

Use of morphological and molecular data to identify three new sibling species of the genus *Munida* Leach, 1820 (Crustacea, Decapoda, Galatheidae) from New Caledonia

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Abstract

Three cryptic species of the genus *Munida* from New Caledonia, previously identified as *M. tuberculata* Henderson, 1885, *M. notata* Macpherson, 1994 and *M. clinata* Macpherson, 1994, are described and illustrated. The three species are identified by subtle and constant morphological characters, which match clear differences in molecular sequences (16S rDNA and COI genes). The results also confirm the importance of several of these characters (e.g. length of the antennular and antennal spines) in the taxonomy of the genus *Munida*.

Keywords: *Cryptic species, molecular data, morphology, Munida, new species, Pacific ocean, taxonomy*

Introduction

The area of New Caledonia and adjacent islands (e.g. Chesterfield, Loyalty, Matthew and Hunter) contains a very rich fauna and is considered a “hot spot” in marine biodiversity (Richer de Forges et al. 2000; Bouchet et al. 2002). Among the distinct groups of decapod crustaceans in the area, the genus *Munida* Leach, 1820 is one of the most diverse, with around 80 species (Macpherson 1994, 2004). These crustaceans are found from ca 20 m to ca 2000 m, and have a variety of habitats (i.e. rocks, corals, sponges, mud).

Molecular data provide a complementary approach to discriminate species separated by subtle morphological characters (Knowlton 1993; Avise 1994; Sarver et al. 1998). Recently, Macpherson and Machordom (2001) identified species of the genus *Raymunida* (formerly in the genus *Munida*), distinguished by small morphological differences, which matched clear differences in mitochondrial nucleotide sequences. These studies and other related papers (e.g. Chan and Chu 1996; Sarver et al. 1998; Mathews et al. 2002; Goetze 2003) have confirmed the usefulness of molecular data, in addition to morphology, as supplementary support for species identification.

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During two recent cruises in the sea-mounts of New Caledonia, SW Pacific Ocean (NORFOLK 1, June 2001, and NORFOLK 2, October to November 2003), several representatives of *Munida*, initially identified as *M. tuberculata* Henderson, 1885, *M. clinata* Macpherson, 1994 and *M. notata* Macpherson, 1994 (Henderson 1888; Macpherson 1994), were collected. These specimens showed slight morphological differences with type material, which were considered part of the intraspecific variability of these characters. However, the molecular analysis of these specimens for phylogenetic purposes (Machordom and Macpherson 2004) revealed significant genetic divergences with topotypic individuals, indicating cryptic species and the taxonomic validity of several subtle, but constant, morphological characters. Here the three new sibling species are described.

Material and methods

For morphological descriptions, we examined the specimens collected during the NORFOLK 1 and 2 cruises (see below) and deposited in the Muséum national d'Histoire naturelle of Paris (MNHN). For comparative material, we used type specimens of *M. notata* and *M. clinata* (see Macpherson 1994), specimens collected during the cruises, and specimens of *M. tuberculata* collected in the type locality, Fiji Islands (see Macpherson 2004) and during the cruises. The terminology used follows that of previous papers (see Macpherson 1994; Baba and de Saint-Laurent 1996). The measurements given are the carapace length, excluding rostrum.

For molecular analysis, we used specimens collected during the cruise NORFOLK 1, preserved in absolute alcohol and deposited in the MNHN. One to four individuals for each putative species were analysed. From each specimen, a pereopod or a small piece of the abdomen muscle was removed. The list of specimens used, locations and GenBank accession numbers are given in Table I.

Total DNA was extracted after eliminating the alcohol, grinding the samples to a powder in liquid nitrogen or mincing the muscle, before adding 600 µl of CTAB lysis buffer (2%

Table I. Species studied for mitochondrial DNA sequences collected during the NORFOLK 1 cruise, including station data.

Species	Station	Latitude	Longitude	Depth (m)	GenBank accession no.		
					COI	16S	Code
<i>M. tuberculata</i>	1694	24°39'69S	168°38'61E	575–589	AY351015	AY351184	Fo102
<i>M. parile</i>	1695	24°39'66S	168°38'812E	562–587	AY351024	AY351193	Fo111
<i>M. parile</i>	1697	24°39'500S	168°38'273E	569–616	AY351025	AY351194	Fo117
<i>M. parile</i>	1701	24°40'228S	168°39'296E	564–586	AY351026	AY351195	Fo120
<i>M. notata</i>	1683	24°43'144S	168°07'695E	248–272	AY350976	AY351144	Fo67
<i>M. notata</i>	1683	24°43'144S	168°07'695E	248–272	AY350977	AY351145	Fo68
<i>M. notata</i>	1712	23°22'58S	168°02'5E	180–600	AY350978	AY351146	Fo125
<i>M. notata</i>	1718	23°23'728S	168°01'386E	260–373	AY350979	AY351147	Fo164
<i>M. simulatrix</i>	1721	23°19'249S	168°00'862E	416–443	AY351027	AY351196	Fo157
<i>M. clinata</i>	1727	23°17'377S	168°14'346E	190–212	AY350942	AY351113	Fo219
<i>M. clinata</i>	1681	24°43'835S	168°09'699E	228–240	AY350940	AY351111	Fo220
<i>M. clinata</i>	1726	23°18'009S	168°14'852E	185–207	AY350941	AY351112	Fo221
<i>M. pectinata</i>	1727	23°17'377S	168°14'346E	190–212	AY351028	AY351197	Fo249
<i>M. pectinata</i>	1727	23°17'377S	168°14'346E	190–212	AY351029	–	Fo250

cetyl trimethylammonium bromide, 1.4 M NaCl, 0.2% β -mercaptoethanol, 20 mM EDTA, 0.1 M Tris, pH 8) and digested with proteinase K ($100 \mu\text{g ml}^{-1}$) at 55°C for several hours to 2 days. The rest of the extraction followed the phenol/chloroform method (Sambrook et al. 1989).

