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DESCRIPTION OF *HENNEGUYA JACZOI* SP. N. (MYXOSPOREA, MYXOBOLIDAE) FROM *PERCA FLUVIATILIS* (L.) (PISCES, PERCIDAE) WITH SOME REMARKS ON THE SYSTEMATICS OF *HENNEGUYA* SPP. OF EUROPEAN FISHES

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A new *Henneguya* species, *H. jaczoi* sp. n., is described from perch (*Perca fluviatilis*) from Lake Balaton, Hungary. This species infects the palatal region of the fish, forming large plasmodia in the thickened caudal part of the buccal cavity and at the dorsal ends of the cartilaginous gill arches. The species differs from the gill-dwelling *Henneguya* species of perch and pike (*Esox lucius*) both morphologically and in molecular aspects. The authors conclude that the type species *H. psorospermica* Thélohan is a specific parasite of pike, while the species forming plasmodia in the gills of perch corresponds to *H. texta* Cohn, which was hitherto regarded as a synonym of *H. psorospermica*. Besides the above-mentioned species, *H. creplini* was frequently found in pikeperch (*Sander lucioperca*) and Volga pikeperch (*Sander volgensis*), but no *Henneguya* infection has been recorded in ruffe (*Gymnocephalus cernua*), which is a common percid fish of the lake and is known to be the type host species for *H. creplini*.

Key words: Myxozoa, perch, pikeperch, pike, occurrence, molecular phylogeny, histology

Henneguya spp. are common myxozoan parasites of pike (*Esox lucius* L.) and percid fishes (Table 1). The type species, *H. psorospermica* was described by Thélohan (1895) from the gills of both pike and perch (*Perca fluviatilis* L.). Of them the pike, mentioned first by Thélohan, should be regarded as type host. Other pike-infecting *Henneguya* species were recorded from the gills (*H. lobosa* Cohn, 1895), the ovary (*H. oviperda* Cohn, 1895), the epidermis (*H. schizura* Gurley, 1893) and the intestine (*H. periintestinalis* Cépéde, 1906) (Table 1). From perch, *H. texta* Cohn, 1895 and *H. minuta* Cohn, 1895 were described from the gills and *H. wolinensis* Romuk-Wodoracki, 1990 from the epidermis beneath the scales (Cohn, 1895; Romuk-Wodoracki, 1990). The latter author also mentioned the occurrence of *H. lobosa* and *H. creplini* Gurley, 1894 in perch, although the original descriptions of these parasites were from different fish hosts

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(Kudo, 1919). *Henneguya creplini* was first observed in the gills of ruffe (*Gymnocephalus cernua* L.) by Gurley (1894). From this fish two more *Henneguya* species were described: *H. acerinae* Schröder, 1906 from the gills and *H. tenuis* Vaney & Conte, 1901 from the intestine of ruffe (Kudo, 1919; Donec and Shulman, 1984). *Henneguya gigantea* Nemeczek, 1911 and *H. nemeczeki* Tripathi, 1952 were described and the occurrence of *H. creplini* was reported from the gills of pikeperch (*Sander lucioperca* L.) and Volga pikeperch (*Sander volgensis* Gmelin) (Donec and Shulman, 1984; Lom and Dyková, 1992, 2006) (Table 1). In addition to these European species, Fantham et al. (1939) described *H. percae* in Canada from yellow perch [*Perca flavescens* (Mitchill)], and another species, *H. dogieli* Akhmerov, 1960, was recorded from a Far Eastern percid fish, *Siniperca chuatsi* (Basilewsky) (Donec and Shulman, 1984).

Table 1

Henneguya spp. described from pike and percid fishes

Pike *(Esox lucius)*	Perch *(Perca fluviatilis)*	Pikeperch *(Sander lucioperca)*	Volga-pikeperch *(Sander volgensis)*	Ruffe *(Gymnocephalus cernua)*
H. psorospermica (Thélohan, 1895) G	*H. psorospermica* (Thélohan, 1895) G	***H. nemeczeki*** (Tripathi, 1952) G	*H. creplini*	***H. creplini*** (Gurley, 1894) G
H. oviperda (Cohn, 1895) O	***H. texta*** (Cohn, 1895) G	***H. gigantea*** (Nemeczek, 1911) G		***H. acerinae*** (Schröder, 1906) G
H. lobosa (Cohn, 1895) G	***H. minuta*** (Cohn, 1895) G	*H. psorospermica*		***H. tenuis*** (Vaney et Conte, 1901) I
H. schizura (Gurley, 1893) E	***H. wolinensis*** (Romuk-Wodoracki, 1990)	*H. oviperda*		
H. periintestinalis (Cépéde, 1906) I	*H. creplini*	*H. creplini*		
H. gigantea	*H. lobosa*	*H. acerinae*		
H. nemeczeki	***H. dogieli*** (Akhmerov, 1960) G *(Siniperca)*			

G = gills, I = intestine, O = ovary, E = eyes; Names with bold letters = good hosts; names with non-bold letters = inadequate identifications

In Hungary, the myxozoans of Lake Balaton fishes were first studied by Jaczó (1940) who found a *Myxobolus* and a *Chloromyxum* spp. in the gills and the gallbladder, respectively, and a *Henneguya* species in the tongue of perch but did not describe it. On the basis of illustrations, however, this last mentioned

species appears to be the same as the one we recorded and present here. Based on morphological data, habitat preference, tissue specificity and molecular data, in this paper we describe a new species, *Henneguya jaczoi* sp. n. from perch, and re-describe *H. texta* and *H. psorospermica* spp. from perch and pike, respectively.

