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
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, *Micropterus salmoides* (Lacepède, 1802); smallmouth bass, *M. dolomieui* 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 Janě 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 Koenigsammer 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, (Helpūn – 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 Koenigsammer (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 Helpūn 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 Koenigsammer 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 Helpūn 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 BXS50 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. heterodontata* 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 pointed 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 anterior prominence. 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 described 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 – dential diagram. Scale: 20 μm.

Fig. 2. *Trichodina cf. heterodentata* Dunkan, 1977. A – silver impregnated photomicrograph; B – dential 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 Koenigsracker pond (France) were observed, though poor quality of impregnation did not allow appropriate descriptions.

*Trichodina lepomi* n. sp.

urn:lsid:zoobank.org:act:0A8BFCCF-DB2A-4C1D-AC45-35C7DBAE84FD

Medium trichodinids (40.2–55.0 µm) identified as *T. lepomi* were observed on the fins of several pumpkinseed from two localities: Koenigsracker 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: Centrarchidae)

Site: fins

Locality: Koenigsracker 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*);
B) absence of notch/indentation opposing ray apophysis (*T. nigra*, *T. mutabilis*, *T. kazubski*);
B) absence of ray apophysis (*T. nigra*, *T. mutabilis*, *T. kazubski*);
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

---

**Fig. 3.** *Trichodina lepomi* sp. n. **A** – silver impregnated photomicrograph of an individual from Koenigsmacker pond; **B** – denticle diagram of an individual from Koenigsmacker pond; **C** – silver impregnated photomicrograph of an individual from pond in Třeboň; **D** – denticle diagram of an individual from private pond Třeboň; **E** – adoral spiral. Scale: 20 μm.
| Species     | T. lepomi                      | Trichodina nigra Lom, 1961 | Trichodina nigra sensu Lom et Hoffman, 1964 | Trichodina nigra Lom, 1961 | Trichodina mutabilis Kazubski et Migala, 1968 | Trichodina mutabilis Kazubski et Migala, 1968 | Trichodina ka-zubski Van As et Basson, 1989 | Trichodina tu-mefaciens Davis, 1947 |
|-------------|--------------------------------|-----------------------------|---------------------------------------------|-----------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Reference   | This study                     | Lom (1961)                  | Kazubski and Migala (1968)                  | Basson and Van As (1994)    | Basson and Van As (1994)                    | Bauer (1984)                                | Van As and Basson (1989)                    | Wellborn (1967)                             |
| Host        | Lepomis gibbosus               | different                   | Cyprinus carpio                            | Carassius auratus, Cyprinus carpio | Carassius auratus, Cyprinus carpio, Hypophthalmichthys molitrix | Carassius auratus, Cyprinus carpio, Hypophthalmichthys molitrix | Carassius auratus, Cyprinus carpio, Hypophthalmichthys molitrix | Carassius auratus, Cyprinus carpio, Hypophthalmichthys molitrix |
| Locality    | Metz, France                   | South Bohemia               | Bohemia                                     | Chupei Fishery Station, Taiwan | different waterbodies                       | different waterbodies                       | different waterbodies                       | different waterbodies                       |
| Position on host | fins                        | skin, rarely                | skin, fins, gills                           | skin, fins, gills            | gills                                        | gills                                       | 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 trichodinid with a disc-shaped body   | medium trichodinid with a disc-shaped body   | medium trichodinid with a disc-shaped body   |
| Body diam.  | 44.7–74.5 (58.8±7.6, 27)       | 61–79                       | 42.6–68.6 (55.6)                           | 46–55 (50)                  | 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)                    |
| A.d. diam.  | 31.6–43.3 (38.4±2.9, 35)       | 43–54                       | 38.5–58.2 (48.8)                           | 32–42 (36)                  | 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)                    |
| B.m. width  | 3.6–5.6 (4.8±0.5, 27)          | 3.5–5                       | 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)                      |
| D.r. diam.  | 17.5–28.0 (23.2±2.5, 35)       | 27–33                       | 25.0–36.4 (18–23)                          | 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)                    |
| D.r. ext. diam. | 29.1–41.1 (35.7±3.1, 35) | 15.0–61.5                   |                                              |                             |                                              |                                              |                                              |                                              |
| Centre of adhesive disc | texture the same as rest of adhesive disc | dark centre | dark centre | 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                                      | 21–36                                      | 22–26 (23, 23).                             |
| 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).                               |
| Denticle length | 5.3–6.9 (5.9±0.4, 75) | 6.0–7.5 (6.7±0.5, 6) | 10.0–12.0 (9.2±0.8, 75) | 11.5–14.0 (12.6±0.9, 6) | 15.0–18.0 (16.4±0.8, 13) | 5.2–20.7 | 5.2–20.7 |
| 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 | 5.2–20.7 |

