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## Genetic and morphological evidence for cryptic species in *Macrobrachium australe* and resurrection of *M. ustulatum* (Crustacea, Palaemonidae)

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**Abstract.** *Macrobrachium australe* is an amphidromous prawn living in the insular freshwater systems of the Indo-Pacific. Because it possesses few informative morphological characters, that often vary from one habitat to another, *M. australe* has produced much taxonomic confusion and has historically been described under eight synonyms. Here, 53 specimens collected throughout the Indo-Pacific under the name *M. australe* were phylogenetically and morphologically examined. Results revealed that what has been called *M. australe* belongs to at least two distinct species: *M. australe*, distributed from the Southwest Indian Ocean to the Central Pacific Ocean, and a cryptic species potentially restricted to the Northwest Pacific Ocean, here identified as *M. ustulatum*, which until now was considered as a junior synonym. Although they are not quite found in the same habitat (lentic-lotic), the presence of these distinct, and reciprocally monophyletic entities in the same rivers on the islands of Palau and Santo strongly favors the hypothesis of two reproductively isolated entities. Six morphological characters, including the proportions of the joints of the male second pereopod, the shape of the epistome lobe and the armature of the fourth thoracic sternite, are evidenced as diagnostic. A neotype of *M. australe* is designated and deposited in the Muséum national d'Histoire naturelle in Paris.

**Keywords.** Amphidromous prawn, Indo-West Pacific, multi-locus phylogeny, morphology.

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## Introduction

*Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838) is a large-sized prawn species living in the insular freshwater systems of the tropical Indo-Pacific. The species possesses an amphidromous life cycle in which the larvae develop in the marine plankton before returning as juveniles to rivers to grow and reproduce. This specific life cycle is considered a critical element to the persistence of the populations and the viability of the species in insular freshwater habitats (Keith 2003), which are usually sparse and of an unstable nature. *Macrobrachium australe*, which is one of the most widespread amphidromous prawns, distributed from the Seychelles and Madagascar eastwards to French Polynesia, is considered as a “Least Concern” species in conservation priority (Jayachandran 2001). However, an unpublished primary molecular sequencing of specimens collected by the Muséum national d’Histoire naturelle (MNHN) over a range that spans the Indo-Pacific suggested the possible existence of distinct evolutionary units (species) within what was morphologically assigned to *M. australe*. This raised questions about the exact distribution of *M. australe* and its actual conservation status.

The genus *Macrobrachium* Spence Bate, 1868 is considered as one of the most challenging decapod crustacean groups for species delineation and alpha taxonomy (Holthuis 1950; Chace & Bruce 1993; Short 2004). While taxonomically informative morphological traits (e.g., on the rostrum and the second pereopods) are mostly present only in fully developed males, they also often vary within species (Holthuis 1950; Short 2004). Indeed, different environmental conditions have been shown to induce carapace shape variation in the South American prawn *M. borellii* (Nobili, 1896) (Torres *et al.* 2014) and the Asian prawn *M. nipponense* (De Haan, 1849 in De Haan 1833–1850) (Chen *et al.* 2015). Similarly, in populations of *M. australe* from Reunion Island, the rostral shape varies with water velocity, being long, thin and orientated upward in lentic populations and short, robust and straight in lotic populations (Zimmermann *et al.* 2012). Taking into account these data, it is likely that the carapace plasticity in *Macrobrachium* has contributed to taxonomic confusion. In this context, species delimitation in the genus should not be solely based on carapace features but on the integration of complementary sources of evidence (e.g., morphometric or molecular data).

Addressing these issues, however, requires a reasonably complete sampling size, ideally covering the whole distribution range of the target species. Indeed, the broad geographical distribution of *M. australe*, combined with the difficulty of accessing tropical insular freshwater systems, has led to geographic sampling gaps blurring the distinction between geographical and/or ecological variations and species-level divergences. As a consequence, *M. australe* has historically been described under eight synonyms (De Grave & Franssen 2011), each more or less corresponding to an isolated location on the edge of the species range: 2 synonyms were described from the Central and Southwestern Pacific (*Palaemon australis* Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838 from Tahiti and *P. danae* Heller, 1865 from Sydney, Australia (but probably erroneous, see Short 2004)); 3 synonyms were described from the Central Indo-Pacific (*P. dispar* von Martens, 1868 from Adonara Island, Indonesia; *P. (Eupalaemon) ustulatus* Nobili, 1899 from Rigo, Papua New Guinea; *Leander lepidus* de Man, 1915 from Jos Sudarso Bay, West Indonesian Papua); and 3 synonyms were described from the Western Indian Ocean (*P. alphonsonianus* Hoffman, 1874 from Reunion Island; *P. parvus* Hoffman, 1874 from Nossy-Faly Island, Madagascar; and *P. malliardi* Richters, 1880 from Mauritius).

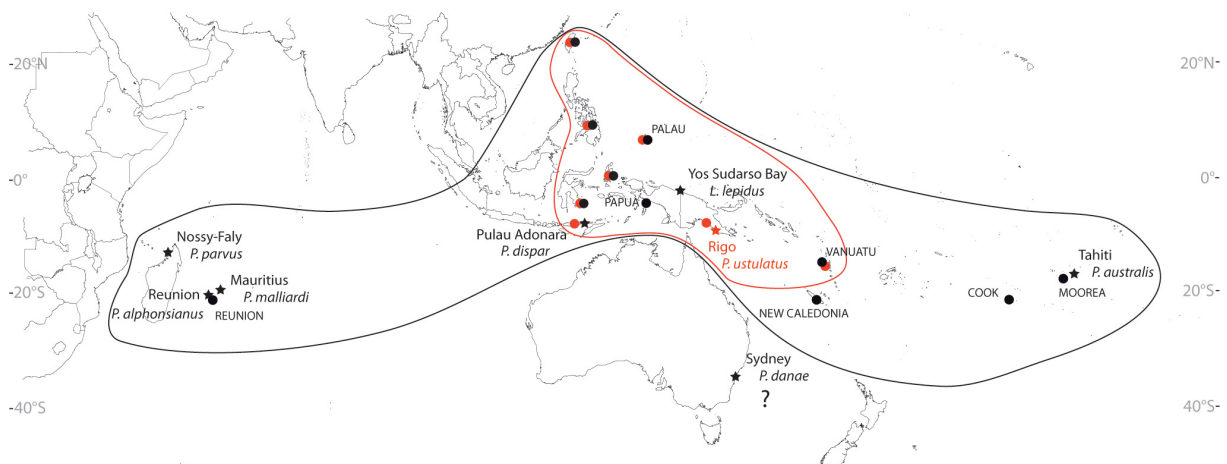
This study aims to clarify the evolutionary independence of lineages within the complex “*M. australe*”, using both molecular and morphological characters. A molecular phylogeny was performed based on the DNA sequencing of two unlinked molecular markers (16S and 28S rRNA) in 53 specimens sampled over an area that covers the range of “*M. australe*”, and includes localities close to the type localities of the synonymous species. To construct a robust molecular taxonomic framework, all published sequences of *Macrobrachium* were included in the phylogenetic analyses. This allowed the assessment of the status of “*M. australe*” lineages relative to one another and relative to other species of the genus *Macrobrachium*. Next, in order to assign molecular clusters to correct species names, morphological characters within and among the divergent genetic clusters identified as “*M. australe*” were examined and compared to all museum data (from collections of voucher specimens and from original descriptions or taxonomic revisions) available on *M. australe* and its seven associated synonyms. Diagnostic morphological characters of identified species were compiled to facilitate future identifications within the “*M. australe*” complex.

## Material and methods

### Collection, molecular data and analyses

Specimens were collected in the Indo-West Pacific (Fig. 1, Table 1) by electrofishing (portable Dekka 3000 electric device, Germany). In the field, most of the specimens were released back into the river after a piece of pereopod was clipped off and fixed in 95% ethanol for molecular analyses. Only some specimens (mostly corresponding to mature males presenting morphological features enabling species-level taxonomic identification) were kept intact and fixed in 75% ethanol for morphological analyses.

A fragment of the mitochondrial 16S rRNA gene and a fragment of the nuclear 28S rRNA gene, including the D1 and D2 domains (Hassouna *et al.* 1984; Palumbi *et al.* 1991), were amplified using the universal primers 16Sa-L and 16Sb-H2 (Palumbi 1996) and C1 and D2 (Jovelin & Justine 2001), respectively. Polymerase chain reaction (PCR) protocols were described in Castelin *et al.* (2013). PCR products were sequenced in both directions to insure the accuracy of base calls. Chromatograms were edited



**Fig. 1.** Map of the Indo-Pacific showing localities where *Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838) (black area) and *M. ustulatum* (Nobili, 1899) (red area) were collected and/or recorded. Capitalized locality names correspond to the 7 localities sampled for this study. Non-capitalized locality names correspond to the localities reported from the literature. Stars shows the type localities of the synonyms of *M. australe* (black stars) and *M. ustulatum* (red star).

