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Dos nuevas especies de protista: *Trypanoplasma ojedae* sp. n. (Mastigophora: Kinetoplastida) y *Trichodina lascrucensis* sp. n. (Ciliophora: Peritrichida) en un pez blénido *Scartichthys viridis*, de la costa de Chile

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Resumen.- Dos protistas parásitos fueron observados en un pez blénido, *Scartichthys viridis*, que habita en pozas de mareas en Las Cruces, Chile central. En la sangre, *Trypanoplasma ojedae* sp. n. (Mastigophora: Kinetoplastida) se caracteriza por poseer pequeño tamaño (29,8 ± 5,0 x 5,1 ±1,2 μm), un corto flagelo anterior (4,9 ± 0,6 μm), situado anterior al kinetoplasto (2,4 ± 0,2) y una prominente membrana ondulante. De 18 peces todos estaban infectados y el 67 % se encontraba parasitado en las branquias por una especie de sanguijuela no identificada de la familia Piscicolidae que albergaba estados en desarollo e infectantes del biflagelado. *Trichodina lascrucensis* sp. n. (Ciliophora: Peritrichida) parasita los filamentos branquiales de todos los peces. El ciliado se caracteriza por su tamaño corporal (70,8 ± 8,4 μm), disco adhesivo (53 ± 5,4 μm), anillo denticular (33 ± 3,1 μm), número promedio de dentículos (32,8 ± 3,0), número promedio de pins (9,5 ± 1,0) forma de los dentículos y micronúcleos ovoides localizados cerca del armamento macronuclear. Debido a que ninguno de los dos parásitos es similar a cualquier especie descrita para hábitats de pozas de mareas del Océano Pacífico, ambas son consideradas nuevas especies con las características descritas acá.

Palabras clave: Peces intermareales, hemoparásito, ciliado branquial, Pacífico sureste

Abstract.- Two new protistan species were observed in a blenniid fish, *Scartichthys viridis*, inhabiting tide pools at Las Cruces, central Chile. *Trypanoplasma ojedae* sp. n. (Mastigophora: Kinetoplastida) in the blood is characterized by its small size (29.8 ± 5.0 x 5.1 ±1.2 μm), short anterior flagellum (4.9 ± 0.6 μm), anterior located kinetoplast (2.4 ± 0.2) and a prominent undulating membrane. All of 18 fish were infected and 67% were parasitized on the gills by an unidentified piscicolid leech that harbored developing and infective biflagellated stages. *Trichodina lascrucensis* sp. n. (Ciliophora: Peritrichida) parasitized the gill fillaments of all fish. The ciliate is characterized by its body size (70.8 ± 8.4 μm), adhesive disc (53 ± 5.4 μm), denticular ring (33 ± 3.1 μm), mean number of denticles (32.8 ± 3.0), mean number of denticular pins (9.5 ± 1.0) and ovoid micronucleus located near to one of the macronuclear arms. Since none of the two parasites was similar to any species described from marine tide pool habitats as well as from the Pacific Ocean, both are considered new species with the characteristics described herein.

Key words: Intertidal fish, haemoparasite, gill ciliate, Southeast Pacific

Introduction

A few studies account for the records and descriptions of protistan parasites in autochtonous marine animals off Chile (Moser & Noble 1976, Oliva 1982, Morillas *et al*. 1987, Castro & Burgos 1996, Khan *et al*. 2001, Díaz & George-Nascimento 2003, George-Nascimento *et al*. 2004, Ibáñez *et al*. 2005, Pardo-Gandarillas *et al*. 2004, 2007). Only one trypanosome, *Trypanosoma humboldti* Morillas *et al*., 1987, and only one trypanoplasm, *Trypanoplasma* (= *Cryptobia*) *neghmei* Khan *et al*., 2001, are known from the southeastern Pacific (Morillas *et al*. 1987, Khan *et al*. 2001). No reports or descriptions of parasitic ciliates have been published from the southeastern Pacific Ocean.

