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Comparative exploration of the morphological plasticity of *Trichodina centrostrigeata* (Peritrichia: Mobilida), ectoparasite from the gills of two tilapia species (*Oreochromis niloticus* and *O. mossambicus*) in a global context

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Abstract: *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983 from *Oreochromis mossambicus* (Peters) and *O. niloticus* (Linnaeus) from different host populations from Argentina, Mexico and South Africa was reviewed. Although *T. centrostrigeata* has a distinct denticle structure that makes morphological taxonomic inferences uncomplicated, variation of the denticles within and among individuals and populations were still observed. While traditional taxonomy of mobilines is heavily reliant on morphometrics, and recently even more so on molecular analysis, this paper proposes the use of geometric morphometry, specifically elliptical Fourier analysis, to address morphological conflicts that arise when comparing different populations. By applying this technique, combined with traditional taxonomy, it was found that *T. centrostrigeata* in this study can be grouped into two separate morphotypes, the first (type *a*) from aquaculture farms in Argentina and Mexico and the second (type *b*) from a natural habitat in Glen Alpine Dam, South Africa. This study supports the validity of geometric morphometry as an additional technique to distinguish not only between species but also evolutionary plasticity of the same species from different localities and habitats.

Keywords: Ciliates, geometric morphometry, Fourier analysis, Trichodinidae, morphology

Trichodinid ciliates are common parasites or symbionts of aquatic invertebrates and vertebrates (Van As and Basson 1989). These ciliates are among the main groups of fish parasites (Valladão et al. 2013), affecting the skin, fins, gills, and less frequently, the eyes, mouth, gastrointestinal tract, urinary tract and gonads (Van As and Basson 1987, Noga 2010, Valladão et al. 2014). Around 40 species from the family Trichodinidae have globally been reported from cichlid hosts (Islas-Ortega et al. 2020), among these, *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983, is considered to have an association with cichlid fishes, especially tilapia (Van As and Basson 1989, Paperna 1991, Valladão et al. 2016, Abdel-Baki et al. 2017). Some of these mobilids have been introduced into new areas through the anthropogenic dispersal of cichlids worldwide (Van As and Basson 1989, Valladão et al. 2016).

Traditional taxonomy based on the analyses of morphological features has been a fundamental tool to distinguish between trichodinid species. However, these studies could be hampered by the high degree of variation that denticles present within a population of some species, or even within the same individual (Marcotegui et al. 2018). Traditional trichodinid species' delimitation is mainly based on the dimensions and shape of denticles in the adhesive disc (Van As and Basson 1989). However, some authors such as Tang et al. (2017), Islas-Ortega et al. (2018), Marcotegui et al. (2018) and Wang et al. (2019) considered this alone as insufficient, leaving several unresolved taxonomic issues, such as the overlapping of morphological features of distinctly different species, or *vice versa*, an overestimation of the diversity caused by the misinterpretation of small differences in shape or size, or the developmental stage of the individual mobilid.

In recent years, molecular identification has helped to distinguish between remarkably similar species. However, in many cases, multiple infections occur on the same host, and often the different species are morphologically similar, making accurate isolation challenging. In this sense, the

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Table 1. Information of samples of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983. Acronyms of scientific collections: CRPH– Colección de Referencia Parásito-Hospedero, Laboratorio de Zoología Acuática, Facultad de Ciencias, UNAM, Mexico. Catalogue number provided refers to single slide considered as representative of the population.

Locality	Host	No. specimens analysed	Scientific Collection
Aquaculture farm, San Vicente, Buenos Aires, Argentina	*Oreochromis niloticus*	20	CRPH-C0009
"El Pescadito" farm, Hudacareo, Michoacán, Mexico	*Oreochromis niloticus*	20	CRPH-C0008
Glen Alpine dam, South Africa	*Oreochromis mossambicus*	20	Laboratory of Aquatic Ecology and Parasitology, UFS, Bloemfontein, South Africa
Luphephe dam, South Africa	*Oreochromis mossambicus*	20	Laboratory of Aquatic Ecology and Parasitology, UFS, Bloemfontein, South Africa

use of the geometric morphometry arises as an alternative method to provide information regarding changes in the shape of the anatomical structures, independently of the size of the analysed individual, and can be used in cases with multiple infections (Marcotegui et al. 2018). This can be very helpful when endeavouring to quantify variation in species that have large size ranges, e.g., as presented by de Jager and Basson (2019) for *Trichodina hypsilepis* Wellborn, 1967.

Marcotegui et al. (2018) differentiated between trichodinid species for the first time using geometric morphometry through elliptical Fourier analysis (EFA), which quantitatively describes the variation of denticle shapes by using a Fourier transformation of a denticle outline to obtain a set of quantitative variables (called harmonics), each described by four coefficients (Bonhomme et al. 2014). The foundation of the EFA is to separate the *x* and *y* coordinates of the denticle outline and calculate a discrete Fourier series based on these two periodic functions using the number (*n*) of harmonics high enough to capture the satisfactory geometry of the described shape (Bonhomme et al. 2013). These coefficients may then be subjected to multivariate statistical methods, primarily principal component analysis (PCA), which is a statistical method that reduces the number of variables (dimensions) without much loss of information. Moreover, EFA combined with PCA allows visualisation and reconstruction of shapes via inverse Fourier transformation, a powerful tool when considering the potential taxonomic significance of the shape (Tomaszewski and Górzkowska 2016).

Geometric morphometry is a promising alternative to address taxonomic conflicts where samples for molecular biology are not available. Since the ectoparasite *T. centrostrigeata* has a wide distribution due to the introduction of their host (tilapia) to numerous places of the world, this study aimed to quantitatively determine the morphological variation of *T. centrostrigeata* from this species' original region of occurrence (South Africa) and two new regions of distribution, i.e., Mexico and Argentina. This variation, which has been modified anthropogenically by recent introductions of tilapia into new geographical habitats, was revealed by using the novel approach of the EFA.

