A NEW SQUAT LOBSTER OF THE GENUS *RAYMUNIDA* (DECAPODA: GALATHEIDAE) FROM TAIWAN

Chia-Wei Lin, Tin-Yam Chan, and Ka Hou Chu

(CWL and TYC) Institute of Marine Biology, National Taiwan Ocean University, Keelung 202, Taiwan, R.O.C.; (KHC) Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (corresponding author (TYC) tychan@mail.ntou.edu.tw)

ABSTRACT

The galatheid genus *Raymunida* Macpherson and Machordom, 2000, is reported for the first time from Taiwan, and the species collected is also new to science. The new species is most closely related to *R. confundens* Macpherson and Machordom, 2001, but differs in having a more robust cheliped and walking legs covered with distinct squammae. The coloration of the new species is probably unique in the genus by both the carapace and abdomen being uniform in color. Analysis of the mitochondrial cytochrome oxidase I gene sequences also supports the specific status of this Taiwanese form.

The Indo-Pacific galatheid genus Raymunida Macpherson and Machordom, 2000, was recently separated from Munida Leach, 1820, and contains seven species, of which five were described in the last few years (Macpherson and Machordom, 2000, 2001). With the aid of molecular data, Macpherson and Machordom (2001) noted that species of Raymunida are morphologically very similar but with distinct coloration, and most of them have restricted distributions. No Raymunida has been reported from Taiwan before, but four specimens belonging to this genus were collected during recent surveys of deep-water decapod crustaceans of Taiwan. They belong to a species closely related to R. confundens Macpherson and Machordom, 2001, and R. dextralis Macpherson and Machordom, 2001, but show a different coloration. Direct comparison with the type material of these relatives and molecular analysis show that the Taiwanese form represents an undescribed species. This new species is here reported, and its coloration is illustrated.

MATERIALS AND METHODS

The specimens are deposited at the National Taiwan Ocean University, Keelung (NTOU), and the Muséum national d'Histoire naturelle, Paris (MNHN). The measurements given are the carapace length (cl.) measured along the dorsal midline from the base of the rostrum to the posterior margin of the carapace.

For molecular analysis, muscle (~ 25 mg) from the cheliped or fifth pereiopod were isolated from the holotype and a paratype (NTOU P-2001-76) of the new species, preserved in 95% ethanol, and used for DNA extraction using QIAamp DNA Mini Kit (QIAGEN). Partial segments of the mitochondrial genes coding for 16S rRNA and

cytochrome oxidase I (COI) were amplified by polymerase chain reaction (PCR) using the primers 16Sar (Simon *et al.*, 1994) and 1472 (Crandall and Fitzpatick, 1996) and LCO 1490 and HCO 2198, respectively (Folmer *et al.*, 1994). Contained in 50 μ L PCR reaction were 100 ng of the DNA extract, 1× buffer (QIAGEN), 0.2 μ M of each primer (Integrated DNA Technologies), 0.2 mM dNTP (GIBCO BRL), and 2.5 units of *Taq* polymerase (5 units/ μ L, QIAGEN). The PCR conditions were: 90 sec at 94°C, 33 cycles of 30 sec at 94°C, 30 sec at 45.0°C for 16S and 47.8°C for COI, 40 sec at 72°C, and final extension for 5 min at 72°C. Purified PCR product (with QIAGEN QIAquick PCR purification kit) was used as template for cycle sequencing in ABI 310 Genetic Analyzer (Applied Biosystems).

The COI sequence of the new species was aligned with those of other Raymunida species and species of Munida and Agononida from Macpherson and Machordom (2001) using ABI SeqEd Version 1.0.3. Although sequences from more than one individual were available for some of these species, the intraspecific divergences were small (averaged 0.24 percent, Macpherson and Machordom, 2001) so that only a single sequence from each species was used in the phylogenetic analysis (see Table 1 for GenBank accession numbers). Phylogenetic trees were constructed based on three methods, BIO neighbor-joining (Gascuel, 1997), parsimony, and maximum likelihood, with PAUP* (Swofford, 2000). The most appropriate model of DNA substitution was assessed using Modeltest (version 3.06, Posada and Crandall, 1998). The model was used in BIO neighbor-joining and maximum likelihood analyses. In all three analyses, branch support was assessed using 1000 bootstrap replicates.

