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# A New *Trichodina* Species (Peritrichia: Mobilida) from Anuran Tadpole Hosts, *Sclerophrys* spp. in the Okavango Panhandle, Botswana, with Comments on this Taxon

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**Abstract.** Mobiline taxonomic data is mostly inferred from populations collected in and on hosts associated with aquaculture. Even though these conditions may be conducive for studies relating to the hosts, accurate taxonomic inferences of the symbionts will be problematic. The site for the present study was the Okavango Panhandle region in Northern Botswana, an isolated, natural area with minimal anthropogenic influences. Morphometric and molecular evidence revealed that anuran tadpole trichodinids, up to now reported as *Trichodina heterodentata* Duncan 1977 and *T. hypsilepis* Wellborn 1967 from multiple host types, are in fact a new, more host specific species. This study includes comprehensive denticle descriptions of both the anuran hosted trichodinid and the morphologically similar *T. hypsilepis* restricted to teleost hosts (previously *T. heterodentata*).

**Keywords**: *Trichodina koloti* sp. nov.*, Sclerophrys* spp.*,* morphology*,* 18S ribosomal DNA*,* southern Africa.

## INTRODUCTION

The protist microcosm is one of the richest groups in regards to their morphological variation, and exceeds that of all the other eukaryotic kingdoms (Sogin and Silberman 1998). They are predominantly unicellular organisms that inhabit a heterogenous array of environments, ranging from free living to parasitic in nature. Within this complex group, the ciliated protists, with their characteristic rows of cilia for locomotory actions, are the most identifiable (Lynn 2017). Trichodinids are members of the family Trichodinidae Raabe, 1959 and together with its two sister families (Urceolariidae Dujardin, 1840 and Trichodinopsidae Kent, 1881) make up the order Mobilida Kahl, 1933 (Oligohymenophorea de Puytorac *et al.*, 1974: Peritrichia Stein, 1859). The largest group within this family (and order) is the genus *Trichodina* Ehrenberg, 1838 that consists of more than 300 described species to date (Tang *et al*. 2013), all of them symbiotic on or in a diverse range of hosts.

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Trichodinids are usually associated with teleost fishes, especially as ectosymbionts, but have been described from the gills and skin of anuran tadpoles (Raabe 1950, Lom 1961, Chen 1963, Arthur and Lom 1984, Kazubski 1988 and Kruger *et al.* 1995), however, a recent wave of anuran symbiont research from South America (Dias *et al*. 2009, Fernandes *et al*. 2011, Pala *et al*. 2018) has illustrated that some species are more abundant than previously thought. During parasitological surveys in the Nxamasere Floodplain in the Okavango Panhandle, Botswana by the Aquatic Ecology group from the University of the Free State, it was noticed that certain tadpoles have a single trichodinid species infestation on their gills and skin. The geographically isolated Okavango River System has hitherto no known introduced aquatic fish and anuran species, implying that all collected hosts and their symbionts are indigenous to this system. The Nxamasere Floodplain located on the eastern banks of the Okavango River in the system's panhandle in northern Botswana creates seasonal refugia for aquatic life during the dry periods of the year, temporarily trapping fish and tadpoles that in turn become an important food source for the assorted water birds that surround the plain.

The Guttural Toad, *Sclerophrys gutturalis* (Power, 1927), is a large (140 mm in length) pale ground coloured toad with dark patches over its ventral side and a distinct vertebral line along the midline of the back (du Preez and Carruthers 2009) that has a distribution throughout East and sub-Saharan Africa. The tadpoles of *S. gutturalis* naturally occur in any permanent or semi-permanent pools, usually in the shallow water during the day, moving to the deeper patches at night. *Sclerophrys gutturalis* tadpoles usually reach metamorphoses after ten weeks, when their front legs break through and their gills are completely resorbed. The Western Olive Toad, *Sclerophrys poweri* (Hewitt, 1935), on the other hand, is a thickset and robust toad that reaches a maximum size of 100 mm (du Preez and Carruthers 2009) found in southern Angola, northern Namibia, Botswana and central South Africa (Channing *et al*. 2012). These tadpoles prefer the peripheral edges of temporary shallow pools and according to Channing *et al.* (2012) the whole development of *S. poweri* takes about ten weeks from hatching to adulthood.

The present study illustrated that the Botswanan trichodinid species from African anuran tadpoles is indeed the same species described as *T. heterodentata* Duncan, 1977 from anuran tadpoles in other parts of the world. The study also revealed that this anuran hosted trichodinid, although superficially similar to *T. hypsilepis* Wellborn, 1967 (syn. *T. heterodentata Duncan*, 1977) from teleost hosts (de Jager and Basson 2019), differ morphologically (specifically in denticle structure) and molecularly (18S SSU rDNA). These distinctions led to the description of a new freshwater trichodinid taxon, specific to amphibian tadpole hosts.

## MATERIALS AND METHODS

Tadpole specimens, *Sclerophrys gutturalis* and *S. poweri* were collected during the winter of 2015 and both the winter and summer seasons of 2016, from six isolated standing pools in the Nxamasere Floodplain using shrimp nets (Fig. 1). After collection, hosts were separated into aerated tanks, as per locality. Water used for the aquariums was collected from the collection localities, to keep the pH and conductivity the same as the natural environment. Hosts were euthanised according national ethics regulations. All sampling localities was recorded with a Garmin Geographical Positioning System (GPS) (Table 1).

The nuclear apparatus was stained using Mayer's haematoxylin straining method, as suggested by Wellborn (1967) and Basson *et al*. (1983). Silver impregnation for morphometric comparison of the aboral denticle structure was adapted from Klein's (1926) "dry silver" impregnation technique, as described by Lom (1958), Wellborn (1967) and Basson *et al.* (1983). The prescribed measurements initially proposed by Lom (1958) and later adapted by van As and Basson (1989) were done for all sampled populations. Twenty five suitable adult trichodinids per collected population were measured and photographed using a Zeiss Axiophot compound microscope fitted with an AxioCam ICc 5 digital camera. All morphometric measurements are provided in µm, and represented as minimum to maximum (mean ± standard deviation). In the case of the number of denticles and number of radial pins per denticle the mode was used rather than the mean. Body diameter is determined as the adhesive disc plus the border membrane. Denticles of individual representative trichodinids from every population were re-drawn, analysed and described using the method devised by van As and Basson (1989).

