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Trichodinid Ectoparasites (Ciliophora: Peritrichia) of Non-native Pumpkinseed (*Lepomis gibbosus*) in Europe

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Abstract. Three species of trichodinid ciliates, common parasites or symbionts of aquatic invertebrates and vertebrates, have been reported from pumpkinseed (*Lepomis gibbosus*, Centrarchidae) in both their native (*Trichodina fultoni*, *T. tumefaciens*) and introduced (*T. fultoni*, *Trichodinella epizootica*) ranges. In this study, we report five additional trichodinid taxa collected from invasive *L. gibbosus* in France and two regions of the Czech Republic. We describe a new species, *Trichodina lepomi* sp. n., recorded in *L. gibbosus* from both countries. The new species differs from *T. nigra* by absence of posterior projection of blade, absence of notch/ indentation opposing ray apophysis, absence of ray apophysis and the blade not being displaced anteriorly relative to ray as significantly as in *T. nigra*. Two widely distributed species, identified as *Trichodina acuta* and *T*. cf. *heterodentata*, were observed on juvenile fish in the Dyje river basin (Czech Republic). Finally, two undescribed species of *Trichodina* sp. and *Tripartiella* sp. are reported. Detailed description of the new species and comparison with other congeneric species are presented.

Keywords: parasitic ciliates, invasive fish, morphology, Trichodinidae

INTRODUCTION

The pumpkinseed, *Lepomis gibbosus* (L., 1758), is a centrarchid fish (Actinopterygii: Centrarchidae) native to eastern and central North America (Scott and Crossman 1973). Since the late 19th century, the species has been intentionally introduced to Europe as an aquarium and garden pond fish (Vivier 1951). The first individuals transported to France came from Canada in 1877 and North America a few years later, following which several introductions of hundreds to thousands of fish took place in Germany (Stansch 1914). During the 20th century, *L. gibbosus* spread throughout Europe as a result of ornamental fish trade, accidental release to open waters or unintentional introduction with commercial fish (summarised in Wiesner et al. 2010). Currently, the species is established in most European countries, though frequently with patchy distribution (Copp and

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Fox 2007), and is showing increasing invasive potential in southern countries (Fox et al. 2007).

Trichodinid ciliates are common parasites or symbionts of aquatic invertebrates and vertebrates (Van As and Basson 1989). A number of *Trichodina* species are known from centrarchids in their native range, with *Trichodina fultoni* Davis, 1947 reported from green sunfish, *Lepomis cyanellus* Rafinesque, 1819; pumpkinseed; bluegill, *L. macrochirus* Rafinesque, 1819; largemouth black bass, *Mircopterus salmoides* (Lacepède, 1802); smallmouth bass, *M. dolmieui* Lacepède, 1802, and black crappie, *Pomoxis nigromaculatus* (Lesueur, 1829) (Lom and Hofmann 1964, Hofmann and Lom 1967); *Trichodina discoidea* Davis, 1947 from bluegill, rock bass, *Ambloplites ruprestris* (Rafinesque, 1817), and black crappie (Wellborn 1967); and *Trichodina tumefaciens* Davis, 1947 from pumpkinseed (Li and Desser 1985, McDonald and Margolis 1995). In addition, several reports of *Trichodina* spp. have been documented in the non-native range of pumpkinseed, including Cuba (Arthur and Lom 1984), Ukraine (Kostenko 1972, 1981), several central and eastern European countries (Lom 1970, Grupcheva et al. 1989, Nikolić and Simonović 1999) and, more recently, England (Hockley et al. 2011). As most of these remain unidentified to species level, it is difficult to assess whether the parasites were co-introduced with the fish host or were acquired in the new environment.

The current study provides a description of several trichodinid species sampled from non-native pumpkinseed in three different regions (two in the Czech Republic and one in France) and provides comments on their determination and taxonomic position.

MATERIALS AND METHODS

The fish were sampled from (1) a private pond near the Pension u Janů located in the Lužnice river basin (River Elbe drainage) near the town of Třeboň, Czech Republic (coordinates 48.9801 N, 14.8613 E) in May 2018; (2) the Koenigsmacker pond, located in the floodplain of the River Moselle (River Rhine drainage) near the town of Cattenom, France (coordinates N 49.4019, E 6.2634) in June 2018; and (3) two borrow pits (artificial pools created during dyke construction) located in the River Dyje floodplain (Danube river drainage), Czech Republic, (Helpůn – N 48.6688, E 16.9275; D6 – N 48.7173, E 16.8888), in August 2018. The fish were sampled by seine net at the Třeboň (7 m length, 5 mm mesh size) and Koenigsmacker (11 m length, 2 mm mesh size) ponds and the D6 borrow pit, and by electrofishing gear (battery-operated SEN backpack unit; Bednář Ltd., Czech Republic) at the Helpůn borrow pit, depending on habitat conditions. Fish standard length (SL, mm) was measured immediately after sampling.