The partial COI was amplified with the same primers and polymerase chain reaction (PCR) cycles, following Macpherson and Machordom (2001). In the case of the partial 16S rDNA, the conditions were the same as those described in Machordom and Macpherson (2004).

Each strand was sequenced using “Big Dye Terminator” (Applied Biosystems, Inc.) sequencing reactions. Sequence gels were run on an ABI Capillary 3700 Genetic Analyzer (Applied Biosystems).

The DNA sequences obtained were cleaned at the primer ends, aligned and controlled using the Sequencer program (Gene Code Corporation). The CLUSTAL W program (Thompson et al. 1994) was used to align the 16S gene sequences. Additionally, all alignments were controlled by eye. The MacClade 3.06 package was used to translate COI to protein (Maddison and Maddison 1992).

Results

The three new cryptic species (*M. sp1*, *M. sp2* and *M. sp3*) were previously classified as *Munida tuberculata*, *M. notata* and *M. clinata*, respectively, on the basis of their morphological features (Machordom and Macpherson 2004). The molecular data on two mitochondrial genes indicated the following pairs of sister taxa: *M. tuberculata* and *M. sp1*, *M. notata* and *M. sp2*, and *M. clinata* and *M. sp3*. These data consisted of two fragments of 657 and 526 aligned base pairs for the COI and 16S fragments, respectively. The divergences between each pair of taxa (the putative cryptic species and the species in which the new species were previously included) ranged from 1.4% to 5.2% for 16S and from 8.2% to 14% for COI (Table II). These values correspond to a range from 15 substitutions for the 16S to 92 for the COI.

Taxonomy

Munida parile sp. nov.

(Figure 1)

Material examined

NORFOLK 1, stn 1695, 24 June 2001, $24^\circ39.665'S$, $168^\circ38.812'E$, 562–587 m: two males 4.0–5.1 mm; stn 1697, 24 June 2001, $24^\circ39.500'S$, $168^\circ38.273'E$, 569–616 m: one male 3.5 mm; stn 1699, 24 June 2001, $24^\circ39.679'S$, $168^\circ39.978'E$, 581–600 m: one male 7.6 mm; stn 1701, 24 June 2001, $24^\circ40.228'S$, $168^\circ39.296'E$, 564–586 m: one ovigerous female 3.7 mm (holotype, MNHN-Ga 4616).

NORFOLK 2, stn 2061, 25 October 2003, $24^\circ39.50'S$, $168^\circ40.32'E$, 620–1040 m: one female 4.8 mm; stn 2069, 26 October 2003, $25^\circ20.07'S$, $168^\circ57.60'E$, 795–852 m: one male 5.1 mm; stn 2078, 27 October 2003, $25^\circ20.70'S$, $168^\circ18.60'E$, 654–877 m: one male 3.9 mm.

Table II. Divergences (uncorrected “p” distances, per unit) among the specimens analysed: 16S (above diagonal) and COI (below diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. <i>M. clinata</i> (Fo219)	–	0.000	0.000	0.052	*****	0.065	0.065	0.065	0.066	0.073	0.113	0.110	0.110	0.110
2. <i>M. clinata</i> (Fo220)	0.008	–	0.000	0.052	*****	0.065	0.065	0.065	0.066	0.073	0.113	0.110	0.110	0.110
3. <i>M. clinata</i> (Fo221)	0.008	0.000	–	0.052	*****	0.065	0.065	0.065	0.066	0.073	0.113	0.110	0.110	0.110
4. <i>M. pectinata</i> (Fo249)	0.099	0.096	0.096	–	*****	0.045	0.045	0.045	0.045	0.051	0.111	0.108	0.108	0.108
5. <i>M. pectinata</i> (Fo250)	0.100	0.097	0.097	0.001	–	*****	*****	*****	*****	*****	*****	*****	*****	*****
6. <i>M. notata</i> (Fo67)	0.123	0.123	0.123	0.103	0.102	–	0.000	0.000	0.000	0.014	0.097	0.089	0.089	0.089
7. <i>M. notata</i> (Fo68)	0.123	0.123	0.123	0.103	0.102	0.000	–	0.000	0.000	0.014	0.097	0.089	0.089	0.089
8. <i>M. notata</i> (Fo125)	0.123	0.123	0.123	0.103	0.102	0.000	0.000	–	0.000	0.014	0.097	0.089	0.089	0.089
9. <i>M. notata</i> (Fo164)	0.123	0.123	0.123	0.103	0.102	0.000	0.000	0.000	–	0.014	0.099	0.089	0.089	0.089
10. <i>M. simulatrix</i> (Fo157)	0.134	0.134	0.134	0.116	0.114	0.082	0.082	0.082	0.082	–	0.092	0.081	0.081	0.081
11. <i>M. tuberculata</i> (Fo102)	0.186	0.187	0.187	0.195	0.193	0.178	0.178	0.178	0.178	0.167	–	0.043	0.043	0.043
12. <i>M. parile</i> (Fo111)	0.192	0.192	0.192	0.177	0.175	0.181	0.181	0.181	0.181	0.193	0.140	–	0.000	0.000
13. <i>M. parile</i> (Fo117)	0.193	0.193	0.193	0.178	0.177	0.181	0.181	0.181	0.181	0.193	0.140	0.001	–	0.000
14. <i>M. parile</i> (Fo120)	0.192	0.192	0.192	0.177	0.175	0.181	0.181	0.181	0.181	0.193	0.140	0.000	0.001	–

