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POSGRADO EN CIENCIAS DEL MAR Y LIMNOLOGÍA

ZOOPLANCTON DE AGUAS EPICONTINENTALES: ESPECIES ANFIATLÁNTICAS O ESPECIES CRÍPTICAS. UN ANÁLISIS BASADO EN LA TAXONOMÍA INTEGRATIVA.

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

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MÉXICO, CD. MX., MAYO, 2018

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“La ciencia será siempre una búsqueda, jamás un descubrimiento real. Es un viaje, nunca una llegada”. Karl Raimund Popper (1902-1994)

Dedicado a mis padres y hermana por su apoyo incondicional.

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RESUMEN

Se seleccionaron tres especies de microcrustáceos de aguas epicontinentales considerados cosmopolitas, dos cladóceros (*Moina micrura* y *Moina macrocopa*) y un copépodo (*Mesocyclops pehpeiensis*), en base a su abundancia en el continente europeo y americano, para ser estudiados desde el enfoque de la taxonomía integrativa que consiste en utilizar diferentes herramientas complementarias para delimitar las especies putativas. En este caso, utilizamos la morfología, la genética, usando una secuencia de aproximadamente 650 pb de la subunidad 1 del gen mitocondrial de la citocromo oxidasa (COI), y la biogeografía, para delimitar las especies seleccionadas. En total se recolectaron y estudiaron unas 200 muestras crudas de zooplancton principalmente de España y México.

Mesocyclops pehpeiensis, resultó ser una especie oportunista que se está extendiendo por todo el mundo debido posiblemente a una reciente translocación de origen antropogénico relacionada con el cultivo del arroz. Sin embargo, los cladóceros, resultaron ser complejos de especies crípticas con patrones de distribución muy restringidos. El complejo *Moina micrura*, está formado por siete especies diferentes, dos en España habitando en simpatría en la Albufera de Valencia y cinco en México dispersas por toda la República. *Moina macrocopa* tenía hasta el momento dos subespecies descritas, una europea (*Moina macrocopa macrocopa* o *Moina macrocopa* s.l.) y una subespecie americana *(Moina macrocopa americana*). En este trabajo se demostró gracias al uso de la taxonomía integrativa que son dos especies distintas que presentan grandes diferencias morfológicas, genéticas y biogeográficas. La especie americana, *Moina americana* (antes *Moina macrocopa americana*) que se distribuye en el continente americano desde Canadá a Argentina y la especie europea *Moina macrocopa* s.l. que por sí misma es un complejo de especies distribuido por todo el continente Euroasiático.

ABSTRACT

In base of their abundance in the European and American continent, three different species of freshwater microcrustaceans, considered cosmopolite, were selected: two cladocerans (*Moina micrura* y *Moina macrocopa*) and one copepod (*Mesocyclops pehpeiensis*), to study them from an integrative approach which include the use of distinct tools to delimitate the putative species. In this case, we used morphology, genetics (using a sequence of 650 pb of the subunit 1 of the mitochondrial gene of cytochrome oxidase - COI), and biogeography, to delimitate the selected species. In total, we collected and studied 200 raw samples of zooplankton mainly, from Mexico and Spain.

Mesocyclops pehpeiensis, is an opportunistic species that is expanding all over the world due possibly to a recent anthropogenic translocation associated to rice cultivation. However, the cladoceran resulted to be complexes of cryptic species with restricted distributions. The *Moina micrura* complex, studied in this research, is formed by seven distinct species, two in Spain inhabiting in sympatry at Albufera de Valencia Lake, and five in Mexico scattered throughout the Republic.

Moina macrocopa has two subspecies, one European (*Moina macrocopa macrocopa* o *Moina macrocopa* s.l.) and other, American (*Moina macrocopa americana*). This research demonstrates by the integrative taxonomy, that they are two different species with deep morphological, genetical, and biogeographical differences. The American species, *Moina americana* (named before *Moina macrocopa americana*) is distributed in the American continent from Canada to Argentina and the European species, Moina macrocopa s.l., is a complex by itself distributed all over the European continent.

CAPÍTULO I. INTRODUCCIÓN

La clasificación taxonómica de los grupos principales de zooplancton, clase Brachiopoda y Maxillopoda, Suborden Cladocera y Copepoda, respectivamente, es bastante confusa (Van Damme et al. 2011). Desde hace más de 30 años Frey (1982) empezó a cuestionar el conocimiento que se tenía sobre la distribución de estos organismos. A pesar de que el tiempo transcurrido desde entonces ha sido relativamente largo, las técnicas tradicionales de clasificación taxonómica solo han permitido de manera limitada, determinar si las especies que encontramos en ambas partes del océano Atlántico principalmente del continente americano y europeo son realmente anfiatlánticas, o bien representan una amplia diversidad críptica (Michael & Frey 1983; Michael & Frey 1984; Frey 1985; Frey 1986; David G Frey 1987; David G. Frey 1987; Frey 1988a; Frey 1988b; Forró et al. 2008; Smirnov & Kotov 2009; Kotov & Alonso 2010; Van Damme et al. 2013; Nédli et al. 2014; Bekker et al. 2016). Debido a esto, recientemente se ha recurrido a la inducción artificial de machos para analizarlos con detalle ya que, presentan caracteres morfológicos más claros para distinguir las especies (Kim et al. 2006). Sin embargo, estas técnicas requieren una inversión elevada de tiempo y la estabilización de las especies en condiciones de cultivo, lo cual no siempre se puede lograr.

Una alternativa interesante son los métodos moleculares. Entre estos destacan los códigos de barras, que utilizan una secuencia de aproximadamente 650 pares de bases (pb) de la subunidad 1 del gen mitocondrial citocromo c oxidasa (COI), el cual fue propuesto hace quince años como un sistema de identificación universal para animales (Hebert et al. 2003). En el caso del zooplancton, esta metodología demostró que puede ser una primera aproximación para el descubrimiento de especies crípticas, sobre todo de cladóceros y copépodos (Elías-Gutiérrez & Valdez-Moreno 2008; Bekker et al. 2016). Sin embargo, la información molecular en si misma constituye un solo carácter más, que no siempre funciona de manera efectiva. Por ejemplo, en ciertos grupos como la familia Characidae de peces, los códigos de barras no distinguen las especies del complejo *Astyanax-Bramocharax* (Valdez-Moreno et al. 2009). Debido a estos problemas, la mejor aproximación para el reconocimiento de la biodiversidad deriva de un nuevo concepto

denominado taxonomía integrativa, que surgió a partir de las controversias originadas al usar solo datos moleculares para delimitar las especies (Dayrat 2005; Will et al. 2005). Es en México donde por primera vez se describieron dos especies de cladóceros (Elías-Gutiérrez & Valdez-Moreno 2008; Quiroz-Vázquez & Elías-Gutiérrez 2009) y una de copépodos (Montiel-Martínez et al. 2008) utilizando esta nueva aproximación taxonómica. Esta tendencia se está consolidado cada vez más en el descubrimiento de nuevas especies en diversos grupos (Bertolani et al. 2011; Boyer et al. 2011; Galimberti et al. 2012; Cruz-Barraza et al. 2012; Ceccarelli et al. 2012; Razo-Mendivil et al. 2013; Lavinia et al. 2017; Bataille et al. 2013).

Por otro lado, en el caso del zooplancton, la integración de los protocolos moleculares para la obtención de códigos de barras se ha visto limitada por problemas metodológicos para amplificar la región de interés, que apenas hasta hace cinco años aparentemente han sido resueltos (Prosser et al. 2013). A pesar de lo anterior, los avances logrados en el conocimiento del zooplancton mexicano se han convertido en un modelo de referencia a nivel global (Van Damme & Sinev 2013,Makino et al. 2017). Conforme se avanza en el conocimiento de estos grupos, diversos autores coinciden en señalar que el siguiente paso es la comparación intercontinental de las especies de microcrustáceos, a fin de reconocer su diversidad real.

Por esta razón, la base principal de este trabajo será la comparación anfiatlántica de algunas especies de cladóceros y copépodos ciclopoides, que siguiendo la taxonomía actual en las claves regionales se están identificando con el mismo nombre en América y Europa, a fin de confirmar si realmente se trata de una misma especie o son en realidad especies diferentes.

Antecedentes

Para el caso de los Cladóceros libres de las aguas epicontinentales, el libro “Fauna Ibérica” (Alonso 1996) cita 90 especies de cladóceros en España de las cuales, comparándolas con el libro “Guía ilustrada de Cladocera y Copepoda de las aguas continentales de México”

(Elías-Gutiérrez, Suárez-Morales, et al. 2008), observamos que 26 especies son aparentemente anfiatlánticas.

En el caso de los Copépodos libres de las aguas continentales, más concretamente, la Familia de los Cyclopidae, a los que se va a dedicar también parte de este estudio, nos encontramos ante la misma problemática, de las 62 especies encontradas en Europa continental, citadas por (Dussart 1969), 13 aparentemente también son anfiatlánticas.

Por ejemplo, *Acanthocyclops robustus* (Sars), ha sido considerado cosmopolita, pero hay evidencias de que se trata de un complejo de especies crípticas distribuido por todo el mundo. Por ejemplo, ciertas poblaciones americanas previamente consideradas como *A. robustus* resultaron ser una nueva especie denominada *Acanthocyclops rebecae* (Fiers et al. 2000). Otra especie confundida con *A. robustus* resultó ser *A. americanus*, el cual además representa una amplia distribución anfiatlántica, derivada a partir de una posible introducción reciente en Europa (Miracle et al. 2013).

En el caso de cladóceros, el género *Moina,* está representado por varias especies anfiatlánticas bien definidas como *Moina macrocopa* (Straus) y *Moina micrura* (Kurz). Goulden (1968) reconoce dos subespecies de *M. macrocopa*, una americana y otra europea. Creemos que en realidad se trata de dos especies distintas y no de subespecies. En cuanto *Moina micrura*, es considerada un complejo de especies. En México se han detectado genéticamente tres subgrupos diferentes, que son también consistentes con su distribución geográfica (Elías-Gutiérrez, Martínez-Jerónimo, et al. 2008) En el caso de España, *Moina micrura* ha sido registrada en por todo el país incluyendo las Islas Baleares (Armengol, 1978; Alonso, 1996).

Lo anterior permite reconocer la evidencia de especies crípticas y la aparente distribución restringida de la mayoría de las especies de zooplancton de las aguas epicontinentales, lo cual se demostró en un gradiente norte-sur en Norteamérica (Jeffery et al. 2011). Esto apoya la idea de que la mayor parte de la fauna de cladóceros sigue siendo muy subestimada (Forró et al. 2008) y que la mayoría de los nuevos descubrimientos serán en latitudes más bajas, particularmente en los trópicos (Adamowicz & Purvis 2005), o las

regiones más cálidas de Europa (ver el caso de la *Moina* anteriormente mencionado). Es así que de 1996 a 2003 se descubrieron 17 nuevas especies de Cladóceros en México de estas, 7 son endémicas (Elías-Gutiérrez & Suárez-Morales 2003). En el caso de España, a pesar de que se ha considerado que el inventario faunístico es completo desde del trabajo de Alonso (1996), desde entonces se han descrito tres especies nuevas: dos endémicas de *Leydigia* (Kotov & Alonso 2010) y una de Alona (Sinev et al. 2012).

Desde el punto de vista molecular se utiliza de manera universal una divergencia genética del 3% para considerar dos especies como diferentes (Jeffery et al. 2011), este valor podría ser variable. La divergencia intraespecífica calculada por Costa et al. (2007) para Daphnia promedió 1.32%, en cambio Gutiérrez-Aguirre et al. (2014) encontraron más del 4% en el género *Mastigodiaptomus*, lo que no le permitió concluir respecto a la identidad de las especies encontradas. Elías-Gutiérrez et al. (2008) encontró una divergencia intraespecífica promedio del 0.82% para cladóceros y 0.79%. Sin embargo, encontró valores superiores al 4% en unos pocos casos y concluyó que posiblemente se tratara de especies crípticas. Los valores máximos por encima del 4% que encontraron otros autores que se han abocado al problema del valor de corte entre las especies de crustáceos (Costa et al. 2007; Lefébure et al. 2006) son cuestionables, pues están basados en gran medida en secuencias obtenidas del GenBank, donde se ha demostrado que un gran número de identificaciones son erróneas (Fritz et al. 2012; Hsu et al. 2013), siendo este problema aún más grave en copépodos, donde la identificación es más difícil (Karanovic & Krajicek 2012). Desafortunadamente los errores incluidos en GenBank aparentemente no tienen solución viable y expedita (Fritz et al. 2012).

Justificación e Importancia

La base de cualquier análisis ecológico, fisiológico o toxicológico radica en una correcta identificación de las especies. El zooplancton comprende una gran variedad de organismos que se encuentran cerca de la base de la cadena trófica en los ecosistemas acuáticos, siendo muy importantes para las poblaciones de peces. Estos organismos se utilizan como bioindicadores de contaminación, y también para proporcionar información sobre las

características del medio en un gran número de trabajos de ecología aplicada. Por lo tanto, es imprescindible una identificación acertada de las especies, a fin de poder describir y estudiar el sistema ecológico en el que habitan.

Los resultados de este trabajo son fundamentales en cualquier estudio que requiera la correcta identificación de las especies del zooplancton epicontinental.

Actualmente existe la base de datos de GenBank, donde la técnica de BLAST, para un gen específico, permite asociar una secuencia con alguna especie que ya exista en esta base de datos. Sin embargo, la mayor parte del material disponible en este sistema no cuenta con una identificación confiable, ni se puede asociar a una región geográfica en particular.

Una buena alternativa es la base de datos BOLD (Barcode of Life Data base). Los códigos de barras, asociados a esta base de datos y las nuevas metodologías en metagenómica (secuenciación de segunda generación) podrían permitir el reconocimiento de las especies con más del 90% de certeza a un costo cada vez más bajo.

En la actualidad, la principal limitante es la biblioteca de secuencias que, a pesar de tener más de 5,997,719 especímenes secuenciados (www.boldsystems.org, consultada el 19 de marzo del 2018) apenas representa una mínima fracción de la biodiversidad global. A pesar de lo anterior, existe una buena base para partir con el análisis de grupos de zooplancton (cyclopoides 1,873 especímenes con código de barras y cladóceros3 8,797 especímenes con código de barras).

Por otra parte, la mayoría de las especies de cladóceros y copépodos que se conocen actualmente han sido descritas por primera vez en Europa. Gran parte de ese material se ha perdido, o los métodos de preservación solo permiten el análisis de morfología comparada, con bastantes limitaciones dependiendo de la forma en que se han conservado los especímenes. Por lo que, para la realización de este proyecto, es necesario la obtención de topotipos (o visitar localidades lo más cercanas posibles a las localidades tipo) para esclarecer la clasificación taxonómica de estos grupos.

Preguntas e hipótesis

1. ¿Son o no anfiatlánticas las especies de microcrustáceos epicontinentales que actualmente se encuentran en los registros para México y España?
    - Hipótesis: Las especies seleccionadas (Moina micrura, Moina macrocopa y Mesocyclops pehpeiensis) que actualmente se presentan como comunes para España y México son especies crípticas y diferentes.

2. Si se tratara de especies crípticas, entonces ¿existirá una correspondencia directa entre los haplotipos con base al gen COI y los fenotipos?
    - Hipótesis: Al tratarse de posibles especies crípticas, el gen COI permitirá su diferenciación e identificación

3. ¿Cuáles son los límites de divergencia genética, que nos permiten establecer que se trata de especies diferentes?
    - Hipótesis: Un 3% de divergencia genética permitirá distinguir las especies, tal como lo señala Jeffery et al. (2011).

4. ¿Es posible aplicar los principios de taxonomía integrativa (sensu Razo-Mendivil et al. 2013a) en el caso de las especies que no sean anfiatlánticas?
    - Hipótesis: La mejor aproximación para establecer nuevas hipótesis respecto a las especies y su ámbito de distribución geográfica es a través de la taxonomía integrativa.

5. ¿Estos métodos modernos son compatibles con el sistema Linneano de clasificación jerárquica?
    - Hipótesis: Los resultados que se obtengan a partir de análisis con la taxonomía integrativa son compatibles con la actual clasificación jerárquica de los seres vivos basada en el sistema de Linneo.

Objetivos

Objetivo General:

Analizar la diversidad, basados en caracteres moleculares con el gen CO1, su distribución y su morfología. de tres especies de copépodos y cladóceros epicontinentales aparentemente anfiatlánticos, con énfasis especial en México y la Península Ibérica Con base a esta información, identificar los patrones reales de distribución de las especies estudiadas.

Objetivos específicos:

1. Contrastar la identidad taxonómica de las especies seleccionadas con base a caracteres morfológicos,
2. Analizar el grado de variabilidad fenotípica en las especies analizadas y determinar que caracteres morfológicos facilitarían la mejor diferenciación de estas.
3. Determinar empíricamente que el umbral de divergencia conocido en las secuencias genéticas del COI (3%), es el más adecuado.
4. Establecer la correlación entre los haplotipos encontrados y el fenotipo de las especies analizadas y su correspondencia con el sistema de clasificación de Linneo.
5. Con base a la información anterior, realizar inferencias sobre los posibles patrones de distribución de las especies estudiadas.

CAPÍTULO II. *Mesocyclops pehpeiensis* Hu, 1943

Montoliu, L., Miracle, M. R., & Elías-Gutiérrez, M. (2015). Using DNA barcodes to detect non-indigenous species: the case of the Asian copepod *Mesocyclops pehpeiensis* Hu, 1943 (Cyclopidae) in two regions of the world. *Crustaceana 88* (12-14), 1323-1338

BRILL

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CRUSTACEANA

USING DNA BARCODES TO DETECT NON-INDIGENOUS SPECIES: THE CASE OF THE ASIAN COPEPOD *MESOCYCLOPS PEHPEIENSIS* HU, 1943 (CYCLOPIDAE) IN TWO REGIONS OF THE WORLD

BY

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ABSTRACT

To date, little attention has been paid to analyses of copepods as exotic species. The genus *Mesocyclops*, a freshwater cyclopoid, has a worldwide distribution, but individual species within the genus have a quite restricted geographical range. *Mesocyclops pehpeiensis* Hu, 1943 is a Central-East Asian species, rarely found outside of this area, and when it appears should be considered as non-native. Based on morphology and DNA barcode analyses, using the COI gene, we confirmed records of *M. pehpeiensis* in two ponds in Mexico and in a rice paddy near Valencia, Spain. The morphology of this species, based on morphometric analyses, was found to be variable, but DNA barcoding confirmed the same identity for specimens from two continents. The extremely low COI genetic divergence among these disjunct populations of *M. pehpeiensis* strongly evidences anthropogenic translocations. DNA barcoding can be a fast and useful analytical tool to accurately identify exotic species across the world.

RESUMEN

Poca atención se ha prestado al análisis de los copépodos como especies exóticas. El género *Mesocyclops* de aguas continentales tiene una amplia distribución mundial pero las especies que lo componen tienen distribuciones bastante restringidas. *Mesocyclops pehpeiensis* Hu, 1943 es una especie del Centro-Este de Asia, raramente encontrado fuera de esta área, y cuando se ha registrado se considera como no-nativo. Basados en la morfología y el ADN mitocondrial confirmamos la presencia de *M. pehpeiensis* en dos estanques en México y en un arrozal cercano a Valencia, España. La morfología de esta especie es variable en caracteres morfométricos, pero los llamados códigos de barras del ADN confirmaron la misma identidad para los especímenes encontrados en ambos continentes. La baja divergencia genética entre estas poblaciones tan disyuntas evidencia la existencia de translocaciones antrópicas. Proponemos el uso de códigos de barras como una herramienta analítica rápida y confiable para detectar especies exóticas en todo el mundo.

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INTRODUCTION

The genus *Mesocyclops* Sars, 1914 comprises 71 species from around the world (Hołyńska, 2006). It is especially common in the tropics, from where most species have been described in recent years. The first described species, *Mesocyclops leuckarti* Claus, 1857, was thought to be cosmopolitan. Since its description from Germany at the end of the 19th century until the work of Kiefer (1981), who showed that this species was restricted to Europe and West Asia, misidentifications of *Mesocyclops* from other parts of the world have occurred (Menendez-Díaz et al., 2006). Van de Velde (1984) performed a taxonomic revision of the African populations of this genus that revealed all records of *M. leukarti* as misidentifications and confirmed that this species did not occur on that continent. Because of the poor knowledge on the taxonomy of this genus at that time, and reliance on European identification keys, *M. leuckarti* appeared to be the only species found in European waters, except for a taxon named as *M. ruttneri* by Kiefer (1981), recorded from one locality, a greenhouse in Lunz (Austria). This species was later also found in North America (Reid, 1993). Subsequently, *M. ruttneri* was synonymized by Guo (2000) with *M. pehpeiensis* Hu, 1943, who accurately redescribed it from its terra typica: Central and East China. On the basis of this information, all records of *M. ruttneri* can be considered as introduced populations of *M. pehpeiensis*.

Interestingly, the genus *Mesocyclops* has not been recorded from the Iberian Peninsula (Dussart, 1969; Dussart & Defaye, 2006), the westernmost end of Europe, except for a single and recent record under the name of *M. leuckarti* in the south of Spain (Frisch et al., 2006). Considering the fact that it is the only species of the genus included in Western European keys, this record could be a misidentification.

Mesocyclops pehpeiensis is a widespread tropical species from central and southeast Asia with confirmed records from Uzbekistan, Kazakhstan, India, Sri Lanka, Indochina, China, Japan (Hołyńska et al., 2003; Dussart & Defaye, 2006; Resmi & Jayachandran, 2014) and Taiwan (record mined from GenBank, see table II). This species was introduced in the Atlantic and Gulf coasts of the United States (Mississippi, Louisiana and the District of Columbia), associated with rice fields and aquatic gardens (Reid, 1993; Reid & Marten, 1995; Hołyńska et al., 2003; Wyngaard et al., 2010). Later, in 2005, this species was also found in Southeast Mexico (Suárez-Morales et al., 2005), in two ponds located near to the Pacific Coast, and it was suggested to be a human introduction in a similar way as the above-mentioned records from the United States. In 2006 it was recorded from Cuba (Menendez-Díaz et al., 2006) and in 2014 it was also recorded from Crimea (Russia) (table I). This latter record is the first finding of the species

TABLE I

Records confirmed of *Mesocyclops pehpeiensis* Hu, 1943 in localities outside its original range

Country	Exact locality	Date	Coordinates	No. of specimens	Reference
Austria	Glashaus, Lunz	4 December 1926	48°16′59″N 14°18′43″E*	25+	Kiefer (1981)
USA	Joe Brown Park, New Orleans, LA	July 1987	30°02′11″N 89°58′08″E*	41+	Reid (1993)
USA	Ricefield, Compton Farm, New Orleans, LA	10 August 1990	NA	2	Reid (1993)
USA	Ricefields near Jennings, LA	9 August 1990; 26 July 1991	30°12′03″N 92°32′43″W*	21+	Reid (1993)
USA	Ricefields near Cleveland, MS	10 August 1991	33°46′26″N 90°41′52″W*	20+	Reid (1993)
USA	Kenilworth Aquatic Gardens, DC	1 June 1996	38°54′46″N 76°56′31″W	3	Wyngaard et al. (2010)
Mexico	Fishpond, Simón Bolívar, Tapachula	29 August 2003	14°37′37″N 92°16′08″W	50	Suárez-Morales et al. (2005)
Mexico	Pond, highway to Ciudad Hidalgo Cosalapa, Chiapas	23 September 2003	14°30′23″ 92°16′1″W	14 1	Suárez-Morales et al. (2005)
Cuba	El Cacao reservoir, La Habana	2005	23°04′08″N 82°16′08″W	–	Menéndez-Díaz et al. (2006)
Russia	Kuchuk-Adjigol, Crimea	8 August 2012	45°06′00″N 35°27′00″E	–	Anufriieva et al. (2014)

* Approximate coordinates, not provided in the original source of information.

in a natural water body in Europe (Anufriieva et al., 2014). All these records, except the one from GenBank, have been based only on morphological analyses. The main problem with this type of analysis is the need for an expert to study the minute anatomical details of the morphology, which could be why *Daphnia lumholtzi* Sars, 1885 is the only well-documented exotic micro-crustacean species. This cladoceran is easily recognizable due to its long spines on the head and the valves (see Frisch et al., 2013).

Here, based on comparative morphology and DNA barcodes, we confirm the presence of *M. pehpeiensis* in America (Mexico) and Europe (Iberian Peninsula), and discuss the potential of molecular analyses to detect invasive or non-indigenous species that are difficult to identify. The possible causes of today's disjunct distribution of the species are also analysed.

MATERIAL AND METHODS

Collections of *Mesocyclops* were made in Spain and Mexico in 2011 and 2012. In Spain, specimens were collected from a rice paddy on the Mediterranean coast of the Iberian Peninsula (L'Estell fields, in the Albufera of Valencia Natural Park), and in Mexico from two ponds located in Yucatan Peninsula (near Palizada town, Campeche State) (table III). Samples were taken in the littoral zone of the water bodies with a 50 μm plankton net attached to a handle and fixed immediately with 96% non-denatured ethanol.

In the laboratory the samples were sorted under a Zeiss SV6 binocular stereomicroscope. Specimens were placed on slides (in a drop of a glycerol-formaldehyde mixture) and studied under an Olympus model BX51 optical microscope using a differential interference and/or phase contrast. The organisms were measured with an eyepiece micrometer and morphologically identified using the descriptions given by Guo (2000), Hołyńska et al. (2003) and Suárez-Morales et al. (2005).

DNA analyses based on mitochondrial barcodes (*cytochrome c oxidase I*, *COI*) were made following the protocols reported by Elías-Gutiérrez et al. (2008). DNA was extracted using the HOTSHOT method (Montero-Pau et al., 2008) from detached eggs sacs of single females or a male cephalothorax (see table II). The remains of each specimen were used for identification and preserved as a voucher in the collections in the University of Valencia (Spain), and El Colegio de la Frontera Sur (ECOSUR), Chetumal, Mexico. Primers used for Polymerase Chain Reaction (PCR) were LCO1490-HCO2198 (Folmer et al., 1994) and ZplankF1_t1-ZplankR1_t1 (Prosser et al., 2013).

Sequence data, electropherograms, trace files, primer details, photographs of the main taxonomical characters and accession numbers for all specimens are available

in the project "*Mesocyclops*" in the Barcode of Life Data System-BOLD (Ratnasingham & Hebert, 2007; http://www.boldsystems.org) and GenBank database (http://www.ncbi.nlm.nih.gov/) (see Appendix for ID tree).

All analyses were conducted using MEGA 6 software (Tamura et al., 2013). The obtained sequences were checked manually and aligned using the MUSCLE algorithm (Edgar, 2004) with default parameters. The best-fit model of nucleotide substitution was established with the option Find Best Fit Model (Maximum Likelihood, ML). Once the best model was selected, we calculated the ML tree using the bootstrap method, with 1000 replications and the nearest-neighbour interchange heuristic method. For comparison, we also obtained the ID Tree using as distance model Kimura 2 parameter (K2P; Kimura, 1980) and the BOLD Aligner provided in BOLD (http://www.boldsystems.org). As an outgroup we included a *COI* sequence of *Mesocyclops edax* Forbes, 1891 (table II), an American congener of *M. pehpeiensis*.

RESULTS

Comparative morphology

We found *Mesocyclops pehpeiensis* outside its native area of distribution in two very distinct localities. One was a rice paddy in the Albufera Lake Natural Park, Valencia in the fields of L'Estell (table II). This park is a wetland area comprising Albufera Lake (24 km^2) and surrounding marshland (10 times the lake area) that has been converted to rice fields. The aforementioned rice paddy and Albufera Lake are interconnected by irrigation channels (Carrera de la Reina), but *M. pehpeiensis* has not yet been found in the lake or in other sites in that area. The other localities were two ponds in Campeche State (Mexico), an important rice producer in this country. A morphological comparison with specimens and drawings after Guo (2000) and Suárez-Morales et al. (2005) confirmed the identity of this species as *M. pehpeiensis*. One diagnostic character of this species is the spinule ornamentation on the caudal surface of the P4 coxopodite, which is relatively short and stout in comparison to the spinules in the same position for other congeners (Hołyńska et al., 2003). In table III morphometric ratios from our specimens are compared with those found in the literature.

MOLECULAR ANALYSES

The *COI* sequences for the 15 specimens of *Mesocyclops pehpeiensis* processed in this work were between 546 and 658 bp of length.

TABLE II
Confirmed records for *Mesocyclops pehpeiensis* Hu, 1943 for GenBank and BOLD searches

Species	Location	Coordinates	Date	BOLD sample ID	GenBank accession no.	Sex and life stage sequenced
Mesocyclops pehpeiensis Hu, 1943	Rice field, Albufera Natural Park, Spain	39°17′45″N 0°18′57″W	21 July 2012	EES104	KT962940	♀ Adult
				EES106	KT962939	♂ Adult
				EES108	KT962938	♀ Adult
				EES126	KT962937	♀ Adult
				EES135	KT962936	♀ Adult
				EES137	KT962935	♀ Adult
				EES142	KT962934	♀ Adult
				EES149	KT962933	Egg sac
				EES150	KT962932	♂ Adult
				EES151	KT962931	Egg sac
				EES156	KT962930	Egg sac
M. pehpeiensis	Palizada, pond I, Mexico	18°03′47″N 92°01′01″W	26 June 2011	HE580.1	KC617307	♀ Adult
				HE580	KC617278	♀ Adult
M. pehpeiensis	Palizada, pond II, Mexico	18°02′53″N 92°53′16″W	26 June 2011	HE313.1	KC617566	Egg sac
M. pehpeiensis	Taiwan	–	–	–	KJ020571	Mined from GenBank
Mesocyclops edax Forbes, 1891	Tecualilla, Mexico	22°45′80″N 105°40′10″W	18 February 2011	–	JQ284449	Mined from GenBank

TABLE III

Comparison of morphometric ratios (mean values and standard deviation) of different *Mesocyclops pehpeiensis* Hu, 1943 populations

	n	L/W		Si/Sme	Sme/Smi	Si/Se	CRL/Sd	A17/A16
		CR	L4END3					
Published ranges		2.81-4.0	2.6-3.3	0.63-0.73	0.51-0.71	3.1-3.6	1.05-1.38	0.68-0.79
Mexico (present study)	5	3.06 ± 0.25	*2.49 ± 0.37*	0.67 ± 0.24	*0.95 ± 0.33*	*2.63 ± 0.29*	1.33	*0.91 ± 0.1*
Spain (present study)	5	3.34 ± 0.69	2.73 ± 0.45	0.66 ± 0.02	0.7 ± 0.03	*2.91 ± 0.11*	–	0.76 ± 0.05

Characters based on Guo (2000): CR, caudal rami; L4EN3, third endopodal segment of the fourth swimming leg; TL, total length to the distal end of CR; Sd, dorsal seta; Se, outermost terminal seta; Si, innermost seta; Sme, outer medial seta; Smi, inner medial seta; A, antennular segment; L, length; W, width. Italicised numbers correspond to values outside ranges reported by Hu (1943), Guo (2000), Reid (1993), Suárez-Morales et al. (2005) and Anufriieva et al. (2014). n = number of measured specimens (2 measurements for each animal, at both sides of the body).

In our sequence compositions, the mean GC% in position 1, 2 and 3 was 41.93, 43.15 and 10.85%, respectively. The mean composition was: G 18.32% (SE = 0.11), C 13.68% (SE = 0.05), A 26.86% (SE = 0.06) and T 41.14% (SE = 0.09).

The model that provided the best description of the nucleotide substitution pattern was the Hasegawa-Kishino-Yano model (HKY) (Hasegawa et al., 1985), and this was used to calculate the ML tree shown in fig. 1. A discrete Gamma distribution was used to model evolutionary rate differences among sites (+G, parameter = 0.2508). The ML and ID Tree calculated in BOLD were identical (see Appendix).

The sequence of *M. pehpeiensis* species from Taiwan was equal to three of the Spanish specimens (see fig. 1). The other sequences differed from the one from Taiwan in positions 166 and 173 where the guanine and cytosine from our sequences were replaced by adenine and thymine, respectively. The Mexican specimen with Sample ID HE313.1 in BOLD presents the same variation in position 166 and a guanine instead of thymine, in position 463.

The *COI* sequences from Spanish and Mexican specimens of *M. pehpeiensis* cluster together with Taiwan specimen (see referred project in http://www.boldsystems.org or table II for GenBank accession numbers to all sequences). The maximum divergence within species is only 0.59% and the maximum divergence within the genera 16.99%. On the other hand, the Barcode Index Number (BIN) assigned in BOLD (http://www.boldsystems.org) for the *M. pehpeiensis* species is ABA8110. The BIN system is an online framework that clusters barcode sequences algorithmically, generating a web page for each cluster. Since clusters show high concordance with species, this system can be used to verify species identifications as well as document diversity when taxonomic information is lacking or unreliable (Ratnasingham & Hebert, 2013).

DISCUSSION

There are only few occurrences of *Mesocyclops pehpeiensis* reported from outside its native area of distribution (Central-East Asia) (table I). Our analysis of the COI sequence (fig. 1, table II) showed that all haplotypes found were extremely similar, with the same haplotype found in the Mediterranean (Spain) and Campeche (Mexico) populations. Furthermore, the haplotype recorded from a population in the original area of East Asia is the same as several found in the West European population. These results can only be explained by recent invasions, most probably via anthropogenic translocations.

In this work, we provide a record from two ponds close to the southern Gulf of Mexico, near Palizada town, in the state of Campeche (table II). Previous American

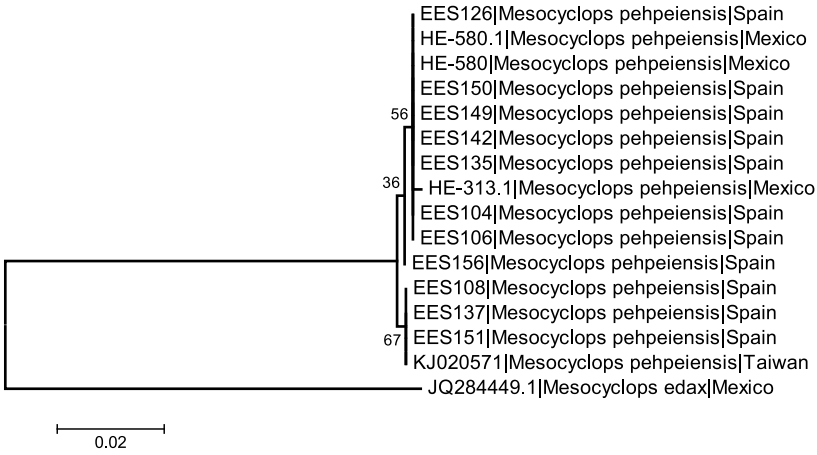

Fig. 1. Maximum likelihood tree based on the Hasegawa et al. (1985) model for *Mesocyclops pehpeiensis* Hu, 1943 from America and Europe. The outgroup corresponds to *Mesocyclops edax* Forbes, 1891. Numbers preceding the species name are the specimen ID in BOLD, except for the Taiwan and *Mesocyclops edax* specimens, that are the GenBank Accession numbers.

records were mostly from rice fields in Louisiana and Mississippi (U.S.A.) (Reid, 1993; Reid & Marten, 1995), all of them along the northern Gulf of Mexico coast. We found it in two isolated ponds in Palizada, which is also an important rice crop area. Other records from Mexico (Suárez-Morales et al., 2005) and Cuba (Menendez-Díaz et al., 2006) are related to aquaculture ponds of the Malayan prawn (*Macrobrachium rosenbergii* De Man, 1879). The species has also been detected in urban ponds (District of Columbia; Reid, 1996). Finally, the single record from Austria (Kiefer, 1981) was reported after the introduction of exotic plants in a greenhouse.

On the other hand, on the Iberian Peninsula the genus *Mesocyclops* has not been found, and besides our record of *M. pehpeiensis*, in a rice paddy near Valencia (table II), only a single recent record of *M. leuckarti* exists from a man-made pond in a coastal wetland (Doñana National Park), where rice is also produced. This locality is further south than Valencia, and the occurrence of *M. leuckarti* is questionable because it could be misidentified with *M. pehpeiensis*, not included in West European identification keys. The oldest previous record of *M. pehpeiensis* in Europe is the above-mentioned pond in a greenhouse (Kiefer, 1981), but it has not been reported again since. The less extreme weather in Spain may have allowed the settlement of this subtropical-tropical species, probably combined with the eutrophication of the water (Vicente & Miracle, 1992) that is still ongoing (Miracle, pers. obs.). In the rice field, subject to periodic desiccations, *M. pehpeiensis* was found associated with dense populations of

filamentous cyanobacteria and other algae. It agrees with the observation that *M. pehpeiensis* is often associated with high abundances of these blue-green algae (Sarma et al., 2013). Furthermore, Albufera Natural Park, Valencia is an active agricultural area where other exotic microcrustaceans, such as ostracoda, have been also recorded (Valls et al., 2014). These authors consider rice agricultural practices to be the most probable vector for introduction and establishment of exotic ostracods.

However, other factors could be involved in the introduction of this copepod in other places. Anthropogenic translocation associated with shipping activities, through ballast water discharge, is considered one of the major vectors of copepod dispersion (Karanovic & Krajicek, 2012). On the other hand, a recent Crimean record of *M. pehpeiensis* has been attributed to transportation by birds (Anufriieva et al., 2014). In spite of this, we consider that *M. pehpeiensis* has spread recently in Europe and America owing to human-derived translocation, as can be inferred from the extremely small divergences in the DNA barcodes. At the same time, the ongoing expansion of its range may be a result of the global change (i.e., climatic changes and man's impacts on water bodies).

Due to the complexity of the characters used for the genus *Mesocyclops* in classical taxonomy (Hołyńska, 2000; Suárez-Morales et al., 2005), and the variability of morphological characters as we have noted here for *M. pehpeiensis*, genetic analyses can be an important alternative for correct identification. The main morphometric characters of the Spanish specimens of *M. pehpeiensis* are within the ranges known for this species, except the ratio of the antennular segments (A17/A16) (Suárez-Morales et al., 2005). However, Mexican specimens from Palizada showed small differences between some of the ratios, such as L/W CR (length/width caudal rami), L/W L4END3 (length/width third endopodal segment of fourth swimming leg), Si/Se (innermost seta/outermost terminal seta) and A17/A16 (last antennular segment/before last antennular segment). This variation of relative lengths in some structures within one species can be related to ecological factors, but, in general, these processes are subtle and not well understood for this group of animals (Williamson & Reid, 2001). Commonly *M. pehpeiensis* coexisted with other congeners autochthonous to the collection sites (Reid, 1996) and it was difficult to detect their presence.

Therefore, we applied the barcoding technique that has been demonstrated to be effective for the identification of freshwater microcrustaceans, including the copepods (Elías-Gutiérrez et al., 2008). Another example of the usefulness of this technique to clarify distribution and taxonomical problems in copepod species is the study of *Acathocyclops*. The first interpretation of invasion of *Acanthocyclops americanus* Marsh, 1892 from North America made by Lowndes (1926) could

only be confirmed by molecular studies (Miracle et al., 2013). This work showed that specimens of *A. americanus* from Europe and America were genetically very close, sharing haplotypes, but differed greatly from other morphologically closely-related European species of the genus (*A. robustus robustus* (Sars G. O., 1863) and *A. vernalis robustus* (Sars G. O., 1863)). Synonymization of *A. americanus* with *A. robustus*, including the recent description of species that are junior synonyms of *A. americanus*, has caused a lot of confusion. Again it could be only clarified with molecular genetic analyses. Indeed, recent molecular genetic studies on other freshwater zooplanktons, such as cladocerans and rotifers, have also demonstrated that many species thought to be cosmopolitan are possibly cryptic with restricted distributions (Elías-Gutiérrez & Valdez-Moreno, 2008; García-Morales & Elías-Gutiérrez, 2013; Karanovic, 2015).

These techniques have only recently been applied to different groups of aquatic organisms (Mineur et al., 2012). Even though their potential has been highlighted (Blanchet, 2012), especially in species difficult to identify (Porco et al., 2014), their use still is in preliminary or in experimental phases.

It is important to note that for future identifications it is not necessary to get a full 640-bp barcode, as a mini-barcode of about 100 bp can provide species identification with 90% accuracy (Meusnier et al., 2008). The cost of and time needed for these mini-sequences is quite low (Ivanova et al., 2009). In the short term, with the popularization of second-generation sequencing, routine environmental barcoding will be a reality, allowing the discovery of exotic fauna before it will become widespread (Bronnenhuber & Wilson, 2013). The sequences presented here can be used as a reference to compare and accurately identify organisms of the same species from any part of the world.

The development of these tools will help to expand new techniques, such as the environmental barcodes or eDNA (Rees, 2014), which will be much more accurate methods to monitor all kinds of waters; nevertheless, the bottleneck in all these studies still is the incompleteness or lack of reliable identifications in reference databases such as BOLD or GenBank (Crocetta et al., 2015).

Finally, the consequences or ecological impacts of *M. pehpeiensis* introduction could be very important. It is well known that some species of *Mesocyclops* and other copepods are useful as a biological control for mosquitoes (*Aedes* sp.) (Soto et al., 1999; Dieng et al., 2003). *Mesocyclops pehpeiensis* is also an omnivorous tactile predator that naturally may influence the density and species composition of its preys (cladocerans, rotifers and dipteran larvae) (Dieng et al., 2003; Nagata & Hanazato, 2006; Sarma et al., 2013). These alien predatory cyclopids may change the zooplankton composition in their new habitats, further destabilizing

the ecosystems and opening ways for new incomers (Anufriieva et al., 2014). To date, this has not been studied to our knowledge. Thus, the next step will be to analyse the impact on the plankton community once they are introduced in a new site.

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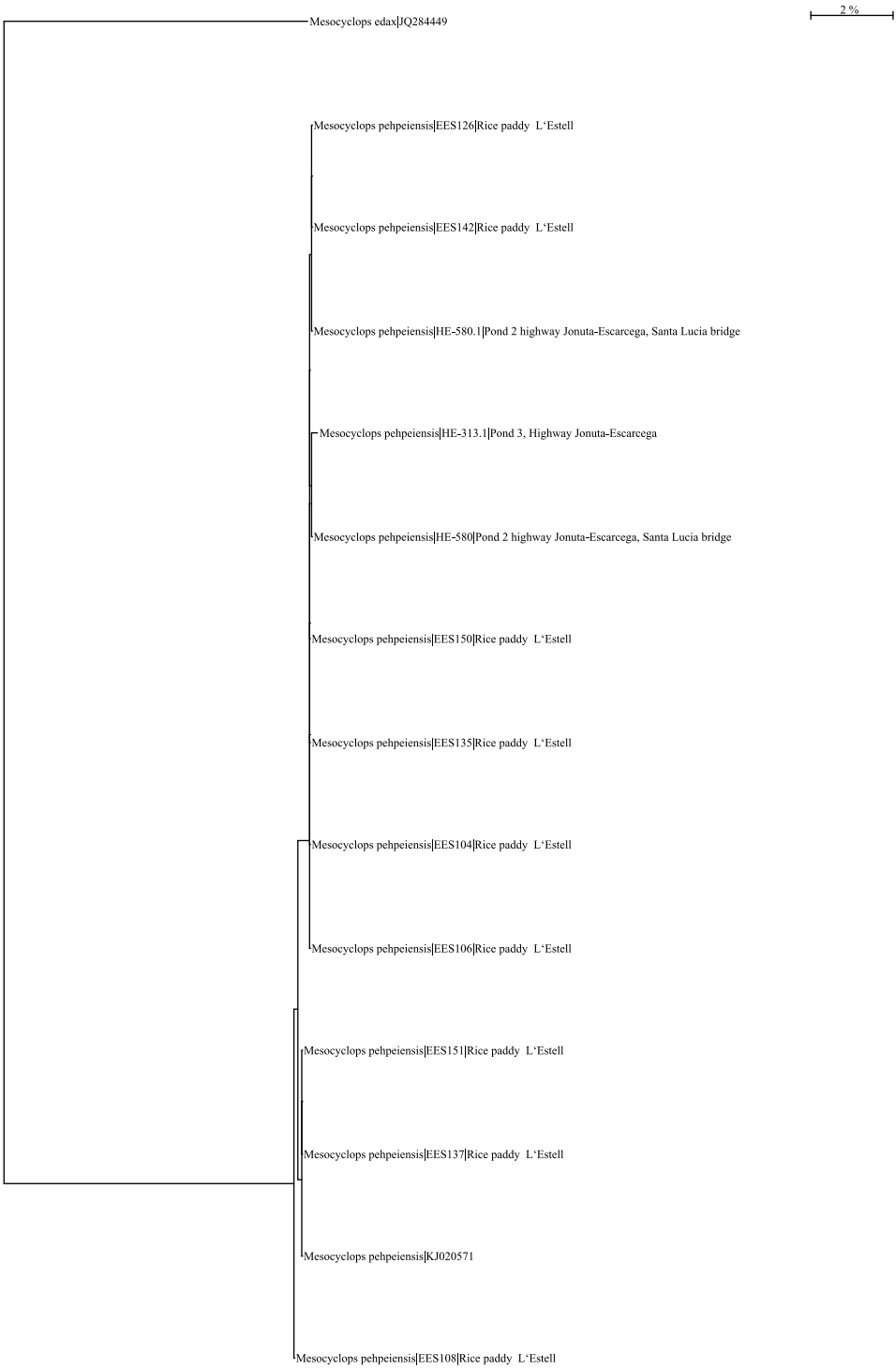
APPENDIX

ID tree from BOLD tools from Sequence Analysis link (see http://www.boldsystems.org, project MESO; Mesocylops)

Bold TaxonID tree

```
Title           : SEARCH: Tax(Mesocyclops),
                  Include public records [SEARCH2]
Date            : 16-October-2015
Data Type       : Nucleotide
Distance Model  : Kimura 2 Parameter
Marker          : COI-5P
Codon Positions : 1st, 2nd, 3rd
Labels          : Site, SampleID
Filters         : Length > 200
Colorization    : [blue]=Stop Codons
                  [red]=Contamination or misidentification
Sequence Count  : 14
Species count   : 2
Genus count     : 1
Family count    : 1
Unidentified    : 0
```


2 %
Mesocyclops edax|JQ284449
Mesocyclops pehpeiensis|EES126|Rice paddy L'Estell
Mesocyclops pehpeiensis|EES142|Rice paddy L'Estell
Mesocyclops pehpeiensis|HE-580.1|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge
Mesocyclops pehpeiensis|HE-313.1|Pond 3, Highway Jonuta-Escarcega
Mesocyclops pehpeiensis|HE-580|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge
Mesocyclops pehpeiensis|EES150|Rice paddy L'Estell
Mesocyclops pehpeiensis|EES135|Rice paddy L'Estell
Mesocyclops pehpeiensis|EES104|Rice paddy L'Estell
Mesocyclops pehpeiensis|EES106|Rice paddy L'Estell
Mesocyclops pehpeiensis|EES151|Rice paddy L'Estell
Mesocyclops pehpeiensis|EES137|Rice paddy L'Estell
Mesocyclops pehpeiensis|KJ020571
Mesocyclops pehpeiensis|EES108|Rice paddy L'Estell

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CAPÍTULO III. *Moina macrocopa,* Straus 1820

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minthes. We performed a phylogenetic placement of the clustered operational taxonomic units (OTUs) from the different environmental datasets in order to retain sequences with interesting phylogenetic position within, or close to, Platyhelminthes. We then used these sequences to reconstruct the phylogeny of the phylum. Furthermore, we combined our phylogenetic results with data of abundance and other ecological parameters to assess the worldwide distribution of the different clades within Platyhelminthes. **Significance:** To our knowledge, this is the first effort to compile such a rich and diverse dataset in order to address the question of hidden diversity within the phylum Platyhelminthes. Apart from the well-studied planarians, polyclads, and neodermatans, very little is known about the rest of the flatworm orders that are usually collectively referred to as "microturbellarians". We expect not only to better understand the diversity patterns of all Platyhelminthes but to also unravel previously undescribed free-living clades, to gain a better understanding of the microturbelarian "dark matter" and to make inferences of their ecology.

DNA barcoding for identification of small-sized beetles from steppe areas of the Republic of Moldova

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Background: Steppes in the Republic of Moldova are part of the unique Eurasian steppe ecosystem, supporting a rich flora and fauna, and providing invaluable ecosystem services. Steppes are little involved in the network of protected areas, and their degradation continues. Insect communities of the steppes are poorly known. Particularly, identification of small-sized beetles represents a major challenge. For many taxonomic groups, DNA barcoding proved to be a useful, standardized tool for species identification. **Results:** This study is the first attempt to reveal the small-sized beetle diversity of the steppe areas of the Republic of Moldova using the DNA barcoding tool for identification. DNA was extracted from 95 specimens and depending on the size of the sampled individual, one or two legs were used. Voucher specimens are deposited in the collection of the Entomological Museum, Institute of Zoology of the Academy of Sciences of Moldova. COI sequences were obtained from 77 specimens, of which 61 (79%) were barcode compliant. Based on the Barcode of Life Data System (BOLD), 39 (51%) specimens were identified at species level (18 species), 34 (44%) at genus level (15 genera), and 4 (1%) were not assigned to a lower-level taxon. **Significance:** The current study indicates that the DNA barcoding tool is effective for identifying species of small-sized beetles and facilitates the discovery of new beetle species. Obtained sequences will contribute to extending the reference library of Coleoptera. The availability of barcode data will help to solve taxonomic confusion and reduce the time needed for species identification.

Construction of a baseline for zooplankton from the biggest karstic sinkhole in the south of Yucatan Peninsula (Mexico)

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Background: Even though DNA barcoding has become extremely popular since Hebert's proposal in 2003, until now, there has not been a single baseline created for zooplankton in any freshwater system in the world. This is due to the fact that zooplankton species are often hard to barcode and that sampling methods have not changed in the past 100 years. In this study, we established a baseline of the Cenote Azul using new sampling methods and one single set of primers (Zplank) for all groups collected. **Results:** The Cenote Azul is a karstic, oligotrophic system that is 74 m deep and 200 m in diameter without a littoral zone. We used a combination of plankton nets (50 and 300 μm mesh size) and light traps of our own design for collections. We registered a total of 40 taxa including 4 cladocerans, 4 copepods, 2 ostracods, 1 palaemonid, 2 fish larvae, 2 rotifers, 1 isopod, 1 bivalve, 7 chironomids, and 13 arachnids. We believe that this number will increase with additional sampling. At least half of all the species that we found have not been registered in the Barcode of Life Data System (BOLD) or in previous species lists from this location. There was a significant difference in the number of taxa collected with the plankton nets and with the light traps. Only eight taxa were collected in the nets, while almost all the taxa recorded were present in the light traps. **Significance:** The results of this study demonstrate that light traps were an effective method for a rapid evaluation of zooplankton in this system. Combining DNA barcoding and next-generation methods will enable us to perform rapid evaluations to determine the conservation status of these aquatic systems. For this reason, we believe that it is fundamental to first elaborate species baselines in these ecosystems.

Moina macrocopa: another complex of species in a common Cladocera

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Background: The genus *Moina* encompasses many complexes of sibling species with worldwide distributions as in the case of *Moina micrura*, *Moina brachiata*, and the target of this study, *Moina macrocopa*. Many of these species have been described in Europe during the 19th century, and the material used for these descriptions is lost. Most of the keys for identification all over the world are based on a single species, usually European in origin, leading to large confusion as to their real identity. **Results:** Molecular analyses based on COI demonstrate that *M. macrocopa* is a complex of at least two species. Moreover, we found *M. macrocopa macrocopa*, the European species, in a temporal pond in Calderitas, México, living in sympatry with some congeners of the *M. micrura* complex. The COI gene shows a mean divergence of 7.04% between the American *M. macrocopa americana* and the European *M. macrocopa macrocopa* sequences. Morphological analyses and molecular results indicate that they are clearly distinct species. **Significance:** The significance of this study is to reorder one of the most common cladocerans in the world, belonging to the complex *M. macrocopa* and demonstrates that it is possible to distinguish them. This study reaffirms that an integrative taxonomical approach is necessary and useful to delimit species.

Key limitations to aquatic eDNA metabarcoding: a cautionary case study from a diverse public aquarium

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Background: Environmental DNA (eDNA) and DNA metabarcoding techniques have been widely touted as powerful new tools for monitoring biodiversity in aquatic ecosystems. However, these techniques are still in their infancy and require thorough validation because there are still several key uncertainties surrounding eDNA metabarcoding. These uncertainties include both methodological and analytical limitations that must be addressed before there is wider adoption of eDNA metabarcoding in biodiversity monitoring. In this study, we assess both the ability for eDNA metabarcoding to capture biodiversity in a highly diverse closed system at the Ripley's Aquarium of Canada in Toronto, Ontario, as well as address a number of knowledge gaps pertaining to eDNA metabarcoding to open a discussion on current issues limiting this tool. **Results:** This study found that eDNA metabarcoding recovered 62 of 107 (58%) target species and 30 of 44 (68%) target genera from a closed system when using a multimarker (COI, 16S, 12S) approach. Additionally, individual markers showed great disparity in off-target identification noise, with COI pro-

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1 ***Moina macrocopa* (Straus, 1820): another complex of species in a common Cladocera,**
2 **highlighted by morphology and DNA barcodes**

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12

13 Abbreviated title: *Moina macrocopa* complex

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RESUMEN

***Moina macrocopa* (Straus, 1820): otro complejo de especies en un cladócero común, resaltado por morfología y códigos de barras de ADN**

Los microcrustáceos de aguas continentales son uno de los grupos más diverso y menos estudiado del reino animal. Un enfoque integrativo, que incluya al menos, datos morfológicos, moleculares y geográficos, es esencial para delimitar las especies de estos invertebrados, como es el caso del complejo *Moina macrocopa*. En este estudio, utilizamos tres tipos de caracteres: morfológicos, genéticos (códigos de barras del ADN) y geográficos, para demostrar que *Moina macrocopa* (Straus, 1820), el clado del Viejo Mundo, considerada aquí como *M. macrocopa* s.l., y *Moina macrocopa americana* (Goulden, 1968), el clado americano, son especies distintas, no subespecies. Además, confirmamos que *Moina macrocopa* s.l. es un complejo de especies, formado por un mínimo de 3 clados diferentes.

Palabras clave: zooplancton, *Moina macrocopa*, especies crípticas, taxonomía integrativa, Códigos de Barras de la Vida, COI

ABSTRACT

***Moina macrocopa* (Straus, 1820): another complex of species in a common Cladocera, highlighted by morphology and DNA barcodes**

Freshwater microcrustaceans are one of the most diverse and understudied groups of the animal kingdom. The use of an integrative approach, including morphological, molecular and geographical data at least, is essential to delimitate the species of these invertebrates, as the case of the Cladocera *Moina macrocopa* complex. In this study, we demonstrate, with three different types of characters, including detailed morphological analyses, DNA barcodes (COI gene) and geographical distribution to study *Moina macrocopa* (Straus, 1820), the Old-World clade, here considered as *M. macrocopa* s.l. and *Moina macrocopa americana* (Goulden, 1968), the American clade. We found that they are different species, not subspecies. Moreover, we confirm that *Moina macrocopa* s.l. is a complex of species, formed by minimum three different clades.

Key words: zooplankton, *Moina macrocopa*, cryptic species, integrative taxonomy, DNA Barcodes, COI

INTRODUCTION

Moina is one of the most confusing genera of cladocerans and is the second most diverse Anomopod (Goulden, 1968; Elías-Gutiérrez *et al.*, 2008a; Nédli *et al.*, 2014; Bekker *et al.*, 2016). *Moina macrocopa macrocopa* (Straus, 1820) was drawn for the first time by Joblot in 1754 who referred to it as "pou aquatique" (water flea) (Joblot, 1754). Late, after 65 years, in 1819, the French zoologist Hercule Eugène Straus-Durckheim illustrated this species again, and finally, in 1820, he described it as *Daphnia macrocopus* (Straus, 1820). After his publication, too many synonyms have been described due to the lack of details on his description and illustration (Straus, 1819, 1820; Goulden, 1968) and it was not recognized as *Moina macrocopa,* the actual name until the early 1900's (Goulden, 1968).

Goulden (1968) after his review of the genus, proposed two subspecies: *Moina macrocopa macrocopa* (Straus, 1820) as the typical form restricted to the Old World, and described *Moina macrocopa americana* limited to North America, specifically the United States. This proposed subspecies was collected from a roadside ditch, along a county road one mile east of the Cheyenne Bottoms Waterfowl Refuge in Barton County, Kansas. He found between both morphos, the American and Old World, several differences being the first clues of the non-cosmopolitanism of Cladocera suggested later by Frey (1980, 1987) and afterward presented by Bekker *et al*. (2016) on their figure 2.

It was Arevalo (1920), who cited *Moina macrocopa macrocopa* (Straus, 1820) for the first time in the Iberian Peninsula, from Gandía (Comunidad Valenciana, Spain) under the name *Moina rectrirostris var. casañi*. Then, Alonso (1996) on his revision of the Iberian Fauna, describes and illustrates *Moina macrocopa macrocopa* (Straus, 1820) from Albufera de Valencia (Comunidad Valenciana, Spain), being the only place at the Iberian Peninsula where he found this species. This

71 subspecies was reported in many different sites all over the world: in U.K, France, Norway, former
72 Czechoslovaki, Hungary, Azores, Algeria, Israel, Iran, Western Russia, Mongolia, Manchuria, Japan,
73 India, Philippines, Bolivia, Argentina, Brazil, South Korea and possibly Algeria (Goulden, 1968; Elías-
74 Gutiérrez & Zamuriano- Claros, 1994; Paggi, 1997; Petrusek, 2002; Mangas-Ramírez *et al.*, 2004;
75 Elmoor-Loureiro *et al.*, 2010; Kotov, Jeong & Lee, 2012; Rietzler *et al.*, 2014; Ghaouaci *et al.*, 2018).