MATERIALS AND METHODS

Taxonomic morphometrics and denticle description

Four populations of *Trichodina centrostrigeata* collected from the gills of *Oreochromis niloticus* (Linnaeus) from Argentina (San Vicente aquaculture facility) and Mexico (Huandacareo aquaculture facility), and *Oreochromis mossambicus* (Peters) from South Africa (Glen Alpine and Luphephe Dams, both in the Limpopo River drainage system) were measured, using the uniform characteristics initially proposed by Lom (1958) and later adapted by Van As and Basson (1989), both based on silver-impregnated specimens. Taxonomic identification of these populations of the *T. centrostrigeata* from Argentina and Mexico was reported by Islas-Ortega et al. (2020), and South African specimens by Basson et al. (1983). Micrographs of *T. centrostrigeata* specimens were obtained using Leica DM500® photomicroscope equipment, with Leica ICC50 HD® capture imaging system, a microscope Olympus DP71, and a Zeiss Axiophot compound microscope fitted with an AxioCam ICc 5 digital camera. The measurements of the populations were acquired from the micrographs, using ZEN core v3.1 imaging software. All morphometric measurements are provided in μm and represented as minimum to maximum (mean ± standard deviation), for the number of denticles and number of radial pins per denticle the mode was used rather than the arithmetic mean. Body diameter is determined as the adhesive disc plus the border membrane. Denticles were described using the method devised by Van As and Basson (1989).

Analysis of geometric morphometry

Analyses were based on the outlines (silhouettes) of 20 randomly selected *T. centrostrigeata* denticles per population for each of the four localities. A summary of the collection data is given in Table 1. One denticle per individual trichodinid was used for the analyses and the silhouette of the denticle was then drawn from micrographs using GIMP (2.10.2) editing software.

Elliptical Fourier analysis (EFA) and statistical analyses

Elliptical Fourier analysis was done through RStudio (1.3.1056) (RStudio Team 2020) using the Momocs package (version 0.2-01), dedicated to shape analysis (Bonhomme et al. 2014). Silhouettes were imported into RStudio and denticle outlines were extracted into a "Coo" class object as a list of x; y pixel coordinates. The pixel coordinates were obtained using an algorithm by Claude (2008) extracted from a black denticle silhouette on a white background. An EFA was performed on 80 denticle outlines. Before performing elliptical Fourier descriptor analysis, an estimation of the number of harmonics to perform was calculated using the *hquant* function. The optimal number of harmonics to reach approximately 99% of the maximum calculated Fourier power was determined from the relationship between the harmonic numbers and their cumulated power (Fourier power spectrum). Qualitative analysis of the ability for different numbers of harmonics to recapitulate the original shape outline

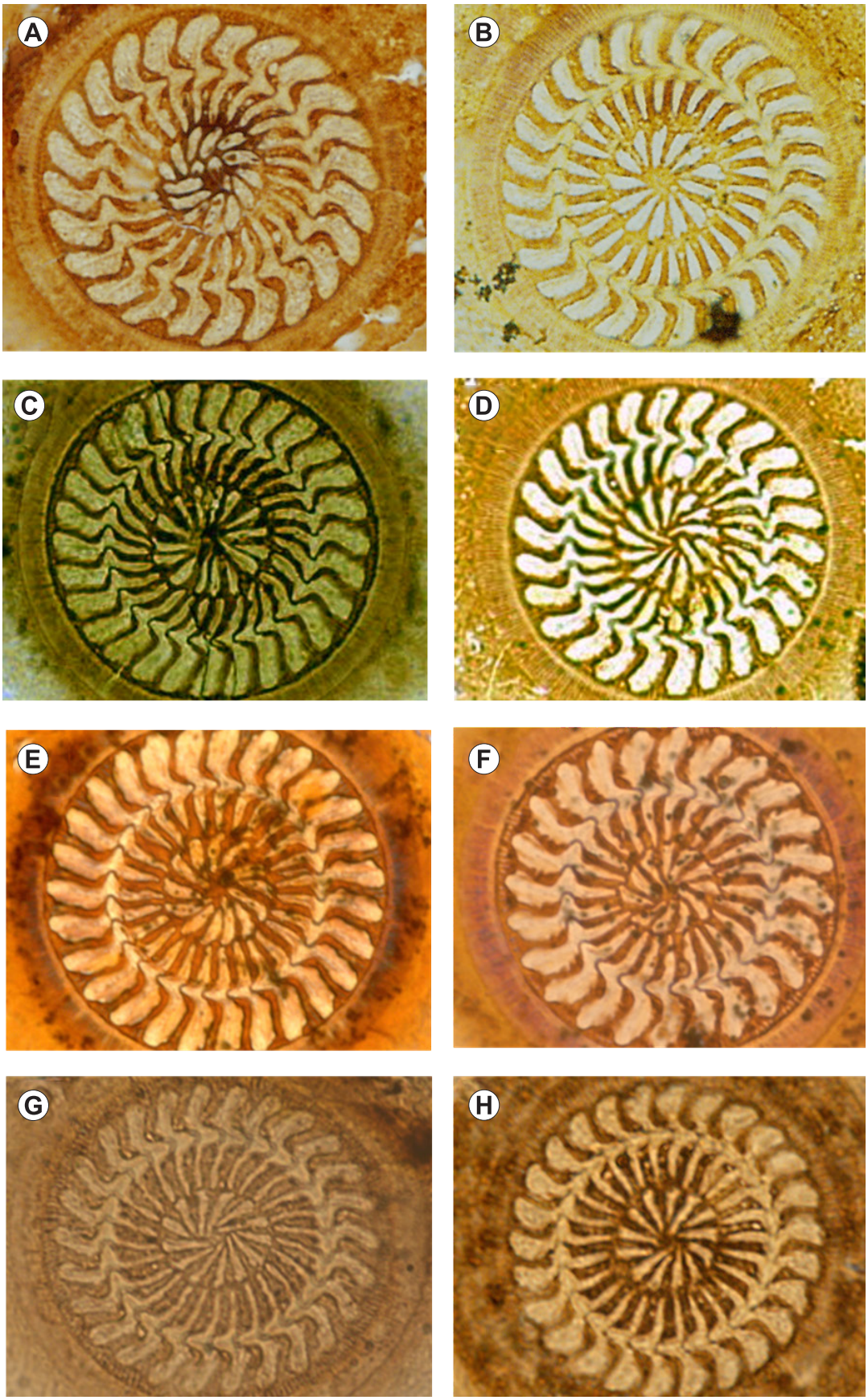

Fig. 1. Micrographs of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983 from *Oreochromis niloticus* (Linnaeus), San Vicente, Argentina (A, B) and Huandacareo, Mexico (C, D), and from *O. mossambicus* (Peters), Luphephe Dam, South Africa (E, F) and Glen Alpine Dam, South Africa (G, H).