A total of 20 fish (1 to 3 years old) were collected from the Třeboň and Koenigsmacker ponds, with sizes ranging from 53–74 mm and 55–100 mm of standard length (SL), respectively; and 30 fish per site (0+ juveniles) from the borrow pits, with lengths ranging from 15–29 mm SL in Helpůn and 24–42 mm SL in D6. All fish were placed into aerated tanks and transported to the laboratory alive, where they were measured and dissected for parasites two days after sampling (Kvach et al. 2016). Air-dried smears impregnated with silver nitrate were prepared for study of adhesive discs (Klein 1958), the smears being mounted in Canada balsam as permanent slides and examined under a BX50 Olympus light microscope fitted with differential interference contrast (Olympus Optical Co.). Trichodinid ciliates were identified following the methodology of Van As and Basson (1989). Measurements of 17 features (Bauer 1984, Lom and Dyková 1992) were obtained using Olympus MicroImage™ Digital Image Analysis software. External diameter in text and Table 1 corresponds denticle ring diameter in Bauer, 1984; this feature has been measured as distance between distal points of two opposite denticles (diameter of adhesive disc without length of pins). This measurement was made to include in analysis some *Trichodina* descriptions (Bauer 1984). All measurements were made in micrometres; and range, mean ± standard deviation and number of specimens measured are provided in the text. Nuclear apparatus was not studied as haematoxylin stained material was not available. Adoral spiral was measured using AxioVision Software 4.8.

For the comparative analysis of *Trichodina lepomi* n. sp., we used data from elaborated descriptions of *Trichodina nigra* by Lom (1961), Kazubski and Migala (1968), the description of *Trichodina* sp. *sensu* Lom et Hoffman, 1964 from American centrarchids (likely *T. nigra*), the description presented in Bauer (1984) and a diagnosis of *T. nigra* from introduced fishes in Taiwan, based on the modern approach to denticle morphology (Van As and Basson 1989). In addition, we used descriptions of other species with similar metric data and shape of denticle and species from pumpkinseed and centrarchid fishes from North America (Wellborn 1967). In order to clearly diagnose and compare similar *Trichodina* species, we use tables of metric data (see Table 1) and a linear description of denticle morphology (see the comparative diagnosis) alongside a comparison of discrete, or relatively discrete, continuous characters of denticle morphology, which represent important features according to current approaches to the taxonomy of this ciliate group (Table 2).

RESULTS

As a result of the study, five trichodinid ciliate taxa were identified from the three different European regions, including three species (*Trichodina acuta* Lom, 1961, *Trichodina* cf. *heterodentata* Dunkan, 1977, a new species, *Trichodina lepomi* sp. n.), and two unidentified taxa (*Trichodina* sp. and *Tripartiella* sp.).

Class Oligohymenophorea de Puytorac et al. 1974

Order Mobilida Kahl, 1933
Family Trichodinidae Claus, 1951
***Trichodina acuta* Lom, 1961** (Figs.1 A, B)

Locality. Helpůn borrow pit, Danube river drainage, Czech Republic

Comparative description: Large trichodinid with flattened, disc-shaped body, 53.0–64.5 (58.1, 5) in diameter. Adhesive disc concave, 43.7–54.7 (48.1, 5) in diameter; surrounded by finely striated border membrane 4.6–6.1 (5.0, 5) wide. Diameter of denticle ring 25.8–32.0 (28.7, 5). Centre of adhesive disc bears a clear or not so intensively impregnated area 8.8–11.7 (9.9, 5) in diameter, containing some darker spots. Number of denticles 18–22 (20, 5). Length of denticle 8.2–9.9 (8.9); span 11.1–14.8 (13.1); length of ray 5.6–7.7 (6.3); width of central part 2.7–3.2 (2.9); length of blade 4.3–5.6 (5.0). The blade is broad, filling large portion of sector between Y and Y+1 axes. Tangent point sharp, with distal margin slightly higher or at same level as tangent point. Posterior margin indentation forms small semi-lunar arch with Y axis, with deepest point slightly lower than blade apex. Blade apophysis developed and angular, coinciding with posterior projection in previous denticle. The distinction between the central part, ray and blade is pronounced. Central part robust and widely triangular with bluntly rounded tip which extends more than halfway past Y-axes and fitted tightly into next denticle. Section of central part above and below X-axis similar. Indentation in lower section of central part is distinct. Ray connection well developed. Rays robust and mostly straight, parallel to Y-axis or slightly directed posteriorly with sharply pointed end. Point of ray does not reach clear area. Ray apophysis prominent. Individuals with visible adoral spiral were not observed.

Comments: Biometric data corresponds to the description of the species (Lom 1961, Van As and Basson 1989). In one specimen, the denticle morphology was similar to *Trichodina compacta* Van As et Basson, 1989, the distal surface having a flat section running parallel to the border membrane.

***Trichodina* cf. *heterodentata* Dunkan, 1977 (Figs. 2 A, B)**

Locality. D6 borrow pit, Danube river drainage, Czech Republic

Comparative description: Medium-sized trichodinid with saucer-shaped body, 53.9–56.3 (54.5, 3) in diameter. Adhesive disc concave, 43.2–47.2 (45.6, 3) in diameter; surrounded by finely striated border membrane 3.6–4.6 (4.2, 3) wide. Diameter of denticle ring 28.7–29.9 (29.3, 3). Centre of adhesive disc with texture identical to rest of adhesive disc, diameter of central area 9.3–14.0 (11.6, 3). Number of denticles 23–25. Number of radial pins per denticle: (10–11, 2). Length of denticle 6.8–7.4 (7.1); span 12.1–15.4 (14.1); length of ray 6.3–8.0 (6.2); width of central part 1.9–3.0 (2.8); length of blade 5.0–5.2 (5.1). Individuals with visible adoral spiral were not observed. Blade is narrow and sickle-shaped, apex does not extend beyond the Y+1 axis. Apophysis of the blade is well-developed and prominent. Tangent point sharp, with distal margin at same level or slightly higher than tangent point. Posterior margin forming deep curve with deepest point in lower half of curve, slightly lower than blade apex. The central part is delicate, with no visible posterior projection, tapering to rounded point fitting tightly into preceding denticle. Central part extends more than halfway to Y axis. Section above and below X axis similar in shape. Ray connection short and broad. Ray apophysis distinct.

