



## Research note

# *Trichodina mutabilis* (Protozoa: Ciliophora: Trichodinidae) from the characid fish *Astyanax mexicanus* in the Cuatro Ciénegas region, northern Mexico

## *Trichodina mutabilis* (Protozoa: Ciliophora: Trichodinidae) en el pez carácido *Astyanax mexicanus* de la región de Cuatro Ciénegas, norte de México

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**Abstract.** As a part of a series of ongoing studies on trichodinids of freshwater fishes of Mexico, several species from diverse localities in the Cuatro Ciénegas region, Coahuila, northern Mexico, were examined. Wet smears of gills and skin were made fresh and screened for the presence of trichodinids under light microscopy. Additional specimens were fixed for SEM. The only trichodinid species found was *Trichodina mutabilis*, a parasite of the characid fish *Astyanax mexicanus*. This ciliate protozoan has been introduced from Asia to many countries, including Mexico, with its original host, the Asian carps. The present record is the first for Mexico and the second in North America, being the first record of a *Trichodina* species for native wild freshwater fish in a natural environment in Mexico, since all previous records were from exotic cultured fishes.

Key words: Trichodinidae, ciliates, ecto-parasitism.

**Resumen.** Como parte de un proyecto de investigación a largo plazo sobre tricodínidos parásitos de peces dulceacuícolas de México, varios ejemplares de diversas especies de peces se recolectaron y examinaron en Cuatro Ciénegas, Coahuila, México. Se revisaron muestras frescas de la piel y las branquias bajo microscopio óptico. Algunas muestras adicionales se fijaron para microscopía electrónica. La única especie encontrada fue *Trichodina mutabilis*, la cual se registra por primera vez en México, parasitando al pez *Astyanax mexicanus*. Esta especie de protozoo ha sido introducida a diferentes partes del mundo con su huésped original, la carpa asiática. El presente registro es el primero para México y el segundo para Norteamérica, siendo también el primero en México de una especie de *Trichodina* en peces nativos en su ambiente natural, ya que los registros previos fueron hechos en peces exóticos cultivados.

Palabras clave: Trichodinidae, ciliados, ectoparasitismo.

Ciliates of the genus *Trichodina* Ehrenberg, 1831 are epibionts and often ectoparasites on animals including fish, where they are pathogenic, causing damage to skin and gills (Sleigh, 1991), often leading to death of the infested fish (Hoffman, 1999). Its proliferation might be promoted by changes in the relationship among host, parasite, and environment caused by nutritional deficiency, poor water quality, and infectious or parasitic diseases, causing severe epidermal lesions and disease outbreaks (Khan, 2004; Martins et al., 2010).

Studies of *Trichodina* in Mexico are scarce. Most have been carried out on exotic cultured fishes such

as *Carassius auratus* (L.), *Ctenopharyngodon idella* (Valenciennes), *Cyprinus carpio* (L.) (Cyprinidae), and *Oreochromis niloticus* (L.) (Cichlidae) (Aladro-Lubel et al., 2006). To date there are not records of this ciliate in native populations of freshwater fishes inhabiting natural localities.

Cuatro Ciénegas is a small intermontane valley edge of the Sierra Madre Oriental in the Mexican state of Coahuila, northern Mexico. The approximately 1 000 km<sup>2</sup> desert valley of Cuatro Ciénegas possess the greatest number of endemic species of any place in North America (Stein et al., 2000), holding a large biodiversity and more than 70 endemic species. Its springs, marshes, rivers, and lakes support a diverse fish fauna for a North American desert region, including at least 16 native fish species, 8

of which are endemic (Minckley, 1984). A wide inventory of the helminth species parasitizing these fish taxa have been provided recently by Aguilar-Aguilar et al. (2014); however, the occurrence of protozoan parasites of these fishes has not been documented. During a prospective study addressed to establish the diversity of ciliate parasites of freshwater fishes occurring in arid regions of Mexico, particularly associated with the Cuatro Ciénegas region, we analyzed several fish species. This paper provides the record of *Trichodina mutabilis* Kazubski and Migala, 1968 for the freshwater fish *Astyanax mexicanus* (De Filippi, 1853), which represents the first record of this trichodinid in Mexico and the second in North America.