A recent study reported that *S. viridis* was a host for ten species of parasites, eight metazoans and two protistan (Díaz & George-Nascimento 2003). The latter included unidentified species of *Trypanoplasma (= Cryptobia)*, a biflagellate that occurred in the blood and a trichodinid ciliate attached to its gill filaments. Comparison of the

morphology of these two protozoan parasites revealed distinct differences from others described from blenniids or other fish species inhabiting intertidal zones. *Scartichthys viridis* (Valenciennes 1836) is an abundant blenniid fish species occurring in tide pools (Muñoz & Ojeda 1997, 2000; Berríos & Vargas 2004, Pulgar *et al.* 2005), from Bahía Independencia, Perú, up to Valparaíso, Chile (Chirichigno 1974). This species feeds mainly on green and red algae (Muñoz & Ojeda 1997, 2000; Berríos & Vargas 2004), attaining up to 310 mm in total body length (Muñoz & Ojeda 2000).

The present study provides evidence that both taxa recorded in *S. viridis* are new species occurring in this fish species from the southeastern Pacific Ocean.

Material and methods

Scartichthys viridis was collected from tide pools at Las Cruces (33°27'S, 71°37'W) by seine and held for no longer than one day in the laboratory in flow-through aquaria until examination. A total of 18 specimens were examined. To detect protozoans from the blood, a sample was taken from the caudal vein with a needle and heparinised syringe and thick and thin smears prepared on glass slides from each fish. After drying, the slides were incubated at 56°C for 30 minutes. Thin smears were fixed in methanol and all smears stained subsequently with Giemsa's stain (1:20 dilution in phosphate buffer, pH 7.6) for 45 minutes. Gill smears, three to four from each fish, were also prepared and one from each stained with Klein's 2% silver nitrate, Feulgen's stain and Heidenhain's hematoxylin. Staining and taxonomic criteria followed those outlined by Lom & Laird (1969).

Results and discussion

***Trypanoplasma ojedae* sp. n.** (Mastigophora: Kinetoplastida)

Description is based on Giemsa-stained specimens observed in 10 infected fish. Measurements in ìm include mean ± standard deviation (x ± s. d., µm).

Genus *Trypanoplasma* Laveran & Mesnil 1901. *Trypanoplasma ojedae* sp. n. (Figs. 1a-c, and Fig. 2). Diagnosis: Body slender, elongate 29.8 ± 5.0 x 5.0 ± 1.2, rounded anteriorly and tapering posteriorly; undulating membrane extending full length of body. Both flagella, longer anterior (4.9 ± 0.3) and shorter posterior (3.1 ± 0.2), nucleus ovoid 3.9 ± 0.3, located 6.0 µm from anterior extremity and 3.0 µm from kinetoplast; kinetoplast slightly elongates 5.2 ± 0.4 x 2.1 ± 0.2, located near anterior end.

Development stages in an unidentified piscicolid leech attached to gill filaments (Figs. 1d-g). Ovoid forms, 5.3

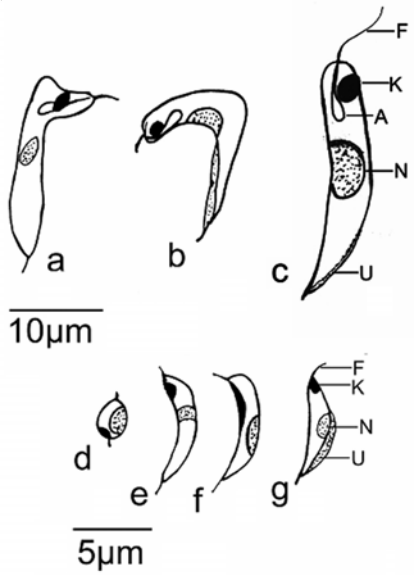

Figure 1

Line diagrams of *Trypanoplasma* (= *Cryptobia*) *ojedae* sp. n. in the blood of *Scartichthys viridis* (a-c) and in the crop of an unidentified leech (d-g). Abbreviations: A, aciculum; F, anterior flagellum; K, kinetoplast, N, nucleus; U, undulating membrane

