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Molecular identification of Antarctophthirus microchir and detection of Dirofilaria immitis DNA in lice from California sea lions in Mexico

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Short Report

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Abstract

The seal louse *Antarctophthirus microchir* (Anoplura: Echinophthiriidae) is an obligate hematophagous ectoparasite of pinnipeds, but molecular records from Mexican populations and information on microorganisms detected in this species remain limited. This study aimed to confirm the molecular identity of A. microchir collected from California sea lions (*Zalophus californianus*) in the Gulf of California, Mexico, and to screen these ectoparasites for selected bacterial and parasitic agents. Twenty lice were collected from six of 30 sea lion pups examined at Los Islotes; Baja California Sur. Morphological identification was supported by sequencing a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. All specimens were morphologically identified as *A. microchir*, and COI sequences showed 98–99% nucleotide identity with reference sequences. Individual DNA extracts were screened by PCR for selected bacterial, protozoan, and filarial agents. *Dirofilaria immitis*-compatible DNA was detected in four lice collected from a single pup, whereas no amplification was obtained for the other screened agents. Three positive amplicons were successfully sequenced, revealing two ITS2 haplotypes with 99.08% and 99.31% nucleotide identity to reference *D. immitis* sequences. Phylogenetic analysis supported their placement within the *D. immitis* clade. This study provides the first molecular confirmation of *A. microchir* in Mexico and, to our knowledge, the first report of *D. immitis* DNA in an anopluran louse. Because all positive lice originated from a single host that was not independently tested for heartworm infection, this finding represents a single host-associated detection event and does not establish infection of the louse or its role in parasite transmission. These results expand the molecular information available on pinniped ectoparasites and provide a basis for further investigation of microorganisms detected in marine mammal lice.

Introduction

Sucking lice (Psocodea: Anoplura) are obligate hematophagous ectoparasites that complete their entire life cycle on mammalian hosts (Durden and Musser 1994). Their permanent association with hosts and blood-feeding behavior make them relevant to veterinary parasitology, particularly because they may acquire microorganisms circulating in host blood. Several bacterial and parasitic agents have been detected in hematophagous ectoparasites, although their detection does not necessarily indicate biological vector competence (Bartlett and Anderson 1987; Roux and Raoult 1999).

Within the Anoplura, the family Echinophthiriidae comprises specialized lice adapted to parasitize pinnipeds and other semiaquatic mammals (Leonardi and Palma 2013; Leonardi et al. 2022). The genus *Antarctophthirus* includes species associated with otariid and phocid hosts, including *Antarctophthirus microchir*, which has been reported from several sea lion species, particularly in the eastern Pacific (Aznar et al. 2009; Leonardi et al. 2009; Leonardi and Palma 2013). Despite its association with California sea lions (*Zalophus californianus*), geographical records of this ectoparasite in Mexico remain limited, and molecular confirmation from Mexican specimens has been lacking. Molecular characterization is therefore important to complement morphological identification and improve the taxonomic and geographical documentation of this species (Sánchez-Montes et al. 2013).

Information regarding microorganisms detected in pinniped lice is also limited. Previous molecular investigations of the seal louse *Echinophthirius horridus* have reported *Anaplasma phagocytophilum*, *Mycoplasma* sp., and the filarial nematode *Acanthocheilonema spirocauda* (Hirzmann et al. 2021; Lehnert et al. 2023; Wilhelm et al. 2026).

Considering the limited information available on microorganisms associated with pinniped lice, molecular screening of these ectoparasites may contribute to identifying previously undocumented pathogen DNA and expanding knowledge of their parasitological associations. Therefore, this study aimed to molecularly confirm the identity of *A. microchir* collected from California sea lion pups in the Gulf of California, Mexico, and investigate the presence of selected bacterial and parasitic agents, including *Anaplasma/Ehrlichia* spp., *Bartonella* spp., *Coxiella* spp., *Mycoplasma* spp., *Rickettsia* spp., *Babesia*/*Theileria* spp., and filarial nematodes. The findings provide the first molecular confirmation of *A. microchir* in Mexico and document, to our knowledge, the first detection of *D. immitis* DNA in an anopluran louse.