RESULTS

Systematics *Raymunida formosanus*, new species Figs. 1–3

Material Examined.—Holotype: Taiwan, Tai-Shi fishing port, I-Lan County, commercial trawler, about 300 m, Feb 1999, 1 male 15.4 mm CL (NTOU H-1999-2).

	R. formosanus	R. dextralis	R. confundens	R. insulata	R. erythrina	R. cagnetei	R. elegantissima	M. rhodonia	M. rubridigitalis	A. incerta
R. formosanus	_	0.0845	0.1045	0.1167	0.1459	0.1167	0.1214	0.1644	0.1644	0.1782
R. dextralis	0.1260	_	0.0737	0.1029	0.1183	0.1152	0.1198	0.1567	0.1582	0.1628
R. confundens	0.1847	0.1080	_	0.1106	0.1214	0.1152	0.1290	0.1674	0.1659	0.1736
R. insulata	0.1976	0.1581	0.1817	_	0.1306	0.1183	0.1183	0.1613	0.1644	0.1920
R. erythrina	0.3453	0.2407	0.2771	0.2830	_	0.1137	0.1167	0.1843	0.1720	0.1813
R. cagnetei	0.2382	0.2198	0.2476	0.2463	0.2299	_	0.0783	0.1736	0.1644	0.1751
R. elegantissima	0.2290	0.2192	0.2734	0.2129	0.2319	0.1165	_	0.1644	0.1782	0.1720
M. rhodonia	0.3793	0.3529	0.4244	0.3790	0.4560	0.4376	0.3684	_	0.0645	0.1659
M. rubridigitalis	0.3963	0.3714	0.4456	0.3945	0.4425	0.4232	0.4242	0.0863	_	0.1613
A. incerta	0.4878	0.4203	0.4545	0.5070	0.5298	0.5141	0.4609	0.4351	0.4126	-

Table 1. Divergence of cytochrome oxidase I gene among *Raymunida* species and three outgroup taxa.

Values above the diagonal are uncorrected "p" distances, and those below the diagonal are distances based on the general time reversible model (Rodriguez *et al.*, 1990) with a proportion of invariable sites and with a gamma distrbution, "GTR+G+I", with the following parameters: A = 0.269, C = 0.163, G = 0.187, T = 0.381; substitution model rate matrix; [A-C] = 1.820, [A-C] = 9.729, [A-T] = 12.342, [C-G] = 1.478, [C-T] = 5.7624, [G-T] = 1.000; proportion of invariable sites = 0.594; gamma shape parameter = 2.090. The GenBank accession numbers of the sequences are AY288292 (*R. formosanus*), AF283874 (*R. dextralis*), AF283872 (*R. confundens*), AF283884 (*R. insulata*), AF283882 (*R. erythrina*), AF283869 (*R. cagnetei*), AF283875 (*R. elegantissima*), AF283885 (*M. rhodonia*), AF283887 (*M. rubridigitalis*), and AF283888 (*A. incerta*).

Paratypes: Taiwan, Tai-Shi fishing port, I-Lan County, commercial trawler, about 300 m, 05 Jan 1999, 1 female 19.3 mm CL (NTOU P-1999-01-05); 12 Mar 2003, 1 female 12.5 mm CL (NTOU P-2003-03-12).—TAIWAN 2001, stn CP 76, 24°56.54'N, 122°01.51'E, beam trawl, 115–170 m, 07 May 2001, 1 ovigerous female 15.0 mm CL (NTOU P-2001-76).

