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The systematics of the amphidromous shrimp *Macrobrachium hobbsi* Nates & Villalobos, 1990 (Decapoda: Caridea: Palaemonidae) from the Mexican Pacific slope

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ABSTRACT

Six amphidromous species of *Macrobrachium* Spence Bate, 1868 have been recorded on the Pacific slope of Mexico, including the two morphologically related *M. hobbsi* Nates & Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990 and the Atlantic species *M. olfersii* (Wiegmann, 1836). The hypothesis that the formation of the Isthmus of Panama in the Late Pliocene produced a vicariant event leading to the formation of two groups of *Macrobrachium* species, one on the Atlantic slope and the other on the Pacific slope, has raised doubts about the amphiamerican occurrence of *M. hobbsi* and *M. olfersii*. Recent molecular studies have supported that hypothesis that there are no amphiamerican species of *Macrobrachium*, but rather amphiamerican species groups. In a previous publication, we proposed that one of the groups occurring along the Mexican slopes is the *olfersii* group, comprising the *digueti* subgroup, and the *olfersii* subgroup represented by *M. hobbsi* in the Pacific and *M. olfersii* in the Atlantic. We reviewed the systematics of the *olfersii* subgroup from the Pacific slope by analyzing individuals obtained from 104 sites in 44 drainage basins in nine Mexican states. Morphological and molecular genetic analyses with newly generated fragments of the mitochondrial genes 16S (470 bp) and COI (542 bp) confirm that, in the *olfersii* subgroup, only one morphologically plastic species, *M. hobbsi*, occurs on the Mexican Pacific slope. It is the most common species of *Macrobrachium* on this slope and is genetically different from the *M. olfersii* Atlantic populations. The results also indicate that disjunct populations of the Baja California Peninsula and the mainland belong to the same genetic lineage.

Key Words: 16S gene, COI gene, Baja California Peninsula, *olfersii* group, *olfersii* subgroup, Mexican Pacific slope, phylogenetics

INTRODUCTION

Six morphological amphidromous species of *Macrobrachium* Spence Bate, 1868 have been recognized along rivers, estuaries, and lagoons of the Mexican Pacific slope, including the Baja California Peninsula, hereafter peninsula: *M. americanum* Spence Bate, 1868, *M. digueti* (Bouvier, 1895), *M. hobbsi* Nates & Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990, *M. occidentale* Holthuis, 1950, *M. olfersii* (Wiegmann, 1836), and *M. tenellum* (Smith, 1871) (García-Velazco *et al.*, 2014, 2017). Rathbun (1902) reported *M. olfersii* (as *Bithynis olfersii*) from the peninsula, even if the type locality of this species is Brazil. Villalobos (1969) also reported the species from the Pacific Isthmus of Tehuantepec and Hernández *et al.* (2007) from the peninsula. *Macrobrachium hobbsi*, although its type locality is on the Mexican Pacific slope, was also

recorded from the Mexican Atlantic slope by Villalobos Hiriart & Nates Rodríguez (1990), noting that the closest species of *M. hobbsi* is *M. olfersii*, with the main morphological difference being the fingers of the largest chela of the second periopod, straight with no gap in *M. hobbsi* and curved and gapping in *M. olfersii*.

Anger (2013) proposed that the formation of the Isthmus of Panama in the Late Pliocene (about 2 million ybp) was a vicariant event that led to the formation of two groups of *Macrobrachium* species, one on the Atlantic slope and its counterpart on the Pacific. He therefore concluded that the amphi-isthmian occurrence of *M. hobbsi* and *M. olfersii* was controversial, and that both forms may represent a geminate pair of sister species. The molecular analyses of García-Velazco *et al.* (2017) on Mexican material of *Macrobrachium* support that concept that there are no amphiamerican species, but rather amphiamerican species groups. They proposed four groups of amphiamerican species: 1) *olfersii* group, represented by the *digueti* subgroup with *M. digueti* and *M. hancocki* Holthuis, 1950 on the Pacific and *M. crenulatum* Holthuis, 1950 on the Atlantic and the *olfersii* subgroup with *M. hobbsi* in the Pacific and *M. olfersii* in the Atlantic; 2) *acanthurus* group, with *M. tenellum* in the Pacific and *M. acanthurus* (Wiegmann, 1836) in the Atlantic; 3) *heterochirus* group, with *M. occidentale* in the Pacific and *M. heterochirus* (Wiegmann, 1836) in the Atlantic; and 4) *carcinus* group, with *M. americanum* in the Pacific and *M. carcinus* (Linnaeus, 1758) in the Atlantic.

The aim of this study was to review the systematics of *Macrobrachium hobbsi* from the Mexican Pacific slope. Extensive field collections from the Baja California Norte state in the north to the Guerrero state in the south of the mainland were carried out. We performed morphological and molecular genetic analyses with newly generated fragments of the mitochondrial genes 16S ribosomal RNA (16S) and cytochrome oxidase subunit I (COI). The main focus was to test the hypotheses that *M. hobbsi* is the representative species of the *olfersii* subgroup for the Mexican Pacific and that individuals from the peninsula belong to the same lineage found on the Pacific slope of mainland Mexico.

MATERIALS AND METHODS

Field collections

Field surveys were made from 2008 to 2013 along the Pacific slope, from Baja California to the state of Guerrero on the mainland. Additional material from an Atlantic site was collected from a drainage basin in the state of Tamaulipas, Mexico. We used hand nets and casting nets to collect shrimp and measured water temperature, total dissolved solids (TDS), and pH using EcoSense handheld instruments (EC300, pH100; YSI, Yellow Springs, OH, USA) from most of the sites. The specimens were preserved in 100% ethanol and deposited in the crustacean collection at Centro de Investigaciones Biológicas del Noroeste (CIBNOR). We recorded the geographic position and altitude (meters above sea level (masl)) of the sites using GPS unit. The names of the drainage basins used are those published by the Comisión Nacional del Agua (www.conagua.gob.mx/CONAGUA07/.../TM(Cuencas_Hidrológicas).xls). García-Velazco *et al.* (2014) provide information on the absence of species of *Macrobrachium*.

Revision of morphology

The type specimens of *M. hobbsi*, deposited in the Colección Nacional de Crustáceos, Universidad Nacional Autónoma de México, were examined (Hernández *et al.*, 2007). The characters in the key to species of Hernández *et al.* (2007) were used to identify the specimens collected in the field and specimens previously deposited at CIBNOR. For the *olfersii* subgroup, we selected specimens having unequal chelae on the second pair of pereiopods, straight rostrum, and larger second pereiopod with the carpus as long as or longer than the merus and evident pubescence and setae over the palm and cutting edges of fingers, and fingers being closed (*M. hobbsi* morphology) or gapping (*M. olfersii* morphology) (Hernández *et al.*, 2007). Individuals bearing other morphological characters were identified as *M. digueti* and *M. occidentale* (García-Velazco *et al.*, 2014, 2017) or as *M. americanum* or *M. tenellum* (unpublished data). We separated males from females by relying on the appendix masculina on the second pleopods and confirmed by the morphology of the thoracic sternite 8 (T8). Morphometry and other morphological characters were recorded following García-Velazco *et al.* (2017): total length (TL) from tip of rostrum to posterior end of telson; carapace length (CL) from tip of rostrum to posterior dorsal margin of carapace; length and height of merus, carpus and propodus (palm); length of dactylus of the larger chela of the second pair of pereiopods; merus to carpus lengths (MeL:CaL); length to height of propodous (PrL:PrH); number of teeth on both margins of the rostrum (without apical tooth); shape of inferior orbit, bec ocellaire, epistome, thoracic sternite 4 (T4), T8, and the pre-anal carina on inter-uropodal sclerite.