### PCR amplification of small rRNA gene, sequencing and phylogenetic analyses

Material for molecular analysis was collected in the field with a fine glass pipette, fixed in absolute ethanol and kept at 4°C for the duration of the expedition and analysed back at the laboratory on the university campus. Genomic DNA extraction and PCR amplification was performed using the REDExtract-N-Amp Tissue PCR Kit according to the manufacturer's instructions. The 18S SSU rRNA gene region was PCR amplified, using the following primer set: ERIB 1 EukA forward primer (ACC TGG TTG ATC CTG CCA G); ERIB 10 EukA reverse primer (CTT CCG CAG GTT CAC CTA CGG) as per Medlin *et al.* (1988). Amplification cycling parameters were as follows; initial denaturation (94°C) for 5 minutes followed by 35 cycles each of denaturation (94°C) for 40 seconds, annealing (56°C) for one minute and extension (72°C) for 1 minute 30 seconds. Final extension occurred at 72°C for 10 minutes. PCR

**Table 1.** Longitude and latitude of collection pools in the Nxamasere Plain. Collection sites NX5 and NX6 share the same coordinates, as these are two separate pools during the drier latter months of the year, but are connected during the earlier months of the year.

| Standing pool (Population) | LONGITUDE | LATITUDE |
|---|---|---|
| Nxamasere 1 (NX1) | S 18° 35, 770’ | E 22° 01, 551’ |
| Nxamasere 2 (NX2) | S 18° 36, 007’ | E 22° 01, 349’ |
| Nxamasere 3 (NX3) | S 18° 35, 396’ | E 22° 00, 766’ |
| Nxamasere 4 (NX4) | S 18° 35, 247’ | E 22° 00, 198’ |
| Nxamasere 5 (NX5) | S 18° 34, 984’ | E 22° 00, 035’ |
| Nxamasere 6 (NX6) | | |

products were sub-cloned into the pSMART® vector (Lucigen) and sequenced using the BigDye® Terminator (v 3.1) Cycle Sequencing Kit (Applied Biosystems®) as per manufacturer’s instructions, using SR1 and SL2 primers. Data was collected using a 3130xl Genetic Analyser (Hitachi).

## Phylogenetic analysis

The extracted sequences were imported into Geneious v.7.1.3 (Biomatters, Auckland, New Zealand), where the cloned sequences were De Novo assembled into a consensus sequence which in turn was multiple aligned, using Geneious Alignment default settings (93% similarity cost matrix) with 18S SSU rRNA mobiline sequences acquired from the GenBank/NCBI database (Clark *et al.* 2016) (Table 2). Members from the Urceolariidae family (*U. korschelti*, *U. parakorschelti* and *U. urechi*) were chosen as outgroup. Sequences from the above mentioned alignment were used for constructing phylogenetic trees, using Maximum-Likelihood (ML) (Saitou and Nei 1987) and Bayesian Inference (BI) methods. The optimal evolutionary model for both Maximum Likelihood and Bayesian Inference was the GTR+I+G model. Bayesian Inference (BI) completed using MrBayes 3.1 (Ronquist and Huelsenback 2003) with 1,000,000 generations, sampling every 100 generations. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar 2000), where the bootstrap test (1,000 replicates) was used for the percentage of replicate trees in which the associated taxa clustered together (Felsenstein 1985). The tree with the highest log likelihood (–11,634.29) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1668)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 36.34% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 40 nucleotide sequences. There were a total of 2,251 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*. 2018). Evolutionary distances of representative consensus sequences from different localities and hosts were computed using the Kimura 2-parameter method (Kimura 1980) in MEGA X (Kumar *et al*. 2018) and are in the units of the number of base substitutions per site (Table 3).

# RESULTS

Type species: *Trichodina koloti* sp. nov.

Type locality and type host: *Sclerophrys gutteralis* (Power, 1923), Nxamasere Floodplain, Okavango panhandle, Botswana (S 18° 35, 770’ E 22° 01, 551’)

Additional locality and host: *Sclerophrys powerii* (Hewitt, 1926), Nxamasere Floodplain, Okavango panhandle, Botswana (S 18° 34, 984’ E 22° 00, 035’)

Type-specimens: Holotype, slide 2016/12/15-01 and paratype, slides 2015/07/09-01 and 2015/07/12-09

18S nucleotide accession number: MT214940

Etymology: *koloti* (noun); [Tswana, a language spoken in southern Africa and the Okavango Delta region] – meaning tadpole

## Morphology and morphometrics

Adult trichodinid specimens collected from tadpoles had a C-shaped macronucleus with a mean external diameter of 31.1–57.4 µm (47.0±9.5), thickness of 4.5–11.3 µm (7.2±2.5) and length of sector between terminations of macronucleus 3.8–38.5 µm (21.1±13.7) with no micronucleus were observed (Fig. 2). The adoral spiral followed a course of 370°–405°, which falls within the variation for the genus *Trichodina*. Based on the comparative morphological dimensions of all six populations, with only the minimum and maximum values given, the trichodinids had a convex body diameter ranging from 43.5–62.6 µm; adhesive disc diameter between 37.3–51.2 µm with a poorly to well-developed border membrane with a width of 2.7–5.7 µm (Table 5). The denticle ring diameter is between

