Journal of Crustacean Biology

The Crustacean Society

Journal of
Crustacean Biology

Journal of Crustacean Biology, 37(2), 168–186, 2017. doi:10.1093/jcbiol/rux008

The systematics of the Mexican populations of *Macrobrachium digueti* (Bouvier, 1895) (Decapoda: Caridea: Palaemonidae)

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(Received 27 September 2016; accepted 20 December 2016)

ABSTRACT

Macrobrachium digueti (Bouvier, 1895), an amphidromous shrimp with unequal chelae, was described from Mulegé River in Baja California Peninsula, Mexico. A controversy on the morphological identity of *M. digueti* started six decades ago. Some authors point out that the redescription given in 1952 by L. Holthuis in his important revision of the Palaemonidae of the American continent does not correspond to Bouvier's species. A controversy also exists on the taxonomy of the species because morphological and molecular studies have indicated great similarity of *M. acanthochirus* Villalobos, 1967 and *M. michoacanus* Nates and Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990 with *M. digueti* (*sensu* Bouvier), suggesting their synonymy with the last species. We review the systematics of the Mexican populations of the putative *M. digueti* (Bouvier, 1895) consisting by individuals with unequal chelae, a carpus shorter than the merus and scarce pubescence on the palm and cutting edges of the fingers of the larger male second pereiopod. Extensive field surveys of the species of *Macrobrachium* Spence Bate, 1868 were made along the Pacific slope of Mexico, from the Baja California Peninsula in the north to Guerrero state in the south, an area that covered the type localities of the three nominal species. Results of the morphological revision and molecular genetic analyses with fragments of the mitochondrial genes 16S and COI confirmed the morphological identity of *M. digueti* as described by Bouvier and indicate that this entity is a morphologically plastic genetic species with a disjunct distribution along the peninsula and the Pacific mainland. Our study also confirms that *M. acanthochirus* and *M. michoacanus* are junior synonyms of *M. digueti*. Implication of present findings on the amphiamerican species groups of *Macrobrachium* is discussed.

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

INTRODUCTION

Macrobrachium digueti (Bouvier, 1895) is an amphidromous shrimp and thus occurring along coasts as well as in upstream freshwater environments. Bouvier (1895) described it as *Palemon digueti* using specimens collected in the Mulegé River, Baja California Peninsula. He also reported the presence of *M. americanum* Spence Bate, 1868 (cited as "*Palemon jamaicensis* Herbst") and *M. tenellum* (Smith, 1871) (as *Palemon forceps* H. Milne Edwards, 1837) in the same river. Prior to this, Lockington (1878) had already recorded *M. tenellum* (as a new species, *Palaemon longipes* Lockington, 1878) from Río Mulegé. On the southern part of the peninsula, Rathbun (1902) reported *M. olfersii* (Wiegmann, 1836) (as *Bithynis olfersii*) from La Paz and Cabo San Lucas. The three species

recorded by Bouvier (1895) were later listed occurring on the peninsula by Holthuis (1952), Rodriguez De la Cruz (1968), Wicksten & Hendrickx (1992, 2003), and Hendrickx (1994). Hernández *et al.* (2007) increased that number of species to six by adding *M. hobbsi* Nates and Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990 and *M. michoacanus* Nates and Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990. More recently García-Velazco *et al.* (2014) recorded the seventh species, *M. occidentale* Holthuis, 1950. Another species of *Macrobrachium* Spence Bate, 1868, *M. acanthochirus* Villalobos, 1967 from the states of Colima and Oaxaca was proposed as a synonym of *M. digueti* by Hernández *et al.* (2007) because of their morphological similarities. Thus, seven morphologically-distinct species of *Macrobrachium* that occur on the Pacific slope of the Mexican mainland also occupy the peninsula (García-Velazco *et al.*, 2014).

A controversy on the morphological identity of *M. digueti* started six decades ago. Bouvier (1895) described *M. digueti* with the second large chela lacking large setae over the external face and between the fingers and drew the larger second pereiopod of the male with gaping fingers and the carpus shorter than the merus. Holthuis (1952), in his important revision on the Palaemonidae of the American continent, redescribed *M. digueti* with the merus slightly shorter than the carpus or as long as that joint, fingers gaping and bearing tufts of hairs along the cutting edges and the propodus with a large, thickly pubescent large area at each of the lateral surfaces. Villalobos (1969) and Hernández *et al.* (2007) examined specimens from the peninsula (type locality area) and concluded that their morphology fit well with Bouvier's diagnosis and that the redescription given by Holthuis (1952) does not correspond to the original species of Bouvier. Villalobos (1969) advanced the hypothesis of the existence of a natural species cluster (*olfersii* group) deduced from the morphology of the male second pair of pereiopods. The cluster is composed of six species: *M. olfersii* (considered the trunk of the group) distributed on the Atlantic slope of the American continent from Tamaulipas, Mexico to southern Brazil and on the Pacific slope in Oaxaca, Mexico; *M. digueti* from the Baja California Peninsula, *M. faustinum* (De Saussure, 1857) from the West Indies; *M. acanthochirus* from the west coast of Mexico from Colima to Oaxaca; *M. crenulatum* Holthuis, 1950 on the Atlantic slope in Panama, Venezuela, Jamaica, Trinidad, and Dominican Republic; and *M. hancocki* Holthuis, 1950 on the Pacific slope in Costa Rica, Colombia, Panama, and the Galapagos and Cocos islands. Holthuis (1950, 1952) had previously mentioned the great morphological similarities in *M. digueti* and *M. olfersii* and *M. crenulatum* and *M. hancocki* pairs of species. The affinity of *M. hobbsi* and *M. michoacanus*, distributed on the Pacific slope, to the *olfersii* group was noted by Villalobos Hiriart & Nates Rodríguez (1990) in the morphology of the propodus of the larger second pereiopod of males. These authors also recognized that the external ornamentation pattern of the propodus of *M. michoacanus* is very similar to *M. acanthochirus* and *M. digueti*, whereas the propodus ornamentation in *M. hobbsi* is more related to *M. olfersii*. Acuña Gómez *et al.* (2013), using partial sequences (385 bp length) of the mitochondrial gene 16S rRNA (16S), inferred the phylogenetic relationship among Mexican representatives of 16 nominal *Macrobrachium* species and reported 100% genetic similarity between *M. acanthochirus, M. digueti*, and *M. michoacanus*.

In his identification key to the American species of *Macrobrachium*, Holthuis (1952) gave much taxonomic importance to the proportion of the length between the carpus and the merus of the second pereiopod; thus, as a first step, his key indicated the identification of *M. digueti* and *M. olfersii* with a carpus of the second leg as long as or longer than the merus and to *M. crenulatum* and *M. hancocki* with a carpus distinctly shorter than the merus. The key to the *Macrobrachium* species of northwestern Mexico by Hernández *et al.* (2007) separates the four Pacific slope species of the *olfersii* group on the basis of three main morphological characteristics of the larger male second pereiopod: 1) shape of the fingers being closed or gaping, 2) pubescence of the palm, and 3) length of the carpus respect to the length of the merus.

We revised the geographical distribution of these four species within the Mexican Pacific slope drainage area, including the Baja California Peninsula, performed molecular genetic analyses using mitochondrial gene fragments of 16S and cytochrome oxidase subunit I (COI), and confirmed the findings of Acuña Gómez *et al.* (2013) that the so called *olfersii* group is composed of two subclusters, which correspond to the morphological species groups noted by Villalobos Hiriart & Nates Rodríguez (1990).

The distribution of the species of *Macrobrachium* along their northern Pacific coastal range is not continuous. There is a gap for about 2000 km of records along the northern part of the Gulf of California slope separating geographically the peninsular populations from those of the mainland (Hendrickx, 1995; Hernández *et al.*, 2007; García-Velazco *et al.*, 2014). In spite of this, García-Velazco *et al.* (2014) reported the presence of the same genetic entity of *M. occidentale* in the peninsula and the Pacific mainland slopes and concluded that the disjunct coastal distribution of this species is better explained by oceanic dispersal than by vicariance. We reviewed the Mexican populations of the putative *M. digueti* formed by individuals with unequal chelae, carpus shorter than the merus, and scarce pubescence over the palm and cutting edges of the fingers of the larger second pereiopod of males. We made an extensive survey along the Pacific slope from the Baja California (Norte) state in the north to the mainland Guerrero state in the south. The study was carried out on material obtained in 104 sites in 44 drainage basins across nine Mexican states and the type localities of *M. acanthochirus, M. digueti*, and *M. michoacanus* and specimens from the collection of Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Baja California Sur. We performed molecular genetic analyses with newly generated fragments of the mitochondrial genes 16S (494 bp) and COI (560 bp) and determined whether individuals from the Pacific slope of mainland Mexico belong to the same entity found on the peninsula.

MATERIALS AND METHODS

Field collections

An extensive field survey for the species of *Macrobrachium* was made from 2008 through 2013 along the Pacific slope of Mexico, from the Baja California Peninsula to Guerrero state on the mainland. We collected shrimps using hand nets and casting nets. At the time of sampling at most of the sites, temperature, total dissolved solids and pH of water were measured (EC300, pH100; YSI, Yellow Springs, OH, USA). The geographic position of the sites was determined with a GPS unit. The specimens were fixed in 100% ethanol and deposited in the crustacean collection at Centro de Investigaciones Biológicas del Noroeste (CIBNOR).

Revision of morphology

Hernández *et al.* (2007) examined the type materials of *M. digueti*, deposited in the Muséum national d'Historie naturelle, Paris, and of *M. acanthochirus* and *M. michoacanus*, deposited in the Colección Nacional de Crustáceos, Universidad Nacional Autónoma de México. We revised the morphology of additional specimens and specimens previously deposited at CIBNOR by following the key to species of Hernández *et al.* (2007), focusing on specimens having unequal chelae on the second pair of pereiopods, straight rostrum, and larger second pereiopod with carpus shorter than the merus and scarce pubescence and setae over the palm. Individuals with other morphological features were identified as *M. occidentale*, which was revised by García-Velazco *et al.* (2014), or as *M. americanum*,

M. hobbsi, or *M. tenellum* (unpublished data). Males and females were distinguished by the presence or absence of the appendix masculina on the second pleopods and confirmed by the morphology of the thoracic sternite 8 (T8). The following measurements were recorded using a digital caliper: total length (TL; tip of rostrum to posterior end of telson), carapace length (CL; tip of rostrum to posterior dorsal margin of carapace), length and height of merus, carpus, and propodus (palm), and length of dactylus of the larger chela of the second pair of pereiopods. Ratios of merus length to carpus length (MeL:CaL) and propodus length to propodus height (PrL:PrH) were calculated. We recorded the number of teeth on both margins of the rostrum (without apical tooth). We also recorded the inferior orbit shape, bec ocellaire, epistome, thoracic sternite 4 (T4), T8, and the pre-anal carina on inter-uropodal sclerite as proposed by Short (2004). A database containing all morphometric and meristic data was compiled and was deposited in the crustacean collection at CIBNOR.

DNA extraction, amplification, and sequencing

Muscle tissue was extracted from the ventral side of shrimps and their genomic DNA was isolated using the Gentra Puregene kit (Qiagen, Minneapolis, MN, USA) with some modifications. Amplifications of a fragment of 16S with primers 1471B and 1472B (Liu *et al*, 2007) and a fragment of COI with primers COI-a and COI-f (Palumbi & Benzie, 1991) were carried under thermocycling: initial denaturation at 94 °C for 4 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 5 min (García-Velazco *et al*., 2014). Amplified products were verified and sequenced with forward primers. To validate accuracy, a few samples were sequenced with reverse primers and a few samples were also resequenced bi-directionally. To edit sequences, we used DNA Baser 3.5 (www.dnabaser.com) and Clustal X software to generate multiple alignments of the sequences under default settings (Thompson *et al*., 1997).