Base of ray broad, tapering to sharp point. Ray mostly straight, parallel to Y-axis or slightly directed posteriorly or anteriorly. Section of denticle above X axis to section below, <1 (ratio 0.75 to 0.85).

Comments: The biometric data and denticle shape of *Trichodina* cf. *heterodentata* generally corresponds with *T. heterodentata* from other localities (Van As and Basson 1989) and *Trichodina pediculus* Ehrenberg, 1838, with a wide range of biometric variation described by some authors (Bauer 1984). In accordance to Kazubski and Migala (1968), *T. pediculus* is characterised by large size (adhesive disc 48.8–59.4), numerous denticles (28–29) and long rays. Differences in blade shape ("broad" in most part of species descriptions) can be connected with relatively "younger" age of descripted not adult individuals. Among the trichodinids, *T. heterodentata* is a cosmopolitan species and more than 35 species of fishes in 14 families have now been recognised as hosts for this parasite (Martins et al. 2010) since it was first described by Duncan (1977). It is believed that this species originated from the African continent and has been dispersed to other countries together with shipments of cichlids destined for aquaculture (Van As and Basson 1989). Some congeneric species (*Trichodina paraheterodentata* Tang et Zhao, 2013 and *Trichodina pseudoheterodentata* Tang et al., 2017) with similarities in denticle morphology have recently been described (Tang and Zhao 2013, Tang et al. 2017).

Other trichodinids

Ciliates with a dark centre to the adhesive disc have been observed in all localities, though no specimens

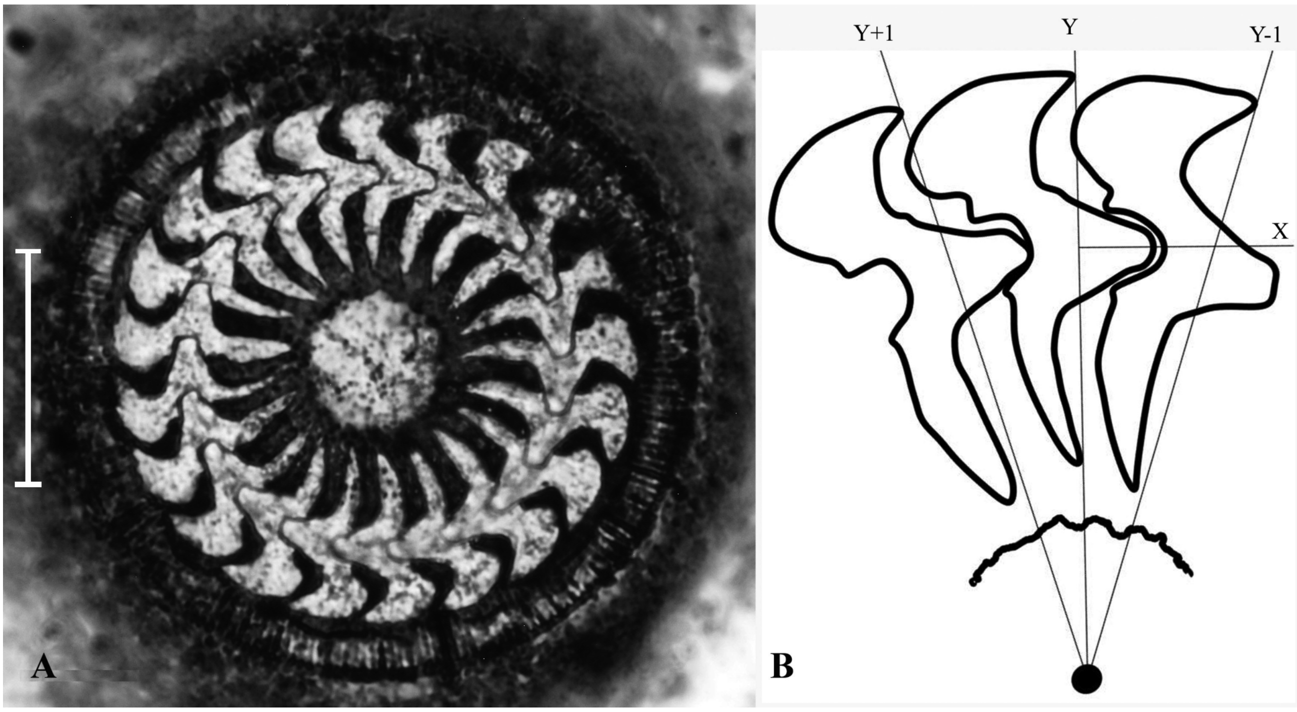

Fig 1. *Trichodina acuta* Lom, 1961. **A** – silver impregnated photomicrograph; **B** – dentical diagram. Scale: 20 μm.

