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## ORIGINAL ARTICLE /ARTÍCULO ORIGINAL

# A TAXONOMIC STUDY OF ASPIDOGASTREANS OF THE GENUS *LOBATOSTOMA* ECKMANN, 1932 (TREMATODA) DEPOSITED IN THE UNITED STATES NATIONAL PARASITE COLLECTION (USNPC)

# ESTUDIO TAXONÓMICO SOBRE LOS ASPIDOGASTREOS DEL GÉNERO *LOBATOSTOMA* ECKMANN, 1932 (TREMATODA) DEPOSITADOS EN LA UNITED STATES NATIONAL PARASITE COLLECTION (USNPC)

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## ABSTRACT

The helminthological collections are depository of the type/voucher specimens and contribute to the maintenance of the history of the species. In this study, a reassessment of the taxonomic status of *Lobatostoma* (*a priori*) species is performed based on specimens deposited in the United States National Parasite Collection (USNPC). Nine slides (six species) of stained specimens of *L. albulae* Yamaguti, 1968; *L. kemostoma* (MacCallum & MacCallum, 1913); *L. manteri* Rohde, 1973; *L. pacificum* Manter, 1940; *L. ringens* (Linton, 1905); *L. veranoi* Oliva & Luque, 1989, including the photomicrographs from the USNPC, were analyzed. The specimens under accession numbers USNPC 36394.02 (assumed to be *L. ringens*) and USNPC 083016/B7.30, B7.36 (assumed to be *L. kemostoma*), are identified herein as *Cotylogaster* sp. and *Lobatostoma* sp., respectively. Further, we provide taxonomic comments on each taxon studied and outline the need of caution when assessing the taxonomic identification of deposited material in the helminthological collections.

**Keywords:** Aspidogastrea – *Cotylogaster* - helminthological collections – *Lobatostoma* – taxonomy - USNPC.

## RESUMEN

Las colecciones helmintológicas son depositarios de los especímenes tipo/muestras representativas y contribuyen al mantenimiento de la historia de la especie. En este estudio, se proporciona una reevaluación de la situación taxonómica de las especies de *Lobatostoma* (*a priori*) basado en especímenes depositados en la United States National Parasite Collection (USNPC). Nueve láminas (seis especies) con muestras teñidas de *L. albulae* Yamaguti, 1968; *L. kemostoma* (MacCallum & MacCallum, 1913); *L. manteri* Rohde, 1973; *L. pacificum* Manter, 1940; *L. ringens* (Linton, 1905); *L. veranoi* Oliva & Luque, 1989, incluyendo las fotos enviadas por USNPC, fueron analizadas. Los especímenes bajo los números de acceso USNPC 36394.02 (que se supone *L. ringens*) y USNPC 083016/B730, B7.36 (que se supone *L. kemostoma*), se identificaron en este estudio como *Cotylogaster* sp. y *Lobatostoma* sp., respectivamente. Además, se proporcionan comentarios taxonómicos acerca de cada taxón estudiado y se enfatiza la necesidad de evaluar conservadoramente la identificación taxonómica de material depositado en las colecciones helmintológicas.

**Palabras clave:** Aspidogastrea - colecciones helmintológicas – *Cotylogaster* – *Lobatostoma* – taxonomía - USNPC.

## INTRODUCTION

Aspidogastrea Faust & Tang, 1936 is a small group of trematodes parasitizing molluscs as obligate hosts and vertebrates (fishes and turtles) as facultative or obligate final hosts (Rohde, 2002). It represents the most basal group of flukes (Olson *et al*., 2003). The aspidogastreans are characterized by having a ventral holdfast organ with rows of alveoli or suckerlets, or just presenting a row of rugae or suckers (Rohde, 2002).