DNA extraction, amplification, and sequencing

Genomic DNA of selected specimens of the putative *M. hobbsi* was extracted, using Gentra Puregene kit (Qiagen, Minneapolis, MN, USA), and fragments of 16S and COI from mitochondrial genome were amplified with primers 1471B and 1472B (Liu *et al.*, 2007), and COI-a and COI-f (Palumbi & Benzie, 1991), respectively. By implementing the thermocycling conditions of García-Velazco *et al.* (2014) in the polymerase chain reaction, 16S and COI fragments were amplified. Amplified products were sequenced with forward primers. Complementary strands of the amplified product of some samples were sequenced to validate the sequencing.

Molecular analysis

DNA chromatograms were examined in the DNA Baser 4.5 program (www.dnabaser.com). Edited DNA sequences from the DNA Baser were aligned in Clustal X software under default settings (Thompson *et al.*, 1997). Species identity of the morphologically identified specimens was evaluated on the basis of genetic identity (haplotype). We also estimated the number, frequency, and diversity of haplotypes for each gene. Differentiation of populations in the peninsula and the mainland was evaluated using the analysis of molecular variance (AMOVA) by treating them as two groups.

Haplotypic diversity and analysis of molecular variance

The genetic diversity of each gene fragment of 16S and COI was assessed by the number of variable sites, number of haplotypes, and haplotype diversity using the software DnaSP 5.10 (Librado & Rozas, 2009). MEGA 7.0 software (Kumar *et al.*, 2015) was used to estimate genetic divergences (uncorrected pairwise *p*-distance) between the haplotypes of 16S, COI, and concatenated 16S-COI of putative *M. hobbsi* from the Mexican Pacific slope. Genealogical relationships of 16S and COI haplotypes were constructed using the median-joining algorithm (Bandelt *et al.*, 1999) in PopART software (Leigh & Bryant, 2015). The effect of the geographical isolation of the peninsula on genetic differentiation of putative *M. hobbsi* populations was tested in a hierarchical analysis of molecular variance (AMOVA) by subdividing concatenated 16S and COI haplotype frequencies as the peninsula group and the mainland group in the program Arlequin 3.5 (Excoffier & Lischer, 2010) with 10,000 permutations.

Phylogenetic analysis

Analyses of phylogenetic relationship of putative *M. hobbsi* with other species of *Macrobrachium* from the Americas were carried out with combined 16S and COI sequences. We used maximum parsimony (MP), maximum likelihood (ML), and Bayesian Inference

(BI) methods to reconstruct the phylogeny. Maximum parsimony reconstruction was carried out using PAUP 4.0a153 (Swofford, 2002) under a heuristic tree-searching option with tree bisection-reconnection branch swapping and random stepwise addition of 100 replicates per bootstrap; the consensus tree was generated by applying the 50% majority rule. For the model-based phylogenetic reconstruction methods, a best-fit model of nucleotide substitution from 88 models in jModelTest 2.1.4 software (Darriba *et al.*, 2012) was calculated for the combined data (16S-COI), based on the Bayesian Information Criterion (BIC). The optimal model was incorporated in the ML and BI analyses. The analysis of ML performed in PAUP was set with heuristic tree-searching option, using tree bisection-reconnection branch swapping with 1000 pseudoreplicates. Fifty percent majority rule was applied to construct a consensus tree. The reconstruction of BI was executed in MrBayes 3.2 software (Ronquist *et al.*, 2012) with settings of two runs and eight independent chains. Analysis was run for 15 million generations, sampling every 100 generations. By using the burn-in option, 25% of the initial trees were eliminated and a majority rule consensus tree was generated from the remaining trees.

SYSTEMATICS

The synonymy is restricted to publications referring to Mexican material of the Pacific slope. The diagnosis is based on the descriptions of Villalobos Hiriart & Nates Rodríguez (1990) and Hernández *et al.* (2007), but updated with the morphological variations found in the 496 specimens examined.

Palaemonidae Rafinesque, 1815
Macrobrachium Spence Bate, 1868
Macrobrachium hobbsi Nates & Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990.
(Figs. 1–5)

Bithynis olfersii (Wiegmann, 1836) – Rathbun (1902) (part).
Macrobrachium olfersii (Wiegmann, 1836) – Villalobos, 1969; Villalobos Hiriart & Nates Rodríguez, 1990; Villalobos-Hiriart *et al.*, 1993, 2003, 2010; Wicksten & Hendrickx, 2003; Hernández *et al.*, 2007; Acuña Gómez *et al.*, 2013; García-Velazco *et al.*, 2014; Pérez-Tello *et al.*, 2016.
Macrobrachium hobbsi Nates & Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990; Villalobos-Hiriart *et al.*, 1993, 2010; Hernández *et al.*, 2007; Wicksten & Hendrickx, 2003; Hendrickx & Wicksten, 2011; García-Velazco *et al.*, 2014.

Type locality: Río El Naranjo, Chiapas (Villalobos Hiriart & Nates Rodríguez, 1990).

Diagnosis: Rostrum narrow, straight (Figs. 1–2), reaching or passing last joint of antennular peduncle; upper margin with 12–18 teeth, 3–8 of them postorbital; lower margin with 2–5 teeth; antennules, eyes normal in shape. Second pereiopods similar in shape but unequal in adult males (Figs. 1–2). Propodus of largest chela little compressed laterally, PrL:PrH variable, normally 1.8–3.1 (range 1.7–4.0); dorsal, lateral sides with distinct spines; lateral, inner sides with pubescence, setae and spinules; dorsal, ventral margins slightly curved or straight (Figs. 1–4); ventral side of propodus, dactylus with small spines, tubercles. Fingers shorter or as long as propodus, with highly variable morphology, bearing denticles, setae, tufts of hairs along cutting edges; dactylus straight, not leaving gap between fingers, or curved upward leaving narrow to large gap between fingers; fixed finger straight, or curving downward leaving gap between fingers (Figs. 1–4); proximal half of cutting edge of fixed finger, dactylus normally with series of rounded teeth, denticles (Figs. 2–4); carpus slightly longer or as long as merus, MeL:CaL normally 0.9–1.0 (range 0.8–1.2); carpus, merus slightly shorter or as long as propodus (Figs. 1, 2). Pleopods, uropods normal in shape. Carapace length of largest male, largest female examined 38.2, 40.1 mm, respectively, both from La Purísima Basin, Baja California Sur.