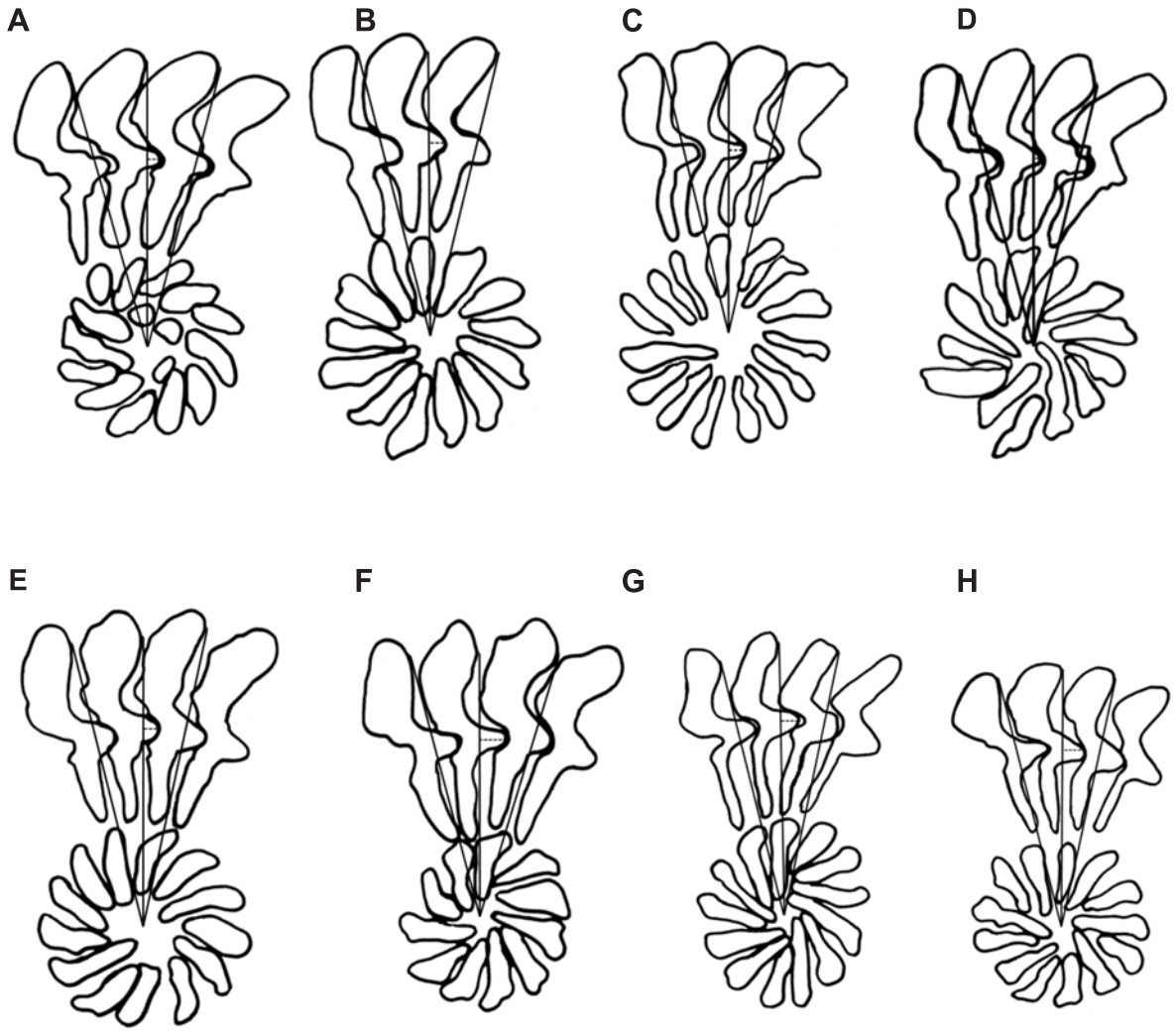

Fig. 2. Diagrammatic drawings of the denticles of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983 from *Oreochromis niloticus*, San Vicente, Argentina (A, B); from *O. niloticus*, Huandacareo, Mexico (C, D); from *O. mossambicus*, Luphephe Dam, South Africa (E,F); and from *O. mossambicus*, Glen Alpine Dam, South Africa (G, H).

was performed using the *hqual* function. Fourier analysis was performed using the *eFourier* function, parameters of the first harmonic were used to normalise coefficients so that they were invariant to size, rotation and starting point of the outline trace. Harmonic coefficients from the resulting "Coe" object were used for subsequent statistical analyses.

All statistical analyses were performed using RStudio (1.3.1056). Principal Component Analyses (PCA) and multivariate analyses of variance (MANOVA) were used to discriminate between shape variations and to test for differences. Linear Discriminant Analysis (LDA) on the harmonic coefficient was performed using the LDA function.

RESULTS

Morphological analysis (based on traditional taxonomy): denticle description for all four populations (Figs. 1, 2, Table 2)

Blade shape varies from angular and truncated in most denticles to roundly spatulate in some, where blades vary from broad and flattened head (distal part of the blade) tapering towards central part, to others with narrower head broadening slightly towards central part (these variations often within the same population). In some, anterior and posterior blade edges parallel. Most populations with narrow blades, varying from none to 18%. Distal surface ranges from flattened to slightly rounded, very few with flattened distal edge. Distal surface mostly parallel to border membrane in some specimens, in others definitely not parallel to border membrane. In cases where not parallel, distal surface slopes in anterior direction. Contact surface of tangent point varies, in some forms short line rather than point, tangent point situated lower than distal point. Anterior margin varies from curved to angular, some straight.

