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Rapid radiation and cryptic speciation in squat lobsters of the genus *Munida* (Crustacea, Decapoda) and related genera in the South West Pacific: molecular and morphological evidence

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Abstract

Squat lobsters (genus *Munida* and related genera) are among the most diverse taxa of western Pacific crustaceans, though several features of their biology and phylogenetic relationships are unknown. This paper reports an extensive phylogenetic analysis based on mitochondrial DNA sequences (cytochrome *c* oxidase subunit I and 16S rRNA) and the morphology of 72 species of 12 genera of western Pacific squat lobsters. Our phylogenetic reconstruction using molecular data supports the recent taxonomic splitting of the genus *Munida* into several genera. Excluding one species (*M. callista*), the monophyly of the genus *Munida* was supported by Bayesian analysis of the molecular data. Three moderately diverse genera (*Onconida*, *Paramunida*, and *Raymunida*) also appeared monophyletic, both according to morphological and molecular data, always with high support. However, other genera (*Crosnierita* and *Agononida*) seem to be para- or polyphyletic. Three new cryptic species were identified in the course of this study. It would appear that the evolution of this group was marked by rapid speciation and stasis, or certain constraints, in its morphological evolution.

Keywords: Phylogeny; Taxonomy; Galatheidae; COI; Mitochondrial DNA; 16S rRNA; Morphology; Cryptic species

1. Introduction

One of the most diverse families of anomuran decapod crustaceans, the Galatheidae, includes crabs found in all marine habitats world-wide (Baba, 1988). Its species are commonly found living on corals, gorgonians, and sponges in rocky or muddy bottoms. Despite their ecological importance and high diversity, many aspects of the biology of squat lobsters are poorly understood, and there is still much debate regarding their systematics and phylogenetic history (McLaughlin and Lemaitre, 1997; Schram, 2001). Until recently, the family Galatheidae was divided into 16 genera, *Munida* (ca. 95 species) being the most speciose genus in the continental shelf and slope around the world (Baba, 1988). The number of described species is clearly higher in the waters of the West Pacific (42 species) than in other oceans. After numerous expeditions across the West Pacific—from the Philippines to New Caledonia (Richer de Forges et al., 2000)—some unknown species of *Munida* and related genera have been discovered in the last 15 years (ca. 122 new species have been described in the area since 1988). The genus has thus been split into 5 genera: *Agononida*, *Crosnierita*, *Munida*, *Paramunida*, and *Raymunida* (Baba, 1988; Baba and de Saint Laurent, 1996; Macpherson, 1994; Macpherson and Machordom, 2001) (see Table 1). A closely related genus, *Bathymunida*, which until the last decade was comprised of 8 species, was also enriched by the material obtained in these expeditions and now 12 new species and 5 new genera (*Anoplonida*, *Heteronida*, *Neonida*, *Onconida*,

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Table 1
Family Galatheidae

Genus	World	SWP	Analyzed here
Agononida[a]	(24)	24	9
Alainius	1	1	1
Allogalathea[b]	1	1	—
Allomunida[b]	1	1	—
Anomoeomunida[d]	1	0	—
Anoplonida	2	1	—
Bathymunida	13	8	2
Cervimunida	2	0	1
Coralliogalathea[b]	1	1	—
Crosnierita[a]	4	4	3
Fennerogalathea	2	0	—
Galathea	(55)	(20)	—
Heteronida[c]	2	2	1
Janetogalathea[b]	1	0	—
Lauriea[b]	2	1	—
Leiogalathea[b]	1	1	1
Munida	(210)	(79)	36
Munidopsis	(160)	(63)	—
Nanogalathea[b]	1	0	—
Onconida	5	5	2
Paramunida[a]	21	21	8
Phylladiorhynchus[b]	(5)	(3)	—
Plesionida	2	2	1
Pleuroncodes	2	0	1
Raymunida[a]	7	(4)	6
Sadayoshia[a]	(4)	(1)	—

Number of species per genus, including all known species in the world and southwestern Pacific (SWP), and those analyzed in the present study. Numbers indicated in parentheses are currently under revision and should be considered approximate. Genera formerly included in another genus are indicated.

[a] Genus *Munida*.
[b] Genus *Galathea*.
[c] Genus *Bathymunida*.
[d] Genus *Phylladiorhynchus*.

and *Plesionida*) are recognized in the *Bathymunida* group. This high number of species is probably still an underestimate of the family's true diversity and there are numerous yet undescribed cryptic species (Macpherson and Machordom, 2001, and see below).

Aside from their taxonomy, the phylogenetic affinities among the squat lobsters are poorly understood. The systematics of the group has not been fully resolved, and current taxonomic treatments divide genera into several large groups based on the number of male pleopods, general spinulation and the shape of the carapace and abdomen (Baba, 1988; Baba and de Saint Laurent, 1996; Macpherson and Machordom, 2001).

The present study is a first attempt at elucidating the phylogenetic relationships of Southwest Pacific species of squat lobsters with the help of molecular markers. Previous molecular studies on this group are scarce, and only include a study on *Munidopsis* species based on protein electrophoresis (Creasey et al., 2000) and an analysis of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) for cryptic species of *Raymunida* (Macpherson and Machordom, 2001). Further molecular studies on anomuran crabs have been mainly restricted to the families Porcellanidae (Stillman and Reeb, 2001; Werding et al., 2001), Aeglidae (Pérez-Losada et al., 2002), Hippidae (Haye et al., 2002) or miscellaneous taxa (Cunningham et al., 1992; Schubart et al., 2000).

In this study, we use DNA sequences and morphological data to phylogenetically analyze a collection of species from the West Pacific belonging to the genus *Munida* and to recently erected genera. We included 62 species of the genera *Agononida*, *Crosnierita*, *Munida*, *Paramunida*, and *Raymunida*, all previously assigned to the genus *Munida*. To complete the phylogenetic picture and assess the phylogenetic significance of the number of pleopods and other morphological characters, we also analyzed 10 species of related genera (i.e., *Alainius*, *Bathymunida*, *Cervimunida*, *Heteronida*, *Leiogalathea*, *Onconida*, *Plesionida*, and *Pleuroncodes*) (Table 1). On average, these numbers represent 45% of the known species of the former *Munida* in the West Pacific area (Table 1). The mitochondrial genes selected for analysis were the cytochrome *c* oxidase subunit I (COI) and 16S rRNA (16S) genes. These markers have been used extensively for elucidating relationships among species and genera for different phyla (e.g., Avise, 1994), and have been used in other studies on decapod crustaceans (Baldwin et al., 1998; Crandall et al., 2000; Fratini and Vannini, 2002; Knowlton and Weigt, 1998; Maggioni et al., 2001; Tong et al., 2000).

2. Materials and methods

2.1. Material analyzed

Samples were obtained from specimens deposited in the collections of the "Muséum National d'Histoire Naturelle," Paris, collected during different oceanographic expeditions carried out by the IRD ("Institut de Recherche pour le Développement") in New Caledonia and adjacent waters. One to seven specimens were analyzed per locality and species to give a total of 208 specimens used in the molecular analyses. We analyzed specimens (Table 2) of *Agononida* (9 species, $N=22$), *Alainius* (1 species, $N=4$), *Bathymunida* (2 species, $N=6$), *Cervimunida* (1 species, $N=1$), *Crosnierita* (3 species, $N=9$), *Heteronida* (1 species, $N=7$), *Leiogalathea* (1 species, $N=1$), *Munida* (36 species, $N=110$), *Onconida* (2 species, $N=5$), *Paramunida* (8 species, $N=24$), *Plesionida* (1 species, $N=1$), *Pleuroncodes* (1 species, $N=1$), and *Raymunida* (6 species, $N=16$). Besides these species, we included *Eumunida sternomaculata* (Anomura, Chirostylidae) as outgroup. *Eumunida* has been considered the sister taxon of *Munida* (Morrison et al., 2001).

For the morphological analysis, one to several hundred specimens of each of the previously cited species were used to define the characters that diagnose the species.

Table 2
Species included in the present study

Genus species (code)	*N*	Locality[a]	Station	Depth	GenBank Accession No.	
					COI	16S
Munida complex						
Agononida						
alisae (Fo132,133)	2	New Caledonia	1719	385–440	—	AY351064-5
incerta (AIN1,2)	2	New Caledonia[(1)]	824	17–720	AF283888-9	AY351066-7
laurentae (Fo14,15)	2	New Caledonia	1660	260–610	—	AY351068-9
laurentae (Fo80)	1	New Caledonia	1685		—	AY351070
marini (Fo74,76)	2	New Caledonia	1685	463–600	AY350914	AY351071-2
ocyrhoe (Fo16,17)	2	New Caledonia	1661	420–650	—	AY351073-4
ocyrhoe (Fo32)	1	New Caledonia	1670		—	AY351075
pilosimanus (Fo204)	1	Taiwan	—	250–582	—	AY351076
procera (Fo92)	1	New Caledonia	1688	450–620	AY350916	AY351077
procera (Fo119)	1	New Caledonia	1699		AY350917	AY351078
similis (ASI)	1	Indonesia[(2)]	STN 82	146–494	AY350915	—
sphecia (Fo1)	1	New Caledonia	1651	59–520	AY350918	AY351079
sphecia (Fo9)	1	New Caledonia	1654		AY350919	AY351080
sphecia (Fo43)	1	New Caledonia	1671		AY350920	AY351081
sphecia (Fo121)	1	New Caledonia	1705		AY350921	AY351082
sphecia (Fo135,136)	2	New Caledonia	1719		AY350922-3	AY351083-4
Crosnierita						
dicata (Fo163)	1	New Caledonia	1718	283–440	AY350924	AY351085
urizae (Fo24-27)	4	New Caledonia	1669	230–610	—	AY351086-9
urizae (Fo41,42)	2	New Caledonia	1671		—	AY351090-1
yante (Fo4)	1	New Caledonia	1651	95–460	—	AY351092
yante (Fo130)	1	New Caledonia	1718		—	AY351093
Munida						
acantha (Fo8)	1	New Caledonia	1654	59–460	AY350925	AY351094
acantha (Fo139,141)	2	New Caledonia	1729		AY350926-7	AY351095-6
acantha (Fo143)	1	New Caledonia	1737		AY350928	AY351097
alonsoi (Fo173)	1	New Caledonia	1659	448–680	AY350929	AY351098
alonsoi (Fo178)	1	New Caledonia	1662		AY350930	AY351099
alonsoi (Fo19,20)	2	New Caledonia	1664		AY350931-2	AY351100-1
alonsoi (Fo23)	1	New Caledonia	1667		AY350933	AY351102
alonsoi (Fo70)	1	New Caledonia	1684		AY350934	AY351103
alonsoi (Fo109)	1	New Caledonia	1695		AY350935	AY351104
alonsoi (Fo118)	1	New Caledonia	1697		AY350936	AY351105
armilla (Fo22)	1	New Caledonia	1667	233–700	AY350937	AY351106
armilla (Fo199)	1	New Caledonia	1681		AY350938	AY351107
callista (Fo29)	1	New Caledonia	1670	335–590	—	AY351108
callista (Fo51,52)	2	New Caledonia	1680		AY350939	AY351109-10
clinata (Fo220)	1	New Caledonia	CP 1681	28–245	AY350940	AY351111
clinata (Fo221)	1	New Caledonia	DW 1726		AY350941	AY351112
clinata (Fo219)	1	New Caledonia	DW 1727		AY350942	AY351113
compressa (Fo224,225)	2	Salomon Is.[(3)]	CP 1795	224–668	AY350943-4	—,AY351114
congesta (Fo91)	1	New Caledonia	1689	536–668	AY350945	AY351115
distiza (Fo53)	1	New Caledonia	1680	400–590	AY350946	AY351116
distiza (Fo63)	1	New Caledonia	1682		AY350947	AY351117
distiza (Fo128)	1	New Caledonia	1716		AY350948	—
distiza (Fo129,131)	2	New Caledonia	1718		AY350949-50	AY351118-9
eclepsis (Fo81)	1	New Caledonia	1687	515–520	AY350951	AY351120
eclepsis (Fo84,85)	2	New Caledonia	1688		AY350952-3	AY351121-2
gordoae (Fo47,48)	2	New Caledonia	1676	80–283	AY350954-5	AY351123-4
gordoae (Fo59,62)	2	New Caledonia	1681		AY350956-7	AY351125-6
gordoae (Fo138)	1	New Caledonia	1724		AY350958	AY351127
guttata (Fo45,46)	2	New Caledonia	1675	170–320	AY350959-60	AY351128-9
guttata (Fo60,61)	2	New Caledonia	1681		AY350961-2	AY351130-1
leagora (Fo171)	1	New Caledonia	1661	265–580	AY350963	AY351132
leagora (Fo36,37,38,195)	4	New Caledonia	1670		AY350964-7	AY351133-6
leagora (Fo54)	1	New Caledonia	1680		AY350968	AY351137
leagora (Fo95)	1	New Caledonia	1691		AY350969	AY351138

