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# Molecular evidence linking the larval and adult stages of *Mexiconema cichlasomae* (Dracunculoidea: Daniconematidae) from Mexico, with notes on its phylogenetic position among Dracunculoidea

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**Abstract**

We describe the larval developmental stages and life cycle of the dracunculid nematode *Mexiconema cichlasomae* in both the intermediate, *Argulus yucatanus* (Crustacea: Branchiura), and definitive hosts, *Cichlasoma urophthalmus* (Perciformes: Cichlidae), from the Celestun tropical coastal lagoon, Yucatan, Mexico. The morphological analyses showed significant differences between the total length of L1 found in *M. cichlasomae* gravid female and L2–L3 in *A. yucatanus*. This result indicates that the *M. cichlasomae* larval development occurs in the intermediate host. We obtained sequences from the small subunit (SSU) ribosomal marker from larval stages of *M. cichlasomae* in *A. yucatanus* and adult nematodes in *C. urophthalmus*. Our morphological and molecular results support conspecificity between *M. cichlasomae* larvae in *A. yucatanus* and the adult stages in *C. urophthalmus*. We briefly discuss the phylogenetic position of *M. cichlasomae* among the Daniconematidae, and provide evidence of the monophyly of the daniconematids associated with branchiurid intermediate hosts. Based on the phylogenetic results, we support the transfer of the *Mexiconema* genus to the family Skrjabillanidae and do not support the lowering of family Daniconematidae to subfamily.

## Introduction

The taxonomy and geographical distribution of the parasitic nematode fauna of aquatic organisms is poorly known in the Neotropics (e.g. Moravec, 1998; Salgado-Maldonado *et al.*, 2000; Caspeta-Mandujano, 2005). The life cycles of these nematodes are even less well known, with only 13 papers on the subject published for the Neotropics, compared to the 61 life cycles described for the Palearctic realm (supplementary table S1). Knowledge of the life cycles of parasitic nematodes of Neotropical aquatic organisms in most cases is restricted to Anisakidae and Camallanidae families (supplementary table S1).

With respect to dracunculid nematodes parasitizing fish, there exist 192 species belonging to eight families (Anguillicolidae, Daniconematidae, Guyanemidae, Lucionematidae, Micropleuridae, Philometridae, Skrjabillanidae and Tetanonematidae) (Moravec, 2004; Moravec and de Buron, 2013). Of eight families belonging to the superfamily Dracunculoidea, only 29 nematode life cycles have been described, 15% of which are members of the families Philometridae, Angullicolidae, Skrjabillanidae and Daniconematidae (Moravec, 2004). The life cycles of dracunculid nematodes parasitizing fish in temperate latitudes have been reported by Moravec (2004) (supplementary table S1); however, there is a lack of information on the life cycle of dracunculid nematodes parasitizing fish in the tropical zone.

One of the few partial nematode life cycles described in Mexico is that of the dracunculid *Mexiconema cichlasomae* Moravec *et al.* (1992), for which the larval stage has been reported in the parasitic branchiurid *Argulus yucatanus* (Moravec *et al.*, 1999), and the adult stages in the Mayan cichlid *Cichlasoma urophthalmus* (Moravec *et al.*, 1992), both from the Celestun coastal lagoon, Yucatan, Mexico (May-Tec *et al.*, 2013). However, the lack of distinguishing characteristics in the larval stages described by Moravec *et al.* (1999) casts doubt about whether they truly belong to *M. cichlasomae*. Furthermore, despite the careful description of the adult stages of *M. cichlasomae* in its definitive host *C. urophthalmus*, the larval stages present in this host have not been properly described up to now. An alternative to overcome the problem of linking the larval stages of nematode parasites is the use of molecular markers, which have been

used on parasites related to human and animal health (Klimpel and Palm, 2011; Borges *et al.*, 2012; Liu *et al.*, 2015). However, molecular marker studies linking larval stages and adult nematodes of wildlife organisms are scarce (Loung and Hudson, 2012; Blasco-Costa and Poulin, 2017). In addition, molecular studies using the small subunit (SSU) ribosomal marker in wildlife nematodes to explore phylogenetic relationships have been particularly useful for establishing the phylogenetic position among nematode families of the superfamily Dracunculoidea, such as Daniconematidae, Philometridae and Skrjabillanidae (Blaxter *et al.*, 1998; Holterman *et al.*, 2006; Nadler *et al.*, 2007; Černotíková *et al.*, 2011; Choudhury and Nadler, 2016; Pereira *et al.*, 2017). In this context, it is plausible to use this marker to link larval and adult nematodes to find species boundaries and to determine whether there are biological reasons to support their phylogenetic relationships. Furthermore, based on molecular phylogenetic analyses, several members of the paraphyletic families Daniconematidae, Skrjabillanidae and Philometridae form a monophyletic group infecting the serosa of freshwater, brackish and marine fishes, and develop in blood-sucking branchiurids, e.g. genera *Mexiconema* (Moravec *et al.*, 1992), *Molnaria* (Moravec, 1968), *Skrjabillanus* (Shigin and Shigina, 1958), *Esocinema* (Moravec, 1977) and *Philonema* (Kuitunen-Ekbaum, 1933) (Černotíková *et al.*, 2011). However, *M. cichlasomae* was not included in these analyses, and therefore its phylogenetic identity was not tested as a member of the Daniconematidae family associated with branchiurid intermediate hosts (Černotíková *et al.*, 2011; Mejía-Madrid and Aguirre-Macedo, 2011).

Therefore, our aims were threefold: (1) test the possible life-cycle links of *M. cichlasomae* between larval stages in *A. yucatanus* and adults in *C. urophthalmus* using the SSU marker; (2) describe morphologically the larval stages of *M. cichlasomae* in both its intermediate and definitive hosts; and (3) re-evaluate the molecular phylogenetic position of *M. cichlasomae* into the Daniconematidae family.

