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PROSPECCIÓN MOLECULAR DE *Neoechinorhynchus brentnickoli* (ACANTHOCEPHALA: NEOECHINORHYNCHIDAE) UN PARÁSITO DE *Dormitator latifrons* DEL PACIFICO MEXICANO

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QUE PARA OBTENER EL GRADO ACADÉMICO DE

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P R E S E N T A

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 28 de noviembre de 2011, se aprobó el siguiente jurado para el examen de grado de **MAESTRO EN CIENCIAS BIOLÓGICAS (SISTEMÁTICA)** del alumno **PINACHO PINACHO CARLOS DANIEL** con número de cuenta **510020792** con la tesis titulada **"Prospección molecular de *Neoechinorhynchus brentnickoli* (Acanthocephala: Neoechinorhynchidae) un parásito de *Dormitator latifrons* del Pacífico Mexicano",** realizada bajo la dirección del **DR. JOSÉ MARTÍN GARCÍA VARELA:**

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Sin otro particular, me es grato enviarle un cordial saludo.

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ÍNDICE

	Pág.
ÍNDICE DE FIGURAS……..	**2**
ÍNDICE DE TABLAS……	**3**
I.RESUMEN…….....	**4**
II. ABSTRACT……	**6**
III. INTRODUCCIÓN……..	**8**
Delimitación de especies…….....	**8**
Especies crípticas en acantocéfalos…….	**9**
Marcadores moleculares utilizados en helmintos parásitos……..	**10**
Neoechinorhynchus brentnickoli Monks, Pulido-Flores y Violante-González, 2011 (Acanthocephala: Eoacanthocephala: Neoechinorhynchidae)……....	**13**
Ciclo de vida……..	**14**
IV. JUSTIFICACIÓN……	**19**
V. OBJETIVOS……..	**20**
VI. RESULTADOS……	**21**
VII. MANUSCRITO……	**21**
Abstract……..	**22**
1. Introduction……	**24**
2. Material and methods……..	**25**
3. Results……...	**29**
4. Discussion…….	**36**
5. Conclusions……..	**49**
Acknowledgements……..	**40**
References……	**40**
VII. CONCLUSIONES……...	**56**
IX. REFERENCIAS……	**58**

ÍNDICE DE FIGURAS

Pág.

Figura 1. Molécula de ADN mitocondrial de animales…….. **12**

Figura 2. Organización de genes del RNA ribosomal en eucariotas…….. **13**

Figura 3. Ciclo de vida de *Neoechinorhynchus rutili* (Müller 1780) Stiles y Hassall 1905……. **15**

Figura 4. Microfotografía de *Neoechinorhynchus brentnickoli*…… **17**

Figura 5(1). Sampling sites of specimens of *Neoechinorhynchus mamesi* n. sp. and the other 4 congeneric species reported from Mexico **51**

Figura 6(2). *Neoechinorhynchus mamesi* n. sp……. **52**

Figura 7(3).Trees inferred with the combined (*cox* 1 + LSU) data set…… **53**

Figura 8(4).Trees inferred with the maximum parsimony and maximum likelihood methods and Bayesian Inference. A. *cox* 1 data set. B. LSU data set……. **54**

Figura. 9(5). Median-joining network of *Neoechinorhynchus mamesi* n. sp., *N. golvani*, *N. brentnickoli*, *N. roseus* and *N. saginatus* building with cytochrome c oxidase subunit I (*cox* 1) gene……. **55**

ÍNDICE DE TABLAS

Pág.

Table 1. Specimens information, collection sites (CS), Sample number, species analyzed, specimens analyzed (N), host species, locality name, geographical coordinates, Genbank accession number, and catalog number (CNHE) for specimens studied in this work……. **45**

Table 2**.** Primers sequences information……... **47**

Table 3. Tree statistics for LSU and *cox* 1, and combined (LSU + *cox* 1) data set. Number of informative characters, C.I., and tree length refer to parsimony inference…….. **48**

Table 4. Hook sizes for *Neoechinorhynchus mamesi* n. sp. and the other congeneric species from Mexico……. **49**

Table 5. Genetic divergence estimated among 5 clades (species) and intraclade, with the LSU gene (LSU; lower matrix) and *cox* 1 gene (*cox* 1; upper matrix)…….. **50**

I. RESUMEN

La especie *Neoechinorhynchus mamesi* n. sp., es descrita del pez estuarino *Dormitator latifrons* de tres localidades de las costas del estado de Chiapas, México. La nueva especie se caracteriza por tener un tronco pequeño, una proboscis pequeña con ganchos apicales largos y ganchos medios y basales pequeños, dos núcleos gigantes ventrales, los machos tienen los testículos más pequeños que la glándula de cemento. Las secuencias del DNA de dos genes, citocromo oxidasa subunidad I y de los dominios D2 y D3 de la subunidad mayor del RNA ribosomal (LSU) fueron empleadas para corroborar las diferencias morfológicas. La distancias genéticas estimadas entre las poblaciones de *N. brentnickoli* y *N. mamesi* n. sp., mostraron un rango del 10.14 al 10.55 % en el LSU y del 20.53 al 22.06 % en el *cox* 1. La divergencia genética entre *N. golvani* y *N. mamesi* n. sp., tuvo un rango del 20.31 al 21.03 % para el LSU y del 22.24 al 24.95 % para el *cox* 1. Los análisis filogenéticos fueron llevados a cabo con los métodos de Máxima verosimilitud, Máxima parsimonia e Inferencia bayesiana, para cada base de datos y el combinado de ambas (LSU + *cox* 1). Todos los análisis filogenéticos mostraron que los especímenes de tres localidades de las costas del estado de Chiapas, México, representan un clado con altos valores de bootstrap y probabilidades posteriores. La red de haplotipos inferido con el gen *cox* 1 indicó que *N. mamesi* n. sp., ésta separado con 84 substituciones de *N. brentnickoli* y con 69 de *N. golvani*. La evidencia morfológica en combinación con la divergencia genética estimada con dos genes, la monofília reciproca en los análisis filogenéticos y la red de haplotipos sugieren que los acantocéfalos encontrado en el aparato digestivo de *D. latifrons* del sureste de México es una

nueva especie y representa la segunda especie del género *Neoechinorhynchus* asociado al pez estuarino *D. latifrons* de las costas del Pacífico Mexicano.

Palabras claves: Acanthocephala, *Neoechinorhynchus mamesi* n. sp., *Dormitator latifrons, cox* 1, LSU, Filogenia, red de haplotipos, México.

II. ABSTRACT

Neoechinorhynchus mamesi n. sp., is described from the estuarine fish *Dormitator latifrons* collected in 3 localities along the coast of Chiapas State in Southwestern Mexico. The new species is characterized by possesing a small trunk, a very small proboscis with relatively very long apical proboscis hooks and small middle and posterior hooks, 2 giant nuclei in the ventral body wall, and males with testes smaller than the cement gland. DNA sequences of 2 genes, cytochrome oxidase subunit 1 (*cox* 1) of the mitochondrial DNA and the domains D2 and D3 of the large subunit of the nuclear ribosomal RNA (LSU) were used to corroborate the slight morphological distinction. The genetic divergence estimated among populations of *N. brentnickoli* and *N. mamesi* n. sp., ranged from 10.14 to 10.55 % for LSU and from 20.53 to 22.06 % for *cox* 1, whereas the genetic divergence between *N. golvani* and the *N. mamesi* n. sp., ranged from 20.31 to 21.03 % for LSU and from 22.24 to 24.95 % for *cox* 1. Maximum likelihood, maximum parsimony and Bayesian Inference analyses were performed for the combined data sets (LSU + *cox* 1) and each data set alone. All the phylogenetic analyses showed that the specimens from 3 coastal lagoons of Chiapas State in Southwestern Mexico represented a monophyletic clade with strong bootstrap support and Bayesian posterior probabilities. The haplotype network based on the analysis of the *cox* 1 indicated that *N. mamesi* n. sp., is separated by 84 substitutions from *N*. *brentnickoli,* and with 69 substitutions from *N. golvani*. The morphological evidence, in combination with the genetic divergence estimated with two genes, the reciprocal monophyly in all the phylogenetic analyses, and the haplotype network, suggested that the acanthocephalans found in the intestine of

D. latifrons in Southwestern of Mexico is a new species, named *N. mamesi* n. sp., and it represents the second species of the genus *Neoechinorhynchus* associated with the Pacific fat sleeper in the Pacific coast of Mexico.

Keywords: Acanthocephala, *Neoechinorhynchus mamesi* n. sp., *Dormitator latifrons, cox* 1, LSU, Phylogeny, haplotype network, Mexico.

III. INTRODUCCIÓN

Delimitación de especies

El concepto de especie es uno de los temas más debatidos en la biología evolutiva (Mayr, 1970; Mayden, 1999). Es importante conocer los métodos que permiten delimitar correctamente una especie para detectar los procesos, patrones y mecanismos evolutivos que influyen en una especie (Sites y Marshall, 2003). En la literatura taxonómica, sistemática y evolutiva se han desarrollado al menos 22 conceptos de especie que pueden ser agrupados en tres grandes grupos: 1) conceptos que requieren similitud, 2) los que se basan en la monofilia y 3) los que se busca su aislamiento reproductivo (Hull, 1997; Mayden, 1997, 1999). Con base en conceptos operacionales (concepto de especie taxonómica) y conceptos teóricos (concepto de especie evolutiva) se han reconocido linajes (aislamiento reproductivo, características ecológicas, monofilia, genealogía), capaces de establecer con claridad límites entre las especies (Mayden 1997; De Queiroz 1998; Sites y Marshall 2003; Miura *et al*., 2005). Dada la necesidad de contar con un cuerpo teórico y práctico que permita manejar la diversidad biológica, es importante una revisión más a fondo sobre los conceptos de especie para lo cual se recomienda consultar a Mayden (1997, 1999), Mayr (1970), Claridge *et al*. (1997) y Cerritos-Flores (2007). Los datos morfológicos se han utilizado tradicionalmente para delimitar y describir a una especie de helminto, sin embargo, muchos estudios recientes han utilizado alternativamente secuencias de DNA mitocondrial y nuclear para reconfirmar las descripciones taxonómicas (Pérez-Ponce de León *et al*., 2008, Razo-Mendivil *et al*., 2004, 2008). En otros trabajos para delimitar especies de parásitos se ha utilizado el concepto evolutivo de

especie, es decir como un linaje (secuencia de poblaciones ancestro-descendiente) que evoluciona separadamente de otros linajes y que tiene una estructura evolutiva propia. Particularmente en acantocéfalos donde recientemente se han realizado trabajos que describen la variación genética entre poblaciones (Steinauer *et al*., 2007; Martínez-Aquino et al., 2009).

Especies crípticas en acantocéfalos

Estudios recientes han empleado caracteres moleculares para delimitar especies particularmente en parásitos y se ha detectado una subestimación de la biodiversidad cuando se detectan complejos de especies crípticas (Brooks y Hoberg 2000; Poulin y Morand 2004; Bensch *et al*., 2004; Westenberger *et al*., 2004). Un complejo de especies crípticas se define como linajes que son morfológicamente similares pero genéticamente distintos (Mayden 1999; Criscione *et al*., 2005). Se ha propuesto que a partir de los estudios de prospección molecular se puede inferir la presencia de especies crípticas empleado porcentajes de divergencia genética y análisis filogenéticos, que permite detectar linajes con historias evolutivas independientes y con ausencia de flujo génico (Blouin 2002; Criscione et al., 2005; Vilas et al., 2005). Una revisión más reciente sobre el concepto de especies crípticas sugiere que a estas especies se les asigne un nombre de manera provisional hasta que nuevos estudios morfológicos que examinen diferentes sistemas de caracteres revelen diferencias que permitan diagnosticar morfológicamente a las especies. Para evitar confusiones taxonómicas en el futuro, se recomienda que las especies crípticas se describan

empleando la nomenclatura taxonómica universal (Pérez-Ponce de León y Nadler, 2010).