(*continued on next page*)

Table 2 (*continued*)

Genus species (code)	*N*	Locality[a]	Station	Depth	GenBank Accession No.	
					COI	16S
leagora (MMO, MMOL)	2	New Caledonia[4]	CP 736		AY350970-1	AY351139-40
lenticularis (Fo210)	1	French Polynesia		200	AY350972	—
leptosyne (MLEP)	1	New Caledonia[5]	Banya Sud	6–100	AY350973	AY351141
leviantennata (MLE2)	1	New Caledonia[2]	CP 889	300–1250	AY350974	AY351142
militaris (MML1)	1	New Caledonia[6]	ST 168	100–1280	AY350975	AY351143
notata (Fo67,68)	2	New Caledonia	1683	120–850	AY350976-7	AY351144-5
notata (Fo125)	1	New Caledonia	1712		AY350978	AY351146
notata (Fo164)	1	New Caledonia	1718		AY350979	AY351147
ofella (M13)	1	Fiji Is.[7]	1355	210–500	AY350980	—
ommata (Fo5)	1	New Caledonia	1651	205–610	AY350981	AY351148
ommata (Fo39)	1	New Caledonia	1671		AY350982	AY351149
ommata (Fo58)	1	New Caledonia	1680		AY350983	AY351150
ommata (Fo169)	1	New Caledonia	1707		AY350984	AY351151
pagesi (MPA)	1	New Caledonia[6]	STN 239	250–600	AY350985	AY351152
proto (M14)	1	Fiji Is.[7]	CP 1326	155–610	AY350986	AY351153
psamathe (Fo89,90)	2	New Caledonia	1688	500–700	AY350987-8	AY351154-5
psamathe (Fo103)	1	New Caledonia	1694		AY350989	AY351156
psamathe (Fo115,116)	2	New Caledonia	1697		AY350990-1	AY351157-8
psylla (Fo73)	1	New Caledonia	1684	380–573	AY350992	AY351159
rhodonia (MRH1,2)	2	New Caledonia[8]	854	459–705	AF283885-6	AY351160-1
rogeri (MROG)	1	Chesterfield Is.[9]	ST 288	245–400	AY350993	—
rosula (M11)	1	New Caledonia[8]	CP 867	465–860	AY350994	AY351162
rubrodigitalis (MRU1)	1	New Caledonia[10]	52	285–650	AF283887	AY351163
rufiantennulata (Fo206)	1	New Caledonia		167–705	AY350995	AY351164
spilota (Fo152,165)	2	New Caledonia	1718	220–400	AY350996-7	AY351165-6
stia (Fo175)	1	New Caledonia	1654	360–610	AY350998	AY351167
stia (Fo179)	1	New Caledonia	1662		AY350999	AY351168
stia (Fo18)	1	New Caledonia	1663		AY351000	AY35116-
stia (Fo100)	1	New Caledonia	1664		AY351001	AY351170
taenia (Fo126,127)	2	New Caledonia	1712	200–400	AY351002-3	AY351171-2
taenia (Fo159,160)	2	New Caledonia	1716		AY351004-5	AY351173-4
taenia (Fo140)	1	New Caledonia	1729		AY351006	AY351175
taenia (Fo144)	1	New Caledonia	1737		AY351007	AY351176
taenia (Fo147)	1	New Caledonia	1738		AY351008	AY351177
thoe (Fo30)	1	New Caledonia	1670	220–430	AY351009	AY351178
thoe (Fo40)	1	New Caledonia	1671		AY351010	AY351179
thoe (Fo72)	1	New Caledonia	1684		AY351011	AY351180
thoe (Fo98)	1	New Caledonia	1692		AY351012	AY351181
thoe (Fo142)	1	New Caledonia	1733		AY351013	AY351182
tiresias (MTIR)	1	New Caledonia[11]	BT 60	1140–2049	AY351014	AY351183
tuberculata (Fo102)	1	New Caledonia	1694	240–650	AY351015	AY351184
tyche (MTY)	1	Vanuatu[12]	DW 1042	127–400	AY351016	AY351185
tyche (MTY2)	1	New Caledonia[6]	ST 153		AY351017	AY351186
zebra (Fo2,6)	2	New Caledonia	1651	200–610	AY351018-9	AY351187-8
zebra (Fo12,13)	2	New Caledonia	1659		AY351020-1	AY351189-90
zebra (Fo79)	1	New Caledonia	1685		AY351022	AY351191
zebra (MZA)	1	New Caledonia[13]	DW 01		AY351023	AY351192
sp1 (Fo111)	1	New Caledonia	1695	564–616	AY351024	AY351193
sp1 (Fo117)	1	New Caledonia	1697		AY351025	AY351194
sp1 (Fo120)	1	New Caledonia	1701		AY351026	AY351195
sp2 (Fo157)	1	New Caledonia	1721	416–443	AY351027	AY351196
sp3 (Fo249)	1	New Caledonia	1727	190–212	AY351028	AY351197
sp3 (Fo250)	1	New Caledonia	1727		AY351029	—
Paramunida						
belone (Fo196)	1	New Caledonia	1719	245–437	AY351030	AY351198
belone (Fo154)	1	New Caledonia	1721			AY351199
granulata (Fo106)	1	New Caledonia	1694	400–650	AY351031	AY351200
labis (Fo64)	1	New Caledonia	1683	245–440	AY351032	AY351201
labis (Fo151)	1	New Caledonia	1718		AY351033	AY351202
luminata (Fo33,35,185)	3	New Caledonia	1670	400-440	—, AY351034-5	AY351203-5
luminata (Fo104)	1	New Caledonia	1694		—	AY351206

Table 2 (*continued*)

Genus species (code)	*N*	Locality[a]	Station	Depth	GenBank Accession No.	
					COI	16S
pictura (Fo176,177)	2	New Caledonia	1658	205–600	AY351036-7	AY351207-8
pictura (Fo155,156)	2	New Caledonia	1721		AY351038-9	AY351209-10
pronoe (Fo182,184)	2	New Caledonia	1670	500–510	—, AY351040	AY351211-2
stichas (Fo34)	1	New Caledonia	1670	210–590	—	AY351213
stichas (Fo71)	1	New Caledonia	1684		AY351041	AY351214
stichas (Fo107)	1	New Caledonia	1694		AY351042	AY351215
stichas (Fo114)	1	New Caledonia	1697		AY351043	AY351216
thalie (Fo28)	1	New Caledonia	1669	245–283	AY351044	AY351217
thalie (Fo49,50)	2	New Caledonia	1676		AY351045,—	AY351218-9
thalie (Fo65)	1	New Caledonia	1683		AY351046	AY351220
thalie (Fo150)	1	New Caledonia	1718		AY351047	AY351221
Raymunida						
cagnetei (RCA1-3)	3	Marquesas Is.[14]	1177	53–112	AF283869-71	AY351222-4
confundens (RCO1)	1	Chesterfield Is.[9]	315	80–400	AF283872	AY351225
confundens (RCO2)	1	New Caledonia[15]	178		AF283873	AY351226
dextralis (RDE1)	1	Loyalty Is.[16]	419	285	AF283874	AY351227
elegantissima (REL1)	1	Bellona Is.[17]	—	25–440	AF283875	AY351228
elegantissima (REL2)	1	Wallis Is.[18]	498		AF283876	AY351229
elegantissima (REL3)	1	Philippines[19]	57		AF283877	AY351230
elegantissima (REL4,5)	2	Vanuatu[12]	966		AF283878-9	—
elegantissima (REL6)	1	New Caledonia[20]	640		AF283880	AY351231
elegantissima (REL7)	1	Fiji[7]	1363		AF283881	AY351232
erythrina (RER1)	1	Futuna Is.[18]	515	180–252	AF283882	AY351233
erythrina (RER2)	1	Vanuatu[12]	1077		AF283883	AY351234
insulata (RIN1)	1	Seychelles Is.[21]	—	200	AF283884	AY351235
Related genera						
Alainius						
crosnieri (Fo11)	1	New Caledonia	1651	90–600	AY351048	AY351236
crosnieri (Fo122-124)	3	New Caledonia	1706		AY351049-51	AY351237-9
Bathymunida						
nebulosa (Fo44)	1	New Caledonia	1675	300–610	—	AY351240
nebulosa (Fo192)	1	New Caledonia	1670		—	—
nebulosa (Fo55,56)	2	New Caledonia	1680		—, AY351052	AY351241,—
sibogae (O5)	1	Fiji Is.[22]	CP 1411	118–345	—	AY351242
sibogae (NEG)	1	Vanuatu[12]	CP 1137		—	AY351243
Cervimunida						
johni (A17)	1	Chile	—	0–100	AY351054	AY351244
Heteronida						
aspinirostris (Fo57)	1	New Caledonia	1680	345–930	—	AY351245
aspinirostris (Fo94)	1	New Caledonia	1691		—	AY351246
aspinirostris (Fo99,101)	2	New Caledonia	1694		—	AY351247-8
aspinirostris (Fo108)	1	New Caledonia	1695		—	AY351249
aspinirostris (Fo166,167)	2	New Caledonia	1707		—	AY351250-1
Leiogalathea						
laevirostris (O2)	1	New Caledonia[23]	DW 73	160–805	AY351055	AY351252
Onconida						
alaini (Fo137)	1	New Caledonia	1722	200–575	AY351056	AY351253
alaini (Fo189,191)	2	New Caledonia	1670		AY351057-8	AY351254-5
tropis (Fo228,229)	2	New Caledonia	CP 1831	210–480	AY351059-60	AY351256-7
Plesionida						
aliena (P26)	1	Fiji Is.[22]	CP 1433	545	AY351061	AY351258
Pleuroncodes						
monodon (Fo212)	1	Chile	—	94–523	AY351062	AY351259

(*continued on next page*)

Table 2 (*continued*)

Genus species (code)	*N*	Locality[a]	Station	Depth	GenBank Accession No.	
					COI	16S
Outgroup						
Eumunida sternomaculata (Fo205)	1	New Caledonia	—	420–560	AY351063	AY351260

N = number of specimens analyzed. [a]All specimens obtained in the Norfolk expedition except: [1]Bathus 3, [2]Bathus 4, [3]Salomon 1, [4]Bathus 2, [5]Ouvea, [6]Musorstom 4, [7]Musorstom 10, [8]Halipro 1, [9]Musorstom 5, [10]Biocal, [11]Halipro 2, [12]Musorstom 8, [13]Halical 1, [14]Musorstom 9, [15]Smib 8, [16]Musorstom 6, [17]Corail 1, [18]Musorstom 7, [19]Musorstom 1, [20]Lagon Est, [21]Cepros, [22]Bordau 1, [23]Chacal 2.