**Table 1.** Specimens collected in the present study. Coordinates annotated with an asterisk are only approximates. 28S = 28S rRNA GenBank accession number; 16S = 16S rRNA GenBank accession number; na = not available. [continued next page]

Species	Locality	Latitude	Longitude	Collection date	Working ID no.	MNHN Voucher no.	Specimen	28S	16S
<i>M. ustulatum</i> (Nobili, 1899)	Vanuatu, Santo, Jourdain, Ora River	15.1666° S	166.8999° E	3 Oct. 1994	MC1235	MNHN-IU-2013-13201	yes	na	KY039511
					MC1236	MNHN-IU-2013-13202	yes	na	KY039512
	Palau, Negmeskang, unnamed stream	7.5413° N	134.5778° E	1 Mar. 2011	MC4060	MNHN-IU-2013-19677	yes	KY039560	na
					MC4061	MNHN-IU-2013-13204	yes	KY039561	KY039515
					MC4062	MNHN-IU-2013-13203	yes	KY039562	KY039516
					MC4074	MNHN-IU-2013-19678	yes	na	KY039519
<i>M. australe</i> (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838)	Palau, Meseketat, unnamed stream	7.5413° N	134.5778° E	3 Mar. 2011	MC1027	MNHN-IU-2013-19679	no	KY039520	KY039501
					MC1028	MNHN-IU-2013-19680	no	KY039521	na
	New Caledonia, Tibarama	20.938° S	165.378° E	Oct. 2010	MC1029	MNHN-IU-2013-19681	no	KY039522	na
					MC1030	MNHN-IU-2013-19682	no	KY039523	KY039502
	Cook, Avana	21.2461° S	159.7316° W	Jul. 2010	MC1075	MNHN-IU-2013-19683	no	KY039524	na
					MC1076	MNHN-IU-2013-19684	no	KY039525	na
					MC1077	MNHN-IU-2013-19685	no	KY039526	na
					MC1079	MNHN-IU-2013-19686	no	KY039527	na
					MC1080	MNHN-IU-2013-19687	no	KY039528	na
					MC1081	MNHN-IU-2013-19688	no	KY039529	na
					MC1082	MNHN-IU-2013-19689	no	KY039530	na
					MC1083	MNHN-IU-2013-19690	no	KY039531	KY039503
					MC1084	MNHN-IU-2013-19691	no	KY039532	KY039504
MC1085					MNHN-IU-2013-19692	no	KY039533	na	
MC1086					MNHN-IU-2013-19693	no	KY039534	na	
MC1087	MNHN-IU-2013-19694	no	KY039535	na					
MC1088	MNHN-IU-2013-19695	no	KY039536	KY039505					
MC1089	MNHN-IU-2013-19696	no	KY039537	na					
MC1090	MNHN-IU-2013-19697	no	KY039538	na					
MC1092	MNHN-IU-2013-19698	no	KY039539	KY039506					
MC1093	MNHN-IU-2013-19699	no	KY039540	na					

Species	Locality	Latitude	Longitude	Collection date	Working ID no.	MNHN Voucher no.	Specimen	28S	16S
					MC1094	MNHN-IU-2013-19700	no	KY039541	na
					MC1095	MNHN-IU-2013-19701	no	KY039542	na
					MC1096	MNHN-IU-2013-19702	no	KY039543	na
					MC1097	MNHN-IU-2013-19703	no	KY039544	na
					MC1098	MNHN-IU-2013-19704	no	KY039545	KY039507
					MC1099	MNHN-IU-2013-19705	no	KY039546	KY039508
					MC1100	MNHN-IU-2013-19706	no	KY039547	na
					MC1106	MNHN-IU-2013-19707	no	KY039548	KY039509
					MC1108	MNHN-IU-2013-19708	no	KY039549	KY039510
					MC1109	MNHN-IU-2013-19709	no	KY039550	na
					MC1110	MNHN-IU-2013-19710	no	KY039551	na
	Papua, Pouyaud Creek	04.0070° S	134.4785° E	26 Nov. 2010	MC1202	MNHN-IU-2013-19711	yes	KY039552	na
	Vanuatu, Santo, Tasmate Village, Mamasa River	15.1557° S	166.6381° E	8 Nov. 2006	MC16415	MNHN-IU-2013-19712	yes	KY039553	na
	Vanuatu, Santo, Penaorou Village, Penaorou River	14.9777° S	166.6029° E	16 Nov. 2006	MC16423	MNHN-IU-2013-19713	yes	KY039554	na
	Vanuatu, Santo, Patunar River under a bridge	NA	NA	15 Sept. 2006	MC16443	MNHN-IU-2013-19714	yes	KY039555	na
	Vanuatu, Santo, Blue hole CIRAD	NA	NA	5 Sept. 2006	MC16478	MNHN-IU-2013-19715	yes	KY039556	na
	Palau, unnamed stream	7.4984° N	134.6349° E	28 Feb. 2011	MC4047	MNHN-IU-2013-19716	yes	KY039557	KY039513
					MC4051	MNHN-IU-2013-19717	yes	KY039558	na
					MC4052	MNHN-IU-2013-19718	yes	KY039559	KY039514
	Palau, Meseketat, unnamed stream	7.4384° N	134.5720° E	3 Mar. 2011	MC4071	MNHN-IU-2013-19719	yes	KY039563	na
					MC4072	MNHN-IU-2013-19720	yes	KY039564	KY039517
					MC4073	MNHN-IU-2013-19721	yes	na	KY039518
	French Polynesia, Moorea, Oponuhu River	17.5202° S	149.8483° W	Jun. 2011	MC4088	MNHN-IU-2013-19722	yes	KY039565	na
	Palau, unnamed stream	7.4984° N	134.6349° E	2 Mar. 2011	MC4116	MNHN-IU-2013-19723	yes	KY039565	na
	Reunion, St Jean	20.9133° S	55.6306° E	13 Sept. 2007	MC4136	MNHN-IU-2013-19724	yes	KY039567	na
					MC4138	MNHN-IU-2013-19725	yes	KY039568	na

using CodonCode Aligner v. 4.1.1 (CodonCode Corporation, Dedham, MA, USA) and sequences were deposited in GenBank (accession numbers: KY039501–KY039568, Table 1).

Sequences were used to search the NCBI database for related sequences using the Sequence Search (MegaBlast algorithm) tool implemented in Geneious software (Biomatters Ltd) for highly similar sequences (Zhang *et al.* 2000). Related sequences (Appendix) were downloaded and aligned with our sequences using the MUSCLE Server (Edgar 2004). Sequences from *P. debilis* Dana, 1852 were used as outgroup. Poorly aligned sites in the 28S dataset were identified using Gblocks Server v. 0.91b (Castresana 2000) and removed from analyses. Parameters used in Gblocks allowed for smaller final blocks and gap positions within the final blocks. Identical sequences were identified using DnaSP v. 5 (Librado & Rozas 2009) and one representative sequence retained for each haplotype. Nucleotide substitution models were determined using BIC in jModelTest v. 2.1.6 (Darriba *et al.* 2012).

A phylogeny was inferred for each gene using the Bayesian inferences (BI) implemented in BEAST v. 1.8.2 (Drummond *et al.* 2012). BEAST input files were generated using BEAUti v. 1.8.0 (Drummond *et al.* 2012). The Yule model was used as a prior for the speciation process. When an optimal nucleotide substitution model was not available in BEAUti we selected a similar but more complex near-optimal model (Huelsenbeck & Rannala 2004). The heterogeneity of the mutation rate across lineages was set under an uncorrelated, relaxed log-normal clock and mean rate was fixed to one. Each analysis ran for 100 000 000 generations with sample frequency of 1000. The final trees were calculated based on 99 000 trees (after burning 1001 generations) with maximum clade credibility and median node heights. Length of burning was determined by examination of traces in Tracer v. 1.6 (Rambaut *et al.* 2014). In addition, to better assess the robustness of nodes, a Maximum Likelihood (ML) tree using the GTR+ $\Gamma$  model of rate substitution (the most inclusive model) was inferred for each gene using RAxML HPC2 (Stamatakis 2006) on Teragrid v. 7.2.7, implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v. 3.1 (Miller *et al.* 2011). The best-scoring ML tree was estimated from 100 independent searches, each starting from distinct random trees. Robustness of nodes was assessed using the rapid bootstrapping algorithm (1000 replicates) (Felsenstein 1985; Stamatakis *et al.* 2008).

After checking for congruency between 16S and 28S tree topologies, a concatenated dataset was built using BI and ML analyses similar to those described above. Sequence variation was partitioned between genes and gene-specific nucleotide substitution model parameters were used, with each gene allowed to evolve at a different rate. Nucleotide substitution models were similar to those used in the single-gene phylogenies.

### **Morphological study**

Morphological observations were made on the specimens sampled in this study, as well as on specimens from various European museum collections corresponding to *M. australe* and its synonyms. The rostrum, the general cephalon, the second pereopods and the abdomen were observed using a stereoscopic microscope. The carapace length and the proportions of the various joints of the second chelipeds were measured using callipers (under stereoscopic microscope when needed). Drawings were made using the “Digital Inking” method (Coleman 2003; Coleman 2006) by tracing vectorial paths on high resolution photographs with Adobe Illustrator (CS6) equipped with a WACOM PTZ-1230 graphic tablet.

Abbreviations:

- CAL = carpus length
- CED = cutting edge of dactylus
- CEFF = cutting edge of fixed finger
- CHL = chela length



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CL	=	carapace length, measured laterally from postorbital margin to posterior margin of carapace
FL	=	finger length
FF	=	fixed finger
IL	=	ischium length
MAL	=	manus length
MEL	=	merus length
P2	=	pereiopod 2, or cheliped or second cheliped
PL	=	palm length
TL	=	body total length, measured from tip of rostrum to tip of telson
TS4	=	thoracic sternite 4

Museum abbreviations:

MNHN	=	Muséum national d'Histoire naturelle, Paris, France
MSNG	=	Museo Civico di Storia Naturale di Genova, Genoa, Italy
NHMW	=	Naturhistorisches Museum Wien, Vienna, Austria
RMNH	=	Naturalis Biodiversity Center, Leiden, the Netherlands
ZMB	=	Zoologisches Museum Berlin, Germany

## Results

### Molecular analyses

Fifty-three specimens were successfully sequenced for their 16S or 28S gene, or both (Table 1). The 19 amplified 16S sequences included 520 bp containing 36 variable sites. The 49 amplified 28S sequences included 743 bp containing 33 variable sites. After alignment with 53 sequences from Genbank (Appendix), the 16S dataset of 520 bp of the ingroup included 299 variable sites, of which 167 were phylogenetically informative. The 28S dataset of 774 bp of the ingroup included 305 variable sites, of which 245 were phylogenetically informative. After alignment with 62 sequences from Genbank (Appendix) and removal of ambiguous blocks, 504 bp of sequence remained to be used in phylogenetic analyses (65% of the original 774 positions), of which 144 bp were variable and 123 bp phylogenetically informative.