Additional morphological characters: Shape of inferior orbit distinctly convex, moderately produced (Figs. 1, 2); ocular cornea large, well pigmented with accessory pigment spot (Fig. 5A); bec ocellaire strongly developed with truncated apex (Fig. 5B); epistome with rounded lobes; T4 with well-developed median process with large antero-central protuberance, 2 smaller postero-lateral protuberances; T8 in males with joined lobes (Fig. 5C, E), widely separated lobes in females (Fig. 5D); inter-uropodal sclerites with well-developed preanal carina, normally without dorsal setae (Fig. 5F).

Haplotypic identity: We extracted genomic DNA from 174 *M. hobbsi* specimens and obtained 166 sequences of 16S gene fragments, 54 sequences of specimens from the peninsula distributed among nine drainage basins, and 112 of specimens from the mainland distributed among 16 drainage basins. The edited size of the 16S fragments was 470 bp with 36 variable sites segregated into 47 haplotypes (Supplementary material Table S1). The haplotypes showed 94.4% transition type polymorphic variation and their genetic distance ranged 0.21–1.49% (Supplementary material Table S2). Nine of the 11 shared haplotypes were found in the two regions, the peninsula and the mainland.

For the COI gene fragment, 68 sequences were generated, 25 distributed among nine drainage basins in the peninsula and 43 distributed among 13 drainage basins on the mainland. The 542 bp length of COI sequences showed 57 polymorphic variable sites and 45 haplotypes (Supplementary material Table S3). The number of polymorphic sites ranged 1–16 between haplotypes, whose genetic distance ranged 0.18–2.95% (Supplementary material Table S4). Two COI haplotypes (35% of sequences) were found on both the peninsula and the mainland.

Haplotypic diversity and analysis of molecular variance: Haplotype network analyses showed that most of the *M. hobbsi* haplotypes (16S and COI) are separated by a single mutation step (Fig. 6A, B). Few haplotypes were separated by two mutational steps in the 16S, whereas three mutational steps were found among some COI haplotypes. Putative haplotypes were not detected in 16S, but COI sequences presented 13 putative undiscovered haplotypes (Fig. 6A, B). Shared haplotypes represented 78.3% of the total sequences analyzed in the 16S (Supplementary material Table S1). Only Plutarco Elías Calles Basin on the peninsula and Río San Pedro Basin on the mainland did not share their haplotype (Supplementary material Table S1, Fig. 6A). Private haplotypes were represented by 36 sequences and the 16S haplotypic diversity was 0.86. Two of the 11 haplotypes were only shared by populations from the mainland. Two predominant haplotypes were recorded: Hap 17 (haplotype) was shared by individuals from five peninsular and 11 mainland basins; the Hap 36 was shared by individuals from four peninsular and 13 mainland basins (Supplementary material Table S1). The Hap 17 showed less than 1% variation with 46 haplotypes (Supplementary material Table S2). Haplotype network provided no evidence of a geographical separation of haplotypes (Fig. 6A).

One of the three shared haplotypes in the COI is from the Río Verde Basin on the mainland (Supplementary material Table S3). The predominant Hap 9 was shared by individuals from five peninsular and seven mainland basins, and individuals from one peninsular and three mainland basins shared the other predominant Hap 15 (Supplementary material Table S3, Fig. 6B). Over 93% of the haplotypes were private haplotypes represented by 61% of sequences (42 sequences). Fifty percent of the COI haplotypes showed minimum variation (< 1%) with the predominant Hap 9

Figure 1. Adults of *Macrobrachium hobbsi*: males of same 16S haplotype 36 (**A**–**C**); males of same COI haplotype 8 (**D**, **E**); males of same COI haplotype 9 (**F**–**G**). Body with larger chela of second pair of pereiopods in dorsal-lateral view (CIB-834.1), Rancho El Cardalito, Las Pocitas-San Hilario Basin, Baja California Sur (**A**); body with larger chela of second pair of pereiopods in left lateral view (CIB-825.4) from El Caracol, Las Pocitas-San Hilario Basin, Baja California Sur (**B**); body with second pair of pereiopods in dorsal view (CIB-1133.1) from La Poza, Todos Santos Basin, Baja California Sur (**C**); body with larger chela of second pair of pereiopods in right lateral view (CIB-1133.2) from La Poza, Todos Santos Basin, Baja California Sur (**D**); body with smaller chela of second pair of pereiopods in left lateral view (CIB-1137.1) from Vado Guasave, Río Sinaloa 2 Basin, Sinaloa (**E**); body with larger chela of second pair of pereiopods in dorsal view (CIB-839.1) from Oasis Santa Rosa, San José del Cabo Basin, Baja California Sur (**F**); body with larger chela of second pair of pereiopods in right lateral view (CIB-823.1) from San Basilio, Santa Rita Basin, Baja California Sur (**G**); body with second pair of pereiopods in dorsal (**H**) and ventral (**I**) views of female (CIB-854.1) from Los Salazares, Río Santiago 4 Basin, Nayarit. Numbers refer to the carapace length in mm.

(Supplementary material Table S4). Haplotype diversity estimates for this gene was 0.92. Haplotype network of COI also provided no evidence on the geographical structure of haplotypes (Fig. 6B).

Sixty-four sequences of 16S and COI were concatenated. Combined sequences (1012 bp) produced 48 haplotypes (Supplementary material Table S5), and their genetic distance

Figure 2. Anterior part of the body with second pair of pereiopods of adult males of *Macrobrachium hobbsi* from drainage basins of the Baja California Peninsula. Specimens of same 16S haplotype 36 (**A, B**); specimens of same COI haplotype 9 (**C, D**). Dorsal view (specimen CIB-825.3) from El Caracol, Las Pocitas-San Hilario Basin (**A**); right lateral view (CIB-819.1) from San Isidro, La Purísima Basin (**B**); dorsal view (CIB-821.4) from San Pedro de La Presa, Santa Rita Basin (**C**); dorsal view (CIB-859.1) from Poza El Mechudo, La Paz Basin (**D**). Numbers refer to the carapace length in mm.

ranged from 0.18–1.78% (Supplementary material Table S6). Two haplotypes (Hap 9 and Hap 13) were shared by peninsular and mainland basins (Supplementary material Table S5). Haplotype frequencies of the combined 16S and COI sequences were subdivided into peninsular and mainland groups. The hierarchical AMOVA of this data provided no evidence for subdivisions in different drainage basins or the two groups. Variations within drainage basins were higher than other sources of variation ($P > 0.05$) (Table 1).

Phylogenetic analyses: Seven 16S-COI haplotypes of *M. hobbsi* (Hap 1, Hap 7, Hap 9, Hap 13, Hap 22, Hap 23, and Hap 32) with higher genetic variation (Supplementary material Table S6) were compared with other species of *Macrobrachium* from the Americas (Supplementary material Table S7). Phylogenetic reconstruction using MP and the ML and BI methods implemented with the TIM1 model with invariant sites (I) and gamma-distributed rates (G) produced phylogenetic trees with almost similar topologies (Fig. 7). The seven *M. hobbsi* haplotypes were grouped with the GenBank sequences labeled as *M. digueti* (*M. digueti*1, *M. digueti*2, and *M. digueti*3). This node was strongly supported by bootstrap values of MP and ML (100% and 99%) and BI posterior probabilities (1) and clustered as a sister group to the GenBank sequences of *M. ofersii* with significant bootstrap values (MP 98%, ML 93%), and posterior probabilities (BI 1). The clade consisting of the *olfersii* subgroup species clustered as a sister clade with the *digueti* subgroup, formed by *M. crenulatum* (*M. crenulatum*1 and 2), *M. digueti* (*M. digueti*4–7), and *M hancocki* (*M. hancocki*1 and 2). The *olfersii* group node was strongly supported by ML (82%) and BI posterior probabilities (0.99). Closely related to the *olfersii* group are *M. americanum* and *M. carcinus* (Fig. 7).

