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Research Article

# Molecular Characterization of Two Myxosporean Species, *Henneguya namae* Haldar *et al*. 1983 and *Myxobolus sophorae* Jayasri, 1982 (Myxosporea: Myxobolidae)

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**Abstract.** In Indian freshwater fish myxosporean infections are among the most cosmopolitan parasites, they are relatively well studied morphologically but their phylogenetic relationships were unclear and the genetic data is limited only to a few species. The study aims to present molecular data for two myxosporean species, *Henneguya namae* Haldar *et al*. 1983 and *Myxobolus sophorae* Jayasri, 1982 collected from Indian freshwater fish, the elongate glass-perchlet *Chanda nama* (=*Ambassis nama*) and pool barb *Puntius sophore*, respectively. In the present study molecular data are provided for *H. namae* and *M. sophorae* using nested PCR. The obtained partial 18S rDNA gene sequences were analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. The 18S rDNA gene sequences of *H. namae* showed similarity with the sequences of *H. chaudhuryi*, *Henneguya* sp. RA-2015, *H. voronini* and *H. setiuensis* about 72.1 to 78% and *M. sophorae* with *Myxobolus ticto* was about 90% respectively. The aim of this paper was to identify *H. namae* and *M. sophorae* morphologically and using molecular methods.

**Keywords:** fish, *Henneguya*, India, *Myxobolus*, phylogeny, 18S rDNA.

## INTRODUCTION

Myxozoans are a diverse group of endoparasites inhabiting vertebrates generally fish, sometimes amphibians and mammals also (Okamura *et al.* 2015). In Indian fish, more than 23 species of *Henneguya* and about 130 *Myxobolus* species has been reported from freshwater habitats, mostly were described on the basis of myxospore shape and size morphology (Eiras 2002, Eiras *et al.* 2005, Kalavati and Nandi 2007, Kaur and Singh 2011, Eiras *et al.* 2014) and this number of species is going on increasing continuously (Székely *et al.* 2015, Gupta and Kaur 2017, Chaudhary *et al.* 2018, Ahmed *et al.* 2019). From India to the date, molecular sequences of 56 *Myxobolus* and 7 *Henneguya* species have been deposited in the GenBank. Recently, frequent occurrence of fish myxospores hastened us to investigate their diversity and infection in the present study area.

*Chanda nama* Hamilton, 1822 and *Puntius sophore* (Hamilton, 1822) distributes throughout Pakistan, India, Nepal, Bangladesh and Myanmar, also contributes

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significantly to the nutritional as well as the livelihood security of the rural mass. Both the fish have high economic value as these are appropriate for supplementing the part of diets among the developed and developing countries and also of ornamental value (Roos *et al.* 2003, Mohanty *et al.* 2013, Choudhary *et al.* 2015). In India, species of myxobolids were focused only based on spore morphology (Kalavati and Nandi 2007) and sometimes hard to discriminate between closely related species, therefore, molecular approaches are needed for correct identification.

In the present work, morphological redescription and molecular biological examinations of *H. namae* and *M. sophorae* were performed to support their validity.

## MATERIALS AND METHODS

### Specimens and morphological analysis

A total of 40 and 34 specimens of elongate glassy perchlet *Chanda nama* (=*Ambassis nama*) Hamilton, 1822 (Actinopterygii: Ambassidae) and pool barb *Puntius sophore* (Hamilton, 1822) (Actinopterygii: Cyprinidae) were collected respectively from the Ganga River at Bairaj, Bijnor (29° 23' N, 79° 11' E) from the local vendors that collected fish and from local fishermen in Meerut (29° 01' N, 77° 45' E), in the state of Uttar Pradesh (U.P.), India during the period between October 2018 to February 2019. They were transported in icebox to the laboratory at the Department of Zoology, Chaudhary Charan Singh University, Meerut, U.P., India for routine parasitological examination. Fish were euthanized by clove oil, fresh preparations of kidney, liver, gill filaments, gall bladder and muscles were examined for myxozoan infection under a Motic SMZ-168 series stereomicroscope (Motic, Xiamen, People's Republic of China). Infection was found in the gill filaments and kidney of *C. nama* and *P. sophore* respectively. Cysts from the gill filaments of *C. nama* and plasmodia within kidney tissue of *P. sophore* was examined as fresh preparations under a Nikon eclipse Ts2 microscope (Nikon Corporation, Tokyo, Japan) for morphology of the spores. A subset was fixed in 95% ethanol for subsequent molecular study. Photographs of the spores were taken with a Nikon eclipse (Ts2) microscope using Nikon NIS Elements Imaging software version 5.10. Measurements of fresh myxospores were taken according to the guidelines of Lom and Arthur (1989). All measurements reported here are in micrometers (μm) unless stated otherwise. Photos of spores were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India (Coll. No. HSS/ZOO/MYX/01/19 and HSS/ZOO/MYX/02/19).

### PCR and DNA sequencing

For DNA extraction, preserved sample in 95% ethanol were centrifuged at 8,000 × *g* for 5 min and then the ethanol was removed. Genomic DNA was extracted using the Qiagen DNeasy™ Blood & Tissue Kit (Qiagen, Germany), following the manufacturer's protocol. The partial 18S rDNA was amplified using the primers ERIB1 and ERIB10 (Table 1) in a 25 μl reaction mixture comprising 3 μl genomic DNA, 4 μl 1 mM deoxyribonucleotide triphosphates (dNTPs, Biotools, Spain), 0.50 μl of each primer, 2.5 μl of 10 × Taq buffer (Biotools, Spain), 0.50 μl of Taq polymerase (1 U; Biotools, Spain), and 14 μl of distilled water. The PCR cycle consisted of an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of at 95 °C for 50 s, 56 °C for 1 min, 72 °C for 1 min and a final extension at 72 °C for 7 min. This was followed by a second round of PCR with the Myx1F-SphR primer pair (Table 1). A total volume of the reaction was 50 μl, containing 2 μl of amplified DNA, 0.8 μl of each primer, 5 μl of 10 × Taq buffer (Biotools, Spain), 10 μl of the 1 mM dNTPs (Biotools, Spain), 0.9 μl of Taq polymerase (1 U; Biotools, Spain) and 30.5 μl of distilled water. PCR amplification protocol for the second round as follows: 95 °C for 3 min, then 35 cycles at 95 °C for 50 s, 56 °C for 1 min, 72 °C for 1 min, terminated with an extension at 72 °C for 10 min and then resting at 4 °C. The obtained PCR products were electrophoresed, separated by 1% agarose gel in Tris-acetate-EDTA buffer stained with 1% ethidium bromide and observed under ultraviolet light. They were then purified with a Purelink™ Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen, Löhne, Germany). Then purified PCR products were sequenced with the primers listed in Table 1, with the ABI Big Dye Terminator v3.1 Cycle Sequencing Kit in ABI 3100 Genetic Analyzer, Applied Biosystems (Foster City, California, USA).

### DNA sequence analyses

Sequences from closely related myxozoans were found out by BLASTn search and downloaded for further analysis. Sequences were aligned in BioEdit (Hall 1999) and ambiguous bases clarified using corresponding ABI chromatograms. For phylogenetic relationships of the present species with the related myxozoans, sequences retrieved from GenBank were aligned using the Clustal W program (Thompson *et al.* 1994) with defaulting setting, implementing in MEGA7 (Kumar *et al.* 2016). Phylogenetic analysis was conducted using maximum likelihood (ML) analysis performed in MEGA7 (Kumar *et al.* 2016) and Bayesian analyses were conducted in Topali 2.5 (Milne *et al.* 2009). For maximum likelihood (ML) and Bayesian analysis, the best evolutionary model was determined by jModelTest 3.0 (Posada 2008) which identified the general time reversible model (GTR + I + G) as the best evolutionary model, using Akaike information criteria. DNA pairwise sequence distances were calculated using the p-distance model in MEGA7. Bootstrap values based on 1,000 resampled datasets were generated for ML. For Bayesian inference (BI) analyses, posterior probabilities were estimated over 1,000,000 generations via five independent runs of four simultaneous MCMCMC (Metropolis-coupled Markov chain Monte Carl) chains with every 100th tree saved. The "burn in" was set to 25%. *Myxobolus cerebralis* (MN266293) was designated as outgroup.

## RESULTS

### *Henneguya namae* Haldar *et al.* 1983

Type host: *Chanda nama* (=*Ambassis nama*) Hamilton, 1822; chanda (local name).

**Table 1.** Primers used for PCR and sequencing in the present study.

| Primer | Sequence (5'–3') | Application | Source |
|---|---|---|---|
| ERIB1 | ACCTGGTTGATCCTGCCAG | 1st round PCR | Barta *et al.* 1997 |
| ERIB10 | CTTCCGCAGGTTCACCTACGG | 1st round PCR | Barta *et al.* 1997 |
| Myx1F | GTGAGACTGCGGACGGCTCAG | 2nd round PCR and sequencing | Hallet and Diamant 2001 |
| SphR | GTTACCATTGTAGCGCGCGT | 2nd round PCR and sequencing | Eszterbauer and Székely 2004 |
| MC5 | CCTGAGAAACGGCTACCACATCCA | Sequencing | Molnár *et al.* 2002 |
| MC3 | GATTAGCCTGACAGATCACTCCACGA | Sequencing | Molnár *et al.* 2002 |
| ACT1r | AATTTCACCTCTCGCTGCCA | Sequencing | Hallet and Diamant 2001 |

Site of infection: Gill filaments.

Locality: Ganga River at Bairaj, Bijnor (29° 23' N, 79° 11' E) in the state of Uttar Pradesh (U.P.), India.

Prevalence of infection: A total of 40 specimens of *Chanda nama* shows prevalence of infection 34/40 (of the 5–6 cm size in length with a prevalence of 85%; Intensity of infection: High).

Material deposited: Digital images (Photos) of spores were deposited in the parasitological collection of the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India collection no. (Coll. No. HSS/ZOO/MYX/01/19). The 18S rDNA sequence was deposited in GenBank under accession numbers MN218392 and MN218393.

Description: *H. namae* cyst present in the lamellae of gill filaments roundish in shape, interlamellar measuring 70–150 µm, small and large due to synchronicity development and contained myxospores that clearly showed high infection (Fig. 1 A, B). Spore body elongated with two caudal appendages (Fig. 1 C, D; Fig. 2 A, B). In frontal view, anterior end of spores appears blunt while the caudal end somewhat rounded and gradually continued into long, bifurcated caudal appendages (Fig. 2 A, B). Total spores length, 27.82–33.17 (30.6 ± 1.71) (N = 30); spore body length, 12.34–15.6 (14.15 ± 0.96) (N = 30); caudal appendages length, 15.12–17.92 (16.64 ± 1.03) (N = 30); spore width, 4.94–5.98 (5.41 ± 0.32) (N = 30) and spore thickness, 3.9–4.34 (4.07 ± 0.15) (N = 20). Spore wall smooth, composed of two uniformly thin valves, sutural line prominent and thick. Polar capsule two in number, elongated, pear shaped, pointed at anterior end and unequal in size. Larger capsule 3.18–4.16 (3.66 ± 0.34) (N = 30) long and 1.0–1.14 (1.08 ± 0.05) (N = 15) wide. Smaller capsule 2.84–3.73 (3.27 ± 0.31) (N = 30) long and 0.98–1.1(1.04 ± 0.04) (N = 15) wide. Number of polar filament coils seen 8–9 in large and 6–7 in small one. *H. namae* was identified on basis of the above characteristics. All the morphometrical measurements with closely related species listed in the supplementary table 1.