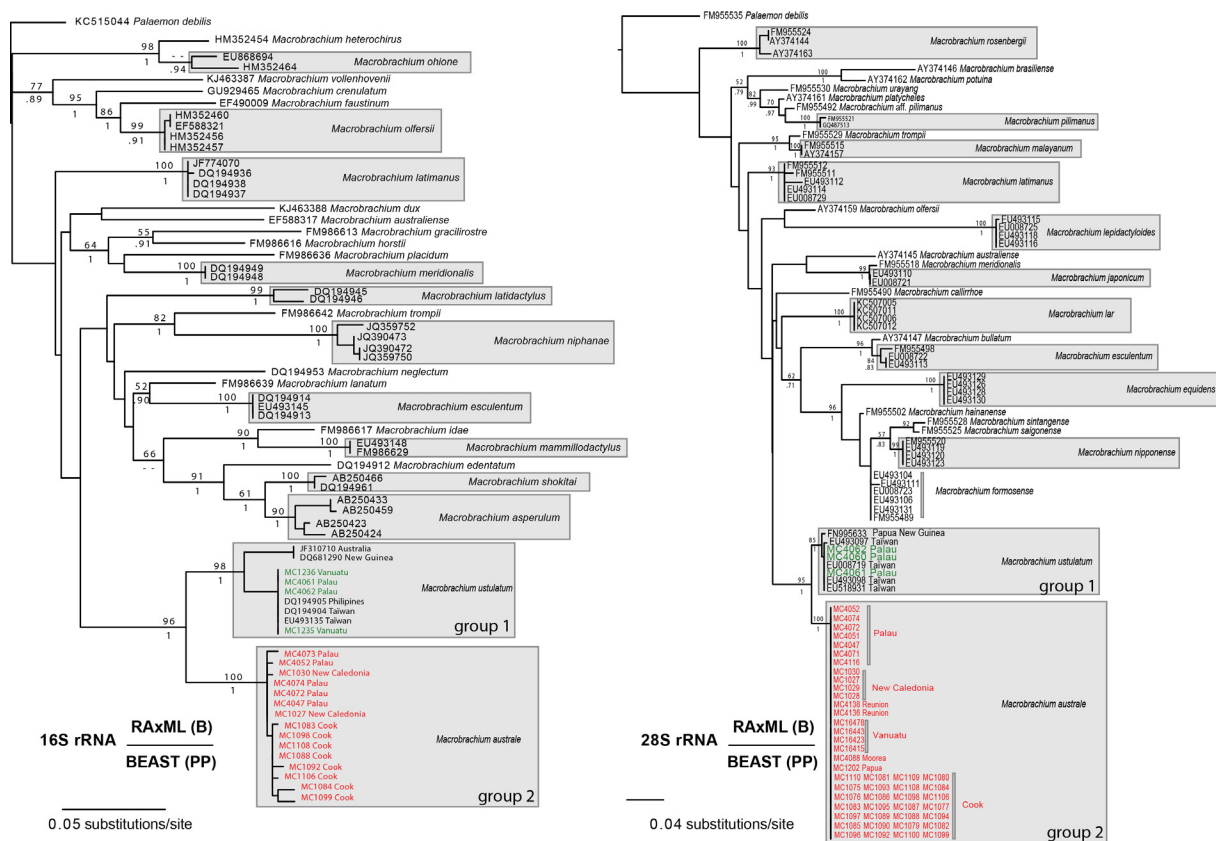
In each of the 16S and 28S trees, specimens originally assigned to “*M. australe*” were segregated into two groups (hereafter named group 1 and group 2, Fig. 2) that appeared as sister taxa, and were genetically distinct from one another and from all other species included in the tree. Indeed, group 1 and group 2 formed a monophyletic group, highly supported by posterior probabilities (PP) and bootstrap (B) analyses (16S: PP=1, B=96; 28S: PP=1, B=95) (Fig. 2), and the monophyly of each group was supported by both PP and B with both genes (16S: group 1: PP=1, B=98; group 2: PP=1, B=100; 28S: group 1: PP=1, B=85; group 2: PP=1; B=100). Analysis based on the concatenation of 16S and 28S could only be performed on the *M. australe* complex and *M. lar* (Fabricius, 1798), for which the 2 genes were available. In this phylogeny, although the monophyly of the two groups was supported (group 1: PP=1, B=100; group 2: PP=1, B=100), the relationship of the two groups as sister taxa was not highly supported (PP=0.56, B=100) (data not shown).

Based on the 16S dataset, pairwise genetic distances between group 1 and group 2 varied from 5.2 to 5.9%. Pairwise genetic distances within group 1 varied from 0 to 1.4%, while no genetic variation was observed within group 2. Pairwise genetic distances between group 1 and palaemonid sequences ranged from 3.7 to 9.2%, while genetic distances between group 2 and other palaemonid sequences ranged from 5.9 to 8.5%. Based on the 28S dataset, pairwise genetic distances between group 1 and group 2 varied from 2.2 to 2.4% (variation of 13 nucleotide sites out of 504 bp). Pairwise genetic distances within

group 1 varied from 0 to 0.6%, while no genetic variation was observed within group 2. Pairwise genetic distances between group 1 and sequences of *Macrobrachium* ranged from 2.2 to 12.2%, while genetic distances between group 2 and other sequences of *Macrobrachium* ranged from 2.2 to 11.9%.

### Specimens morphologically examined from museum collections or from the literature

For *Palaemon danae* from the NHMW, we used data from De Man (1892), and the comments of Holthuis (1950) and Short (2004). For *P. dispar* \_ENREF\_42 from the RMNH, we used data from De Man (1887, 1892) and Roux (1933). For *P. malliardi* from the ZMB, we used the original description by Richters (1880) and the comments of Holthuis (1950). For *Leander lepidus* from the RMNH, we used the comments of Holthuis (1950). The type specimens of *P. australis*, deposited in an unknown collection, *P. alphonsianus* and *P. parvus* (both originally deposited at the RMNH) could not be examined, as the specimens are lost. For *P. ustulatus* from the MSNG, two syntypes from Rigo (Papua New Guinea) were examined. These corresponded to a male (specimen VII-106) and a female (specimen VII-105). In



**Fig. 2.** Single-gene molecular phylogenies for *Macrobrachium* Spence Bate, 1868 based on the mitochondrial 16S and the nuclear 28S genes with *Palaemon debilis* Dana, 1852 as outgroup. Maximum likelihood (ML) and Bayesian (B) analyses, produced with BEAST and RAxML, respectively. Support values from Maximum likelihood and Bayesian analyses are posterior probabilities (PP) and bootstraps (B), respectively. GenBank accession numbers and working ID numbers are given at nodes. Species names are given on the right-hand side. The name of the sequences produced in the present study are colored in red for *M. australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838) and in green for *M. ustulatum* (Nobili, 1899). For these, sampling locality are given at nodes. See Table 1 and Appendix for details.



addition, five specimens from the MNHN, recorded under the name of *M. australe*, were examined: two specimens (MNHN-IU-2013-13201 and MNHN-IU-2013-13202) were collected in the Ora River on Santo Island (Vanuatu), one specimen (MNHN-IU-2013-13199) was collected from Anjouan (Comoros Islands), three specimens (MNHN-IU-2013-13196, MNHN-IU-2013-13197, MNHN-IU-2013-13198) were collected on Rarotonga (Cook Islands), and one specimen (MNHN-IU-2013-13200) on Pentecost Island (Vanuatu).

### Morphological study

In order to assign molecular clusters to correct species name, morphological characters within and among the genetic clusters, identified in phylogenetic analyses as “*M. australe*”, were examined and compared to all available museum data on “*M. australe*” and its eight associated synonyms (i.e., collections of voucher specimens and descriptions or taxonomic revisions). Specimens of group 1 were assigned to *Macrobrachium ustulatum* (Nobili, 1899) and specimens of group 2 to *M. australe* (Table 2). The holotype of *Palaemon ustulatus* from MSNG is represented by one male only with the minor second cheliped (Table 2). The proportions of the various joints of the cheliped do not match with the morphology of the specimens belonging to *M. australe*: CAL/CHL=0.69 (vs 0.79–1.04); CAL/PL=1.15 (vs 1.19–1.60) and FL/PL=0.66 (vs 0.46–0.53) (Table 2). Moreover, these characteristics fit well with those observed on specimens identified as *M. ustulatum*: CAL/CHL=0.67–0.75; CAL/PL=1.01–1.25 and FL/PL=0.49/0.66 (Table 2). The holotype shows an epistome with lobes strongly produced antero-ventrally (vs not strongly diverging anteriorly) and a fourth thoracic sternite armed with a rounded median process (vs unarmed). Overall, six characters were identified as diagnostic to distinguish the two species (Table 3).

Out of the seven synonyms of *M. australe*, six were confidently identified as junior synonyms based on the rostrum shape, the position of the hepatic spine toward the antennal spine and the proportions of the male second pereopod (Table 4). The seventh synonym, *P. ustulatus* is no longer considered as such.

### Systematic Account

Infraorder Caridea Dana, 1852  
Superfamily Palaemonoidea Rafinesque, 1815  
Family Palaemonidae Rafinesque, 1815  
Subfamily Palaemoninae Rafinesque, 1815

***Macrobrachium*** Bate, 1868

***Macrobrachium australe*** (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838)

Fig. 3, Table 2

*Palaemon australis* Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838: 37 (“Île de Taïti” = Tahiti).  
*Palaemon danae* Heller, 1865: 120, pl. 11, fig. 4 (“Sydney”; synonymy considered erroneous by Short 2004).