Figure 3. Larger chela of second pair of pereiopods in lateral view of adult males of *Macrobrachium hobbsi* from different drainage basins in the Baja California Peninsula and mainland Mexico. All specimens were molecularly characterized, and their states of origin (and identification code) are as follows: Baja California Sur (CIB-1133.2) (**A**); Oaxaca (CIB-1147.2) (**B**); Nayarit (CIB-1183.2) (**C**); Sinaloa (CIB-1029.1) (**D**); Sinaloa (CIB-1178.2) (**E**); Nayarit (CIB-1183.1) (**F**); Oaxaca (CIB-1187.2) (**G**); Sinaloa (CIB-1178.4) (**H**); Nayarit (CIB-1144.2) (**I**); Sinaloa (CIB-1181.3) (**J**); Oaxaca (CIB-1147.1) (**K**); Baja California Sur (CIB-859.4) (**L**); Sinaloa (CIB-1180.5) (**M**); Sinaloa (CIB-1171.7) (**N**); Nayarit (CIB-1183.4) (**O**); Sinaloa (CIB-1181.4) (**P**); Sinaloa (CIB-1180.8) (**Q**); Sinaloa (CIB-1140.1) (**R**); Sinaloa (CIB-1029.2) (**S**); Sinaloa (CIB-1178.3) (**T**). Numbers refer to the carapace length in mm.

Distribution on the Mexican Pacific slope: Morphological and molecular genetic analyses indicate that of the *olfersii* subgroup along the Mexican Pacific slope, there is only one morphologically plastic species, *M. hobbsi*, which is genetically different than the Atlantic species *M. olfersii*. The geographical distribution of *M. hobbsi* (including the records of *M. olfersii* for this slope) therefore ranges from the Baja California Sur and Sonora states in the north to the state of Chiapas in the south (Fig. 8). The states and drainage basins where this species has been recorded are: Baja California Sur: La Purísima, Santo Domingo, Santa Rita, Las Pocitas-San Hilario,

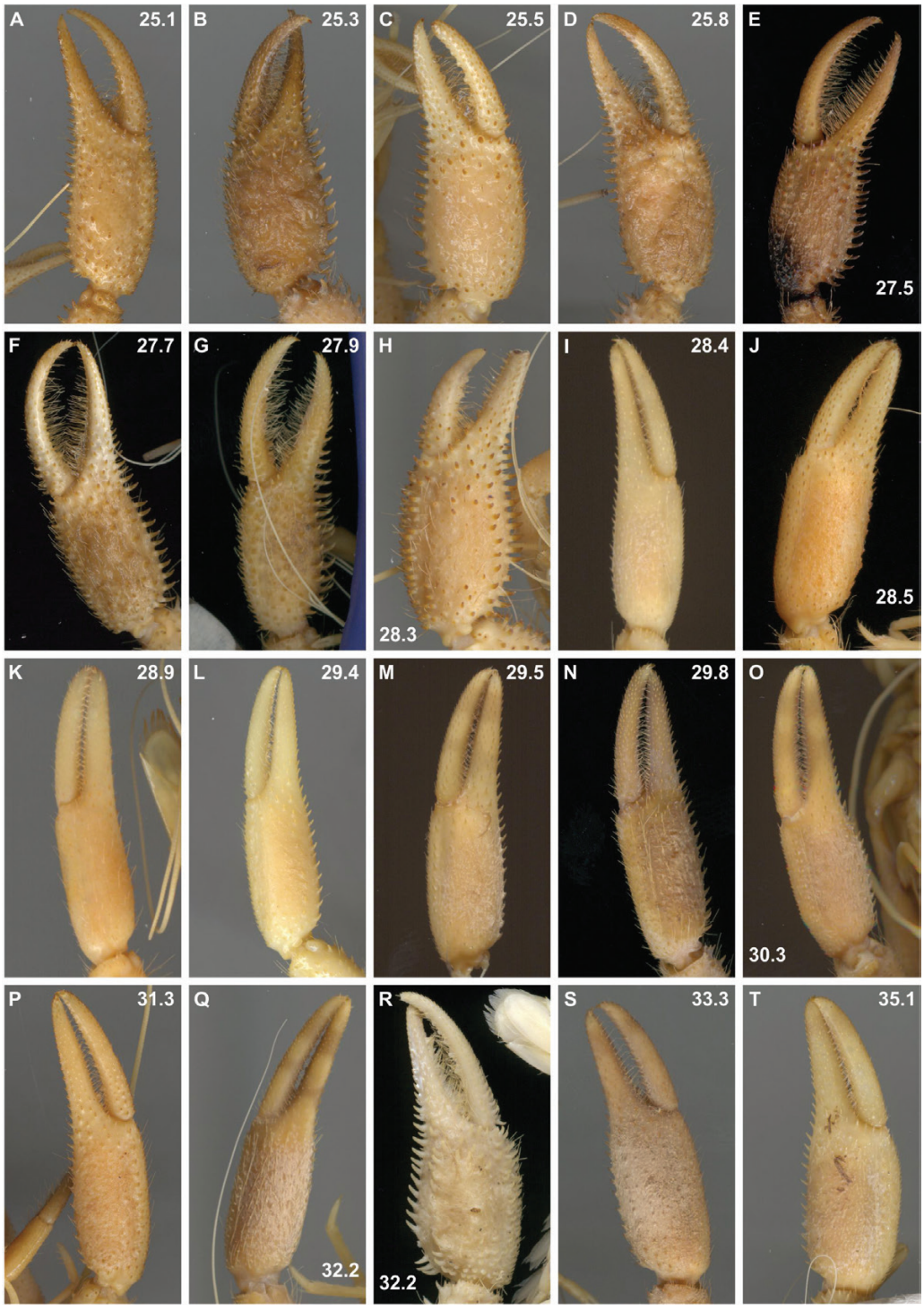

Figure 4. Larger chela of second pair of pereiopods in lateral view of adult males of *Macrobrachium hobbsi* from different drainage basins in the Baja California Peninsula and mainland Mexico. All specimens were molecularly characterized and their states of origin (and identification code) are as follows: Baja California Sur (CIB-834.1) (**A**); Nayarit (CIB-1144.1) (**B**); Baja California Sur (CIB-859.3) (**C**); Baja California Sur (CIB-839.2) (**D**); Nayarit (CIB-1143.1) (**E**); Baja California Sur (CIB-839.1) (**F**); Baja California Sur (CIB-836.2) (**G**); Baja California Sur (CIB-859.1) (**H**); Sinaloa (CIB-1171.2) (**I**); Baja California Sur (CIB-825.4) (**J**); Baja California Sur (CIB-821.2) (**K**); Baja California Sur (CIB-1165.1) (**L**); Sinaloa (CIB-1171.4) (**M**); Sonora (CIB-1136.1) (**N**); Sinaloa (CIB-1177.2) (**O**); Nayarit (CIB-854.2) (**P**); Sinaloa (CIB-1177.1) (**Q**); Guerrero (CIB-1146.1) (**R**); Baja California Sur (CIB-822.1) (**S**); Baja California Sur (CIB-1139.1) (**T**). Numbers refer to the carapace length (in mm).