With this aim, freshwater fishes of the species *Astyanax mexicanus* (n= 85) (Characidae), *Gambusia marshi* Minckley and Craddock, 1962 (Poeciliidae) (n= 122), *Cyprinodon atrorus* Miller, 1968 (n= 108), *C. bifasciatus* Miller, 1968 (n= 28) (Cyprinodontidae), and *Herichthys minckleyi* (Kornfield and Taylor, 1983) (n= 19) (Cichlidae), were collected from several localities in the Cuatro Ciénegas region. Fish were kept alive and studied for ectoparasitic ciliates no more than 6 hours after capture. Squash slides of skin and gills were made from live specimens. Slides were impregnated by silver-nitrate to observe the adhesive disc, as described by Lom (1958). Air-dried slides of trichodinids were stained with either Harris-Lillie's hematoxylin or Ehrlich's acid hematoxylin to studying the nuclear apparatus. Voucher slides were deposited at the Colección del Grupo de Trabajo en Parasitología de Organismos Acuáticos at the Facultad de Ciencias, UNAM, Mexico (No. CPOA-012 to 038). All measurements are presented in micrometers following the recommendations of Lom (1958) and Van As and Basson (1989). They were made on photomicrographs obtained using an Olympus BX40® photomicroscope equipped with a Leica Pixlink PL-A662® image capture system. The measurements of the parasites were made with the aid of the Image-Pro Plus® 4.1 software. For SEM study, samples of gills and skin were fixed using glutaraldehyde 3%, and post fixed in 1% OsO4 for 1 hr, dehydrated through a graded series of ethyl alcohol and then critical point dried with CO<sub>2</sub>. Specimens were mounted on metal stubs, coated with gold, and examined in a Hitachi Stereoscan Model S-2469N.

The only ectoparasitic ciliate species found was *Trichodina mutabilis* (Figs. 1-4). This species was recovered in high numbers (more than 100 individuals per fish, prevalence= 100%) from the skin and gills of *Astyanax mexicanus* from the Anteojo San Juan spring in the Cuatro Ciénegas region (26°58'07" N, 102°07'38" W). Remaining host species were free of infection. Ciliates have a medium size disc-shaped body (Fig. 1) measuring

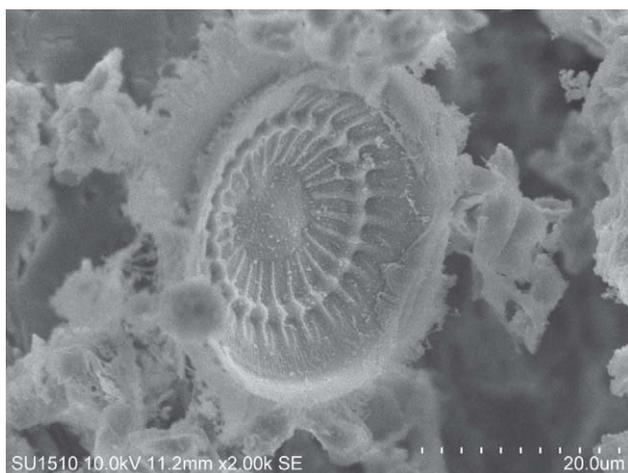


**Figure 1.** Klein's silver impregnated *Trichodina mutabilis*, bar= 15µm.

38.31 ± 2.07 diameter, adhesive disc 36.22 ± 2.07; absence of granules in silver-impregnated specimens. A horseshoe-shaped macronucleus (Fig. 2) is observed, with an external diameter of 64.5 ± 6.37, and a distance between the ends of 7.67 ± 2.37. Denticle ring (Fig. 3): 21.77 ± 2.02, number of denticles 23 - 28, number of radial pins per denticle: 7



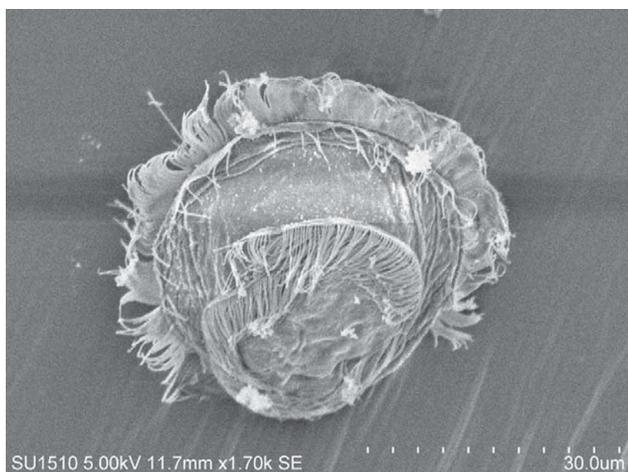
**Figure 2.** Ehrlich's acid hematoxylin stained nuclear apparatus of *T. mutabilis*, bar= 10µm.