*Palaemon dispar* von Martens, 1868: 41 (“Insel Adenare, unweit Flores” = Adenare Island, near Flores).

*Palaemon alphonsonianus* Hoffmann, 1874: 33, pl. 9, figs 63–65 (“l’île de la Réunion” = Reunion Island).

*Palaemon parvus* Hoffmann, 1874: 35, pl. 7, fig. 59 (“l’île de Nossy-Faly” = Nosy Faly Island).

*Palaemon malliardi* Richters, 1880: 166, pl. 18, figs 1–3 (“Creole River, Black River, Mauritius Is.”).

*Leander lepidus* de Man, 1915: 410, pl. 28, figs 6–6d (“Mündung des kleinen Flusses zu Oinaké, einem Dorfe an der Küste östlich von der Humboldt-Bai” = mouth of a small river at Oinaké, a coastal village east of Humboldt Bay (Yos Sudarso Bay), Papua).

**Table 2.** Measurements of the various joints of the male P2 in *Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838) and its synonyms, and in *M. ustulatum* (Nobili, 1899). TL = total body length. Other abbreviations: see Material and methods. Missing data are represented by dashes.

Species	Specimen	Sample location	TL (mm)	IL		MEL		CAL		CHL		CAL/CHL		PL		CAL/PL		FL		FL/PL	
				max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.
<i>M. ustulatum</i>	<b>Holotype, GENOVA Grande vaso VII-106</b>	<b>Rigo (Papua New Guinea)</b>	<b>86</b>	–	–	–	<b>12.5</b>	–	<b>19</b>	–	<b>27.5</b>	–	<b>0.69</b>	–	<b>16.5</b>	–	<b>1.15</b>	–	<b>11</b>	–	<b>0.66</b>
	MNHN-IU-2013-13201	Santo (Vanuatu)	74	11.4	9.2	17.7	12.4	29	20	42.6	29.5	0.68	0.67	28.7	19.7	1.01	1.01	13.9	9.8	0.48	0.49
	MNHN-IU-2013-13203	Palau (Rep. of Palau)	65	8.9	7.1	11.7	9.3	21.5	12.8	31.3	18.8	0.68	0.68	21.4	12.2	1.00	1.05	9.9	6.6	0.46	0.54
	<i>P. dispar</i> in de Man 1892	Selayar (Indonesia)	73	–	–	14	–	22	–	30	–	0.73	–	19.5	–	1.12	–	10.5	–	0.54	–
	<i>P. dispar</i> in de Man 1902	Halmahera (Indonesia)	78	–	–	15	–	26	–	38	–	0.68	–	24.5	–	1.06	–	13.5	–	0.55	–
	<i>P. dispar</i> in de Man 1915	Papua (Armo R., Indonesia)	84	12	10	14	9.5	25	15	36.5	20	0.68	0.75	23	12	1.08	1.25	13.5	8	0.58	0.66
	<i>P. dispar</i> in de Man 1893	Flores (Indonesia)	90	–	–	–	11	–	17	–	23.5	–	0.72	–	15	–	1.13	–	8.5	–	0.56
	<b>Neotype, MNHN-IU-2013-13198</b>	<b>Rarotonga (Cook Isl.)</b>	<b>60</b>	<b>10.3</b>	<b>7.3</b>	<b>15.4</b>	<b>9</b>	<b>29</b>	<b>12.7</b>	<b>31.5</b>	<b>16</b>	<b>0.92</b>	<b>0.79</b>	<b>23</b>	<b>10.6</b>	<b>1.26</b>	<b>1.19</b>	<b>8.4</b>	<b>5.4</b>	<b>0.36</b>	<b>0.5</b>
	MNHN-IU-2013-13199	Anjouan (Comoros Isl.)	67	9.2	7.2	15.2	9.4	28.1	13.8	32.4	16.9	0.86	0.81	23.4	11.5	1.2	1.2	9	5.4	0.38	0.46
	MNHN-IU-2013-13200	Pentecost (Vanuatu)	61	10.1	–	21	–	37.4	–	40.8	–	0.91	–	30.8	–	1.21	–	10	–	0.32	–
<i>P. dispar</i> in de Man 1887	Ambon (Indonesia)	63	–	–	15.5	–	28	–	31.25	–	0.89	–	21	–	1.33	–	10.25	–	0.49	–	
<i>P. dispar</i> in de Man 1892	Timor (Koimino R., Indonesia)	66	13	–	17	–	33	–	42	–	0.78	–	27	–	1.22	–	15	–	0.55	–	
<i>P. dispar</i> in de Man 1892	Flores (Nargi R., Indonesia)	62	–	–	14	–	23	–	29.5	–	0.78	–	19.5	–	1.18	–	10	–	0.51	–	
<i>P. dispar</i> in Roux 1933	Salawati (Indonesia)	65	9.5	7	13	9	22.5	12	23	11.5	0.97	1.04	16	7.5	1.40	1.60	7	4	0.43	0.53	

**Table 3.** Traits of six characters identified as diagnostic to distinguish *Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838) from *M. ustulatum* (Nobili, 1899).

Diagnostic characters	<i>Macrobrachium australe</i>	<i>Macrobrachium ustulatum</i>
1 Proportion between major and minor pereopod lengths	1.48–2	1.37–1.59
2 Proportions of the joints of the major male P2	CAL/CHL 0.78–0.97 CAL/PL 1.18–1.40 FL/PL 0.32–0.55	CAL/CHL 0.68–0.73 CAL/PL 1.00–1.12 FL/PL 0.46–0.58
3 Proportions of the joints of the minor male P2	CAL/CHL 0.79–1.04 CAL/PL 1.19–1.60 FL/PL 0.46–0.53	CAL/CHL 0.67–0.75 CAL/PL 1.01–1.25 FL/PL 0.49–0.66
4 Velvety pappose setal pubescence of the minor male P2	palm and bases of fingers only densely packed with velvety setae	palm and carpus densely packed with velvety setae
5 Epistome lobes	not strongly produced anteriorly	strongly produced antero-ventrally
6 Fourth thoracic sternite	unarmed	armed with a round median process

#### Material examined

##### Neotype

COOK ISLANDS: 1 ♂, Rarotonga Island, Avana River, 21.2461° S, 159.7316° W, 11 Jul. 2010, 15.3 mm CL (MNHN-IU-2013-13198).

##### Other material

COOK ISLANDS: 1 ♂, same collection data, 12 mm CL (MNHN-IU-2013-13196); 1 ♂, 14.5 mm CL (MNHN-IU-2013-13197).

COMOROS: 1 ♂, Anjouan Island, Jomani River, 12.246° S, 44.529° E, 2 Nov. 2005, 16.2 mm CL (MNHN-IU-2013-13199).

VANUATU: 1 ♂, Pentecost Island, Warbot River, 15.957° S, 168.195° E, 26 Jan. 2010, 14.3 mm CL (MNHN-IU-2013-13200).

#### Description

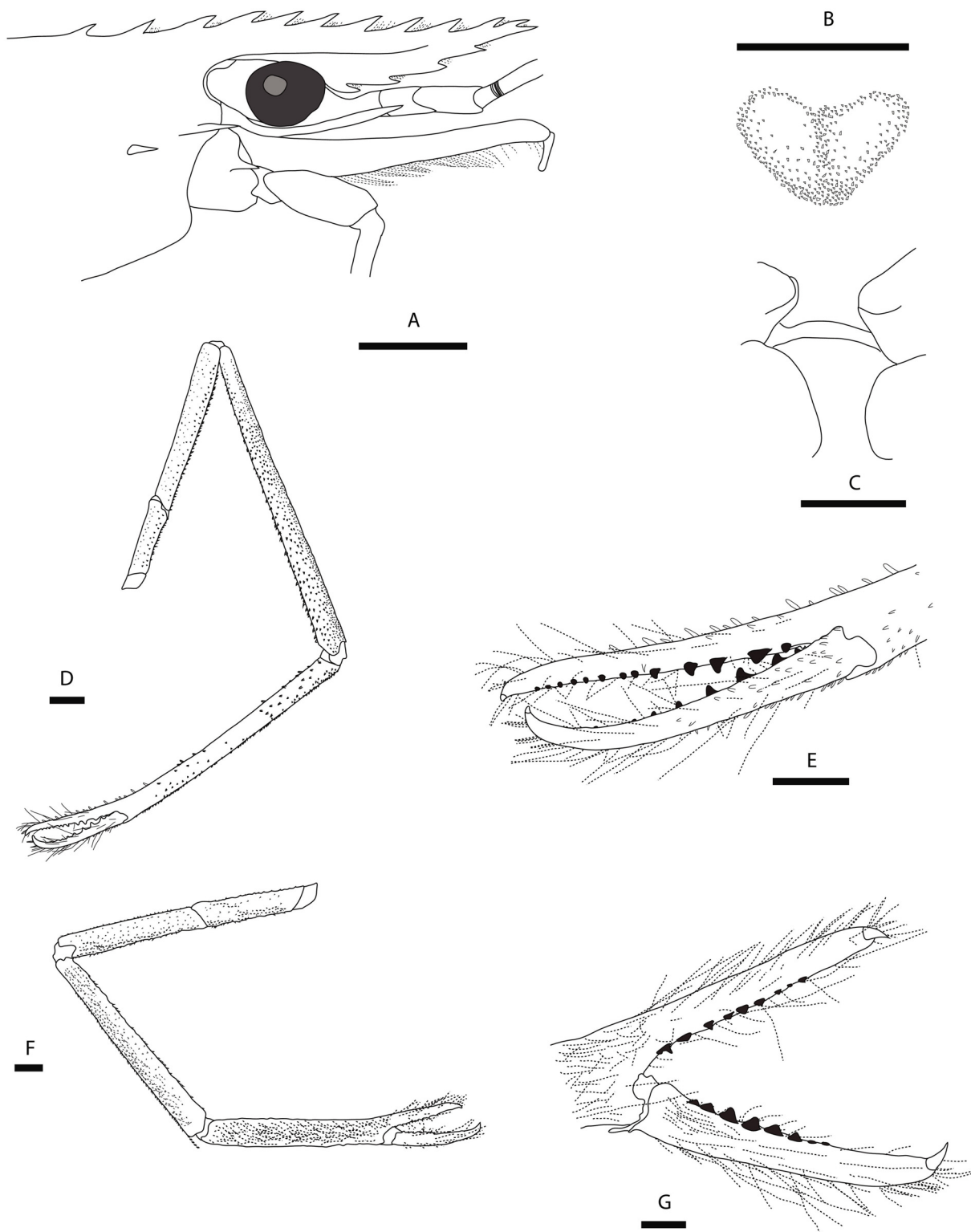
**ROSTRUM.** Moderately long, reaching to or slightly beyond end of scaphocerite (Fig. 3A); tip slightly up-curved; dorsal margin with 10–11 teeth, including 2–3 teeth on carapace; ventral margin with 3–4 teeth; teeth large, evenly spaced; sometimes distance between ultimate and penultimate teeth much larger than distances between other teeth; sometimes distance between 1<sup>st</sup> and 2<sup>nd</sup> teeth larger than between 2<sup>nd</sup> and 3<sup>rd</sup> teeth.