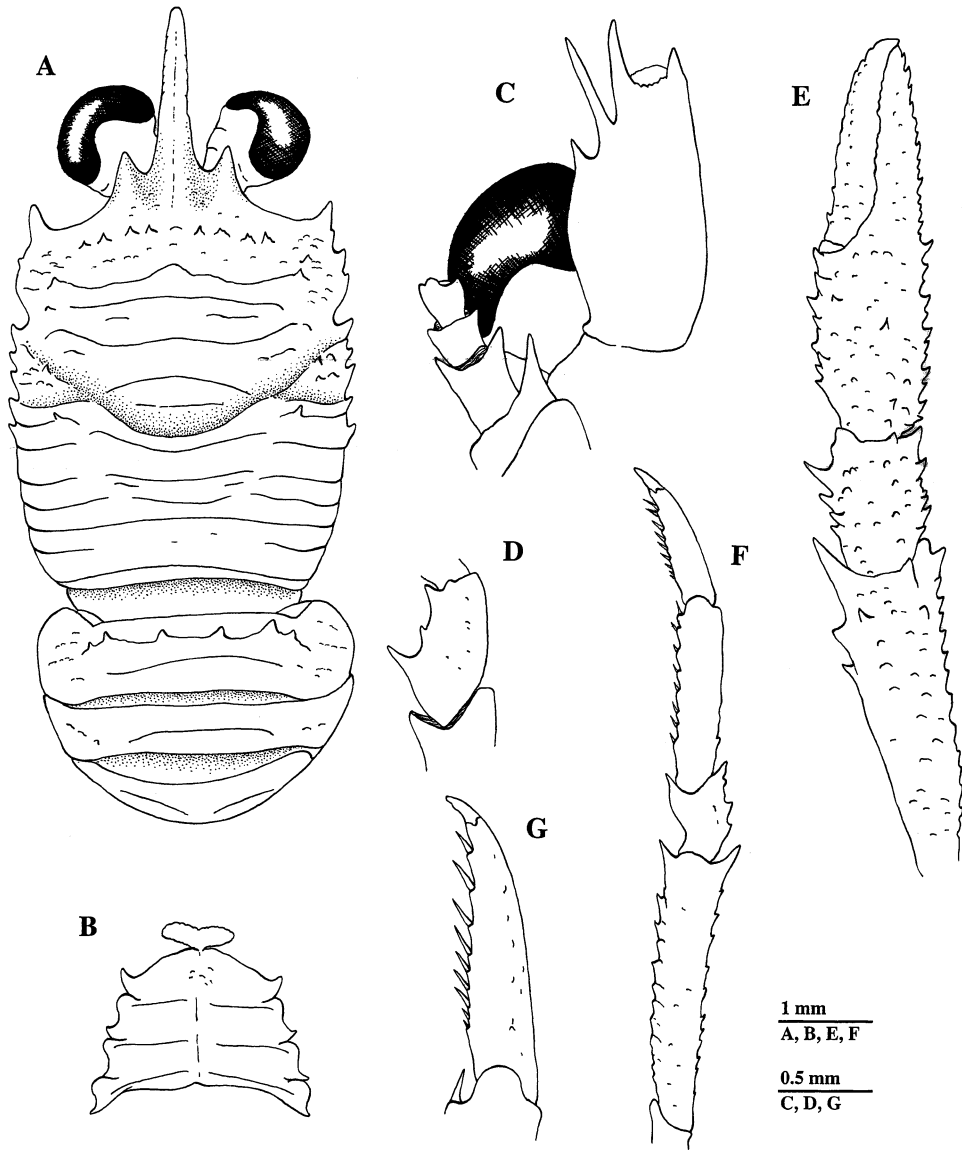


Figure 1. *Munida parile* sp. nov., ovigerous female 3.7 mm, holotype from stn 1701 (NORFOLK 1). (A) Carapace, dorsal view; (B) sternal plastron; (C) ventral view of cephalic region, showing antennular and antennal peduncles; (D) left third maxilliped, lateral view; (E) right cheliped, dorsal view; (F) left first walking leg, lateral view; (G) dactylus of left first walking leg, lateral view.

Etymology

From the Latin, *par*, equal, like, in reference to the similarity to *M. tuberculata*.

Description

Carapace slightly longer than wide. Transverse ridges mostly interrupted on cardiac region, with few short, non-iridescent setae. Main transverse striae on posterior part of carapace

interrupted in cardiac region. Small scales on hepatic and anterobranchial regions. Intestinal region without striae. Gastric region with row of small epigastric spines. One parahepatic, one or two anterobranchial and one postcervical spine on each side. Frontal margins transverse. Lateral margins slightly convex. Anterolateral spine well developed, situated at anterolateral angle, not reaching level of sinus between rostrum and supraocular spines. One to two small marginal spines before cervical groove. Branchial margins with five small spines. Rostrum spiniform, half as long as remaining carapace, horizontal. Supraocular spines short, not reaching midlength of rostrum or end of corneas, parallel, directed upwards (Figure 1A).

Thoracic sternites smooth, without granules or carinae. Few short scales on fourth sternite. Anterior part of fourth sternite narrower than third (Figure 1B).

Anterior ridge of second abdominal somite with six spines. Second to fourth somites each with one transverse stria.