Molecular analysis

Sequences of 16S gene fragments were obtained from 105 specimens, 65 from seven drainage basins on the Baja California Peninsula and 40 from seven basins on mainland Mexico. Sequences of COI gene fragments were obtained from 60 individuals, 26 from seven basins on the peninsula and 34 from six basins on the mainland (see below for material examined and locations). All the sequences were deposited in the GenBank under accession numbers KY111030–KY111194. We determined the genetic identity of these individuals through haplotype delineation; haplotypic diversity was estimated as well. The genetic information of these individuals was used to analyze the population structure and demographic history by separately treating the peninsular and mainland groups. We tested the monophyly and species status of the studied populations through phylogenetic analyses using GenBank sequences of selected freshwater shrimps of the American continent.

Haplotypic diversity, population structure, and demographic history

Sequence polymorphisms and genetic diversity analyses, including number of variable sites, number of haplotypes, and haplotype diversity were estimated using DnaSP 5.10 software (Librado & Rozas, 2009). Uncorrected pairwise genetic distances (*p*-distance) among the haplotypes were calculated with MEGA5 software (Tamura *et al*., 2011). Relationships between haplotypes of each gene were estimated using a median-joining haplotype network with Network 4.6 software (Fluxus Technology, Sudbury, UK) (Bandelt *et al*., 1999). We tested whether geographical isolation is manifested in genetic differentiation by separating the concatenated 16S and COI sequence data from the peninsula as one group and the data from the mainland as another group with the analysis of molecular variance (AMOVA) (Excoffier & Lischer, 2010). To explore the demographic history of populations, mismatch distribution and raggedness index based on the observed and simulated differences among haplotypes, DnaSP was used (Slatkin & Hudson, 1991; Rogers & Harpending, 1992).

Phylogenetic analysis

Phylogenetic reconstruction was carried out with combined partial sequences of 16S and COI genes to assess whether the putative *Macrobrachium digueti* populations form a monophyletic clade and to study its phylogenetic relationship with other *Macrobrachium* species from the American continent. In the phylogenetic analyses we included eight 16S-COI haplotypes of *M. digueti*, Hap 2, Hap 6, Hap 13, Hap 14, Hap 17, Hap 23, Hap 24, and Hap 28, that our study (Table 3) showed to have a broad range of genetic distances. We added the following GenBank sequences in the analyses: *M. acanthochirus* from Huatulco, Oaxaca, Mexico (16S-JQ805798, COI-JQ805897); *M. acanthurus* (Weigmann, 1836), 1) Guaraqueçaba, Paraná, Brazil (16S-HM352444, COI-HM352485) and 2) Bocas del Toro, Panama (16S-KM101467, COI-KM101541); *M. amazonicum* (Heller, 1862), 1) Itacoatiara, Amazonas, Brazil (16S-HM352443, COI-HM352488) and 2) Atlantic slope, Panama (16S-KM101468, COI-KM101542); *M. americanum*, 1) Pacific slope, Costa Rica (16S-HM352447, COI-HM352489) and 2) Río Cabuya, Panama (16S-KM101469, COI-KM101543); *M. borellii* (Nobilii, 1896), Buenos Aires, Argentina (16S-HM352426, COI-HM352480); *M. brasiliense* (Heller, 1862), Serra Azul, São Paulo, Brazil (16S-HM352429, COI-HM352481); *M. carcinus* (Linnaeus, 1758), 1) Santana, Amapá, Brazil (16S-HM352448, COI-HM352490) and 2) Cahuita, Costa Rica (16S-KM101474, COI-KM101549); *M. crenulatum*, 1) Isla Margarita, Venezuela (16S-HM352463, COI-HM352498) and 2) Atlantic slope, Costa Rica (16S-JQ805804, COI-JQ805900); *M. digueti*, 1) Pacific slope, Costa Rica (16S-JQ805806, COI-JQ805903), 2) Río Aranjuez, Costa Rica (16S-KM101476, COI-KM101551) and 3) Pacific slope, Mexico (16S-JQ805808, COI-JQ805906); *M. hancocki*, 1) Pacific slope of Panama (16S-JQ805817, COI-JQ805915) and 2) Costa Rica (16S-JQ805822, COI-JQ805919); *M. heterochirus* (Weigmann, 1836), 1) Ilha de São Sebastião, São Paulo, Brazil (16S-HM352454, COI-HM35249) and 2) Veracruz, Mexico (16S-KM101479, COI-KM101554; *M. jelskii* (Miers, 1877), Pereira Barreto, São Paulo, Brazil (16S-HM352437, COI-HM352484); *M. michoacanus*, Oaxaca, Mexico (16S-KM101480, COI-KM101555); *M. occidentale*, 1) Río Aranjuez, Costa Rica (16S-KM101482, COI-KM101557), 2) El Chucarro, Plutarco E. Calles Basin, Baja California Sur, Mexico (16S-KF636830, COI-KF636901), and 3) Oaxaca, Mexico (16S-KM101481, COI-KM101556); *M. olfersii*, 1) Ilha de São Sebastião, São Paulo, Brazil (16S-HM352459, COI-HM352496), 2) Reserva Veragua, Costa Rica (16S-KM101483, COI-KM101560), and 3) Isla Margarita, Venezuela (16S-HM352460, COI-KM101559); *M. panamense* Rathbun, 1912, 1) Río Tempisque, Costa Rica (16S-KM101484, COI-KM101561) and 2) Guanacaste, Costa Rica (16S-KM101486, COI-KM101563); *M. tenellum*, 1) Guanacaste, Costa Rica (16S-KM101489, COI-KM101568) and 2) Oaxaca, Mexico (16S-KM101487, COI-KM101566); *Exopalaemon carinicauda* (Holthuis, 1950), China (16S-EF560650, COI-EF560650), and *E. modestus* (Heller, 1862), China (16S-DQ194971, COI-AB235307). Two GenBank sequences were included in the analyses for most species of *Macrobrachium* depending on the availability of sequences. We determined the best fit model of nucleotide substitution based on the Bayesian Information Criterion (BIC) for the combined data (16S–COI) using jModelTest 2.1.4 software (Darriba *et al*., 2012). The selected model was implemented in phylogenetic

reconstruction methods, maximum likelihood (ML) and Bayesian inference (BI). The ML analysis was performed with PAUP*4 beta 10 software (Swofford, 2002) with 500 pseudoreplicates and the BI was performed with MrBayes 3.2 software (Ronquist *et al.*, 2012) for 10 million generations with sampling at every 1000 generations. A consensus tree was generated based on majority rule. In the BI, the consensus tree was generated after the elimination of the first 25% of trees, using the burn-in option. Maximum parsimony (MP) analysis was also performed in PAUP*4 beta 10 with 10,000 pseudoreplicates. Tree-searching in MP included heuristic search option with tree bisection-reconnection branch-swapping and 100 random taxon additions per bootstrap; majority rule was used to generate a consensus tree.

SYSTEMATICS

The synonymy of species is restricted to species from Mexican material. The diagnosis of *Macrobrachium digueti* (Bouvier, 1895) is based on the descriptions given by Bouvier (1895), Villalobos (1969), and Hernández *et al.* (2007), and is updated with the morphological variations found in the examined material.

The geographical information of sites where *Macrobrachium* species were absent is available in Appendix 1 of García-Velazco *et al.* (2014).

Palaemonidae Rafinesque, 1815
Macrobrachium Spence Bate, 1868
Macrobrachium digueti (Bouvier, 1895)

(Figs. 1–8)

Palemon digueti Bouvier, 1895

Macrobrachium digueti (Bouvier, 1895) — Holthuis, 1952, 1954, 1980; Rodriguez De la Cruz, 1968; Villalobos, 1969; Hendrickx *et al.*, 1983; Wicksten, 1983, 1989; Abele & Kim, 1989; Román-Contreras, 1991; Wicksten & Hendrickx, 1992, 2003; Villalobos-Hiriart *et al.*, 1993; Hendrickx, 1994; Hernández *et al.*, 2007; Villalobos-Hiriart *et al.*, 2010; Hendrickx & Wicksten, 2011; De Grave & Fransen, 2011; Anger, 2013; García-Velazco *et al.*, 2014.

Macrobrachium acanthochirus Villalobos, 1967 — Villalobos, 1969; Wicksten, 1989; Villalobos Hiriart & Nates Rodríguez, 1990; Wicksten & Hendrickx, 1992, 2003; Román *et al.*, 2000; Wicksten & Hendrickx, 1992, 2003; Hernández *et al.*, 2007; Villalobos-Hiriart *et al.*, 2010.

Macrobrachium michoacanus Nates & Villalobos in Villalobos Hiriart & Nates Rodríguez, 1990 — Villalobos-Hiriart *et al.*, 1993; Wicksten & Hendrickx, 2003; Hernández *et al.*, 2007; Villalobos-Hiriart *et al.*, 2010; García-Velazco *et al.*, 2014.

Type locality: Río Mulegé and irrigation channels, Baja California Sur, Mexico (Bouvier, 1895).

Diagnosis: Rostrum narrow, straight (Figs. 1–3), reaching last joint of antennular peduncle; upper margin with 11–18 teeth, 3–8 of which are postorbital; lower margin with 2–8 teeth; antennules, eyes normal in shape. Second pereiopods similar in shape but unequal in size in adult males (Figs. 1–5). Propodus of largest chela little compressed laterally, ratio of length to height (PrL:PrH) variable, normally 1.0–1.9 (range 0.9–2.8); dorsal, lateral sides with distinct spines, scarce pubescence, setae; dorsal, ventral margins slightly curved or straight (Figs. 1–5); ventral, inner sides of propodus, dactylus with small spines, tubercles (Fig. 1 C, E). Fingers as long as propodus, with highly variable morphology; dactylus can be straight, not leaving a gap between fingers, or curved upward leaving narrow to a large gap between fingers; fixed finger may be curved downward leaving gap between fingers (Figs. 1–7); proximal half of cutting edge of fixed finger normally with series of large teeth and denticles, dactylus with denticles, 1, 2 distinct rounded teeth (Fig. 1 A–C); cutting edges with no or scarce pubescence, setae. Carpus globose at distal half (Figs. 1–4), shorter than merus, MeL:CaL normally 1.3–1.7 (range 1.0–2.3); merus as long as propodus. Pleopods, uropods normal in shape. Carapace length of largest male examined 40.1 mm (Santa Rita Basin, Baja California Sur), largest female examined 37.3 mm (La Paz Basin, Baja California Sur).

Additional morphological characters: Shape of inferior orbit distinctly convex, moderately produced (Fig. 2C); ocular cornea large, well pigmented with accessory pigment spot (Fig. 8A); bec ocellaire strongly developed with apex truncated (Fig. 8B); epistome with lobes rounded; T4 with well-developed median process with 2, posteriorly separated, small protuberances, larger anterior protuberance; T8 in males with joined lobes (Fig. 8C, E), widely separated lobes in females (Fig. 8D); inter-uropodal sclerites with well-developed preanal carina, normally without dorsal setae (Fig. 8F).