**GENERAL CEPHALON.** Eyes well developed; cornea as long as, but broader than peduncle; stylocerite distinctly pointed, reaching beyond basal segment; inferior orbit bluntly angular (Fig. 3A); post-antennular carapace margin straight or slightly concave; protective setation absent on carapace; epistome lobes narrowly separated, not strongly diverging anteriorly (Fig. 3B); TS4 unarmed (Fig. 3C).

**SECOND CHELIPEDS** (fully developed ♂). Very unequal sizes, non-isomorphic. Major P2 (Fig. 3D) long, subcylindrical, merus extending beyond scaphocerite; carpus 0.78–0.97 CHL, 1.18–1.40 PL. Fingers slender, 0.32–0.55 PL; dactylus shorter than FF. Cutting edges (Fig. 3E) with 4–5 conical teeth at base

**Table 4.** Measurements of the various joints of the second chelipeds (mm) in *Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838), as *Palaemon australis*, and in some of its synonyms. ss = slightly shorter. Missing data are represented by dashes.

Species (synonym)	Source	TL (mm)		Ischium		Merus		Carpus		Chela		Carpus/Chela		Palm		Carpus/Palm		Finger		Finger/Palm	
		max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.
<i>Palaemon australis</i>	Guérin-Méneville 1838	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Macrobrachium australe</i>	Holthuis 1950	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4– 0.5
<i>Palaemon dispar</i>	von Martens 1868	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.28– 0.33
<i>Palaemon malliardi</i>	Richters 1880	55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Palaemon alphonsonianus</i>	Hoffmann 1874	110	16	9	28	18	56	30	38	69	38	0.81	0.79	46	26	1.21	1.15	23	12	0.5	0.46
<i>Palaemon parvus</i>	Hoffmann 1874	51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Palaemon sundaticus</i>	de Man 1892	50	-	-	-	-	-	7	9	-	0.77	-	5	-	1.4	-	4	-	-	-	0.8
<i>Palaemon danae</i>	de Man 1892	65.5	-	-	-	7.1	-	10.3	19.9	-	0.94	-	6.4	-	1.70	-	4.5	-	-	-	0.7



**Fig. 3.** *Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838), MNHN-IU-2013-13198. **A.** Cephalothorax. **B.** Epistome. **C.** Fourth thoracic sternite. **D.** Major second pereiopod. **E.** Major second pereiopod finger. **F.** Minor second pereiopod. **G.** Minor second pereiopod finger. Scale bars: A, E, G = 2 mm; B–C = 1 mm; D, F = 4 mm.



(3<sup>rd</sup> largest) followed by 9–14 evenly spaced, small, blunt teeth along entire length; teeth diminishing in size distally; FF elongated, curved inwards; teeth arrangement similar to that of dactylus; basal part armed with 5–6 conical teeth (3<sup>rd</sup> largest) followed by 9–16 evenly spaced, small, blunt teeth along entire length; teeth diminishing in size distally. Largest tooth of FF lies after largest tooth of dactylus. Cheliped with numerous large spiniform setae on inner edge, smaller and denser on outer edge. Some scattered long setae present on cheliped, but very distinct on fingers. Merus 0.51–0.60 CAL, ischium 0.48–0.76 MEL. Minor P2 (Fig. 3F) with entire carpus beyond scaphocerite. Carpus 0.79–1.04 CHL, 1.19–1.60 PL. Fingers slender, 0.46–0.53 PL. Palm and bases of fingers densely packed with velvety setae. CED (Fig. 3G) with 6–13 teeth along entire length diminishing in size. CEFF with 9–15 teeth, similar teeth arrangement to that of dactylus, but teeth smaller. Pereiopods with numerous spiniform setae, denser on lower edge. MEL 0.60–0.81 CAL, IL 0.76–0.81 MEL.

ABDOMEN. Smooth, inter-uropodal sclerite with preanal carina.

### Remarks

Since the type specimen of *P. australis* is lost, and because of the taxonomic problems discussed above, a specimen from the Avana River on Rarotonga Island (Cook Islands, Central Pacific), which is geographically close to the type locality of *P. australis* (Tahiti), is herein designated as the neotype of *M. australe* in order to stabilize the taxonomy of the species. One specimen in our sample was collected from Moorea, which is closer to Tahiti. However, the specimen was in a less good condition than specimens from Rarotonga; thus, diagnostic morphological characters defined in this study were less obvious. Specimens from Rarotonga and from Moorea, however, showed no genetic variation in both the 28S and the 16S genes.

### Distribution

From the Indian Ocean (Madagascar, Comoros and Mascarenes Islands) to the Central Pacific Ocean (French Polynesia and Cook Islands). Syntopic with *M. ustulatum* in the Northwest Pacific Ocean (Taiwan, Philippines, Indonesia, Papua New Guinea and Palau) and the Southwest Pacific Ocean (Vanuatu) (Fig. 1).

### Habitat

Lentic habitats in the lower courses of rivers and at the edge of rivers, in water plant communities (Keith *et al.* 2013).

### Color patterns

Body colors (Fig. 4A) tend to match the substrate, varying from grey to brown, rather translucent, with three reddish stripes on each side of the cephalothorax.

### *Macrobrachium ustulatum* (Nobili, 1899)

Fig. 5, Table 2

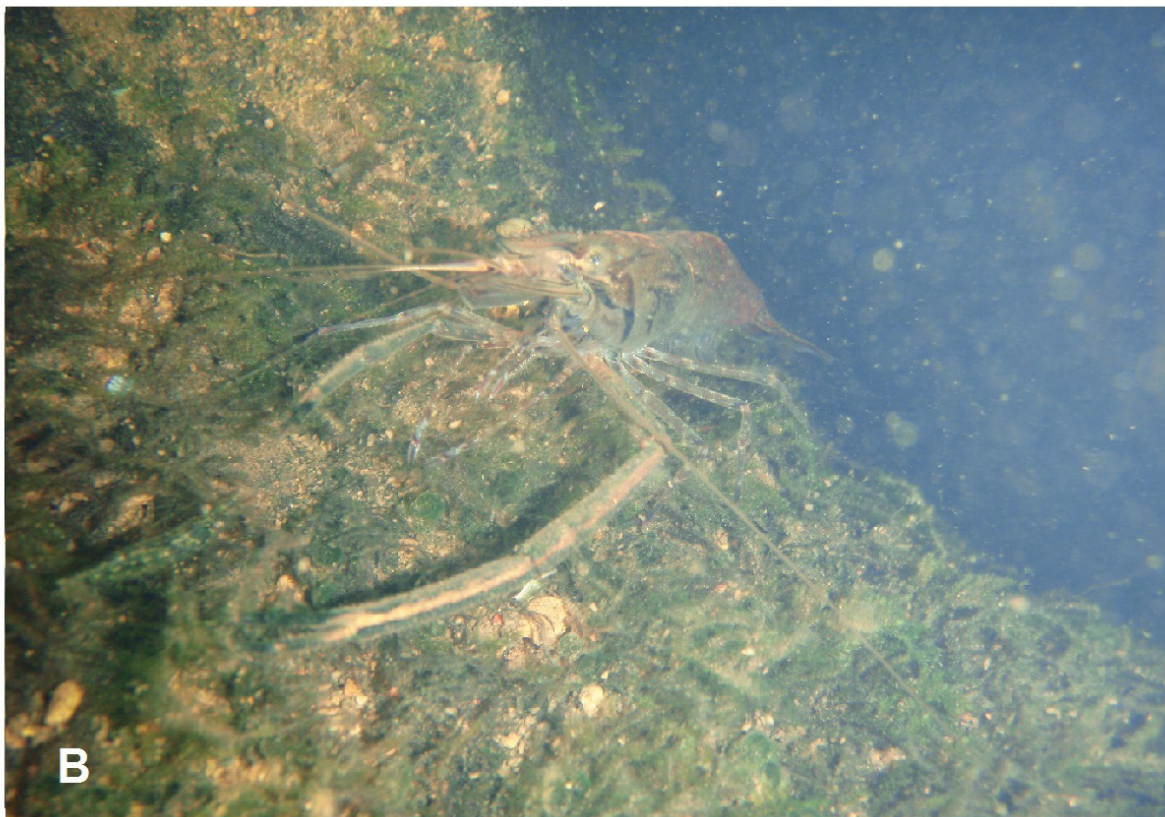
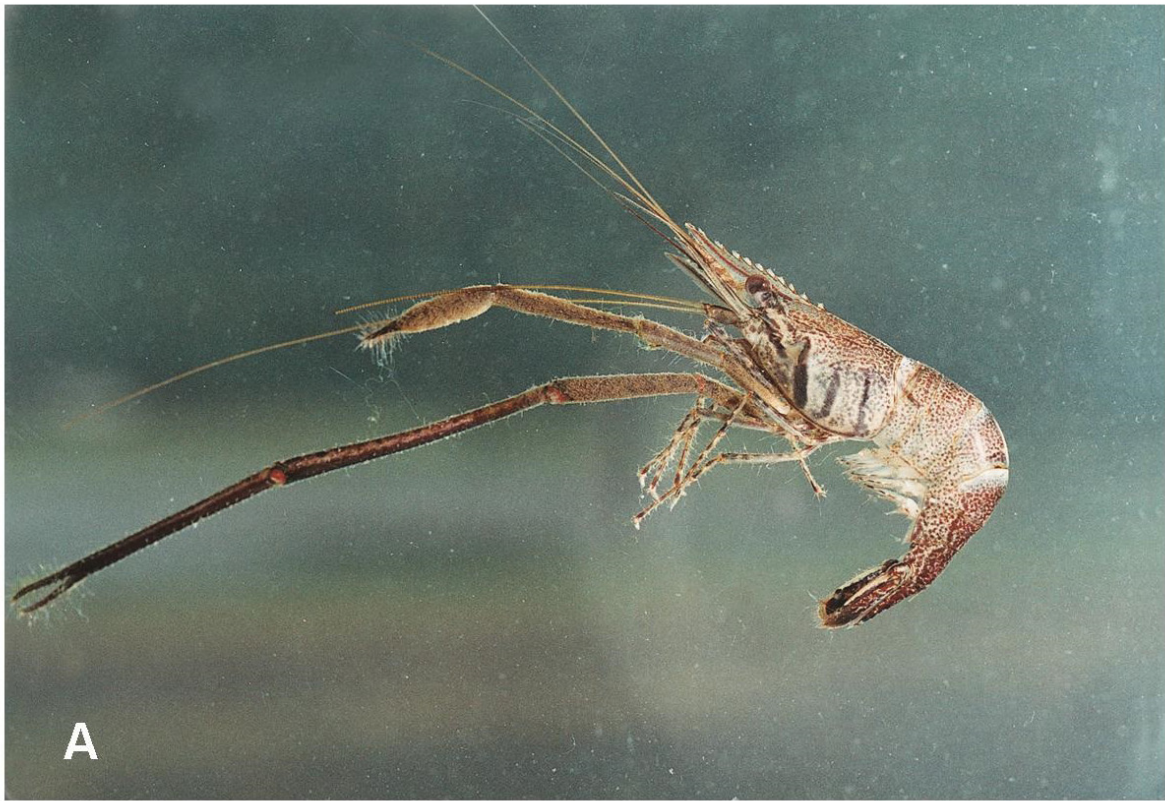
*P.[alæmon]* (*Eupalæmon*) *ustulatus* Nobili, 1899: 241 (Rigo, SE Papua).