**Table 2.** List of 18S SSU rDNA mobiline sequences from GenBank (Clark et al. 2016) used for phylogenic inference.

| Species selected | Accession nr. | Author/Collector (Year) | Locality |
|---|---|---|---|
| *Trichodina acuta* | KX904932 | Wang *et al*. (2017) | Freshwater fish |
| *T. bellotti* | MH730162 | Marcotegui *et al*. (2018) | Freshwater fish |
| *T. centrostrigeata* | KP295473 | Wang *et al*. (2015) | Freshwater fish |
| *T. compacta* | MF135183 | Abdelkhalek *et al*. (2018) | Freshwater fish |
| *T. domerguei* | KY596037 | Irwin *et al*. (2017) | Euryline fish |
| ***T. heterodentata*** **(syn.)** | AY88099 | Gong *et al.* (2006) | Freshwater fish |
| *T. hyperparasitis* | KX904933 | Wang *et al*. (2017) | Freshwater fish |
| ***T. hypsilepis*** **(syn.)** | EF524274 | Gong *et al*. (unpublished) | Freshwater amphibians |
| *T. meretricis* | FJ499387 | Zhan *et al.* (2013) | Marine molluscs |
| *T. modesta* | GU906245 | Tang *et a*l. (2013) | Freshwater fish |
| *T. nobilis* | AY102172 | Gong *et al.* (2006) | Freshwater fish |
| *T. paraheterodentata* | GU906244 | Tang *et al*. (2013) | Freshwater fish |
| *T. paranigra* | MG198569 | Wang *et al*. (2018) | Freshwater fish |
| *T. pectenis* | JQ663868 | Zhan *et al.* (2013) | Marine molluscs |
| *T. pseudoheterodentata* | JQ821348 | Tang *et al***.** (2017) | Freshwater fish |
| *T. reticulata* | MG198568 | Wang *et al*. (2018) | Freshwater fish |
| *T. ruditapicis* | FJ499385 | Zhan *et al.* (2009) | Marine molluscs |
| *T. sinipercae* | EF599255 | Gong *et al.* (unpublished) | Freshwater fish |
| *T. sinonovaculae* | FJ499386 | Zhan *et al.* (2013) | Marine molluscs |
| *T. tenuidens* | GU906245 | Irwin *et al.* (2017) | Euryline fish |
| *T. truttae* | LC186029 | Mizuno *et a*l. (unpublished) | Freshwater fish |
| *T. uniforma* | HQ407383 | Tang *et al*. (2013) | Freshwater fish |
| *T. unionis* | KY596041 | Irwin *et al*. (2017) | Freshwater molluscs |
| *T. unionis* | MN08236 | Wiroonpan and Purivirojkul (2019) | Freshwater molluscs |
| *Urceolaria korschelti* | JQ663870 | Zhan *et al*. (2013) | Marine molluscs |
| *U. parakorschelti* | KP698205 | Irwin and Lynn (2015) | Marine molluscs |
| *U. urechi* | FJ499388 | Zhan *et al*. (2009) | Marine molluscs |