76 In the case of the American subspecies, *Moina macrocopa americana* (Goulden, 1968), as
77 mentioned above, in its description was considered restricted to the United States but in 2011, it
78 was recorded in Manitoba (Canada) (Jeffery *et al.*, 2011), and in central Mexico, in reservoirs from
79 the cities of Aguascalientes and Mexico (Elías-Gutiérrez, 1995; Elías-Gutiérrez *et al.*, 2008b;
80 Prosser, Martínez-Arce & Elías-Gutiérrez, 2013) expanding its distribution area all over North
81 America.

82 This cladoceran has been mainly recorded in water bodies severely eutrophicated under heavy
83 anthropogenic influences (sewage, fertilizers). It is a species whose populations play a key role in
84 food webs of epicontinental aquatic environments (Vignatti *et al.*, 2013) and it is of economic
85 importance due to its use as live food for fishes (Valdivia-Villar, 1988; Elías-Gutiérrez, 1995) and as
86 bioindicator (Nandini *et al.*, 2004).

87 Due to the ubiquity, small size, phenotypic plasticity, genetic variability, cryptic taxa and lack of
88 taxonomical identification keys for many parts of the world, an integrative approach must be
89 made to delimitate with accuracy the species from this complex. In the other regard, since DNA
90 barcoding (cytochrome oxidase c subunit 1 – COI gene) has been proposed as fast, reliable and
91 cost-effective tool to delimitate the animal species (Hebert *et al*., 2003a). After his proposal, a vast
92 cryptic diversity has been discovered among the invertebrates (Mills *et al.*, 2016; Chertoprud *et*
93 *al.*, 2017 and Lavinia *et al.*, 2017) and in general in all the animal groups (i.e. Álvarez-Castañeda *et*

94 *al.*, 2012; Lima *et al.*, 2017). In particular, within *Moina*, Bekker *et al*., (2016) demonstrated, with
95 DNA barcodes, a possible high cryptic diversity of the Old World *Moina macrocopa* (Straus, 1820)
96 and they did not discuss but presented a tree with differences among Eurasian clades. Because of
97 this reason, we will call all specimens from the Old World as *M. macrocopa* s.l. (sensu lato).

98 Other authors proved the value of DNA Barcoding for the early detection of exotic species in many
99 groups (Valdez-Moreno *et al.*, 2012; Gutiérrez-Aguirre *et al*., 2014; Montoliu *et al*., 2015). Finally,
100 the biogeographical signal of this gene shows a high correspondence between haplotypes and
101 restricted distributions permitting us to delimitate with accuracy the species (Mills *et al.*, 2016).

102 In this work, we studied *Moina macrocopa* complex using morphological, genetical (DNA
103 Barcodes) and biogeographical analyses to re-describe *Moina macrocopa americana (*Goulden,
104 1968) and demonstrate that it is a distinct species, not a subspecies, of *Moina macrocopa* s.l.

105 **MATERIAL AND METHODS**

106 **Sampling**

107 Specimens of *Moina macrocopa* s.l. and *Moina macrocopa americana* Goulden, 1968 were
108 collected with a plankton net with mesh size of 45 µm, and a hand net with mesh size of 90 µm,
109 from Albufera Lake in Valencia (Spain), a small pond in Calderitas (Quintana Roo, Mexico), Texcoco
110 Lake (Mexico State, Mexico), Los Gringos dam and Niagara dam (Aguascalientes, Mexico) (see
111 table S1, available at http://www.limnetica.net/en/limnetica). All samples were preserved in 96%
112 alcohol (non-denaturated) following the procedure suggested by Prosser *et al*. (2013) and stored
113 in the freezer a minimum of 72h, to preserve the DNA.

114 **Morphological observations**

115 Specimens were sorted from the ethanol samples under a stereomicroscope and placed in a drop
116 of glycerol. Several females and males were dissected. Whole animals and dissected sections were
117 examined and measured under a compound microscope. The organisms were morphologically
118 identified following descriptions by several authors, i.e., Straus (1820), Goulden (1968), Alonso
119 (1996), Paggi (1997), Elías-Gutiérrez *et al.* (2008b). Some specimens were prepared for Scanning
120 Electron Microscopy (SEM) for the observation of microcharacters. We made part of the images in
121 a JEOL microscope Model JSM6010 Plus at Chetumal Unit of ECOSUR at 10 KV and the other part
122 in a JEOL microscope Model LB5900 at 12 KV in the Autonomous University of Aguascalientes.

123 Mexican specimens are deposited in the reference collection of El Colegio de la Frontera Sur
124 (ECOSUR) Chetumal unit, in Mexico, and Spanish ones, are in the collection of Freshwater
125 zooplankton at the Institut Cavanilles de Biodiversitat i Biologia Evolutiva (University of Valencia,
126 Spain).

127 **Molecular markers**

128 COI gene was selected as molecular marker for this study for its fast-evolving mitochondrial
129 protein-coding genes, which reflect the evolutionary history of invertebrate populations
130 (Audzijonyte & Väinölä, 2006). Also, it is quite used to discriminate cladoceran species, becoming
131 in these last years, a standard to compare the fauna to species level (Machida *et al.*, 2004;
132 Dasmahapatra *et al.*, 2010; Gutiérrez-Aguirre, Cervantez-Martínez & Elías-Gutiérrez, 2014;
133 Montoliu, Miracle & Elías-Gutiérrez, 2015), and has been used as a model in species delimitation
134 (Puillandre *et al.*, 2012).

135 **DNA isolation, PCR amplification, and sequencing**

136 DNA was extracted using AcroPrep 96 Filter Plate 3 µm GF/ 0.2 µm BioInert (Pall Corporation, Port
137 Washington, NY, USA) according to the manufacturer's instructions. The Polymerase Chain

Reaction (PCR) was used to amplify approximately 600-658 bp of the COI gene using Zplank primers suggested by (Prosser *et al.*, 2013). The 12.5 µl PCR reaction mixes included 6.25 µl of 10% trehalose stabilizer, 2 µl of ultrapure water, 1.25 µl of 10X PCR buffer, 0.625 of MgCl2 (50 mM), 0.125 µl of each primer (0.01 mM), 0.0625 µl of each dNTP mix (0.05 mM), 0.625 µl of Taq polymerase (5 U/µl) (New England Biolabs or Invitrogen), and 2.0 µl of DNA template. Thermocycler program was as follows: 94°C for 1 minute, 5 cycles of 94°C for 40 seconds, 45°C for 40 seconds, 72°C for 1 minute, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 51°C for 40 seconds, elongation at 72°C for 1 minute, and a final extension at 72°C for 5 minutes, with a final hold at 4°C. The PCR products were visualized on a 2% agarose gels (E-gel® 96 system, Invitrogen Inc.). Amplicons were bidirectionally sequenced using BigDye Terminator Cycle Sequencing Kit (v3.1) on an ABI 3730XL DNA Analyzer. The forward and the reverse sequences were assembled, edited and aligned using CodonCode Aligner v. 5.0.1 (CodonCode Corporation, USA) and tested in MEGA (V_6.0) to verify that they were free of stop codons and gaps.

We prepared a dataset under the name DS-MMACRO with all specimens and sequence information in the Barcode of Life (BOLD). It includes all sequences generated in this study and all public sequences available and previously published, including those in GenBank. In particular, we also used the sequences of *M. macrocopa* (Straus, 1820) published by Elías-Gutiérrez *et al*. (2008a).

Sequence analysis

COI sequences obtained in this study were combined with those for *M. macrocopa* s.l. and *M. macrocopa americana* (Goulden, 1968) available in GenBank and BOLD (see table S1, available at http://www.limnetica.net/en/limnetica) to corroborate the identity of our sequences and to gain a

161 better understanding of the geographical patterning of the genetic diversity for both, *Moina*
162 *macrocopa americana* and *Moina macrocopa* s.l.

163 COI sequences were aligned with BOLD aligner (Amino Acid based HMM) and the non-overlapping
164 sequence regions at the 5' - and 3' -ends were trimmed; then sequences were downloaded for
165 further analyses.

166 As the first approach, genetic divergence between groups was calculated using K2P model
167 (Kimura, 1980) with pairwise deletion of gaps and missing data.

168 To perform the Maximum Likelihood (ML) tree distances, a best-fitting model of nucleotide
169 substitution was selected in MEGA, based on the likelihood for 24 different nucleotide substitution
170 models and the Akaike information criterion (Posada & Buckley, 2004). The best model was
171 Tamura 3-parameters with a specific fraction of sites evolutionarily invariable (T92+I) (Tamura,
172 1992). The analyse was performed using 500 bootstraps, and partial deletion of gaps and missing
173 data. Finally, as an outgroup, a sequence of *Moina* cf. *micrura 2* from Mexico was used (See table
174 S1, available at http://www.limnetica.net/en/limnetica). The subtree for each group was collapsed
175 with the "compress/ expand subtree" function on MEGA software. Monophyly was confirmed with
176 the web service "Monophilizer" (Mutanen *et al.*, 2016).

177 *Delimitation of M. macrocopa s.l. and M. macrocopa americana complex groups by different*
178 *clustering algorithms*

179 Molecular operational taxonomic units (MOTU's) have frequently been used to infer putative
180 species boundaries where morphological identifications are difficult (Ashfaq *et al.*, 2015). To
181 assess the presence of cryptic taxa, we implemented four different clustering algorithms to assign
182 the COI sequences from *M. macrocopa* s.l. *and M. macrocopa americana* complex species to
183 MOTU's: the Refine Single Linkage algorithm (RESL, Barcode Index Number, or BIN)

184 (Ratnasingham & Hebert, 2013), Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.*,
185 2012), multi-rate Poisson tree processes (mPTP) (Triantafyllidis *et al.*, 2011) and statistical
186 parsimony networks (TCS)(Clement *et al.*, 2002) were used.

187 The BIN system uses the RESL algorithm to reach decisions on the number of MOTU's in a
188 sequence dataset through a three-phase analysis based on, sequence variation with a 2.2%
189 threshold of maximum divergence allowed within a cluster.

190 ABGD employs a multi-phase system which initially divides sequences into MOTUs based on a
191 statistically inferred barcode gap (i.e., initial partitioning), and subsequently conducts additional
192 rounds of splitting (i.e., recursive partitioning). It is a statistical method that explores the
193 distribution of all pairwise distances looking for the gap between intra- and interspecific distances.
194 An online version of ABGD (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.htm) was
195 employed using default parameters and K2P as the distance metric.

196 The mPTP is a multi-rate Poisson tree processes for single-locus species delimitation under
197 maximum likelihood and Markov chain Monte Carlo (Kapli *et al.*, 2017). For this analysis, also an
198 online version was used (http://mptp.h-its.org/#/tree).

199 Finally, TCS applies the statistical parsimony method to construct haplotype networks. It has been
200 shown that the count of Linnaean species present in a COI alignment greatly matches that of
201 independent statistical parsimony networks inferred by the software. A nexus file was generated
202 to calculate a TCS network (Clement *et al.*, 2002) in PopART (http://popart.otago.ac.nz) (Leigh &
203 Bryant, 2015). This Interface permits to visualize in a graph the network of relationships between
204 the haplotypes and geographical distributions.

205 *Genetic diversity analysis*

206 The alignments in FASTA format were imported into DnaSP v5 to perform haplotype diversity
207 analyses and verify results obtained by TCS network (Librado & Rozas, 2009).

208 **RESULTS**

209 **Morphological observations**

210 Family Moinidae Goulden, 1968

211 Genus *Moina* Baird, 1850

212 *Moina macrocopa* s.l. (Fig. 1A-P)

213 Type locality: unknown, Europe. Considering that the majority of the species described by Straus
214 where from France, as *Daphnia magna* Straus, 1820 described from Paris in the same publication,
215 and the proximity among France (*terra typica*) and Spain. We consider that the population shared
216 between Spain, Russia and Calderitas (Mexico) corresponds to the real *Moina macrocopa* s. str.
217 (Straus, 1820).

218 Material examined: from a rain pond in Calderitas (Quintana Roo state, Yucatan Peninsula,
219 Mexico) (18.6308 N; 88.2251 W) probably recently introduced, and from a rice field of Albufera de
220 Valencia Lake (Spain) (39.296 N; 0.316 W).

221 <u>Parthenogenetic female</u>

222 Size: Mean: length 1.3±0.103 mm; wide 81±0.08 mm; ratio: 0.656±0.008 mm (N=10). Color of
223 specimens: brownish.

224 The Mexican (from Calderitas) and Spanish specimens' features, of this European form (*Moina*
225 *macrocopa* s.l.), agrees with those of the typical form (Fig. 1). The head and shell surface are
226 covered with scattered long hairs, the head broad with a slightly supraocular depression (Fig. 1A,

arrow) and there is an oval "nucal pore" close to the dorsal end of the head (illustrated in *Moina americana*, Fig. 2B,C), as the Argentinean and Spanish specimens studied by Paggi (1997) and Alonso (1996) respectively, but not mentioned by Goulden (1968) and it is possibly the most significant character of the complex because it has not been reported in other congeners as i.e. *Moina micrura*.

The shell is deeply reticulated (visible at SEM) with hairs appearing on the border of the polygons. The hairs are denser at the anterior part of the shell and disappear at the dorsal part. The number of setae of the ventral rim of the shell ranges from fifty-five to seventy (Fig. 1D,E), the posterior edge has a submarginal, continuous row of fine spinules (Fig. 1E,F), and at the dorsum there is a pair of hooks, one on each valve (Fig. 1G). The setae of the anterior rim are slightly feathered.

The antennule is cylindrical (mean length: 0.214 mm; mean ratio width/length: 0.22), ciliated, slightly curved on the tip and in its surface has many short rows of spinules around it (Fig. 1B). At the tip of the antennule, there are nine aesthetascs (only eight visible at Fig. 1C) with flat tip and without projections as in *Moina micrura*. The sensory seta is long, but not as long as Goulden (1968) and Paggi (1997) remarked, and it is inserted in the mid-point of the antennule.

The antennae are robust, covered with many rows of spinules. Both rami have medial rows of long and fine setulae and the usual distribution of swimming setae (1-1-3/0-0-1-3) and spines (0-0-1/0-1-0-1). The sensory setae of the basipodite are approximately equal in length, about one-third the length of the segment. The basipodite covered by a row of spines parallel to it, and one stout spine at the tip (Fig. 1H).

The first trunk limb has the setation pattern common to most species of Moina (3-2-2-3), eight feathered setae, two toothed setae, and two ejector hooks (Fig. 1I). The anterior seta of the penultimate segment has a row of stout and distal teeth that may be longer that the seta's width.

(Fig. 1I). The teeth on the seta of the last segment are much smaller but higher in number and closer than the penultimate tooth seta.

Limb II with the two setae of the proximal group of the gnathobase of the same size as Alonso (1996) remarked. We did not consider it as a significant taxonomical feature because it is similar in other congeners and does not present any variation in the studied populations.

In Limb IV Alonso (1996) cited that the setae of the filter plate are less and more separated than the other congeners, but we did not find any significant difference with other congeners i.e. populations of *Moina micrura* complex (pers. obs.).

The postabdomen is long and robust with many short rows of spinules at the dorsal and lateral sides (Fig. 1J) with two biarticulated natatory setae with long hairs at the distal part (Fig. 1K). The conical distal part of the postabdomen carries 7-10 feathered teeth, as Goulden (1968) described instead of 9-11 teeth cited by Paggi (1997), and one bifid tooth (Fig. 1L). The branches of the bifid tooth are distinctly unequal. There are some scattered tiny spinules near the base of the bifid tooth. The inner side of the claw has a continuous row of fine setae a little thicker than those on the outer side. On the ventral base, there is a pecten with seven teeth (Fig. 1L) only visible at SEM .

Ephippium

The surface of the ephippium is covered with thick rounded cells and contains two eggs (Fig. 1N-P). It is sub rectangular in lateral view (fig. 1N).

Male (Fig. 1Q-U)

Size: 0.52 mm (N=1). Color of specimens: brownish.

The body male and head are covered with hairs (Fig.1Q,R). These are longer and denser, on the body than in the head. The head lacks the supraocular depression.

The long antennules originate below the eye, are bent at the mid-point and have two sensory setae arising at or near the knee of this bend (Fig. 1S). The distal halves of the antennules are curved inward and have four to six short hooks at the tip. The hooks form a semicircle around a group of sensory papillae that project from the end of the antennule.

The surface of the shell is reticulated and covered with hairs, as mentioned above. The ventral rim has from thirty-five to forty marginal setae. The shorter spines along the posterior margin of the shell are ungrouped and scattered as in the female (Fig. 1T).

The first leg of the male has a long, recurved hook originating from the penultimate segment (Fig. 1U). The terminal segment carries three setae; the middle seta is long and hook-like. The other two setae are feathered. The penultimate segment covered with many short hairs along the medial margin, and there is a seta arising from this surface opposite the hook. The first leg has an exopod segment that terminates with a very long seta which is longer than the leg and reaches to the posterior margin of the shell. This seta is usually bent ventrally and projects well beyond the ventral margin as Goulden described in his review (Goulden, 1968).

The postabdomen and the claw of the male, are similar in general form, to that of the female. However, the conical part of the postabdomen is much broader, and the claw projects from the middle of the distal margin of the postabdomen (Fig. 1V).

Biology: In Spain, this species has only appeared in the Albufera of Valencia, and related water bodies (Arévalo, 1920; Alonso, 1996) as the rice fields at the south part of the lake. In these rice fields, the quality and stationarity of the water mass depend on the rice cultivation cycle. During the summer waters are transparent, but loaded with organic matter from rice fertilizers, shallow (about 25 cm depth) and come from Jucar River, so they are always renewing. In Calderitas

294 (Mexico), we collected the specimens possibly recently introduced, from a temporary pond
295 formed by the rain, with a high organic load.

296

297 *Moina americana* s.str. (Goulden, 1968) (= *Moina macrocopa americana* (Goulden, 1968))

298 Type locality: According to Goulden (1968), a roadside ditch, along a county road one mile east of
299 the Cheyenne Bottoms Waterfowl Refuge in Barton County, Kansas (USA). Cheyenne Bottoms is
300 the largest marsh in the interior of the United States, deeply modified by man, was designated a
301 Wetland of International Importance in 1988 by the Ramsar Convention on Wetlands. The area is
302 considered the most important shorebird migration point in the western hemisphere.
303 Approximately 45 percent of the North American shorebird population stops at the Bottoms
304 during spring migration.

305 Sequenced specimens from terra typica where collected in a pond in Denver (40.73 N, -104.40W).

306 Type specimens of this subspecies are in the United States National Museum (Catalogue Numbers
307 Holotype 123203; Paratypes 123204).

308 Material examined: Texcoco Lake (State of Mexico, Mexico) (19.45 N, -99.00 W) and Los Gringos
309 dam (Aguascalientes, Mexico) (21.91 N, -102.26 W).

310 *Differential diagnosis* (see Table 1)

311 *Moina americana* can be easily distinguished from *Moina macrocopa* s.l. for the following
312 characters: the sensory seta of the antennule is longer, and it is inserted in the first third (Fig. 2D);
313 the setae on the posterior rim formed three groups increasing in size (Fig. 2D), following these
314 setae at the dorsal part, there is a row of stout and robust spinules instead of fine spinules as is
315 present in *Moina macocopa* (Fig. 2I); the first trunk limb presents the anterior seta of the

316 penultimate segment with a row of stout teeth, smaller and more numerous than in *M.*
317 *macrocopa* s.l. (Fig. 2J). The ephippium is bigger than *M. macrocopa*, its surface covered with
318 trapezoidal cells instead of round and it seems a water drop with the point slightly curved upwards
319 (Fig. 2O). The male's first leg has a very large recurved hook, originating from the penultimate
320 segment more prominent than in the Old-World form and presents several tiny spinules on the tip
321 (Fig. 3F).

322 *Parthenogenetic female* (Fig. 2A-L)

323 Size: Length 1.14±0.05 mm; width 0.83±0.05 mm and ratio: 0.729±0.005 (N=10). Color of
324 specimens: whitish. In life sometimes red, due to the presence of hemoglobin.

325 The revised material agrees with the typical form described by Goulden (1968): the setae along
326 the posterior shell rim grouped, some being much larger than the others and there are usually two
327 or three groups with large teeth located just posterior to the ventral row of setae.

328 Long and dense hairs cover the body and head, more than the European clade (Fig. 2A,B). The
329 head presents a broad and slightly supraocular depression and an oval "nucal pore", cited by
330 Alonso (1996) and Paggi (1997) in the Old World form but never cited before for American form
331 (Fig. 2B,C). Hairy labrum covered with many scatered fine and long hairs, the proximal part very
332 bulky (Fig. 2F).

333 The shells are sub rectangular and rounded in lateral view (Fig. 2G), with a row of stiff feathered
334 marginal setae (Fig. 2H). The setae of the posterior rims are grouped and ordered in increasing in
335 size (Fig. 2I) followed by a continuous row of fine spinules.

336 The antennule is cylindrical, shorter than the observed in *M. macrocopa* s.l. (mean length: 0.25
337 mm; mean ratio width/length: 0.188 mm), ciliated, slightly curved on the tip and in its surface

present long rows of spinules around it (Fig. 2D,E). The sensory seta is also longer than the European specimens analyzed and is inserted in the first third of the antennule (Fig. 2D).

The antennae are robust, covered with many rows of spinules. Both rami with medial rows of long and fine setulae and the usual distribution of swimming setae (1-1-3/0-0-1-3) and spines (0-0-1/0-1-0-1) (Fig.2A, H). The sensory setae of the basipodite are approximately equal in length, about half the length of the segment. The basipodite covered by a row of spines parallel to the segment, and one stout spine at the tip. (Fig. 2A).

The first trunk limb presents eight feathered setae, two toothed setae, and two ejector hooks of different size. The anterior seta of the penultimate segment has a row of stout teeth, smaller and more numerous than in *M. macrocopa* s.l. (Fig. 2J).

The postabdomen is similar to the Old World specimens but more hairy (Fig. 2K) It is very long and robust with many short rows of spinules at the dorsal and lateral sides. The conical distal part carries nine feathered teeth and one bifid tooth (Fig. 2L). The branches of the bifid tooth are distinctly unequal. There are small groups of spinules near the base of the bifid tooth. The inner side of the claw has a continuous row of fine setae a little thicker than those on the outer side. On the ventral base, there are thirteen teeth (Fig. L).

Ephippium

The surface of the ephippium composed of flat cells that are more trapezoidal then squared in cross section and contains two eggs. It is bigger than in *Moina macrocopa* s.l. and seems like a water drop with the point slightly curved upwards (Fig. 2M,N).

Male (Fig. 3A-L)

The males mean size: length 0.92 mm (N=10).

The body male and head are hairy. The shell is deeply reticulated, the setules grow, mainly, in the contours of these polygons. The head lacks the supraocular depression (Fig. 3A,M) but also presents reticulation on dorsal view.

The long antennules originated below the eye, they are bent at the mid-point and have two sensory setae arising at or near the knee of this bend (Fig. 3D). The distal halves of the antennules are curved inward with a row of tiny spinules on the tip and four to six short brush-like setae (Fig. 3E). The brush-like setae form a semicircle around a group of sensory papillae that project from the end of the antennule (Fig.3D).

The second antennae are robust, covered with many rows of spinules. Both rami with middle rows of long and fine setulae and the general distribution of swimming setae (1-1-3/0-0-1-3) and spines (0-0-1/0-1-0-1). The sensory setae of the basipodite are approximately equal in length, about one-third the length of the segment. The basipodite covered by a row of spines parallel to the segment, and one stout spine at the tip. (Fig. 3G).

The surface of the shell is reticulated and covered with hairs, as mentioned above (Fig.3A). The ventral rim has from 34 to 43 marginal setae. The setae along the posterior margin of the shell are grouped and ordered in increasing in size (see 1 in Fig. 3H) followed by a continuous row of fine spinules (Fig.3I). At the dorsal rim, there are some stiff spinules, stronger than in the European form, followed by two hooks (Fig. 3J).

The first leg of the male has a large recurved hook, originating from the penultimate segment, bigger than Old World specimens (Fig 3F). The terminal segment carries three setae; the middle seta is very long and hooklike without spinules on the tip (see a in Fig. 3F) as in other congeners as *Moina micrura*. The other two setae are feathered. The penultimate segment covered with many grouped short hairs along the medial margin, and there is a seta arising from this surface opposite

383 the hook. The first leg has an exopod segment that terminates with a very long seta which is
384 longer than the leg and reaches to the posterior margin of the shell and presents several little
385 teeth on the tip (see b in Fig. 3F). These latter are absent in *M. macrocopa* s.l.

386 The postabdomen and the claw of the male, are similar in general form to that of the female.
387 However, the conical part of the postabdomen is much broader, and the claw projects from the
388 middle of the distal margin of the postabdomen (Fig. 3K). The two genital openings are just at the
389 end of the feathered teeth row, in lateral view, one on either side of the postabdomen not as in
390 *Moina macrocopa* s.l. where they are ventral to the claws (see arrow and magnification in Fig.3L).

391 Biology: Los Gringos dam is in central highlands of Mexico, in the city of Aguascalientes (21.91 N;
392 102.268 W). The waters of this dam are highly eutrophicated due to the fact that it received the
393 sewage water from the nearby neighborhood. Texcoco Lake is in the east of Mexico City (19.452
394 N; 98.995 W), located at 2.236 meters above the sea level in the TransMexican volcanic belt
395 (Alcocer & Bernal-Brooks, 2010). This saline soda-lake is highly polluted and eutrophicated and
396 supports an active anthropogenic pressure since the arrival of the Aztecs (Alcocer & Bernal-
397 Brooks, 2010).

398 Notes

399 We have found morphological differences between the two populations of the European clade
400 inhabiting in Calderitas, as the spinulation pattern of the posterior shell rim and the antennule,
401 which have direct correspondence with genetical results commented later. But to describe this
402 possible new species, further genetical and morphological comparative studies must be
403 done.Alcocer, J. and Bernal-Brooks, F. W. (2010) 'Limnology in Mexico', *Hydrobiologia*, 644(1), pp.
404 15–68. doi: 10.1007/s10750-010-0211-1.

405 **Genetical analyses**

406 We created a dataset of 60 sequences under the name of DS-MMACRO in BOLD Systems database.
407 It includes 57 sequences of *Moina macrocopa* complex and three sequences of *Moina cf. micrura*
408 2, (Elías-Gutiérrez *et al.*, 2008a), used as an outgroup.

409 The "barcode identification request" in BOLD showed five different BINs. Sequences matching
410 European clade (possibly including the real *Moina macrocopa* (Straus, 1820), assigned to three
411 different BINs, BIN: BOLD:ACH4664 (population of Hungary, Russia and Mexico, Calderitas), BIN:
412 BOLD:ACA1705 (populations of Spain, Russia and Mexico, Calderitas) and BIN: BOLD:AAK6825
413 (population from European Russia). Sequences matching the American clade, including specimens
414 near the original type locality, *Moina americana* s. str. Goulden, 1968 were assigned to a unique
415 BIN: BOLD:AAC3108 and finally, sequences assigned to BIN: BOLD:ADF9261 correspond to the
416 Asiatic clade found in Russia, shown by Bekker *et al*. (2016).

417 Instead, MPTP and ABGD yield other results, three groups instead of five as Barcode Identification
418 request showed (Table 2). These three groups correspond to European clade (possibly the real
419 *Moina macrocopa* (Straus, 1820), American clade *Moina americana* Goulden, 1968 and the Asiatic
420 clade (*Moina* cf. *macrocopa*). Differences in the number of groups delimitated by BIN system and
421 the other tools (MPTP and ABGD) five and three respectively, is due that the BIN system is more
422 sensitive than the other tools. It uses a threshold of maximum divergence of 2.2% to delimitate
423 the clusters and it should be mentioned that is continuously evolving in new BINs if more
424 information is added (Ratnasingham & Hebert, 2007, 2013).

425 Kimura 2-parameters (K2P) mean intraspecific distances for each population in *Moina macrocopa*
426 s.l. *(*European clade) are: 0.41% in BOLD:ACH4664, 0% in BOLD:AAK6825 and 0.75% in
427 BOLD:ACA1705. In the case of *Moina americana* (Goulden, 1968) (American Clade, BIN number
428 BOLD:AAC3108), the mean intraspecific distance is 0.64%. (Table 3). Interspecific distances ranged

429 from 3.72% to 13.36%. The European clade showed the minimum mean interspecific distance
430 between its populations (Russian population has not been considering for this analysis, due to the
431 low number of available sequences in BOLD). The biggest mean interspecific distance is between
432 the Asiatic clade (BOLD:ADF9261) evidenced by Bekker *et al*. (2016) and the European population
433 with BIN number BOLD:ACA1705 that it is distributed in Hungary, Mexico and Russia.

434 The low intraspecific divergence showed by the American clade (BOLD:AAC3108) which includes
435 sequences from *terra typica* in North America and other places from North and South America
436 suggest the incipient expansion of this species.

437 The high divergence in the European clade indicates that *Moina macrocopa* s.l. is a complex
438 composed by at least three distinct species, two of them confirmed by morphology, genetics and
439 distribution that corresponds to BIN numbers ACH4664 and ACA1705, the last one corresponding
440 to the real *Moina macrocopa* (Straus, 1820).

441 The high divergences showed between the clades from Europe and America corroborates our
442 hypothesis that they are different species.

443 Regarding the GC content at the sequence composition (Table 4), it ranges from 34.97 to 36.59.
444 Populations from cooler regions show low GC content than the other clades.

445 *Neighbor-Joining and Maximum Likelihood analysis*

446 ML analysis of COI gene is consistent with previous studies, showing that each clade is a
447 monophyletic cluster. The branch of each cluster is highly robust (Fig. 4). We emphasize that the Id
448 tree is not a complete phylogenetical analysis. This tree delimitates five groups, but we cannot
449 ascertain in deep the relationships between these groups.

450 *Genetic Diversity and haplotype analyses*

451 Genetic diversity indexes and the results of neutrality test for COI are shown in Table 5. The
452 average number of pairwise nucleotide differences (K), nucleotide diversity (π) and haplotype
453 diversity (Hd) varies among the clades showing elevated levels of diversity inside the clades. A
454 negative Tajima's D showed by the Asiatic clade demonstrates low levels of both low and high-
455 frequency polymorphism relative to expectation.

456 In total, 18 COI haplotypes were detected including four in the American clade (from two
457 countries) (Fig. 5). The Asiatic clade includes two haplotypes from Sakhalin area (Russia), and
458 finally, the European clade contains eight haplotypes from four different regions, with the
459 dominant haplotype, shared by Hungary and Mexico (Calderitas) and the American clade also
460 shows eight haplotypes disseminated from Canada to Bolivia.

461

462 **DISCUSSION**

463 Due to the high number of characters, analyzed in this paper, (morphological, genetical
464 divergences and geographical distribution), and the differences found between *Moina macrocopa*
465 s.l. (Old World clade) and *Moina macrocopa americana* Goulden, 1968 (the American clade) and
466 following the rules of the International Code of Zoological Nomenclature (ICZN) (Jäch, 2000), a
467 taxonomical act can be made to elevate to species level the subspecies *Moina macrocopa*
468 *americana* (Goulden, 1868). So, we propose the name *Moina americana* Goulden, 1968, with a
469 BIN number BOLD:AAC3108 to facilitate its identification, in place of *Moina macrocopa americana*
470 Goulden, 1968.

471 The detailed morphological studies using tools as the Scanning microscope allowed verification of
472 some dubious characters, as the "nucal pore" and the claw pecten, not clearly visible using
473 traditional techniques. The "nucal pore" according to Alonso (1996), it is one of the most

474 significant characters of the species not seen previously in other congeners, and it did not appear
475 in the descriptions of the species of Goulden (1968) and Elmoor-Loureiro *et al.*, (2010). One other
476 variable character of the species, the faint claw pecten, has also contributed to the confusion.
477 Depending on the view of this setation on the claw, one might either conclude that a pecten is, or
478 is not, present. This accounts for the different description of *Moina banffyi* (Daday, 1883) which
479 was said to lack a pecten while *Moina esau* was described as having one (Brehm, 1936). Both
480 forms are definitely the same species. In this study we confirm the presence of these characters in
481 the *Moina macrocopa* complex species and we remark the importance of the "nucal pore" to
482 identify the species along with the anterior seta on the penultimate segment of the female's first
483 trunk limb toothed. Differences found by Elmoor-Loureiro *et al.* (2010) between South American
484 populations of *Moina macrocopa* s.l. must be confirmed by genetical analysis will allow
485 establishing their real identity of this *Moina.* In case of genetic characters, our results are
486 coincident with the morphology, and the maximum divergence threshold of 3% proposed by
487 several authors to delimitate the species level (Hebert *et al.*, 2003b; Bekker *et al.*, 2016). These
488 results also agree with the morphological differences previously published (Straus, 1820; Arévalo,
489 1920; Goulden, 1968; Smirnov, 1976; Elías-Gutiérrez & Zamuriano- Claros, 1994; Alonso, 1996;
490 Paggi, 1997; Elmoor-Loureiro *et al.*, 2010) between the American and Old World morphos, and add
491 some taxonomical remarks that should be taken into account for further descriptions of new
492 species belonging to these complexes of species. Specimens with genetic divergence lower than
493 3% do not present morphological differences, so we can conclude, that BIN numberAAC3108,
494 representing *Moina americana* Goulden, 1968 is present in Canada, USA (from *terra typica*, near
495 the original type locality, see specimens with Process ID: BCRUS111-10, BCRUS107-10, and
496 BCRUS106-10 in BOLD), and is distributed in Mexico and Bolivia. The low variability of the
497 American species, the high number of haplotypes (8 haplotypes) and the broad geographical area

498 of distribution, shown by our results, indicates that this species is beginning an expansion that
499 started less than 250.000 years ago (according to Ratnasingham & Hebert 2007).

500 The high existent divergence between the populations in the Old World can be interpreted as a
501 longer time of separation represented by the BIN numbersACH4664 andACA1705) with a
502 maximum divergence of 3.72%. This divergence also is coincident with morphological traits as
503 mentioned above.

504 The use of molecular markers helped us to delimitate species, but the sole use of these tools, will
505 cause the loss of essential information (Will, Mishler & Wheeler 2005). The phenotypical plasticity
506 present in different geographical areas for invertebrate species evidence high cryptic speciation
507 and not morphotypes or cosmopolitanism, as it has been considered (Alcántara-Rodríguez *et al.*,
508 2012; Karanovic 2015; Lavinia *et al.*, 2017).

509 This example is one more of the so-called integrative taxonomy (sensu Dayrat, 2005), but the
510 information we have until now is not enough to conclude about the Old-World clades previously
511 seen (Nédli *et al.*, 2014; Bekker *et al.*, 2016).

512 In other regard, haplotypes shared between far geographical sites evidence a recent translocation
513 of both species, *Moina macrocopa* s.l. and *Moina americana* Goulden, 1968*,* possibly due to
514 human activities such as it has been documented several times for the European clade of this
515 species and for other microinvertebrate groups by several researchers (Elías-Gutiérrez &
516 Zamuriano- Claros, 1994; Miracle *et al.*, 2013; Vignatti, Cabrera & Echaniz, 2013; Montoliu, Miracle
517 & Elías-Gutiérrez, 2015) and also, due to biotic (birds) or/and abiotic factors. It is widely
518 documented that cladocerans during the glaciations of Pleistocene survived in small isolated
519 refugia during the Ice Age. Since the thaw, they colonized many new areas due to the ephippia
520 stuck in the legs of birds (Adamowicz *et al.*, 2002; Korovchinsky, 2006), and given their

opportunistic and potentially invasive nature (Vignatti, Cabrera & Echaniz, 2013) they became common in new habitats or environments. In this regard, climate change drives birds to change their migration routes, and with them, *Moina* and other invertebrates will benefit to disperse to new warm regions, where before it was not possible to survive.

The proximity existent (morphological, genetical and geographical) between the different populations of the American clade and the lack of interest in this American species (because the European species is used as bioindicator and food for aquaculture) suggest that their distribution can be due to biotic or abiotic factors and not for an anthropogenic translocation.

Instead, the Old-World species found in Calderitas (Mexico) seems to be a recent human mediated translocation owing to the little divergence intraspecific existent between the Mexican populations and the Europeans (ACH4664: 0.41% and ACA1705: 0.75%). We hypothesize that a European student of Amphibia, who surveyed the ponds before us, introduced the resistant ephippia inadvertently with his net in search of tadpoles. Both species were found inhabiting in sympatry in two close, isolated temporary pools, with no fish. Otherwise, there is no explanation, because *Moina macrocopa* s.l. was included in a list of 94 potential invaders of inland waters, coastal and littoral lakes in Mexico (Okolodkov *et al.*, 2007), but it has not been previously found in the tropics (Elías-Gutiérrez *et al.*, 2001; Elías-Gutiérrez *et al.*, 2008a; Elías-Gutiérrez *et al.*, 2008b).

In this work, we increased the distributional area of *Moina americana* s. str. Its new range is from 58.77°N to 18.24°S, but nowadays, only restricted to the American continent instead of just at the USA as Goulden (1968) stated.

Moina americana Goulden, 1968 seems to prefer permanent water bodies, highly eutrophic and with an excess of food supply as Los Gringos dam and Texcoco Lake.

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736

737 **TABLES**

738 **Table 1. Morphological differences.**

739 **Tabla 1. Diferencias morfológicas.**

Characters	***Moina macrocopa* (Straus, 1820)**	***Moina americana* (Goulden, 1968)**
Female		
Antennulae (A1)	Sensory seta inserted in the middle	Sensory seta longer, inserted in the first third
Setae of posterior shell rim	No grouped	Forming three groups
Setae of dorsal shell rim	Fine spinules	Stout and robust spinules
Toothed seta of LI	Fine and separate teeth	A row of stout teeth, smaller and more numerous
Ephippium	Round cells	Trapezoidal cells
Male		
Limb I	Large recurved hook	Very large recurved hook
Genital openings	Ventral to the claw	At the end of the feathered teeth

740

741 Table 2. OTUs of *Moina macrocopa* complex by three different species delimitation methods.

742 Tabla 2. UTOs del complejo *Moina macrocopa* obtenidos a través de la aplicación tres métodos

743 diferentes para la delimitación de las especies

Marker	BIN System	mPTP Model	ABGD											
			0.0017		0.0028		0.0077		0.0129		0.0215		0.0359	
			I	R	I	R	I	R	I	R	I	R	I	R
COI	5	3	3	3	3	3	3	3	2	3	2	3	2	3

744

745 Table 3. K2P Mean Genetic divergences (intra and interspecific) of *Moina macrocopa* complex.
746 Bold capitals: Mean Intraspecific distances.

747 Tabla 3. Divergencias genéticas K2P medias (intra e interespecíficas) del complejo *Moina*
748 *macrocopa*. Letra en negrita: Distancias intraespecíficas medias.

		Moina macrocopa European Clade		*Moina* cf *macrocopa* Asiatic Clade	*Moina americana* American Clade
		H, MX, RS BOLD:ACH4664	SP, MX, RS BOLD:ACA1705	RS BOLD:ADF9261	CA, MX BOLD:AAC3108
Moina macrocopa European Clade	H, MX, RS BOLD:ACH4664	**0.41**			
	SP, MX, RS BOLD:ACA1705	3.72	**0.75**		
Moina cf *macrocopa* Asiatic Clade	RS BOLD:ADF9261	13.36	12.64	**0**	
Moina americana American Clade	CA, MX BOLD:AAC3108	12.74	13.17	6.62	**0.64**

749

750

751 Table 4. Mean GC% content at the sequence composition of the COI Gene.

752 Tabla 4. Contenido medio de GC% en la composición de las secuencias del gen COI.

	European clade (36.3 ± 0.0826)			Asiatic clade	American Clade
BIN	ACH4664	AAK6825	ACA1705	ADF9261	AAC3108
Mean GC% content (± SE)	36.03 ± 0.0831	35.31 ± 0	36.59 ± 0.0496	34.97 ± 0.033	35.81 ± 0.092

753

754 Table 5. Genetic diversity index and neutrality test (Fu & Tajima's D) on the mitochondrial COI
755 sequences of *Moina macrocopa* complex. Sequences show no diversity. *n*: number of sequences;
756 S: number of polymorphic sites; h: number of haplotypes; Hd: haplotype diversity; π: nucleotide
757 diversity; k: average number of pairwise nucleotide differences. Tajima's D: A negative Tajima's D

758 signifies an excess of low frequency polymorphisms relative to expectation. A positive Tajima's D
759 signifies low levels of both low and high frequency polymorphisms. Statistical significance: Not
760 significant, P> 0.1.

761 Tabla 5. Índices de diversidad genética y test de neutralidad (Fu y Tajima's D) de las secuencias del
762 gen mitocondrial COI del complejo *Moina macrocopa*. Las secuencias muestran baja diversidad. *n*:
763 número de secuencias; S: número de sitios polimórficos; h: número de haplotipos; Hd: diversidad
764 haplotípica; π: diversidad nucleotídica; k: número promedio de diferencias de nucleótidos por
765 pares. Tajima's D: un valor negativo en el test significa un exceso de polimorfismos de baja
766 frecuencia en relación con las expectativas un valor positivo en el test significa bajos niveles de
767 polimorfismos de baja y alta frecuencia. Significancia estadística: No significante, *P*> 0.1.

768

769

	n	S	*h*	Hd	π	K	Tajima's D (Significance)		Fu &Li (Significance)	
EUROPA	28	19	8	0.86	0,02506	7,57	1,57	*P* > 0.10	0,96	P > 0.10
ASIA	4	1	2	0.5	0,00166	0,5	-0,75	*P* > 0.10	1,44	P > 0.10
AMERICA	21	6	8	0.855	0,00760	2.286	0.18769	*P* >0.10	0.18769	P >0,10
TOTAL	64	59	18	0.937	0.07934	24.04	2.28	*P* < 0.5		

770

771 **LIST OF FIGURES**

772 Figure 1. SEM observations of *Moina macrocopa* s.l. (Female). A. Habitus, arrow: magnification of
773 hairs. B. Antennule. C. Tip of the antennule. D. Anterior shell rim and first limb. E. Posterior shell
774 rim, exterior view. F. Posterodorsal shell rim, interior view. G. Dorsal shell rim and hooks. H.
775 Antenna. I. First limb, 1: toothed seta, 2: ejector hooks. J. Postabdomen. K. Postabdomen and

swimming setae. L. Claw and feathered teeth. M. Claw and pecten. N. Ephippium, lateral view. O. Head and ephippium. P. Surface of ephippium. *Moina macrocopa* s.l. (Male). Q. Habito, illustration of Alonso (1996). R. Habito. S. Antennula. T. Posterior shell rim. U. First limb, immature form. V. Claw. All specimens are from Calderitas (Mexico).

Figura 1. Observaciones de *Moina macrocopa* s.l (hembra) con el Microscópio Electrónico de Barrido. A. Habito. B. Anténula. C. Punta de la anténula. D. Margen anterior del caparazón y pata I. E. Margen posterior del caparazón, vista exterior. F. Margen posterodorsal del caparazón, vista interior. G. Margen dorsal del caparazón y ganchos. H. Antena. I. Pata I, 1: seta dentada, 2: ganchos eyectores. J. Postabdomen. K. Postabdomen y setas natatorias. L. Garra y dientes plumosos. M. Garra y pecten. N. Efipio, vista lateral. *Moina macrocopa* s.l. (Macho). L. Habito. M. Anténulas. N. Margen posterior del caparazón. O. Cabeza y efipio. P. Surface of ephippium. *Moina macrocopa* s.l. (Male). Q. Habito, ilustración de Alonso (1996). R. Habito. S. Anténula. T. Margen posterior del caparazón, vista interior. U. Pata I, forma inmadura. V. Garra. Todos los especímenes fueron recolectados en Calderitas (México).

Figure 2. SEM observations of *Moina americana* (Goulden, 1968) (Female). A. Habitus. B. Nucal pore on head. C. Magnification of the nucal pore. D, E. Antennula. F. Labrum. G. Shell, lateral view. H. Anterior shell rim. I. Groups of setae present at the posterior shell rim. J. First limb. K. Postabdomen. L. Claw and feathered teeth. M. Head and ephippium. N. Ephippium, lateral view. Specimens from Texcoco lake (City of Mexico) and Los Gringos dam (Aguascalientes, Mexico).

Figura 2. Observaciones de *Moina americana* (Goulden, 1968) (Hembra) con el Microscopio Electrónico de Barrido. A. Habito. B. Órgano nucal. C. Magnificación órgano nucal. D,E. Anténula. Caparazón vista lateral. H. Margen anterior del caparazón. I. Grupos de setas presentes en el margen posterior del caparazón. J. Pata I. K. Postabdomen. L. Garra y dientes plumosos. M. Cabeza

799 y efipio. N. Efipio, vista lateral. Especímenes recolectados en el Lago Texcoco (Ciudad de Mexico) y
800 en la presa de Los Gringos (Aguascalientes, México).

801 Figure 3. SEM observations of *Moina americana* (Male). A. Habitus. B. Nucal pore on head. C.
802 Magnification of the nucal pore. D. Antennules. E. Tip of the antenne, brush-like setae and
803 aesthetascs. F. First limb, a: tip of the hook, b: tip of the middle seta of the penultimate segment.
804 G. Antenne. H. Shell lateral view. I. Posterior shell rim, interior view. J. Dorsal shell rim and hooks.
805 K. Postabdomen, claw and feathered teeth. L. Gonopores, arrow: shows the structure. M. Draw
806 from Goulden (1968). Specimens from Texcoco Lake (City of Mexico).

807 Figura 3. Observaciones de *Moina americana* (Goulden, 1968) (macho) con el Microscopio
808 Electrónico de Barrido. A Habito. B. Órgano nucal. C. Magnificación órgano nucal. D. Anténulas. E,
809 Punta de la antena, setas tipo brocha y aestetascos. F. Pata I, a: detalle del gancho, b: punta de la
810 seta del medio del penúltimo segmento. G. Antena. H. Caparazón, vista lateral. I. Margen posterior
811 del caparazón, Vista interior. J. Margen dorsal del caparazón y ganchos. K. Postabdomen, garra y
812 dientes plumosos. L. Gonoporos, flecha: muestra la estructura. M. Dibujo de Goulden (1968).
813 Especímenes recolectados en el Lago Texcoco (Ciudad de Mexico).

814 Figure 4. Id tree inferred by using the ML cluster analysis. Bootstrap values (500 replicates) are
815 shown above the branches. The scale bar shows K2P distances. The node of each clade with
816 multiples specimens is collapsed to a vertical triangle, with the horizontal depth indicating the
817 level of intraclade divergence.

818 Figura 4. Árbol de identificación inferido a través del análisis de clústeres de ML. Los valores de
819 Bootstrap (500 réplicas) están sobre las ramas. La escala muestra las distancias K2P. El nodo de
820 cada uno de los clados está colapsado en un triángulo vertical, la profundidad horizontal indica el
821 nivel divergencia dentro del clado.

822 Figure 5. COI Haplotype network of *Moina macrocopa* complex. Each circle indicates a unique
823 haplotype and variation in circle reflects the number of sequences assigned to haplotypes. Colors
824 represent the countries of each haplotype.

825 Figura 5. Red de haplotipos del gen COI del complejo *Moina macrocopa*. Cada círculo indica un
826 haplotipo único y la variación en el tamaño del círculo refleja el número de secuencias asignadas a
827 cada haplotipo. Los colores representan los países en donde se encuentra cada haplotipo.

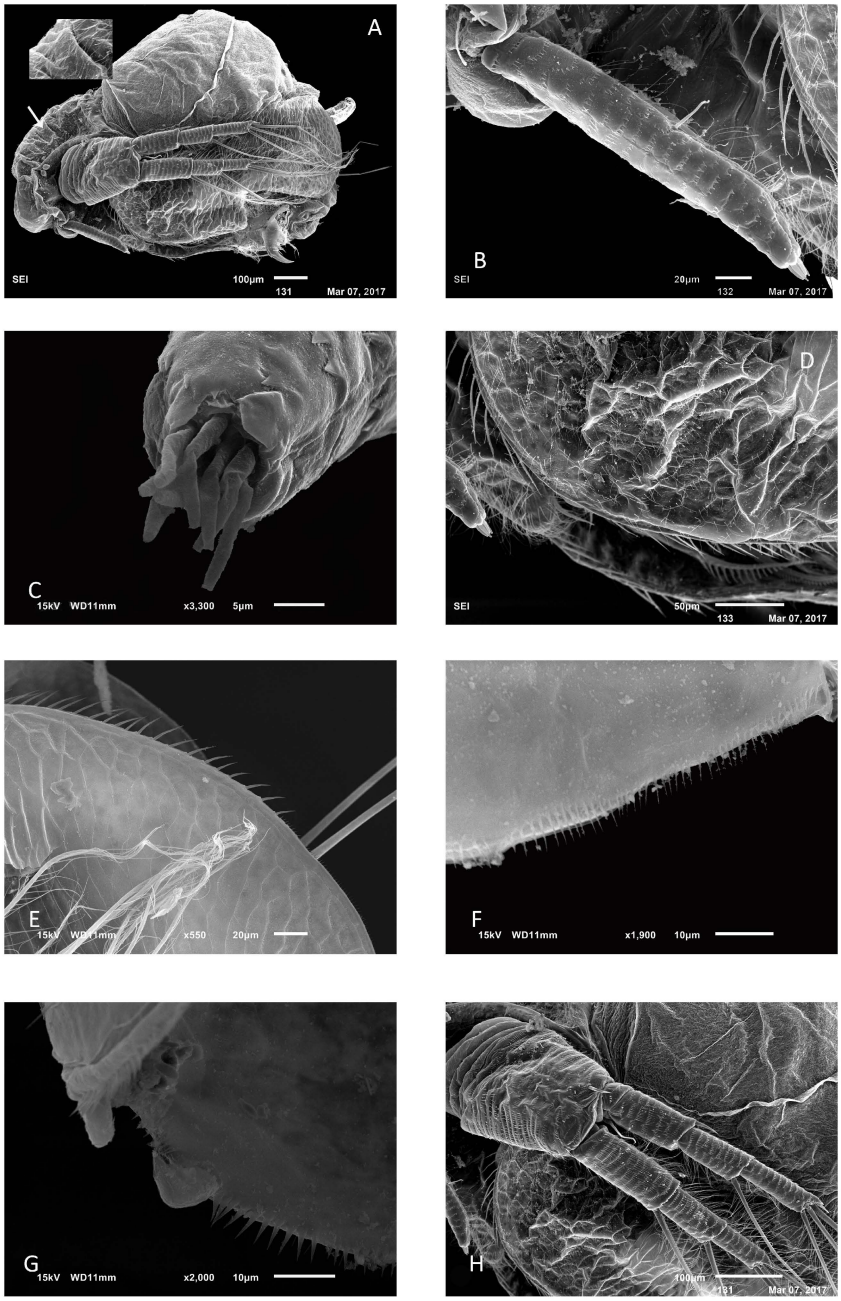
828 **Supplementary material**

829 S1. Table. Complete list of sequences generated in this study and public sequences from BOLD and
830 GenBank used in the analyses.

831 S1. Tabla. Lista completa de secuencias generadas en este estudio y secuencias públicas de BOLD Y
832 GenBank usadas en este análisis.