Apex prominent, less prominent in specimens with rounded anterior margin than in case of angular and straight forms. In latter cases anterior blade margin shows angular, almost triangular shape with apex, as well as distal and proximal sides of anterior blade. Anterior surface extends beyond y + 1 axis in some individuals; particularly in case of angular shaped denticles; whilst touching y + 1 axis with some close to, but not touching y + 1. Blade apophysis

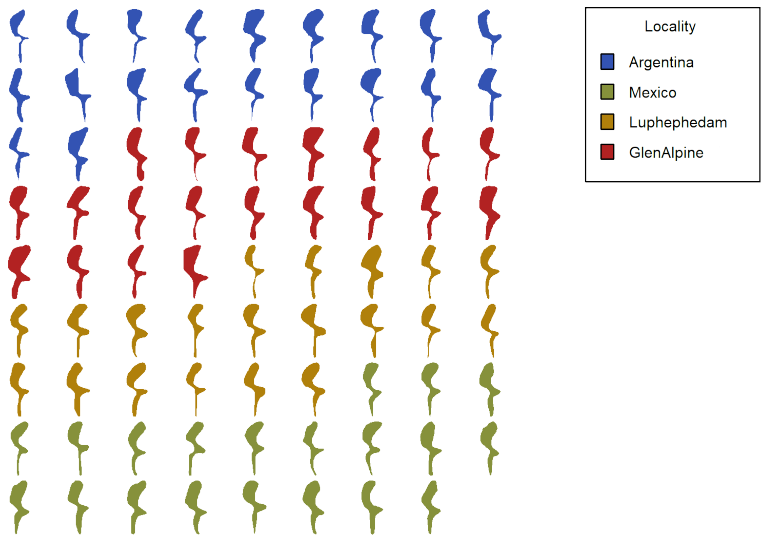

Fig. 3. Denticles silhouettes of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983 utilised with Fourier analysis.

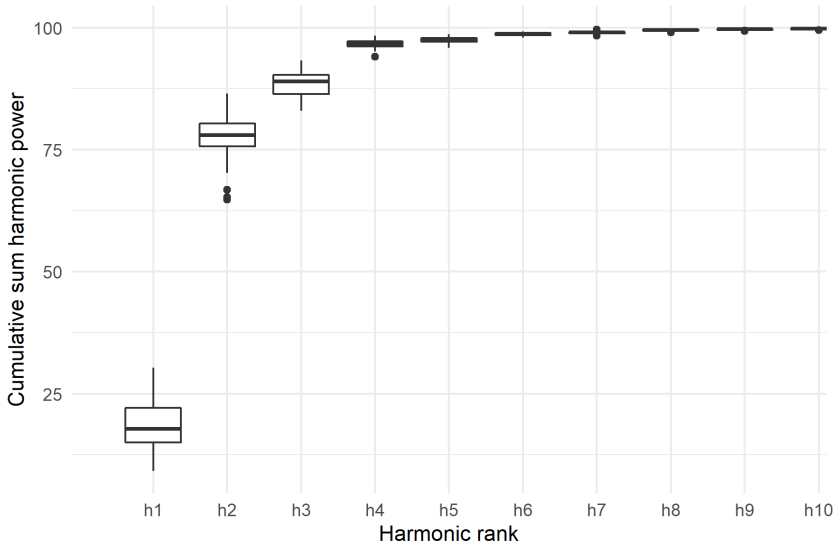

Fig. 4. Fourier harmonic power spectrum based on elliptical Fourier analysis of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983. Harmonics sufficient to reach approximately 99% of the maximum cumulated.

consistently present, never large, but prominent. Posterior margin forms characteristic deep L-shaped curve, varying from shallow to deep, with deepest point at lowest end (adjacent to central part), mostly lower than apex of anterior surface. Posterior projection present, but not prominent in all specimens.

Blade length varies from 1.8–7.5. Blade connection narrow to well developed, but thinner than blade. Central part conical, some longer and narrower than others that are wider and squatter, small number with almost triangular central part. Central part tapering from base to sharply rounded point in some, fitting tightly into preceding denticle. Section above and below x-axis similar in shape. Central part extends less than halfway to y–1 axis in majority, but some halfway to y–1 axis. Central part width 1.0–4.8. Ray connection short, well-developed in some, others of same thickness or slightly thicker than ray.

Ray apophysis present in majority, small, situated high on ray, making contact with tip of central part of following denticle. Rays straight in vast majority of cases, of varying thickness, thinner rays tapering gradually to sharp rounded point, whilst others with broad, well-developed rays ending in rounded tips. Rays run parallel to and between y axes in most cases, some rays directed in anterior direction with tips extending past y+1 axis. Some rays lightly touch y–1 and some y+1 axes. Rays' length 3.7–6.3. Ridged line visible and prominent in blade and rays of many specimens, a typical characteristic of this species.

Ridged line in centre of blade, with both anterior and posterior parts of blade evidently thinner in individuals of some populations. Centre ridges vary in number, collectively from 10 to 17 (mode 13 in all populations); although varying in thickness. Mostly strongly developed, more or less of same thickness as rays. Arrangement of centre ridg-

Table 2. Morphometric comparison of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983 of the type population and the four populations in this study