Materials and methods

Most of the fish were collected at a fish trap built into the water gate of Lake Balaton, while some others were captured with a seine at the city of Siófok (46°54'29.4"N 18°02'46.4"E) from 12 March to 1 April 2015 and from 24 February to 12 April 2016). A total of 8 pikes [33–41 cm standard length (SL)] and 104 percid fishes (9 to 20 cm SL) were collected and examined for myxozoans. The percid fishes included 48 perch, 17 pikeperch, 7 Volga perch and 32 ruffe specimens.

The fish were brought to the laboratory alive in oxygenated plastic bags, kept in aerated aquaria and subjected to complete parasitological dissection within three days. They were sedated with a drop of clove oil into their water and euthanised by a cervical cut. When mature plasmodia were found, some of the spores were studied as fresh preparations. Subsamples of spores from mature plasmodia were studied as fresh preparations, collected and stored in 70% ethanol in Eppendorf tubes until further molecular analysis or preserved as glycerine-gelatin slide preparations. Tissue samples from organs infected by mature plasmodia were fixed in Bouin's solution, processed for histology through a series of ethanol, embedded in paraffin (Molar Chemicals Ltd., Hungary), cut to 4–5 μm thick sections, and stained with haematoxylin and eosin. The maturity of plasmodia and the vitality of the spores were assessed by adding a few hundred spores into a 0.4% solution of urea in water (Lom and Dyková, 1992). Plasmodia were considered mature when at least 90% of the spores extruded polar filaments in this solution. Unfixed spores were studied using Nomarski differential interference contrast with an Olympus BH2 microscope. The spores were photographed with an Olympus DP 20 digital camera. All measurements are expressed in micrometres and given as the range followed by the mean ± standard deviation, with the number of measurements in parentheses.

Molecular data

Myxospores preserved in ethanol were centrifuged at 10,000 *g* for 10 min and the supernatant was removed. Genomic DNA was extracted from the pelleted spores using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Amplification and sequencing of the 18S rDNA were conducted as described by Cech et al. (2015).

The sequence fragments were assembled using MEGA 5.2 software (Tamura et al., 2011). The contiguous 18S rDNA sequences and the most similar myxozoan sequences from the GenBank based on BLAST matches were aligned with the software CLUSTAL W (Thompson et al., 1994). DNA pairwise distances were calculated with the MEGA 5.2 software using the Maximum Composite Likelihood model. Phylogenetic analysis was performed via Maximum Likelihood (ML) *Ceratonova shasta* (Noble, 1950) was used as the outgroup. The dataset was tested using MEGA 5.2 for the nucleotide substitution model of best fit and the model shown by the Akaike Information Criterion (AIC) as the best-fitting one was chosen (TN93+G model). Bootstrap values based on 1,000 resampled datasets were generated.

Results

Large, globular myxosporean cysts, about 1 to 2 mm in diameter, were found in the palatal region of the mouth and in the gill arches of perch. The cysts contained mature spores of a *Henneguya* species and occurred exclusively in this specific habitat. No similar infection was found in other percid fishes, e.g. in pikeperch, Volga pikeperch and ruffe (Molnár, 1998; present study). Based on the shape and size of spores, the specific habitat of cysts and molecular data of the 18S rDNA of spores, this parasite is described here as *Henneguya jaczoi* sp. n. Twelve out of the examined 48 perch specimens were infected with *H. jaczoi* sp. n. plasmodia. Among fish of different size only specimens measuring 13 to 16 cm in length showed infection. The plasmodia of *H. jaczoi* sp. n. were located in a specific sclerotic area of the palate located dorso-caudally in the buccal cavity. This area, divided into a right and a left side, is a thickened part of the buccal cavity covered by stratified epithelium containing mucous cells and small ossified spines (Fig. 1). The epithelium is supported by a dense connective tissue under which a zone of loose connective tissue and a muscle layer are located. This specific part of the palate incorporates the dorsal ends of the cartilaginous gill arches (Fig. 2). Two types of plasmodia were found. Some large globule-shaped plasmodia 1 to 2 mm in diameter were located between the dense and the loose connective tissue. Other, less regular shaped plasmodia were located at the end of the cartilaginous gill arches, where they joined the muscle. Some of the large plasmodia surrounded by a very thin connective tissue were recovered in the muscular layer. Less frequently round plasmodia were found also in the cartilaginous gill arch, which is also regarded as part of the caudal portion of the oral cavity in its function.

Besides infection of the palatal region, small plasmodia containing *Henneguya* spores but differing in shape and size from *H. jaczoi* sp. n. were found in the gill lamellae of four perch specimens collected with a seine. By their size and shape these spores corresponded to *H. texta*. In addition to perch, *Henneguya* in-

fection was also found in 2 out of the 8 pike specimens examined. In this fish, large plasmodia were located in the gill filaments. Spores found in these plasmodia were identified as the species *H. psorospermica*.