833

834 Fig. 1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131
Mar 07, 2017
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20µm
132
Mar 07, 2017
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5µm
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SEI
50µm
133
Mar 07, 2017
E
15kV WD11mm
x550
20µm
F
15kV WD11mm
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10µm
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100µm
Mar 07, 2017

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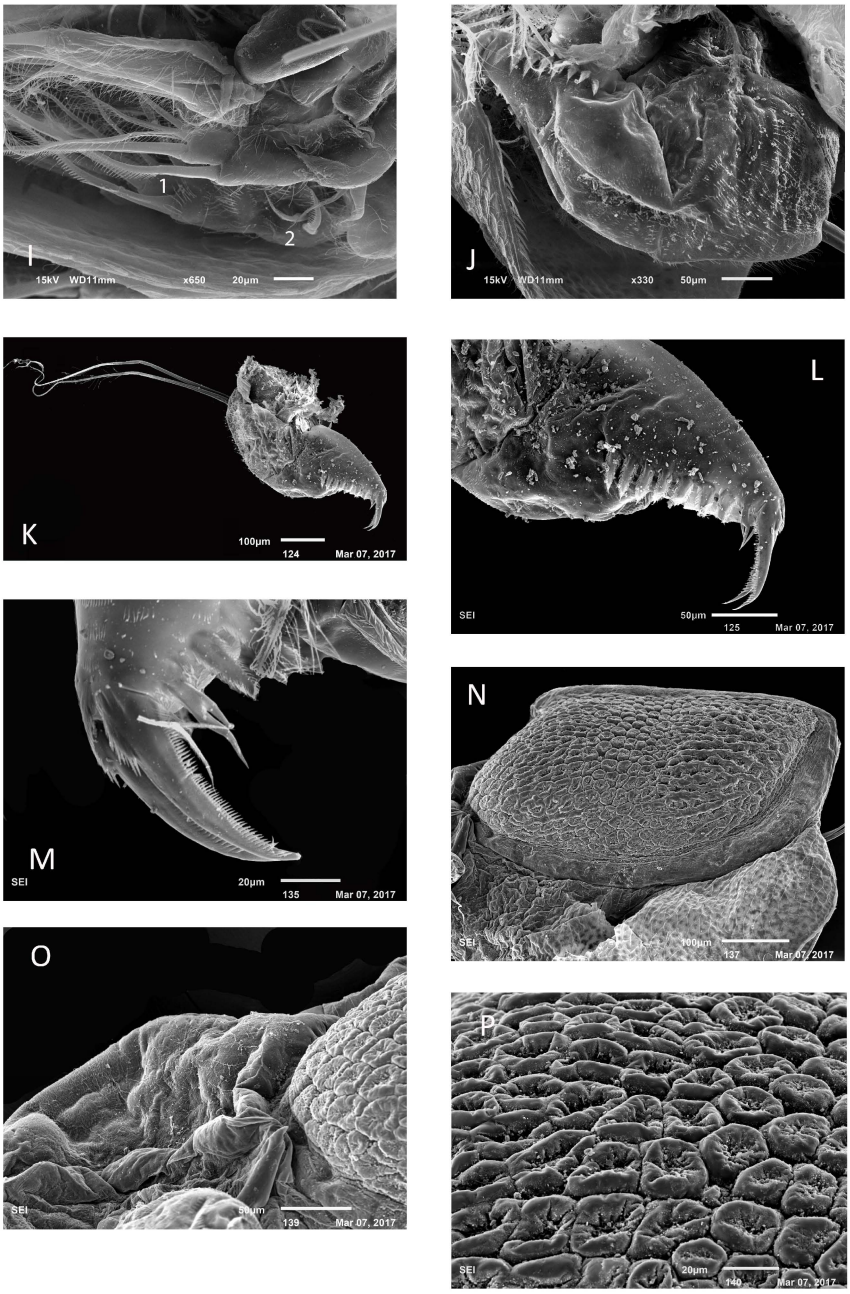
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Mar 07, 2017
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Mar 07, 2017
M
SEI
20µm
135
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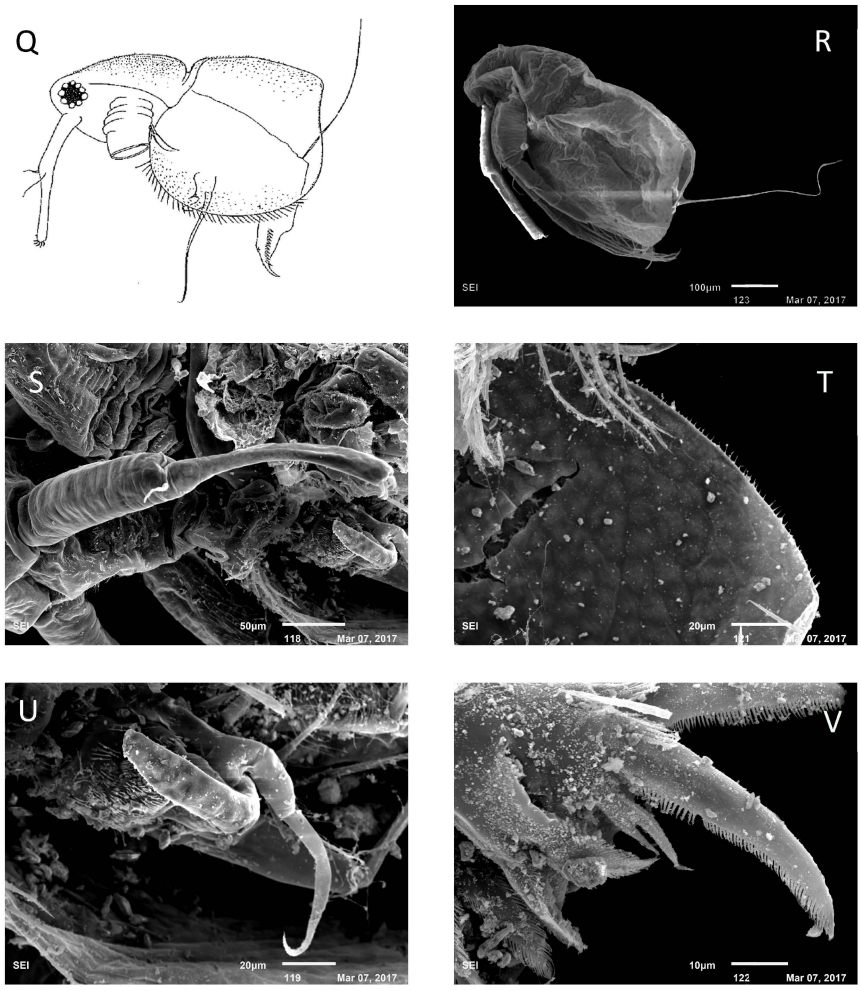
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10µm
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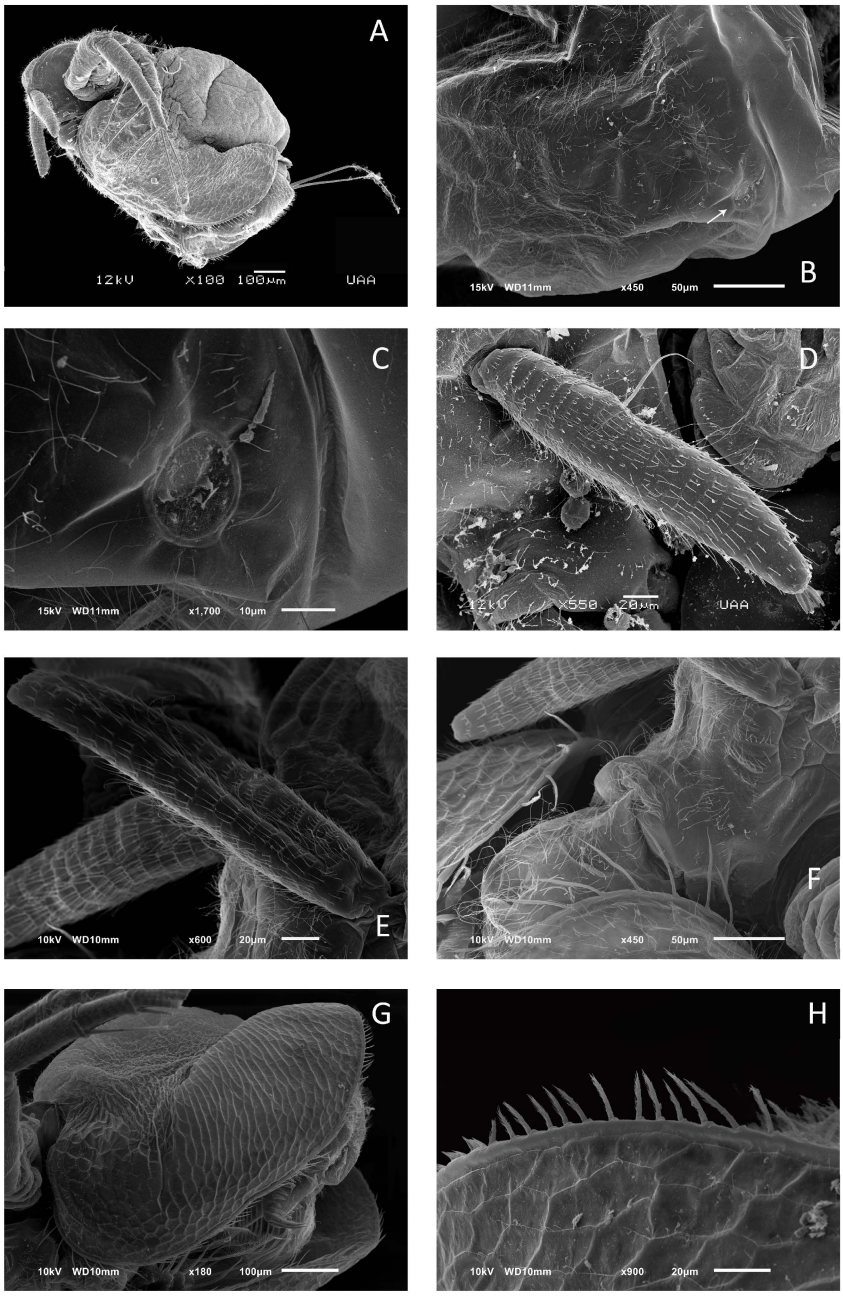
841 Fig. 2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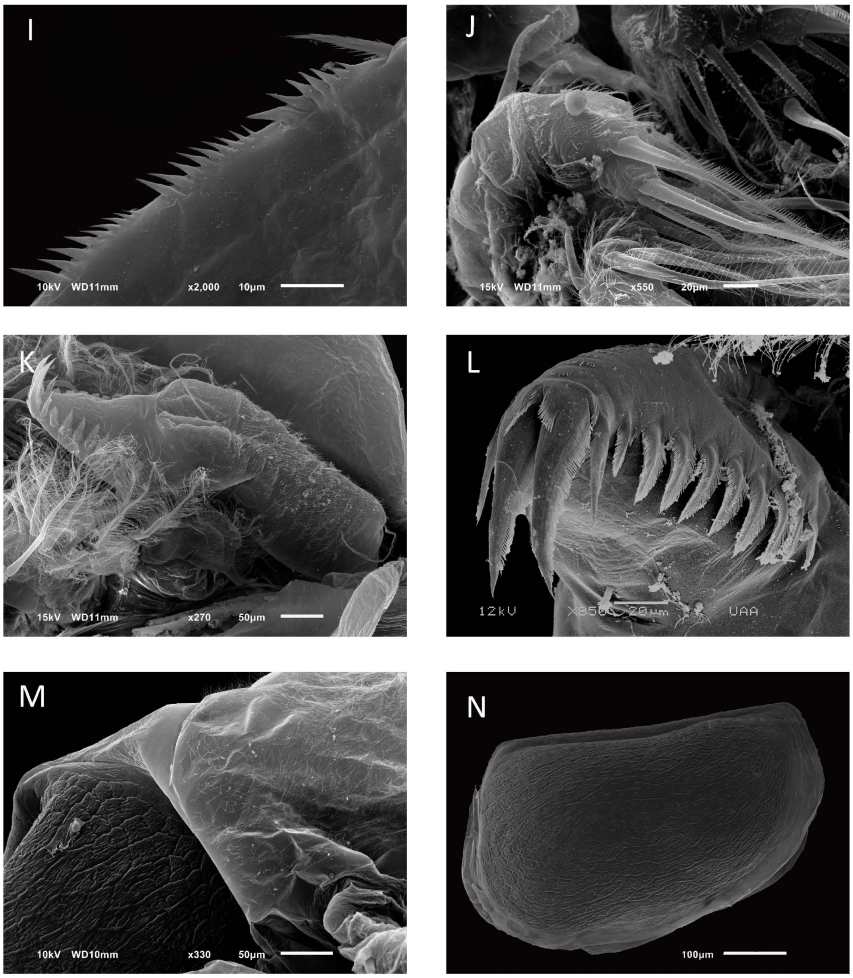
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N
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843

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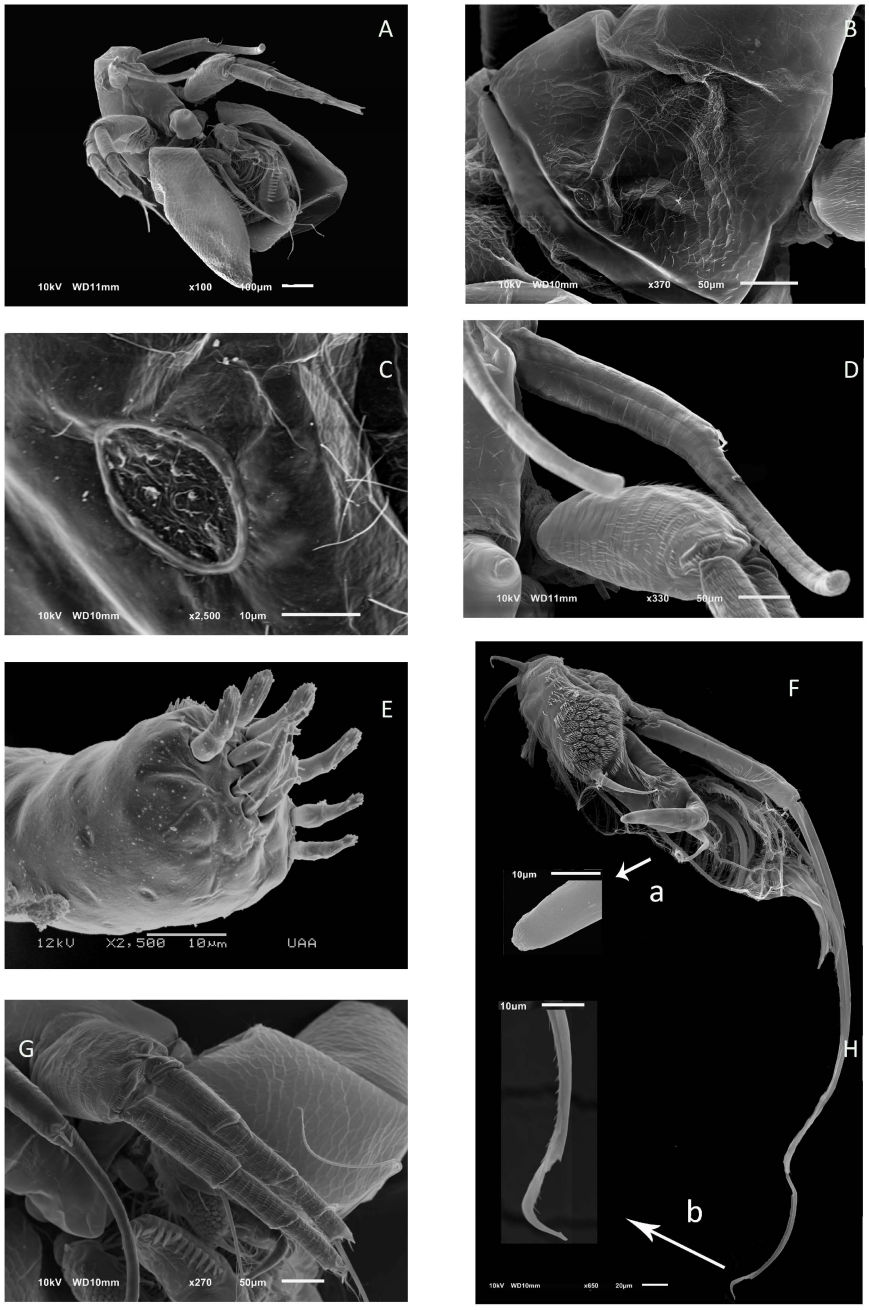
845 Fig. 3



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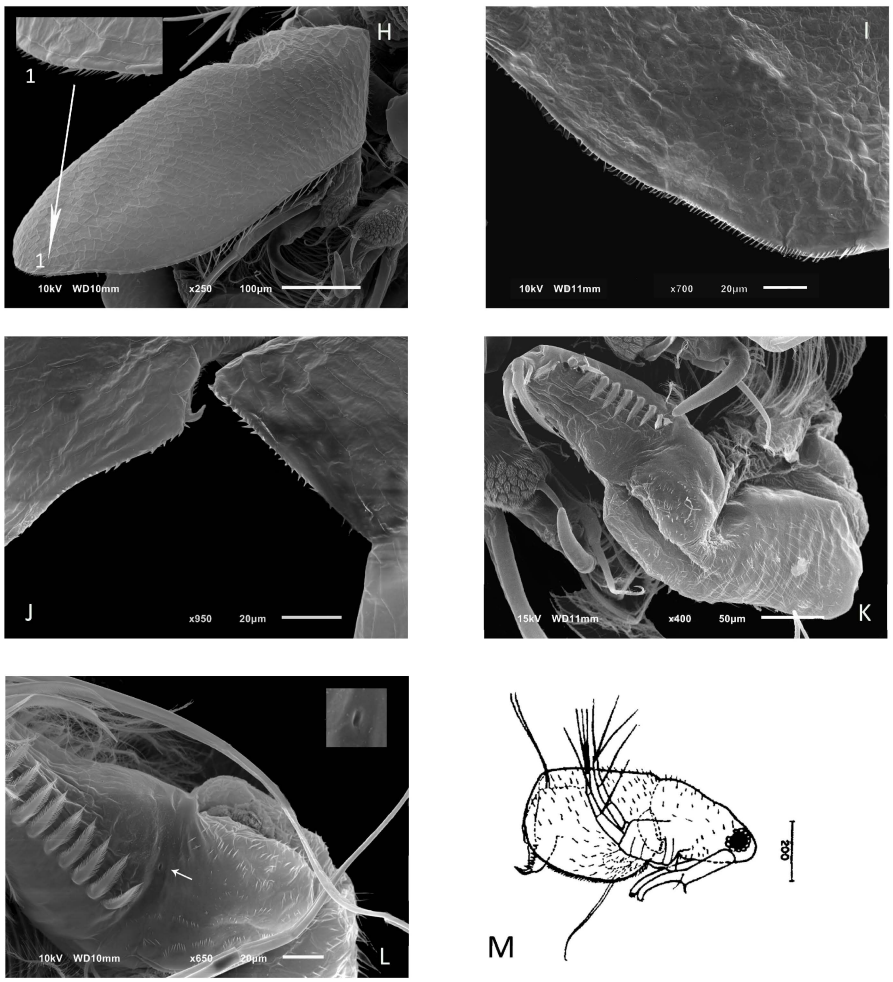

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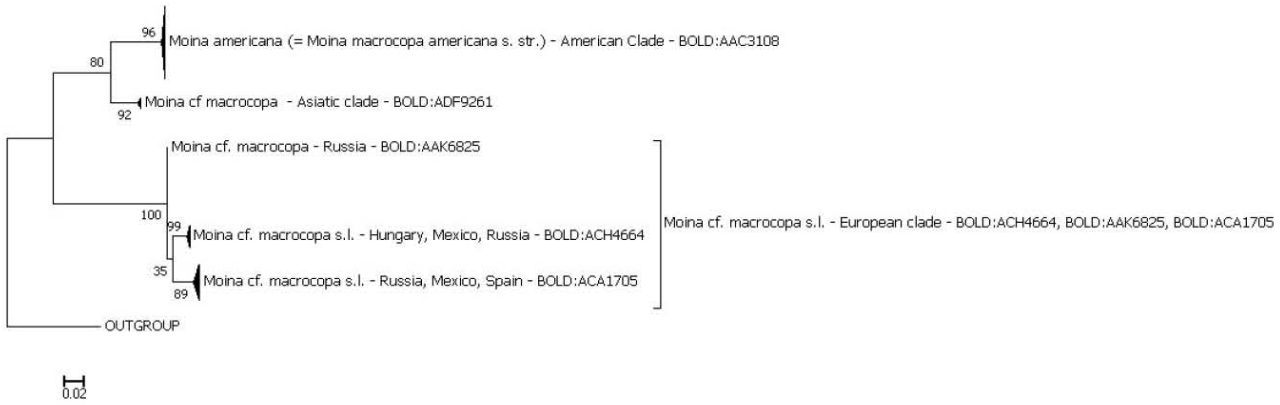
852 Fig. 4



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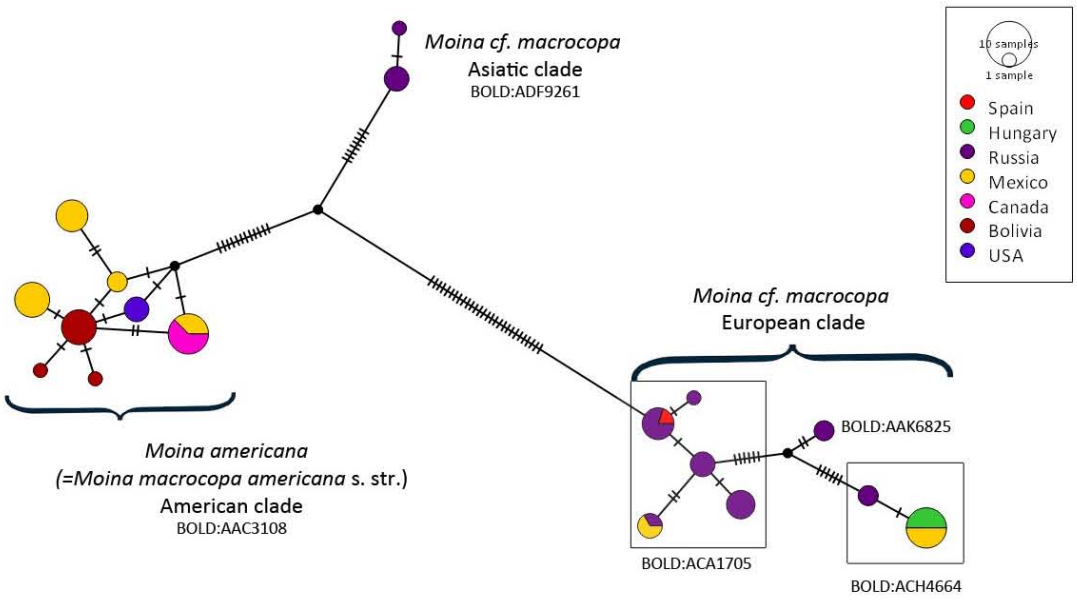
855 Fig. 5



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Montoliu-Elena, L., Elías-Gutiérrez, M., Miracle Sole, M. R., & Korinek, V. 2015. Who is *Moina micrura*? An example of how barcodes can help to clarify highly confused species. GENOME Vol. 58, No. 5, pp. 215-215

Elías-Gutiérrez, M., Juračka, P.J., Montoliu - Elena, L., Miracle, M.R. and Korinek, V. 2018. Who is *Moina micrura*? Redescription of one of the most confusing cladocerans from terra typica, based on integrative taxonomy. *Limnética* (Aceptado)

Montoliu-Elena, L., Almeyda-Osorio, J.K. and Elías-Gutiérrez, M. 2018. Seven species in one: solving *Moina Micrura* complex in Mexico and Spain, with morphological and biogeographical insights. (In revision)

Montoliu-Elena, L., Elias-Gutierrez, M., Miracle Sole, M. R., & Korinek, V. 2015. Who is *Moina micrura*? An example of how barcodes can help to clarify highly confused species. GENOME Vol. 58, No. 5, pp. 215-215

Testing primer bias and biomass – sequence relationships in metabarcoding: implications for monitoring of freshwater invertebrate communities

Vasco Elbrecht and Florian Leese

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Corresponding author: Vasco Elbrecht (e-mail: vasco.elbrecht@rub.de).

Background: Metabarcoding combines DNA barcoding with next-generation sequencing to reliably identify hundreds of specimens from an environmental sample at once. However, detection rates for species-rich stream invertebrate samples, as well as the capability to quantify biomass or species abundances, have not been systematically tested. We developed a cytochrome c oxidase subunit I (COI) metabarcoding protocol that uses the Illumina MiSeq platform and performed two controlled experiments (with 10 replicates each) using stream invertebrate samples. **Results:** In the first experiment we used 31 specimens of a single stonefly species that differed by up to four orders of magnitude in biomass. We found a clear biomass – sequence abundance relationship, but even the smallest specimens were reliably detected. In the second experiment, recovery of 52 different freshwater invertebrate taxa was tested using similar amounts of biomass per specimen as template. With a single universal primer pair we could recover 83% of the taxa. However, sequence abundance varied by four orders of magnitude between taxa. **Significance:** Our experiments show that although biomass can be estimated if only a single species is present in a sample, reliable species biomass or abundance estimates from environmental samples are impossible due to primer bias. Thus, DNA-based ecosystem assessments should rely on presence-absence rather than abundance data.

PrimerMiner: An R package for the development of universal barcoding primers and mini barcodes using partial COI sequences

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Corresponding author: Vasco Elbrecht (e-mail: vasco.elbrecht@rub.de).

Background: DNA barcoding for species identification is increasingly applied in ecological research and biodiversity monitoring. Conserved "universal" primers are used to PCR-amplify a specific gene region of the mitochondrial cytochrome c oxidase subunit I (COI) in animals. Unfortunately, the universal primers do not amplify all taxa equally well and may even fail. Therefore, many more group-specific, degenerate primers have been developed. While many COI barcode sequences are readily available in online databases such as the Barcode of Life Data Systems (BOLD), sequence information about the primer-binding region is often limited or even contains errors. Until now, mitochondrial genomes have mostly been used to design improved universal barcoding primers for animals. However, mitochondrial genomes are still not available for many groups, which limits the applicability. **Results:** Here we developed improved degenerate COI primers for freshwater invertebrates targeting the traditional "Folmer region", using a novel approach that also utilizes the full potential of partial COI sequences. COI sequences for important freshwater taxa were obtained from online databases, clustered, and mapped against the COI consensus from available mitochondrial genomes. Many partial COI sequences overlapped with the Folmer region and could thus be used for designing degenerate primers. The alignments created are also useful for the development of mini-barcodes that lie within the Folmer region. **Significance:** With this novel approach, we were able to design reliable barcoding primers despite the few mitochondrial genomes available for freshwater invertebrate taxa. Our approach of including partial barcode sequences can be used to design and verify optimized degenerate primers for all taxonomic groups with unprecedented coverage. An R package for downloading and processing sequences is available on GitHub: https://github.com/VascoElbrecht/PrimerMiner.

Who is *Moina micrura*? An example of how barcodes can help to clarify highly confused species

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[1]*Posgrado de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México and El Colegio de la Frontera Sur - Chetumal Unit, Chetumal, México.*
[2]*El Colegio de la Frontera Sur - Chetumal Unit, Chetumal, México.*
[3]*Departamento de Microbiología y Ecología, Universitat de Valencia, Valencia, Spain.*
[4]*Department of Ecology, Charles University, Prague, Czech Republic.*
Corresponding author: Lucia Montoliu Elena (e-mail: luciamontoliuelena@gmail.com).

Background: *Moina micrura* seems to be one of the most ubiquous cladocerans, with many studies on ecology, ecotoxicology, cultures, distribution, etc. It is really one of the most confused species of freshwater cladocerans. As a result, all studies on this taxon cannot be compared or repeated between different laboratories. With this study, based on morphology, molecular data, and distribution we try to establish the identity of the real *Moina micrura*. **Results:** *Moina micrura* was one common species recorded in Albufera de Valencia (Spain). Detailed analyses and a comparison with specimens from the type locality demonstrated the presence of two taxa there, neither one of them belonging to this species. *M. micrura* presents specific morphological traits at Limb II, the large lobe, the arrangement of the seta and sensilla between the large lobe and the gnathobase, the ornamentation of the basal corner of the gnathobase, the exopodite and the accessory setae of Limb V. All these traits are not included at the original description, and they are of great taxonomic importance to delineate species. Molecular data, namely COI and *12S* sequences, clearly allow us to differentiate it from its congeners. **Significance:** The significance of this study is to establish a baseline to identify this species and discriminate it from all other similar taxa. This study opens a new field to understand the taxonomy and general relationships within the anomopods.

After 10 years of DNA barcoding in Mexico – where are we?

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[2]*Estacion de Biologia Chamela, Instituto de Biologia, UNAM, Mexico.*
Corresponding author: Manuel Eliás-Gutiérrez (e-mail: melias@ecosur.mx).

Background: The 2 000 000 km^2 of territory occupied by the Mexican Republic is the fourth most biodiverse country in the world. Regardless of this outstanding "natural capital", as the National Commission for Knowledge and Use of Biodiversity (CONABIO) has called it, support for research in this area has been quite limited by the federal agencies such as the National Council of Science and Technology (CONACYT), compared to other countries. **Results:** In spite of this resourcing challenge, several strategies were developed by Mexican researchers when they realized that DNA barcoding became an important tool to overcome the taxonomic impediment: the Mexican Barcode Network (MEXBOL) was established, including a National Laboratory focused on reducing the costs of DNA analyses; alliances between interested institutions were built; and main biorepositories got involved. The confidence of the academicians allowed this country to rank among the top 10 nations in terms of DNA barcoding of the national biota, with results in almost all important groups of animals, fungi, and plants. New species of fish, echinoderms, crustaceans, insects, polychaetes, leeches, platyhelminths, and acanthocephalans highlighted by the barcodes have been described. New insights into the diversity of rotifers, molluscs, and the above-mentioned groups have been discovered, with a much higher diversity than expected. Diverse applications of barcoding, such as analyses of seafood, exotic species, disease vectors, and a database of endangered species to help in the control of trafficking, have been developed. With a group of well-trained taxonomists, we are adopting new strategies, considering next-generation sequencing, and looking to integrative taxonomy in response to new questions. Finally, we started an educational program with a mobile PCR laboratory in schools. **Significance:** In summary, Mexican researchers have developed creativity and efficiency in order to use to maximal effect the limited support provided by national science policies and funders. These advances are particularly important

Elias-Gutierrez, M., Juračka, P.J., Montoliu - Elena, L., Miracle, M.R. and Korinek, V. 2018. Who is *Moina micrura*? Redescription of one of the most confusing cladocerans from terra typica, based on integrative taxonomy. Limnética (aceptado)

1 Who is *Moina micrura*? Redescription of one of the most confusing cladocerans from
2 *terra typica*, based on integrative taxonomy

3

4 Manuel Elías-Gutiérrez, Petr Jan Juračka*, Lucía Montoliu-Elena**, Maria Rosa
5 Miracle†, Adam Petrusek*, Vladimír Kořínek*

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13 México

14 †University of Valencia (deceased)

15

16 Corresponding author: melias@ecosur.mx

17

18 Short title: Who is Moina micrura?
19

RESUMEN

Moina micrura Kurz, 1875 pertenece a una de las especies de cladoceres menos definidas en el mundo. Esta especie ha sido considerada cosmopolita y se usa ampliamente para experimentos de laboratorio, ecotoxicología o estudios de ecología. Sin embargo, análisis moleculares recientes corroboraron la idea de que es un complejo diverso de especies estrechamente relacionadas. Los persistentes problemas sistemáticos con *M. micrura* se derivan del hecho de que no se ha redescrito en detalle, y además estánperdidos tanto su material tipo así como la localidad tipo. Con este estudio, tratamos de proporcionar una redescripción de *M. micrura* s. str, utilizando la morfología de hembras partenogenéticas y efipiales y machos, así como caracteres modernos como los códigos de barras de ADN. El material procede de un estanque situado no lejos de su localidad tipo original en la República Checa. En primer lugar, secuenciamos genes mitocondriales para la citocromo c oxidasa subunidad I (COI o códigos de barras) y 12S rDNA para establecer la identidad de *M. micrura* s. str. Después de este análisis, comparamos los datos genéticos con todas las secuencias disponibles en bancos de datos del mundo. La especie parece restringida al Paleártico occidental, con los registros confirmados genéticamente localizados más al este provenientes de Kazajstán e Israel. El linaje más próximo a *M. micrura* s. str. se encontró en España y necesita un análisis exhaustivo para establecer su identidad. *M. micrura* s. str. tiene características únicas en la espinulación posterior de las valvas de la hembra partenogenética, y la ornamentación del efipio. En el macho, las espinulas en la punta de la antena y el gancho del primer toracópodo también son únicos. Con esta descripción, esperamos que los especialistas tengan una referencia clara para descubrir la diversidad de este complejo, así como comprender su biogeografía y diversidad.

ABSTRACT

Moina micrura Kurz, 1875 belongs among the most poorly defined cladoceran species in the world. This species has been considered cosmopolitan and is widely used for laboratory experiments, ecotoxicology, or ecology studies. Nevertheless, recent molecular analyses corroborated the idea that it is a diverse complex of closely related species. Persisting systematic problems with *M. micrura* stem from the fact that it has not been redescribed in detail, and its type material as well as the type locality are lost. With this study, we try to provide a redescription, using morphology of females, males and ephippial females and the DNA barcodes for *M. micrura* s. str. from the pond situated not far from its original type locality in the Czech Republic. Firstly, we sequenced mitochondrial genes for cytochrome c oxidase subunit I (COI) and 12S rDNA to establish the identity of *M. micrura* s. str. After this analysis, we compared the genetic data with all available sequences across the world. The species seems restricted to Western Palearctic, with the most easterly located genetically confirmed records originating from Kazakhstan and Israel. The closest related lineage to *M. micrura* s. str. was found in Spain and needs a thorough analysis to establish its systematic status. *M. micrura* s. str. has unique features in the posterior spinulation of the valves of the parthenogenic female, and the ornamentation of the ephippium. In the male, spinules on the tip of the antennule and the hook on the first thoracopod are also unique. With this description, we hope to inspire specialists to start uncovering the diversity of this complex, to understand its biogeography and diversity, as well as the real range of *M. micrura* s. str.

67 **INTRODUCTION**

68 *Moina micrura*, seemingly one of the most ubiquitous cladocerans with apparently
69 worldwide distribution, has been also one of the most taxonomically confused species.
70 It was described by Kurz (1875) from Bohemia (the present-day Czech Republic), in
71 short paragraphs, where no presently relevant taxonomic characters were used.
72 Although the description and accompanying drawings (Fig. 1) were exceptionally good
73 for those times, they lacked fundamental diagnostic characters, and no characters of
74 males or ephippial females were mentioned. Furthermore, the type material is lost and
75 the original *locus typicus*, a fish pond Mlýnský (Muehlteich) at Malešov village, has
76 been dried out decades ago and the surrounding area forested (Petrusek 2002). The first
77 attempt to review contemporary knowledge on *M. micrura* in Bohemia, its *terra typica*,
78 was published by Šrámek-Hušek (1940), as the validity of Kurz' species was doubted
79 (Wagler, 1937). Šrámek-Hušek (1940) presented a substantial amount of evidence to
80 support the distinct status of the species, based on the ample material collected in the
81 vicinity of the original type locality.

82 Later, due to the apparent intraspecific variability within *M. micrura*, at least three
83 subspecies were described and accepted by specialists. Goulden (1968) in his classic
84 monograph included seven formerly separate species as younger synonyms of *M.*
85 *micrura*, and later, two more were synonymized by Smirnov (1976). Since then, a great
86 confusion has been apparent, and regional faunal books display *M. micrura* descriptions
87 and drawings that do not match each other. For example, Alonso (1996) used Spanish
88 material to illustrate *M. micrura*, but it differs in details, mainly in the thoracopods
89 (second and fifth limb) when compared with the descriptions by Goulden (1968), who
90 studied 28 different populations across the globe, but only one European (from an

Italian rice field), or with the recently published cladoceran fauna of Slovakia by Hudec (2010). The latter publication contains a very good morphological description and figures of *M. micrura* s. str. based on Central European material from the Danube basin, and also provides diagnostic characters to distinguish it from a superficially similar species also found in the region, *Moina weismanni* Ishikawa, 1896. Unfortunately, Hudec's description of *M. micrura* does not include all thoracopods and other important details of the shell.

M. micrura, presumably widely distributed in Europe, has been also recorded from all other continents except Antarctica. In Asia, it has been reported for example in Turkey (Bekleyen *et al*., 2008), southern China (Li *et al.,* 2012), Malaysia (Idris, 1983) and India (Chatterjee *et al.,* 2013). The last-mentioned authors regarded *Moina dubia* Guerne & Richard, 1892 as a valid species distinct from *M. micrura*. In the American continent, Elías-Gutiérrez *et al.* (2008) compiled the records related to this taxon in Mexico and regarded *M. micrura* as a complex of species even within that single country. Particularly in Brazil, presumed *M. micrura* has been recorded in diverse environments, from freshwaters to a brackish hyper-eutrophic estuary (Paranhos *et al.,* 2013; Paranaguá *et al.,* 2005). It has also been reported from Australia (Smirnov *et al.,* 1983; but see Petrusek *et al.,* 2004) and from the whole African continent (Dumont *et al.,* 1981).

As detailed morphology of most branchiopods remains not satisfactory described, it is considered that molecular data could help to understand diversity and phylogeny of this group (DeWaard *et al.,* 2006). In the case of *M. micrura,* as far as we know, at least three studies involved some molecular analyses, but they are partial and do not clarify the taxonomy of this species sufficiently. Petrusek *et al.* (2004) questioned the apparent

115 cosmopolitanism of *M. micrura*, demonstrating by crossing experiments and molecular
116 characters (sequence divergence of the mitochondrial gene for 12S rRNA) a distinctness
117 of *M. micrura* from the Czech Republic (sampled about 90 km from the original type
118 locality) from Australian *M. micrura*-like specimens. However, the genetic comparisons
119 were based only on one clone of both species, and their morphology was not described.
120 Elías-Gutiérrez *et al.* (2008) found in Mexican populations morphologically similar to
121 *M. micrura* three related but distinct lineages based on the DNA barcodes, i.e.,
122 fragments of the cytochrome c oxidase subunit I gene, COI (Hebert *et al.,* 2003a, b).
123 However, they could not compare these lineages with the European populations,
124 because there were no sequences of *M. micrura* from *terra typica* available for this
125 gene, which has become the standard marker for identification of animal species,
126 including crustaceans (Eischeid *et al.,* 2016). A recent DNA barcoding-based study
127 uncovered 21 phylogenetic groups of *Moina* within the Palearctic, with at least three
128 clades of *micrura*-like taxa from this region (Bekker *et al.,* 2016).

129 Despite the taxonomical problems, many papers dealing with culture, ecotoxicology,
130 ecology and other topics related to *M. micrura* have been published recently (58 papers
131 are recorded in the Web of Knowledge (Thomson Reuters) between 2010 and 2017),
132 obviously due to a wide range and ubiquity of this taxon. However, many – possibly
133 most – of these studies probably do not deal with *M. micrura* in a strict sense, and
134 consequently results of such studies are not fully comparable.

135 The aim of our study is therefore to establish the identity of *Moina micrura* from its
136 type locality, and to provide a good reference for future comparison and identification
137 of this species. To achieve this, we used an integrative taxonomy approach (Dayrat,
138 2005), combining detailed morphological analyses and additional DNA sequencing. We

also provide a short description of differential characters of *Moina weismanni* Ishikawa, 1896, as it often co-occurs in ponds with *M. micrura* within its *terra typica* (Petrusek, 2002), and their parthenogenetic females are superficially similar. Furthermore, we explored variation in ultrastructure of ephippia, as a potentially highly relevant taxonomic trait (Goulden, 1968; Juračka *et al.,* 2016) in populations of the *Moina micrura* complex across the globe, and compared them with several other species of the genus.

MATERIAL AND METHODS

Field Sampling

Material of *Moina micrura* was collected with a plankton (45 μm) and a hand net (90 μm) from a small fish pond named Sádka (49.963 N, 15.329 E; area 0.85 ha) situated between Ovčáry and Nové Dvory villages, 4.6 km east from Kutná Hora, in the Czech Republic, very close to the original (now dried up) type locality (Supplementary information Table S1).

Material examined

All material (see Supplementary information Tables S1 and S2) was preserved with non-denatured ethanol and since 2012, the procedure suggested by Prosser *et al.* (2013) was followed. Samples for intercontinental comparison are from V. Kořínek's collection of Cladocera (www.cladocera-collection.cz) and were originally preserved in 4% formaldehyde solution and stored in 70% ethanol.

As *Moina micrura* s. l. is widely distributed, we examined ephippial ultrastructure by scanning electron micrography on the material sampled from various continents, including Europe, Asia, Africa, South America, and Australia (see Supplementary

information Table S2) to evaluate morphological variation among populations isolated by large inter-continental distances. However, this variability within the species complex has to be compared also with the material from other well-defined species of the genus to evaluate the extent of interspecific variation. Therefore, we also included in the comparison *M. belli*, *M. brachiata*, *M. macrocopa*, *M. mongolica*, *M. reticulata*, *M. tenuicornis*, as well as morphologically clearly distinct but apparently undescribed lineage from Australia (Supplementary information Table S2).

Analyses of the material

Morphological observations

Specimens were sorted from the ethanol-preserved samples under a stereomicroscope and placed in a drop of a glycerol. Several females were dissected. Whole animals and dissected sections were examined and measured under a differential interference contrast microscope and/or phase contrast microscope. They were morphologically identified following descriptions by several authors (Goulden, 1968; Alonso, 1996; Hudec, 2010).

Selected individuals were prepared for scanning electron microscopy (SEM). To remove unwanted biofilm covering surface of studied individuals, examined material was cleaned for 10 minutes with hot 10% potassium hydroxide prior to its dehydration. Dehydration series followed standard graded series of acetone solutions in alcohol 30, 50, 70, 80, 90, 95 and 97%, followed by two immersions in 100% acetone. Afterwards, we replaced acetone with the hexamethyldisilazane for 20 minutes and left the material overnight in the desiccator. The dried samples were gold-coated and then observed with JEOL JSM-6380 LV scanning electron microscope at 15 kV. Further details of the

methods are described in Juračka *et al.* (2016). Ephippial ultrastructure was always studied above the center of the egg chamber.

Selected molecular markers

As mentioned above, two mitochondrial genes (12S and COI) were selected as molecular markers for this study. A fragment of the gene for 12S rRNA (343 bp) was selected to allow comparison with the previously published sequence of *M. micrura* s. str. by Petrusek *et al.* (2004), and the gene for COI (620 bp) due to its status as a standard barcoding gene in animal kingdom (Hebert *et al.,* 2003; Eischeid *et al.,* 2016), its frequent use to discriminate cladoceran species (Elías-Gutiérrez *et al.,* 2008), and its availability for various *Moina* lineages (Elías-Gutiérrez *et al.,* 2008; Bekker *et al.,* 2016).

*DNA isolation, PCR amplification and sequ*encing

DNA was extracted from whole body homogenates using a mix of Proteinase K with invertebrate lysis buffer and digested overnight at 56ºC for material processed before 2011. Genomic DNA was subsequently extracted using a membrane-based approach. After 2011, we used the HotShot extraction protocol (Montero-Pau *et al.,* 2008). Approximately 600-658 bp were amplified for the COI using LCO1490 and HCO2198 primers (Folmer *et al.,* 1994) and/or ZPLK primers suggested by Prosser *et al.* (2013).

The polymerase chain reaction (PCR) reagents used were as follows: 12.5 µL of PCR reaction mix included 6.25 µl of 10% trehalose, 2 µl of distilled deionized water and 1.25 µl of 10× PCR buffer for the enzyme Taq Platinum, 0.625 µl of MgCl2 (50 mM), 0.125 µl of each primer, forward and reverse (0.01 mM), 0.0625 µl (10 mM) dNTP mix, 0.06 µl Platinum Taq polymerase (5 U/µl), and 2.0 µL of template DNA.

208 The thermocycler program included initial denaturation at 94°C for 1 minute, 5 cycles
209 of 94°C for 40 seconds, 45°C for 40 seconds, 72°C for 1 minute, 35 cycles of 94°C for
210 40 seconds, 51°C for 40 seconds, 72°C for 1 minute, and a final extension at 72°C for 5
211 minutes.

212 For 12S, the PCR reaction mix was identical to that of COI but primers L13337-12S
213 and H13842-12S were used (Machida *et al.,* 2004). The PCR was performed under
214 following conditions: 95ºC at 4 min, followed by 40 cycles of 94ºC for 45 seconds,
215 60ºC for 45 seconds, 72ºC for 90 seconds, and the final extension step at 72ºC for 6
216 minutes. PCR products were visualized on pre-cast agarose gels (E-Gels©, Invitrogen).
217 They were then sequenced bidirectionally using an ABI 3730 (Applied Biosystems)
218 capillary sequencer using the BigDye© Terminator v.3.1 Cycle Sequencing Kit (Applied
219 Biosystems), as described in Hajibabaei *et al.* (2005).

220 *Sequence analysis*

221 COI sequences obtained in this study (Supplementary information Table S1) were
222 combined with those for *M. micrura* and other members of this genus available in
223 GenBank and Barcode of Life Database (BOLD, boldsystems.org) (Supplementary
224 information Table S1), to examine clade diversity.

225 Alignments and calculations were made with the tools provided by BOLD
226 (Ratnasingham *et al.,* 2007). COI sequences were aligned with BOLD aligner (Amino
227 Acid based HMM) and 12S sequences were aligned with Kalign algorithm (Lassmann
228 *et al.,* 2005). Nucleotide divergence was calculated using the Kimura two-parameter
229 (K2P) algorithm (Kimura, 1980) with complete deletion of gaps and missing data.
230 Neighbour-joining (NJ) trees (Saitou *et al.,* 1987) based on K2P distances (ID Tree)

were created to provide a graphic representation of divergence patterns among species and lineages. For COI-based analyses, a sequence of *Moina macrocopa* from Texcoco Lake (Mexico) was used as outgroup; a sequence of *Moina macrocopa* from Kostelec (Czech Republic) was used for the same purpose when analysing 12S sequences (see Supplementary information Table S1).

We also performed a maximum likelihood analysis with the MEGA6 software (Tamura *et al.,* 2013). A maximum likelihood tree was constructed using the General Time Reversible model with Gamma distributed and invariant sites (GTR G+I) as the best fitting model of substitution. Gamma distribution was approximated using five rate categories and nearest-neighbour interchange was uses as heuristic method for tree inference. Nodal support for the resulting branches was estimated with 1000 bootstrap replications. The subtrees for terminal clades were collapsed for visualization in MEGA6.

Delimitation of groups within the *M. micrura* complex

Molecular operational taxonomic units (OTU's) have frequently been used to infer putative species boundaries where morphological identifications are difficult (Ashfaq *et al.,* 2015). We used two approaches to assign the 12S and COI sequences presumably belonging to the *M. micrura* species complex to OTU's: Barcode Index Number (BIN) system (Ratnasingham *et al.,* 2013) and Automatic Barcode Gap Discovery (ABGD; Puillandre *et al.,* 2012). The BIN system uses the Refined Single Linkage (RESL) algorithm to reach decisions on the number of OTU's in a sequence dataset through a three-phased analysis. ABGD employs a multi-phase system which initially divides sequences into OTUs based on a statistically inferred barcode gap (i.e., initial partitioning), and subsequently conducts additional rounds of splitting (i.e., recursive

255 partitioning). The COI and 12S sequences were analyzed with an online version of
256 ABGD (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) using K2P as the
257 distance metric, employing a relative gap (X) of 1.5, a minimal intraspecific distance
258 (Pmin) of 0.001, and a maximal intraspecific distance (Pmax) ranging from 0.02 to 0.1 .

259 **RESULTS**

260 **Sequence analysis**

261 Kimura-2-parameter (K2P) distances among the 12S and COI sequences of putative *M.*
262 *micrura* from various regions ranged from 0 to 11.3% and from 0.27 to 18.09%,
263 respectively (Table 1, A and B). COI gene is less conservative than 12S, hence
264 divergences are bigger. COI sequences with divergences exceeding 3% were considered
265 to belong to different groups (Hebert *et al.,* 2003).

266 Analyzing the results of both genes and using the above-mentioned divergence
267 threshold of 3%, *Moina micrura* s. str. has been confirmed in the Czech Republic, Israel
268 (in this case using the 12S fragment, with 0% divergence) and Kazakhstan (near
269 Ushkempir). The other groups represent nine divergent lineages, potentially cryptic
270 species, of the *M. micrura* complex, the most closely related one originating from
271 Spain.

272

273 **Comparison of 12S and COI sequence variation**

274 The results of the ID tree analysis in BOLD (Fig. S3) and the maximum likelihood
275 method (Figs. 2a, b and S4) were similar for both genes.

In the 12S tree (Figs 2a, S4), the first group belongs to the species *M. micrura* s. str., it includes specimens from *terra typica* (Nové Dvory, CZ). The specimen sequenced by Petrusek *et al.* (2004) from a sandpit near Dobříň (CZ) (Supplementary information Table S1) was grouped together with these, as well as specimens from Slovakia and Israel, in the same way as in the BOLD Id tree (Fig. S3). The closest relative to this clade is *M. cf. micrura* 1 ES from Spain. The second group forms a consistent cluster and is apparently restricted to Spain (ES). The two next groups which include sequences from Ethiopia (ET) and Australia (AU) are singletons, so further sequencing is needed to assess the distribution and variation within these groups. The fourth group is another species of the *micrura*-complex, occurring in sympatry with *M.* cf. *micrura* 1 ES in the Albufera of Valencia lake (Spain). Next cluster groups together a sequence from Hungary (HU) and another from Zahillo, South Spain (*M.* cf. *micrura* 3 ES).

The COI tree (Figs 2b, S4), which allowed us to compare sequences of other parts of the world, included eleven groups that were recognized as distinct OTUs by both BIN and ABGD approaches. This wider comparison gave related results (comparable to those of Bekker *et al.,* 2016): the first group is *Moina micrura* s. str. and include sequences from the Czech Republic and Kazakhstan. The nearest group, as in 12S tree, is the cluster represented by the Spanish *Moina* cf. *micrura* 1 ES. The remaining nine groups are possibly distinct species, each with a geographically restricted distribution. The localities where *Moina micrura* s. str. can be found according to our results are shown in figure 3.

Molecular analyses thus confirm that *Moina micrura* s. l. is a diverse group of species, with *M. micrura* s. str. apparently restricted only to a limited region of the Old World, probably western part of the Palearctic region only. However, before any nomenclatural

act is taken to describe all these presumably new cryptic species or resurrect old names at present considered synonymous to *M. micrura*, it is necessary a complete an adequate morphological description of *Moina micrura* s. str. Because of this reason, we provide in the next part a detailed description of *M. micrura* s. str. from *terra typica*

Redescription of *Moina micrura* Kurz, 1875

Synonymy uncertain

Neotype: adult parthenogenetic female from Sádka pond stained with lignin pink and chlorazol black E; mounted in Canada balsam.

Type locality: Czech Republic, fish pond Sádka, at Nové Dvory village, NE of Kutná Hora, 49.963 N, 15.329 E. Repeated sampling from 2002 to 2015 & laboratory culture of specimens collected in the same pond (A. Petrusek & M. Miracle lgt.).

- Distribution of specimens from "*terra typica*" (watershed of the Elbe River in Central Europe) among museums:

- National museum, Natural history collections, Prague:

1. One permanent mount of a *Moina micrura* Kurz, 1875 female – *neotype* specimen. Stained with a mixture of lignin pink & chlorazol black E and mounted in Canada balsam. Sádka Pond, Kutná Hora, Nové Dvory. 27.6.2002. A. Petrusek legit. Cat. Nr. P6E 4160

2. Four permanent mounts stained and mounted as above, specimens collected in the same locality. Cat. Nr. P6E 4161 & P6E 4162 (zooplankton sample) and cat. Nr. P6E 4163 (females) & P6E 4164 (males). Laboratory culture.

3. One permanent mount of *Moina micrura* from Upper Školní Pond, near Chabařovice. 8.8.2004. K. Pilařová legit; stained as above, mounted in Hydro-Matrix synthetic water miscible resin. P6E 4165.

4. One permanent mount of *Moina micrura* from a sand pit at Dobříň, Roudnice nad Labem. 19.9.1999. A. Petrusek legit. Specimens cleared in hot lactic acid, stained as above, mounted in Canada balsam. P6E 4166.

5. One vial with zooplankton sample from the neotype locality, 27.6.2002, A. Petrusek legit. Preserved in 70% ethanol. The species *Moina micrura* predominant, *Moina weismanni* Ishikawa, 1896 accessory. P6E 4169.

- Natural History Museum UK, London

1. One vial with zooplankton sample from the neotype locality, 27.6.2002. A. Petrusek legit. Preserved in 70% ethanol Cat. Nr. NHMUK 2017.38-47

Diagnosis

Parthenogenetic female

With round body, dorsum of valves elevated behind head when embryos present, convex or slightly straight in posterior part, no hairs on head and valves. Head with marked supraocular depression, no rostrum; ocellus without pigment. Denticles at posterior margin of valves organized in groups, each group with 7-10 elements increasing in size posteriorly. Postabdomen with row of 4-5 scale-like teeth fringed with row of marginal setae, and bident tooth with distal branch always significantly longer than proximal branch. Row of fine setae at the base of bident. Three groups of denticles and setules (pectens) on outer face of postabdominal claw; basal and middle pectens consisting of 4-5 and 8-12 strong thick spines, respectively, arranged fan-like; distal pecten with fine spinules. Basal and middle pectens often fused in continuous row. Antennule rod-like, with line of long setules on posterior face and sensory seta in middle, groups of minute spines with variable arrangements. Antenna with two coxal sensory setae, one short and other long reaching nearly to top of basal segment. Basal

segment with minute distal spine on dorsal face, and remarkably long distal sensory seta on ventral face. Thoracopod I (Th1) with inner distal lobe (IDL) bearing a single strong anterior seta and 2 posterior soft setae. Endite 3 (= "penultimate segment") with anterior seta, endite 1 with three posterior setae and two ejector hooks, one large and other small.

Ephippial female

Ephippium with one egg. Free ephippium from lateral aspect broadly rounded anteriorly, posteriorly slightly tapering. Ephippium with robust dorsal ridge, its surface covered with hexagonal pattern.

Male

Body elongated. Dorsal margin nearly straight or feebly convex. Head small, wedge-like; filled up by optic vesicle. Ocellus without pigmentation. Ventral face slightly convex with antennule inserted laterally. Antennule long, apical sensory setae relatively long, two more setae (male seta and a sensillum) near first third of antennule. Four apical hook-like spines of different sizes. Trunk thoracopod I with a long, curved copulatory hook, its tip with two small claw-like projections and wart-like outgrow near inner base of its curvature. Large basal part of hook rounded and covered with dense mat of short bristles. Postabdomen with row of 4 plumose teeth and bident tooth, length of both branches of bident as in female. Row of fine setae at base of bident. Pectens on outer face of postabdominal claw as in female. Group of three oblique denticles near base of claw on the ventral side. Lateral gonopores situated on each side of postabdomen close to respective proximal-most plumose teeth.

372 **Detailed description**

373 *Adult parthenogenetic female* (Fig. 4A)

374 Length: 0.44-0.76 mm (n=25). Body nearly rounded in lateral aspect, high (body height
375 to length ratio 0.54-0.68), maximum height in middle-posterior portion, depending on
376 number of embryos. Dorsum of valves strongly convex, projected much higher than
377 head, almost straight in posterior part, dorsal depression between head and rest of body
378 present. Postero-dorsal angle well-marked, rounded. Posterior margin more or less
379 straight, continuing into widely convex ventral margin. No sculpture on surface of
380 valves. No cuticular hairs on head and valves, without dorsal keel. Live animals are
381 whitish in colour.

382 Head – relatively large, rounded, without any rostral projection; with marked supra-
383 ocular depression and large compound eye. Optical vesicle contiguous with prominent
384 top of head. Ventral margin depressed under compound eye and bulging at level of
385 antennules. Ocellus without visible pigmentation or with few grains of pigment (Fig.
386 4A, B).

387 Labrum – with fleshy main body, its ventral margin slightly convex.

388 1st maxilla – with three long strongly curved setae and one short stump-like outgrow, all
389 with secondary setules.

390 Mandible – with asymmetrical, bifurcated anchoring projection.

391 Antennule (A1) – rod-like, about four times longer than wide, almost cylindrical, with
392 transverse rows of scattered denticles on anterior face and row of long setules on
393 posterior face (Fig. 4P). Antennular sensory seta conical, elongated, arising

394 approximately in middle of lateral face. Distal tip bordered with small thick spinules.
395 Nine short aesthetascs, two of them slightly longer. Tip of each aesthetasc with small
396 projections giving an aspect of crown.
397 Antenna (A2) – coxal part with two setulated sensory setae, one long reaching up to ¾
398 of basal segment length, the other short and thin (Fig. 4N). Basal segment robust, with
399 distal spine at dorsal face as long as first segment of 4-segmented antennal branch; long
400 distal sensory seta on ventral face (Fig. 4O). Transverse rows of numerous, minute
401 spinules on surface. Antennal branches elongated, 4-segmented exopodite slightly
402 shorter than 3-segmented endopodite, all segments cylindrical, with rows of minute
403 spinules and rows of long and thin setae. Antennal formula: setae 0-0-1-3/1-1-3, spines
404 0-1-0-1/1-0-1. Three long, apical swimming setae on both antennal branches, all with
405 basal and distal segments bilaterally armed with fine, long setules. Lateral seta on third
406 segment of dorsal branch with similar armature. Basal and distal lateral setae of ventral
407 branch armed in another manner: basal segments unilaterally setulated and distal ones
408 asymmetrically setulated. Spine on first endopodite segment short, like apical spines.
409 Valves – large, sub-ovoid, with row of 14 to16 setae along anterior ventral margin (Fig.
410 4A). Posterior part with row of marginal denticles, organized in groups of 7-10,
411 increasing in size distally within each group. Denticles in posterior most margin form
412 uniform row. Two posterior hooks supporting postabdominal setae (*setae natatoriae*)
413 present.
414 Thoracopods (Th)
415 Th1 (Fig. 5A) – distal lobe or endite 4 with single anterior thicker seta, bearing short
416 setules and two soft setae. Endite 3 with short, strong, single anterior seta, bearing
417 noticeable lateral spinule-like projections in both sides, and single long and strong

418 posterior seta. Endite 2 with two posterior setae. Endite 1 with three posterior setae.
419 Two ejector hooks of remarkably different sizes. No maxillary process on limb base.

420 Th2 (Figs 4T-V, 5B-D) – with large cylindrical lobe bearing long apical seta, its distal
421 part regularly setulated. Distal endite with two setulated setae, other two endites with
422 only one setulated seta each. One small seta near gnathobase plate (Fig. 5B, D),
423 followed anteriorly by small hook-like outgrow (Fig. 5I). Rest of gnathobase margin
424 with row of setae arranged fan-like. Posterior-most long single seta and neighboring
425 more anteriorly located, long seta separated by large gap. Both unilaterally setulated
426 with long peg-like setules facing proximally; posterior-most seta setulated along its
427 whole length, neighbouring seta on distal portion only. Above-mentioned two long setae
428 followed by row of 10 to 12 gradually shortening and curved setae. Fan-like
429 arrangement of setae is closed by group of three anterior brush-like setae, curved
430 proximally with unilateral setulation facing distally, posterior one strongly reduced.
431 Two shorter and thick setae with apical setulation inserted parallel to group of three
432 anterior-most setae (Fig. 4T, V and a-I in Fig. 5B). Fan-like setation is arranged in two
433 planes. Two long posterior setae plus two thick anterior *sensilla* and anterior brush-like
434 seta in ventral plane, remaining setae in dorsal plane (Fig. 4V and Fig. 5B, C).

435 Th3 – exopodite large, flat, with four distal setae (c-f in Fig. 5E) and two short proximal
436 setae, one short and one long (a and b in Fig. 5E), all of them bilaterally setulated. Inner
437 distal portion with three endites. Endite 3 with single anterior seta (3 in Fig. 5E); endite
438 2 with one anterior and one posterior seta (2, and arrow in Fig. 5E). Endite 1, with one
439 anterior seta (1, in Fig. 5E). Rest of endopodite with 28-29 soft setae (Fig. 5E).

440 Th4 (Figs 5G) – exopodite like Th3, but more elongated (Fig. 5F), with four long setae
441 (c-f in Fig. 5F) in distal part and two setae in proximal part, one short and other long (a-

442 b in Fig. 5F). Inner distal portion of thoracopod with two endites: endite 1 with long
443 articulated and bisetulated seta. Endite 2 with two long setulated setae (Fig. 5G). Filter
444 plate with 20-22 setae (Fig. 5G).

445 Th5 – with large ovoid lobe, with setulated margin, large distal seta and small proximal
446 seta, both bi-setulated (Fig. 4S and 5H). Inner thoracopod portion with elongated lobe
447 with long setulae followed by two setae (1-2 in Fig. 5H).

448 Postabdomen – elongated, conically narrowing distally. Ventral margin almost straight,
449 with numerous rows of minute setules in transversal rows (Fig. 4Q). Large anus located
450 closer to base of postabdomen than to its distal extremity, preanal margin long, straight.
451 Post-anal part tapering to claws. Preanal angle not well expressed. Laterally, row of 4-5
452 large, triangular teeth, fringed with marginal setules. Rows of setules on distal portion
453 continue along base of bident tooth; distal branch of bident tooth significantly longer
454 than proximal branch.

455 *Setae natatoriae* longer than postabdomen; basal part naked, distal part with two rows
456 of fine setules (Fig. 1).

457 Postabdominal claw slightly curved, with sharp, pointed tip (Fig. 4Q, R). Two
458 successive pectens along dorsal margin: basal pecten consisting of 5 to 8 slender spines
459 huddled together, followed by middle pecten of 8-12 strong, thick spines diverging to
460 both sides in fan-like arrangement; distal pecten consisting of smaller spines forming
461 row from mid-claw to naked tip. Basal and middle pectens fused in some individuals.
462 Uniform row of setules along inner face of claw. 4-7 large denticles on ventral margin,
463 near base of claw.
464

Ephippial female (Figs 4B, D-G)**:**

Ephippium broadly rounded, tapering only slightly in posterior part. Central part over egg chamber embossed. Dorsal ridge strongly sclerotized. Lateral surface with marked hexagonal reticulated pattern (Fig.4F). Ultrastructure of ephippial surface (magnification up to 1000x – 2000x) formed by complex structure of polygons interlocked with many thin tentacle-like projections of various length. Some projections cover also inner surface of polygons (Fig. 4G).

Male

Body – smaller than female, body length 0.45-0.54 mm (n=10), more elongated as compared to female (Fig. 4C). Dorsal margin of valves almost straight, postero-dorsal angle clearly distinct. Antero-ventral portion of valves fringed with long, thin setules. Denticles along posterior margin more distinct than in female, arranged in groups similarly.

Head – more elongated than in female, rostrum absent, labrum less fleshy than that of female. Optical vesicle fills top of head. No trace of pigment in ocellus.

Antennule – long, antennular sensory seta relatively long, male seta and sensillum inserted in first third of antennule. Nine aesthetascs and three to four hooks of distinct size and orientation, with bifurcated tips, present distally (Figs 4H, I).

Antenna – similar in armature to female. Distal margin of all segments with row of thick, short spinules.

Carapace surface without any hairs or setules (Fig. 4J).

Th1 – with thick copulatory hook, its tip with two spinules, endite 4 (IDL) with four setae, endite 3 with one seta. Basal part short and rounded (width:length ratio about 1:1), with row of stout bristles on ventral face. Small wart-like outgrow at inner base of hook (Figs 4K,L).

Postabdomen – as in female, shorter, with about 4-5 setulated teeth, distalmost tooth thin and with sharp tip. Distal bident tooth as in female. About 4-5 teeth in basal pecten of postabdominal claw, up to 10 in middle pecten (arranged fan-like) and 5-7 on dorsal side at basal part of claw (Fig. 4R). Gonopores in lateral position situated close to proximal triangular tooth (Fig. 4M).

Differential diagnosis

Within *terra typica*, the species *Moina micrura* may be confused probably with only one other species: *Moina weismanni* Ishikawa, 1896 (Figs 6A, B). Both species frequently co-occur in the same habitats and their parthenogenetic females look superficially similar. The list of easily recognized individual characters which differentiate *M. weismanni* from *M. micrura* are listed in Tab. 2. Full differential diagnosis from other members of the *micrura*-like species complex requires detailed systematic revision of populations from other biogeographic regions and the lineages detected by molecular methods.

