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Gill Monogeneans of Potentially Cultured Tilapias and First Record of *Cichlidogyrus mbirizei* Bukinga *et al*., 2012, in Thailand

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Abstract

Monogeneans are pathogenic flatworms commonly found in many fish species. To avoid the risk of economic loss, parasitological investigation is necessary. A survey of gill monogeneans from 45 red hybrid tilapias (*Oreochromis* sp.) in Kanchanaburi province, Thailand, was conducted. Fish samples were infested with 6 gill monogenean species, viz., *Cichlidogyrus sclerosus*, *C. thurstonae*, *C. halli, C. mbirizei*, *C. tilapiae*, and *Scutogyrus longicornis*. Morphologically, unidentified *Cichlidogyrus* species from cultured red tilapia (*Oreochromis niloticus* × *O. mossambicus*) in Nakhon Si Thammarat province, southern Thailand, collected in 2012 and *C. mbirizei*, found in this study, were similar. This finding provides new host and locality records for *C. mbirizei* as a new pathogenic gill monogenean in Thailand. According to the original type host (*Oreochromis tanganicae*) and habitat of *C. mbirizei*, it was strongly suggested that *C. mbirizei* can be translocated with the host during fish trading. For practical identification, a key of gill monogeneans on tilapia in Thailand is also provided herein.

Keywords: Monogenean, *Cichlidogyrus*, *Scutogyrus*, tilapia, Thailand

Introduction

To date, 3 species of tilapia- Nile tilapia (*Oreochromis niloticus*), *Oreochromis niloticus* × *O. mossambicus*, and *Oreochromis* sp.- have been recognized as important fish species for economic consumption in Thailand. *Oreochromis niloticus* was introduced to Thailand in 1965 from Japan, while the other 2 species were created by breeding in 1984 and 1998, respectively [1]. The latter 2 species (*Oreochromis niloticus* × *O. mossambicus* and *Oreochromis* sp.) were crossbred from *Oreochromis* in order to improve quality in the fish product. However, diseases in tilapia cultures have been continuously reported, especially the problems of ectoparasites, such as monogeneans, which are usually found in tilapia culture systems. Monogeneans were reported in *Oreochromis niloticus* for the first time in Thailand in 1996 with the genera *Dactylogyrus* and *Gyrodactylus*, and then, subsequently, in Nile tilapia in 2001, with new records of five species- *Cichlidogyrus sclerosus*, *C. thurstonae*, *C. tubicirrus*, *C. tilapiae*, and *Scutogyrus longicornis* (formerly *Cichlidogyrus longicornis*) [2].

In April 2013, a parasitological study of red tilapia was conducted in Kanchanaburi province, Thailand. An additional species of *Cichlidogyrus* was discovered and identified as *C. mberizei*. Therefore,

the aims of this study were to present taxonomic information, identify the route of invasion of *C. mberizei*, and the distribution of the other gill monogenean species found in tilapia species of economic value for consumption in Thailand.

Materials and methods

Forty-five red hybrid tilapia (*Oreochromis* sp.) {25.9 (23.5-29.2) cm length, 458.8 (300-580) g weight} were collected from cultured cages in Kanchanaburi province (13°58, 16.42″ N 99° 34, 46.33 E) in April 2013 using a scoop net. Gills were removed from the fish samples, gently scraped and examined for gill parasites under a dissecting microscope. Gill monogeneans were pipette, dropped onto glass slides, and covered with a cover-slip. Fixation and preservation were processed with ammonium-picrate glycerine for study of the sclerotized hard parts. The internal anatomy or soft parts were observed by flattening specimens, fixing in 5 % formalin, staining with Heidenhain's iron hematoxylin, alum carmine, dehydrating in a graded ethanol series, clearing in xylene, and mounting with permount. Parasites were viewed with phase contrast and photographed with a Nikon Eclipse Ci phase contrast 0.90 dry (Nikon Corporation, Japan).