Eyes large, maximum corneal diameter a third of the distance between bases of anterolateral spines.

Basal segment of antennule (distal spines excluded), about one-third carapace length, elongate, over-reaching cornea, with two distal spines, mesial spine shorter than lateral spine; two spines on lateral margin, proximal one short, located at midlength of segment, distal one long, reaching end of distolateral spine (Figure 1C).

Antennal peduncle reduced. First segment with one short distal spine on mesial margin, nearly reaching end of second segment, and reaching base of basal antennular segment; second segment with two short distal spines, mesial spine not reaching end of third segment; third segment with small distomesial spine (Figure 1C).

Ischium of third maxilliped about twice length of merus measured along dorsal margin, distoventrally bearing spine. Merus of third maxilliped with three spines on flexor margin, median smaller; extensor margin unarmed (Figure 1D).

Chelipeds subequal, squamous, with a few short uniramous, non-iridescent setae. Palm slightly shorter than fingers. Merus armed with some spines, strongest spine on distal border short, reaching proximal third of carpus. Carpus with some spines on dorsal side and several spines scattered on mesial and ventral sides. Palm with several spines scattered on mesial and dorsal sides and one row of lateral very short spines, continuing along fixed finger. Fingers distally curving and crossing, ending in a sharp point; movable, with one basal spine on mesial border; cutting edges with small teeth of distinct sizes (Figure 1E).

Second pereopod slightly shorter than twice carapace length; merus shorter than carapace, about five times as long as high, about three times carpus length and 1.6 times as long as propodus; propodus four times longer than height, as long as dactylus (Figure 1F). Merus with small spines on dorsal border, increasing in size distally, ventral margin with several spines and one long distal spine. Carpus with few dorsal spines and one distoventral spine. Propodus with eight to nine movable ventral spines. Dactylus slightly curving distally, with eight to nine movable spinules along entire ventral margin (Figure 1G). Third pereopod similar to second; fourth pereopod shorter than second and third. Merus of fourth pereopod two-thirds length of second pereopod.

Remarks

Munida parile belongs to the group of species with the following features: five spines on the lateral margins of the carapace behind the cervical groove, large eyes, spines along the anterior ridge of the second abdominal somite, no granules or carinae on the lateral parts of

the posterior thoracic sternites, spiniform rostrum, a longer distolateral than distomesial spine of the basal antennular segment, small antennal peduncle and a row of spinules along the entire ventral border of the dactylus of the walking legs. The new species is closely related to *M. tuberculata* Henderson, 1885 found between 435 and 650 m in New Caledonia, Matthew and Hunter Islands and Fiji Islands (type locality) (Henderson 1888; Macpherson 1994, 2004). For comparison, the material of *M. tuberculata* listed in Macpherson (1994, 2004) and specimens collected from distinct localities, including type material (Table I) was examined.

The two species are distinguished by several subtle morphological characters as follows.

The rostral spine is thicker in *M. tuberculata* than in *M. parile*. The rostral spine (measured at level of end of corneae) is as wide as or narrower than the second segment of the antennular peduncle (measured at terminal level) in *M. parile*, whereas the rostral spine is clearly wider in *M. tuberculata*.

The anterolateral angle of the carapace is more convex in *M. tuberculata* than in *M. parile*. Furthermore, the anterolateral spine is situated just on the anterolateral angle in *M. parile*, while in *M. tuberculata* it is placed on the frontal margin (see Macpherson 1994, Figure 58).

The granules on the hepatic regions are more numerous and the epigastric spines are more granulated in *M. tuberculata* than in *M. parile*.

The molecular data showed a nucleotidic divergence of about 9.7% when the two genes were considered together (4.3% for 16S sequences and 14% for COI; Table II), the greatest divergence of the three pairs of taxa. This divergence corresponds to 27 and 92 diagnostic positions in the 16S and COI, respectively. For the latter, two haplotypes for the three specimens of the new species were found, which differed in only one position among the 657 analysed.

Distribution

New Caledonia, between 562 and 616 m.

***Munida simulatrix* sp. nov.**

(Figure 2)

Munida notata Macpherson 1994, p 500 (in part).

Material examined

New Caledonia. BIOCAL, stn 108, 9 September 1985, 22°02.55'S, 167°05.68'E, 335 m: 6 males 5.7–7.7 mm, four ovigerous females 5.6–7.8 mm.

NORFOLK 1, stn 1721, 26 June 2001, 23°19.249'S, 168°00.862'E, 416–443 m: one male 7.7 mm (holotype, MNHN-Ga 4617).

NORFOLK 2, stn 2092, 29 October 2003, 24°45.17'S, 168°07.23'E, 320–345 m: three males 7.6–9.6 mm, one ovigerous female 7.8 mm; stn 2118, 1 November 2003, 23°22.87'S, 168°00.86'E, 383–393 m: one ovigerous female 9.3 mm; stn 2119, 1 November 2003, 23°22.75'S, 168°01.64'E, 300 m: three males 7.9–9.8 mm, one ovigerous female 8.6 mm; stn 2127, 2 November 2003, 23°16.03'S, 168°14.60'E, 379–381 m: two males 10.5–11.0 mm; stn 2130, 2 November 2003, 23°15.90'S, 168°13.54'E, 375–427 m: one female 10.9 mm; stn 2135, 3 November 2003, 23°01.61'S, 168°21.35'E,