Todos Santos, Pescaderos, Plutarco Elías Calles, Mulegé, La Paz, Santiago, and San José del Cabo basins (Rathbun, 1902; Hernández *et al.*, 2007; García-Velazco *et al.*, 2014); Sonora: San Carlos, Guaymas (Pérez-Tello *et al.*, 2016), and Río Mayo 3 Basin; Sinaloa: Baluarte 2 (Wicksten & Hendrickx, 2003), Culiacán, Sinaloa 2, Mocorito, Elota, Piaxtla 2, Presidio 2, and Cañas 2 rivers; Nayarit: Santiago 4 (Hernández *et al.*, 2007; Guzmán-Arroyo *et al.*, 2009), San Pedro, and Ameca Ixtapa B rivers; Jalisco: Tomatlán-B rivers;

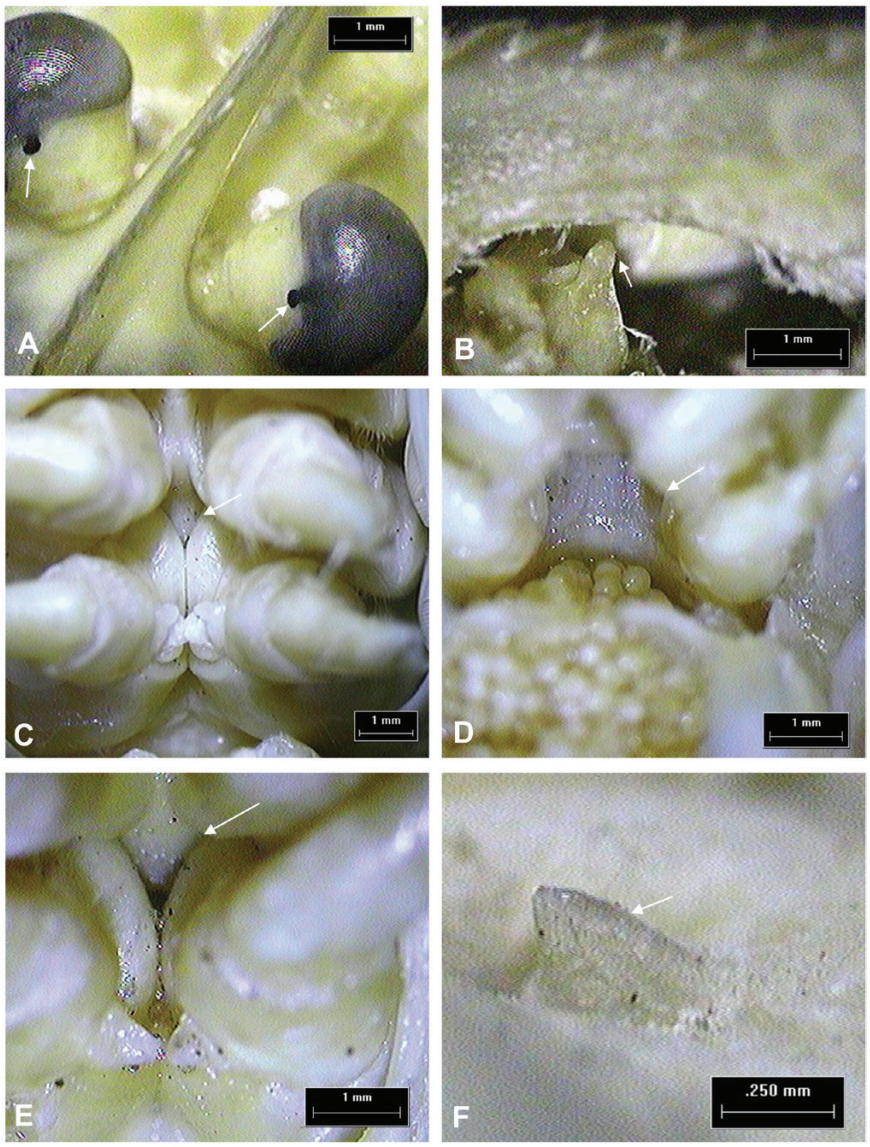

Figure 5. Males and female of *Macrobrachium hobbsi*. Female (CIB-836) from Todos Santos, Todos Santos Basin, Baja California Sur (**A, D**); male (CIB-1148) from San Isidro, La Purísima Basin, Baja California Sur (**B, E**); male (CIB-836) from Todos Santos, Todos Santos Basin, Baja California Sur (**C**); male (CIB-1147) from Río Viejo, Río Verde Basin, Oaxaca (**F**). Rostrum and compound eyes with large and well pigmented ocular cornea and accessory pigment spot (**A**); bec ocellaire with apex in right lateral view (**B**); thoracic sternite 8 (T8) showing joined lobes in ventral view (**C, E**); thoracic sternite 8 (T8) showing widely separated lobes in ventral view (**D**); inter-uropodal sclerite with well-developed preanal carina in ventral lateral view (**F**).

Michoacán: Río Bajo Balsas Basin, Balsas (Hernández *et al.*, 2007) river; Guerrero: Río Murga (Villalobos Hiriart & Nates Rodríguez, 1990), Presa Morelos, Zihuatanejo (Hernández *et al.*, 2007), and Río Coyuca 2; Oaxaca: Río Astata (Villalobos Hiriart & Nates Rodríguez, 1990), Merced del Potrero, San Lorenzo, Zimatán, Tehuantepec, and Coyula, Copalita, Zimatán (Hernández *et al.*, 2007; Villalobos-Hiriart *et al.*, 2010), and Verde rivers; Chiapas: Arroyo Ocuilapa, Lagartero, Cintalapa, Chacamax, El Naranjo (Villalobos Hiriart & Nates Rodríguez, 1990; Wicksten & Hendrickx, 2003), Grande, Novillero, and Urbina rivers (Hernández *et al.*, 2007).

Material examined: The material examined is listed in Supplementary material Table S8. Material of *M. hobbsi* was found at 54 sites along the Mexican Pacific slope in 26 drainage basins of eight states: 29 sites from 10 basins in Baja California Sur, and 25 sites from 16 basins along the mainland slope in seven states: Sonora, Sinaloa, Nayarit, Jalisco, Michoacán, Guerrero, and Oaxaca.