En acantocéfalos se han realizado estudios en donde se han detectado complejos de especies crípticas. O'Mahony et al. (2004) detectaron una variación genética en *Pomphorhynchus laevis* del 2.2% para *cox* 1 entre poblaciones de Irlanda y Gran Bretaña. El primer trabajo en detectar y reconocer un complejo de especies crípticas en acantocéfalos fue el de Steinauer et al. (2007); estos autores estimaron la divergencia genética entre poblaciones de *Leptorhynchoides thecatus* Linton 1891, encontrando valores de 1 al 8.7% para los espaciadores trascritos internos (ITS's) del DNA nuclear y entre 6.3 al 11.6% para *cox* 1. Recientemente, Martínez-Aquino et al. (2009) revelaron un complejo de especies en *Neoechinorhynchus golvani* de México. En este trabajo los autores mencionan que *N. golvani* se compone de al menos tres linajes distintos, el primero (*N. golvani*, *sensu estricto*) asociado con peces de la familia Ciclhidae, mientras que los otros dos linajes están asociados con peces de la familia Eleotridae distribuidos en las vertientes del Pacífico y del Golfo de México.

Marcadores moleculares utilizados en helmintos parásitos

Los marcadores moleculares son biomoléculas que aportan información genética en términos genealógicos (Avise, 2004). Los biólogos evolutivos usan tanto datos morfológicos como moleculares para establecer hipótesis de relaciones filogenéticas entre organismos, para estimar la variación dentro de las poblaciones y para probar hipótesis de adaptaciones ecológicas (Rentería-Alcántara, 2007). Los marcadores moleculares son una herramienta alternativa en

diversos campos de la biología, incluyendo evolución, ecología, biomedicina, ciencias forenses y estudios de biodiversidad.

Los marcadores moleculares pueden ser clasificados en tres grupos: 1. Marcadores con base en la hibridación del DNA, v. gr. polimorfismos en la longitud de fragmentos de restricción del DNA (RFLP, restriction fragment length polymorphisms); 2. Análisis de las secuencias del DNA nuclear y mitocondrial; y 3. Marcadores mixtos, v. gr. polimorfismo en la longitud de fragmentos amplificados de DNA, pueden considerarse como una combinación de RFLP y RAPD´s (Picca *et al*., 2002; Avise 2004; Rentaría-Alcántara 2007). Una de las moléculas más ampliamente utilizadas en análisis filogenéticos es el ADN mitocondrial (Figura 1). Ésta es una molécula circular covalente de aproximadamente 16-20 kilobases. Este genoma generalmente contiene un total de 37 genes dependiendo del organismo (13 RNA mensajeros, 2 RNA ribosomales y 22 RNA de transferencia) (Avise et al., 1987). Las propiedades más interesantes en términos filogenéticos y filogeográficos son su alta tasa de evolución (sustitución) a nivel de secuencias de nucleótidos, su nula recombinación, variación intraespecífica, y más importante, su herencia materna (con escasas excepciones). Estas características permiten describir la historia matrilineal de organismos coespecíficos y con ello aplicar estimaciones de reloj molecular y hacer análisis de coalescencia (Vázquez-Domínguez, 2005; Vázquez-Domínguez *et al*., 2009). En helmintos parásitos se han utilizado algunos genes mitocondriales para inferir la evolución de los grupos y separar especies (Macnish *et al*., 2002; Bensh *et al*., 2004; Miura *et al*., 2005; Grillo *et al*., 2007).

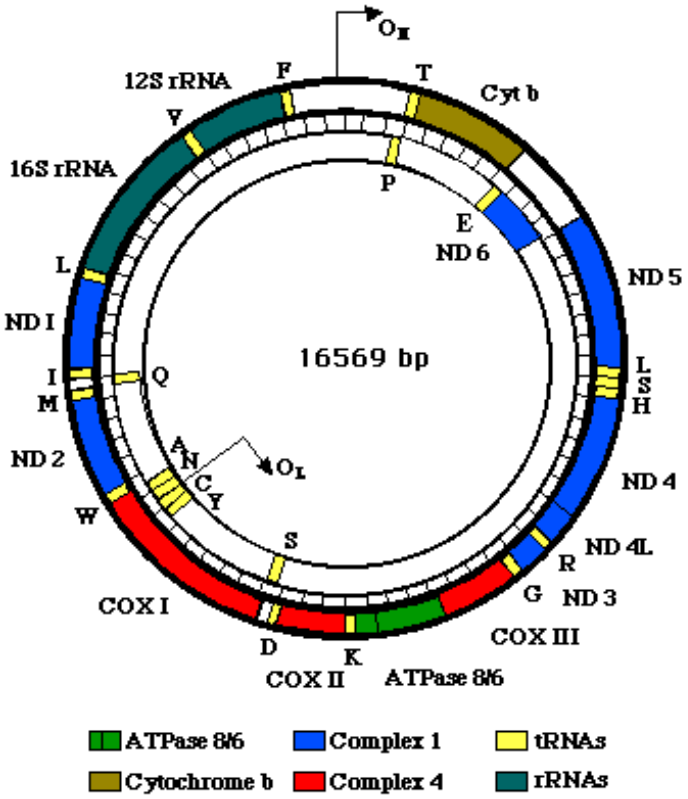

Figura 1. Molécula de ADN mitocondrial de animales.

Otro de los marcadores ampliamente utilizado en helmintos parásitos son los genes nucleares del ADN nuclear ribosomal. El rADN se presenta en repeticiones tándem y está formado por tres subunidades altamente conservadas (18 rADN, 5.8 rADN y 28 rADN), separadas por dos espaciadores transcritos internos con elevadas tasas de sustitución (ITS1 e ITS2) (Eickbush y Eickbush, 2007). Estas repeticiones en tándem se encuentran conservadas a lo largo de todo un genoma y evolucionan concertadamente, lo que se atribuye a eventos recombinatorios como entrecruzamiento desigual y conversión génica (Figura 2) (Rentaría-Alcántara, 2007, Eickbush y Eickbush, 2007). Genes del RNA ribosomal (rRNA) (ITS1, 5.8S, ITS2 y de los dominios D2-D3 del 28S) se han utilizado para

delimitar especies crípticas en helmintos parásitos (Blouin 2002; Luo *et al*., 2002, 2003; Macnish *et al*., 2002; Král'ová-Hromadová *et al*., 2003; Miura *et al*., 2005; Vilas *et al*., 2005; Marques *et al*., 2007; Steinauer *et al*., 2007; Martínez-Aquino *et al*., 2009).

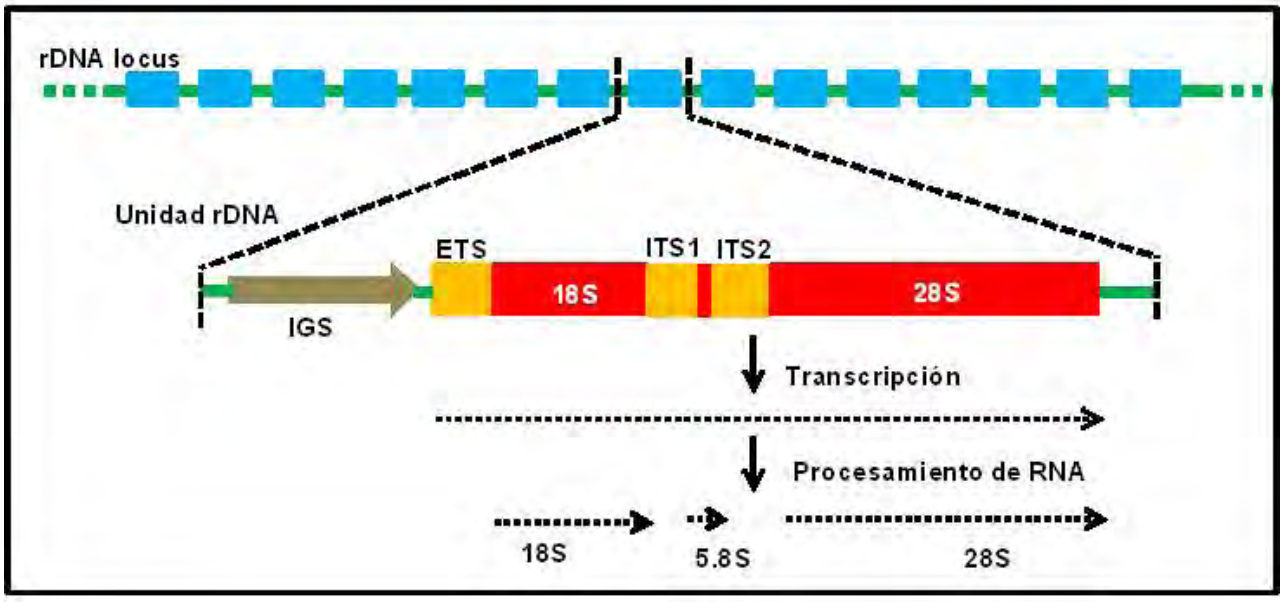

Figura 2. Organización de genes del RNA ribosomal en eucariotas.

***Neoechinorhynchus brentnickoli* Monks, Pulido-Flores y Violante-González, 2011**

Neoechinorhynchus Stiles y Hassall 1905 es uno de los géneros más diversos del phylum Acanthocephala, con 91 especies descritas distribuidas en todo el mundo, de las cuales 63 son parásitas intestinales de peces dulceacuícolas, 13 de peces marinos, 10 de tortugas y una de anfibio (Bullock 1970; Amin 2000, 2002; Amin *et al*., 2003; Barger *et al*., 2004; Amin y Christison 2005). Taxonómicamente el género *Neoechinorhynchus* es clasificado en la familia

Neoechinorhynchidae Ward 1917, de la clase (Eoacanthocephala). Filogenéticamente, el género *Neoechinorhynchus* es considerado como uno de los grupos más basales del phylum Acanthocephala (Amin, 1985; Near *et al*., 1998; García-Varela *et al*., 2000; Monks, 2001; Near, 2002).

Las especies del género *Neoechinorhynchus* se caracterizan por presentar una proboscis pequeña, globular o subcilíndrica, armada con 18 ganchos dispuestos en tres hileras horizontales de seis ganchos cada una, o en seis filas espirales de tres ganchos cada una, un receptáculo de la proboscis, ganglio cerebral ubicado en la base del receptáculo de la proboscis, un tronco sin espinas, con una sola pared muscular, los machos presentan una sola glándula de cemento sincicial (Amin 2002). Amin (2002) sugirió que el género *Neoechinorhynchus* está dividido en dos subgéneros: *Hebesoma* Van Cleave 1928 y *Neoechinorhynchus* Stiles y Hassall 1905, con base en un solo caracter morfológico (forma de los huevos); sin embargo, la carencia de un análisis filogenético del género *Neoechinorhynchus* deja esta sugerencia taxonómica como una hipótesis que debe ponerse a prueba.

Ciclo de vida

El ciclo de vida de los neoechinorhínchidos es indirecto y alternativamente usan vertebrados como huéspedes definitivos y crustáceos como huéspedes intermediarios. Las formas adultas sexualmente maduras habitan el intestino de varios tipos de vertebrados (e.g. peces, tortugas, anfibios); las hembras liberan huevos al medio ambiente acuático a través de las heces de sus huéspedes. Los huevos son ingeridos por un crustáceo en el cuál se desarrollan tres fases

larvarias en el siguiente orden: (1) acantor, (2) acantela y (3) cistacanto o fase infectiva. Al ser ingerido el crustáceo con la fase infectiva llamada cistacanto por el huésped definitivo, el ciclo de vida es completado (Figura 3) (Schmidt 1985, 1988; Kennedy 2006).

