Morphology, histology and phylogeny of *Henneguya sinova* sp. nov. (Myxobolidae: Myxozoa) infecting gills of *Parablennius tentacularis* in the Black Sea, Turkey

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ABSTRACT: Myxosporeans of the genus *Henneguya* have a global distribution and infect organs and tissues of both marine and freshwater fishes. Here we describe the morphological, histological and molecular characteristics of *Henneguya sinova* sp. nov. parasitizing the gill arches of tentacled blenny *Parablennius tentacularis* (Perciformes: Blenniidae) collected from the coast of Sinop on the Black Sea in Turkey. Several oval whitish plasmodia of different sizes in the gill arches of fish were found. The mature spores were rounded oval in frontal view, with a mean (range) total length 57.5 (51.5–68.0) µm; the spore body was 11.7 (11.3–12.0) µm in length by 7.6 (7.3–8.3) µm in width and 6.7 (6.6–6.8) µm in thickness. The caudal appendages, measuring 46.0 (40.0–55.0) µm in length, were very thin at the tapered end. The prevalence of infection by *H. sinova* sp. nov. was 35.5%. Phylogenetic analysis of nuclear small subunit ribosomal DNA (SSU rDNA) clearly suggested *H. sinova* as a new species which is clustered within the marine *Henneguya* lineage. Pairwise nucleotide similarities and DNA distance values of SSU rDNA between *H. sinova* sp. nov. and other related *Henneguya* species also supported this suggestion.

KEY WORDS: *Parablennius tentacularis* · Tentacled blenny · *Henneguya sinova* · Black Sea · Turkey

INTRODUCTION

Myxosporea represent a major group of fish parasites, and their impact on wild and cultured fish is significant (Kent et al. 2001). A total of about 2180 species of myxosporean parasites have been reported in 60 genera of fishes (Carriero et al. 2013). Among myxosporeans, the genus *Henneguya* Thélohan, 1892 includes a total of 189 species (Eiras 2002, Eiras & Adriano 2012) and is the second largest group within the phylum Myxozoa. *Henneguya* spp. are common parasites of marine and mostly fresh-

water fish and can infect different organs and tissues, particularly the gills, skin, kidney, musculoskeletal system or gastrointestinal tract (Kent et al. 2001, Eiras 2002, Bahri & Marques 2008), and are relatively less common in the bulbus arteriosus of fish (Yokoyama et al. 2005). *Henneguya* species may cause strong, significant pathological effects on host tissues, such as degenerative cardiomyopathy (Yokoyama et al. 2005), the loss of normal appearance of gill tissues and deleterious effects on the hosts by decreasing the respiratory surface of the gills (Haaparanta et al. 1994); by causing epithelial prolifera-

tion and formation of granulation tissue around infected secondary lamella of gills after the maturation of spores and the disruption of plasmodia (Molnár 1998); and by causing some cell modifications around the cysts (Khlifa et al. 2012). The tentacled blenny *Parablennius tentacularis* (Brünnich, 1768) is a shallow-water species inhabiting rocky substrates in the Mediterranean Sea, Black Sea and Atlantic Ocean (Zander 1986). To date, 3 myxosporean species have been reported from *P. tentacularis* in the Black Sea, but none is a member of the genus *Henneguya* (Yurakhno 2009). Studies on myxosporean parasites of fish along Turkish coasts are scarce (Altunel 1983, Özer 2003, Umur et al. 2010, Özak et al. 2012, Özer et al. 2014), and until now, no *Henneguya* species have been reported parasitizing any fish species from Turkish coastal areas.

Here we report detailed morphological, histological and molecular characteristics of *H. sinova* sp. nov. infecting the gill arches of *P. tentacularis*.

MATERIALS AND METHODS

In total, 31 tentacled blenny, 5.2 to 11.2 cm in length, were caught by hook and line off the coast of Sinop on the Black Sea in June 2013, transported to the laboratory and examined for parasites. Skin, gills, gall bladder, stomach, intestine, kidney, gonads and liver were investigated. All organs were dissected and placed separately in Petri dishes to determine infected organs and parasites. Plasmodia and fresh spores were examined under an Olympus BX51 microscope with phase-contrast and differential interference contrast attachments and photographed with a DP-25 digital camera using data-processing software. Spores (n = 30) were measured and described according to the criteria established by Lom & Arthur (1989). Infection prevalence (%) was determined according to Bush et al. (1997). For histological analysis, fragments of gill containing young and mature plasmodia were fixed in 4% neutral buffered formalin for 24 h and embedded in paraffin. Thin sections (5 μm) obtained with a microtome were stained with haematoxylin & eosin and later observed under a light microscope following the histological protocol of Bahri et al. (2010).