2.2. DNA extraction and amplification

Tissue samples preserved in ethanol were ground to powder in liquid nitrogen or minced before adding 600 µl of CTAB lysis buffer [2% CTAB, 1.4 M NaCl, 0.2% β-mercaptoethanol, 20 mM EDTA, 0.1 M Tris (pH 8)] and digested with proteinase K (100 µg/ml) for 2–5 h at 60 °C or 1–2 days at 50–55 °C. Total DNA was extracted according to standard phenol/chloroform procedures (Sambrook et al., 1989).

The COI and 16S partial sequences were amplified by polymerase chain reaction (PCR) using the following primers: 16Sar-L and 16Sbr-H (Palumbi et al., 1991) for 16S; and LCO1490 (Folmer et al., 1994) and COI-H 5′-TCA GGG TGA CCA AAA AAT CA-3′ (6 bases shorter than the HCO2198 of Folmer et al., 1994) for COI. The COI fragment of some of the species analyzed here could not be amplified using this pair of primers. Thus, we designed a new forward primer, COI-543, which provided a fragment longer than the previous one and allowed us to amplify the markers in a greater number of species: COI-543 5′-CCA ATT GCT ATT ATA GC-3′. Unfortunately, 35 specimens sequenced for the 16S gene yielded no results in the COI amplifications even using the COI-543 primer (amplification mainly failed in specimens within the genera *Agononida* and *Crosnierita*). In a final volume of 50 µl, the PCR mix contained DNA template, 0.16 µM of both primers, 0.2 mM of each dNTP, 2 mM $MgCl_2$, 1 U *Tth* DNA polymerase (Biotools), the corresponding buffer and ddH_2O. The following PCR conditions were used in the partial COI amplification: 92 °C (5 min), 40 cycles of 94 °C (45 s), 45–50 °C (1 min), 72 °C (1 min), and a final extension at 72 °C (10 min). Amplification of the 16S gene was performed under the same conditions except for a lower annealing temperature (40–45 °C). The amplified fragments (around 700 bp) were purified by ethanol precipitation prior to sequencing both strands using "BigDye Terminator" (Applied Biosystems, ABI) sequencing reactions. Sequence gels were run on an ABI 3700 Genetic Analyzer (Applied Biosystems).

The forward and reverse DNA sequences obtained for each specimen were aligned and checked using the Sequencher program (Gene Code) after removing the primers regions. CLUSTAL X (Thompson et al., 1994) was employed to align the 16S gene sequences, with several gap-opening and gap-extension penalties, and finally selecting the values 15 and 7, respectively. Additionally, all alignments were checked by eye. To choose among alternatives, alignments were used in parsimony analyses to find the shortest tree. The selected alignment is available from the MPE website. COI translation to protein was undertaken using the package MacClade 3.06 (Maddison and Maddison, 1992).

2.3. Morphological data

The morphological dataset was comprised of 79 characters for 76 taxa, including the outgroup (Appendix A). Data were gathered on all the genus- and species-differentiating characters commonly used by the different authors (e.g., Baba, 1988; Baba and de Saint Laurent, 1996; Macpherson, 1994; Macpherson and Machordom, 2001 and references cited therein); no behavioral characters or color details (unknown in many species) were considered. The specimens examined were obtained from the collections of the "Muséum National d'Histoire Naturelle" in Paris, where they are preserved in ethanol. There were no missing entries in any of the species.

2.4. Phylogenetic analyses

Nucleotide saturation was evaluated by plotting transition and transversion changes against uncorrected ("p") divergence values. Sequence analysis was based on the principles of parsimony (MP), neighbor-joining (NJ), and maximum likelihood (ML). The evolutionary molecular model that best fitted our data was selected using MODELTEST 3.06 (Posada and Crandall, 1998) under the Akaike information criterion (Akaike, 1974). Parsimony analysis was performed by heuristic searches under TBR branch swapping and 10 random taxon addition using the PAUP* 4.0b10 package (Swofford, 2002). Maximum likelihood analyses were also run in PAUP, using the model and parameters selected by MODELTEST through neighbor-joining or heuristic searches, with the same protocols indicated before. We estimated support in the MP, NJ, and ML analyses by bootstrapping (100 pseudo replications) (Felsenstein, 1985).

Each gene was analyzed independently. For the 16S locus, two types of tests were run in which we either took

into account or did not consider the most variable regions (in which alignments between separate groups were most difficult). Nevertheless, the results revealed these regions to have a phylogenetic signal. Thus, our phylogenetic proposals were based on complete 16S sequences. To consider the information of both genes together, congruence among tree topologies of COI and 16S rRNA genes was assessed by the partition homogeneity test implemented in PAUP* (Farris et al., 1994; Mickevich and Farris, 1981).

We also performed Bayesian analyses to estimate the posterior probability of the nodes in the phylogenetic trees. MrBayes (Huelsenbeck and Ronquist, 2001) was run with 6 substitution types (nst = 6). This procedure is based on a GTR model and considers gamma-distributed rate variation as well as the proportion of invariable positions for the two genes combined (but independently analyzed). For the COI gene, it also indicates partition by codon position. The MCMCMC (Metropolis-coupled Markov chain Monte Carlo) algorithm with four Markov chains was used for 2,000,000–5,000,000 generations, with a sampling frequency every 100 generations, and eliminating 10–20% of the first trees obtained since they did not reach the stationarity of the likelihood values.

To test whether speciation rates changed through time we performed CR tests (constant rate tests, Pybus and Harvey, 2000). Given the possible bias due to incomplete taxon sampling, the significance of the test outcome was assessed by the Monte Carlo constant rate test (MCCR-Test; Pybus, 2000). The γ-statistic (a measure of the relative position of internal nodes within a phylogeny) (Pybus and Harvey, 2000) was calculated using GENIE v.3.0 (Pybus and Rambaut, 2002). These CR–MCCR tests are based on the assumption of homogeneous diversification among lineages. Thus, we followed the recommendations of Pybus and Harvey (2000) to test this assumption. First, tree imbalance was assessed by the $B1$ index (Kirkpatrick and Slatkin, 1993) using MeSA v.1.5.3, (Agapow, 2003). Second, lineages with higher than the expected rate of cladogenesis were identified using relative cladogenesis statistics as implemented in End-Epi v.1.01 (Rambaut et al., 1997). This program was also used to generate a semilogarithmic plot of lineage through time (LTT). Since our data showed significant differences in branch lengths as assessed by the relative rate test (PHYLTEST, Kumar, 1996), an ultrametric tree was constructed using the non-parametric rate smoothing method (NPRS; Sanderson, 1997) as implemented in TreeEdit (Rambaut and Charleston, 2001). These analyses were performed on interspecific data only.

Morphological data were analyzed both by parsimony and Bayesian methods. Characters were treated as unordered. The number of equally parsimonious trees was enormous, due to the relationship between the number of taxa examined and the number of parsimony informative characters. Thus, the parsimony analysis was only conducted in PAUP by heuristic search under TBR branch swapping and random taxon addition (2 replicates), finding the consensus tree among the equally parsimonious trees obtained. Looking for the shortest tree by this approach might be not the best way, or performing Bootstrap replicates not possible in real time. Thus, we also carried out the analysis of the morphological data through the parsimony ratchet method (Nixon, 1999), implemented in WinClada (Nixon, 2002). The characters were coded as nonadditive, and the ''island hopping'' conducted through 400.000 iterations, one tree held from each, amb-poly = option, 10% of the characters sampled, and a random constraint = 10. The bootstrap analysis was also performed in WinClada, using TBR option, 1000 replications with 10 searches and one starting tree per replicate. For the Bayesian analysis, the Markov k model (Lewis, 2001) was combined with gamma-distributed rates across characters, considering variable characters only. The other parameters were similar to those used for the molecular analyses: MCMCMC algorithm with four Markov chains, 5,000,000 generations, with a sample frequency every 100 generations, and 10% burn-in of the first trees obtained. Decay indices (Bremer, 1988, 1994) were also calculated using AutoDecay (Eriksson, 1998).

3. Results

3.1. Sequence characteristics and variation

After alignment, 1203 bp were used in the analyses: 657 for COI and 546 for 16S. Non-aligned 16S sequences were 506–523 bp in length (from *Leiogalathea* and *Paramunida*, respectively), the two most variable regions occurring between positions 232 and 292 and between 364 and 376. We obtained 197 new sequences for 16S and 150 for COI, and used 21 COI sequences from a previous study (Macpherson and Machordom, 2001). We finally managed to obtain sequences for both genes in 161 specimens. Base composition was homogenous in all the taxa analyzed except for third codon positions of the COI gene (Table 3). Both gene fragments showed an AT bias, especially in COI third codon positions. Saturation tests indicated no saturation when we plotted all the substitutions together, but signs of a saturation tendency were shown for transitions in third codon positions of the COI gene for divergence values above 0.15 (or 15%) (Fig. 1). The extreme conservation of the second codon position of this gene is worth noting, with a maximum of only three substitutions (transitions or transversions) found in comparisons between ingroup and outgroup taxa. Out of the 14,706 pairwise substitutions screened, only one pair, *Raymunida insulata* vs. *Crosnierita dicata*, showed five substitutions in total (adding transitions and transversions). Direct estimation

Table 3
Number of characters analyzed, nucleotide proportions, and transition/transversion (ts/tv) ratios for all the taxa analyzed according to COI and 16S rRNA sequences

	COI				16S
Codon position:	1st	2nd	3rd	All	
Characters					
Total	219	219	219	657	546
Constant	150	207	1	358	268
(%)	68.49	94.52	0.46	54.49	49.08
Parsimony informative	49	5	217	271	246
(%)	22.37	2.28	99.09	41.25	45.05
A (%)	27.78	13.31	40.54	27.21	34.42
C (%)	19.53	25.17	11.26	18.65	9.46
G (%)	31.08	17.66	4.66	17.80	18.32
T (%)	21.61	43.86	43.54	36.34	37.80
P	1.00	1.00	0.00	0.77	1.00
ts/tv ratio	7.82	0.50	0.97	1.23	1.68

P, homogeneity. χ^2 test probability of base composition.