	Basson et al. (1983)	Basson and Van As (1994)	Present study			
	South Africa	Taiwan	Mexico	Argentina	Luphephe (South Africa)	Glen Alpine (South Africa)
	Pseudocrenilabrus philander	*Oreochromis mossambicus*	*O. niloticus*	*O. niloticus*	*O. mossambicus*	*O. mossambicus*
Body diameter	37.7–54.4 (44.6±3.8; 100)	38.0–51.0 (43.8±4.4; 16)	40.8–56.7 (47.0 ± 4.4; 23)	38.7–50.3 (44.1±2.7; 30)	41.6–55.0 (47.9±3.8; 29)	40.2–51.9 (46.0±3.4; 16)
Adhesive disc	31.2–45.8 (37.6±3.6; 100)	30.0–45.0 (36.0±4.2; 16)	33.2–47.0 (39.2±4.1; 23)	32.3–44.4 (36.9±2.9; 30)	33.4–45.7 (39.2±3.5; 29)	32.1–43.5 (37.8±3.2; 16)
Denticle ring diameter	18.7–33.3 (23.2±2.4; 100)	18.5–26.0 (21.7±2.4; 16)	20.8–28.6 (23.7±2.1; 23	19.9–26.7 (22.7±1.9; 30)	19.8–28.2 (23.3±2.5; 29)	20.3–27.3 (23.2±2.1; 16)
Border membrane	2.0–4.4 (3.4±0.4; 100)	3.0–4.0 (3.6±0.5; 16)	2.9–4.8 (3.9±0.5; 23)	2.3–5.1 (3.8±0.6; 30)	2.9–5.1 (4.2±0.6; 29)	3.0–5.3 (4.0±0.6; 16)
Blade length	2.8–6.4 (5.2±0.7; 100)	5.0–6.0 (5.5±0.5; 15)	4.7–7.1 (5.8±0.6; 23)	1.8–6.5 (5.2±1.1; 30)	4.5–7.5 (6.2±0.7; 29)	4.2–6.3 (5.2±0.6; 16)
Central width	1.1–3.0 (1.9±0.3; 100)	1.0–1.5 (1.1±0.2; 15)	1.2–2.3 (1.6±0.3; 23)	1.0–4.8 (2.0±0.8; 30)	1.7–2.8 (2.2±0.5; 29)	1.4–2.9 (2.0±0.4; 16)
Ray length	3.2–6.0 (4.5±0.6; 100)	3.5–5.0 (4.3±0.6; 15)	3.7–6.2 (4.8±0.5; 23)	3.8–6.1 (4.6±0.5; 30)	4.3–6.3 (5.0±0.5; 29)	3.8–5.8 (4.9±0.6; 16)
Denticle span	–	10.0–13.0 (11.3±0.9; 12)	10.6–14.0 (12.3±0.9; 23)	9.7–13.5 (11.7±; 30)	11.4–15.6 (13.3±1.0; 29)	10.1–14.1 (12.0±1.2; 16)
Denticle width	3.0–6.2 (4.1±0.6; 100)	3.0–5.0 (4.3±0.6; 13)	3.1–4.7 (4.1±0.4; 23)	2.3–5.2 (3.8±0.7; 30)	3.5–5.0 (4.2±0.5; 29)	3.0–5.0 (3.9±0.6; 16)
Number of denticles	26–30 (28*;100)	24–29 (27*;16)	24–29 (27*; 23)	23–29 (26*; 30)	23–31 (25*; 29)	22–28 (26*; 16)
Radial pins per denticle	6–7 (7*; 100)	7–8 (8*; 7)	7–14 (10*; 14)	8–14 (11*; 26)	8–15 (9*; 29)	9–15 (12*; 15)
Number of central ridges	12–16 (14*; 100)	13–16 (14*; 12)	10–14 (13*; 23)	10–17 (13*; 30)	10–16 (13*; 29)	11–14 (13*; 16)

es not symmetrical but fanning out from roughly centre point. Centre ridges mostly extended rod-shaped, in others rods of varying lengths, some half length of others, and some almost circular structures. Presence of centre ridges constant feature, occurring in immature and adult specimens. Ratio of denticle above x-axis to section below vary slightly among populations, majority slightly more than one (1.1–1.4). In one population minimum ratio just less than one (0.9).

The morphometrics of each population, including the type population from *Pseudocrenilabrus philander* (Weber) as reference, are provided in Table 2.

Analysis of geometric morphometry (EFA)

The Elliptical Fourier Analysis represents a valid method to compare the shape of denticles and found significative differences among populations. In this study, the analysis provides the evidence of the morphological similarities among some populations, but also exhibits statistical differences for one population in relation with others. Figure 3 presents the 80 denticle silhouettes of *Trichodina centrostrigeata* from four populations (20 silhouttes per population) used in this analysis.

Eight harmonics were found to be sufficient to reach approximately 99% of the maximum cumulated Fourier power for the denticles of *T. centrostrigeata* (Fig. 4). Results of the PCA analysis indicate that over 99% of the observed outline shape variation of *T. centrostrigeata* can be represented on 18 orthogonal principal component axes. Table 3 illustrates the per cent of variance contribution of the principal components to morphological variation. To further assess the variation between the four populations, we performed a linear discriminant analysis (LDA) to determine if this variation could be distinguished quantitatively based on PCA axes 1 to 18.

Based on the LDA, the population of Argentina could correctly be predicted by 50%, the Mexican population by 75%, the Luphephe Dam population by 35%, and the Glen Alpine Dam population by 70% (Fig. 5, Table 4). PCA scores were analysed under a multivariate analysis of variance (MANOVA) pairwise. Shapes between Argentina and Mexico do not present differences, while the rest of the populations have significant differences (Fig. 6, Table 5). Based on the LDA and MANOVA results we found two different morphotypes for *T. centrostrigeata*; morphotype *a*, mainly found in Argentina and Mexico, and morphotype *b*, mainly found in Glen Alpine Dam, South Africa.

Figure 6 shows that populations of Argentina and Mexico are similar but with slight variations in the posterior margin of the blade. The Glen Alpine Dam population presents morphological differences in the shape of the ray and in the general shape of denticle with the Americas and Luphephe dam population respectively; this last population also exhibits morphological differences in the central part of the denticle with the Americas populations.

Our results suggest that there are two main morphotypes for *T. centrostrigeata*, which present detectable variations in the denticles. Population from the Americas (Argentina and Mexico) constituted a single morphological group, by possessing a close similarity in the shape of the denticles. According to the present results, it is possible to differentiate this morphological group from the Glen Alpine Dam population, South Africa, which represents a morphologically separate group characterising the African morphotype (Fig. 6).

Table 3. Principal components analysis scores based on Fourier coefficients from 8 harmonics from *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983.

Importance of components:									
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Standard deviation	0.05767	0.03869	0.03622	0.0338	0.02737	0.02228	0.01763	0.01461	0.01274
Proportion of variance	0.33646	0.15147	0.13271	0.1156	0.07581	0.05024	0.03144	0.02161	0.01641
Cumulative proportion	0.33646	0.48794	0.62065	0.7362	0.81204	0.86229	0.89373	0.91534	0.93175
	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18
Standard deviation	0.01180	0.01010	0.008977	0.00807	0.007425	0.00717	0.006252	0.005696	0.004773
Proportion of variance	0.01408	0.01032	0.008150	0.00659	0.005580	0.00520	0.003960	0.003280	0.002310
Cumulative proportion	0.94583	0.95615	0.964310	0.97090	0.976470	0.98168	0.985630	0.988910	0.991220

Both morphotypes are described below in the remarks based on the method proposed by Van As and Basson (1989).