Remarks: *H. namae* was compared with other *Henneguya* spp. described parasitizing freshwater fish. Originally *H. namae* was described by Haldar *et al*. 1983 from gills of *C. nama*. Approximately > 20 species described thus far in Indian fish, the spores of *H. namae* infecting the gills of *C. nama* revealed the greatest similarity to the spores of *H. ophiocephali* Chakravarty 1939, *H. notopterae* Lalitha Kumari 1965, *H. qadrii* Qadri 1965, *H. singhi* Lalita Kumari 1969 and *H. thermalis* Seenappa *et al.* 1981. Moreover, the strict morphological comparisons showed that the shape, size of spore body and the length of caudal appendage of above respective species can be easily differentiated *H. namae* from others (Supplementary table 1). In the Supplementary table 1, we add *Henneguya* species reported from India that was similar to *H. namae* and shows species with unequal polar capsules i.e., *H. ophiocephali*, *H. notopterae*, *H. qadrii*, *H. singhi* and *H. thermalis*. *H. namae* could be distinguished from *H. ophiocephali* in the size and shape of the spore body as it is more rounded anteriorly in *H. ophiocephali*. *H. notopterae* have more pointed at the anterior end in spore shape in comparison to *H. namae* and additionally with a long duct in polar capsule. Spores of *H. qadrii* are smaller in size as compared to *H. namae* but size of polar capsules of *H. qadrii* is larger as compared to *H. namae*. Moreover, the spore body of *H. namae* is more elongated as compare to *H. singhi* as well as distinguished with each other in polar capsule shape. However, *H. thermalis* have a more rounded shape of spores while *H. namae* have little roundish and more pointed, both differ in the shape and size of polar capsules too. With regard to *H. chaudhuryi* (Bajpai and Haldar 1982) and *Henneguya* sp. RA-2015 (KR704889) Bala (2015), the differences are the presence of equal polar capsules while *H. namae* comprise unequal polar capsules. There are

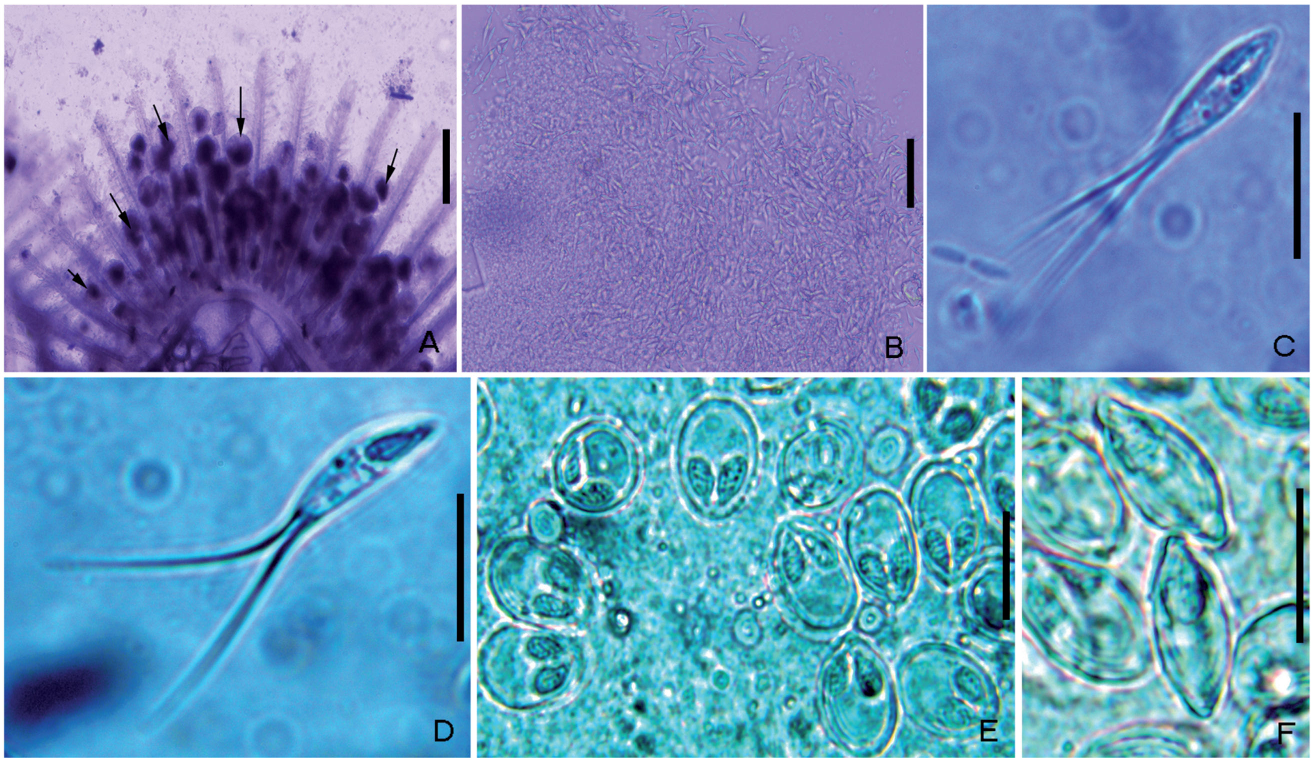



**Fig. 1.** Photographs of myxobolids: A – Cysts of *H. namae* of different sizes between gill filaments of the host fish show by arrows, B – Spores released from ruptured cysts of *H. namae*, C – *H. namae* frontal view, D – *H. namae* sutural view, E – *M. sophorae* frontal view, F – *M. sophorae* sutural view. Scale bars (A) 300 µm, (B) 50 µm, (C–F) 10 µm.

no molecular data available for the species *H. ophiocephali*, *H. notopterae*, *H. qadrii*, *H. singhi* and *H. thermalis*. Therefore, on the basis of above mentioned characteristics of *H. namae*, it can be readily distinguished from other species (see in supplementary table 1).

Molecular analysis: 18S rDNA of two different pools of isolates of *H. namae* were sequenced (1305 and 1315 bp). No intraspecific divergence was found among the newly generated sequences from isolates of *H. namae* and shown to be closely related with other *Henneguya* species described from Perciformes and Cypriniformes hosts.

### ***Myxobolus sophorae*** **Jayasri, 1982**

Type host: *Puntius sophore* (Hamilton, 1822); punti (local name).

Site of infection: Kidney.

Locality: Meerut (29° 01' N, 77° 45' E), in the state of Uttar Pradesh (U.P.), India.

Prevalence of infection: A total of 34 specimems of *Puntius sophore* shows prevalence of infection: 11/34 (of the 5–6 cm size in length with a prevalence of 32%; Intensity of infection: Low) during the present study.

Material deposited: Digital images (Photos) of spores were deposited in the parasitological collection of the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India collection no. (Coll. No. HSS/ZOO/MYX/02/19). The 18S rDNA sequence was deposited in GenBank under accession numbers MN595207 and MN595208.

Description: Plasmodia filled with spores and scattered spores were found in the kidneys. Spores ovo-rounded shaped in frontal view, anterior and posterior ends blunt, anterior end narrower than the posterior end (Fig. 1 E, F; Fig. 2 C, D). Spore length 14.0–15.1 (14.57 ± 0.33) (N = 30); width, 10.4–11.4 (10.91 ± 0.34) (N = 30) and thickness, 6.2–7.4 (6.91 ± 0.35) (N = 20). Polar capsules two in number, pyriform in shape and slightly unequal in size, obliquely located on either side of the midline, filling around half of the spore cavity. Larger capsule length 5.72–6.5 (6.04 ± 0.27) (N = 30) and width, 2.6–3.2 (2.89 ± 0.22) (N = 30). Smaller cap-

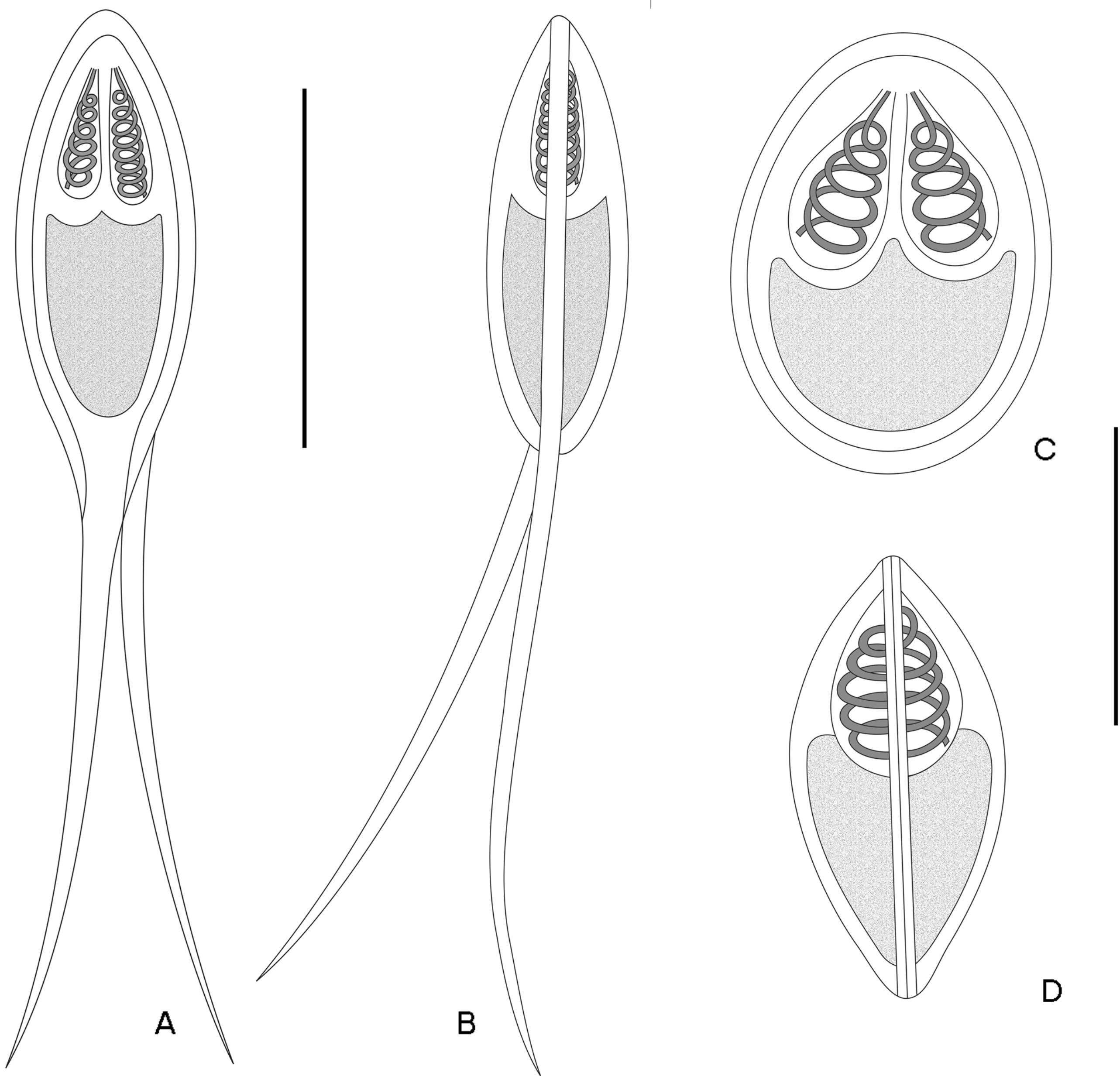



**Fig. 2.** A schematic drawing of *Henneguya namae* and *Myxobolus sophorae* myxospores found infect *Chanda nama* and *Puntius sophore*. In frontal view: A – *H. namae*, C – *M. sophorae*. In sutural view: B – *H. namae*, D – *M. sophorae*. Scale bars (A–D) 10 µm.

sule length 4.94–6.14 (5.48 ± 0.34) (N = 30) and width, 2.34–3.12 (2.69 ± 0.24) (N = 30). Polar capsules open to exterior of spores on either side of midline pointing towards each other, equipped with polar filaments which are distinctly seen. Number of filament coils seen is 5–6 in both the polar capsules, polar filament threadlike and uniform in their thickness throughout the length. *M. sophorae* identified morphologically on the basis of above described characteristics and the details of morphometrical data of *M. sophorae* with other related species are presented in supplementary table 2.