Haplotypic identity: Haplotypes of the 16S and COI genes were shared by individuals from the Baja California Peninsula and the mainland (Tables 1 and 2). Fifteen 16S haplotypes, based on polymorphisms at 14 nucleotide sites in the 494 bp fragments of 105 individuals from 14 drainage basins were obtained (Table 1). Most of the haplotypes presented polymorphism at two nucleotide sites. Maximum number of polymorphic sites observed between haplotypes was four. The uncorrected pairwise genetic distances range between 16S haplotypes was 0.2–0.81%. Haplotype (Hap) 7 was obtained from two sites, one in the Plutarco E. Calles Basin in Baja California Sur and the second in the Río Ameca Ixtapa B Basin in Nayarit state, mainland Mexico, and showed a maximum genetic distance of 0.81%, with Hap 8 represented by a single sequence from the Río Baluarte 2 Basin in Sinaloa state. All polymorphic nucleotide sites were of transition types. Specific 16S haplotype sequences are shown in Supplementary Material Table S1. The three shared 16S haplotypes Hap 1, 6 and 7 were found on the peninsula and the mainland (Table 1). The COI had 21 haplotypes from the 560 bp fragments of 60 individuals from 13 drainage basins (Table 2). These haplotypes differed at 17 nucleotide sites and most of them presented two to three polymorphic sites. About 90% of the variable sites were of the transition type. Maximum number of polymorphic sites observed between haplotypes was seven. The uncorrected pairwise genetic distances range between COI haplotypes was 0.18–1.25%. One sequence from the Río Verde Basin in Oaxaca state representing Hap 2 and another sequence representing the Hap 12 from the Río Purificación Basin in Jalisco state had the maximum genetic distance of 1.25%. Specific COI haplotype sequences are shown in Supplementary Material Table S2. The six shared COI haplotypes Hap 1, 3, 5, 6, 9, and 13 were found on the peninsula and the mainland (Table 2).

Distribution in Mexico (Fig. 9): Baja California Sur: The species had been reported from Mulegé, La Paz, Santiago, Boca de La Sierra, and Los Potreros (Bouvier, 1895; Villalobos, 1969; Hernández *et al.*, 2007). New site records are San Pedro de la Presa, Santa Rita Basin, Todos Santos, Todos Santos Basin, Oasis San Pedrito, Pescaderos Basin and San Venancio, Plutarco E. Calles Basin; Sinaloa: Río Baluarte, Laguna Caimanero (Hernández *et al.*, 2007); new records from El Roble, La Cruz de Elota, Río Elota Basin, and Estación Dimas, Río Piaxtla 2 Basin; Nayarit: El Colomo, Río Ameca Ixtapa B Basin (Hernández *et al.*, 2007); Jalisco: Río Cuitzmala, Chamela and Río Las Aletas, and Río Los Cuartones (Hernández *et al.*, 2007); new record from La Huerta, Río Purificación Basin; Colima: Tecomán (Villalobos, 1967) and Puerto Juárez (Hernández *et al.*, 2007); Michoacán: Presa Morelos (La Villita), Río Mexcalhuacán, Río Chucatitlán, Río Papoyutla Mexcaltitlán, and Río Murga (Villalobos Hiriart & Nates Rodríguez, 1990; Hernández *et al.*, 2007); new record from La Angostura, Río Balsas; Guerrero: Acapulco, Río Coyuca (Villalobos Hiriart & Nates Rodríguez, 1990; Hernández *et al.*, 2007); Oaxaca: Río Valdeflores (Villalobos, 1967), Río Copalita, Río Coyula, Río Zimatán (Villalobos Hiriart & Nates Rodríguez,

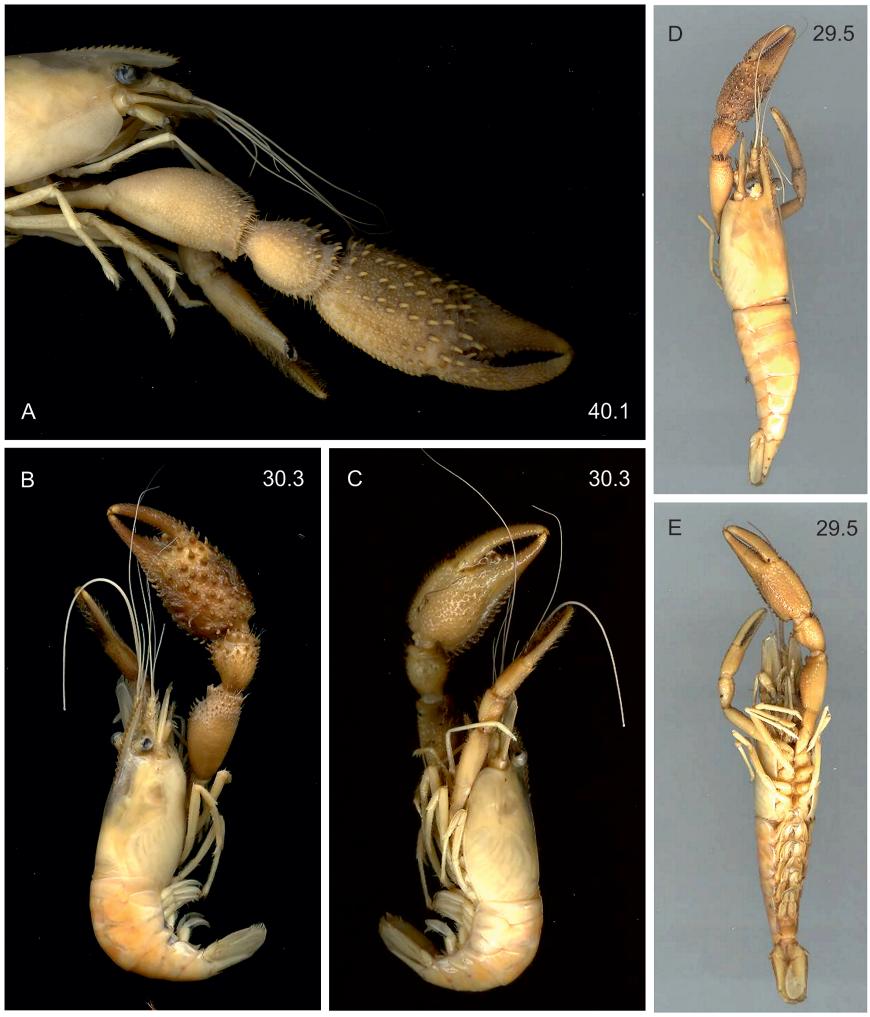

Figure 1. Adult males of *Macrobrachium digueti* (Bouvier, 1895). A, anterior part of body with the larger chela of second pair of pereiopods in right lateral view of specimen (CIB-1103.1), San Pedro de la Presa, Santa Rita Basin, Baja California Peninsula; B–E, specimens (CIB-1114.5, CIB-1130.4) from Río Coyuca, Vado Aguas Blancas, Coyuca de Benítez, Río Coyuca 2 Basin, Guerrero, mainland Mexico. Numbers refer to the cephalic length (in mm). This figure is available in color at *Journal of Crustacean Biology* online.

1990; Hernández *et al.*, 2007; Villalobos *et al.*, 2010), new record from Río Viejo in Río Verde Basin.

Several authors who recorded *Macrobrachium* in Mexico followed the morphological description of *M. digueti* given by Holthuis (1952). This description, however, does not correspond to the original species of Bouvier (1895) (Villalobos, 1969; Hernández *et al.*, 2007; this study). The following records of *M. digueti* need to be revised: Baja California Sur (Holthuis, 1952; Hendrickx, 1994), Sonora (Rodriguez De la Cruz, 1968), Sinaloa (Hendrickx *et al.*, 1983; Wicksten, 1983; Hendrickx & Wicksten, 2011), Guerrero (Holthuis, 1952; Wicksten & Hendrickx, 2003), and Oaxaca (Wicksten & Hendrickx, 2003).

General geographical distribution: *Macrobrachium digueti* has been reported from Mexico to Peru, but we propose that the following records also require a revision based on the problem mentioned about the morphological description of *M. digueti* of Holthuis (1952): Guatemala: Río Camaya, Río Matapa between Escuintla and Chiquimulilla (Holthuis, 1952); El Salvador: Río Zunzal, Río Coyol, Río Chimalapa, Río Banderas, Río Mandinga, Río Comasagua, Río Huiza, Río Jiboa, Río Lempa, San Vicente, Lomas de La Coyotera, Río Sirama (Holthuis, 1954); Costa Rica: Río Grande de Terraba (Rólier-Lara & Wehrtmann, 2011); Panama: Río Chamé, Chorrera, Araijan, Pedro Miguel, Río Mamoni, Río Trinidad, Río Chucunaque, Río Yape, Río Cupe, Isla San José, Archipiélago de Las Perlas, Río Santa María, Río Los Chorros, Río Cobre, Río La Villa and Río Parita, Panama Canal (Holthuis, 1952; Abele & Blum, 1977; Abele & Kim, 1989; Vega *et al.*, 2006); Colombia: Río Calima (Valencia & Campos, 2007; Campos, 2014); Ecuador: Porto Viejo (Holthuis, 1952: 106); Peru: Río Zarumilla, Río Tumbos, Río Lambayoque, Río Reque, Río Chicama and Río Moche (Amaya & Guerra, 1976).

Material examined: The examined material includes the number of individuals used for DNA analysis (in parentheses), TL or CL (in parentheses), and the characteristics of water. The names of the drainage basins are from the classification published by the Comisión Nacional del Agua (www.conagua.gob.mx/CONAGUA07/.../TM(Cuencas_Hidrológicas).xls). Mexico: Baja California Sur: Santa Rita Basin: Oasis Santa María de Toris, 24°53′00.0″N, 111°02′17.0″W, 227 masl, 02 August 2009, A. Maeda coll., CIB 1198, 8 (6) males (CL 19.6–27.6 mm); 23 May 2010, A. Maeda coll., CIB 1199, 5 (1) males (CL 11.0–27.4 mm), 2 females (CL 18.3 and 20.3 mm), water 28.9 °C, TDS 0.51 g l^{-1}, pH 8.3; San Pedro de La Presa, 24°50′57.9″N, 110°59′28.1″W, 224 masl, no date, G. Talamantes coll., CIB 1103, 1 (1) male (TL 84.3 mm); 02 August 2009, A. Maeda coll., CIB 1188, 18 (10) males (CL 21.9–29.8 mm); 23 May 2010, A. Maeda coll., CIB 1195, 5 (2) males (CL 11.0–27.0 mm), 2 (1) females (16.5–19.8 mm), water 26.8 °C, TDS 0.40 g l^{-1}; 13 June 2011, A. Maeda coll., CIB 1196,

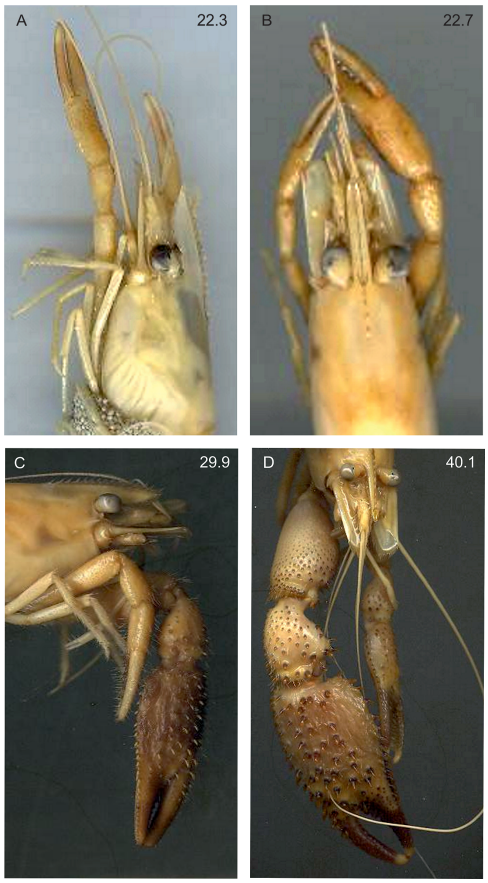

Figure 2. Female and males of *Macrobrachium digueti* (Bouvier, 1895) of same COI haplotype 13 from different drainage basins in Baja California Peninsula and mainland Mexico. A, anterior part of body in left lateral view of adult female (CIB-1153.2) from La Poza, Todos Santos Basin, Baja California Peninsula; B, anterior part of body in dorsal view of adult male (CIB-1130.1) from Río Coyuca, Vado Aguas Blancas, Coyuca de Benítez, Río Coyuca 2 Basin, Guerrero, mainland Mexico; C, anterior part of body in right lateral view of adult male (CIB-1110.3) from Canal El Colomo, Bahía Banderas, Río Ameca Ixtapa B Basin, Nayarit, mainland Mexico; D, anterior part of body in dorsal view of adult male (CIB-1103.1) from San Pedro de la Presa, Santa Rita Basin, Baja California Peninsula. Numbers refer to cephalic length (in mm). This figure is available in color at *Journal of Crustacean Biology* online.