Molecular analyses

Genomic DNA was extracted from *Henneguya* using the Qiagen DNeasy Blood & Tissue Kit accord-

ing to the manufacturer's instructions, and stored at −20°C prior to use. Nuclear small subunit ribosomal DNA (SSU rDNA) was used to infer phylogenetic relations, as nuclear SSU rDNA haplotypes of different *Henneguya* species are available in international databases. To avoid host DNA contamination, the following primer set was designed specifically for marine *Henneguya* and *Myxobolus* species: Henn_Myx_120For (AAT CTG CTC GAT TGT AAG GG) and Henn_Myx_2100Rev (CCG CTC CCA AGG TAT TAT). For amplification, a 50 μl PCR reaction was prepared using genomic DNA (<1 μg), 1.5 mM $MgCl_2$, 1.25 U *Taq* polymerase (New England Bio-Labs), 2.5 mM dNTP mix (Thermo Scientific), 5 μl of 10× PCR buffer, 0.5 pmol (final concentration) of each primer and ddH_2O. Amplifications were made using a Techne (TC-Plus) thermal cycler, with initial denaturation at 95°C for 3 min; followed by 40 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2.5 min; and final extension at 72°C for 10 min. The PCR product was electrophoresed on 1% agarose gel (peqGOLD Universal Agarose) prepared in 1× TBE buffer. After staining with ethidium bromide, the gel was visualized with a Vilber Lourmat imaging system.

Nucleotide sequencing was performed commercially by Macrogen Inc. (Korea). For sequencing, 2 internal primers, viz. NS3 and NS4 (White et al. 1990), were used in addition to the primers above. Nucleotide sequences were assembled using BioEdit (Hall 1999), and ClustalX (Thompson et al. 1997) was used to generate multiple nucleotide sequence alignments of the current nuclear SSU rDNA haplotype together with those obtained from GenBank (Table 1). Akaike's information criterion (AIC) and Bayesian information criterion (BIC) tests were applied with the jModelTest v. 0.1 package (Guindon & Gascuel 2003, Posada 2008) to determine the most

Table 1. Nuclear SSU rDNA haplotypes of *Henneguya* spp. and corresponding GenBank accession numbers for comparison with new sequence for *H. sinova* sp. nov.

Species	Acc. no.	Reference
H. lateolabracis	AB183747	Yokoyama et al. (2005)
H. pagri	AB183748	Yokoyama et al. (2005)
H. tunisiensis	GQ340975	Bahri et al. (2010)
H. mauritaniensis	JQ687060	Khlifa et al. (2012)
H. akule	EU016076	Work et al. (2008)
H. cynoscioni	JN017203	Dykova et al. (2011)
H. ogawai	AB693051	Li et al. (2012)
H. yokoyamai	AB693053	Li et al. (2012)
H. jocu	KF264964	Azevedo et al. (2014)

fitting DNA substitution model for the data set. To evaluate the evolutionary relationships among species, neighbour-joining (NJ) (Saitou & Nei 1987), maximum parsimony (MP) and maximum likelihood (ML) methods were applied. The first 2 approaches were conducted using the software program PAUP* v. 4.0b10 (Swofford 1998), whereas ML was conducted with PhyML 3.0 (Guindon & Gascuel 2003). MP analyses were performed with the heuristic search approach using the TBR swapping algorithm (10 random repetitions). Bootstrap tests (Efron 1982, Felsenstein 1985) were conducted with 10000 pseudo-replicates for NJ and ML trees and with 1000 pseudo-replicates for the MP tree. Nucleotide sequence identity and DNA distance values among species were calculated using BioEdit (Hall 1999) and PAUP* v. 4.0b10, respectively. The novel nuclear SSU rDNA sequence obtained in this study was deposited in GenBank under accession number KU726089.

