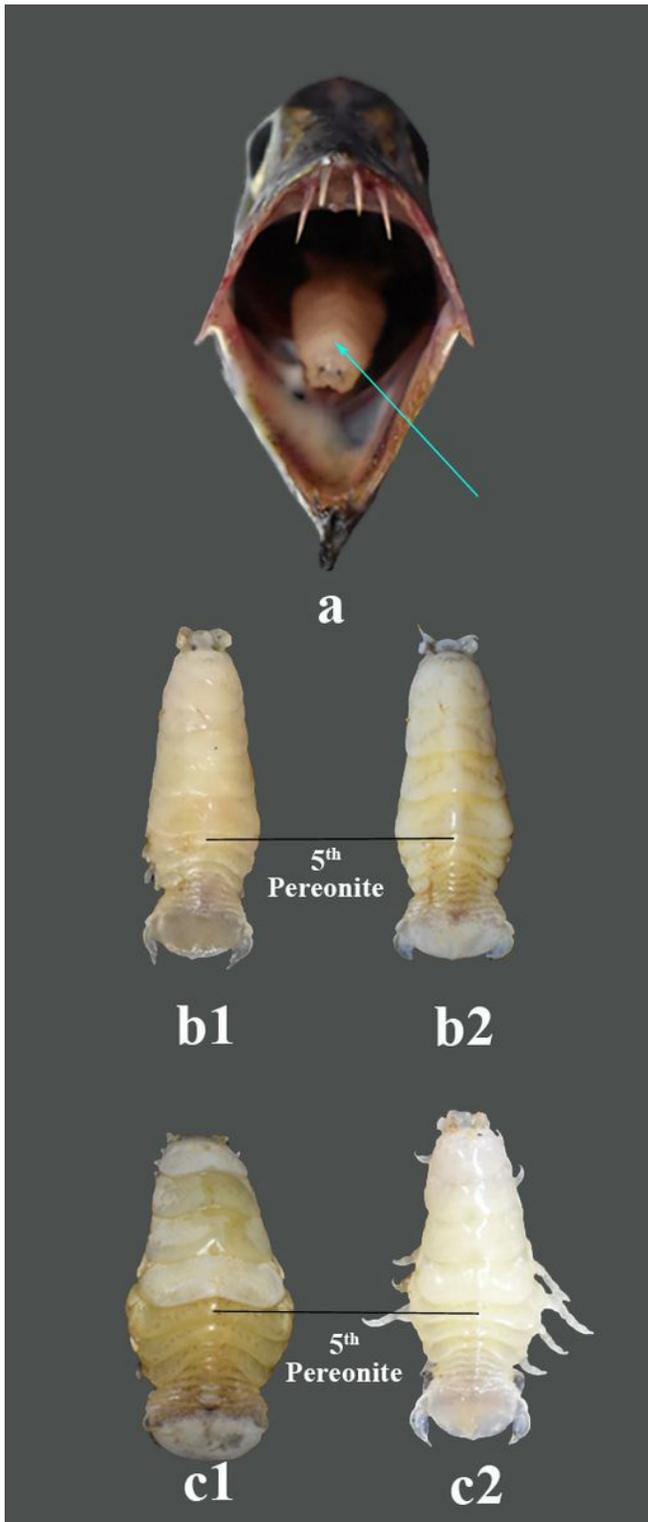


Figure 1

a: *L. typus* inside the buccal cavity of *T. lepturus*; b1 and b2: *L. typus* (Gravid Female); c1 and c2: *L. typus* (Intermoult female)

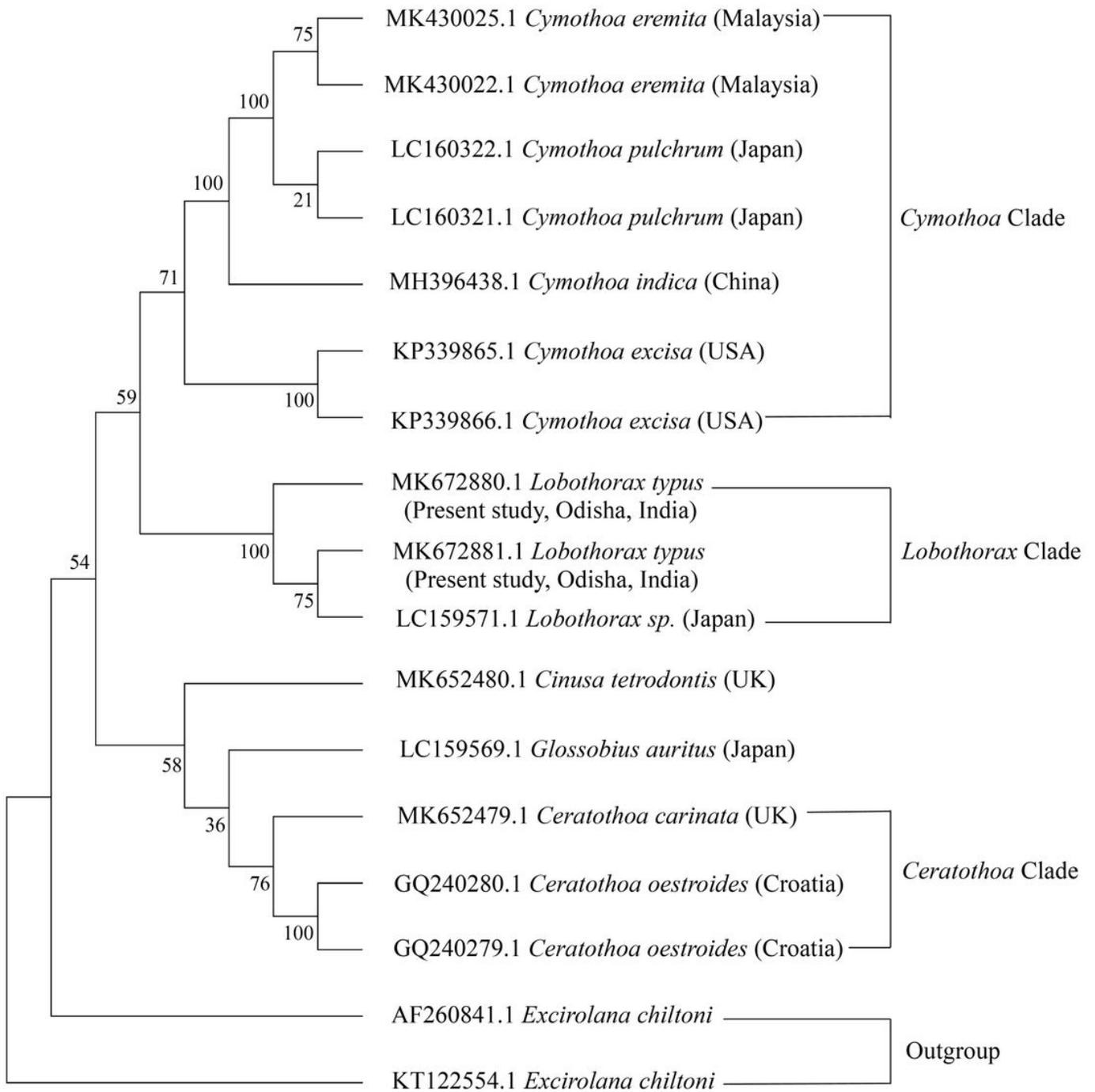


Figure 2

Maximum likelihood (ML) tree based on COI gene sequences of the buccal attaching cymothoidae