2 males (CL 24.7 and 27.9 mm), water 30.0 °C, TDS 0.40 g l^{-1}, pH 8.6; 18 March 2012, A. Maeda coll., CIB 1197, 2 females (CL 21.6 and 25.4 mm), water 26.0 °C, TDS 0.38 g l^{-1}, pH 8.2. Las Pocitas-San Hilario Basin: Rancho Las Cuevas, 24°49′02.2″N, 110°52′45.1″W, 350 masl, 30 May 2004, L. Hernández coll., CIB 829, 11 (3) males (TL 48.7–75.3 mm), 3 females (TL 46.4–57.3 mm), water 30.0 °C, TDS 0.50 g l^{-1}, pH 8.1; La Cuchilla, 24°48′37.1″N, 110°51′01.3″W, 331 masl, 26 July 09, A. Maeda coll., CIB 1200, 1 male (CL 23.8 mm), water 34.2 °C, TDS 0.68 g l^{-1}, pH 8.1; Huatamote, 24°40′34.2″N, 110°59′22.9″W, 182 masl, 27 June 2009, A. Maeda coll., CIB 1201, 4 males (CL 10.4–13.7 mm), water 34.7 °C, TDS 0.78 g l^{-1}; 25 July 2009, A. Maeda coll., CIB 1202, 7 (3) males (CL 12.4–17.1 mm); 29 May 2010, A. Maeda coll., CIB 1203, 2 (1) females (CL 13.8–16.5 mm), water 26.4 °C, TDS 0.84 g l^{-1}; El Pilar, 24°28′22.7″N, 111°01′34.0″W, 98 masl, 27 June 2009, A. Maeda coll., CIB 1204, 11 (6) males (CL 12.9–22.3 mm), water 20.3 °C, TDS 2.87 g l^{-1}; 26 June 2011, A. Maeda coll., CIB 1205 1 (1) male (CL 25.3 mm) and 1(1) female (CL 10.3 mm), water 33.9 °C, TDS 0.53 g l^{-1}, pH, 8.5. Todos Santos Basin: Todos Santos, 23°28′35.6″N, 110°12′41.4″W, 15 masl, February 2003, L. Hernández coll., CIB 1118, 1 (1) female (TL 72.6 mm), water 25.5 °C, TDS 0.1 g l^{-1}, pH 7.3; 12 October 2007, J. Salcido coll., CIB 1105, 2 (1) males (TL 46.4 and 47.9 mm); CIB 1106, 1 (1) male (TL 50.3 mm); CIB 1119, 2 (2) males (TL 46.4 and 56.7 mm); 13 December 2007, G. Murugan coll., CIB 1027, 17 males (TL 46.3–67.0 mm); Canales Todos Santos, 23°27′16.6″N, 110°13′28.8″W, 17 masl, 19 November 2008, H. García coll., CIB 1156, 2 (2) males (TL 34.2 and 35.7 mm), 2 (1) females (TL 37.2–62.6 mm), water 24.9–25.2 °C, TDS 0.34–0.87 g l^{-1}, pH 8.1–8.3; La Poza, Todos Santos, 23°26′13.4″N, 110°14′17.3″W, 18 November 2008, H. García coll., CIB 1104, 2 (1) males (TL 46.6 and 57.8 mm); CIB 1117, 1 (1) female (TL 64.6 mm); CIB 1153, 5 (4) females (TL 46.6–63.6 mm). Pescaderos Basin: Oasis San Pedrito, 23°23′23.5″N, 110°12′40.6″W, 10 masl, 12 September 2006, A. Maeda coll., CIB 1121, 1 (1) male (TL 37.1 mm), water 25.0 °C, TDS 2.0 g l^{-1}, pH 7.3; 19 November 2008, H. García coll., CIB 1107, 2 (2) males (TL 32.6 and 36.9 mm). Plutarco E. Calles Basin: Las Vinoramas, 23°17′33.2″N, 110°01′16.4″W, 236 masl, 28 December 2010, G. Murugan coll., CIB 1122, 2 (2) males (TL 49.2 and 64.2 mm), 3 (1) females (TL 55.9–58.2 mm), water 24.6–24.8 °C, TDS 0.42 g l^{-1}; El Chucarro, 23°17′18.8″N, 110°01′47.3″W, 220 masl, 06 October 2002, A. Maeda coll., CIB 802, 2 (2) males (TL 77.6 and 82.2 mm); CIB 827, 2 females (TL 54.7 and 68.3 mm), water 32.6 °C, TDS 0.42 g l^{-1}; 14 July 2003, L. Hernández coll., CIB 831, 1 (1) male (TL 64.7 mm), 2 (2) females (TL 46.6 and 72.7 mm); San Venancio, 23°16′31.8″N, 110°02′12.9″W, 191 masl, 20 November 2008, H. García coll., CIB 1108, 2 (1) males (TL 68.7 and 76.0 mm), water 31.7 °C, TDS 0.27 g l^{-1}; San Pedro de La Soledad, 23°14′33.6″N, 109°59′29.2″W, 152 masl, 25 November 2004, L. Hernández coll., CIB-828, 1 (1) female (TL 57.8 mm), water: 25.8 °C, TDS 0.3 g l^{-1}, pH 8.0. La Paz Basin: Las Vinoramas, 24°11′53.09″N, 110°12′12.11″W, 114 masl, 29 January 2009, A. Maeda coll., CIB 1125, 5 (2) females (TL 54.9–81.4 mm), water in 21 November 2003 24.0 °C, TDS 1.5 g l^{-1}. Santiago Basin: Arroyo Santiago, 23°28′30.09″N, 109°44′20″W, May 2004, F. Cota coll., CIB 1123, 8 males (TL 41.7–69.0 mm). San José del Cabo Basin: Boca de La Sierra, 23°23′10″N, 109°49′11″W, 152 masl, 08 September 2004, L. Hernández coll., CIB 801, 2 (2) males (TL 69.9 and 78.0 mm), 2 (2) females (TL 57.7 and 81.6 mm), water 25.3 °C, TDS 0.1 g l^{-1}, pH 7.6; Oasis Santa Rosa, San José del Cabo, 23°05′18.8″N, 109°41′58.0″W, 122 masl, 15 May 2007, A. Maeda coll., CIB 1124, 1 male (TL 22.2 mm), water 22.5 °C, TDS 0.25 g l^{-1}. Sinaloa: Río Elota Basin: Río Elota, Puente El Roble, La Cruz de Elota, 23°55′11.3″N, 106°48′57.2″W, 20 masl, 28 April 2011, G. Murugan coll., CIB 1126, 1 female (TL 54.2 mm), water 25.6 °C, TDS 0.18 g l^{-1}. Río Piaxtla 2 Basin: Río Piaxtla, Estación Dimas, 23°43′39.8″N, 106°47′09.6″W, 20 masl, 29 April 2011, G. Murugan coll., CIB 1127, 2 (1) males (TL 34.2 and 39.6 mm), 1 female (TL 56.5 mm). Río Presidio 2 Basin: Río Presidio, Villa Unión 2, 23°10′57.7″N, 106°13′50.1″W, 25 masl, 02 May 2011, G. Murugan coll., CIB 1109, 1 (1) male (TL 36.6 mm), water 29.6 °C, TDS 0.14 g l^{-1}. Río Baluarte 2 Basin: Río Baluarte, El Rosario, 22°58′49.3″N, 105°51′01.9″W, 26 masl, 03 May 2011, G. Murugan coll., CIB 1128, 2 (2) females (TL 64.8 and 65.4 mm), water 27.6 °C, TDS 0.13 g l^{-1}. Nayarit: Río Ameca Ixtapa B Basin: El Colomo, 13 August 2003, L. Hernández coll., CIB 866, 6 (3) males (TL 76.1–86.5 mm), 3 (1) female (TL 62.2–68.1 mm); Canal El Colomo, Bahía Banderas, 20°53′16.5″N, 105°08′25.9″W, 60 masl, 05 May 2011, A. Maeda coll., CIB 1110, 6 (5) males (TL 62.0–69.9 mm); CIB 1129, 5 (5) males (TL 45.8–57.4 mm), water 28.2 °C, TDS 0.37 g l^{-1}. Jalisco: Río Purificación Basin: Río Purificación, La Huerta, 19°29′43.0″N, 104°40′20.2″W, 250 masl, 07 May 2011, A. Maeda coll., CIB 1111, 9 (5) males (TL 70.9–80.0 mm), water 28.8 °C, TDS 0.39 g l^{-1}, pH 7.5. Michoacán: Río Bajo Balsas Basin: La Angostura, October 2006, J. Bautista coll., CIB 1112, 1 male (TL 45.9 mm); Río Balsas, 11 February 2006, J. Bautista coll., CIB 1113, 1 male (TL 59.3 mm). Guerrero: Río Coyuca 2 Basin: Río Coyuca, Vado Aguas Blancas, Coyuca de

Figure 3. Anterior part of body with larger chela of second pair of pereiopods of adult males of *Macrobrachium digueti* (Bouvier, 1895) of same 16S haplotype 1 from different basins in mainland Mexico. A and B, males (CIB-1110.4, CIB-1110.5) from Canal El Colomo, Bahía Banderas, Río Ameca Ixtapa B Basin, Nayarit; C and D, males (CIB-1111.3, CIB-1111.1) from Río Purificación, La Huerta, Río Purificación Basin, Jalisco; E and F, males (CIB-1114.4, CIB-1114.5) from Río Coyuca, Vado Aguas Blancas, Coyuca de Benítez, Río Coyuca 2 Basin, Guerrero. Numbers refer to cephalic length (in mm). This figure is available in color at *Journal of Crustacean Biology* online.