DISCUSSION

There are several records of *M. olfersii* along the Pacific slope of Mexico from Baja California Sur, Sonora, Jalisco, and the Isthmus of Tehuantepec (Rathbun, 1902; Villalobos, 1969; Hernández *et al.*, 2007; Acuña Gómez *et al.*, 2013; Pérez-Tello *et al.*, 2016). Based on the morphology of the male second pair of pereiopods, Villalobos (1969) suggested the existence of an "*olfersii* group" formed by *M. olfersii* distributed on the Atlantic and Pacific slopes, *M. digueti* from Baja California Sur, *M. faustinum* (De Saussure, 1857) from the West Indies, *M. acanthochirus* Villalobos, 1967 from the Mexican Pacific slope, *M. crenulatum* from the Atlantic slope, and *M. hancocki* from the Pacific slope. *Macrobrachium hobbsi* recorded from the Mexican Pacific and Atlantic slopes was described as very similar to *M. olfersii*, with the shape of the fingers (closed *versus* gapping) as the main morphological difference between them (Villalobos Hiriart & Nates Rodríguez, 1990). Acuña Gómez *et al.* (2013), using one specimen each of *M. hobbsi*

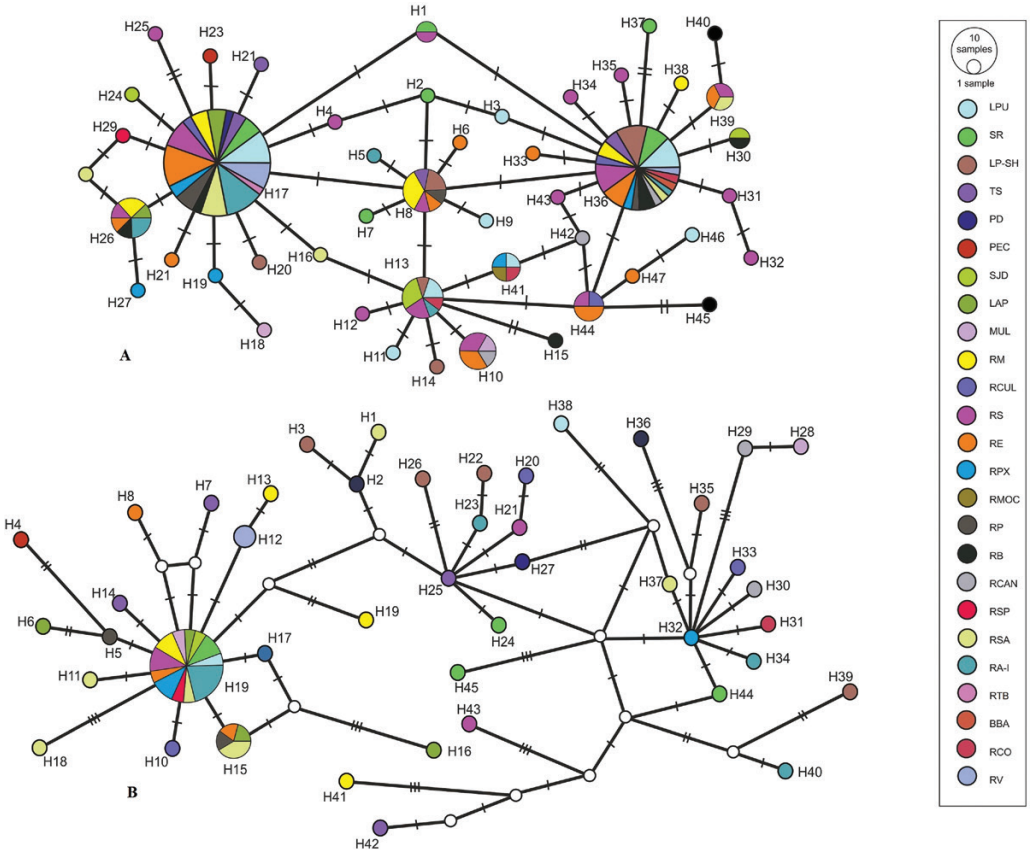

Figure 6. Median-joining network showing relationships among 16S haplotypes of *Macrobrachium hobbsi* from 14 drainage basins (**A**) and COI haplotypes of *M. hobbsi* from 13 drainage basins (**B**). Colors indicate basins and the size of circles and colored segments are proportional to the haplotype frequencies. Black circles represent mutational step between haplotypes. Baja California Peninsula basins: LPU, La Purísima; SR, Santa Rita; LP-SH, Las Pocitas-San Hilario; TS, Todos Santos; PD, Pescaderos; PEC, Plutarco Elías Calles; SJD, San José del Cabo; LAP, La Paz; MUL, Mulegé. Mainland Mexico basins: RM, Río Mayo 3; RCUL, Río Culiacán; RS, Río Sinaloa 2; RE, Río Elota; RPX, Río Piaxtla 2; RMOC, Río Mocorito; RP, Río Presidio 2; RB, Río Baluarte 2; RCAN, Río Cañas 2; RSP, Río San Pedro; RSA, Río Santiago 4; RA-I, Río Ameca Ixtapa B; RTB, Río Tomatlán-B; BBA, Río Bajo Balsas; RCO, Río Coyuca 2; RV, Río Verde.

Table 1. Analysis of molecular variance (AMOVA) of the combined 16S and COI genes sequence data of *Macrobrachium hobbsi* Nates & Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990 divided in two groups: eight drainage basins on the Baja California Peninsula, Mexico and 13 drainage basins on the mainland Pacific slope of Mexico.

Source of variation	*df*	Sum of squares	Percentage of variation	Fixation indices	*P*
Among regions	1	0.470	–0.38	–0.00378	0.5488
Among basins, within regions	19	9.749	2.53	0.02516	0.0965
Within basins	43	20.500	97.85	0.02147	0.1078

and *M. olfersii* from the Pacific slope, reported 99% of genetic similarity in 16S fragments (385 bp length) of these nominal species. All specimens from the Pacific slope we examined with 16S (470 bp) and COI (542 bp) having the typical morphology of both nominal *M. hobbsi* and *M. olfersii*, constituted a single genetic lineage at the species level (Supplementary material Tables S2, S4). A comparison of our 166 sequences of 16S from the Pacific slope with 27 GenBank sequences of *M. olfersii* from the Atlantic slope indicated that our samples belong to a distinct lineage (Supplementary material Table S9). As expected, our three closed fingers specimens from a site in Tamaulipas (Gulf of Mexico) grouped within the *M. olfersii* lineage, two of them segregated with a predominant 16S haplotype that comprised 23 sequences from the Atlantic slopes of Brazil, Costa Rica, Panama, and Venezuela (Supplementary material Table S9). Analysis of 68 COI sequences from the Pacific slope, with 46 GenBank sequences of *M. olfersii* from the Atlantic slope also confirmed that our *M. hobbsi* samples form a distinct lineage (Supplementary material Table S10). One of the *M. olfersii* haplotypes comprised nine *M. olfersii* GenBank sequences and one of our sequences from Tamaulipas. The Pacific lineage showed a minimum genetic distance of 1.30% in 16S and 9.2% in COI (Supplementary material Tables S9, S10) with the original Atlantic species *M. olfersii*.