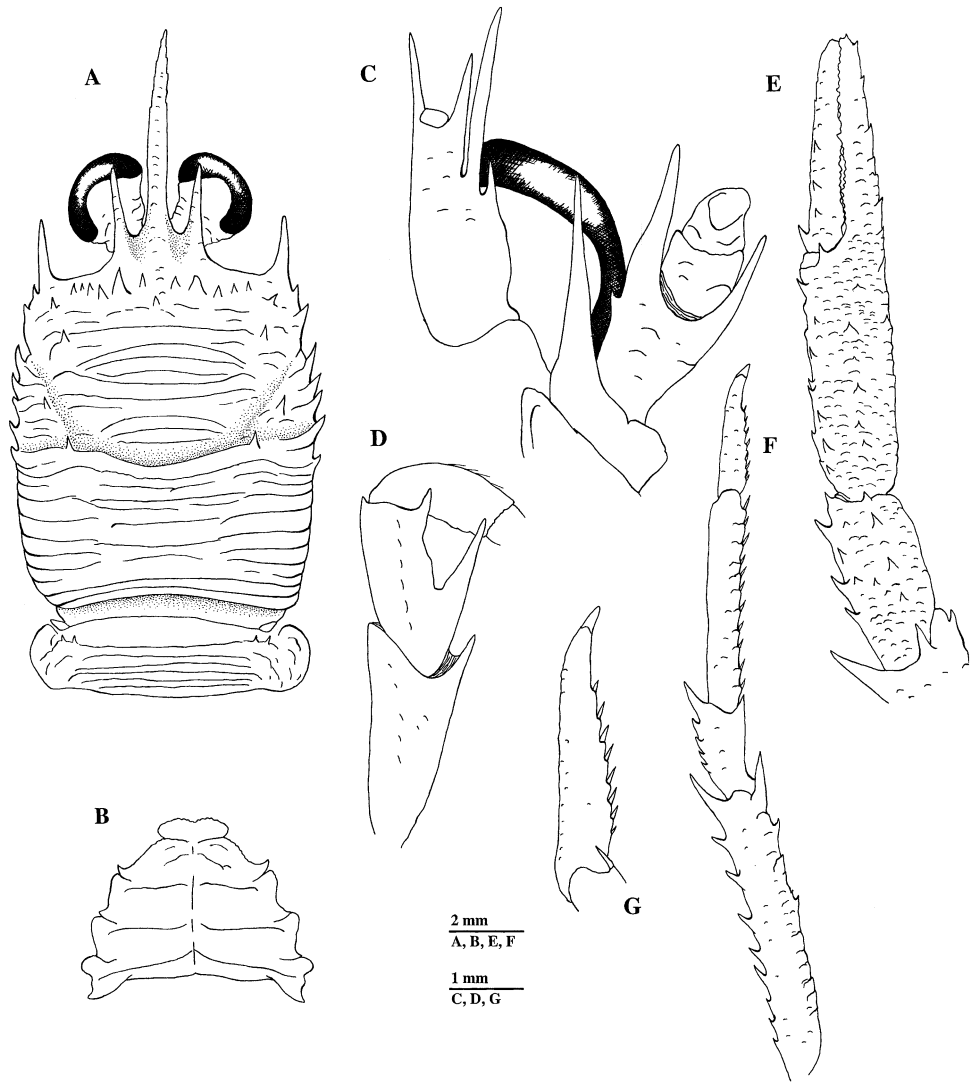


Figure 2. *Munida simulatrix* sp. nov., male 7.7 mm, holotype from stn 1721 (NORFOLK 1). (A) Carapace, dorsal view; (B) sternal plastron; (C) ventral view of cephalic region, showing antennular and antennal peduncles; (D) right third maxilliped, lateral view; (E) right cheliped, dorsal view; (F) right first walking leg, lateral view; (G) dactylus of right first walking leg, lateral view.

295–330 m: one male 6.7 mm; stn 2139, 3 November 2003, 23°00.56'S, 168°22.80'E, 372–393 m: one male 8.5 mm, one ovigerous female 8.4 mm; stn 2148, 4 November 2003, 22°44.20'S, 167°15.97'E, 386–391 m: three males 6.3–7.7 mm, four ovigerous females 7.4–8.7 mm, one female 7.4 mm; stn 2151, 5 November 2003, 22°42.75'S, 167°14.10'E, 353–368 m: one male 9.6 mm, two ovigerous females 8.4–8.6 mm; stn 2152, 5 November 2003, 22°44.72'S, 167°13.89'E, 380–390 m: one male 11.2 mm, one ovigerous female 11.3 mm.

Loyalty Islands. MUSORSTOM 6, stn 398, 13 February 1989, 20°47.19'S, 16705.65'E, 370 m: four males 9.2–10.1 mm, three ovigerous females 9.0–9.5 mm.

Etymology

From the Latin *simulatrix* in reference to the similarity to *M. notata*.

Description

Carapace slightly longer than wide, longitudinally convex. Transverse ridges mostly interrupted, with dense, very short, non-iridescent setae and a few long setae. Few scales and secondary striae between main striae. One stria on intestinal region. Gastric region with 12 epigastric spines. One hepatic, one parahepatic, one anterior branchial and one postcervical spine on each side. Frontal margins transverse. Lateral margins subparallel. Anterolateral spine well developed, reaching level of sinus between rostrum and supraocular spines. Two small marginal spines before cervical groove, posterior spine larger than preceding one and about three times smaller than anterolateral spine. Branchial margins with five spines (Figure 2A).

Rostrum spiniform, three-quarters as long as remaining carapace, straight and slightly upwards directed. Supraocular spines reaching midlength of rostrum and not reaching end of corneae, parallel, directed upwards.

Thoracic sternites smooth. Fourth sternites with few short arcuate striae, concave medially. Anterior part of fourth sternite as wide as third (Figure 2B).