Remarks. Morphotype *a* (Fig. 1A-D, Fig. 2A-D, Fig. 6) presents an angular blade, truncated in most denticles. Narrow blades occur in 8–9% of all specimens. The distal surface ranges from flattened to slightly rounded, being parallel to the border membrane in the majority of cases, with only small numbers not parallel to the border membrane. In cases where the distal margin is not parallel, the distal surface slopes in an anterior direction. Blades vary with broad and flattened heads, tapering towards the central parts in the vast majority of cases, while these broaden slightly towards the central parts in some with narrower heads. The contact surface of the tangent point forms a point rather than a short line, with the tangent point situated lower than the distal point. The anterior margin varies from curved to angular, some with a straight margin. In specimens with a rounded anterior margin, the apex is less prominent than in cases of angular and straight forms. In the latter cases, the anterior blade margin shows an angular, almost triangular shape with the apex, as well as the distal and proximal sides of the anterior blade. The anterior surface extends beyond the y+1 axis in most denticles, not just in those with angular denticles. The blade apophysis is consistently present, varying from slight to somewhat prominent. The posterior margin forms a characteristic deep L-shaped curve, with the deepest point at the lowest end (adjacent to the central part), mostly lower than the apex of the anterior surface. The posterior projection is present, but not prominent in all specimens. The blade connection is narrow in the majority of cases, while only some possess a well-developed connection. The central part is conical in shape, some longer and narrower than others that are wider and squatter, but a small number have almost a triangular central part. The central part tapers from the base to a sharply rounded point in some, fitting tightly into the preceding denticle. The sections above and below the x-axis are similar in shape and the central part extends less than halfway to the y–1 axis. The ray connection is short, but well-developed in most; in the majority of cases this connection is of the same thickness as the ray. The ray apophysis is small, situated high on the ray, making contact with the tip of the central part of the following denticle. Rays are straight in the vast majority of cases, of varying thickness, with roughly 40–50% of the specimens having thin rays, where these taper gradually to sharp rounded points. In the other half of the cases the rays are broad and well developed, ending in rounded tips. Rays run along the y axes in most cases, mostly directed in an anterior direction with the tips extending past the y+1 axes. A ridged line is visible in both the blade and ray of many specimens, a typical characteristic of this species (Fig. 1A,C). The centre ridges vary in number from 10 to 17 (mode 13), also varying in thickness most are thick, more or less of the same thickness as the rays. In some cases, the arrangement of the centre ridges is not symmetrical, with no clear pattern distinguishable. These centre ridges are sometimes extended rod-shaped, fanning out from a rough centre point, in others the rods are of varying lengths, with some half the length of others. Some centre ridges are almost circular in structure. The ratio of the denticle above the x-axis to the section below is slightly more than one (1.2–1.3).

In the morphotype *b* (Fig. 1G–H, Fig. 2G–H, Fig. 6) the blade is angular and truncated in most denticles, but roundly spatulate in a few. Narrow blades occur in 18% of the cases. The distal surface is mostly slightly rounded, while very few have flattened distal edges. The distal surface is mostly parallel to the border membrane, with only a small number that are not parallel to the border membrane. In those cases where the distal surface is not parallel, it slopes in an anterior direction. The blade shape varies, from those possessing broad and flattened heads (distal part) that taper slightly towards the central part, to some with narrower blades, where the anterior and posterior edges are parallel. The contact surface of the tangent point forms a short line rather than a point, with the tangent point situated slightly below the distal point. The anterior margin is angular; some possess a straight margin. The apex is prominent with an angular anterior blade margin that forms an almost triangular shape with the apex, as well as the distal and proximal sides of the anterior blade. The anterior surface barely touches the y + 1 axis with a few being close, but not touching the y + 1. The blade apophysis is not large, but prominent and is consistently present. The posterior margin forms a characteristic shallow L-shaped curve, with the deepest point at the lowest end (adjacent to the central part), being mostly lower than the apex of the anterior surface. The posterior projection is present, but not prominent

Table 4. Confusion matrix from linear discriminant analysis (LDA) based on PC1-PC18 axes from *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983.

Current identification\predicted identification	San Vicente, Argentina	Glen Alpine dam, South Africa	Luphephe dam, South Africa	Huadacareo Mexico	Total	% Correct
San Vicente, Argentina	10	5	2	3	20	50%
Glen Alpine dam, South Africa	0	14	4	2	20	70%
Luphephe dam, South Africa	3	5	7	5	20	35%
Huandacareo, Mexico	3	0	2	15	20	75%