The gill lamellae of 17 pikeperch and 2 Volga pikeperch specimens harboured immature cysts of *H. creplini*, while none of the ruffe specimens showed *Henneguya* infection.

Description of the new species:

***Henneguya jaczoi* sp. n. (Figs 3 and 4)**

Type-host: Perch, *Perca fluviatilis* (L.)

Type locality: Lake Balaton, Siófok (46°54'29,8''N, 18°2',46,2''E)

Habitat of tissue development: Palatal region of the buccal cavity, gill arches

Prevalence: 25% of examined perches, 12/48, and 100% of specimens measuring 13–16 cm in standard length, 12/12

Type-material: Spores in glycerine-gelatin, photo-types and histological sections were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-19792.

Etymology: The name of the species comes from the name of the late Hungarian fish parasitologist, Imre Jaczó, who first reported this infection.

Vegetative stages: Large round or roundish plasmodia 1.0–2.0 mm in diameter developed in the dorso-caudal part of the palate and in the gill arches. Irregular-shaped plasmodia were also found around the cartilaginous ends of the gill arches.

Spores: Spores elongated, spindle-shaped both in frontal and sutural view (Figs 3a, 4 and 3b). Length of the spore body 13.2–15.2 (14.1 ± 1.1) (n = 50), width 5.2–7 (6.2 ± 0.64) (n = 50), thickness 4.8–5.2 (5.1 ± 0.19) (n = 11). Polar capsules elongated, equal or somewhat different in size, tapering posteriorly, 4.8–7.2 (6.4 ± 0.83) long (n = 50) and 1.6–2.4 (2 ± 0.23) wide (n = 50). Twelve to 13 filament coils arranged perpendicular to the capsule length, coiled densely in the polar capsule. The straight, continuously tapering caudal extensions 20.8–27.2 (24 ± 2.4) long. They are in most cases equal in length, less frequently somewhat different. Sutural edge markings not seen. No intercapsular appendix found. In the sporoplasm no iodinophilous vacuole found, but a distinct, single, bright nucleus present (Fig. 4).

Histology: Large, round plasmodia developed in the connective tissue of the thickened dorso-caudal region of the buccal cavity at the border of the dense and the loose connective tissue. They extended deep in the muscular layer (Fig. 1). Some other smaller irregular-shaped plasmodia were also found in the dense connective tissue joining the cartilaginous ends of the gill arches (Fig. 2) and in the gill arch.

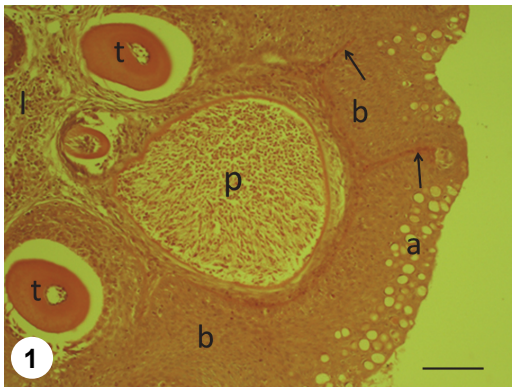

Fig. 1. Palatal region of a perch infected with *H. jaczoi* sp. n.: a) layer of epithelial cells and goblet cells, b) dense connective tissue, p) *M. jaczoi* plasmodium, t) ossified spines, l) loose connective tissue. Arrows = blood vessels. Histological section, haematoxylin and eosin (HE). Bar = 100 µm

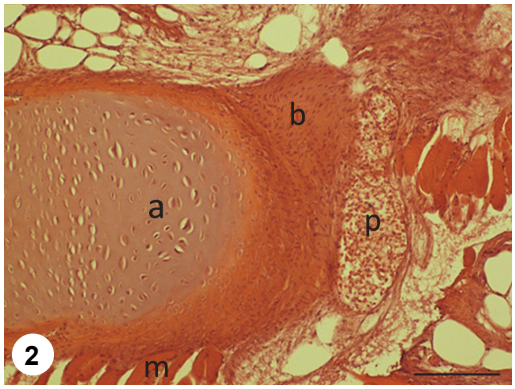

Fig. 2. Palatal region at the dorsal end of the cartilaginous gill arch: a) end of the cartilaginous gill arch, b) dense connective tissue covering the gill arch, m) muscle cells attaching to the gill arch, p) plasmodium of *H. jaczoi*. Histological section, HE. Bar = 100 µm

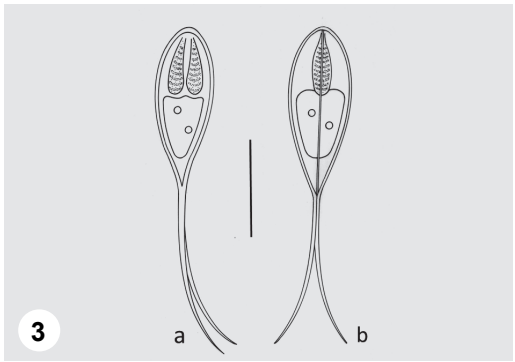

Fig. 3. Schematic drawing of *Henneguya jaczoi* sp. n.: a) spore in frontal view, b) spore in sutural view. Bar = 10 µm

