Indian Journal of Geo Marine Sciences
Vol. 53 (04), April 2024, pp. 212-218
DOI: 10.56042/ijms.v53i04.9017

First occurrence of *Metanophrys sinensis* Song & Wilbert, 2000 (Protozoa: Ciliophora: Scuticociliatida) outbreak in a marine ornamental fish: Morphological and phylogenetic analysis

N B Dhayanithi, A Sudhagar, T T Ajith Kumar* & U K Sarkar

ICAR-National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh – 226 002, India

*[E-mail: ttajith87@gmail.com]

Received 22 January 2024; revised 19 April 2024

A scuticociliatosis outbreak with high mortality was recorded in the clownfish breeding facility of the ICAR-NBFGR, Maharashtra, India. Moribund fish juveniles showed abnormal swimming and high mucus secretion with energy loss. Microscopic investigation found that all moribund juveniles had dense parasites on their gills, fins, and almost the whole-body surface. Thin to elongated oval-shaped ciliates *Metanophrys* sp. with pointy anterior end (25-50 × 10-20 µm) were detected and collected for further observation. The causative parasite was characterised by 18 SSU rRNA gene sequencing method using universal and specific primers. The causative ciliate was confirmed as *Metanophrys sinensis* Song & Wilbert, 2000, based on 97 % gene similarity in BLAST analysis results. The accession codes OM055651.1 (universal primer) and ON329185.1 (specific primer) were acquired after gene sequences were uploaded to GenBank. On the basis of available literature, this is the first occurrence record of scuticociliatosis outbreak caused by *Metanophrys sinensis* in the marine ornamental fish hatchery in India and elsewhere.

[**Keywords**: 18 SSU rRNA, Clownfish, *Metanophrys sinensis*, Morphology, Scuticociliatosis]

Introduction

All ecosystems on the earth consist of parasites, which typically have a negligible effect on the fitness of healthy fish. However, when raised in captivity, fish can experience stressful circumstances that cause parasite issues. Improper water quality and dense stockings can create ideal circumstances for parasite infection and reproduction, which soon rises to pathogenic levels[1]. Parasites (ecto & endo) are causing severe problems in marine ornamental fish aquaculture and can result in substantial economic losses in the aquarium and production units[2].

Scuticociliates belong to the class Oligohymenophorea and subclass Scuticociliatia. They are single-celled eukaryotic marine organisms with a ubiquitous distribution. Almost 20 members of the group have been connected with disorder known as scuticociliatosis, in which ciliates are the parasites[3]. Scuticociliate species (Ciliophora) act as facultative parasites in farmed fish, causing significant mortality. In addition to creating skin lesions, disease-causing scuticociliates can enter the body, feed on tissue, multiply in the bloodstreams, and infect the majority of internal organs in a few days[4]. The scuticociliates *Philasterides dicentrarchi*, *Miamiensis avidus*, and *Uronema marinum* are the cause of mass mortality in cultured marine food fishes, including sea bass, *Dicentrarchus labrax*, turbot, *Scophthalmus maximus* and olive flounder, *Paralichthys olivaceus*[5-8].

The genus *Metanophrys* (de Puytorac, Grolière, Roque & Detcheva, 1974) is one of the Scuticociliatia, including four species, namely *M. elongate*, *M. echini*, *M. sinensis* and *M. similis*. Several descriptions have been made of *M. sinensis* and *M. similis*, which have been discovered in Chinese waters[7,9,10]. *Metanophrys* morphologically resembles *Paranophrys* and *Mesanophrys*. Key differentiating traits to distinguish between them is the anterior and posterior ends. In general, *Metanophrys* species were recorded in seawater as free-living stages. However, there was no proper evidence of its infection stages or potency recorded earlier. Using certain staining techniques, oral and physical infraciliature morphometry is used to diagnose the scuticociliatosis microscopically. Morphometry and taxonomic differentiation suggest that targeting small subunit rRNA is more effective in preventing misinterpretation[10].

Coral reef fishes that are kept in public and private aquaria for their appealing colours and unusual patterns are known as marine ornamental fishes[11]. Marine aquarium trade is a multibillion-dollar business that began in the 1930s and has seen tremendous growth in recent decades[12-14]. Clownfishes are the most widely traded marine aquarium fish in the global marine ornamental fish trade. Diseases are the major challenge in the marine ornamental fish culture systems, creating heavy mortality[15]. In general, parasites have unique mechanisms that limit the strength of a healthy host. However, when fish are raised in captivity, it can cause issues under unfavourable circumstances[16]. In this line, the present study is conducted to find out the reason for the large scale mortality in marine ornamental fish hatchery. The causative agent was identified using both morphological & molecular methods and conformed as *M. sinensis*. Based on the literature, this is the first report of *M. sinensis* infection in marine ornamental fish worldwide.

