**New *Echinocephalus* species from an Amazonian stingray suggests co-origin of host and parasite in late Oligocene to early Miocene**

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**SUPPLEMENTARY MATERIAL**

Supplementary Table 1: Nematodes included in the phylogenetic analyses from SSU and COI. New sequences obtained for the present study are in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species** | ***Host species*** | **Collection site** | **Gene** | **GenBank Acession No.** | **Source** |
| *Acanthocheilus rotundatus* | *Rhizoprionodon acutus* | China | SSU | MF072699 | Li et al. 2018 |
| *Anguillicola crassus* |  |  | SSU | DQ118535 | unpublished |
| *Anguillicola crassus* |  | Czech Republic | SSU | DQ490223 | Wijova et al. 2005 |
| *Anguillicola crassus* | *Ameiurus nebulosus* | USA | COI | MF458546 | El-Shehabi et al. 2018 |
| *Anguillicola crassus* | *Ameiurus nebulosus* | USA | COI | MF458547 | El-Shehabi et al. 2018 |
| *Anisakis simplex* | *Carcharhinus sorrah* | China | SSU | MF072711 | Li et al. 2018 |
| *Anisakis pregreffi* | *Caretta caretta* | Italy | SSU | EF180082 | Nadler et al 2007 |
| *Anisakis pregreffi* | *Conger myriaster* | China | SSU | MF072697 | Li et al. 2018 |
| *Ascaris suum* | *Canis lupus familiares* | China | SSU | MN558962 | Xie et al. 2020 |
| *Ascarophis arctica* |  |  | SSU | DQ094172 | unpublished |
| *Aspiculuris tetraptera* | *Mus musculus* | Saudi Arabia | SSU | MT755640 | Omer et al. 2020 |
| *Baylisascaris transfuga* | *Ursus maritimus* | China | SSU | JN256988 | Li et al. 2012 |
| *Baylisascaris schroederi* | *Ailuropoda melanoleuca* | China | SSU | JN256992 | Li et al. 2012 |
| *Brugia malayi* | *Canis lupus familiares* | Thailand | COI | MK250753 | unpublished |
| *Cosmocercoides tonkiensis* | *Acanthosaura lepidogaster* | Vietnan | SSU | AB908160 | Tran et al. 2015 |
| *Cosmocercoides qingtianens* | *Bufo gargarizans* | China | SSU | MH178321 | Chen et al. 2018 |
| *Cosmocercoides pulcher* | *Bufo japonicus* | Japan | SSU | MH178326 | Chen et al. 2018 |
| *Cucullanus baylisi* | *Synodontis* sp. | Sudan | SSU | JF803935 | Moravec 2007 |
| *Cucullanus dodsworthi* | *Sphoeroides testudineus* | Mexico | SSU | HQ241923 | Mejia-Madrid and Aguirre-Macedo 2011 |
| *Cucullanus robustus* |  |  | SSU | JF934726 | Laetsch et al. 2012 |
| *Dentostomella sp.* |  |  |  | AF036590 | Blaxter et al. 1998 |
| *Dujardinascaris gigantea* | *Alligator sinensis* | China | SSU | MF072701 | Li et al. 2018 |
| *Echinocephalus overstreeti* | *Heterodontus portusjacksoni* | Australia | SSU | JF934729 | Laetsch et al. 2012 |
| *Echinocephalus* cf. *pseudouncinatus* | *Atrina maura* | Mexico | SSU | MN514178 | unpublished |
| *Echinocephalus sp.1* |  | Egypt | SSU | KY911549 | unpublished |
| *Echinocephalus sp.2* | *Saurida undosquamis* | Egypt | SSU | KY972321 | unpublished |
| ***Echinocephalus spinosus n. sp.*** | ***Potamotrygon motoro*** | **Brazil** | **SSU** | **Xxxx**  **xxxxx** | **This study** |
| ***Echinocephalus spinosus n. sp.*** | ***Potamotrygon motoro*** | **Brazil** | **COI** | **Xxxxxxxx**  **Xxxxxxx**  **Xxxxxx**  **xxxxxxx** | **This study** |
| *Gnathostoma binucleatum* |  |  | SSU | Z96946 | unpublished |
| *Gnathostoma binucleatum* |  |  | COI | AB037131 | unpublished |