RESULTS

Taxonomic summary

Name: *Henneguya sinova* sp. nov.
Type host: Tentacled blenny *Parablennius tentacularis* (Brünnich, 1768) (Perciformes: Blenniidae)
Type locality: Coast of Sinop, Black Sea, Turkey (42° 02′ 51″ N, 35° 02′ 56″ E)
Site of infection: Gill arches
Prevalence of infection: 35.5% (n = 31 fish)
Type material: One holotype (NHMUK 2015.1) and 1 paratype (NHMUK 2015.2) were deposited in the Natural History Museum, London.
Etymology: The specific epithet 'sinova' recalls the historical name 'Sinop', the name of the city where this parasite was found.

Description

Vegetative stages

Whitish oval plasmodia formed by myxosporeans were found in the gill arches of the tentacled blenny (Figs. 1 & 2). Plasmodia ranged between 233 and 550 μm in size, with typically 1 to 5 plasmodia per arch. Light microscopic observations of smears made from plasmodia revealed many mature and immature spores belonging to the genus *Henneguya* (Figs. 3 & 4).

Spores

Mature fresh spores are rounded, wide and oval in frontal view, with rounded anterior and posterior poles (Figs. 5, 6A & 7A), and ellipsoidal in sutural view (Figs. 6B & 7B). The anterior spore pole is without protrusion. Spore caudal appendages are separated along the entire length, are very thick and straight, gradually narrowing toward the back end. Polar capsules are pyriform and equal-sized. Tips of polar capsules are closely located. The length of the polar capsules comprises ca. 1/3 of the spore's body length. The length of the caudal appendages is over 4 times greater than the spore body length. Total length of mature spores (mean, range) is 57.5 (51.5–68.0) μm; the spore body is 11.7 (11.3–12.0) μm in length by 7.6 (7.3–8.3) μm in width and 6.7 (6.6–6.8) μm in thickness. Polar capsules are 4.0 (3.9–4.1) μm long by 2.2 (2.0–2.3) μm wide. Polar filaments are coiled, with 4−5 turns, situated perpendicularly to the longitudinal axis of the polar capsule. Extended length of polar filaments is 22−24 μm. The caudal processes are 46.0 (40.0−55.0) μm in length and taper through the end.

Remarks on differential diagnosis

Based on its form and size, the *H. sinova* sp. nov. spore is close to the freshwater species *H. cutanea* Dogiel et Petruschewsky, 1933 (Schulman 1984, Iskov 1989) from the freshwater hosts *Abramis brama*, *Leuciscus idus*, *L. leuciscus*, *Pelecus cultratus*, *Barbus ciscauscaicus* and *Cyprinus carpio*, but differs from *H. cutanea* by having a rounded, not narrowing, spore posterior pole. The pinched front ends of *H. cutanea* polar capsules overlap at the anterior pole of the spore, forming a small cross. Conversely, vertices of *H. sinova* sp. nov. polar capsules lie next to each other. The length of the spores, the spore body length and caudal appendages of *H. sinova* sp. nov. are slightly different from the width and polar capsule length of *H. cutanea* (Table 2). Moreover, both species have quite different localizations within the host's body.