## Materials and methods

### Collection of hosts, ectoparasite branchiurids and endoparasite nematodes

As part of our study on the life cycle of *M. cichlasomae*, from January to July 2016 a total of 105 *C. urophthalmus* (15 fish examined each month) were caught by hook and line from the middle zone of the Celestun tropical lagoon, Yucatan Peninsula (20°52′46.68″N, 90°21′15.4″W) (fig. 1). We collected *A. yucatanus* branchiurids from the body of each *C. urophthalmus* caught, and examined them for nematode larvae (May-Tec *et al.*, 2013; Sosa-Medina *et al.*, 2015). During the study period we collected 473 *A. yucatanus* and 29 *M. cichlasomae* larvae (supplementary table S2). For molecular studies, from 45 *C. urophthalmus* collected during January–March 2016, we collected a total of 124 *A. yucatanus* parasitized with nine *M. cichlasomae* larvae. The live fish captured were transported to the laboratory in a tank of 200 l of lagoon water and oxygen. Once there, the body surface of each fish was examined under a stereomicroscope, looking for *A. yucatanus*, and each *A. yucatanus* was examined for *M. cichlasomae* larvae. The parasitic specimens for morphological studies were collected and fixed in 96% ethanol, and for molecular analysis with 100% ethanol. Mexican authorities, in this case the National Committee of Fisheries and Aquaculture (PPF/DGOPA-070/16) issued the collecting permits.

### Morphological data and morphometric analyses

The protocols for the morphological study of *M. cichlasomae* larvae were based on the taxonomic description of nematode larvae of Skrajabillanidae family, given their taxonomical and biological similarities such as the measurements of the larval stages, the use of branchiurid ectoparasites *Argulus* sp. as an intermediate host and the absence of free-living stages (Tikhomirova, 1970, 1975, 1980; Molnár and Székely, 1998; Černotíková *et al.*, 2011). The morphological terminology for each stage of maturity followed that of Moravec *et al.* (1992, 1994), Hugot and Quentin (2000) and Caspeta-Mandujano and Mejía-Mojica (2004). The morphological examination of the nematodes was performed using an optical microscope (Olympus BX 50) equipped with a digital camera (Evolution MP). The measurements were in micrometers (μm), presented here as the ranges followed by the mean and standard deviation in parentheses, and were obtained using the Image J 1.50e software (Schneider *et al.*, 2012). Statistica v. 8.0 software (www.statsoft.com) was used for statistical analysis of the morphometric data. Lastly, several of our morphological measurements for the larval stages of *M. cichlasomae* were compared with those of other members of families Philometridae and Skrjabillanidae (supplementary table S3) to determine their phylogenetic affinity.

Eggs (ω), embryos (E) and first larval stage (L1) were obtained from gravid *M. cichlasomae* females removed from mesenteries and body cavities of *C. urophthalmus*. The second (L2) and third larval stages (L3) of *M. cichlasomae* were collected from *A. yucatanus*; the juvenile stage (L4) was found in the mesenteries of *C. urophthalmus*. Eggs, embryos and larvae were cleared in glycerin (1 : 2) and then mounted on glass slides with glycerine jelly. Measurements were based on at least 10 specimens of each developmental stage, slightly flattened under cover-glass pressure. The morphological measurements of *M. cichlasomae* embryos and larvae (L1, L2, L3 and L4) were compared by one-way analysis of variance (ANOVA) to examine differences in size of these larval stages (Sokal and Rohlf, 2009). The significance of all statistical analyses was established at $\alpha < 0.05$.

### DNA extraction, PCR amplification and sequencing

To obtain a small fraction of the genetic variability of *M. cichlasomae* we used samples of worms of different host individuals from the same locality (avoiding sequencing all the individuals from the same host). Deoxyribonucleic acid (DNA) was extracted from one individual adult male nematode and one individual adult female nematode from *C. urophthalmus*. We also extracted DNA of four larvae obtained from *A. yucatanus*. DNA extraction was performed using the DNA easy blood and tissue extraction kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The SSU rDNA gene fragment was amplified by polymerase chain reaction (PCR) (Saiki *et al.*, 1988), using D–1F forward (5′–GCC TAT AAT GGT GAA ACC GCG AAC–3′) and D–1R reverse (5′–CCG GTT CAA GCC ACT GCG ATT A–3′) (Wijová *et al.*, 2005). The reactions were prepared using the Green GoTaq Master Mix (Promega). This procedure was carried out using an Axygen MaxyGene thermocycler. PCR cycling conditions were as follows: an initial denaturing step of 5 minutes at 94°C, followed by 35 cycles of 92°C for 30 s, 54°C for 45 s, 72°C for 90 s, and a final extension step at 72°C for 10 minutes. The PCR products were analysed by electrophoresis in 1% agarose gel using TAE 1X buffer and observed under UV light using

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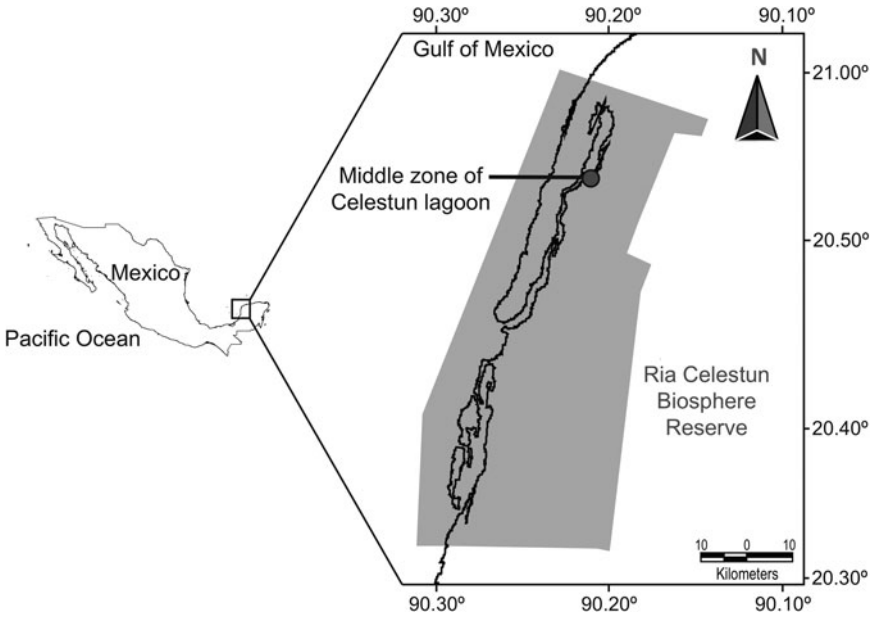


**Fig. 1.** Map of the study area, the middle zone of the Celestun coastal lagoon, Yucatan, Mexico.

the QIAxcel®Advanced System. PCR products were purified and sequencing carried out in a specialized laboratory, Genewiz, South Plainfield, NJ, USA (https://www.genewiz.com/).