Remarks: This species was originally described from the gills and kidney of *P. sophore* by Jaysari, 1982. Myxospores of *M. sophorae* differentiated from the other species that infected host of the genus *Puntius* from India based on morphology and morphometrics

**Table 2.** Pairwise distance (above diagonal) and identity values (below diagonal) among myxozoan species of the ribosomal 18S gene sequences.

| | MN595207 | MN595208 | KJ476887 | MK412935 | MN218392 | MN218393 | KT279402 | KR704889 | MH743110 | MH743111 | KP030765 | AF306794 | KU516662 | JF311899 | MG253820 | MH329614 | MG520368 | MG520366 | HM624024 | KM092529 | KM401439 | MH119080 | KU160631 | KY911463 | GU574808 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MN595207 | | 0.00 | 0.07 | 0.27 | 0.41 | 0.41 | 0.29 | 0.39 | 0.27 | 0.28 | 0.18 | 0.30 | 0.25 | 0.22 | 0.18 | 0.16 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.18 | 0.15 | 0.19 | 0.18 |
| MN595208 | 100 | | 0.07 | 0.27 | 0.41 | 0.41 | 0.29 | 0.39 | 0.27 | 0.28 | 0.18 | 0.30 | 0.25 | 0.22 | 0.18 | 0.16 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.18 | 0.15 | 0.19 | 0.18 |
| KJ476887 | 90 | 90 | | 0.29 | 0.37 | 0.37 | 0.29 | 0.34 | 0.28 | 0.31 | 0.13 | 0.28 | 0.21 | 0.20 | 0.15 | 0.12 | 0.13 | 0.13 | 0.12 | 0.15 | 0.13 | 0.14 | 0.15 | 0.15 | 0.14 |
| MK412935 | 68.9 | 68.9 | 68.2 | | 0.42 | 0.42 | 0.38 | 0.39 | 0.36 | 0.36 | 0.26 | 0.36 | 0.33 | 0.30 | 0.27 | 0.26 | 0.26 | 0.26 | 0.27 | 0.27 | 0.27 | 0.26 | 0.27 | 0.24 | 0.24 |
| MN218392 | 53.1 | 53.1 | 56.5 | 50.9 | | 0.00 | 0.21 | 0.23 | 0.24 | 0.24 | 0.36 | 0.31 | 0.35 | 0.34 | 0.36 | 0.36 | 0.35 | 0.36 | 0.36 | 0.36 | 0.37 | 0.36 | 0.35 | 0.36 | 0.36 |
| MN218393 | 53.1 | 53.1 | 56.5 | 50.9 | 100 | | 0.21 | 0.23 | 0.24 | 0.24 | 0.36 | 0.31 | 0.35 | 0.34 | 0.36 | 0.36 | 0.35 | 0.36 | 0.36 | 0.36 | 0.37 | 0.36 | 0.35 | 0.36 | 0.36 |
| KT279402 | 67.6 | 67.6 | 65.9 | 58.6 | 78 | 78 | | 0.17 | 0.22 | 0.22 | 0.30 | 0.23 | 0.27 | 0.27 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.29 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| KR704889 | 57.7 | 57.7 | 61.7 | 56.1 | 76.4 | 76.4 | 80.6 | | 0.26 | 0.25 | 0.34 | 0.27 | 0.34 | 0.32 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.32 | 0.34 | 0.33 | 0.34 | 0.33 | 0.34 |
| MH743110 | 70.3 | 70.3 | 67.8 | 60.8 | 72.1 | 72.1 | 74 | 69.5 | | 0.13 | 0.29 | 0.23 | 0.27 | 0.27 | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 | 0.28 | 0.28 | 0.28 | 0.27 | 0.28 | 0.28 |
| MH743111 | 68.8 | 68.8 | 64.4 | 60.6 | 72.7 | 72.7 | 73.6 | 70.2 | 85 | | 0.31 | 0.24 | 0.27 | 0.26 | 0.31 | 0.30 | 0.31 | 0.31 | 0.32 | 0.28 | 0.31 | 0.31 | 0.26 | 0.31 | 0.31 |
| KP030765 | 81.4 | 81.4 | 85 | 71.2 | 57.5 | 57.5 | 65.8 | 62.4 | 67.7 | 63.8 | | 0.27 | 0.22 | 0.19 | 0.09 | 0.10 | 0.08 | 0.08 | 0.10 | 0.09 | 0.07 | 0.08 | 0.13 | 0.14 | 0.13 |
| AF306794 | 66.3 | 66.3 | 67.6 | 59 | 74.5 | 74.5 | 72.3 | 68.1 | 73.2 | 72.6 | 69.2 | | 0.28 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.28 | 0.27 | 0.27 | 0.27 | 0.29 | 0.27 | 0.28 |
| KU516662 | 70.1 | 70.1 | 75 | 62.9 | 56.8 | 56.8 | 67.3 | 60.7 | 68.3 | 68.5 | 74.3 | 66.5 | | 0.16 | 0.22 | 0.21 | 0.21 | 0.21 | 0.21 | 0.20 | 0.21 | 0.21 | 0.21 | 0.22 | 0.22 |
| JF311899 | 75.5 | 75.5 | 77.2 | 66.5 | 59.6 | 59.6 | 68.1 | 63.2 | 69.5 | 69.9 | 78.9 | 69.1 | 79.9 | | 0.19 | 0.19 | 0.19 | 0.19 | 0.18 | 0.20 | 0.19 | 0.19 | 0.21 | 0.19 | 0.18 |
| MG253820 | 80.8 | 80.8 | 83.5 | 69.9 | 57 | 57 | 65.9 | 61.8 | 66.9 | 64.4 | 90.2 | 69 | 74.4 | 78.2 | | 0.12 | 0.09 | 0.09 | 0.12 | 0.11 | 0.09 | 0.10 | 0.15 | 0.16 | 0.14 |
| MH329614 | 82.4 | 82.4 | 86 | 71 | 57.6 | 57.6 | 65.2 | 62.4 | 68.2 | 65.4 | 88.5 | 69 | 75.8 | 79.4 | 86.8 | | 0.10 | 0.10 | 0.05 | 0.12 | 0.10 | 0.11 | 0.06 | 0.14 | 0.13 |
| MG520368 | 81.6 | 81.6 | 85 | 71 | 57.7 | 57.7 | 65.4 | 61.9 | 67.9 | 64.5 | 91.7 | 68.2 | 75.5 | 78.5 | 89.9 | 87.9 | | 0.03 | 0.11 | 0.10 | 0.08 | 0.05 | 0.12 | 0.14 | 0.13 |
| MG520366 | 81.5 | 81.5 | 85.1 | 71.6 | 57.3 | 57.3 | 65.9 | 61.5 | 67.6 | 64.2 | 91.2 | 68.3 | 75.7 | 78.6 | 89.6 | 87.7 | 96.9 | | 0.12 | 0.10 | 0.08 | 0.04 | 0.13 | 0.15 | 0.14 |
| HM624024 | 82.2 | 82.2 | 85.9 | 70.4 | 57.5 | 57.5 | 65.4 | 62.2 | 67.5 | 63.6 | 88.8 | 68 | 75.7 | 79.4 | 86.7 | 94.2 | 87.8 | 87.6 | | 0.13 | 0.11 | 0.12 | 0.05 | 0.14 | 0.13 |
| KM092529 | 81.7 | 81.7 | 83.1 | 70.7 | 57.8 | 57.8 | 66.6 | 63.5 | 68.3 | 68.2 | 90.1 | 68.7 | 75.9 | 77.4 | 87.8 | 86.5 | 88.9 | 88.7 | 85.9 | | 0.01 | 0.10 | 0.13 | 0.16 | 0.15 |
| KM401439 | 81.4 | 81.4 | 85 | 70.5 | 56.6 | 56.6 | 65.4 | 62.1 | 68.2 | 64.3 | 92.4 | 68.8 | 75.4 | 78.8 | 89.8 | 87.8 | 91.1 | 90.8 | 88.3 | 98 | | 0.09 | 0.13 | 0.14 | 0.13 |
| MH119080 | 81 | 81 | 84.4 | 71 | 57.2 | 57.2 | 65.9 | 62.2 | 68.4 | 65.3 | 91.5 | 69.1 | 75.4 | 78.4 | 89.1 | 87.9 | 94.9 | 95.3 | 86.6 | 88.8 | 90.2 | | 0.12 | 0.15 | 0.14 |
| KU160631 | 83.5 | 83.5 | 82.6 | 70.6 | 58.2 | 58.2 | 66.1 | 62.3 | 70.4 | 70.5 | 85.3 | 67.2 | 75.7 | 76.8 | 82.9 | 93.1 | 85.7 | 85.5 | 94.3 | 84.7 | 84.9 | 85.7 | | 0.17 | 0.16 |
| KY911463 | 79.7 | 79.7 | 82.8 | 74 | 57.2 | 57.2 | 65.7 | 63.1 | 68.4 | 63.9 | 84.3 | 68.5 | 74.2 | 78.1 | 82.8 | 83.6 | 84.6 | 84.1 | 84.4 | 83.2 | 84.3 | 83.4 | 81.7 | | 0.06 |
| GU574808 | 80.6 | 80.6 | 83.9 | 74.2 | 57.4 | 57.4 | 66 | 62.6 | 68.6 | 64.6 | 84.6 | 68.6 | 74.7 | 79.1 | 84 | 85.2 | 84.5 | 84.2 | 84.5 | 83.4 | 84.4 | 84.3 | 82.6 | 92.7 | |
| MK412938 | 83.9 | 83.9 | 84.4 | 69.8 | 57.8 | 57.8 | 65.7 | 62.6 | 66.8 | 63.5 | 85.4 | 68.3 | 75.6 | 76.7 | 84.1 | 90.5 | 84.8 | 84.9 | 89.8 | 84.4 | 85.2 | 85 | 88.5 | 82.6 | 82.5 |
| KJ725075 | 81.2 | 81.2 | 82.3 | 71.1 | 58 | 58 | 66.5 | 61.8 | 68.8 | 69.4 | 89.3 | 66.3 | 75.7 | 77.9 | 88.4 | 86.3 | 97 | 95.9 | 85.2 | 88.1 | 88.5 | 93.9 | 85.1 | 81.3 | 82.8 |
| HM146129 | 80.4 | 80.4 | 83.5 | 74.3 | 57.5 | 57.5 | 66 | 63.1 | 68.3 | 64.5 | 85 | 68.5 | 74.3 | 79.1 | 83.8 | 84.6 | 85.1 | 84.6 | 85 | 83.7 | 85.3 | 84 | 82.2 | 94.3 | 95.8 |
| MH329618 | 82.3 | 82.3 | 81.4 | 74.4 | 57.9 | 57.9 | 66.5 | 63.1 | 70.1 | 70.4 | 81.6 | 67 | 73.8 | 76.7 | 81 | 82.2 | 82.3 | 82 | 81.8 | 81.6 | 81.8 | 81.7 | 81.6 | 92.8 | 96 |
| KC843624 | 80.6 | 80.6 | 84.8 | 71.2 | 57.5 | 57.5 | 66.6 | 62.4 | 68 | 64.7 | 88.1 | 69 | 75.1 | 78.3 | 86.4 | 91.2 | 88.1 | 88 | 91 | 85.3 | 87.2 | 87.3 | 88.3 | 84.2 | 84.2 |
| MH119079 | 81.2 | 81.2 | 84.7 | 70.5 | 56.7 | 56.7 | 65.3 | 61.8 | 68.2 | 65.3 | 91 | 69.1 | 75.3 | 78.9 | 89.5 | 88.2 | 95.1 | 95 | 87.3 | 88.7 | 90.3 | 95.8 | 85.2 | 83.6 | 84.9 |
| MG520367 | 81.5 | 81.5 | 84.9 | 71.4 | 56.8 | 56.8 | 66.2 | 61.9 | 67.7 | 64.2 | 91.2 | 68.9 | 75.4 | 79.5 | 89.7 | 88.1 | 95.1 | 94.9 | 88.3 | 88.9 | 91.1 | 94.3 | 85.9 | 84.3 | 84.5 |
| AB447994 | 66.5 | 66.5 | 68 | 59.1 | 75.5 | 75 | 74.9 | 69.4 | 71.5 | 68.5 | 69.7 | 75.5 | 67.1 | 68.3 | 68.8 | 69.2 | 69.3 | 69.3 | 69 | 67.9 | 69.1 | 69.9 | 67.9 | 69.3 | 69.3 |
| AB447992 | 66.6 | 66.6 | 68.1 | 59.4 | 75.1 | 75.1 | 75.3 | 69.2 | 71 | 68.3 | 70.1 | 75.2 | 66.8 | 68.7 | 69.3 | 69.3 | 69.6 | 69.4 | 69.2 | 68 | 69.2 | 69.9 | 68.2 | 69.7 | 69.8 |
| AB447993 | 66.6 | 66.6 | 68.6 | 59.3 | 75 | 75 | 72.6 | 68.4 | 72.4 | 70.1 | 69.8 | 75.5 | 67.5 | 69.5 | 69.9 | 70 | 69.3 | 69.2 | 69.6 | 68.7 | 69.4 | 70.3 | 68.3 | 68.7 | 69.7 |
| EU643628 | 75.1 | 75.1 | 74.8 | 65 | 58.1 | 58.1 | 66.8 | 62.3 | 68.5 | 69.7 | 75.8 | 68.5 | 78.9 | 88.9 | 75.8 | 76.8 | 76.1 | 76.1 | 76.5 | 75.5 | 76 | 76.1 | 76.2 | 76.8 | 76.8 |
| MK371243 | 77.4 | 77.4 | 78.2 | 67.5 | 56.4 | 56.4 | 65.9 | 62.6 | 67 | 64.4 | 80.7 | 68.1 | 76.1 | 78.2 | 80.3 | 79.6 | 81.1 | 80.7 | 79.9 | 78.5 | 79.8 | 80.3 | 76.8 | 80.2 | 79.6 |
| KC711053 | 66.6 | 66.6 | 65.9 | 59 | 60.3 | 60.3 | 70.4 | 65.7 | 70.9 | 71.7 | 66.5 | 70.1 | 66.2 | 67.1 | 65.4 | 67 | 66.2 | 66 | 67.2 | 67.7 | 66.1 | 66.3 | 67.4 | 67.2 | 67.2 |
| AB693052 | 67.2 | 67.2 | 67 | 59.1 | 59.4 | 59.4 | 68.6 | 64.5 | 68.7 | 65.1 | 66.3 | 72.7 | 65.6 | 68.3 | 66.2 | 67.5 | 66.3 | 66.7 | 66.8 | 68.5 | 66 | 67.7 | 67.9 | 66.6 | 67 |
| KP030761 | 70 | 70 | 69.1 | 61.6 | 74 | 74 | 74.4 | 69.4 | 77.1 | 71.3 | 71.1 | 75.8 | 68.8 | 70.4 | 69.9 | 70.5 | 70.8 | 70.7 | 70.4 | 71.2 | 71.1 | 71 | 70 | 70.7 | 69.7 |
| KJ725078 | 65.2 | 65.2 | 63.2 | 57.2 | 58.8 | 58.8 | 70.1 | 64.6 | 66.6 | 67.6 | 63.5 | 68.1 | 65.2 | 65.4 | 63.2 | 64.4 | 64.2 | 64.3 | 64.1 | 64.6 | 63.2 | 63.8 | 64.8 | 64.7 | 64.8 |
| KF264964 | 66.7 | 66.7 | 67.5 | 57.9 | 59.9 | 59.9 | 68.5 | 65.2 | 69.4 | 67.1 | 68.1 | 73 | 66.1 | 67 | 67.1 | 68 | 67.3 | 67.1 | 68 | 68.9 | 67 | 67.3 | 68.4 | 67.2 | 67.2 |
| AB693050 | 66.9 | 66.9 | 66.7 | 59.5 | 60 | 60 | 69.1 | 65 | 68.6 | 65.5 | 66.5 | 73 | 66 | 68.2 | 66.7 | 67.2 | 66.4 | 66.7 | 66.8 | 68.8 | 66.4 | 67.8 | 68.3 | 66.4 | 66.6 |
| MH300136 | 59.1 | 59.1 | 57.5 | 51 | 50.8 | 50.8 | 60.2 | 56.8 | 61.4 | 61.9 | 57.6 | 59.9 | 59.5 | 58.5 | 57.4 | 56.8 | 56.3 | 55.8 | 56 | 60.7 | 56.5 | 56.9 | 59.3 | 56.1 | 58.1 |
| MF118774 | 67.2 | 67.2 | 71.6 | 59.4 | 59.8 | 59.8 | 74.9 | 64.7 | 77.2 | 77 | 70.9 | 75.8 | 66.8 | 73.5 | 71 | 71.7 | 71.3 | 71.6 | 71.4 | 73.8 | 70.3 | 72 | 74.9 | 71.9 | 72 |
| KY172846 | 65.3 | 65.3 | 65.9 | 59.4 | 60.8 | 60.8 | 71.7 | 65.7 | 72 | 72.5 | 67.5 | 73.8 | 66 | 68.7 | 67.4 | 68.1 | 67.5 | 67.5 | 68.1 | 69.1 | 67.7 | 67.1 | 69 | 66.6 | 66.5 |
| KJ849240 | 66.3 | 66.3 | 67.6 | 58.1 | 61 | 61 | 71.4 | 66.6 | 71.3 | 68.7 | 68.2 | 75.1 | 66.5 | 68.4 | 67.6 | 69.1 | 67.9 | 67.9 | 69.4 | 68.1 | 67.6 | 67.6 | 68.8 | 68.6 | 68.8 |
| AB183747 | 66.3 | 66.3 | 66.3 | 58.1 | 59.8 | 59.8 | 68.6 | 64.6 | 68.9 | 65.5 | 66.5 | 71.8 | 65.4 | 68.2 | 66.4 | 66.4 | 66.1 | 65.8 | 65.1 | 67.8 | 65.5 | 67 | 65.5 | 66.1 | 66.2 |
| KY172851 | 65.7 | 65.7 | 66.1 | 59.3 | 60.4 | 60.4 | 71.2 | 65.5 | 71.4 | 72.1 | 67.1 | 72.2 | 66.2 | 68.6 | 67.1 | 67.7 | 67 | 66.9 | 68.1 | 68.8 | 67.1 | 66.7 | 68.7 | 66.2 | 66.4 |
| KP099967 | 85.1 | 85.1 | 71.5 | 57.4 | 55.2 | 55.2 | 72.8 | 54.9 | 76 | 74.4 | 72.5 | 77.3 | 74.3 | 70.9 | 70.5 | 70.7 | 70.9 | 70 | 70.1 | 71.1 | 71 | 70.9 | 71.1 | 72.1 | 71.2 |
| MF582545 | 69 | 69 | 71.1 | 60.1 | 57.2 | 57.2 | 67 | 63.2 | 67.7 | 66.5 | 71.6 | 69.6 | 72.5 | 71.4 | 70.5 | 71.5 | 71.6 | 71.8 | 71.4 | 70.9 | 70.9 | 71.5 | 68.8 | 71.7 | 72 |
| MN266293 | 71.6 | 71.6 | 72.1 | 61.9 | 58.2 | 58.2 | 68.5 | 63.7 | 67.8 | 65.2 | 72.4 | 70.3 | 74.2 | 71.2 | 71.6 | 72.6 | 72.2 | 72.6 | 72.7 | 71.5 | 72.3 | 72.3 | 70.4 | 72.6 | 72.8 |