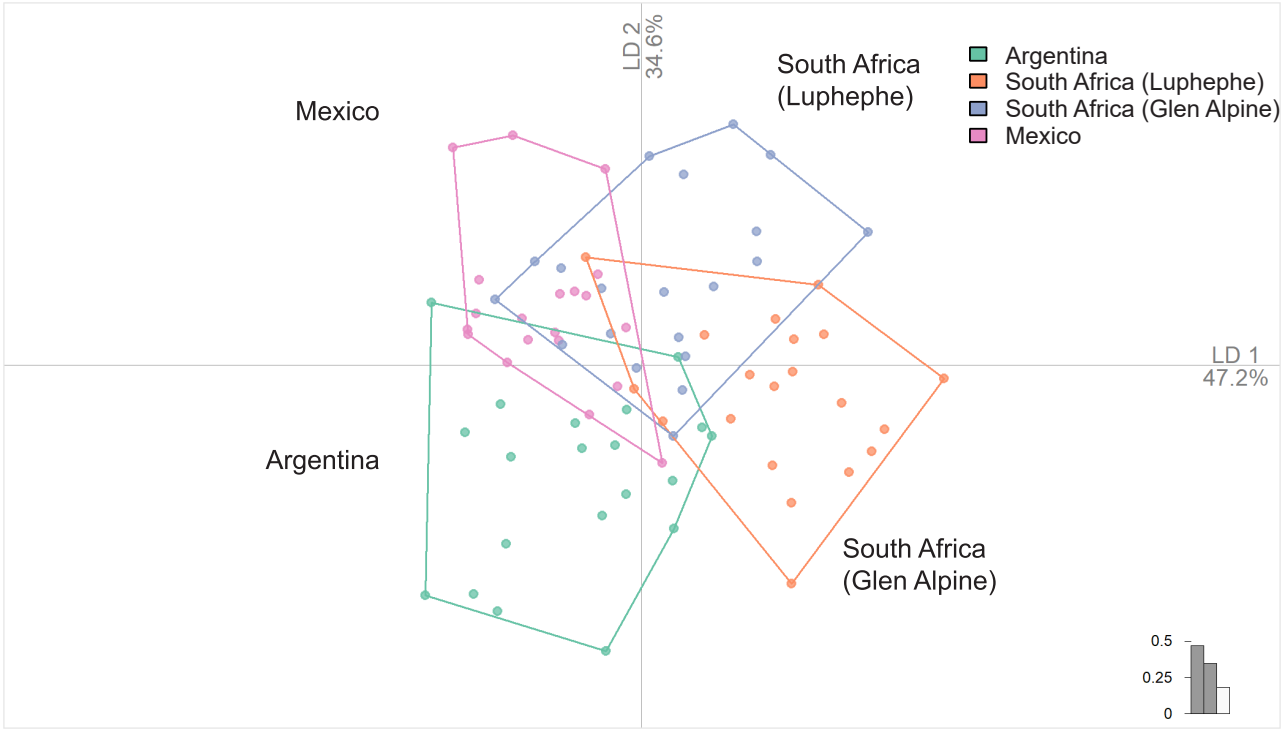

Fig. 5. Linear discriminant analysis (LDA) of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983 using normalised elliptical Fourier descriptors. Percentages indicate the proportion of the trace captured in each LD component.

in all specimens. The blade connection is narrow in the majority of cases, with only some showing a well-developed connection. The central part is conical-shaped, some are longer and narrower than others that are wider and squatter, the central parts are almost triangular in shape, but in a small number of cases. The central part tapers from the base to a sharply rounded point in some specimens, fitting tightly into the preceding denticle. The sections above and below the x-axis are similar in shape. The central part extends either less than halfway, or at least halfway to the y–1 axes. Ray connections are short, but well-developed in most denticles, while slightly thicker than the ray in the majority of cases. The ray apophysis is small but present in most individuals where it is situated high on the ray, making contact with the tip of central part of the following denticle. Rays are straight in the majority of cases, with more than 55% having thin rays. Thinner rays taper gradually to sharp rounded points, whilst well-developed thicker rays tend to end in rounded tips. Rays run parallel to y-axes, with none extending past the y-axes, although some touch the y–1 and some y+1 axes. Ridged lines are prominent in blades and rays of many specimens, a typical characteristic of this species (Fig. 1G). Centre ridges vary in number from 11 to 14 (mode 13); varying slightly in thickness, with the majority strongly developed and more or less of the same thickness as the rays. The centre ridges in the majority of cases are extended rod-shaped, where these clearly fan out from a rough centre point. The ratio of the denticle above the x-axis to the section below is slightly less to slightly more than one (0.9–1.1).

DISCUSSION

Trichodina centrostrigeata is found associated with a wide variety of tilapia hosts within a large geographical range (Islas-Ortega et al. 2020). One of the main morphological methods of differentiating this species is commonly based on the presence of central ridges, although similar ridges are also found in other species such as *Trichodina miranda* Stein, 1979 and *Trichodina frenata* Van As et Basson, 1992 (Mitra and Bandyopadhyay 2006). Based on traditional morphological features, the shape and measurements of the analysed organisms presented minimal variations in some denticle structures such as blade and ray length and central width. However, some slight differences are detected when particular populations are compared. For example, the blade varies with broad and flattened head tapering towards the central part between trichodinid populations from Glen Alpine Dam and Mexico. Also, Glen Alpine Dam or-

Table 5. MANOVA table with the statistics summary of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983. DF = degrees of freedom; Pillai = values of Pillai´s test; AproxF = approximate F-statistic; NumDF = numerator degrees of freedom; Den DF = denominator degrees of freedom; Pr(>F) = P-value.(*** Significance differences).

	DF	Pillai	Aprox F	Num DF	Den DF	Pr(>F)
Argentina – Glen Alpine dam	1	0.7985	4.623	18	21	0.0005693***
Argentina – Luphephe dam	1	0.6378	2.055	18	21	0.0576787***
Argentina – Mexico	1	0.5925	1.696	18	21	0.1227139
Glen Alpine dam – Luphephe dam	1	0.7299	3.153	18	21	0.0066018***
Glen Alpine dam – Mexico	1	0.7720	3.950	18	21	0.0016453***
Luphephe dam - Mexico	1	0.6873	2.564	18	21	0.0203626***

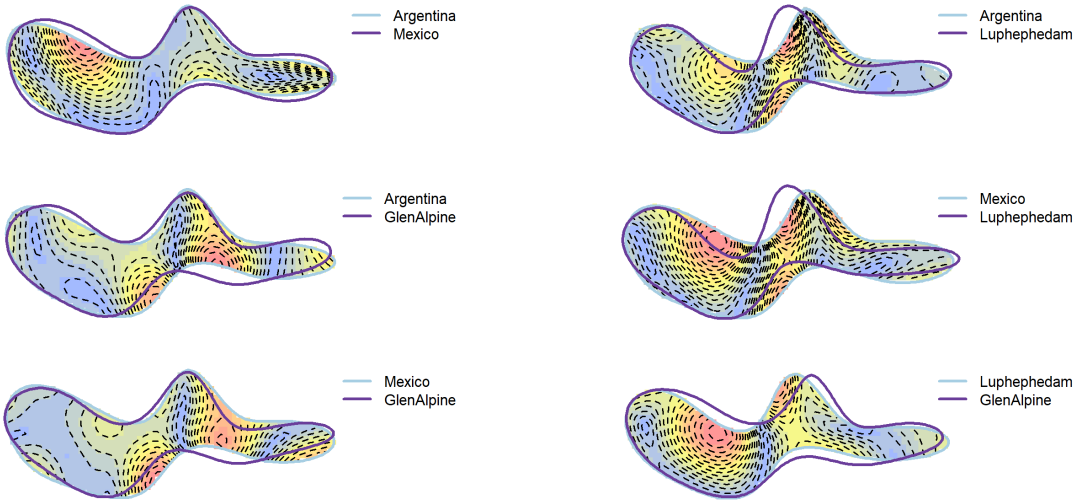

Fig. 6. Isodeformation lines of *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983. Red colour – high variation in the shape; yellow colour – medium variation in the shape; blue colour – low variation in the shape.

ganisms exhibit a slightly more and rounded distal surface of the blade in comparison with the remaining populations. Additionally, the Mexican population presents differences in the tangent point of the blade, which forms a point rather than a short line like the other populations; and the blade of Argentine population exhibits a well-developed connection, but thinner than those of the other populations, and the central ridges of the same population are thinner.