Materials and Methods

Field sampling was conducted in July 2025 at the Los Islotes California sea lion (*Z. californianus*) breeding colony (24°35'N, 110°23'W), located on Partida Island within the Espíritu Santo Archipelago National Park and the Islands of the Gulf of California Flora and Fauna Protection Area, Baja California Sur, Mexico (Fig. 1.).

Thirty California sea lion pups were examined for ectoparasites. Animals were physically restrained using mesh nets, and their bodies were examined for lice. Specimens were manually collected using fine-toothed combs and individually preserved in 70% ethanol until laboratory processing. All animal handling procedures were performed under the supervision of trained veterinarians and technical personnel to minimize animal stress and ensure compliance with applicable animal welfare regulations. The number of lice collected from each pup was recorded.

Lice were prepared for morphological examination using a modified slide-mounting technique based on Kim et al. (1986). Specimens were subsequently mounted in Canada balsam following Palma (1978) and examined under a light microscope. Species identification was based on the taxonomic keys and morphological descriptions provided by Kim et al. (1986) and Leonardi (2014b). Diagnostic morphological characters were evaluated to establish the taxonomic identity and developmental stage of the specimens.

Genomic DNA was extracted individually from each louse using a 10% Chelex® 100 solution. Before extraction, each specimen was punctured in the abdominal region with a sterile insulin needle to facilitate tissue digestion. Samples were incubated in 400 µL of Chelex solution at 56°C for 12 h, followed by protein denaturation at 94°C. After centrifugation at 8,000 × g for 15 min, the supernatant containing genomic DNA was transferred to sterile microcentrifuge tubes.

Molecular identification of the lice was performed by amplifying an approximately 650-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene using the primers described by Folmer et al. (1994). In addition, individual DNA extracts were screened by PCR for selected bacterial and parasitic agents. The bacterial targets included *Anaplasma/Ehrlichia* spp., *Bartonella* spp., *Coxiella* spp., *Mycoplasma* spp., and *Rickettsia* spp.; protozoan screening targeted *Babesia*/*Theileria* spp.; and filarial nematodes were screened. The primer sequences, target genes, expected amplicon sizes, and corresponding references are presented in Supplementary Table 1.

PCR amplification conditions were those established for the respective primer sets. PCR products were separated by electrophoresis on 2% agarose gels stained with SmartGlow and visualized using an Accuris gel documentation system.

PCR products showing the expected amplification pattern were purified and bidirectionally sequenced by Macrogen Inc. (Seoul, Republic of Korea). Forward and reverse chromatograms were assembled into consensus sequences and manually edited before analysis. Consensus sequences were compared with those available in GenBank using BLASTn to assess nucleotide identity and taxonomic assignment.

Phylogenetic analyses were conducted separately for the COI sequences obtained from lice and the ITS2 sequences assigned to filarial nematodes. Multiple sequence alignments were generated using homologous reference sequences retrieved from GenBank. Phylogenetic relationships were inferred using the Maximum Likelihood method implemented in IQ-TREE. The best-fitting nucleotide substitution model for each dataset was selected according to the Bayesian Information Criterion (BIC), and branch support was assessed using ultrafast bootstrap replicates.

Results

Thirty California sea lion pups were examined, and 20 sucking lice were recovered from six individuals. All specimens were morphologically identified as *A. microchir*. Diagnostic characters included piercing-sucking mouthparts, well-developed abdominal spiniform setae, and characteristic chaetotaxy, consistent with the morphological descriptions of this species. All specimens were identified as third-instar nymphs based on the presence of four-segmented antennae, well-developed thoracic phragmata, and the distribution of dorsal scales and setae (Fig. 2).