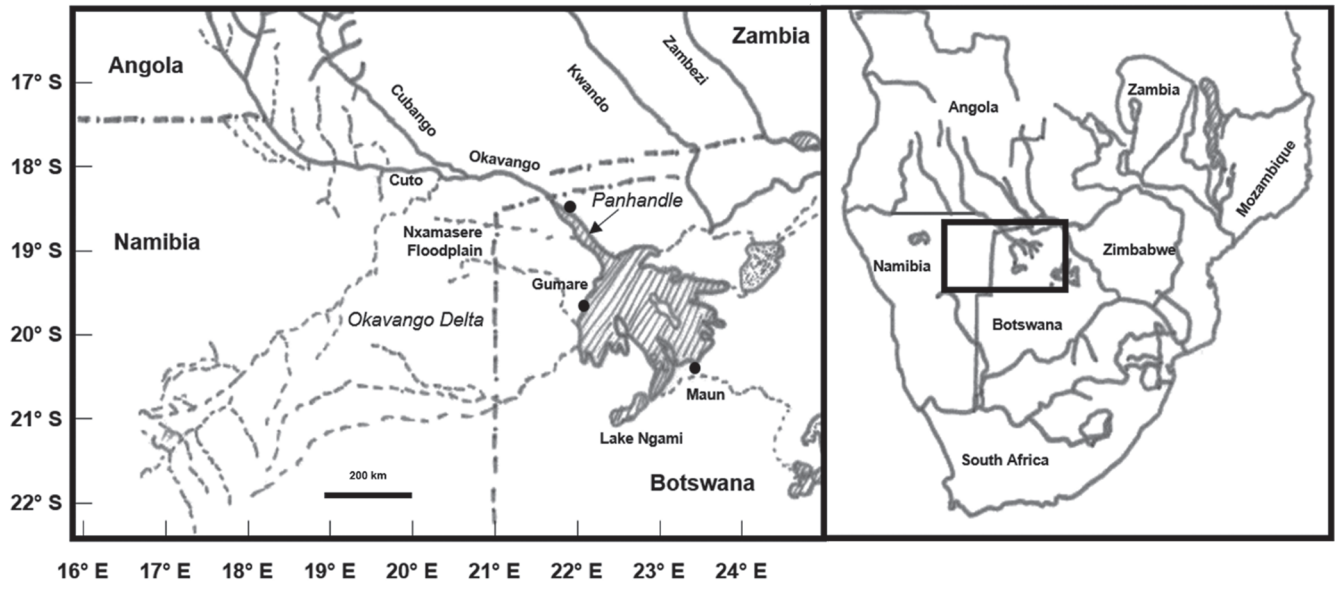
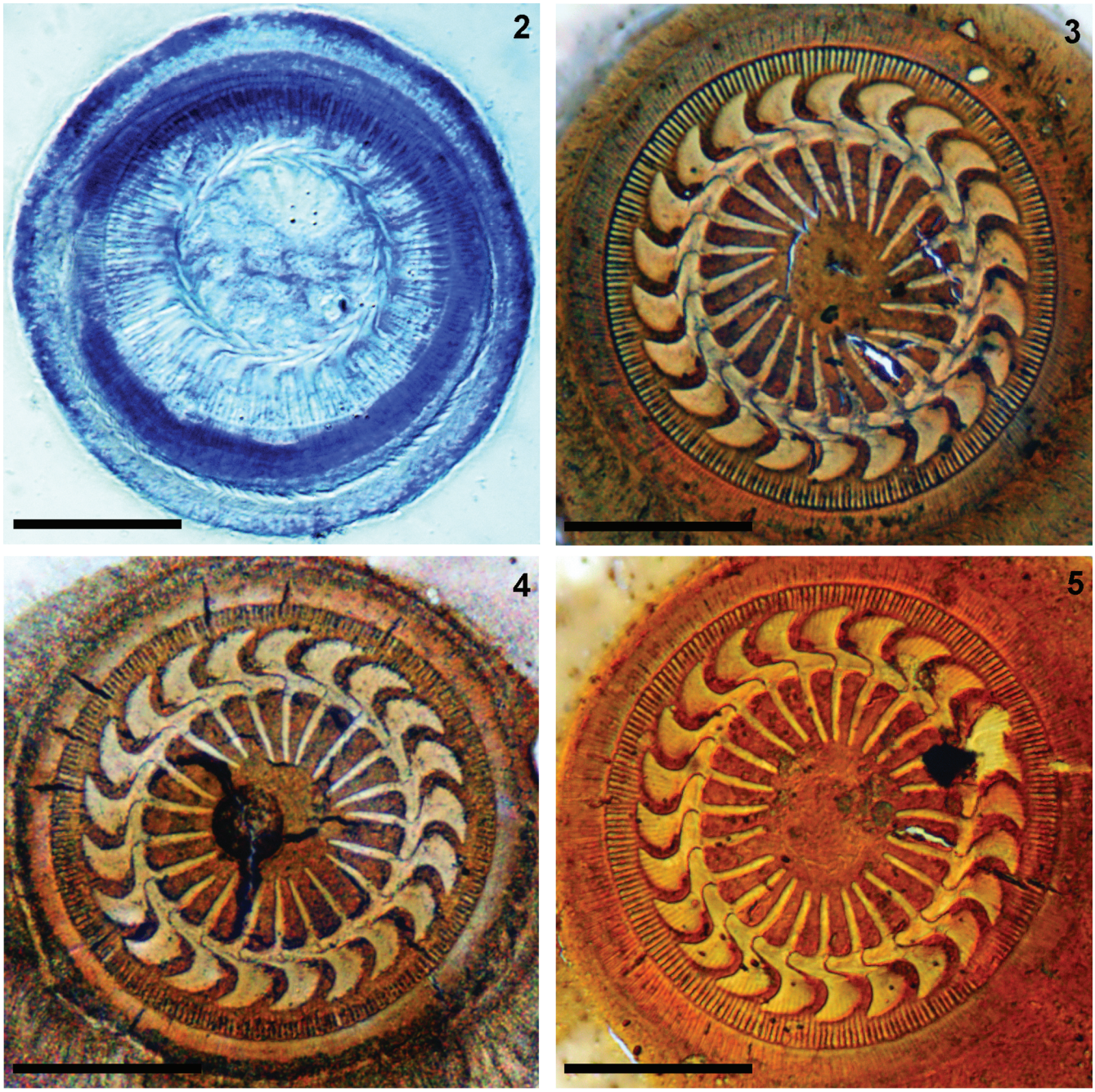



**Fig. 1.** Map of the Okavango River System in southern Africa, including the Nxamasere Floodplain where *Sclerophrys gutturalis* (Power, 1923) and *S. poweri* (Hewitt, 1953) were collected (redrawn and adapted from West *et al.* 2015) (scale = 200 km).




**Figs 2–5.** Micrographs of representative *Trichodina koloti* sp. nov. specimens from each of the six populations measured from the Nxamasere Floodplain; **2** – Haematoxylin stained nuclear material; **3–5** – collected from the skin and gills of *Sclerophrys gutturalis* (Power, 1927) (scale = 10 µm).

21.3–35.3 µm, consisting of 20–28 denticles and 8–13 radial pins per denticle. The denticle blades are strong, semi-circular with a prominent apophysis on the anterior side with a length from 4.3–7.1 µm, tapering off towards a pointing tip. The ray of the denticle is strong, generally straight and tapers off towards the tip, it has a length ranging from 4.5–7.4 µm. The central part width is from 1.2–2.7 µm and the total denticle span is between 5.9–16.0 µm. There is little to no biometric variation between trichodinids collected from the different pools and anuran hosts, also not for seasonality (Figs 3–9).


**Figs 6–9.** Micrographs of representative *Trichodina koloti* sp. nov. specimens from each of the six populations measured from the Nxamasere Floodplain; **6**–**8** – collected from the skin and gills of *Sclerophrys gutturalis* (Power, 1927); **9** – collected from the skin and gills of *S. poweri* (Hewitt, 1935) tadpoles during the 2016 winter (July to August) expedition (scale = 10 µm).

By analysing the denticle dimensions for the Botswanan tadpole ecto trichodinids, according to the van As and Basson (1989) method (Figs 10a–f): The blade region was the most constant with minor differences between the specimens examined. Almost all had large, broad blades filling a large part of the section between the y and y+1 axes with the tangent point being slightly more proximal than the distal blade margin. The distal blade margin (surface) was generally curved, gradually sloping towards the proximal direction and parallel to the border membrane. Most of the posterior blade margins (surface) were smoothly curved in a shallow L-shape,

with a few exceptions that were more deeply curved. In all the specimens the deepest point of the curve was more proximal than the apex of the blade, although some were almost on the same plain. The apexes of the blades were generally rounded, with some being slightly more pointed, and most of them extended past the y+1 axis. The anterior blade apophysis had slightly varying degrees of prominence, with the majority of specimens being somewhat prominent. No posterior projections on any specimens were observed. The central parts were slender and elongated. The distal surface was smaller, sloping more than the proximal. For the majority of the specimens the central part extended halfway past or more than halfway to the y axes. The form of the ray was mostly delicate. Almost all rays were of straight and equal width for the whole length, ending in a blunt tip. The majority of rays touched or ran parallel to the y–1 axis, in some specimens (Figs 10a, b) the rays were angled in a posterior direction, extending past the y–1. In most cases a delicate ray apophysis was present and in some specimens the ray apophyses were slightly more prominent (Figs 10b, c). The ray connections of the examined specimens were either marginally narrower than the width of the rest of the ray or of equal thickness of the ray. The ratio of the denticle above and below the x axis fell in a range between 0.5 and a maximum of 0.74, with most of the ranges clustering around 0.54.