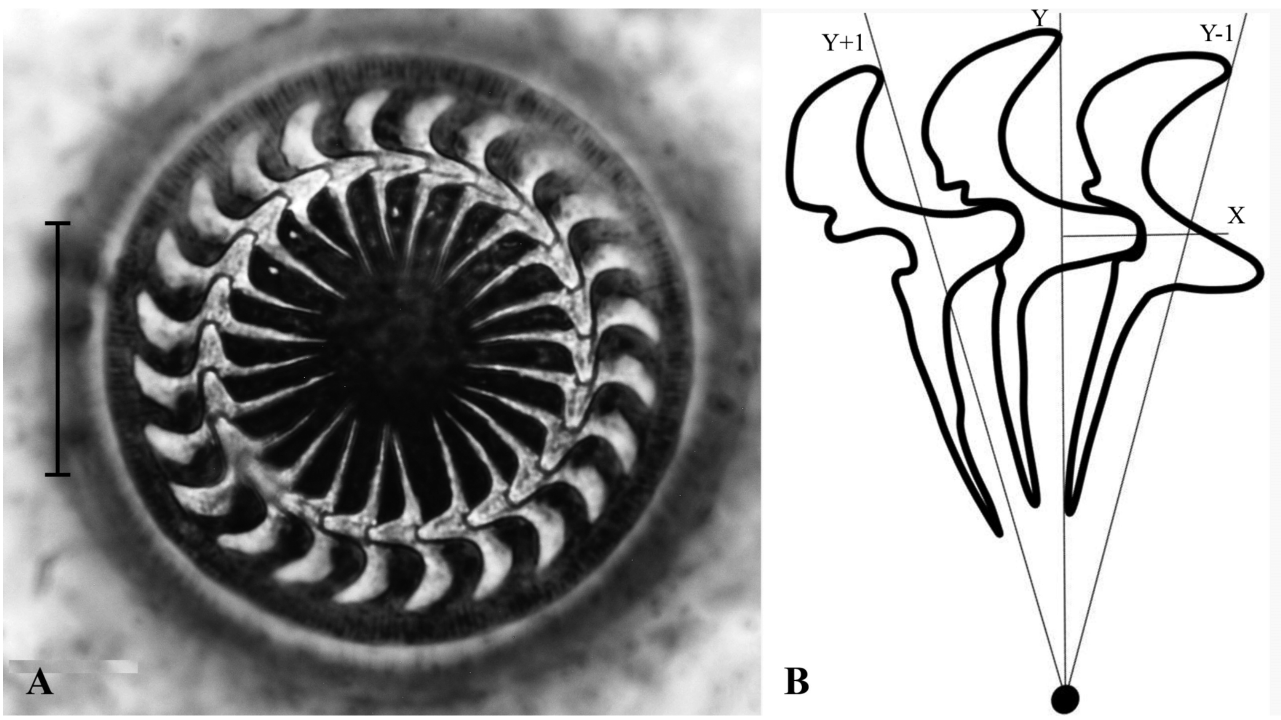

Fig. 2. *Trichodina* cf. *heterodentata* Dunkan, 1977. **A** – silver impregnated photomicrograph; **B** – dentical diagram. Scale: 20 μm.

had parameters completely corresponding to particular *Trichodina* species. Ciliates similar to those described as *Trichodina* sp. *sensu* Lom et Hoffman, 1964 (possibly *Trichodina nigra* Lom, 1961) from American centrarchids (see Lom and Hoffman 1964) were observed in the D6 sample. Smaller sized individuals with non-completely developed denticles and, in some cases, remnants of a resorbed denticle ring in the central zone were considered as young individuals and not included in the analysis.

Several specimens *Tripatriella* sp. from the Koenigsmacker pond (France) were observed, though poor quality of impregnation did not allow appropriate descriptions.

Trichodina lepomi n. sp.

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Medium trichodinids (40.2–55.0 μm) identified as *T. lepomi* were observed on the fins of several pumpkinseed from two localities: Koenigsmacker pond in France (Figs. 3A, B) and the private pond near Třeboň in the Czech Republic (Figs. 5C, D). Several differences in denticle morphology compared with other trichodinids according to the criteria of Van As and Basson (1989), suggest that the ciliates represent new ciliate species. The observed morphological differences reflect host-induced intraspecific variability. A more detailed comparative diagnosis of this form follows.

Description

Trichodina lepomi n. sp. (Figs. 3A, B, C, D, E)

Body: medium-large trichodinid, disc shaped, size 40.2–55.0 (48.3±3.7, 27)

Adhesive disc: 31.6–43.3 (38.4±2.9, 35)

Border membrane: 3.6–5.6 (4.8±0.5, 27)

Denticle ring: diameter 17.5–28.0 (23.2±2.5, 35); external diameter of denticle ring 29.1–41.1 (35.7±3.1, 35).

Centre of adhesive disc: texture identical to rest of adhesive disc, diameter of central area 6.4–12.6 (9.9±1.6).

Number of denticles: 21–27 (24, 25).

Number of radial pins per denticle: 7–9 (8, 25).