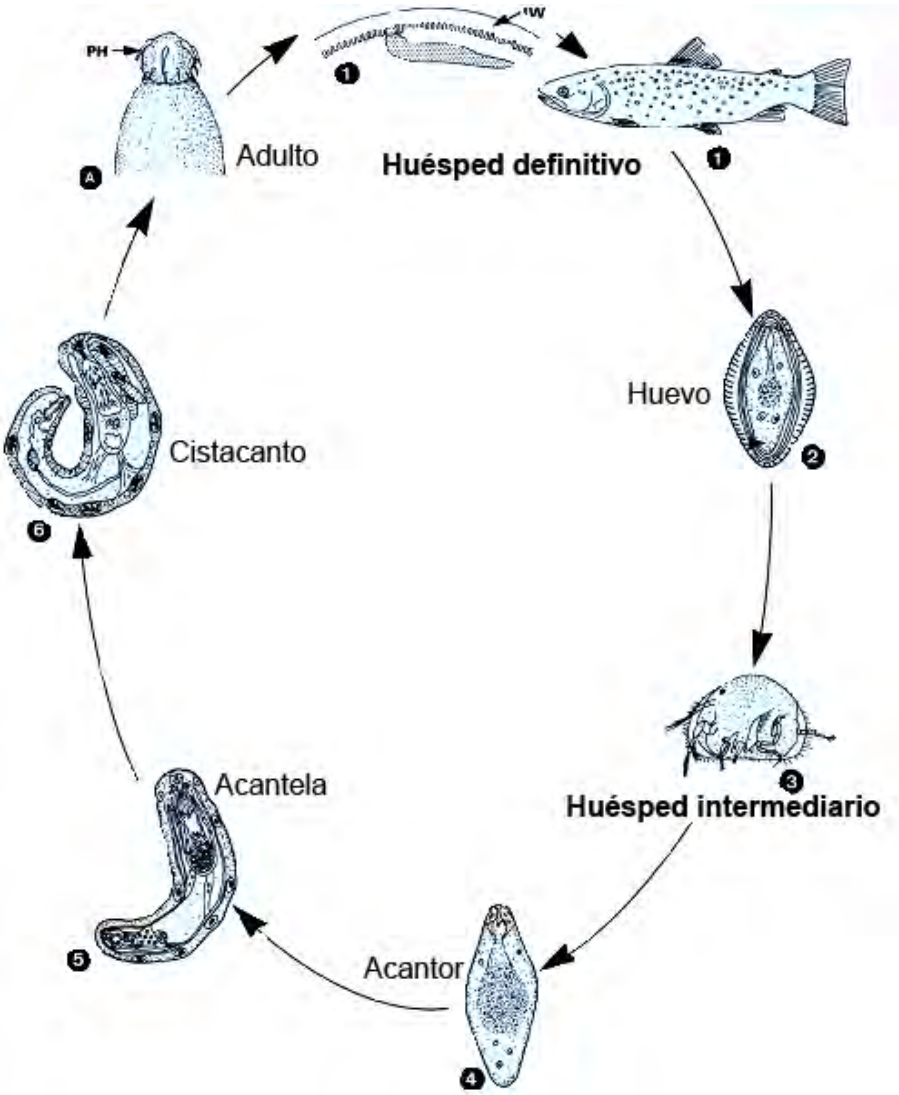

Figura 3. Ciclo de vida de *Neoechinorhynchus rutili* (Müller 1780) Stiles y Hassall 1905. **A**) adulto con ganchos (**PH**), **1**) los adultos se pegan a la pared intestinal (**IW**) de su huésped definitivo, carpas y otros peces, **2**) los embriones salen al medio por medio de las heces, **3-6**) el huésped intermediario *Asellus aquaticus* es infectado por la captación de huevos, donde se desarrollan tres estadios, 4) Acantor, 5) Acantela y 6) Cistacanto, finalmente cuando el huésped intermediario parasitado es ingerido por el huésped definitivo el ciclo de vida es completado. Tomado y modificado de: *http://parasitology.informatik.uni wuerzburg.de/login/n/h/0008.html*.

En México se han descrito cuatro especies del género *Neoechinorhynchus* asociados con peces dulceacuícolas, estuarinos y marinos: *N. roseum* Salgado-Maldonado, 1978; *N. golvani* Salgado-Maldonado, 1978; *N. chimalapasensis* Salgado-Maldonado et al., 2010 y *Neoechinorhynchus brentnickoli* Monks, Pulido-Flores and Violante-González, 2011. Además se han descrito otras dos especies del género asociadas con tortugas dulceacuícolas del género *Trachemys* en el Golfo de México*, N. emyditoides* Fisher, 1960, y *N. schmidti* Barger, Thatcher y Nickol, 2000 (Amin, 2002; Barger et al., 2004, García-Prieto et al., 2010; García-Varela et al., 2011).

N. brentnickoli fue descrita como parásito de *Dormitator latifrons* (Richardson, 1844) de tres localidades de las costas del Pacifico mexicano, laguna de Tres Palos, Guerrero, Bahía de Chamela, Jalisco y Mazatlán, Sinaloa. Morfológicamente *N. brentnickoli* se caracteriza por presentar una proboscis subcilíndrica recubierta con 18 ganchos dispuestos en tres hileras horizontales de seis ganchos en cada una. Adicionalmente, poseen un tronco ovoide con un marcado dimorfismo sexual entre machos y hembras; pared del cuerpo con cinco núcleos gigantes dorsales y dos núcleos ventrales gigantes, proboscis pequeña, largo receptáculo de la proboscis, lemniscos en forma de saco, mismos que sobrepasan el receptáculo de la proboscis (Figura 4) (Monks *et al*., 2011).

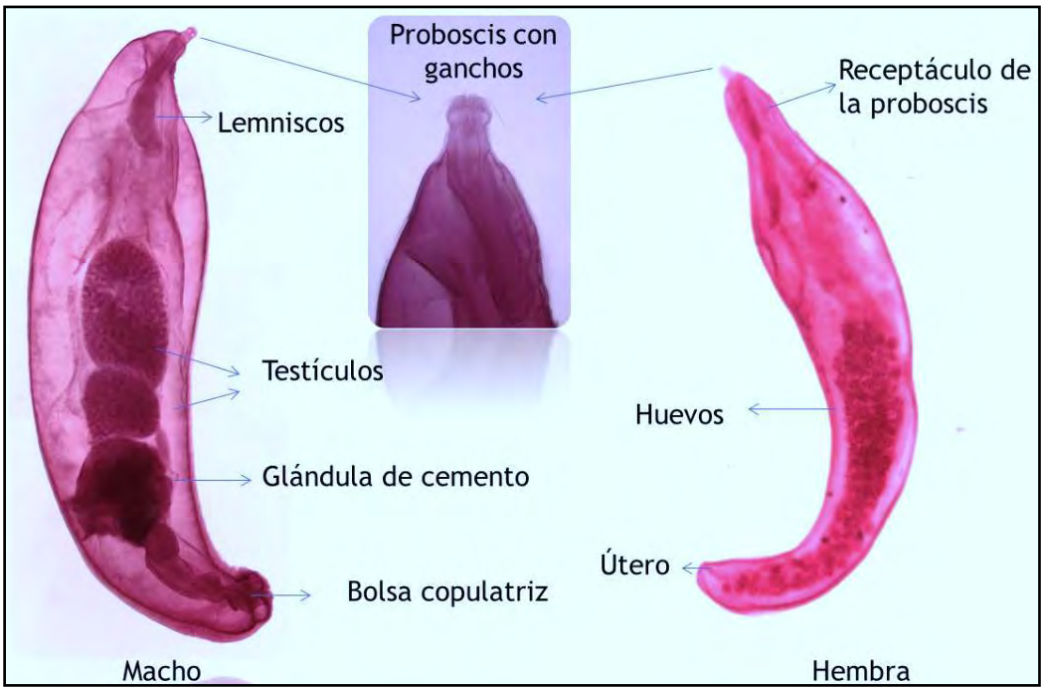

Figura 4. Microfotografía de *Neoechinorhynchus brentnickoli*

Adicionalmente *N. brentnickoli* se ha registrado en nueve especies de peces, de siete familias: Ariidae, Centropomidae, Gerridae, Cichlidae, Eleotridae, Gobiidae y Lutjanidae en la laguna Tres Palos Guerrero (Garrido-Olvera *et al*., 2004; Violante-González *et al*., 2007, 2008 y Monks *et al*., 2011).

Martínez-Aquino *et al*. (2009) realizaron un análisis de la variación genética interpoblacional del acantocéfalo *N. golvani* en México utilizando genes nucleares. La divergencia genética y los análisis filogenéticos sugirieron que *N. golvani* representa un complejo de especies crípticas que se compone de al menos tres linajes. El primer linaje de *N. golvani* indica una distribución amplia hacia el noreste, sur, centro y sureste de México asociado a peces de la familia Cichlidae, estrictamente de agua dulce. Los linajes 2 y 3 están asociados a peces de la familia Eleotridae que se distribuyen en el Golfo de México y en la vertiente del Pacífico y representan dos especies crípticas. El linaje 3 distribuido en las costas

del Pacífico mexicano, asociado al pez eleótrido *Dormiator latifrons* se describió más adelante como una nueva especie, como *N. brentnickoli* (Monks et al., 2011).

IV. JUSTIFICACIÓN

El acantocéfalo *Neoechinorhynchus brentnickoli* (denominado linaje 3, ver Martínez-Aquino *et al*., 2009) asociado con el pez estuarino *Dormiator latifrons* del Pacífico mexicano, exhibe cierta variación morfológica intraespecífica a lo largo de su área de distribución lo que sugiere ausencia de flujo genético entre sus poblaciones. Por lo tanto, este parásito representa un excelente modelo para realizar un estudio de prospección molecular que permita delimitar las poblaciones o las posibles especies.

V. OBJETIVOS

Objetivo general

- Determinar molecular y morfológicamente si *Neoechinorhynchus brentnickoli*, asociado con *Dormitator latifrons* del Pacífico mexicano, representa una sola especie o un complejo de especies.

Objetivos particulares.

- Estimar las distancias genéticas intrapoblacional y entre poblaciones de *Neoechinorhynchus brentnickoli* empleando genes nucleares (28S del RNA ribosomal) y mitocondriales (Citocromo Oxidasa Subunidad I).

- Inferir las relaciones filogenéticas entre las poblaciones de *Neoechinorhynchus brentnickoli* utilizando marcadores mitocondriales y nucleares.

VI. RESULTADOS

Description of a new species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) a parasite of *Dormitator latifrons* from Southwestern Mexico based on morphological and molecular characters

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Los resultados del presente estudio se presentan en forma de un manuscrito que ya fue enviado para su publicación a la revista (**Parasitology International**).

Abstract

Neoechinorhynchus mamesi n. sp., is described from the estuarine fish *Dormitator latifrons* collected in 3 localities along the coast of Chiapas State in Southwestern Mexico. The new species is characterized by possesing a small trunk, a very small proboscis with relatively very long apical proboscis hooks and small middle and posterior hooks, 2 giant nuclei in the ventral body wall, and males with testes smaller than the cement gland. DNA sequences of 2 genes, cytochrome oxidase subunit 1 (*cox* 1) of the mitochondrial DNA and the domains D2 and D3 of the large subunit of the nuclear ribosomal RNA (LSU) were used to corroborate the slight morphological distinction. The genetic divergence estimated among populations of *N. brentnickoli* and *N. mamesi* n. sp., ranged from 10.14 to 10.55 % for LSU and from 20.53 to 22.06 % for *cox* 1, whereas the genetic divergence between *N. golvani* and the *N. mamesi* n. sp., ranged from 20.31 to 21.03 % for LSU and from 22.24 to 24.95 % for *cox* 1. Maximum likelihood, maximum parsimony and Bayesian Inference analyses were performed for the combined data sets (LSU + *cox* 1) and each data set alone. All the phylogenetic analyses showed that the specimens from 3 coastal lagoons of Chiapas State in Southwestern Mexico represented a monophyletic clade with strong bootstrap support and Bayesian posterior probabilities. The haplotype network based on the analysis of the *cox* 1 indicated that *N. mamesi* n. sp., is separated by 84 substitutions from *N. brentnickoli,* and with 69 substitutions from *N. golvani*. The morphological evidence, in combination with the genetic divergence estimated with two genes, the reciprocal monophyly in all the phylogenetic analyses, and the haplotype network, suggested that the acanthocephalans found in the intestine of

D. latifrons in Southwestern of Mexico is a new species, named *N. mamesi* n. sp., and it represents the second species of the genus *Neoechinorhynchus* associated with the Pacific fat sleeper in the Pacific coast of Mexico.