### *Molecular data and phylogenetic reconstruction*

Sequences of *M. cichlasomae* obtained in this study were edited using the platform Geneious Pro v.5.1.7 (Drummond *et al.*, 2012). All sequences, together with published representative outgroup (OG) sequences of Daniconematidae, Skrjabillanidae, Philometridae and Camallanidae (supplementary table S4), used previously by Mejía-Madrid and Aguirre-Macedo (2011) and Černotíková *et al.* (2011), were aligned using an interface available with MAFFT v.7.263 (Katoh and Standley, 2016), an "auto" strategy and a gap-opening penalty of 1.53 within Geneious Pro, and a final edition by eye in the same platform. The best substitution model for the DNA dataset was chosen under the Bayesian Information Criterion (BIC; Schwarz, 1978) using the "greedy" search strategy in Partition Finder v.1.1.1 (Lanfear *et al.*, 2012, 2014). The nucleotide substitution model that best fit was K80 + I (Kimura, 1980). The Gblocks website (Castresana, 2000; Talavera and Castresana, 2007) was used to detect ambiguously aligned hypervariable regions in the SSU dataset, according to a secondary structure model; these were excluded from the analyses. Additionally, the proportion ($p$) of absolute nucleotide sites ($p$-*distance*) (Nei and Kumar, 2000) was obtained to compare the genetic distance between species of Dracunculoidea nematodes (without outgroups, i.e. *Camallanus oxycephalus*, *Camallanus hypophthalmichthys* and *Procamallanus pintoi*). The *P*-value matrix was obtained using MEGA v.7.0 (Kumar *et al.*, 2016), with variance estimation with the bootstrap method (1000 replicates) and with a nucleotide substitution (transition + transversions) uniform rate.

Phylogenetic reconstruction was carried out using Bayesian Inference (BI) through MrBayes v.3.2.3 (Ronquist *et al.*, 2012). Phylogenetic trees were reconstructed using two parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMC) for $20 \times 10^6$ generations each, to estimate the posterior probability (PP) distribution. Topologies were sampled every 1000 generations and the average standard deviation of split frequencies was observed to be less than 0.01, as suggested by Ronquist *et al.* (2012). The robustness of the clades was assessed using Bayesian Posterior Probability (PP), where PP > 0.95 was considered to be strongly supported. A majority consensus tree with branch lengths was reconstructed for the two runs after discarding the first 5000 sampled trees. The Bayesian phylogenetic reconstruction was run through the CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010).

## Results

### *Morphological characteristics of eggs, embryos and larvae (L1) in* Mexiconema cichlasomae *gravid female*

The uterus of an *M. cichlasomae* gravid female is prodelphic, and L1 larvae were found from the posterior to the anterior ends of the uterus. The mature eggs (n = 10) were almost spherical, thin-walled, and in a cell division process; 21.96–36.02 (26.27 ± 4.68) long, 10.38–22.18 (16.40 ± 4.14) wide (fig. 2a). Developed embryos (n = 10) were localized in the middle of the uterus, longer than eggs (57.95–78.25 (70.79 ± 11.63) long, 11.27–16.68 (13.80 ± 1.54) wide), but without evidence of organ development (fig. 2b, c). In the anterior third of gravid females, close to the vulva, we found *M. cichlasomae* L1 (n = 10) presenting a slender, translucent body, with rounded head, sharply pointed tail and measuring 122.23–173.21 (134.00 ± 11.63) long and 6.08–11.12 (8.49 ± 1.33) wide (fig. 2d). The gravid females (n = 10) had, on average, 189–468 (339.71 ± 107.82) L1 larvae.

### Mexiconema cichlasomae *larvae (L2–L3) in* Argulus yucatanus

The *M. cichlasomae* L2 larvae (fig. 2e) were found in the haemocoel and natatory appendages of *A. yucatanus*. Their measurements were 153.00–227.68 (188.27 ± 24.35) long, 5.96–9.59 (7.20 ± 1.33) wide (n = 10). This larval stage presented a smooth cuticle, rounded anterior end and conical tail (fig. 2e). The body of the L3 was 324.02–347.93 (331.93 ± 11.51) long, 7.00–7.6 (7.26 ± 0.30) wide (n = 6) (fig. 2f), with an oesophagus not clearly divided into muscular and glandular parts (48.40–52.85

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**Fig. 2.** Morphology of *Mexiconema cichlasomae* larval stages present in uterus of gravid females in *Cichlasoma urophthalmus* and its intermediate host *Argulus yucatanus*. (a) Egg (150×), (b, c) embryos (40×) and (d) first larval stage (L1) in gravid females of *M. cichlasomae* (40×); (e, f) second and third larval stages (L2–L3) of *M. cichlasomae* in *A. yucatanus* (40×); (g) tail of *M. cichlasomae* (L3) with two button-like processes (indicated by black arrows) at the tip (100×). Scale bars = 20 μm.

(50.41 ± 2.25) long, 3.6–4.49 (4.03 ± 0.44) wide) and a tail with two button-like processes (fig. 2g). In the mesenteries of *C. urophthalmus*, we found L4 stage larvae (2060.00–2490.00 (2292.00 ± 210.16) long, 35.00–42.00 (39.20 ± 2.77) wide; n = 5). There were significant differences in the total length of L1 found in gravid females and L3 in *A. yucatanus* (one-way ANOVA $F_{2, 27} = 32.46$, $P < 0.05$) (supplementary fig. S1). We observed that only female *A. yucatanus* (18 of 231 females examined) with *M. cichlasomae* larvae (n = 29), and not males, were parasitized (242 males examined). The size of *A. yucatanus* did not present a statistically significant association with the number of *M. cichlasomae* larvae ($R^2 = 0.04$, $P > 0.05$).

### *DNA sequences and phylogenetic tree*

A total of six SSU assembly sequences (forward and reverse) were obtained from two adult *M. cichlasomae* specimens (male and female) and four *M. cichlasomae* larval specimens from *C. urophthalmus* and *A. yucatanus*, respectively (supplementary table S4). Sequences of SSU gene fragments were obtained with a range of 1668–1702 base-pairs (bp). The SSU sequences of adult nematodes from *C. urophthalmus* were identical to those of larval nematodes from *A. yucatanus*. Therefore, both nematode stages correspond to *M. cichlasomae*. Nucleotide sequence variation in the SSU alignment from dracunculids to the phylogenetic reconstruction had 1214 conserved sites, 347 variables sites, 278 parsimony-informative sites and 69 singleton sites.