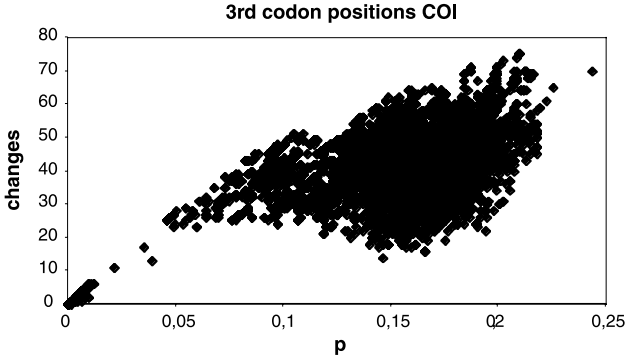

Fig. 1. Saturation plot: relationships between uncorrected mean divergence (*p*) between pairs of taxa and the number of transitional changes in the third codon position of the COI gene sequence.

of the transition/transversion ratio gave a higher value for 16S (ts/tv ratio = 1.68) than COI (ts/tv ratio = 1.23).

Intrageneric sequence divergence for both genes ranged from 0.39 or 0.78% (between 16S sequences of *Munida rosula* vs. *M. militaris* and *M. guttata* vs. *M. distiza*, respectively) to 22.53% (interspecific divergence of COI between *M. callista* and *M. tiresias*). The mean values were similar across taxa, and almost always several times higher for COI than for 16S (Table 4). Nevertheless, we found some discordant cases in which intraspecific divergences, in particular, greatly exceeded the usual ranges, and might therefore reflect interspecific differences. Within the *Bathymunida* samples, two specimens considered as *B. nebulosa* showed 9.79% divergence in the 16S gene and two other specimens showed 3.89% in COI. This disagreement was more evident for some *Munida* specimens, among which we conducted over 5100 pairwise comparisons. Some of the specimens previously considered as *M. notata*, *M. clinata* or *M. tuberculata* showed much greater intraspecific values for both COI and 16S than expected (up to 14 or 4.71%, while the usual ranges reach 2 or 0.4%, respectively, Table 4). According to these results, we considered such specimens as representing "misidentified cryptic species." These will hereafter be referred to as *Munida* sp1, sp2, and sp3.

The models and the associated parameters, selected by MODELTEST and applied in the ML and NJ analyses for each molecular dataset, were as follows:

16S: TrN + I + Γ (Tamura–Nei model, Tamura and Nei, 1993; with correction for the proportion of invariable sites—I, and for among-site rate variation—Γ), base frequencies = (A = 0.4024, C = 0.0419, G = 0.1286, T = 0.4271), rate matrix = (1.0000, 9.6388, 1.0000, 1.0000, 12.7524), α = 0.6705, I = 0.414.

COI: GTR + I + Γ (General Time Reversible model, Lavane et al., 1984; Rodríguez et al., 1990), base frequencies = (A = 0.3600, C = 0.1419, G = 0.0569, T = 0.4412), rate matrix = (0.2745, 9.2380, 0.2833, 0.9790, 5.2361), α = 0.4133, I = 0.4841.

16S + COI: TVM + I + Γ (Traversion Model), base frequencies = (A = 0.4038, C = 0.1086, G = 0.0828, T = 0.4048), rate matrix = (0.3055, 7.4211, 0.6030, 0.4594, 7.4211), α = 0.4599, I = 0.4520.

Table 4
Average intra and interspecific divergences (%) in the genera analyzed

	N	16S rRNA				COI			
		Intra		Inter		Intra		Inter	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Agononida	(8/21, 5/12)	0.13	0.00–0.39	9.28	0.58-11.51	0.09	0.00–0.30	16.48	14.00–17.96
Alainius	(1/4, 1/4)	0.00	0.00–0.00	—	—	0.30	0.15–0.46	—	—
Bathymunida	(2/4, 1/2)	**4.89**	0.00–**9.79**	11.54	11.05–12.02	**3.89**	**3.89**	—	—
Crosnierita	(2/9, 1/1)	0.22	0.00–0.39	7.76	4.49–12.47	—	—	—	—
Heteronida	(1/7, —)	0.05	0.00–0.19	—	—	—	—	—	—
Munida	(33/104, 36/108)	0.25	0.00–**4.71**	7.16	0.39–14.93	0.83	0.00–**14.00**	14.46	3.50–22.53
Munida (2)	(36/104, 39/108)	0.04	0.00–0.38	7.15	0.39–14.93	0.18	0.00–1.22	14.45	3.50–22.53
Onconida	(2/5, 2/5)	0.19	0.00–0.38	4.31	4.05–4.64	0.27	0.15–0.46	8.55	8.37–8.83
Paramunida	(8/24, 8/18)	0.06	0.00–0.38	5.65	3.11–10.56	0.23	0.00–0.91	12.96	10.20–16.89
Raymunida	(6/14, 6/16)	0.09	0.00–0.19	3.65	2.35–5.72	0.25	0.00–2.13	11.22	7.31–13.09

N = number of species/specimens analyzed from each genus (for 16S, for COI). In the genus indicated as *Munida* (2), some divergent specimens became separated from the rest of those considered the same species (see text). Numbers in bold emphasize figures that exceed most ranges or averages.

3.2. Phylogenetic relationships based on molecular data

Fig. 2 shows a ML tree obtained for the 16S sequences and the indices indicating the posterior probabilities and the bootstrap values for the ML, MP, and NJ methods (2,000,000 replicates in the Bayesian analysis, 20,000 of which were sampled, and 100 bootstrap replicates for the other methods). Posterior probabilities and bootstrap values obtained for the terminal branches (not shown in the figure), mainly representing relationships among the

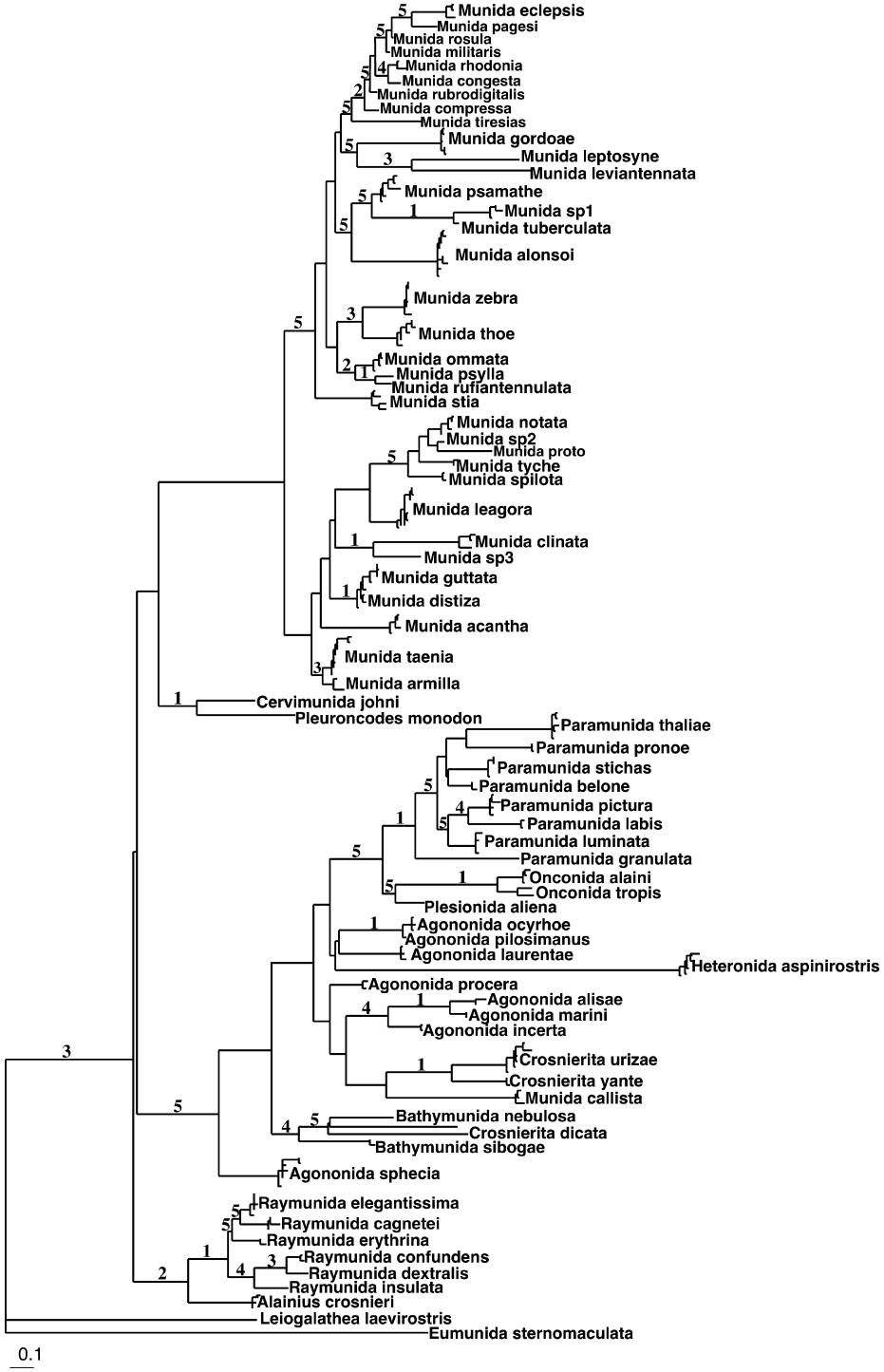

Fig. 2. Phylogenetic hypothesis based on 16S sequences represented by a tree obtained by Bayesian inference (2,000,000 replicates, 20,000 trees sampled, and 20% burn-in). Numbers on branches indicate posterior probabilities (=Pp) and the bootstrap values (=Bv) for MP, NJ, and ML coded as follows: 1: Pp = 1–0.95, Bv $\geqslant$ 70; 2: Pp = 1–0.95, Bv $\geqslant$ 60; 3: Pp = 1–0.95, Bv $\geqslant$ 55 (at least in two of the methods); 4: Pp = 1–0.85, Bv $\geqslant$ 55 (at least in one of the methods); and 5: Pp = 50 or at least two Bv $\geqslant$ 60.

different specimens within each species, were highly significant (posterior probabilities (Pp), 0.95–1.00; bootstrap values (Bv) for the three other treatments: maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ), 80–100) except in the case of specimens of *M. guttata* (Pp = 0.71, Bv-ML = 70), *A. ocyrhoe* (Pp = 0.83) and *B. nebulosa* (Pp = 0.81, not recovered with ML, NJ or MP). The monophyletic ingroup, excluding *Leiogalathea laevirostris*, that occupied a basal position and the outgroup *Eumunida sternomaculata* showed 4 main clades, with different supports: (1) *Raymunida* + *Alainius*; (2) *Agononida* + *Bathymunida* + *Crosnierita* + *Heteronida* + *Plesionida* + *Onconida* + *Paramunida* + *Munida callista*; (3) *Pleuroncodes* + *Cervimunida*; and (4) *Munida* (except for *M. callista*). The second group included all species with a single pair of male pleopods, except in the case of *Munida callista*. Except *Munida*, all these groups were supported by high posterior probability values (between Pp = 0.96 and Pp = 1.00), but only the *Raymunida* + *Alainius* and *Pleuroncodes* + *Cervimunida* groups were supported in all the analytical methods. Nevertheless, the relationships among these four groups were mainly unresolved. Similarly, internal relationships in the second and fourth groups were not clearly established by analysis of the 16S sequence data.

The monophyly of *Paramunida* and of *Onconida* in the second group (*Agononida* + *Bathymunida* + *Crosnierita* + *Heteronida* + *Plesionida* + *Onconida* + *Paramunida* + *Munida callista*) were each supported (Pp = 1.00; Bv = 80–100), although we only examined two of the four extant species of *Onconida*. *Crosnierita* appeared as non-monophyletic, since *C. dicata* clustered with species of the genus *Bathymunida*; *M. callista* being the sister taxon of the remaining *Crosnierita* species analyzed. Finally, *Agononida* clustered in different groups, with uncertain phylogenetic relationships. Thus, the monophyly of the genus *Agononida* was rejected by the 16S data.