Fig. 4. Spores of *Henneguya jaczoi* sp. n. from the palate of a perch. A single nucleus can be seen in the sporoplasm. Bar = 10 µm

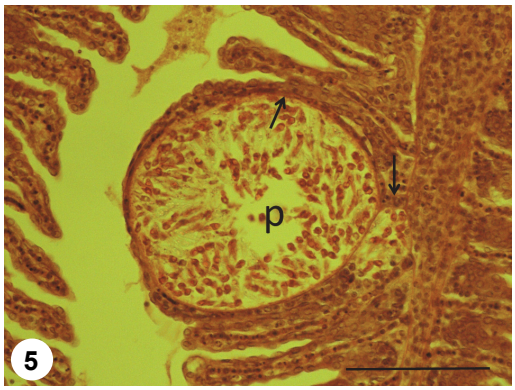

Fig. 5. Gill of a perch infected with an intralamellar plasmodium (p) of *Henneguya texta*. The capillary (arrows) runs at one side of the plasmodium. The other side is covered only by a single layer of membrana basalis. Histological section, HE. Bar = 100 µm

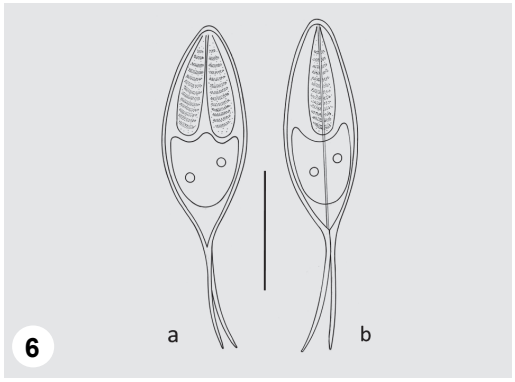

Fig. 6. Schematic drawing of *Henneguya texta*: a) spore in frontal view, b) spore in sutural view. Bar = 10 µm

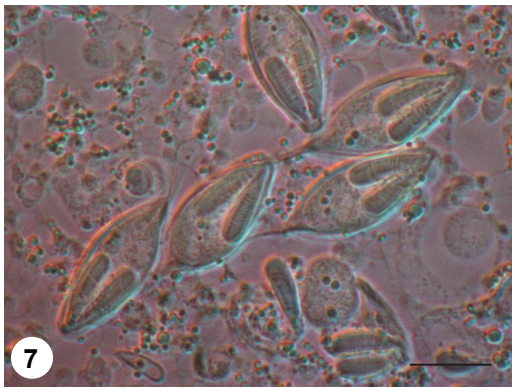

Fig. 7. Spores of *Henneguya texta* from the gills of a perch. Two or three nuclei can be seen in the sporoplasms. Bar = 10 µm

Fig. 8. Gill filaments of a pike infected by plasmodia of *H. psorospermica*. Fresh mount picture. Bar = 2 cm

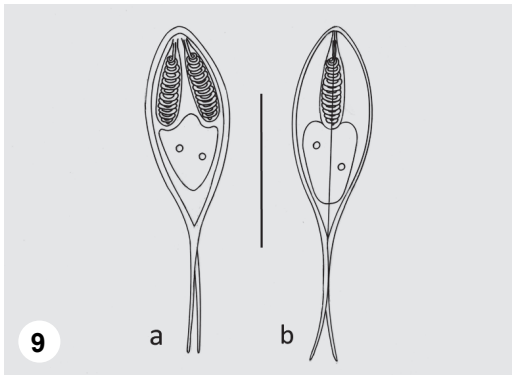

Fig. 9. Schematic drawing of *Henneguya psorospermica*: a) spore in frontal view, b) spore in sutural view. Bar = 10 µm

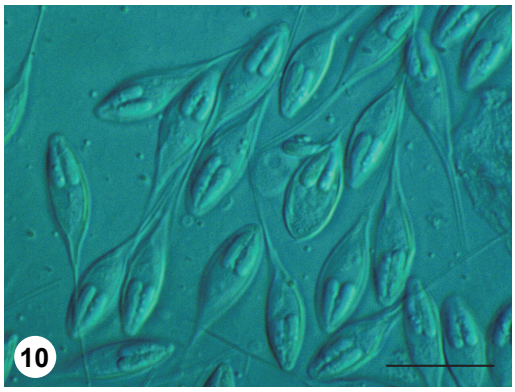

Fig. 10. Spores of *Henneguya psorospermica* from the gills of a pike. Bar = 10 µm

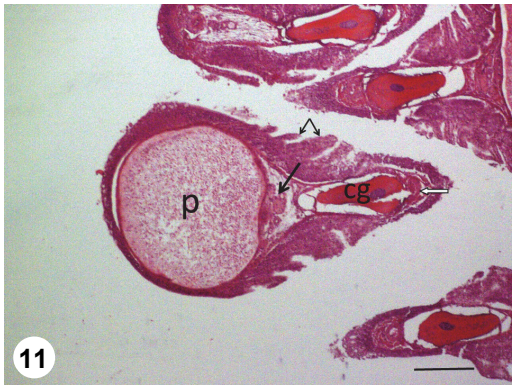

Fig. 11. Gill filament of a pike with a cross-sectioned *Henneguya psorospermica* plasmodium (p). The plasmodium is located in the outer part of the filament close to the efferent branchial artery (arrow). The cartilaginous gill ray (cg) and the afferent branchial artery (open arrow) can be found in the inner part of the filament. The edges of the filament are covered by multilayered epithelium, but in the middle some lamellae are seen (small arrows). Histological section, HE. Bar = 200 µm