Keywords: Acanthocephala, *Neoechinorhynchus mamesi* n. sp., *Dormitator latifrons, cox* 1, LSU, Phylogeny, haplotype network, Mexico.

1. Introduction

Neoechinorhynchus Stiles and Hassall, 1905 is one of the most diverse genera within Acanthocephala with approximately 101 describe species [1, 2, 3, 4, 5, 6, 7, 8]. All these species are characterized by possessing a small globular or sub-cylindrical proboscis, armed with 3 circles of 6 hooks each, a single-walled proboscis receptacle and a cerebral ganglion located at the base of proboscis receptacle, males possessing 2 spherical to oblique testes, equatorial or post-equatorial, a single syncytial cement gland, genital pore terminal in both sexes or sub-terminal in females, and oval eggs, elliptical or elongate, with concentric shells or with polar prolongation of fertilization membrane [2].

In Mexico, 6 species of the genus *Neoechinorhynchus* have been described, 2 of these occur in freshwater turtles, i.e., *N. schmidti* Barger, Thatcher and Nickol, 2004 and *N. emyditoides* Fisher, 1960, and other 4 species occur in marine, brackish and freshwater fishes, i.e., *N. roseus* Salgado-Maldonado, 1978; *N. golvani*, Salgado-Maldonado, 1978, *N. chimalapasensis* Salgado-Maldonado, Caspeta-Mandujano and Martínez-Ramírez, 2010, and *N. brentnickoli* Monks, Pulido-Flores and Violante-González, 2011 [7, 9, 10]. Recently, molecular and morphological data revealed that the acanthocephalan *N. golvani* actually comprises a complex of cryptic species [9]. One lineage corresponded with *N. golvani sensu stricto* and is associated with cichlid fishes in strictly freshwater environments. Another two lineages are distributed in brackish water systems along the Gulf of Mexico and Pacific Sea slopes, and are associated with eleotrid fishes *Dormitator maculatus* and *Dormitator latifrons*, respectively [9]. A

morphological analysis of the specimens of *Neoechinorhynchus* associated with the fish *D. latifrons* from a few localities along the Pacific Sea slope, allowed authors to describe one of the allegedly cryptic species (lineage 3) as *N. (N) brentnickoli* [7].

A thorough sampling of the eleotrid host (*D. latifrons*) in coastal lagoons along Southwestern Mexico (from Chiapas, northwards to Colima States) allowed us to detect the presence of an undescribed species of *Neoechinorhynchus.* In the current study we describe the new species based on morphological and molecular evidence.

2. Materials and methods

2.1 Specimen collection

Adult acanthocephalans were collected from the intestines of their definitive hosts from 19 localities of Mexico (Table 1; Fig. 1). Fish were examined for parasites immediately after their capture. The acanthocephalans recovered were placed in distilled water to relax the specimens. Later, all the specimens were preserved in 100% ethanol, and stored at 4 ° C. For taxonomic identification, some specimens were stained with Mayer's paracarmine, dehydrated in a graded ethanol series, cleared with methyl salicylate, and mounted on permanent slides with Canada balsam, and were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, México City, (Table 1). The acanthocephalans collected in this study were assigned to genus *Neoechinorhynchus* and were compared with type species deposited at the CNHE as follows: *N. chimalapasensis* (holotype CNHE-5018, allotype CNHE-5019,

12 paratypes CNHE-5020); *N. golvani* (CNHE-0603); *N. roseus* (holotype CNHE-633, CNHE-634 paratypes) and *N. brentnickoli* (holotype CNHE-7537, allotype CNHE-7538, paratypes CNHE-7539-7540).

2.2 Morphological analyses

The specimens collected in this study were identified as *N. brentnickoli*, *N. golvani N. roseus*, and *Neoechinorhynchus* sp., were drawing with the aid of a drawing tube attached to the microscope. Measurements of the trunk, proboscis hooks, proboscis receptacle, lemnisci, uterine bell, vagina, testes, cement gland were taken from sexually mature specimens. Measurements are given in micrometers (μm); for some morphological traits, ranges are given, followed in parentheses by mean values ± standard deviation and sample size (n). Measurements and drawings of eggs were made from fully developed eggs measured in situ through the body wall of female worms. The specimens collected in the current study were deposited in the Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México (Table1).

2.3 Amplification and sequencing of DNA

Eighty three acanthocephalans from 19 populations were digested overnight at 56 °C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM Na_2 EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions. The two genes; *cox* 1 and LSU were amplified using the polymerase chain reaction

(PCR). A fragment of the mitochondrial DNA *cox* 1*,* was amplified using the forward 5'-AGTTCTAATCATAA(R)GATAT(Y)GG-3' and the reverse primer 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. However specific primers were designed for each species of *Neoechinorhynchus* (Table 2). The domains D2 + D3 from LSU rDNA were amplified using the forward 5´ CAAGTACCGTGAGGGAAAGTTGC 3´ and the reverse primer 5´ GTCGATAGGACTCCCTTTG 3´ [11].

PCR reactions (25 µl) consisted of 10 µM of each primer, 2.5 µl of 10X buffer, 2 mM $MgCl_2$, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters for rDNA amplifications included denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 10 min. PCR cycling conditions for the *cox* 1 amplifications included denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, annealing at 40–50 °C for each species of *Neoechinorhynchus* (Table 2) for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 10 min. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 3.5.4 (Codoncode Corporation, Dedham, Massachusetts). The sequences have been deposited in the Genbank data set (Table 1).

2.4 Alignments and phylogenetic analyses

Sequences obtained in the current research from LSU and *cox* 1 was aligned separately using the software Clustal W [12] and adjusted manually with the MacClade program [13]. Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference analyses were performed for each data set and the combined of both data sets (LSU + *cox* 1). MP tree was inferred using the program PAUP*4.0b10 [14]. The ML tree was inferred using RAxML 7.0.4., for each and combined of both data set [15]. The modeltest program version 3.0 [16] was used for inferring the best model of evolution for all the data sets (Table 3). Tree searches were performed using 1,000 (MP) random taxon addition heuristic searches. Clade support was assessed by bootstrap resampling with 10,000 replicates by MP and ML trees. Additionally, Bayesian analyses were performed with the program MRBAYES version 3.1.2 [17]. The settings were two simultaneous runs of the Markov chain Monte Carlo (MCMC) for 10 million generations, sampling every 200 generations, a heating parameter value of 0.2 and a 'burn-in' of 10%. Trees were drawn using FigTree program version 1.3.1 [18].

2.5 Haplotype network construction

Gene genealogies were inferred using two approaches for haplotype network construction. Median-joining networks [19] were calculated with the program NETWORK version 4.2.0.1 (www.fluxusengineering. com) keeping the parameter ε = 0. This method starts with minimum spanning trees combined within a single network and then, to reduce tree length, median vectors (consensus

sequences) are added. Such vectors can be interpreted as possibly extant unsampled sequences or extinct ancestral sequence [19]. In addition, TCS program version 1.21 [20] was employed to infer the haplotype networks using statistical parsimony [21] with a confidence of 95%.

3. Results

3.1. Morphological description

3. 1.1. Neoechinorhynchus mamesi n. sp. (Fig. 2)

Description based on 21 specimens (10 male and 11 female). Trunk cylindrical, 1,383−3,542 (2,714 ± 717, n=18) long, 350−1,110 (746 ± 252, n=18) wide, swollen in anterior region, slender in posterior region (Fig. 2: A, B). Trunk with a thick wall in both dorsal and ventral sides, containing 5 dorsal giant subcuticular nuclei and 2 ventral nuclei (n=21). Proboscis wider than long, arranged in 3 circular rows of 6 hooks (n=21) (Fig. 2: C). Apical hooks with strong roots. Apical, middle, basal hooks are similar in size in both sexes (Table 4). Small sensory papillae located near the neck (Fig. 2: C). Neck wider than long. Proboscis receptacle attached to the junction of the neck with the trunk. Cerebral ganglion triangular, slightly conspicuous. Binucleate lemniscus shorter than uninucleate lemniscus. Both lemnisci extend beyond the proboscis receptacle. Lemnisci nuclei are ovoid fragmented in some specimens (Fig. 2: D). Striated ligament sac connected to the uterine bell in females. Uterine bell narrow at anterior opening, vagina elongate, wide at posterior end. Females with one pair of moderately developed bands of vestibular muscle attached ventrally at genital pore, extending

to dorsal body wall (Fig. 2: E). Testes oval, tandem, overlapping (Fig. 2: A), posterior testis overlapping cement gland (Fig. 2: A). Anterior testis slightly larger than the posterior one. Efferent ducts connecting each testicle with each seminal gland. Seminal glands oval, possessing an ejaculatory duct. Single cement gland with 8 unfragmented nuclei. Reservoir of the cement gland located behind the cement gland. Saefftingen's pouch located immediately posterior to cluster of cement gland. Genital pore slightly ventral in both sexes. Mature eggs oval, elongation of fertilization membranes absent. Eggs measured through body wall 22–25 (23 ± 1, n=6) long, 6–9 (7 ± 1, n=6) wide (Fig. 2: F).

Male (Fig. 2: A, D.): body 1,230–2,360 (1,993 ± 470, n=9) long, 430–820 (641±132, n=9) wide. Trunk 1,165–2,284 (1,847 ± 500, n= 7) long, 430–820 (637 ± 141, n=8) wide. Proboscis 26–44 (36 ± 6, n=8) long, 50–150 (68 ± 30, n=9) wide. Proboscis hooks in anterior circle, dorsal hooks 38–52 (45 ± 5, n=8) long, 7–9 (8 ± 0.8, n=8) wide at base, root of dorsal hooks 20–23 (21 ± 1, n=5) long, 6–8 (7 ± 0.7, n=5) wide; lateral hooks 38–53 (44 ± 5, n=7) long, 8–9 (8 ± 0.4, n=7) wide at base, root of lateral hooks 22–22 (22 ± 0, n=2) long, 8–8 (8 ± 0, n=2) wide; ventral hooks 40–52 (45 ± 4, n=7) long, 8–9 (8 ± 0.8, n=7) wide at base, root of ventral hooks 23–23 (23 ± 0, n=2) long, 7–7 (7 ± 0, n=2) wide. Hooks of middle circle 12–17 (14 ± 1, n= 27) long, 4–9 (4 ± 1, n= 27) wide; posterior circle 13–17 (15 ± 1, n=24) long, 3–9 (4 ± 0.6, n= 24) wide. Neck 18–40 (30 ± 7, n=9) long, 55–212 (82 ± 49, n=9) wide. Proboscis receptacle 205–275 (241 ± 21, n=10) long, 5–8 (6 ± 10, n=10) wide. Longer lemniscus 235–500 (405 ± 87, n=10) long, 30–87

(59 ± 21, n=10) wide; shorter lemniscus 167−470 (366 ± 95, n=10) long, 20−100 (55 ± 24, n=10) wide. Reproductive system almost fully occupies posterior 2/3 of body length, 750−1,830 (1,386 ± 414, n=9) long. Anterior testis 177−660 (419 ± 160, n=9) long, 155−400 (310 ± 94, n=9) wide. Posterior testis 152−440 (317 ± 109, n=8) long, 167−430 (325 ± 93, n=8) wide. Seminal vesicle 62−425 (187 ± 115, n=7) long, 10−600 (156 ± 185, n=8) wide. Cement gland 115−580 (343 ± 188, n=10) long, 165−450 (310 ± 114, n=10) wide. Copulatory bursa opens terminally 57−130 (104 ± 40, n=3) long, 57−117 (83 ± 30, n=3) wide.