The fourth group was comprised of the remainder *Munida* species. Only the cited *M. callista* group outside of this clade. However, resolution within this group shows low support using the 16S sequences alone. *Munida* splits into some major divisions: for instance, one formed by species with a massive carapace and moderately long chelipeds and walking legs (*M. eclepsis*, *M. militaris*, *M. pagesi*, *M. rosula*, *M. rhodonia*, *M. congesta*, *M. rubrodigitalis*, and *M. compressa*), another including species with carinae on the sternum, a character probably related to reproductive behavior (*M. ommata*, *M. psylla*, and *M. rufiantennulata*), a group including species with smooth chelipeds, antennular spines strongly different and legs with short dactyli (*M. alonsoi*, *M. psamathe*, and *M. tuberculata*), and a group including species with elongate carapace, and walking legs (*M. leagora*, *M. notata*, and *M. spilota*, among others).

In the trees based on COI sequences (Fig. 3), most of the dichotomies found were only supported by the Bayesian approach. Only some terminal associations were recovered by the four analytical methods used. Most of these groups were the same ones proposed for the phylogenetic analyses of the 16S sequences. Some species were not included in the previous analysis: *Munida lenticularis*, *M. rogeri*, *M. ofella* and *Agononida similis*. *Munida lenticularis* clustered within the *M. ommata* + *M. psylla* + *M. rufiantennulata* group; *M. rogeri* was the sister species of *M. gordoae*; but the relationships of *M. ofella* were ambiguously established. *Agononida similis* appeared as a sister species of *A. procera*. None of the genera analyzed, except *Onconida* (with only two species studied), were recovered as monophyletic by all the treatments, while three of the treatments (NJ, ML, and MP) supported the monophyly of *Raymunida* + *Alainius* cluster (Bv = 86, 91, and 69, respectively).

Despite the differences stated, the partition homogeneity test indicated no significant differences in the topologies obtained separately from each gene ($P = 0.56$). Taking together both sets of data, certain unclear phylogenetic relationships were clarified (Fig. 4) and some of the basal polytomies were resolved. For example, *Munida callista* (the only *Munida* species not forming part of the *Munida* group in the 16S results) appeared basal to the cluster that included all species with a single pair of male pleopods. The internal relationships of the previously considered genera were also better resolved or supported.

Using the Bayesian procedure on combined data for the two genes to independently search for the best model for each gene along with the other analytical methods, all the genera considered, with the exception of *Agononida* and the placement of *M. callista*, appeared as monophyletic groups and were well supported. Nevertheless, as in the independent analyses, not all the species of the genus *Munida* clustered as a single group (this is only supported by a Pp = 0.79). The first cluster next to the outgroup was the genus *Raymunida* associated with the species *Alainius crosnieri*. After this first assemblage, we found a well supported and diverse group of genera (*Crosnierita* + *Bathymunida* + *Agononida* + *Heteronida* + *Plesionida* + *Onconida* + *Paramunida*, all of them with one pair of male pleopods, and in a basal position *Munida callista*). The rest of the species grouped as follows: *Cervimunida* and *Pleuroncodes* and the species belonging to the genus *Munida* in a polytomy (strictly speaking, if we take into account the results of all the methods used), where some groups of *Munida* species showed clear phylogenetic relationships with other *Munida* species (Fig. 4).

Some of the relationships within *Munida* were well supported by some of the treatments. Thus, the *M. rhodonia*, *M. congesta*, *M. militaris*, *M. rosula*, *M. compressa*, *M. rubrodigitalis*, *M. eclepsis*, *M. pagesi*, and *M. tiresias* cluster was supported by: Pp = 1; Bv-NJ = 68, ML = 79, MP = 74, though the relationships

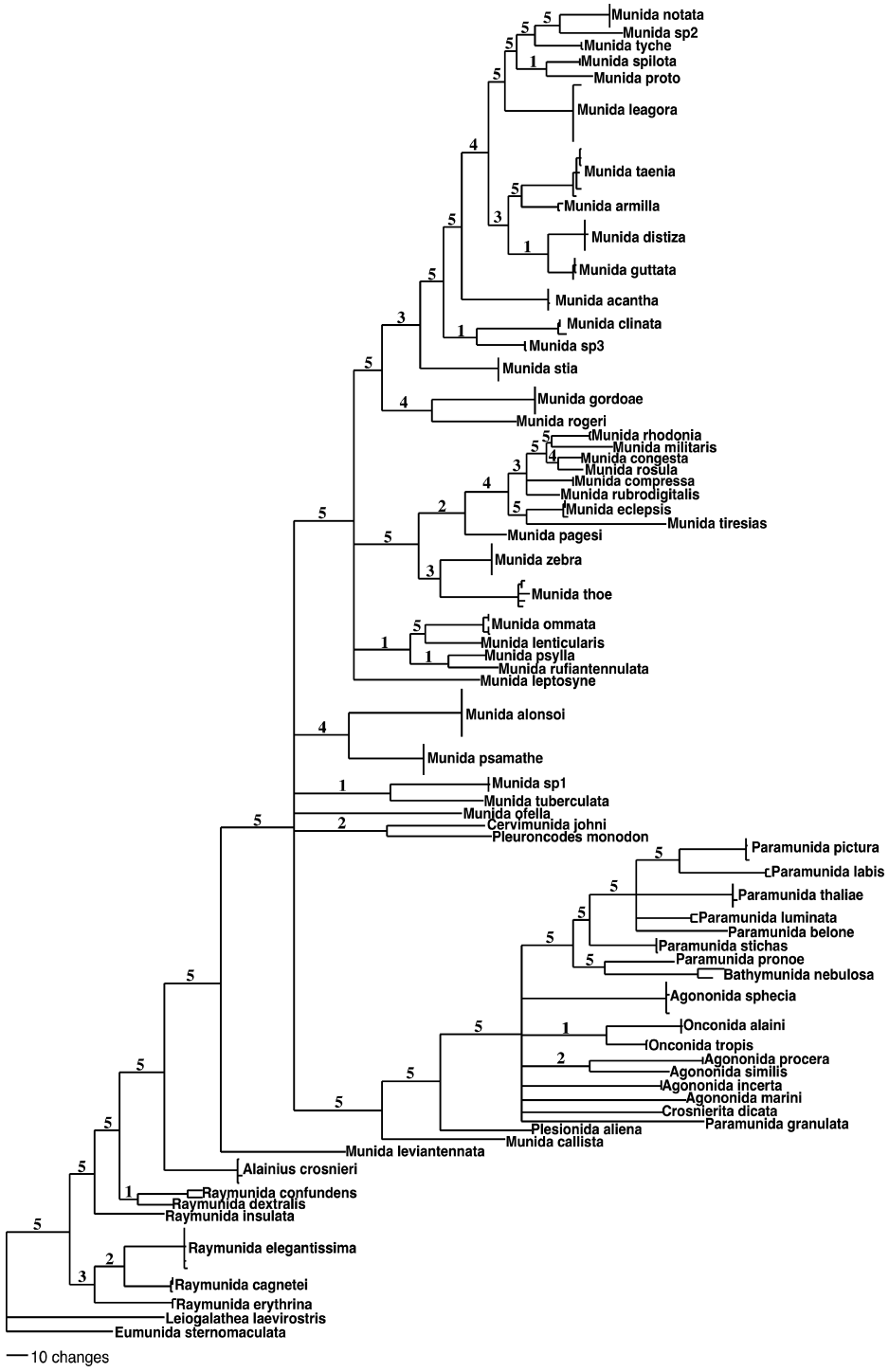

Fig. 3. Phylogenetic hypothesis based on COI sequences represented by a consensus tree obtained by Bayesian inference (2,000,000 replicates, 20,000 trees sampled, and 10% burn-in). Numbers on branches indicate posterior probabilities (=Pp) and the bootstrap values (=Bv) for MP, NJ, and ML coded as follows: 1: Pp=1–0.95, Bv $\geqslant$ 70; 2: Pp=1–0.8, Bv $\geqslant$ 60; 3: Pp=1–0.7, Bv $\geqslant$ 60 (at least in two of the methods); 4: Pp=1–0.6, Bv $\geqslant$ 50 (at least in one of the methods); and 5: only Pp>50.

among the different species were not always well supported. Another clear relationship was the one established among *M. taenia*, *M. armilla*, *M. distiza*, and *M. guttata* (Pp=0.90; Bv-NJ=76, ML=56, MP=66), but the relationships among this clade and *M. clinata* and *M. acantha* could not be resolved.

This is also the case for the genus *Paramunida*, which formed a monophyletic group (Pp=1; Bv-ML=53, MP=61), yet intragroup posterior probabilities or bootstrap values were not always high for all the dichotomies. The best resolved internal relationships were those of the *Raymunida* species.