Molecular data: The partial sequence of the 18S rRNA gene of four samples of *H. jaczoi* sp. n. were identified. HG4 and HG5 samples (KY172847) were identical, HG6 (KY172848) and HG9 (KY172849) differed in 2 nucleotides (0.1%), so they can be considered the same myxozoan species. Their sequences showed 94.5% similarity to *H. texta* and *Henneguya* sp. ex *Perca fluviatilis* (EU732599), parasites of the gill from the same host species. They were also similar (94.7%) to the *H. creplini*-like spores from pikeperch of the present study and to *H. creplini* sequences deposited in the GenBank (EU732597-8). The sequences of the new species showed, however, only very low similarity (82.8%) to *H. psorospermica* of the pike. The 18S rDNA sequences of *H. jaczoi* sp. n. were deposited in the GenBank under the accession number KY172847-9 (Fig. 12).

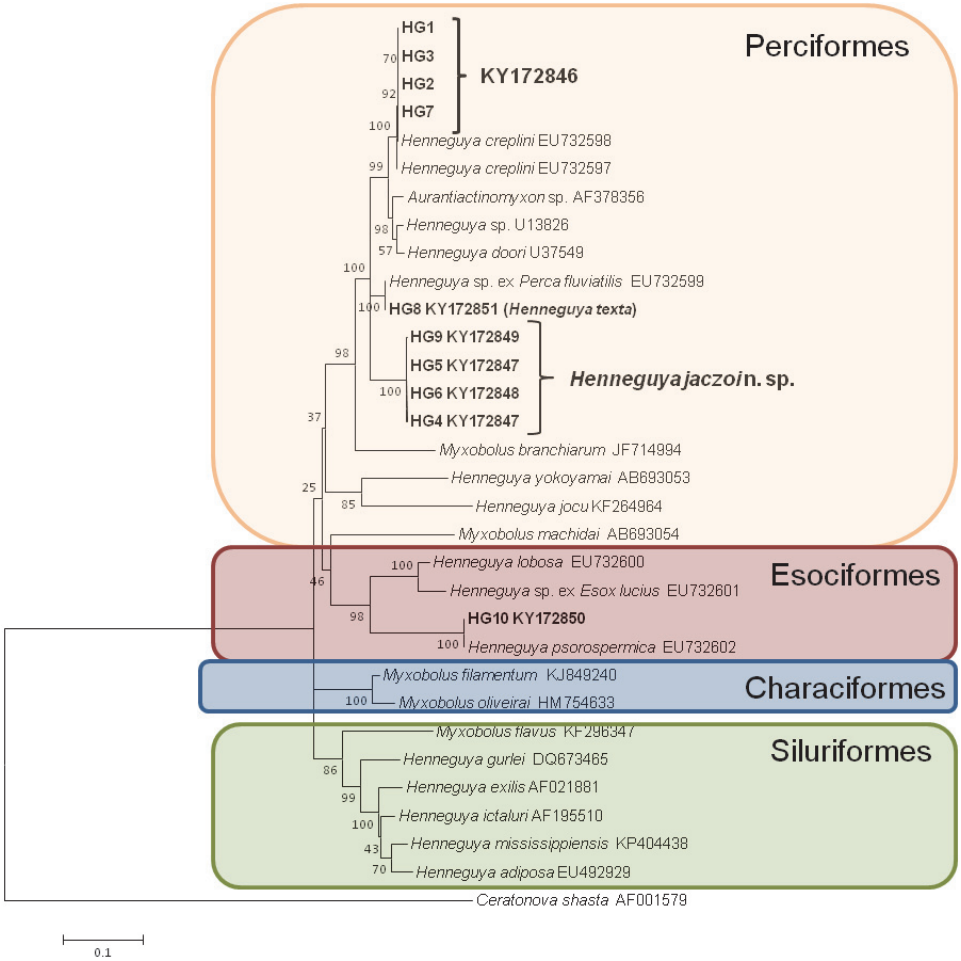

Fig. 12. Phylogenetic position of the new species *Henneguya jaczoi*, *H. texta* and *H. psorospermica* based on 18S rDNA analysis by the Maximum Likelihood algorithm. *Ceratonova shasta* was used as the outgroup. Bootstrap values are given at the nodes. The scale bar indicates the number of expected substitutions per site