Molecular identification based on mitochondrial COI sequences supported the morphological identification. BLASTn comparisons showed 98–99% nucleotide identity with a previously deposited *A. microchir* sequence from *Z. californianus* (GenBank accession number MW803093.1). Maximum Likelihood phylogenetic analysis grouped the Mexican sequences within the *A. microchir* clade (Fig. 3).

PCR screening detected filarial nematode DNA in four of the 20 examined lice. All positive specimens were collected from the same California sea lion pup. Three of the four positive amplicons yielded sequences suitable for further analysis. BLASTn comparisons identified the sequences as *D. immitis*. No amplification was obtained for the other bacterial and protozoan agents included in the molecular

screening, namely *Anaplasma/Ehrlichia* spp., *Bartonella spp*., *Coxiella* spp., *Mycoplasma* spp., *Rickettsia* spp., and *Babesia/Theileria* spp.

Sequence analysis of the three ITS2 amplicons identified two haplotypes. The first haplotype, represented by one sequence, showed 99.08% nucleotide identity (433/437 bp), whereas the second haplotype, represented by two sequences, showed 99.31% nucleotide identity (434/437 bp) with reference *D. immitis* sequences. The closest matches included sequences obtained from *Aedes albopictus* in the United States and domestic dogs (*Canis lupus familiaris*) from Brazil and Mexico (GenBank accession numbers PQ496879.1, PV699119.1, and KX932115.1). Maximum Likelihood phylogenetic analysis placed both haplotypes within the *D. immitis clade* (Fig. 4).

Discussion

Molecular characterization provides an important complement to morphological identification, particularly for ectoparasites whose taxonomic and geographical records remain limited. In the present study, COI sequence analysis supported the morphological identification of *A. microchir* collected from California sea lions in Mexico. The 98–99% nucleotide identity with previously reported A. microchir sequences and the clustering of the Mexican sequences within the corresponding phylogenetic clade provide additional molecular evidence supporting this identification. These findings represent the first molecular confirmation of *A. microchir* in Mexico and complement previous records based primarily on morphological evidence, including those compiled by Sánchez-Montes et al. (2013). Beyond confirming the species identity, the sequences generated in this study provide reference data for future taxonomic and molecular investigations of echinophthiriid lice in the eastern Pacific. Nevertheless, the available sequences are insufficient to draw conclusions about population-level genetic diversity or geographical differentiation.

An additional finding was the detection of filarial nematode DNA in four louse specimens, three of which yielded sequences identified as *D. immitis*. Sequence analysis revealed two ITS2 haplotypes, both of which clustered within the *D. immitis* clade and showed high nucleotide identity with reference sequences previously obtained from mosquitoes and domestic dogs. To our knowledge, this represents the first report of *D. immitis* DNA detected in an anopluran louse. This finding is noteworthy in the context of previous reports of *D. immitis* in pinnipeds (Alho et al. 2017), including California sea lions from the Gulf of California (Farriols et al. 2020). However, the sequence similarity with isolates from other hosts and geographical locations does not establish an epidemiological connection between these records. The relevance of the present finding lies in documenting the presence of *D. immitis* DNA in whole-louse extracts, rather than demonstrating a biological association between the nematode and *A. microchir* (Livingston et al., 2024).

The origin of the detected DNA remains uncertain. Given the hematophagous feeding behavior of sucking lice, one possible explanation is the acquisition of parasite DNA during a blood meal from a host carrying circulating microfilariae. However, this hypothesis cannot be confirmed because the California

sea lion from which the positive lice were collected was not independently tested for *D. immitis*. Therefore, neither the infection status of the pup nor the origin of the DNA detected in the lice could be established. Moreover, PCR detection does not provide evidence of parasite viability, survival, development, or migration within the ectoparasite. Previous investigations have detected microorganisms in pinniped lice, including *A. phagocytophilum* and *Mycoplasma* spp. in *E. horridus* (Hirzmann et al. 2021), while more recent work has investigated *A. spirocauda* larvae in this louse as a presumed intermediate host (Wilhelm et al. 2026). These studies provide a relevant context for investigating microorganisms in seal lice; nevertheless, they do not demonstrate that *A. microchir* participates in the development or transmission of *D. immitis*. Accordingly, the present results do not support classifying this louse as an intermediate host or vector of the nematode.