*Lobatostoma* Eckmann, 1932 is one of the most speciose aspidogastreans and their members are found in molluscs and teleost fish distributed worldwide (Rohde, 1994). Alves *et al*. (2015a) listed nine valid species in the genus, namely, *L. anisotremum* Oliva & Carvajal, 1984; *L. hanumanthai* Narasimhulu & Madhavi, 1980; *L. jungwirthi* Kritscher, 1974; *L. kemostoma* (MacCallum & MacCallum, 1913); *L. manteri* Rohde, 1973; *L. pacificum* Manter, 1940; *L. platense* Mañé-Garzon & Holcman-Spector, 1976; *L. ringens* (Linton, 1905); and *L. veranoi* Oliva & Luque, 1989. Of them, six have been described from carangid fish, while the remaining have been described from fish of the families Haemulidae, Sciaenidae and Cichlidae (only freshwater host). All species possesses head lobes, single testis and the presence of a cirrus-sac as differential diagnosis of the genus (Rohde, 2002).

Alves *et al*. (2015b) redescribed *L. kemostoma*, based on the type and newly collected material from the type host *Trachinotus carolinus* (Linnaeus, 1766) (Carangidae) off the Brazilian coast. They also designated a lectotype from the syntypes deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland. So far, this collection has been of assistance for parasitologists all over the world with loan of specimens, lots of which serve as source of information on biodiversity and systematics of parasites (see Hoberg, 2002). Recently, the operational responsibility for curation and the management of specimens deposited in the USNPC has been transferred to the National Museum of Natural History (NMNH), Washington, D.C. (Hoberg & Phillips, 2014).

As a part of an ongoing research project on *Lobatostoma*, we had access to specimens deposited in the USNPC/NMNH. Herein, we reassess the taxonomic identification of some species, with comments on each taxon.

## MATERIAL AND METHODS

The type and voucher specimens of *Lobatostoma* examined were lent by the staff of USNPC. Photomicrographs of holotypes, kindly sent by Ms. Patricia A. Pilitt (Associate Curator of USNPC), were also studied. A list of the material examined is shown in Table 1.

The specimens were studied according to their original descriptions and the present literature on aspidogastreans. Photomicrographs were taken using a CELL D™ software set up in the Olympus™ BX51 microscope. The names of fish follow FishBase (Froese & Pauly, 2014).

## RESULTS

A total of nine slides containing stained specimens of *Lobatostoma* spp. (*a priori*), including those photographs sent by the staff of USNPC, were analyzed. Taxonomic inconsistencies were observed in the specimens under accession number USNPC 36394.02 labeled as *L. ringens*. In fact, the five specimens on the slide are reassigned to *Cotylogaster* Monticelli, 1892. Similarly, the two slides USNPC 083016/B7.30, B7.36 with two specimens on each, previously labeled as

*L. kemostoma* are now identified as *Lobatostoma* sp. The comments on each taxon are provided below:

*Lobatostoma albulae* Yamaguti, 1968 (Fig. 1A)

This species was briefly described based on two specimens from *Albula vulpes* (Linnaeus, 1758) (Albuliformes: Albulidae) off the Pacific Ocean. Several important morphological features, such as the arrangement and the total number of alveoli on the ventral disc were incompletely described. In addition, the holotype (Fig. 1A) illustrated in the original description was deformed due the excessive number of eggs, which made impossible its specific identification. Apparently, the second specimen studied by Yamaguti was not deposited (see Yamaguti, 1968).

Narasimhulu & Madhavi (1980) suggested that *L. albulae* is probably junior synonym of *L. ringens*, but there is no evidence for this and Oliva & Luque (1989) considered as valid only nine species, excluding *L. albulae*. Despite of that, new material from the type host is necessary to reassess the taxonomic status of this doubtful species. Therefore, *L. albulae* is considered to be a *species inquirenda*.

