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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

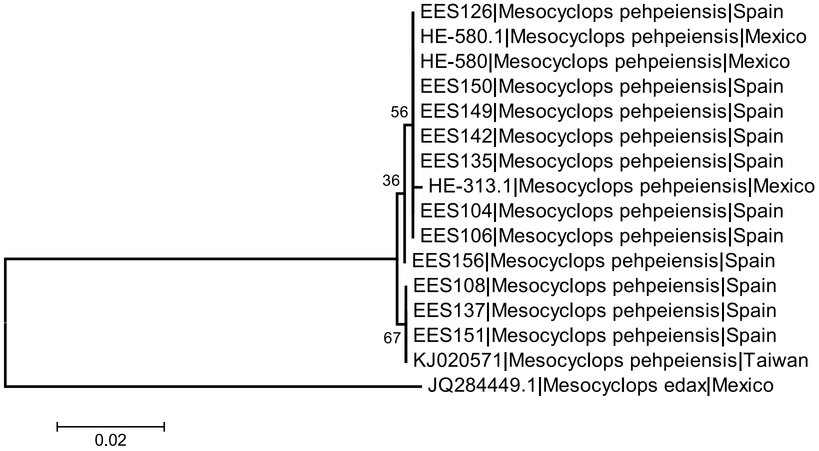

Fig. 1. Maximum likelihood tree based on the Hasegawa et al. (1985) model for *Mesocyclops pehpeiensis* Hu, 1943 from America and Europe. The outgroup corresponds to *Mesocyclops edax* Forbes, 1891. Numbers preceding the species name are the specimen ID in BOLD, except for the Taiwan and *Mesocyclops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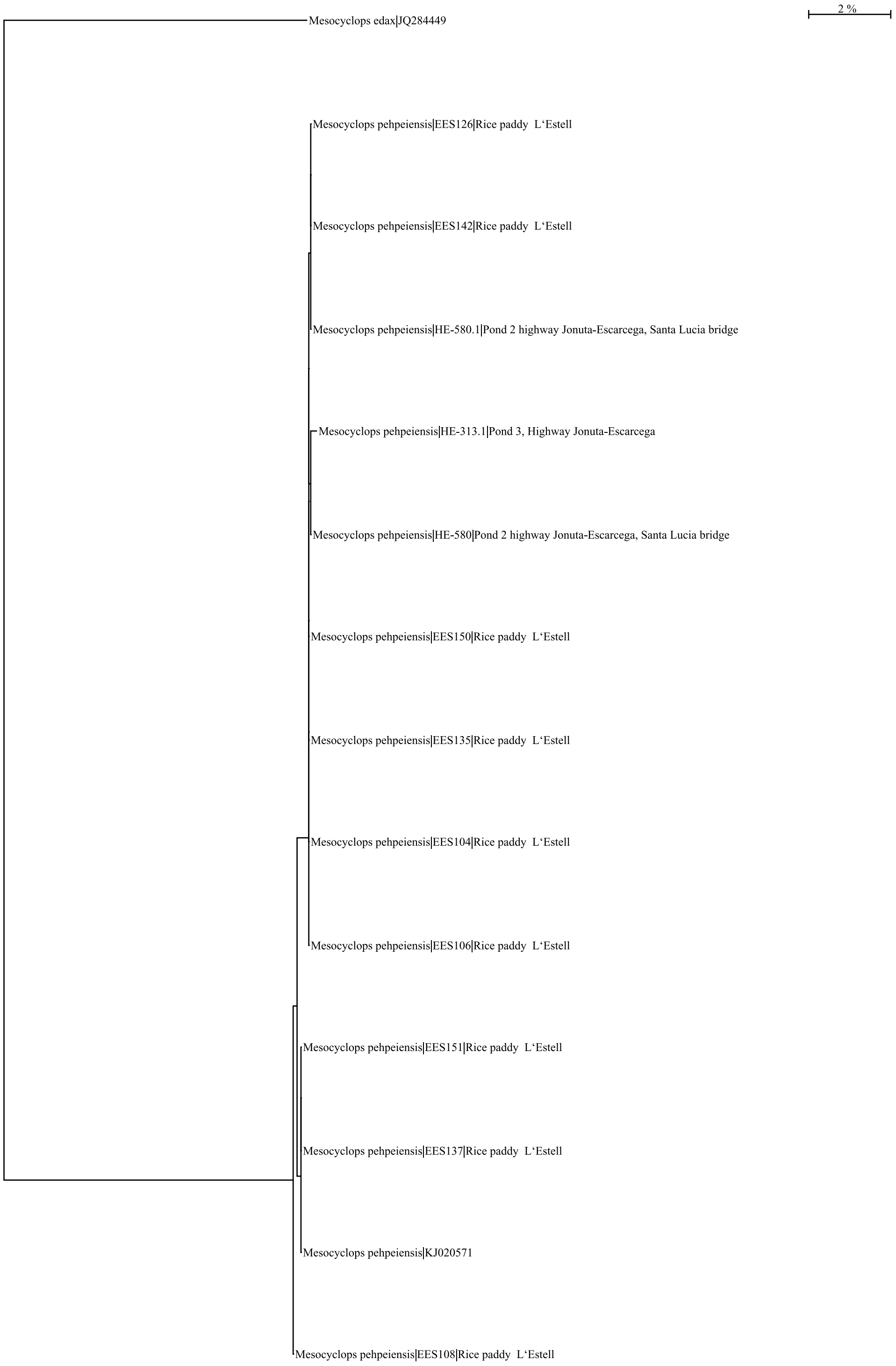
APPENDIX

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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