Remarks: The species seems to have an affinity to the connective tissue, forming large plasmodia in this palatal region of the mouth. The morphology of the spores of *H. jaczoi* sp. n. was very similar to that of *H. texta,* the specific parasite of the gill lamellae of perch, but they had a smaller spore body size (Table 2) and longer caudal extensions, and their plasmodia had a different location in the organs and tissues of the host. The approximately 5% differences between the 18S rDNA sequences of *H. jaczoi* sp. n. and those of *H. texta* and *H. creplini*, respectively, indicate that *H. jaczoi* sp. n. should be regarded as a new species. The size and shape of *H. jaczoi* sp. n. spores also resembled the spores of *H. pso-*

rospermica, which infects the pike, but their caudal extensions were longer and they substantially differed genetically. Besides genetic differences of the hosts, there were differences in the location of plasmodia, tissue affinity and molecular sequences of the spores. In infecting the connective tissue, *H. jaczoi* sp. n. resembles *H. wolinensis*, which forms large plasmodia and develops in similar tissues under the scales. However, the spores of *H. jaczoi* sp. n. are significantly smaller than those of *H. wolinensis*. *Henneguya percae* has been described from the gills of a genetically closely related fish, *Perca flavescens,* but the polar capsules in the spores of this American species are short and differ from the elongated capsules of the European *Henneguya* species of percid fishes. In the morphology of spores, *H. jaczoi* sp. n. also resembles *H. creplini,* but this latter species has longer caudal extensions and its spores are formed during the winter (Molnár, 1998). Moreover, the 18S rDNA sequences of the two species show 5% difference.

Redescription of *Henneguya texta* Cohn, 1895 (Figs 5, 6 and 7)

Host: Perch, *Perca fluviatilis* (L.)

Locality: Lake Balaton, Siófok (46°54'29,8'' N, 18°2',46,2''E)

Habitat of tissue development: Gill lamellae

Prevalence: 8.3% of examined perches, 4/48

Material: Spores in glycerine-gelatin, photo-types and histological sections were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-19793. The 18S rDNA sequences of *H. texta* were deposited in the GenBank under the accession number KY172851.

Vegetative stages: Small round plasmodia located in the gill lamellae (Fig. 5)

Spores: Spores elongated, spindle-shaped both in frontal and sutural view (Figs 6a,b and 7). Length of the spore body 17.5–22 (19.7 ± 1.8) (n = 50), width 7.2–9.6 (8.3 ± 1) (n = 50), thickness 7.2–9.6 (8 ± 0.9) (n = 11). Polar capsules elongated, equal or somewhat different in size, tapering posteriorly, 8.1–12 (9.5 ± 1.4) long (n = 50) and 2.4–3.2 (2.9 ± 0.4) wide (n = 50). Twelve to 13 filament coils arranged perpendicular to the capsule length, coiled densely in the polar capsule. The straight, continuously tapering caudal extensions are shorter than the spore body, they measure 11–20 (15 ± 1.4) in length. They are in most cases equal in length, less frequently somewhat different. Sutural edge markings, intercapsular appendix and iodinophilous vacuole in the sporoplasm not found, but 2 or 3 nuclei in the sporoplasm present (Fig. 7).

Histology: Small, ellipsoidal plasmodia located dissymmetrically in the capillary network, so that the rest of the capillary with blood cells runs at one side of the plasmodium, while the other side is covered only by a thin basement membrane (Fig. 5).

Molecular data: The 18S rDNA sequence of *H. texta* was 100% identical with that of the specimen from *Perca fluviatilis* deposited in the GenBank

(EU732599). A relatively high similarity (94.9%) exists to *H. doori* from yellow perch (*Perca flavescens*) and to sequences of *H. creplini* of the pikeperch (95.6%) and an Aurantiactinomyxon (AF378356, 94.7%). Its similarity to *H. jaczoi* n. sp. was 94.5% (Fig. 12).

Remarks: Henneguya texta differs from *H. jaczoi* by its larger spores and its specific location in the gill lamellae, and their molecular similarity is 94.5%. It also differs from *M. psorospermica* of the pike by its larger spores and the 84% differences of their 18S rDNA sequences. Although Thélohan (1895) described *H. psorospermica* from pike and perch, and Donec and Shulman (1984) regarded *H. texta* as a synonym of the above species, our molecular data support that *H. texta* described by Cohn (1895) is a valid species from perch.

Redescription of *Henneguya psorospermica* Thélohan, 1895 (Figs 8, 9, 10 and 11)

Host: Pike, *Esox lucius* (L.)

Locality: Lake Balaton, Siófok (46°54’29,8’’N, 18°2’,46,2’’E)

Site of tissue development: gill filaments

Prevalence: 25% of examined pikes (2/8)

Type-material: Spores in glycerine-gelatin, photo-types and histological sections were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-19794. The 18S rDNA sequence of *H. psorospermica* from our study was deposited in the GenBank under the accession number KY172850.

Vegetative stages: Oval-shaped plasmodia measuring 1–2 mm in length and 0.4–0.6 mm in width are located in the gill filaments (Fig. 8).

Spores: Spores elongated, spindle-shaped both in frontal and sutural view (Figs 9a,b and 10). Length of the spore body 9.6–14.4 (12.4 ± 1.02) (n = 50), width 4.8–7.2 (6.3 ± 0.66) (n = 50), thickness 7.6–7.8 (7.7 ± 0.08) (n = 11). Polar capsules elongated, equal or somewhat different in size, tapering posteriorly, 5–7.2 (6.3 ± 0.74) long (n = 50) and 1.6–2 (1.9 ± 0.19) wide (n = 50). Twelve to 13 filament coils arranged perpendicular to the capsule length, coiled densely in the polar capsule. The straight, continuously tapering caudal extensions are 12.8–16 (13.8 ± 1) long. They are in most cases equal in length, less frequently somewhat different. Sutural edge markings, intercapsular appendix and iodinophilous vacuole in the sporoplasm not seen.