Intercontinental and interspecific comparison of *Moina* ephippial ultrastructure

Specific ultrastructure on the ephippial surface from the *terra typica* (Fig. 4G), irregular hexagonal pillow-like cells, observed on the material from the Sádka pond, is morphologically similar to the structure observed on the material from other European

localities (see Supplementary information Table S2). However, we did not observe such structure on any of the *M. micrura* s. l. material from Africa, Asia, South America or Australia (Fig. 7A-H), whose ephippial structures vary from very flat hexagonal porous patterns (e.g., Figs 7C, H) to well delineated polygonal shapes (Fig. 7G). Ephippial ultrastructure of other *Moina* species (Fig. 8A-H) was even more variable, ranging from almost smooth surfaces (Figs 8A, B) to rounded cells (Figs 8G, H) or even prominent protuberances (Fig. 6E).

DISCUSSION

Moina micrura has been one of the most poorly defined cladoceran species, recorded under that name in almost any part of the world from the temperate regions to the tropics. Even worse, animals identified as *M. micrura* have been widely used as models for culturing or as ecotoxicology biomarkers. Results of studies employing this taxon, however, cannot be directly compared or replicated, as mentioned in the introduction. There was thus an urgent need to establish the correct assignment for *M. micrura* as a valid species, including the neotype, genetic analyses and detailed morphological description of all forms within the life cycle.

According to the International Code of Zoological Nomenclature (ICZN) (2000), designation of a neotype is justified when there is an exceptional need to clarify the taxonomy of a species (as is the case when several poorly defined sibling species exist), or when there is a reasonable certainty that the original type material does not exist. In case of *M. micrura*, both these conditions are met. Our search for the type material of *M. micrura* was unsuccessful. Kurz's original samples or specimens are not available either in the National Museum in Prague (curator's personal communication, P. Dolejš, 2014) or in Naturhistorisches Museum, Wien (curator P. Dworschak, 2015), which

533 would be most likely institutions for depositing types of animal species described in the
534 19th century from Bohemia.

535 Considering the documented existence of several genetically well-defined lineages, so
536 far labelled as *Moina micrura*, it is necessary to establish the neotype and the new type
537 locality as a reference for future studies. We chose the new type locality as close as
538 possible from the original one studied by Kurz (1875). However, typical *M. micrura*
539 localities in *terra typica,* the watersheds of the Elbe and Danube rivers, are fish ponds
540 and other relatively small water bodies with rapidly changing environments. Therefore,
541 it is highly probable that many present-day *Moina* populations will disappear or re-
542 occur in the near future. Hence, we added some more localities from *terra typica* as a
543 comparative material.

544 Despite rapidly changing natural environments of Europe, we managed to collect *M.*
545 *micrura* no more than 10 km from the Kurz's original type locality. Petrusek *et al.*
546 *(*2004) used material located about 90 km from the *locus typicus*; to confirm the
547 conspecifity of our population, we sequenced the gene for 12S rRNA originally studied
548 by Petrusek *et al.* (2004). Both studied Czech populations indeed belonged to the same
549 species, and thus provide a solid base for future comparisons.

550 The geographical distribution of *M. micrura* s. str. remains unclear, but the available
551 data (base on both genetic analyses and morphological comparisons) suggest that this
552 species is restricted to Europe and the western part of the Palearctic. Apart from Central
553 Europe, genetic data confirmed the presence of the same lineage also in the Middle East
554 (Israel) and Central Asia (Kazakhstan). Molecular analyses nevertheless showed that
555 within Eurasia, numerous other lineages, presumably species, of the *M. micrura*
556 complex reside (Bekker *et al.,* 2016; this study), this is also true for Europe alone where

557 distinct lineages were detected in Hungary or Spain. Sequenced American (Elías-
558 Gutiérrez *et al.,* 2008; Prosser *et al.,* 2013) and Australian populations (Petrusek *et al.,*
559 2004) compared with all Eurasian sequences indicate that they are all distinct species as
560 well, with K2P divergences at COI or 12S exceeding 13% and 8%, respectively. The
561 distinctness of divergent clades of the complex are also supported by a hybridization
562 experiment that demonstrated an apparent reproductive isolation between European and
563 Australian clones (Petrusek *et al.,* 2004).

564 A detailed redescription of morphology of *M. micrura* s.str. presented here provides a
565 necessary step for detangling of this rich species complex. Furthermore, we highlight a
566 potential importance of ephippial ultrastructure for *Moina* taxonomy. This feature that
567 was for long suspected as taxonomically relevant: Goulden (1966) suggested the
568 possibility that the structure of the ephippial surface in *Moina* is crucial in the male
569 selection of the partner during mating, and is thus species specific. As available
570 identification keys cover only partially up to date known members of the family
571 Moinidae, we have selected 15 gamogenetic populations of *Moina micrura s.l.* from
572 available samples collected on different continents, in which ephippia were present. To
573 assess further the diversity of ephippial ultrastructures within the genus *Moina*, we
574 added SEM pictures of nine additional species distinct from *micrura*-like populations
575 (Figs. 7 and 8). Comparison of the ephippial surfaces revealed several distinct patterns
576 of ornamentation. None of them resembles that of *Moina micrura* Kurz, 1875 and the
577 feature seems to be a good differential character in species identification. Such
578 substantial variation in the ephippial ultrastructure seems to support the Goulden's
579 original hypothesis, although the potential role of this morphological character feature
580 in establishing or maintenance of reproductive barriers between *Moina* species needs
581 testing. In any case, these apparently species-specific characteristics of *Moina* ephippia

582 open a possibility to develop keys for their routine identification in various limnological
583 projects.
584 Most of above-mentioned morphological traits have not been described before, at least
585 for the *micrura* complex. Future analysis of them is recommendable, to understand the
586 evolution, adaptation and success of these organisms in different regions of the world.
587 Any detailed study of these complexes also requires a detailed knowledge of the males
588 and ephippial female morphology, because these often present more relevant species-
589 specific traits to recognize the species than parthenogenetic females (as also shown for,
590 e.g., *Daphnia*; Juračka *et al.*, 2010; Popova *et al.*, 2016). Although gamogenetic
591 populations may occur only sporadically in the field, there is a possibility to induce
592 sexual individuals in a laboratory culture, including hormonal stimulation to obtain
593 males (Kim *et al.*, 2006).
594 Combining detailed studies of morphology, with distribution data and molecular
595 markers have been very useful to detangle complex groups of sibling species (Popova *et*
596 *al.,* 2016). The *M. micrura* complex would deserve a similar treatment in the future,
597 redescription of *M. micrura* s. str. should only be the beginning.

598 Sequences from the COI gene will likely play a useful role in future identifications, as
599 molecular techniques are becoming widespread even in routine biodiversity surveys and
600 limnological projects. Unfortunately, in most cases when possible hidden taxa of
601 cladocerans are uncovered by molecular tools, no nomenclatural act is realized, often
602 due to the lack of reference material to compare (e.g., Elías-Gutiérrez *et al.,* 2008), or
603 due the focus of the study different to taxonomy (e.g., Xu *et al.,* 2016). However, to
604 foster further research and appropriately recognize the diversity of groups in question in
605 other biological fields (e.g., ecotoxicology), formally naming well-defined lineages,
606 even cryptic species, should be considered (Fišer *et al.*, 2018).

607

608 **CONCLUSIONS**

609 We can conclude that *Moina micrura s. str.* is distributed at least from Central Europe
610 (with *terra typica* in the Czech Republic) to Central Asia (Kazakhstan) but it seems to
611 overlap in distribution with other related species (as a genetically distinct lineage has
612 been detected in Hungary). *Moina* cf. *micrura* from Albufera Lake in Spain and Sobrón
613 reservoir possibly is another, not yet described species that requires further attention. Is
614 clear that European *Moina micrura* s. str. is distinct from analyzed populations from
615 North and South America, Australia, Africa, Korea or Russia. The *M. micrura*-like
616 populations from American continent are a complex of species, consisting of at least
617 five distinct lineages that also require further, more detailed analyses. We hope this
618 work, based on integrative taxonomy, establishes a baseline for future comparisons of
619 *M. micrura* s. str. with other members of this complex, in order to facilitate its critical
620 revision and the delimitation of species within.

621

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638

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779

780 **Table 1. Genetic divergences (K2P) among the 12S (A) and COI (B) sequences for**

781 ***M. micrura* s.l.** All divergences are calculated as comparisons to *M. micrura* s. str. from

782 the fishpond Sádka at Nové Dvory, i.e., a locality from which a neotype was selected.

783 *Needs a revision to evaluate its distinctness or conspecifity with *M. micrura* s. str.

784 **Only one sequence is available.

785 **(A)**

Country	Locality (clade)	12S divergence
Czech Republic	Sádka	0
	Dobříň	0.19
	Ovčáry	0
Spain	Albufera lake (1 ES)	1.32*
	Sobron reservoir (1 ES)	1.32*
	Albufera lake (2 ES)	8.96
	Zahillo (3 ES)	11.31
Israel	Kinneret	0
Ethiopia	Dibdibo	6.46
Slovakia	Hrhov	0.19
Hungary	Czista Puszta	10.22
Australia	Albury	8.95

786 **(B)**

Country (lineage)	COI Divergence	
	Min Dist (%)	Max Dist (%)
Czech Republic	0**	0**
Kazakhstan	0.46	0.46
Spain (1 ES)	4.64	4.64
India	11.58	11.58
Spain (2 ES)	15.34	15.34
Russia	17.68	18.09
South Korea, Russia, Hungary	15.99	15.99
Mexico (MX 1)	15.24	16.37
Mexico (MX 2)	15.11	15.46
Mexico (MX 3)	13.28	14.30
Mexico (MX 4)	14.70	14.70
Mexico (MX 5)	13.52	13.52

787 **Table 2.** Differential diagnosis between *Moina micrura* Kurz and *Moina weismanni*

788 Ishikawa

Character	***Moina micrura***	**Fig.**	***Moina weismanni***	**Fig.**
Female				
Shape of antenna I.	straight	4P	curved	6M
Spines in middle pecten on postabdominal claw	short, robust; 1:4 *)	4R	long, slender; 1:11-12*)	6L
Ephippial surface	reticulated	4F	distinctly raised knobs	6C,D
Ephippial ultrastructure (SEM)	polygons interlocked with many tentacle-like projections	4G	distinct knobs without projections	6E
Male				
Anterior surface of carapace	Naked	4J	covered with dense "fur" of short hairs	6J
Length of basal part of antennule	about 1/3 of its length	4H	About 1/5 of its length	6F
Setae along anterior ventral margin	about same length	4J	growing in length distally	6K
1st thoracopod	short basal part (without hook); 1:1 *)	4K,L	long basal part; 1:2 *)	6H,I
	hook robust	4K	hook feebly developed	6H
	small outgrow at base of hook	4K	broad scale-like seta at base of hook	6H

789 *) Ratio width:length (width of spines in pecten measured at their base)

790 **Figure 1**. *Moina micrura* drawn by Kurz (1875) in the original species description.

791 **Figure 2**. **Maximum Likelihood trees of the *M. micrura* complex**. Bootstrap values
792 (1000 replicates) are shown above the branches. The scale bar shows K2P distances.
793 The node for each clade with multiple specimens is collapsed, expanded tree is found as
794 supplementary material S4. Bracketed numbers next to each group name indicate the
795 number of individuals analyzed. A. Represents 29 sequences of 12S gene. M.
796 macrocopa from the Czech Republic was used as an outgroup. B. Represents 148 COI
797 sequences from the *M. micrura* complex. In this case *M. macrocopa* from Mexico was
798 used as an outgroup.

799 **Figure 3.** Distribution of *Moina micrura* s. str. based on the genetic analyses of the
800 genes for 12S rRNA and COI.

801 **Figure 4**. **Morphological features of *Moina micrura* s.str.** Specimens were sampled at
802 three localities in the Czech Republic: fishpond Sádka at Nové Dvory (27th June 2002,
803 images A, B, C, F, G, I, Q, R, S, T, U & V), fishpond Školní at Chabařovice (2nd
804 August 2004, images D, E, H, J, K, M & N), and flooded sandpit near Dobříň (5th
805 October 1999, image P). A-C: light microscope, focus stacked images. A) Adult
806 parthenogenetic female. B) Adult ephippial female. C) Adult male. D-R: SEM. D) Adult
807 ephippial female in the dorsal view. E) Adult ephippial female in lateral view. F) Free
808 ephippium in the lateral view. G) Free ephippium ultrastructure detail. H) Adult male,
809 head in the ventral view. I) Adult male, tips of first antennae (detail). J) Adult male,
810 ventral carapace margin. K) Adult male, 1st thoracopode, arrow indicates small outgrow
811 at base of the hook. L) Adult male, first thoracopode. M) Adult male, postabdomen,
812 arrow indicates gonopore. N) Ephippial female, basipodite of second antenna. O)
813 Ephippial female, second antenna. P) Adult female, first antenna. Q) Adult female,
814 postabdomen. R) Adult female, base of postabdominal claw with pectens. S) Adult

female, 5th thoracopode. T) Adult female, 2nd thoracopode. U) Adult female, 2nd thoracopode. V) Adult female, 2nd thoracopode gnathobase.

Figure 5. **Thoracic limbs of female of *Moina micrura* s.str.** (drawings based on individuals from Sádka pond at Nové Dvory). A: Th1 general view; B: Th2 general view; C: Th2 – anterior setae; D: Th2 – detail of the seta; E: Th3 general view; F: Th4, gnathobase; G: Th4, exopod; H: Th5, general view; I: Th2, detail of the sensilia.

Figure 6. **Morphological features of *Moina weismanni*.** Specimens sampled at Radíkovice, Czech Republic (3rd July 1957, images A & B) and at Krasnodar, Russia (21st July 1952, all other images). A-B: light microscope, focus stacked images. A) Adult female in the lateral view. B) Adult male in the lateral view. C-M: SEM. C) Adult ephippial female in the lateral view. D) Ephippial female in the dorsal view. E) Ephippium ultrastructure of the ephippial female. F) Adult male, first antennae (detail of tips in inset). G) Adult male, basipodite of first antenna. H) Adult male, 5th thoracopode in ventral aspect. I) Adult male, 5th thoracopode in dorsal aspect. J) Adult male, ventral carapace margin and postabdomen. K) Adult female, carapace ventral margin. L) Adult female, claw. M) Adult female, first antennae.

Figure 7. Intercontinental comparison of ephippial ultrastructure of Moina micrura s.l. Ephippia were mostly positioned in the ephippial female (with the exception a free ephippium in M), all images are of the same magnification. A) Costa Rica (18th May 1981). B) Bordo ala Colorado, Aguascalientes, Mexico (18th January 1989). C) Lima, Lima Campos, Ceara, Brazil (23rd May 1989). D) Relfs, NSW, Australia (25th January 1968). E) Lake Albufera, Valencia, Spain (2015). F) Mwanza, Tanzania (7th April 1985). G) Borena, Oromia, Ethiopia (1st October 1983). H) Jabaddi Ludhiana, Punjab, India (13th September 1977).

Figure 8. Variation of ephippial ultrastructure among different *Moina* species images are from the same magnification. A) Bač, Slovakia (27 July 1954). B) North Victoria highways, Australia (5th February 1982). C) Avis Dam, Namibia (27th April 1972). D) Yihe Modoto, Mongolia (6th September 1988). E) Schantrapay, Russia (26th July 1974). F) Lago Jecateringa, Brazil (2nd 1980). G) Alexandria, Australia (26th September 1973). H) Komárno, Slovakia (27th July 1954).

Supporting Information

S1 Table. Localities, GPS Coordinates, BOLD Sample ID, GenBank accession numbers and sequenced gen for *Moina micrura* complex in this study

S2 Table. Material examined

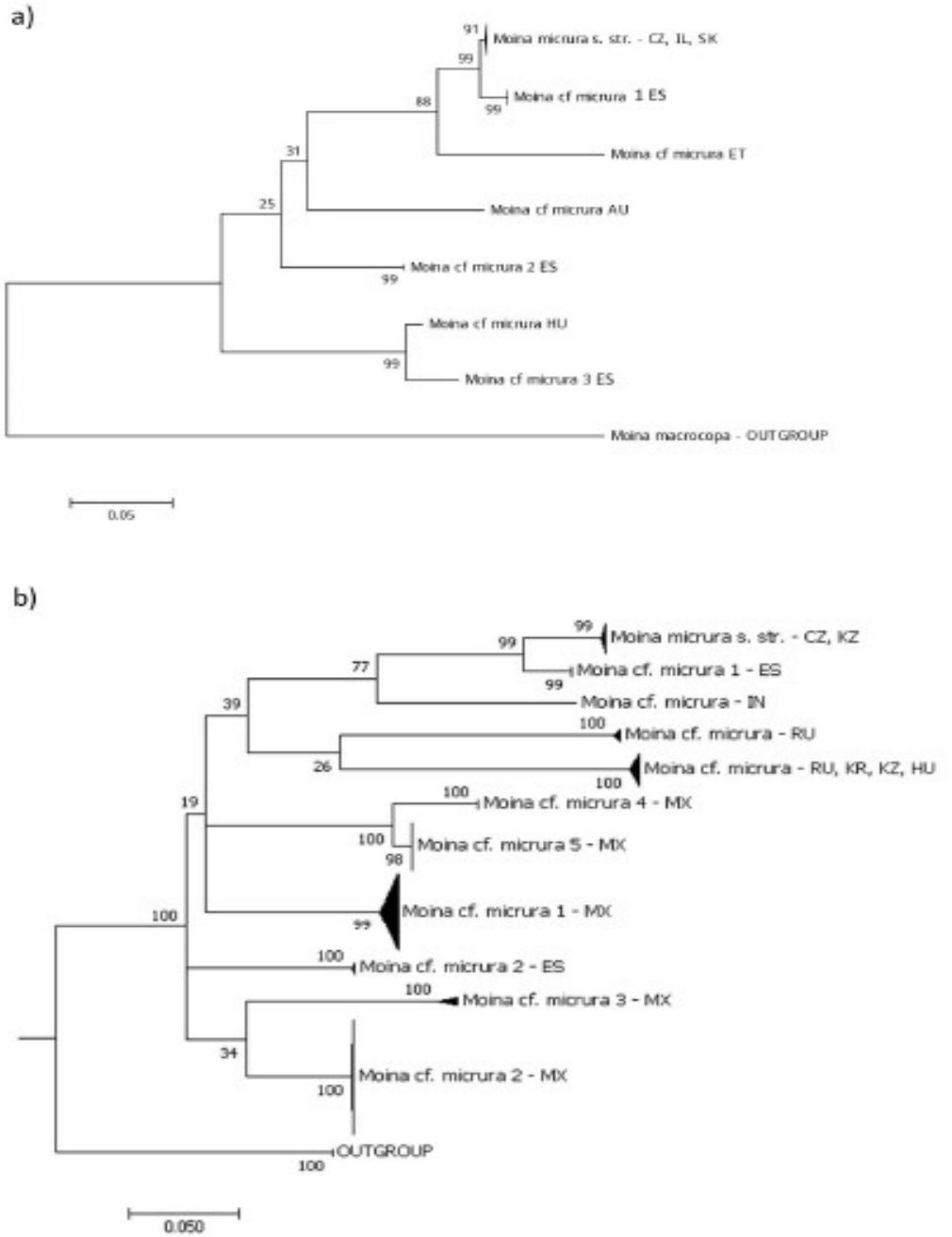
S4 Figure. 12S and COI ID Tree

S6 Figure. 12S and COI ML expanded Tree

852 Fig. 1

853

854 Fig. 2



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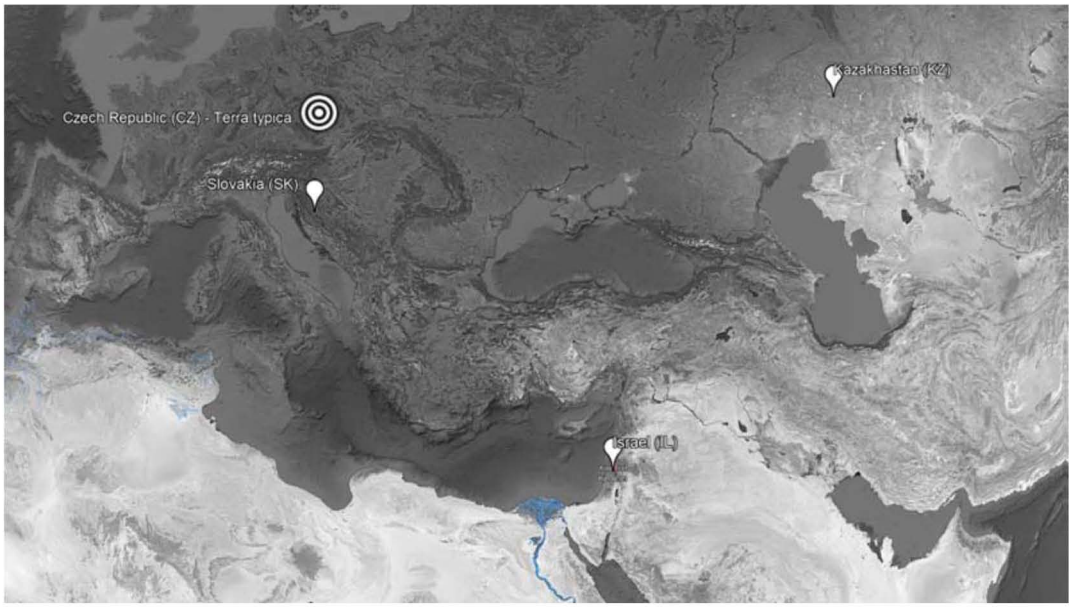

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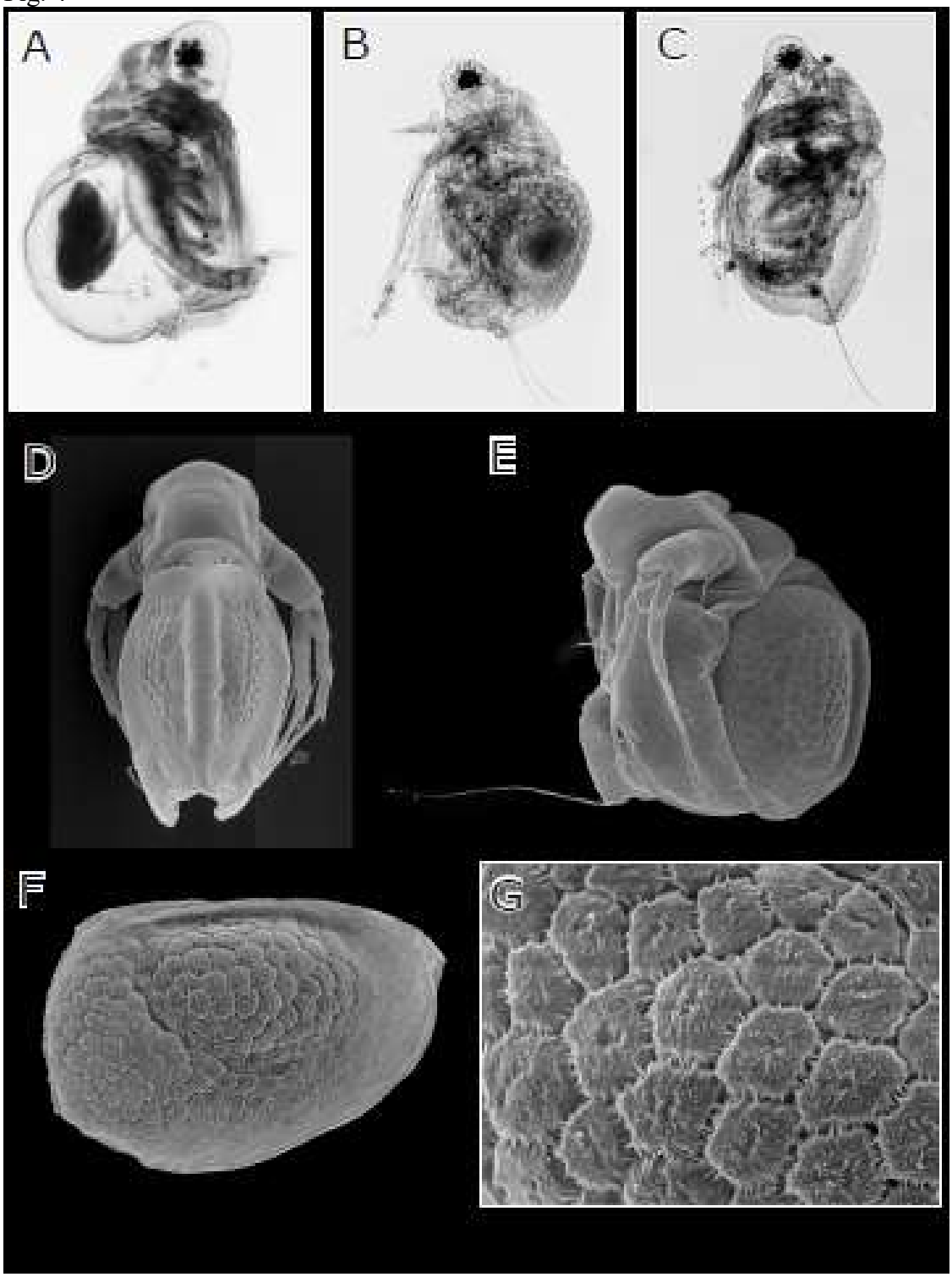
872 Fig. 4



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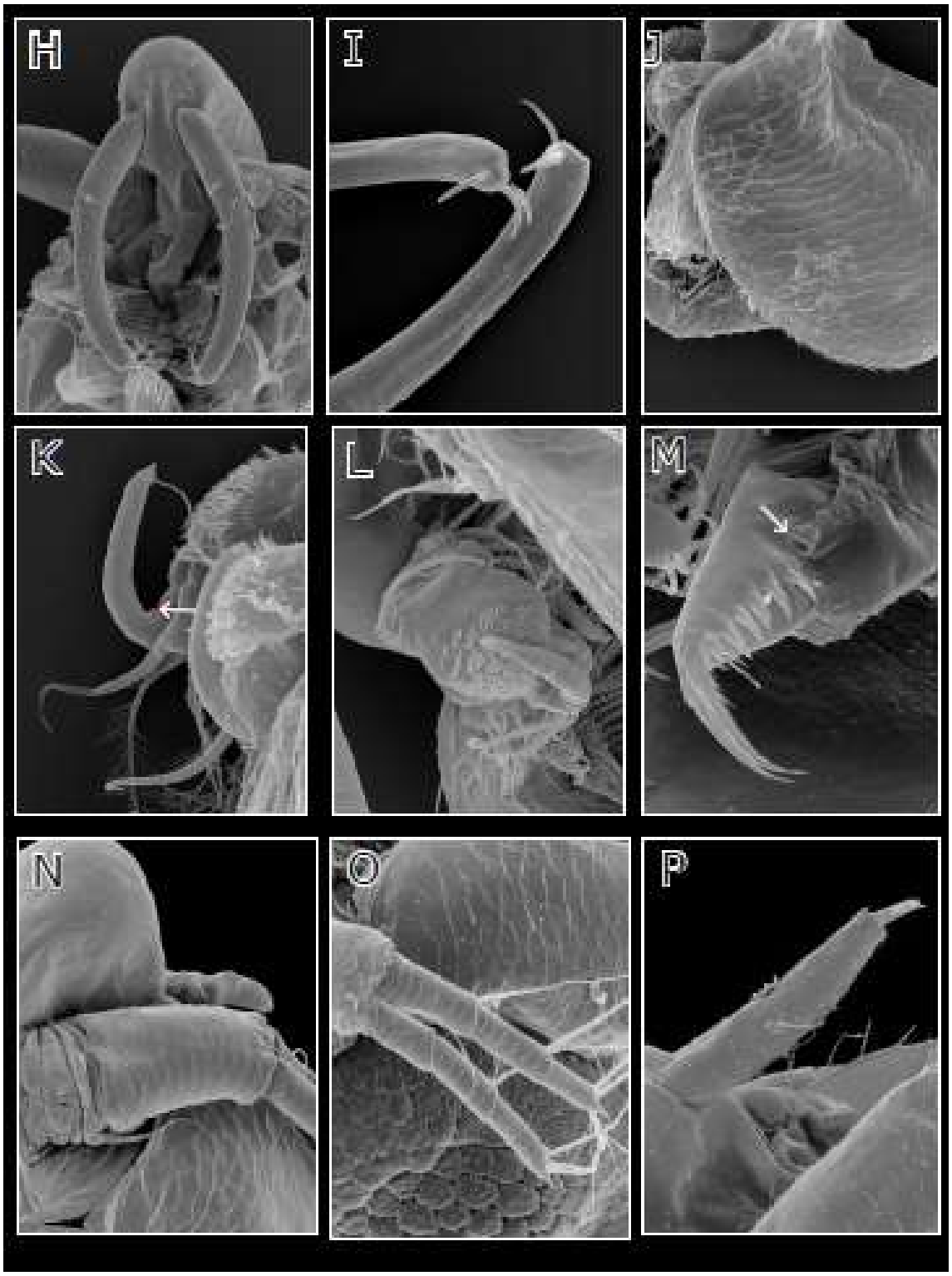

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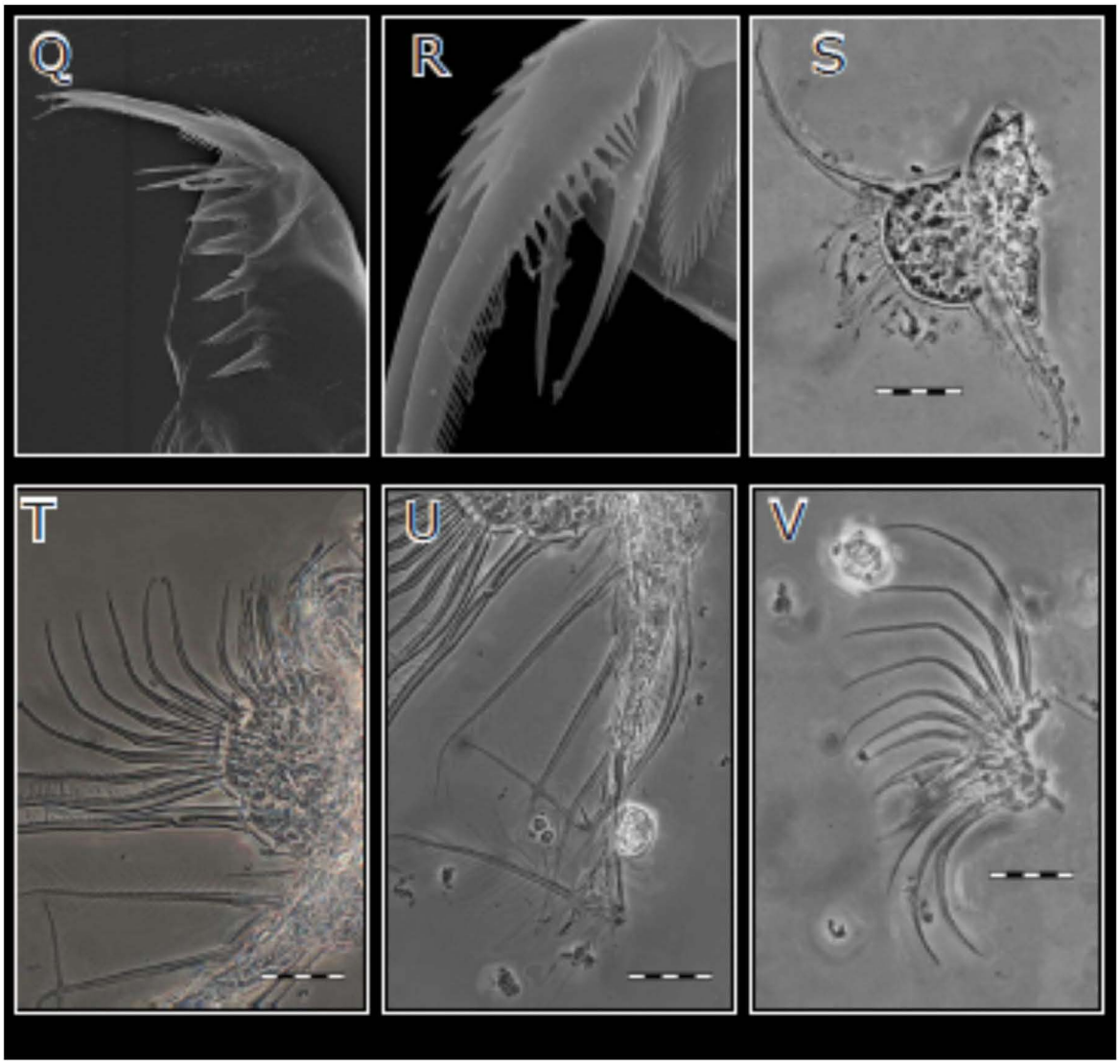

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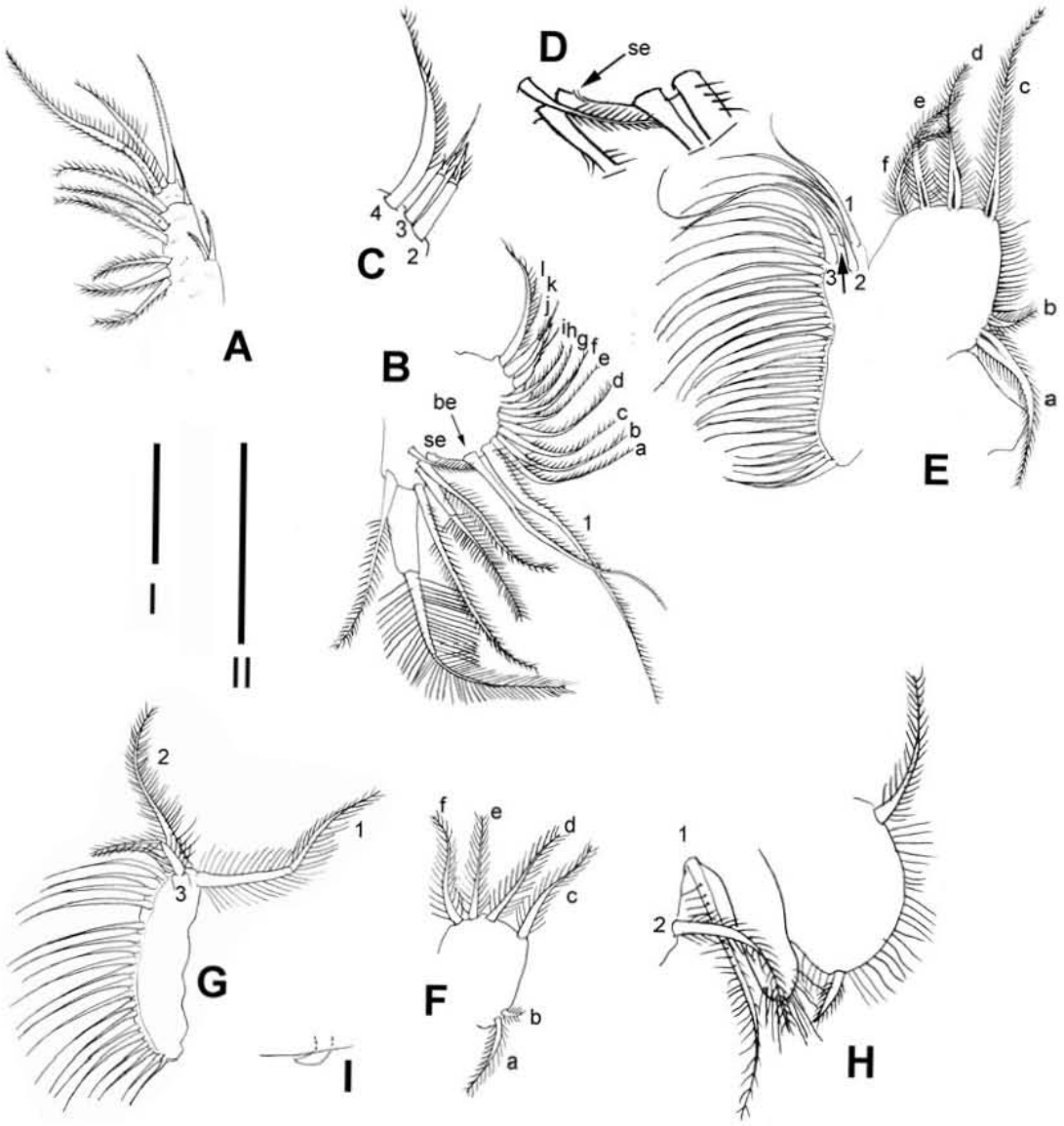
879 Fig. 5



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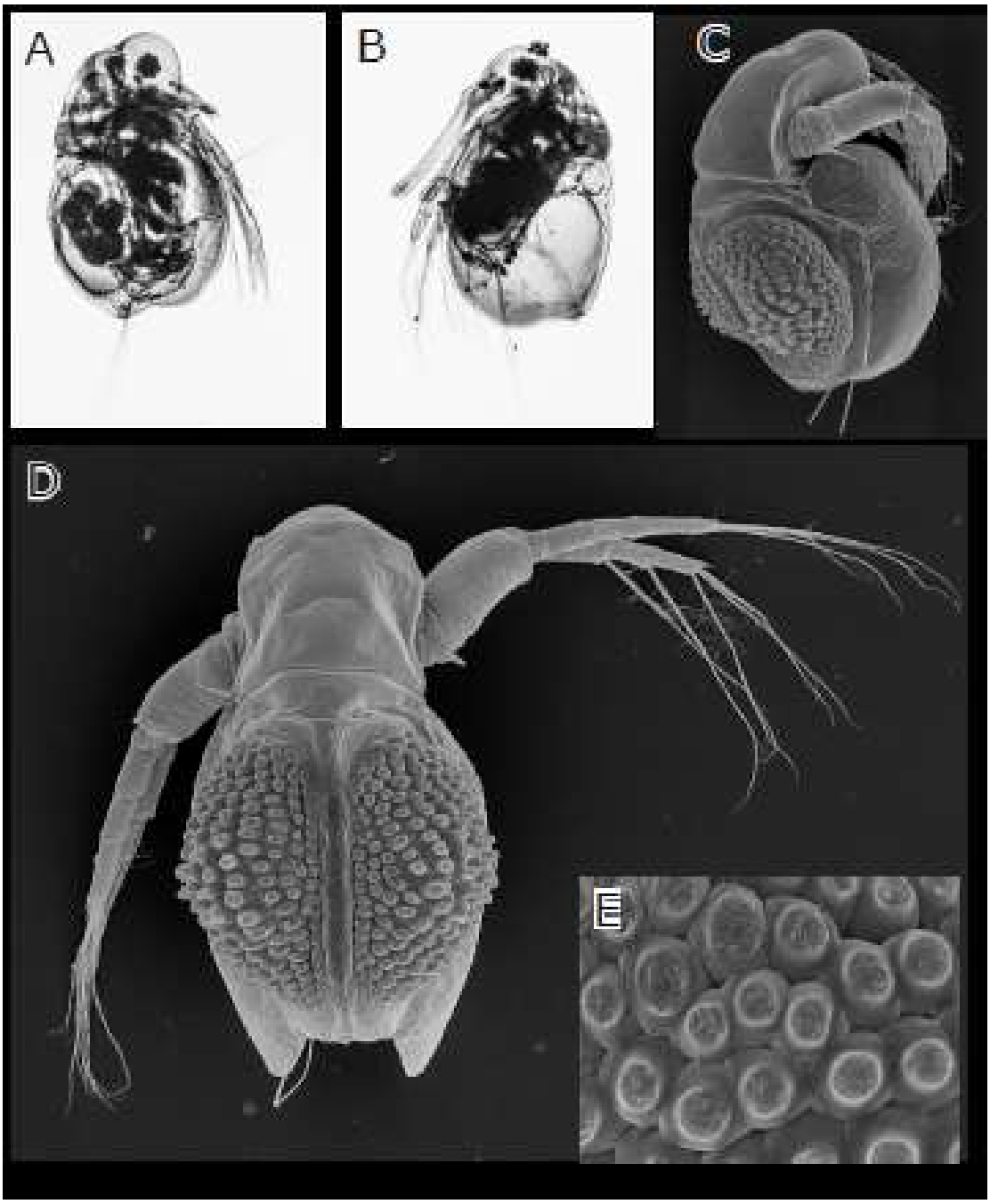
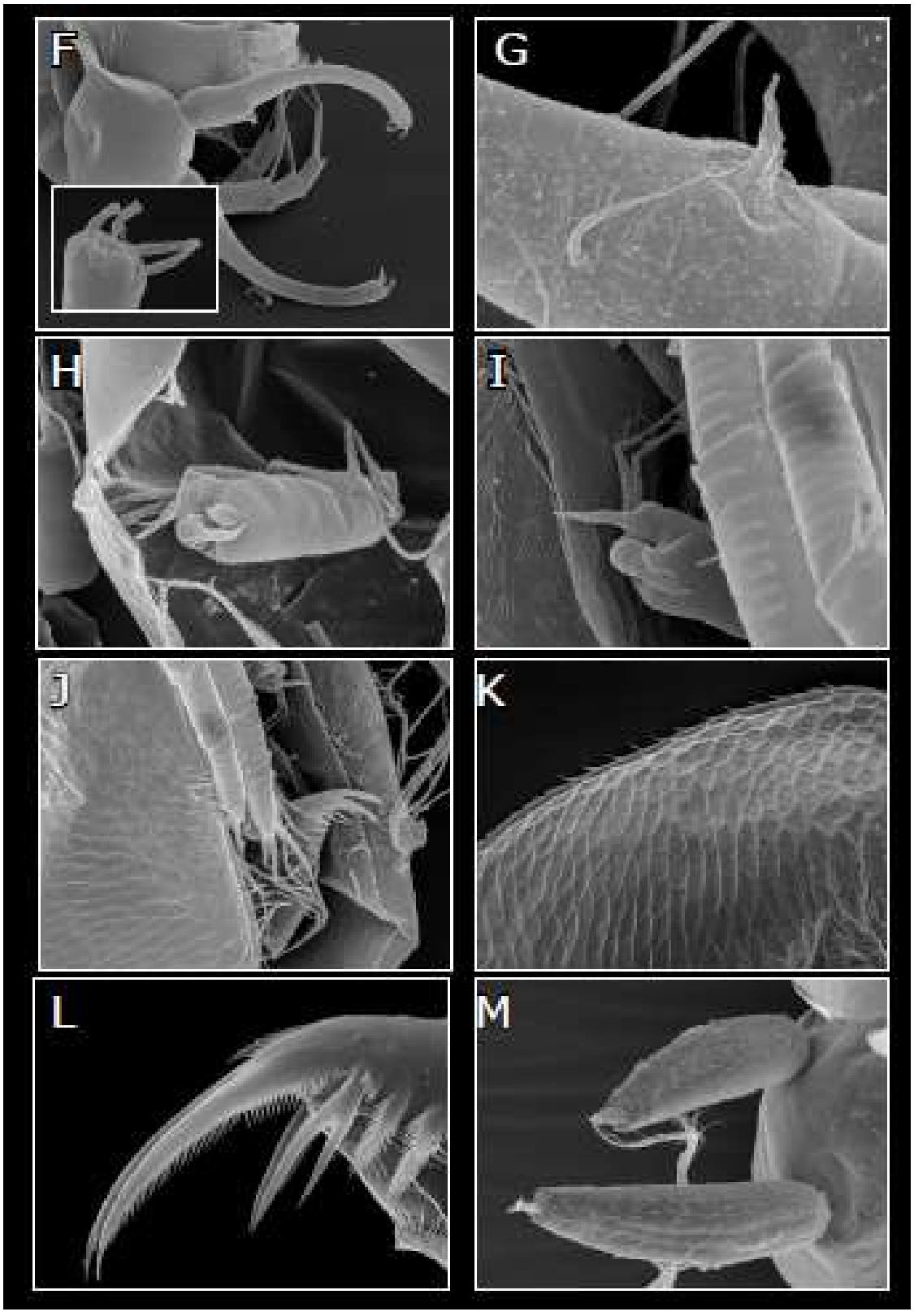
882 Fig. 6





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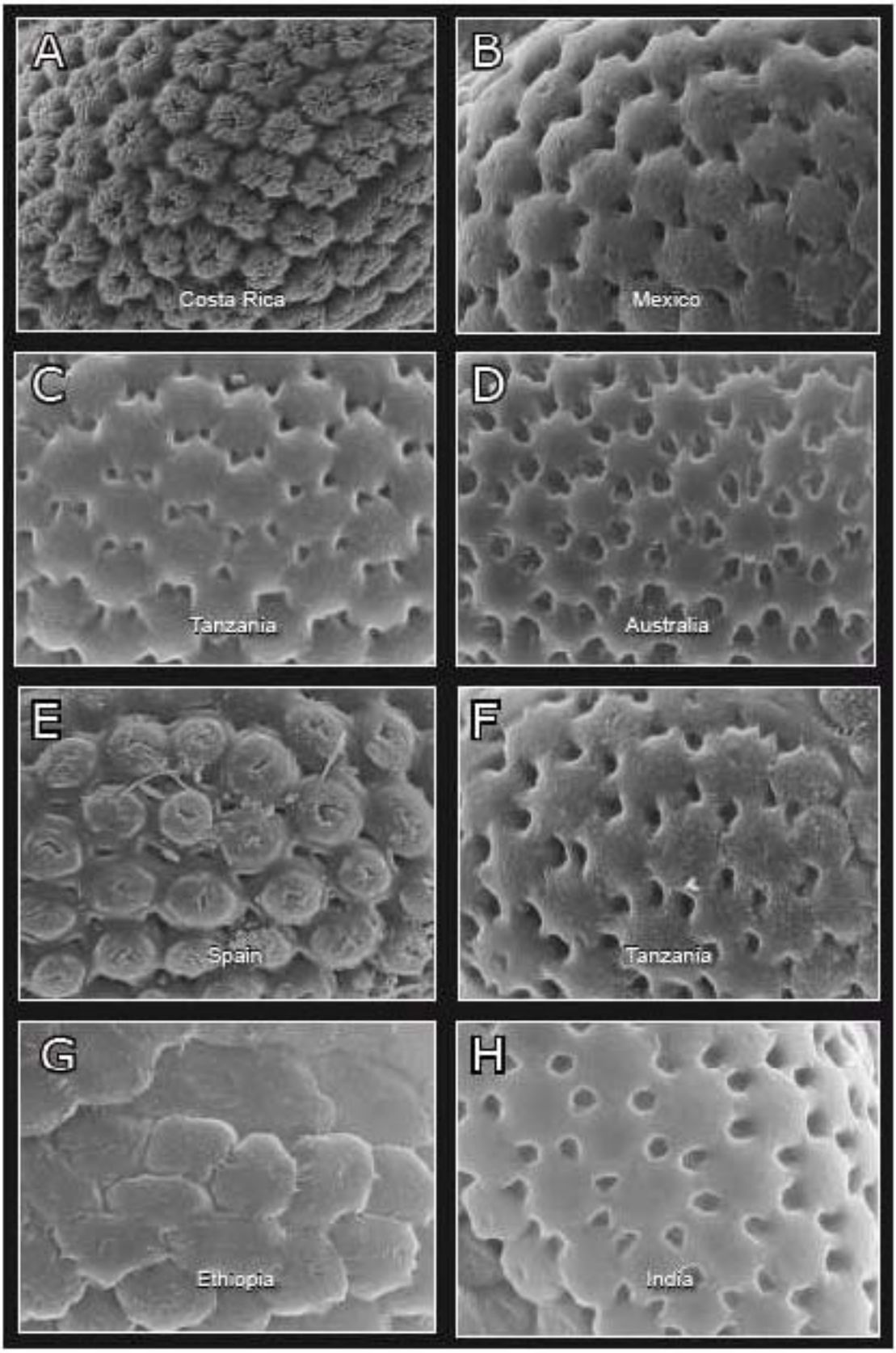
886 Fig. 7



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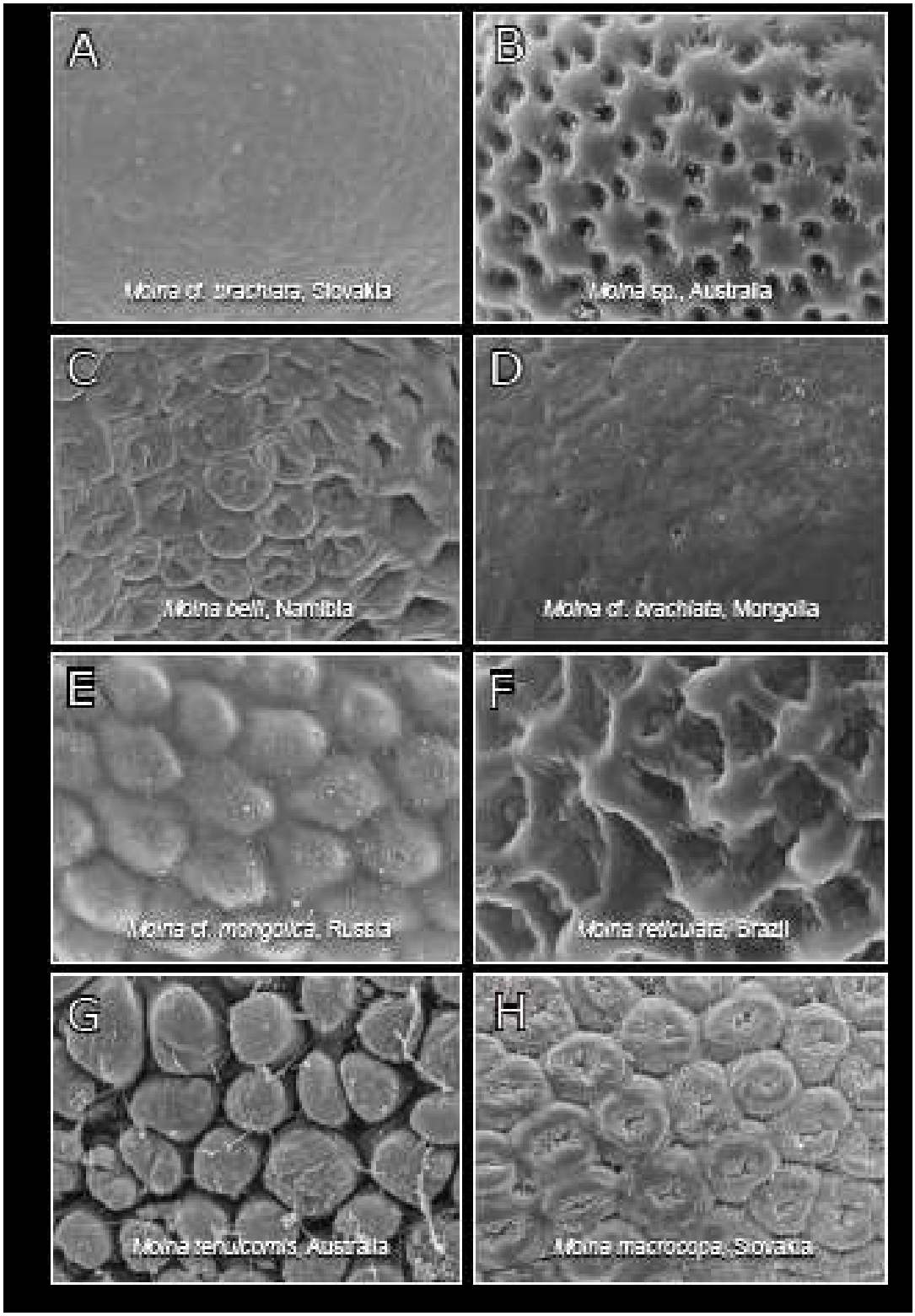
889 Fig. 8


A
Moina cf. brachiata, Slovakia
B
Moina sp., Australia
C
Moina belli, Namibia
D
Moina cf. brachiata, Mongolia
E
Moina cf. mongolica, Russia
F
Moina reticulata, Brazil
G
Moina tenuicornis, Australia
H
Moina macrocopa, Slovakia

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Observations	Phylogroup name	Sequence unique ID	Country	GenBank accession numbers	Reference
Terra typica	*Moina micrura* s. str.	EES614	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES615	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES620	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES622	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES623	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES624	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES625	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES626	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES627	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES628	Czech Republic		This study
Terra typica	*Moina micrura* s. str.	EES629	Czech Republic		This study
	Moina cf. *micrura*	EES056	Spain		This study
	Moina cf. *micrura*	EES069	Spain		This study
	Moina cf. *micrura*	EES093	Spain		This study
	Moina cf. *micrura*	EES094	Spain		This study
	Moina cf. *micrura*	EES054	Spain		This study
	Moina cf. *micrura*	EES089	Spain		This study
	Moina cf. *micrura*	EES518	Spain		This study
	Moina cf. *micrura*	EES519	Spain		This study
	Moina cf. *micrura 2*	ZPLKA028	Mexico		This study
	Moina cf. *micrura 2*	ZPLKA123	Mexico		This study
	Moina cf. *micrura*	EES646	Spain		This study
	Moina cf. *micrura*	EES647	Spain		This study
	Moina cf. *micrura*	EES648	Spain		This study
Terra typica	*Moina micrura* s. str.	CZ001	Czech Republic		This study
	Moina micrura s. str.	CZ002	Slovakia		This study
	Moina micrura s. str.	CZ003	Israel		This study
	Moina cf. *micrura*	CZ004	Ethiopia		This study
	Moina cf. *micrura*	CZ005	Hungary		This study
	Moina cf. *micrura*	CZ006	Spain		This study
Terra typica	*Moina micrura* s. str.	AF339093	Czech Republic	AF339093	Petrusek *et al.*, 2004
	Moina cf. micrura	CZ007	Australia	AF339092	Petrusek *et al.*, 2004
	Moina cf. micrura	KC479042	India	KC479042	Dumont *et al.*, direct submission
	Moina cf. *micrura*	HQ336797.1	Russia	HQ336797.1	Frolova *et al.*, direct submission
	Moina cf. *micrura*	HE-643.1	South Korea	KC617390.1	Prosser *et al.*, 2013
	Moina cf. *micrura*	HE-647.1	South Korea	KC617391.1	Prosser *et al.*, 2013
	Moina cf. *micrura*	HE-645.1	South Korea	KC617392.1	Prosser *et al.*, 2013
	Moina cf. *micrura*	HE-646.1	South Korea	KC617393.1	Prosser *et al.*, 2013

Name	Collection date	Country	Locality	Habitat	Latitude, °N	Longitude, °E	Collector
Moina micrura Kurz, 1875	27/07/2002	Czech Republic	Sádka pond (Nové Dvory)	Fishpond	49.97	15.33	A. Petrusek
Moina micrura Kurz, 1875	13/8/2013	Czech Republic	Sádka pond (Nové Dvory)	Fishpond	49.97	15.33	M. Miracle
Moina micrura Kurz, 1875	10/2015	Czech Republic	Sádka pond (Nové Dvory	Fishpond	50.06	15.24	A. Petrusek
Moina micrura Kurz, 1875	19/09/1999 5/10/1999	Czech Republic	Dobříň near Roudnice nad Labem	Sand pit	50.44	14.31	A. Petrusek
Moina micrura Kurz, 1875	02/08/2004	Czech Republic	Školní pond at Chabařovice	Fishpond	50.67	13.937	K. Pilařová
Moina micrura Kurz, 1875	09/09/1960	Slovakia	Michalovce, Vinianské Lake	Lake	48.818	21.987	V. Kořínek
Moina micrura Kurz, 1875	02/07/1957	Slovakia	Štúrovo	Pool at Danube River	47.81	18.73	M. Legner
Moina micrura Kurz, 1875	06/2002	Slovakia	Hrhovske ponds	Fishpond	48.595	20.757	A. Petrusek
Moina micrura Kurz, 1875	11/10/1987	Bulgaria	S. of Blagoevgrad	Fishpond	41.98	23.08	V. Korinek
Moina micrura Kurz, 1875	2002	Israel	Lake Kinneret	Lake	32.8	35.6	K.D. Hambright
Moina cf. *micrura*	25/07/2012	Spain	Sobrón reservoir	River reservoir	42.77	-3.10	M.R. Miracle
Moina cf. *micrura*	21/07/2012	Spain	Albufera de Valencia	Coastal lagoon	39.33	-0.37	M.R. Miracle
micrura-like	25/01/1968	Australia	NSW, Relfs via Gloucester	Irrigation Dam	-31.98	151.95	B. Timms
micrura-like	09/07/1973	Australia	Weering (Victoria)	Pond	-38.12	143.63	C.H. Fernando
micrura-like	05/1981	Costa Rica	Pond #18 at Nunez	Pond			M. Dickman
micrura-like	28/03/1966	Cuba	Laguna Sabanilla (Habana)	Lake			J. Fott
micrura-like	18/01/1989	Mexico	La colorada pond (Aguascalientes)	Pond	21.985	101.986	M. Silva-Briano
micrura-like	23/05/1989	Brazil	Ceara, Pond at reservoir Lima Campos	Pond	-5.54	38.82	C.H. Fernando
micrura-like	03/04/1972	Brazil	Pindamonhangaba	Fish tank	-22.939	45.462	C.H.