Second abdominal somite with one pair of spines on each side of anterior ridge. Second to fourth somites with several transverse striae.

Eyes large, maximum corneal diameter nearly half distance between bases of anterolateral spines.

Basal segment of antennule (distal spines excluded), about one-quarter carapace length, elongate, slightly over-reaching end of corneae, with two distal spines, mesial longer than lateral; two spines on lateral margin, proximal one short, located at midlength of segment, distolateral spine long, reaching end of distal spines (Figure 2C).

First segment of antennal peduncle with one strong distal spine on mesial margin, slightly over-reaching end of third segment and reaching base of long lateral spine of basal segment of antennule; second segment with two long distal spines, mesial spine longer than lateral spine, slightly over-reaching end of antennal peduncle; penultimate segment unarmed (Figure 2C).

Ischium of third maxilliped slightly longer than merus measured along dorsal margin, distoventrally bearing spine. Merus of third maxilliped with two well-developed spines on flexor margin, distal smaller, extensor margin with small distal spine (Figure 2D).

Chelipeds squamous, with long uniramous setae, most of them iridescent, denser on mesial and lateral borders of articles. Palm slightly longer than fingers. Merus armed with some spines, strongest spine on distal border short, reaching proximal third of carpus. Carpus with several spines on dorsal side and several spines scattered on mesial and ventral sides. Palm with spines scattered on mesial and dorsal sides and row of spines on lateral border. Fixed finger with some spines along lateral margin, movable finger with spines along mesial border, fingers distally curving and crossing, ending in a sharp point (Figure 2E).

Second pereopod about two times carapace length; merus shorter than carapace, about six times as long as high, about three times carpus length and 1.5 times as long as propodus; propodus about 5.5 times as long as high, 1.5 times longer than dactylus (Figure 2F). Merus with well-developed spines along dorsal and ventral borders, increasing in size distally. Carpus with some dorsal spines and one distoventral spine. Propodus with 11

movable ventral spines. Dactylus slightly curving distally, with eight movable spinules along ventral margin, distal quarter unarmed (Figure 2G). Third pereopod slightly shorter than second; fourth pereopod clearly shorter than second and third. Merus of fourth pereopod two-thirds length of second pereopod.

Remarks

Munida simulatrix belongs to the group of species with the following features: five spines on the lateral margins of the carapace behind the cervical groove, smooth thoracic sternites, large eyes, abdominal somites that are unarmed or have spines on each side of the anterior ridge on the second somite, spiniform rostrum, a shorter distomesial than distolateral spine of the basal antennular segment and a distal spine on the extensor margin of the merus of the third maxilliped. The new species is very close to *M. notata* Macpherson, 1994, found between 59 and 850 m in New Caledonia, Loyalty Islands, Chesterfield Islands, Vanuatu, Wallis and Futuna Islands, Fiji Islands and Tonga Islands (Macpherson 1994, 2004). For comparison, material of *M. notata* collected during the NORFOLK 1 and 2 cruises and additional material, included in the original description (Macpherson 1994), was used.

However, these two species are distinguished by the following morphological and molecular differences.

The dorsal surface of the branchial regions in *M. notata* have more secondary striae than *M. simulatrix*.

The distomesial spine of the basal antennal segment reaches the base of the long lateral spine of the basal antennular segment in *M. simulatrix*, whereas this does not occur in *M. notata* (see Macpherson 1994, Figure 34c). Furthermore, this distomesial spine of the basal segment clearly over-reaches the third segment in the new species, whereas in *M. notata* it only slightly exceeds this segment.

Molecular data allowed distinction between *M. simulatrix* and *M. notata*, even though these species are the most closely related of the three pairs of taxa studied. Fifteen substitutions were found in the 16S sequences analysed and 67–68 in the COI, which leads to a global divergence of 5.2% (1.4% for 16S and 8.2 for COI).

Distribution

New Caledonia at 295–443 m and Loyalty Islands (370 m).

***Munida pectinata* sp. nov.**

(Figure 3)

Material examined

New Caledonia. NORFOLK 1, stn 1681, 22 June 2001, 24°43.835'S, 168°09.699'E, 228–240 m: one ovigerous female 9.1 mm; stn 1727, 27 June 2001, 23°17.377'S, 168°14.346'E, 190–212 m: two males 7.6–7.9 mm; four ovigerous females 6.4–10.0 mm (holotype, ovigerous female 6.9 mm, MNHN-Ga 4618); three females 6.0–6.9 mm.

NORFOLK 2, stn 2096, 29 October 2003, 24°43.91'S, 168°08.88'E, 230–240 m: two males 7.1–11.2 mm, three ovigerous females 10.4–11.1 mm, one female 7.9 mm.