Benítez, 17°03′20.7″N, 100°01′48.5″W, 80 masl, 11 May 2011, A. Maeda coll., CIB-1114, 5 (5) males (TL 57.0–65.9 mm); CIB-1130, 9 (5) males (TL 47.7–63.8 mm), 4 females (TL 44.8–62.9 mm); CIB-1185, 8 (2) males (TL 35.4–53.0 mm), 6 females (TL 39.1–50.3 mm), water 30.6 - 32.1 °C, TDS 1.28 g l^{-1}, pH 8.2. Oaxaca: Río Verde Basin: Río Viejo, 16°05′56.41″N, 97°43′41.60″W, May 2008, J. Bautista coll., CIB 1115, 1 (1) males (TL 64.2 mm); 09.07.2008, J. Bautista coll., CIB 1116, 2 (2) males (TL 51.3 and 60.7 mm) and CIB 1131, 2 (2) males (TL 55.0 and 62.2 mm).

Haplotypic diversity, population structure, and demographic history

Most of the haplotypes in the 16S and COI were separated by a single mutation step; only two mutations separated one haplotype in the 16S and two haplotypes in the COI (Fig. 10; Supplementary Material Tables S1 and S2). Two haplotypes included 86.7% of the individuals analyzed in the 16S gene. Single individuals representing private haplotypes constituted 80% of the total (15) haplotypes (Table 1). The 16S haplotype diversity was 0.39. The predominant Hap 1 was found in all drainage basins except one, shared by 82 individuals. The median-joining haplotype network placed this haplotype as a central node with all private haplotypes around it indicating the absence of a geographical structure (Fig. 10A). Hap 6 was shared by five populations; all of them from the peninsula (Table 1). In the COI, 33.3% of haplotypes was found in more than one individual and haplotype diversity was 0.86. Six of 21 haplotypes were shared by individuals from the peninsula and the mainland (Table 2). The predominant Hap 5 was found in 20 specimens found in 10 drainage basins, five each from both regions (Table 2) and formed a central node of star-like clusters in the median-joining haplotype network (Fig. 10B). Other COI haplotypes occurring in both regions were 1, 3, 6, 9, and 13 (Table 2). Except for Hap 6, the other haplotypes formed a node for the derivation of haplotypes found in both regions. The COI haplotypes network provided no indication of geographical structure of *M. digueti* populations (Fig. 10B).

The combination of the 16S and COI fragments from 56 individuals yielded 28 haplotypes of 1054 bp length. Five frequent haplotypes occurred on the peninsula and mainland; Hap 2 was in three

Figure 4. Adult males of *Macrobrachium digueti* (Bouvier, 1895) of same 16S haplotype 1 from a single basin, Río Verde, Oaxaca, mainland Mexico. A-D, anterior part of body with larger chela of second pair of pereiopods in dorsal view (A, specimen CIB-1116.1; B, specimen CIB-1131.2; C, specimen CIB-1131.1; D, specimen CIB-1115.1). Numbers refer to cephalic length (in mm). This figure is available in color at *Journal of Crustacean Biology* online.

peninsular and four mainland basins and Hap 17 was in one peninsular and three mainland basins (Table 3). The hierarchical AMOVA of combined 16S and COI sequences subdivided into the peninsular and mainland groups provided no evidence for subdivisions in different drainage basins or different groups. It indicated that all variations were related to differences within drainage basins ($P > 0.05$); the fixation indices also supported genetic homogeneity (Supplementary Material Table S3). Mismatch analyses based on frequency distributions of pairwise differences between sequences provided unimodal curve, suggesting recent population demographic and range expansion (Fig. 11); the mismatch distribution was supported by the Harpending's raggedness index (0.0526, $P > 0.05$).

Phylogenetic analyses

jModeltest calculated Kimura 3-parameter model (TPM1uf) with unequal base frequencies, invariant sites (I) and gamma distributed rates (G) as the best fit model of nucleotide substitution for the concatenated 16S-COI data. This model was implemented in the ML and BI methods of phylogenetic inference. The three methods of phylogenetic reconstruction, MP, ML, and BI recovered almost identical topologies and placed *M. digueti* haplotypes in a monophyletic clade (Fig. 12). This clade also included GenBank sequences of *M. acanthochirus* and *M. michoacanus* and was strongly supported by the bootstrap values of MP and ML (100%, 94%, respectively) and the posterior probability of BI (1). The *M. digueti* clade formed a monophyletic group with the sister clades of *M. crenulatum* and *M. hancocki*. This group was also strongly supported by MP and ML (each with 100% bootstrap) and BI posterior probability of 1. The three *M. digueti* sequences from the GenBank database did not cluster with our *M. digueti* sequences, rather they were associated with the GenBank sequences of *M. olfersii* and formed a clade with high MP (99%), ML (98%) and BI (1) supports (Fig. 12). This clade was a sister clade with our *M. digueti* sequences, forming a monophyletic group (*olfersii* group), which was placed as a sister clade to *M. americanum* and *M. carcinus* species (from GenBank sequences). This node was fairly supported by MP (60%) and ML (67%), but strongly supported by the BI (1). The node that included the *M. heterochirus* and *M. occidentale* branch and the branch with the *olfersii* group, *M. americanum* and *M. carcinus*, was nevertheless well supported by the three methods of phylogenetic analyses (Fig. 12).

DISCUSSION

In agreement with Villalobos (1967) and Hernández *et al.* (2007), our morphological and molecular analyses confirmed the validity and morphological identity of *Macrobrachium digueti* as described by Bouvier (1895). The species is mainly characterized by a straight rostrum, second pereiopods with unequal chelae, carpus typically shorter than merus, and scarce pubescence and setae over the palm and cutting edges of fingers. The redescription of *M. digueti* given by Holthuis (1952) thus treated the morphology of a different entity that does not correspond to Bouvier's species. Holthuis (1952) mentioned that his *M. digueti* is most closely related to *M. olfersii* and described that its merus is slightly shorter than the carpus

Figure 5. Larger chela of second pair of pereiopods in lateral view of adult males of *Macrobrachium digueti* (Bouvier, 1895) from different drainage basins in the Baja California Peninsula and mainland Mexico. A and B, males of same 16S haplotype 7 from the Plutarco E. Calles Basin, Baja California Sur (CIB-802.1) and Río Ameca Ixtapa B Basin, Nayarit (CIB-866.2); C and D, males of same combined 16S-COI haplotype 6 from Río Ameca Ixtapa B Basin, Nayarit (CIB-1110.1) and San José del Cabo Basin, Baja California Sur (CIB-801.4); E and F, female and male of same combined 16S-COI haplotype 23 from Río Baluarte 2 Basin, Sinaloa (CIB-1128.1) and Río Purificación Basin, Jalisco (CIB-1111.1). Numbers in the figures refer to cephalic length (in mm). This figure is available in color at *Journal of Crustacean Biology* online.

or as long as that joint, the fingers are gaping and bearing tufts of hairs along the cutting edges and the propodus with a distinct, large, thickly pubescent large area at each of the lateral surfaces. He also commented that "the specimen of which Bouvier figured the second cheliped must be an exceptionally large male. It exceeds all my material in length. The carpus of the second leg of Bouvier's male seems to be shorter in relation to the merus than in my males, in which the merus moreover is less swollen than figured by Bouvier. The pubescence of the second legs in my males is more distinct than in Bouvier's specimen … The young specimens from Acapulco are referred with some doubt to the present species as they have the carpus distinctly shorter than the merus…" (Holthuis, 1952: 106). The variation of the joints of the second pereiopods is a well-known phenomenon; however, it has been recognized long ago that the relation between the carpus and the merus is constant during growth (Holthuis, 1952). As Holthuis (1952) recognized in his species key, the results of this work support the concept that the proportion of these joints is of great taxonomic importance in *Macrobrachium*. The genetic and morphological data demonstrate phenotypic plasticity of propodus and fingers of the larger male second pereiopod in *M. digueti*. The proportion of length and height of the propodus (PrL:PrH) is very variable (Figs. 6, 7), with an observed range of 0.9 to 2.8. The fingers vary from closed straight fingers up to strongly widely arched fingers. We observed this variation from small (LC 15.0 mm) to large (LC 40.1 mm) specimens that were molecularly characterized (Figs. 6, 7). There was no apparent geographical pattern in relation to the morphology of the fingers. Specimens with closed and gaping fingers were found occurring (and often co-occurring) along the whole range of *M. digueti* in Mexico, sharing the same 16S, COI and 16S-COI haplotypes (Figs. 2, 3, 5). This geographical range included the type locality areas of the nominal species *M. acanthochirus* (Colima and Oaxaca) (Villalobos, 1967) and *M. michoacanus* (Michoacán) (Villalobos Hiriart & Nates Rodríguez, 1990). *Macrobrachium acanthochirus* with gaping fingers (Villalobos, 1967) was previously synonymized with *M. digueti* by Hernández *et al.* (2007). We propose, based on our results, to maintain this synonymy and also propose that *M. michoacanus*, described with closed fingers (Villalobos Hiriart & Nates Rodríguez, 1990), also represents a junior synonym of *M. digueti*. Acuña Gómez *et al.* (2013) reported 100% genetic similarity in fragments of the 16S gene of these three forms and agreed to synonymize of *M. acanthochirus* and suggested that, by agreeing with the Hernandez *et al.* (2007) findings of distinct morphology, more studies are needed to determine whether *M. michoacanus* is a

Figure 6. Larger chela of second pair of pereiopods in lateral view of adult males of *Macrobrachium digueti* (Bouvier, 1895) 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: A, Baja California Sur (CIB-1107.2); B, Baja California Sur (CIB-1121.1); C, Oaxaca (CIB-1116.2); D, Guerrero (CIB-1130.5); E, Baja California Sur (CIB-1105.1); F, Oaxaca (CIB-1116.1); G, Baja California Sur (CIB-1106.1); H, Nayarit (CIB-1129.3); I, Oaxaca (CIB-1131.2); J, Oaxaca (CIB-1131.1); K, Guerrero (CIB-1114.3); L, Baja California Sur (CIB-1104.1); M, Baja California Sur (CIB-1122.1); N, Nayarit (CIB-1110.2); O, Guerrero (CIB-1130.4); P, Guerrero (CIB-1130.2); Q, Nayarit (CIB-1110.4); R, Guerrero (CIB-1114.5); S, Nayarit (CIB-1110.5); T, Baja California Sur (CIB-831.3). Numbers in the figures refer to cephalic length (in mm). This figure is available in color at *Journal of Crustacean Biology* online.

valid species. Morphological and molecular analyses (16S) carried out by Pileggi & Mantelatto (2010, 2012) on Brazilian populations of *Macrobrachium* have also demonstrated the existence of genetic entities (species) which exhibit great morphological variation in the second pereiopods, especially in the propodus and fingers of the larger chela, sustaining the synonymy of *M. holthuisi* Genofre & Lobão, 1978 and *M. birai* Lobão, Melo & Fernandes, 1986 with *M. olfersii* and *M. petronioi* Melo, Lobão & Fernandes, 1986 with *M. potiuna* (Müller, 1880).