Diagramas de *Trypanoplasma* (= *Cryptobia*) *ojedae* sp. n. en la sangre de *Scartichthys viridis* (a-c) y en el cultivo de una sanguijuela no identificada (d-g). Abreviaturas: A, aciculum; F, flagelo anterior; K, kinetoplasto; N, núcleo; U, membrana ondulante

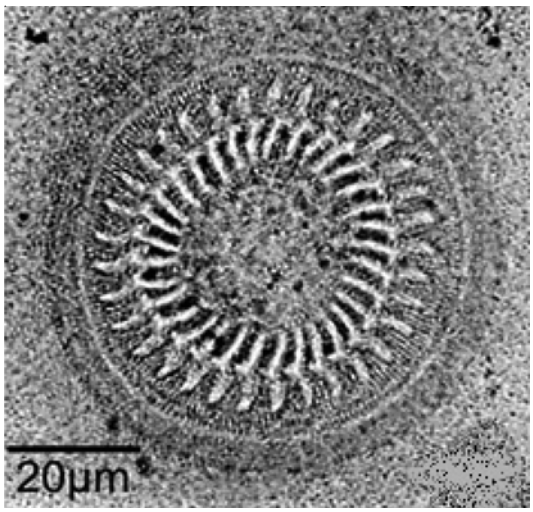

Figure 2

Photomicrograph of *Trichodina lascrucensis* sp. n. (in silver nitrate preparation) from the gills of *Scartichthys viridis*

Fotomicrografía de *Trichodina lascrucensis* sp. n. (en preparación de nitrato de plata) de las branquias de *Scartichthys viridis*

± 0.3; nucleus 2.4 ± 0.1; kinetoplast and two flagella visible (Fig. 1d). Elongate forms, body slender, 10.6 ± 0.7 x 2.5 ± 0.3 µm; prominent undulating membrane; nucleus elongate 3.2 ± 0.2 ± 1.6 ± 0.1; kinetoplast ovoid to elongate 2.4 ± 0.3 x 1.6 ± 0.1; anterior and posterior flagella present but barely visible (Figs. 1e-g).

Taxonomic remarks

Four species of *Trypanoplasma* have been described from the blood of marine fish previously, two from the Pacific and one from the Atlantic Ocean (see Burreson 2007). *Trypanoplasma beckeri* was described from *Scorpaenichthys marmoratus* in Oregon coastal waters in the North Pacific (Burreson 1979a). The parasite was characterised by an acicular-like process located at its anterior extremity (Table 1) This organelle was not observed in either *T. bullocki* from *Pleuronectes* (= *Pseudopleuronectes) americanus* inhabiting Chesapeake Bay, U.S.A. in the North Atlantic or *T. neghmei* in *Paralichthys* spp. off coastal Chile in the South Pacific (Burreson 1979b, Khan *et al*. 2001). Both species of flagellates, *T. beckeri* and *T. neghmei,* infecting fish from the Pacific are considerably larger than *T. ojedae* sp. n. observed in the intertidal fish *S. viridis* from the coast of Chile (Table 1). In addition, the morphology of stages in an unidentified leech-vector possesses a distinct undulating membrane and an organelle resembling an acicular-like process not observed in the other species. We suggest that these differences are adequate to consider *T. ojedae* sp. n. distinct from the other species mentioned previously.

Taxonomy summary

Type host*: Scartichthys viridis* (Valenciennes 1836).

Geographical location: Coast of Central Chile at Las Cruces, 33°27'S, 71°37'W.

Site of infection: Blood.

Prevalence: 100% of 18 fish infected.

Etymology: The species is named after Professor F. Patricio Ojeda for his contributions to fish ecology in Chile.