Description.—Carapace slightly longer than wide, bearing few long setae dorsally. Transverse striae with dense but short setae. Few secondary striae between main striae. Posterior two main transverse striae on carapace not interrupted in cardiac region. Intestinal region with scales. Five pairs of epigastric spines present, median pair distinctly smaller. One or two parahepatic, 3-5 branchial anterior, and 1-2 postcervical spines on each side of carapace. Frontal margin somewhat oblique, bearing 1 small spine between supraocular spine and anterolateral spine. Lateral margins slightly convex. Anterolateral spine (first marginal spine) well developed but not reaching level of sinus between rostrum and supraocular spines. Second marginal spine in front of cervical groove much smaller than anterolateral spine. Branchial margin bearing 4 spines, posteriormost spine distinctly smaller than others. Rostrum spiniform, about one-third of remaining carapace length, directed slightly downward. Supraocular spines horizontal, subparallel, and slightly overreaching midlength of rostrum and eyes.

Lateral parts of fifth to seventh thoracic sternites smooth. Anterior part of fourth thoracic sternite slightly narrower than third sternite.

Abdominal tergites with some short setae, unarmed on anterior border. Second and third tergites each with 1 uninterrupted transverse stria. Eyes moderately large. Basal segment of antennule (distal spines excluded) reaching cornea, with 2 distal spines, mesial spine distinctly shorter than lateral spine; 2 (only 1 on left side of holotype, probably regenerated) spines on lateral margin, proximal one short, distal one very long and overreaching distolateral spine. Antennal peduncle with mesial margin of first segment bearing 1 extremely long distal spine reaching distal end of basal segment of antennular peduncle (distal spines excluded); second segment with 2 long distal spines, mesial spine longer than lateral spine and overreaching third antennal segment; distal segment unarmed.

Third maxilliped with ischium as long as merus; merus with 2 strong spines on flexor margin, basal one slightly longer; extensor margin unarmed.

Cheliped robust, depressed, with numerous dense long, simple (i.e., not plumose) setae, distinctly more massive in male (chela 3.4 times longer than wide in male holotype) than in female (chela 4.9 times longer than wide in the ovigerous female paratype but chelae missing in the other two paratype females). Palm more or less as long as fingers, 1.7 times as long as wide in male, 2.4 times as long in female. Palm with several spines scattered on mesial and dorsal surfaces, bearing 1 row of strong dorsolateral spines running continuously to about middle of fixed finger. Fingers bearing distinct dorsal ridges, distally curving and crossing, ending in sharp points; movable finger with 3-4 mesial spines on proximal third; cutting edge with 1 large and a few small teeth situated on proximal third. Merus and carpus densely covered with spines.



Fig. 1. *Raymunida formosanus* sp. nov. a–d, holotype male 15.4 mm cl; e–f, paratype ovig. female 15.0 mm CL (NTOU-P-2001-76). a, e, carapace, dorsal view; b, ventral view of right cephalic region, showing antennular and antennal peduncles; c, sternal plastron; d, right third maxilliped, lateral view; f, ventral view of left cephalic region, showing antennular and antennal peduncles. Scale bars equal 1 mm.



Fig. 2. *Raymunida formosanus* sp. nov. a–e, holotype male 15.4 mm CL; f, paratype ovig, female 15.0 mm CL (NTOU-P-2001-76). a, f, right chela, dorsal view; b, first walking leg; c, dactylus of first walking leg; d, second walking leg; e, third walking leg. All legs in lateral views. Scale bars equal 1 mm.

Second pereiopod 2.5 times carapace length; merus more than 6.3 times as long as wide, nearly as long as carapace and 3 times longer than carpus; propodus 10 times as long as wide, 2.7–3.0 times as long as carpus and 2.6–3.0 times dactylus length. Merus with entire surfaces covered with distinct squammae, bearing 9–12 spines (increasing in size distally) along dorsal border, ventral margin with some distal spines. Carpus bearing 4–5 dorsal spines and 1 small distoventral spine, lateral surface with distinct longitudinal ridge and some squammae. Propodus having 4–5 movable ventral spines. Dactylus short, curving distally, ventral border indented, armed with 6–8 movable spinules. Third pereiopod similar to second but slightly longer, merus about 5–6 times as long as wide, bearing 1–2 rows of dorsal spines. Fourth pereiopod similar to but shorter than second and third, propodus with 4 movable ventral spines, merus 4 times as long as wide, merocarpal articulation overreaching frontal margin of carapace but not reaching (sometime just behind) level of sinus between rostrum and supraocular spines.