Materials and Methods

Case history

In August 2021, mass mortality was observed in the juveniles of clownfish, *Amphiprion percula* (Lacepède, 1802) (1.5±0.5 cm) in the marine ornamental hatchery facility of ICAR-NBFGR located at the Coastal and Marine Biodiversity Centre, Airoli, Maharashtra, India (19°08'54.8" N; 72°59'06.7" E). However, no mortality or clinical signs of disease were observed in the broodstocks.

Clinical investigation and microscopic observation

The post-settlement stages of 25 to 45 days aged clownfish *A. percula* (1.5±0.5 cm) were stocked in the grow-out facility in 2000 L fiber-reinforced plastic (FRP) tanks with canister and biological filtrations and skimmer. Salinity, temperature, pH, ammonia, nitrite, nitrate, and dissolved oxygen were maintained as 30 ppt, 27 ºC, 8, < 0.05 mg/ml, < 0.025 mg/ml, < 0.5 mg/ml, and 8 mg/L, respectively. During the middle of the monsoon, some juveniles showed abnormal swimming initially and high mucus secretion with energy loss. The unusual things were extended to almost entire juvenile rearing section tanks, resulting in mass mortality within 36 to 48 h. Infected moribund juveniles were collected and shifted to the laboratory attached to the facility for macroscopic and microscopic (ESAW, India; 5.0 Vdc@600mA) investigations. The body surface, skin scrapings and gills biopsy were observed under the microscope.

Sample preparation & DNA extraction

Sterilised distilled water was used to separate parasites from the gills, skin scrapings, and body surfaces. The skin mucus was washed with distilled water and the fluid was centrifuged for the separation of parasites from mucus and other contaminants. The DNeasy Blood & Tissue Kit was used to extract DNA as per the instructions from the manufacturer (Qiagen). NanoDrop spectrophotometer (Thermo Scientific) was used to measure the quality and quantity of the DNA[17].

Polymerase Chain Reaction (PCR) amplification and sequencing

Polymerase Chain Reaction (PCR) and sequencing were done to generate a 1733 bp sequence of the small subunit ribosomal RNA (SSU rRNA) gene of the pathogen using the list of primers provided in Table 1. For all the PCR's, the reaction mixture had 1 µl of 100 ng total DNA, 1.5 µl (10 pmol) of each primer, 0.25 µl of Taq DNA polymerase (5 U-1 µl), 2.5 µl of 10x Taq buffer A, 0.5 µl of dNTPs (2 mM), and double-distilled H_2O to make up the final volume of 25 µl of the PCR reaction mixture. Initially, 648 bp SSU rRNA gene of the parasite was amplified using the universal primers Euk A and Euk B (Table 1), according to an earlier study[17]. The PCR amplification was done under the following conditions: 1 cycle (94 °C, 5 min); 30 cycles (94 °C for 1 min; 62 – 50 °C touch down for 1 min; and 72 °C for 1 min 30 s); and 1 cycle (72 °C for 10 min)[17]. Furthermore, to reaffirm the molecular identity of the parasite, two pairs of specific primers (Met_OF, Met_OR, Met_IF, and

Table 1 — List of primers used in the study

Primer ID	Sequence (5′-3′)	Amplicon	Reference/ Accession Number
Euk A	AACCTGGTTGATCCTGCCAGT	1754 bp	Gao *et al.*[17] / HM236336.1
Euk B	TGATCCTTCTGCAGGTTCACCTAC		
Met_OF	AACCTGGTTGATCCTGCCAGTA	1733 bp	ON329185.1 (present study)
Met_OR	TGATCCTTCTGCAGGTTCAC		
Met_IF	GGCATTAGTACTTAACAGTCAG		
Met_IR	CCAAGTATTTCACCACTGAC		

Met_IR) were designed to amplify the SSU rRNA gene of the parasite. A total of 35 cycles of PCR amplification were performed under the following conditions: 30 s at 98 °C, 10 s at 98 °C, 15 s at 55 °C, 20 s at 68 °C, and 2 min at 68 °C. The amplified PCR product was visualised using agarose gel electrophoresis and was sequenced in the ABI Prism 3700 Big Dye sequencer platform (Agrigenome Labs, Kochi, India).