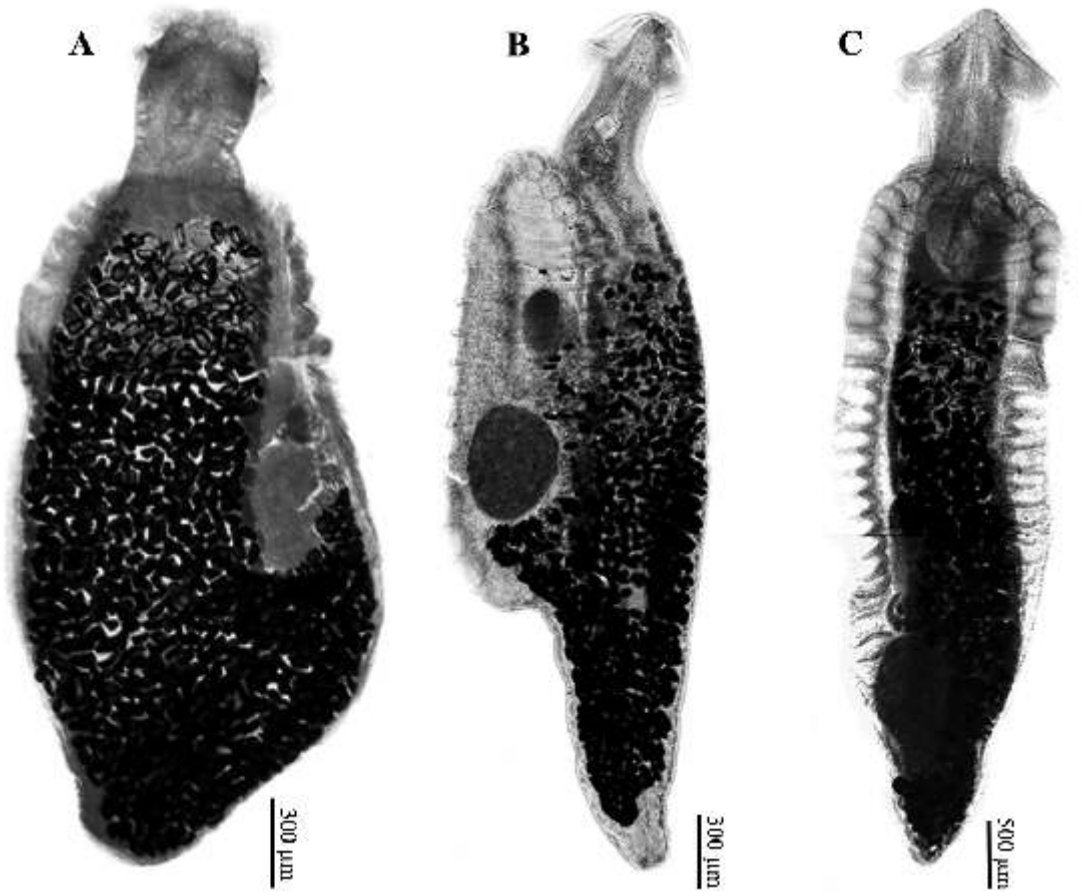


**Figure 1.** Holotypes of *Lobatostoma* deposited in the USNPC: A) *L. albulae* from *Albula vulpes* – ventral view (USNPC 063691.00); B) *L. pacificum* from *Trachinotus paitensis* – ventro-lateral view (USNPC 09321.00); C) *L. manteri* from *T. blochii* – ventral view (USNPC 71056.00).

*Lobatostoma pacificum* Manter, 1940 (Fig. 1B)

*Lobatostoma pacificum* was the first species of the genus described from the Pacific Ocean parasitizing *Trachinotus paitensis* (Cuvier, 1832) (syn. of *T. paloma*) in the Galapagos Islands. It is differentiated from the similar *L. kemostoma*, by having higher number of marginal alveoli on the ventral disc (32–34 *vs* 30), cephalic lobes with different shape and size and by having a hindbody (from distal base of the ventral disc to posterior end) smaller than the ventral disc. *L. pacificum* is also differentiated from the other species of the genus by possessing the hindbody at least one third of the body length.