Name	Collection date	Country	Locality	Habitat	Latitude, °N	Longitude, °E	Collector
			(Sao Paulo)				Fernando
micrura-like	01/10/1983	Ethiopia	Borkena swamp Oromia)	Swamp			C.H. Fernando
micrura-like	07/03/1985	Tanzania	Mwanza	Small lake	-2.74	32.52	Mr. Kovarik
micrura-like	07/08/1987	India	Raani Taal Pond (Madhya Pradesh)	Pond	23.173	79.918	C.H. Fernando
micrura-like	13/09/1977	India	Jabadi Ludhiana (Punjab)	Ditch	30.90	75.81	C.H. Fernando
micrura-like	22/06/1977	Indonesia	Sindang Barang, Bogor	Fish pond	-6.58	106.76	C.H. Fernando
micrura-like	18/09/1973	Malaysia	Experimental station, Sayopal				C.H. Fernando
micrura-like	09/04/1968	Philippines	Lake Lanao (Mindanao)	Lake			C.H. Fernando
Moina weismanni Ishikawa, 1896		Czech Republic	Fishpond Radíkovický (Benešov)	Fish pond	49.771	14.708	Z. Brandl
Moina weismanni Ishikawa, 1896	19/08/1943	Czech Republic	Village pond in Ohrobec	Pond	49.942	14.432	R. Šrámek-Husek
Moina weismanni Ishikawa, 1896	14/08/1931	Czech Republic	Bohumileč, village pond	Pond	50.10	15.859	R. Šrámek-Husek
Moina weismanni Ishikawa, 1896	19/08/2013	Czech Republic	Strakonice, Fish Pond Pýcha	Fish pond	49.434	13.910	V. Kořínek
Moina weismanni Ishikawa, 1896	21/07/1952	Russia	Goryachiy Klyuch, experimental pond #5	Fish farm	44.64	39.14	Hydrobiol. Expedition Zool. Inst. R. Acad. Sc
Moina weismanni Ishikawa, 1896	16/07/1938	China	Hulun Lake (=Dalai nuur lake)	Outflow to Argun River	49.30	117.67	T. Kawamura & D. Miyadi
Moina cf. *australiensis* Sars, 1896	29/09/1973	Australia	Weering (Victoria)	Roadside pond			C.H. Fernando
Moina belli Gurney, 1904	27/04/1972	Namibia	Avis Dam (Windhoek region)	Dam	– 22.573	17.131	S. Bethune
Moina brachiata Jurine, 1820	6/09/1988	Mongolia	Yihe Modoto	Ditch	48.189	111.192	J. Seďa
Moina macrocopa Straus,	27/07/1954	Slovakia	Komárno town	Flooded fields			E. Balon

Name	Collection date	Country	Locality	Habitat	Latitude, °N	Longitude, °E	Collector
1820							
Moina reticulata Daday, 1905	02/09.1980	Brazil	Lake Jacaretinga (Amazonas)	Lake	-5.412	60.419	A. Duncan
Moina mongolica Daday, 1901	26/07/1974	Russia	Lake Bol'schoy Schantrapay	Lake	54.796	61.970	E. Makartseva
Moina cf *flexuosa* Sars, 1897	05/07/1982	Australia	75km S. of the junction - Great Northern Hwy x Victoria Hwy	Roadside pond	-21.195	118.722	P.E. Tyler
Moina tenuicornis Sars, 1896	26/09/1973	Australia	Alexandria (Victoria)	Small puddle			C.H. Fernando
Moina sp.	14/05/1974	Mongolia	Bulgan	River			Dash Dorzh

BOLD TaxonID Tree

Title : Tree Result - DS-MICRURA1 (28 records selected)
Date : 15-May-2017
Data Type : Nucleotide
Distance Model : Kimura 2 Parameter
Marker : 12S
Colourization : [blue]=Stop Codons [red]=Contamination or misidentification

Label : Sample ID
Label : Species
Label : Country
Label : Exact Site

Filter : length > 200bp only
Filter : exclude records flagged as misidentifications
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Genus count : 1
Family count : 1
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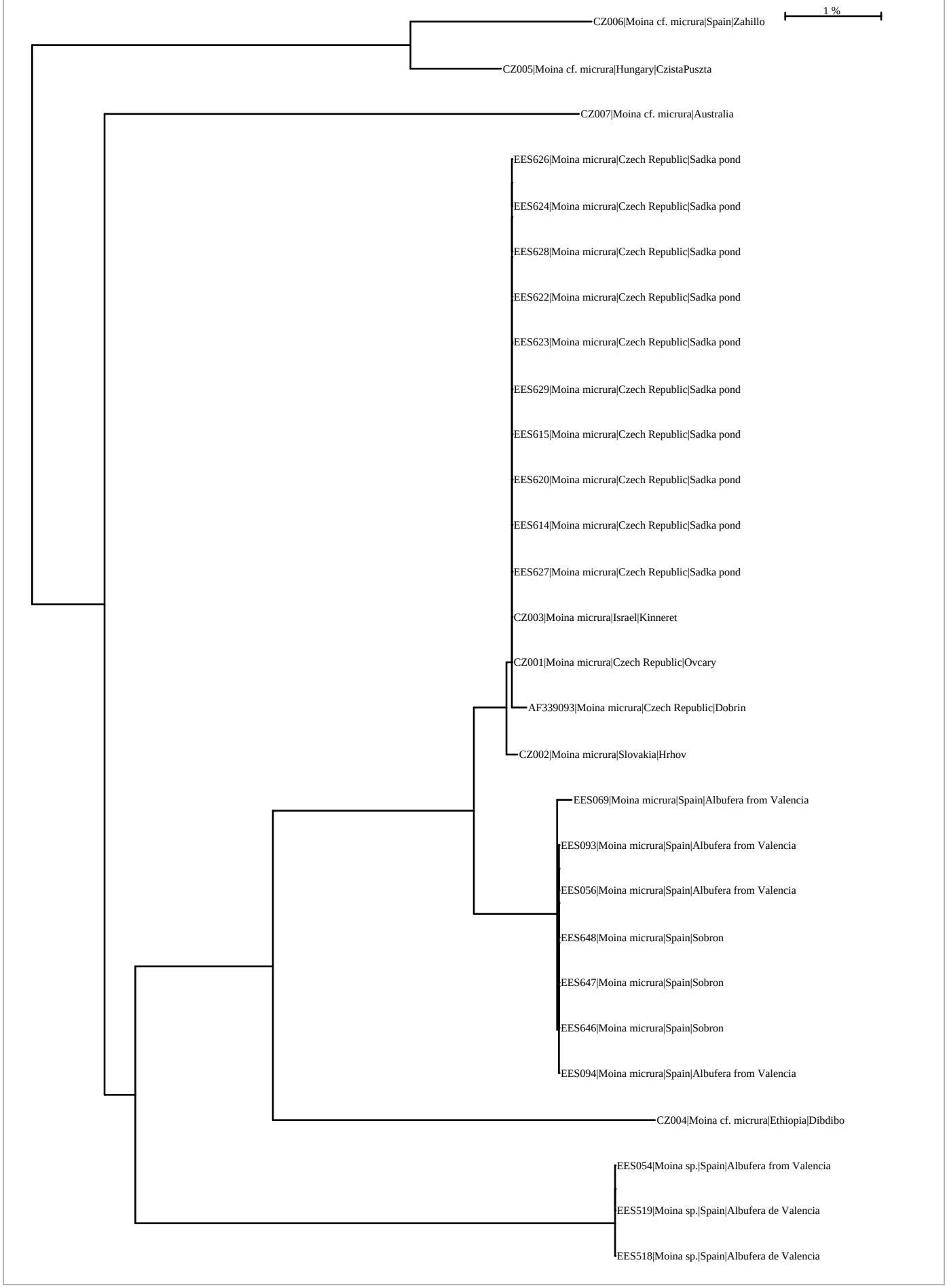
BIN Count : 3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CZ007|Moina cf. micrura|Australia
EES626|Moina micrura|Czech Republic|Sadka pond
EES624|Moina micrura|Czech Republic|Sadka pond
EES628|Moina micrura|Czech Republic|Sadka pond
EES622|Moina micrura|Czech Republic|Sadka pond
EES623|Moina micrura|Czech Republic|Sadka pond
EES629|Moina micrura|Czech Republic|Sadka pond
EES615|Moina micrura|Czech Republic|Sadka pond
EES620|Moina micrura|Czech Republic|Sadka pond
EES614|Moina micrura|Czech Republic|Sadka pond
EES627|Moina micrura|Czech Republic|Sadka pond
CZ003|Moina micrura|Israel|Kinneret
CZ001|Moina micrura|Czech Republic|Ovcary
AF339093|Moina micrura|Czech Republic|Dobrin
CZ002|Moina micrura|Slovakia|Hrhov
EES069|Moina micrura|Spain|Albufera from Valencia
EES093|Moina micrura|Spain|Albufera from Valencia
EES056|Moina micrura|Spain|Albufera from Valencia
EES648|Moina micrura|Spain|Sobron
EES647|Moina micrura|Spain|Sobron
EES646|Moina micrura|Spain|Sobron
EES094|Moina micrura|Spain|Albufera from Valencia
CZ004|Moina cf. micrura|Ethiopia|Dibdibo
EES054|Moina sp.|Spain|Albufera from Valencia
EES519|Moina sp.|Spain|Albufera de Valencia
EES518|Moina sp.|Spain|Albufera de Valencia

BOLD TaxonID Tree

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Label : Sample ID
Label : Species
Label : Country
Label : Exact Site
Label : Barcode Cluster (BIN)

Filter : length > 200bp only
Filter : exclude records flagged as misidentifications
Filter : composed of <1% N
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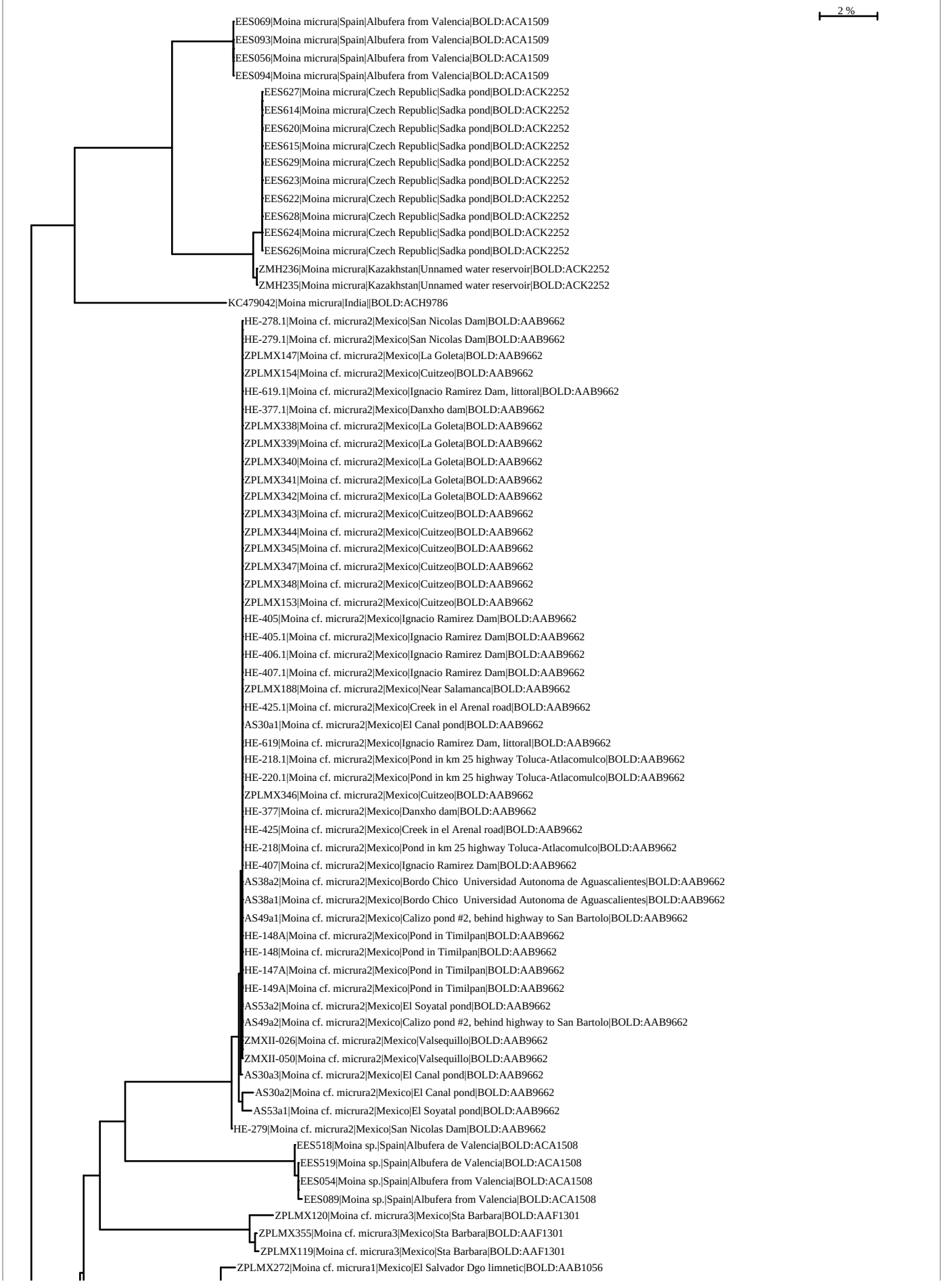
Filter : exclude contaminants

Sequence Count : 143
Species count : 7
Genus count : 1
Family count : 1
Unidentified : 5

BIN Count : 11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EES056|Moina micrura|Spain|Albufera from Valencia|BOLD:ACA1509
EES094|Moina micrura|Spain|Albufera from Valencia|BOLD:ACA1509
EES627|Moina micrura|Czech Republic|Sadka pond|BOLD:ACK2252
EES614|Moina micrura|Czech Republic|Sadka pond|BOLD:ACK2252
EES620|Moina micrura|Czech Republic|Sadka pond|BOLD:ACK2252
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EES628|Moina micrura|Czech Republic|Sadka pond|BOLD:ACK2252
EES624|Moina micrura|Czech Republic|Sadka pond|BOLD:ACK2252
EES626|Moina micrura|Czech Republic|Sadka pond|BOLD:ACK2252
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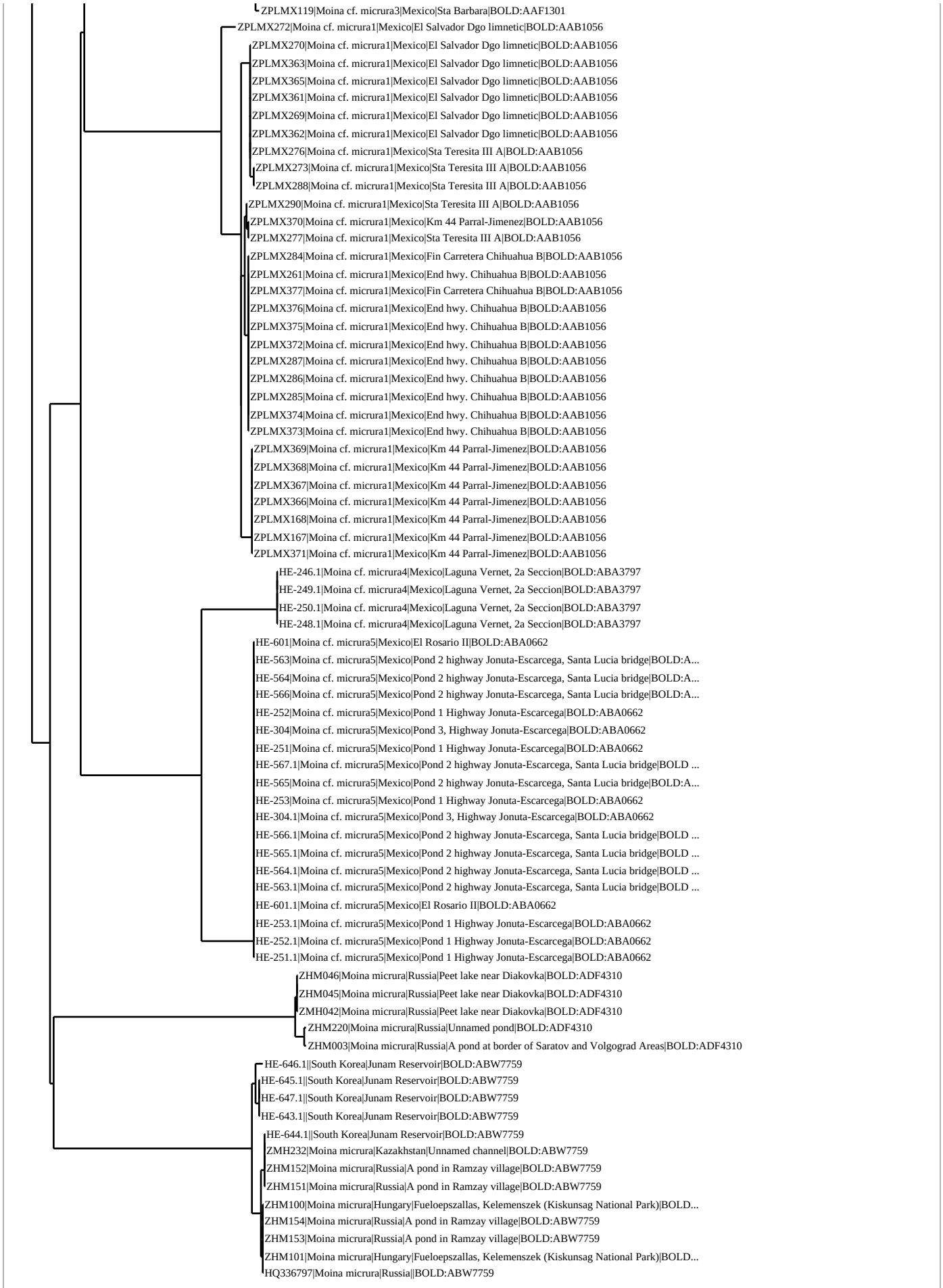
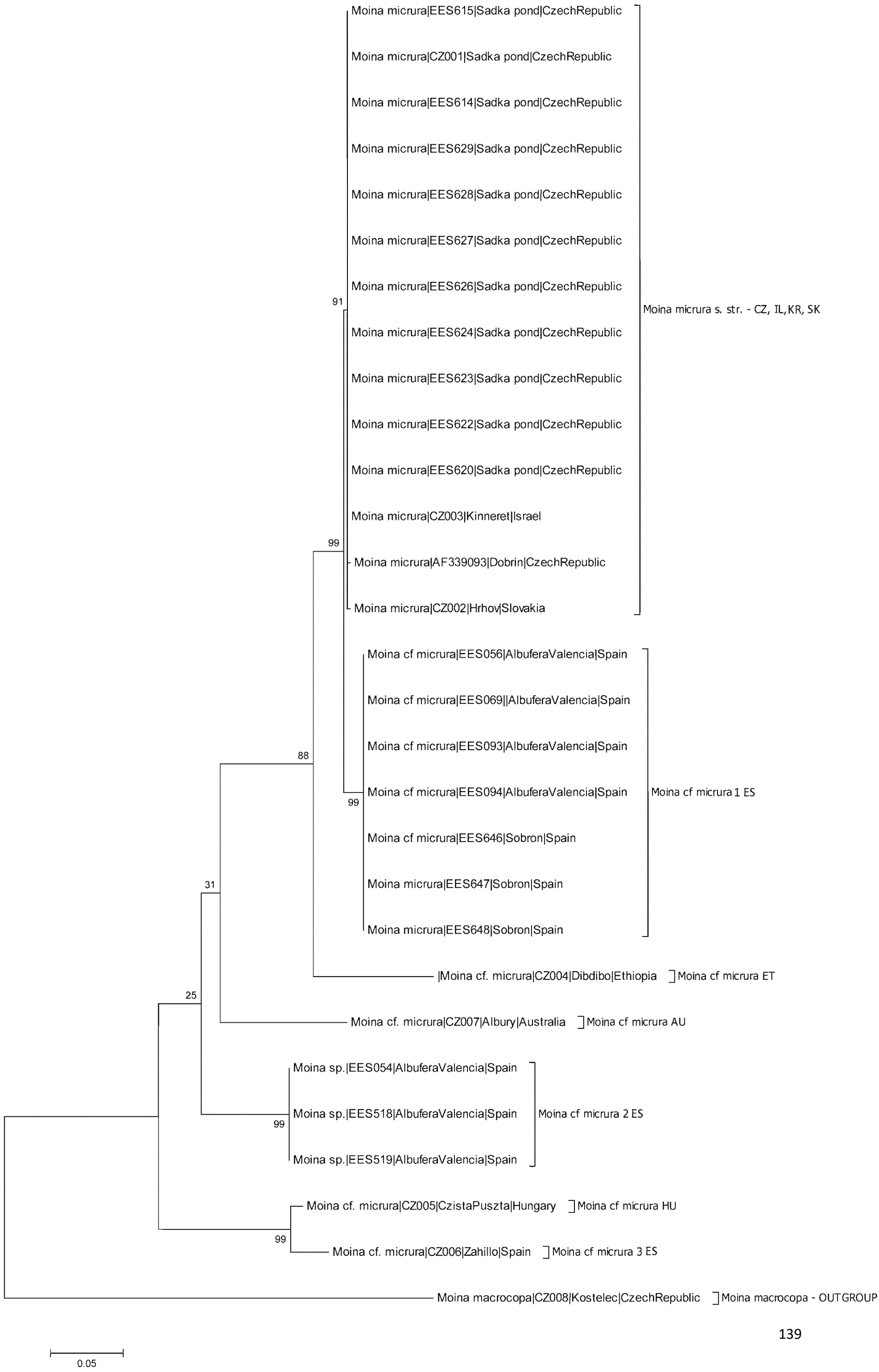
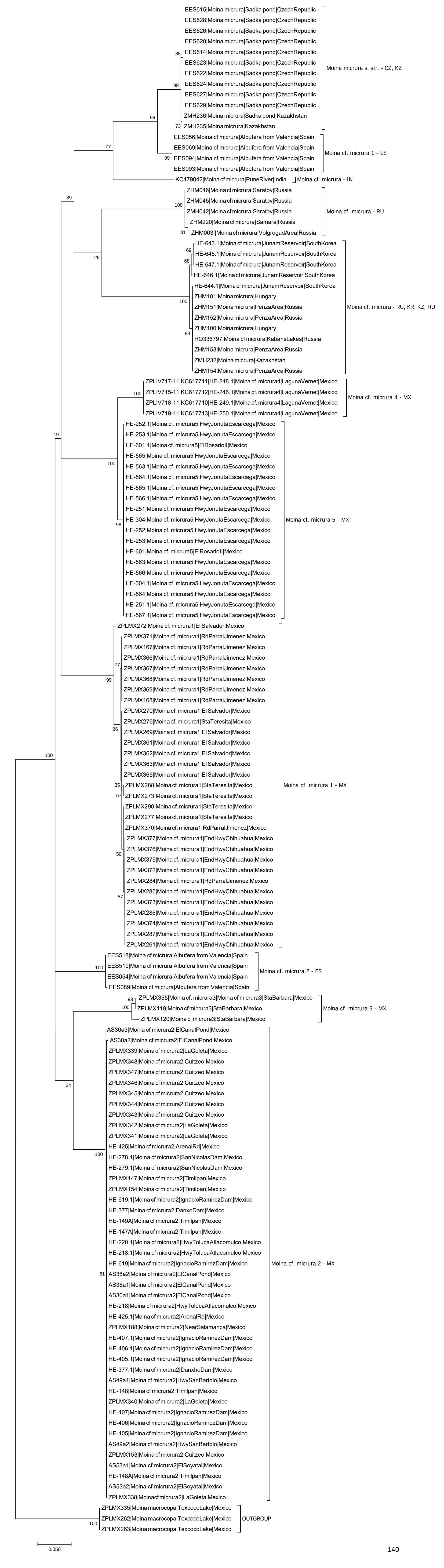
ZMH235|Moina micrura|Kazakhstan|Unnamed water reservoir|BOLD:ACK2252
KC479042|Moina micrura|India||BOLD:ACH9786
HE-278.1|Moina cf. micrura2|Mexico|San Nicolas Dam|BOLD:AAB9662
HE-279.1|Moina cf. micrura2|Mexico|San Nicolas Dam|BOLD:AAB9662
ZPLMX147|Moina cf. micrura2|Mexico|La Goleta|BOLD:AAB9662
ZPLMX154|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
HE-619.1|Moina cf. micrura2|Mexico|Ignacio Ramirez Dam, littoral|BOLD:AAB9662
HE-377.1|Moina cf. micrura2|Mexico|Danxho dam|BOLD:AAB9662
ZPLMX338|Moina cf. micrura2|Mexico|La Goleta|BOLD:AAB9662
ZPLMX339|Moina cf. micrura2|Mexico|La Goleta|BOLD:AAB9662
ZPLMX340|Moina cf. micrura2|Mexico|La Goleta|BOLD:AAB9662
ZPLMX341|Moina cf. micrura2|Mexico|La Goleta|BOLD:AAB9662
ZPLMX342|Moina cf. micrura2|Mexico|La Goleta|BOLD:AAB9662
ZPLMX343|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
ZPLMX344|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
ZPLMX345|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
ZPLMX347|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
ZPLMX348|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
ZPLMX153|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
HE-405|Moina cf. micrura2|Mexico|Ignacio Ramirez Dam|BOLD:AAB9662
HE-405.1|Moina cf. micrura2|Mexico|Ignacio Ramirez Dam|BOLD:AAB9662
HE-406.1|Moina cf. micrura2|Mexico|Ignacio Ramirez Dam|BOLD:AAB9662
HE-407.1|Moina cf. micrura2|Mexico|Ignacio Ramirez Dam|BOLD:AAB9662
ZPLMX188|Moina cf. micrura2|Mexico|Near Salamanca|BOLD:AAB9662
HE-425.1|Moina cf. micrura2|Mexico|Creek in el Arenal road|BOLD:AAB9662
AS30a1|Moina cf. micrura2|Mexico|El Canal pond|BOLD:AAB9662
HE-619|Moina cf. micrura2|Mexico|Ignacio Ramirez Dam, littoral|BOLD:AAB9662
HE-218.1|Moina cf. micrura2|Mexico|Pond in km 25 highway Toluca-Atlacomulco|BOLD:AAB9662
HE-220.1|Moina cf. micrura2|Mexico|Pond in km 25 highway Toluca-Atlacomulco|BOLD:AAB9662
ZPLMX346|Moina cf. micrura2|Mexico|Cuitzeo|BOLD:AAB9662
HE-377|Moina cf. micrura2|Mexico|Danxho dam|BOLD:AAB9662
HE-425|Moina cf. micrura2|Mexico|Creek in el Arenal road|BOLD:AAB9662
HE-218|Moina cf. micrura2|Mexico|Pond in km 25 highway Toluca-Atlacomulco|BOLD:AAB9662
HE-407|Moina cf. micrura2|Mexico|Ignacio Ramirez Dam|BOLD:AAB9662
AS38a2|Moina cf. micrura2|Mexico|Bordo Chico Universidad Autonoma de Aguascalientes|BOLD:AAB9662
AS38a1|Moina cf. micrura2|Mexico|Bordo Chico Universidad Autonoma de Aguascalientes|BOLD:AAB9662
AS49a1|Moina cf. micrura2|Mexico|Calizo pond #2, behind highway to San Bartolo|BOLD:AAB9662
HE-148A|Moina cf. micrura2|Mexico|Pond in Timilpan|BOLD:AAB9662
HE-148|Moina cf. micrura2|Mexico|Pond in Timilpan|BOLD:AAB9662
HE-147A|Moina cf. micrura2|Mexico|Pond in Timilpan|BOLD:AAB9662
HE-149A|Moina cf. micrura2|Mexico|Pond in Timilpan|BOLD:AAB9662
AS53a2|Moina cf. micrura2|Mexico|El Soyatal pond|BOLD:AAB9662
AS49a2|Moina cf. micrura2|Mexico|Calizo pond #2, behind highway to San Bartolo|BOLD:AAB9662
ZMXII-026|Moina cf. micrura2|Mexico|Valsequillo|BOLD:AAB9662
ZMXII-050|Moina cf. micrura2|Mexico|Valsequillo|BOLD:AAB9662
AS30a3|Moina cf. micrura2|Mexico|El Canal pond|BOLD:AAB9662
AS30a2|Moina cf. micrura2|Mexico|El Canal pond|BOLD:AAB9662
AS53a1|Moina cf. micrura2|Mexico|El Soyatal pond|BOLD:AAB9662
HE-279|Moina cf. micrura2|Mexico|San Nicolas Dam|BOLD:AAB9662
EES518|Moina sp.|Spain|Albufera de Valencia|BOLD:ACA1508
EES519|Moina sp.|Spain|Albufera de Valencia|BOLD:ACA1508
EES054|Moina sp.|Spain|Albufera from Valencia|BOLD:ACA1508
EES089|Moina sp.|Spain|Albufera from Valencia|BOLD:ACA1508
ZPLMX120|Moina cf. micrura3|Mexico|Sta Barbara|BOLD:AAF1301
ZPLMX355|Moina cf. micrura3|Mexico|Sta Barbara|BOLD:AAF1301
ZPLMX119|Moina cf. micrura3|Mexico|Sta Barbara|BOLD:AAF1301
ZPLMX272|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056


ZPLMX119|Moina cf. micrura3|Mexico|Sta Barbara|BOLD:AAF1301
ZPLMX272|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056
ZPLMX270|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056
ZPLMX363|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056
ZPLMX365|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056
ZPLMX361|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056
ZPLMX269|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056
ZPLMX362|Moina cf. micrura1|Mexico|El Salvador Dgo limnetic|BOLD:AAB1056
ZPLMX276|Moina cf. micrura1|Mexico|Sta Teresita III A|BOLD:AAB1056
ZPLMX273|Moina cf. micrura1|Mexico|Sta Teresita III A|BOLD:AAB1056
ZPLMX288|Moina cf. micrura1|Mexico|Sta Teresita III A|BOLD:AAB1056
ZPLMX290|Moina cf. micrura1|Mexico|Sta Teresita III A|BOLD:AAB1056
ZPLMX370|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
ZPLMX277|Moina cf. micrura1|Mexico|Sta Teresita III A|BOLD:AAB1056
ZPLMX284|Moina cf. micrura1|Mexico|Fin Carretera Chihuahua B|BOLD:AAB1056
ZPLMX261|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX377|Moina cf. micrura1|Mexico|Fin Carretera Chihuahua B|BOLD:AAB1056
ZPLMX376|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX375|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX372|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX287|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX286|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX285|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX374|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX373|Moina cf. micrura1|Mexico|End hwy. Chihuahua B|BOLD:AAB1056
ZPLMX369|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
ZPLMX368|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
ZPLMX367|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
ZPLMX366|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
ZPLMX168|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
ZPLMX167|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
ZPLMX371|Moina cf. micrura1|Mexico|Km 44 Parral-Jimenez|BOLD:AAB1056
HE-246.1|Moina cf. micrura4|Mexico|Laguna Vernet, 2a Seccion|BOLD:ABA3797
HE-249.1|Moina cf. micrura4|Mexico|Laguna Vernet, 2a Seccion|BOLD:ABA3797
HE-250.1|Moina cf. micrura4|Mexico|Laguna Vernet, 2a Seccion|BOLD:ABA3797
HE-248.1|Moina cf. micrura4|Mexico|Laguna Vernet, 2a Seccion|BOLD:ABA3797
HE-601|Moina cf. micrura5|Mexico|El Rosario II|BOLD:ABA0662
HE-563|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD:A...
HE-564|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD:A...
HE-566|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD:A...
HE-252|Moina cf. micrura5|Mexico|Pond 1 Highway Jonuta-Escarcega|BOLD:ABA0662
HE-304|Moina cf. micrura5|Mexico|Pond 3, Highway Jonuta-Escarcega|BOLD:ABA0662
HE-251|Moina cf. micrura5|Mexico|Pond 1 Highway Jonuta-Escarcega|BOLD:ABA0662
HE-567.1|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD ...
HE-565|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD:A...
HE-253|Moina cf. micrura5|Mexico|Pond 1 Highway Jonuta-Escarcega|BOLD:ABA0662
HE-304.1|Moina cf. micrura5|Mexico|Pond 3, Highway Jonuta-Escarcega|BOLD:ABA0662
HE-566.1|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD ...
HE-565.1|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD ...
HE-564.1|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD ...
HE-563.1|Moina cf. micrura5|Mexico|Pond 2 highway Jonuta-Escarcega, Santa Lucia bridge|BOLD ...
HE-601.1|Moina cf. micrura5|Mexico|El Rosario II|BOLD:ABA0662
HE-253.1|Moina cf. micrura5|Mexico|Pond 1 Highway Jonuta-Escarcega|BOLD:ABA0662
HE-252.1|Moina cf. micrura5|Mexico|Pond 1 Highway Jonuta-Escarcega|BOLD:ABA0662
HE-251.1|Moina cf. micrura5|Mexico|Pond 1 Highway Jonuta-Escarcega|BOLD:ABA0662
ZHM046|Moina micrura|Russia|Peet lake near Diakovka|BOLD:ADF4310
ZHM045|Moina micrura|Russia|Peet lake near Diakovka|BOLD:ADF4310
ZMH042|Moina micrura|Russia|Peet lake near Diakovka|BOLD:ADF4310
ZHM220|Moina micrura|Russia|Unnamed pond|BOLD:ADF4310
ZHM003|Moina micrura|Russia|A pond at border of Saratov and Volgograd Areas|BOLD:ADF4310
HE-646.1||South Korea|Junam Reservoir|BOLD:ABW7759
HE-645.1||South Korea|Junam Reservoir|BOLD:ABW7759
HE-647.1||South Korea|Junam Reservoir|BOLD:ABW7759
HE-643.1||South Korea|Junam Reservoir|BOLD:ABW7759
HE-644.1||South Korea|Junam Reservoir|BOLD:ABW7759
ZMH232|Moina micrura|Kazakhstan|Unnamed channel|BOLD:ABW7759
ZHM152|Moina micrura|Russia|A pond in Ramzay village|BOLD:ABW7759
ZHM151|Moina micrura|Russia|A pond in Ramzay village|BOLD:ABW7759
ZHM100|Moina micrura|Hungary|Fueloepszallas, Kelemenszek (Kiskunsag National Park)|BOLD...
ZHM154|Moina micrura|Russia|A pond in Ramzay village|BOLD:ABW7759
ZHM153|Moina micrura|Russia|A pond in Ramzay village|BOLD:ABW7759
ZHM101|Moina micrura|Hungary|Fueloepszallas, Kelemenszek (Kiskunsag National Park)|BOLD...
HQ336797|Moina micrura|Russia||BOLD:ABW7759


Moina micrura|EES615|Sadka pond|CzechRepublic
Moina micrura|CZ001|Sadka pond|CzechRepublic
Moina micrura|EES614|Sadka pond|CzechRepublic
Moina micrura|EES629|Sadka pond|CzechRepublic
Moina micrura|EES628|Sadka pond|CzechRepublic
Moina micrura|EES627|Sadka pond|CzechRepublic
Moina micrura|EES626|Sadka pond|CzechRepublic
91
Moina micrura|EES624|Sadka pond|CzechRepublic
Moina micrura|EES623|Sadka pond|CzechRepublic
Moina micrura|EES622|Sadka pond|CzechRepublic
Moina micrura|EES620|Sadka pond|CzechRepublic
Moina micrura|CZ003|Kinneret|Israel
99
Moina micrura|AF339093|Dobrin|CzechRepublic
Moina micrura|CZ002|Hrhov|Slovakia
Moina micrura s. str. - CZ, IL, KR, SK
Moina cf micrura|EES056|AlbuferaValencia|Spain
Moina cf micrura|EES069||AlbuferaValencia|Spain
Moina cf micrura|EES093|AlbuferaValencia|Spain
88
Moina cf micrura|EES094|AlbuferaValencia|Spain
99
Moina cf micrura|EES646|Sobron|Spain
Moina micrura|EES647|Sobron|Spain
Moina micrura|EES648|Sobron|Spain
Moina cf micrura 1 ES
31
|Moina cf. micrura|CZ004|Dibdibo|Ethiopia
Moina cf micrura ET
25
Moina cf. micrura|CZ007|Albury|Australia
Moina cf micrura AU
Moina sp.|EES054|AlbuferaValencia|Spain
Moina sp.|EES518|AlbuferaValencia|Spain
99
Moina sp.|EES519|AlbuferaValencia|Spain
Moina cf micrura 2 ES
Moina cf. micrura|CZ005|CzistaPuszta|Hungary
Moina cf micrura HU
99
Moina cf. micrura|CZ006|Zahillo|Spain
Moina cf micrura 3 ES
Moina macrocopa|CZ008|Kostelec|CzechRepublic
Moina macrocopa - OUTGROUP
0.05


EES615|Moina micrura|Sadka pond|CzechRepublic
EES628|Moina micrura|Sadka pond|CzechRepublic
EES626|Moina micrura|Sadka pond|CzechRepublic
EES620|Moina micrura|Sadka pond|CzechRepublic
EES614|Moina micrura|Sadka pond|CzechRepublic
EES623|Moina micrura|Sadka pond|CzechRepublic
EES622|Moina micrura|Sadka pond|CzechRepublic
EES624|Moina micrura|Sadka pond|CzechRepublic
EES627|Moina micrura|Sadka pond|CzechRepublic
EES629|Moina micrura|Sadka pond|CzechRepublic
ZMH236|Moina micrura|Sadka pond|Kazakhstan
ZMH235|Moina micrura|Kazakhstan
Moina micrura s. str. - CZ, KZ
EES056|Moina cf micrura|Albufera from Valencia|Spain
EES069|Moina cf micrura|Albufera from Valencia|Spain
EES094|Moina cf micrura|Albufera from Valencia|Spain
EES093|Moina cf micrura|Albufera from Valencia|Spain
Moina cf. micrura 1 - ES
KC479042|Moina cf micrura|PuneRiver|India
Moina cf. micrura - IN
ZHM046|Moina cf micrura|Saratov|Russia
ZHM045|Moina cf micrura|Saratov|Russia
ZMH042|Moina cf micrura|Saratov|Russia
ZHM220|Moina cf micrura|Samara|Russia
ZHM003||Moina cf micrura|VolgrogadArea|Russia
Moina cf. micrura - RU
HE-643.1|Moina cf micrura|JunamReservoir|SouthKorea
HE-645.1|Moina cf micrura|JunamReservoir|SouthKorea
HE-647.1|Moina cf micrura|JunamReservoir|SouthKorea
HE-646.1|Moina cf micrura|JunamReservoir|SouthKorea
HE-644.1|Moina cf micrura|JunamReservoir|SouthKorea
ZHM101|Moina micrura|Hungary
ZHM151|Moina micrura|PenzaArea|Russia
ZHM152|Moina micrura|PenzaArea|Russia
ZHM100|Moina micrura|Hungary
HQ336797|Moina cf micrura|KabansLakes|Russia
ZHM153|Moina micrura|PenzaArea|Russia
ZMH232|Moina micrura|Kazakhstan
ZHM154|Moina micrura|PenzaArea|Russia
Moina cf. micrura - RU, KR, KZ, HU
ZPLIV717-11|KC617711|HE-248.1|Moina cf. micrura4|LagunaVernet|Mexico
ZPLIV715-11|KC617712|HE-246.1|Moina cf. micrura4|LagunaVernet|Mexico
ZPLIV718-11|KC617710|HE-249.1|Moina cf. micrura4|LagunaVernet|Mexico
ZPLIV719-11|KC617713|HE-250.1|Moina cf. micrura4|LagunaVernet|Mexico
Moina cf. micrura 4 - MX
HE-252.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-253.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-601.1|Moina cf. micrura5|ElRosarioII|Mexico
HE-565|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-563.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-564.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-565.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-566.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-251|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-304|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-252|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-253|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-601|Moina cf. micrura5|ElRosarioII|Mexico
HE-563|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-566|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-304.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-564|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-251.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
HE-567.1|Moina cf. micrura5|HwyJonutaEscarcega|Mexico
Moina cf. micrura 5 - MX
ZPLMX272|Moina cf. micrura1|El Salvador|Mexico
ZPLMX371|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX167|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX366|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX367|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX368|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX369|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX168|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX270|Moina cf. micrura1|El Salvador|Mexico
ZPLMX276|Moina cf. micrura1|StaTeresita|Mexico
ZPLMX269|Moina cf. micrura1|El Salvador|Mexico
ZPLMX361|Moina cf. micrura1|El Salvador|Mexico
ZPLMX362|Moina cf. micrura1|El Salvador|Mexico
ZPLMX363|Moina cf. micrura1|El Salvador|Mexico
ZPLMX365|Moina cf. micrura1|El Salvador|Mexico
ZPLMX288|Moina cf. micrura1|StaTeresita|Mexico
ZPLMX273|Moina cf. micrura1|StaTeresita|Mexico
ZPLMX290|Moina cf. micrura1|StaTeresita|Mexico
ZPLMX277|Moina cf. micrura1|StaTeresita|Mexico
ZPLMX370|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX377|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX376|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX375|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX372|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX284|Moina cf. micrura1|RdParralJimenez|Mexico
ZPLMX285|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX373|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX286|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX374|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX287|Moina cf. micrura1|EndHwyChihuahua|Mexico
ZPLMX261|Moina cf. micrura1|EndHwyChihuahua|Mexico
Moina cf. micrura 1 - MX
EES518|Moina cf micrura|Albufera from Valencia|Spain
EES519|Moina cf micrura|Albufera from Valencia|Spain
EES054|Moina cf micrura|Albufera from Valencia|Spain
EES089|Moina cf micrura|Albufera from Valencia|Spain
Moina cf. micrura 2 - ES
ZPLMX355|Moina cf. micrura3|Moina cf micrura3|StaBarbara|Mexico
ZPLMX119|Moina cf micrura3|StaBarbara|Mexico
ZPLMX120|Moina cf micrura3|StaBarbara|Mexico
Moina cf. micrura 3 - MX
AS30a3|Moina cf micrura2|ElCanalPond|Mexico
AS30a2|Moina cf micrura2|ElCanalPond|Mexico
ZPLMX339|Moina cf micrura2|LaGoleta|Mexico
ZPLMX348|Moina cf micrura2|Cuitzeo|Mexico
ZPLMX347|Moina cf micrura2|Cuitzeo|Mexico
ZPLMX346|Moina cf micrura2|Cuitzeo|Mexico
ZPLMX345|Moina cf micrura2|Cuitzeo|Mexico
ZPLMX344|Moina cf micrura2|Cuitzeo|Mexico
ZPLMX343|Moina cf micrura2|Cuitzeo|Mexico
ZPLMX342|Moina cf micrura2|LaGoleta|Mexico
ZPLMX341|Moina cf micrura2|LaGoleta|Mexico
HE-425|Moina cf micrura2|ArenalRd|Mexico
HE-278.1|Moina cf micrura2|SanNicolasDam|Mexico
HE-279.1|Moina cf micrura2|SanNicolasDam|Mexico
ZPLMX147|Moina cf micrura2|Timilpan|Mexico
ZPLMX154|Moina cf micrura2|Timilpan|Mexico
HE-619.1|Moina cf micrura2|IgnacioRamirezDam|Mexico
HE-377|Moina cf micrura2|DanxoDam|Mexico
HE-149A|Moina cf micrura2|Timilpan|Mexico
HE-147A|Moina cf micrura2|Timilpan|Mexico
HE-220.1|Moina cf micrura2|HwyTolucaAtlacomulco|Mexico
HE-218.1|Moina cf micrura2|HwyTolucaAtlacomulco|Mexico
HE-619|Moina cf micrura2|IgnacioRamirezDam|Mexico
AS38a2|Moina cf micrura2|ElCanalPond|Mexico
AS38a1|Moina cf micrura2|ElCanalPond|Mexico
AS30a1|Moina cf micrura2|ElCanalPond|Mexico
HE-218|Moina cf micrura2|HwyTolucaAtlacomulco|Mexico
HE-425.1|Moina cf micrura2|ArenalRd|Mexico
ZPLMX188|Moina cf micrura2|NearSalamanca|Mexico
HE-407.1|Moina cf micrura2|IgnacioRamirezDam|Mexico
HE-406.1|Moina cf micrura2|IgnacioRamirezDam|Mexico
HE-405.1|Moina cf micrura2|IgnacioRamirezDam|Mexico
HE-377.1|Moina cf micrura2|DanxhoDam|Mexico
AS49a1|Moina cf micrura2|HwySanBartolo|Mexico
HE-148|Moina cf micrura2|Timilpan|Mexico
ZPLMX340|Moina cf micrura2|LaGoleta|Mexico
HE-407|Moina cf micrura2|IgnacioRamirezDam|Mexico
HE-406|Moina cf micrura2|IgnacioRamirezDam|Mexico
HE-405|Moina cf micrura2|IgnacioRamirezDam|Mexico
AS49a2|Moina cf micrura2|HwySanBartolo|Mexico
ZPLMX153|Moina cf micrura2|Cuitzeo|Mexico
AS53a1|Moina cf micrura2|ElSoyatal|Mexico
HE-148A|Moina cf micrura2|Timilpan|Mexico
AS53a2|Moina cf micrura2|ElSoyatal|Mexico
ZPLMX338|Moinacf micrura2|LaGoleta|Mexico
Moina cf. micrura 2 - MX
ZPLMX335|Moina macrocopa|TexcocoLake|Mexico
ZPLMX262|Moina macrocopa|TexcocoLake|Mexico
ZPLMX263|Moina macrocopa|TexcocoLake|Mexico
OUTGROUP
0.050

Montoliu-Elena, L. Almeyda-Osorio, J.K. and Elías-Gutiérrez, M. 2018. Seven species in one: *solving Moina micrura* complex in Mexico and Spain, with morphological and biogeographical insights. (En revisión)

SEVEN SPECIES IN ONE: SOLVING *Moina Micrura* COMPLEX IN MEXICO AND SPAIN, WITH MORPHOLOGICAL AND BIOGEOGRAPHICAL INSIGHTS.

INTRODUCTION

The most part of the cladocerans species were described in the Old World mainly, during XIX-XX centuries (Goulden, 1968). The first author who described a *Moina* was Jurine in 1820 under the names Monocle à bec droit (straight beak) or *Monoculus rectrirostris*, Monocle à gros brás (big arms) or *Monoculus brachiatus* and Monocle à long cou (long neck) or *Monoculus longicollis* (Fig. 1) (Jurine, 1820). His drawings are impressive for his time.

Goulden (1968) in his revision of the genera verified 17 species of *Moina* from a total of 50 species described until this time. He considered *Moina micrura* as the most ubiquitous, the most variable morphologically and the most widely distributed of all species of genus *Moina*. He cited that it is distributed anywhere, in the semiarid and arid regions of the world (Old and New world) (Fig. 2).

In the Iberian Peninsula, the first list of crustaceans was published by Bolívar in 1892 but in it, any *Moina* was mentioned (Bolívar y Urrutia, 1892). Arévalo (1916) in his work about the plankton in the Albufera de Valencia Lake reviewed the Branchiopods registered in the Iberian Peninsula and the Balearic Islands, and listed only 20 species. It was 4 years later, in 1920, when he cited for the first time the genus *Moina* in the Iberian Peninsula, exactly in Gandia (Community of Valencia, Spain). He called it *Moina rectirostris* var. *casañi* in honor of teacher Casañi, who collected the samples (Arévalo, 1920), actually known as *Moina macrocopa* s. l. (Montoliu-Elena et al., submitted). Armengol (1978), registered *Moina micrura* in seven different dams from 103 sampled all around Spain (Fig.3) (Armengol, 1978).

Finally, in 1996, Alonso published his work where cited and describes with detail the morphology, biology, and distribution of the Branchiopods from the Iberian Peninsula and Balearic Islands (Alonso, 1996). In the case of the genus Moina, he described four species: *Moina brachiata*, *Moina salina*, *Moina macrocopus* and *Moina micrura*. He illustrated specimens of *Moina micrura*, our species of interest, from a pond next to Villerin (Zamora, Spain) and he assigned them to the subspecies *Moina micrura dubia* proposed by Goulden (1968) for specimens collected from Guatemala, due to the size of the claw's proximal pecten and the sensory setae of the basipodite

of the second antennae, both structures were more developed than in *Moina micrura* s. str. and mentioned the presence of this species widely distributed in Spain and also, in Mallorca (Balearic Islands) (Fig. 4). Goulden in his revision cited this subspecies from Guatemala, but he didn't say it is valid or not (Goulden, 1968).

In the case of Mexico, the study of Cladocera started at the beginning of the last century, when some foreign scientific expeditions where done (see Elias-Gutiérrez et al., 2008 for the history). Juday (1915) was the first who recorded 14 species from three localities near the City of Mexico. By the second half of the XX century, Van de Velde et al (1978) recorded the Genus *Moina* by the first time in Mexico. They cited three species: *Moina reticulata, Moina micrura,* and *Moinodaphnia macleayi*. *Moina micrura* was registered in two different sites of the six that were sampled by them, in San José near Tulancingo (in several large but shallow water basins) and in a tributary at the Usumacinta river in the road Villahermosa - Palenque (around this site, samples were collected in the river and in some surrounding swamps) (Van de Velde et al., 1978). Finally, the first *Moina* described from Mexico was *Moina dumonti*, distributed from the Yucatán Peninsula to Cuba and Tabasco (Kotov et al., 2005). Nowadays, Elías-Gutiérrez et al (submitted) re-described *Moina micrura s. s.tr.* from a pond near the original type locality due that original material was lost. They used molecular, morphological and biogeographical tools in their work.

The use of molecular tools, concretely DNA Barcoding, evidenced the huge possible cryptic diversity of *Moina micrura*, comprising 11 different clades (Elías-Gutiérrez et al., submitted).

Next step is to test the hypothesis (at least in part) if the clades found are real true full species, with the use of integrative taxonomy (sensu Dayrat, 2005) as it was done for other cladocerans (Elías-Gutiérrez & Valdez-Moreno, 2008; Quiroz-Vázquez & Elías-Gutiérrez, 2009).

Moina micrura s. l. is commonly associated with small, temporary water bodies but may also be found, in the plankton of large freshwater lakes (Goulden, 1968). It is more frequent in permanent or semi-permanent environments than in temporal ponds; it prefers turbid water (muddy or eutrophic). In Spain it was found in dams with less of 1 m of Secchi and it is considered thermophile, with an optimum of temperature between 22-33°C, although it has even been found at more of 37°C (Alonso, 1996).

The goal of this work is to elucidate about the identity of the *M. micrura* group found in Mexico (Elías-Gutiérrez, 2008; Elías-Gutiérrez, submitted) and establish if they are different from the

specimens found in Spain. For all comparisons, we will use *M. micrura* s. str., collected from Sadka pond in Czech Republic (Elías-Gutiérrez et al., submitted).

MATERIAL AND METHODS

Specimens were collected in several places in Mexico, Spain and Czech Republic (S1, Fig. 6). In this study we analyzed seven, different species from Mexico and Spain from *Moina micrura* complex called *Moina* cf *micrura* 1-5MX for Mexican populations and *Moina* cf *micrura* 1-2ES for Spanish ones.

Morphological observations

Specimens were sorted from the ethanol samples under a stereomicroscope and placed in a drop of a glycerol. Several females and males were dissected from various places of Mexico and Spain (S1 supplementary material).

Whole animals and dissected sections were examined and measured under a differential interference contrast microscope and/or phase contrast microscope. The organisms were morphologically identified following descriptions by several authors i.e. Goulden (1968), Alonso (1996), Elías-Gutiérrez, et al. (2008), Elías-Gutiérrez et al. (submitted). Structures of taxanomic interest were illustrated with a camera lucida attached to a compound microscope Olympus BX51. Some specimens were prepared for SEM (Scanning Electron Microscopy) for the observation of microcharacters. The microscope used was the JEOL Model JSM6010 Plus at Chetumal Unit of ECOSUR.

In this article we used the terminology proposed by Kotov et al., (2005). Abbreviations used in the descriptions are: A1, antennula; A2, antenna; L I-V, limbs I-V; E1-3, endites.

Statistical studies

Two Principal Components Analysis (PCA) were performed in RStudio (Team, 2015), one morphometrical and other, environmental to characterize each species and its habitat and to determinate the correlation with the other species.

In females, 13 morphological ratios were considered in the PCA: ratio Habitus (length (L)/Width(W)); ratio A1 (L/W) ; ratio Length of basipodite A2 /Length Long Proximal Seta (A2); ratio Length of basipodite A2/ length of Small Proximal Seta A2; ratio Length of basipodite A2/ length Distal Seta A2; ratio A2 setae (Long/Small); ratio LIII exopod (L/W); ratio length Long seta LIII /

length Small Setae LIII; ratio LIV exopod (L/W); ratio length Long seta LIV/ length Small Seta LIV; ratio length exopodite LIV/ length Small seta LIV; length exopodite LIV/ length Long seta LIV; ratio setae length Small seta LIV/ length long seta LIV. Abbreviatures used for analysis are respectively: RatioHabito, RatioA1, ratioA2L/LPS, ratioA2L/SPS, ratioA2L/LDS, ratioA2Setae, RatioL3, ratioSetasL3, ratioL4, ratioSetasL4, ratioL5/SS, ratioL5/LS and ratioL5Ss.

The environmental parameters used were seven (in parenthesis abbreviatures used in the analysis and units): mean latitude, mean altitude, depth (cm), Secchi disk (cm) (Secchi) and water temperature (°C) (Temp).

Haplotype network, intraspecific divergences and Barcode Index Number (BIN number)

A nexus file with a dataset including sequences of all over the world compiled from the Barcode of Life Database (BOLD, www.boldsystems.org), and published by Elías-Gutiérrez et. al., 2018 (submitted) was used to calculate a TCS network (Clement et al., 2002) in PopART (http://popart.otago.ac.nz) (Leigh and Bryant, 2015). TCS applies the statistical parsimony method to construct haplotype networks. It has been shown that the count of Linnaean species present in a COI alignment greatly matches that of independent statistical parsimony networks inferred by the software. This network allows to visualize graphically the network of relationships between the haplotypes and geographical distributions.

To establish a global comparison of the Intraspecific divergences of Mexican and Spanish populations with the remaining *micrura* group, we used the MEGA Software (Tamura et al., 2013) using the public dataset named DS-MMICRO available in BOLD, using the Kimura two-parameter (K2P) algorithm (Kimura, 1980) with complete deletion of gaps and missing data.

Barcode Index number (BIN) (Ratnasingham and Hebert, 2013) published by Elías-Gutiérrez et al., (2018), and available in BOLD systems database (Ratnasingham and Hebert, 2007) were included in our study to compare with groups delimitated by the haplotype network.

RESULTS

Morphological observations (Fig, 6)

Figures of *Moina micrura* s. str. from Sadka pond, Czech Republic, near the original type locality (Elías-Gutiérrez et al., submitted). The figures are for comparisons proposes, and to show the different parts of the structures. Only taxonomical differences not mentioned before for this species group are going to be described.

Moina micrura s. str. sensu Elías-Gutiérrez et al., (acepted)

Habit: Fig. 7K-L

Antennulas: Fig. 9I-J. Male Fig. 9M.

Antenna: Fig. 11F-G

Valves: Fig. 12M-N

Limb I: Fig. 13H

Limb II: Fig.14H

Limb III: Fig. 15H. At the base of the filter plate at the gnathobase there is a row of big spinules and some hairs, not illustrated before for other taxonomist (Fig. 15cont K remarked box, it is show limb IV but limb III has the same pattern). This spinules are the biggest in all the species studied and for the variability showed, they should be used to differentiate the species of the complex.

Limb IV: Fig. 16H. As in limb III, there are big spinules and hairs at the base of the filter plate at the gnathobase (Fig. 15cont.K). Other difference found in this limb not illustrated either described before, is the hairy seta at endite 2 there are two setae: one bitesulated soft seta and one hairy seta, only present in *Moina* cf *micrura* 1ES, genetically, the closest species (Fig.16F in black).

Limb V: Fig. 17H

Postabdomen and claw: Fig. 19N

Ephippia: Figs. 20H-J

Moina cf micrura 1MX (Fig. 7A)

Material examined: Chihuahua: El Salvador (26.067°N, 104.974°W), End highway Chihuahua B (26.907°N, 104.559°W), Km 44 Parral - Jimenez road (27.047°N, 105.218°W), and Sta. Teresita IIIA (26.351°N, 105.265°W).

Detailed description

Adult parthenogenetic female (Fig. 7A): Length: 0.65-0.85 mm (n=40). Body nearly rounded in lateral aspect, high (ratio: 1.73), maximum height in middle-posterior portion, depending on number of embryos (Fig. 7A). Dorsum of valves strongly convex, projected much higher than head, almost straight in posterior part, dorsal depression between head and rest of body present. Postero-dorsal angle well-marked, rounded. Posterior margin straight, continuing into widely

convex ventral margin. No slight sculptures on surface of valves. No cuticular hairs on head and valves, without dorsal keel. Live animals are whitish in color.

Head – Relatively large, rounded, without any rostral projection; with marked supra-ocular depression and large compound eye. Optical vesicle contiguous with prominent top of head. Ventral margin depressed under compound eye and bulging at level of antennules.

Antennule (A1) (Fig. 8A, 9A) – Rod-like, with the base wider than the tip, with transverse rows of scattered and highly sclerotized denticles of different size on anterior face (only visible with SEM) and row of long setules on posterior face. Antennular sensory seta elongated, arising approximately in middle of lateral face. Distal tip bordered with small thick spinules (arrow in Fig. 9A). Nine short aesthetascs (1 in Fig. 9A).

Antenna (A2) (Fig. 10A-B) – Coxal part with two setulated sensory setae, one long, the other short and thin (ratio between setae: 2.5) (see c in Fig.10A, B). Basal segment robust, with distal spine at dorsal (see b in Fig.10A), and a long distal sensory seta on ventral face (see a in Fig.10A). Transverse rows of numerous, big and highly sclerotized spinules on surface of similar size and fine hairs. Antennal branches elongated, 4-segmented exopodite slightly shorter than 3-segmented endopodite, all segments cylindrical, with rows of minute spinules and rows of long and thin setae. Antennal formula: setae 0-0-1-3/1-1-3, spines 0-1-0-1/0-0-1. Three long, apical swimming setae on both antennal branches, all with basal and distal segments bilaterally armed with fine, long setules and small denticles visible at SEM. Lateral seta on third segment of dorsal branch with similar armature. peg

Valves (Fig. 12A) – Large, sub-ovoid, with row of 13 to17 setae along anterior ventral margin. Posterior part with row of marginal denticles, organized in groups of 8-13, increasing in size distally within each group. Denticles in posterior most margin form uniform row. Two posterior hooks supporting postabdominal setae (setae natatoriae) present.

