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# Redescription of *Henneguya chaudhuryi* (Bajpai & Haldar, 1982) (Myxosporea: Myxobolidae), infecting the gills of the freshwater fish *Channa punctata* (Bloch) (Perciformes: Channidae) in India

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**Abstract** During a survey of myxosporean parasites of freshwater fishes in Meerut, Uttar Pradesh (UP), India, spores of *Henneguya chaudhuryi* (Bajpai & Haldar, 1982) were found in the gill lamellae of the spotted snakehead fish *Channa punctata* (Bloch) (Perciformes: Channidae). This species was described lacking several characteristics in the original description, which makes challenging the accurate diagnosis. Here, we supplemented its description based on morphological, histological and molecular data. Plasmodia of *H. chaudhuryi* are oval, measuring 60–100 × 40–68 µm, located intralamellarly. Mature spores are elongate, measuring 10.5–13.2 × 3.6–4.2 µm, with two slightly unequal polar capsules with 6–7 filamental turns and two straight, equal caudal appendages, 10–17 µm long. Scanning electron microscopy revealed a flat surface. The 18S rDNA sequence for *H. chaudhuryi* did not show a close relationship with those of any other *Henneguya* spp., represented in the GenBank.

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## Introduction

Among the parasites of class Myxozoa Grassé 1970, *Henneguya* Thélohan, 1982 is the second largest genus. This genus comprises about 189 species; the majority of these are parasites of freshwater fishes (Eiras, 2002; Eiras & Adriano, 2012; Azevedo et al., 2014) and several species cause economic impacts (Feist, 2008). Twenty-three species of *Henneguya* have been reported in India (Kalavati & Nandi, 2007). However, most species have been described only on the basis of their morphology under light microscopy (Chakravarty, 1939; Tripathi, 1952; Bhatt & Siddiqui, 1964; Lalitha-Kumari, 1965, 1969; Qadri, 1965, 1970; Narasimhamurti & Kalavati, 1975; Sarkar, 1985; Haldar et al., 1997, 1983; Hemanand et al., 2008) thus making the accurate diagnosis challenging.

The spotted snakehead *Channa punctata* (Bloch) is an economically important fish, available throughout the year in India (Rohankar et al., 2012). Its myxosporean fauna is well studied in India based on spore morphology, but the descriptions available are less informative to identify unambiguously the myxosporeans at the species level. Accurate identification of these parasites in India is problematic due to the typically poor morphological descriptions and the lack of molecular data. However, recent publications (Molnár et al., 2010; Liu et al., 2012; Székely et al., 2015) suggest that molecular approaches are necessary for correct species identification. In India, molecular tools have barely been applied in studies





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of myxozoans, therefore, no data are available on *Henneguya* spp. from India in the GenBank database.

In the present study, we conducted morphological (both light and scanning electron microscopy) and molecular approach to identify *Henneguya chaudhuryi* (Bajpai & Haldar, 1982) infecting the gills of spotted snakehead *Channa punctata* in Meerut, Uttar Pradesh (UP), India.

## Materials and methods

### *Sampling and microscopy*

Fish were collected from the local fish market Sotiganj, Meerut (28°59′0″N, 77°42′0″E), Uttar Pradesh (UP), India from November to December 2014. *Channa punctata* (n = 32) were examined for myxozoan infections in the laboratory at the Department of Zoology, Chaudhary Charan Singh University, Meerut, UP, India. The gill filaments of each hemibranchium were checked for myxozoan plasmodia under a Motic stereomicroscope (SMZ-168 series). Plasmodia were carefully removed from the gills, opened with a fine needle on a slide and observed under Olympus microscope (CH30). A subset of the spores obtained from mature plasmodia was fixed in 80% ethanol in vials and sent to further morphological examinations to Hungary. Another subset of the spores collected were placed into 1.5 ml tubes and stored at −20°C for subsequent molecular study in India.

Spores were also fixed in 5% glutaraldehyde in sodium cacodylate buffer (pH 7.2) at 4°C for scanning electron microscopy (SEM) in India. For SEM, spores were washed in the buffer, dehydrated through ascending ethanol series, dried by critical point drier, mounted on stubs, coated with a thin layer of metallic gold and observed with a Neoscope JCM5000 SEM at an accelerating voltage of 15 kV.