The specimen USNPC 09321.00 is registered in the USNPC as "type", without any mention of what kind of type it is, recognized by the International Commission on Zoological Nomenclature (ICZN), article 72.1. (ICZN, 1999). Manter deposited all holotypes of the several described species in the USNPC and the paratypes in the Allan Hancock Foundation at The University of Southern California, consequently the studied specimen is the holotype (see pag. 329 in Manter, 1940).

*Lobatostoma manteri* Rohde, 1973 (Fig. 1C)

This species was described from *Trachinotus blochii* (Lacepède, 1801) off the Australian coast (Pacific Ocean part). It differs from the other eight congeneric species by having 56–62 marginal alveoli on the ventral disc (Rohde, 1973). Despite of the author also differentiates the species by the ratio between the cirrus sac and pharynx, *L. platense* shows similarly the cirrus sac twice the size, at least, of the pharynx (Mañé-Garzon & Holcman-Spector, 1976).

Among the *Lobatostoma* species, *L. manteri* has been widely studied regarding the biology of the genus. For instance, studies on life cycle (Rohde, 1973), morphology (Rohde, 1973), including ultrastructure (Rohde, 1989; Rohde & Watson, 1989a; Rohde *et al*., 1991), sensorial system (Rohde & Watson, 1989b, 1992), parasite-host system (Rohde & Sandland, 1973; Rohde, 1981) and pathogenicity (Rohde, 1975) were performed on this species.

*Lobatostoma ringens* (Linton, 1905) (Fig. 2 A–B)

The species was described as *Aspidogaster ringens* Linton, 1905 from *T. carolinus* and *Micropogonias undulatus* (Linnaeus, 1766) in the North Atlantic Ocean; the type host was not given. Eckmann (1932) erected *Lobatostoma* to accommodate *L. ringens* and *L. kemostoma* based on features already mentioned. Since then, *L. ringens* has been reported from several unrelated groups of fishes (nine different families) across the western Atlantic Ocean, with the major number of records in the genus (Hendrix & Overstreet, 1977; Chinchilla & Mago, 2005). Moreover, there are two records on molluscs, although its life cycle has not been completely elucidated as for *L. manteri* and *L. jungwirthi* (Rohde, 1973; Zylber & Ostrowski de Núñez, 1999).

The identification of the species has been broadly based on the relative position of testis, the ratio hindbody/ventral disc and pharynx/cirrus sac, number of marginal alveoli and the arrangement of the vitellaria (Rohde, 1973; Mañé-Garzon & Holcman-Spector, 1976; Lunaschi, 1984; Oliva & Carvajal, 1984). However, the intraspecific variation, the degree of contraction, as well as the mounting of the material, can alter some of these features (Oliva & Luque, 1989; Alves *et al*., 2015a). Therefore, *L. ringens* can be differentiated from its congeners, but three by having 32–40 marginal alveoli. It differs from *L. pacificum* and *L. kemostoma* by a smaller hindbody (less than one third of the body *vs* at least one third of the body). It is also differentiated from *L. jungwirthi*, the only freshwater species of the genus, due to the lacking of a hindbody in the last taxon.