Limb I (Fig. 13A) – Distal lobe or endite 4 with single anterior thicker seta, bearing short setules and two soft setae. Endite 3 with short, strong, single anterior seta, bearing noticeable lateral spinule-like projections in both sides, and single long and strong posterior seta. Endite 2 with two posterior setae. Endite 1 with three posterior setae. Two ejector hooks of different size.

Limb II (Fig. 14A) – Exopodite with large cylindrical lobe bearing long apical seta, its distal part regularly setulated, and with a thick lateral seta not visible at the illustration. Distal endite with

two setulated setae, other two endites with only one setulated seta each. One small seta near gnathobase plate, followed anteriorly by small hook-like outgrow. Rest of gnathobase margin with row of setae arranged fan-like. Above-mentioned two long setae followed by row of 13 to 16 gradually shortening and curved setae. Fan-like arrangement of setae is closed by group of three anterior brush-like setae, curved proximally with unilateral setulation facing distally, posterior one strongly reduced. Two shorter and thick setae with apical setulation inserted parallel to group of three anterior-most setae one of them with a very long prolongation. Fan-like setation is arranged in two planes.

Limb III (Fig. 15A) – Exopodite large, flat, with four distal setae and two short proximal setae, one short and one long and thick, all of them bilaterally setulated. Inner distal portion with three endites. Endite 3 with single anterior seta; endite 2 with one anterior and one posterior seta Endite 1, with one anterior seta and seta "unica" (arrow in Fig. 15 A). Rest of endopodite with 40-45 soft setae. At the base of the filter plate there are some rows of medium denticles and long hairs

Limb IV (Fig. 16A) – Exopodite like III, but more elongated, with four long setae in distal part and two setae in proximal part, one short and another long. Inner distal portion of limb with two endites: endite 1 with long articulated and bisetulated seta. Endite 2 with two long setulated setae. Filter plate with 30-35 setae. At the base of the filter plate there is a dense row of big denticles and long hairs, bigger than in the other species of the complex (arrow in Fig 16A).

Limb V (Fig. 17A) – With a large ovoid lobe, with setulated margin, large distal seta and small proximal seta, both bi-setulated. Inner thoracopod portion with elongated lobe with long setulae followed by two setae.

Postabdomen (Fig. 18A, 19A-B) –Elongated, conically narrowing distally, more than in *Moina micrura* s. str. Ventral margin almost straight, with numerous rows of minute setules in transversal rows. Ventral margin long, straight. Laterally, row of 7 – 9 large, triangular teeth, fringed with marginal setules. Rows of setules on distal portion continue along base of bident tooth; distal branch of bident tooth significantly longer than proximal branch.

Natatory setae – Articulated, longer than the postabdomen, with the proximal part shorter and without setae. Distal part longer, bilaterally setulated with few and fine setae.

Postabdominal claw (Fig. 19B) slightly curved, with sharp, pointed tip. Two successive pectens along dorsal margin: basal pecten consisting of 6 to 10 slender spines huddled together, followed by a middle pecten of 8-12 strong spines. Basal and middle pectens seem to be only one in some individuals. Uniform row of setules along inner face of claw.

Ephippium

Ephippium ovoid with only one egg (Fig.20A-C). Dorsal ridge strongly sclerotized. Lateral surface with marked semi-circular reticulated pattern. Ultrastructure of ephippial surface formed by complex structure of semi- circles interlocked with many thin tentacle-like projections of various length. Some projections cover also inner surface of polygons.

Male

Body – Smaller than female but more elongated. Dorsal margin of valves almost straight, postero-dorsal angle clearly distinct. Antero-ventral portion of valves fringed with long, thin setules. Denticles along posterior margin more distinct than in female, arranged in groups similarly. Carapace surface without any hairs or setules.

Head – More elongated than in female, rostrum absent, labrum less fleshy than that of female. Optical vesicle fills top of head. No trace of pigment in ocellus.

Antennule (Fig. 8H) – Long, male seta and sensillum inserted in first third of antennule, at the exit of the sensilia there is a group of about five aesthetascs forming like a star. Nine aesthetascs and three hooks of distinct size and orientation, with bifurcated tips, present distally.

Antenna – similar in armature to female (Fig. 10A-B). Distal margin of all segments with row of thick, short spinules.

Trunk limb I (Fig. 21A) – With thick copulatory hook, its tip with two spinules (1 in figure 21A), endite 4 (IDL) with three bisetulated setae, endite 3 with two bisetulated setae. Endite 2 with one bisetulated seta, one stiff seta with denticles on the tip, and one little plumose seta (3 in figure 21A). Endite 1 has three setae, one long unsetulated seta with fine denticle on its tip (3 in figure 21A) and two bisetulated setae of same length (4 in figure 21A). Basal part short and rounded with marked groups of stout bristles on ventral face and two ejector hooks of different size.

Limb II (Fig. 22A) – Same morphology as in female (Fig. 14A).

Limb III (Fig. 23A) – Same morphology as in female, but with less number of seta et the filter plate, 34-36 instead of 40-45 and less denticles at the base of the filter plate.

Limb IV (Fig. 24A)– as in limb III, there is a row of the denticles at the base of the filter plate clearly remarkable in both sexes with too many hairs.

Limb V (Fig. 25A)– as in females.

Postabdomen (Fig. 26A)– as females.

Moina cf micrura 2 MX (Fig. 7B, C)

Material: In Mexico, Bordo Chico - UAA (Aguascalientes) (21.912°N,-102.317°W), Creek in Arenal road (Chiapas) (16.107°N, 90.99°W), Cuitzeo Lake (Michoacan) (19.925°N, 101.141°W), Danxho dam (State of Mexico) (19.879°N, -99.559°W), El Canal pond (Aguascalientes) (22.148°N, 102.346°W), El Soyatal pond (Aguascalientes) (21.909°N, 102.166°W), Ignacio Ramirez dam (State of Mexico) (19.461°N, 99.797°W), La Goleta (State of Mexico) (20.07°N, 99.556°W), °Near Salamanca city (Querétaro) (20.542°N, 101.162°W), San °Nicolas dam (State of Mexico) (19.924°N, -99.788°W), and Valsequillo dam (Puebla) (18.909°N, -98.16°W).

Detailed description

Adult parthenogenetic female (Fig. 7B-C): Length: 0.57-0.9 mm (n=20). Body nearly rounded in lateral aspect, high (ratio: 1.65), maximum height in middle-posterior portion, depending on number of embryos. Dorsum of valves strongly convex, projected much higher than head, almost straight in posterior part, dorsal depression between head and rest of body present. Postero-dorsal angle well-marked, rounded. Posterior margin straight, continuing into widely convex ventral margin. No slight sculptures on surface of valves. No cuticular hairs on head and valves, without dorsal keel. Live animals are whitish in color.

Head – Relatively large, rounded, without any rostral projection; with marked supra-ocular depression and large compound eye. Optical vesicle contiguous with prominent top of head. Ventral margin depressed under compound eye and bulging at level of antennules.

Antennule (A1) (Fig. 8B, 9B) – Cylindrical, with scattered denticles of different size on anterior face (only visible with SEM) and row of long setules on posterior face. Antennular sensory seta

elongated, arising approximately in middle of lateral face. Distal tip bordered with small thick spinules. Nine short aesthetascs.

Antenna (A2) (Fig. 10C-D) – coxal part with two setulated sensory setae, one long, the other short and thin (ratio between setae: 2.1) (Fig. 10D). Basal segment robust, with distal spine at dorsal, and a long distal sensory seta on ventral face setulated with long hairs on the tip (Fig. 10D). Transverse rows of numerous spinules on surface less sclerotized than in *Moina* cf *micrura* 1MX, with different size and rows of long fine dense hairs. Antennal branches elongated, 4-segmented exopodite slightly shorter than 3-segmented endopodite, all segments cylindrical, with rows of minute spinules and rows of long and thin setae. Antennal formula: setae 0-0-1-3/1-1-3, spines 0-1-0-1/1-1-1, different to the other species of the complex. Three long, apical swimming setae on both antennal branches, all with basal and distal segments bilaterally armed with fine, long setules and diminutes denticles visible at SEM (Fig.12D-1). Lateral seta on third segment of dorsal branch with similar armature.

Valves (Fig. 12D) – large, sub-ovoid, with row of 10 to 20 setae along anterior ventral margin. Posterior part with row of marginal denticles, organized in groups of 8-15, each group of the same size (see 2 in Fig. 12D). Denticles in posterior most margin form uniform row (see 1 in Fig. 12D). Two posterior hooks supporting postabdominal setae (seta natatoria).

Limb I (Fig. 13B) – distal lobe or endite 4 with single anterior thicker seta, bearing short setules and two setae (one of them not visible at the illustration). Endite 3 with short, strong, single anterior seta, bearing noticeable lateral spinule-like projections in both sides, and one bisetulated seta. Endite 2 with two posterior setae. Endite 1 with three posterior setae. Two ejector hooks of different sizes. The surface of the limb is not ornamented.

Limb II (Fig. 14B) – with large cylindrical lobe bearing long apical seta, its distal part regularly setulated, and with a thick lateral seta (in black on fig 14B). Distal endite with two setulated setae, other two endites with only one setulated seta each. One small seta near gnathobase plate, followed anteriorly by small hook-like outgrow. Rest of gnathobase margin with row of setae arranged fan-like. Posterior-most long single seta and neighboring more anteriorly located, long seta separated by large gap. Both unilaterally setulated with long peg-like setules facing proximally; posterior-most seta setulated along its whole length, neighbouring seta on distal portion only. Above-mentioned two long setae followed by row of 10 - 13 gradually shortening and curved setae. Fan-like arrangement of setae is closed by group of three anterior brush-like

setae, curved proximally with unilateral setulation (setulation as in *Moina micrura* s.str. Fig. 14H,4) facing distally, posterior one strongly reduced. Two shorter and thick setae with apical setulation inserted parallel to group of three anterior-most setae one of them with a very long prolongation. Fan-like setation is arranged in two planes. Two long posterior setae plus two thick anterior sensilla and anterior brush-like seta in ventral plane, remaining setae in dorsal plane.

Limb III (Fig. 15B) – Exopodite large, flat, with four distal setae and two short proximal setae, one short and one long, all of them bilaterally setulated. Inner distal portion with three endites. Endite 3 with single anterior seta; endite 2 with one anterior and one posterior seta. Endite 1, with one anterior seta. Rest of endopodite with 40-45 soft setae. At the base of the filter plate there are some rows of medium denticles and long hairs.

Limb IV (Fig. 16B) – Exopodite like III, but more elongated, with four long setae in distal part and two setae in proximal part, one short and another long. Inner distal portion of limb with two endites: endite 1 with long articulated and bisetulated seta. Endite 2 with two long setulated setae. Filter plate with 14-30 setae. At the base of the filter plate there are some long hairs but there is not any denticle as in *Moina* cf *micrura* 1MX.

Limb V (Fig. 17B) – With large ovoid lobe, with setulated margin, large distal seta and small proximal seta, both bi-setulated. Inner thoracopod portion with elongated lobe with long setulae followed by two setae.

Postabdomen (Fig. 18B, 19D) – Elongated, conically narrowing distally. Ventral margin almost straight, with numerous rows of minute setules in transversal rows. Ventral margin long, straight. Laterally, row of 5-6 large, triangular teeth, fringed with marginal setules. Distal branch of bident tooth significantly longer than proximal branch.

Natatory setae - Articulated, longer than the postabdomen, with the proximal part shorter and without setae. Distal part longer, bilaterally setulated with few and fine setae.

Postabdominal claw (Fig. 19E) slightly curved, with sharp, pointed tip. Ventral pecten consisting of 6 to 9 slender spines huddled together. Basal and middle pectens fused, but spines of basal pectin are considerably bigger than the middle ones.

Ephippial female (Fig.20D)

Ephippium ovoid with only one egg (Fig. 20D-E). Dorsal ridge strongly sclerotized. Lateral surface with marked semi-circular reticulated pattern grapes-like. Ultrastructure of ephippial surface formed by complex structure of semi- circles interlocked with many thin tentacle-like projections of various length. Some projections cover also inner surface of polygons.

Male

Body – Smaller than female but more elongated. Dorsal margin of valves almost straight, postero-dorsal angle clearly distinct. Antero-ventral portion of valves fringed with long, thin setules. Denticles along posterior margin more distinct than in female, arranged in groups similarly.

Head – More elongated than in female, rostrum absent, labrum less fleshy than that of female. Optical vesicle fills top of head. No trace of pigment in ocellus.

Antennule (Fig. 8I) – long, ornamentated with big and stout denticles with one setae on the first third and one spine on the last third of the antennule (see arrows in Fig.8I) not present in the other species of the complex. Nine aesthetascs and three hooks of distinct size and orientation, with bifurcated tips, present distally.

Antenna– similar in armature to female (Fig. 10C-D). Distal margin of all segments with row of thick, short spinules.

Carapace surface without any hairs or setules.

Trunk limb I (Fig. 21B) – with thick copulatory hook, its tip with two spinules, endite 4 (IDL) with four setae (not visible at the figure), endite 3 with one stout spine, and one long bisetulated seta, instead of two setae. Endite 3 with one bisetulated seta and one stiff seta. Endite 2 has three setae, one long unsetulated seta (in black in Fig. 21B) and two bisetulated setae of same length. Basal part short and rounded with covered with big denticles with different arrangement on ventral face.

Limb II (Fig. 21B) – same morphology as in female.

Limb III (Fig. 23B) – same morphology as in female, with no denticles at the base of the filter plate.

Limb IV (Fig. 24B) – as in limb III, there are some big denticles at the base of the filter plate and a dense row of hairs.

Limb V (Fig. 25B) – as in females.

Postabdomen (Fig. 26B) as in females.

Moina cf micrura 3 MX (Fig. 7D)

Material revised: Santa Bárbara (Durango, Mexico) (23.917°N, 104.952°W).

Detailed description

Adult parthenogenetic female (Fig. 7D): Length: 0.77-0.98 mm (n=20). Body nearly rounded in lateral aspect, high (ratio: 1.58), maximum height in middle-posterior portion, depending on number of embryos. Dorsum of valves strongly convex, projected much higher than head, almost straight in posterior part, dorsal depression between head and rest of body present. Postero-dorsal angle well-marked, rounded. Posterior margin straight, continuing into widely convex ventral margin. No slight sculptures on surface of valves (Fig.12E). No cuticular hairs on head and valves, without dorsal keel. Live animals are whitish in color.

Head – Relatively large, rounded, without any rostral projection; with marked supra-ocular depression and large compound eye. Optical vesicle contiguous with prominent top of head. Ventral margin depressed under compound eye and bulging at level of antennules.

Antennule (A1) (Fig. 8C, 9C) – Cylindrical, with scattered denticles of different size on anterior face and rows of long setules on posterior face. Antennular sensory seta elongated, arising approximately in middle of lateral face. Distal tip bordered with small thick spinules. Nine short aesthetascs.

Antenna (A2) (Fig. 10E) – coxal part with two setulated sensory setae, one long, the other shorter and thinner (ratio between setae: 1.8). Basal segment robust, with distal spine at dorsal, and a long distal sensory seta on ventral face. Antennal branches elongated, 4-segmented exopodite slightly shorter than 3-segmented endopodite, all segments cylindrical, with rows of minute spinules and rows of long and thin setae. Antennal formula: setae 0-0-1-3/1-1-3, spines 0-1-0-1/0-0-1. Three long, apical swimming setae on both antennal branches, all with basal and distal segments bilaterally armed with fine and long setules.

Valves (Fig.12E) – large, sub-ovoid, with row of 20-23 setae along anterior ventral margin. Posterior part with row of marginal denticles, organized in groups of 5-10, each group with one big spine followed by denticles decreasing in size (see 2 in Fig.12E). Denticles in posterior most margin

form uniform row (see 3 in Fig.12E). Two posterior hooks supporting postabdominal seta (setae natatoria).

Limb I (Fig. 13C) – distal lobe or endite 4 with single anterior thicker seta, bearing short setules and two soft setae. Endite 3 with short, strong, single anterior seta, bearing noticeable lateral spinule-like projections in both sides, and single long and strong posterior seta. Endite 2 with two posterior setae. Endite 1 with three posterior setae. Two ejector hooks of different sizes. The surface of the limb is slightly ornamentated.

Limb II (Fig. 14C) – with large cylindrical lobe bearing long apical seta, its distal part regularly setulated, and with a thick lateral seta. Distal endite with two setulated setae, other two endites with only one setulated seta each. One small seta near gnathobase plate, followed anteriorly by small hook-like outgrow. Rest of gnathobase margin with row of setae arranged fan-like. Posterior-most long single seta and neighboring more anteriorly located, long seta separated by large gap. Both unilaterally setulated; posterior-most seta setulated along its whole length, neighbouring seta on distal portion only. Above-mentioned two long setae followed by row of 10 - 15 gradually shortening and curved setae. Fan-like arrangement of setae is closed by group of three anterior brush-like setae, curved proximally with unilateral setulation facing distally, posterior one strongly reduced. Two shorter and thick setae with apical setulation inserted parallel to group of three anterior-most setae one of them with a very long prolongation. Fan-like setation is arranged in two planes. Two long posterior setae plus two thick anterior sensilla and anterior brush-like seta in ventral plane, remaining setae in dorsal plane.

Limb III (Fig. 15C) – Exopodite large, flat, with four distal setae and two short proximal setae, one short and one long, all of them bilaterally setulated. Inner distal portion with three endites. Endite 3 with single anterior seta; endite 2 with one anterior and one posterior seta. Endite 1, with one anterior seta and the seta “unica”. Rest of endopodite with 53 soft setae. At the base of the filter plate there are some rows of medium denticles and some long hairs (Fig.15cont. I, J).

Limb IV (Fig. 16C) – Exopodite like III, but more elongated, with four long setae in distal part and two setae in proximal part, one short and other long. Inner distal portion of limb with two endites: endite 1 with long articulated and bisetulated seta, and endite 2 with two long setulated setae. Filter plate with 37-44 setae. At the base of the filter plate there is a dense row of long hairs and a row of big denticles.

Limb V (Fig. 17C) – With large ovoid lobe, with setulated margin, large distal seta and small proximal seta, both bi-setulated. Inner thoracopod portion with elongated lobe with long setulae followed by two setae.

Postabdomen (Fig. 18C, 19C) – Elongated, conically narrowing distally. Ventral margin almost straight, with numerous rows of minute setules in transversal rows. Ventral margin long, straight. Laterally, a row of 6-11 large, triangular teeth, fringed with marginal setules. Rows of denticles on distal portion continue along base of bident tooth; distal branch of bident tooth significantly longer than proximal branch.

Postabdominal claw (Fig. 18C, 19C) slightly curved, with sharp, pointed tip. Ventral pecten consisting of 4 slender spines huddled together. Basal, middle and distal pectens fused.

Natatory seta – Articulated, longer than the postabdomen, with the proximal part shorter and without setae. Distal part longer, bilaterally setulated with few and fine setae.

Ephippium

Ephippium ovoid with only one egg (Fig. 20F-G). Dorsal ridge strongly sclerotized. Lateral surface with marked polygonal reticulated pattern. Ultrastructure of ephippial surface formed by complex structure of polygons interlocked with many thin tentacle-like projections of various length. Some projections cover also inner surface of polygons.

Male

Body – smaller than female but more elongated. Dorsal margin of valves almost straight, postero-dorsal angle clearly distinct. Antero-ventral portion of valves fringed with long, thin setules. Denticles along posterior margin more distinct than in female, arranged in groups similarly. Carapace surface without any hairs or setules.

Head – more elongated than in female, rostrum absent, labrum less fleshy than that of female. Optical vesicle fills top of head. No trace of pigment in ocellus.

Antennule (Fig. 8J) – long with two setae on the first third. Nine aesthetascs and four hooks of distinct size and orientation, with bifurcated tips, present distally.

Antenna – similar in armature to female (Fig. 10E).

Trunk limb I (Fig. 21C) – With thick copulatory hook, its tip with two spinules, endite 4 (IDL) with three setulated setae, endite 3 with two bisetulated setae, endite 2 with one long bisetulated seta, one stiff seta with denticles on the tip, and endite 1 has three setae, one long unsetulated seta and two bisetulated setae of same length. Basal part short and rounded with covered with big denticles with different arrangement on ventral face.

Limb II (Fig. 22C) – same morphology as in female.

Limb III (Fig. 23C) – same morphology as in female, with less denticles and hairs at the base of the filter plate.

Limb IV (Fig. 24C) – as females.

Limb V (Fig. 25C) – as in females.

Postabdomen (Fig. 26C) – as females.

Moina cf micrura 4 MX (Fig. 7E)

Material examined: Lake Vernet 2nd section (Tabasco, Mexico) (17.877°N, -92.55°W).

Detailed description

Adult parthenogenetic female (Fig. 7E): Length: 0.47-0.60 mm (n=5). Body nearly rounded in lateral aspect, high (ratio: 1.62), maximum height in middle-posterior portion, depending on number of embryos. Dorsum of valves strongly convex, projected much higher than head, almost straight in posterior part, dorsal depression between head and rest of body present. Postero-dorsal angle well-marked, rounded. Posterior margin straight, continuing into widely convex ventral margin. No slight sculptures on surface of valves. No cuticular hairs on head and valves, without dorsal keel. Live animals are brownish in color.

Head – relatively large, rounded, without any rostral projection; with marked supra-ocular depression and large compound eye. Optical vesicle contiguous with prominent top of head. Ventral margin depressed under compound eye and bulging at level of antennules.

Antennule (A1) (Fig. 8D, 9D) – Rod-like, with scattered denticles of different size on anterior and a row of long setules on posterior face. Antennular sensory seta elongated, arising approximately in middle of lateral face. Distal tip bordered with small thick spinules. Nine short aesthetascs.

Antenna (A2) (Fig. 10F) – Coxal part with two setulated sensory setae, one long, the other short and thin (ratio between setae: 1.8). Basal segment robust, with distal spine at dorsal, and a long distal sensory seta on ventral face. Antennal branches elongated, 4-segmented exopodite slightly shorter than 3-segmented endopodite with no spinulation visible with optical microscopy. Antennal formula: setae 0-0-1-3/1-1-3, spines 0-0-0-1/0-0-1. Three long, apical swimming setae on both antennal branches, all with basal and distal segments bilaterally armed with fine and long setules.

Valves (Fig. 12B-C) – Large, sub-ovoid, with row of 12-13 setae along anterior ventral margin. Posterior part with row of marginal denticles, organized in groups of 5-8, each group of distinct size. Denticles in posterior most margin form uniform row. Two posterior hooks supporting postabdominal setae (setae natatoriae).

Limb I (Fig. 13D) – Distal lobe or endite 4 with single anterior thicker seta, bearing short setules and two soft setae. Endite 3 with short, strong, single anterior seta, bearing noticeable lateral spinule-like projections in both sides, and single long and strong posterior seta. Endite 2 with two posterior setae. Endite 1 with three posterior setae. Two ejector hooks of different sizes. The surface of the limb is slightly ornamented.

Limb II (Fig. 14D) (due to the lack of material of this group, this structure is not clear at the illustration but characters described were checked) – With large cylindrical lobe bearing long apical seta, its distal part regularly setulated, and with a thick lateral seta (not visible at the illustration). Distal endite with two setulated setae, other two endites with only one setulated seta each. One small seta near gnathobase plate, followed anteriorly by small hook-like outgrow. Rest of gnathobase margin with a row of setae arranged fan-like. Posterior-most long single seta and neighboring more anteriorly located, long seta separated by large gap. Both unilaterally setulated with long peg-like setules facing proximally; posterior-most seta setulated along its full length, neighbouring seta on distal portion only. Above-mentioned two long setae followed by row of 6-7 gradually shortening and curved setae. Fan-like arrangement of setae is closed by group of three anterior brush-like setae, curved proximally with unilateral setulation facing distally, posterior one strongly reduced. Two shorter and thick setae with apical setulation inserted parallel to group of three anterior-most setae one of them with a very long prolongation. Fan-like setation is arranged in two planes. Two long posterior setae plus two thick anterior sensilla and anterior brush-like seta in ventral plane, remaining setae in dorsal plane.

Limb III (Fig. 15D) – Exopodite large, flat, with four distal setae and two short proximal setae, one short and one long, all of them bilaterally setulated. Inner distal portion with three endites. Endite 3 with single anterior seta; endite 2 with one anterior and one posterior seta. Endite 1, with two anterior setae and the seta "unica". Rest of endopodite with 23 soft setae. At the base of the filter plate there is a row of small denticles and no hairs.

Limb IV (Fig. 16D) – exopodite like III, but more elongated, with four long setae in distal part and two setae in proximal part, one short and other long. Inner distal portion of limb with two endites: endite 1 with long articulated and bisetulated seta; endite 2 with two long setulated setae. Filter plate with 13-15 setae. At the distal part of the gnathobase there are some scattered big denticles and some long hairs.

Limb V (Fig. 17D) – With large ovoid lobe, with setulated margin, large distal seta and small proximal seta, both bi-setulated. Inner thoracopod portion with elongated lobe with a long setula followed by two setae.

Postabdomen (Fig. 18D, 19F) – elongated. Ventral margin almost straight, with numerous rows of minute setules in transversal rows. Ventral margin long, straight. Laterally, row of 4 large, triangular teeth, fringed with marginal setules. Distal branch of bident tooth significantly longer than proximal branch.

Natatory setae (Fig. 18D) – articulated, longer than the postabdomen, with the proximal part shorter and without setae. Distal part longer, bilaterally setulated with few and fine setae.

Postabdominal claw (Fig. 18D, 19F) slightly curved, with sharp, pointed tip. Ventral pecten with 3 dorsal slender spines. Basal and middle pectens separated by a gap.

Moina cf micrura 5 MX (Fig. 7F, G)

Material examined: In Mexico, El Rosario II, (Baja California Sur) (23.758°N, -110.092°W), pond 1, 2and 3 at Jonuta-Escárcega road (Campeche) (18.109°N, -92.079°W; 18.063°N, -92.017°W; 18.048°N, -91.888°W, respectively).

Detailed description

Adult parthenogenetic female: Length: 0.55-0.77 mm (n=5). Body nearly rounded in lateral aspect, high (ratio: 1.45), being the smallest ratio of the complex, maximum height in middle-posterior

portion, depending on number of embryos (Fig.7F-G). Dorsum of valves strongly convex, projected much higher than head, almost straight in posterior part, dorsal depression between head and the rest of body present. Postero-dorsal angle well-marked, rounded. Posterior margin straight, continuing into widely convex ventral margin. Sculptures on surface of valves only visible with SEM. No cuticular hairs on head and valves, without dorsal keel. Live animals are whitish in color.

Head – Relatively large, rounded, without any rostral projection; with marked supra-ocular depression and large compound eye. Optical vesicle contiguous with prominent top of head. Ventral margin depressed under compound eye and bulging at level of antennules.

Antennule (A1) (Fig. 8E, 9E) – Cylindrical, with some tiny scattered, denticles on anterior face (only visible with SEM) and row of long setules on posterior face. Antennular sensory seta elongated, arising approximately in middle of lateral face. Distal tip bordered with small thick spinules. Nine short aesthetascs.

Antenna (A2) (Fig. 11A - C) – Coxal part with two setulated sensory setae, one long, the other short and thin (ratio between setae: 1.8) (see arrows 1, 2 in fig. C). Basal segment robust, with distal spine at dorsal side (see arrows Fig. A and Fig. B), and a long distal sensory seta on ventral face. Antennal branches elongated, 4-segmented exopodite slightly shorter than 3-segmented endopodite, all segments cylindrical, with rows of minute spinules and rows of long and thin setae. Antennal formula: setae 0-0-1-3/1-1-3, spines 0-0-0-1/0-0-1. Three long, apical swimming setae on both antennal branches, all with basal and distal segments bilaterally armed with fine and long setules.

Valves (Fig.7F-G) – Small, sub-ovoid, with row of 13-16 setae along anterior ventral margin. Posterior part with row of marginal denticles, organized in groups of 6-7, each group of distinct size. Denticles in posterior most margin form uniform row. Two posterior hooks supporting postabdominal setae (setae natatoria).

Limb I (Fig. 13E) – Distal lobe or endite 4 with single anterior thicker seta, bearing short setules and two soft setae. Endite 3 with short, strong, single anterior seta, bearing noticeable lateral spinule-like projections, and single long and strong posterior seta. Endite 2 with two posterior setae. Endite 1 with three posterior setae. Two ejector hooks of different sizes.

Limb II (Fig. 14E) – With large cylindrical lobe bearing long apical seta, its distal part regularly setulated, and with a thick lateral seta. Distal endite with two setulated setae, other two endites

554 with only one setulated seta each. One small seta near gnathobase plate, followed anteriorly by
555 small hook-like outgrow. Rest of gnathobase margin with row of setae arranged fan-like. Posterior-
556 most long single seta and neighboring more anteriorly located, long seta separated by large gap.
557 Both unilaterally setulated with long peg-like setules facing proximally; posterior-most seta
558 setulated along its full length, neighbouring seta on distal portion only. Above-mentioned two long
559 setae followed by row of 10 gradually shortening and curved setae. Fan-like arrangement of setae
560 is closed by group of three anterior brush-like setae, curved proximally with unilateral setulation
561 facing distally, posterior one strongly reduced. Two shorter and thick setae with apical setulation
562 inserted parallel to group of three anterior-most setae one of them with a very long prolongation.
563 Fan-like setation is arranged in two planes. Two long posterior setae plus two thick anterior
564 sensilla and anterior brush-like seta in ventral plane, remaining setae in dorsal plane.

565 Limb III (Fig. 15E) – Exopodite large, flat, with four distal setae and two short proximal setae, one
566 short and one long, all of them bilaterally setulated. Inner distal portion with three endites. Endite
567 3 with single anterior seta; endite 2 with one anterior and one posterior seta. Endite 1, with one
568 anterior seta and the seta “unica”. Rest of endopodite with 25 soft setae. At the base of the filter
569 plate there some scattered small denticles.

570 Limb IV (Figs 16E) – Exopodite like III, but more elongated, with four long setae in distal part and
571 two setae in proximal part, one short and another long. Inner distal portion of limb with two
572 endites: endite 1 with long articulated and bisetulated seta. Endite 2 with two long setulated
573 setae. Filter plate with 13-15 setae. At the distal part of the gnathobase there is a row of scattered
574 big denticles and some long hairs.

575 Limb V (Fig. 17E) – With large ovoid lobe, with setulated margin, large distal seta and small
576 proximal seta, both bi-setulated. Inner thoracopod portion with elongated lobe with long setulae
577 followed by two setae.

578 Postabdomen (Fig. 18E, 19G) – Elongated, ventral margin almost straight, with numerous rows of
579 minute setules in transversal rows. Ventral margin long, straight. Laterally, row of 6 large,
580 triangular teeth, fringed with marginal setules. Rows of denticles on distal portion continue along
581 base of bident tooth; distal branch of bident tooth significantly longer than proximal branch.

582 Postabdominal claw (Fig. 19H) - slightly curved, with sharp, pointed tip. Ventral pecten consisting
583 of 6 slender spines huddled together. Basal, middle, and distal pectens fused.

Natatory setae – articulated, longer than the postabdomen, with the proximal part shorter and without setae. Distal part longer, bilaterally setulated with few and fine setae.

Moina cf micrura 1 ES (Fig. 7I, J)

Material revised: In Spain: Albufera de Valencia Lake (39.33°N, 0.37°W) and Sobrón dam (42.77°N, 3.10°W).

Detailed description

Adult Parthenogenetic female: Small animals, length: 0.610-698 mm (n=10). Body roundish in lateral view, convex in the dorsum and not high (body height/ length = 0.54-0.66) (Fig. 7I, J). A small dorsal depression between the head and the rest of the body. Postero-dorsal angle well-marked, as an angle. Posterior margin with a dorso posterior angle present. Sculpture on the surface of the valves resembling scales. No integumental hairs on head and valves, with a dorsal keel. Live animals are whitish in colour.

Valves - large, subovoid, with setae along ventral margin (Fig. 12H). Posterior to the last marginal seta, a row of marginal denticles, organized in groups with 7-10 members increasing in size uniformly distally. These denticles in the dorsalmost part form a uniform row that becomes submarginal with thin and long elements towered to the dorsal hooks (Fig. 12I). Between the hooks there are present a series of submarginal spine-like setulae (Fig. 12I). The hook is setulated and works for the setae natatoria, found in the dorsal most internal part of the valves.

Postabdomen - elongated, conically narrowing distally, posterior view with scattered lines of spinules in lateral side, smooth (Fig. 18F, 19I). Ventral margin almost straight. Preanal angle, well expressed. Laterally, a row of 5-6 large, triangular, plumose teeth, and the bident tooth, with the distal branch 1/3 larger than the basal branch (Fig. 18F, 19I).

Postabdominal claw (Figs. 18F, 19J) - curved, externally with three successive pectens along the dorsal margin, basal pecten consisting of 4-5 smaller spines, middle pecten with 9 stronger spines, distal pecten consisting with smaller denticles forming a row from the mid-claw. On the ventral margin there are 5-6 strong denticles, near the base of the claw.

Seta natatorial - longer than the postabdomen; its distal segment longer than the basal one with lateral setulae.

Antenna I (Figs. 8F, 9F) - elongated, slightly wider in the last third (length about 4 diameters), with transverse rows of scattered spinules. A longitudinal row of long setules on the posterior face. Antennular sensory seta conical, elongated, arising in the middle of the lateral face. Distal tip bordered with a scattered line of small thick spinules. Nine aesthetascs two of them slightly larger, tip of each aesthetascs with small projections, giving an aspect of a crown (Fig. 9F). This species has the biggest ratio of the A1 (Mean ratio: 7.21, i.e. Moina micrura s. str. has a mean ratio of 2.9) of all the studies groups.

Antenna II (Fig. 11D) - coxal part with two setulated sensory setae, one big and the other smaller and thinner. Basal segment, with a short spine at anterior face in distal side, and a distal sensory seta on posterior face. Spinulation of all segments similar to *M. micrura* s. str. but with denser and stronger lines of spinules. Antennal branches of both, endopod and exopod with a row of long and thin setae in the posterior face. Antennal formula: setae 0-0-1-3/1-1-3, spines 0-1-0-1/0-0-1.

Limb I (Fig. 13F) - inner distal lobe, or endite 4 with a single anterior thicker seta, bearing short setules, and two soft setae. Endite 3 with a long anterior seta, bearing lateral spinule-like projections in both sides, and a single long and strong posterior seta. Endite 2, with two posterior setae. Endite 1 with three posterior setae. Two ejector hooks of different size. No maxillar process on limb base.

Limb II (Fig. 14F) - A large lobe bearing a long-articulated seta, similar to *M. micrura*, with the distal part regularly setulated, followed by a single endite, bearing two soft, setulated setae and two

endites with one setulated seta. In the opposite side a soft seta short setulated seta close to the gnathobase. A small setulated sensilla follows this small seta and a long “beating seta”. A long anterior seta. Gnathobase clearly defined, with nine anterior setae (and seven posterior setae in two rows, four posterior and three anterior, one of them with distal part covered by setulae.

Limb III (Fig. 15F) - Exopodite large, flat, with four distal setae and two proximal setae all of them bilaterally setulated, one short and the other long. Inner distal portion with three endites. Endite 3 with single anterior seta; endite 2 with one anterior and one posterior seta. Endite 1, with one anterior seta and the seta “unica”. Rest of endopodite with 27-28 setae. Distal corner with one long seta.

Limb IV (Fig. 16F). - Exopodite similar to Limb III, but more elongated, with four long setae in distal part and two setae in proximal side, one short and one long, all of them bilaterally setulated. Inner distal portion of the limb with two endites: endite 2 (see Fig. 16F named as 2 in *Moina micrura s. s.tr.* fig.16H) with two setae, one of them long, soft and hairy as *in Moina micrura*, s.s.tr. no described before seta (see Fig. 16F in black named as 3 in *Moina micrura s. s.tr.* fig.16H). Endite 1 with one anterior (see Fig. 16F named as 1 in *Moina micrura s. s.tr.* fig.16H) and one posterior setae. Filter plate with 26-28 setae.

Limb V. (Fig.17F) - With large ovoid lobe, with setulated margin, a large distal seta and a small proximal seta. Inner limb portion with a rounded lobe and two soft setae, one long and one short and thin.

Ephippial female: No material available

Detailed description of males

Body – smaller than female, more elongated as compared to female. Dorsal margin of valves almost straight, postero-dorsal angle clearly distinct. Antero-ventral portion of valves fringed with

long, thin setules. Denticles along posterior margin more distinct than in female, arranged in groups similarly, as in *Moina micrura* s. str.

Head – more elongated than in female, rostrum absent, labrum less fleshy than that of female. Optical vesicle fills top of head. No trace of pigment in ocellus.

Antennule (A1) (Fig. 8K) – long, antennular sensory not too long, male seta and sensillum inserted in first third of antennule, the sensilium inserted under the sensory seta in different points. Nine aesthetascs and two hooks of distinct size and orientation, with bifurcated tips, present distally. The surface is full of denticles on the first half and long setulas in the second half part.

Antenna (A2) (Fig. 11D) – similar in armature to female. Distal margin of all segments with row of thick, short spinules.

Carapace surface without any hairs or setules.

Limb I (Fig.21D) – with thick copulatory hook, its tip with two spinules, endite 4 (IDL) with three setae, endite 3 with one seta and one stiff seta with little denticles on the margin, and endite 2 with three setae two bilaterally setulated and one long soft seta without setaluation (see fig. 21D remarked in black). Basal part short and rounded, with row of stout bristles on ventral face.

Limb II (Fig. 22D) – same morphology as in female (Fig. 14F).

Limb III (Fig. 23D) – same morphology as in female (Fig.15F), this species shows the biggest denticles at the base of the filter plate, clearly different to the other species of the complex.

Limb IV (Fig. 24D) – as in limb III, the denticles at the base of the filter plate, are clearly remarkable in both sexes but in males, there are no hairs over the structure.

Limb V – we couldn't observe this limb and there was not more material available.

Postabdomen (Fig. 26D) – as in female, shorter, with about 4-5 setulated teeth, distalmost tooth thin and with sharp tip. Distal bident tooth as in female. About 4-5 teeth in basal pecten of postabdominal claw - up to 10 in middle pecten (arranged fan-like) and 5-7 on dorsal side at basal part of claw.

Moina cf micrura 2 ES (Fig. 7H)

Material examined: Albufera de Valencia Lake (Spain) (39.33°N, 0.37°W).

Detailed description

Adult Parthenogenetic female: Length bigger than *M. micrura* s. str.: mean length 0.831 mm (n=11). Body more elongated in lateral view, slightly convex in the dorsum, not so high, same ratio *as Moina micrura* s. s.tr. (Lentgh/Height: 1.69) (Fig. 7H). A small dorsal depression between the head and the rest of the body. Postero-dorsal angle well-marked, as an angle. Posterior margin straight. Sculpture on the surface of the valves resembling scales (Fig. 12J-K). No integument hairs on head and valves, with a dorsal keel. Live animals are whitish in color.

Valves (Figs. 12J-L) - large, subovoid, with a row of 15 setae along ventral margin (Fig. 12J, K). Posterior to the last marginal seta, a row of marginal denticles, organized in groups with 7-10 members increasing in size uniformly distally. These denticles in the dorsalmost part form a uniform row that becomes submarginal and after a curve again appear as marginal. A remarkable setulated hook for the postadominal setae in the dorsalmost part of the valves (Fig. 12L).

Postabdomen (Figs. 18G, 19K) - elongated, conically narrowing distally, with numerous lines of spinules in lateral side, and an appearance of scale-like dots, just visible in the SEM (Fig. 19K). Ventral margin almost straight. Laterally, a row of 5-8 large, triangular, plumose teeth, and the bident tooth, with the distal branch larger than the basal branch and with a row of fine seta on the base.

Postabdominal claw (Fig. 19L) - curved, externally with two successive pectens along the dorsal margin, basal pecten consisting of 7-11 thick spines, distal pecten consisting with smaller denticles forming a row from the mid-claw. Internally, there is a uniform row of denticles. On the ventral margin there are 7-8 strong denticles, near the base of the claw.

Seta natatorial - longer than the postabdomen; its distal segment longer than the basal one with lateral setulae.

Antennula I (Fig. 8G, 9G-H) - elongated, rod-like (length about 5 diameters), cylindrical, with transverse rows of surrounding it a longitudinal row of long setules on the posterior face. Also, some long setules around the first third of the antenna. Antennular sensory seta conical, elongated, arising slightly before the middle of the lateral face. Distal tip bordered with a line of small thick spinules (Fig.9H1). Nine aesthetascs two of them slightly shorter, tip of each aesthetascs with small projections, giving an aspect of a crown.

Antenna II (Fig. 11E) - coxal part with two setulated sensory setae one big and the other smaller and thinner. Basal segment, with a short spine at anterior face in distal side, and a distal sensory seta on posterior face. Spinulation of all segments like *M. micrura*, but with denser and stronger lines of spinules. Antennal branches elongated, 3-segmented endopod with rows of long and thin setae in the posterior face. Antennal formula: setae 0-0-1-3/1-1-3, spines 0-1-0-1/0-0-1.

Limb I (Fig. 13G) - inner distal lobe, or endite 4 with a single anterior thicker seta, bearing short setules, and two soft setae. Endite 3 with a variable in length anterior seta, bearing lateral spinule-like projections in both sides, and a single long and strong posterior seta. Endite 2 with two posterior setae. Endite 1 with three posterior setae. Two ejector hooks of distinct size. No maxillar process on limb base.

Limb II (Fig.14G) - A large lobe bearing a long single and thin bisetualted seta, followed by four endites, each with bisetulated setae, decreasing in size. One sensilia close to the gnathobase, but longer and thinner than that in *M. micrura*. A long, setulated seta-like follows this smaller seta, the long "beating" seta present. Gnathobase with 7 anterior setae and five posterior setae, two of them covered with strong setules in the last third.

Limb III (Fig. 15G) - Exopodite large, roundish, with four setae along the distal margin and two proximal setae, one of them strong and long, all of them bilaterally setulated. Inner distal portion with three endites. Endite 3 with a single, very strong seta; endite 2 with one anterior, articulated seta. Endite 1 (very remarked), with two anterior setae, and the seta "unica". Rest of endopodite with 29-37 setae. At the base of the filter plate there is a row of little denticles and hairs.

Limb IV (Fig. 16G) - Exopodite similar to Limb III, with four long setae decreasing in size distally, and two setae in proximal side, one short and one long. Inner distal portion of the limb with two endites: endite 1 with anterior setae and one posterior seta. Endite 2 with one anterior seta and endite 3 with 1 anterior seta. Filter plate with 17-27 setae.

Limb V (Fig. 17G) - With large ovoid lobe, with setulated margin, a large, bisegmented distal seta and a small proximal seta. Inner limb portion with a lobe bearing a thin seta and a long seta.

Detailed description of males

Body – Smaller than female, more elongated as compared to female. Dorsal margin of valves almost straight, postero-dorsal angle clearly distinct. Antero-ventral portion of valves fringed with

long, thin setules. Denticles along posterior margin more distinct than in female, arranged in groups similarly, as in *Moina micrura* s. str.

Head – More elongated than in female, rostrum absent, labrum less fleshy than that of female. Optical vesicle fills top of head. No trace of pigment in ocellus.

Antennule (Fig. 8L) – Long, antennular sensory not too long, male seta and sensillum inserted in first third of antennule, the sensilia is inserted under the sensory seta. Nine aesthetascs and three hooks of distinct size and orientation, with bifurcated tips, present distally. There is not any ornamentation on the surface.

Antenna (Fig. 11E) – Similar in armature to female. Distal margin of all segments with row of thick, short spinules.

Carapace surface without any hairs or setules.

Limb I (Fig. 21E) – With thick copulatory hook, its tip with two spinules, endite 4 (IDL) with three setae, endite 3 with one seta and one stiff seta with little denticles on the margin, and endite 2 with three setae two bilaterally setulated and one long soft seta without setulation. Basal part short and rounded, with row of stout bristles on ventral face.

Limb II (Fig. 22E) – same morphology as in female (Fig.14G).

Limb III (Fig. 23E) – Same morphology as in female, this species presents some rows of medium denticles at the base of the filter plate.

Limb IV (Fig. 24E) – As in limb III, there is a row of the denticles at the base of the filter plate clearly remarkable in both sexes with too many hairs.

Limb V (Fig. 25D) – As in females, with large ovoid lobe, with setulated margin, a large, bisegmented distal seta and a small proximal seta. Inner limb portion with a lobe bearing a thin seta.

Postabdomen (Fig. 26E) – As in female, shorter, with about 6 setulated teeth, distalmost tooth thin and with sharp tip. Distal bident tooth as in female. A dense row of teeth in the basal pecten of postabdominal claw.

Morphological comparison

All species of the complex present differences in the following characters (Table 1 and table 2, in other files due their size):

Females:

- Size (Fig. 7): *Moina* cf *micrura* 3MX is the biggest (Mean length: 0.86 mm) followed by *Moina* cf *micrura* 2ES, *Moina* cf *micrura* 2MX, *Moina* cf *micrura* 1MX, *Moina* cf *micrura* 5MX, *Moina* cf *micrura* 1ES, *Moina* cf *micrura* 4MX, and the smallest one, *Moina micrura s. s.tr.* (Mean length: 0.86 mm).
- Antennules (A1) (Fig. 8, 9): ratio A1 (L/W): *Moina* cf *micrura* 1ES, has the longest antennule, surface's ornamentation, position of sensorial seta.
- Antennes (A2) (Fig. 10, 11): Ratio long Proximal seta / short proximal seta being *Moina micrura* s. str. the one which presents the biggest ratio and *Moina* cf *micrura* 1ES the smallest one, surface's ornamentation, Spanish populations present the greatest spinulation, and antennal formula (variable in the number of spines).
- Valves (Fig. 13): type of spinulation and sculptures on shell (see Table 2).
- Limb II (Fig. 14): number of setae on filter plate (see table II).
- Limb III (Fig. 15): ratio setae, number of setae on filter plate, hairs and spinulation of gnathobase.
- Limb IV (Fig. 16): ratio setae, number of setae on filter plate, hairs and spinulation of gnathobase, seta of endite 1. In *Moina* cf *micrura* 4MX, it seems to have one more seta on endite 1 than the others.
- Limb V (Fig. 17): ratio setae.
- Post abdomen (Fig.18, 19): basaldorn spines and feathered teeth, Basal and middle pecten of postabdominal claw in *Moina* cf *micrura* 4MX is separated instead of fusions as in the others species.
- Ephippium (Fig. 20): circular egg as in *Moina* cf *micrura* 3MX or an ovoid egg as in *Moina micrura s. s.tr., Moina* cf *micrura* 1MX, and *Moina* cf *micrura* 2MX. Polygonal cells or circular cells.

The main differences in males are in:

- A1 (Fig. 8): presence of aesthetascs at the exit of the lateral seta, ornamentation of surface as the, presence of spines in *Moina* cf *micrura* 2MX (Fig. 8I) and the number of the hooks from five, like in *Moina micrura* s. str. to two in *Moina* cf *micrura* 1ES.

- Limb I (Fig. 21): Ornamentation of endites, the tip of seta without setulation on endite 1 of *Moina* cf *micrura* 1MX presents spinulation, and also in *Moina* cf *micrura* 1MX, there is a plumose seta on endite 2 not presented in the others (Fig. 21A).
- Limb II (Fig. 22): Number of setae on gnathobase.
- Limb III (Fig. 23): Number of setae on gnathobase and ornamentation
- Limb IV (Fig. 24): Number of setae on gnathobase and ornamentation
- Limb V (Fig. 25): No differences
- Post abdomen (Fig. 26): basaldorn spines and feathered teeth.

Taxonomical remarks

Females of *Moina* cf *micrura* 1MX present the most marked pattern of spinulation and hairs at the base of the Gnathobase mainly in limb IV of all Mexican species. Males of *Moina* cf *micrura* 1MX, can be easily distinguished by the plumose seta on endite 2 and tip of unsetulad seta of endite 1 of the trunk limb I, and in the antennule for the aesthetascs present at the exit of the sensory seta. These characters are not shared with the other species of the complex.

Males of *Moina* cf *micrura* 2MX, present a stout spine in the last third of the antennule and its trunk limb I presents one soft seta and a stout spine instead of two soft setae.

Moina cf *micrura* 3MX is the biggest species of the complex and *Moina* cf *micrura* 4MX is the smallest found in the American continent. Also, *Moina* cf *micrura* 3MX has a round egg instead of ovoid as in *Moina* cf *micrura* 1MX,2MX and *Moina micrura* s. s.tr.

Moina cf *micrura* 4MX, present one seta more one endite 1 of limb IV not present in the other species of the complex.

Moina cf *micrura* 5MX, has the biggest antennula.

Females of *Moina* cf *micrura* 1 ES can be distinguished from the other studied species of the complex by the hairy seta on endite 1 of limb IV. This character is shared with *Moina micrura* s. str. the closest, genetically, species of the complex.

The body of females of *Moina* cf *micrura* 2 ES is more elongated in lateral view, its postabdomen presents an appearance of scale-like dots, just visible in the SEM and the form of the postabdomen is straighter than the others.

Statistical analyses

The PCA of the environmental variables (Table 3) shows two groups (Fig. 27A): the first group represents the Old World (European) species and is composed by 1, 2, and 3, *Moina micrura s. s.tr.*; *Moina* cf *micrura* 2ES; and *Moina* cf *micrura* 1ES, respectively (right circle on Fig.27A). And the second group, the American one, is composed by all Mexican species (left circle on Fig.27A numbers 4-8, *Moina* cf *micrura* 1- 5 MX, respectively). The habitat of European group is characterized by high temperature, latitude and depth, and the habitat of the American group is characterized by altitude and Secchi (Fig.27A).

Especies	AltMin	AltMax	LatMin	LatMax	Depth	Secchi	Temp
Mmsstr	216.00	216.00	50.00	50.00	1500.00	0.00	24.00
Mcfm2ES	0.00	509.00	39.00	39.00	1300.00	290.00	24.60
Mcfm1ES	0.00	509.00	39.00	42.00	17300.00	230.00	24.00
Mcfm1MX	1416.00	1823.00	26.00	27.00	900.00	470.00	23.70
Mcfm2MX	310.00	2811.00	16.00	22.00	2350.00	270.00	19.50
Mcfm3MX	2131.00	2131.00	23.00	23.00	150.00	150.00	26.90
Mcfm4MX	10.00	10.00	17.00	17.00	150.00	150.00	31.00
Mcfm5MX	3.00	60.00	18.00	23.00	900.00	200.00	18.00

Table 3. Environmental parameters used for the analysis. AltMin: Minimum Altitude; AltMax: Maximum Altitude; LatMin: Minimum Latitude; LatMax: Maximum Altitude; Depth (cm); Secchi (cm); Temp: temperature (°C).

The PCA of the morphological parameters of females Table 2 and Fig.27B). shows four groups correlated by their morphology. The first group (*Moina* cf *micrura* 1 and 4 MX) is characterized by these ratios: habit (L/W); length of basipodite A2/Length Distal Setae of A2; and Limb III Length Long seta/ Length Small seta. The second group (*Moina micrura s. s.tr.*, *Moina* cf *micrura* 2 and 5 MX) is characterized by: length of basipodite A2/Long Proximal Setae of A2 and Exopod limb IV (length/width); Limb V Long seta/ Small seta.

Haplotype network and intraspecific divergence and BIN numbers

The analyses of the COI dataset detected 24 haplotypes (Fig. 28) showing restricted distribution. Genetical intraspecific divergences of Mexican and Spanish populations ranged from 0 to 1.78%, being the putative species *Moina* cf *micrura* 1MX, the species with more divergence. This greater divergence in this species is due to the high number of haplotypes showed by the haplotype network related with the geographical history of the Mexican Sierra Madre.

The clade A (BOLD:ACK2252) represents *Moina micrura s. s.tr.*, includes 2 haplotypes, one haplotype in Czech Republic (Terra typica) and the other in Kazakhstan. Clade B (BOLD:ACA1509) is represented only by 1 haplotype restricted to Spain (*Moina* cf *micrura* 1 ES) and is the closest to *M. micrura* s. str. Clade C (BOLD:ACH9786), is one haplotype from India (*Moina* cf *micrura* IN). Clade D (BOLD:ACA1508) is another Spanish haplotype (*Moina* cf *micrura* 2 ES) coexisting in sympatry with specimens of Clade B in Albufera de Valencia Lake.

In Mexico, there are 14 haplotypes in 5 different clades (E, F, G, I, K), *Moina* cf *micrura* 1 MX, clade I (BOLD:AAB1056), is the group with more haplotypes (Fig. 28).

Clade H (BOLD:ABW7759) include two different haplotypes, one exclusively in South Korea and the others are shared between South Korea, Russia, Kazakhstan and Hungary (*Moina* cf *micrura* SK, RUS, KZ, HU).

Finally, Clade J (BOLD:ADF4310) is restricted to Russia showing three different haplotypes (*Moina* cf *micrura* RUS).

DISCUSSION

The detailed analyses of the morphology of the five-putative species of *Moina micrura* complex in Mexico (*Moina* cf *micrura* 1-5MX) and two from Spain (*Moina* cf *micrura* 1-2ES) described above (Figs.7-24) showed significant differences in agreement with barcoding and patterns of distribution.

In these detailed analyses, new characters not considered before, as limbs, were included. These latter characters, present many significant differences i.e. the ornamentation limbs III-IV in both sexes and the ephippium and the hairy seta on limb IV present in *Moina* cf *micrura* 1ES and *Moina micrura* s. str. It is also important to highlight, the importance of the first trunk limb in males as one of the most taxonomical informative limbs due to its variability in each studied species of the complex. The ornamentation found on the gnathobase of limbs III and IV (in males and females)

had not been described or illustrated before for *Moina micrura*. A similar pattern of spinulation was illustrated by Kotov et al., (2005), in the description of *Moina dumonti*, but they did no comment about this. The phenotypical plasticity showed by this group, allows them to adapt to new habitats in response to changing abiotic and biotic pressures (Burge et al., 2018).

These descriptions based on morphology, molecular data, and distribution patterns, are part of the integrative taxonomy (Dayrat, 2005), an approach where different types of evidence converge to support the species discovery that currently is revitalizing this discipline (Agnarsson and Kuntner, 2007).

Species of *Moina micrura* complex studied in this work, share some morphological characters with other species of the genera i.e. *Moina* cf *micrura* 1MX has similar habitus to *Moina dumonti* but they have differences in the claw and in the spinulation of the valves (Kotov et al., 2005), and *Moina* cf *micrura* 2MX can be confused with *Moina ephemeralis* from South Slovakia (Central Europe) but they can be differentiated by the spinulation of the valves, the length of the branches of the bident tooth (Hudec, 1997) and clearly by its distribution.

Haplotypes with genetical divergences of COI gene minor of 2% (Lefébure et al., 2006) cannot be distinguished morphologically. Morphological differences are consistent genetic differentiation in the COI gene among species (Elías-Gutiérrez et al., submitted). Both genetic analyses and morphology, have been employed to successfully discriminate sibling species of cladocerans (Quiroz-Vázquez and Elías-Gutiérrez, 2009), marine and freshwater copepods (Gutiérrez-Aguirre et al., 2014; Miracle et al., 2013), rotifers (Mills et al., 2016; García-Morales and Elías-Gutiérrez, 2013), and others.

Environmental PCA showed two main groups, the Old-World group, and the American group, and confirmed the restricted distribution of each putative species shows by the haplotype network, BIN numbers and results obtained by Elias-Gutiérrez et al., (sumitted, 2018), underlying the importance of morphology and environment to delimitate the species.

Shared records between distant areas, as clade A and clade H, are possibly due to the dispersal capacity of this group, through ephippia (this structure has 600 years of viability) (Burge et al., 2018), and to a men-mediated introduction (Montoliu-Elena et al., sumitted, 2018).The huge number of haplotypes showed by Mexican populations evidence the possibility of the American continent as the center of speciation of the *Moina micrura* complex. As several authors had

mentioned before (Adamowicz and Purvis, 2005; Forró et al., 2008), biodiversity in cladocerans is understudied and hides a high cryptic diversity (Bekker et al., 2016).

Finally, this study remarks the importance to use a universal and standard molecular marker to differentiate and compare the species, as DNA barcoding is being, and the importance of study genetically the type material or specimens from terra typica, when type material is not available and to include the DNA barcode as an easy tool to facilitate the identification and delimitation of the species. So, to finally solve this complex, further analyses must be done to compare these seven new species with the ancient synonyms of *Moina micrura* and possibly with all the species of the genera.