Table 1. Biometric data for T. lepomi sp. n. compared with descriptions of T. nigra and other similar trichodinids.
Trichodinids of Lepomis gibbosus in Europe

D6 and that of Trichodina sp. sensu Hoffman. 1964 supports the possible introduction of parasitic Trichodina ciliates from North America, which was indeed 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 in question are due to 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 studies 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). Trichodina 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 surface | 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 to Y+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 | not distinct | distinct | very small | slight indentation on central part opposite apophysis visible |
| Ray apophysis      | mostly straight, parallel to Y-axis or slightly directed anteriorly | rays straight, following Y axes mostly straight, but directed anteriorly | prominent and directed anteriorly | parallel to Y axis |
| 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.

*Trichodina lepomi* 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.

**Funding.** This study was supported by the Czech Science Foundation (Grant No. P505/12/G112).

**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.

**REFERENCES**

Arthur J. R., Lom J. (1984) Some trichodinid ciliates (Protozoa: Peritrichida) from Cuban fishes, with a description of *Trichodina cubanensis* n. sp. from the skin of *Cichlasoma tetracantha*. *Trans. Am. Microsc. Soc.* **103**: 172–184

Bauer O. N. (1984) *Opredelitel parazitov presnovodnykh ryb fauny SSSR*. Nauka, Leningrad (in Russian)

Basson L., Van As J. G. (1994) Trichodinid ectoparasites (Ciliophora: Peritrichida) of wild and cultured freshwater fishes in Taiwan, with notes on their origin. *Syst. Parasitol.* **28**: 197–222

Copp G., Fox M. G. (2007) Growth and life history traits of introduced pumpkinseed (*Lepomis gibbosus*) in Europe, and the relevance to its potential invasiveness. In: Freshwater bioinvaders: profiles, distribution, and threats (Ed.: F. Gherardi). Springer, Berlin, 289–306
Trichodinids of Lepomis gibbosus in Europe

Duncan B. L. (1977) Urceolarid ciliates, including three new species, from cultured Philippine fishes. Trans. Am. Microsc. Soc. 96: 76–81

Fox M. G., Vila-Gispert A., Copp G. H. (2007) Life-history traits of introduced Iberian pumpkinsseed Lepomis gibbosus relative to native populations. Can differences explain colonization success? J. Fish Biol. 71: 56–69

Gong Y. C., Yu Y., Feng W., Shen Y. (2005) Phylogenetic relationships among Trichodinidae (Ciliata: Peritricha) derived from the characteristic values of denticles. Acta Protozool. 44: 237–243

Grupcheva G., Lom J., Dykova I. (1989) Trichodinids (Ciliata: Urceolariidae) from gills of some marine fishes with the description of Trichodina zaikai sp. n. Folia Parasitol. 36: 193–207

Hockley F. A., Williams C. F., Reading A. J., Taylor N. G., Cable J. (2011) Parasite fauna of introduced pumpkinsseed fish Lepomis gibbosus; first British record of Onchoceleidus dispar (Monogenea). Dis. Aquat. Organ. 97: 65–73

Hoffman L., Lom J. (1967) Observations on Tripartiella bursiformis, Trichodina nigra and a pathogenic trichodinid, Trichodina fultoni. Bull. Wildlife Dis. Ass. 3: 156–159

Irwin N. A. T., Sabatrashek M., Lynn D. H. (2017) Diversification and phylogenetics of mobild peritrichs (Ciliophora) with description of Urceolaria parakorschelli sp. nov. Protist 168: 481–493. doi: 10.1016/j.protis.2017.07.003

Klein B. M. (1958) The dry silver method and its proper use. J. Protozool. 5: 99–103. doi: 10.1111/j.1550-7408.1958.tb02535.x

Kostenko S. M. (1972) Urceolaridae – parazity ryb sovetskogo uchastka Dunaya. Gidrobiologicheskij Zhurnal 8: 107–112 (in Russian)

Kostenko S. M. (1972) Urceolaridy (Peritricha, Mobiliidae). Naukova Dumka, Kyiv (in Russian)