**Figure 3.** Scanning electron micrograph of *T. mutabilis*, view of a denticulate ring.

-10, dimensions of denticle: length  $4.85 \pm 0.25$ ; blade  $4.33 \pm 0.32$ ; central part  $1.46 \pm 0.15$ ; ray  $5.52 \pm 0.67$ ; span  $11.55 \pm 0.73$ . Adoral ciliary spiral  $400^\circ$  (Fig. 4).

The ciliate species *Trichodina mutabilis* was first described by Kazubski and Migala (1968). Since its original description, various records from diverse fish hosts have been published around the world (Basson and Van As, 2006), and a high morphological variability has been documented (Lom, 1970; Basson and Van As, 2006). Specimens analyzed in this study exhibit some morphological variation with respect to the size of the body, being smaller than those described from other parts of the world (see Kazubski and Migala, 1968; Lom, 1970;



**Figure 4.** Scanning electron micrograph of *T. mutabilis*, adoral view.

Basson et al., 1983; Özer and Erdem, 1998; Albaladejo and Arthur, 1989; Özer, 2000; Dove and O'Donoghue, 2005; Hu, 2012); however, in general terms our specimens fit with most of the characteristics described for *T. mutabilis* (Basson and Van As, 1994), and have a similar size to a population described by Mitra and Bandyopadhyay (2005).

*Trichodina mutabilis* is a cosmopolitan species recorded from diverse host fish species, mainly cyprinids in the Europe, Asia, South Africa, and North America (Lom, 1970; Basson et al., 1983; Albaladejo and Arthur, 1989; Özer and Erdem, 1998; Özer, 2000; Kritsky and Heckmann, 2002; Dove and O'Donoghue, 2005; Mitra and Bandyopadhyay, 2005; Basson and Van As, 2006; Hu, 2012). According to Basson and Van As (2006), this is a ciliate species with a worldwide distribution, probably attained via transcontinental introductions of fish. Particularly, this species has been dispersed to other countries together with shipments of carps (Cyprinidae) destined for aquaculture. It possesses a large dispersal capability and it is now found not only in introduced hosts but also in the native freshwater fish fauna. Similar introductions of parasitic species such as exotic digeneans and cestodes have been documented for freshwater fish species in Mexico (see Aguilar-Aguilar et al., 2014), where the low host specificity and great capability to adapt to different environmental conditions allow the invasive species to thrive and to expand its host and distributional range. The report of an additional introduced parasite species affecting freshwater fish fauna in Mexico must be considered in terms of suggesting accurate conservation strategies for this fauna.

The present record of *Trichodina mutabilis* in *Astyanax mexicanus* represents a new host record and the first for Characidae. The present finding, along with previous records of this ciliate species in non-cyprinid fish (Kritsky and Heckmann, 2002; Mitra and Bandyopadhyay, 2005), throws new light on the biodiversity and host preference of the species (Mitra and Bandyopadhyay, 2005), and allows us to suggest a successful colonization from exotic cultured carps to native characid fish. Additionally, this is the first record of this ciliate species in Mexican fish and the second for North America, where it was previously recorded infesting the gills of carps of the species *Cyprinus carpio* in Utah, USA (Kritsky and Heckmann, 2002). Furthermore, this is the first record of ciliates of the genus *Trichodina* parasitizing native wild fishes in Mexico, since previous reports were for exotic cultured fishes (Aladro-Lubel et al., 2006).

