

First molecular identification of the trematode *Maritrema bonaerense* Etchegoin & Martorelli, 1997 (Plagiorchiida, Microphallidae) from its intermediate hosts, the gastropod *Heleobia australis* (d'Orbigny, 1835) (Littorinimorpha, Cochliopidae) and the crab *Neohelice granulata* (Dana, 1851) (Decapoda, Varunidae) in Argentina

Lorena Martinez¹, Carmen Gilardoni², Cintia Medina³, Juan José Lauthier⁴, Florencia Cremonte², Jorge Etchegoin¹

1 Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM), CONICET-UNMdP, Centro de Asociación Simple CIC-PBA, Juan B. Justo 2550 (7600), Mar del Plata, Argentina

2 Instituto de Biología de Organismos Marinos (IBIOMAR-CONICET), Boulevard Brown 2915, (9120), Puerto Madryn, Chubut, Argentina

3 Instituto de Diversidad y Evolución Austral (IDEAus-CONICET), Boulevard Brown 2915, (9120), Puerto Madryn, Chubut, Argentina

4 Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM), Facultad de Medicina, Universidad de Buenos Aires (UBA-CONICET), Paraguay 2155, Piso 12, (1121) Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina

<https://zoobank.org/EC975AED-6AE7-4848-9A6F-63C0C84C916D>

Corresponding author: Carmen Gilardoni (gilardoniacarmen@cenpat-conicet.gob.ar)

Academic editor: Pavel Stoev ♦ Received 8 August 2022 ♦ Accepted 8 November 2022 ♦ Published 26 January 2023

Abstract

The genus *Maritrema* Nicoll, 1907 (Platyhelminthes, Trematoda, Plagiorchiida, Microphallidae) comprises cosmopolitan species that predominantly parasitize birds. Although approximately 65 species have been described worldwide, including 6 for Argentina, molecular data referring to *Maritrema* species are still scarce worldwide, especially in South America. Unfortunately, this lack of references for nucleotide sequences is an obstacle to understanding the taxonomy and life cycles of trematodes, and impedes advancing our studies on the phylogeny and geographical distribution of these parasites. For that reason, we performed the molecular study of developmental stages of *Maritrema bonaerense*: cercariae (collected from the snail first intermediate host *Heleobia australis*, inhabiting Mar Chiquita lagoon) and metacercariae (collected from the crab second intermediate host *Neohelice granulata*, inhabiting Mar Chiquita lagoon and San Antonio Oeste, Argentina). The accordance between the ITS2 sequence of *M. bonaerense* cercaria from the snail *H. australis* and the sequences of metacercariae from the crab *N. granulata* was 100%, supporting previous findings of the life cycle of *M. bonaerense* based on morphological data. All *Maritrema* species are included in a monophyletic and well-supported clade. *Maritrema bonaerense* grouped more closely with *Maritrema graciosum*. These findings contribute to the knowledge of digeneans in coastal marine ecosystems.

Key Words

digeneans, ITS2 sequence, life cycle, South America

Introduction

The genus *Maritrema* Nicoll, 1907 (Platyhelminthes, Trematoda, Plagiorchiida, Microphallidae) comprises

cosmopolitan species that predominantly parasitize birds in brackish, marine and to a lesser extent, freshwater ecosystems (Deblock 2008; Capasso et al. 2019). Their life cycles also involve gastropods and crustaceans as first

and second intermediate hosts, respectively (Yamaguti 1975). To date, approximately 65 species of this genus have been described worldwide (Presswell et al. 2014), including 6 species from Argentina: *Maritrema bonaerense* Etchegoin & Martorelli, 1997; *M. orensense* Cremonte & Martorelli, 1998; *M. madrynense* Díaz & Cremonte, 2010; *M. formicae* Díaz, Gilardoni & Cremonte, 2012; *M. patagonica* Rauque, Flores & Brugnì, 2013, and *M. pichi* Capasso D'Ámico & Díaz, 2019.

As with other digeneans, molecular data referring to *Maritrema* species are still scarce in South America. For example, the only DNA sequences available in Argentina are from *M. madrynense* (Bagnato et al. 2015). Unfortunately, this lack of references for nucleotide sequences for South America is an obstacle to understanding the taxonomy and life cycles of trematodes (López-Hernández et al. 2019). Likewise, an increase in genetic data would be a significant step forward in our studies on the phylogeny and geographical distribution of these parasites in the region.