| MK412938 | KJ725075 | HM146129 | MH329618 | KC843624 | MH119079 | MG520367 | AB447994 | AB447992 | AB447993 | EU643628 | MK371243 | KC711053 | AB693052 | KP030761 | KJ725078 | KF264964 | AB693050 | MH300136 | MF118774 | KY172846 | KJ849240 | AB183747 | KY172851 | KP099967 | MF582545 | MN266293 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.15 | 0.18 | 0.18 | 0.17 | 0.18 | 0.18 | 0.17 | 0.29 | 0.29 | 0.29 | 0.23 | 0.21 | 0.28 | 0.27 | 0.27 | 0.28 | 0.28 | 0.27 | 0.34 | 0.27 | 0.29 | 0.28 | 0.28 | 0.29 | 0.13 | 0.27 | 0.24 |
| 0.15 | 0.18 | 0.18 | 0.17 | 0.18 | 0.18 | 0.17 | 0.29 | 0.29 | 0.29 | 0.23 | 0.21 | 0.28 | 0.27 | 0.27 | 0.28 | 0.28 | 0.27 | 0.34 | 0.27 | 0.29 | 0.28 | 0.28 | 0.29 | 0.13 | 0.27 | 0.24 |
| 0.13 | 0.16 | 0.15 | 0.17 | 0.14 | 0.14 | 0.13 | 0.27 | 0.27 | 0.27 | 0.23 | 0.19 | 0.28 | 0.26 | 0.26 | 0.30 | 0.27 | 0.26 | 0.35 | 0.23 | 0.28 | 0.27 | 0.27 | 0.28 | 0.22 | 0.25 | 0.23 |
| 0.27 | 0.26 | 0.24 | 0.23 | 0.26 | 0.27 | 0.26 | 0.36 | 0.36 | 0.36 | 0.32 | 0.30 | 0.36 | 0.35 | 0.35 | 0.37 | 0.38 | 0.34 | 0.41 | 0.35 | 0.36 | 0.37 | 0.37 | 0.36 | 0.40 | 0.36 | 0.34 |
| 0.36 | 0.35 | 0.36 | 0.35 | 0.36 | 0.37 | 0.36 | 0.29 | 0.30 | 0.30 | 0.36 | 0.37 | 0.32 | 0.32 | 0.31 | 0.31 | 0.33 | 0.32 | 0.40 | 0.32 | 0.31 | 0.31 | 0.33 | 0.31 | 0.34 | 0.35 | 0.34 |
| 0.36 | 0.35 | 0.36 | 0.35 | 0.36 | 0.37 | 0.36 | 0.29 | 0.30 | 0.30 | 0.36 | 0.37 | 0.32 | 0.32 | 0.31 | 0.31 | 0.33 | 0.32 | 0.40 | 0.32 | 0.31 | 0.31 | 0.33 | 0.31 | 0.34 | 0.35 | 0.34 |
| 0.30 | 0.29 | 0.30 | 0.30 | 0.30 | 0.30 | 0.29 | 0.21 | 0.21 | 0.23 | 0.29 | 0.28 | 0.24 | 0.24 | 0.22 | 0.24 | 0.26 | 0.24 | 0.32 | 0.19 | 0.23 | 0.23 | 0.25 | 0.23 | 0.22 | 0.29 | 0.27 |
| 0.33 | 0.34 | 0.33 | 0.33 | 0.34 | 0.34 | 0.34 | 0.26 | 0.26 | 0.27 | 0.33 | 0.33 | 0.28 | 0.28 | 0.27 | 0.28 | 0.28 | 0.27 | 0.35 | 0.28 | 0.27 | 0.27 | 0.29 | 0.27 | 0.41 | 0.32 | 0.31 |
| 0.30 | 0.28 | 0.29 | 0.27 | 0.29 | 0.28 | 0.29 | 0.24 | 0.25 | 0.23 | 0.28 | 0.29 | 0.23 | 0.25 | 0.20 | 0.28 | 0.26 | 0.25 | 0.31 | 0.18 | 0.22 | 0.23 | 0.25 | 0.23 | 0.18 | 0.29 | 0.28 |
| 0.33 | 0.27 | 0.31 | 0.27 | 0.31 | 0.31 | 0.31 | 0.27 | 0.27 | 0.26 | 0.27 | 0.30 | 0.23 | 0.28 | 0.23 | 0.26 | 0.28 | 0.27 | 0.31 | 0.18 | 0.22 | 0.26 | 0.28 | 0.22 | 0.20 | 0.30 | 0.31 |
| 0.12 | 0.10 | 0.14 | 0.17 | 0.10 | 0.08 | 0.08 | 0.26 | 0.26 | 0.26 | 0.22 | 0.17 | 0.28 | 0.26 | 0.25 | 0.30 | 0.26 | 0.26 | 0.35 | 0.24 | 0.27 | 0.26 | 0.26 | 0.27 | 0.21 | 0.25 | 0.23 |
| 0.28 | 0.30 | 0.28 | 0.29 | 0.27 | 0.27 | 0.27 | 0.21 | 0.21 | 0.21 | 0.27 | 0.27 | 0.24 | 0.21 | 0.20 | 0.27 | 0.22 | 0.21 | 0.32 | 0.17 | 0.21 | 0.19 | 0.22 | 0.22 | 0.17 | 0.27 | 0.26 |
| 0.21 | 0.21 | 0.22 | 0.23 | 0.22 | 0.21 | 0.21 | 0.27 | 0.27 | 0.27 | 0.17 | 0.20 | 0.27 | 0.27 | 0.27 | 0.27 | 0.28 | 0.26 | 0.32 | 0.26 | 0.27 | 0.26 | 0.28 | 0.26 | 0.23 | 0.23 | 0.21 |
| 0.21 | 0.20 | 0.19 | 0.21 | 0.19 | 0.19 | 0.18 | 0.27 | 0.26 | 0.26 | 0.11 | 0.19 | 0.27 | 0.26 | 0.26 | 0.28 | 0.27 | 0.26 | 0.34 | 0.21 | 0.26 | 0.26 | 0.25 | 0.25 | 0.23 | 0.24 | 0.24 |
| 0.13 | 0.11 | 0.14 | 0.18 | 0.12 | 0.10 | 0.09 | 0.27 | 0.27 | 0.26 | 0.22 | 0.17 | 0.29 | 0.27 | 0.26 | 0.30 | 0.27 | 0.26 | 0.35 | 0.23 | 0.27 | 0.27 | 0.27 | 0.27 | 0.23 | 0.26 | 0.23 |
| 0.08 | 0.13 | 0.13 | 0.17 | 0.08 | 0.11 | 0.10 | 0.27 | 0.27 | 0.26 | 0.22 | 0.18 | 0.27 | 0.26 | 0.25 | 0.30 | 0.26 | 0.26 | 0.35 | 0.22 | 0.26 | 0.26 | 0.27 | 0.27 | 0.23 | 0.25 | 0.23 |
| 0.13 | 0.03 | 0.14 | 0.17 | 0.11 | 0.04 | 0.05 | 0.27 | 0.26 | 0.26 | 0.22 | 0.17 | 0.28 | 0.26 | 0.25 | 0.29 | 0.26 | 0.26 | 0.35 | 0.22 | 0.27 | 0.27 | 0.26 | 0.27 | 0.23 | 0.25 | 0.23 |
| 0.13 | 0.04 | 0.14 | 0.17 | 0.11 | 0.05 | 0.05 | 0.27 | 0.27 | 0.26 | 0.22 | 0.17 | 0.28 | 0.26 | 0.26 | 0.29 | 0.27 | 0.26 | 0.35 | 0.22 | 0.27 | 0.27 | 0.27 | 0.27 | 0.25 | 0.25 | 0.23 |
| 0.09 | 0.14 | 0.14 | 0.17 | 0.08 | 0.11 | 0.11 | 0.27 | 0.27 | 0.26 | 0.22 | 0.18 | 0.27 | 0.26 | 0.26 | 0.30 | 0.26 | 0.26 | 0.35 | 0.22 | 0.26 | 0.24 | 0.25 | 0.24 | 0.24 | 0.24 | 0.23 |
| 0.14 | 0.11 | 0.15 | 0.17 | 0.13 | 0.10 | 0.10 | 0.28 | 0.28 | 0.27 | 0.22 | 0.19 | 0.27 | 0.26 | 0.25 | 0.29 | 0.27 | 0.26 | 0.32 | 0.21 | 0.26 | 0.24 | 0.25 | 0.24 | 0.23 | 0.24 | 0.22 |
| 0.12 | 0.10 | 0.13 | 0.17 | 0.11 | 0.09 | 0.08 | 0.27 | 0.27 | 0.26 | 0.22 | 0.18 | 0.28 | 0.26 | 0.25 | 0.30 | 0.27 | 0.26 | 0.35 | 0.23 | 0.26 | 0.24 | 0.24 | 0.24 | 0.23 | 0.23 | 0.22 |
| 0.13 | 0.06 | 0.14 | 0.17 | 0.11 | 0.04 | 0.05 | 0.26 | 0.26 | 0.25 | 0.22 | 0.17 | 0.28 | 0.26 | 0.25 | 0.30 | 0.27 | 0.26 | 0.35 | 0.22 | 0.27 | 0.23 | 0.24 | 0.24 | 0.23 | 0.23 | 0.22 |
| 0.10 | 0.13 | 0.17 | 0.17 | 0.11 | 0.13 | 0.12 | 0.28 | 0.27 | 0.27 | 0.22 | 0.21 | 0.27 | 0.27 | 0.27 | 0.30 | 0.27 | 0.26 | 0.33 | 0.20 | 0.26 | 0.23 | 0.24 | 0.23 | 0.23 | 0.22 | 0.21 |
| 0.15 | 0.17 | 0.06 | 0.07 | 0.14 | 0.15 | 0.15 | 0.27 | 0.26 | 0.27 | 0.21 | 0.18 | 0.27 | 0.25 | 0.26 | 0.29 | 0.27 | 0.26 | 0.36 | 0.22 | 0.28 | 0.23 | 0.24 | 0.23 | 0.23 | 0.22 | 0.21 |
| 0.15 | 0.16 | 0.03 | 0.04 | 0.14 | 0.14 | 0.13 | 0.27 | 0.26 | 0.27 | 0.21 | 0.18 | 0.27 | 0.26 | 0.26 | 0.29 | 0.27 | 0.26 | 0.35 | 0.22 | 0.28 | 0.22 | 0.23 | 0.23 | 0.23 | 0.22 | 0.21 |