H. tunisiensis Bahri, Marton, Marques, Eszterbauer, 2010 (Bahri et al. 2010), found on the gill arches of the marine fish species *Symphodus tinca* off Tunisia, is also similar to the species described herein. While the spore body of *H. sinova* sp. nov. is more oval, it is more rounded than that of *H. tunisiensis*. Moreover, *H. sinova* sp. nov. also differs from *H. tunisiensis* by having straight caudal appendages,
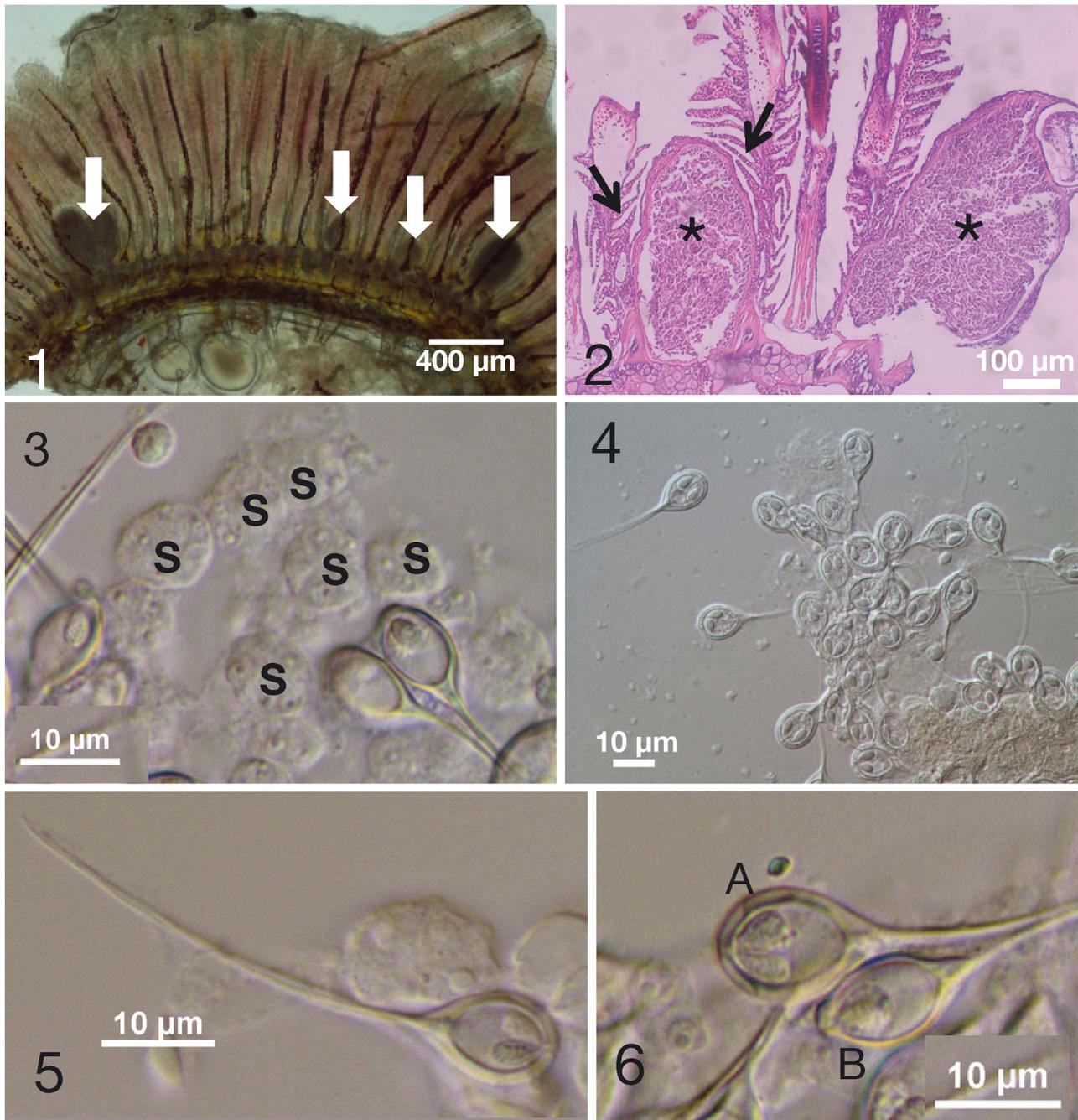
Figs. 1 to 6. *Henneguya sinova* sp. nov. parasitizing the gill arches of *Parablennius tentacularis*. Fig. 1. Plasmodia (arrows) of different sizes between gill filaments of the host fish. Fig. 2. Histological sections of several plasmodia (*) located between gill filaments of the fish and mature plasmodium (arrows) compressing the neighbouring gill filaments (H&E). Fig. 3. Immature spores (S) in a fresh preparation. Fig. 4. Developing spores released from plasmodium. Fig. 5. Complete view of mature spore. Fig. 6. Mature spore in (A) frontal and (B) sutural view

whereas the latter has curved filamentous ends. The length, width and thickness of the spore body of *H. sinova* sp. nov. are smaller than those of *H. tunisiensis* spores. On the other hand, the caudal appendages are longer than those of *H. tunisiensis*.