Our diagnosis of *M. digueti* is updated according to the variation of morphological characters found in the Mexican material. The number of teeth of the rostrum reported by Hernández *et al.* (2007) is now increased, 11 to 18 in the dorsal carina, of which 3 to 8 are postorbital, and 2 to 8 in the ventral carina. We also describe for the first time several additional morphological characters proposed by Short (2004), such as the shape of the inferior orbit, bec ocellaire, epistome, T8, and the pre-anal carina. These structures were already described for Mexican specimens of *M. occidentale* (García-Velazco *et al.*, 2014). As in *M. occidentale*, *M. digueti* has a distinctly 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. 8). As in *M. occidentale* (García-Velazco *et al.*, 2014), *M. olfersii* (Pileggi & Mantelatto, 2012), and many Australian species of *Macrobrachium* (Short, 2004), the epistome of *M. digueti* is divided into two anteriorly-rounded lobes. Short (2004) proposed the morphology

Figure 7. Larger chela of second pair of pereiopods in lateral view of adult males of *Macrobrachium digueti* (Bouvier, 1895) 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: A, Guerrero (CIB-1114.1); B, Baja California Sur (CIB-801.4); C, Baja California Sur (CIB-1198.3); D, Baja California Sur (CIB-802.1); E, Baja California Sur (CIB-1198.1); F, Nayarit (CIB-866.4); G, Jalisco (CIB-1111.2); H, Baja California Sur (CIB-1198.4); I, Nayarit (CIB-866.2); J, Baja California Sur (CIB-1205.1); K, Baja California Sur (CIB-802.2); L, Jalisco (CIB-1111.5); M, Baja California Sur (CIB-801.1); N, Baja California Sur (CIB-1188.9); O, Baja California Sur (CIB-1199.1); P, Baja California Sur (CIB-1195.1); Q, Baja California Sur (CIB-1188.7); R, Nayarit (CIB-866.1); S, Baja California Sur (CIB-1103.1);T, Baja California Sur (CIB-1188.6). Numbers in the figures refer to cephalic length (in mm). This figure is available in color at *Journal of Crustacean Biology* online.

of the T4 as part of the differential diagnostic features of the Australian species of *Macrobrachium*, given that some species bear a well-developed median process, but it is greatly reduced or absent in other species. We previously reported *M. digueti* and *M. hobbsi* with a similar T4, having a well-developed median process consisting of two posteriorly-separated, small protuberances and a larger anterior protuberance (García-Velazco *et al.*, 2014; fig. 4). Pileggi & Mantelatto (2012) described the T4 of *M. olfersii* as a well-developed median process forming an acute tip. The T4 has a taxonomic value, given that in *M. americanum* this structure has a median process with a large anterior central protuberance and two posteriorly-separated smaller protuberances, whereas *M. occidentale* has a median process with two posteriorly-separated protuberances and a small, anterior-central protuberance and *M. tenellum* a median process showing only a large acute anterior-central protuberance (García-Velazco *et al.*, 2014: fig. 4).

The T8 has been proven to be a useful sexually dimorphic character in *M. occidentale* (García-Velazco *et al.*, 2014). It is also a sexually dimorphic character in *M. digueti*, where the lobes are joined postero-medially in males and widely separated in females (Fig. 8). Short (2004) proposed that the presence (or absence) as well as the morphology of the pre-anal carina in the inter-uropodal sclerite is part of the diagnostic features of the Australian species of *Macrobrachium*. As reported by García-Velazco *et al.* (2014) for

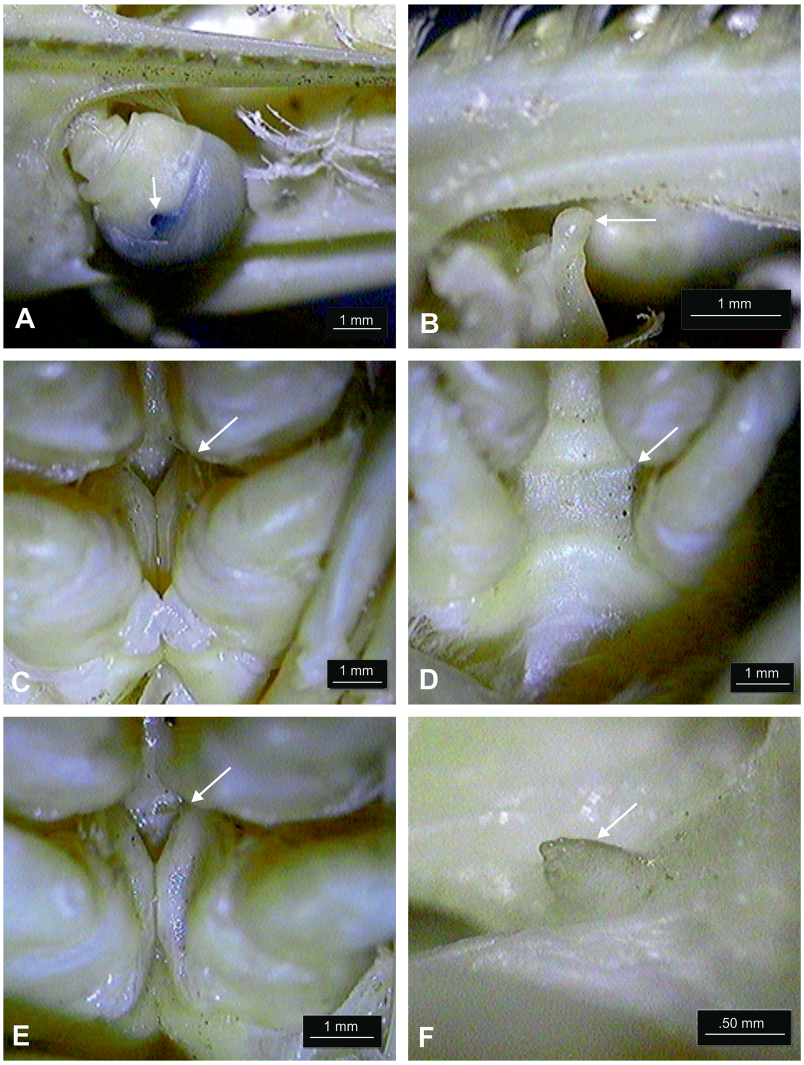

Figure 8. Males and female of *Macrobrachium digueti* (Bouvier, 1895) from the Baja California Peninsula and mainland Mexico. A, B and E, male (CIB-801.4), Boca de la Sierra, San José del Cabo Basin, Baja California Sur; C, male (CIB-866.2), El Colomo, Río Ameca Ixtapa B Basin, Nayarit, mainland Mexico; D, female (CIB-866.3), El Colomo, Río Ameca Ixtapa B Basin, Nayarit, mainland Mexico; F, female (CIB-1117.1), La Poza, Todos Santos, Todos Santos Basin, Baja California Sur. A, rostrum and right compound eye with large and well pigmented ocular cornea and accessory pigment spot; B, bec ocellaire with apex truncated in right lateral view; C, thoracic sternite 8 (T8) showing joined lobes in ventral view; D, thoracic sternite 8 (T8) showing widely separated lobes in ventral view; E, thoracic sternite 8 (T8) showing joined lobes in ventral view; F, inter-uropodal sclerite with well-developed preanal carina in ventral view. This figure is available in color at *Journal of Crustacean Biology* online.

M. occidentale, males and females of *M. digueti* also bear a well-developed preanal carina, but normally without dorsal setae (Fig. 8). Detailed description of more features of *M. digueti* related to the morphology and external ornamentation of the first and second pairs of pereiopods were given by Villalobos (1967) and Villalobos Hiriart & Nates Rodríguez (1990) in the original descriptions of the junior synonyms *M. acanthochirus* and *M. michoacanus*, respectively.

The genetic analyses indicate that the *M. digueti* populations from both the Baja California Peninsula and the mainland belong to the same genetic lineage. The intraspecific genetic distances found among the 16S and COI *M. digueti* haplotypes (0.2–0.81% and 0.18–1.25%, respectively) are comparable to those reported for other *Macrobrachium* species. In populations of *M. amazonicum*, Vergamini *et al.* (2011) found maximum distances of 1.1% in 16S and 3.3% in COI haplotypes, and Rossi & Mantelatto (2013) reported populations of *M. olfersii* on the Atlantic slope of Costa Rica, Panama, Venezuela, and Brazil with a maximum distance of 0.18% for 16S and 0.95% for COI haplotypes. The shared, frequent haplotypes, three in the 16S and seven in the COI were found occurring in the peninsula and on the mainland (Tables 1 and 2). The five most frequent haplotypes of the combined 16S-COI haplotypes (Hap 2, 6, 17, 23, and 28) were also found occurring in the peninsula and on the mainland (Table 3). The predominant 16S Hap 1 occurred in almost all drainage basins where the species was collected. The median-joining network placed this haplotype as the central node, with all private haplotypes around it, indicating the absence of a geographical structure. The peninsula Hap 2, 3, 4, 5, 11, 13, 14, and 15 and the mainland haplotypes Hap 8, 9, 10, 12, and 14 are derived from Hap 1 (Fig. 10A). In the COI gene, the predominant Hap 5 was found in 21 specimens among 10 drainage basins, five each from both regions (Table 2) and forming a central node of star-like clusters in the median-joining haplotype network (Fig. 10B). A maximum genetic distance of 0.81% was observed between the Hap 7 and Hap 8 in the 16S from drainage basins in Baja California Sur, Nayarit, and Sinaloa states. The genetic distance between the most common Hap 1 and the others ranged 0.20–0.40%. For the COI, a maximum genetic distance of 1.25% was

Table 1. Geographical distribution of 16S haplotypes (494-bp length) of *Macrobrachium digueti* (Bouvier, 1895) obtained from 105 individuals, 65 from seven drainage basins of the Baja California Peninsula and 40 from seven drainage basins of the mainland Pacific slope of Mexico distributed in the states Sinaloa, Nayarit, Jalisco, Guerrero and Oaxaca. The drainage basins from the peninsula are: SR = Santa Rita; LP-SH = Las Pocitas-San Hilario; TS = Todos Santos; PD = Pescadero; PEC = Plutarco E. Calles; SJD = San Jose del Cabo, and LAP = La Paz. The drainage basins from the mainland are: RPX = Río Piaxtla 2; RP = Río Presidio 2; RB = Río Baluarte 2; RA-I = Río Ameca Ixtapa B; RPU = Río Purificación; RCO = Río Coyuca 2; RV = Río Verde. The number of individuals for each haplotype found in the basins is indicated.

Haplotype	Baja California Peninsula							Sinaloa			Nayarit	Jalisco	Guerrero	Oaxaca	Total number of individuals (basins)
	SR	LP-SH	TS	PD	PEC	SJD	LAP	RPX	RP	RB	RA-I	RPU	RCO	RV	
Hap 1	18	14	5	2	6	3		1	1	1	12	5	9	5	82 (13)
Hap 2			1												1 (1)
Hap 3						1									1 (1)
Hap 4					1										1 (1)
Hap 5	1														1 (1)
Hap 6	1	1	4	1			2								9 (5)
Hap 7					1						1				2 (2)
Hap 8										1					1 (1)
Hap 9											1				1 (1)
Hap 10													1		1 (1)
Hap 11			1												1 (1)
Hap 12													1		1 (1)
Hap 13	1														1 (1)
Hap 14													1		1 (1)
Hap 15			1												1 (1)

Table 2. Geographical distribution of COI haplotypes (560-bp length) of *Macrobrachium digueti* (Bouvier, 1895) obtained from 60 individuals, 26 from seven drainage basins of the Baja California Peninsula and 34 from six drainage basins of the mainland Pacific slope of Mexico distributed in the states Sinaloa, Nayarit, Jalisco, Guerrero and Oaxaca. The drainage basins from the peninsula are: SR = Santa Rita; LP-SH = Las Pocitas-San Hilario; TS = Todos Santos; PD = Pescadero; PEC = Plutarco E. Calles; SJD = San Jose del Cabo, and LAP = La Paz. The drainage basins from the mainland are: RPX = Río Piaxtla 2; RB = Río Baluarte 2; RA-I = Río Ameca Ixtapa B; RPU = Río Purificación; RCO = Río Coyuca 2; RV = Río Verde. The number of individuals for each haplotype found in the basins is indicated.