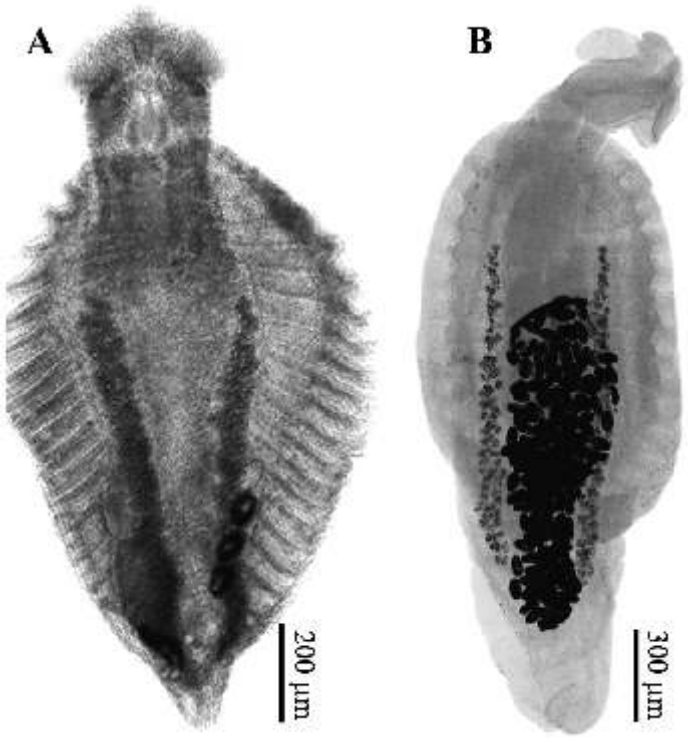


**Figure 2.** Vouchers and type specimens of *Lobatostoma ringens* deposited in the USNPC: A) Young adult from *Calamus calamus* – ventral view (USNPC 08538.02); B) Adult from *Trachinotus carolinus* – ventral view (USNPC 035560.00).

The unique specimen which bears the name of the taxon, as recommended by ICZN, is still lacking. According to records of USNPC, in 1996, Ms. Meryl A. Ferguson redetermined the specimens of *L. ringens* USNPC 035556.02, 035557.02, 035559.02 deposited by G.W. MacCallum in 1911 and 1912 as "types", although she has not published any work to validate it (P. Pilitt, personal communication). A redescription of *L. ringens*, based on morphological and molecular studies of specimens collected across the western Atlantic Ocean, is needed along with a critical review of the material deposited in the USNPC.

Gomes & Fábio (1976) redescribed *L. ringens* arguing that there were no records of this species in the South America, but Szidat (1961) and Suriano (1966) have already reported it (with taxonomic description) from *Oncopterus darwinii* Steindachner, 1874 and *Micropogonias furnieri* (Desmarest, 1823), respectively, in the Argentine waters. Moreover, they overlooked the number of median alveoli on the ventral disc, thus underestimating the total number of them.

*Lobatostoma veranoi* Oliva & Luque, 1989 (Fig. 3A–C)

*Lobatostoma veranoi* was described from *Menticirrhus ophicephalus* (Jenyns, 1840) in the Peruvian coast. It represents the third species described from a host which does not belong to the *Trachinotus* genus. This species is distinguished from the others but one by having a higher number of marginal alveoli (64–66 *vs* 62 at maximum). Oliva & Luque (1989) pointed out that *L. veranoi* differs from the similar species in the number of marginal alveoli *L. anisotremum* (64), by its smaller cirrus sac than the pharynx, but they did not

provide any measurement of the cirrus sac to corroborate this statement.

In the present study, new measurements were taken from the paratype under accession number USNPC 80460.00. Indeed, the cirrus sac (160µm long) is smaller than the pharynx (217 µm long), leading us to agree with Oliva & Luque (1989).

*Lobatostoma* sp. (Fig. 4 A–B)
Bunkley-Williams *et al.* (1996) reported *L. kemostoma* from *T. carolinus* off the Caribbean Sea and also off the Dauphin Island, northern Gulf of Mexico. However, the specimens do not have any autapomorphic character of the referred species as stated by Alves *et al.* (2015a). Thus, they were identified in the present study just as *Lobatostoma* sp. since its degree of contraction and preservation precluded the observation of the main morphological characters.