In comparison with *H. pagri* Yokoyama, Itoh, Tanaka, 2005 (Yokoyama et al. 2005), *H. sinova* sp. nov. has a somewhat greater length and thickness of the spore body, greater length and width of the polar capsules and longer caudal appendages. On the other hand,
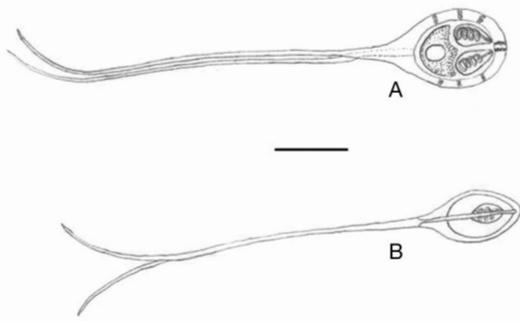
Fig. 7. Illustrations of *Henneguya sinova* sp. nov. mature spore. (A) frontal and (B) sutural view. Scale bar = 10 µm

when compared with *H. mauritaniensis* Khlifa, Miller, Adlard, Faye, Sasal, 2012 (Khlifa et al. 2012), *H. sinova* sp. nov. has a somewhat shorter length and width of the spore body, smaller polar capsule width and longer caudal appendages. *H. jocu* Azevedo, Rocha, Matos P., Matos E., Oliveira, Al-Quraishy and Casal, 2014 (Azevedo et al. 2014) is also close to *H. sinova* sp. nov. by being found only on the gill lamellae of its marine host fish *Lutjanus jocu* from Brazil. On the other hand, *H. sinova* sp. nov. differs from *H. jocu* by having oval cysts, no ellipsoidal spores and polar capsules, greater spore body length and thickness, wider polar capsules, smaller width of spore bodies and shorter polar capsules. *H. sinova* sp. nov. also differs from *H. lateolabracis* Yokoyama, Kawakami, Yasuda, Tanaka, 2003 (Yokoyama et al. 2003) by having slightly larger spores and polar capsules and differs from *H. akule* Work, Takata, Whipps, Kent, 2008 (Work et al. 2008) by having greater total spore length, width and thickness of the spore body, length and width of the polar capsules and shorter length of the spore body. Similarly, in comparison with *H. cynoscioni* Dyková, de Buron, Roumillat, Fiala, 2011 (Dyková et al. 2011), *H. sinova* sp. nov. has greater total spore length, spore body length and thickness and caudal appendage length, and smaller spore body width and polar capsule size. *H. lobosa* Cohn, 1895 and *H. creplini* Gurley, 1894 are the only representatives of the genus *Henneguya* previously reported from the Black Sea prior to our investigation, and only in the Dnieper liman (Ukraine) in freshwater fish species (Karataev & Iskov 1981, 1983).

Histology

Our histological study revealed the presence of plasmodia located between gill filaments in the carti-laginous gill arches. Young plasmodia were rounded, being constrained by a wall formed by stratified columnar epithelium cells of the gill arch. When plasmodia grew, they compressed the neighbouring tissue, inducing the atrophy of distal portions of the infected area (Fig. 2). No or only a weak inflammatory reaction could be detected in the infected gills, with the only lesions being distortions of the lamellar structure and gill stasis due to compression of large developed cysts.

Molecular analyses

We sequenced approximately 1750 bp of the nuclear SSU rDNA from our *Henneguya* sample. Phylogenetic analysis of our new haplotype together with the ones belonging to different marine *Henneguya* species obtained from GenBank (species which gave the highest BLAST scores) were carried out with 1737 aligned nucleotides containing 518 variable sites. AIC and BIC tests suggested TIM3+G (G: 0.173) and TPM3uf+G (G: 0.172) substitution models, respectively. Because the TIM3+G model gave higher bootstrap values, we used the NJ and ML trees produced with this model. In MP analyses, we determined a single most parsimonious tree with 1074 steps (consistency index = 0.696; retention index = 0.431; homoplasy index = 0.304). All 3 phylogenetic trees created with NJ (Fig. 8), ML and MP algorithms showed the same topology, with minor differences. In all trees, our haplotype formed a lineage with *H. pagri* with 90.5% nucleotide sequence similarity and 0.12 DNA distance value. This lineage was supported with 91, 72 and 96% bootstrap values in NJ, MP and ML trees, respectively. The lineage comprised of *H. tunisiensis* and *H. mauritaniensis* appeared as sister to the lineage described above, with 78, 54 and 62% bootstrap values in NJ, MP and ML trees, respectively. DNA distance and nucleotide sequence similarity values among *Henneguya* species used for phylogenetic analysis are given in Table 3.