Nucleotide analysis and phylogenetic tree construction

The generated sequences were further analysed using Applied Biosystems sequence scanner software version 1.0 and Bioedit ver. 7.2.5[ref. 18] and were assembled into a consensus sequence. The homology of nucleotide sequences was evaluated using NCBI's Basic Local Alignment Search Tool (BLAST). Additionally, using MEGA X software, a phylogenetic tree was created utilising the small subunit (SSU) rRNA gene sequence (1733 bp) of *M. sinensis* from the current study as well as other representatives (gathered from the NCBI database)[19]. After using MUSCLE to align the sequences, the Tamura-Nei model with separate Gamma allocation and invariable sites (T93+G+I) was determined to be the most suitable model to create the phylogenetic tree. It had the lowest Bayesian Information Criterion score (13912.51) and the Maximum Likelihood value (-6506.21). The phylogenetic tree was generated using the Maximum Likelihood technique with the T93+G+I model, and the bootstrap method with 1000 repetitions was used to test the phylogeny. The interactive Tree Of Life (iTOL) was used to visualise the final product tree[20].

Results and Discussion

Significant mortality rates in farmed fish worldwide can be caused by scuticociliates, which can infect fish and function as histophagous parasites[4]. There have been reported cases of fish scuticociliatosis in North America, Europe, Australia, and Asia. Most scuticociliates are located in areas with high concentrations of microbes, vitamins and nutrients, and organic debris, even though they can tolerate a wide variety of salinities[21].

During disease outbreaks, marine ornamental fish have frequently been found with *Uronema marinum* and *Philasterides dicentrarchi*[22]. According to Noga[23], Scuticociliatida can affect tropical marine fish and acts as an opportunistic pathogen frequently under stressed condition. In freshwater or saltwater, *Metanophrys* ciliates have survived freely or momentarily on the shallow surface of tissues of newly dead juvenile shrimp, *Penaeus vannamei*[9] and *Macrobrachium rosenbergii* larvae[24]. In line with this, the present study has documented the scuticociliatosis outbreak at the clownfish hatchery, which was caused by *M. sinensis*.

The infected percula clownfish juveniles showed slightly darkened colour, epidermal desquamation on the head and body surface, and reddened fins and gill operculum (Fig. 1). The symptomatology of the condition was in line with earlier reports of scuticociliatosis. According to Ramos *et al.*[25], the afflicted fish displayed skin ulcers encircled by a whitish halo, reddening at the bottom of the fins, and darkening of the ventral skin. A few of these ulcers extended into the muscle, revealing the fin rays, which showed an abundance of ciliates when examined under a light microscope.

Gasping for oxygen, fast gill movement, excessive mucus secretion, and laying on the bottom were all indications of infected fish. The microscopic examination revealed that the moribund fish had dense parasites on their gills, fins, and practically the whole-body surface. According to an earlier study, microscopic examination typically reveals an invasive infection with a large number of parasites consuming host tissues in the epidermis, gills, muscles of the skeleton, brain, and infrequently other organs[26-28]. Scuticociliatosis can be diagnosed by microscopic inspection of infected tissues, and the parasite species can also be recognised using specialised staining

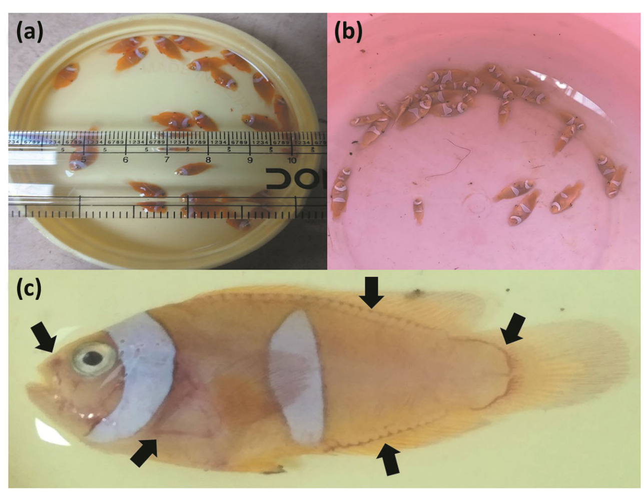

Fig. 1 — Clinical investigation during the scuticociliatosis outbreak: (a) & (b) Mass mortality of *A. percula* juveniles (1.5±0.5 cm) during the outbreak; (c) Epidermal desquamation at head and gill operculum, reddening at the base of the caudal fin, dorsal fin and pectoral fins (arrow)

procedures based on morphometric properties of cutaneous and oral infraciliature[3,5,6,29-32].