The permanent mounts of previously relevant works from various collections in Thailand were borrowed, namely *Cichlidogyrus* sp.A, *C. halli*, *C. tubicirrus*, *C. sclerosus, C. thurstonae*, and *Scutogyrus longicornis*. Specimens were re-examined and compared with the present study. The unidentified gill monogenean species collected from cultured red tilapia (*Oreochromis niloticus* × *O. mossambicus*) in Nakhon Si Thammarat province, southern Thailand, in 2012 by [3] were also re-examined and re-identified in this study. Drawings were made with the aid of a drawing tube attached to an Olympus BX51 compound microscope. Average measurements were recorded (all in micrometers) followed by the range in parentheses. Monogenean identification was made using keys [4,5]. The morphological terminology followed [6]. The ecological terms were defined by [7]: prevalence is a percentage of infected fish in a sample, and mean intensity is the average number of parasites per host examined. Monogenean specimens were deposited in the Zoological Museum of Kasetsart University, Thailand (ZMKU-PM-1001-1004 for *C. mbirizei*, ZMKU-PM-1005-1008 for *C. halli*, ZMKU-PM-1009-1012 for *C. sclerosus*, ZMKU-PM-1013-1016 for *C. tilapiae*, ZMKU-PM-1017-1020 for *C. thurstonae* and ZMKU-PM-1021-1024 for *Scutogyrus longicornis*).

Table 1 Gill monogeneans from Nile tilapia (*Oreochromis niloticus*) in Thailand.

Monogene	Location	Year collected	P*	MI*	References
Cichlidogyrus sclerosus	Nakhon Si Thammarat province	2001	60	9	[2]
	Chonburi province	2006	30	4	[16]
	Loie province	2008-2009	100	19.4	[1]
	Sakon Nakhon province	2008-2009	100	8	[1]
	Chaiyaphum province	2008-2009	67	19.4	[1]
	Nakhon Ratchasima province	2008-2009	100	47	[1]
		2009	33.33	3	[18]
	Nakhon Pathom province	2008-2009	100	7	[1]
Cichlidogyrus thurstonae	Nakhon Si Thammarat province	2004	50	7	[2]
	Chonburi province	2006	20	2	[17]
	Loie province	2008-2009	100	16.6	[1]
	Chaiyaphum province	2008-2009	67	4	[1]
	Nakhon Ratchasima province	2008-2009	100	16	[1]
Cichlidogyrus tilapiae	Nakhon Si Thammarat province	2004	40	3	[2]
	Chonburi province	2006	25	2	[17]
	Loie province	2008-2009	50	1	[1]
	Sakon Nakhon province	2008-2009	60	4.8	[1]
	Nakhon Ratchasima province	2008-2009	50	2	[1]
	Nakhon Nayok province	2008-2009	100	16	[1]
Cichlidogyrus halli (published as C. *tubicirrus*)	Nakhon Si Thammarat province	2001	20	10	[2]
Cichlidogyrus halli	Loie province	2008-2009	83	16.4	[1]
	Chaiyaphum province	2008-2009	33	3.5	[1]
	Nakhon Ratchasima province	2008-2009	100	9	[1]
Scutogyrus longicornis	Nakhon Si Thammarat province	2001	50	4	[2]
	Loie province	2008-2009	100	8.6	[1]
	Sakon Nakhon province	2008-2009	60	2.4	[1]
	Chaiyaphum province	2008-2009	67	6.6	[1]
	Nakhon Ratchasima province	2008-2009	100	6	[1]
		2009	33.33	1	[18]
	Nakhon Nayok province	2008-2009	100	2	[1]

*P = prevalence / MI = mean intensity

Table 2 Gill monogeneans from red tilapia (*Oreochromis niloticus* × *O. mossambicus*) in Thailand.

Monogene	Location	Year collected	P*	MI*	References
Cichlidogyrus sclerosus	Nakhon Si Thammarat province	2012	60.86	5.39	[3]
Cichlidogyrus thrustonae	Nakhon Si Thammarat province	2012	62.28	7.08	[3]
Cichlidogyrus tilapiae	Nakhon Si Thammarat province	2012	20.09	2.03	[3]
Cichlidogyrus halli (published as *C. tubicirrus*)	Nakhon Si Thammarat province	2012	60.42	7.86	[3]
Cichlidogyrus mbirizei (published as *Cichlidogyrus* sp. A)	Nakhon Si Thammarat province	2012	6.04	0.85	[3]

*P = prevalence / MI = mean intensity

Table 3 Gill monogeneans from red hybrid tilapia (Tubtim fish) (*Oreochromis* sp.) in Thailand.