| *Gnathostoma neoprocyonis* |  |  | SSU | Z96947 | unpublished |
| *Gnathostoma spinigerum* |  |  | COI | AB037132 | unpublished |
| *Gnathostoma turgidum* |  |  | SSU | Z96948 | unpublished |
| *Heligmosomoides polygyrus* | *Apodemus mystacinus* | Turkey | COI | AF303153 | Zalesny et al. 2014 |
| *Hysterotylacium deardorffoverstreetorum* | *Paralichthys isosceles* | Brazil | SSU | JF718550 | Knoff et al. 2012 |
| *Hysterotylacium tetrapteri* | *Kajikia audax* | China | SSU | MF072705 | Li et al. 2018 |
| *Ichtyobronema hamulatum* | *Lota lota* | Russia | SSU | KY476351 | Sokolov et al. 2017 |
| *Mawsonascaris zhoui* | *Glaucostegus granulatus* | China | SSU | MF072706 | Li et al. 2018 |
| *Necator americanus* | Human | China | COI | AF303153 | Hawdon et al. 2001 |
| *Neoascarophis macrouri* |  |  | SSU | DQ442660 | Wijova et al 2006 |
| *Oxiurys equi* | *Equus caballus* | USA | SSU | EF180062 | Nadler et al. 2007 |
| *Physaloptera alata* |  |  | SSU | AY702703 | unpublished |
| *Physaloptera hispida* | *Sigmodon hispidus* | USA | COI | MH782845 | unpublished |
| *Physaloptera thalacomys* | *Perameles gunnii* | Australia | SSU | JF934734 | Laetsch et al. 2012 |
| *Plectus aquatilis* |  | South Africa | SSU | GQ892827 | unpublished |
| *Porrocaecum reticulatum* | *Egretta garzetta* | China | SSU | MF072700 | Li et al. 2018 |
| *Procamallanus spiculogubernaculus* | *Heteropneustes fossilis* | India | COI | KU292358 | unpublished |
| *Proleptus trygonorrhini* | *Trygonorrhina fasciata* | Australia | SSU | JF934733 | Laetsch et al. 2012 |
| *Pseudanisakis rajae* | *Raja pulchra* | China | SSU | MF072707 | Li et al. 2018 |
| *Raphidascaroides brasiliensis* | *Platydoras costatus* | Brazil | SSU | KP726276 | Pereira et al. 2015 |
| *Raphidascaroides moraveci* | *Platydoras armatulus* | Brazil | SSU | KP726278 | Pereira et al. 2015 |
| *Rhadochona acuminatus* | *Brycon guatemalensis* | Mexico | COI | MK341636 | SantaCruz et al. 2019 |
| *Rhabdochona guerreroensis* | *Sicydium multipunctatum* | Mexico | SSU | JF934732 | Laetsch et al. 2012 |
| *Spiroxys hanzaki* | *Andrias japonicus* | Japan | SSU | AB818383 | Hasegawa et al. 2013 |
| *Spiroxys japonica* | *Rana nigromaculata* | Japan | SSU | AB818382 | Hasegawa et al. 2013 |
| *Spinitectus humbertoi* | *Profundulus punctatus* | Guatemala | COI | MK024434 | Barrios-Gutierrez et al. 2019 |
| *Tanqua tiara* | *Varanus indicus* | Australia | SSU | JF934728 | Laetsch et al. 2012 |
| *Terranova caballeroi* |  |  | SSU | U94381 | unpublished |
| *Toxocara canis* | *Alopex lagopus* | China | SSU | JN256976 | Li et al. 2012 |
| *Toxocara cati* | *Felis domesticus* | USA | SSU | EF180059 | Nadler et al. 2007 |
| *Toxocara vitulorum* | *buffalo* | Sri Lanka | SSU | EF180078 | Nadler et al. 2007 |
| *Toxascaris leonina* | *Panthera tigris altaica* | China | SSU | JN256979 | Li et al. 2012 |
| *Truttaedactinis clitellari* | *Acipenser fulvescens* | USA | SSU | KP275681 | Choudhury & Nadler 2015 |
| *Truttaedactinis heterodonti* | *Heterodontus francisci* | USA | SSU | KP275680 | Choudhury & Nadler 2016 |
| *Truttaedactinis truttae* | *Onchorhynchus mykiss* | USA | SSU | EF180063 | Nadler et al. 2007 |
| *Turgida* sp. |  | Mexico | COI | KC130680 | Prosser et al. 2013 |
| *Tylocephalus auriculatus* |  |  | SSU | AF202155 | unpublished |