The taxonomic work of trichodinid ciliates is based on the shape and dimensions of the denticles in the adhesive disc (Van As and Basson 1989). The standard procedure started with the proposal of Lom (1958) modify by Van As and Basson (1989), which is based on a series of measurements obtained from the analysis of micrographs of silver-impregnated specimens. This method seems to be useful to accurately discriminate genera and species, and because of its effectiveness, there is not an alternative proposal to replace it. Additionally, the difficulties to collect and preserve these organisms for studies based on DNA-taxonomy, reinforce the use of morphological methods described above. Notwithstanding the universal acceptation of the taxonomic criteria, some taxonomic issues still make the discrimination between morphologically similar species difficult due to the high variation of parasites from different host species, in morphometric data, and some morphological characters (Lom 1958, Tang et al. 2017, Islas-Ortega et al. 2018, Marcotegui et al. 2018, Wang et al. 2019). These issues remain unsolved mainly because of the lack of numerical support confirming or discarding each proposal. In this sense, the use of geometric morphometric arises as a numerical method, complementing morphological studies. While traditional morphometry uses lengths, width, and angles, geometric morphometrics uses the shape as a whole, considering all the geometrical relationships of the input data (Bonhomme et al. 2014). Geometric morphometric data have the advantage of providing a consistent set of shape variables for hypothesis testing and provide graphic analyses that quantify and visualise morphometric variation within and between organisms (Tracey et al. 2006). Elliptical Fourier functions represent a precise method for describing and characterising outlines, efficiently capturing shape information in a quantifiable manner (Kuhl and Giardina 1982, Lestrel 1997, Marcotegui et al. 2018).

Some authors have suggested that *T. centrostrigeata* possesses a morphological uniformity (Basson et al. 1983, Basson and Van As 1994, Mitra and Bandyopadhyay 2006, Abdel-Baki et al. 2017). For this reason, this species is easily distinguishable for other species of the family. In present paper, we used EFA to detect the morphological variations in the shape of the denticles of *T. centrostrigeata* associated with tilapia from geographically separate populations. This work reveals slight differences among the analysed populations, which can be attributed to natural variations in the shape of denticles in this species. These differences are numerically recorded in traditional morphological studies, but occasionally, slight differences could be hidden

by structures with coincident measurements. For example measurements of the central part and the ray of the denticle shown in Table 2 exhibit some slight differences, but in general terms, all of these measurements are in the same range, hindering to discriminate populations. However, the analysis of the shape of these structures by EFA helps to detect some significative differences hidden behind the numerical values. In this sense, the EFA prevents confusions derived from comparisons of numerical data, by an analysis of the shape, where individual silhouettes are the basis to perform one single silhouette representative of each population, susceptible to be compared to find significative differences, indicating the degree of variation among populations. Once detected, a formal description of these variations is desirable to objectively unify the criteria recognising some trichodinid taxa.

Differences between these morphotypes are measurable, and remarkably one of these is distributed in the Americas, while the other is confined to a region in South Africa. There are extensive morphological variations within the Luphephe dam population, most of the individuals share morphological denticular traits with the other three populations. The similarity of Luphephe population with those from Argentina is 15%, and 25% respect to Mexico and Glen Alpine Dam; this prevents its inclusion as a separate morphotype, although fitting within the general description for the species.

Morphotypes suggested in this study are based on results of the MANOVA, where populations from Argentina and Mexico do not present any significant differences in the shape of the denticle. Once detected, the description of each morphotype follows the proposals by Van As and Basson (1989).

Some natural factors could be considered to explain the detected variation. *Oreochromis mossambicus* and *O. niloticus* are indigenous to African freshwater environments, and due to their effective adaption to diverse habitats, it led to their expansion across the world as a financially viable food source (Trewavas 1982, Welcomme 1988, García et al. 2010). In both cases, a wide route of introductions, into and out of many continents and countries, occurred before ending up in the aquaculture farms in the Americas from which the hosts were sampled for this study. Although all of this happened within a very short time scale, their introduction into these new habitats may have had some effect on the niche (as defined by Whittaker et al. 1973) of their protistan symbionts, which could have led to small morphological adaptations to their new environments. Another important factor is that specimens of *T. centrostrigeata* obtained from Argentina and Mexico were collected from the introduced *O. niloticus* acquired from aquaculture farms, meanwhile, the South African populations were obtained from indigenous *O. mossambicus* from natural systems. Morphological variation may also be possible due to the artificial nature of the aquaculture environment where the extra stresses of this environment may indirectly promote adaptational changes of their parasites. Additional explanations could consider environmental factors influencing variations. Some studies suggest that the morphology of the trichodinids may change over the seasons or host species (Kazubski and Migala 1967, Van As and Basson 1989, Basson and Van As 1994, Ogut and Altuntas 2011). The relevance of these factors must be verified with further studies, including other tools used for taxonomic validation such as molecular taxonomy.

In this study we attempted to verify whether the current distribution range of the populations of *T. centrostrigeata* has induced a morphological variation that can be measured. Even though there are statistically significant differences in most of the analysed populations, the method itself does not allow one to determine when populations are so far from each other to consider that they might actually represent different species. In this sense, we consider that the immediate future in the taxonomy of this ciliate group must be based on a meticulous morphological analysis such as the descriptive method proposed by Van As and Basson (1989), including tools of geometric morphometry, especially in species where taxonomic differentiation is problematic and also in species that present a wide distribution or a wide range of hosts, to begin a study of a possible relationships of morphological variation with factors such as the host or the environment should be performed, complemented with molecular characterisations where possible. This is crucial for the species delimitation of slightly differing populations that belong to the same species (i.e. Islas-Ortega et al. 2018). If the differences observed coincide with a high value of genetic divergence, then the suggestion of new species is justified (Marcotegui et al. 2018).

Authors' contribution. A.G.I.O and P.M. performed the analysis of geometric morphometric. A.G.I.O., L.B., G.J. and R.A.A. performed the measurements and denticle descriptions. A.G.I.O. and P.M. captured the micrographs. A.G.I.O. and G.J. edited the figures. R.A.A. supervised the study. All authors have contributed to the data analysis and the discussion. A.G.I.O., L.B., G.J. and R.A.A. wrote the manuscript.

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