Monogene	Location	Year collected	P*	MI*	References
Cichlidogyrus sclerosus	Nakhon Si Thammarat province	2001	70	4	[2]
	Kanchanaburi province	2010-2011	57.78	34.07	[8]
		2013	100	81.11	present work
	Samut Songkhram province	2010-2011	55.99	42.27	[8]
	Chiang Mai province	2008-2009	100	28	[1]
	Uttaradit province	2008-2009	100	73.2	[1]
	Nakhon Ratchasima province	2008-2009	100	62.6	[1]
	Kamphaeng Phet province	2008-2009	100	58	[1]
	Nakhon Sawan province	2008-2009	100	14.6	[1]
	Uthai Thani province	2008-2009	100	12	[1]
	Phatthalung province	2008-2009	100	13.4	[1]
Cichlidogyrus thurstonae	Nakhon Si Thammarat province	2001	80	4	[2]
	Kanchanaburi province	2010-2011	42.22	8.55	[8]
		2013	100	18.9	present work
	Samut Songkhram province	2010-2011	45.98	5.76	[8]
	Chiang Mai province	2008-2009	70	3.6	[1]
	Uttaradit province	2008-2009	25	16	[1]
	Kamphaeng Phet province	2008-2009	100	20	[1]
	Nakhon Sawan province	2008-2009	75	8	[1]
	Uthai Thani province	2008-2009	50	1	[1]
	Phatthalung province	2008-2009	33	28	[1]
Cichlidogyrus tilapiae	Nakhon Si Thammarat province	2001	40	12	[2]
	Kanchanaburi province	2010-2011	14.44	2.38	[8]
		2013	6	1	present work
	Samut Songkhram province	2010-2011	16.61	2.79	[8]
	Nakhon Ratchasima province	2008-2009	33	0.6	[1]
	Nakhon Sawan province	2008-2009	38	1.6	[1]
	Suphan Buri province	2008-2009	50	5	[1]
Cichlidogyrus halli	Kanchanaburi province	2013	42.96	12.71	[8]

Monogene	Location	Year collected	P*	MI*	References
(published as *C. tubicirrus*)	Samut Songkhram province	2013	35.47	11.99	[8]
Cichlidogyrus halli	Kanchanaburi province	2013	100	25.21	present work
	Chiang Mai province	2008-2009	80	7	[1]
	Uttaradit province	2008-2009	50	1	[1]
	Nakhon Ratchasima province	2008-2009	100	15.4	[1]
	Kamphaeng Phet province	2008-2009	100	16.8	[1]
	Nakhon Sawan province	2008-2009	88	6	[1]
	Uthai Thani province	2008-2009	50	1	[1]
	Prachin Buri province	2008-2009	100	6	[1]
	Suphan Buri province	2008-2009	100	2	[1]
	Phatthalung province	2008-2009	33	5.4	[1]
Cichlidogyrus mbirizei	Kanchanaburi province	2013	100	115.2	present work
Scutogyrus longicornis	Kanchanaburi province	2010-2011	40.37	7.33	[8]
		2013	100	19.3	present work
	Samut Songkhram province	2010-2011	30.66	6.55	[8]
	Chiang Mai province	2008-2009	10	0.6	[1]
	Uttaradit province	2008-2009	50	4	[1]
	Kamphaeng Phet province	2008-2009	100	6.8	[1]
	Nakhon Sawan province	2008-2009	50	2.8	[1]
	Phatthalung province	2008-2009	67	5.4	[1]

*P = prevalence / MI = mean intensity

Results and discussion

Forty five red hybrid tilapia collected from Kanchanaburi province were infested with 6 species of gill monogenean. The infection level differed between the species for *C. tilapiae* (overall prevalence and mean abundance: 6 % and 1.0, respectively), *C. halli* (100 %, 25.2), *C. sclerosus* (100 %, 81.1), *C. thurstonae* (100 %, 18.9), *C. mbirizei* (100 %, 115.2), and *Scutogyrus longicornis* (100 %, 19.3). All except *C. mbirizei* have been previously reported and described in Thailand in *Oreochromis niloticus* (**Table 1**), *Oreochromis niloticus* × *O. mossambicus* (**Table 2**), *Oreochromis* sp. (**Table 3**). *C. mbirizei* which was originally found in *Oreochromis tanganicae* (endemic to Lake Tanganyika, West Africa) was also observed on tilapia fish cultured in Thailand. The re-description as well as illustrations of *C. mbirizei* from Thailand are given below.