Dimensions of denticle: Length 5.3–6.9 (5.9±0.4, 75), blade 4.6–6.6 (5.6±0.4, 75), ray 4.5–7.1 (5.7±0.6, 75), width of central part 1.4–2.2 (1.8±0.2, 75), length of central part 2.4–3.4 (3.1±0.3, 75), span 10.6–14.8 (12.9±0.9, 75).

Adoral spiral: 370–400° (Fig. 5 E).

Denticle morphology. Blade broad, distal surface flat, parallel to border membrane or slightly slant. Tangent point on the same level or slightly lower than distal margin. Anterior and posterior margins forming extended curves. No prominent apex. Blade extends to and sometimes beyond Y+1 axis. Blade apophysis present but not clearly distinguishable. Posterior margin forming shallow, flat curve. Deepest point situated on lower third of curve. Connection between blade and central part delicate. Central part robust, extending near than halfway to Y-1 axis. Central part above X axis similar to part below X axis. Point of central part rounded, closely associated with following denticle. Ray connection short and broad. Rays mostly straight, parallel to Y-axis or slightly directed anteriorly, thickened in base and tapers gradually to a blunt point. Ratio between denticle above and denticle below X-axis close to one (0.99±0.09).

Taxonomic summary

Host: *Lepomis gibbosus* (Actinopterygii: Centrarchiidae)

Site: fins

Locality: Koenigsmacker pond, Moselle river basin near Cattenom, France (N49.4019, E6.2634), private pond, Třeboň region, Czech Republic (N48.9801, E14.8613).

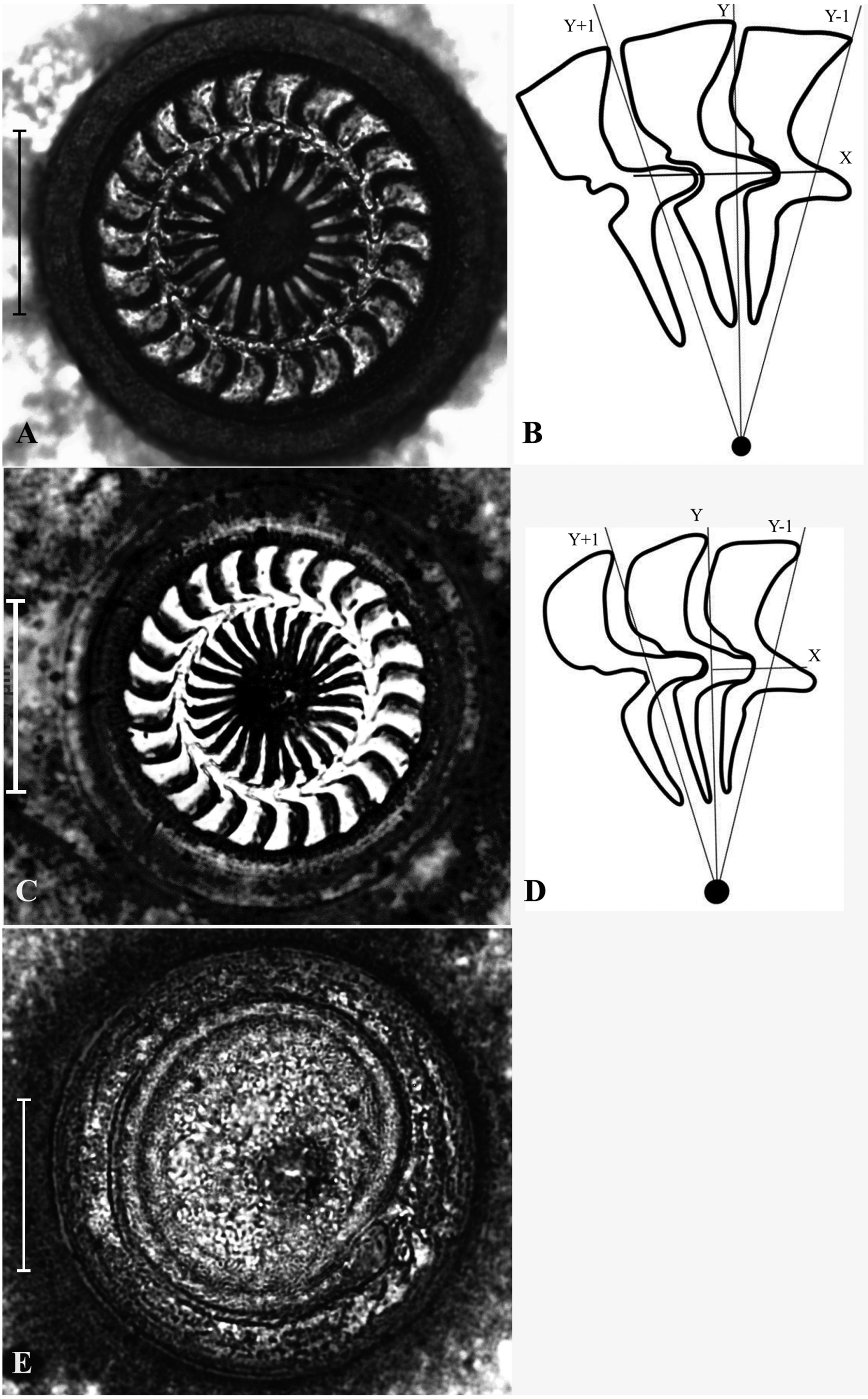
Collection date: May-June 2018

Specimens deposited: National Museum of Natural History at the National Academy of Sciences of Ukraine. Holotype No 2341, paratypes: 2342, 2343.