*Palaemon ustulatus* – de Man 1915: 431, pl. 29, fig. 32 (Rigo, SE Papua).

### Material examined

#### Holotype

PAPUA NEW GUINEA: 1 ♂, Rigo District, 9.8767° S, 147.7938° E, unknown date, 23.5 mm CL (MSNG VII-106).



**Fig. 4.** **A.** Live coloration of *Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838) (photo: E. Vigneux). **B.** Live coloration of *M. ustulatum* (Nobili, 1899) (photo: P. Keith).



### Other material

VANUATU: 1 ♂, Espiritu Santo Island, Ora Jourdain River, 15.1666° S, 166.8999° E, 3 Oct. 1994, 20.7 mm CL (MNHN-IU-2013-13201); 1 ♂, 22 mm CL (MNHN-IU-2013-13202).

REPUBLIC OF PALAU: 1 ♂, Palau Island, Negmeskang River, 7.5413° N, 134.5778° E, 1 Mar. 2011, 20.7 mm CL (MNHN-IU-2013-13203).

### Description

**ROSTRUM.** Moderately long, reaching end of scaphocerite (Fig. 5A); tip horizontal; dorsal margin nearly straight, faintly convex, with 10–12 teeth, including 3 teeth on carapace; ventral margin with 3–4 teeth; teeth large, generally evenly spaced, with gap near anterior end of dorsal series; sometimes distance between 1<sup>st</sup> and 2<sup>nd</sup> teeth larger than between 2<sup>nd</sup> and 3<sup>rd</sup> teeth.

**GENERAL CEPHALON.** Eyes well developed; cornea as long as, but broader than peduncle; stylocerite distinctly pointed, reaching beyond basal segment; inferior orbit bluntly angular (Fig. 5A); post-antennular carapace margin straight or slightly concave; protective setation absent on carapace; epistome lobes narrowly separated, strongly produced anteroventrally (Fig. 5B); TS4 with rounded median process (Fig. 5C).

**SECOND CHELIPEDS** (fully developed ♂). Unequal sizes, non-isomorphic. Major P2 (Fig. 5D) long, subcylindrical, with small part of merus extending beyond scaphocerite; carpus 0.68–0.73 CHL, 1–1.12 PL. Fingers slender 0.46–0.58 PL; dactylus shorter than FF. Cutting edges (Fig. 5E) with 4–5 conical teeth (3<sup>rd</sup> largest) followed by 6–10 evenly spaced, very small, blunt teeth, not along entire length; teeth diminishing in size distally; CEFF with teeth arrangement similar to that of dactylus; basally armed with ridge formed by 2 small teeth and 3–4 larger, conical teeth followed by 4–6 evenly spaced, small, blunt teeth, not along entire length; teeth diminishing in size distally. Largest tooth of FF lies just after largest tooth of CED when fingers closed. Cheliped with numerous spiniform setae, larger on inner edge, smaller and denser on outer edge. Numerous long setae present on manus and outer edge of fingers. Merus 0.54–0.61 CAL, ischium 0.64–0.85 MEL. Minor P2 (Fig. 5F) with entire carpus beyond scaphocerite. Carpus 0.67–0.75 CHL, 1.01–1.25 PL. Fingers slender 0.49–0.66 PL. CEFF with 11–14 teeth, gradually diminishing in size distally (Fig. 5G). CED with 13–15 teeth, slightly smaller and more numerous than those of major P2, similar teeth arrangement to that of dactylus. Fingers, palm and carpus densely packed with velvety setae. MEL 0.62–0.72 CAL, IL 0.74–1.05 MEL.

**ABDOMEN.** Smooth, inter-uropodal sclerite with preanal carina.

### Distribution

Syntopic with *M. australe* in the Northwest Pacific Ocean (Taiwan, Philippines, Papua New Guinea, Indonesia and Palau) and in the Southwest Pacific Ocean (Vanuatu) (Fig. 1).

### Habitat

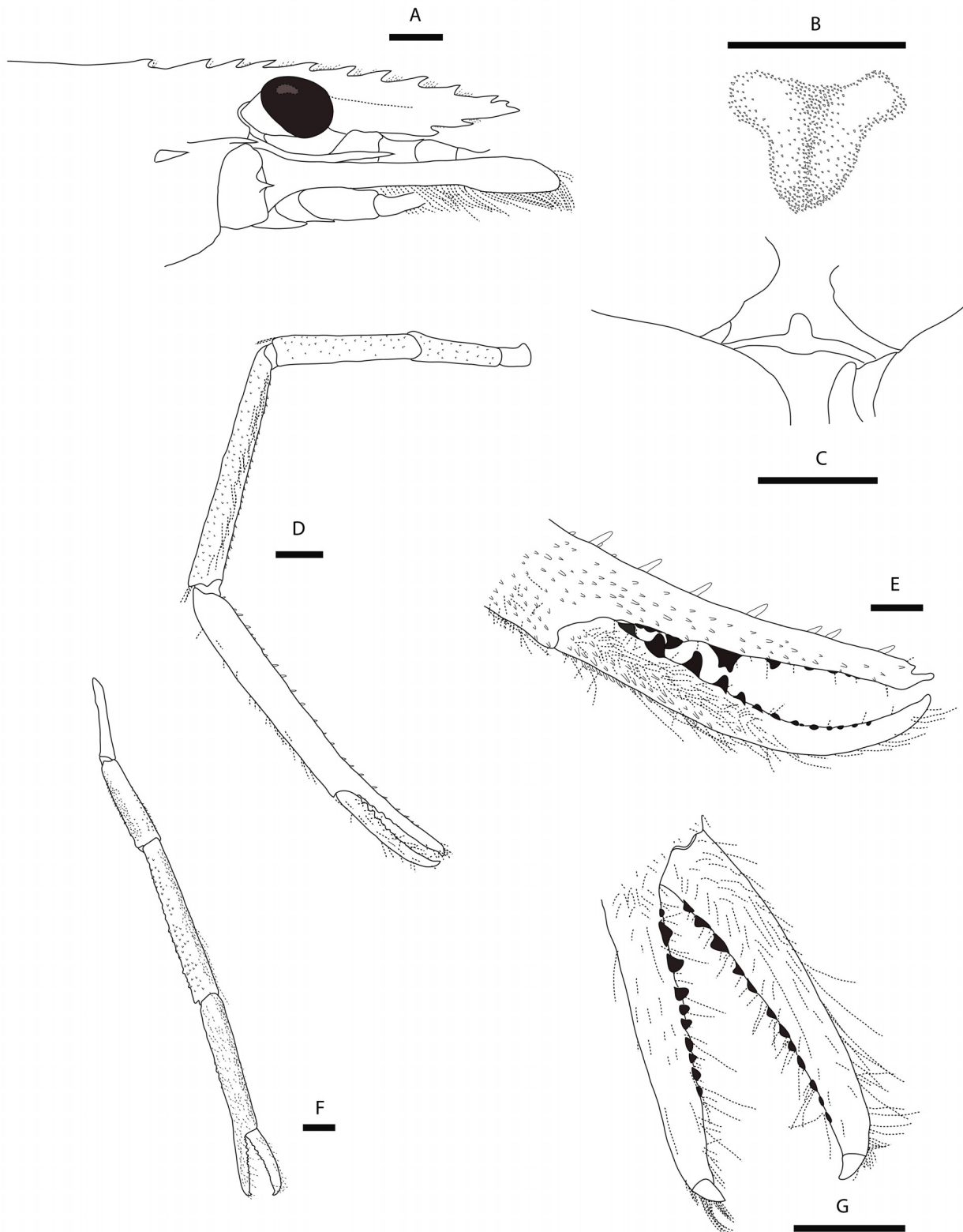
Lotic habitats in the middle course of rivers, clear and oxygenated waters, sometimes above waterfalls. Rocky substrates, boulders, pebbles and pools in countercurrents.