**Remarks**. All tadpole trichodinid populations presented by Arthur and Lom (1984), Kruger *et al*. (1993a), Dias *et al*. (2009), Pala *et al*. (2018) and the populations from the current study show similarities in the biometrical data (Tables 4 and 5) and denticle shape (Figs 10 and 11). All of the above-mentioned populations represent the same species and based on the comparative similarities the denticle plan of this typical tadpole trichodinid is as follows:

Rays are always delicate and of equal thickness throughout the length of the ray.

Ray connections are thin, of the same thickness or slightly narrower than the rest of the ray.

Rays all terminate in rounded points.

The y axes' relationship to the rays vary from touching the complete proximal side of the ray (Figs 11c, d, f) to running parallel, but not touching the ray (Figs 10 c, e, f; 11a, b, e). Variation noted in some individuals of the Botswana population includes rays angled posteriorly where some cross the y axes (Figs 10a,b) and angled anteriorly where some touch the y axes (Fig. 10e). However in all cases, the ray connection and base of the ray never cross the y axes posteriorly.

The central parts of all these trichodinid populations are narrow, elongated and of equal width throughout.

The base of the central part is of the same width as the central part for all populations. Posterior projections are observed in the population described by Pala *et al*. (2018) (Figs 11e, f), however, the above mentioned characteristics for the central part still apply.

The posterior termination of the central part is distinctly rounded.

In the majority of cases, bar the population of Kruger *et al*. (1993a) (Fig. 11c) the central part leans in a proximal direction.

In contrast, the typical denticle plan for *T. hypsilepis* from fish hosts is as follows:

Rays are always robust and never of equal thickness throughout the length of the ray (Figs 12a–f).

Ray connections vary from broad, narrowing after the connection (Figs 12a, c, f) and ray connection is well developed, but still narrower than the base of the ray (Figs 12b, e). Both of these characteristics can sometimes be observed in the same individual, but in different denticles (Fig. 12d).

Rays all narrow perceptibly along their length ending in a narrow/sharp point.

The y axes' relationship to the rays vary from touching the complete proximal side of the ray (Figs 12b, c, f), running parallel, but not touching the ray (Figs 12a, d); to running though the midsection of the ray (Fig. 12e). Again, these characteristics can vary in different denticles of the same individual (Figs 12d, e, f).

The central parts of all *T. hypsilepis* populations are squat, varying in shape.

The base of the central part is always significantly wider than the central part proper.

The central part in the majority of cases is triangular.

In the majority of cases, bar the population of Pala *et al*. (2018) (Fig. 12e) the central part exhibits no slant.

**Molecular phylogeny**

Because phylogenetic inferences from ML and BI analysis were very similar, an amalgamated tree was constructed based on the ML tree (Fig. 13). The consensus sequence from the current study illustrates that *T. koloti* sp. nov. from Botswana clustered with strong support (95%) in the ML tree and even stronger support (100%) in the BI tree with the *T. hypsilepis* sequence deposited by Gong *et al*. (unpublished), while both these sequences form a fully supported clade (100% for both trees) with *T. bellotti*, as deposited by Marcotegui *et al*. (2018). The statistical p-values (genetic distances)

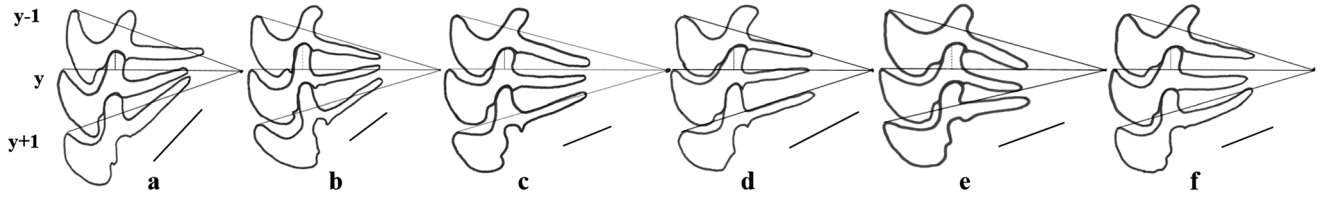
**Table 3.** P-values for 18SSU sequences for representative cloned inserts from trichodinids collected from different localities and hosts in the Nxamasere Floodplain, modelled for *Trichodina heterodentata* Duncan, 1977 and *T. hypsilepis* Wellborn, 1967 sequences obtained from the NCBI database (p > 0.05 is significantly different). (**T. heterodentata* as synonym *toT. hypsilepis; ***T. hypsilepis* as synonym to *T. koloti*)

| Tadpole host | Population | *T. heterodentata** | *T. hypsilepis*** |
|---|---|---|---|
| *Sclerophrys gutturalis* | NX4 | 0.091 | 0.002 |
| *S. gutturalis* | NX5 | 0.093 | 0.004 |
| *S. gutturalis* | NX 6 | 0.091 | 0.002 |
| *S. powerii* | NX 6 | 0.090 | 0.001 |