Infected gills were fixed in 4% formalin and sent to Hungary for histological examinations. For histology, infected gills were fixed in Bouin's solution for 4 h, washed in 80% ethanol several times, embedded in paraffin wax, cut into 5–8 µm thick sections and stained with haematoxylin and eosin. Photos of fixed spores as well as of histological sections were taken in Hungary with an Olympus BH-2 microscope equipped with a DP-10 digital camera. Measurements of fresh myxospores were taken with a calibrated eyepiece micrometer according to the guidelines of Lom & Arthur (1989). Additional spores were measured on the photomicrographs. All measurements are in micrometres and are presented as the range followed by the mean and standard deviation in parentheses.

### *Molecular methods*

For DNA extraction, samples preserved in 95% ethanol were centrifuged at 8,000×*g* for 5 min to pellet the spores and then the ethanol was removed. The DNA was extracted using a QIAGEN DNeasy™ tissue kit (animal tissue protocol, Qiagen, Hilden, Germany) and eluted in 50 µl buffer. The 18S rDNA was amplified using primers ERIB1 and ERIB10 (Table 1) in a 25 µl reaction mixture comprising 2 µl genomic DNA, 5 µl 1 mM deoxyribonucleotide triphosphates (dNTPs, Biotools, Spain), 0.45 µl of each primer, 2.5 µl of 10× Taq buffer (Biotools), 0.40 µl of Taq polymerase (1U; Biotools) and 14.20 µl of distilled water. The PCR cycle consisted of an initial denaturation step at 95°C for 3 min, followed by 35 cycles at 95°C for 40 s, 56°C for 1 min, 72°C for 1 min, completed with a final extension step at 72°C for 7 min. This was followed by a second round of PCR with the Myx1f-SphR primer pair (Table 1). Nested PCR reactions were conducted with a volume of 50 µl consisting of 1 µl of amplified DNA, 10 µl of 1 mM dNTPs (Biotools), 0.90 µl of each primer, 5 µl of 10× Taq buffer (Biotools), 0.80 µl of Taq polymerase (1U; Biotools) and 31.40 µl of distilled water. Amplifications conditions in the second round were carried out with the following profile: 95°C for 3 min, followed by 35 cycles at 95°C for 50 s, 56°C for 50 s, 72°C for 1 min, and a final extension step at 72°C for 7 min. Both PCR cycles were performed in a Mastercycler personal-2231 (Eppendorf, Germany). The PCR products were electrophorised in 1% agarose gel in Tris-Acetate-EDTA buffer gel stained with 1% ethidium bromide. Amplified DNA was purified with the Purelink™ Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen, Löhne, Germany). Purified PCR products were sequenced with the primers listed in Table 1, using the Big Dye Terminator vr. 3.1 cycle sequencing kit in ABI 3130 Genetic Analyzer, Applied Biosystems.

The various forward and reverse sequences were assembled in the software MEGA 6 (Tamura et al., 2013) and ambiguous bases were clarified using







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**Table 1** Primers used for PCR and sequencing

| 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 | Hallet & Diamant (2001) |
| SphR | GTTACCATTGTAGCGCGCGT | 2nd round PCR and sequencing | Eszterbauer & Székely (2004) |
| MC5 | CCTGAGAAACGGCTACCACATCCA | Sequencing | Molnár et al. (2002) |
| MC3 | GATTAGCCTGACAGATCACTCCACGA | Sequencing | Molnár et al. (2002) |
| MB5r | ACCGCTCCTGTTAATCATCACC | Sequencing | Eszterbauer (2004) |
| ACT1r | AATTTCACCTCTCGCTGCCA | Sequencing | Hallet & Diamant (2001) |
| NSF573/19 | CGCGGTAATTCCAGCTCCA | Sequencing | Wuyts et al. (2004) |