*Cotylogaster* sp. (Fig. 5)
The five specimens on the slide (USNPC 36394.02) were identified as *L. ringens* by Ms. M. A. Ferguson, but further analysis on this material showed that they do not belong to the

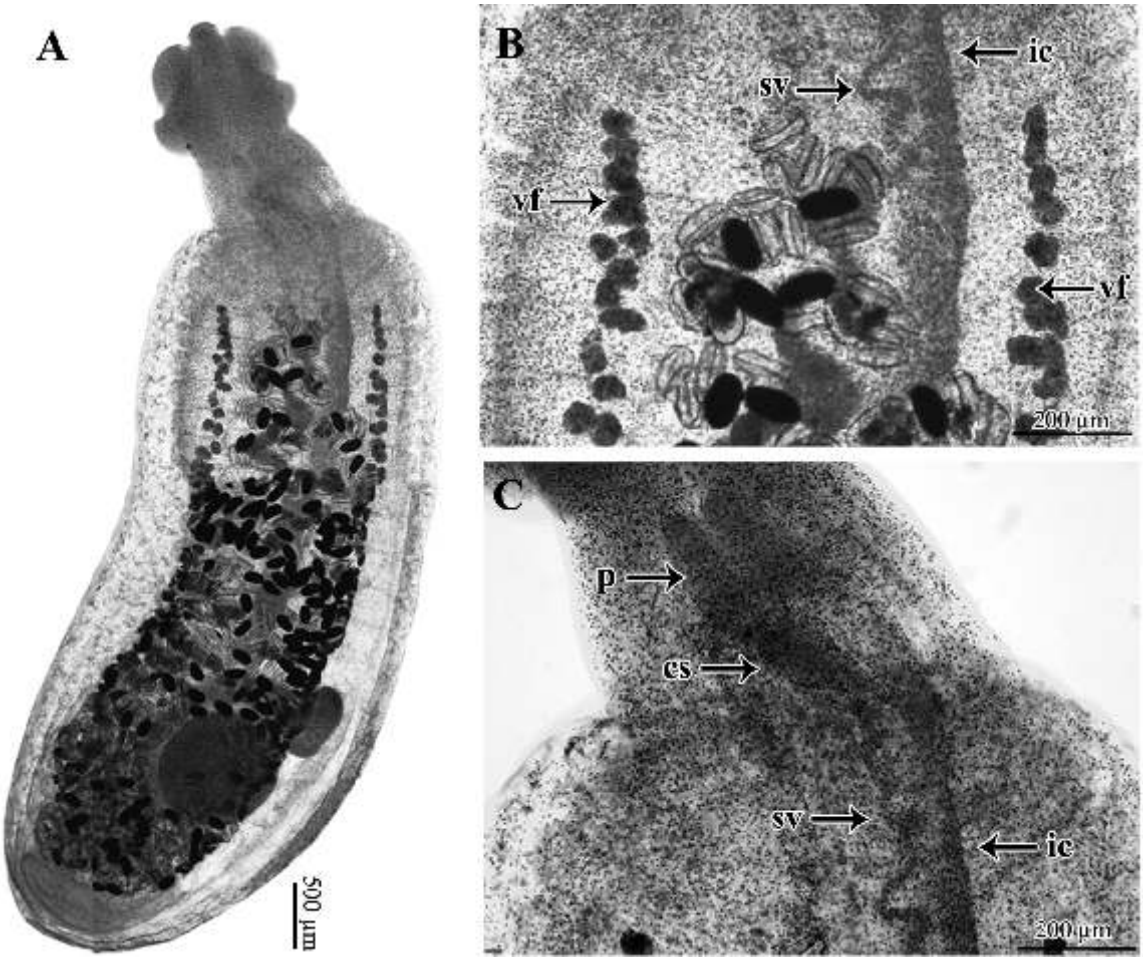


**Figure 3.** Paratype of *Lobatostoma veranoi* (USNPC 80460.00) deposited in the USNPC: A) Whole-mount – ventral view; B) Detail of the internal organs, second quarter of the body – ventral view; C) Detail of the internal organs, first quarter of the body – ventral view. Abbreviations: cirrus sac (cs), intestinal caecum (ic), pharynx (p), seminal vesicle (sv), vitellaria follicular (vf).

subfamilyAspidogastrinae. These specimens are now placed in Cotylaspidinae Chauhan, 1954 because they have three longitudinal rows of alveoli on the ventral disc as stated by Rohde (2002). In this subfamily, just *Cotylogaster* Monticelli, 1892 is found in teleost fish; *Cotylaspis* Leidy, 1857 and *Lissemysia* Sinha, 1935 are primarily parasites of molluscs and turtles (Rohde, 2002), although *L. pandei* Rai, 1970 and *L. agrawali* Singh and Tewari, 1985 have been described in teleost fish (Rai, 1970; Singh & Tewari, 1985).