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LIST OF TABLES, FIGURES, AND SUPLEMENTARY MATERIAL

	SPECIES							
MORPHOLOGICAL CHARACTERS	***Moina micrura* s. str**	***Moina cf micrura* 1 MX**	***Moina cf micrura* 2 MX**	***Moina cf micrura* 3 MX**	***Moina cf micrura* 4 MX**	***Moina cf micrura* 5 MX**	***Moina cf. micrura* 1 ES**	***Moina cf. micrura* 2 ES**
BODY								
Size (Length-Width) (mm)	L: 0.44 -0.76 / W: 0.54 - 0.68	L: 0.65 - 0.85 / W: 0.45 - 0.55	L: 0.57 - 0.9 / W: 0.3 - 0.55	L: 0.77 - 0.98 / W: 0.41 - 0.61	L: 0.47 - 0.60 / W: 0.28 - 0.36	L: 0.57 - 0.77 / W: 0.37 - 0.58	L: 0.64 / W: 0.39	L: 0.81 / W: 0.48
Mean Length (micras)	488.7	738 ± 71	781 ± 117	861.6±51.2	543.451±53.24	668 ± 74.6	642.8	813.3
Size Ratio (L/W)	1.69	1.73	1.65	1.58	1.62	1.45	1.63	1.69
ANTENULAS (A1)								
Mean Length (micras)	115.46	130.3	143.07	132.96	83.9 ± 5.14	147.09 ± 13.6	277.18	161.73
Ratio	0.34	0.14	0.24	0.22	0.28	0.25	0.16	0.24
Anterior face with denticles (1 = few, 2 = many)	1	2	1	1	1	1	2	2
Sensorial seta on the middle	first 1/3	first 1/3	middle	middle	middle	middle	middle	middle
ANTENNAS (A2)								
Basipodite								
Length (micras)	179-259 micras	195 - 307 micras	219 - 289 micras	153 - 221 micras	123.6 - 161.7 micras	199 - 293 micras	176.18 micras	278 - 218 micras
Mean Length (micras) and Standar deviation	198.17 ± 26.5	266.8 ± 62.5	245 ± 38.21	184.174 ± 25.2	154.187 ± 13.95	233.9 ± 33.15	176.18	248.3 ± 28.5
Basal segment								
Long proximal seta (LPS) / Short proximal seta (LPS)	2.5	1.5	2.1	1.8	2.2	1.8	1	1.7
LPS respect basipodite / SPS respect Basipodite	1.75 / 4.3	1.75 / 2.7	1.6 / 3.5	1.1 / 2	1.5 / 3.3	136 / 3	1.14 / 1.13	1.73 / 2.9
Terminal segment								
Ratio: Basipodite length /Long distal seta	1.2	1.8	1.4	1.3	2.1	1.3	1.2	1.7
Surface								
Denticles (1 = small, 2 = medium, 3 = big)	1	3	2	2	1	2	2	2
Branches								
Antennal formula Spines	0-1-0-1 / 0-0-1	1-1-0-1 / 0-0-1	0-1-0-1 / 1-1-1	0-1-0-1 / 0-0-1	0-1-0-1 / 0-0-1	0-1-0-1 / 0-0-1	0-1-0-1 / 0-0-1	0-1-0-1 / 0-0-1
VALVES								
Ventral margin								
Number of big spines	14 - 16	13 - 17	10 a 20	20 - 23	de 12 a 13	13 - 16	17	15
Number of groups of little spines	7 - 10	8 - 13	8 to 15	13	5 to 10	8	7 to 10	7 to 10
Increasing in size	X	X	NO	X	X	X	X	X
Nº of spines in each group		8 to 10	5 to 10	4 to 9	5 to 8	6 to 8	15	10 to 11
Sculptures on shell (0 = no, 1 = little marked, 2 = very marked)	0	1	2	Not observed	Not observed	2	1	2
LIMB II								
Gnathobase								
Unic and first long seta next to the beating seta	X	X	X	X	X	X	X	X
Number of posterior setae (filter plate of gnathobase)	b	13 to 15	10 to 13	12 to 15	6 to 7	10	10	12
LIMB III								
Exopodite big and flat								
Ratio	1.19	1.16	1.24	1.26	1.14	1.15	1.22	1.32
Ratio setae (Long/short)	5.78	5.23	3.91	3.3	3.07	2.81	4.41	2.59
Gnathobase								
Number of setae	28 to 29	40 to 45	35 to 45	53	22	25	27-28	35 - 38
Presence of hairs (1=few, 2=medium, 3=many)	1	3	3	2	1	1	3	2
Type of spines (Small few=1, Big few=2, Many small=3, Many medium=4, Many big=5)	5	4	4	4	4	2	5	2
LIMB IV								
Smaller and flat exopodite								
Ratio	1.37	1.19	1.17	1.36	1.29	1.15	1.13	1.06
Ratio setae (Long/short)	5.6	5.34	4.7	3.73	3.16	6.72	2.43	2.72
Distal intern face								
Endite 1								
Number of anterior setae long and articulated	1 hairy and unarticulated seta	1	1	1	1	1	1 hairy and unarticulated seta	1
Gnathobase								
Number of setae	20 to 22	30 to 35	14 to 30	37 to 44	13 to 15	18	26 to 28	17 to 27
Spines (Small few=1, Big few=2, Many small=3, Many medium=4, Many big=5)	5	5	1	5	3	3	5	5
LIMB V								
Exopode								
Ratio long seta / Exopode	1.94	2.24	1.56	1.38	2.5	1.64	2.6	2.11
Endopode								
Ratio Setas	1.65	2.12	3.49	1.83	1.32	1.75	2.87	1.82
POSTABDOMEN								
Lateral view								
Number of teeth	4 to 5	7 to 9	5 to 6	6 to 11	4	6	4 to 6	8
Postabdominal claw								
Number of spines								
Number of ventral spines (at the top of the claw)	4-5	6 to 10	5 to 6	4	3	6	5 to 6	3 to 6
Basal - middle pected fused	X	X	X	X	NO	X	X	X
EPHIPIUM								
Lateral surface								
Hexagonal reticulum	X	X	X	X				
Relief of ephippial surface								
Complex structure of polygons interlocked with many thin tentacle-like projections	X	X	Circular	Grapes-like				
Projections cover inner surface of polygons	1	1	0	1				
MALE								
A1								
Aesthetascs at the exit of the seta	0	1	0	0			0	0
Number of hooks	4 to 5	3	3	4			2	3
Spine in the last thrid	0	0	1	0			0	0
Denticles (0=No denticles, 1 = few, 2 = many)	0	1	2	0			2	0
Presence of hairs (0=No, 1=yes)	0	0	0	0			1	0
LIMB I								
Endite 1								
Seta without setulation with spines on the tip	1	0	0	0			0	0
Endite 2								
Plumose seta	0	1	0	0			0	0
Endite 3								
Num. setae	2	2	1	2			2	2
Spines	0	0	1	0			0	0
Endite 4								
Num. setae		3	2	3			3	2
Hairs or spines	Spines	Spines	Spines	Big Spines			Spines	Spines
Making groups	0	1	1	0			0	1
Desigual hooks	X	X	X	X			X	X

1033 Fig.1. Jurine's original drawings (Jurine, 1820).

1034 Fig.2. Distribution of *Moina micrura* sensu Goulden (1968).

1035 Fig.3. Distribution of *Moina micrura* sensu Armengol (1978).

1036 Fig.4. Description of *Moina micrura* sensu Alonso (1986).

1037 Fig. 5. Map of sampling points

1038 Fig. 6. Morphological scheme of *Moina micrura*

1039 Fig.7. Habit of females *Moina micrura* complex. Optical microscope (MO) photographs and
1040 Scanning Electronic Microscope (SEM) photographs. A) *Moina* cf *micrura* 1MX; B,C) *Moina*
1041 cf *micrura* 2MX; D) *Moina* cf *micrura* 3MX; E) *Moina* cf *micrura* 4MX; F,G) *Moina* cf *micrura*
1042 5MX; H) *Moina* cf *micrura* 2ES; I, J) *Moina* cf *micrura* 1ES; K,L) *Moina micrura* s. str.

1043 Fig. 8. A1 ♀ and ♂. ♀: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf
1044 *micrura* 3MX; D) *Moina* cf *micrura* 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura*
1045 1ES; G) *Moina* cf *micrura* 2ES. ♂: H) *Moina* cf *micrura* 1MX; I) *Moina* cf *micrura* 2MX; J)
1046 *Moina* cf *micrura* 3MX; K) *Moina* cf *micrura* 1ES; L) *Moina* cf *micrura* 2ES; M) *Moina*
1047 *micrura* s. str. (SEM).

1048 Fig. 9. SEM photographs of A1 ♀. A) *Moina* cf *micrura* 1MX, 1: detail of tip and
1049 aesthetascs; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura*
1050 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura* 1ES; G,H) *Moina* cf *micrura* 2ES; I,J)
1051 *Moina micrura* s. str.

1052 Fig.10. SEM and MO photographs of A2 ♀. A,B) *Moina* cf *micrura* 1MX: a: distal seta; b:
1053 distal spine; c: proximal setae; C,D) *Moina* cf *micrura* 2MX; E) *Moina* cf *micrura* 3MX; F)
1054 *Moina* cf *micrura* 4MX.

1055 Fig.11. SEM photographs of A2 ♀.A,B,C) *Moina* cf *micrura* 5MX: B: distal spine; C) 1: distal
1056 seta; 2: proximal setae; D) *Moina* cf *micrura* 1ES; E) *Moina* cf *micrura* 2ES; F,G) *Moina*
1057 *micrura* s. str.

Fig.12. SEM and MO photographs of Valves ♀. A) *Moina* cf *micrura* 1MX; B,C) *Moina* cf *micrura* 4MX; D) *Moina* cf *micrura* 2MX: 1: detail of the end of the valves, 2: detail of spinulation; E) *Moina* cf *micrura* 3MX: 1, 2, 3: details of spinulation pattern;.F,G) *Moina* cf *micrura* 5MX; H,I) *Moina* cf *micrura* 1ES; J-L) *Moina* cf *micrura* 2ES; M,N) *Moina micrura* s. str.

Fig. 13. Limb I ♀: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura* 1ES; G) *Moina* cf *micrura* 2ES; H) *Moina micrura* s. str.

Fig. 14. Limb II ♀: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura* 1ES; G) *Moina* cf *micrura* 2ES; H) *Moina micrura* s. str.: se: sensilia, be: beating seta, 1: long seta, 2,3: setae with apical spinulation; 4: anterior brush-like setae; a-l:filter plate.

Fig. 15. Limb III ♀: A) *Moina* cf *micrura* 1MX, arrow: seta única; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura* 1ES; G) *Moina* cf *micrura* 2ES; H) *Moina micrura* s. str.: 1,2,3: Endites, a,b: distal setae, c-f: setae of exopod.

Fig. 15cont.: Spinulation pattern and hairs present at the base of the filter plate at gnathobase. I,J) *Moina* cf *micrura* 3MX: 1,2: rows of spines, 3: hairs. K) *Moina micrura* s. str.

Fig. 16. Limb IV ♀: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura* 1ES; G) *Moina* cf *micrura* 2ES; H) *Moina micrura* s. str.: 1: seta endite1, 2: seta endite2, 3: hairy seta.

Fig. 17. Limb V ♀: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura* 1ES; G) *Moina* cf *micrura* 2ES; H) *Moina micrura* s. str.

Fig. 18. Postabdomen ♀: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 4MX; E) *Moina* cf *micrura* 5MX; F) *Moina* cf *micrura* 1ES; G) *Moina* cf *micrura* 2ES; H) *Moina micrura* s. str.

Fig.19. SEM and light photographs of Postabdomen and claw ♀. A,B) *Moina* cf *micrura* 1MX; C) *Moina* cf *micrura* 3MX; D,E) *Moina* cf *micrura* 2MX; F) *Moina* cf *micrura* 4MX; G,H) *Moina* cf *micrura* 5MX; I,J) *Moina* cf *micrura* 1ES; L,M) *Moina* cf *micrura* 2ES; N) *Moina micrura* s. str.

Fig.20. SEM photographs of Ephippia and ephippial females. A,B,C) *Moina* cf *micrura* 1MX; D,E) *Moina* cf *micrura* 2MX; F,G) *Moina* cf *micrura* 3MX; H,I,J) *Moina micrura* s. str.

Fig. 21. Limb I ♂: A) *Moina* cf *micrura* 1MX, 1: Tip of the hook, 2: Detail of the spinulation of the unsetulated seta (in black), 3: Plumose seta 4: bisetulated seta; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 1ES; E) *Moina* cf *micrura* 2ES.

Fig. 22. Limb II ♂: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 1ES; E) *Moina* cf *micrura* 2ES.

Fig. 23 Limb III ♂: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 1ES; E) *Moina* cf *micrura* 2ES.

Fig. 24 Limb IV ♂: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 1ES; E) *Moina* cf *micrura* 2ES.

Fig. 25. Limb V ♂: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 2ES.

Fig. 26. Illustrations of Postabdomen ♂: A) *Moina* cf *micrura* 1MX; B) *Moina* cf *micrura* 2MX; C) *Moina* cf *micrura* 3MX; D) *Moina* cf *micrura* 1ES; E) *Moina* cf *micrura* 2ES.

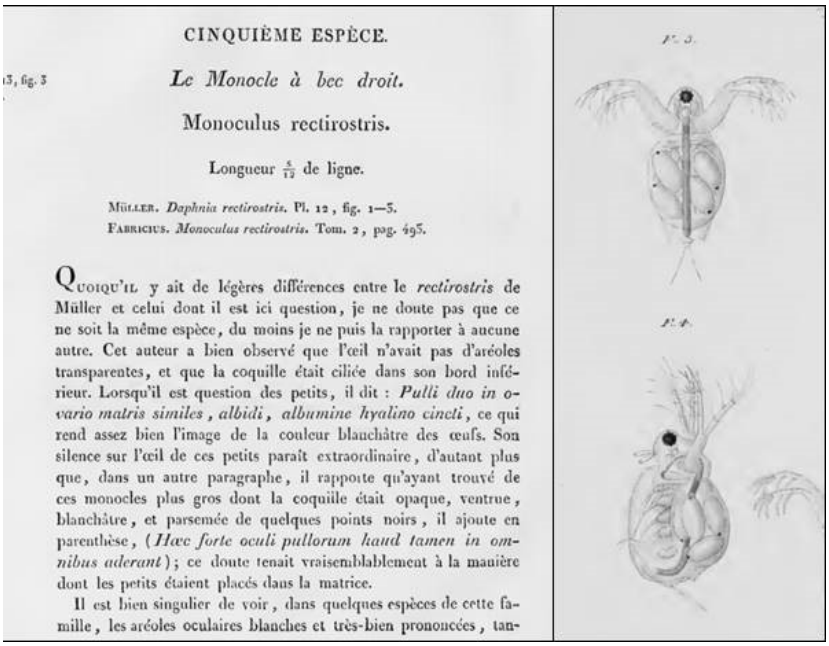
Fig. 27. PCA. A) Environmental PCA. B) Morphometrical PCA. Numbers in boxes represent species. 1 = *Moina micrura s. s.tr.*; 2 = *Moina* cf *micrura* 2ES; 3 = *Moina* cf *micrura* 1ES; 4 =

Moina cf *micrura* 1MX; 5 = *Moina* cf *micrura* 2MX; 6 = *Moina* cf *micrura* 3MX; 7 = *Moina* cf *micrura* 4MX; 8 = *Moina* cf *micrura* 5MX.

Fig. 28. COI Haplotypes network of M. micrura complex. Each circle indicates a unique haplotype and variation in circle size reflects the number of sequences assigned to it. Colors and sizes in circles represent the countries of collection and the number of sequence records for each haplotype. In parenthesis, intraspecific divergences of COI gene from *Moina micrura* complex in Mexico and Spain. % Minimum divergence = Min., % Mean divergence = Mean., % Maximum divergence = Max. Bin numbers assigned to each clade. Clades in grey boxes are sequences from different parts of the world only used for intercontinental comparison. Clades in blue boxes are populations analyzed in this study.

S1. Supplementary material. Sampling points data.

Fig. 1



CINQUIÈME ESPÈCE.

3, fig. 3

Le Monocle à bec droit.

Monoculus rectirostris.

Longueur $\frac{5}{12}$ de ligne.

Müller. *Daphnia rectirostris.* Pl. 12, fig. 1—5.
Fabricius. *Monoculus rectirostris.* Tom. 2, pag. 495.

Quoiqu'il y ait de légères différences entre le *rectirostris* de Müller et celui dont il est ici question, je ne doute pas que ce ne soit la même espèce, du moins je ne puis la rapporter à aucune autre. Cet auteur a bien observé que l'œil n'avait pas d'aréoles transparentes, et que la coquille était ciliée dans son bord inférieur. Lorsqu'il est question des petits, il dit : *Pulli duo in ovario matris similes, albidi, albumine hyalino cincti*, ce qui rend assez bien l'image de la couleur blanchâtre des œufs. Son silence sur l'œil de ces petits paraît extraordinaire, d'autant plus que, dans un autre paragraphe, il rapporte qu'ayant trouvé de ces monocles plus gros dont la coquille était opaque, ventrue, blanchâtre, et parsemée de quelques points noirs, il ajoute en parenthèse, (*Hæc forte oculi pullorum haud tamen in omnibus aderant*); ce doute tenait vraisemblablement à la manière dont les petits étaient placés dans la matrice.

Il est bien singulier de voir, dans quelques espèces de cette famille, les aréoles oculaires blanches et très-bien prononcées, tan-

Fig. 2.

1122

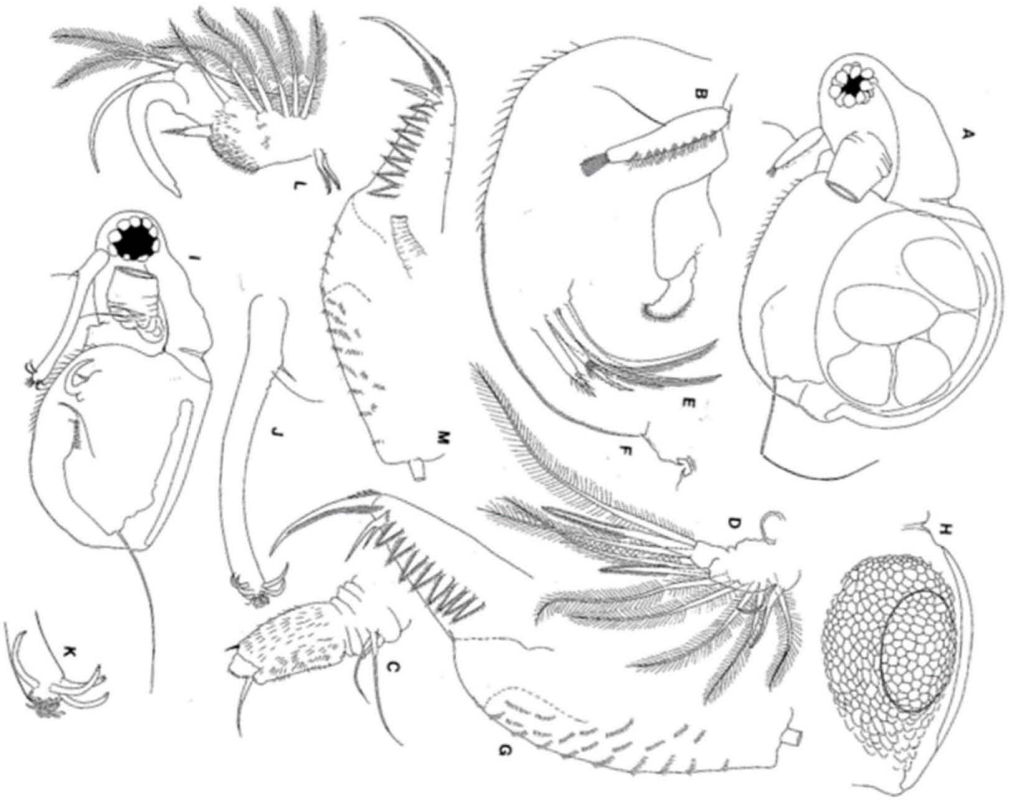
1123 Fig. 3

1124

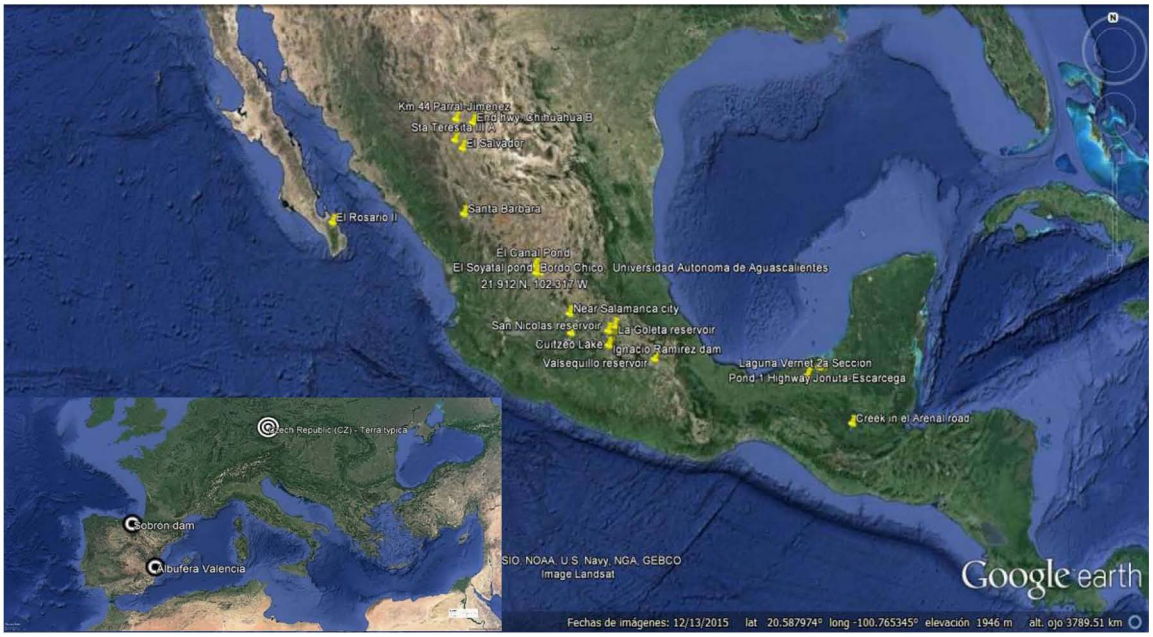
1125

1126 Fig. 4.

1127

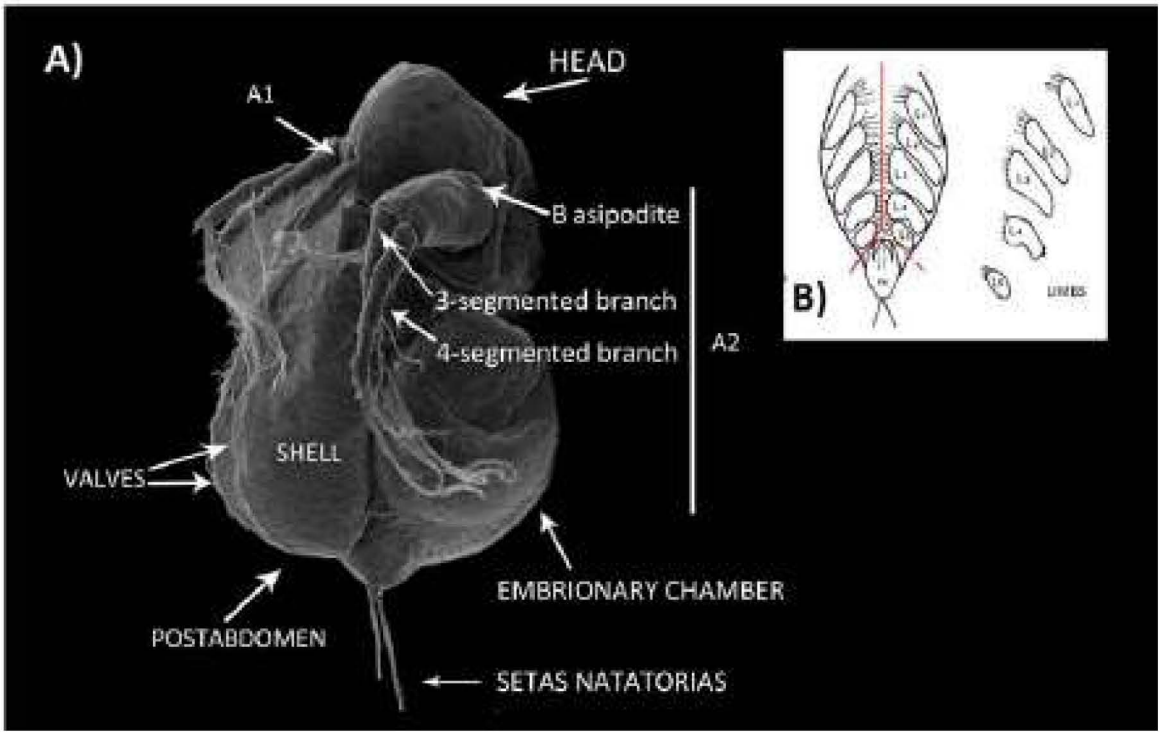


1128 Fig.5



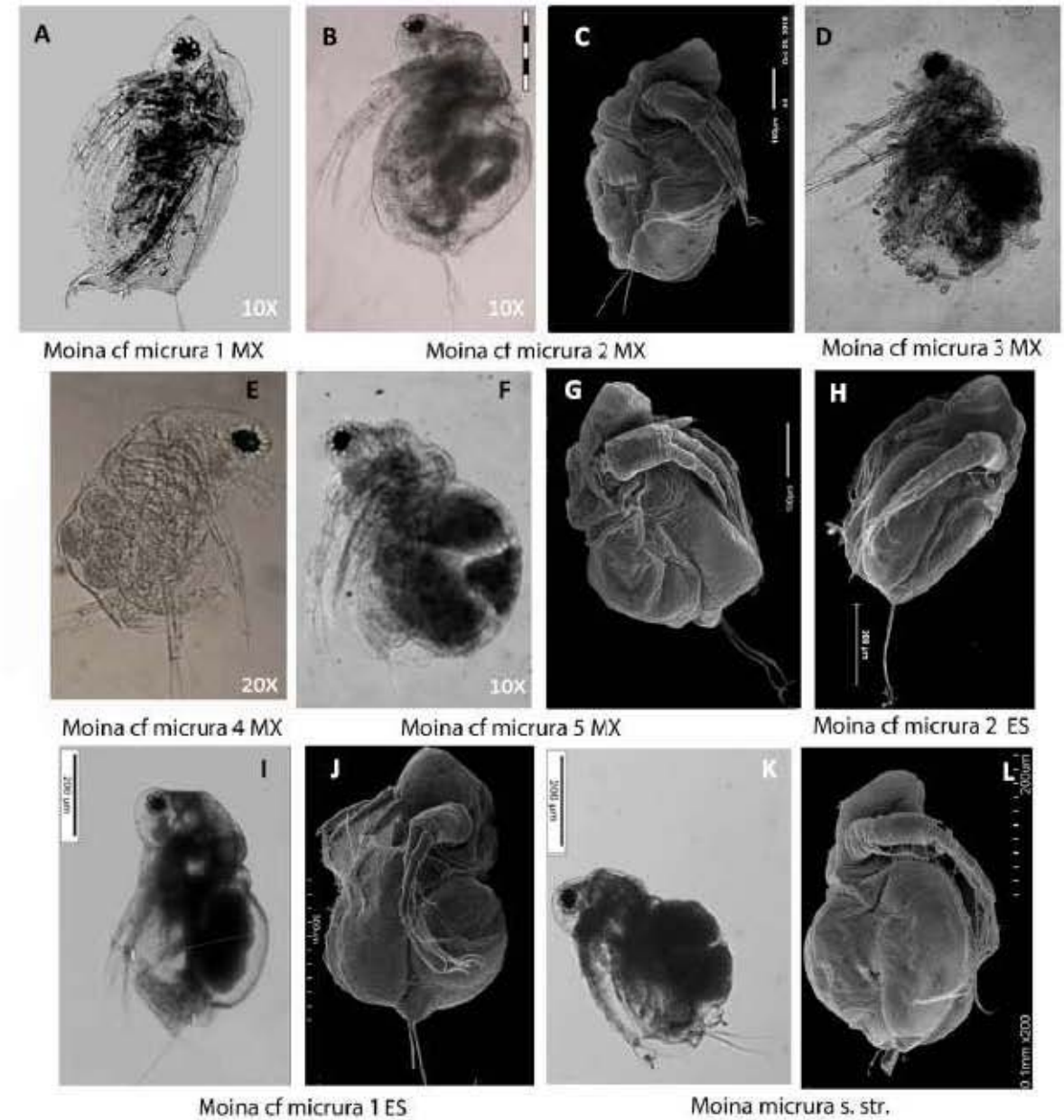
1129

1130 Fig.6



1131

1132 Fig. 7



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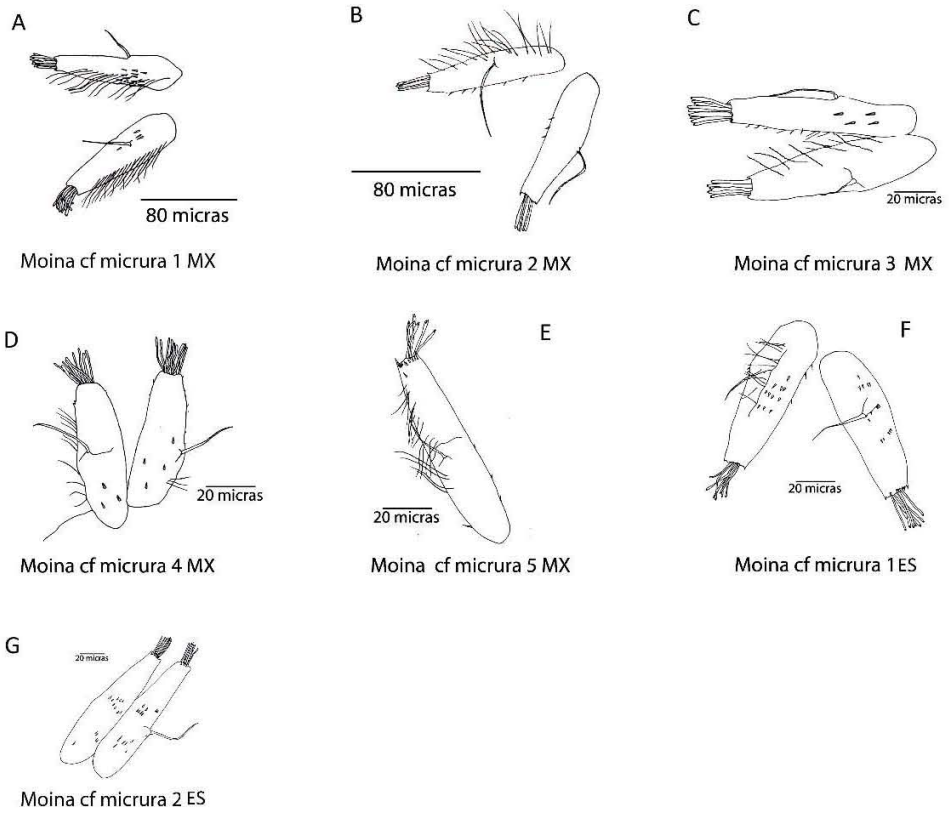
1135

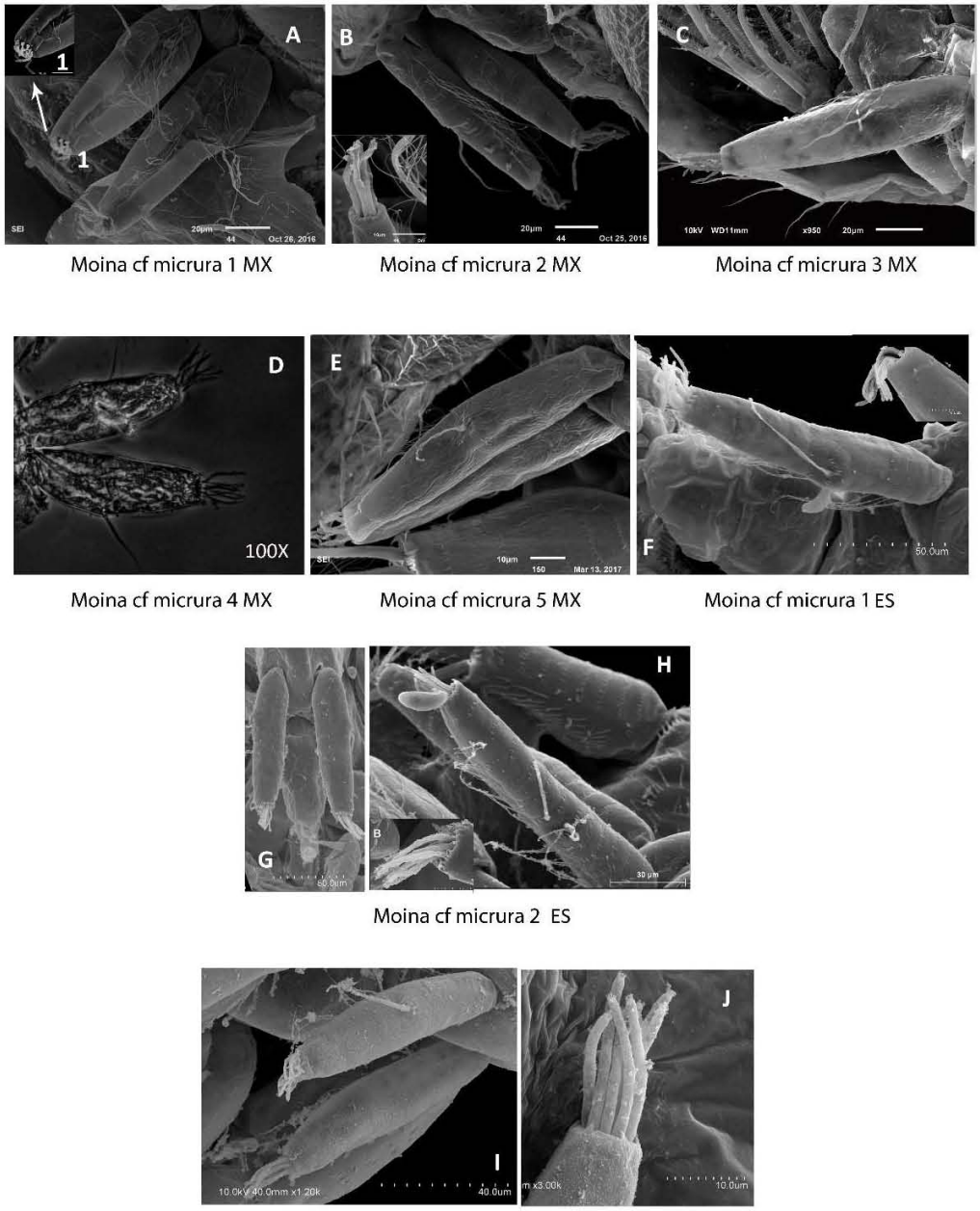
1136 Fig. 8

1137



1138

1139 Fig. 9



Moina cf micrura 1 MX Moina cf micrura 2 MX Moina cf micrura 3 MX

Moina cf micrura 4 MX Moina cf micrura 5 MX Moina cf micrura 1 ES

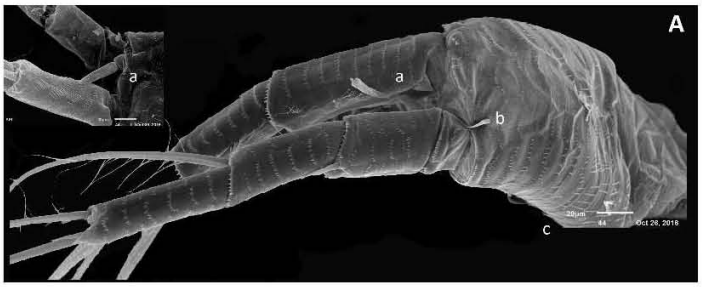
Moina cf micrura 2 ES

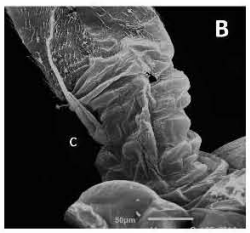
1140 Moina micrura s. str.

1141

1142 Fig. 10

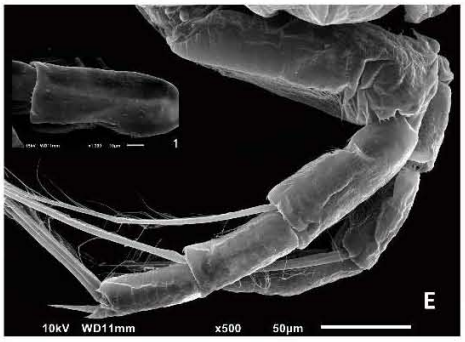
Antennes (A2)



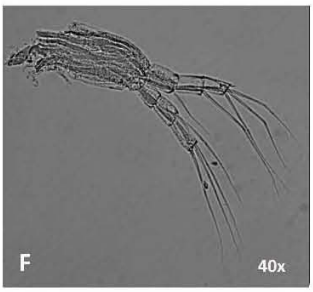


Moina cf micrura 1 MX

Moina cf micrura 2 MX



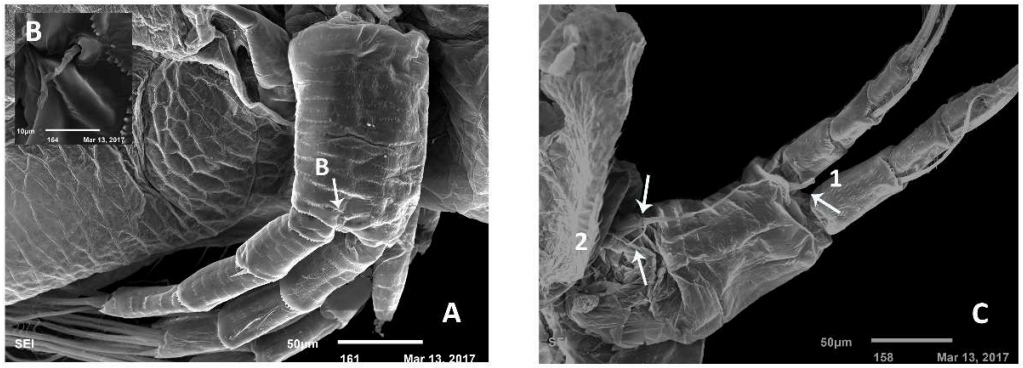
Moina cf micrura 3 MX



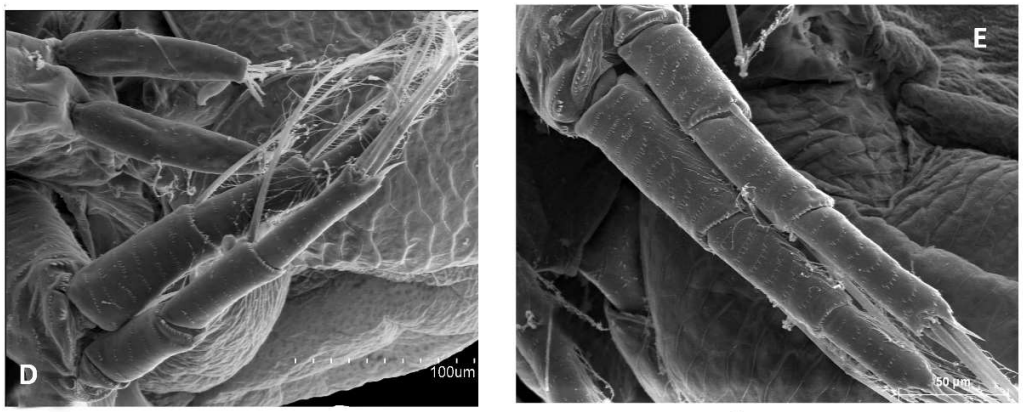
Moina cf micrura 4 MX

1143

1144 Fig. 11

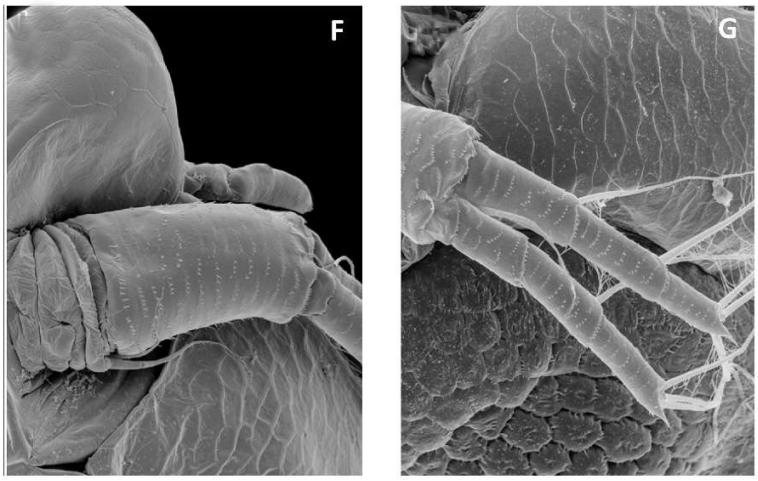


Moina cf micrura 5 MX



Moina cf micrura 1 ES

Moina cf micrura 2 ES



Moina micrura s. str.

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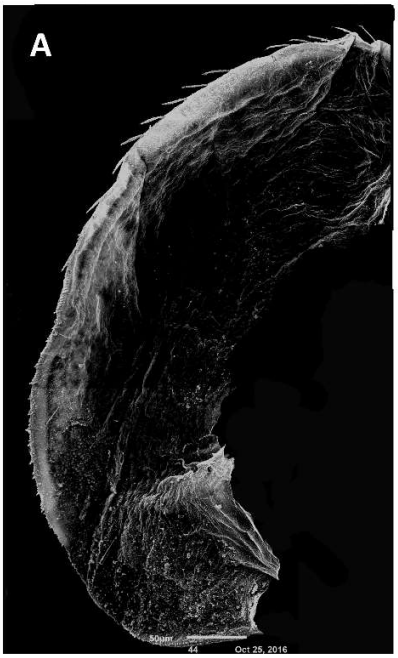
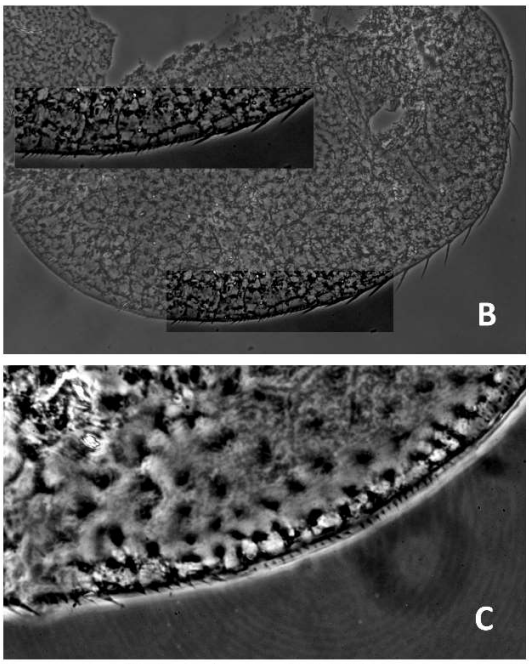
1146

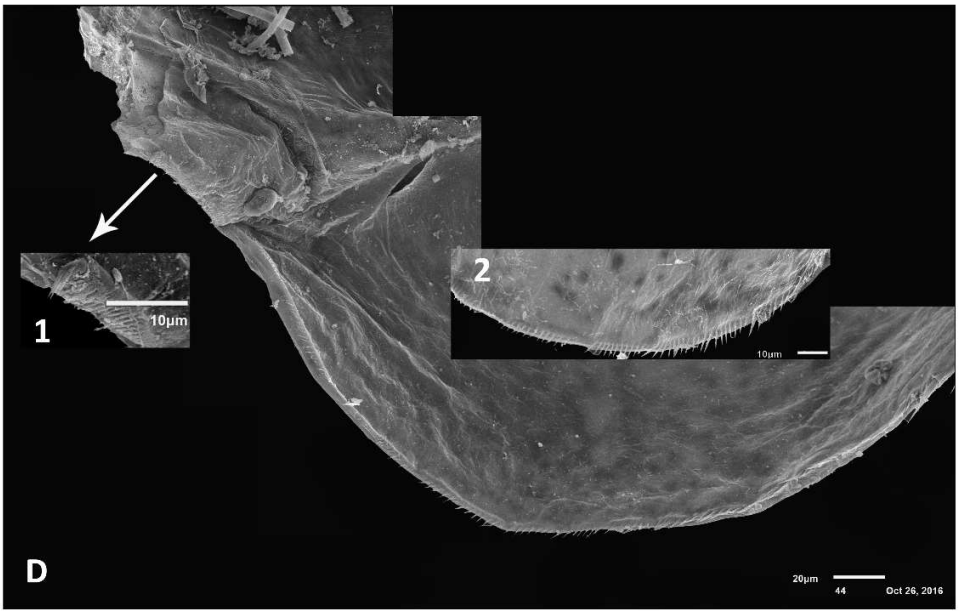
1147 Fig. 12



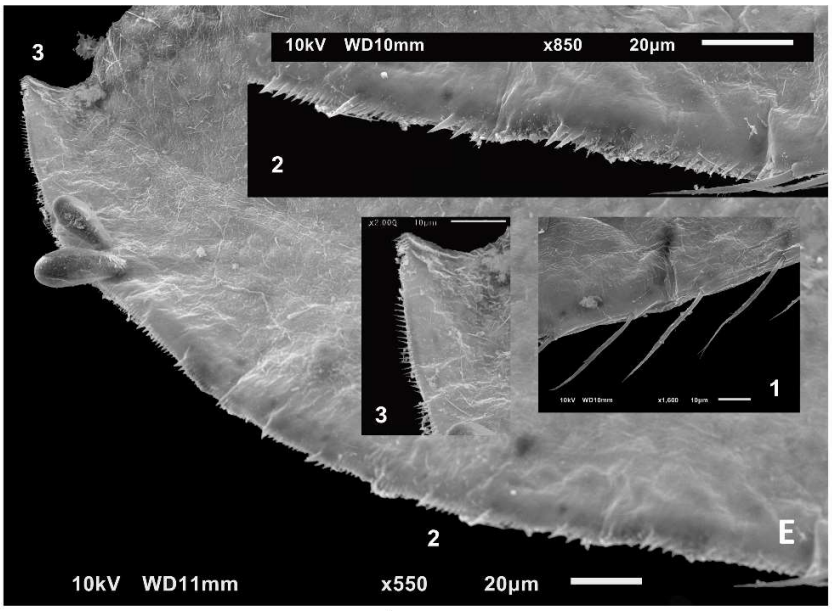
Moina cf micrura 1 MX



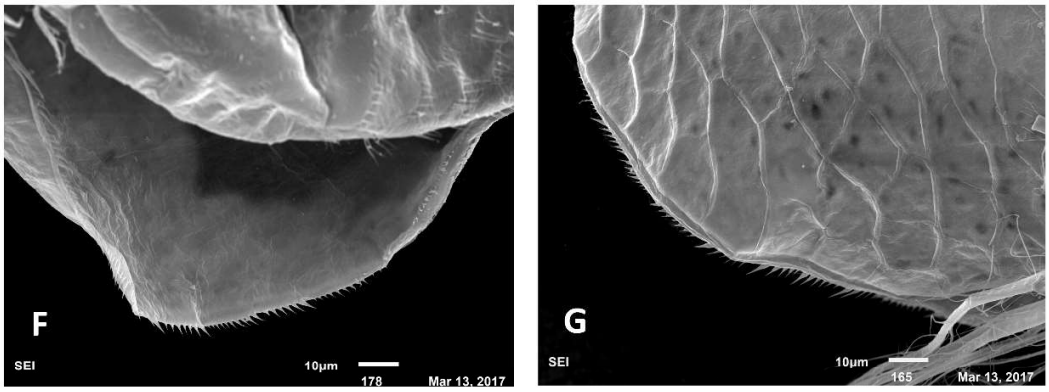
Moina cf micrura 4 MX



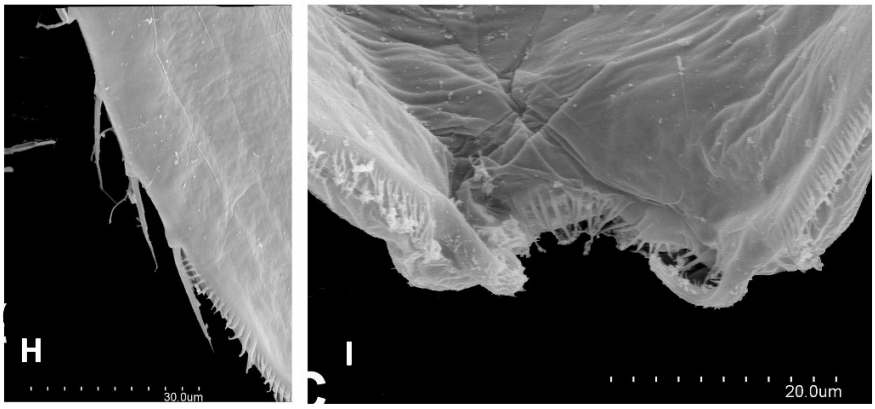
Moina cf micrura 2 MX



Moina cf micrura 3 MX



Moina cf micrura 5 MX



Moina cf micrura 1 ES

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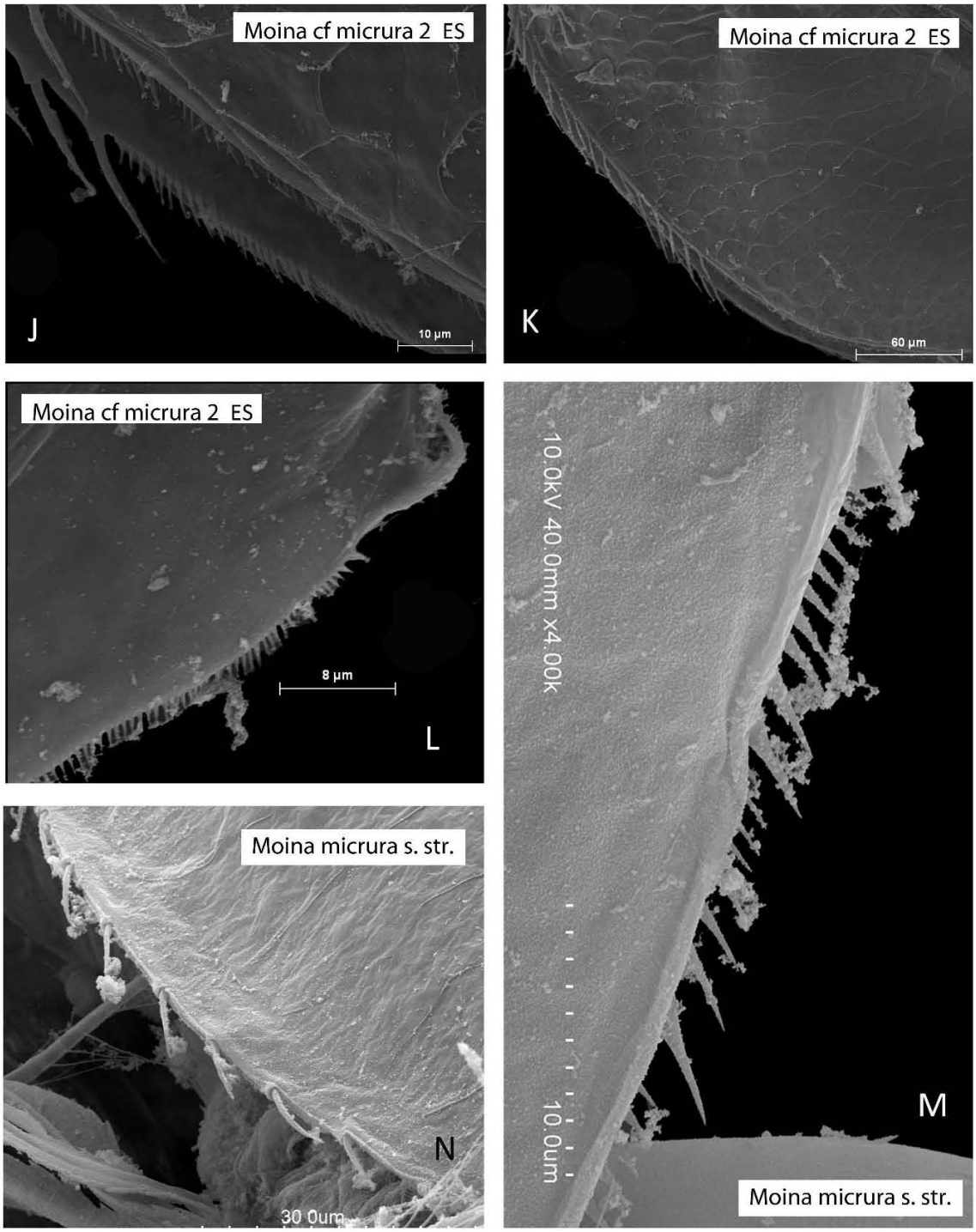
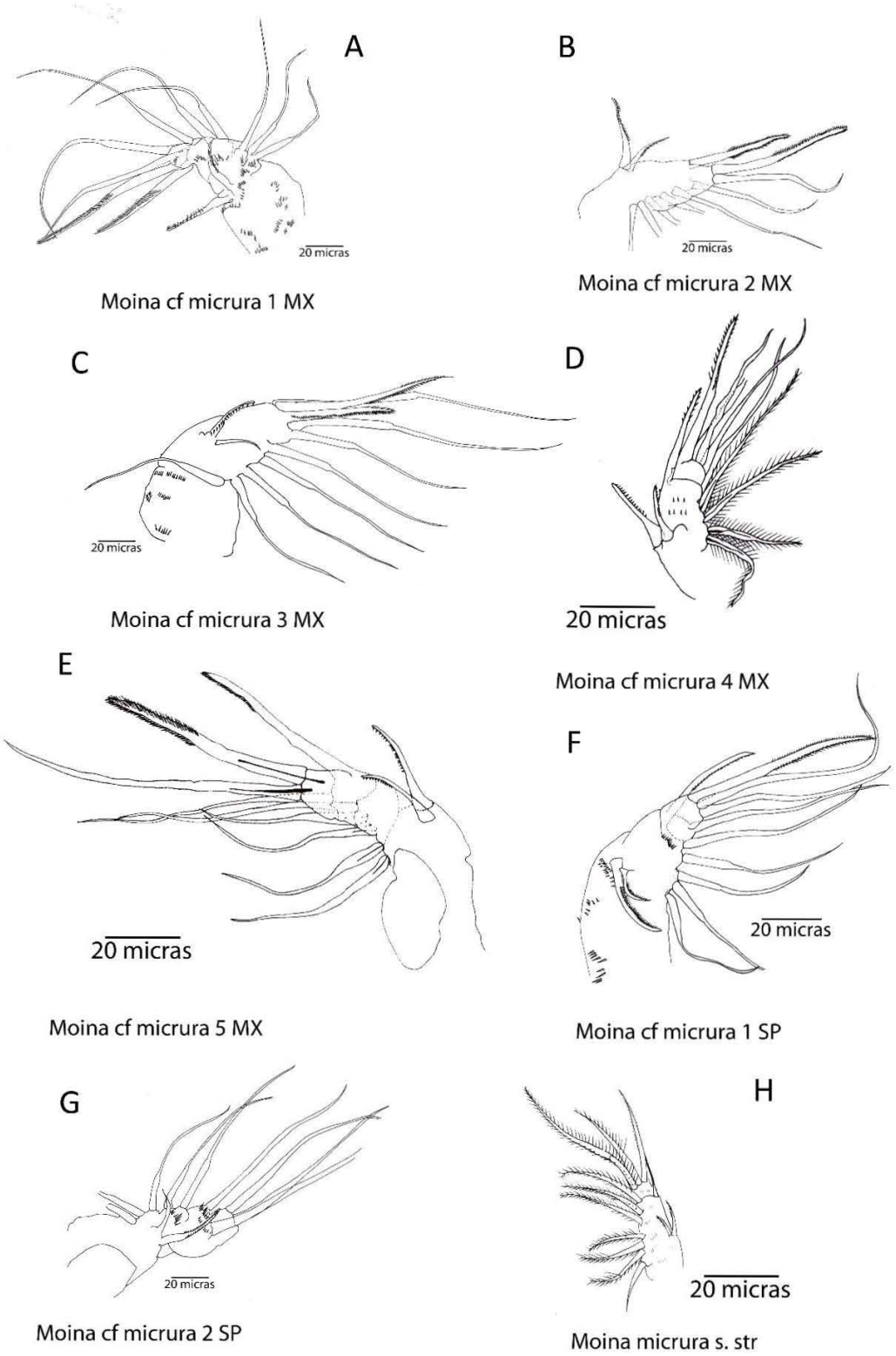

Moina cf micrura 2 ES
J
10 µm
Moina cf micrura 2 ES
K
60 µm
Moina cf micrura 2 ES
8 µm
L
10.0kV 40.0mm x4.00k
10.0um
M
Moina micrura s. str.
Moina micrura s. str.
N
30 0um