The microscopic examination found a pointy anterior end (25-50 × 10-20 µm sized) with a thin to elongated oval-shaped parasite (Fig. 2). Earlier studies reported that the size of *M. sinensis* (27-40 × 11-15 µm), *M. similis* (25-50 × 10-15 µm), and *Paranophrys marina* (33-46 × 8-15 µm) was between 25-50 × 10-20 µm[17,33,34]. Song *et al.*[10] highlighted that morphologically, *Metanophrys* is similar to *Paranophrys* and *Mesanophrys*. However, the terminal position of the anterior end of the paroral membrane, extending anteriorly to the middle of adoral membranelles (*Metanophrys*), the anterior end (*Paranophrys*) or the posterior end (*Mesanophrys*) serves as the main distinguishing feature.

In total, 648 bp size of 18 SSU rRNA gene was amplified by using universal primers (Euk A & Euk B). BLAST results highlighted that it had 98 % similarity to the previously reported 18 SSU rRNA of *M. sinensis* (GenBank No-HM236336.1). 18 SSU rRNA gene was amplified using universal primers, deposited the sequence to the GenBank and accession number was received (GenBank accession number:

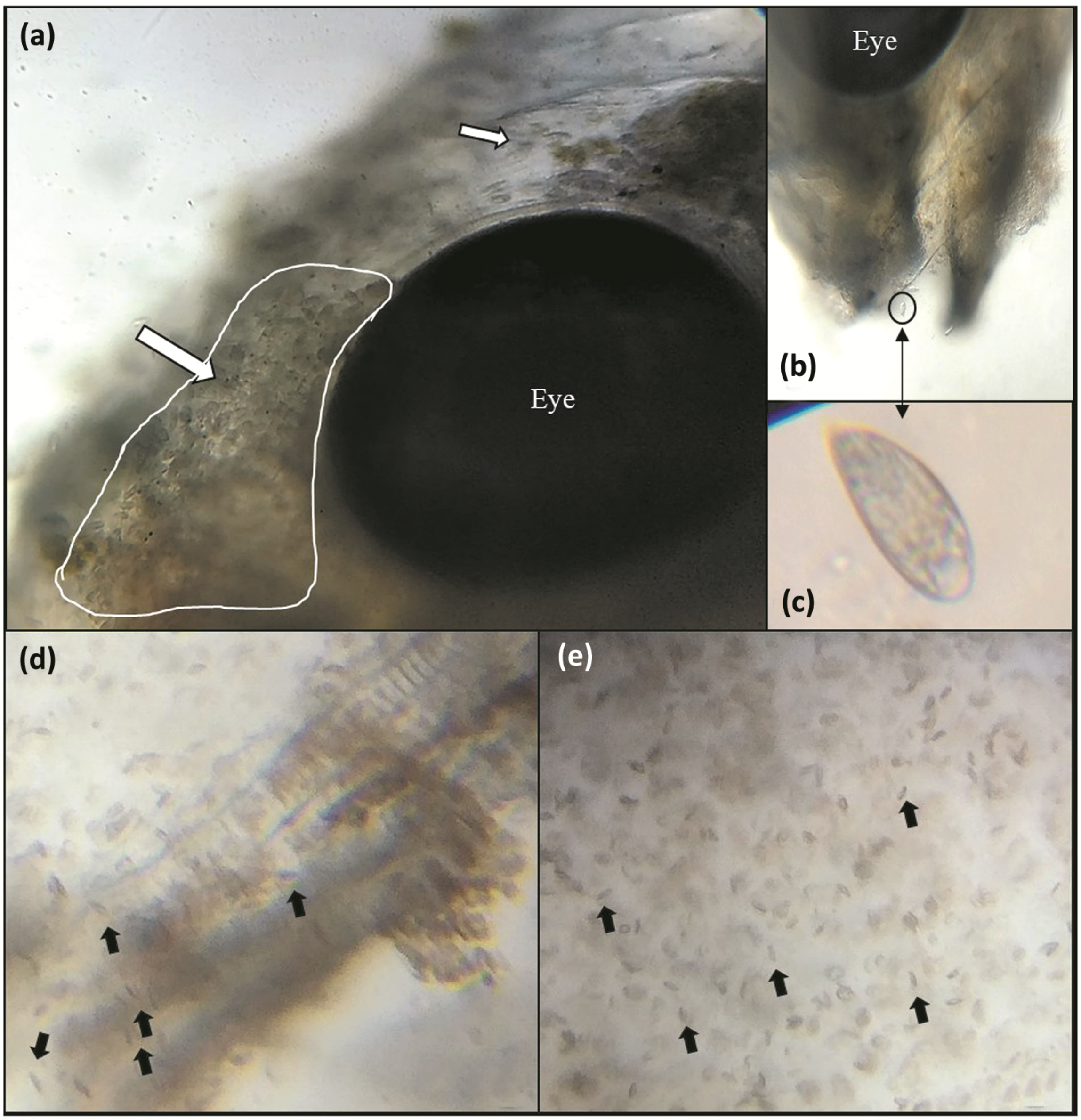

Fig. 2 — Microscopic investigation of infected *A. percula* juvenile: (a) Dense ciliate (25-50 × 10-20 µm in size), (*M. sinensis*) on the head region of moribund fish; (b) Excess mucus secretion with ciliate in the mouth region; (c) Individual ciliate, *M. sinensis*, (d) Dense parasite attachment on the gill filament (arrow); and (e) Occurrence of the parasite in gill wet mount