The morphology of the specimens agrees with *Cotylogaster* which is characterized by possessing two testes; the presence of the cirrus sac in the genus is controversial. Rohde (2002) pointed out that this feature is present in the group and Yamaguti (1963) differentiated *Cotylogaster* from *Cotylogasteroides* Yamaguti, 1963 (synonym of the former), by the presence or absence of the cirrus sac, respectively. However, following works on redescription and description of new species did not mention a true cirrus sac. Rather, it corresponds to the prostatic complex (Fredericksen, 1972; Hendrix & Overstreet, 1977), which is the case of the present forms.

Four species are currently recognized in the genus, *C. basiri* Siddiqi and Cable, 1960; *C. dinosoides* Hendrix and Overstreet, 1977; *C. michaelis* Monticelli, 1892; and *C. occidentalis* Nickerson, 1902. Of these, three have been reported from marine fish, only *C. occidentalis* have been found in molluscs and

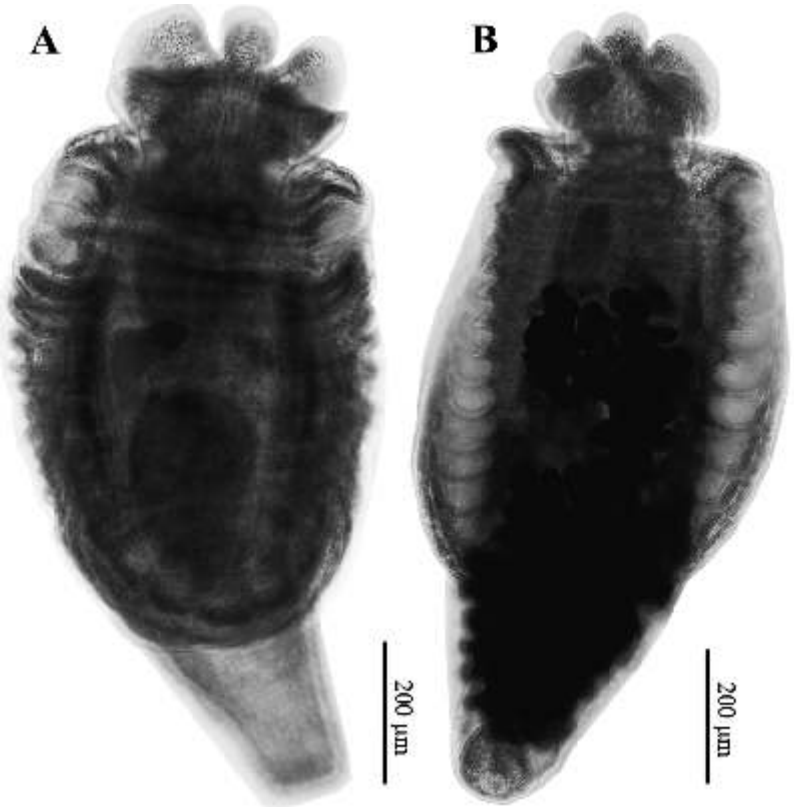


**Figure 4.** *Lobatostoma* sp. deposited in the USNPC: A) Young adult – ventral view (USNPC 083016. B7.36); B) Adult – ventral view (USNPC 083016.B7.30).

in the freshwater fish *Aplodinotus grunniens* Rafinesque, 1019 from North America (Fredericksen, 1972).