Haplotype	Baja California Peninsula							Sinaloa		Nayarit	Jalisco	Guerrero	Oaxaca	Total number of individuals (basins)
	SR	LP-SH	TS	PD	PEC	SJD	LAP	RPX	RB	RA-I	RPU	RCO	RV	
Hap 1				1					1		1	3		6 (4)
Hap 2													1	1 (1)
Hap 3				1	1					1	1			4 (4)
Hap 4			1											1 (1)
Hap 5		1	5		2	2	1	1	1	4		2	1	20 (10)
Hap 6					1					2		1	1	5 (4)
Hap 7											1	1		2 (2)
Hap 8												1		1 (1)
Hap 9						1				2		2		5 (3)
Hap 10					1									1 (1)
Hap 11												1		1 (1)
Hap 12											1			1 (1)
Hap 13	1		1							1		1		4 (4)
Hap 14			1											1 (1)
Hap 15			1											1 (1)
Hap 16					1									1 (1)
Hap 17													1	1 (1)
Hap 18						1								1 (1)
Hap 19											1			1 (1)
Hap 20					1									1 (1)
Hap 21			1											1 (1)

observed between Hap 2 from Oaxaca and Hap 12 from Jalisco. Uncorrected pairwise distance between the most common COI Hap 5 and the others ranged 0.18–0.71%. In *Macrobrachium*, as in other animal groups, unique haplotypes may be of recent origin, whereas the more frequent and more dispersed haplotype is likely the oldest and ancestral (Cook *et al.*, 2002; Vázquez-Domínguez *et al.*, 2009; Ferreri *et al.*, 2011). The presence of the same haplotypes in the two regions indicates a constant genetic flow in both

Table 3. Geographical distribution of combined 16S and COI haplotypes (1054-bp length) of *Macrobrachium digueti* (Bouvier, 1895) obtained from 56 individuals, 22 from seven drainage basins of the Baja California Peninsula and 34 from six drainage basins of the mainland Pacific slope of Mexico distributed in the states Sinaloa, Nayarit, Jalisco, Guerrero and Oaxaca. The drainage basins from the peninsula are: SR = Santa Rita; LP-SH = Las Pocitas-San Hilario; TS = Todos Santos; PD = Pescadero; PEC = Plutarco E. Calles; SJD = San Jose del Cabo, and LAP = La Paz. The drainage basins from the mainland are: RPX = Río Piaxtla 2; RB = Río Baluarte 2; RA-I = Río Ameca Ixtapa B; RPU = Río Purificación; RCO = Río Coyuca 2; RV = Río Verde. The number of individuals for each haplotype found in the basins is indicated.

Haplotype	Baja California Peninsula							Sinaloa		Nayarit	Jalisco	Guerrero	Oaxaca	Total number of individuals (basins)
	SR	LP-SH	TS	PD	PEC	SJD	LAP	RPX	RB	RA-I	RPU	RCO	RV	
Hap 1						1								1 (1)
Hap 2		1	3			1		1		4		2	1	13 (7)
Hap 3						1								1 (1)
Hap 4													1	1 (1)
Hap 5					1									1 (1)
Hap 6						1				2		1		4 (3)
Hap 7												1		1 (1)
Hap 8												1		1 (1)
Hap 9					1									1 (1)
Hap 10	1													1 (1)
Hap 11										1				1 (1)
Hap 12											1			1 (1)
Hap 13			1									1		2 (2)
Hap 14			1											1 (1)
Hap 15			1											1 (1)
Hap 16							1							1 (1)
Hap 17					1					2		1	1	5 (4)
Hap 18					1									1 (1)
Hap 19												1		1 (1)
Hap 20									1					1 (1)
Hap 21			1											1 (1)
Hap 22											1	1		2 (2)
Hap 23				1					1		1	2		5 (4)
Hap 24													1	1 (1)
Hap 25											1			1 (1)
Hap 26												1		1 (1)
Hap 27			1											1 (1)
Hap 28				1	1					1	1			4 (1)

directions between the Baja California Peninsula and the mainland (Tero *et al.*, 2003; de Bruyn *et al.*, 2005; Page *et al.*, 2008; Hughes *et al.*, 2009). The hierarchical AMOVA of combined *M. digueti* 16S and COI sequences provided no evidences for subdivisions in different drainage basins or between the peninsula and the mainland. Mismatch analyses, based on the frequency distribution of pairwise differences between sequences, showed a unimodal curve, suggesting recent population and range expansion (Fig. 11).

The phylogenetic reconstruction through MP, ML, and BI of combined sequences of 16S and COI fragments gave almost identical topologies with high bootstrap values and high posterior probability, placing our *M. digueti* haplotypes in a monophyletic clade (*digueti* subgroup) along with the Mexican *M. acanthochirus* and *M. michoacanus* GenBank sequences (Fig. 12). As expected, this *M. digueti* clade formed a well-supported monophyletic group with two species, which according to Holthuis (1952) have a carpus that is distinctly shorter than the merus: *M. hancocki* (from Costa Rica and Panama on the Pacific slope) and *M. crenulatum* (from Costa Rica and Venezuela on the Atlantic slope). In agreement with its geographical distribution, *M. digueti* haplotypes showed closer genetic distances (3.1–3.6%) with *M. hancocki* than with *M. crenulatum* (5.8–6.5%). Surprisingly, the three GenBank sequences registered as *M. digueti* (two specimens from Costa Rica and one from Mexico) (Rossi & Mantelatto, 2013; Pileggi *et al.*, 2014) did not cluster with our *M. digueti* clade; rather, they were strongly associated

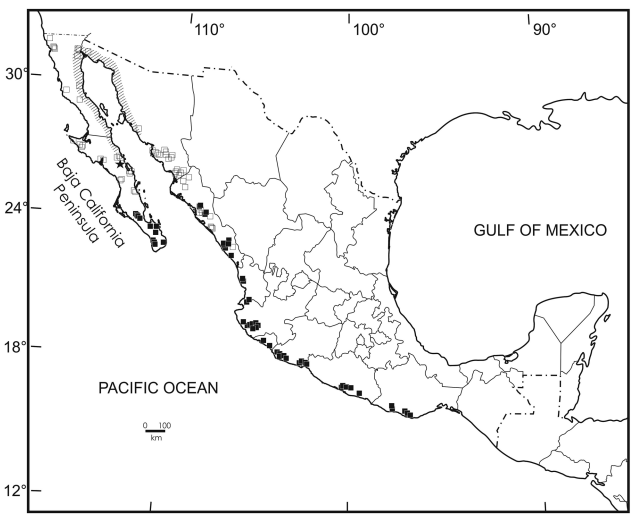

Figure 9. Geographical distribution of *Macrobrachium digueti* (Bouvier, 1895). Star represents type locality. Solid squares represent records of the species in Mexico. Empty squares represent sampled areas without records of the species. Area with dashed lines show the disjunct distribution of the genus *Macrobrachium* along the coastal plains of the northern part of the Gulf of California.

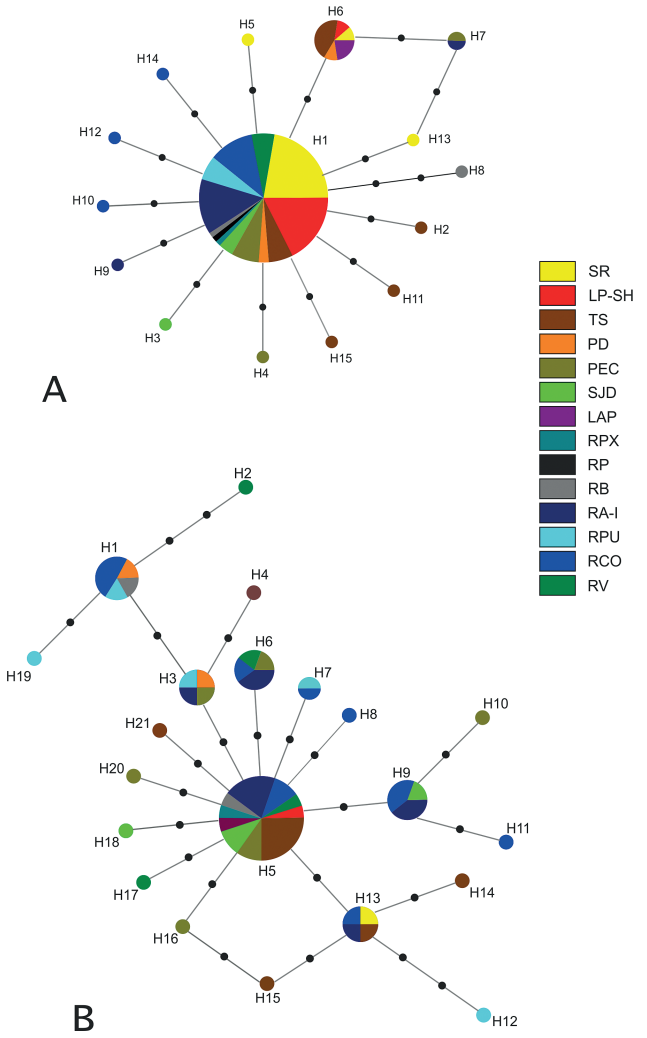

Figure 10. Median-joining network showing relationships among 16S haplotypes of *Macrobrachium digueti* (Bouvier, 1895) from 14 drainage basins (A) and COI haplotypes of *M. digueti* from 13 drainage basins (B). Colors indicate basins and the size of circles and colored segments are proportional to the haplotype frequencies. Black circles represent mutational step between haplotypes. Baja California Peninsula basins: SR, Santa Rita; LP-SH, Las Pocitas-San Hilario; TS, Todos Santos; PD, Pescadero; PEC, Plutarco E. Calles; SJD, San Jose del Cabo; LAP, La Paz. Mainland Mexico basins: RPX, Río Piaxtla 2; RP, Río Presidio 2; RB, Río Baluarte 2; RA-I, Río Ameca Ixtapa B; RPU, Río Purificación; RCO, Río Coyuca 2; RV, Río Verde.

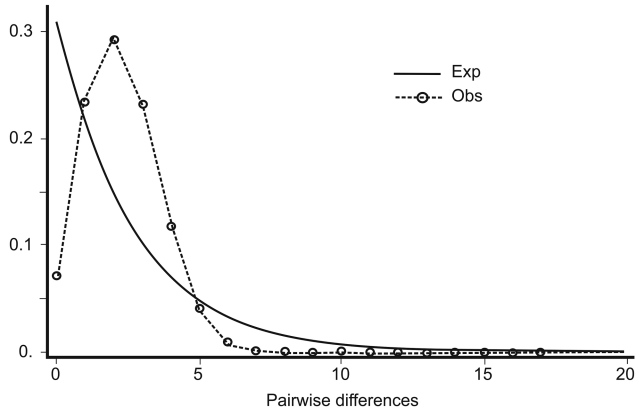

Figure 11. Mismatch-distribution for the combined 16S and COI data of *Macrobrachium digueti* (Bouvier, 1895) haplotypes representing the observed (Obs) and expected (Exp) pairwise differences under the sudden population expansion model.

with the *M. olfersii* sequences (Fig. 12) with genetic distances of 5.9–6.7% among them. The genetic distance ranges observed between our *M. digueti* sequences and the *M. digueti* and *M. olfersii* GenBank sequences were 10.0–11.2% and 9.6–10.2%, respectively. These distances are at the species level; therefore, these specimens (source of sequence) in the GenBank as *M. digueti* need taxonomic revision.