#### References regarding S Table 1:

#### Barrios-Gutierrez, J. J., Santacruz, A., Martinez-Ramirez, E., Rubio-Godoy, M., Pinacho-Pinacho, C. D. (2019). *Spinitectus mixtecoensis* sp. nov. (Nematoda: Cystidicolidae), from the Oaxaca killifish *Profundulus punctatus* (Osteichthyes: Profundulidae) from Mexico, with comments on the distribution of *Spinitectus humbertoi* in the genera *Profundulus* and Tlaloc. *Revista Mexicana de Biodiversidad*. 90. <https://doi.org/10.22201/ib.20078706e.2019.90.2684>

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P.,Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T., Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature*. 392 (6671), 71-75.

Choudhury, A. & Nadler, S. A. (2015). Phylogenetic relationships of cucullanidae (nematoda), with observations on seuratoidea and the monophyly of *Cucullanus*, *Dichelyne* and *Truttaedacnitis.* *Journal of Parasitology*. 102(1), 87-93.  [10.1645/15-806](https://doi.org/10.1645/15-806)

Chen, H. X., Zhang, L. P., Nakao, M., Li, L. (2018). Morphological and molecular evidence for a new species of the genus *Cosmocercoides* Wilkie, 1930 (Ascaridida: Cosmocercidae) from the Asiatic toad *Bufo gargarizans* Cantor (Amphibia: Anura). *Parasitology Research*. 117(6), 1857-1864. doi: 10.1007/s00436-018-5877-8.

El-Shehabi, F., Marcogliese, D. & Oliveira, K. (2018). New North America paratenic hosts of Anguillicola crassus and molecularly inferred source of invasion. *Aquatic Invasions*. 13(2), 231-246.

Hasegawa, H., Sato, A., Kai, M., Uchida, A. (2013). Helminth parasites of bullfrogs, Lithobates catesbeianus (Shaw,1802), in Kanto District, Japan, with special reference to those introduced from North America. *Japan Journal of Veterinary Parasitology*. 12, 1-10.

Hawdon, J. M., Li, T., Zhan, B., Blouin, M. S. (2001). Genetic structure of populations of the human hookworm, *Necator americanus*, in China. *Molecular Ecology.* 10 (6), 1433-1437.

Knoff, M., Felizardo, N. N., Iniguez, A. M., Maldonado, A. Jr., Torres, E. J., Magalhaes Pinto, R., Gomes, D. C. (2012). Genetic and morphological characterisation of a new species of the genus *Hysterothylacium* (Nematoda) from *Paralichthys isósceles* Jordan, 1890 (Pisces: Teleostei) of the Neotropical Region, state of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*. 107 (2), 186-193.

Laetsch, D. R., Heitlinger, E. G., Taraschewski, H., Nadler, S. A., Blaxter, M. L. (2012). The phylogenetics of Anguillicolidae (Nematoda: Anguillicolidea), swimbladder parasites of eels. *BMC Evolutionary Biology*. 12 (1), 60.

Li, Y., Niu, L., Wang, Q., Zhang, Z., Chen, Z., Gu, X., Xie, Y., Yan, N., Wang, S., Peng, X., Yang, G. (2012). Molecular characterization and phylogenetic analysis of ascarid nematodes from twenty-one species of captive wild mammals based on mitochondrial and nuclear sequences. *Parasitology*. 139 (10), 1329-1338.

Li, L., Lue, L., Nadler, S. A., Gibson, D. I., Zhang, L. P., Chen, H. X., Zhao, W. T., Guo, Y. N. (2018). Molecular Phylogeny and Dating Reveal a Terrestrial Origin in the Early Carboniferous for Ascaridoid Nematodes. *Systematic Biology*. 67, 888-900.  [10.1093/sysbio/syy018](https://doi.org/10.1093/sysbio/syy018)

Mejia-Madrid, H. H. & Aguirre-Macedo, M. L. (2011). Redescription and Genetic Characterization of *Cucullanus dodsworthi* (Nematoda: Cucullanidae) from the Checkered Puffer *Sphoeroides testudineus* (Pisces: Tetraodontiformes). *Journal of Parasitology*. 97 (4), 695-706.