Histology: The plasmodia of *H. psorospermica* were formed in the outer edge of the gill filament close to the efferent branchial artery (arteria branchialis efferens) (Fig. 11). Plasmodia in all cases were surrounded by a relatively thin, dense connective tissue wall, but in the lamella-free parts of the filaments they were covered by stratified epithelium as well. Although the filaments were greatly deformed by the large plasmodia in their central region, gill lamellae could also be seen.

Table 2

Comparative measurements of *Henneguya* spp. of pike and percid fishes. Data from: (1) Donec & Shulman, 1984; (2) Romuk-Wodoracki, 1990; (3) Wegener, 1910, cit. by Kudo (1919); (4) Eiras, 2002; (5) present data (in micrometres)

Name of *Henneguya* sp.	Host	Location	Length of the spore body	Width of the spore body	Thickness of the spore body	Length of the tail	Length of the polar capsule	Width of the polar capsule
H. psorospermica (1)	*Esox lucius, Perca fluviatilis*	gills	10–15	6.2–9	4–6	14–30	6.2–11	3.5–5
H. psorospermica (5)	*Esox lucius*	gills	9.6–14.4 (12.4 ± 1.02)	4.8–7.2 (6.3 ± 0.66)	–	12.8–16 (13.8 ± 1)	5–7.2 (6.3 ± 0.74)	1.6–2 (1.9 ± 0.19)
H. lobosa (1)	*Esox lucius*	gills	10–27	4–8	4–5	20–30	6–10	1.5–2.5
H. creplini (1)	*Gymnocephalus cernua*	gills	13–22	6.2–9	5–7	13–66	6.3–10	2–3
H. gigantea (1)	*Sander lucioperca*	gills	10	4		77–100		
H. percae (4)	*Perca flavescens*	gills	11.5	7		3.5	2.5	
H. doori (4)	*Perca flavescens*	gills	20.1 (15–24)	8.7 (6–16)	7.1 (4–8.4)	18.7 (6–27)	9.1 (7–9.6)	1.5–3
H. texta (3)	*Perca fluviatilis*	gills	15–18	7–8		15–25		
H. texta (5)	*Perca fluviatilis*	gills	17.5–22 (19.7 ± 1.8)	7.2–9.6 (8.3 ± 1)	7.2–9.6 (8 ± 0.9)	11–20 (15 ± 1.4)	8.1–12 (9.5 ± 1.4)	2.4–3.2 (2.9 ± 0.4)
H. jaczoi sp. n. (5)	*Perca fluviatilis*	palate	13.2–15.2 (14.1 ± 1.1)	5.2–7 (6.2 ± 0.64)	4.8–5.2 (5.1 ± 0.19)	20.8–27.2 (24 ± 1.5)	4.8–7.2 (6.4 ± 0.83)	1.6–2.4 (2 ± 0.23)
H. wolinensis (2)	*Perca fluviatilis*	skin	22.3–27.2 (25.1)	7.4–8.7 (8)	5.8–7.7 (6.9)	34.6–44 (41.3)	11.1–15.1 (13)	2.4–3.5 (3)

Molecular data: The 18S rDNA sequences of *H. psorospermica* found by us were completely identical with those of the *Henneguya* species from *Esox lucius* deposited in the GenBank (EU732602). Their similarity to *H. lobosa* (EU732600), another parasite of the pike, is only 85.9% and to a *Henneguya* sp. of the pike (EU732601) it is 85.4% (Fig. 12).

Remarks: Henneguya psorospermica differs from *H. texta* by its smaller spores and by the 84.0% difference between their 18S rDNA sequences. Data obtained in this study suggest that *H. psorospermica* is a specific species of the pike and, despite morphological similarities of the spores, it does not infect percid fishes.

Spores of a *Henneguya* species were found also in the intestine of a perch. Molecular studies of these spores, however, revealed that the sequences of this species had 100% similarity to the species *H. cutanea*, a parasite of cyprinid fishes, indicating that the spores originated from a cyprinid prey fish (most probably a young bream, *Abramis brama*) consumed by the perch.

Comparative measurements of our data and those given by some relevant authors on *Henneguya* spp. are indicated in Table 2.