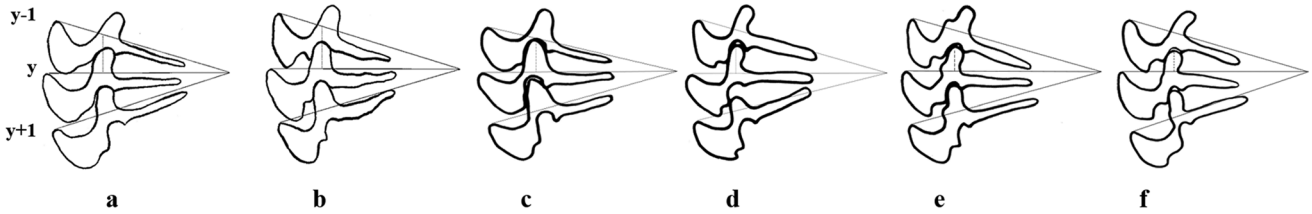
**Table 4.** Biometrical data (in µm) of all published tadpole host populations of *Trichodina hypsilepis* Wellborn, 1967 (*syn. *T. heterodentata* Duncan, 1977), *Trichodina koloti* sp. nov. (**syn. *T. hypsilepis* Wellborn, 1967) and *T. koloti* (ADD – Adhesive disc diameter, BD – Body diameter, BMW – Border membrane width, CL – Collection locality, DBL – Denticle blade length, DCPW – Denticle central part width, DL – Denticle length, DRD – Denticle ring diameter, DRL – Denticle ray length, DS – Denticle span, HS – Host species, LoH – Location on host, n – population size, nD – Number of denticles, nRP/D – Number of radial pins per denticle) (*pertains to the mode, rather than the mean).

| | *Trichodina hypsilepis* Wellborn, 1967* | | | *Trichodina koloti* sp. nov.** | *Trichodina koloti* sp. nov. |
|---|---|---|---|---|---|
| | Kruger *et al.* (1993a) | Dias *et al.* (2009) | Pala *et al.* (2018) | Arthur and Lom (1984) | Current study |
| CL | South Africa | Brazil | Brazil | Cuba | Botswana (Nxamasere 4) |
| LoH | Skin and Gills | Skin | | Skin | Skin and Gills |
| HS | *Xenopus laevis laevis* | *Rhinella pombali* | *R. schneideri* | *Unknown tadpole* | *Sclerophrys gutturalis* |
| BD | – | (55.1) | 38.6–59.3 (54.5±6.2) | 55.1–85.7 (67.4 ± 6.9) | 47.0–57.4 (53.3 ± 2.6) |
| ADD | 41.1–64.3 (49.3±5.6) | 38.9–60.0 (49.8±5.3) | 39.5–54.1 (48.0±5.4) | 39.8–56.1 (47.6 ± 3.9) | 40.4–48.2 (44.7 ± 2.2) |
| DRD | 24.4–41.2 (31.4±3.8) | 24.6–33.2 (29.2±3.2) | 25.0–32.3 (30.3±2.5) | 25.5–34.2 (29.4 ± 2.3) | 24.5–32.4 (28.0 ± 1.8) |
| DL | 7.4–9.9 (8.4±0.8) | 5.4–8.7 (6.9±0.7) | 5.9–8.6 (6.5±1.1) | 10.2–14.8 (12.4 ± 1.1) | 5.7–7.8 (6.7 ± 0.5) |
| DBL | 3.9–6.2 (5.1±0.6) | 3.0–5.2 (4.2±0.4) | 4.0–6.2 (4.4±0.7) | 4.5–5.6 (5.2 ± 0.8) | 5.0–6.6 (5.7 ± 0.4) |
| DRL | 5.9–9.2 (7.2±0.8) | 4.9–8.8 (6.7±0.7) | 1.3–8.6 (6.2±0.9) | 6.6–11.2 (8.2 ± 0.8) | 5.3–8.0 (6.7 ± 0.6) |
| DCPW | 1.8–3.3 (2.6±0.4) | 2.03–3.72 (2.7±0.3) | 2.0–3.1 (2.1±0.3) | 2–3.1 (2.5 ± 0.4) | 1.4–2.3 (1.9 ± 0.2) |
| DS | 11.6–18.7 (14.9) | 10.4–14.6 (12.5) | 10.2–13.3 (12.0±0.8) | 14.3–19.4 (15.9 ± 1.0) | 12.0–14.8 (13.3 ± 0.7) |
| BMW | 3.5–6.1 (4.7) | 2.9–5.1 (4.2±0.4) | 3.2–7.4 (4.3 ±0.7) | 4.1–6.1 (4.9 ± 0.5) | 3.1–5.7 (4.5 ± 0.7) |
| nD | 21–25 (24*) | 19–24 (22*) | 19–23 (22*) | 20–23 (21.0 ± 0.8) | 20–24 (22* ± 1) |
| nRP/D | 7–13 (11*) | 7–10 (7*) | 7–11 (8*) | 9–12 (commonly 10–11) | 10–12 (11* ±1) |
| n | 35 | 50 | 60 | 25 | 25 |

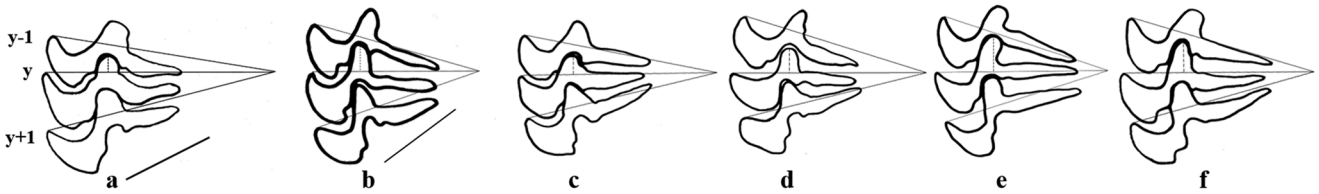
calculated for the number of base pair differences per site on the above mentioned sequences falls between 0.001 and 0.004 for *T. koloti*, while substantially larger when compared with *T. hypsilepis* (syn. *T. heterodentata)* from fish hosts (Table 4).