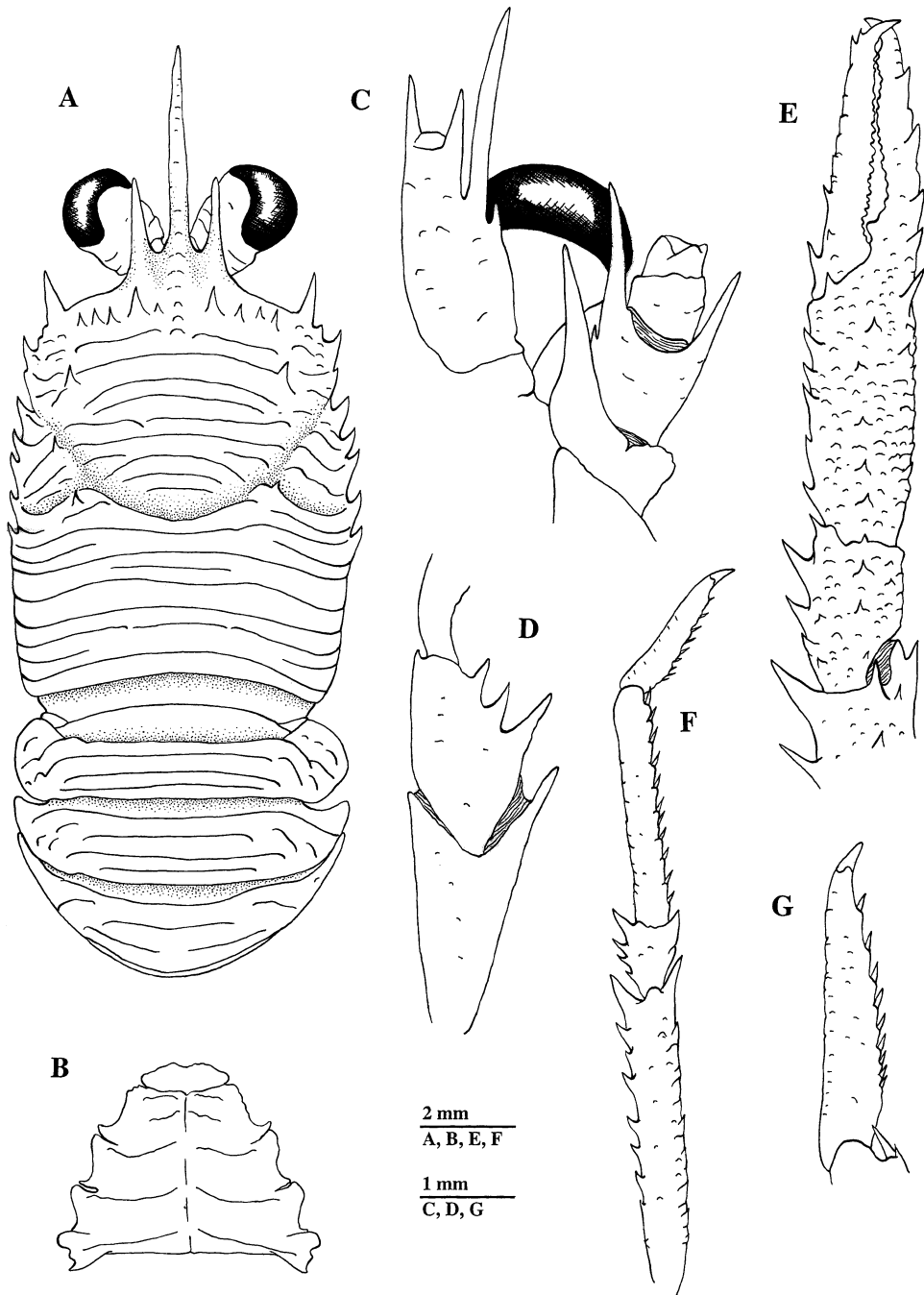


Figure 3. *Munida pectinata* sp. nov., ovigerous female 6.9 mm, holotype from stn 1727 (NORFOLK 1). (A) Carapace, dorsal view; (B) sternal plastron; (C) ventral view of cephalic region, showing antennular and antennal peduncles; (D) right third maxilliped, lateral view; (E) right cheliped, dorsal view; (F) right first walking leg, lateral view; (G) dactylus of right first walking leg, lateral view.

Etymology

From the Latin, *pecten*, comb, in reference to the spinulation of the merus of the third maxilliped.

Description

Carapace longer than wide. Transverse ridges mostly interrupted, with dense, very short, non-iridescent setae, and few long non-iridescent setae. Main transverse striae on gastric area interrupted. Few scales and secondary striae between main striae. Scales on intestinal region absent. Gastric region with 8–10 epigastric spines. One parahepatic, one anterobranchial (sometimes absent) and one postcervical spine on each side. Frontal margins transverse. Lateral margins slightly convex. Anterolateral spine well developed, at anterolateral angle, not reaching level of sinus between rostrum and supraocular spines; two marginal spines before cervical groove, anterior one three times shorter than anterolateral spine. Branchial margins with five spines. Rostrum two-thirds as long as remaining carapace, horizontal, tip slightly upwards directed. Supraocular spines well developed, reaching midlength of rostrum, and not exceeding end of corneae (Figure 3A).

Thoracic sternites smooth, without striae, granules or carinae. Anterior part of fourth sternite slightly wider than third (Figure 3B).

Second abdominal somite unarmed. Second and third somites each with three transverse striae.

Eyes moderately large, maximum corneal diameter about one-third distance between bases of anterolateral spines.

Basal segment of antennule (distal spines excluded), about one-quarter carapace length, slightly over-reaching corneae, with two distal spines, mesial spine longer than lateral spine; two spines on lateral margin, proximal one short, located at midlength of segment, distal one long, over-reaching distolateral spine (Figure 3C).

First segment of antennal peduncle with one long distal spine on mesial margin, reaching end of third segment and not reaching base of long lateral spine of basal segment of antennule; second segment with two long distal spines, mesial spine clearly over-reaching end of antennal peduncle; penultimate segment unarmed (Figure 3C).

Ischium of third maxilliped about 1.8 times length of merus measured along dorsal margin, distoventrally bearing spine. Merus of third maxilliped with three well-developed spines on flexor margin, distal smaller, extensor margin with small distal spine (Figure 3D).