Systematic account
Phylum Platyhelminthes
Class Monogenea
Order Monopisthocotylea
Family Ancyrocephalidae
Genus *Cichlidogyrus*
Cichlidogyrus mbirizei Bukinga *et al*., 2012
(**Figure 1**)

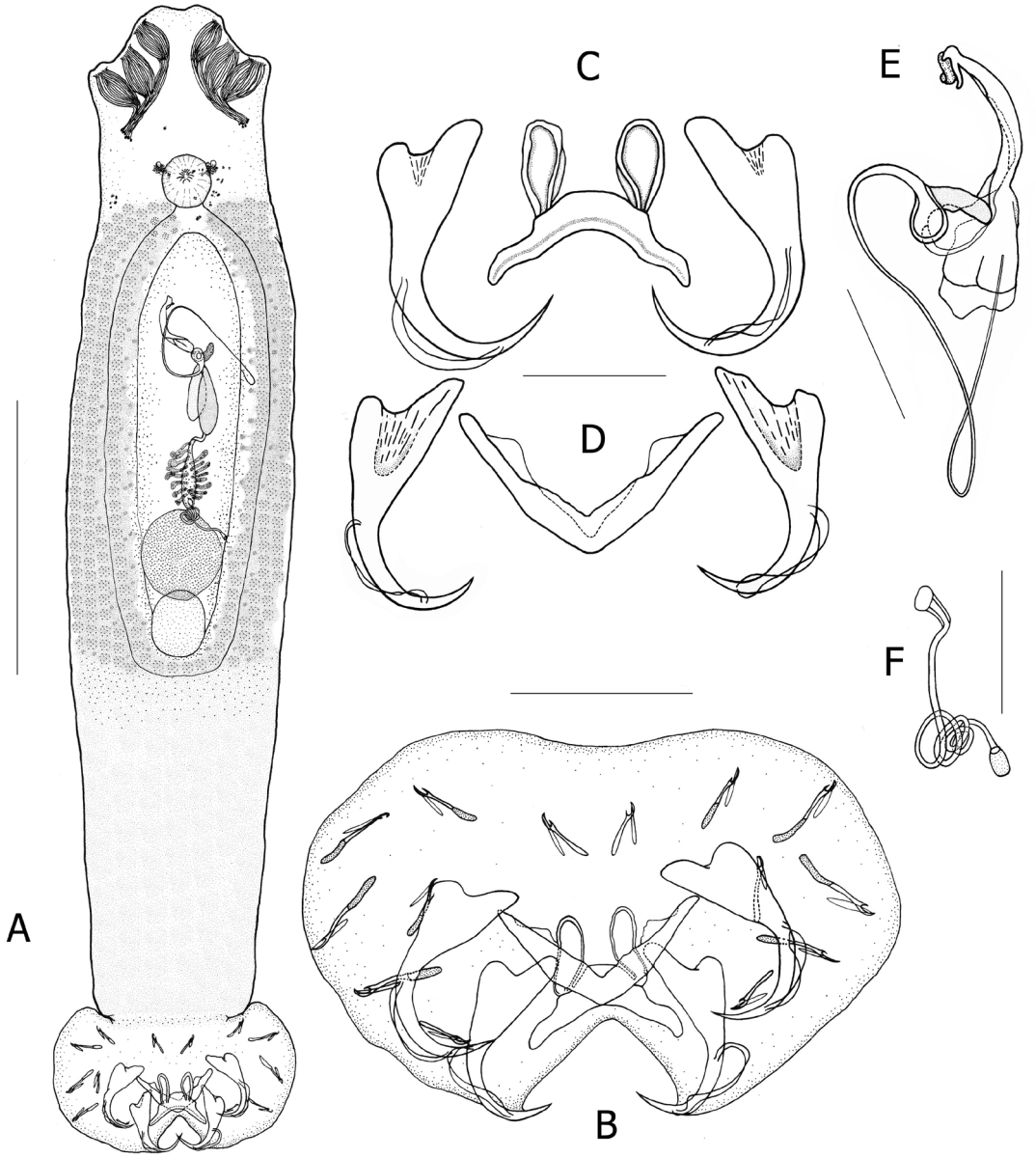

Figure 1 *Cichlidogyrus mbirizei* Bukinga *et al.*, 2012 A, whole body; B, Haptor; C, dorsal bar and dorsal hamulus; D, ventral bar and ventral hamulus; E, copulatory organ; F, vagina. Scale bars: A, 200 µm; B, 50 µm; C-F, 30 µm.