**Figs 10a–f.** Denticle dimensions, as by van As and Basson (1989, 1992), of *Trichodina koloti* sp. nov. representatives from six different populations collected at the Nxamasere Floodplain, where **a**–**e** from *Sclerophrys gutturalis* (Power, 1927) and **f** – *S. poweri* (Hewitt, 1935) tadpoles during the 2016 winter (July to August) expedition (scale = 10 µm).




**Figs 11a–f.** Denticle dimensions, as proposed by van As and Basson (1989, 1992), of *Trichodina koloti* sp. nov. as recorded by and redrawn from: **a** and **b** – Arthur and Lom (1984), **c** – Kruger *et al*. (1993a), **d** – Dias *et al*. (2009), **e** and **f** – Pala *et al*. (2018).




**Figs 12a–f.** Denticle dimensions, as proposed by van As and Basson (1989; 1992), of *Trichodina hypsilepis* Wellborn, 1967 (syn. *T. heterodentata*) as recorded by and redrawn from: **a** – Population A of Duncan (1977), **b** – Population B of Duncan (1977), **c** – from van As and Basson (1989), **d** – from Tang and Zhao (2007), **e** – from Pádua *et al*. (2012), **f** – from Valladão *et al*. (2013).

## DISCUSSION

Arthur and Lom (1984) described *T. hypsilepis* from unknown tadpoles in Cuba, comparing it to the description of the same species by Wellborn (1967) from a freshwater fish, the highscale shiner (*Notropis hypsilepis* Suttkus and Raney, 1955), even though these authors commented on similarities with *T. heterodentata* in the same article. When Duncan (1977) described *T. heterodentata* from cichlid hosts, he either did not know about the publication of Wellborn (1967), an unlikely possibility, or decided that because of the large difference in size (both body and adhesive disc diameter) between his populations and those of Wellborn (1967), *T. heterodentata* is a separate species, even though denticle morphology appears strikingly similar. The similarities of three of Wellborn's (1967) species, *T. hypsilepis* being one of them, and Duncan's (1977) *T. heterodentata* have been discussed and it was proposed that all teleost hosted *T. heterodentata*-like species become a synonym to *T. hypsilepis* (de Jager and Basson 2020). However, *T. heterodentata*-like mobilines have also been described and recorded from anuran tadpoles in the Southern Hemisphere and originally described as *T. heterodentata* (Kruger *et al*. 1993a, 1993b, 1995;