Chelipeds squamous, with some iridescent uniramous setae, denser on mesial borders of articles. Palm as long as fingers. Merus armed with some spines, strongest spine on distal border short, reaching proximal quarter of carpus. Carpus with several spines on dorsal side and several spines scattered on mesial side. Palm with several spines scattered on mesial and dorsal sides, spines along lateral border continuing along fixed finger, some spines on proximal half of movable finger. Fingers distally curving and crossing, ending in a sharp point (Figure 3E).

Second pereopod about 2.5 carapace length; merus shorter than carapace, about six times as long as high, about 1.3 times as long as propodus; propodus 6.5 times as long as high, about 1.5 times dactylus length (Figure 3F). Merus with small spines on dorsal border, increasing in size distally, ventral margin with one long distal spine. Carpus with few dorsal spines and one distoventral spine. Propodus with 10–11 movable ventral spines. Dactylus slightly curving distally, with eight to nine movable spinules along entire ventral margin (Figure 3G). Third pereopod similar to second; fourth pereopod shorter than

second and third. Merus of fourth pereopod two-thirds length of second pereopod. Epipods absent from all pereopods.

Remarks

Munida pectinata belongs to the group of species closely related to *M. clinata* Macpherson, 1994, and has the following features: five spines on the lateral margins of the carapace behind the cervical groove, oblique frontal margins, smooth thoracic sternites, unarmed abdominal somites, spiniform rostrum, and distal spine on the extensor margin of the merus of the third maxilliped.

However, these two species are distinguished by several constant morphological characters as follows.

The frontal margins of the carapace are more oblique in *M. clinata* than in *M. pectinata*.

The distomesial spine of the basal antennular segment is longer than the distolateral spine in *M. pectinata*, whereas in *M. clinata* these spines are subequal.

The distomesial spine of the basal segment of the antennal segment clearly over-reaches third segment in *M. pectinata*. In *M. clinata* this spine reaches only the end of the second segment.

The molecular data indicated a close similarity of *M. pectinata* to *M. clinata* and *M. notata*. Thirty-three substitutions were found between the *M. pectinata* specimens and *M. clinata*, and 37 with *M. notata*, for 16S. For COI, *M. pectinata* specimens have two distinct haplotypes (only one transition change between them), which showed 63–66 diagnostic positions for *M. clinata* and 67–68 for *M. notata*. However, the molecular phylogenetic analyses always consistently showed *M. pectinata* as a sister taxon of *M. clinata*. For the global data, the divergence with respect to *M. clinata* ranged from 7.68% to 7.85% (5.2% for 16S and from 9.6% to 10% for COI; Table II).

Distribution

New Caledonia, between 190 and 240 m.

Discussion

The results confirm the validity of subtle morphological characters to distinguish species of the genus *Munida*, as observed in species of the genus *Raymunida* (Macpherson and Machordom 2001; Lin et al. 2004). Furthermore, it is demonstrated that the presence of cryptic species is common in this group, as occurs in other taxa of pelagic and benthic crustaceans (e.g. Knowlton 1993, 2000; Baldwin et al. 1998; Lee 2000; Goetze 2003).

The divergence ranges between close species are similar to those observed for other species of squat lobsters of the genus *Munida*, *Agononida*, *Paramunida*, and *Raymunida* (Machordom and Macpherson 2004) and for other decapod taxa (e.g. Palumbi and Benzie 1991; García-Machado et al. 1993; Tam et al. 1996; Baldwin et al. 1998; Sarver et al. 1998; Schubart et al. 1998; Tam and Kornfield 1998; Chu et al. 1999; Harrison and Crespi 1999; Shank et al. 1999; Tong et al. 2000). The smallest genetic divergence was observed between *M. simulatrix* and *M. notata* (1.4% for 16S and 8.2% for COI). The criteria used to designate distinct species is always controversial (Cracraft 1989; Avise 1994). As many authors have pointed out, the rationale in distinguishing species on the basis of molecular markers is quite similar as in the case of morphological-based species (Westheide and

Schmidt 2003). In an interesting study on the cryptic speciation of some pelagic copepods, Goetze (2003) used a very conservative genetic divergence (greater than 3% at 16S rRNA). However, the results observed in some clearly distinct species of decapods, e.g. the genetic divergence between *Nephrops norvegicus* (Linnaeus, 1758) and *Homarus americanus* H. Milne Edwards, 1837 is 1.78 at 16S rRNA (Tam and Kornfield 1998), indicate that divergences of between 1% and 1.5% or more at this gene are indicative of distinct species. Other morphologically well-differentiated species of the genus *Munida*, e.g. *M. guttata* Macpherson, 1994 versus *M. distiza* Macpherson, 1994, *M. rosula* Macpherson, 1994 versus *M. compressa* Baba, 1988, *M. rhodonia* Macpherson, 1994 versus *M. congesta* Macpherson, 1994, *M. rosula* Macpherson, 1994 versus *M. rubrodigitalis* Baba, 1994, among other species pairs, exhibit divergences of less than 1.4% at 16S and less than 8% at COI (Machordon and Macpherson 2004). The mean intraspecific divergences in some genera of Galatheidae (e.g. *Agononida*, *Munida*, *Paramunida*, *Raymunida*) are usually lower than 0.2% at 16S and 0.3% at COI, whereas the minimum interspecific divergences are larger than 0.4% and 3.5%, respectively (Machordon and Macpherson 2004).

Finally, on the basis of our results we conclude that a number of subtle morphological characters (e.g. length of the antennular and antennal spines, slight differences in the carapace shape) contribute greatly to the taxonomy of the genus *Munida*.

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