Comparisons between *Trichodina nigra* and congeneric species

Analysis of biometric indices demonstrates similarity between the species compared in different descriptions (*Trichodina mutabilis* Kazubski et Migala, 1968, *Trichodina kazubskii* Van As et Basson, 1989, *T. nigra* and *T. tumefaciens*), which is common for *Trichodina* species (Van As and Basson 1989). Out of the species compared, the new species exhibited maximum similarity to *T. nigra* with shorter length of the central part of a denticle as the only significant difference in metric data available from description of *T. nigra* in Bauer 1984 (Table 1). Denticle morphology allows identification of differences between *Trichodina lepomi* n. sp. and *T. nigra* and other congeneric species such as *T. mutabilis* and *T. kazubskii*, as follows (Table 2):

A) absence of posterior projection of blade (*T. nigra, T. mutabilis*);


A
B
Y+1
Y
Y-1
X
C
D
Y+1
Y
Y-1
X
E

B) absence of notch/ indentation opposing ray apophysis (*T. nigra, T. mutabilis, T. kazubskii*);
B) absence of ray apophysis (*T. nigra, T. mutabilis, T. kazubskii*);
C) blade is not displaced anteriorly relative to ray as significantly as in *T. nigra*.

Some specimens of *Trichodina lepomi* have a prominent border between the anterior and posterior parts of denticle, probably due to their different thicknesses. The border reaches the blade apophysis (see Fig. 3A). Posterior margin of blade is clearly defined; anterior margin is often partially impregnated.

Trichodinids reported from the centrarchids and, in particular, representatives of the genus *Lepomis* in North America, include *T. tumefaciens* (Li and Desser 1985, McDonald and Margolis 1995) and *T. fultoni* (Hofmann and Lom 1967). Measurements of *T. tumefaciens* used for comparison with our specimens (Table 1) were taken from species described from the North American mottled sculpin, *Cottus bairdi* Girard, 1850 (Wellborn 1967), as metric characteristics from pumpkinseed are not available (Li and Desser 1985) and exhibit significant differences in many features (Table 1). Nevertheless, this species is not mentioned in the main summary articles and keys devoted to trichodinids (Lom and Dyková 1992, Bauer 1984, Van As and Basson 1989, Basson and Van As 1994), casting doubt on its validity. Metric data of *Trichodina fultoni* were not included in analysis as this species has a more distinguishable difference in morphology – light central zone of adhesive disk.

Slight differences in denticle morphology reflect ontogenetic variability. Some intra-population variability has been confirmed for *T. lepomi* sp. n. from different localities and host-individuals; however, all differences correspond to parameter ranges in comparative diagnoses.

DISCUSSION

Our study confirmed susceptibility of European populations of pumpkinseed, a non-native fish, to at least two ciliate genera (*Trichodina* and *Tripartiella*) and three species, including the new one. The new species, *Trichodina lepomi* sp. n., was described from fins of pumpkinseed sampled from two different European watersheds, the Elbe river basin in the Czech Republic and the Rhine river basin in France. A detailed comparison of the new described species with previous descriptions of *T. nigra* and other congenic species of similar size and general structure of adhesive disc and denticle, revealed significant differences in denticle morphology.

The comparative analysis between the newly described species and *T. nigra*, a closely related European *Trichodina* species, and American species described from centrarchid fishes showed differences in at least four parameters. We suppose it sufficient for a new species description. In general, literature sources tend to differ in the weight they lay on individual trichodinid morphological features (Lom 1961, Bauer 1984, Van As and Basson 1989) and often postulate high morphological and metric variability in some species, including *T. nigra* (Kazubski and Migala 1968, Bauer 1984). Kazubski and Migala (1968), for example, studied seasonal variability in widely distributed trichodinid species, including *T. nigra* and *T. mutabilis*, and demonstrated a general trend of longer and somewhat narrower denticles in winter individuals compared with those from summer. In this case, however, the authors mention that *Trichodina* sp. *sensu* Lom et Hoffman, 1964 (from North-American centrarchids) did not correspond with *T. nigra sensu* Kazubski et Migala, 1968 (Lom and Hoffman 1964, Kazubski and Migala 1968). The similarity in denticle morphology of *Trichodina* sp. from

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Fig. 3. *Trichodina lepomi* sp. n. **A** – silver impregnated photomicrograph of an individual from Koenigsmacker pond; **B** – dentical diagram of an individual from Koenigsmacker pond; **C** – silver impregnated photomicrograph of an individual from pond in Třeboň; **D** – dentical diagram of an individual from private pond Třeboň; **E** – adoral spiral. Scale: 20 μm.