Discussion

Henneguya is the second most species-rich genus of myxosporeans after *Myxobolus,* with over 195 species (Eiras, 2002; Eiras and Adriano, 2012). Most species described recently from different fishes have proper morphological descriptions supported by histological and molecular data (Székely et al., 2009; Ye et al., 2012; Yokoyama et al., 2012; Carriero et al., 2013; Rocha et al., 2014). Some other species described more than a hundred years ago, however, have only scanty data on morphology, insufficiently designated habitats and more than one host in their original description. To add to the problem, researchers often misidentified the myxozoans they encountered, and consequently the host range of known species was erroneously enlarged. For instance, Thélohan (1895) described the species *H. psorospermica* from pike and perch which belong to two different fish families. When studying the histopathological changes caused in fish gills by *Henneguya* spp., Dyková and Lom (1978) – accepting Thélohan's description – recorded *H. psorospermica* from pike and perch but remarked that in pike large plasmodia of this species developed in the filament arteries while in perch small plasmodia were formed in intralamellar location. In a similar way, Donec and Shulman (1984) regarded *H. texta* and *H psorospermica* as a single species and synonymised the two species. In all probability the above authors came across plasmodia of two different species, namely *H. psorospermica* in pike and *H. texta* in perch. These scantly described species need a complete revision which can now be carried out relatively easily, considering the importance

of location and tissue affinity and owing to the availability of molecular techniques as an identification tool. Morphological differences among *Henneguya* species are relatively scarce and the differences in their elongated spindle-shaped spores often rely on the length of the caudal extensions. Then, species infecting the pike and percids are rather similar with their elongated spindle shape. On the other hand, a relatively large difference exists between *Henneguya* spp. of percids and cyprinids. The spores of *H. cutanea*, the parasite infecting the fins of the cyprinid bream (*Abramis brama*), have a wide spore body resembling *Myxobolus* spp. Some authors (Eszterbauer et al., 2005; Fiala and Bartosová, 2010; Carriero et al., 2013) suggested that *Henneguya* is a polyphyletic group and molecular data support that some of its members, e.g. *H. cutanea* with its spore body resembling a *Myxobolus,* are clustered in a clade composed otherwise only of *Myxobolus* spp. In its spore structure, *H. jaczoi* sp. n. resembles most species known from pike and percids, and it is especially similar to *H. texta*. The specific location of plasmodia in the palatal region of the fish, however, distinguishes it from *Henneguya* spp. typically developing intravasally in the gills (e.g. *H. creplini* and *H. texta*). Histological evidence and molecular data strongly support the validity of *H. jaczoi* sp. n. as a new species. On the other hand, more data are necessary for separating and qualifying some known species described from the pike and from percid fishes. New data on the host specificity of myxobolid myxosporeans (Molnár, 1994; Lom and Dyková, 2006; Cech et al., 2012) seem to support the notion that a given myxosporean infects only one host or some closely related fish of the same tribe, and genetically far standing species like the pike and the perch are not infected by the same myxosporean species. However, in the case of morphologically similar species described from identical organs of percid fishes, the occurrence of more than a single species cannot be excluded, and only molecular evidence can give a definite answer. Besides molecular data, tissue affinity and differences in development can serve as useful taxonomic characteristics. Considering tissue affinity of the parasite, there is no need for molecular data to state that a species developing in the connective tissue, e.g. *H. jaczoi* sp. n., differs from *H. psorospermica* and *H. texta,* which form perivascular and intravascular plasmodia in the gills. In a similar way, differences in developmental cycles help in the proper identification of species. Most *Henneguya* species have a relatively short developmental cycle and their matured plasmodia can be found in different seasons of the year. Others, like *H. creplini* of the pikeperch, have a complete one-year developmental cycle and their unmatured plasmodia can be found in all seasons of the year; however, their spores develop at the end of winter. Molnár (1998) found an almost 100% infection with this parasite in pikeperch, and Lom and Dyková (1992) also reported its common occurrence in that fish species. Although the occurrence of *H. creplini* in pikeperch seems to be well documented, its occurrence in this fish species can still be questioned. The species *H. creplini* was originally described by Gurley (1894) from the gills of

the ruffe (*Gymnocephalus cernua*), which was intensively studied also in Lake Balaton, but showed no *Henneguya* infection (Molnár, 1966, 1991; Molnár et al., 2001). It is quite unrealistic that a myxosporean species described as commonly infecting two genetically close standing fishes in a given habitat would infect only one of them (pikeperch) and would never be found in the other (ruffe). Considering the strict host specificity of *Henneguya* species, *H. creplini* seems to be a host-specific species of the ruffe, and the species infecting the pikeperch and until now called *H. creplini* seems to correspond to *H. nemeczeki* (Tripathi, 1952). While studying *Henneguya* infection of the perch in Finland, Haaparanta et al. (1994) found an intensive infection with a *Henneguya* species which they identified as *H. creplini*. The above authors found small interlamellar plasmodia and large filamental plasmodia in the gills. It is supposed that these authors studied one or two species other than *H. creplini*, since Dyková and Lom (1978), who studied *H. creplini* in the original host (the ruffe), described intralamellar cysts for this species.

Henneguya jaczoi sp. n. differs from the *Henneguya* spp. known from Hungary (*H. psorospermica, H. texta, H. creplini, H. cutanea*) in its tissue habitat. Its location in a specific part of the oral cavity is unusual, but similar habitats have been reported for other myxosporeans. Liu et al. (2014) has described that *Myxobolus oralis* infecting the gibel carp [*Carassius auratus gibelio* (L.)] forms large plasmodia in the palate and induces severe pathogenic effects. Donec and Shulman (1984) also remarked that the plasmodia of *H. psorospermica* and *H. lobosa* can infect the palate of the hosts besides the gill filaments. On the basis of the present investigations we suppose that Donec and Shulman (1984) had misidentified their species and actually found *H. jaczoi* sp. n.

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