The interpretation of these findings is limited by the fact that all four filarial PCR-positive lice were collected from a single California sea lion pup, representing one host-associated detection event rather than independent epidemiological observations. Moreover, the host was not tested for *D. immitis*, and only three of the four positive specimens yielded sequences confirming the presence of this species. Consequently, these results do not allow estimation of infection frequency among sea lions or conclusions regarding parasite circulation within the Los Islotes colony. The exclusive recovery of third-instar nymphs also prevented comparisons among developmental stages. Further studies involving larger numbers of lice from multiple hosts and colonies, together with paired molecular or parasitological testing of sea lions, are needed to clarify the origin of the detected DNA and determine whether seal lice could serve as complementary samples for wildlife pathogen surveillance. Experimental investigations would additionally be required to establish whether *A. microchir* supports any developmental stage of *D. immitis* or plays a role in its transmission.

Overall, this study provides the first molecular confirmation of *A. microchir* in Mexico and documents the detection of *D. immitis* DNA in whole-louse extracts. Although the latter finding represents a previously undocumented observation in an anopluran louse, it does not establish infection of the ectoparasite, infection of the sea lion pup, or vector competence. These findings expand the molecular information available on pinniped ectoparasites and provide a basis for further investigations into the origin and biological significance of parasite DNA detected in marine mammal lice.

Conclusion

In conclusion, this study provides the first molecular confirmation of *A. microchir* in Mexico and documents the detection of *D. immitis* DNA in whole-louse extracts. The latter finding identifies a previously undocumented observation in an anopluran louse, but does not demonstrate infection of the ectoparasite, host infection, or vector competence. Further studies with paired host–ectoparasite sampling are required to clarify the origin and biological significance of this molecular finding.

Declarations

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Declarations statement

The authors declare no conflict of interest.

Animal ethics

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This study was approved by the Bioethics Committee of the Centro Interdisciplinarios de Ciencias Marinas, Instituto Politécnico Nacional (Animals were handled according to National Legislation and Ethics (NOM-012-ZOO-1993). Sampling was conducted under the regulation of the scientific collection permit No. SGPA/DGVS/12,771/16 dated November 23, 2016.

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Author Contributions

V.A.P.-P.: Investigation, Methodology, Formal analysis, Visualization, Writing—original draft. M.P.R.-H.: Conceptualization, Methodology, Investigation, Resources, Supervision, Writing—review and editing. C.I.M.-G.: Investigation, Methodology, Resources, Writing—review and editing. E.R.-F.: Investigation, Resources, Writing—review and editing. R.I.H.-H.: Investigation, Resources, Writing—review and editing. P.S.M.-A.: Investigation, Resources, Writing—review and editing. E.D.R.M.: Investigation, Data curation, Writing—review and editing. E.G.: Methodology, Validation, Formal analysis, Writing—review and editing. B.S.-S.: Methodology, Validation, Investigation, Writing—review and editing. H.M.Z.-I.: Investigation, Resources, Writing—review and editing. J.M.O.: Investigation, Resources, Writing—review and editing. I.B.: Conceptualization, Methodology, Supervision, Writing—review and editing. C.J.H.-C.: Investigation, Resources, Supervision, Writing—review and editing.L.A.O.-C: Investigation. J.LB.-R.: Investigation, Resources, Supervision, Writing—review and editing. S.S.-M.: Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Project administration, Writing—original draft, Writing—review and editing.