Female (Fig. 2: B, C, F, E): body 1,450−3,620 (2, 323 ± 859, n=7) long, 350−1, 110 (746 ± 252, n=10) wide. Trunk 1,383−3,542 (2,414 ± 717, n= 11) long, 350−1,110 (746 ± 252, n=10) wide. Proboscis 26−58 (47 ± 10, n=8) long, 60−71 (66 ± 4, n=8) wide. Proboscis hooks in anterior circle, dorsal hooks 35−54 (46 ± 6, n=8) long, 8−10 (8 ± 0.6, n=8) wide at base, root of dorsal hooks 22−25 (23 ± 1, n=7) long, 7−9 (8 ± 0.6, n=7) wide; lateral hooks 37−54 (44 ± 5, n=8) long, 7−9 (8 ± 0.5, n=8) wide at base, root of lateral hooks 23−23 (23 ± 0, n=2) long, 8−8 (8 ± 0, n=2) wide; ventral hooks 37−55 (45 ± 5, n=8) long, 8−9 (8 ± 0.4, n=8) wide at base, root of ventral hooks 22−23 (22 ± 0.5, n=3) long, 7−8 (7 ± 0.5, n=3) wide. Hooks of middle circle 13−17 (15 ± 1, n= 23) long, 4−6 (4 ± 0.5, n= 23) wide; posterior circle 13−20 (16 ± 2, n=23) long, 4−5 (4 ± 0.7, n=22) wide. Neck 15−35 (25 ± 6, n=8) long, 60−80 (68 ± 6, n=8) wide. Proboscis receptacle 215−300 (248 ± 30, n=8) long, 52−87 (68 ± 14, n=8) wide. Longer lemniscus 187−465 (342 ± 114, n=6) long, 20−87 (58 ± 23, n=6) wide; shorter lemniscus 162−425 (304 ±

102, n=6) long, 20−92 (52 ± 26, n=5) wide. Total length of reproductive system from anterior margin of the uterine bell to terminal genital pore 350−700 (475 ± 156, n=4) long. Uterine bell 150−340 (207 ± 80, n=5) long, 43−120 (62 ± 32, n=5) wide. Uterus 100−223 (158 ± 47, n=6) long, 20−96 (49 ± 29, n=6) wide. Length vagina 25−137 (70 ± 43, n=7) long, 9−35 (22 ± 7, n=7) wide. Genital pore subterminal 20−55 (34 ± 12, n=8) long, 18−52 (34 ± 13, n=8) wide. Eggs elliptical 22−25 (23 ± 1, n=6) long, 6−9 (7 ± 1, n=6) wide.

3.1. 2 Taxonomic summary

Type-host: *Dormitator latifrons* (Richardson, 1844) (Eleotridae: Pacific fat sleeper).

Site of infection: Intestine

Type-locality: Rión Pijijiapan, Chiapas State, Mexico (15° 31' 54.3" N, 93° 09' 39.4" W)

Additional localities: La Conquista, Chiapas State, Mexico (15° 40' 00.20" N, 93° 24' 51.61" W) and Joaquín Amaro, Pijijiapan, Chiapas State, Mexico (15° 46' 16.19" N, 93° 24' 30.11" W,).

Type-material: Holotype CNHE: No. 8180; allotype CNHE: No. 8181; paratypes CNHE: No.8182-8184.

Etymology: The new species is named after the Mayan ethnic group (Mames) that inhabits the coastal region of Chiapas, Mexico and Guatemala.

3.1.3 Remarks

In Mexico 4 species of the genus *Neoechinorhynchus* associated with brackish and freshwater fishes have been described [10]. The new species can be

readily distinguished to *N. chimalapasensis* and *N. roseus* by its smaller size and by having a smaller body length-to-width ratio (LWR) (Table 4). *Neoechinorhynchus mamesi* n. sp., closely resembles *N. golvani* and *N. brentnickoli*. On average, the new species is larger than *N. golvani* but it is smaller than *N. brentnickoli.* However, *N. mamesi* n. sp., can be distinguished to *N. golvani* by having less dimorphism in trunk length between the sexes (males 1,993 long, females 2,323 vs. males 1,046 long, females 3.187, respectively) (Table 4). In addition, the new species possesses 2 giant ventral nuclei instead of only 1, has shorter hooks in the 3 circles in both sexes (anterior 44 to 45 vs. 52 to 78, middle 14 vs.18, and posterior 15 vs.18, respectively in males) and (anterior 44 to 46 vs. 45 to 48, middle 15 vs. 18, and posterior 16 vs. 18 respectively in females), and has larger testes (anterior testis 419 long by 310 wide vs. 260 long by 193 wide and posterior testis 317 long by 325 wide vs. 187 long by 227 wide, respectively).

On the other hand, *N. mamesi* n. sp. is similar to *N. brentnickoli* and they both are found parasitizing Pacific fat sleepers along the Pacific coast of Mexico, but also can be distinguished from by being smaller and by having less dimorphism in trunk length between the sexes (males 1,993 long, females 2,323 vs. males 2,815 long, females 4,214, respectively) (Table 4). Additionally, the new species is distinguished to *N. brentnickoli* because even though they both possesses 2 giant ventral nuclei, in the new species these nuclei are separated and not contiguous as in the latter species, but also because proboscis hooks in *N. mamesi* n. sp. are larger and shorter with respect to those in *N. brentnickoli* (i.e., anterior hooks 44 to 45 vs. 38 to 43, middle hooks 14 vs.17, and posterior hooks 15 vs.16, respectively in males) and (anterior hooks 44 to 46 vs. 38 to 42, middle 15 vs. 15, and posterior

16 vs. 14, respectively in females) (Table 4). Finally, the new species possess smaller testes (anterior testis 419 long by 310 wide vs. 568 long by 361 wide and posterior testis 317 long by 325 wide vs. 428 long by 365 wide, respectively).

3. 2 Base composition and genetic divergence

DNA fragments of the LSU and *cox* 1 were amplified and sequenced for individuals representing five species of the genus *Neoechinorhynchus. N. mamesi* n. sp., *N. brentnickoli*, *N. golvani*, *N. roseus* and *N. saginatus*. PCR products varied from 815 to 824 bp for LSU and from 490 to 620 bp for *cox* 1. Nucleotide frequencies for the combined (LSU + *cox* 1) data set were 0.26 (A), 0.15 (C), 0.23 (G), and 0.34 (T). The total length of each data set and the combined of both (LSU + *cox* 1) data sets are shown in Table 3. The genetic divergence estimated among the populations of *N. mamesi* n. sp., *N. brentnickoli,* and *N. golvani* ranged from 10.14 to 21.03 for LSU and from 20.53 to 25.91 for *cox* 1 (Table 5).

3.3. Combined LSU + cox 1 *data set*

This data set composed of two genes (LSU + *cox* 1), included 49 individuals with 1,292 characters, of which 529 were parsimony informative. Parsimony analysis of this combined dataset yielded 14,520 trees with a C.I.= 0.84 and a length of 1,027 steps (Table 3). The MP strict consensus tree shows the 5 main clades, which are recognized as five species. The 12 specimens collected in Pacific fat sleepers from Southwestern Mexico conform a monophyletic clade with 100 percent bootstrap support. In the combined data set this clade is the sister taxa to *N. (N) brentnickoli*, and both clades received strong nodal support (Fig. 3).

The ML analysis yielded a single tree with -ln =7427.997809. The ML topology also showed the main 5 clades as in the MP tree, but the support among the clades was lower than MP analysis. The bayesian tree also yielded the same branch pattern than the MP and ML trees and it was well supported with posterior probabilities values (Fig. 3). To examine the separate contribution of each data set in the systematic position of the species of *Neoechinorhynchus*, additional phylogenetic analyses were conducted using *cox* 1 and LSU data sets separately as we show next.

3. 4. cox 1 *data set*

This data set included 83 taxa, with 538 characters, of which 218 were parsimony informative (Table 3). Maximum parsimony analysis (Fig. 4: A) yielded 2039 trees with a C.I. = 0.78 and length of 463 steps. The MP strict consensus tree, as well as the ML and Bayesian inference showed 5 major clades. Even though ML and Bayesian trees also yielded the same topology as MP inferred with *cox* 1 data set, the 5 main clades received high bootstrap support and bayesian posterior probabilities (Fig. 4: A). The tree inferred with *cox* 1 data set, placed *N. mamesi* n. sp., as the sister species of *N. golvani* albeit nodal support was low for this sister taxa relationships (Fig. 4: A).

3.5. LSU data set

This data set included 50 taxa, with 754 characters, of which 311 were parsimony informative. Maximum parsimony analysis (Fig. 4:B) yielded 195 trees

with a C.I. = 0.89 and length of 567 steps (Table 3). The MP strict consensus tree yielded the same topology as the ML and Bayesian trees, and also the same topology as the *cox* 1 analyses. Five main clades are recognized, with almost identical sister group relationships, excepting that the new species is nested with *N. brentnickoli*, a relationship strongly supported by bootstrap and posterior probabilities values, and not with *N. golvani*, as suggested by the *cox* 1 analyses.

3.6 Haplotype network construction

In the current study 37 haplotypes were detected in the *cox* 1 analysis, 12 correspond with *N. mamesi* n. sp., 8 with *N. golvani*, 15 with *N. brentnickoli*, 1 with *N. roseus* and 1 with *N. saginatus*. The haplotype clusters were separated into five groups. The haplogroup representing *N. mamesi* n. sp., is separated by 84 substitutions from *N. brentnickoli,* and by 69 substitutions from *N. golvani*. Five specimens of each locality of *N. mamesi* n. sp., were analyzed, representing 3 populations from coastal lagoons from Chiapas 1. Rion Pijijiapan lagoon that showed 4 haplotypes, 2. The Conquista lagoon with 5 haplotypes and 3. Joaquin Amaro Stuary with 4 haplotypes (Fig. 5).

4. Discussion

Neoechinorhynchus mamesi n. sp., is the seventh species of the genus described from Mexico [10, 22] and it represents the second species associated with an estuary fish, the Pacific fat sleeper (*D. latifrons*) from the Pacific Sea slope. The genetic divergence estimated within 3 populations of *N. mamesi* n. sp., ranged from 0.23 to 2.06 % for *cox* 1, whereas the genetic divergence found among 8

populations of *N. brentnickoli* ranged from 0.23 to 3.21 %. These ranges of intraspecific genetic divergence is higher to those previously described for other populations of acanthocephalans, e.g., *Pomphorhynchus laevis* Muller 1776. This species of acanthocephalan showed a range of 0.35 to 0.70 % [23] while individuals of polymorphid acanthocephalans such as *Corynosoma strumosum* Lühe 1904, *Southwellina hispida* Van Cleave 1925, *Polymorphus brevis* Van Cleave, 1916, *Profilicollis altmani* Perry 1942, and *Profilicollis botulus* Van Cleave 1916 exhibited intraspecific genetic variation ranging from 1 to 5% [24]. Likewise, the 17 specimens representing 6 populations of *N. golvani*, associated with cichlid fishes in strictly freshwater environments analyzed in the current study, revealed high genetic divergence ranging from 0.23 to 10.41% with *cox* 1. These values suggested that these specimens could represent a complex of cryptic species, however additional molecular work will be performed to support this finding. Additionally, a nuclear gene (LSU) was used as an additional molecular marker to establish a more robust species delimitation criterion among populations of the genus *Neoechinorhynchus.* The genetic divergence of LSU estimated within the populations of *N. mamesi* n. sp., ranged from 0.1 to 0.13 %, while for *N. brentnickoli* divergence ranged from 0.13 to 0.27 % and for *N. golvani* it ranged from 0.12 to 1.37 %. These ranges of genetic divergence within populations are also similar to those previously described for *Neoechinorhynchus* [9]. All the phylogenetic trees inferred with each molecular marker analyzed independently, and the concatenated data set, yielded that the five species of *Neoechinorhynchus* analyzed in this study, represent independent clades, with extremely high genetic divergence varying between 20.53 and 24.95 % for *cox* 1 and between 10.14 and

21.03 % for LSU (Table 5). The haplotype network obtained in this study showed that five species of *Neoechinorhynchus* conform 5 independent haplogroup and that the species *N. mamesi* n. sp., contains at least 12 haplotypes, which are separated by 1 to 3 substitutions (Fig. 5). The morphological data, in combination with high genetic divergence estimated for each gene, the haplotype network, the reciprocal monophyly of the populations in all the phylogenetic trees inferred with *cox* 1, LSU, and combined of both genes (*cox* 1 + LSU), clearly demonstrate that specimens associated to *D. latifrons* from 3 localities along the Chiapas State along the Southwest Pacific Ocean slope, represents a new taxa for which we propose the name *Neoechinorhynchus mamesi* n. sp.