Deposition of specimens: Holotype deposited in the Museo Nacional de Historia Natural, Santiago, Chile. Catalogue number MNHNCL 3011. Paratypes, catalogue numbers MNHNCL 3012-3016

***Trichodina lascrucensis* sp. n. (Ciliophora: Peritrichida)**

This new species is described by the following measurements (µm) (Figs. 2 and 3): body diameter 70.8 ± 5.1; adhesive disc 53.6 ± 3.4; denticular ring 33 ± 3.2; border membrane 2.2 ± 0.2; number of denticles 32.8 ± 1.8; number of radial pins 8.0 ± 0.4; length of denticle 11.8 ± 1.1; length of blade 3.4 ± 0.3; width of blade 3.2 ± 0.3; length of thorn 3.2 ± 0.3; micronucleus ovoid 2.4 ± 0.1, located near macronuclear arm (+y); macronucleus horseshoe-shaped, diameter 29.6 ± 3.1.

Table 1

Comparison of morphological characteristics (µm) of *Trypanoplasma beckeri, T. bobolsoni, T. neghmei* y *T. ojedae* sp. n. from the blood of marine fish from the Pacific Ocean

Comparación de características morfológicas (µm) de *Trypanoplasma beckeri*, *T. bobolsoni*, *T. neghmei* and *T. ojedae* sp. n. de la sangre de peces marinos del Océano Pacífico

	T. beckeri	*T. bobolsoni*	*T. neghmei*	*T. ojedae* sp. n
Body length	104 (43-165)	16.8 (13.9-18.8)	41.9 ± 3.4*	29.8 ± 3.0 (25-34)
Body width	6.5 (4-11)	3.3 (2.5-5.0)	3.8 ± 0.6	3.1 ± 0.3 (4-6)
Anterior flagellum	8.5 (7-10)	17.2 (14.9-21.5)	10.3 ± 1.1	4.9 ± 0.6 (4-6)
Posterior flagellum	2.5 (1-3)	9.0 (7.1-10.2)	5.2 ± 0.4	3.1 ± 0.3 (2-4)
Nuclear length	8.5 (5-10)	4.9 (4.0-5.9)	3.8 ± 0.2	2.9 ± 0.3 (2-4)
Nuclear width	4.0 (4-6)	0.8 (0.2-1.2)	3.2 ± 0.1	--
Location from anterior end	10.5 (6-14)	--	--	2.4 ± 0.2 (2-4)
Kinetoplast shape	acicular-like	elongate	elongate	elongate

*error in publication; emended

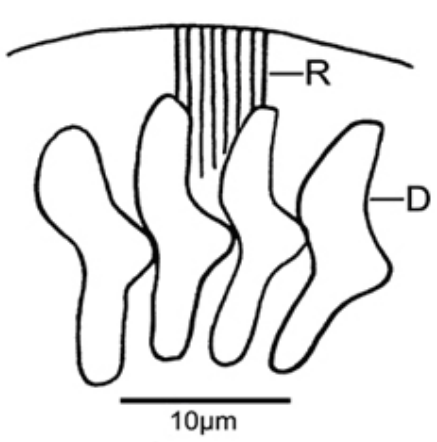

Figure 3

Line diagram of *Trichodina lascrucensis* sp. n. showing denticles (D) and radial pins (R)

Diagrama de *Trichodina lascrucensis* sp. n. mostrando dentículos (D) y pins radiales (R)

Taxonomic remarks

Three species of *Trichodina* have been reported previously from blenniid fish. Fantham (1930) described *T. blennii* from the gills of *Blennius cornutus* from coastal South Africa that was considerably smaller with fewer denticles and radial pins than *T. lascrucensis* sp. n. (Table 2). Tripathi (1948) reported *T. branchicola* from eight species of fish, including two blenniids, captured at Plymouth, U.K. The parasite was notably smaller in size than both *T. blennii* and *T. lascrucensis* sp. n. (Table 2). A third species, *T. multidentis,* infected blenniids inhabiting the intertidal zone at Wellington, New Zealand in the Pacific (Laird 1953). Although several of its morphometric characteristics appear similar to those of *T. lascrucencis* sp. n., the shape of the denticles (Fig. 4), the wide range in size of its adhesive disc and its larger denticular ring separates the two parasites (Table 2). Consequently, we consider *T. lascrucensis* sp. n. distinct from *T. multidentis* and designate it with the characteristics noted herein.