DISCUSSION

Members of the genus *Henneguya* have a worldwide distribution and infect both marine and mainly freshwater fish species (Lom & Dyková 2006). Some species were reported to be important disease agents in both wild and farmed fish, and there has been a considerable increase in the number of newly identi-

Table 2. Site of infection in hosts, geographical localities and dimensions (µm, means and/or ranges) of spores between morphologically close species of the genus *Henneguya* found in marine and freshwater fishes

Species	Total spore length	Spore body Length	Width	Thickness	Caudal appendage length	Polar capsule Length	Width	Site in host	Host species	Locality
Henneguya sinova sp. nov.	57.5 (51.5–68.0)	11.7 (11.3–12.0)	7.6 (7.3–8.3)	6.7 (6.6–6.8)	46 (40.0–55.0)	4 (3.9–4.1)	2.2 (2.0–2.3)	Gills	*Parablennius tentacularis*	Black Sea (coast of Sinop, Turkey; this study)
H. cutanea Dogiel, Petrushevsky, 1933	37.0–50.0	10.5–15.0	8.0–10.0	No data	26.0–39.0	4.5–6.0	No data	Skin, fins, gill covers, muscles, kidney	*Abramis brama, Leuciscus leuciscus, L. idus, Pelecus cultratus, Barbus ciscaucasicus, Cyprinus carpio*	Neva Bay, basins of Pechora, Pregel, Dnieper, Volga, Ural, Terek, Syrdarya, Amur Rivers (Russia, Ukraine, Uzbekistan, Tajikistan, Kazakhstan, Georgia)
H. tunisiensis Bahri, Marton, Marques, Eszterbauer, 2010	41.8 (38.0–50.0)	13.1 (13.0–14.0)	9.1 (9.0–10.0)	8 (7.5–8.5)	28.4 (25.0–32.0)	4 (3.5–4.0)	2 (1.8–2.0)	Gills	*Symphodus tinca*	Mediterranean Sea (Kerkennah Islands, Tunicia)
H. pagri Yokoyama, Itoh, Tanaka, 2005	No data	10.5 (9.9–11.9)	7.5 (6.4–8.4)	5.9 (5.4–6.4)	29.6 (24.8–34.7)	3.1 (2.5–4.0)	1.6 (1.5–2.0)	Bulbus arteriosus of heart	*Pagrus major*	Pacific Ocean (Mie, Japan)
H. mauritaniensis Khlifa, Miller, Adlard, Faye, Sasal, 2012	No data	12.3	8	No data	25.3	4.1	3	Bulbus arteriosus of heart	*Pagrus caeruleostictus*	Atlantic Ocean (Nouakchott, Mauritania)
H. jocu Azevedo, Rocha, Matos P., Matos E., Oliveira, Al-Quraishy, Casal, 2014	45.2 (44.0–55.3)	10.9 (10.3–11.4)	8.2 (7.8–8.6)	2.9 (2.6–3.4)	34.1 (44.0–55.3)	5 (4.6–5.3)	1.4 (1.1–1.6)	Gill lamellae	*Lutjanus jocu*	Atlantic Ocean (State of Pará, Brazil)
H. lateolabracis Yokoyama, Kawakami, Yasuda, Tanaka, 2003	No data	10.7 (9.9–11.9)	7.5 (6.4–7.8)	6.2 (5.9–6.4)	37.7 (30.7–49.5)	3.4 (3.0–4.0)	1.7 (1.5–2.0)	Bulbus arteriosus of heart	*Lateolabrax* sp.	Pacific Ocean (Mie, Japan)
H. akule Work, Takata, Whipps, Kent, 2008	40.8 (29.0–52.0)	12.1 (10.0–14.0)	7.4 (5.0–9.0)	5.3 (3.0–7.0)	No data	3.4 (2.0–6.0)	1.4 (1.0–2.0)	Bulbus arteriosus of heart	*Selar crumenophthalmus*	Pacific Ocean (Hawaii, USA)
H. cynoscioni Dykova, de Buron, Roumillat, Fiala, 2011	38.6 (34.3–44.1)	10.4 (9.8–11.7)	8.8	5.9	28.1 (23.5–33.3)	3.3	2	Bulbus arteriosus of heart	*Cynoscion nebulosus*	Atlantic Ocean (South Carolina, USA)

fied *Henneguya* species in recent years (Eiras & Adriano 2012). Studies on myxosporean parasites of blennid fish are very limited (Yurakhno 2009), and only 3 species of myxosporean parasites have thus far been reported from *Parablennius tentacularis* in the Black Sea, though none of them is a member of the genus *Henneguya* (Yurakhno 2009). Thus, given that no *Henneguya* species has been reported from any blennid fish in the Black Sea or elsewhere, we consider *H. sinova* sp. nov. observed in *P. tentacularis* to be a new species.