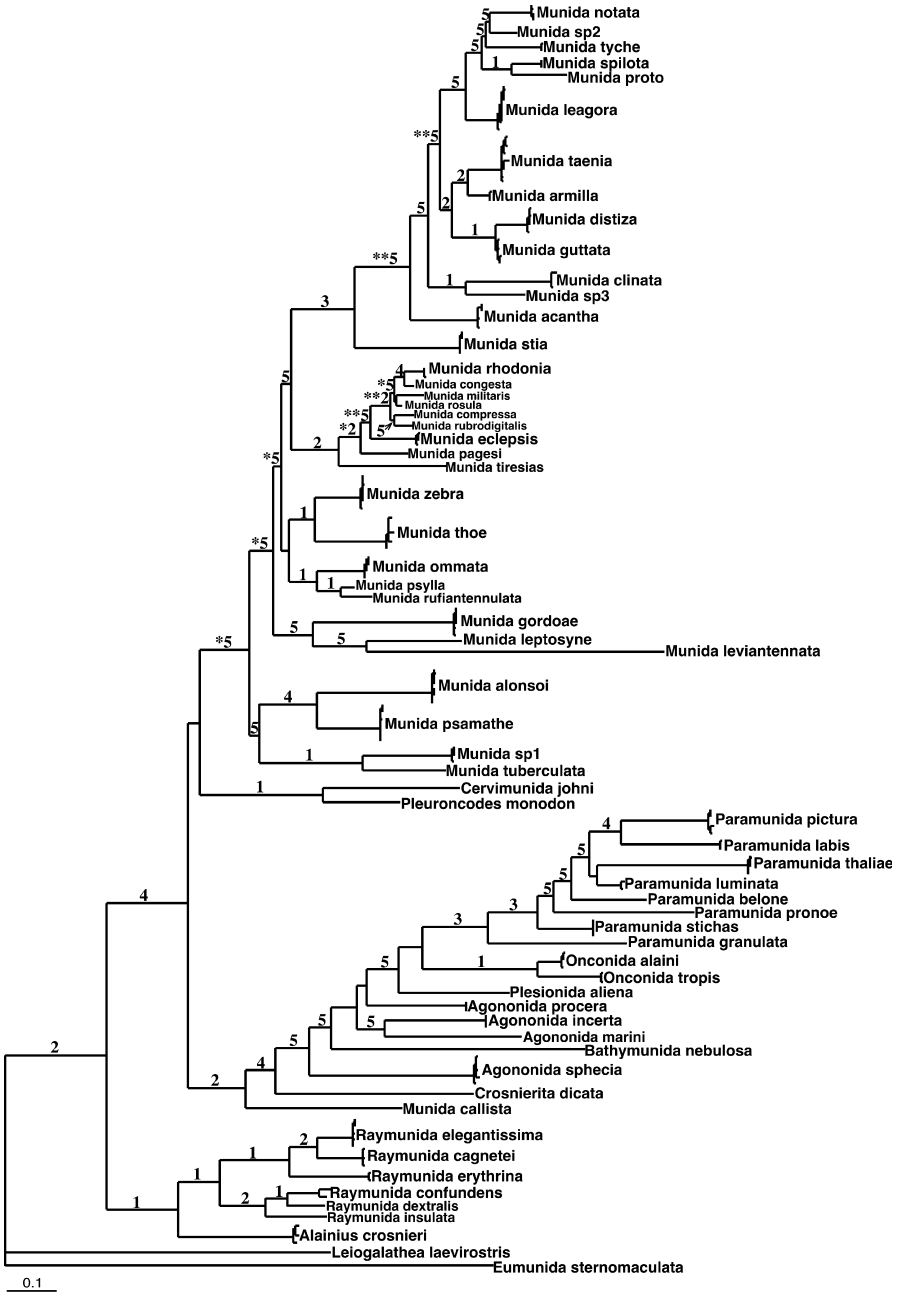

Fig. 4. Phylogenetic hypothesis based on global molecular data (COI and 16S) represented by a tree obtained by Bayesian inference (2,000,000 replicates, 20,000 trees sampled, and 20% burn-in). Numbers on branches indicate posterior probabilities (=Pp) and the bootstrap values (=Bv) for MP, NJ, and ML coded as follows: 1: Pp = 1–0.99, Bv $\geqslant$ 70; 2: Pp = 1–0.95, Bv $\geqslant$ 60; 3: Pp = 1–0.90, Bv $\geqslant$ 55; 4: Pp = 0.99–0.85, Bv $\geqslant$ 50 (at least in one of the methods); and 5: Pp = 0.99–0.50. Asterisks indicate lineages with a significantly higher than expected rate of speciation according to the relative cladogenesis statistic (*$P < 0.05$; **$P < 0.01$).

3.3. Diversification rates

The relative rates test indicated significantly different rates of evolution for some species (e.g., the *Munida rhodonia*, *M. congesta*, *M. militaris*, *M. rosula*, *M. compressa*, *M. rubrodigitalis* clade had significantly shorter branches, while *Bathymunida nebulosa* had a longer one; $P < 0.05$). Given the lack of rate constancy in the galatheids analyzed, we constructed an ultrametric tree using the NPRS method. Before conducting the CR-MCCR tests, the $B1$ test and the relative cladogenesis statistic were used to test the assumption of equal rates of cladogenesis among lineages. The $B1$ test did not reject the symmetry of our tree ($N = 62$; $B1 = 30.05$; $P > 0.05$). The relative cladogenesis statistic showed some branches with higher than expected rates of cladogenesis: the three innermost branches of the

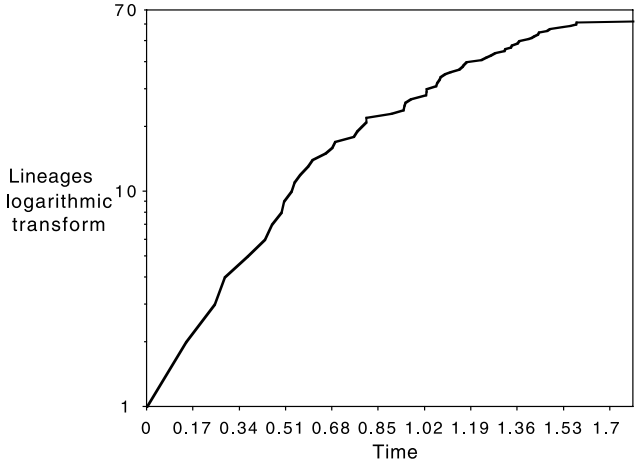

Fig. 5. Semilogarithmic plot of lineages through time using a dataset including 62 ingroup taxa.

Munida cluster showed statistically higher rates than the remaining tree branches ($P < 0.05$) and even certain inner *Munida* branches attained a probability of less than 0.01 (Fig. 4). The LTT plot was convex (Fig. 5), indicating an early burst of speciation. To establish whether this finding was really indicative of rapid speciation or the result of an incomplete taxon sampling effect, we calculated the γ-statistic ($\gamma = -5.51$). The MCCR test showed that the critical value of γ at the 2.5% level was -5.35 (-5.11 at 5% level). For this test, we considered the 62 ingroup taxa and all the 370 Galatheidae species known in the world (Table 1), except the genus *Munidopsis*, since according to our own data (unpublished), this genus cannot be considered as part of the ingroup. The significance of the MCCR test, thus, indicates that the squat lobsters underwent an early speciation burst.

3.4. Phylogenetic relationships based on morphological data

Sixty-four of the 79 morphological characters considered were parsimony-informative. The ratio between this number of the informative characters considered and the number of taxa examined gave rise to more than 100,000 equally parsimonious trees. These trees were 258 steps in length and their consistency index (CI) were 0.27 (retention index RI = 0.73), both with PAUP and WinClada programs.

The bootstrap consensus tree was split into two basal groups (including on the one hand *Onconida*, *Bathymunida*, and *Heteronida*, and on the other *Leiogalathea* and *Alainius*) plus two main clades (Fig. 6), although one of them was only supported by a low posterior probability, Pp = 0.59. The division of the biggest groups corresponded to the presence of one or two pairs of male pleopods, division that was strict in the Bayesian analysis (the "*Munida* cluster" included also *M. leviantennata*, *Leiogalathea laevirostris*, and *Alainius crosnieri*, and *Paramunida* + *Plesionida* + *Crosnieri* + *Agononida*, incorporated *Bathymunida* + *Heteronida* + *Onconida*.

In the group with two pairs of male pleopods, only one genus was monophyletic: *Raymunida*. The rest of the genera were only represented by one species, except *Munida*, which showed different relationships with them. This character (two male pleopod pairs), constituting the synapomorphy that characterizes this main cluster, should be considered phylogenetically informative (contrasting with the molecular data) in the Bayesian analysis but not in the parsimony one. Subdivisions into species groups with two pairs of male pleopods were mainly supported by the length of the spines on the antennular and antennal peduncles.

On the contrary, in the group typified by the presence of only one pair of male pleopods (one of the characters most widely used for the taxonomy of the family Galatheidae), all the genera were monophyletic, except the lack of resolution among *Agononida* species. Both *Agononida* and *Crosnierita* are characterized by a carapace and abdomen with major well-defined transverse ridges. *Paramunida*, *Plesionida*, *Onconida*, *Heteronida*, and *Bathymunida* differ considerably from other species, in some morphological characters (e.g., spinulation of the carapace, abdomen, and antennae).

The three cryptic species detected grouped with *M. tiresias* and *M. tuberculata* (*Munida* sp1), with *M. notata* (*Munida* sp2, with no apomorphies distinguishing them) and with *M. clinata* (*Munida* sp3).

4. Discussion

The present is the first attempt to elucidate phylogenetic relationships among western Pacific squat lobsters of the genus *Munida* and related genera. The phylogenetic trees inferred from morphological and molecular characters support different hypotheses of relationships between genera and species. Further, our phylogenetic reconstructions based on 16S and COI gene sequences clearly support the splitting of the genus *Munida* into several genera, i.e., *Munida*, *Paramunida*, *Agononida*, and *Raymunida* (Baba, 1988; Baba and de Saint Laurent, 1996; Macpherson and Machordom, 2000). However, the position and phylogenetic relationships of some taxa were not fully resolved. For example, the monophyly of other new genera, such as *Crosnierita*, was not supported.

Using the morphological data available, we were able to construct a data matrix that yielded two well-differentiated groups, i.e., species with one or two pairs of male pleopods, plus two basal groups including very morphologically differentiated taxa. However, the internal structure of these groups was different to that recovered using molecular data. Our results demonstrated that the most

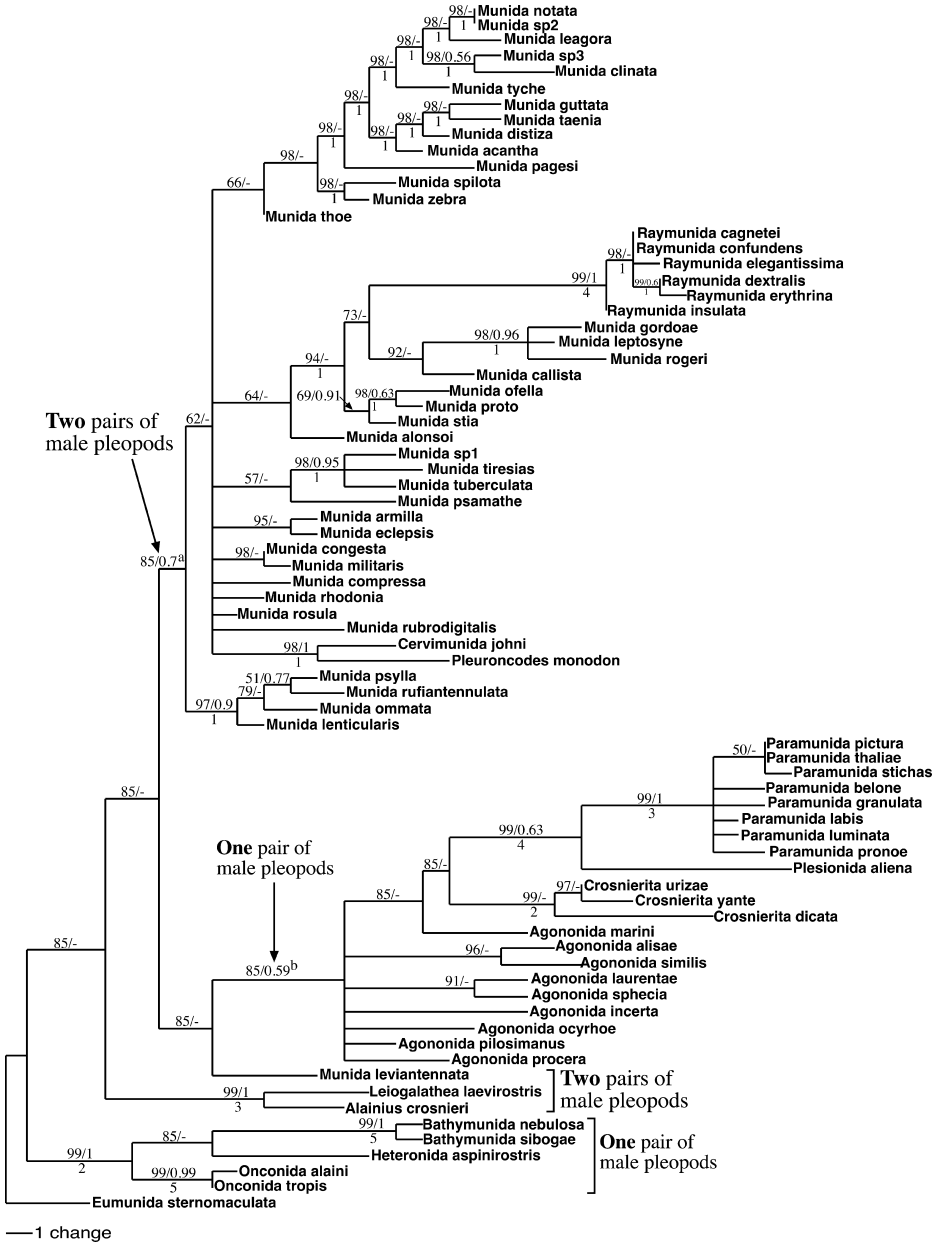

Fig. 6. Bootstrap consensus tree based on morphological characters. Numbers on branches represent those in first position bootstrap values, and numbers in second position, posterior probabilities from Bayesian inference (Pp > 0.5; 5,000,000 replicates, 50,000 trees sampled, and 10% burn-in). When numbers appear under branches, they indicate decay indices different from zero. Hyphens indicate Pp < 0.5. [a]the posterior probability supported this node but including *Munida leviantennata* (with two pairs of male pleopods), *Leiogalathea laevirostris* and *Alainius crosnieri*, and [b]in the Bayesian analysis *Bathymunida*, *Heteronida*, and *Onconida* were included in this cluster.

common morphological characters used in the taxonomy of the family Galatheidae (e.g., spines on the abdominal segments, spines on branchial margins, presence of granules on sternites, or the relative length of the antennule/antennal spines) have not always been of phylogenetic value according to our molecular and morphological reconstructions. On the other hand, some others characters, such as the spinulation of the dorsal carapace surface, the carinae on the sides of the sternum, the robust and massive shape of the carapace, or the presence of epipods on the pereiopods are phylogenetically informative and congruent in both molecular and morphological analyses.