Table 1. Biometric data for *T. lepomi* sp. n. compared with descriptions of *T. nigra* and other similar trichodinids

Species	*T. lepomi*	*Trichodina nigra* Lom, 1961	*Trichodina nigra* Lom, 1961	*Trichodina* sp. sensu Lom et Hoffman, 1964	*Trichodina nigra* Lom, 1961	*Trichodina nigra* Lom, 1961	*Trichodina mutabilis* Kazubski et Migala, 1968	*Trichodina mutabilis* Kazubski et Migala, 1968	*Trichodina kazubski* Van As et Basson, 1989	*Trichodina tumefaciens* Davis, 1947
Reference	This study	Lom (1961)	Kazubski and Migala (1968)	Lom and Hoffman (1964)	Basson and Van As (1994)	Bauer (1984)	Basson and Van As (1994)	Bauer (1984)	Van As and Basson (1989)	Wellborn (1967)
Host	*Lepomis gibbosus*	different	*Cyprinus carpio*	*Lepomis macrochirus*	*Carassius auratus*, *Cyprinus carpio*	more than 80 fish species	*Carassius auratus*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*	more than 30 fish species	*B. paludinosus*, *B. trimaculatus*,	*Cottus bairdi*
Locality	Metz, France South Bohemia, Třeboň	Bohemia	Poland	Kearneys-ville, W.Va	Chupei Fishery Station, Taiwan	different waterbodies	Chupei Fishery Station, Taiwan	different waterbodies	Turfloop Dam., Boskop Dam., SA	Kearneysville, West Virginia
Position on host	fins	skin, rarely – gills	skin	gills	skin, fins, gills	skin, fins, gills	gills	skin, fins, gills	skin, fins, gills	gills
Body shape	saucer- to disc-shaped body				medium trichodinid	disc-shaped	large trichodinid, disc-shaped		medium trichodinid with a disc-shaped body	medium-sized trichodinid
Body diam.	44.7–74.5 (58.8±7.6, 27)	61–79	42.6–68.6 (55.6)	46–55 (50)	42.0–52.0 (47.3±4.3, 6)	30.7–102.8	57.0–70.0 (63.4±4.2, 13)	37.0–160.0	34.3–54.6 (41.2±4.5, 23)	35–45 (40)
A.d. diam.	31.6–43.3 (38.4±2.9, 35)	43–54	38.5–58.2 (48.8)	32–42 (36)	32.0–42.0 (37.6 ±3.9, 6)	27.0–69.0	46.0–58.0 (52.4±4.1, 13)	30.0–74.0	26.7–39.5 (32.9±3.7, 23)	37–35 (32)
B.m. width	3.6–5.6 (4.8±0.5, 27)	3.5–5	5		4.5–5.0 (4.9±0.2, 6)	2.5–6.0	4.5–6.5 (5.6±0.6, 13)	3.0–7.0	3.2–5.9 (4.3±0.6, 23)	1–1.5
D.r. diam. Lom	17.5–28.0 (23.2±2.5, 35)	27–33	25.0–36.4	18–23 (20)	20.0–24.5 (21.8±1.8, 6)	17.0–40.0	29.0–37.5 (32.6±2.5, 13)	15.0–54.0	16.4–26.3 (20.2±2.4, 23)	16–20 (19)
D.r.ext. diam. Stein	29.1–41.1 (35.7±3.1, 35)					15.0–61.5		16.0–62.0		
Centre of adhesive disc	texture the same as rest of adhesive disc	dark centre	dark centre	dark centre	texture the same as rest of adhesive disc	texture the same as rest of adhesive disc	texture identical to rest of adhesive disc	texture the same as rest of adhesive disc	stains uniformly in silver impregnated specimens	
Number of denticles	21–27 (24, 25)	21–23	21–30 (24.5)	22–25 (24)	18–21 (20, 6)	17–33	23–29 (27, 13)	21–36	22–26 (23,23).	24–27 (26)
R.p./d.	7–9 (8, 25)	8–10	9–11		9 (9, 6)	8–16	9–11 (10, 13)	7–12	7–10 (8, 23)	7
Denticle length	5.3–6.9 (5.9±0.4, 75)	9–11			6.0–7.5 (6.7±0.5, 6)		7.0–8.0 (7.4±0.4, 13)		3.6–5.7 (4.3±0.5, 23)	4–6 (5)
Denticle span	10.6–14.8 (12.9±0.9, 75)				11.5–14.0 (12.6±0.9, 6)		15.0–18.0 (16.4±0.8, 13)	5.2–20.7		

Blade length Lom	5.6–6.6 (5.6±0.4, 75)	4.5–7	4.5–5.5 (5.1±0.3, 6)	3.6–7	6.0–7.0 (6.5±0.4, 13)	4.4–10.0	3.5–5.7 (4.6±0.7, 23)	3–5 (4)
Blade length Stein	5.3–6.9 (6.4±0.4, 75)			2.9–10.5		5.5–9.6		
Ray length	4.5–7.1 (5.7±0.6, 75)	5–9	4.0–5.0 (4.6±0.4, 6)	3.5–9.0	6.5–8.0 (7.5±0.5, 13)	4.8–10.4	3.6–6.4 (5.2±0.6, 23)	2.5–4 (3)
Ray length Stein	5.9–7.5 (6.6±0.5, 75)			3.8–15.0		4.8–11.0		
C.p. width	1.4–2.2 (1.8±0.2, 75)	2.0–3.3	2.0–3.0 (2.6±0.5, 6)	0.9–4.0	1.5–2.5 (1.9±0.3, 13)	1.1–3.0	1.5–3.2 (2.0±0.4, 23)	1–2 (1.3)
C.p. length	2.4–3.4 (3.1±0.3, 75)			3.5–11.0				

Notes: A.d., adhesive disc; B.m., border membrane; C.p., central part; diam., diameter; D.r., denticle ring; R.p./d., number of radial pins per denticle. Two values before parentheses indicate variation range, numbers within parentheses represent absolute minimum and maximum values observed (Lom 1961).