OM055651.1). The use of gene-specific primers achieved the sequence of 1733 bp which is also published in GenBank with accession number - ON329185.1. It showed a 97 % resemblance to previously reported *M. sinensis* (GenBank No-HM236336.1). Earlier researchers pointed out that several diagnostic PCR techniques can be performed to identify scuticociliate species at the molecular level, most of which analyse the nucleotide sequence of the gene (using specific gene primers) encoding the small subunit rRNA (SSU rRNA)[35,36]. The SSU rRNA sequence of *M. sinensis* was aligned with SSU rRNA sequences of 37 other ciliates from eight orders (Pseudocohnilembidae, Cohnilembidae, Paralembidae, Parauronematidae, Philasteridae, Entorhipidiidae, Uronematidae and Orchitophryidae) in the GenBank database (Fig. 3). The phylogenetic tree of the present study revealed that *M. sinensis* and *P. magna* are closely related and formed a single clade. The current results are also supported by the findings of Gao *et al.*[17] through the presence of different signatures within a single family of Orchitophryidae.

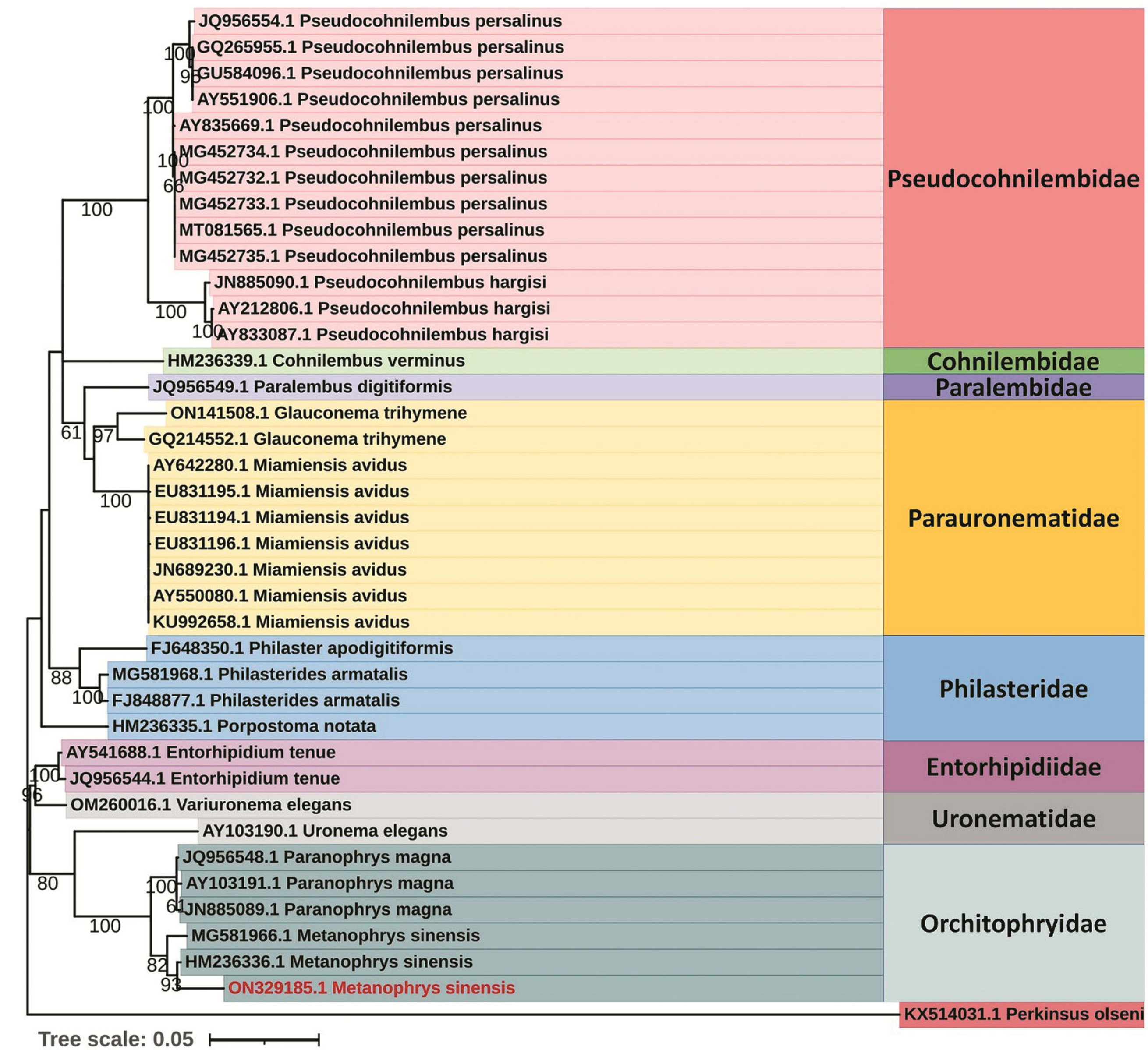

Fig. 3 — Phylogenetic tree analysis of 18 SSU rRNA gene of *Metanophrys sinensis* (ON329185.1) amplified using universal primer, showing a relationship with an earlier reported isolate of *M. sinensis* (HM236336.1 and MG581966.1). The dendrogram was generated by MEGA 6 using the maximum likelihood method. The 18 SSU rRNA sequence of *Perkinsus olseni* was used as an outgroup

Further, *Uronema elegans* belonging to the group Uronematidae have a sister relationship with Orchitophryidae. Moreover, Lynn[37] reported that *Metanophrys*, *Mesanophrys*, *Anophryoides*, and *Paranophrys* were all assigned to the family Orchitophryidae. He reported that four genera were not clustered together but were distributed over three clades: i) *M. sinensis* and *P. magna*; ii) *M. carcini* and *M. similis*; and iii) *A. haemophila* with *M. avidus*.

Conclusion

The present study concluded that the scuticociliatosis outbreak in the clownfish breeding facility was caused by *M. sinensis*. Based on the literature study, this is the first documentation of *M. sinensis* infection in marine ornamental fish.

Acknowledgements