corresponding ABI chromatograms. To evaluate the relationship of *H. chaudhuryi* with other myxozoans, a homology search was performed using BLAST. Based on the blast matches myxozoan sequences were downloaded from the GenBank for further analysis. DNA pairwise distances were calculated using the p-distance model. Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed to determine the phylogenetic position of the analysed sample. Model testing for the nucleotide substitution model of best fit of the dataset was carried out using the Akaike Information Criterion (AIC) in MEGA 6; the selected GTR + G + I model was used in the ML analysis computed by MEGA 6. Bootstrap values based on 1,000 resampled datasets were generated. BI was computed by Topali 2.5 (Milne et al., 2008). The substitution models were tested by the Bayesian Information Criterion and GTR + G + I was chosen. Posterior probabilities were estimated over 1,000,000 generations *via* five independent runs of four simultaneous MCMCMC chains with every 100th tree saved. The 'burn in' was set to 25%. *Myxobolus cerebralis* Hofer, 1903 (AF115255) was selected as the outgroup in the final alignment.

## Results

During the course of this study for myxosporean infections in the spotted snakehead, a single *Henneguya* species was found in the lamellae of the gill filaments. Regarding both the prevalence (59%) and the intensity (1 to 20 cysts/gill hemibranch) a moderate infection was found. Based on the morphology of the spores the material was identified as *Henneguya chaudhuryi* described by Bajpai & Haldar (1982)

**Family Myxobolidae Thélohan, 1892**

**Genus *Henneguya* Thélohan, 1892**

***Henneguya chaudhuryi* (Bajpai & Haldar, 1982)**

*Host*: *Channa punctata* (Bloch); local common name 'sauli' (Perciformes: Channidae).
*Locality*: Sotiganj, Meerut (28°59′0″N, 77°42′0″E), Uttar Pradesh, India.
*Site of tissue development*: Gill lamellae.
*Vouchermaterial*: Digitized photos of spores and histological sections were deposited in the Parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest (Coll. No. HNHM-71590).
*Prevalence of infection*: 59.3% (19 out of 32 fish of the 8–11 cm size group infected).
*Intensity of infection*: Moderate.
*Representative DNA sequence*: The 18S rDNA sequence of *H. chaudhuryi* was deposited in GenBank under accession number KT279402.

Description (Figs. 1–6)

*Trophozoites*
Plasmodia relatively small, interlamellar, with a roundish or ellipsoidal shape. Round plasmodia 40–74 (64) in diameter; ellipsoidal plasmodia measuring 60–100 (74) in length and 40–68 (54) in width.





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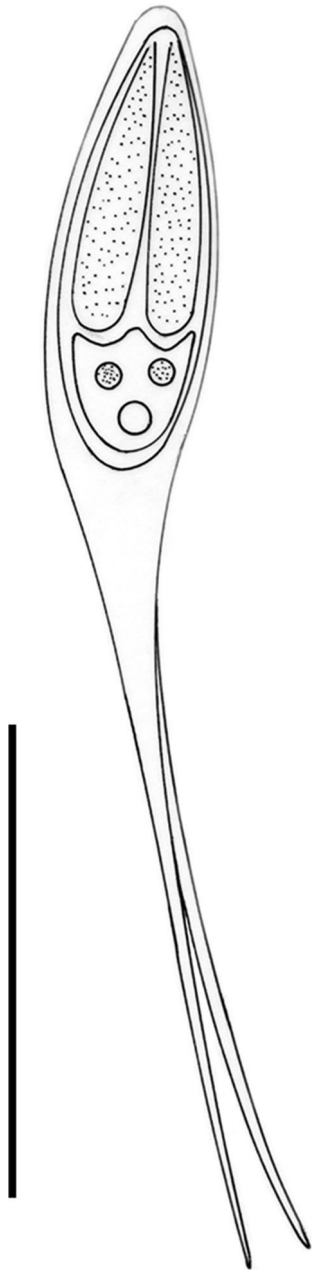

**Fig. 1** Schematic illustration of *Henneguya chaudhuryi* spores, frontal view. *Scale-bar*: 10 μm

*Spores*

Spores elongated, with 2 straight caudal appendages, and elongated polar capsules located side by side (Figs. 1–2). Spore wall thin, smooth, composed of 2 equal valves. Only frontal view of spores recorded. Oral end of spore body blunt in frontal view; caudal end rounded and continued into caudal appendages.