Description (30 specimens measured): Body slender, 730 (500 - 1000) long, and 117 (90 - 150) wide. Cephalic lobe with 3 - 4 pairs of head organs. One pair of eyes with lens. Pharynx spherical, 50 (45 - 55 in diameter; intestine bifurcated posterior to pharynx and becoming confluent posterior to testis. Haptor rounded, with 2 pairs of hamuli and 7 pairs of hooklets. Ventral and dorsal hamuli of similar size and shape, except the inner root of ventral hamuli is truncated while rounded in dorsal hamuli, 38 (34 -

42) and 46 (38 - 45) long, respectively, and has a strongly curved shaft. Hamulus filament present. Ventral bar V-shaped, 34 (26 - 41) in transverse length, with rounded extremities and indented anterior rim. Dorsal bar massive and X-shaped, 35 (30 - 40) in transverse length; branches wide; appendages pyriform with rounded ends. Hooklets short; pair 2 shortest without base, other pairs with short base; hooklet lengths 12 (11 - 15), 11 (10 - 12), 16 (12 - 19), 20 (15 - 23), 21 (18 - 24), 21 (19 - 24) and 18 (16 - 22), respectively, for pairs 1 - 7.

Testis ovoid and located dorso-posterior to ovary. Two prostatic reservoirs situated posterior to copulatory organ. Copulatory organ located one-third from anterior part of the body, comprising a thin copulatory tube, length 256 (240 - 285), with distinctive heel at base and arched accessory piece length, 99 (86 - 120) with bifid at the distal end and petal membranous at base. Ovary ovoid and located anteroventral to testis. Vaginal opening funnel-shaped, connecting to dilated seminal receptacle with coiled vaginal tube. Vitelline follicles well-developed and co-extensive with intestine. Mehlis' gland well developed surrounding the ootype.

Hosts: *Oreochromis* sp., red tilapia (*O. niloticus* × *O. mossambicus*) (Perciformes: Cichlidae).
Site of attachment: Gills.
Localities: Kanchanaburi province, Nakhon Si Thammarat province, Thailand.
Remarks: The finding of *C. mbirizei* in this study represents the first record in Thailand and a new host record for fish of value for economic consumption. This parasite was originally described from the gills of *Oreochromis tanganicae* in the Democratic Republic of the Congo, Africa, by [5].

Although monogenean parasites from *Oreochromis niloticus* were first reported in Thailand in 1996, only the genera *Dactylogyrus* and *Gyrodactylus* were mentioned. Lerssutthichawal and Supamattaya [2] later reported 5 *Cichlidogyrus* species from cultured and wild *Oreochromis* spp. in 2001, representing the first report for *Cichlidogyrus* in Thai cichlids. The parasitic diversity, prevalence, and mean intensity in each location in Thailand seem to be different. Comparatively, a wild population has a lower prevalence than that of a cultured one. After we collected red hybrid tilapia sample in Kanchanaburi province, 6 species of monogeneans as listed above were found. *Cichidogyrus tubicirrus*, reported in some scientific papers in Thailand, was revised. Paperna and Thurston [8] separated *C. tubicirrus* into 3 subspecies- *C. tubicirrus magnum*, *C. tubicirrus minutus*, and *C. tubicirrus longipenis*. The original specimens, collected by several authors [2-3,9], were re-examined, and finally re-assigned to *Cichlidogyrus halli* (personal contact).