Color.—Body including sternal plastron generally orange-red. Eyes pale black. Carapace uniformly orange-red, spines on lateral margins whitish with red tips, anterior margin at base of rostrum and behind eyes whitish, cephalic appendages showing 3 transverse lines of red, white and red when viewed anteriorly. Cheliped with spines and distal part of palm reddish, fingers reddish and covered with two white bands on distal and middle parts. Walking legs and pereiopod V with red and white alternating bands.

Distribution.—Taiwan northeastern coast, 115 to about 300 m deep.

Remarks.-Following the key to species of Raymunida provided by Macpherson and Machordom (2001), the Taiwanese material is keyed out to R. confundens, a species known from two specimens from the New Caledonia and Chesterfield Islands. The general appearance of the Taiwanese form does show a great similarity to the holotype of R. confundens (female 7.6 mm CL from New Caledonia, MNHN-Ga 4376, the other specimen from the Chesterfield Islands is badly damaged and with a much smaller size than the holotype). However, the cheliped of R. confundens is much more slender (chela 6.2 times longer than wide and palm 2.6 times longer than wide), and the walking legs lack distinct squammae. Furthermore, R. confundens has weak ridges on the fingers of the cheliped and the carpus of the walking legs, two pairs of branchial anterior spines, and the body less hairy. The Taiwanese material has a much larger size than that of the holotype of R. confundens, and the above differences are not size related. For instance, *R. dextralis* from the Loyalty Islands is closely related to both R. confundens and the Taiwanese form. The only specimen known for R. dextralis is a holotype ovigerous female (12.3 mm CL, MNHN-Ga 2678). This fully grown specimen, with a general appearance somewhat more similar to R. confundens than the Taiwanese form, also has slender chelipeds (chela 7.2-7.5 times longer than wide and palm 3.1-3.3 times longer than wide, fingers also lacking distinct ridges) and with the walking legs almost devoid of squammae. The distinctiveness of the



Fig. 3. Raymunida formosanus sp. nov., holotype male 15.4 mm CL.

Taiwanese form is also supported by the very different coloration (carapace with white bands and abdomen bearing white spots in R. *confundens*, see Macpherson and Machordom, 2001). Thus, there is little hesitation in treating the Taiwanese form as new.

The robust appearance of *R. formosanus* shows some resemblance to *R. insulata* Macpherson and Machordom, 2001, known only from the holotype from the Seychelles Islands (female 16.5 mm CL MNHN-Ga 4377). However, *R. insulata* has the squammae and ridges on the walking legs rather weak, the chelipeds (chela 4.3-4.5 times longer than wide and palm 1.9–2.0 times longer than wide) being even more hairy and the entire outer margin of the fixed finger spinous. Moreover, the carapace of *R. insulata* appears to be more rounded and vaulted than that of *R. formosanus*.

Of the four specimens collected from Taiwan, the holotype and a paratype (NTOU P-2001-76) have the left cheliped missing or incomplete. One paratype (NTOU P-2003-3-12) has both chelipeds incomplete. The other paratype (NTOU P-1999-1-5) has all the anterior four pereiopods missing except for the left third pereiopod. These specimens are generally very similar but the branchial anterior spines vary from three to five pairs, and this number is somewhat greater than the 1-3 pairs in all the other species of the genus.

The coloration of *R. formosanus* is probably unique in the genus, having the carapace and abdomen uniform in color, whereas all the other *Raymunida* species (only *R. insulata* with coloration unknown) have prominent markings and/or spots on the carapace and/or abdomen. We would also like to mention that a recently published photograph of "*R. elegantissima* (de Man, 1902)" from Hachijo Island, Japan (Kato and Okuno, 2001) appears not to belong to that species or any known species of *Raymunida*. Nevertheless, this Hachijo Island form (two specimens examined, see Acknowledgements) has slender chelipeds and a coloration completely different from *R. formosanus*.

Etymology.—This new species is named after its type-locality, and Formosa is the old name of Taiwan.