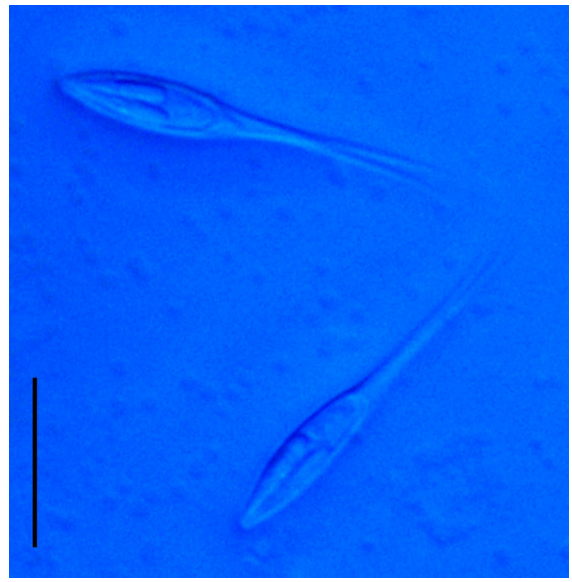

**Fig. 2** Ethanol-fixed spores of *Henneguya chaudhuryi*, frontal view. *Scale-bar*: 10 μm

Spore total length (from anterior end of spore body to end of caudal appendages) 24–30 (27.8 ± 2.7) (n = 50). Spore body 10.5–13.2 (11.6 ± 1.13) (n = 50) long, 3.6–4.2 (3.8 ± 0.21) (n = 50) wide, 3.6–4.2 (3.9 ± 0.24) (n = 8) thick. Polar capsules 2, equal in size, elongated, pointed at anterior end of spore body. Polar capsules 5.5–7.2 (6.5 ± 0.67) (n = 50) long, 1.0–1.3 (1.1 ± 0.1) (n = 50) wide, and 1.0–1.3 (1.2 ± 0.1) thick (n = 8). Polar filaments coiled in 6–7 turns, located perpendicular to long axis of polar capsules; length of extruded filaments 24–30 (27.8 ± 2.7) (n = 22). Suture not observed. Iodinophilous vacuole present in sporoplasm, small, round.

## Histology and SEM examination

Intralamellar plasmodia were located in the gill lamellae filled with mature spores, elongate and well visible (Figs. 3–4). The SEM observation showed that the spore wall is smooth but some depressions were observed on surface. Caudal processes bifurcated extending from the posterior end of the spore body (Fig. 5).

## Molecular data

The partial18S rDNA sequence of *H. chaudhuryi*, composed of 1,650 bp, was deposited in GenBank



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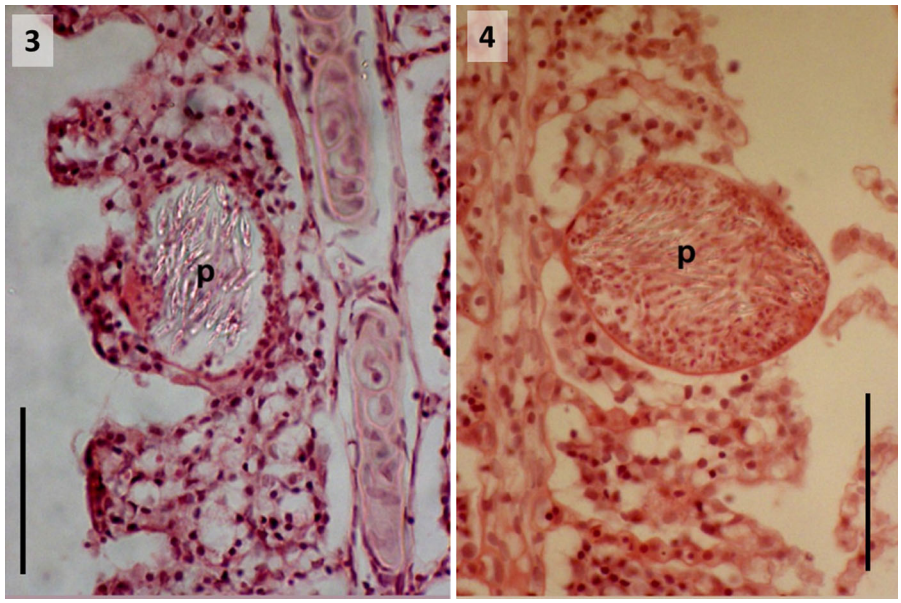