Bayesian phylogenetic analysis was undertaken for seven *M. cichlasomae* individuals and one of *M. africanum*, three skrjabillanid species, two philometrid species plus three camallanid species, to test life-cycle links between the larval stages and adult nematodes with molecular data, and re-evaluate the phylogenetic position of *M. cichlasomae*. The SSU tree clearly shows that all samples of *M. cichlasomae* from *C. urophthalmus* and *A. yucatanus* were nested together (monophyletic group with PP ≥ 0.95). The phylogenetic analysis recovered a monophyletic group comprising three polyphyletic taxa each, i.e. Daniconematidae (*M. cichlasomae* and *M. africanum*; *Mexiconema* genus is not a monophyletic group), Skrjabillanidae (*Esocinema bohemicum*, *Molnaria intestinalis* and *Skrjabillanus scardinii*) and Philometridae (*Philonema oncorhynchi* and *Philonema* sp.) (fig. 3). The genetic distance values of *M. cichlasomae* relative to other dracunculids was 3.93% with *M. intestinalis*, 4.02% with *S. scardinii*, 4.45% with *M. africanum*, 6.46% with *E. bohemicum*, 5.94% with *P. oncorhynchi* and 5.76% with *Philonema* sp. (table 1).

## Discussion

Our molecular and phylogenetic results strongly suggest that the nematode larvae parasitizing *A. yucatanus* are conspecific to those infecting the cichlid fish *C. urophthalmus* as adults, and that both belong to *Mexiconema cichlasomae*. This is relevant because this is the first complete life cycle of a dracunculid nematode parasite of fishes described for the Neotropics. Below, we stress several relevant biological aspects of the larval stages in both *A. yucatanus* and *C. urophthalmus*, compare the life cycle of *M. cichlasomae* to that of other dracunculid nematodes and discuss the systematic classification of *M. cichlasomae* as molecular phylogenetic reconstruction allows.

### *Description of larval stages of* Mexiconema cichlasomae

There is intraspecific variation in the morphological measurements of L1 of *M. cichlasomae* in different species of definitive hosts. The mean length of L1 larvae (134.00 ± 11.63) from *M. cichlasomae* females from *C. urophthalmus* in the present

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**Table 1.** Distance matrix of uncorrected *p*-distances within skrjabillanid nematode species, derived from SSU by Bayesian phylogenetic analyses (percentage values).

| | *M. cichlasomae* | *M. africanum* | *M. intestinalis* | *S. scardinii* | *E. bohemicum* | *P. oncorhynchi* |
|---|---|---|---|---|---|---|
| *Mexiconema cichlasomae* | | | | | | |
| *Mexiconema africanum* | 4.45 | | | | | |
| *Molnaria intestinalis* | 3.93 | 4.28 | | | | |
| *Skrjabillanus scardinii* | 4.02 | 4.37 | 0.61 | | | |
| *Esocinema bohemicum* | 6.46 | 6.29 | 6.38 | 6.46 | | |
| *Philonema oncorhynchi* | 5.94 | 5.50 | 5.50 | 5.76 | 5.24 | |
| *Philonema* sp. | 5.76 | 5.33 | 5.50 | 5.59 | 5.07 | 0.17 |

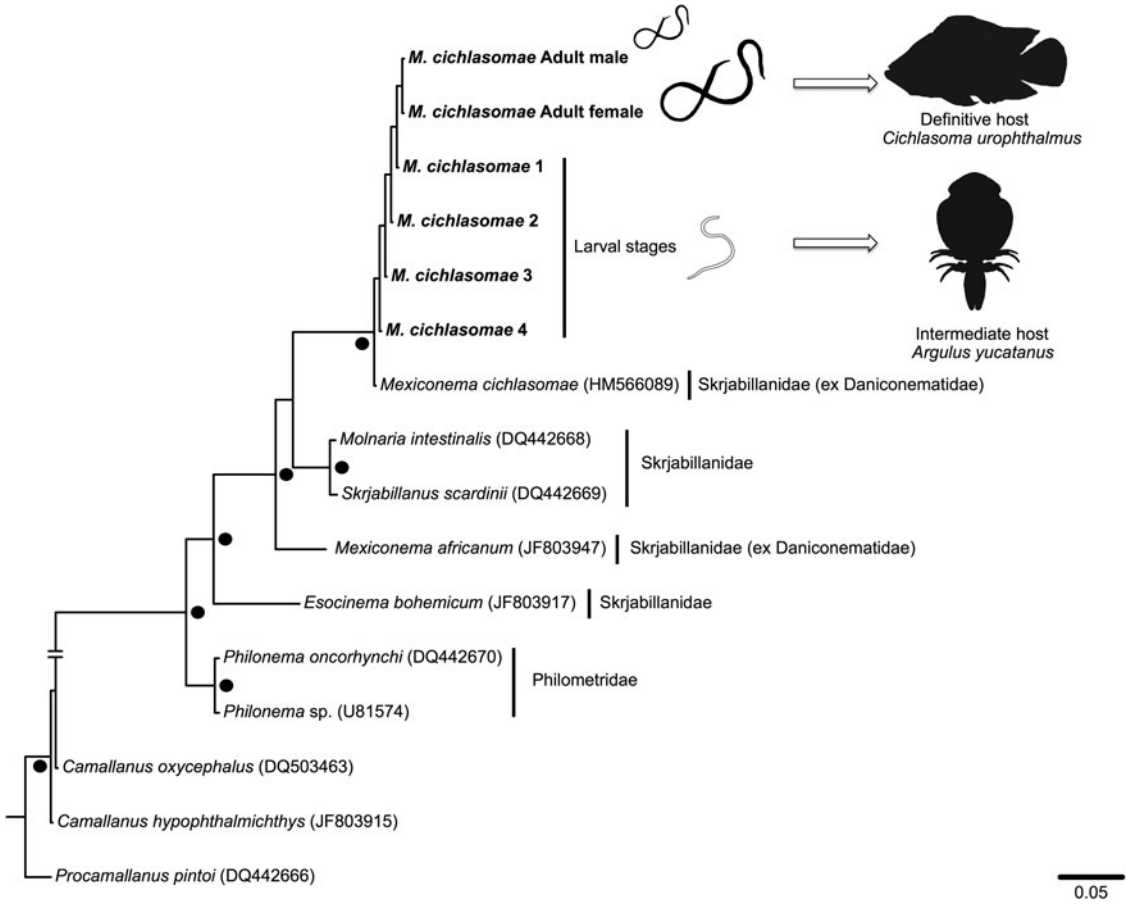


**Fig. 3.** Bayesian tree inferred from the small subunit (SSU) ribosomal DNA of *Mexiconema cichlasomae* adults and larvae. The scale bar represents the number of nucleotide substitutions per site. Filled black circles above/below branches represent Bayesian posterior probability ≥ 0.95. Bold font indicates new sequences generated in the present study; their GenBank accession numbers are provided in supplementary table S4.