Both genes analyzed provided support at different levels. While COI sequences failed to establish relationships at the most basal nodes, the 16S data were incapable of clarifying relationships at terminal nodes. This rendered phylogenetic trees derived from data for both genes the most resolved, as expected by the hypothesis of "overlapping levels of resolution" (Giribet, 2002).

Combining all the recovered data, the situation for each genus could be viewed as detailed below.

4.1. Munida

This is the most diverse group. To consider this group as monophyletic, *M. callista* must be excluded. Even if morphological data assign this species to *Munida*, this should be regarded as convergent evolution of its morphological traits or simply yet a further case of discrepancy between morphological and molecular characters (Patterson et al., 1993). *Munida callista* showed the greatest divergence in both sequenced genes with respect to the other species of the genus. This divergence was even higher than that between *Munida* and *Paramunida* species or between *Munida* and *Raymunida*. Some morphological treatments also argue phylogenetic relationships respect to *Munida leviantennata*, supporting its basal position respect to some genera with one pair of male pleopods instead of to cluster it among its congeneric species.

Some subgroups were strongly supported but, in general, the phylogenetic relationships within the genus *Munida* were not fully established. In some of the well-resolved groups, some morphological synapomorphies became apparent. These morphological characters have not always been considered determinant in taxonomic keys. Thus, some clearly phylogenetically related species according to molecular data represent dwarf lineages (e.g., *M. alonsoi*, *M. tuberculata*). In others, the most relevant feature was their massive size (e.g., *M. rhodonia*, *M. rubrodigitalis*). Nevertheless, some taxonomic characters revealed their phylogenetic value, e.g., marked carinae on the sides of the sternum (*M. ommata*, *M. psylla*, and *M. rufiantennulata*).

There are several possible explanations for the incomplete resolution of internal relationships within *Munida*. On the one hand, the morphological characters of this group seem to show some type of stasis, inertia or convergence, which leads to very similar morphotypes that are genetically clearly divergent. Certain constraints result in indistinguishable species that have been genetically isolated for a long time, as observed in *Cancer* decapods, which show extensive convergence in adult crab morphology (Harrison and Crespi, 1999). On the other hand, we could always argue that the genes screened here were inadequate for analyzing this group, but considering the number of informative positions and the lack of saturation, this seems not to be the case. Alternatively, the polytomies detected (or the lack of resolution in different data treatments prompting us to consider some non-supported nodes as polytomies) could also be explained by rapid radiation. Vicariant events are considered the main speciation forces, but usually by pair of taxa. Nevertheless, sudden changes in habitat conditions followed by isolation, or the colonization of new ecological niches, could lead (in our case in a marine environment) to the splitting of a previous more or less widely distributed taxon into several new taxa. If these changes or isolation phenomena occur sufficiently rapidly, there is no genetic signal reflecting phylogenetic relationships, and the internodes are very short relative to the terminal branches (Shaffer and McKnight, 1996), as in our case. Our diversification rates lend support to rapid radiation in the early evolutionary history of the squat lobsters and particularly the *Munida* lineage. The CR–MCCR tests indicated an early burst of speciation (Fig. 5) and several events of unusually rapid diversification rate occurring in *Munida* were identified by relative cladogenesis statistics (Fig. 4).

4.2. Paramunida

This genus was recognized in all the analyses, both morphological and molecular, as a monophyletic group. Support was always high, confirming the separation made by Baba (1988) of *Paramunida* from the genus *Munida*, based on morphological characters. One of the most differentiated species within the *Paramunida* cluster was *P. granulata* that appeared basal to this group. This subdivision corresponded to a marked morphological difference in the size of the distomesial spine of the second segment of the antennular peduncle; *P. granulata* having a much longer spine than other species of the genus (Baba, 1988). The phylogenetic results also supported *Plesionida aliena*, originally included in *Paramunida*, as a separate taxon (Macpherson, 2004). It appeared as the sister species of the rest of *Paramunida* in the morphological analysis, but differed greatly in genetic terms (mean divergence between *Plesionida aliena* and the *Paramunida* specimens was 13.98% overall for the two genes). In fact, in the molecular phylogenetic analyses, *Plesionida aliena* appeared as sister species of the genus *Onconida* (16S), or basal to *Paramunida* + *Bathymunida* + *Agononida* + *Onconida* + *Crosnierita* (COI) or to the *Paramunida* + *Onconida* cluster (according to both genes together).

4.3. Raymunida

This genus was recently described in the light of both morphological and molecular data (Macpherson and Machordom, 2000, 2001). Although both datasets were easily able to identify these species, previously considered members of the genus *Munida*, the phylogenetic relationships based on morphological characters observed here did not resolve the relationships among the *Raymunida* species and those in the genus *Munida*. Indeed, *Raymunida* forms a well-supported monophyletic assemblage within a large group that includes all the *Munida* species, the *Raymunida* species, *Leiogalathea laevirostris*, *Alainius crosnieri*, *Cervimunida johni*, and *Pleuroncodes monodon* (the latter two species inhabiting the eastern

Pacific ocean). However, molecular data clearly distinguished the *Raymunida* species from the others, placing them in a basal position. Thus, the singularity of this group is always supported, though the morphological characters considered conflict with our molecular data.

4.4. Other genera

The genera recently separated, or created, by morphological differences (*Crosnierita*, *Onconida*, and *Heteronida*) from *Agononida* and *Bathymunida* (Baba and de Saint Laurent, 1996; Macpherson, 2004) were not fully supported by our molecular data.

None of our analyses recovered *Agononida* as a monophyletic entity and the 16S data also showed *Crosnierita* as a non-monophyletic assemblage, but unfortunately, only one of the *Crosnierita* species could be sequenced for both genes. In the overall molecular analysis, we did find that one *Bathymunida* species appeared more closely related to other *Agononida* species than, for instance, *A. sphecia*. Similarly, according to the 16S data, *C. dicata* clustered in a *Bathymunida* group rather than appearing as the sister species of *C. urizae* or *C. yante*. A more detailed analysis of these genera would be needed to resolve these points.

Onconida is morphologically close to *Bathymunida* and *Heteronida*. However, the two species of *Onconida* analyzed always clustered together in the molecular analyses, indicating that their sister taxa were the morphologically highly different *Plesionida* and *Paramunida*. Thus, once again, morphological and molecular characters yielded different phylogenetic reconstructions.

Cervimunida and *Pleuroncodes* are morphologically close to *Munida*, though the species considered in this study came from distant geographic areas (Chile, southeastern Pacific). Our phylogenetic analysis indicated them to be closer to the genus *Munida* than the other genera (*Paramunida* or *Agononida*), but without significant support.

The only known species of the genus *Leiogalathea* always appeared in a basal position in the different molecular phylogenetic analyses, in contrast to morphological relationships with *Alainius crosnieri*. *Leiogalathea* is very different from the other genera and is probably more related to the genus *Galathea* (Baba, 1988), not included here. Further research may clarify these potential relationships.

4.5. Taxonomic implications

The molecular analyses undertaken allowed us to identify several cryptic or sibling species. Owing to their sclerotized exoskeleton, decapod crustaceans have a relatively large number of phylogenetically informative morphological characters (Mathews et al., 2002). However, sibling species are fairly common and sometimes even occur remarkably frequently in marine invertebrates including decapods (Knowlton, 1993; Thorpe et al., 2000) or in other marine organisms (see the review by Knowlton, 2000). Some slight morphological differences found in species of the genus *Munida* were considered as intraspecific character variations (Baba, 1988; Macpherson, 1994). In our study, we related some of these subtle morphological differences to high molecular divergences and independent evolutionary lineages. This was the case for some specimens initially ascribed to *M. notata*, *M. clinata*, and *M. tuberculata* species, for which we found evidence of genetic isolation. The divergence found between each of them and their corresponding previously considered species largely exceeds the average intraspecific divergence found for the other taxa. We consider that three new putative species should be described and have denoted these *Munida* sp1, sp2, and sp3.

The two *Bathymunida nebulosa* haplotypes found (with remarkable divergence between them) may also be cryptic species. However, this genus was not largely represented, and the number of specimens analyzed precludes us drawing valid conclusions.

Munida callista was the only *Munida* species that was not included in the *Munida* molecular cluster. Thus, we recommend removing *M. callista* from the genus *Munida* and verifying its phylogenetic relationships by analyzing this species and closely related species (such as *M. javieri*) (Macpherson, 1994). In the same way, the phylogenetic relationships of *Munida leviantennata* require in-depth analysis. This species was excluded from the cluster in which the rest of *Munida* species grouped in morphological parsimonious trees. In the COI molecular analyses, its relationships were not defined in most of the treatments, while the Bayesian inference situated it basally to most of the species screened here (Fig. 3).

Finally, in the light of our results, the taxonomic status of *Agononida* and *Crosnierita* genera would also need revision.