**Fig. 3-4** Plasmodia (p) of *Henneguya chaudhuryi* in the gills of *Channa punctata*. Histological section. Haematoxylin and eosin staining. *Scale-bar*: 50 µm

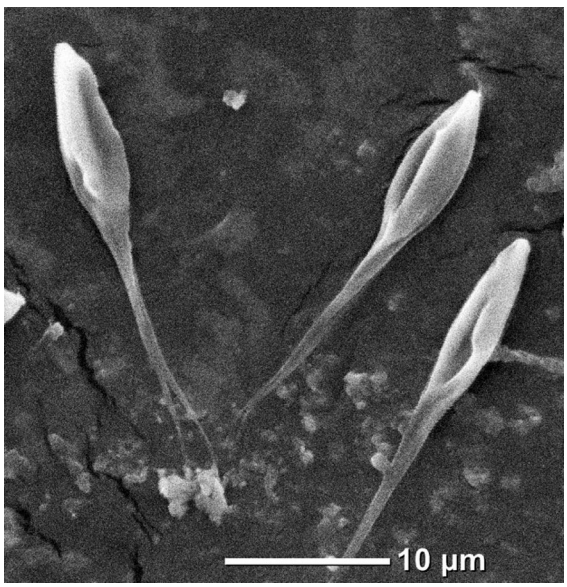


**Fig. 5** Scanning electron micrograph of myxospores of *Henneguya chaudhuryi*, frontal view. *Scale-bar*: 10 µm

(accession number KT279402).The generated ML and BI trees showed an identical topology, therefore, only ML tree is presented here (Fig. 6).The pairwise distances showed remarkable differences, there were no closely related species regarding the 18S rDNA sequence similarities of *H. chaudhuryi*. The phylogenetic tree positioned *H. chaudhuryi* separately from other species supported by high bootstrap values and posterior probabilities (99% and 1.00). *Henneguya chaudhuryi* specifically clustered with the other gill-infecting *Henneguya* spp. (*H. rhinogobii* Li & Nie, 1973; *H. pseudorhinogobii* Kageyama, Yanagida & Yokoyama, 2009; *H. shaharini* Shariff, 2006; and *Henneguya* sp. YFG-SFE1) parasitising hosts in fresh and brackish waters (Fig. 6). Pairwise comparisons among the 18S rDNA sequences for *Henneguya* species showed that *H. chaudhuryi* exhibited sequence similarities with *H. rhinogobii* and *H. pseudorhinogobii* reaching only 87.3 and 87.2%, respectively.

## Remarks

Most of the known *Henneguya* spp. identified have elongate spores, with elongated polar capsules which makes morphological differentiation difficult. Identification of the species, their host specificity, the location of plasmodia in the host and the length of the tail (caudal attachments) should have also been considered. The proper identification has frequently been restricted by the fact that the length of the spores in some original descriptions has been done in different ways. Lom & Arthur (1989) described the total length of the spores (spore body length plus the