The life cycle and developmental stages of *M. bonaerense* were originally described by Etchegoin and Martorelli (1997) from Mar Chiquita lagoon (Buenos Aires province, Argentina). Later, Alda et al. (2013) re-described the adult and metacercaria, and experimentally confirmed the life cycle of this species which includes the cochliopid snail *Heleobia australis* as first intermediate host and the crabs *Cyrtograpsus angulatus* and *Neohelice granulata* as second intermediate hosts, and the birds *Chroicocephalus maculipennis*, *Larus atlanticus* and

L. dominicanus as definitive hosts. Both mentioned studies were conducted using only morphological analyses. For that reason, and taking into account the scarcity of genetic data related to the species of *Maritrema*, we performed the molecular study of cercariae and metacercariae of *M. bonaerense*, collected from the snail *H. australis* inhabiting Mar Chiquita lagoon and from the crab *N. granulata* inhabiting Mar Chiquita lagoon and San Antonio Oeste (Rio Negro province, Argentina).

It is important to mention here that although adult stages from the definitive hosts could not be obtained because Mar Chiquita is a Man and Biosphere Reserve (UNESCO) within which the birds are protected, *M. bonaerense* is the only species of *Maritrema* that parasitizes *H. australis* and *N. granulata* in this location (Etchegoin 2001; Parietti et al. 2013). Therefore, there was no possibility of misidentifications of developmental stages collected for this study.

Materials and methods

The specimens of *H. australis* were collected in Mar Chiquita lagoon, Buenos Aires province, Argentina (37°45'08"S, 57°26'18"W). In the laboratory, molluscs were isolated individually in 45 ml plastic cups and maintained under a 12–12 light-dark photoperiod for 48 h to stimulate shedding of cercariae. Crabs (*N. granulata*) collected in Mar Chiquita lagoon and in San Antonio Oeste (40°43'36"S, 64°54'49"W) (Fig. 1) were transported to

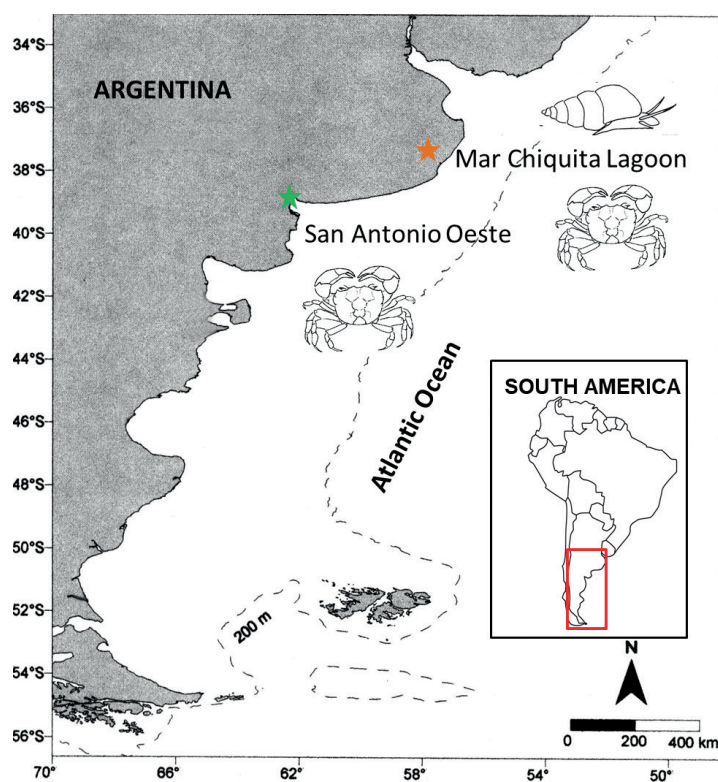


Figure 1. Map of sampling sites from Argentina: Mar Chiquita Lagoon (Buenos Aires province) where the snail *Heleobia australis* and the crab *Neohelice granulata* were collected and San Antonio Oeste (Rio Negro province) where *N. granulata* were collected. Invertebrate drafts extracted from Alda et al. (2013).