4.6. Evolutionary considerations

Phylogenetic analysis of the squat lobsters was able to resolve most of the groups considered today with high support. However, certain intrageneric relationships could not be assessed with confidence. This low support could be the result of a lack of characters showing phylogenetic signal, probably due to a rapid radiation event that left no signs of evolutionary splitting or divergence of taxa. Moreover, given there is no fossil record to clarify the time of divergence of the different groups, nor are there any clear marine barriers that could justify the isolation of the ancestors of these species, we did not undertake molecular clock calibration of the divergences found. Some authors have proposed recent speciation or the existence of cryptic species to explain a species phylogeny that differs from relationships inferred from particular DNA sequences (Mathews et al., 2002; McMillan and Palumbi, 1995; Neigel and Avise, 1986). This could be the

case for the pairs of sibling species we detected, though mean divergence values failed to suggest recent divergences among most of the species analyzed. For instance, mean interspecific divergence found for the genus *Munida* was greater than 14% for the COI, and 6.76% for the 16S data. Considering general mean divergence values (between 1 and 2% per million years for genes as variable as COI) (Bermingham et al., 1997; Brown et al., 1982; Moritz et al., 1987) (0.53–0.88%/My for 16S) (Schubart et al., 2000; Stillman and Reeb, 2001), *Munida* species diversification may have began some 7–14 million years ago. This corresponds to the Middle or Late Miocene, which cannot be considered "recent." The diversification of a pomacentrid fish of the genus *Dascyllus* in the Indo-Pacific area was dated as mid-Miocene (McCafferty et al., 2002). Hence, the most probable hypothesis for the evolution of this group is a rapid or explosive mode of speciation and stasis, or constraints in its morphological evolution. This hypothesis is supported by our LTT plot showing a speciation burst early in the evolutionary history of the squat lobsters followed by a decline in the speciation rate. New data on the biology and ecology of these species and their habitats, and improved knowledge of the palaeogeographical history of the New Caledonia region will help us clarify the possibility of microhabitat and behavioral specialization giving rise to the adaptive radiation of squat lobsters, as established in organisms such as fishes (Danley and Kocher, 2001; Rüber et al., 2003; Streelman et al., 2002).

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Appendix A

Matrix of morphological character states used in this study

	AAAAABBBBBBBCCCDDDDDDDDDDDDDDDDDDDDDDDDDDDEEEEEFGGHIIIIIIIIJJKKKKKLLLLMMMMMMMMMM
	11111111112222222 1
	12345123456712312345678901234567890123456123 45 12345678121234512341234567890
E.sternomaculata	00000000021000000001000000000001000100010000100000000001001000100010000000000
L.laevirostris	01000000000000000000210000000010100001100111000101012001010010110000000000000
A.alisae	10101200000010001100000110010000100000000001100000000000010000110100000001000 01
A.incerta	10101210000010001100000000000000100000000001100000000000010000110100000000000 01
A.laurentae	00101110002010001100000000010000100000000001100000000000010000110100000010000 01
A.marini	11101200001020001100000110010000100000000001100000000000010000110000000000000 01
A.ocyrhoe	01101100001010001100100000010000100000000001100000000000010000010100000100000 01
A.pilosimanus	01101000001010001100000000010000100000000001100000000000010000010000000100000 01
A.procera	11101000000010001100000100011000100000000001100000000000010000010000000100000 01
A.similis	10101100000020001100000100000000100000000001100000000000010000110100000001000 01
A.sphecia	01101110000010001100000001000000100000000001100000000000010000110100000100000 01
Al.crosnieri	01000100001000100002000000000001000001000010001010110010100001000000000000000
B.nebulosa	00101000100020001000200001000001111100000001000000000001001000010000000001001000
B.sibogae	10101000100020001000200001000001111100000001000000000001001000011000000001001000
Ce.johni	00101000000000001010000001001100100000000001000001100000010000100100000000000
C.dicata	11101010000020001000200111000000100000000001100000000000010001110100000001000 00
C.urizae	11101010000020001100200011010000100000000001100000000000010001110000000100000 01
C.yante	11101010000020001100200001000000100000000001100000000000010001110000000100000 01
H.aspinirostris	11111000100000001000200000100001000100010000000000000010000000110000000000000
M.acantha	00100110000010001000000001000000100000000210000001000000010000100000000000000
M.alonsoi	01000000001020001000000001000000100000000000000001000000010000100000000000000
M.armilla	01100010000020001000000001000000100000000201000001000000010000000100000000000
M.callista	00000000000020001000100000100000100000000221000001000000010000000000000000000
M.clinata	00000010000000001000100001000000100000000221000001000000010000101000000000000
M.compressa	01100000000000000000000001000001100000000001000001000100010000000000000000000
M.congesta	01100000000020001000000001000001100000000001000001000000010000000000000000000
M.distiza	00100110000010001000000001000000100000000211000001000000010000000010000000000
M.eclepsis	01100010000020000000000001000000100000000201000001000000010000000000000000000
M.gordoae	01100000000021000001000000000001000000001110000010000000100000000100000000000
M.guttata	00100100000010001000000001000000100000000211000001000000010000101110000000000

(*continued on next page*)

Appendix A (*continued*)

M.leagora	00000010000000001000000001000001000000001110000010000000100000000000001000000
M.lenticularis	01100000000001001000000000000001000000002010000010000001000000000010000000000
M.leptosyne	01100000000011000001000000000001000000022100000100000001000000001000000000000
M.leviantennata	01101000010000011000001000010000000000000110000010000001000001000000000000000
M.militaris	01100000000020001000000001000001100000000101000001000000010000000000000000000
M.notata	00000110000010001000000001000001000000001110000010000000100001000000001000000
M.ofella	01000000000021001000000010000001000000022100000100000001000000000000001000001
M.ommata	01100010000021001000000000000001000000001010000010000001000000000001000000000
M.pagesi	00100110000021001000000011000001000000021100000100000001000000010000001000000
M.proto	00000000000020001000000001000001000000022100000100000001000010000000001000001
M.psamathe	01100000100021001000000000000001000000000000000010000000100001000000000000001
M.psylla	01000010000021001000000000000001000000000010000010000000100000000010000000000
M.rhodonia	00100000000000010000000100000110000000000100000100000001000000000000000000000
M.rogeri	01100100000021000001000000000001000000022100000100000001000000002000000000001
M.rosula	01100000000000001000000010000011000000001000000100000001000000000000000000000
M.rubrodigitalis	00100000000000001000000010000010000000000100000100010001000000010000000000000
M.rufiantennulata	01001100000021001000000000000001000000000100000100000001000000000010000000000
M.spilota	00000110000000001000000010000001000000002000000010000000100001000000000000000
M.stia	01000000000020000000000001000000100000000221000001000000010000100000000010000000
M.taenia	00100110000010001000000010000001000000002010000010000000100001010200000000000
M.thoe	00100000000000001000000010000001000000002010000010000000100000000000000000000
M.tiresias	01000001000210100001000011000001000000000010000110000000100000100000000000000
M.tuberculata	01100001000210100000000010000001000000000010000010000000100001010000000000000
M.tyche	00100110000001001000000010000001000000001110000010000000100001000200000000000
M.zebra	00100110000000001000000000000001000000002000000010000000100000000000000000000
Munida sp1	01100000100020010000000010000001000000000000000010000000100000000000000000000
Munida sp2	00000110000010001000000010000001000000001110000010000000100001000000001000000
Munida sp3	00000110000010001000100001000000100000000111000001000000010000101000000000000
O.alaini	10002001000000001000200000000001100100000000000000000010010000010000000010100
O.tropis	00000200100000001000200000000001100100000000000000000010010000010000000010100
P.belone	10101211000020001101000111010000000000000000000000000010010000010000011000000
P.granulata	10101210000020001101000111010000000000000000000000000010010000010100011000000
P.labis	10101201000020001101000111010000000000000000000000000010010000010000011000000
P.luminata	10101200000020001101000111010000000000000000000000000010010000010000011000000
P.pictura	10101210000020001101000111000000000000000000000000000010010000010000011000000
P.pronoe	10101200000020001001000111000000000000000000000000000010010000010000011000000
P.stichas	10101211000020001101000111000000000000000000000000000010010000010000011000000
P.thaliae	10101210000020001101000111000000000000000000000000000010010000010000011000000
Ples.aliena	11001200001020001000000010000000000001100000000000000010010000010000000100001
Pleuro.monodon	01000000000010000010000001001100100000000010000010000001000001010000001000000
R.cagnetei	01000200000020000000011000000000100000000111001001000000011010100000000000010
R.confundens	01000200000020000000011000000000100000000111001001000000011010100000000000010
R.dextralis	01000200000020000000011000000000100000000111001001000000011010100000000000110
R.elegantissima	00000200000020000000011000000000100000000111001001000000011010100000000000010
R.erythrina	01000200000020000000011000000000100000000111001001000000011010000000000000110
R.insulata	01000200000020000000011000000000100000000211001001000000011010100000000000010

See below for character descriptions.

Characters were coded : (0) absent, (1) present, except where indicated otherwise.

A: Abdomen
- 1: fourth segment with posterior spine
- 2: second segment with less than 2 striae
- 3: second segment with median spines
- 4: strong median process on second and third segments
- 5: third and fourth segments with spines

B: Antenna
- 1: distomesial spine of basal segment: (0) not exceeding second segment, (1) slightly exceeding second segment, (2) exceeding peduncle
- 2: distomesial spine of second segment exceeding antennal peduncle
- 3: distomesial spine of second segment mucronated
- 4: peduncle reduced in size
- 5: peduncle unarmed
- 6: distomesial spine on third segment: (0) absent, (1) small spine, (2) very long
- 7: second segment with scaphocerite

C: Antennule
- 1: distal spines: (0) subequal, (1) mesial > lateral, (2) mesial < lateral
- 2: peduncle exceeding corneae
- 3: three distal spines

D: Caparace
- 1: anterolateral angle convex
- 2: anterolateral spine long
- 3: branchiocardiac spines
- 4: cervical groove in anterior position
- 5: covered with numerous spinules
- 6: front margin: (0) transverse, (1) oblique, (2) concave
- 7: front margin with spine behind antenna
- 8: frontal margin with antennal spine

9: median cardiac spines
10: mesogastric spine
11: number of lateral spines on branchial region: (0) 3–4, (1) 5
12: numerous secondary striae and scales
13: posterior border with spines
14: protogastric spines
15: pterygostomian flap: (0) non-visible, (1) dorsally visible
16: ridges with coarse setae
17: robust and massive
18: striae well differentiated
19: strong cardiac process
20: strong gastric and/or cardiac spines
21: strong gastric process
22: three pairs of hepatic spines in oblique line
23: with long coarse setae
24: without spines
25: without striae
26: triangular shape

E: Cheliped
1: Marginal spines along fixed finger: (0) absent, (1) on proximal half, (2) along entire border
2: Marginal spines along movable finger: (0) absent, (1) on proximal half, (2) along entire border
3: palm with spines
4: spines on merus and palm forming lines
5: ventral pad on propodus

F: Pereiopods: (0) without epipods, (1) with epipods

G: Eyes
1: peduncles short
2: very reduced

H: Male pleopods: (0) one pair, (1) two pairs

I: Rostrum
1: dorsal and ventral spines
2: flatish
3: lateral spines: (0) absent, (1) reduced, (2) well developed
4: laterally compressed
5: supraocular spines short
6: triangular
7: two pairs of supraocular spines
8: well developed

J: Telson
1: endopodites with some long spines
2: one transverse suture

K: Third maxilliped
1: carpus with distal spine on extensor margin
2: merus extremely short
3: merus with distal spine on extensor margin
4: one spine on flexor margin of merus
5: three strong spines on flexor margin

L: Thoracic sternum
1: squamate
2: Small granules on sternites: (0) absent, (1) on sternite 7, (2) on sternites 6 and 7
3: fourth sternite with two spines
4: sternites with lateral carinae

M: Walking legs
1: dactyli distally compressed
2: dactyli with ridge along lateral and mesial sides
3: dactyli with ventral spinules on distal portion
4: dactyli with ventral spinules on proximal 2/3
5: dactyli with ventral spinules on proximal portion or unarmed
6: fifth leg of males with barb-like process
7: fifth leg of males with toothbrush-like process
8: merocarpal articulation reaching supraorbital sinus
9: merus of second leg thinner than third and fourth legs
10: twice carapace length

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