The definitive host of the newly described species of acanthocephalan, *D. latrifons,* has a wide distribution along the Pacific Ocean slope, with a range that extends from southern California, USA to Northern Peru [25]. Other than Mexico, no records of acanthocephalans have been published for this estuarine fish along its distributional range. In this study, 46 Pacific fat sleepers were collected in 3 coastal lagoons along the Oaxaca state coast, i.e., Laguna Superior (2), Laguna Chacahua (31), and Laguna Pastoria (13). This region of Southwestern Mexico seems to represent a gap in the distribution of *Neoechinorhynchus* spp., even though its definitive host is commonly found in coastal lagoons in Oaxaca. The absence of acanthocephalans in populations of Pacific fat sleepers in Oaxaca allow us to speculate that such gap is the result of the absence of the crustacean intermediate host, which is probably restricted to strictly freshwater environments [26], where *D. latifrons* eventually penetrates, and may get infected. Since the fish

definitive host extends along the entire Pacific coast of Mexico, it seems likely that the intermediate host plays a major role in the diversification of *Neoechinorhynchus,* with the Isthmus of Tehuantepec acting in some way as a biogeographical barrier that separated *N*. *brentnickoli* and the new species from their ancestor. This region of Mexico has complex geologic history and is the major responsible for the divergence of numerous animal terrestrial lineages [27, 28, 29]. Future samplings of Pacific fat sleepers fish along Central America Pacific coast, and even samplings in northern South America will determine of the new species is also found there, or if other potential biogeographical barriers determined that other species of *Neoechinorhynchus* will be find in the near future. Still, the hypothesis that the intermediate host is the limiting factor in the evolutionary history of these species of acanthocephalans (and not the definitive host) needs to be determined by proper sampling of potential intermediate hosts, and in addition to that, a more extensive sampling of Pacific fat sleepers in Oaxaca will be needed to corroborate it represents a gap in the distribution of these acanthocephalans.

5 Conclusions

Neoechinorhynchus mamesi n. sp., is the second species of the genus associated to the Pacific fat sleeper (*D. latifrons*) in the Southwestern Pacific Ocean of Mexico. Morphologically, the new species is distinguished from other six congeneric species described from reptiles and fishes in Mexico by possessing a small trunk, a very small proboscis with long apical proboscis hooks and small middle and posterior hooks. Morphological distinction was further demonstrated by sequencing 2 molecular markers. The high levels of genetic divergence, the

topology of phylogenetic trees with evidence of reciprocal monophyly, and the haplotype network support the erection of the new species.

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Table 1. Specimens information, collection sites (CS), sample number, species analyzed, specimens analyzed (N), host species, locality name, geographical coordinates, Genbank accession number, and catalog number (CNHE) for specimens studied in this work. Sequences marked with an asterisk were obtained in the current study. Nd = not determined. The sample number for each locality corresponds with the same number in the Figures 1, 3 and 4.

CS	Sample	Species	N	Host	Locality	Coordinates		Genbank		Specimens deposited (CNHE)
						North	West	*Cox* 1	LSU	
1	1-5	*N. mamesi* n. sp.	5	*Dormitator latifrons*	Rion Pijijiapan Lagoon, Chiapas	15° 31' 54.3"	93° 09' 39.4"	JN830787* JN830788* JN830789* JN830790* JN830791*	JN830763* JN830764* JN830765*	8180, 8181, 8182
2	6-10	*N. mamesi* n. sp.	5	*Dormitator latifrons*	Conquista Lagoon, Chiapas	15° 40' 00.20"	93° 24' 51.61"	JN830792* JN830793* JN830794* JN830795* JN830796*	JN830766* JN830767* JN830768* JN830769*	8184
3	11-15	*N. mamesi* n. sp.	5	*Dormitator latifrons*	Jaquin Amaro Estuary, Chiapas	15° 46' 16.19"	93° 24' 30.11"	JN830797* JN830798* JN830799* JN830800* JN830801*	JN830770* JN830771* JN830772* JN830773* JN830774*	8183
4	16-20	*N. brentnickoli*	5	*Dormitator latifrons*	Tamarindo River , Guerrero	16° 38' 07.5"	99° 08' 26.4"	JN830802* JN830803* JN830804* JN830805* JN830806*		8179
5	21-28	*N. brentnickoli*	8	*Dormitator latifrons*	Tres Palos Lagoon, Guerrero	16° 48' 00"	99° 47' 00"	JN830807* JN830808* JN830809* JN830810* JN830811* JN830812* JN830813* JN830814*	FJ968157 FJ968156 FJ968158 FJ968159 FJ388991	8178
6	29-33	*N. brentnickoli*	5	*Dormitator latifrons*	Coyuca Lagoon, Guerrero	16° 57' 00"	100° 02' 00"	JN830815* JN830816* JN830817* JN830818*	JN830775* JN830776*	8175

								JN830819*		
7	34-38	*N. brentnickoli*	5	*Dormitator latifrons*	Barra de Pichi Estuary, Michoacan	17° 58' 41.5"	102° 19' 30.0"	JN830820* JN830821* JN830822* JN830823* JN830824*		8174
8	39-43	*N. brentnickoli*	5	*Dormitator latifrons*	Mexcalhuacan Estuary, Michoacan	18° 03' 21.5"	102° 39' 29.8"	JN830825* JN830826* JN830827* JN830828* JN830829*	JN830777* JN830778* JN830779* JN830780* JN830781*	8173
9	44-48	*N. brentnickoli*	5	*Dormitator latifrons*	Huahua Estuary, Michoacan	18° 10' 39.7"	103° 00' 26.3"	JN830830* JN830831* JN830832* JN830833* JN830834*		8177
10	49-58	*N. brentnickoli*	10	*Dormitator latifrons*	Boca de Apiza Estuary, Michoacan	18° 41' 14.46"	103° 44' 04.96"	JN830835* JN830836* JN830837* JN830838* JN830839* JN830840* JN830841* JN830842* JN830843* JN830844*	JN830782* JN830783* JN830784* JN830785* JN830786*	8176
11	59-63	*N. brentnickoli*	5	*Dormitator latifrons*	Cuyutlan Lagoon, Colima	19° 02' 58.6"	104° 15' 58.2"	JN830845* JN830846* JN830847* JN830848* JN830849*		
12	64-66	*N. golvani*	3	*Paraneetroplus fenestratus*	Catemaco Lake, Veracruz	18° 25'	95° 07'	JN830850* JN830851* JN830852*	FJ388986 FJ968145 FJ968146	601, 603, 604, 606, 631, 632
13	67-69	*N. golvani*	3	*Cichlasoma pearsei*	Nezahualcoyolt Dam, Malpaso, Chiapas	17° 10' 49"	93° 36' 49"	JN830853* JN830854* JN830855*	FJ388996 FJ968141 FJ968142	6756
14	70-72	*N. golvani*	3	*Cichlasoma pearsei*	Chicoasen Dam, Chiapas	16° 56' 02"	93° 05' 16"	JN830856* JN830857* JN830858*	FJ388995 FJ968136 FJ968137 FJ968138	6755
15	73-75	*N. golvani*	3	*Cichlasoma urophthalmum*	Ilusiones Lake, Tabasco	17° 58' 46"	92° 56' 17"	JN830859* JN830860*	FJ388992 FJ968143 FJ968144	
16	76-78	*N. golvani*	3	*Cichlasoma urophthalmum*	Carrizal River, Tabasco	18° 1' 45"	92° 55' 00"	JN830861* JN830862* JN830863*	FJ388993 FJ968134 FJ968135	6754

17	79-81	*N. golvani*	3	*Parachromis friedrichhstalii*	Canitzan Lake, Tenosique, Tabasco	17° 28' 57"	91° 25' 27"	JN830864* JN830865* JN830866*	FJ388994 FJ968139 FJ968140	6757
18	82	*N. roseus*	1	*Citharichthys gilbertei*	Tovara Estuary, Nayarit	21° 31' 37"	105° 29' 14"	JN830867*	FJ389000	6763
19	83	*N. roseus*	1	*Achiurus mazatlanus*	Caimanero Estuary, Sinaloa	25° 36' 30"	108° 26' 25"	JN830868*	FJ388999	6762
20	84	*N. saginatus*	1	Nd	Nd	ND	ND	DQ089704	AY829091	

Table 2. Primers sequences information

Locus	species	Primer name	Primer sequence (5'–3')	Tm
cox 1	*N. mamesi* n. sp.	509F This study	AGTTCTAATCATAA(R)GATAT(Y)GG	42 °C
	N. roseus	510R This study	TAAACTTCAGGGTGACCAAAAAATCA	
	N. brentnickoli	520F This study	GTGTGAGGAGGGTTAGTTGG	50 °C
		521R This study	AAAGATAATTGTTCTAATTTTAGG	
	N. golvani	512F This study	GGGTTTGTATAACATRGTTG	40 °C
		513R This study	TTAAAATTTCGATCTAACAA	
LSU		502F (García-Varela and Nadler, 2005)	CAAGTACCGTGAGGGAAAGTTGC	50 °C
		536R (García-Varela and Nadler, 2005)	GTCGATAGGACTCCCTTTG	

Table 3. Tree statistics for LSU and *cox 1*, and combined (LSU + *cox* 1) data set. Number of informative characters, C.I., and tree length refer to parsimony inference. Pinv (proportion of invariable sites), Gd (shape of gamma distribution), -ln likelihood refer to maximum likelihood inference and Akaike Information Criterion (AIC) model inferred with Modeltest program.

Data set	Total characters	Uninformative characters	Constant characters	Informative Characters	C.I.	Tree length	-ln likelihood	Pinv	Gd	Model AIC
cox 1	538	30	290	218	0.78	463	2582.838973	0.3598	0.7024	TVM+I+G
LSU	754	64	379	311	0.89	567	3207.672566	0.4101	0	TVM+I
cox 1 + LSU	1,292	94	669	529	0.84	1027	7427.997809	0.1933	0.6046	K81uf+I+G

Table 4. Hook sizes for *N. mamesi* n. sp. and the other congeneric species from Mexico. Sizes are given as average (minimum–maximum). For the new species, dorsal, lateral, and ventral apical hooks were measured, middle and posterior hooks were measured. Measurements for the hooks of *N. chimalapasensis*, *N. golvani*, *N. roseus* and *N. brentnickoli* were taken from the original descriptions [7].

	Apical hook			Middle hook	Posterior hook	Average of Long- wide of the body	length-to-width ratio
	Dorsal	**Lateral**	**Ventral**				
N. mamesi n. sp.							
Male	45 (38–52)	44 (38–53)	45 (40–52)	14 (12–17)	15 (13–17)	1,993-641	3:1
Female	46 (35–54)	44 (37–54)	45 (37–55)	15 (13–17)	16 (13–20)	2, 323-746	3.1
N. brentnickoli							
Male	43 (39–48)	38 (35–43)	42 (39–45)	17 (15–20)	16 (13–19)	2,815-627	4:1
Female	42 (40–45)	38 (35–40)	42 (39–48)	15 (13–20)	14 (13–18)	4,214-912	4:1
N. chimalapasensis							
Male	34.2 (33–35)	34.2 (33–35)	34.2 (33–35)	19 (15–20)	16.4 (15–19)	5,458-587	9:1
Female	38 (36–40)	38 (36–40)	38 (36–40)	20 (19–22)	15 (15)	9,832-724	14:1
N. golvani							
Male	nr (52–78)	nr	Nr	18 (18)	18 (18)	1,046-515	2:1
Female	nr (45–48)	nr	Nr	18 (18)	18 (18)	3,187-885	3:1
N. roseus							
Male	nr (36–41)	nr	Nr	20 (20)	16 (16)	7,104-814	8:1
Female	41 (41)	nr	Nr	20 (20)	16 (16)	9,279-754	12:1

Table 5. Genetic divergence estimated among 5 clades (species) and intraclade, with the LSU gene (LSU; lower matrix) and *cox* 1 gene (*cox* 1; upper matrix). Uncorrected *p* distances are expressed as percentages.