Table 1. Molecular data of *Maritrema* species considered in this study.

| Species | Life stage | Host | Habitat type | Country | ITS2 | p-distance | Reference |
|-----------------------------------|--------------|---------------------------------|--------------|-------------|----------|------------|-----------------------------|
| <i>Maritrema bonaerense</i> | cercaria | <i>Heleobia australis</i> | brackish | Argentina | ON833442 | | this study |
| <i>Maritrema bonaerense</i> | metacercaria | <i>Neohelice granulata</i> | brackish | Argentina | ON833466 | 0.00 | this study |
| <i>Maritrema bonaerense</i> | metacercaria | <i>Neohelice granulata</i> | marine | Argentina | ON833467 | 0.00 | this study |
| <i>Maritrema graciosum</i> | metacercaria | <i>Semibalanus balanoides</i> | marine | Ireland | HM584171 | 0.04 | Galaktionov et al. (2012) |
| <i>Maritrema subdolum</i> | cercaria | <i>Peringia ulvae</i> | brackish | Russia | HM584172 | 0.08 | Galaktionov et al. (2012) |
| <i>Maritrema eroliae</i> | cercaria | <i>Clypeomorus bifasciata</i> | marine | Kuwait | HQ650132 | 0.11 | Al-Kandari et al. (2011) |
| <i>Maritrema novaezealandense</i> | cercaria | <i>Zeacumantus subcarinatus</i> | marine | New Zealand | KJ540203 | 0.10 | Born-Torrijos et al. (2014) |
| <i>Maritrema madrynense</i> | adult | <i>Larus dominicanus</i> | marine | Argentina | KF575167 | 0.10 | Diaz and Cremonte (2010) |
| <i>Maritrema brevisacciferum</i> | metacercaria | <i>Caridina indistincta</i> | freshwater | Australia | KT355824 | 0.09 | Kudlai et al. (2015) |
| <i>Maritrema oocysta</i> | cercaria | <i>Hydrobia ulvae</i> | marine | Ireland | HM584170 | 0.10 | Galaktionov et al. (2012) |
| <i>Microphallus similis</i> | metacercaria | <i>Carcinus maenas</i> | marine | Russia | HM584180 | 0.14 | Galaktionov et al. (2012) |

the laboratory and maintained in aerated water. Posteriorly, infected snails and crabs were necropsied, and the developmental stages (sporocyst, cercariae and metacercariae) were stored in 96% ethanol for molecular studies. Cercariae and metacercariae of *M. bonaerense* were identified according to Etchegoin and Martorelli (1997) and Alda et al. (2013).

The molecular characterization of the developmental stages of *M. bonaerense* was made using rRNA ITS2 sequences. The DNA extraction, PCR amplification, and sequencing were performed using the protocol described in Gilardoni et al. (2020). Newly generated ITS2 sequences were deposited in GenBank and aligned using MAFFT (Katoh et al. 2019) together with available *Maritrema* spp. and with *Microphallus similis* as outgroup (Table 1). Maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted in MEGA X (Kumar et al. 2018) and MrBayes version 3.2.7a (Ronquist et al. 2012) respectively. Genetic divergences amongst taxa were calculated as uncorrected p-distances using MEGA X.

For both, ML and BI, to determine the nucleotide substitution model that gave the best fit to our data set, the program MEGAX which held the JModel test analysis was employed, with model selection based on the Akaike information criterion (AIC). Results indicated that the general time reversible model with an estimate of gamma distributed among-site rate variation (GTR+G) was the most appropriate. For the ML tree, the percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 35 nucleotide sequences. There are a total of 771 positions in the final dataset. For the BI tree, GTR was selected as the substitution model (command lset nst=6). We ran four independent chains of 100 million

generations each, sampling every 5000 generations, with the first 1000 trees discarded as “burn-in”. Chain convergence was confirmed using Tracer v.1.6 (Rambaut et al. 2018). Finally, a 50% majority rule consensus tree was constructed.