The authors are thankful to Mangrove Cell & Mangrove Foundation, Government of Maharashtra, for the financial support. Thanks to the Director, ICAR - National Bureau of Fish Genetic Resources, Lucknow, for the encouragement.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

NBD: Design of the experiment, data collection, analysis and writing of manuscript. AS: Materials preparation, scientific analysis and methodology. TTAK: Review and editing of the manuscript. UKS: Critical evaluation and overall conceptualisation. All authors significantly contributed to bring out this manuscript. All authors read and approved the final manuscript.

References

1 Lieke T, Meinelt T, Hoseinifar S H, Pan B, Straus D L, *et al.*, Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases, *Rev Aquac*, 12 (2) (2020) 943-965. https://doi.org/10.1111/raq.12365

2 Dhayanithi N B, Sudhagar S A, Ajithkumar T T & Lal K K, Study on amyloodiniosis outbreak in captive□bred percula clownfish (*Amphiprion percula*) and improved control regimens, *J Parasit Dis*, 46 (2022) 1103-1109. https://doi.org/10.1007/s12639-022-01530-1

3 Jung S J & Woo P T K, *Miamiensis avidus* and related species, In: *Fish parasites: Pathobiology and protection*, edited by Woo Patrick T K & Buchmann K, (Wallingford, Oxfordshire: CABI), 2012, pp. 73–91. ISBN 9781845938062

4 Piazzon M C, Leiro J & Lamas J, Reprint of fish immunity to scuticociliate parasites, *Dev Comp Immunol*, 43 (2) (2014) 280-289. https://doi.org/10.1016/j.dci.2013.11.015

5 Dragesco A, Dragesco J, Coste F, Gasc C, Romestand B, *et al.*, *Philasterides dicentrarchi*, n. sp., (Ciliophora, scuticociliatida), a histophagous opportunistic parasite of *Dicentrarchus labrax* (Linnaeus, 1758), a reared marine fish, *Eur J Protistol*, 31 (3) (1995) 327-340. https://doi.org/10.1016/S0932-4739(11)80097-0

6 Iglesias R, Parama A, Alvarez M F, Leiro J, Fernandez J, *et al.*, *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain), *Dis Aquat Org*, 46 (1) (2001) 47-55. https://doi.org/10.3354/dao046047

7 Song W & Wilbert N, Reinvestigations of three ‘‘wellknown’’ marine scuticociliates: *Uronemella filificum* (Kahl, 1931) nov. gen., nov. comb., *Pseudocohnilembus hargisi* Evans & Thompson, 1964 and *Cyclidium citrullus* Cohn 1865, with description of the new genus *Uronemella* (Protozoa, Ciliophora, Scuticociliatida), *Zool Anz*, 241 (4) (2002) 317-331. https://doi.org/10.1078/0044-5231-00075