Moravec, F. (2007). Some aspects of the taxonomy and biology of adult spirurine nematodes parasitic in fishes: a review. *Folia Parasitologica*. 54, 239-257.

Nadler, S. A., Carreno, R. A., Mejia-Madrid, H., Ullberg, J., Pagan, C., Houston, R., Hugot, J. P. (2007). Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitismo. *Parasitology*. 134 (PT 10), 1421-1442.

Omer, S. A., Alghamdi, J. M., Alrajeh, A. H., Aldamigh, M., Mohammed, O. B. (2020). Morphological and Molecular Characterization of *Aspiculuris tetraptera* (Nematoda: Heteroxynematidae) from *Mus musculus* (Rodentia: Muridae) in Saudi Arabia. Bioscience Reports. 40(12).  [10.1042/BSR20203265](https://dx.doi.org/10.1042%2FBSR20203265)

Pereira, F. B., Tavares, L. E., Scholz, T., Luque, J. L. (2015). A morphological and molecular study of two species of *Raphidascaroides* Yamaguti, 1941 (Nematoda: Anisakidae), parasites of doradid catfish (Siluriformes) in South America, with a description of *R. moraveci* n. sp. *Systematic Parasitology*. 91 (1), 49-61.

Prosser, S. W., Velarde-Aguilar, M. G., Leon-Regagnon, V., Hebert, P. D. (2013). Advancing nematode barcoding: A primer cocktail for the cytochrome coxidase subunit I gene from vertebrate parasitic nematodes. *Molecular Ecology Resources*. 13 (6), 1108-1115.

Santacruz, A., Ornelas-Garcia, C. P. & Perez-Ponce de Leon, G. (2019). Diversity of *Rhabdochona mexicana* (Nematoda: Rhabdochonidae), a parasite of *Astyanax* spp. (Characidae) in Mexico and Guatemala, using mitochondrial and nuclear genes, with the description of a new species. *Journal of Helminthology*. 1-11.

Sokolov, S. G. & Malysheva, S. V. (2017). Molecular characterization of *Ichtyobronema hamulatum* (Moulton,1931) (Nematoda: Quimperiidae), a common parasite of burbot *Lota lota* (Linnaeus) (Actinopterygii: Lotidae). *Helminthologia*. 54 (3), 183-188 (2017)

Tran, B. T., Sato, H. & Luc, P. V. (2015). A new *Cosmocercoides* species (Nematoda: Cosmocercidae), *C. tonkinensis* n. sp., in the scale-bellied tree lizard (*Acanthosaura lepidogaster*) from Vietnam. *Acta Parasitologica*. 60 (3), 407-416.

Wijova, M., Moravec, F., Horak, A., Lukes, J. (2006). Evolutionary relationships of Spirurina (Nematoda: Chromadorea: Rhabditida) with special emphasis on dracunculoid nematodes inferred from SSU rRNA gene sequences. *International Journal for Parasitology*. 36 (9), 1067-1075.

Xie, Y., Liu, Y., Gu, X., Zhou, X., Peng, X., He, R., Guo, H., Zuo, Z., Yang, G. (2020). First report on aberrant *Ascaris suum* infection in a dog, China. Parasit es & Vectors. 13 (1), 86.

Zalesny, G., Hildebrand, J., Paziewska-Harris, A., Behnke, J. M., Harris, P. D. (2014). *Heligmosomoides neopolygyrus* Asakawa & Ohbayashi, 1986, a cryptic Asian nematode infecting the striped field mouse *Apodemus agrarius* in Central Europe. *Parasites & Vectors*. 7 (1), 457.



Figure S1: Maximum Likelihood topology based on COI mitDNA sequences of Nematoda (Spirurina). GenBank accession numbers are indicated next to species names. Numbers above nodes represent supported nodes by posterior probabilities for Bayesiananalyses and bootstrap for maximum likelihood analyses respectively (posterior probabilities > 0.90 and bootstrap scores > 70). Branch length scale bar indicates number of substitutions per site.