Trichodina lascrucensis sp. n. is considerably larger than any other trichodinids described from intertidal or subtidal habitats in the northwestern Atlantic (Lom & Laird 1969). Additionally, the adhesive disc, denticular ring and number of denticles are greater than in *T. elizabethae, T. jaramilae, T. gahzae, T. domerguei* subsp. *saintjohnsi* and *T. cottidarum*. The only species occurring in a tide pool fish, the radiated shanny (*Ulvaria subbifurcata*) is considerably different from *T. lascrucensis* sp. n. (Table 2). In view of a lack of characters to other species described from marine fish, we consider *T. lascrucensis* as a new species.

Table 2

Comparison of morphometric characteristics (µm) of *Trichodina blennii, T. branchicola, T. multidentis* and *T. lascrucensis* sp. n. from the blood of marine fish from the Pacific Ocean

Comparación de características morfométricas (µm) de *Trichodina blennii*, *T. branchicola*, *T. multidentis* y *T. lascrucensis* sp. n. de la sangre de peces marinos del Océano Pacífico

	T. blennii	*T. branchicola*	*T. multidentis*	*T. lascrucensis* sp. n.
Body diameter	40-45	30-45	73.3†	70.8 ± 5.1 (60-84)
Adhesive disc	24-27	19-23	53.2 (25.3-67.5)	53.6 ± 3.4 (48-60)
Denticular ring	--	10-20	34.8 (10.8-45.4)	33.0 ± 3.2 (28-40)
Border membrane	--	--	2	2.2 ± 0.2 (2-3)
Number of denticles	24-32	20-26	32 (23-45)	32.8 ± 1.1 (30-36)
Number of radial pins	5	6-8	7-9	8.0 ± 0.4 (7-9)
Length of denticle	--	--	~10‡	11.8 ± 1.1 (10-14)
Length of blade	--	--	~5.6‡	3.4 ± 0.3 (3-4)
Width of blade	--	--	~3.9‡	3.2 ± 0.3 (2-4)
Length of thorn	--	--	~3.6‡	3.2 ± 0.2 (2-4)
Macronucleus diameter	--	--	--	29.6 ± 3.1 (26-35)

†based on figure 88 by Laird (1953); ‡based on figure 89 by Laird (1953)

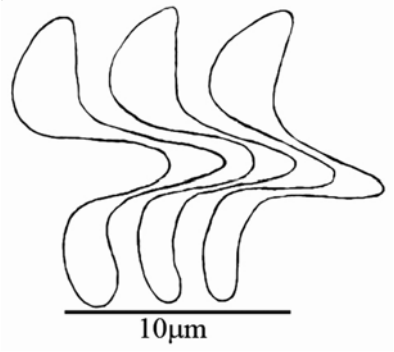

Figure 4

Denticles of *T. multidentis* redrawn from figure 89 in Laird (1953)

Dentículos de *T. multidentis* redibujado según figura 89 en Laird (1953)

Taxonomic summary

Type host: *Scartichthys viridis* (Valenciennes 1836).

Geographical location: coast of central Chile at Las Cruces, 33°27'S, 71°37°W.

Site of infection: gill filaments.

Prevalence: 100% of 18 fish infected.

Etymology: The species is named after the location where its infected host occurred.

Deposition of specimens: Holotype deposited in the Museo Nacional de Historia Natural, Santiago, Chile. Catalogue number MNHNCL Nº 3008. Paratypes, catalogue numbers MNHNCL Nº 3009-3010.

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