study was longer than that of *M. cichlasomae* L1 from *Xiphophorus helleri* Heckel, 1848 (Cyprinodontiformes: Poecilidae) (100 µm long) (Moravec *et al.*, 1998). This difference in total length can be associated with intraspecific variability given the different species of definitive hosts. However, we suggest the need to undertake molecular examination of *M. cichlasomae* from *X. helleri* for comparison with *M. cichlasomae* from *C. urophthalmus* to rule out possible misidentifications.

With respect to the L2 and L3 found in *A. yucatanus*, we found evidence of increased development, as larvae in the crustacean were twice the size compared to L1 in *M. cichlasomae* gravid females in *C. urophthalmus*. This means that *A. yucatanus* probably acts as an intermediate host, in which L3 develop to be able to infect the definitive host (*C. urophthalmus* in this case). This result concurs with Moravec *et al.* (1999), who suggested that *A. yucatanus* acts as intermediate host of *M. cichlasomae* in Yucatan, Mexico.

In addition to the difference in size, the main difference observed between L1 larvae retrieved from uterus and L2 from *A. yucatanus* was that the tapered tail of these larval stages became a rounded tail of L3 larvae, with two small cuticular processes in the tip, which probably become the digital process

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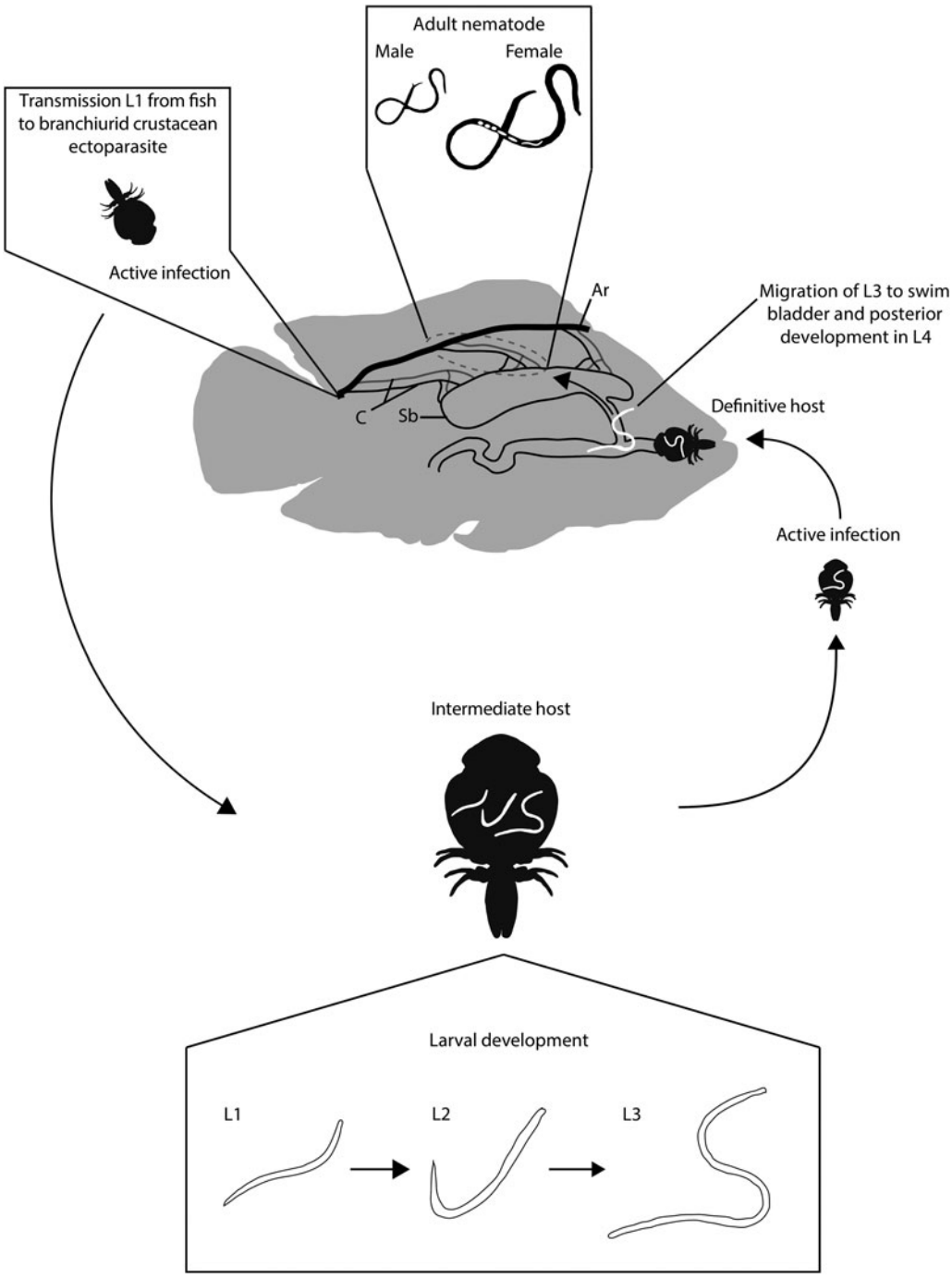


**Fig. 4.** Life cycle of *Mexiconema cichlasomae* in *Cichlasoma urophthalmus* (definitive host) and *Argulus yucatanus* (intermediate host). Ar, Artery; C, Capillaries; Sb, Swim bladder; L1, First larval stage; L2, Second larval stage; L3, Third larval stage (infective stage); L4, Juvenile stage.

typical of adult *M. cichlasomae* (Moravec *et al.*, 1992). Finally, we observed the presence of very small larvae of *M. cichlasomae* in *A. yucatanus*, presumably L1, of < 100 µm. This is not surprising, as infection of L1 of *M. cichlasomae* in *A. yucatanus* has also been reported by Moravec *et al.* (1999).