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Figures

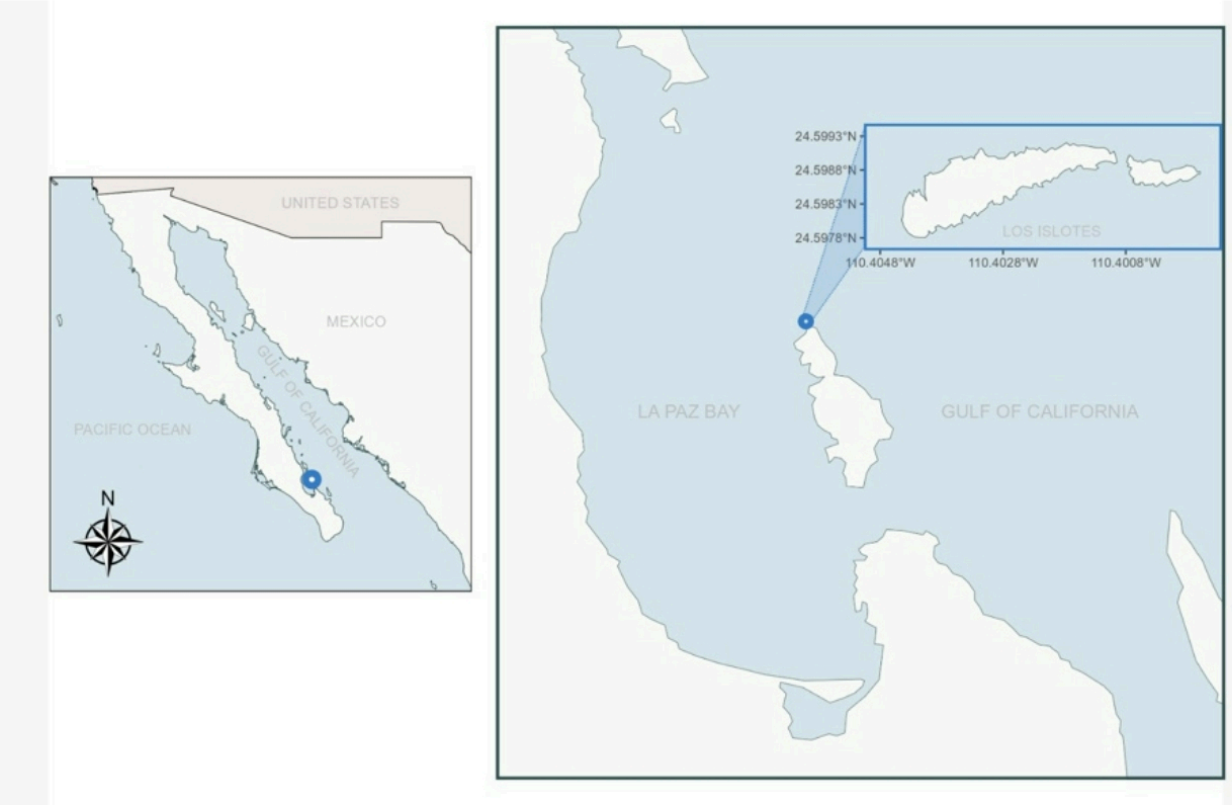


Figure 1

Map of the Gulf of California showing the California sea lion colony sampled in this study to assess the presence of lice.