Holthuis (1952, 1980) restricted *M. olfersii* to Atlantic slopes. Our results support this, and most likely *M. olfersii* does not occur on the Pacific slope. Genetic variations observed between the populations of *M. hobbsi* in the peninsula and the Pacific mainland represent intraspecific variations. The genetic distances calculated for the 16S and COI *M. hobbsi* haplotypes (0.21–1.49% and 0.18–3.14%, respectively) are similar to the variation range reported for other *Macrobrachium* species. Vergamini *et al.* (2011) reported a maximum distance of 1.1% for the 16S in *M. amazonicum*

Figure 7. Bayesian phylogenetic tree topology representing relationships among species of *Macrobrachium* based on the combined data of 16S and COI gene fragments. Numbers above the nodes represent bootstrap values of maximum parsimony and maximum likelihood methods and below the node represents posterior probabilities of Bayesian inference. Numbers following the name of the species represent the origin of the specimen linked to the 16S and COI sequences in the GenBank database.

(Heller, 1862) and Rossi & Mantelatto (2013) reported 0.18% for *M. olfersii* on the Atlantic slopes of Costa Rica, Panama, Venezuela, and Brazil. Maximum distance observed for COI in *M. amazonicum* was 3.3% (Vergamini *et al.*, 2011) and 0.95% in *M. olfersii* (Rossi & Mantelatto, 2013). *Macrobrachium hobbsi* thus represents a single valid species (lineage) for the Pacific slope of the *olfersii* subgroup, with plastic morphology of closed-to-gapping fingers (Figs. 1–4). Our results are in contrast with the designation of *M. olfersii* as an amphiamerican entity based solely on morphology (Villalobos Hiriart & Nates Rodríguez, 1990; Rodríguez-Almaraz & Campos, 1996; Wicksten & Hendrickx, 2003; Hernández *et al.*, 2007).

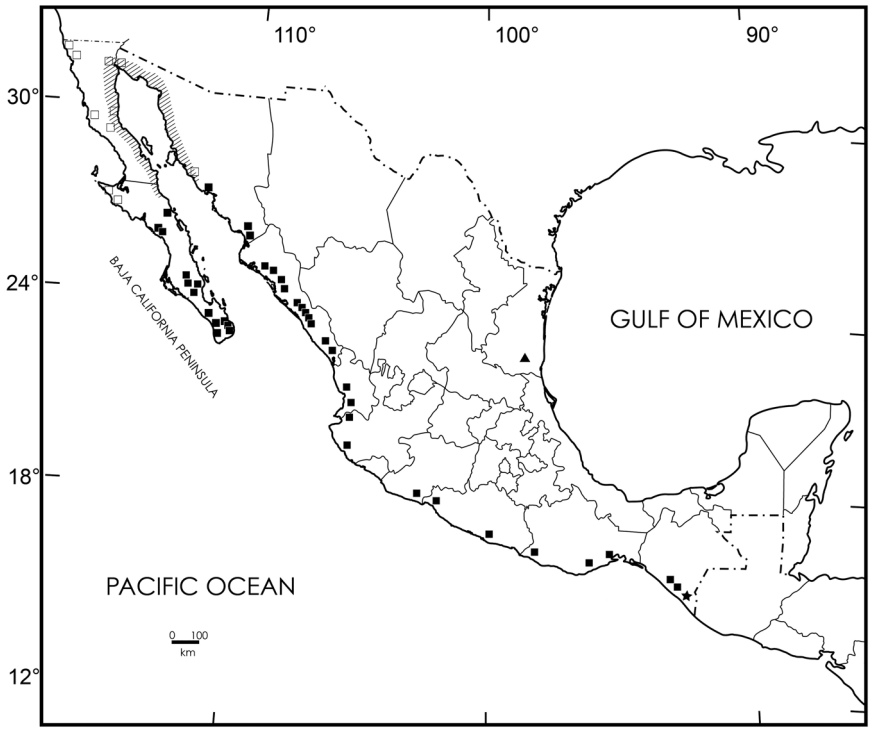

Figure 8. Geographical distribution of *Macrobrachium hobbsi*. Solid squares represent records of the species in the Mexican Pacific slope, a star for type locality and empty squares for sampled areas without records of *Macrobrachium* specimens. Area with dashed lines shows the absence of *Macrobrachium* on the coastal plains of the northern part of the Gulf of California (Mar de Cortés). Solid triangle represents *M. olfersii* sample site in Tamaulipas state.

Nine 16S haplotypes and two COI haplotypes of *M. hobbsi* were found in the peninsula and the mainland (Supplementary material Tables S1, S3). The three most frequent haplotypes of the combined 16S-COI haplotypes were also found on the peninsula and mainland (Supplementary material Table S5). The occurrence of such individuals with common haplotypes might result from migration in both directions (Tero *et al.*, 2003; de Bruyn *et al.*, 2005; Page *et al.*, 2008; Hughes *et al.*, 2009, García-Velazco *et al.*, 2017). Unique haplotypes may be attributed to a recent origins, whereas shared haplotypes are suggested to be old and ancestral as a result of dispersal events (Cook *et al.*, 2002; Vázquez-Domínguez *et al.*, 2009; Ferreri *et al.*, 2011; García-Velazco *et al.*, 2017). Sharing haplotypes between populations of the peninsula and mainland and in the majority of the basins studied was also reflected in the hierarchical AMOVA analysis because there was no evidence of isolation of *M. hobbsi* individuals between the two regions or in different drainage basins.

The absence of *M. olfersii* on the Mexican Pacific slope supports the existence of two vicariant groups (Atlantic and Pacific) after the formation of the Isthmus of Panama, where current *M. olfersii* and *M. hobbsi* represent a geminate pair of sister species (Anger, 2013). The occurrence or absence of *M. hobbsi* on the Atlantic slope of Mexico remains to be confirmed genetically (study in process). The phylogenetic tree topology obtained (Fig. 7) confirmed four well-supported monophyletic amphiamerican groups along the Mexican slope as reported by García-Velazco *et al.* (2017): the *acanthurus* group, with the Pacific *M. tenellum* and the Atlantic *M. acanthurus*; the *heterochirus* group, with the Pacific *M. occidentale* and the Atlantic *M. heterochirus*; the *carcinus* group, with the Pacific *M. americanum* and the Atlantic *M. carcinus*; and the *olfersii* group. These groups, except for the assignation of *M. digueti*, are also supported by the molecular analyses of Acuña Gómez *et al.* (2013) and Pileggi *et al.* (2014).

The *olfersii* and *digueti* subgroups (*olfersii* group) (García-Velazco *et al.*, 2017) are characterized by a straight rostrum and unequal chelae of the second pereiopods. *Macrobrachium olfersii* and *M. hobbsi* normally have a carpus that is slightly longer or as long as the merus, fingers with tufts of hairs along the cutting edges, and a propodus with a distinct, thickly pubescent large area at each of the lateral surfaces (Holthuis, 1952; Villalobos Hiriart & Nates Rodríguez, 1990; Pileggi & Mantelatto, 2012; García-Velazco *et al.*, 2017; this study), whereas *M. digueti* has normally a carpus that is shorter or as long as the merus, and scarce pubescence and setae on the palm and cutting edges of the fingers (García-Velazco *et al.*, 2017).