| | 0.15 | 0.15 | 0.18 | 0.11 | 0.13 | 0.12 | 0.27 | 0.27 | 0.26 | 0.22 | 0.19 | 0.27 | 0.25 | 0.25 | 0.29 | 0.26 | 0.25 | 0.32 | 0.20 | 0.26 | 0.22 | 0.23 | 0.23 | 0.23 | 0.21 | 0.20 |
| 83.1 | | 0.16 | 0.17 | 0.13 | 0.06 | 0.06 | 0.30 | 0.30 | 0.29 | 0.23 | 0.19 | 0.29 | 0.28 | 0.28 | 0.29 | 0.29 | 0.28 | 0.33 | 0.22 | 0.27 | 0.22 | 0.23 | 0.22 | 0.23 | 0.21 | 0.20 |
| 82.3 | 83 | | 0.03 | 0.14 | 0.14 | 0.14 | 0.27 | 0.27 | 0.27 | 0.21 | 0.18 | 0.27 | 0.26 | 0.26 | 0.29 | 0.27 | 0.26 | 0.34 | 0.22 | 0.28 | 0.22 | 0.22 | 0.22 | 0.23 | 0.20 | 0.20 |
| 80.4 | 82.2 | 97.3 | | 0.17 | 0.17 | 0.16 | 0.28 | 0.27 | 0.28 | 0.22 | 0.21 | 0.27 | 0.27 | 0.27 | 0.28 | 0.28 | 0.27 | 0.31 | 0.20 | 0.29 | 0.21 | 0.22 | 0.22 | 0.23 | 0.20 | 0.19 |
| 87.6 | 85.8 | 84.4 | 81.8 | | 0.11 | 0.11 | 0.27 | 0.26 | 0.26 | 0.22 | 0.18 | 0.29 | 0.26 | 0.26 | 0.30 | 0.27 | 0.26 | 0.34 | 0.23 | 0.27 | 0.21 | 0.22 | 0.22 | 0.23 | 0.19 | 0.19 |
| 84.5 | 93.3 | 84.5 | 82.1 | 87.1 | | 0.05 | 0.27 | 0.26 | 0.26 | 0.22 | 0.17 | 0.28 | 0.26 | 0.26 | 0.30 | 0.27 | 0.26 | 0.35 | 0.23 | 0.27 | 0.21 | 0.22 | 0.21 | 0.23 | 0.19 | 0.18 |
| 85.1 | 93.5 | 85 | 82.5 | 87.7 | 94.4 | | 0.27 | 0.26 | 0.26 | 0.21 | 0.17 | 0.29 | 0.26 | 0.26 | 0.30 | 0.27 | 0.26 | 0.35 | 0.22 | 0.27 | 0.21 | 0.21 | 0.21 | 0.23 | 0.19 | 0.18 |
| 69.5 | 65.6 | 69 | 67.1 | 69.3 | 69.4 | 69 | | 0.04 | 0.08 | 0.27 | 0.26 | 0.23 | 0.20 | 0.20 | 0.26 | 0.20 | 0.19 | 0.32 | 0.17 | 0.23 | 0.20 | 0.21 | 0.21 | 0.22 | 0.18 | 0.18 |
| 69.5 | 65.8 | 69.6 | 68.3 | 69.5 | 69.6 | 69.5 | 95.6 | | 0.08 | 0.27 | 0.26 | 0.23 | 0.20 | 0.20 | 0.26 | 0.20 | 0.20 | 0.32 | 0.17 | 0.23 | 0.20 | 0.21 | 0.20 | 0.22 | 0.18 | 0.17 |
| 70.2 | 66.4 | 69.5 | 67.6 | 69.6 | 70.2 | 69.1 | 90.6 | 91 | | 0.26 | 0.26 | 0.22 | 0.19 | 0.19 | 0.26 | 0.21 | 0.19 | 0.32 | 0.18 | 0.23 | 0.20 | 0.20 | 0.20 | 0.22 | 0.17 | 0.17 |
| 75.5 | 75.5 | 76.9 | 76 | 75.9 | 76.2 | 76.7 | 68.4 | 68.7 | 69 | | 0.21 | 0.27 | 0.27 | 0.27 | 0.28 | 0.28 | 0.27 | 0.32 | 0.21 | 0.26 | 0.19 | 0.20 | 0.20 | 0.22 | 0.17 | 0.17 |
| 77.8 | 78.2 | 80.1 | 77.3 | 79.3 | 80.2 | 80.7 | 68.9 | 69.2 | 69.5 | 75.6 | | 0.28 | 0.26 | 0.25 | 0.30 | 0.27 | 0.26 | 0.35 | 0.23 | 0.27 | 0.19 | 0.20 | 0.20 | 0.22 | 0.16 | 0.16 |
| 67.5 | 66 | 67.5 | 68.2 | 65.9 | 66.2 | 65.9 | 71.3 | 71.2 | 72.3 | 67.7 | 65.5 | | 0.11 | 0.24 | 0.22 | 0.18 | 0.11 | 0.26 | 0.09 | 0.20 | 0.19 | 0.20 | 0.19 | 0.22 | 0.16 | 0.16 |
| 68.5 | 66 | 66.5 | 67.5 | 67 | 68 | 66 | 73.8 | 73.9 | 74 | 68.4 | 65.6 | 84.4 | | 0.22 | 0.24 | 0.15 | 0.04 | 0.29 | 0.05 | 0.18 | 0.19 | 0.19 | 0.19 | 0.22 | 0.16 | 0.15 |
| 70.5 | 69 | 70.5 | 69.8 | 70.1 | 70.3 | 70.8 | 76.4 | 76.5 | 76.7 | 69.5 | 70.6 | 70.7 | 70.6 | | 0.26 | 0.21 | 0.21 | 0.32 | 0.19 | 0.23 | 0.18 | 0.19 | 0.19 | 0.22 | 0.15 | 0.15 |
| 65.3 | 64.8 | 64.9 | 66.1 | 63.7 | 63.7 | 63.8 | 69.1 | 69 | 69.2 | 65.6 | 63.3 | 72.7 | 69.5 | 67.6 | | 0.23 | 0.23 | 0.24 | 0.15 | 0.22 | 0.18 | 0.19 | 0.19 | 0.22 | 0.15 | 0.15 |
| 68.8 | 65.4 | 67 | 67.3 | 67.7 | 67.1 | 66.9 | 74.7 | 75 | 74.1 | 67.4 | 66.6 | 77.6 | 80.1 | 73.1 | 71 | | 0.15 | 0.30 | 0.14 | 0.18 | 0.18 | 0.18 | 0.18 | 0.22 | 0.14 | 0.14 |
| 68.5 | 65.9 | 66.2 | 67.2 | 66.8 | 67.9 | 66.1 | 74.3 | 74 | 74.1 | 68.3 | 65.7 | 83.5 | 95.4 | 70.8 | 69.7 | 80.3 | | 0.29 | 0.05 | 0.18 | 0.17 | 0.18 | 0.18 | 0.22 | 0.14 | 0.14 |
| 59.8 | 59.1 | 57.7 | 61.2 | 58.2 | 57.8 | 56.5 | 61.4 | 61.1 | 60 | 59.9 | 56.6 | 67.9 | 63 | 60.6 | 69.9 | 63 | 62.6 | | 0.23 | 0.27 | 0.17 | 0.18 | 0.18 | 0.22 | 0.13 | 0.14 |
| 74.8 | 72.4 | 72.2 | 74.8 | 72.2 | 71.9 | 71.5 | 76.2 | 76.6 | 74.7 | 74.6 | 71.8 | 86.6 | 92.2 | 75.4 | 77.5 | 80.6 | 91.8 | 67.9 | | 0.13 | 0.17 | 0.18 | 0.18 | 0.22 | 0.13 | 0.13 |
| 69.5 | 66.9 | 66.6 | 66.5 | 67.3 | 67.3 | 67.6 | 71.2 | 71.3 | 71.7 | 69.1 | 66.5 | 75.4 | 77.9 | 71.4 | 72.9 | 77.8 | 77.7 | 66.3 | 82 | | 0.17 | 0.17 | 0.17 | 0.21 | 0.13 | 0.13 |
| 70 | 65.6 | 68.2 | 68 | 68.4 | 68 | 67.7 | 73.7 | 74.1 | 74 | 69.4 | 67.3 | 76.4 | 79.9 | 74.7 | 74 | 79.8 | 79.8 | 67.4 | 80.9 | 84.5 | | 0.17 | 0.17 | 0.21 | 0.12 | 0.12 |
| 67.5 | 66.1 | 66.2 | 66.8 | 66.4 | 67.3 | 65.8 | 73.2 | 72.7 | 72.9 | 67.5 | 65.7 | 80.7 | 85.6 | 70.5 | 69.5 | 79.3 | 85.1 | 62.1 | 86.3 | 77.5 | 78.2 | | 0.17 | 0.21 | 0.12 | 0.12 |
| 69.2 | 66.6 | 66.7 | 67.8 | 67.2 | 66.9 | 66.5 | 71.1 | 71.2 | 71.8 | 69 | 65.9 | 76.1 | 77.6 | 71.1 | 73.8 | 77.4 | 77.4 | 66.3 | 80.8 | 93 | 84.6 | 77.1 | | 0.21 | 0.11 | 0.12 |
| 70.6 | 69.9 | 71.7 | 71.3 | 70.2 | 70.7 | 70.4 | 79.5 | 79.1 | 78.7 | 70.1 | 71.4 | 77.3 | 76 | 75 | 81.7 | 76.5 | 75.5 | 80.8 | 93.1 | 77.7 | 77.5 | 75.7 | 77 | | 0.11 | 0.11 |
| 71.1 | 69.3 | 71.7 | 69.3 | 71.6 | 71.7 | 71.7 | 69 | 68.9 | 69.1 | 69.7 | 70.8 | 67 | 68 | 70.1 | 65.7 | 69.1 | 68.2 | 58 | 73 | 68.8 | 70.1 | 67.8 | 68.2 | 69.4 | | 0.11 |
| 71.7 | 70.7 | 72.9 | 70.7 | 72.4 | 72 | 72.2 | 69.6 | 69.6 | 69.4 | 70.4 | 71.4 | 68.9 | 69.8 | 71.1 | 66.8 | 70.4 | 70 | 58.7 | 74.6 | 68.7 | 70.7 | 69.6 | 68.7 | 72 | 77.4 | |