## *The life cycle of* Mexiconema cichlasomae

Based on morphological and molecular links identified in the present study, we suggest that *M. cichlasomae* larvae are ingested by two, probably complementary, processes. During the first process the branchiurid is infested by ingesting L1 while sucking blood from *C. urophthalmus*. The L1 then develops into L3 and is transmitted again during the blood-sucking process. During the second process, the fish host becomes infected by ingesting infected branchiurids with L3, e.g. cleaning symbiosis (fig. 4). This process of active removal of ectoparasites from the body surface has been observed in various fish species (Poulin and Grutter, 1996; Johnson *et al.*, 2010; Quimbayo *et al.*, 2017). In *C. urophthalmus,* once in the gut L3 larvae probably migrate through the pneumatic duct connecting the oesophagus with the swim bladder. However, this ontogenetic migration process

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needs confirmation through histology. In fact, the presence of a pneumatic conduct in *C. urophthalmus* has been corroborated by Cuenca-Soria *et al.* (2013).

The development of *M. cichlasomae* in the definitive host should be as follows. Once in the fish, the L3 should migrate from the peripheral blood into the abdominal cavity, mesenteries, swim bladder and serous membrane covering the intestine (Vidal-Martínez *et al.*, 2001). An alternative way for larval migration from *A. yucatanus* ingested by cleaning symbiosis is through the pneumatic conduct directly into the swim bladder. Once in these microhabitats, the nematode larvae moult into L4, develop secondary sexual characteristics typical of adults, and mate. In the case of gravid females, they burst, releasing approximately 340 ± 108 L1 larvae per individual (authors, pers. obs.), which eventually migrate to the fish blood vessels, circulating until another *A. yucatanus* feeds on this infected fish, acquiring L1 larvae again (fig. 4).

The life cycle of *M. cichlasomae* is similar to that of the daniconematid nematode *S. scardinii*, as females of both species release their first larval stage into the surrounding tissues of fish. These larvae become available in the fish bloodstream to blood-sucking fish lice *Argulus* spp. (Moravec, 2004; Černotíková *et al.*, 2011). The host specificity of *A. yucatanus*, and that of *M. cichlasomae*, is apparently rather low. *Argulus yucatanus* parasitizes several other fish species, such as *Floridichthys carpio* (Günter, 1866) (Cyprinodontiformes: Cyprinodontidae), *Archosargus rhomboidales* (Linnaeus, 1758) (Perciformes: Sparidae) (Sosa-Medina *et al.*, 2015) and *Sphoeroides testudineus* (Linnaeus, 1758) (Tetraodontiformes: Tetraodontidae) (Aguirre-Macedo and Vidal-Martínez, unpublished data at the Laboratory of Aquatic Pathology Cinvestav-Mérida), all of which are marine or brackish-water fishes from Mexican coastal lagoons of the Gulf of Mexico (May-Tec *et al.*, 2013; Sosa-Medina *et al.*, 2015). The adult forms of *M. cichlasomae* have been reported from freshwater and euryhaline fish species of the families Cichlidae, Bagridae (ex Ariidae) (Siluriformes) and Poecillidae from freshwater and coastal lagoons of the Gulf of Mexico (Aguilar-Aguilar *et al.*, 2005; Salgado-Maldonado, 2006; Salgado-Maldonado *et al.*, 2011; Salgado-Maldonado and Quiroz-Martínez, 2013). In fact, adult *M. cichlasomae* have even been reported in a nurse shark *Ginglymostoma cirratum* (Bonnaterre, 1788) (Orectolobiformes: Ginglymostomatidae) (Moravec *et al.*, 1998; Merlo-Serna and García-Prieto, 2016). In this context, it would not be surprising if *M. cichlasome* were found in other fish species occurring in freshwater, brackish water or even marine waters of the Gulf of Mexico.

### *Phylogenetic context of* Mexiconema cichlasomae

The molecular phylogenetic reconstructions showed that *M. cichlasomae* is related to the skrjabillanids *M. intestinalis* and *S. scardinii*, as previously revealed by Mejía-Madrid and Aguirre-Macedo (2011). However, we detected that the genus *Mexiconema* has at least two independent origins, i.e. it is a paraphyletic group (fig. 3). At the moment, the taxonomic categories of the *Mexiconema* genus are variable. For example, based on molecular phylogenetic analysis, Černotíková *et al.* (2011) suggested the transfer of *Mexiconema* from Daniconematidae to Skrjabillanidae. On the other hand, when Černotíková *et al.* (2011) found the family Daniconematidae to be non-monophyletic (that included *Mexiconema* genus), they suggested the family Daniconematidae should be lowered to subfamily level (Daniconematinae) and transferred to the family Skrjabillanidae. In this study, we support the transfer of *M. cichlasomae* and *M. africanum* to the family Skrjabillanidae, based on the values of genetic divergence (3.93–6.46%) between taxa that represent the daniconematids (i.e. *Mexiconema* spp.) and skrjabillanids (table 1). However, we do not support the proposal to lower the family Daniconematidae to Daniconematinae; for such a move, it would be necessary to test the phylogenetic position of two additional monotypic daniconematid genera: *Daniconema* Moravec & Køie, 1987 and *Syngnathinema* Moravec *et al.*, 2001 (Moravec, 2006; Moravec *et al.*, 2009). Additionally, *Mexiconema* as a genus currently includes three species: *M. cichlasomae*, *M. africanum* and *M. liobagri* (Moravec *et al.*, 1992; Moravec and Nagasawa, 1998; Moravec and Shimazu, 2008; Moravec *et al.*, 2009); therefore, it is necessary to include molecular sequences of *M. liobagri* to support or contrast with the paraphyletic pattern detected for the genus *Mexiconema*.