						Intraclade	
cox 1/LSU	*N. mamesi* n.sp.	*N. brentnickoli*	*N. golvani*	*N. roseus*	*N. saginatus*	*cox* 1	LSU
N. mamesi n.sp.	--------	20.53 to 22.06	22.24 to 24.95	27.21 to 27.84	29.53 to 30.46	0.23 to 2.06	0.1 to 0.13
N. brentnickoli	10.14 to 10.55	----------	23.79 to 25.91	22.77 to 24.12	27.61 to 28.07	0.23 to 3.21	0.13 to 0.27
N. golvani	20.31 to 21.03	21.43 to 22.28	----------	26.25 to 28.11	28.42 to 30.77	0.23 to 10.41	0.12 to 1.37
N. roseus	32.07 to 32.38	31.42 to 31.57	32.13 to 32.73	----------		0	0.29
N. saginatus	33.34 to 33.50	33.21 to 33.35	31.86 to 32.15	35.76 to 36.20	----------	0	0

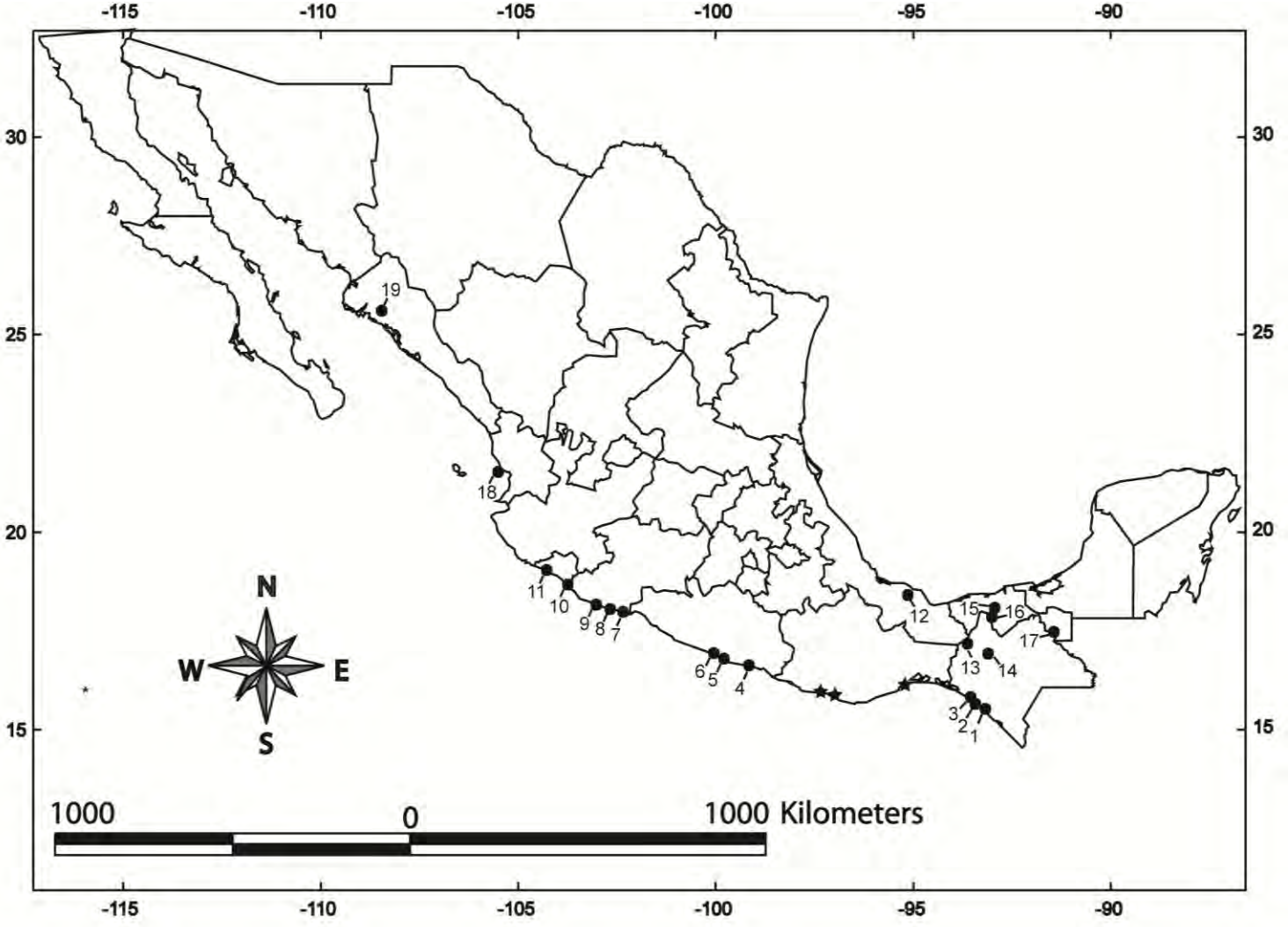

Fig. 5(1). Sampling sites of specimens of *Neoechinorhynchus mamesi* n. sp. and the other 4 congeneric species reported from Mexico. Collection sites are numbered according to Table 1. The asterisk indicates localities where Pacific fat sleeper fishes were studied but negative of the infection.

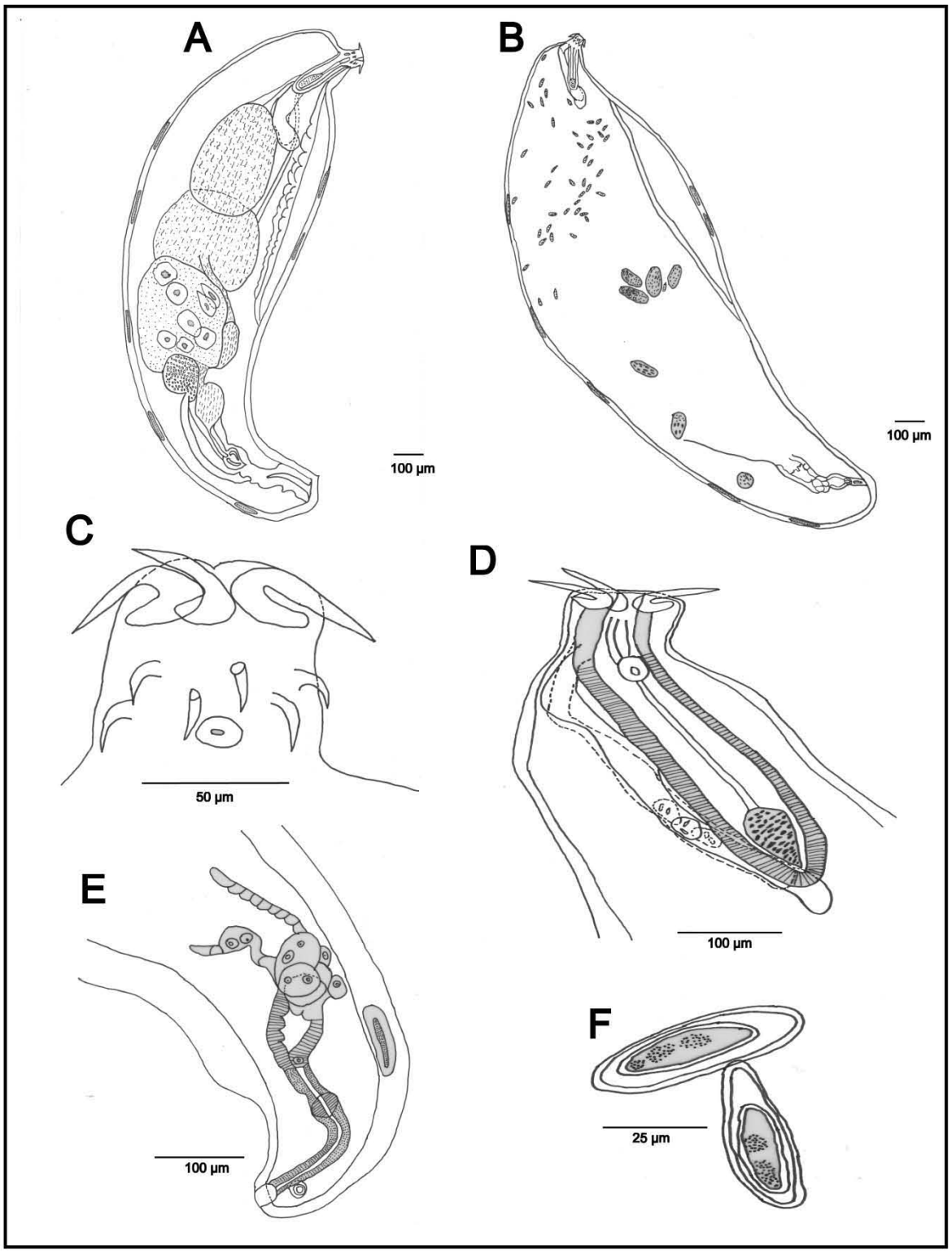

Fig. 6(2). *Neoechinorhynchus mamesi* n. sp. **A**. Male (holotype). **B**. Female (allotype). **C**. Proboscis of (allotype). **D**. Anterior region of the male. **E**. Posterior region of the female. **F**. Eggs (paratypes).

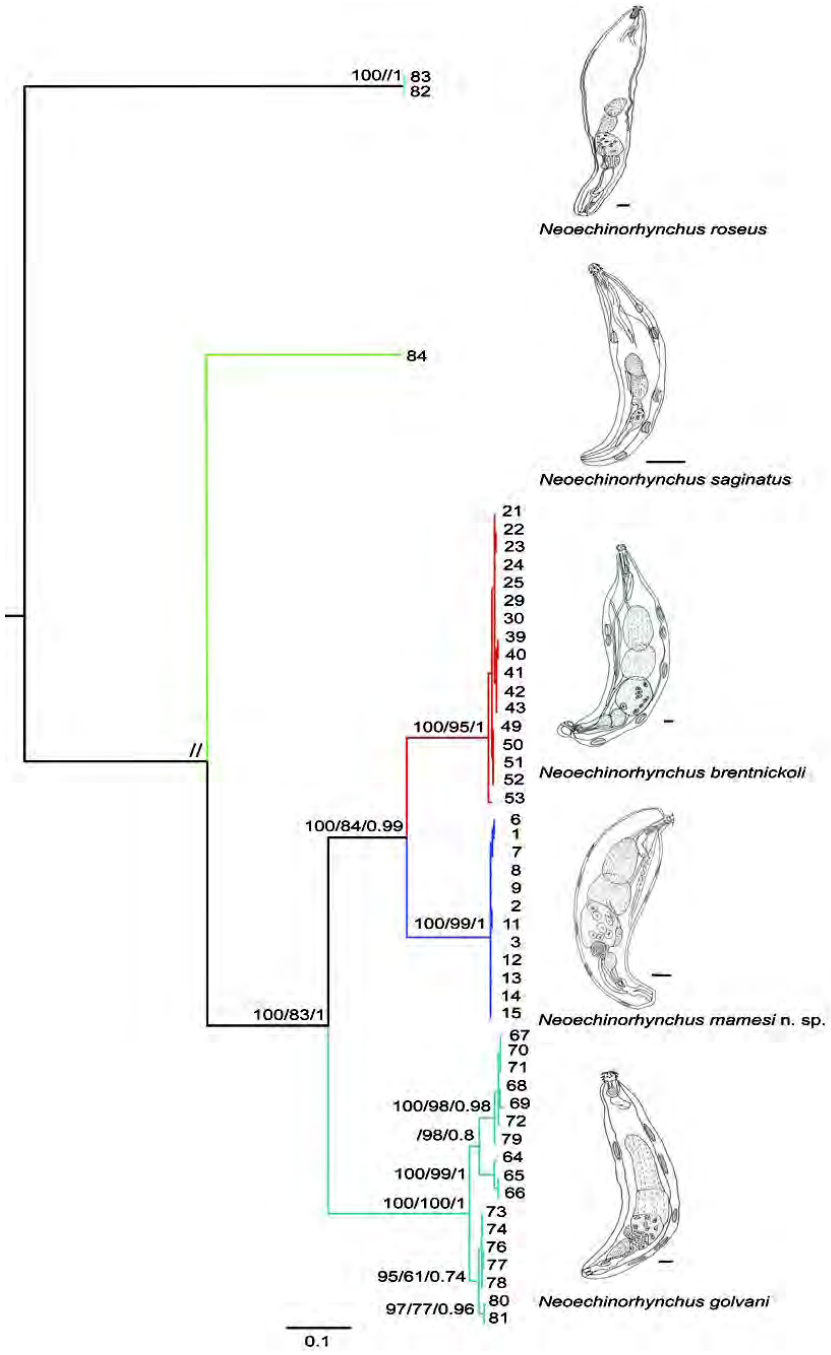

Fig. 7(3). Trees inferred with the combined (*cox* 1 + LSU) data set, using maximum parsimony (1027 steps) and maximum likelihood (–ln likelihood 7427.997809) methods and Bayesian Inference. Numbers near internal nodes show MP/ ML bootstrap clade frequencies and posterior probabilities clade frequencies. Bars=500µm.