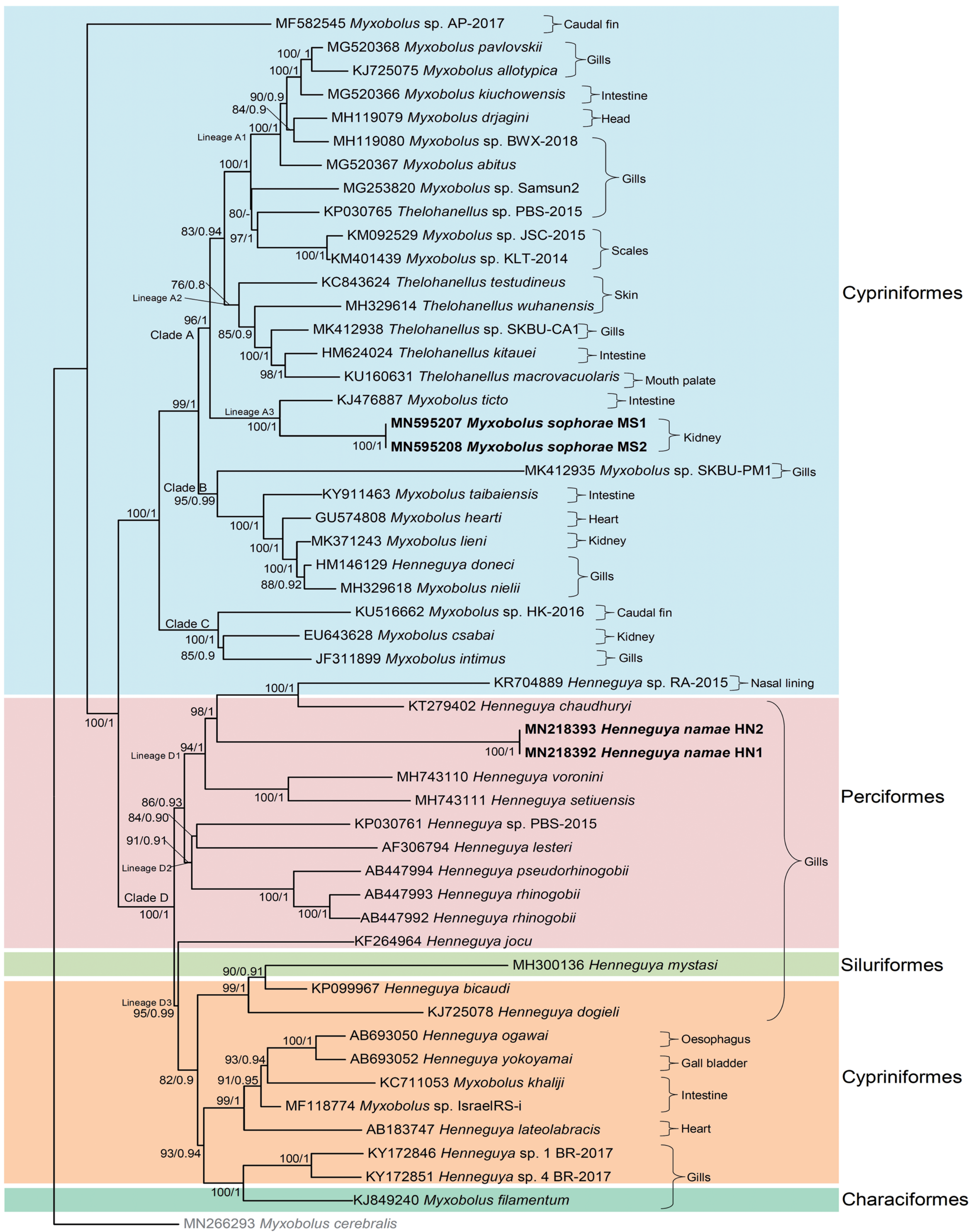
(Supplementary table 2). *M. barbi* Tripathi, 1952 spores show differs from *M. sophorae* in having equal, small sized polar capsules and a intercapsular ridge while *M. saranai* (Tripathi, 1952) emend. Landsberg and Lom, 1991 spores are smaller in size and have unequal polar capsules as compare to *M. sophorae*. *M. ampullaceus* Lalita Kumari, 1969 have spores with oval, smaller sized with equal, flask shaped polar capsules and marked on posterior margin that clearly differentiates from *M. sophorae*. *M. hyderabadense* (Lalita Kumari 1969) emend. Gupta and Khera, 1988 spores differed from *M. sophorae* by pyriform shape, with narrow, pointed anterior end and 4–6 ridges at the posterior end with equal sized polar capsules with filament coils (8–9). Spores of *M. indiae* (Lalita Kumari 1969) emend. Gupta and Khera, 1988 also differed in having narrow, pointed anterior end with having 8–10 filament coils. *M. koli* have spores with small size, truncated anterior end in comparison to *M. sophorae* whereas *M. osmaniae* Lalita Kumari, 1969 shows marked differentiation with *M. sophorae* that comprises narrow, bent anterior end with 8–10 parietal folds on the posterior margin and polar capsules with prominent neck. *M. pinnaurati* Lalita Kumari, 1969 spores are smaller in size as compared to *M. sophorae* though *M. karnatakae* (Hagargi and Amoji, 1981) emend. Landsberg and Lom, 1991 spores are pyriform, larger in size with equal size polar capsules, having 6–7 filament coils that differentiated it from *M. sophorae*. *M. sophorae* can be readily distinguished from *M. curmucae* Seenappa and Manohar, 1980 in having spores more rounded anteriorly in comparison to it. *M. mathuri* Jayasri *et al*. 1981 comprises pointed anterior end with the slightly thick posterior end having 8–9 filament coils in large capsule and 3–4 coils in a smaller capsule as compared to *M. sophorae*. *M. filamentosus* Haldar *et al.* 1985 differentiated from *M. sophorae* (by slightly unequal capsules) as equal capsules are present in *M. filamentosus*. Spores of *M. rohitae* are smaller with a triangular notch at the anterior end and equal sized polar capsules that clearly recognize it from *M. sophorae*. *M. saranae* Gupta and Khera, 1990 differed in having small size spores and unequal polar capsules from *M. sophorae*. Besides the above, *M. sophorae* differed from other species as: *M. ticto* Sheeja and Janardanan, 2006 display different morphology by having 6–8 sutural folds in the posterior one-third of spore with equal polar capsules; while *M. puntiusi* Sheeja and Janardanan, 2006 clearly discriminate in morphology from *M. sophorae* by comprises 12 distinct sutural folds and two unequal polar capsules. Despite sharing some morphometric similarity *M. chittalii* Kaur and Singh, 2011 revealed a difference from *M. sophorae* having spores pear shaped with characteristic nipple-like anterior end, two equal polar capsules and a tongue shaped intercapsular process is also present. Spores of *M. puntiusii* Gupta and Kaur, 2017 significantly differed from *M. sophorae* with having one large and one smaller polar capsule. So, the present collected *Myxobolus* species was identified as *M. sophorae* based on the above mentioned characters.