Results and discussion

The PCR amplification of the ITS2 rRNA from cercaria from Mar Chiquita Lagoon and metacercariae from Mar Chiquita Lagoon and San Antonio Oeste gave products of 540 bp, 557 bp and 543 bp respectively. The accordance between the ITS2 sequence of *M. bonaerense* cercaria from *H. australis* and the sequences of metacercariae from *N. granulata* was 100% (Fig. 2). This result supports previous findings of the life cycle of *M. bonaerense* based on morphological data (Etchegoin and Martorelli 1997; Alda et al. 2013).

The genus *Maritrema* constitutes a monophyletic and well-supported clade. Among all the species of *Maritrema* compared in this work, *M. bonaerense* seems to be more closely related to *M. graciosum* Nicoll, 1907. Both species constitute a well-supported clade separated from the other *Maritrema* spp. The genetic divergence (p-distance) revealed *M. bonaerense* presents 0.04 variation with *M. graciosum*, 0.08–0.11 with the other *Maritrema* spp. and 0.14 with *Microphallus similis* (outgroup). The molecular data support the morphological taxonomy of the genus *Maritrema*, which is distinguished by the vitellarium in symmetrical ribbons reaching close to margin of hindbody, surrounding uterine coils and testes, horse-shoe-shaped with posteriorly directed opening or complete ring (Deblock 2008). Despite the high number of *Maritrema* species morphologically described, molecular data are very scarce: rRNA 18S (6 spp sequenced), 28S (10 spp.), ITS1 (5 spp.), ITS2 (7 spp.), mitochondrial DNA cox1 (1 sp.). To date, most available sequences belong to species infecting marine or brackish hosts (Table 1). However, *Maritrema* species are present in freshwater habitat as *M. brevisacciferum* (Kudlai et al. 2015). Our findings contribute to the development of molecular database that may be used in future studies about these common and widespread parasites infecting birds worldwide.