Genetic Data

The partial segments of the 16S rRNA and COI genes collected from Raymunida formosanus were 541/542 and 657 bp long, respectively. The 16S rRNA sequences of the two individuals of R. formosanus differed only by one single insertion/deletion (GenBank Accession nos. AY288290 and AY288291), and the COI sequences were identical (AY288292). This confirmed that the two specimens analyzed, one male and one female, are truly the different sexes of the same species. In 651 bp of the aligned COI sequences of the seven Raymunida species, there were 162 variable sites of which 83 were parsimony informative. The COI divergence of R. formosanus from the other species varied from 8.5% (from R. dextralis) to 14.6% (from R. erythrina Macpherson and Machordom, 2001), the latter value being the highest observed among all Raymunida species studied (Table 1). The COI divergence of Raymunida formosanus from species of Munida and Agononida averaged 16.9%.

Distance analysis based on BIO neighborjoining separates *Raymunida* spp. into two clades (Fig. 4), one consisting of *R. cagnetei* Macpherson and Machordom, 2000, *R. elegantissima* and *R. erythrina*, and the other *R. confundens*, *R. dextralis*, *R. formosanus*, and *R. insulata*. Yet the inclusion of *R. insulata* in the second clade was not supported by maximum likelihood and parsimony analyses, in which this species was included in the first clade with weak (55 and 42) bootstrap support. No matter to which clade it was clustered, R. insulata was the most distant taxon in the clade. The three methods of phylogenetic reconstruction resolved the same topology in the first clade, with R. cagnetei and R. elegantissima as sister species. For R. confundens, R. dextralis, and R. formosanus, both BIO neighbor-joining and maximum likelihood analyses indicated that R. confundens and R. dextralis clustered together with R. formosanus as their sister group. Yet the most parsimonous tree showed that R. dextralis and R. formosanus were sister taxa (with a bootstrap value of only 40).

DISCUSSION

The analysis of the mitochondrial COI gene sequence from the new species Raymunida formosanus shows that it is genetically distinct from other species of this genus. Macpherson and Machordom (2001) showed that Raymunida species with subtle morphological differences but distinct coloration actually exhibit quite high genetic divergence in the COI gene. Sequence analysis of R. formosanus agrees well with this view. Raymunida formosanus exhibits the lowest COI divergence (8.5%) with R. dextralis in the genus (second lowest 10.5% with R. confundens). The COI divergence among all the species is always higher than 7% (Table 1). This value is higher than the COI divergence reported in some closely related decapod crustacean species such as Sesarma spp. (Schubart et al., 1998), Eriocheir spp. (Chu et al., 2003), Metapenaeopsis spp. (Tong et al., 2000), and Penaeus spp. (Lavery et al., in press).

The phylogenetic tree presented here is essentially similar to that of Macpherson and Machordom (2001: fig. 7), and R. formosanus was found to be most related to the R. confundens-R. dextralis clade. This is in support of the morphological characters proposed by Macpherson and Machordom (2001) in grouping and separating the species of Raymunida. Following the key of Macpherson and Machordom (2001), R. formosanus is grouped with R. confundens while the phylogenetic tree (Fig. 4) also groups R. formosanus with the clade containing R. confundens-R. dextralis. However, the relationships amongst these three species could not be well resolved in the present analysis. Both of the above imply that the squammae on the walking legs and the higher number of



0.05 substitutions/site

Fig. 4. Bio-neighbor-joining tree based on cytochrome oxidase I data. Numbers above branches indicate bootstrap values from maximum likelihood analysis (in bold), parsimony analysis (in italics), and bio-neighbor-joining analysis (normal text). Missing bootstrap value means that the particular branch was not resolved by a certain method.

branchial anterior spines on the carapace in *R*. *formosanus* are likely to be recently derived characters even though they are probably unique in the genus. Thus, Macpherson's and Machordom's (2001) key may be readily augmented to include *R*. *formosanus* as follows:

- 4a. Merocarpal articulation of third walking leg not reaching sinus between rostrum and supraocular spines R. confundens