Molecular analysis: Our 18S rDNA sequences of *M. sophorae* isolates (1260 and 1268 bp) shown to be most similar to *M. ticto*. No intraspecific divergence was found among the newly generated sequences from isolates of *M. sophorae*. Genetic p-distance comparison showed a sequence divergence of *M. sophorae* with *M. ticto* is 0.07% both found from the same fish genera *Puntius*.

**Phylogenetic analysis**

For the analysis, 18S rDNA sequences from isolates of *H. namae* and *M. sophorae* was analyzed in the present study. ML and BI analyses produced an identical topology; therefore, only the ML phylogenetic tree is presented here (Fig. 3). The phylogenetic tree inferred from ML and BI analyses shows that the sequences obtained in the current study of *H. namae* are nested within the lineage D1 in an independent branch with relatively high bootstrap and posterior probability support values (100/1) (Fig. 3). There is no interspecific sequence divergence was found among the two isolates of *H. namae*. Both isolates of *H. namae* are sister to the species that infected fish of the order Perciformes and one that infects Cypriniformes (KR704889). Lineage D1 comprises species all closely aligned with *H. namae* i.e., *H. chaudhuryi* (KT279402), *Henneguya* sp. RA-2015

**Fig. 3.** Phylogenetic relationship of *H. namae* and *M. sophorae* based on the 18S gene sequences. Numbers at nodes indicates ML bootstrap values (1000 replications) and posterior probabilities (BI) respectively. Unsupported nodes by BI are marked with a hyphen. The scale bar indicates the number of substitution per site. Newly generated sequences in this study shown as bold. GenBank accession numbers are listed before the species names.


MF582545 Myxobolus sp. AP-2017
Caudal fin
MG520368 Myxobolus pavlovskii
KJ725075 Myxobolus allotypica
Gills
MG520366 Myxobolus kiuchowensis
Intestine
MH119079 Myxobolus drjagini
Head
MH119080 Myxobolus sp. BWX-2018
MG520367 Myxobolus abitus
MG253820 Myxobolus sp. Samsun2
KP030765 Thelohanellus sp. PBS-2015
Gills
KM092529 Myxobolus sp. JSC-2015
KM401439 Myxobolus sp. KLT-2014
Scales
KC843624 Thelohanellus testudineus
MH329614 Thelohanellus wuhanensis
Skin
MK412938 Thelohanellus sp. SKBU-CA1
Gills
HM624024 Thelohanellus kitauei
Intestine
KU160631 Thelohanellus macrovacuolaris
Mouth palate
KJ476887 Myxobolus ticto
Intestine
MN595207 Myxobolus sophorae MS1
MN595208 Myxobolus sophorae MS2
Kidney
MK412935 Myxobolus sp. SKBU-PM1
Gills
KY911463 Myxobolus taibaiensis
Intestine
GU574808 Myxobolus hearti
Heart
MK371243 Myxobolus lieni
Kidney
HM146129 Henneguya doneci
MH329618 Myxobolus nielii
Gills
KU516662 Myxobolus sp. HK-2016
Caudal fin
EU643628 Myxobolus csabai
Kidney
JF311899 Myxobolus intimus
Gills
KR704889 Henneguya sp. RA-2015
Nasal lining
KT279402 Henneguya chaudhuryi
MN218393 Henneguya namae HN2
MN218392 Henneguya namae HN1
MH743110 Henneguya voronini
MH743111 Henneguya setiuensis
KP030761 Henneguya sp. PBS-2015
AF306794 Henneguya lesteri
AB447994 Henneguya pseudorhinogobii
AB447993 Henneguya rhinogobii
AB447992 Henneguya rhinogobii
KF264964 Henneguya jocu
MH300136 Henneguya mystasi
KP099967 Henneguya bicaudi
KJ725078 Henneguya dogieli
Gills
AB693050 Henneguya ogawai
Oesophagus
AB693052 Henneguya yokoyamai
Gall bladder
KC711053 Myxobolus khaliji
MF118774 Myxobolus sp. IsraelRS-i
Intestine
AB183747 Henneguya lateolabracis
Heart
KY172846 Henneguya sp. 1 BR-2017
KY172851 Henneguya sp. 4 BR-2017
KJ849240 Myxobolus filamentum
Gills
MN266293 Myxobolus cerebralis
Lineage A1
Lineage A2
Lineage A3
Clade A
Clade B
Clade C
Clade D
Lineage D1
Lineage D2
Lineage D3
Cypriniformes
Perciformes
Siluriformes
Cypriniformes
Characiformes
0.02

(KR704889) (78.0%–76.4%) from India and *H. voronini* (MH743110) and *H. setiuensis* (MH743111) from Malaysia (72.7%–72.1%) (Table 2). *H. namae* in clade D was also clustered with other species present in lineage D2: *Henneguya* sp. PBS-2015, *H. lesteri*, *H. rhinogobii* and *H. pseudorhinogobii* (74.0%–75.5%) (Fig. 3, Table 2) which also presented for Perciformes infected hosts. Intraspecific sequence divergence based on 18S dataset was ranging 0.21% (between *H. namae* and *H. chaudhuryi*), 0.23% between *H. namae* and *Henneguya* sp. RA-2015 while for *H. namae* with *H. voronini* and *H. setiuensis* was 0.24% (Fig. 3, Table 2). Phylogenetic tree of the 18S rDNA of the *Myxobolus sophorae* analyzed and showed a 90% similarity with the sequences of *M. ticto* (KJ476887) infecting the host *Puntius ticto* from India in a lineage A3 (Fig. 3). There is no interspecific sequence divergence was found among the two isolates of *M. sophorae*. Other *Myxobolus* species *Myxobolus puntusii* HK-2016 (KU516662), *Myxobolus* sp. SKBU-PM1 (MK412935) and *Myxobolus* sp. AP-2017 (MF582545) also infecting *Puntius sophore*, the same host infected by *M. sophorae* form clades far from *M. sophorae* (Fig. 3). The 18S sequence divergence of *M. sophorae* with sister species *M. ticto* is 0.07%. The intraspecific sequence divergence of *M. sophorae* was ranging 0.25% between *M. sophorae* and *Myxobolus* sp. HK-2016 and it was 0.27% between *M. sophorae* and *Myxobolus* sp. SKBU-PM1. Unfortunately, in comparison to the morphological description available for about 20 *Myxobolus* species from India, molecular data is limited to only few that currently available in the GenBank. The tree revealed well-supported clade (100/1.00) for *M. sophorae*; however, all other *Myxobolus* species infecting *Puntius* species were dispersed in the separate groups (Fig. 3). In the tree (Fig. 3), the myxozoan species shows phylogenetic affinities to the fish host and mainly clustered according to the order of the fish host they belongs.

## DISCUSSION

Among myxozoan, *Henneguya* Thélohan 1892 and *Myxobolus* Bütschli 1882 are the species rich genera and reported worldwide (Eiras 2002, Eiras *et al.* 2005, Lom and Dyková 2006, Eiras and Adriano 2012, Liu *et al.* 2018). However, from the family Myxobolidae, more than 150 species have been described in India and most of the *Henneguya* and *Myxobolus* species were recorded from the freshwater environment in India (Kalavati and Nandi 2007, Kaur *et al.* 2015, Gupta and Kaur 2017, Chaudhary *et al.* 2018, Ahmed *et al.* 2019, Chaudhary *et al.* 2019). Though, in comparing to the large number of species described, the molecular data is available only to a small percentage of them. In India, most of the *Henneguya* species were described on the basis of morphology alone; therefore, the status of Indian *Henneguya* spp. must be questioned. To avoid such situations, for a more valid identification of species, it can be attained with the help of molecular data. Till date, only 05 sequences (*H. mystasi, H. chaudhuryi, H. bicaudi, Henneguya* sp. 1 HK-2016 and *Henneguya* sp. RA-2015) are available on the Genbank database from Indian *Henneguya* species that shows the scarcity of data from this region. When isolates of *H. namae* was compared to *H. chaudhuryi* and *Henneguya* sp. RA-2015 (KR704889) so far as their molecular data is available, they were found to be closely related. In the comparison between *H. namae* with *H. chaudhuryi* and *Henneguya* sp. RA-2015, in addition to the morphological differences pointed out above, 18S rDNA shows a difference of 0.21–0.23% respectively. While pairwise comparisons among sequences of *H. namae* with *H. voronini* and *H. setiuensis* were significantly revealed a difference of 0.24%. The phylogenetic analysis performed by both methods ML and BI revealed that *H. namae* form a separate lineage D1 that comprising most of the species that infect hosts belonging to Perciformes including one from Cypriniformes (*Henneguya* sp. RA-2015 (KR704889). The lineage D1 compiled of four *Henneguya* species, three of them are parasites of hosts from India (*H. namae*, *H. chaudhuryi* and *Henneguya* sp. RA-2015) infecting the gills and nasal lining and two species *H. voronini* and *H. setiuensis* are gills infected parasites from Malaysia respectively. Clustering of parasites, according to the order of the host fish that involving parasites from different genera, *Henneguya* and *Myxobolus* species.

Besides the morphology, *M. sophorae* molecular comparison of the 18S rDNA gene confirmed that this species differ from *Myxobolus puntusii* HK-2016 (KU516662), *Myxobolus* sp. SKBU-PM1 (MK412935) and *Myxobolus* sp. AP-2017 (MF582545) from same host *P. sophore*. In our study, the analyzed *M. sophorae* sequence shares clade with *M. ticto*, but significantly varied in morphology as well as genetically. Besides the above, it is difficult to relate the other species present in supplementary table 2 because there are no 18S rDNA sequence data is available for them. In the both trees for

*H. namae* and *M. sophorae*, if we see the phylogeny, it is represented that in comparison to the infection site, host group is more relevant ancient evolutionary factor during selection can be taken into consideration. Clustering of *Henneguya* species does not appear according to the infected tissue, might be due to reasons of that molecular data available for this genus worldwide contributes a small fraction as compare to their total diversity. Here, we can present the fact that host affinity is more important than tissue tropism as also reported previously for myxobolid species (Carriero *et al.* 2013, Moreira *et al.* 2014, Rocha *et al.* 2019). In general, the phylogenetic tree shows that in case of *H. namae* tree host affinity is stronger evolutionary signal, this contention is also supported by previous studies (Carriero *et al.* 2013, Moreira *et al.* 2014, Rocha *et al.* 2019). In case of *M. sophorae* phylogeny, species that formed tree infected several closely related fish species of order Cypriniformes as also mentioned in a recent study by Rocha *et al.* 2019.

However, future phylogenetic studies with addition of molecular data will demonstrate the accurate relationships of *Henneguya* species as well as other myxobolids in relation to tissue tropism, host affinity and aquatic environments. This is the first report of obtaining partial 18S rDNA sequence of *H. namae* and *M. sophorae* that contributes to the molecular data of Indian myxobolid species and will be helpful to evaluate the risk and to make possible management of severe infection.