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#### Literature cited

- Aguilar-Aguilar, R., A. Martínez-Aquino, H. Espinosa-Pérez and G. Pérez-Ponce de León. 2014. Helminth parasites of freshwater fishes from Cuatro Ciénegas, Coahuila, in the Chihuahuan desert of Mexico: inventory and biogeographical implications. *Integrative Zoology* 9:328-339.
- Aladro-Lubel, M. A., R. Mayén-Estrada and M. Reyes-Santos. 2006. Listados Faunísticos de México XI. Registro actualizado de ciliados (agosto, 2004). Instituto de Biología, UNAM, D. F. 97 p.
- Albaladejo, J. D. and J. R. Arthur. 1989. Some trichodinids (Protozoa: Ciliophora: Peritrichida) from freshwater fishes imported into the Philippines. *Asian Fisheries Science* 3:1-25.
- Basson, L. and J. G. Van-As. 2006. Trichodinidae and other ciliophorans (Phylum Ciliophora). In *Fish diseases and disorders*, 2nd ed. Vol. 1: Protozoan and metazoan infections, P. T. K. Woo (ed.). CABI Publishing, London. p. 154-182.
- Basson, L. and J. G. Van As. 1994. Trichodinid ectoparasites (Ciliophora: Peritrichida) of wild and cultured freshwater fishes in Taiwan, with notes on their origin. *Systematic Parasitology* 28:197-222.
- Basson, L., J. G. Van As and I. Paperna. 1983. Trichodinid ectoparasites of cichlid and cyprinid fishes in South Africa and Israel. *Systematic Parasitology* 5:245-257.
- Dove, A. D. M. and P. J. O'Donoghue. 2005. Trichodinids (Ciliophora: Trichodinidae) from native and exotic Australian freshwater fishes. *Acta Protozoologica* 44:51-60.
- Hoffman, G. L. 1999. *Parasites of North American freshwater fishes*, 2nd ed. Cornell University Press, Ithaca. 539 p.
- Hu, Y. 2012. Ciliate ectoparasites (Ciliophora: Trichodinidae/Chilodonellidae) on gills of *Carassius auratus* from the Yangtze River, China, with the description of *Trichodina luzhoues* sp. n. *Parasitology Research* 111:433-439.
- Kazubski, S. L. and K. Migala. 1968. Urceolariidae from breeding carp *Cyprinus carpio* L. in Zabeniec and remarks on the seasonal variability of trichodinids. *Acta Protozoologica* 6:137-169.
- Khan, R. A. 2004. Disease outbreaks and mass mortality in cultured Atlantic cod, *Gadus morrhua* L., associated with *Trichodina murmanica* (Ciliophora). *Journal of Fish Diseases* 27:181-184.
- Kritsky, D. C. and R. Heckmann. 2002. Species of *Dactylogyrus* (Monogenea: Dactylogyridae) and *Trichodina mutabilis* (Ciliata) infesting koi carp, *Cyprinus carpio*, during mass mortality at a commercial rearing facility in Utah, USA. *Comparative Parasitology* 69:217-218.
- Lom, J. 1958. A contribution to the systematics and morphology of endoparasitic trichodinids from amphibians, with a proposal of uniform specific characteristics. *Journal of Protozoology* 5:251-263.
- Lom, J. 1970. Observations on trichodinid ciliates from freshwater fishes. *Archiv für Protistenkunde* 112:153-177.
- Martins, M. L., N. C. Marchiori, G. Nunes and M. P. Rodrigues. 2010. First record of *Trichodina heterodentata* (Ciliophora: Trichodinidae) from Channel catfish, *Ictalurus punctatus* cultivated in Brazil. *Brazilian Journal of Biology* 70:637-644.
- Minckley, W. L. 1984. Cuatro Cienegas fishes: Research review and a local test of diversity versus habitat size. *Journal of the Arizona-Nevada Academy of Science* 19:13-21.
- Mitra, A. M. and P. K. Bandyopadhyay. 2002. First record of *Trichodina japonica* Imai, Miyazaki et Nomura, 1991 and *Trichodina mutabilis* Kazubski et Migala, 1968 (Ciliophora, Trichodinidae) from Indian fishes. *Protistology* 4:121-127.
- Özer, A. 2000. The occurrence of three species of *Trichodina* (Ciliophora: Peritrichia) on *Cyprinus carpio* in relation to culture conditions, seasonality and host characteristics. *Acta Protozoologica* 39:61-66.
- Özer, A. and O. Erdem. 1998. Ectoparasitic protozoa fauna of the common carp (*Cyprinus carpio* L., 1758) caught in the Sinop region of Turkey. *Journal of Natural History* 32:441-454.
- Sleigh, M. A. 1991. The nature of Protozoa. In *Parasitic Protozoa*, 2nd ed., J. P. Kreier and J. R. Baker (eds.). Academic Press, Inc., San Diego. p. 1-53.
- Stein, B. A., L. S. Kutner, J. S. Adams (eds.). 2000. *Precious heritage: the status of biodiversity in the United States*. Oxford University Press, Oxford. 399 p.
- Van-As, J. G. and L. Basson. 1989. A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. *Systematic Parasitology* 14:157-179.