8 Kim S M, Cho J B, Kim S K, Nam Y K & Kim K H, Occurrence of scuticociliatosis in olive flounder *Paralichthys olivaceus* by *Philasterides dicentrarchi* (Ciliophora: scuticociliatida), *Dis Aquat Organ*, 62 (3) (2004) 233-238. https://doi.org/10.3354/dao062233

9 Song W & Wilbert N, Redefinition and redescription of some marine scuticociliates from China, with report of a new species, *Metanophrys sinensis* nov. spec. (Ciliophora, Scuticociliatida), *Zool Anz*, 239 (1) (2000) 45-74.

10 Song W B, Warren A & Hu X Z, *Free-living ciliates in the Bohai and Yellow Seas, China*, (Supported by the Darwin Initiative Programme, Science Press, Beijing), 2009, pp. 395-396.

11 Ajithkumar T T, Sethu S K, Murugesan P & Balasubramanian T, Studies on captive breeding and larval rearing of clownfish *Amphiprion sebae* (Bleeker, 1835) using estuarine water, *Indian J Geo-Mar Sci*, 39 (1) (2010) 114-119.

12 Rhyne L & Tlusty M F, Trends in the marine aquarium trade: the influence of global economics and technology, *Aquac Aquar Conserv Legis*, 5 (2) (2012) 99-102.

13 Leal M C, Vaz M C, Puga J, Rocha R J, Brown C, *et al.*, Marine ornamental fish imports in the European Union: an economic perspective, *Fish Fish*, 17 (2) (2015) 459-468. https://doi.org/10.1111/faf.12120

14 Prakash S, Ajithkumar T T, Raghavan R, Rhyn A, Tlustye M F, *et al.*, Marine aquarium trade in India: Challenges and opportunities for conservation and policy, *Mar policy*, 77 (2017) 120-129. https://doi.org/10.1016/j.marpol.2016.12.020

15 Dhayanithi N B, Ajithkumar T T, Arockiaraj J, Balasundaram C & Harikrishnan R, Immune protection of *Rhizophora apiculata* in clownfish against *Vibrio alginolyticus, Aquaculture*, 446 (2015) 1-6. https://doi.org/10.1016/j.aquaculture.2015.04.013

16 Assefa A & Abunna F, Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish, *Vet Med Int*, 5432497 (2018) 1-10. https://doi.org/10.1155/2018/5432497

17 Gao F, Katz L A & Song W, Insights into the phylogenetic and taxonomy of philasterid ciliates (Protozoa, Ciliophora, Scuticociliatia) based on analyses of multiple molecular

markers, *Mol Phylogenet Evol*, 64 (2) (2012) 308-317. https://doi.org/10.1016/j.ympev.2012.04.008

18 Hall T A, *BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT*, *Nucleic Acids Symp Ser*, (Oxford University Press), 41 (1999) 95-98.

19 Kumar S, Stecher G, Li M, Knyaz C & Tamura K, MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms, *Mol Biol Evol*, 35 (6) (2018) 1547-1549. https://doi.org/10.1093/molbev/msy096

20 Letunic I, Khedkar S & Bork P, SMART: recent updates, new developments and status in 2020, *Nucleic Acids Res*, 49 (1) (2021) 458-460. https://doi.org/10.1093/nar/gkaa937

21 Urrutxurtu I, Orive E & Sota A D L, Seasonal dynamics of ciliated protozoa and their potential food in an eutrophic estuary (bay of Biscay), *Estuar Coast Shelf Sci*, 57 (5-6) (2003) 1169-1182. https://doi.org/10.1016/S0272-7714(03)00057-X

22 Colorni A & Padros F, Diseases and health management, In: *Sparidae*: *Biology and Aquaculture of Gilthead Sea Bream and other Species*, edited by Pavlidis M A & Mylonas C C, (Wiley-Blackwell, Oxford, UK), 2011, pp. 321-357.