Members of the genus *Henneguya* infect external and internal organs of host fish, including gills, skin, fins, barbels, scales, eye and mouth cavities, bulbus arteriosus, ureter, urinary bladder, pyloric caeca, gall bladder, spleen and swim bladder (Eiras 2002). Of the 189 *Henneguya* species identified so far (Eiras 2002, Eiras & Adriano 2012), gills were the most infected organ. Most *Henneguya* species infecting the gills appear to cause little harm to their hosts, although many are well known pathogens, e.g. *H. psorospermica* Thélohan, 1895 in young perch (Dyková & Lom 1978) and *H. exilis* Kudo, 1929 in channel catfish (Current & Janovy 1978). Our histopathological study of the gills of *P. tentacularis* parasitized by *H. sinova* sp. nov. showed little damage, although compression of the capillaries and retraction of the neighbouring tissue was reported by Bahri et al. (2010) for *H. tunisiensis* in *Symphodus tinca* (L.).

Myxosporean taxonomy is primarily based on morphology and spore structure, and we therefore classified our myxosporean parasite in the genus *Henneguya* using specific morphological criteria. However, Shin et al. (2014) indicated that the traditional classification may not be consistent with many other biological features, such as the life cycle, morphology of actinospores, host specificity and infection-site tropism. Fiala (2006) also indicated that the traditional classification is artificial and does not reflect phylogenetic relationships. We therefore used SSU rRNA as a molecular marker for the construction of the phylogenetic Myxosporea tree as a result of advances in molecular methods. In this context, we performed a molecular phylogenetic analysis using the nuclear SSU rDNA. In the phylogenetic trees created using NJ, MP and ML algorithms, our sample appeared as sister to *H. pagri* with sufficient bootstrap values (Fig. 8) and also showed 90.5 % nucleotide sequence similarity and a DNA distance value of 0.12 (Table 3). These values were highly significant, because nucleotide sequence similarities between sister species of the marine *Henneguya* lineage (Fig. 8) were generally higher than 91 % and DNA distance values were

lower than 0.1, except for *H. lateolabracis* and *H. akule* (Table 3). Values were 91.8 % and 0.0973 between *H. tunisiensis* and *H. mauritaniensis* and 95.5 % and 0.0504 between *H. ogawai* and *H. yokoyamai*. Therefore, our results clearly suggest that our *Henneguya* sample is a new species within the marine *Henneguya* lineage.

Phylogenetic analyses depending on nucleotide sequences of the SSU rDNA gene have been subject to several studies investigating the relationships between biological features such as site selection (Eszterbauer 2004, Holzer et al. 2004, Fiala 2006, Work et al. 2008, Liu et al. 2010). According to our results, *H. sinova* sp. nov. was genetically most similar to *H. pagri*, which parasitizes the bulbus arteriosus of *Pagrus major* off Japan. *H. sinova* sp. nov. has the second closest similarities to *H. tunisiensis* from the gills of *Symphodus tinca* off Tunisia (Bahri et al. 2010) and *H. mauritaniensis* from the bulbus arteriosus of *P. caeruleostictus* off Mauritania (Khlifa et al. 2012). This close phylogenetic relationship of gill-infecting *H. sinova* sp. nov. with heart-infecting *Henneguya* species reveals that site specificity may not be a reliable characteristic for myxosporean identifi-
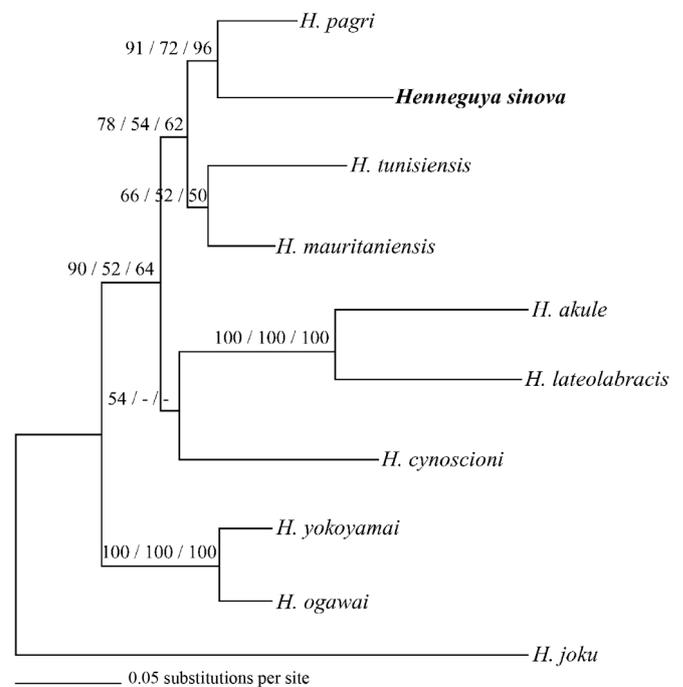