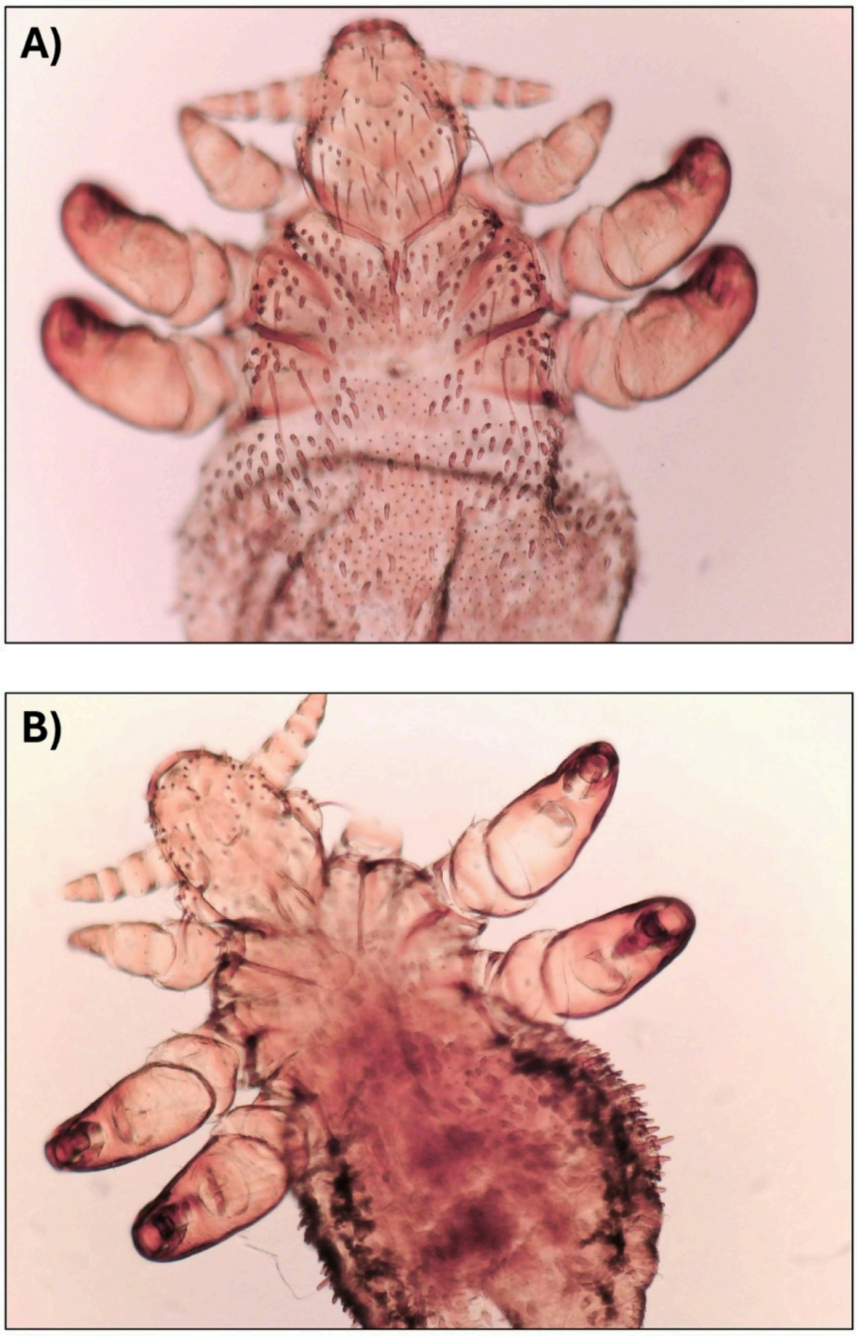


Figure 2

Third-stage nymphs of *Antarctophthirus microchir* from California sea lion pups in Mexico.