Pariselle *et al*. [10] reported 13 monogenean genera infecting the cichlids- *Insulacleidus*, *Ceylonotrema*, *Sclerocleidoides*, *Cichlidogyrus*, *Onchobdella*, *Scutogyrus*, *Gussevia*, *Sciadicleithrum*, *Trinidactylus*, *Tucuranella*, *Gyrodactylus*, *Enterogyrus*, and *Urogyrus*. They also stated that *Cichlidogyrus* and *Scutogyrus* were found in West Africa, East Africa, and the Levant. Accordingly, as many cichlid fish were imported to Thailand under conditions without health guarantees, monogeneans were also transported with their hosts. However, parasitic study has been usually focused on 3 tilapia species of economic importance (Nile tilapia, red tilapia, and hybrid tilapia). Monogeneans found in tilapia culture in Thailand are shown in **Tables 1 - 3**.

Although only 5 gill monogenean species were observed in Nile tilapia (*O. niloticus*) in Thailand [2], other species have been reported in this fish species, viz., *Cichlidogyrus bifurcates* found in *Tilapia nilotica* [11], *C. tiberianus* in Israel and the Philippines [6,12-13], and *Cichlidogyrus rognoni* in West Africa [14]. From these data, we can suggest that the monogenean species in *O. niloticus* depended on fish habitat. A similar pattern of distribution and diversity of gill monogeneans in Thailand has also been reported [2-3,9] (**Tables 1 - 3**). This suggested that *O. niloticus* could accommodate a high variety of gill monogeneans.

Of these 6 monogenen species found, *C. mbirizei* was recorded for the first time in Thailand. When compared with the specimens of *Cichlidogyrus* sp. A from red tilapia (*Oreochromis niloticus* × *O. mossambicus*) in Nakhon Si Thammarat province in 2014 [3], it was determined to be the same species, and this finding also represented a new host record for red tilapia. The mean abundance of *C. mbirizei* in

tilapia samples from Kanchanaburi province was high, while it was low in Nakhon Si Thammarat province. This species was established in 2012, and this parasite was found in *Oreochromis tanganicae* in the Democratic Republic of the Congo, Africa.

Cichlidae originated near Madagascar, and occurs in India and Madagascar [10]. This group of fish was imported into many countries, including Thailand, and this may represent the channel of distribution of the associated monogenean parasites. The distribution and biogeography of the hosts will directly affect their monogeneans; moreover, some monogeneans from South America and Africa showed low host specificity, and can be found in several host species [4]. For this reason, the distribution of monogeneans is likely to come from other species of Cichlidae, which is the most popular group imported to Thailand as ornamental fish [15].

For example, ornamental fish species, such as *Tilapia buttikoferi*, have been imported and cultured in Thailand. Due to the extensive flooding in 2011, this fish might have been released and spread throughout the natural waters of Thailand and become mixed up with cultured tilapia (authors' observation; unpublished data). The negative effects from this fish are a decrease in native fish numbers and the possible introduction of some pathogens to indigenous aquatic species. According to [16], this fish can harbor *C. nuniezi*, *C. bonhommei*, and *C. slembrouchi*. However, after collection of monogenean samples from *T. buttikoferi* from the reservoir in Thailand, we found only *C. tilapiae*, which did not agree with the report of [16]. This parasite was possibly passed to *T. buttikoferi* from *O. niloticus*, which can be found normally in this reservoir. On the other hand, monogeneans from ornamental fish might be a source of contamination in some tilapia in Thailand as well. The possibility of an extended distribution of *C. mbirizei* in Thailand may arise from *O. tanganicae* or other fish species in the family Cichlidae which are imported from their origin countries to Thailand. Therefore, imported fish may be a vector for the monogenean parasites invasion.

Key to identification of the gill monogeneans parasitic on cultured tilapias in Thailand (Figures 2 and 3)

1 dorsal bar with triangular plates connected by narrow bridge supporting 2 extremely long appendages with narrower and rounded extremities. Ventral bar arched, constant width, with round ends, attached with thin, sclerotized bar in form of vault above ventral bar and ending exactly at ends of bar, attached with fan-shaped membranous portion and associated with sclerotized ribbed part
…… *Scutogyrus longicornis*
- dorsal bar without sclerotized connection with ventral bar……..…...…….…….…...2

2 simple shape copulatory organ located one-third of total length from anterior extremity of body; copulatory tube straight and wider at base; accessory piece almost straight with sharp hook at terminal end ……..……..…….……...*Cichlidogyrus tilapiae*
- copulatory organ large and located posterior to intestinal bifurcation…….....…….……...3