Fig. 8. Neighbour-joining (NJ) tree showing the phylogenetic relations of *Henneguya sinova* sp. nov. (in **bold**) and related marine *Henneguya* species based on nuclear small subunit ribosomal DNA (SSU rDNA) nucleotide sequences. GenBank acc. nos. given in Table 1. The tree was created using the TIM3+G (G: 0.173) substitution model, and bootstrap values (> 50 %) are given for NJ / maximum parsimony / maximum likelihood trees

Table 3. Pairwise nucleotide sequence identity (upper right) values and DNA distance values calculated using the TIM3+G substitution model (lower left) among *Henneguya sinova* sp. nov. and marine *Henneguya* species

	H. pagri	*H. sinova*	*H. tuni-siensis*	*H. mauri-taniensis*	*H. yoko-yamai*	*H. oga-wai*	*H. cyno-scioni*	*H. akule*	*H. lateo-labracis*	*H. jocu*
H. pagri	–	90.5	90.3	92.2	88	88.3	89	85.6	86.4	82.1
H. sinova	0.1200	–	87.2	88.3	85.6	85.7	86.2	83.7	83.9	80.1
H. tunisiensis	0.1203	0.1605	–	91.8	85.2	85.6	87.1	84.9	85	81.2
H. mauritaniensis	0.0979	0.1527	0.0973	–	88	87.8	89.5	86	87	82.8
H. yokoyamai	0.1692	0.226	0.2112	0.1560	–	95.5	87	82.3	83.6	80.8
H. ogawai	0.1647	0.2170	0.2073	0.1650	0.0504	–	86.7	82.6	83.7	81.1
H. cynoscioni	0.1729	0.2181	0.2021	0.1626	0.1933	0.2112	–	84.5	86	81.5
H. akule	0.2386	0.2601	0.2425	0.2190	0.2909	0.2863	0.2731	–	88.1	79.5
H. lateolabracis	0.2295	0.2807	0.2647	0.2186	0.2675	0.2752	0.2412	0.1787	–	79.9
H. jocu	0.3812	0.4231	0.3933	0.3544	0.3691	0.3591	0.4084	0.4962	0.5011	–

cation. On the other hand, the spore morphologies of the gill-infecting *H. tunisiensis* and heart-infecting *H. pagri* show similarities with our gill-infecting *H. sinova* sp. nov., especially in the long caudal appendages which taper towards the distal end, although our species was less flexible at the distal end.

With regard to gill infections by *Henneguya* species, plasmodia develop in the epithelium, the capillary lumen of blood vessels and connective tissue elements of gill arches (Molnár 2002, Bahri et al. 2010). Plasmodia of *H. sinova* sp. nov. developed in the connective tissue elements of the gill arches and caused deformation at the neighbouring gill lamellae and pushed them aside, which was also reported by Bahri et al. (2010) for *H. tunisiensis* in *S. tinca* and by Molnár (2002) for *Henneguya* sp. in *Zingel zingel*. Barassa et al. (2003) reported a resulting reduction in the surface available for gas exchange due to compression of the capillaries and retraction of the neighbouring lamellae of *Astyanax altiparanae* caused by *Henneguya* sp. infection. In the present study, the prevalence of infection was 35.5%, and the presence of numerous plasmodia of different sizes causing different histopathological changes suggests that in high numbers, plasmodia of this parasite may compromise the health status of the gills of *P. tentacularis.*

To date, many morphological studies of myxosporean parasites have been performed in Black Sea fishes, and our study included a phylogenetical characterization and analysis along with morphology and histology which revealed a new *Henneguya* species.

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