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**Supplementary Table** 1. Comparison of different *Henneguya* species with unequal polar capsules related with *H. namae*. Data are mean ± SD; measurements given in µm. TLOS = total length of spore, LOSB = length of spore body, WOS = width of spore, TOS = thickness of spore, PC = polar capsule, LOLPC = length of larger polar capsule, LOSPC = length of smaller polar capsule, WOLPC = width of larger polar capsule, WOSPC = width of smaller polar capsule, LOCA = length of caudal appendages, NOPF = number of polar filaments, na = data not available.

| Species | Host | Site of infection | Locality | Source | TLOS | LOSB | WOS | TOS | PC Equal/ Unequal | LOLPC | LOSPC | WOLPC | WOSPC | LOCA | NOPF | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | LPC | SPC |
| *H. namae* | *Chanda nama* (=*Ambassis nama*) | Gill filaments | Bairaj, Bijnor, Uttar Pradesh | Present study (Mean±SD) | 27.82–33.17 (30.6±1.71) | 12.34–15.6 (14.15±0.96) | 4.94–5.98 (5.41±0.32) | 3.9–4.34 (4.07±0.15) | Unequal | 3.18–4.16 (3.66±0.34) | 2.84–3.73 (3.27±0.31) | 1.0–1.14 (1.08±.05) | 0.98–1.1 (1.04±0.04) | 15.12–17.92 (16.64±1.03) | 8–9 | 6–7 |
| | | | Krishna Nagar, West Bengal | Haldar et al. 1983 | na | 17.6–19.3 (18.5) | 5.5–6.6 (6.4) | 4.4–5.0 (4.8) | Unequal | 5.5–6.0 (5.8) | 4.4–5.0 (4.9) | 1.1 | 1.1 | 17.6–18.7 (18.5) | 9–10 | 8–9 |
| *H. ophiocephali* | *Channa punctatus* and *C. gachua* | Gills and muscles | Calcutta and North 24-Parganas districts, West Bengal | Chakravarty 1939 | 41.5–52.5 | na | 6.18–7.2 | na | Unequal | 6.18–9.27 | 5.15–8.24 | 2.06–3.0 | 2.06–3.0 | 26.0–32.0 | na | |
| *H. notopterae* | *Notopterus notopterus* | Gills | Hyderabad, Andhra Pradesh | Qadri 1965 | na | 11.5–13.0 | 4.5–5.0 | na | Unequal | 5.0–5.5 | 3.5–4.5 | 1.0–1.5 | 1.0–1.5 | 40.5–42 | na | |
| *H. qadrii* | *C. gachua* | Intestine | Hyderabad, Andhra Pradesh | Lalitha Kumari 1965 | na | 9.24–12.32 (11.0) | 4.62–5.39 (4.62) | na | Unequal | 4.62–6.16 (5.25) | 3.85–4.62 (4.48) | 1.15–1.82 (1.54) | 1.15–1.92 (1.54) | 6.16–14.3 (9.81) | 9 | |
| *H. singhi* | *Notopterus osmani* | Gills filaments | Hyderabad, Andhra Pradesh | Lalitha Kumari 1969 | na | 11.1–13.6 (12.3) | 3.9–5.7 (4.4) | na | Unequal | 4.8–6.4 (5.7) | 3.5–5.5 (4.1) | 0.8–1.4 (1.1) | 1.4–2.0 (1.6) | 30.0–48.0 (39.0) | 4–5 | |
| *H. thermalis* | *Lepidocephalichthys thermalis* | Brain tissue | Honnenahalli, Chitradurga district, Karnataka | Seenappa et al., 1981 | 47.0–60.0 | 12.0–13.2 | 6.0–8.0 | 5.0 | Unequal | 4.0–5.0 | 3.0–4.0 | 2.0–3.0 | na | 11.0–13.0 | na | |

**Supplementary Table** 2. Comparison of *Myxobolus* species infected different species of *Puntius* from India with *M. sophorae*. Data are mean ± SD; measurements given in µm. LOS = length of spore, WOS = width of spore, TOS = thickness of spore, PC = polar capsule, LOLPC = length of larger polar capsule, LOSPC = length of smaller polar capsule, WOLPC = width of larger polar capsule, WOSPC = width of smaller polar capsule, NOPF = number of polar filaments, na = data not available.

| Species | Host | Site of infection | Locality | Source | LOS | WOS |
|---|---|---|---|---|---|---|
| *M. sophorae* Jayasri,1982 | *Puntius sophore* | Kidney | Meerut, Uttar Pradesh | Present study (Mean±SD) | 14.0–15.1 (14.57±0.33) | 10.4–11.4 (10.91±0.34) |
| | | Gills and kidney | Parvatsar lake, Rajasthan | Jayasri1982 | 6.4–26.6 (14.9) | 5.9–10.1 (7.7) |
| *M. barbi* Tripathi, 1952 | *P. ticto* | Skin | North 24-Parganas district, West Bengal | Tripathi, 1952 | 12.6–13.5 | 9.0 |
| *M. saranai* (Tripathi, 1952) emend. Landsberg and Lom, 1991 | *P. sarana* | Gills | North 24-Parganas district, West Bengal | (Tripathi, 1952) emend. Landsberg and Lom, 1991 | 6.4–7.0 | 4.5–5.0 |
| *M. ampullaceus* Lalitha Kumari, 1969 | *P. kolus* | Dorsal and ventral fins | Hyderabad, Andhra Pradesh | Lalitha Kumari, 1969 | 8.6–10.7 (9.8) | 6.4–7.9 (7.1) |
| *M. hyderabadense* (Lalitha Kumari 1969) emend. Gupta and Khera, 1988b | *P. pinnauratus*, *P. filamentosus* | Gill filaments | Hyderabad city, Andhra Pradesh; Vellayani lake, Kerala | (Lalitha Kumari 1969) emend. Gupta and Khera, 1988b | 9.3–11.5 (10.1) | 5.0–8.0 (5.9) |
| *M. indiae* (Lalitha Kumari 1969) emend. Gupta and Khera, 1988b | *P. sarana* | Gill filaments | Hyderabad city, and Warangal, Warangal districts, Andhra Pradesh | (Lalitha Kumari 1969) emend. Gupta and Khera, 1988b | 12.4–15.0 (13.7) | 6.4–8.6 (7.3) |
| *M. koli* Lalitha Kumari, 1969 | *P. kolus*, *P. filamentosus* | Dorsal and ventral fins | Hyderabad city, Andhra Pradesh; Vellayani lake, Kerala | Lalitha Kumari, 1969 | 7.1–9.6 (8.4) | 5.0–6.4 (6.0) |
| *M. osmaniae* Lalitha Kumari, 1969 | *P. punjabensis* | Liver and intestine | Hyderabad city, Andhra Pradesh | Lalitha Kumari, 1969 | 12.4–15.0 (13.5) | 7.1–10.0 (8.6) |
| *M. pinnaurati* Lalitha Kumari, 1969 | *P. pinnauratus* | Gill filaments | Lake near Hyderabad city, Andhra Pradesh | Lalitha Kumari, 1969 | 8.0–11.4 (9.6) | 6.5–7.9 (7.0) |
| *M. karnatakae* (Hagargi and Amoji, 1981) emend. Landsberg and Lom, 1991 | *P. chola* | Caudal muscles | Gulburga, Karnataka | (Hagargi and Amoji, 1981) emend. Landsberg and Lorn, 1991 | 16.32–19.04 (17.58) | 10.88–13.6 (11.1) |
| *M. curmucae* Seenappa and Manohar, 1980a | *P. curmuca* | Below the scales | Nethravathi River, Bantwal, Karnataka | Seenappa and Manohar 1980 | 8.0–11.0 (9.8) | 7.0–8.0 (7.6) |
| *M. mathuri* Jayasri et al., 1981 | *P. sarana* | Gills | Parvatsar Lake, Rajasthan | Jayasri et al., 1981 | 8.7–23.5 | 5.1–10.1 |
| *M. filamentosus* Haldar et al., 1985 | *P. filamentosa* | Cartilage and brain | Kalyani, Nadia district, West Bengal | Haldaret et al. 1985 | 11.2–17.3 (13.7) | 8.1–12.2 (9.5) |
| *M. rohitae* Haldar et al., 1983 | *P. sarana* | Scales | Krishna nagar, West Bengal; Harike, Nangal, Ropar, Ludhiana, Punjab | Haldar et al., 1983 | 9.9–12.1 (10.6) | 8.8–9.9 (9.0) |
| *M. saranae* Gupta and Khera, 1990 | *P. sarana* | Gills | Ropar and Ludhiana, Punjab | Gupta and Khera, 1990 | 6.0–9.0 (7.72) | 6.0–7.0 (6.2) |
| *M. ticto* Sheeja and Janardanan, 2006 | *P. ticto punctatus* | Gills, muscles, intestine and liver | Malappuram, Kerala | Sheeja and Janardanan 2006 | 12.75–15 (14.55) | 7.75–9.0 (7.8) |
| *M. chittalii* Kaur and Singh, 2011 | *P. sophore* | Gill lamellae | Harike Wetland, Punjab | Kaur and Singh 2011 | 8.8–9.2 (9.0±0.28) | 5.88–6.48 (6.18±0.42) |
| *M. puntusii* Gupta and Kaur, 2017 | *P. sophore* | Caudal fin | Ranjit Sagar Wetland, Punjab | Gupta and Kaur 2017 | 7.56–7.96 (7.76±0.28) | 5.25–5.47 (5.36±0.15) |

| TOS | PC Equal /Unequal | LOLPC | LOSPC | WOLPC | WOSPC | NOPF | |
|---|---|---|---|---|---|---|---|
| | | | | | | Large | Small |
| 6.2–7.4 (6.91±0.39) | Slightly unequal | 5.72–6.5 (6.04±0.27) | 4.94–6.14 (5.48±0.34) | 2.6–3.2 (2.89±0.22) | 2.34–3.12 (2.69±0.24) | 5–6 | 5–6 |
| na | Equal or unequal | na | na | na | na | na | na |
| na | Equal | 3.6–4.5 | | 2.7 | | na | |
| na | Unequal | 3.5 | 1.5 | 1.5 | 1.0 | na | |
| | Equal | 5–6.4 (5.8) | | 2.5–2.9 (2.8) | | 5–6 | |
| na | Equal | 5.0–7.3 (5.8) | | 1.4–3.0 (2.2) | | 8–9 | |
| na | Unequal | 5.7–7.1 (5.9) | 5.0–6.4 (5.2) | 1.4–2.5 (2.1) | 1.4–2.5 (2.1) | 8–10 | 8–10 |
| na | Unequal | 3.9–4.6 (4.3) | 1.4–2.1 (2.0) | 2.1–3.1 (2.8) | 0.7–1.4 (1.2) | 5–6 | |
| na | Unequal | 5.0–7.1 (5.6) | 2.1–3.6 (2.6) | 2.0–3.9 (3.2) | 1.4–2.9 (2.5) | 5–6 | 5–6 |
| na | Unequal | 3.6–6.4 (4.4) | 2.9–5.0 (3.1) | 1.1–2.1 (1.9) | 1.1–2.1 (1.6) | | |
| na | Equal | 7.3–10.88 (11.11) | | 3.58–5.44 (4.78) | | 6–7 | |
| 5.0–6.0 (5.3) | Equal, rarely slightly unequal | 4.5–5.0 (4.9) | 3.0–4.0 (3.9) | 2.0–3.0 (2.5) | 2.0–3.0 (2.4) | na | na |
| na | Unequal | 2.7–11.9 | 2.7–7.8 | 1.8–4.6 | 1.8–4.6 | 8–9 | 3–4 |
| 5.0–6.0 (5.20) | Unequal or equal | 4.0–7.1 (3.6) | | 2.0–4.0 (3.1) | | 5–6 | |
| na | Equal | 6.6 | | 3.3 | | 5–6 | |
| na | Unequal | 4.0–5.0 (4.24) | 1.5–3.0 (1.98) | 2.5–4.0 (3.04) | 1.0–2.0 (1.3) | na | |
| na | Equal | 4.7–7.5 (6.63) | | 2.25–3.0 (2.92) | | 6–8 | |
| na | Equal | 4.0–5.0 (4.5±0.70) | | 2.0–2.8 (2.4±0.56) | | 4–5 | |
| na | Unequal | 2.95–3.07 (3.0±0.08) | 1.60–1.82 (1.71±0.15) | 1.75–1.91 (1.83±0.11) | 0.89–0.99 (0.94±0.07) | 6–7 | 3–4 |