We extend the known distribution of *M. hobbsi* from Mexico to Costa Rica. Sequences of 16S of three specimens deposited as *M. digueti* from Costa Rica in the GenBank are grouped with a shared 16S haplotype of *M. hobbsi* (Supplementary material Table S9) and their COI sequences showed more than 9.59% variation with *M. olfersii* and 0.55–3.32% with *M. hobbsi* (Supplementary material Table S10). *Macrobrachium digueti*1 and *M. digueti*2 sequences from Costa Rica included in the phylogenetic analyses were found to associate with the *M. hobbsi* group (Fig. 7). Three sequences from Mexico in the GenBank (16S: JQ805808 and COI: JQ805905, JQ805906), probably misidentified as *M. digueti*, also belong to *M. hobbsi* (Supplementary material Tables S9, S10; *M. digueti*3 in Fig. 7).

Specimens with closed fingers from the Gulf of Mexico assigned to *M. hobbsi* by Villalobos Hiriart & Nates Rodríguez (1990) most likely correspond to the morphologically plastic species *M. olfersii*. Pileggi & Mantelatto (2012) redescribed the latter species with fingers slightly curved without gap to strongly curved forming a gap. Our *M. olfersii* specimens from Tamaulipas show similar morphology with closed-fingers. We found no apparent geographical pattern of the closed and gapping fingers in *M. hobbsi* because we observed specimens with both morphologies sharing the same 16S and COI haplotypes (Figs. 1, 2). Villalobos Hiriart & Nates Rodríguez (1990) mentioned in the original description of *M. hobbsi* a rostrum having 14–16 teeth on the upper margin, 5 or 6 postorbital and 3 or 4 on the lower margin. That formula is now updated to 12–18 teeth on the upper margin, 3–8 postorbital

and 2–5 on the lower margin. These numbers are similar to those given in the re-description of *M. olfersii* by Pileggi & Mantelatto (2012) with 12–16 teeth on the upper margin, 3–6 postorbital and 2–4 on the lower margin. The PrL:PrH in *M. hobbsi* varies widely, from 1.7 to 4.0 (Figs. 1–4). The fingers vary from closed straight fingers up to widely arched fingers, a variation observed in small (LC 13.3 mm) to large (LC 35.1 mm) specimens that were molecularly characterized (Figs. 3, 4). As in *M. digueti* and *M. occidentale* (García-Velazco *et al.*, 2014, 2017), *M. hobbsi* has a convex inferior orbit, a strongly developed bec ocellaire with a truncated apex, and a large and well-pigmented cornea with an accessory pigmented spot (Fig. 5). The epistome of *M. hobbsi* is divided into two anteriorly-rounded lobes as reported for *M. digueti* and *M. occidentale* (García-Velazco *et al.*, 2014, 2017), *M. olfersii* (Pileggi & Mantelatto, 2012), and for many Australian species of *Macrobrachium* (Short, 2004).

The T4 has been used in the taxonomy of the Australian (Short, 2004) and the Mexican Pacific species of *Macrobrachium* (García-Velazco *et al.*, 2014). We previously reported *M. digueti* and *M. hobbsi* with a similar T4, having a well-developed median process consisting of two small postero-lateral protuberances and a larger antero-central protuberance (García-Velazco *et al.*, 2014). Such morphology is in line with the description by Pérez-Tello *et al.* (2016) for the putative *M. olfersii* from Sonora state. Pileggi & Mantelatto (2012) described the T4 of *M. olfersii* as a well-developed median process forming an acute tip. As in *M. digueti* and *M. occidentale* (García-Velazco *et al.*, 2014, 2017), the T8 in *M. hobbsi* is also a sexually dimorphic character, where the lobes are joined postero-medially in males and widely separated in females (Fig. 5C–E). Short (2004) also proposed that the pre-anal carina in the inter-uropodal sclerite is an important diagnostic feature for the Australian species of *Macrobrachium*. Males and females of *M. hobbsi* have a well-developed preanal carina similar to *M. occidentale* and *M. digueti* (García-Velazco *et al.*, 2014, 2017), but normally without dorsal setae (Fig. 5F), similar to that of the putative *M. olfersii* from Sonora state (Pérez-Tello *et al.*, 2016). A well-developed pre-anal carina also occurs in the Atlantic *M. olfersii* (Pileggi & Mantelatto, 2012). Detailed description of more features of *M. hobbsi* were given in the original description (Villalobos Hiriart & Nates Rodríguez, 1990).

We covered about 3,100 km along the Pacific slope, 1,100 km on the peninsula, and 2,000 km on the mainland, and the most common species of *Macrobrachium* recorded along the Mexican Pacific slope was *M. hobbsi*. It is an amphidromous shrimp that has an extended larval development and occurs on the coast and in upstream freshwater bodies. We found it in waters from 4 to 362 masl, often at remote sites up to 110 km from the coast. Water conditions were 19.0–34.5 °C, TDS 0.10–3.50 g l^{-1}, and pH 6.7–9.3. As in other amphidromous crustaceans, such as the Chilean *Cryphiops caementarius* (Molina, 1782) (Dennenmoser *et al.*, 2010), *M. occidentale* (García-Velazco *et al.*, 2014), and *M. digueti* (García-Velazco *et al.*, 2017), *M. hobbsi* disperses widely in coastal areas, resulting in high haplotype diversity but no significant geographical structuring. Yet, *M. hobbsi*, as its congeners *M. digueti* and *M. occidentale*, could not invade water bodies in the upper part of the Gulf of California (Fig. 8), presenting a disjunct distribution, as recorded for other caridean shrimps (Hendrickx, 1995; Hernández *et al.*, 2007; García-Velazco *et al.*, 2014, 2017). Haplotypes of *M. hobbsi* shared by the peninsula and mainland populations, along with the findings of García-Velazco *et al.* (2014, 2017) for *M. occidentale*, and *M. digueti*, are most likely due to flow patterns within the Gulf of California, cyclonic in summer and anticyclonic the rest of the year (Bray, 1988; Paden *et al.*, 1991), which facilitates a constant genetic flow of these species. Our results support the view that oceanic dispersal is responsible for the wide distribution of the species of *Macrobrachium* rather than by vicariance (de Bruyn *et al.*, 2005; Murphy & Austin, 2005; Chen *et al.*, 2009). The number of species of *Macrobrachium* on the Mexican Pacific slope is here updated to five, *M. americanum*, *M. digueti*, *M. hobbsi*, *M. occidentale*, and *M. tenellum*.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Geographical distribution of 16S rDNA haplotypes (470-bp) of *M. hobbsi*.

S2 Table. Percentage of uncorrected pairwise genetic distances between 16S haplotypes of *M. hobbsi*.

S3 Table. Geographical distribution of COI (542-bp) haplotypes of *M. hobbsi*.

S4 Table. Percentage of uncorrected pairwise genetic distances between COI haplotypes of *M. hobbsi*.

S5 Table. Geographical distribution of combined 16S and COI haplotypes of *M. hobbsi*.

S6 Table. Percentage of uncorrected pairwise genetic distances between 48 haplotypes of combined 16S and COI of *M. hobbsi*.

S7 Table. 16S and COI sequences of *Macrobrachium* and *Exopalaemon* used in the study.

S8 Table. Material examined.

S9 Table. Uncorrected pairwise genetic distances (%) between the 16S haplotypes of *M. hobbsi* and *M. olfersii*.

S10 Table. Uncorrected pairwise genetic distances (%) between the COI haplotypes of *M. hobbsi* and *M. olfersii*.

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