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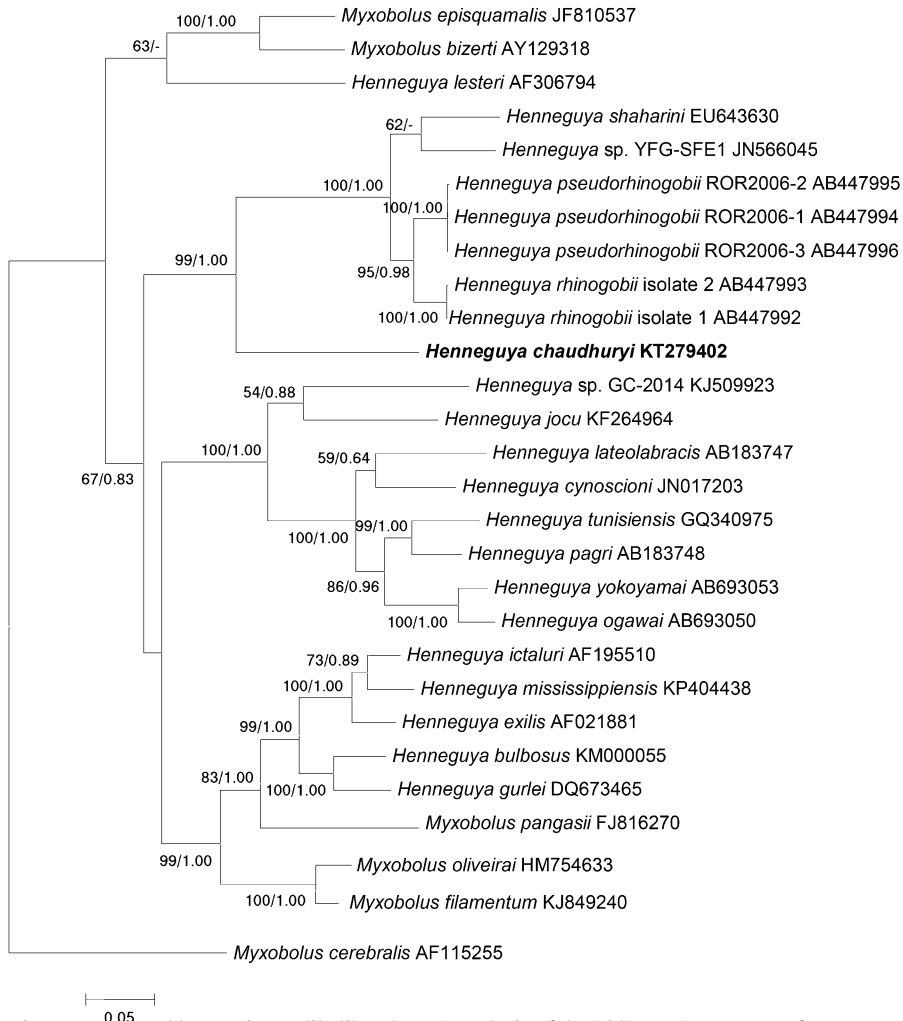


**Fig. 6** Phylogenetic tree generated by maximum likelihood (ML) analysis of the 18S rDNA sequence of *Henneguya chaudhuryi* and some related species. Numbers at nodes indicate the bootstrap values (ML) and posterior probabilities (BI). Unsupported nodes by BI are marked with a hyphen. *Myxobolus cerebralis* was used as the outgroup. *Henneguya* species sequenced in this study is in bold

length of the caudal attachments) as the "length of the spores" and gave correct data on the spore length. The final correct identification of a myxozoan species makes molecular methods necessary. In India, 23 *Henneguya* spp. infecting fishes have been described (Kalavati & Nandi, 2007). Twelve of these infect the gill filaments of different hosts: *H. chaudhuryi*, *H. ophiocephali* (Chakravarty, 1939), *H. zahoori* (Bhatt & Siddiqui, 1964) and *H. waltairensis* (Narasimhamurti & Kalavati, 1975) infect *Channa punctata*; *H. latesi* (Tripathi, 1952) infects *Lates calcarifer*; *H. notopterae* (Qadri, 1965) and *H. ganapatiae* (Qadri, 1970) infect *Notopterus notopterus* (Pallas); *H. singhi* (Lalitha-Kumari, 1969) infects *Notopterus osmani* (Talwar & Jhingran); *H. namae* (Haldar et al., 1983) infects *Ambassis nama* (Hamilton); *H. nandi* (Gupta & Khera, 1987) infects *Nandus nandus* (Hamilton); *H. mystusii* (Sarkar, 1985) and *H. mystasi* (Haldar et al., 1997) infect *Mystus* sp. and *Mystus gulio* (Hamilton), respectively.