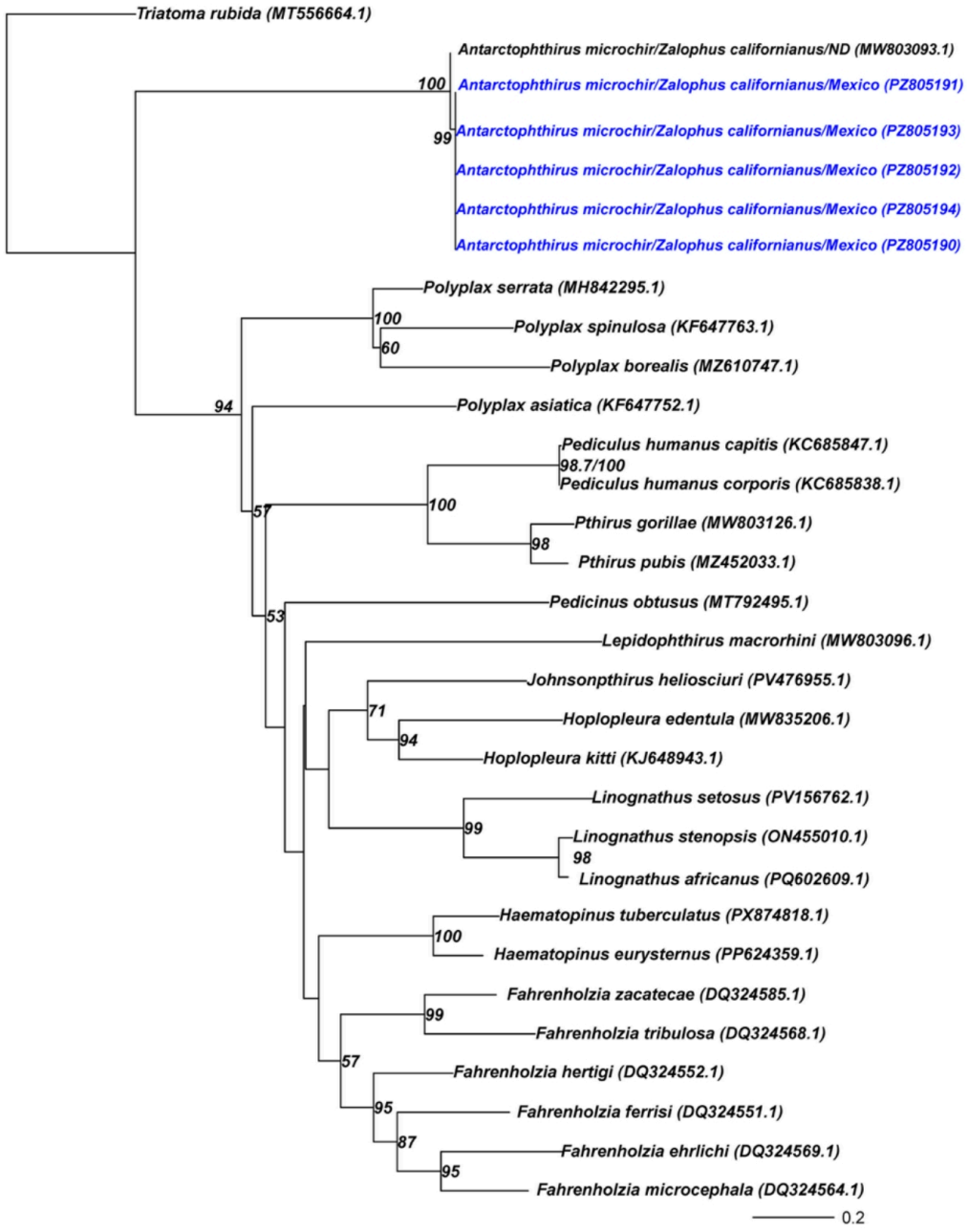


Figure 3

Maximum-likelihood phylogenetic tree inferred from partial Anopluran COI sequences using the Kimura three-parameter model with unequal base frequencies, empirical nucleotide frequencies, a proportion of invariant sites, and gamma-distributed rate heterogeneity with four categories (K3Pu+F+I+G4), selected as the best-fitting substitution model. Sequences generated in the present study are shown in bold and highlighted in blue.