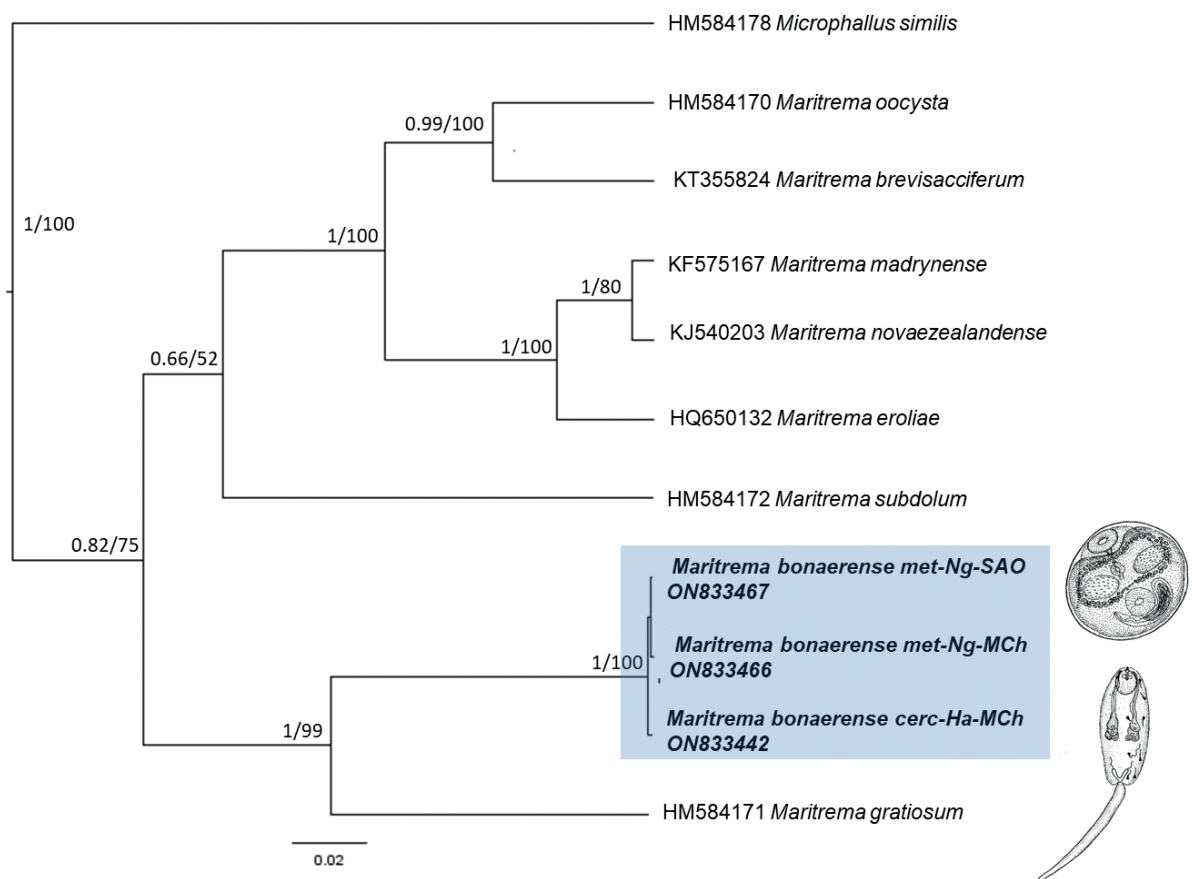


Figure 2. Phylogram for *Maritrema* species (*Microphallus similis* as outgroup), inferred by ML/BI of sequence data for ITS2 of the rRNA genes. The newly generated sequences are indicated in bold. Values on the branches correspond to posterior probabilities > 0.85 followed by bootstrap support > 60. Values below these thresholds were not reported. Abbreviations: cerc-cercaria, met-metacercaria, Ng-*Neohelice granulata*, Ha-*Heleobia australis*, MCh-Mar Chiquita Lagoon, SAO-San Antonio Oeste. Drafts of life stages extracted from Etchegoin and Martorelli (1997).

Acknowledgements

This study was supported by ANPCyT (PICT 2017-1819 to Etchegoin JA, PICT 2019-00837 to Gilardoni C, PICT 2020-2120 to Cremonte F) and from Universidad Nacional de Mar del Plata (Grant number 15/E935 EXA997/20 to Etchegoin JA).

References

- Al-Kandari WY, Al-Bustan SA, Alnaqeeb M (2011) Ribosomal DNA sequence characterization of *Maritrema* cf. *eroliae* Yamaguti, 1939 (Digenea: Microphallidae) and its life cycle. The Journal of Parasitology 97(6): 1067–1074. <https://doi.org/10.1645/GE-2869.1>
- Alda P, Bonel N, Hechinger RF, Martorelli SR (2013) *Maritrema orensense* and *Maritrema bonaerense* (Digenea: Microphallidae): descriptions, life cycles, and comparative morphometric analyses. The Journal of Parasitology 99(2): 218–228. <https://doi.org/10.1645/GE-3238.1>
- Bagnato E, Gilardoni C, Di Giorgio G, Cremonte F (2015) A checklist of marine larval trematodes (Digenea) in molluscs from Argentina, Southwestern Atlantic coast. Check List 11(4): 1706. <https://doi.org/10.15560/11.4.1706>
- Born-Torrijos A, Poulin R, Raga JA, Holzer AS (2014) Estimating trematode prevalence in snail hosts using a single-step duplex PCR: How badly does cercarial shedding underestimate infection rates? Parasites & Vectors 7(1): 1–11. <https://doi.org/10.1186/1756-3305-7-243>
- Capasso S, D'Amico VL, Diaz JI (2019) A new species of *Maritrema* (Trematoda: Microphallidae) parasitizing the Baird's sandpiper *Calidris bairdii*, and comments about diversity of Microphallidae in two Nearctic shorebirds at Patagonian sites in Argentina. Acta Tropica 189: 10–14. <https://doi.org/10.1016/j.actatropica.2018.09.018>
- Deblock S (2008) Family Microphallidae Ward, 1901. In: Bray RA, Gibson DI, Jones A (Eds) Keys to the Trematoda Vol. 3. CABI Publishing, Wallingford-UK, 451–495. <https://doi.org/10.1079/9780851995885.0451>
- Diaz JI, Cremonte F (2010) Development from metacercaria to adult of a new species of *Maritrema* (Digenea: Microphallidae) parasitic in the kelp gull, *Larus dominicanus*, from the Patagonian coast, Argentina. The Journal of Parasitology 96(4): 740–745. <https://doi.org/10.1645/GE-2343.1>
- Etchegoin JA (2001) Dinámica de los sistemas parasitarios. In: Iribarne O (Ed.) Reserva de Biosfera Mar Chiquita: características físicas, biológicas y ecológicas. Editorial Martín, Mar del Plata, Argentina, 171–185.
- Etchegoin JA, Martorelli SR (1997) Description of a new species of *Maritrema* (Digenea: Microphallidae) from Mar Chiquita coastal lagoon