23 Noga E J, *Fish disease: Diagnosis and treatment*, 2nd edn, (Willey- Blackwell, Iowa), 2010, pp. 544. ISBN: 978-0-813-80697-6

24 Sahoo P K, Pattanayak S, Paul A, Sahoo M K, Rajesh Kumar P, *et al*., First record of *Metanophrys sinensis* (Protozoa: Ciliophora: Scuticociliatida) from India causing large scale mortality in a new host *Macrobrachium rosenbergii* larvae, *J Fish Dis*, 41 (8) (2018) 1303-1307. https://doi.org/10.1111/jfd.12809

25 Ramos M F, Costa A R, Barandela T, Saraiva A & Rodrigues P N, Scuticociliate infection and pathology in cultured turbot *Scophthalmus maximus* from the north of Portugal, *Dis Aquat Organ*, 74 (3) (2007) 249-253. https://doi.org/10.3354/dao074249

26 Rossteuscher S, Wenker C, Jermann T, Wahli T, Oldenberg E, *et al*., Severe scuticociliate (*Philasterides dicentrarchi*) infection in a population of sea dragons (*Phycodurus eques* and *Phyllopteryx taeniolatus*), *Vet Pathol*, 45 (4) (2008) 546-550. https://doi.org/10.1354/vp.45-4-546.

27 Jin C N, Harikrishnan R, Moon Y G, Kim M C, Kim J S, *et al*., Histopathological changes of Korea cultured olive flounder, *Paralichthys olivaceus* due to scuticociliatosis caused by histophagous scuticociliate, *Philasterides dicentrarchi*, *Vet Parasitol*, 161 (3-4) (2009) 292-301. https://doi.org/10.1016/j.vetpar.2009.01.033

28 Moustafa E M, Naota M, Morita T, Tange N & Shimada A, Pathological study on the scuticociliatosis affecting farmed Japanese flounder (*Paralichthys olivaceus*) in Japan, *J Vet Med Sci*, 72 (10) (2010) 1359-1362. https://doi.org/10.1292/jvms.10-0026

29 Perez-Uz B & Guinea A, Morphology and infraciliature of a marine scuticociliate with a polymorphic life cycle: *Urocryptum tortum* n. gen., n. comb, *J Eukaryot Microbiol*, 48 (3) (2001) 338-347. https://doi.org/10.1111/j.1550-7408.2001.tb00323.x

30 Kim S M, Cho J B, Lee E H, Won S R, Kim S K, *et al*., *Pseudocohnilembus persalinus* (Ciliophora: Scuticociitida) is an additional species causing scuticociliatosis in olive flounder *Paralichthys olivaceus*, *Dis Aquat Organ*, 62 (3) (2004) 239-244. https://doi.org/10.3354/dao062239

31 Jung S J, Kitamura S, Song J Y & Oh M J, *Miamiensis avidus* (Ciliophora: Scuticociliatida) causes systemic infection of olive flounder *Paralichthys olivaceus* and is a senior synonym of *Philasterides dicentrarchi*, *Dis Aquat Organ*, 73 (3) (2007) 227-234. https://doi.org/10.3354/dao073227

32 Zhang Q, Fan X, Clamp J C, Al-Rasheid K A & Song W, Description of *Paratetrahymena parawassi* n. sp. using morphological and molecular evidence and a phylogenetic analysis of Paratetrahymena and other taxonomically ambiguous genera in the order Loxocephalida (Ciliophora, Oligohymenophorea), *J Eukaryot Microbiol*, 57 (6) (2010) 483-493. https://doi.org/10.1111/j.1550-7408.2010.00501.x

33 Song W, Shang H, Chen Z & Ma H, Comparison of some closely-related Metanophrys-taxa with description of a new species *Metanophrys similis* nov. spec. (Ciliophora, Scuticociliatida), *Eur J Protistol*, 38 (1) (2002) 45-53. https://doi.org/10.1078/0932-4739-00848

34 Kang H S, Whang I & Cho J K, First report of *Paranophrys marina* (Protozoa, Ciliophora, Scuticociliatia) isolated from olive flounder *Paralichthys olivaceus* in Korea: morphological and phylogenetic analysis, *Fish Pathol*, 34 (1) (2021) 047-053. https://doi.org/10.7847/jfp.2021.34.1.047

35 Parama A, Arranz J A, Alvarez M F, Sanmartin M L & Leiro J, Ultrastructure and phylogeny of *Philasterides dicentrarchi* (Ciliophora, Scuticociliatia) from farmed turbot in NW Spain, *Parasitol*, 132 (4) (2006) 555-564. https://doi.org/10.1017/S0031182005009534

36 Gao F, Fan X, Yi Z, Struder-Kypke M & Song W, Phylogenetic consideration of two scuticociliate genera, *Philasterides* and *Boveria* (Protozoa, Ciliophora) based on 18 S rRNA gene sequences, *Parasitol Int*, 59 (4) (2010) 549-555. https://doi.org/10.1016/j.parint.2010.07.002

37 Lynn D H, *The ciliated protozoa: characterization, classification and guide to the literature*, 3rd edn, (Springer Verlag, Dordrecht), 2008, pp. 605. https://doi.org/10.1007/978-1-4020-8239-9