Kazubski S. L., Migala K. (1968) Urceolaridae from breeding carp – Cyprinus carpio L. in Zabieniec and remarks on the seasonal variability of trichodinids. Acta Protozool. 6: 137–169

Kvach Y., Ondračková M., Janáč M., Jurajda P. (2016) Methodological issues affecting the study of fish parasites. I. Duration of live fish storage prior to dissection. Dis. Aquat. Organ. 119: 107–115

Li L., Desser S. S. (1985) The protozoan parasites of fish from two lakes in Algonquin Park, Ontario. Can. J. Zool. 63: 1846–1858

Lom J. (1961) Ectoparasitic trichodinids from freshwater fish in Czechoslovakia. Acta Soc. Zool. Bohemoslovenicae, 25: 215–228

Lom J. (1970) Observations on trichodinid ciliates from freshwater fishes. Arch. Protistenkd. 112: 153–177

Lom J., Dyková L. (1992) Protozoan parasites of fish. Developments in Aquaculture and Fisheries Science, Vol. 26. Elsevier, Amsterdam.

Lom J., Hoffman G. L. (1964) Geographic distribution of some species of trichodinids (Ciliata: Peritricha) parasitic on fishes. J. Parasitol. 50: 30–35

Marcoglui P. S., Montes M. M., Barneche J., Ferrari W., Martorelli S. (2018) Geometric morphometric on a new species of Trichodinidae. A tool to discriminate trichodinid species combined with traditional morphology and molecular analysis. Int. J. Parasitol. Parasites Wildlife 7: 228–236

Martins M. L., Marchiori N., Nunes G., Rodrigues M. P. (2010) First record of Trichodina heterodentata (Ciliophora: Trichodinidae) from channel catfish, Ictalurus punctatus cultivated in Brazil. Brazilian J. Biol. 70: 637–644

McDonald T. E., Margolis L. (1995) Synopsis of the Parasites of Fishes of Canada: Supplement (1978–1993). Canadian Special Publication of Fisheries and Aquatic Sciences, No. 122. National Research Council of Canada, Ottawa

Nikolić V., Simonović P. D. (1999) A survey of ciliate fish-parasite fauna of Yugoslavia. Ichthyologia 31: 37–41

Scott W. B., Crossman E. J. (1973) Freshwater fishes of Canada. Fish. Res. Board Can. Bull. 184: 1–966

Stansch K. (1914) Die exotischen Zierfische in Wort und Bild. Gustav Wenzel & Sohn, Braunschweig

Tang F., Zhao Y. J. (2013) Record of three new Trichodina species (Protozoa, Ciliophora) parasitic on gills of freshwater fishes from Chongqing, China. African J. Microbiol. Res. 7: 1226–1232

Tang F. H., Zhao Y. J., Warren A. (2013) Phylogenetic analyses of trichodinids (Ciliophora, Oligohymenophorea) inferred from 18S rRNA gene sequence data. Curr. Microbiol. 66: 306–313

Van As J. G., Basson L. (1989) A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. Syst. Parasitol. 14: 157–179. doi: 10.1007/BF02187051

Vivier P. (1951) Poissons et crustacés d’eau douce acclimatés en France en eaux libres depuis le début du siècle. Terre et Vie 98: 57–82

Wang S., Zhao Y., Du Y., Tang F. (2019) Morphological redescription and molecular identification of Trichodina reticulata Hirschmann & Partsch, 1955 (Ciliophora, Mobilida, Trichodinidae) with the supplemental new data of SSU rDNA and ITS-5.8S rDNA. J. Eukaryot. Microbiol. 66: 447–459. doi: 10.1111/jeu.12689

Wellborn T. L. (1967) Trichodina (Ciliata: Urceolaridae) of fresh water fishes of the south-eastern United States. J. Protozool. 14: 399–412

Wiesner C., Wolter C., Rabitsch W., Nehring S. (2010) Gebietskundliche Umarbeitung des Trichodinidae (Ciliophora: Trichodi- nidae) von der Donau mit dem Ziel einer besseren Erkennung. Arch. Protistenkd. 157–179. doi: 10.1007/BF02187051

Wellborn T. L. (1967) Trichodina (Ciliata: Urceolaridae) from cultivated in Brazil. Brazilian J. Biol. 70: 637–644

Received on 28th June, 2019; revised on 1st September, 2019; accepted on 5th September, 2019