Of the above 12 species described from different hosts, four have been reported from the gill filaments of *C. punctata*. A comparison of the morphological data for these four *Henneguya* spp. is summarized in Table 2. The present material identified by us as *H. chaudhuryi* corresponds well to the data presented by Gupta & Khera (1987) although the authors of the original description failed to observe the parallel



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**Table 2** Comparative data for *H. chaudhuryi* and *Henneguya* spp. infecting gill filaments of *C. punctata* in India

| Species | *H. chaudhuryi* (Bajpai & Haldar 1982) | | *H. ophiocephali* Chakravarty, 1939 | *H. waltairensis* Narasimhamurti & Kalavati, 1975 | *H. zahoori* Bhatt & Siddiqui, 1964 |
|---|---|---|---|---|---|
| Source | Present study | Bajpai & Haldar (1982); Gupta & Khera (1987) | Chakravarty (1939) | Narasimhamurti & Kalavati (1975) | Bhatt & Siddiqui (1964) |
| | Range (Mean ± SD) | Range (Mean) | Range (Mean) | Range (Mean) | Range (Mean) |
| Spore shape | Elongate | Elongate | Ovoidal or elongate | Oval | Biconvex |
| Spore length | 24–30 (27.8 ± 2.7) | 26.3–33.2 (30.0) | 41.5–52.5 | 14.6–15.5 | 8.0–12.0 (9.6) |
| Spore width | 3.6–4.2 (2.8 ± 0.21) | 3.3–4.1 (3.7) | 6.18–9.27 | 3.2–4.0 | 2.1–3.0 (2.6) |
| Caudal processes length | 24–30 (27.8 ± 2.7) | 14.5–20.0 (17.7) | 26.0–32.0 | 40.0–50.0 | 12.0–18.6 (13.9) |
| Polar capsule length | 5.5–7.2 (6.5 ± 0.67) | 5.0–7.5 (6.0) | 6.18–9.27 | 10.0–12.0 | 4.9–6.7 (5.8) |
| Polar capsule width | 1.0–1.3 (1.1 ± 0.1) | 1.6 | 2.1–3.0 | 1.6–2.5 | 0.7–1.1 (0.9) |
| Polar filament coils | 6–7 | 6–7 | na | 6–7 | na |

*Abbreviation*: na, not available; SD, standard deviation

running pair of caudal attachments and identified this species as *Unicauda* (see Bajpai & Haldar, 1982). *Henneguya waltairensis* clearly differs from *H. chaudhuryi* by its long bifurcated recurving caudal attachment. The spores of *H. ophiocephali* resemble those of *H. chaudhuryi* but have shorter polar capsules and longer caudal attachments. Moreover, *H. zahoori* differs from *H. chaudhuryi* by its biconvex shape of the spore and caudal processes.

## Discussion

During a survey on myxosporean infections in Indian fishes of the four known *Henneguya* species, *H. chaudhuryi*, *H. ophiocephali*, *H. waltairensis* and *H. zahori* infecting the gills of *Channa punctata*, we found plasmodia and spores of *H. chaudhuryi.* Most *Henneguya* species known from the spotted snakehead have only an insufficient description. Therefore we extended the morphological description by adding more details such as histological data on the proper location of plasmodia in the gills and, in addition, 18S rDNA sequence was presented to supplement the morphological observations. At present, analyses of DNA sequences are needed for a proper identification of a new species as well as for redescription of already existing species, and in the case of myxozoans, analysis of 18S rDNA is most commonly used (Molnár et al., 2002; Zhang et al., 2010; Carriero et al., 2013; Moreira et al., 2014, Székely et al., 2015). Molecular methods can refine the traditional taxonomy of these parasites.

Besides this, descriptions should be supported by scanning electron microscopy data (Rocha & Azevedo, 2012). During present study, we tried to fulfil all the requirements including SEM and molecular approaches to characterise the species. Based on its 18S rDNA sequence, *H. chaudhuryi* is related to other gill infecting *Henneguya* species. In addition, the BLAST search indicated that the 18S rDNA sequence of *H. chaudhuryi* does not match closely any other *Henneguya* sequence in GenBank. This might be due to the scarcity of *Henneguya* data available from India





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on GenBank, as also showed by the present investigation; this represents the first phylogenetic study including *Henneguya* spp. from India.

In conclusion, the detailed description made in the present study, based on morphological, histological, scanning electron microscopy and molecular data, indicate that *H. chaudhuryi* is a valid species. The data presented here will facilitate future research of this fish parasite in India.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable institutional, national and international guidelines for the care and use of animals were followed.

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