In this study, *M. cichlasomae* is included in a clade (monophyletic group) with representatives from two paraphyletic families (Skrjabillanidae and Daniconematidae), which include parasites of fishes without free-living stages and using branchiurid ectoparasites, such as *Argulus* sp., as intermediate hosts (Tikhomirova, 1970, 1975, 1980; Černotíková *et al.*, 2011). In this context, this clade with the putative name "Skrjabillanidae" (*sensu laxo* Černotíková *et al.*, 2011) represents a natural group with diversification patterns, particularly regulated at the level of intermediate host (i.e. branchiurids), and host-switching events at the level of the definitive hosts. A future study involving cophylogenetic analyses may shed light on these evolutionary processes (e.g. Martínez-Aquino, 2016; Vanhove *et al.*, 2016).

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## References

**Aguilar-Aguilar R *et al.*** (2005) Aplicación del análisis de parsimonia de endemismos (PAE) en los sistemas hidrológicos de México: Un ejemplo con helmintos parásitos de peces dulceacuícolas. In Llorente-Bousquets J and Morrone JJ (eds), *Regionalización biogeográfica en Iberoamérica y tópicos afines: Primeras Jornadas Biogeográficas de la Red Iberoamericana de Biogeografía y Entomología Sistemática* (RIBES XII. I-CYTED). Mexico City: Las Prensas de Ciencias, UNAM, pp. 227–239.

**Blasco-Costa I and Poulin R** (2017) Parasite life-cycle studies: a plea to resurrect an old parasitological tradition. *Journal of Helminthology* **91**, 647–656.

**Blaxter ML *et al.*** (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**, 71–75.

**Borges JN *et al.*** (2012) Morphological and molecular diagnosis of anisakid nematode larvae from cutlassfish (*Trichiurus lepturus*) off the coast of Rio de Janeiro, Brazil. *PLoS ONE* **7**, e40447.

**Caspeta-Mandujano JM** (2005) *Nematode parasites of freshwater fish in Mexico: key to species, descriptions and distribution*. Cuernavaca, Morelos: Universidad Autónoma del Estado de Morelos, Mexico, Facultad de Ciencias Biológicas.

**Caspeta-Mandujano JM and Mejía-Mojica H** (2004) Seasonal dynamics of the occurrence and maturation of *Rhabdochona canadensis* in its definitive host, *Notropics boucardi* of the Chalma River, State of Morelos, México. *Helminthologia* **41**, 121–123.

**Castresana J** (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**, 540–552.

**Černotíková E, Horák A and Moravec F** (2011) Phylogenetic relationships of some spirurine nematodes (Nematoda: Chromadorea: Rhabditida: *Spirurina*) parasitic in fishes inferred from SSU rRNA gene sequences. *Folia Parasitologica* **58**, 135–148.

**Choudhury A and Nadler SA** (2016) Phylogenetic relationships of Cucullanidae (Nematoda), with observations on Seuratoidea and the monophyly of *Cucullanus*, *Dichelyne* and *Truttaedacnitis*. *Journal of Parasitology* **102**, 87–93.

**Cuenca-Soria CA *et al.*** (2013) Histological development of the digestive system of Mayan cichlid *Cichlasoma urophthalmus* (Günther 1862). *Journal of Applied Ichthyology* **29**, 1304–1312.

**Drummond AJ *et al.*** (2012) Bayesian phylogenetic with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969–1973.

**Holterman M *et al.*** (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology Evolution* **23**, 1792–1800.

**Hugot JP and Quentin JC** (2000) Nemacycle: a coding system for representation of nematode life-cycles. *Research & Review in Parasitology* **60**, 57–67.

**Johnson PTJ *et al.*** (2010) When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends in Ecology and Evolution* **25**, 362–371.

**Katoh K and Standley DM** (2016) A simple method to control over-alignment in the MAFFT multiple sequence alignment program. *Bioinformatics* **32**, 1933–1942.

**Kimura M** (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

**Klimpel S and Palm HW** (2011) Anisakid nematode (Ascaridoidea) life cycles and distribution: increasing zoonotic potential in the time of climate change? In Mehlhorn H (ed.), *Progress in Parasitology, Parasitology Research Monographs*. Berlin, Heidelberg: Springer, pp. 201–222.

**Kuitunen-Ekbaum E** (1933) *Philonema oncorhynchi* g. nov. et sp. nov. *Contributions to Canadian Biology and Fisheries* **4**, 71–75.

**Kumar S, Stecher G and Tamura K** (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

**Lanfear R *et al.*** (2012) Partition finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**, 1695–1701.

**Lanfear R *et al.*** (2014) Selecting optimal partitioning schemes for phylogenomic datasets. *BioMed Central Evolutionary Biology* **14**, 82.

**Liu G-H *et al.*** (2015) The complete mitochondrial genome of the gullet worm *Gongylonema pulchrum*: gene content, arrangement, composition and phylogenetic implications. *Parasites & Vectors* **8**, 100.

**Loung LT and Hudson PJ** (2012) Complex life cycle of *Pterygodermatites peromysci*, a trophically transmitted parasite of the white-footed mouse (*Peromyscus leucopus*). *Parasitology Research* **110**, 483–487.

**Martínez-Aquino A** (2016) Phylogenetic framework for coevolutionary studies: a compass for exploring jungles of tangled trees. *Current Zoology* **62**, 393–403.

**May-Tec AL *et al.*** (2013) Temporal variation of *Mexiconema cichlasomae* (Nematoda: Daniconematidae) in the Mayan cichlid fish *Cichlasoma urophthalmus* and its intermediate host *Argulus yucatanus* from a tropical coastal lagoon. *Parasitology* **140**, 385–395.

**Mejía-Madrid HH and Aguirre-Macedo ML** (2011) Systematics of *Mexiconema cichlasomae* (Nematoda: Daniconematidae) based on sequences of SSU rDNA. *Journal of Parasitology* **97**, 160–162.

**Merlo-Serna AI and García-Prieto L** (2016) A checklist of helminth parasites of Elasmobranchii in Mexico. *ZooKeys* **563**, 73–128.

**Miller MA, Pfeiffer W and Schwartz T** (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop* (*GCE*). *New Orleans*.

**Molnár K and Székely C** (1998) Occurrence of skrjabillanid nematodes in fishes of Hungary and in the intermediate host, *Argulus foliaceus* L. *Acta Veterinaria Hungarica* **46**, 451-463.

**Moravec F** (1968) A new nematode genus *Molnaria* gen. n. (Nematoda: Skrjabillanidae). *Folia Parasitologica* **15**, 322.