D6 and that of *Trichodina* sp. *sensu* Lom et Hoffman, 1964 supports the possible introduction of parasitic *Trichodina* ciliates from North America with centrarchid fish hosts. Molecular genetic analysis, which was out of the scope of this study, would be necessary to resolve this question.

On our opinion the significant difficulties encountered with determining the status of the *Trichodina* species connected with the limited data on intraspecific morphological variability for many similar species.

Unfortunately, there is no clear current resolution of phylogenetic relationships within the genus *Trichodina*, or indeed the family Trichodinidae. An attempt to construct phylogeny based on the degree of development in different parts of the denticle (blade, central part, ray) (Gong et al. 2005), a continuation of traditional morphological research concepts, was contradicted by the first molecular studies (Tang et al. 2013, Wang et al. 2019), which went on to propose a paraphyletic status for the genus *Trichodina*. Moreover, genetic data failed to confirm the original division of all trichodinids into large clades based on the character of the adhesive disc's central zone (Tang et al. 2013, Wang et al. 2019), as proposed by most taxonomists or morphologists in the past (Lom 1961, Bauer 1984, Van As and Basson 1989, Lom and Dyková 1992). Only recently have studies appeared investigating reasons for trichodinid variability at the molecular level (Irwin et al. 2017), and it is to be hoped that future phylogenetic constructions based on new genes will allow advances in the identification of ciliate phylogenetic relationships. Further advances are also likely through the use of new geometric-morphometric methods for denticle structure analysis supported by genetic analysis (Marcotegui et al. 2018).

Few trichodinid species have been reported from European non-native pumpkinseed populations to date. Among these, *Trichodina polystriata* Kostenko, 1969 has been described from pumpkinseed in the Lower Danube basin (Kostenko 1972, 1981), though this species was subsequently synonymised with *T. fultoni* (Bauer 1984), a species parasitising pumpkinseed in North America (Hoffman and Lom 1967). *Trichodinella epizootica* (Raabe, 1950) was recorded as parasitising pumpkinseed in Serbia, though this species was not marked in the table of appropriate references (Nikolić & Simonović, 1999). Both species mentioned above are generalists and widely distributed in populations of autochthonous fish species (Lom and Dyková 1992), as are *T. acuta* and *T.*cf. *heterodentata* found on pumpkinseed in this study. However, while *T. acuta* and *T.* cf.

Table 2. Denticle morphology of *Trichodina lepomi* sp. n. compared with other similar trichodinids.

Species	*T. lepomi*	*Trichodina nigra*	*Trichodina mutabilis*	*Trichodina kazubski*
Reference	this study	Basson and Van As (1994)	Basson and Van As (1994)	Basson and Van As (1989)
Tangent point	on the same level or slightly lower than distal margin	lower than distal surface	on the same level or slightly lower than distal margin	slightly lower than distal surface
Apex AM	**not prominent**	sharp to rounded, situated lower than distal surface	not prominent	slightly pointed
Blade position to Y+1	extends to and sometimes beyond Y+1 axis	extending and beyond Y+1 axis	extends to and sometimes beyond Y+1 axis.	extending toY+1 line and sometimes beyond
Blade apophysis	present, but not clearly distinguishable	present, but not clearly distinguishable	present but not prominent	prominent
Deepest point PM	on lower third of curve	lower half of curve on same level as apex	on lower third of curve	at point where blade connects with central part
Posterior projection	**not present**	present, but not prominent.	small and indistinct	absent
Central part position to Y-1	about halfway to Y-1 axis	less than halfway to Y-1 axis	extending more than halfway to Y-1 axis	
Point of central part	rounded, closely associated with following denticle	tapering to rounded point fitting tightly into preceding denticle	rounded, only tips of central part connected with previous denticle	rounded, loosely associated with following denticle
Notch/ indentation opposing ray apophysis	**not distinct**	distinct	very small	slight indentation on central part opposite apophysis visible
Rays	mostly straight, parallel to Y-axis or slightly directed anteriorly	rays straight, following Y axes	mostly straight, but directed anteriorly	parallel to Y axis
Ray apophysis	**not distinct**	prominent and directed anteriorly	very small and projected in an anterior direction	prominent
Ratio of denticle above X axis to denticle below	0.99±0.09	1	slightly <1 (0.85–0.9)	1

Notes: diagnostic characters are indicated in bold

heterodentata were most probably acquired in Europe from the local fish fauna, the origin of *Trichodina lepomi* remains unclear. The detection of this ciliate in two distant European regions, the Czech Republic and France, suggests its introduction to Europe together with its host, the pumpkinseed. Likewise, it may also indicate possible host-specificity to pumpkinseed, which could be confirmed by findings in other regions and through molecular genetic research.

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Compliance with ethical standards. Methods used for fish sampling, transportation and dissection were in line with the ethical requirements of the Czech Republic (§ 7 Law No. 114/1992 on the protection of nature and the landscape and § 6, 7, 9 and 10 of Regulation No. 419/2012 on the care, breeding and use of experimental animals), and have been approved by the appropriate ethics committee (Expert Committee for Work With Experimental Animals, Project No. MO17/1).

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

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

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