Our results contribute to the knowledge of the amphiamerican *Macrobrachium* species groups. Holthuis (1952) pointed out that most species on the Pacific slope are closely related to species of the Atlantic slope, forming species pairs with few morphological differences between them. In relation to the *olfersii* group *sensu* Villalobos (1969), Holthuis (1952) listed the pairs formed by *M. digueti* with *M. olfersii* and *M. hancocki* with *M. crenulatum*. According to our studies, however, the morphology of the entity assigned by Holthuis (1952) as *M. digueti* fits well with *M. hobbsi* (unpublished results), a species closely related to *M. olfersii* (Villalobos Hiriart & Nates Rodríguez, 1990). Therefore, contrary to the indication of Villalobos (1969) that *M. olfersii* (type locality on the Brazilian Atlantic slope) also occurs on the Pacific slope, we agree with Holthuis (1952, 1980) that its natural geographical distribution is restricted to the Atlantic slope. *Macrobrachium hobbsi* occurs only on the Pacific slope, which is contrary to Villalobos Hiriart & Nates Rodríguez (1990) statement that the species also occurs on the Atlantic slope. According to Anger (2013), the formation of the Isthmus of Panama in the Late Pliocene (about 2 million ybp) is a vicariant event leading to the formation of two groups of *Macrobrachium* species, one on the Atlantic and the other on the Pacific. The amphi-isthmian occurrence of *M. hobbsi* and *M. olfersii* is puzzling and may represent a geminate pair of sister species. In a more recent study on the American trans-isthmian species of *Macrobrachium* using fragments of 16S, COI, and 18S nDNA, Pileggi *et al.* (2014) reported a monophyletic clade, morphologically characterized by a straight rostrum and usually with more than 10 teeth on the dorsal margin. This clade is composed of two species groups, one consisting of *M. digueti* and *M. olfersii* and the second of *M. hancocki*, *M. crenulatum*, and *M. michoacanus*. They concluded that such grouping corroborates the morphological proximity of the species pairs proposed by Holthuis (1952) and suggests that *M. digueti* and *M. olfersii* are sibling species. As previously noted, however, the specimens used by Pileggi *et al.* (2014) registered in the GenBank as *M. digueti* need to be revised. In line with the phylogenetic analyses of Acuña Gómez *et al.* (2013), who used 16S fragments of 16 Mexican *Macrobrachium* species, our results support the existence of the *olfersii* group composed of two clades, the *digueti* subgroup and the *olfersii* subgroup. Our studies indicate that there are no amphiamerican species, but amphiamerican species groups, which confirms a close phylogenetic relationship of several species pairs advanced by Holthuis (1952). The amphiamerican groups occurring along the Mexican coasts (Pacific and Atlantic slopes) are supported by our results (Fig. 12). These groups, except *M. digueti*, are also supported by the molecular analyses of Acuña Gómez *et al.* (2013) and Pileggi *et al.* (2014). The amphiamerican groups occurring along the Mexican coasts therefore consists of four groups: 1) *olfersii* group, represented by the *digueti* subgroup with *M. digueti* and *M. hancocki* in the Pacific and *M. crenulatum* in 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* in the Atlantic, 3) *heterochirus* group, with *M. occidentale* in the Pacific and *M. heterochirus* in the Atlantic, and 4) *carcinus* group, with *M. americanum* in the Pacific and *M. carcinus* in the Atlantic.

Macrobrachium digueti has a long larval development and is an amphidromous shrimp because it occurs in coastal areas as well as in upstream freshwater environments (Villalobos-Hiriart *et al.*, 2010; Bauer, 2013; this study). Of 104 sites among 44 drainage

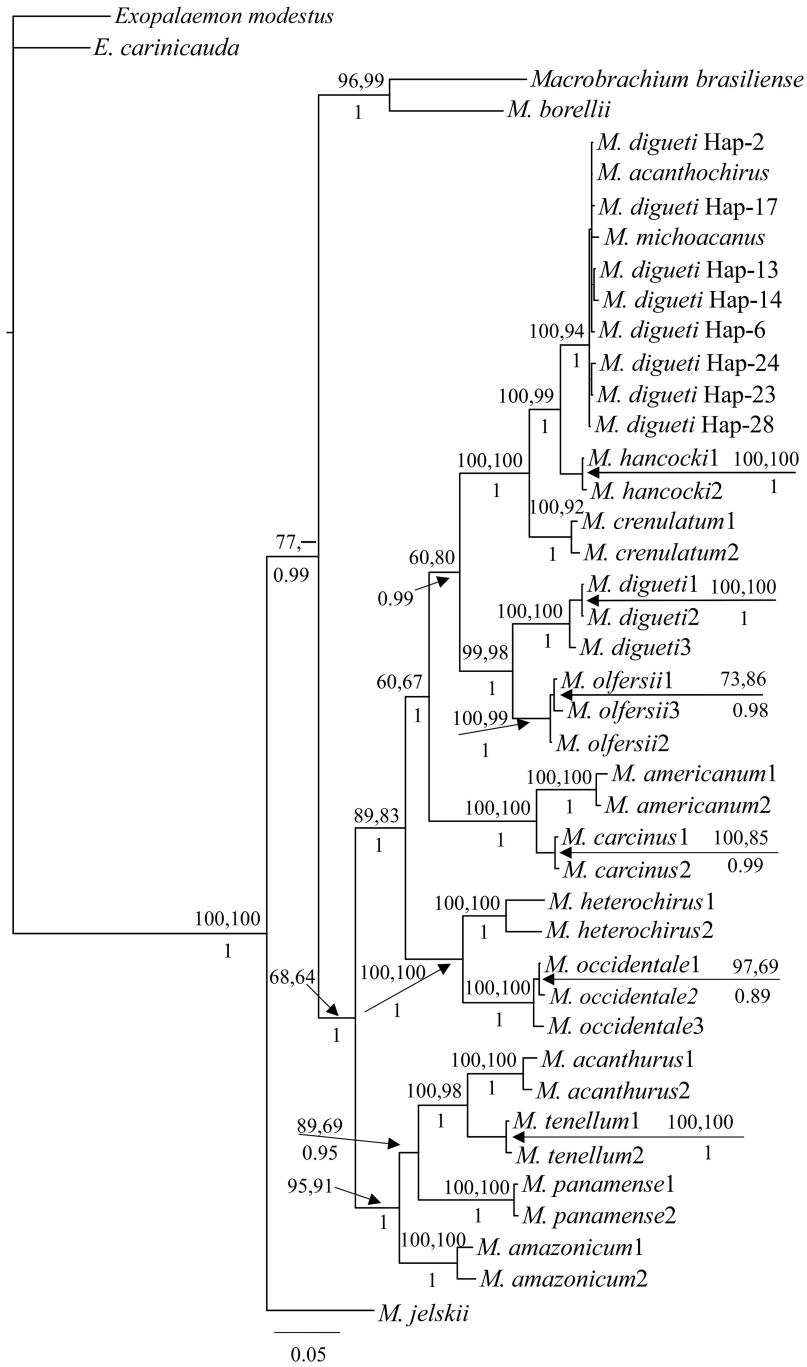

Figure 12. Bayesian phylogenetic tree topology representing relationships among species of *Macrobrachium* based on the combined data of 16S and COI gene fragments. Numbers above the nodes represent bootstrap values of maximum parsimony and maximum likelihood methods and below the node represents posterior probabilities of Bayesian inference. Numbers following *Macrobrachium* species name represent specimen origin linked to the 16S and COI sequences in the GenBank database.

basins in nine Mexican states, we obtained and identified 231 specimens of *M. digueti* from 27 sites in 17 drainage basins in seven states, covering a distance of about 2,900 km along the coast, 1100 km on the peninsula and 1800 km on the mainland. These shrimps were found in waters ranging from 15 to 359 masl, some of them at remote sites up to 80 km from the coast, as in San Pedro de la Presa, Santa Rita Basin, Baja California Sur. The water conditions at the time of collection were 20.3–34.7 °C, TDS 0.1–2.87 g l^{-1}, and pH 7.3–8.6. Amphidromous crustaceans may disperse widely in coastal areas during planktonic larval stages. Dennenmoser *et al.* (2010) used COI fragments of the amphidromous shrimp *Cryphiops caementarius* (Molina, 1782) from five drainage basins in north-central Chile covering about 700 km, finding high haplotype diversity but no significant geographical structuring, suggesting wide dispersal and gene flow rather than "stepping-stone" dispersal between estuaries. *Macrobrachium digueti* also shows a wide but disjunct distribution in the upper part of the gulf (Fig. 9) as recorded for other caridean shrimps in the Gulf of California (Hendrickx, 1995; Hernández *et al.*, 2007; García-Velazco *et al.*, 2014).

We attribute oceanic dispersal events to the presence *M. digueti* on the Baja California Peninsula instead of the vicariance hypothesis suggested by Hernández *et al.* (2007). The occurrence of identical

haplotypes of *M. digueti* on the peninsula and mainland, as in the findings of García-Velazco *et al.* (2014) for *M. occidentale*, indicates a constant genetic flow, which is most likely determined by flow patterns within the Gulf of California, cyclonic in summer and anticyclonic the rest of the year (Bray, 1988; Paden *et al.*, 1991). Several species of *Macrobrachium* show wide interoceanic distribution in the Indo-West Pacific region (Short, 2004) and mtDNA analyses supports the mechanism of oceanic dispersal rather than vicariance events (de Bruyn *et al.*, 2005; Murphy & Austin, 2005; Chen *et al.*, 2009).

SUPPLEMENTARY MATERIAL

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

S1 Table. Variable nucleotide sites among unique sequences of fifteen 16S haplotypes from a 494-bp fragment obtained from 105 individuals of *Macrobrachium digueti* (Bouvier, 1895) from Mexico.

S2 Table. Variable nucleotide sites among unique sequences of 21 COI haplotypes from a 560-bp fragment obtained from 60 individuals of *Macrobrachium digueti* (Bouvier, 1895) from Mexico.

S3 Table. Analysis of molecular variance of the combined 16S and COI genes sequence data of individuals of *Macrobrachium digueti* (Bouvier, 1895) from seven drainage basins of the Baja California Peninsula as one group, and from six drainage basins of the mainland Pacific slope of Mexico as another group.

ACKNOWLEDGEMENTS

We thank José Ángel Camacho Durán, Enrique Calvillo Espinoza, Alejandro Maeda Obregón, Guadalupe Talamantes, Franco Cota, Juan Carlos Sainz, Francisco Leyva, José Ignacio Camacho Durán, José Moreno García, Ambrosio Hernández de la O, Rosa Hernández Díaz, Jesús Bautista, Lesain Cortés Jacinto, and Ahiezer Hernández Valencia for their assistance in field sampling and Gerardo Hernández García for editing the figures and map. Ira Fogel (CIBNOR) provided detailed editing services. HGV is a recipient of a doctoral fellowship (CONACYT 1504086) and other support from the Centro de Bachillerato Tecnológico Agropecuario 198 and Dirección General de Educación Tecnológica Agropecuaria, Subsecretaría de Educación Media Superior, SEP of Mexico to carry out this research. Collection permit was given by the Secretaría de Agricultura, Ganadería, Desarollo Rural, Pesca y Alimentación (DGOPA 07345.010910.4127). Funding was provided by CONACYT grant 62359, directed by G.M.

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