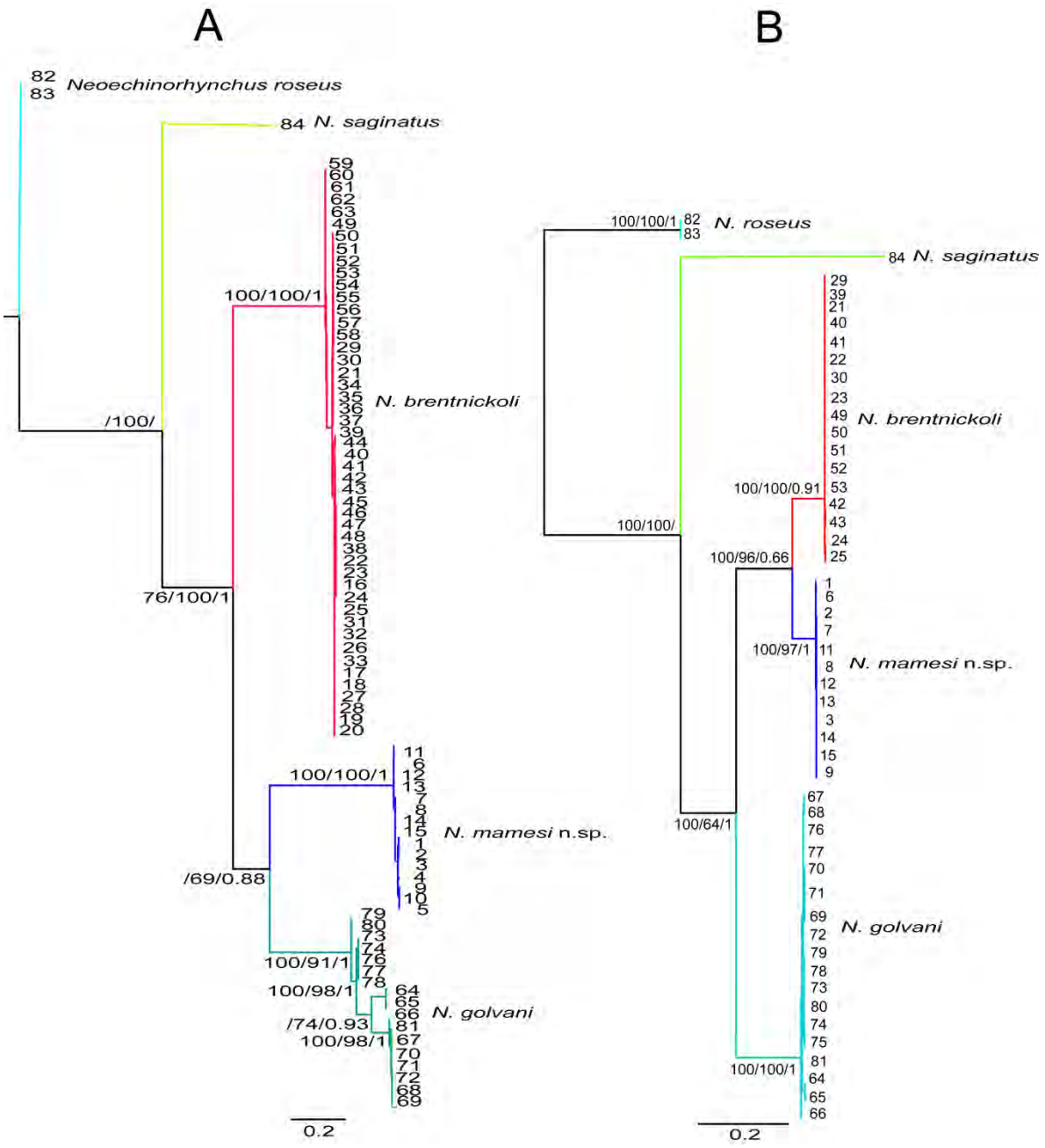

Fig. 8(4). Trees inferred with the maximum parsimony and maximum likelihood methods and Bayesian Inference. A. *cox* 1 data set. B. LSU data set. Numbers near internal nodes show MP/ML bootstrap clade frequencies and posterior probabilities clade frequencies.

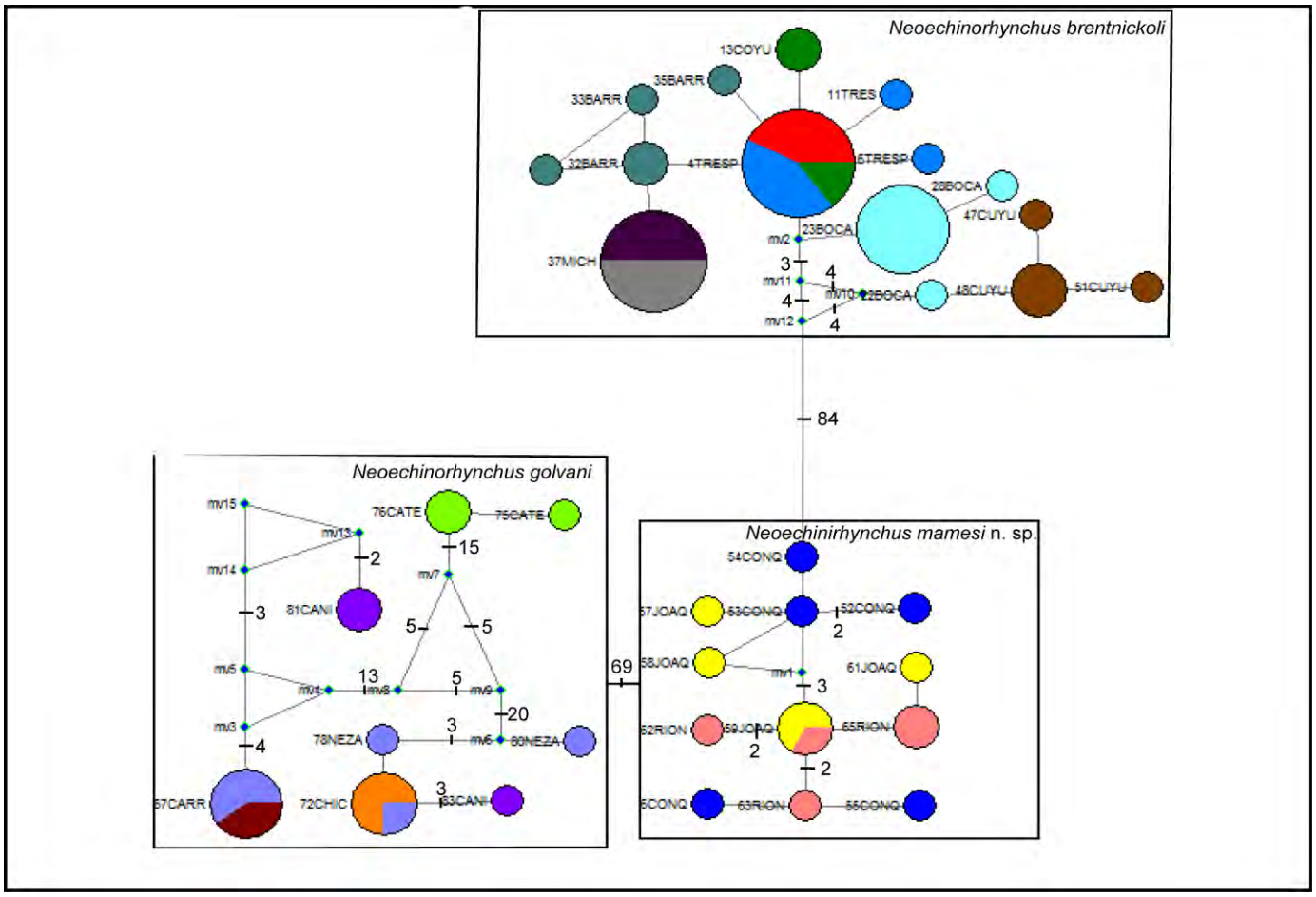

Fig. 9(5). Median-joining network of *Neoechinorhynchus mamesi* n. sp., *N. golvani*, *N. brentnickoli*, *N. roseus* and *N. saginatus* building with cytochrome c oxidase subunit I (*cox* 1) gene. Each circle represents a haplotype, with size proportional to the haplotype's frequency in the population. Numbers among the haplogroups indicate the number of steps. MV1–MV20 indicates haplotypes no detected.

VII. CONCLUSIONES

1.- Con base en el análisis de los datos morfológicos, las distancias genéticas obtenidas para cada gen, la red de haplotipos para las poblaciones, los árboles filogenéticos obtenidos para cada gen y el combinado de ambos genes (*cox* 1+LSU), inferidos con los métodos de Máxima Parsimonia, Máxima Verosimilitud e inferencia Bayesiana, se reconoce claramente la existencia de una nueva especie (aquí denominada como *Neoechinorhynchus mamesi* n. sp.) en tres lagunas costeras del estado de Chiapas.

2.- El porcentaje de divergencia genética estimada entre *N. mamesi* n. sp. y *N. brentnickoli* (su especie hermana) osciló entre el 20.53 a 22.06% para *cox 1* y entre 10.14 a 10.55% para LSU; entre *N. mamesi* n. sp. y *N. golvani* ésta osciló entre el 22.24 a 24.95% para *cox* 1 y entre 20.31 a 21.03% para LSU; y entre *N. mamesi* n. sp. y *N. roseus* ésta osciló entre en 27.21 a 27.84% para *cox* 1 y entre 32.07 a 32.38% para LSU. Finalmente entre *N. mamesi* n. sp y *N. saginatus* la divergencia varió de 29.53 a 30.46% para *cox* 1 y de 33.34 a 33.50% para LSU.

3.- *Neoechinorhynchus mamesi* n. sp. y *Neoechinorhynchus brentnickoli* están asociadas a *Dormitator latifrons* en la vertiente del Pacífico de México, muestran un alto porcentaje de divergencia genética, lo que sugiere una supresión del flujo génico entre ambos linajes debido a su aislamiento geográfico posiblemente por el Istmo de Tehuantepec.

4.- Al parecer la distribución del huésped intermediario es la principal causa que podría explicar el proceso de especiación entre estas dos especies en la vertiente del Pacífico mexicano.

5.- Se reconoció el rango de distribución de *Neoechinorhynchus brentnickoli* que incluye lagunas costeras de los estados de Guerrero, Michoacán y Colima, México. Mientras que la nueva especie descrita en este trabajo tiene un rango de distribución restringida a tres localidades de lagunas costeras del estado de Chiapas.

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