1152

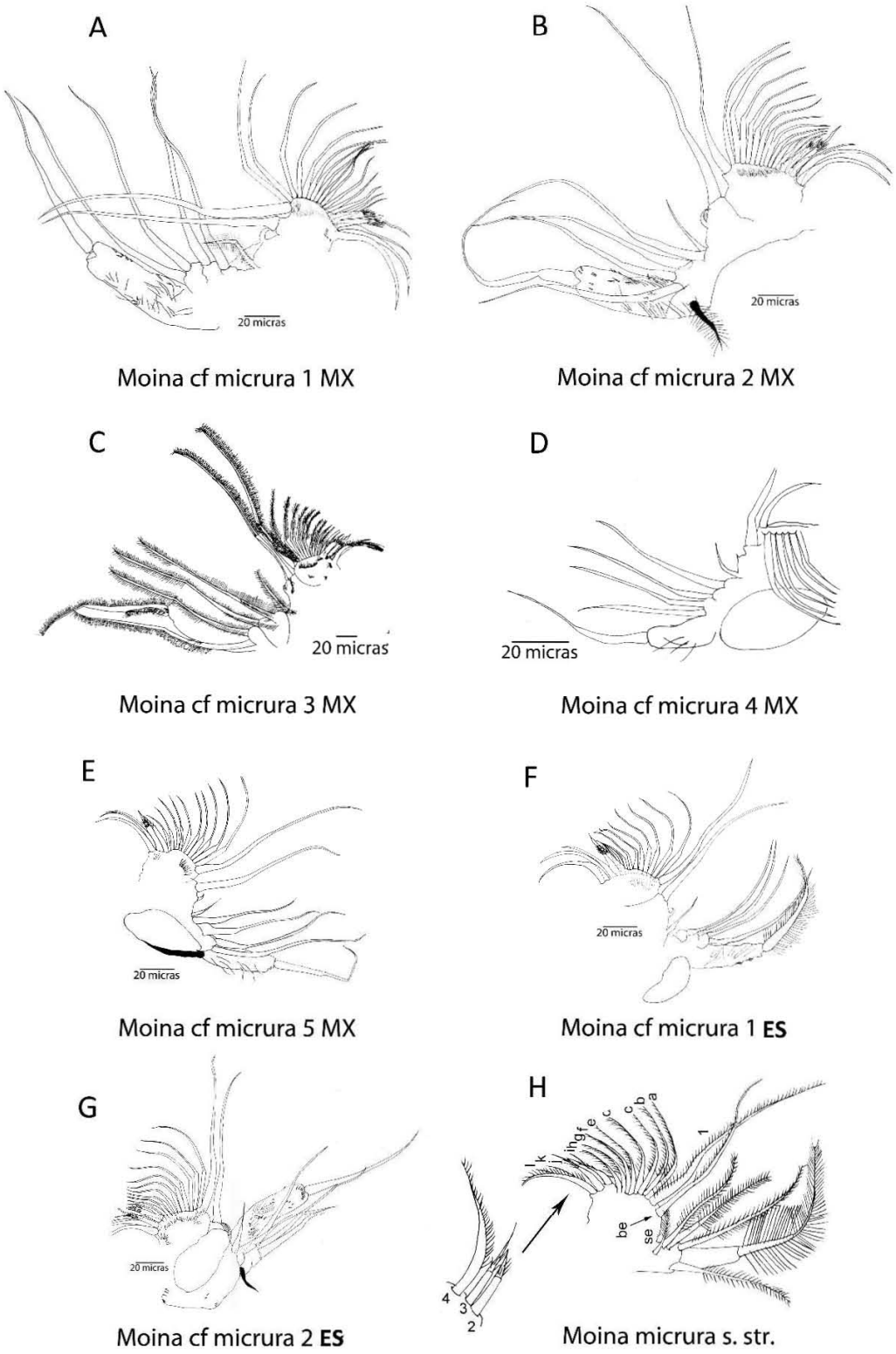
1153

1154 Fig. 13
1155



1156
1157

1158 Fig. 14
1159



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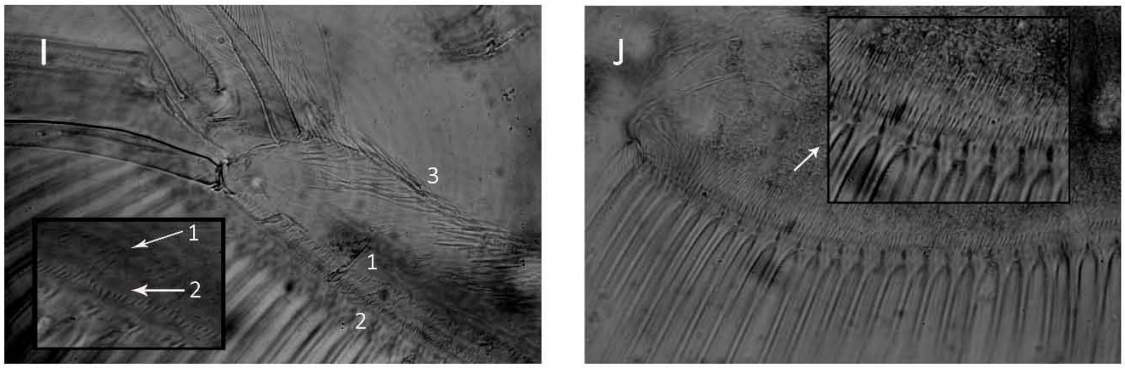
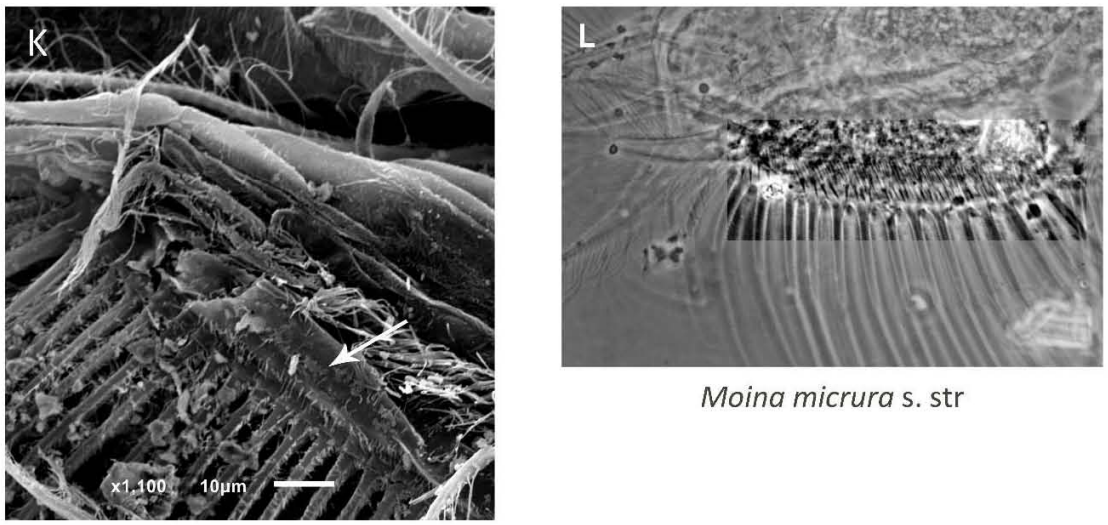


Moina cf micrura 3MX

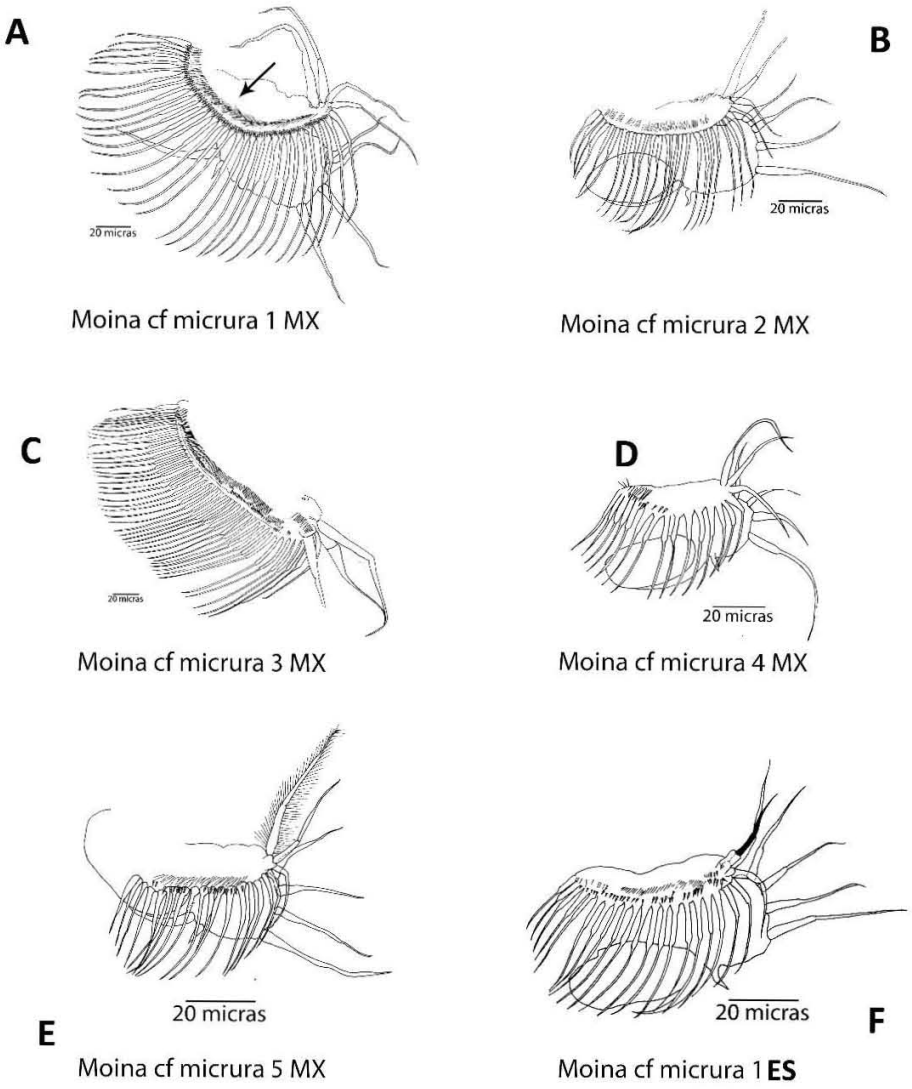


Moina micrura s. str

1163

1164

1165 Fig. 16



1166

1167 Fig. 17

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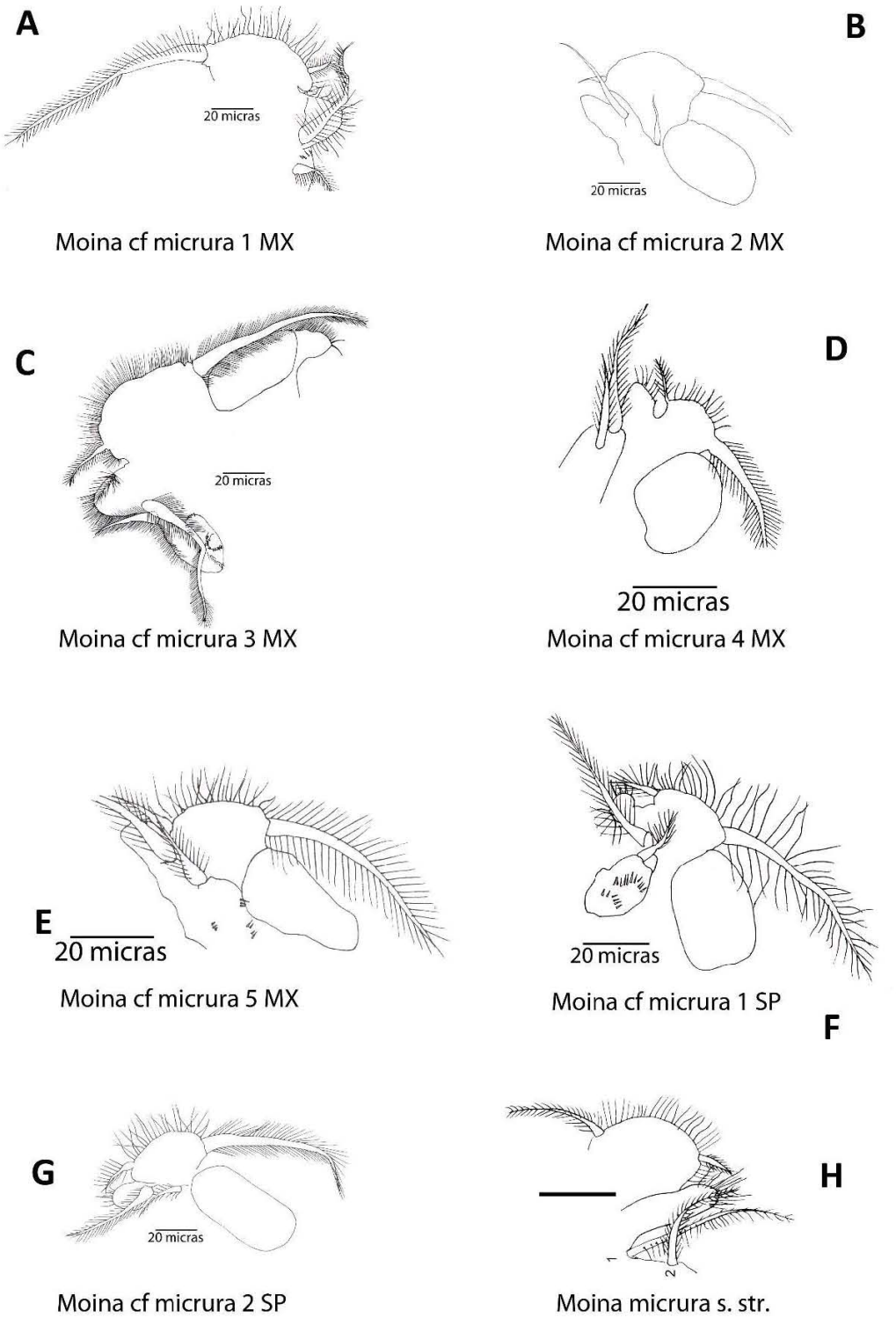
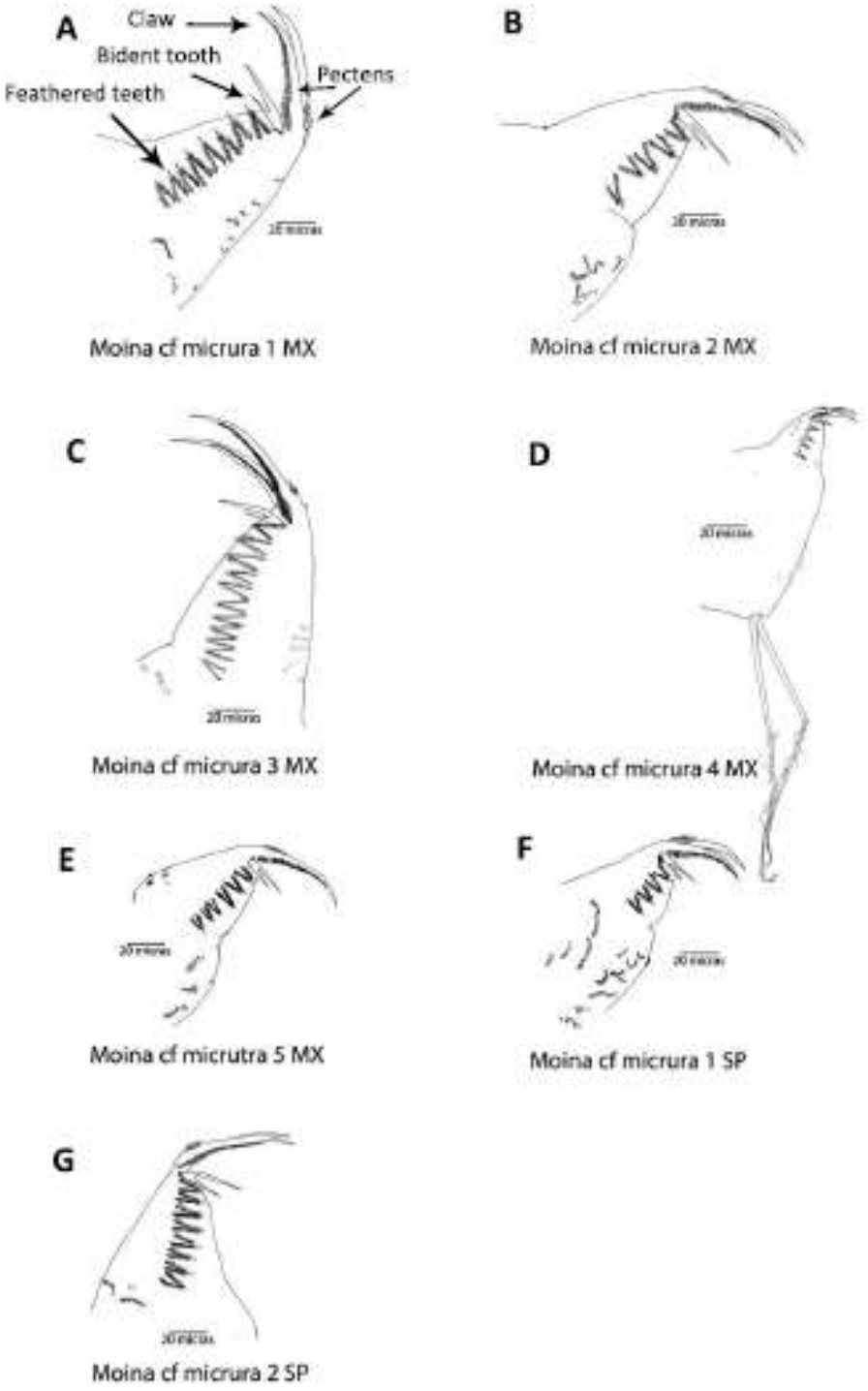


1169

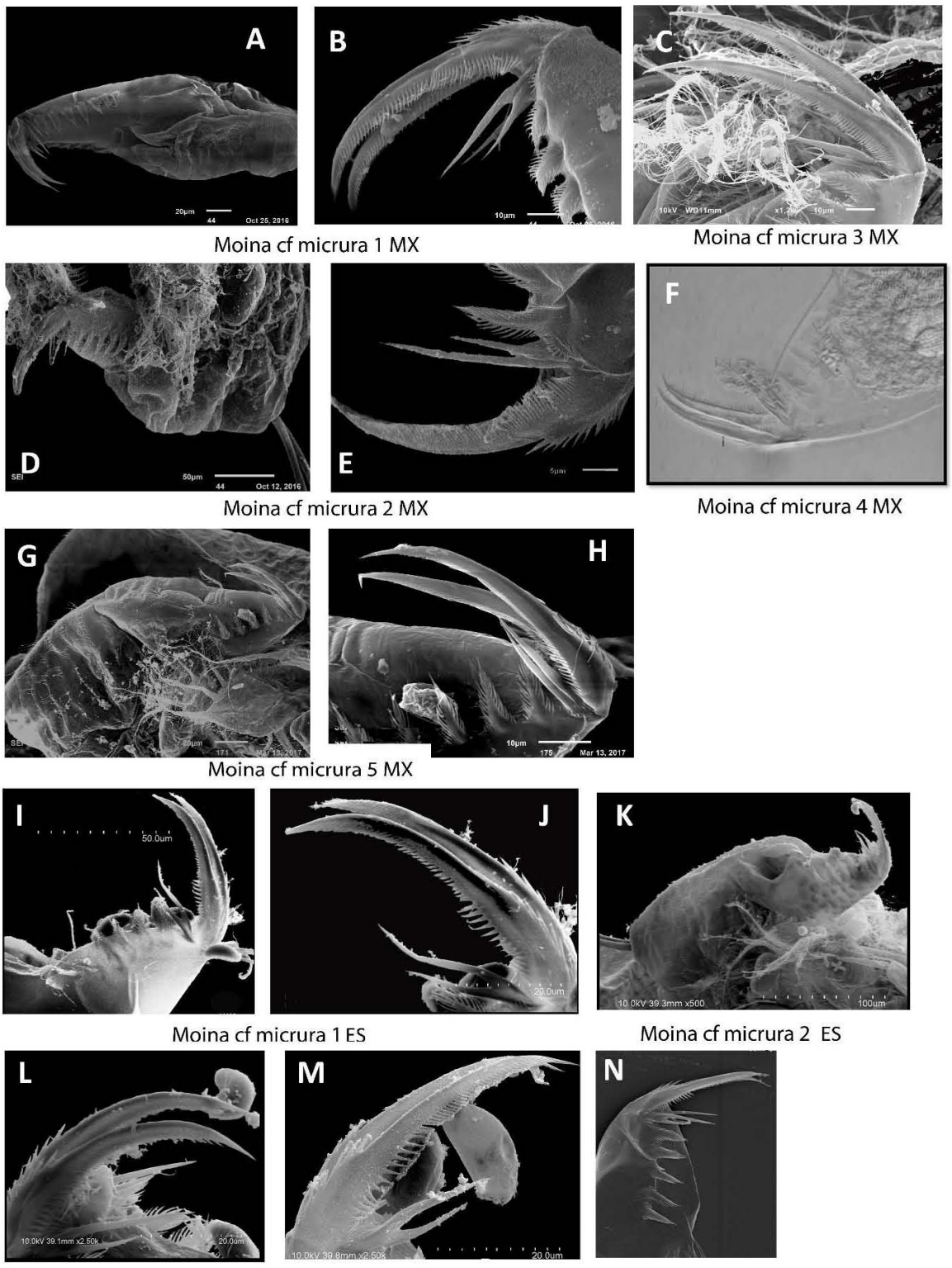
1170 Fig.18

1171



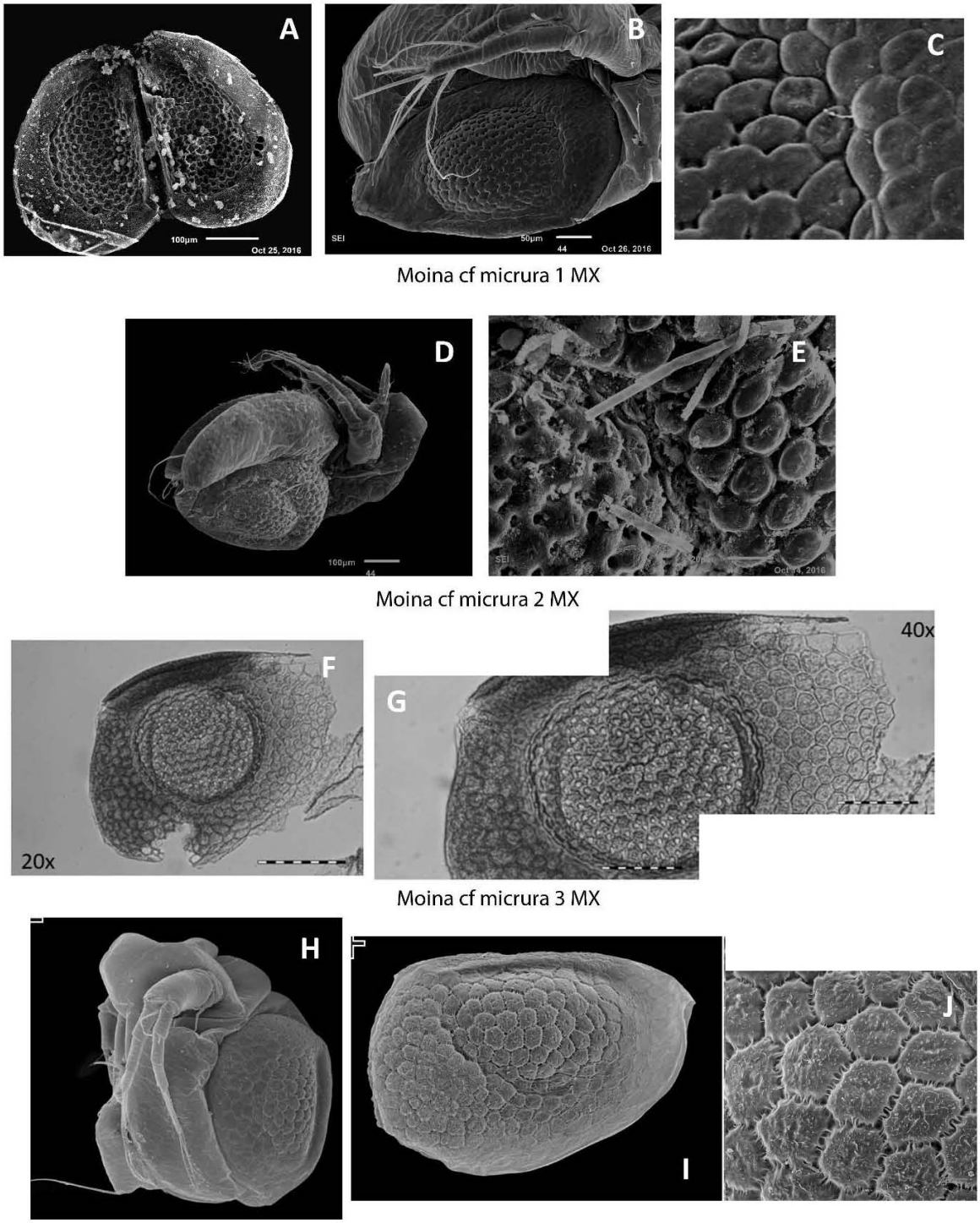
1172

1173 Fig. 19
1174



1175
1176

1177 Fig. 20



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1179

1180 Fig. 21

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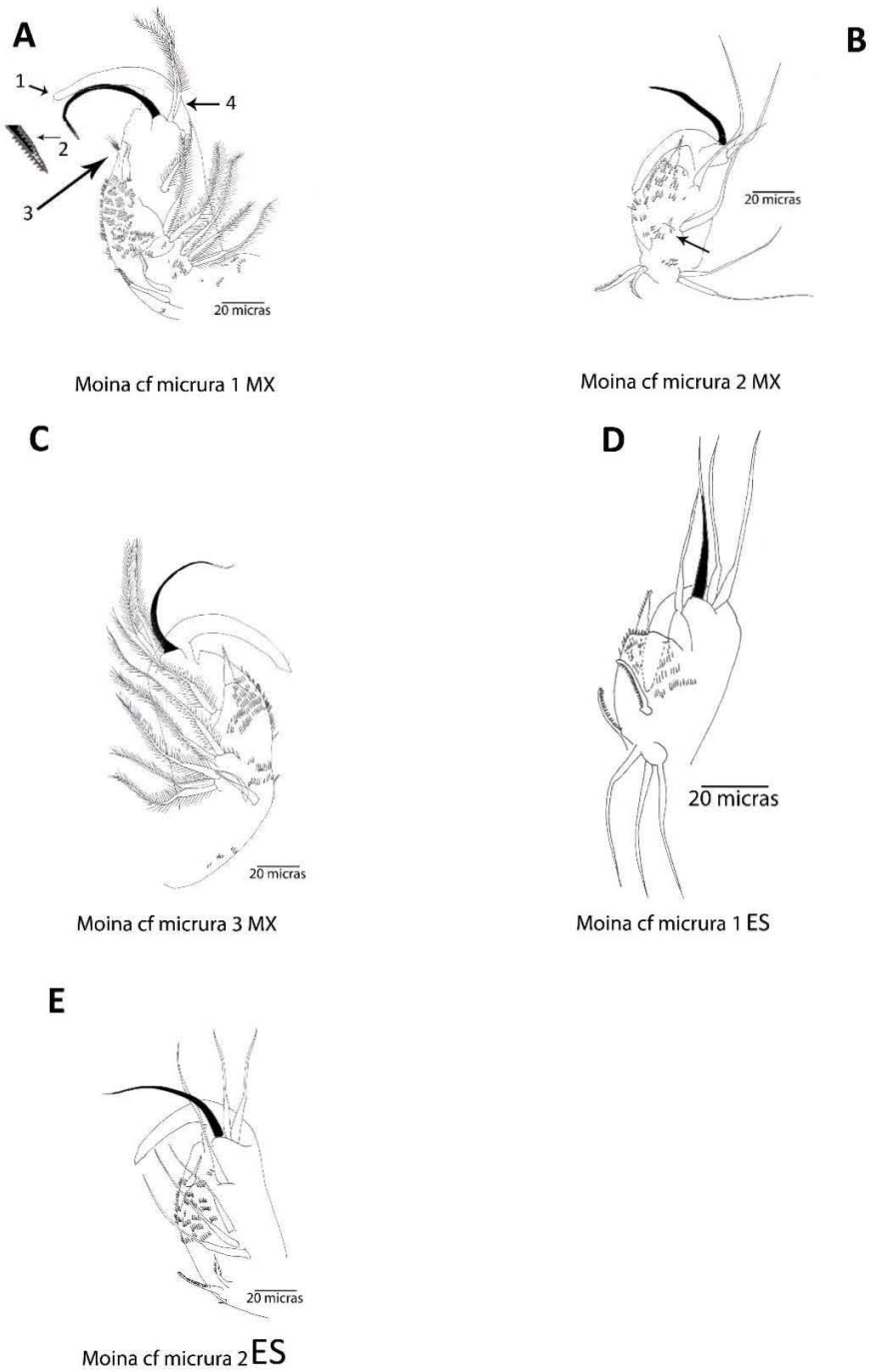

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1184 Fig. 22

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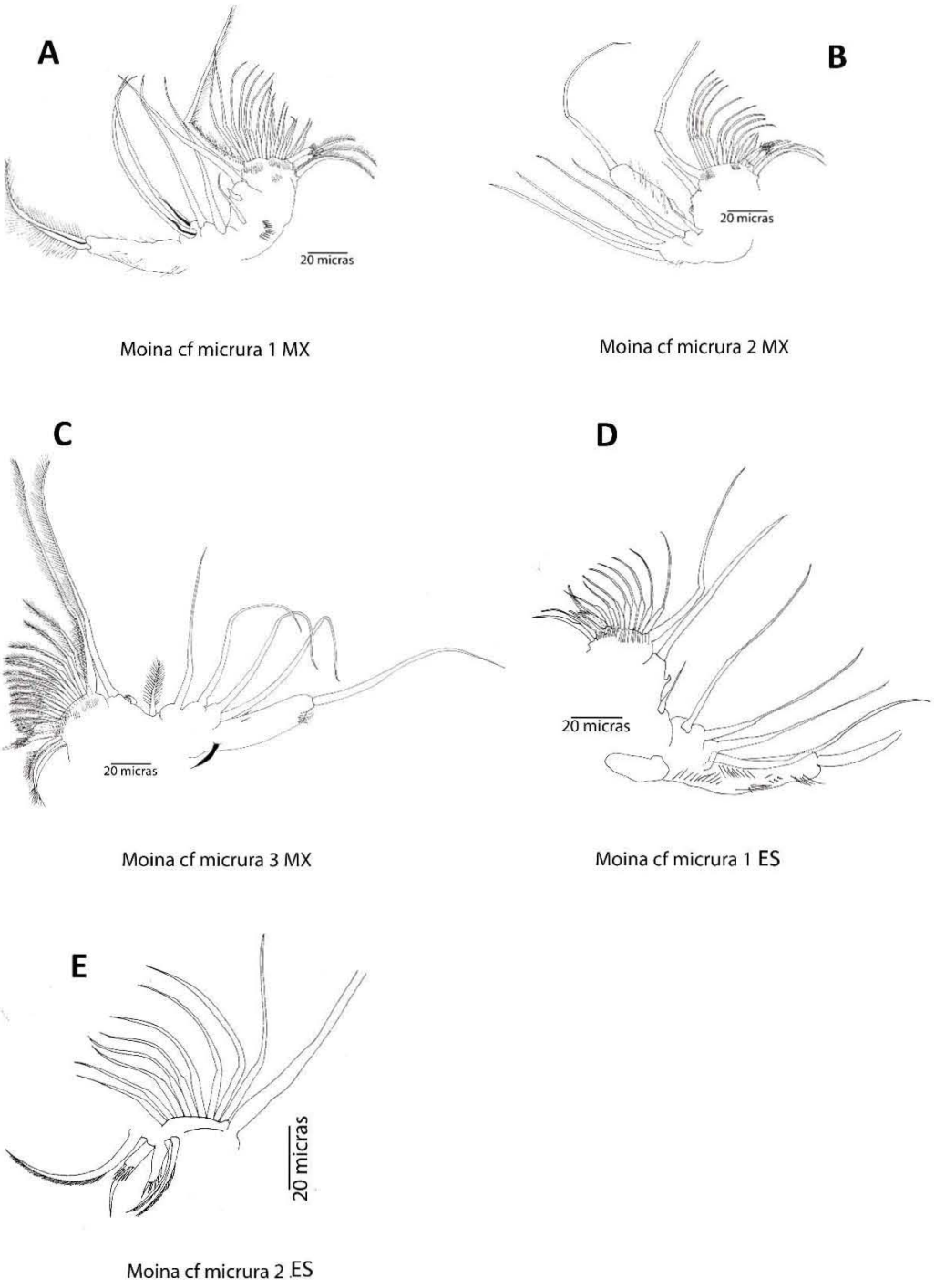

1186

1187

1188 Fig. 23

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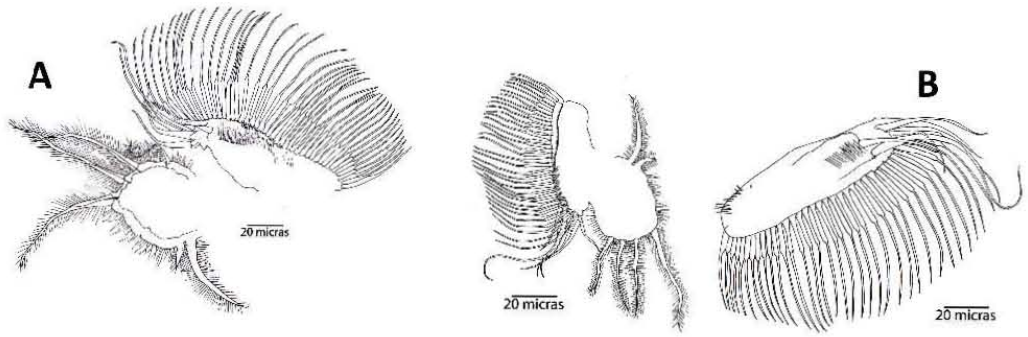
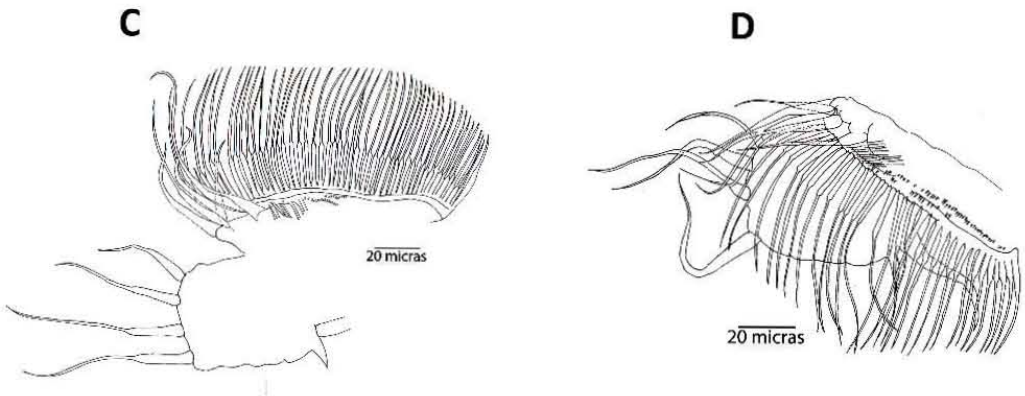


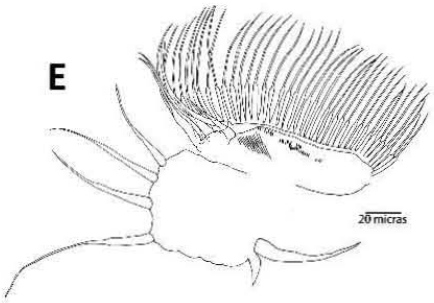
Moina cf micrura 1 MX

Moina cf micrura 2 MX



Moina cf micrura 3 MX

Moina cf micrura 1ES



Moina cf micrura 2 ES

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1192 Fig. 24

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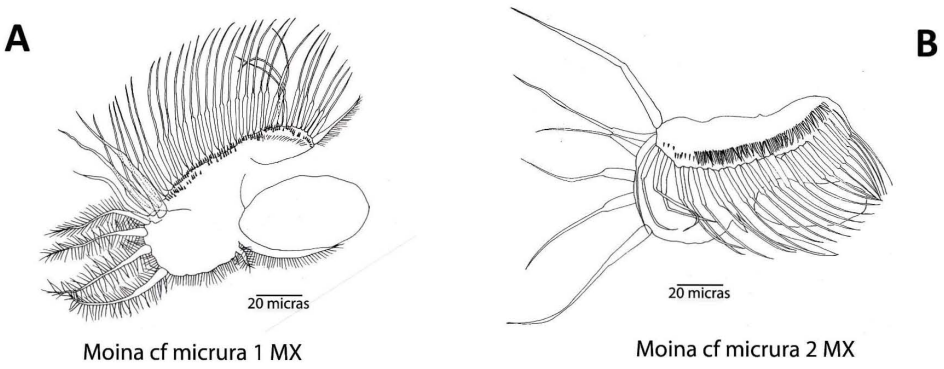
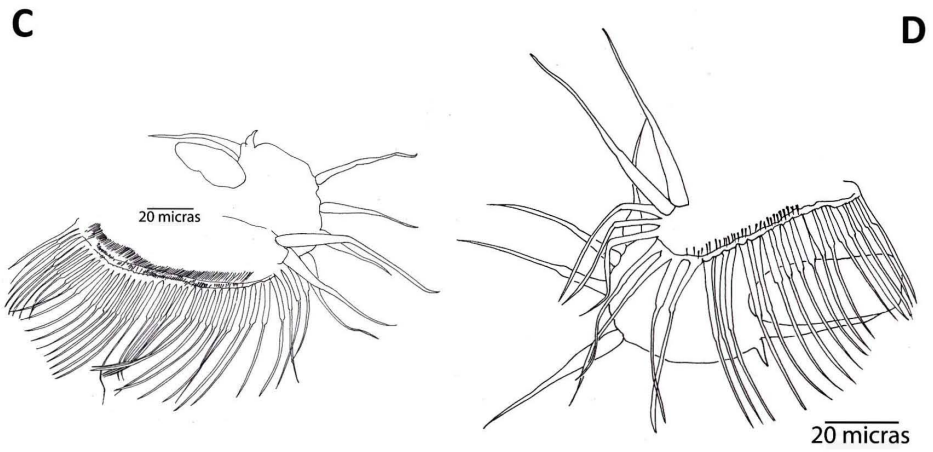


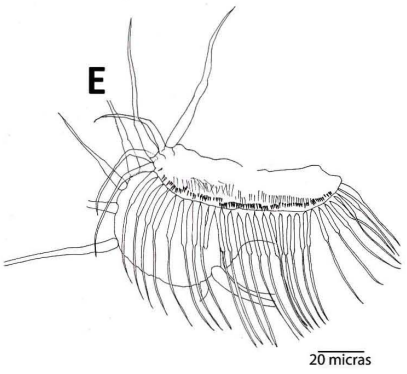
Moina cf micrura 1 MX

Moina cf micrura 2 MX



Moina cf micrura 3 MX

Moina cf micrura 1ES



Moina cf micrura 2 ES

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1196 Fig. 25

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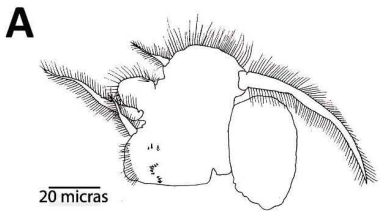
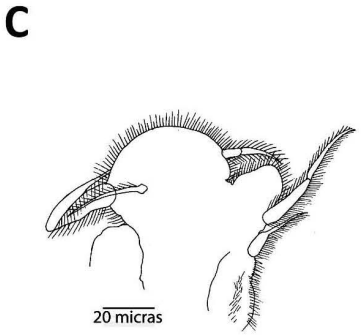
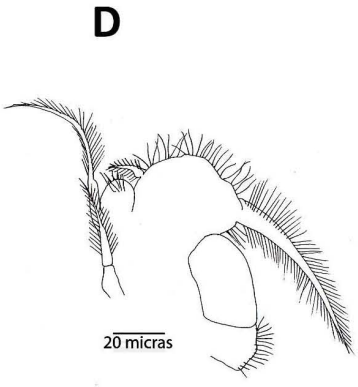


Moina cf micrura 1 MX

Moina cf micrura 2 MX



Moina cf micrura 3 MX



Moina cf micrura 2 ES

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1200 Fig. 26

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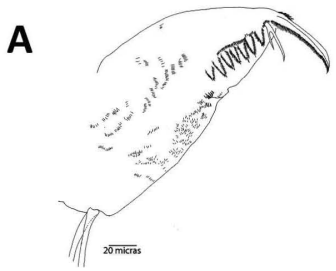
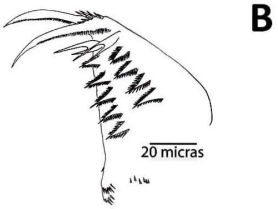
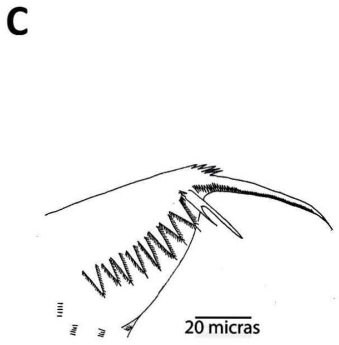


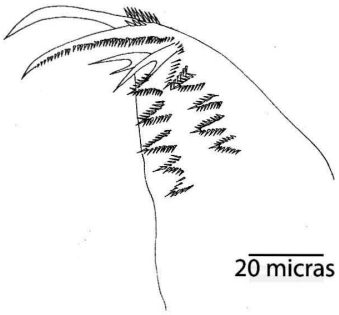
Moina cf micrura 1 MX



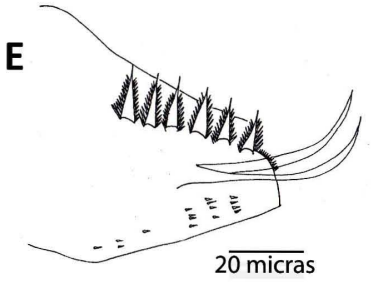
Moina cf micrura 2 MX



Moina cf micrura 3 MX



Moina cf micrura 1 ES

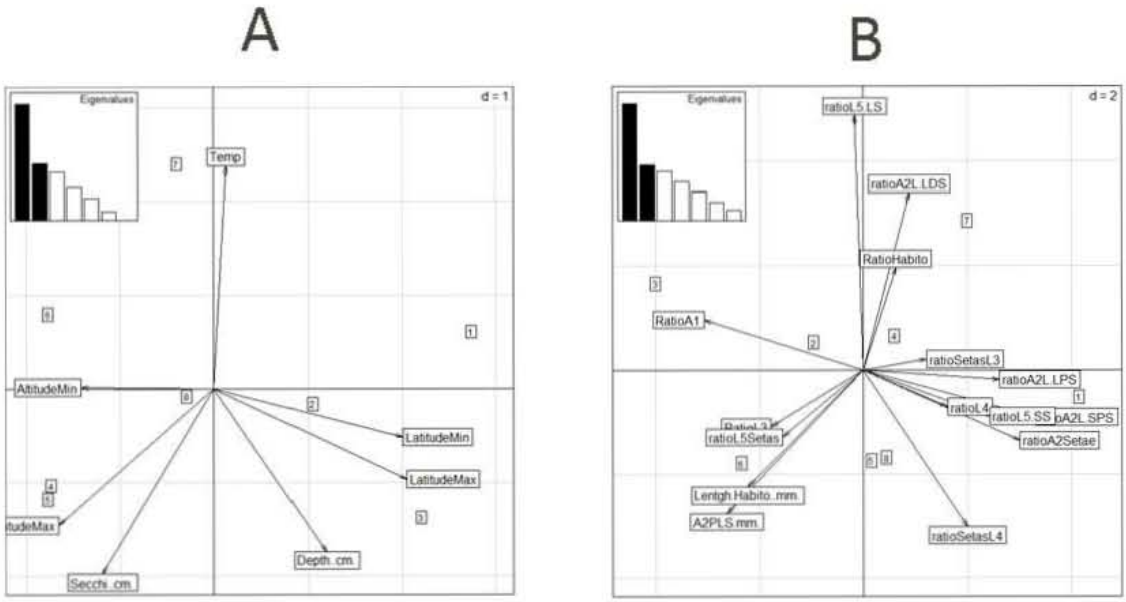


Moina cf micrura 2 ES

1202

1203

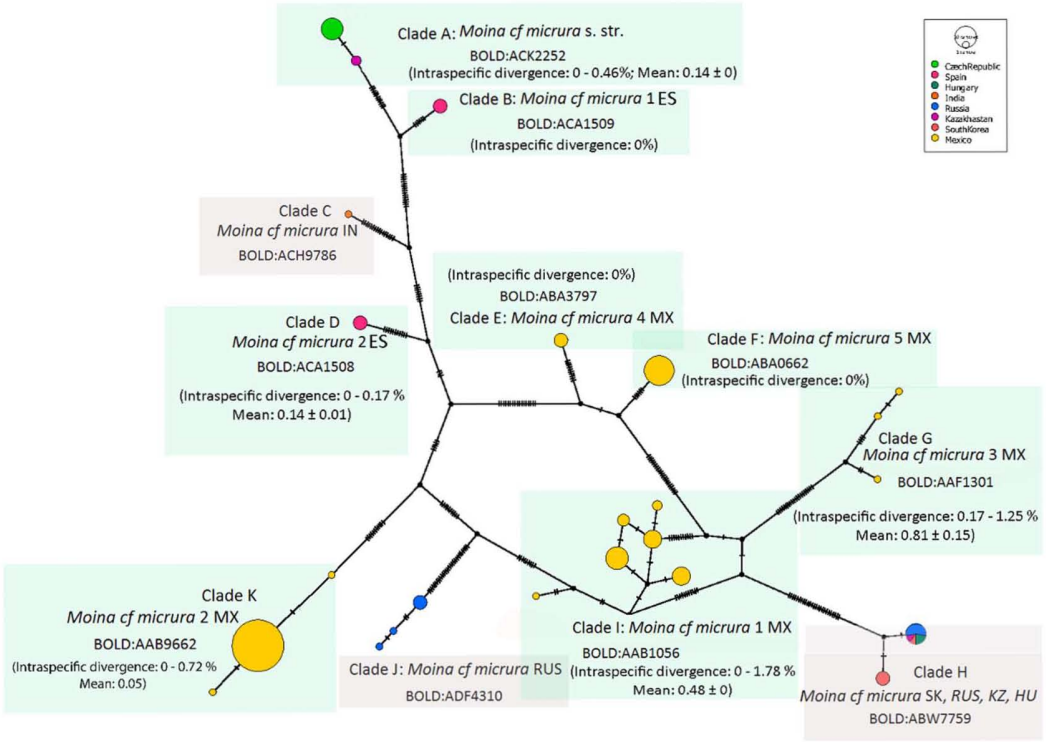
1204 Fig. 27



1205

1206

1207 Fig. 28



1208

S1. Table. Complete list of sequences generated in this study and public sequences from BOLD and GenBank used in the analyses.

Phylogroup name	**Sample ID (BOLD)**	**Genbank accession number**	**Country of origin**	**Reference**
Moina cf macrocopa macrocopa	CH-659-G10		Mexico	In this study
Moina cf *macrocopa macrocopa*	CH-659-G09		Mexico	In this study
Moina cf *macrocopa macrocopa*	CH-659-G01		Mexico	In this study
Moina cf *macrocopa macrocopa*	CH-659-G06		Mexico	In this study
Moina cf *macrocopa macrocopa*	ESP067-12		Spain	In this study
Moina macrocopa americana	BIOUG01705-C03		Canada	Jeffery et al., 2011
Moina macrocopa americana	BIOUG01705-C02		Canada	Jeffery et al., 2011
Moina macrocopa americana	BIOUG01705-A02		Canada	Jeffery et al., 2011
Moina macrocopa americana	BIOUG01701-F09		Canada	Jeffery et al., 2011
Moina macrocopa americana	BIOUG01701-F02		Canada	Jeffery et al., 2011
Moina macrocopa americana	AS22a9	KC617126	Mexico	Prosser et al., 2013
Moina macrocopa americana	AS22a6	KC617127	Mexico	Prosser et al., 2013
Moina macrocopa americana	AS22a3	KC617130	Mexico	Prosser et al., 2013
Moina macrocopa americana	ZPLMX262	EU702249	Mexico	Elías-Gutiérrez et al., 2008
Moina macrocopa americana	ZMXIII_227	KC617408	Canada	Jeffery et al., 2011
Moina macrocopa americana	ZMXII-146	JN233944	Canada	Jeffery et al., 2011

Moina macrocopa americana	ZMXII-134	JN233945	Canada	Jeffery et al., 2011
Moina macrocopa americana	ZPLMX335	EU702246	Mexico	Elías-Gutiérrez et al., 2008
Moina macrocopa americana	ZPLMX334	EU702247	Mexico	Elías-Gutiérrez et al., 2008
Moina macrocopa americana	ZPLMX263	EU702248	Mexico	Elías-Gutiérrez et al., 2008
Moina macrocopa americana	ZMXII-579	KC617128	Mexico	Prosser et al., 2013
Moina macrocopa americana	ZMXII-568	KC617129	Mexico	Prosser et al., 2013
Moina macrocopa americana	ZMXII-649	KC617131	Mexico	Prosser et al., 2013
Moina macrocopa americana	ZMXII-637	KC617132	Mexico	Prosser et al., 2013
Moina macrocopa americana	ZMXII-625	KC617133	Mexico	Prosser et al., 2013
Moina macrocopa americana	ZMXII-613	KC617134	Mexico	Prosser et al., 2013
Moina cf *macrocopa macrocopa*	JN657688	JN657688	Hungary	Nédli et al., 2014
Moina cf *macrocopa macrocopa*	JN657689	JN657689	Hungary	Nédli et al., 2014
Moina cf *macrocopa macrocopa*	JN657690	JN657690	Hungary	Nédli et al., 2014
Moina cf *macrocopa macrocopa*	JN657691	JN657691	Hungary	Nédli et al., 2014
Moina cf *macrocopa macrocopa*	KX168582	KX168582	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168581	KX168581	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168556	KX168556	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168524	KX168524	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168523	KX168523	Russia	Bekker et al., 2016

Moina cf *macrocopa macrocopa*	KX168546	KX168546	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168545	KX168545	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168519	KX168519	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168518	KX168518	Russia	Bekker et al., 2016
Moina cf *macrocopa macrocopa*	KX168571	KX168571	Russia	Bekker et al., 2016
Moina macrocopa americana	09BBCRU-0155		USA (terra typica)	
Moina macrocopa americana	09BBCRU-0151		USA (terra typica)	
Moina macrocopa americana	09BBCRU-0150		USA (terra typica)	
Moina macrocopa americana	SJA-0058		Bolivia	
Moina macrocopa americana	SJA-0048		Bolivia	
Moina macrocopa americana	SJA-0104		Bolivia	
Moina macrocopa americana	SJA-0088		Bolivia	
Moina macrocopa americana	SJA-0091		Bolivia	
Moina macrocopa americana	SJA-0071		Bolivia	
Moina macrocopa americana	SJA-0074		Bolivia	
Moina macrocopa americana	SJA-0076		Bolivia	

CAPÍTULO V. DISCUSIÓN

La delimitación e identificación precisa de las especies sigue siendo un problema central en el conocimiento de la diversidad biológica. Delimitar las especies depende de los datos utilizados y del concepto de especie aplicado (Schwentner, Timms y Richter, 2011). Tradicionalmente, la taxonomía, se ha basado principalmente en caracteres morfológicos. Sin embargo, descripciones basadas sólo en caracteres morfológicos a menudo conllevan una delimitación errónea de los límites de las especies, principalmente de microinvertebrados. Este problema se agudiza con las especies crípticas, es decir aquellas estrechamente relacionadas y que a menudo son demasiado parecidas morfológicamente para ser identificadas sin error. Goulden (1968) en su revisión del género Moina realizó una comparación exhaustiva basada en la morfología de todas las especies de Moina descritas y concluye que muchas de las especies reconocidas eran sinónimos unas de otras, invalidando de este modo un gran número de ellas. Atribuyó la plasticidad fenotípica observada en las poblaciones como adaptaciones locales a diferentes tipos de hábitats. Por estas razones, el uso de un enfoque integrativo, de acuerdo con los estándares modernos para delimitar las especies, basados en la combinación de datos de otro tipo, como los genéticos, biogeográficos, fisiología, etc. permiten una mejor resolución de los límites estas y por lo tanto una hipótesis con mayor soporte.

En este estudio, el uso de este enfoque integrativo para resolver los complejos de las especies de los cladóceros Moina micrura y Moina macrocopa, permitió descubrir y delimitar una gran diversidad críptica, siete especies diferentes en el caso del complejo Moina micrura y tres para el complejo Moina macrocopa, que presentan patrones de distribución muy restringidos tal y como otros autores ya habían evidenciado anteriormente pero sólo con datos derivados de datos genéticos (Bekker et al., 2016; Elías-Gutiérrez, 2008b). Cabe remarcar que una de las piezas fundamentales en la resolución de estos complejos de especies ha sido reconocer la identidad "real" de los holotipos (datos morfológicos, genéticos y geográficos) o en su caso de material topotipo, que por cierto no es reconocido por el Código Internacional de Nomencaltura Zoológica, para que a final de cuentas permiten realizar comparaciones más detalladas entre las

poblaciones que componen un taxón y poder establecer hipótesis más robustas en relación a los límites de las especies.

También se evidenció el éxito del zooplancton de aguas dulces para colonizar nuevos hábitats. Tanto en el caso del copépodo Mesocyclops pehpeiensis (Hu, 1943), como en el de Moina, el estudio de sus patrones de distribución demostró que existen traslocaciones de las poblaciones por todo el mundo, por lo que las encontramos viviendo en simpatría en un sin fin de hábitats. Estas nuevas distribuciones confusas desde el punto de vista biogeográfico son debidas principalmente al hombre y en segunda instancia a otros factores bióticos y abióticos (Montoliu, Miracle and Elías-Gutiérrez, 2015).

Una de las ventajas de utilizar diferentes tipos de datos y herramientas de última generación, es el potencial de determinar la variación intra e interespecífica dentro de cada uno de estos tipos de datos y entre ellos, las divergencias genéticas obtenidas fueron concurrentes con las observaciones morfológicas y geográficas en general pues existe una correspondencia directa entre todas las observaciones. Es importante resaltar que no se encontraron diferencias morfológicas en poblaciones con divergencias menores del 3% en el gen COI (Hebert, Ratnasingham and DeWaard, 2003).

Con respecto a este gen, conocido comúnmente como códigos de barras de la vida (Hebert et. Al., 2003), ya ha sido demostrado que por sí solo no es del todo adecuado para construir un árbol filogenético ni para realizar la taxonomía basada exclusivamente en el ADN, sino para permitir una herramienta de identificación universal respaldada por el conocimiento taxonómico basado en la taxonomía integrativa y recopilada en una biblioteca de referencia disponible en línea. El éxito indiscutible del proyecto de códigos de barras de ADN, que actualmente queda representado por más de 900 publicaciones al año en el Web of Science (webofknowledge.com) se debe principalmente al hecho de que los estándares de códigos de barras de ADN mejoran considerablemente las prácticas actuales para la identificación de los seres vivos a partir de una estandarización que ofrece aplicaciones que van más allá de lo que podemos concebir en este momento para todos los usuarios interesados en el reconocimiento de las especies (Teletchea, 2010).

Pero ahora que hemos resuelto un problema, tenemos otro problema, en relación a la biodiversidad. ¿Realmente existen tantas nuevas especies? o ¿se corresponden con especies previamente invalidadas? Esto ha llevado a un nuevo concepto: la inflación de especies (Isaac et al., 2004). En este caso las dos preguntas planteadas toman especial relevancia, por lo tanto para poder responderlas es necesaria una exhaustiva revisión del género, y para ello, es esencial una descripción integrada de las especies tipo o en su caso, tal y cómo se ha citado anteriormente, de los topotipos, que incluya información no sólo de los organismos sino del ecosistema en el que se encuentran, que incluyan tanto factores bióticos y abióticos, de esta forma no sólo seremos capaces de delimitar con más precisión las especies sino que podremos detectar de una forma temprana, rápida y sencilla los cambios que se están produciendo en el ecosistema.

Finalmente, remarcar que para el descubrimiento de esta inmensa biodiversidad es necesario el uso de herramientas de última generación como el microscopio electrónico de barrido, la metagenómica y la actualización de las metodologías de muestreo, principalmente en el caso del zooplancton de aguas epicontinentales. Elías-Gutiérrez et al. (2018) demostraron que el uso combinado de los códigos de barras del ADN, trampas de luz y mejoras en la preservación de los especímenes les permitió evaluar la comunidad entera de zooplancton. La creación de líneas base de zooplancton nos permitirá un biomonitoreo más preciso que permitirá el desarrollo de estrategias más precisas de conservación y gestión de los recursos ante el reto que presenta el crecimiento desordenado de las actividades humanas, falta de regulación y en última instancia el cambio global.

CAPÍTULO VI. CONCLUSIONES GENERALES

En base a las preguntas de investigación propuestas, podemos concluir que:

Las especies de cladóceros analizadas en este estudio son especies crípticas y diferentes con distribuciones muy restringidas y por lo tanto, no son anfiatlánticas.

El gen mitocondrial COI delimita con exactitud las especies crípticas. Su amplia variación interespecífica permite una correspondencia directa entre la identificación molecular y la identificación basada en caracteres morfológicos de las especies. Esto es debido a su alta tasa de evolución molecular que se manifiesta en la gran cantidad de haplotipos detectados. La variabilidad fenotípica observada en las especies es resultado de la adaptación a nuevos hábitats como consecuencia principalmente de la depredación y de las presiones medioambientales.

El umbral de divergencia genética del gen COI del 3% nos permite delimitar los grupos analizados con precisión. Los diferentes algoritmos utilizados (ML, NJ, ABGD, mPTP) para establecer este umbral de divergencia, fueron concluyentes y obtuvimos los mismos resultados. Estos resultados fueron consistentes con la morfología ya que, no se observaron diferencias morfológicas en poblaciones con divergencias menores al 3%.

La taxonomía integrativa nos permite delimitar las especies con más exactitud que si utilizamos solo un enfoque. Todos los análisis (morfológicos, genéticos y biogeográficos) fueron concordantes y se obtuvieron los mismos resultados, ocho especies en el caso de *Moina micrura*, dos especies en el complejo *Moina macrocopa* mientras que, *Mesocyclops pehpeiensis* es una especie oportunista que se está extendiendo asociada al cultivo del arroz.

El uso de herramientas de última tecnología como la Microscopía Electrónica de Barrido, facilita la identificación de las especies ya que, permite observar diferencias en micro caracteres de gran importancia taxonómica, que no fueron descritos anteriormente al no ser visibles utilizando las herramientas tradicionales. Los resultados muestran diferencias morfológicas en algunos caracteres que han sido usados para la identificación del grupo micrura y la diferenciación con sus congéneres.

Las especies registradas en diferentes partes del mundo muy alejadas unas de otras, y con baja divergencia genética, como *Mesocyclops pehpeiensis*, alguna de las especies del complejo *Moina micrura* y el complejo *Moina macrocopa* s.l. son debidas principalmente a dos razones: actividades antropogénicas, por ejemplo, asociadas a cultivos, agua de lastre y a las aves que, durante su migración van transportando efipios de un lugar a otro, cómo se ha demostrado anteriormente para otros cladóceros.

Los resultados obtenidos en este análisis son totalmente compatibles con la actual clasificación jerárquica de los seres vivos basada en el sistema de Linneo.

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