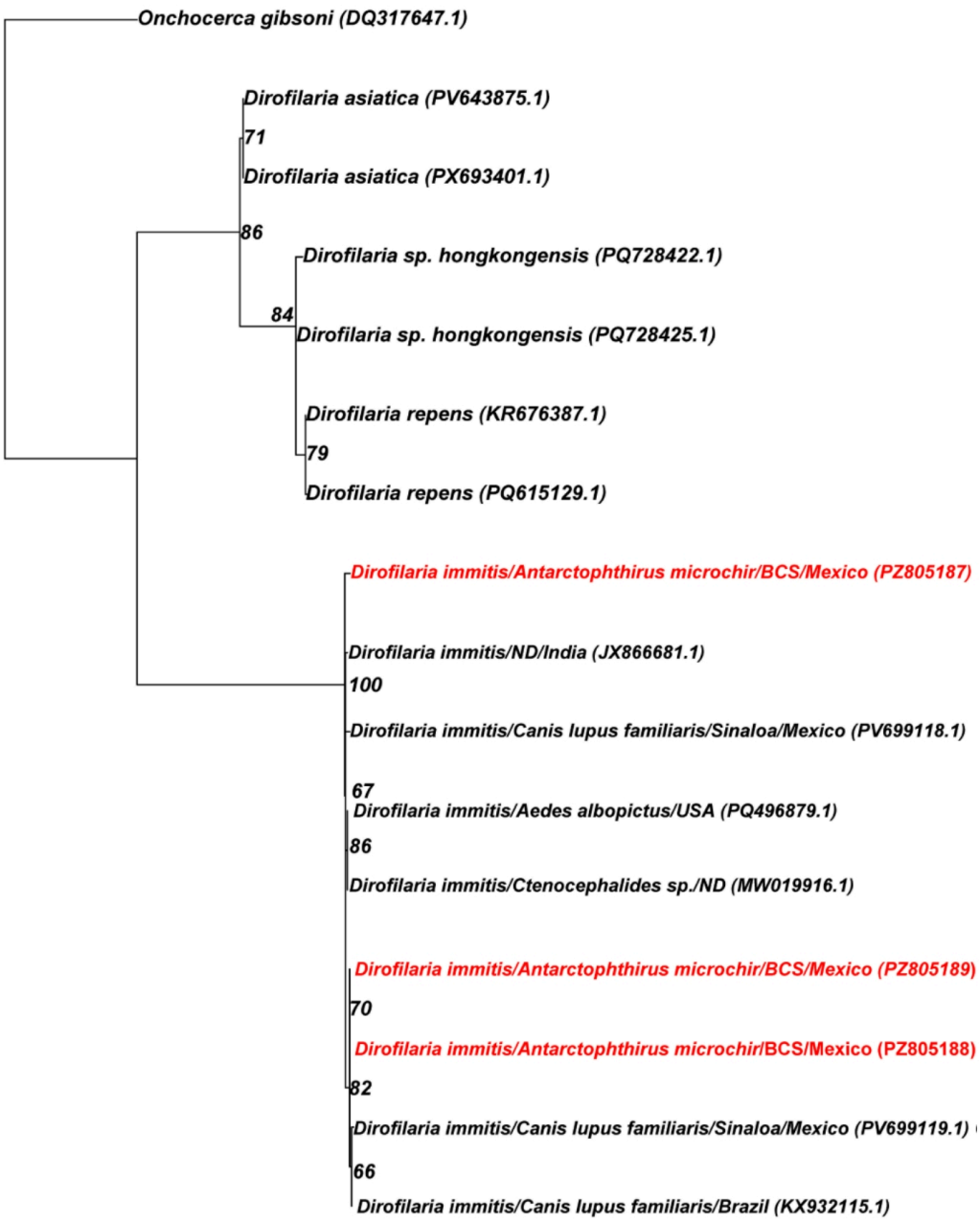


Figure 4

Maximum-likelihood phylogenetic tree inferred from partial nematode ITS2 sequences using the Felsenstein 1981 model with empirical nucleotide frequencies (F81+F), selected as the best-fitting substitution model. Sequences generated in the present study are shown in bold and highlighted in red.

Supplementary Files

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- SupplementaryTable110.docx