ACKNOWLEDGEMENTS

Grateful acknowledgement is extended to A. Crosnier, N. Ngoc-Ho, and R. Cleva of the Muséum national d'Histoire naturelle, Paris (MNHN) for kindly allowing the first and second authors to examine the *Raymunida* collection at the MNHN and their warmest hospitalities during their visits;

and E. Macpherson of the Centro de Estudios Avanzados de Blanes, Girona, for kindly discussing with us on the status of the present new species and kindly reviewing this manuscript. We also thank J. Okuno of the Natural History Museum & Institute, Chiba, for sending us on loan two specimens of "R. elegantissima" illustrated in his book, M. Osawa of the National Science Museum, Tokyo, for discussing with us the Raymunida species of Japan, and C. P. Li and W. L. Chu of the Chinese University of Hong Kong for their assistance in DNA sequence analysis. The cruise "TAIWAN 2001" was supported by the National Museum of Marine Science & Technology (Keelung), National Science Council (Taiwan, R.O.C.), MNHN, and the IRD (Institut de Recherche pour le Développement, France). This work was partially supported by grants from the National Science Council, Taiwan, R.O.C. to TYC, and the Research Grants Council of the Hong Kong Special Administrative Region (Project No. CUHK4157/01M) to KHC.

LITERATURE CITED

- Chu, K. H., H. Y. Ho, C. P. Li, and T. Y. Chan. 2003. Molecular phylogenetics of the mitten crab species in *Eriocheir sensu lato* (Brachyura, Grapsidae).—Journal of Crustacean Biology 23: 738–746.
- Crandall, K., and J. E. Fitzpatrick, Jr. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny.—Systematic Biology 45: 1–26.

- de Man, J. G. 1902. Die von Herrn Professor Kukenthal im indischen Archipel gesammelten Dekapoden und Stomatopoden. Pp. 467–929, pls. 19–27 in Kükenthal, Ergebnisse einer zoologischen Forschungsreise in den Molukken und Borneo.—Abhandlungen herausgegeben von der Senckenbergischen Naturforschenden Gesellschaft 25.
- Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates.—Molecular Marine Biology and Biotechnology 3: 294–299.
- Gascuel, O. 1997. BIONJ: An improved version of the NJ algorithm based on a simple model of sequence data.— Molecular Biology and Evolution 14: 685–695.
- Kato, S., and J. Okuno. 2001. Shrimps and crabs of Hachijo Island. Tbs-Britannica Press, Tokyo. 157 pp.
- Lavery, S., T. Y. Chan, Y. K. Tam, and K. H. Chu. (In press.) Phylogenetics relationships and evolutionary history of the shrimp genus *Penaeus s. l.* derived from mitochondrial DNA.—Molecular Phylogenetics and Evolution.
- Leach, W. E. 1820. Galatéadées.—Dictionaire des Sciences Naturelles, Paris 18: 49–56.
- Macpherson, E., and A. Machordom. 2000. *Raymunida*, new genus (Decapoda : Anomura : Galatheidae) from the Indian and Pacific ocean.—Journal of Crustacean Biology 20, Special Number 2: 253–258.

—, and —, 2001. Phylogenetic relationships of species of *Raymunida* (Decapoda: Galatheidae) based on

morphology and mitochondrial cytochrome oxidase sequences, with the recognition of four new species.— Journal of Crustacean Biology 21: 696–714.

- Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution.—Bioinformatics 14: 817– 818.
- Rodriguez, F., J. L. Oliver, A. Marin, and J. R. Medina. 1990. The general stochastic model of nucleotide substitution.—Journal of Theoretical Biology 142: 485–501.
- Schubart, C. D., R. Diesel, and S. B. Hedges. 1998. Rapid evolution to terrestrial life in Jamaican crabs.—Nature 393: 363–365.
- Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers.—Annals of the Entomological Society of America 87: 652–701.
- Swofford, D. L. 2000. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Tong, J. G., T. Y. Chan, and K. H. Chu. 2000. A preliminary phylogenetic analysis of *Metapenaeopsis* (Decapoda: Penaeidae) based on mitochondrial DNA sequences of selected species from the Indo-West Pacific.—Journal of Crustacean Biology 20: 541–549.

RECEIVED: 23 May 2003. ACCEPTED: 27 August 2003.