3 copulatory tube S-shaped, with irregular base; accessory piece lancet-shaped and shorter than copulatory tube. Vagina sub-median…….……..…..…….*Cichlodogyrus halli*
- copulatory tube not S-shaped ……..……..……....…….…… 4

4 copulatory organ with large serrated plate; copulatory tube thin and arched, with tapered end; accessory piece, with finger-like extension. Vagina sub-median……..*Cichlidogyrus sclerosus*
- copulatory tube thin and arched, with oval base; accessory piece slightly S-shaped, ending bottle-opener-like. Vagina short tube and arched……...……...*Cichlidogyrus thurstonae*
- copulatory organ with very long and thin penis and appendix heel at the penis base; tubular accessory piece, bifid terminal end, with membranous sheaths at based, sub-median thin and coiled vagina ……..……..…...*Cichlidogyrus mbirizei*

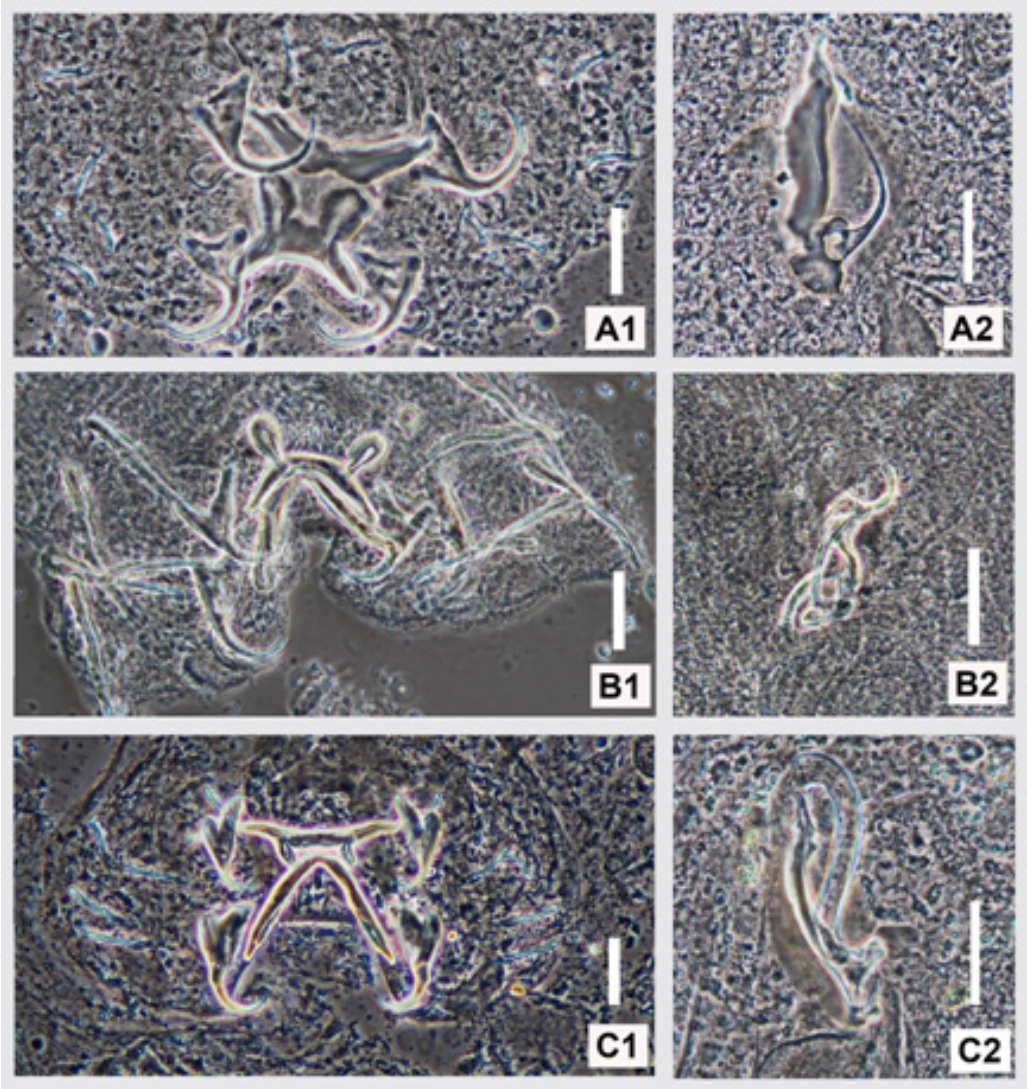

Figure 2 *Cichlidogyrus sclerosus* (A), *C. thurstonae* (B), and *C. halli* (C); 1 = opishaptor, 2 = copulatory organ. Scale bar: 50 µm.