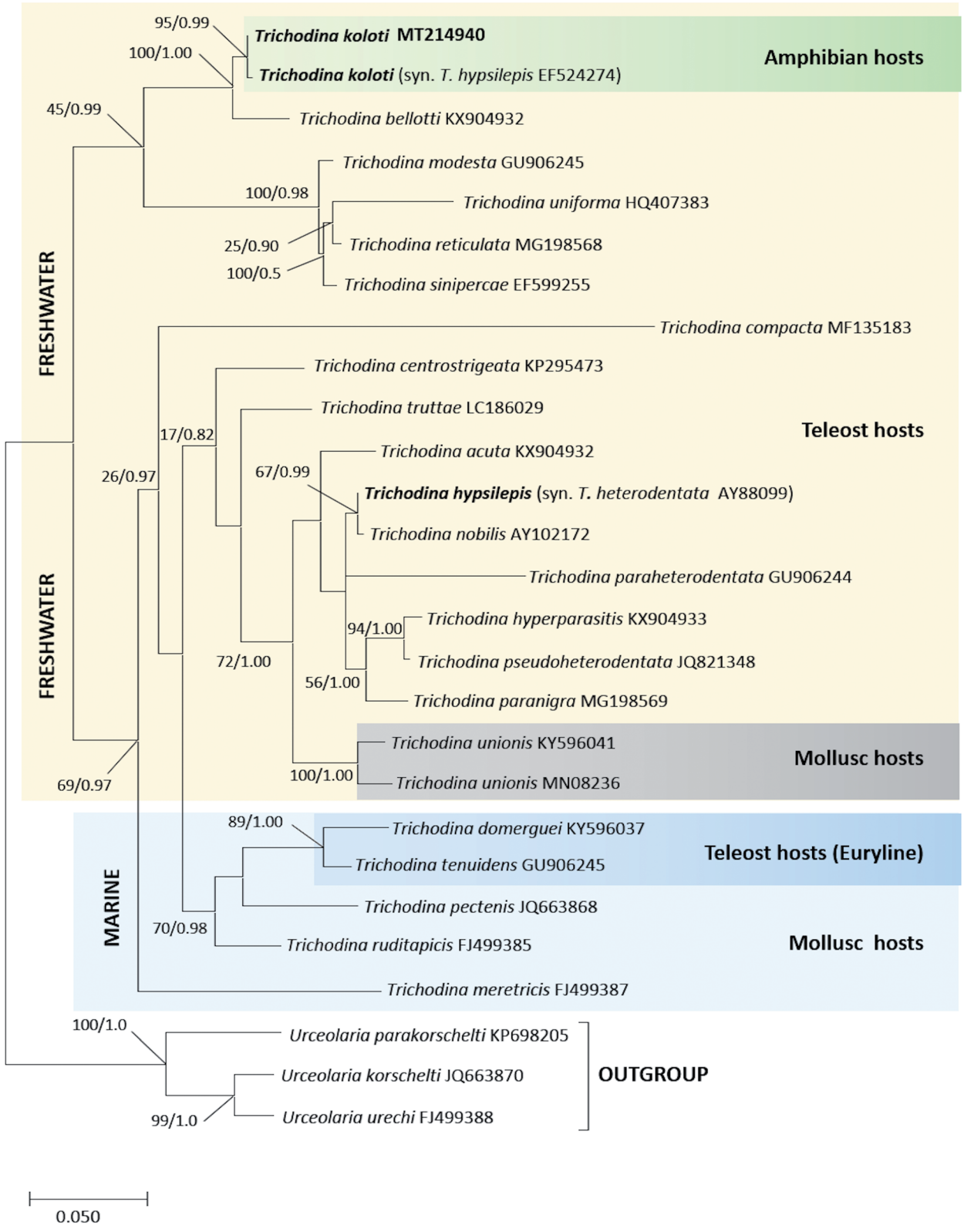



**Fig. 13.** Unrooted Maximum-likelihood (ML) consensus tree inferred from 18S SSU rDNA sequences illustrating the phylogenetic position of *Trichodina koloti* sp. nov. (at the top of the tree in **bold**), derived from 2551 nucleotide positions. Support values at the nodes are for bootstrap values/Bayesian posterior probabilities (ML/BI). Synonyms for both *T. koloti* and *T. hypsilepis* Wellborn, 1967 are indicated in brackets.

Dias *et al*. 2009; Fernandes *et al*. 2011; Pala *et al*. 2018). Even though the morphological similarities between the *T. heterodentata*-like trichodinids from tadpole and *T. hypsilepis* seem prominent, these similarities are restricted to the blade. Significant differences in the rest of the denticle features for the new taxon *Trichodina koloti,* are identified that are clearly distinctive: The rays of *T. koloti* are always delicate and of equal thickness ending in rounded points, while *T. hypsilepis* exhibits robust rays, not of equal thickness throughout, tapering towards a sharper tip. *Trichodina koloti* is characterised by narrow, elongated central parts of equal width with a round termination. The central parts of *T. hypsilepis*, on the other hand, is squat and triangular. All three of the denticle elements (blade, central part and ray) are robust for *T. hypsilepis*, whereas the central parts and rays of *T. koloti* are delicate and slender.

Denticle ray thickness was not included by Lom (1958) as part of his 15 characteristics for morphologic description, but could conceivably be an important characteristic to examine for future morphometric analysis if working within a species complex or with cryptic species. Another characteristic to look into is the proportional comparison of all three the denticle elements, i.e. *T. koloti* seems to have a larger blade than *T. hypsilepis*, although in fact, they of similar proportions. This misconception is due to the smaller proportions of the central part and ray for *T. koloti*.

This new taxonomic arrangement also shows these two species, *T. hypsilepis* and *T. koloti*, being more host specific than previously perceived. By comparing the morphological measurements of the ectozoic trichodinids found by Arthur and Lom's (1984) from unidentified Cuban tadpoles (Table 4), *T. heterodentata* from *Rhinella pombali* (Baldissera, Caramaschi and Haddad, 2004) tadpoles from Brazil (Dias *et al*. 2009, Fernandes *et al.* 2011), and on *Xenopus laevis laevis* (Daudin, 1802) (Kruger *et al.* 1993a) (Table 4), with those from the southern African *Sclerophrys* species, it is suggested that they are all in fact *T. koloti*.

The tadpole populations in the current study show little biometric variation between trichodinids collected from the different pools and different anuran hosts (Table 5). All biometric data fall into the size category for *T. koloti* (previously recorded as *T. heterodentata* Group III from southern African and South American anuran tadpole hosts), including the *T. hypsilepis* record from Cuba (Table 4). Despite the fact that mobilines from two different anuran host species (Figs 8 and 9), during alternating seasons were examined, these specimens illustrated uniformity with very little variation. *Trichodina koloti* appears to be morphologically more consistent than *T. hypsilepis* from fish hosts (de Jager and Basson 2019), lending more weight to the suggestion that this species cannot be *T. hypsilepis*, as one of *T. hyspilepis'* key features is that this is a highly variable species.

Morphology and morphometrics alone support the taxonomic distinction between *T. hypsilepis* and *T. koloti*, as proposed in this study. Nevertheless, where morphological distinction between species is not obvious or taxonomic doubt is still present, molecular analysis becomes inevitable. The molecular analysis of the populations in the present study indicates that the Botswana tadpole-hosted trichodinids all represent the same species, furthermore they form a robust clade around the NCBI deposited sequence of *T. hypsilepis* (accession number: EF524274) (which is now considered to be *T. koloti*), also from an amphibian host (deposited, but not published by Gong *et al.* (unpublished)) (Figure 13). Tang and Zhao (2016) proposed that for 18S rDNA data the minimum and maximum genetic distances (p-values) should be between 0.000–0.005 for intraspecific level, 0.005–0.15 for genus-species levels and higher than 0.15 for family level, of which the specimens from the present study falls exactly in the genetic distances for intraspecies level (Table 3). The genetic distance indicates that *T. koloti* (the tadpole trichodinid) and *T. heterodentata* (accession number: AY88099) (the fish trichodinid now considered *T. hypsilepis*) are genetically not closely related, despite superficial similarities, which may be a consequence of host predilection. The final piece in this taxonomic puzzle must be that a southern African population of *T. hypsilepis* (from African cichlid hosts) be analysed molecularly and compared. This has proven problematic thus far, as it is known that *T. hypsilepis* normally occurs in multispecies infestations under African conditions.

Accepting the above mentioned hypothesis regarding "*T. heterodentata*"-like trichodinids, the final conclusion is that there are two distinct species from different host assemblages; the morphologically variable *T. hypsilepis* limited to freshwater teleost hosts and the more uniform *T. koloti* from freshwater anuran tadpoles. The following trichodinid populations are synonyms for *T. koloti*: *T. hypsilepis* Wellborn, 1967 from Arthur & Lom 1983, from Gong *et al*. unpublished; *T. heterodentata* Duncan, 1977 from Kruger *et al.* 1993a, 1993b; from Dias *et al.* 2009; from Pala *et al*. 2018.

Compliance with ethical standards Ethical clearance obtained from the University of the Free State (ethical clearance number: UFS-AED-2017/0017).

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

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