**Moravec F** (1977) A new nematode parasite, *Esocinema bohemicum* gen. et sp. nov. (Skrjabillanidae) of the European pike. *Folia Parasitologica* **24**, 86–90.

**Moravec F** (1998) *Nematodes of Freshwater Fishes of the Neotropical Region*. Prague: Academia.

**Moravec F** (2004) Some aspects of the taxonomy and biology of dracunculoid nematodes parasitic in fishes: a review. *Folia Parasitologica* **51**, 1–13.

**Moravec F** (2006) *Dracunculoid and Anguillicoloid Nematodes Parasitic in Vertebrates*. Prague: Academia.

**Moravec F and de Buron I** (2013) A synthesis of our current knowledge of philometrid nematodes, a group of increasingly important fish parasites. *Folia Parasitologica* **60**, 81–101.

**Moravec F and Nagasawa K** (1998) Helminth parasites of the rare endemic catfish, *Liobagrus reini* in Japan. *Folia Parasitologica* **45**, 238–294.

**Moravec F and Shimazu T** (2008) Redescription of the female of *Mexiconema liobagri* (Nematoda: Daniconematidae), a little-known parasite of the rare endemic catfish *Liobagrus reinii* (Amblycipitidae), in Japan. *Helminthologia* **45**, 106–108.

**Moravec F, Vidal-Martínez VM and Salgado-Maldonado G** (1992) *Mexiconema cichlasomae* gen. et sp. (Nematoda: Daniconematidae) from *Cichlasoma* spp. (Pisces) from México. *Folia Parasitologica* **39**, 33–40.

**Moravec F *et al.*** (1994) Present occurrence of *Anguillicola novaezelandiae* (Nematoda: Dracunculoidea) in Europe and its development in the intermediate host. *Folia Parasitologica* **41**, 203–208.

**Moravec F, Jiménez-García MI and Salgado-Maldonado G** (1998) New observations of *Mexiconema cichlasomae* (Nematoda: Dracunculoidea) from fishes in Mexico. *Parasite* **5**, 289–293.

**Moravec F, Vidal-Martínez VM and Aguirre-Macedo ML** (1999) Branchiurids (*Argulus*) as intermediate hosts of the daniconematid nematode of *Mexiconema cichlasomae*. *Folia Parasitologica* **46**, 79.

**Moravec F *et al.*** (2009) *Mexiconema africanum* sp. n. (Nematoda: Daniconematidae) from the catfish *Auchenoglanis occidentalis* from Lake Turkana, Kenya. *Parasitology Research* **105**, 1047–1052.

**Nadler SA *et al.*** (2007) Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. *Parasitology* **134**, 1421–1442.

**Nei M and Kumar S** (2000) *Molecular Evolution and Phylogenetics*. Oxford: Oxford University Press.

**Pereira FB, Pereira AN and Luque JL** (2017) Redescription and genetic characterization of *Cystidicoloides vaucheri*, including first description of male and current status on the phylogeny of Cystidicolidae (Nematoda: Habronematoidea). *Journal of Helminthology* **92**, 387–394.

**Poulin R and Grutter AS** (1996) Cleaning symbioses: proximate and adaptive explanations. *BioScience* **46**, 512–517.

**Quimbayo JP *et al.*** (2017) Fish cleaning interactions on a remote island in the Tropical Eastern Pacific. *Marine Biodiversity* **47**, 603–608.

**Ronquist F *et al.*** (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across large model space. *Systematic Biology* **61**, 539–542.

**Saiki RK *et al.*** (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.

**Salgado-Maldonado G** (2006) Checklist of helminth parasites of freshwater fishes from Mexico. *Zootaxa* **1324**, 1–357.

**Salgado-Maldonado G and Quiroz-Martínez B** (2013) Taxonomic composition and endemism of the helminth fauna of freshwater fishes of Mexico. *Parasitology Research* **112**, 1–18.

**Salgado-Maldonado G, García-Aldrete AN and Vidal-Martinez V** (2000) *Metazoan Parasites in the Neotropics: A Systematic and Ecological*

*Perspective*. Mexico City: Instituto de Biología, Universidad Nacional Autónoma de México.

**Salgado-Maldonado G *et al.*** (2011) Helminth parasites of freshwater fish in Chiapas, Mexico. *Parasitology Research* **108**, 31–59.

**Schneider CA, Rasband WS and Eliceiri KW** (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671–675.

**Schwarz G** (1978) Estimating the dimension of a model. *Annals of Statistics* **6**, 461–464.

**Shigin AA and Shigina NG** (1958) A new parasite of tench – *Skrjabillanus tincae* nov. gen., nov. sp. (Nematoda: Camallanata). In *"Raboty po gelmintologii" posviashch*. 80–letiyu akad. K.I. Skryabina. Moscow: Publishing House of the USSR Academy of Sciences, pp. 395–399. (In Russian).

**Sokal RR and Rohlf FJ** (2009) *Introduction to Biostatistics*. 2nd edn. San Francisco, CA: W.H. Freeman.

**Sosa-Medina T, Vidal-Martínez VM and Aguirre-Macedo ML** (2015) Metazoan parasites of fishes from the Celestun coastal lagoon, Yucatan, Mexico. *Zootaxa* **4007**, 529–544.

**Talavera G and Castresana J** (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**, 564–577.

**Tikhomirova VA** (1970) Elucidation of the developmental cycle of the nematode *Skrjabillanus seardinii* Molnar, 1965. *Doklady Akademii Nauk SSSR* **195**, 510–511.

**Tikhomirova VA** (1975) Life cycles of nematodes of the family Skrjabillanidae. In *Voprosy ekologii zhivotnykh Gosudarstvennyi Universitet*. Vol. 2. Kalinin, pp. 118–122. (In Russian).

**Tikhomirova VA** (1980) On nematodes of the family Skrjabillanidae (Nematoda: Camallanata). *Pamzitologiya* **14**, 258–262. (In Russian).

**Vanhove MPM *et al.*** (2016) Cichlids: a host of opportunities for evolutionary parasitology. *Trends in Parasitology* **32**, 820–832.

**Vidal-Martínez VM *et al.*** (2001) *Atlas of the Helminth Parasites of Cichlid Fishes of Mexico*. Prague: Academia.

**Wijová M *et al.*** (2005) Phylogenetic position of *Dracunculus medinensis* and some related nematodes inferred from 18S rRNA. *Parasitological Research* **96**, 133–135.