- (Buenos Aires, Argentina) with notes on its life cycle. The Journal of Parasitology 83(4): 709–713. <https://doi.org/10.2307/3284251>
- Galaktionov KV, Blasco-Costa I, Olson PD (2012) Life cycles, molecular phylogeny and historical biogeography of the ‘*pygmaeus*’ microphallids (Digenea: Microphallidae): widespread parasites of marine and coastal birds in the Holarctic. Parasitology 139(10): 1346–1360. <https://doi.org/10.1017/S0031182012000583>
- Gilardoni C, Etchegoin JA, Cribb T, Pina S, Rodrigues P, Diez ME, Cremonte F (2020) Cryptic speciation of the zoogonid digenean *Diptherostomum flavum* n. sp. demonstrated by morphological and molecular data. Parasite 27: 1–11. <https://doi.org/10.1051/parasite/2020040>
- Katoh K, Rozewicki J, Yamada K (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Kudlai O, Cutmore SC, Cribb TH (2015) Morphological and molecular data for three species of the Microphallidae (Trematoda: Digenea) in Australia, including the first descriptions of the cercariae of *Maritrema brevisacciferum* Shimazu et Pearson, 1991 and *Microphallus minutus* Johnston, 1948. Folia Parasitologica 62: 1. <https://doi.org/10.14411/fp.2015.053>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- López-Hernández D, Locke SA, Costa Alves de Assisa J, Drago FB, Lane de Melo A, Leite Rabeloa EM, Alves Pinto H (2019) Molecular, morphological and experimental-infection studies of cercariae of five species in the superfamily Diplostomoidea (Trematoda: Digenea) infecting *Biomphalaria straminea* (Mollusca: Planorbidae) in Brazil. Acta Tropica 199: 105082. <https://doi.org/10.1016/j.actatropica.2019.105082>
- Parietti MM, Merlo J, Etchegoin JA (2013) Can the studies at a spatial scale of 100s meters detect the spatiotemporal fluctuations of a parasite assemblage? Acta Parasitologica 58(4): 577–584. <https://doi.org/10.2478/s11686-013-0184-0>
- Presswell B, Blasco-Costa I, Kostadinova A (2014) Two new species of *Maritrema* Nicoll, 1907 (Digenea: Microphallidae) from New Zealand: morphological and molecular characterisation. Parasitology Research 113(5): 1641–1656. <https://doi.org/10.1007/s00436-014-3809-9>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Rauque CA, Flores VR, Brugni NL (2013) *Maritrema patagonica* n. sp. (Digenea: microphallidae) cultured from metacercariae from freshwater anomuran, *Aegla* spp. (Decapoda: Aeglidae), in Patagonia. Comparative Parasitology 80(2): 196–202. <https://doi.org/10.1654/4624.1>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Systematic Biology 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Yamaguti S (1975) A synoptical review of the life history of digenetic trematodes of vertebrates, with a special reference to the morphology of their larval forms. Keigaku Publishing Co, Tokyo-Japan, 590 pp.

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Zoosystematics and Evolution](#)

Jahr/Year: 2023

Band/Volume: [99](#)

Autor(en)/Author(s): Martinez Lorena, Gilardoni Carmen, Medina Cintia, Lauthier Juan Jose, Cremonte Florencia, Etchegoin Jorge

Artikel/Article: [First molecular identification of the trematode *Maritrema bonaerense* Etchegoin & Martorelli, 1997 \(Plagiorchiida, Microphallidae\) from its intermediate hosts, the gastropod *Heleobia australis* \(d'Orbigny, 1835\) \(Littorinimorpha, Cochliopidae\) and the crab *Neohelice granulata* \(Dana, 1851\) \(Decapoda, Varunidae\) in Argentina 117-121](#)