The specimens studied herein resemble *C. dinosoides* by possessing a simple buccal funnel with acuminate end without lateral lobes, but they are similar with *C. basiri* by the presence of eyespots in the anterior end; these features are unique for each taxon. Regardless of records of *C. basiri* in *T. carolinus, C. dinosoides* was described based on immature specimens (Hendrix & Overstreet, 1977), which may explain the absence of eyespots near to the pharynx. Thus, new sampling effort focused on aspidogastreans from *T. carolinus* and *Pogonias cromis* (Linnaeus, 1766) (type host of *C. dinosoides*) in the North Atlantic are needed in order to elucidate these taxonomic divergences.


**Figure 5.** *Cotylogaster* sp. (USNPC 36394.02) deposited in the USNPC.

**Table 1.** Collection data from the United States National Parasite Collection (USNPC), with accession number of the studied material. Abbreviations: H = holotype, P = paratype, S = syntype, V = voucher.

| Species | Hosts (Family host) | Accession number | References |
|---|---|---|---|
| *L. ringens* (Linton, 1905) | *Trachinotus carolinus* (Linnaeus, 1766) (Carangidae) | 035560 (S) | MacCallum & MacCallum (1913) |
| | *Calamus calamus* (Valenciennes, 1830) (Sparidae) | 08538.02 (V)[a] | Linton (1910) |
| *L. pacificum* Manter, 1940 | *Trachinotus paitensis* (Cuvier, 1832) (Carangidae) | 09321.00 (H)[a] | Manter (1940) |
| *L. albulae* Yamaguti, 1968 | *Albula vulpes* (Linnaeus, 1758) (Albulidae) | 063691.00 (H)[a] | Yamaguti (1968) |
| *L. manteri* Rohde, 1973 | *Trachinotus blochii* (Lacepède, 1801) (Carangidae) | 71056.00 (H)[a] | Rohde (1973) |
| *L. veranoi* Oliva & Luque, 1989 | *Menticirrhus ophicephalus* (Jenyns, 1840) (Sciaenidae) | 80460.00 (P) | Oliva & Luque (1989) |
| *Cotylogaster* sp. as *L. ringens* | *Trachinotus carolinus* (Linnaeus, 1766) (Carangidae) | 36394.02 (V) | Dataset of USNPC. |
| *Lobatostoma* sp. as *L. kemostoma* | *Trachinotus carolinus* (Linnaeus, 1766) (Carangidae) | 083016/B7.30, B7.36 (V) | Bunkley-Williams *et al.* (1996) |

[a]Photomicrographs released by Ms Patricia Pillit.

## DISCUSSION

The relevance of parasites within studies on conservation and management of the biodiversity have been increased since the last decade, due to the recognition of the essential role that these organisms play in the ecosystems (Brooks & Hoberg, 2000; Pérez-Ponce de León & García-Prieto, 2001; Nichols & Gómez, 2011; Gómez *et al*., 2012). Moreover, Marcogliese (2005) stated that resource managers interested in environmental quality should consider the studies on parasitology along with other disciplines in ecosystem assessments.

In this context, the helminthological collections or museums repositories are very useful to maintain the type and voucher specimens as an empirical record, which validates all taxonomic research (Hoberg *et al*., 2009). In addition, taxonomic works on specimen-based data can improve the knowledge about the global composition, spatial distribution, ecology, systematics, and organismal history (Krishtalka & Humphrey, 2000; Hoberg *et al*., 2009). Despite of its importance, shortcomings of the data deposited or gaps in the information can appear in the museums dataset (Ponder *et al*., 2001) as well as presented by the specimens under accession numbers USNPC 36394.02 and USNPC 083016/B7.30, B7.36.

In this work, we added new information on morphology of some species of *Lobatostoma* and one of *Cotylogaster* deposited in the USNPC, but further studies using both fresh and deposited material of the genera are warranted to better clarify our knowledge on the group. It is clear that type/voucher materials are very useful for taxonomic studies, even though they must be critically examined since specimens with questionable identification can be found among the deposited material.

## ACKNOWLEDGEMENTS

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