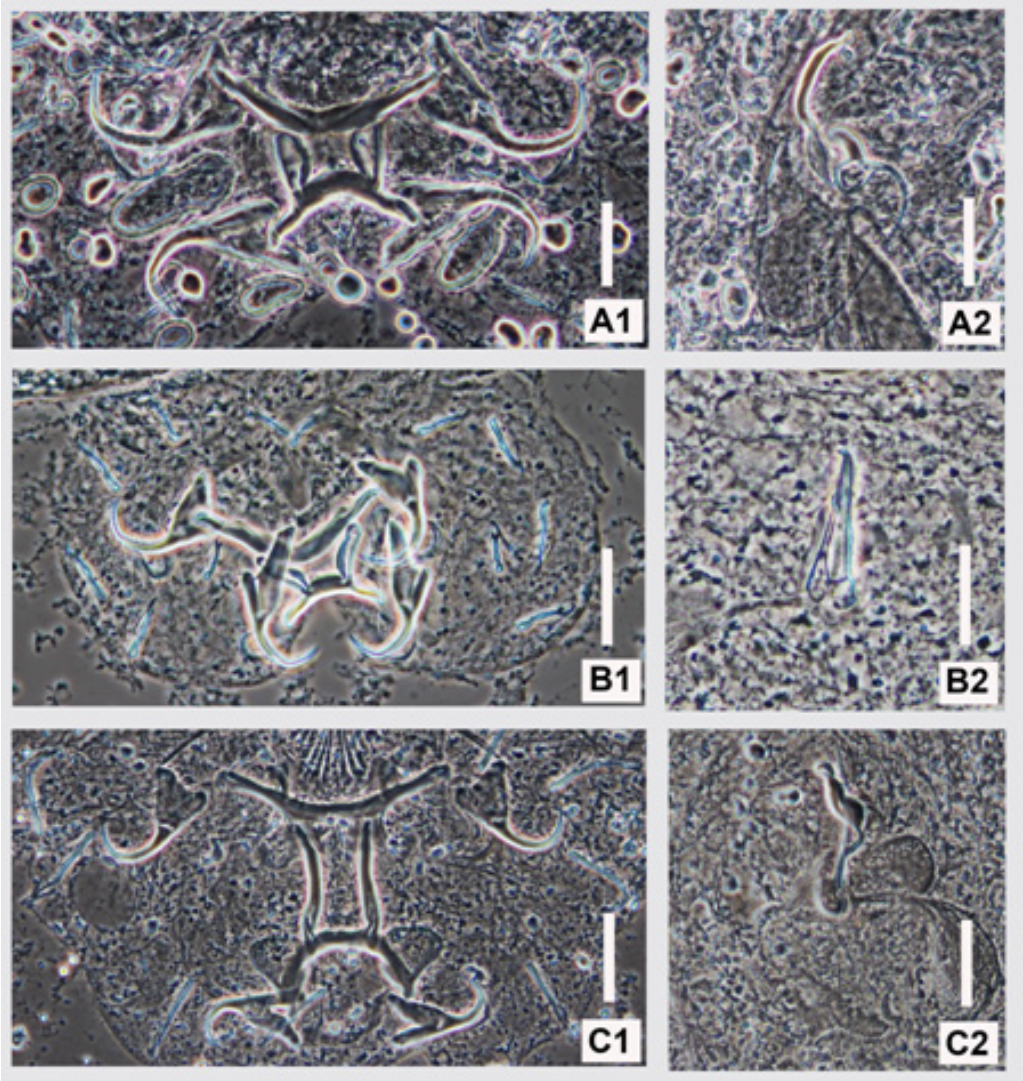

Figure 3 *Cichlidogyrus mbirizei* (A), *C. tilapiae* (B), and *Scutogyrus longicornis* (C); 1 = opishaptor, 2 = copulatory organ. Scale bar: 50 µm.

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