### Color patterns

Body colors (Fig. 4B) tend to match the substrate, varying from black to brownish red, with three reddish stripes on each side of the cephalothorax.

### Remarks

Some specimens originally assigned to *P. dispar* may correspond to *M. ustulatum*. This applies to de Man 1892: 435, pl. 26, fig. 44d (Salayar Island, Indonesia); de Man 1893: 304 (Flores, Indonesia);



**Fig. 5.** *Macrobrachium ustulatus* (Nobili, 1899). – **A–B, E.** MNHN-IU-2013-13202. **A.** Cephalothorax. **B.** Epistome. **E.** Major second pereiopod finger. – **C, G.** MNHN-IU-2013-13201. **C.** Fourth thoracic sternite. **G.** Minor second pereiopod finger. – **D, F.** MNHN-IU-2013-13203. **D.** Major second pereiopod. **F.** Minor second pereiopod. Scale bars: A, E, G = 2 mm; B–C = 1 mm; D, F = 4 mm.

de Man 1902: 766 (Halmahera, Indonesia); and de Man 1915: 435, pl. 29, figs 14–14a (Papua New Guinea).

## Discussion

The DNA sequencing of two unlinked molecular markers in specimens collected from a large geographic range throughout the Indo-Pacific, covering the entire known distribution range of *M. australe* and including localities close to most of the type localities of its synonyms, suggested the existence of at least two distinct species under what is commonly assigned to “*M. australe*”: *M. australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838), with a large distribution range from the Southwest Indian Ocean to the Central Pacific Ocean, and a cryptic species found syntopically at some islands of the Western Pacific Ocean (Taiwan, Philippines, Indonesia, Papua New Guinea, Palau and Vanuatu). Although they are not quite found in the same habitat (i.e., lentic vs lotic), the presence of these distinct genetic entities in the same rivers in Palau and the Santo Islands strongly favors the hypothesis that these genetic entities are reproductively isolated from one another. In addition, the two single-gene phylogenetic reconstructions, which included several species of *Macrobrachium* available from GenBank, suggested that the two species are closely related and could even be sister species. However, this hypothesis remains to be tested using a more comprehensive molecular dataset.

One of the main difficulties encountered in this study was to assign the genetic groups to the correct scientific name. Indeed, probably because of its large geographic range and morphological plasticity (Zimmermann *et al.* 2012), *M. australe* is currently also known under seven associated synonyms. Moreover, the lack of consensus on the diagnostic characters to use in the discrimination of species within this genus has led to descriptions that are difficult to compare. For instance, *P. australis* was described based on the shape of the rostrum; *P. danae* on the proportions of the various joints of the minor second pereopod (see De Man 1892); *P. dispar* on the proportions of the major second pereopod; *P. alphonsianus* on the second pereopods; *P. parvus* on the rostrum, the position of the hepatic spine and the proportions of the minor second pereopod; *P. malliardi* on the rostrum and the minor second pereopod; *P. ustulatus* on the minor second cheliped; and *L. lepidus* on the rostrum, the cephalic region and the pereopods I, II, III and IV (in very young specimens as well). In this context, to assign a scientific name to the cryptic species, we first sequenced specimens collected from or close to the type localities of all these synonymous species. For example, we studied samples from Moorea (French Polynesia) and Rarotonga (Cook Islands), which are close to the type locality of *P. australe*, Palau and Papua (close to the type localities of *P. dispar*, *P. ustulatus* and *L. lepidus*) and the Mascarene Islands (close to the type localities of *P. alphonsianus*, *P. parvus* and *P. malliardi*). We then examined the morphological characters of sequenced specimens, with particular attention toward mature male specimens, and compared these characters to museum samples and/or taxonomic descriptions of available synonyms.

Overall, molecular and morphological results both suggested that among the eight synonyms of *M. australe*, one (*M. ustulatum*) corresponds to a valid species, which therefore needs to be resurrected, one (*P. australis*) corresponds to the original species name, and six are junior synonyms of *M. australe* (*P. danae*, *P. dispar*, *P. alphonsianus*, *P. parvus*, *P. malliardi*, and *L. lepidus*). Indeed, based on rostrum shape, the position of the hepatic spine toward the antennal spine and the proportions of the male second pereopod, *P. danae*, *P. dispar*, *P. alphonsianus*, *P. parvus*, *P. malliardi* and *L. lepidus* can be considered as junior synonyms of *M. australe* (Holthuis 1950; Chace & Bruce 1993; de Grave & Fransen 2011). On the contrary, the proportions of the various joints of the minor second cheliped (i.e., carpus/chela, carpus/palm and finger/palm) of the holotype of *P. ustulatus* from MCSN do not match with the morphology of specimens belonging to *M. australe*, but fit well with those corresponding to the cryptic species identified in this study, *M. ustulatum*. We also emphasized our morphological analyses on the epistome and fourth thoracic sternite, two features known to be very consistent between sexes and among individuals at different development stages (Short 2004).



*M. ustulatum* lives in lotic habitats in the middle course of rivers. Indeed, *P. dispar* of de Man (1902) (most likely *M. ustulatum*) was from Halmahera, at an altitude of 600 m.

*Macrobrachium ustulatum* was reported as *P. dispar* by de Man in 1892, 1893, 1902 and 1915. In 1915 de Man examined *M. ustulatum* from Papua New Guinea and gave a good illustration of its rostrum (de Man 1915: pl. 29, fig. 12). However he continued to report specimens from Papua New Guinea as *P. dispar* although they had the typical chelipeds of *M. ustulatum* (de Man 1915: pl. 29, figs 14, 14a). Later, *P. dispar* was always cited as one among several synonyms of *M. australe*, notably by Holthuis (1950), Chace & Bruce (1993) and de Grave & Franssen (2011). In addition, *M. ustulatum* has until now been confused with *M. australe* because of the similar shape of their rostrum, the numerous teeth on the cutting edges of the fingers of the male second pereopods and the similar life color pattern.

## Conclusion

Overall, genetic and morphological results show that *M. australe* and *M. ustulatum* belong to two distinct species. *Macrobrachium australe* (Guérin-Méneville, 1838 in Guérin-Méneville 1829–1838) has a widespread distribution, from Mascarene to French Polynesia, whereas *M. ustulatum* (Nobili, 1899) could be more restricted, from Taiwan to Vanuatu. Further studies should be conducted on the known geographic range of *M. ustulatum*, in order to assess more precisely its distribution. While the biology and ecology of *M. australe* is relatively well known (e.g., r-reproductive strategy; Ito *et al.* 2002; Wowor *et al.* 2009), the life history traits of *M. ustulatum* are totally unknown, and it is not unlikely that the life history traits of these two species have been mixed in previous studies. This raises questions about the exact distribution of *M. ustulatum* and its actual species conservation status. Unlike *M. lar*, the most widespread amphidromous prawn, which so far has revealed no cryptic species (Castelin *et al.* 2013), *M. australe* with a similar widespread distribution shows one. Chace & Bruce (1993) and Wowor *et al.* (2009) all voiced their suspicions about the potential existence of many undescribed cryptic species in *Macrobrachium*. Furthermore, Liu *et al.* (2007) highlighted five cryptic species among the widespread East Asian species, namely *M. equidens* (Dana, 1852), *M. jaroense* (Cowles, 1914), *M. latidactylus* (Thallwitz, 1891), *M. latimanus* (von Martens, 1868) and *M. placidulum* (de Man, 1892). Similarly, Angre *et al.* (1998) described three species which are referred to the “*M. equidens* complex”.

## Acknowledgements

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**Appendix.** List of the 28S and 16S rRNA sequences recovered from GenBank. 28S = 28S rRNA GenBank accession number; 16S = 16S rRNA GenBank accession number.

Species	Locality	28S	16S	Source
<i>M. asperulum</i> (von Martens, 1868)	Taiwan	–	AB250423	Liu <i>et al.</i> 2011
	Taiwan	–	AB250424	Liu <i>et al.</i> 2011
	Taiwan	–	AB250433	Liu <i>et al.</i> 2011
	Taiwan	–	AB250459	Liu <i>et al.</i> 2011
<i>M. australiense</i> Holthuis, 1950	Australia	AY374145	–	Murphy & Austin 2005
	Australia	–	EF588317	Page <i>et al.</i> 2008a
<i>M. brasiliense</i> (Heller, 1862)	Brazil	AY374146	–	Murphy and Austin 2005
<i>M. bullatum</i> Fincham, 1987	Australia	AY374147	–	Murphy and Austin 2005
<i>M. callirrhoe</i> (de Man, 1898)	Sumatra	FM955490	–	Wowor <i>et al.</i> 2009
<i>M. crenulatum</i> Holthuis, 1950	Venezuela	–	GU929465	Vergamini <i>et al.</i> 2011
	Nigeria	–	KJ463388	Unpubl.
<i>M. edentatum</i> Liang & Yan, 1986	China	–	DQ194912	Liu <i>et al.</i> 2007
<i>M. equidens</i> (Dana, 1852)	Taiwan	EU493126	–	Chen <i>et al.</i> 2009
	Taiwan	EU493128	–	Chen <i>et al.</i> 2009
	Taiwan	EU493129	–	Chen <i>et al.</i> 2009
	Taiwan	EU493130	–	Chen <i>et al.</i> 2009
<i>M. esculentum</i> (Thallwitz, 1891)	Taiwan	EU008722	–	Chen <i>et al.</i> 2009
	Taiwan	EU493113	EU493145	Chen <i>et al.</i> 2009
	Taiwan	–	DQ194913	Liu <i>et al.</i> 2007
	Philippines	–	DQ194914	Liu <i>et al.</i> 2007
	S Sulawesi	FM955498	–	Wowor <i>et al.</i> 2009
<i>M. faustinum</i> (de Saussure, 1857)	Puerto Rico	–	EF490009	Page <i>et al.</i> 2008b
<i>M. formosense</i> Spence Bate, 1868	Taiwan	EU008723	–	Chen <i>et al.</i> 2009
	Taiwan	EU493104	–	Chen <i>et al.</i> 2009
	Taiwan	EU493106	–	Chen <i>et al.</i> 2009
	Taiwan	EU493111	–	Chen <i>et al.</i> 2009
	Taiwan	EU493131	–	Chen <i>et al.</i> 2009
	Japan	FM955489	–	Wowor <i>et al.</i> 2009
<i>M. gracilirostre</i> (Miers, 1875)	N Sulawesi	–	FM986613	Wowor <i>et al.</i> 2009
<i>M. hainanense</i> (Parisi, 1919)	Hainan	FM955502	–	Wowor <i>et al.</i> 2009
<i>M. heterochirus</i> (Wiegmann, 1836)	Brazil	–	HM352454	Pileggi & Mantelatto 2010
<i>M. holthuisi</i> Genofre & Lobão, 1978	Brazil	–	HM352456	Pileggi & Mantelatto 2010
<i>M. horstii</i> (de Man, 1892)	Borneo	–	FM986616	Wowor <i>et al.</i> 2009
<i>M. idae</i> (Heller, 1862)	Tioman Island	–	FM986617	Wowor <i>et al.</i> 2009
<i>M. japonicum</i> (De Haan, 1849 in De Haan 1833–1850)	Taiwan	EU008721	–	Chen <i>et al.</i> 2009
	Taiwan	–	–	Chen <i>et al.</i> 2009
	Taiwan	EU493110	–	Chen <i>et al.</i> 2009



Species	Locality	28S	16S	Source
<i>M. lanatum</i> Cai & Ng, 2002	Sumatra	–	FM986639	Wowor <i>et al.</i> 2009
<i>M. lar</i> (Fabricius, 1798)	Cook Island	KC507005	KC506861	Castelin <i>et al.</i> 2013
	Cook Island	KC507006	KC506865	Castelin <i>et al.</i> 2013
	Cook Island	KC507007	KC506866	Castelin <i>et al.</i> 2013
	Cook Island	KC507011	–	Castelin <i>et al.</i> 2013
	Cook Island	KC507012	KC506871	Castelin <i>et al.</i> 2013
<i>M. latidactylus</i> (Thallwitz, 1891)	Philippines	–	DQ194945	Liu <i>et al.</i> 2007
	Thailand	–	DQ194946	Liu <i>et al.</i> 2007
<i>M. latimanus</i> (von Martens, 1868)	Taiwan	EU008729	–	Chen <i>et al.</i> 2009
	Taiwan	EU493112	–	Chen <i>et al.</i> 2009
	Taiwan	EU493114	–	Chen <i>et al.</i> 2009
	Taiwan	–	DQ194936	Liu <i>et al.</i> 2007
	Philippines	–	DQ194937	Liu <i>et al.</i> 2007
	Japan	–	DQ194938	Liu <i>et al.</i> 2007
	India	–	JF774070	Unpubl.
	West Java	FM955511	–	Wowor <i>et al.</i> 2009
<i>M. lepidactylodes</i> (de Man, 1892)	Bt. Ciawi Tali, W Java or Lanyu	FM955512	–	Wowor <i>et al.</i> 2009
	Taiwan	EU008725	–	Chen <i>et al.</i> 2009
	Taiwan	EU493115	–	Chen <i>et al.</i> 2009
	Taiwan	EU493116	–	Chen <i>et al.</i> 2009
<i>M. malayanum</i> (Roux, 1935)	Taiwan	EU493118	–	Chen <i>et al.</i> 2009
	Singapore	AY374157	–	Murphy & Austin 2005
	Nee Soon	FM955515	–	Wowor <i>et al.</i> 2009
	Taiwan	–	EU493148	Chen <i>et al.</i> 2009
<i>M. mammillodactylus</i> (Thallwitz, 1892)	East Kalimantan	–	FM986629	Wowor <i>et al.</i> 2009
	China	–	DQ194948	Liu <i>et al.</i> 2007
<i>M. meridionale</i> Liang & Yan, 1983	Malaysia	–	DQ194949	Liu <i>et al.</i> 2007
	Hong Kong	FM955518	–	Wowor <i>et al.</i> 2009
	Malaysia	–	DQ194953	Liu <i>et al.</i> 2007
<i>M. neglectum</i> (de Man, 1905)	Thailand	–	JQ359750	Pinpart <i>et al.</i> 2010
<i>M. niphanae</i> Shokita & Takeda, 1989	Thailand	–	JQ359752	Pinpart <i>et al.</i> 2010
	Thailand	–	JQ390472	Pinpart <i>et al.</i> 2010
	Thailand	–	JQ390473	Pinpart <i>et al.</i> 2010
	Thailand	–	JQ390473	Pinpart <i>et al.</i> 2010
<i>M. nipponense</i> (De Haan, 1849 in De Haan 1833–1850)	Taiwan	EU493119	–	Chen <i>et al.</i> 2009
	Taiwan	EU493120	–	Chen <i>et al.</i> 2009
	Taiwan	EU493123	–	Chen <i>et al.</i> 2009
<i>M. ohione</i> (Smith, 1874)	Yizhang, Hunan	FM955520	–	Wowor <i>et al.</i> 2009
	Gulf of Mexico	EU868694	EU868694	Bracken <i>et al.</i> 2009
	Louisiana	–	HM352464	Pileggi & Mantelatto 2010

Species	Locality	28S	16S	Source
<i>M. olfersii</i> (Wiegmann, 1836)	Brazil	–	EF588321	Murphy & Austin 2005
	Brazil	AY374159	–	Murphy & Austin 2005
	Brazil	–	HM352457	Pileggi & Mantelatto 2010
	Venezuela	–	HM352460	Pileggi & Mantelatto 2010
<i>M. pilimanus</i> (de Man, 1879)	Central Indo-Pacific	GQ487513	–	Bracken <i>et al.</i> 2010
	Khammouane	FM955492	–	Wowor <i>et al.</i> 2009
	Khammouane	FM955521	–	Wowor <i>et al.</i> 2009
<i>M. placidum</i> (de Man, 1892)	Sumatra	–	FM986636	Wowor <i>et al.</i> 2009
<i>M. platycheles</i> Ou & Yeo, 1995	Singapore	AY374161	–	Murphy & Austin 2005
<i>M. potuina</i> (Müller, 1880)	Brazil	AY374162	–	Murphy & Austin 2005
<i>M. risenbergii</i> (de Man, 1879)	Tonle Sap	FM955495	–	Wowor <i>et al.</i> 2009
<i>M. rosenbergii</i> (de Man, 1879)	Australia	AY374144	–	Murphy & Austin 2005
	Thailand	AY374163	–	Murphy & Austin 2005
	Irian Jaya	FM955524	–	Wowor <i>et al.</i> 2009
<i>M. saigonense</i> Nguyễn, 2006	Tonle Sap	FM955525	–	Wowor <i>et al.</i> 2009
<i>M. shokitai</i> Fujino & Baba, 1973	Ryukyus, Japan	–	AB250466	Liu <i>et al.</i> 2007
	Japan	–	DQ194961	Liu <i>et al.</i> 2007
<i>M. sintangense</i> (de Man, 1898)	Thailand	FM955528	–	Wowor <i>et al.</i> 2009
<i>M. trompii</i> (de Man, 1898)	Sumatra	FM955529	–	Wowor <i>et al.</i> 2009
	Sumatra	–	FM986642	Wowor <i>et al.</i> 2009
<i>M. urayang</i> Wowor & Short, 2007	E Kalimantan	FM955530	–	Wowor <i>et al.</i> 2009
<i>M. ustulatum</i> (Nobili, 1899)	Taiwan	EU008719	–	Chen <i>et al.</i> 2009
	Taiwan	–	EU493135	Chen <i>et al.</i> 2009
	Taiwan	EU493097	–	Chen <i>et al.</i> 2009
	Taiwan	EU493098	–	Chen <i>et al.</i> 2009
	Taiwan	EU518931	–	Chen <i>et al.</i> 2009
	Taiwan	–	DQ194904	Liu <i>et al.</i> 2007
	Philippines	–	DQ194905	Liu <i>et al.</i> 2007
	Papua New Guinea	–	JF310710	Page & Hughes 2011
	New Guinea	–	DQ681290	Page <i>et al.</i> 2007
	Papua New Guinea	FN995633	–	Page & Hughes 2011
<i>M. vollenhovenii</i> (Herklots, 1857)	Nigeria	–	KJ463387	Unpubl.
<i>P. debilis</i> Dana, 1852	China	–	KC515044	Kou <i>et al.</i> 2013
	Sumatra	FM955535	–	Wowor <i>et al.</i> 2009

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