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Molecular Identification of "Gum Gum": a Food Mole Crab *Hippa adactyla* from Papua New Guinea

Makoto Urata*, Hayato Tanaka and Susumu Ohtsuka

Takehara Marine Science Station, Setouchi Field Science Center, Graduate School of Biosphere Science, Hiroshima University, 5-8-1 Minato-machi, Takehara, Hiroshima, 725-0024, Japan.

Abstract A specimen of *Hippa adactyla* Fabricius, 1787, was collected from Blanche Bay, Papua New Guinea. Nuclear 18S and mitochondrial 16S ribosomal RNA gene sequences were examined for interspecies and intraspecies comparisons. The result suggests some genetic similarity and diversification of this species distributed widely in the Indo-Pacific. We also report a local culture of eating the animal under the name "Gum Gum". This is a pioneering report on the genetic perspective and fishing of a mole crab in southwest Pacific.

Key words: Anomura, fishery, *Hippa adactyla,* mole crab, 16S, 18S.

INTRODUCTION

Mole crabs are benthic crustaceans of the family Hippidae (Anomura, Hippoidea) widely distributed on intertidal and shallow subtidal sandy ocean beaches. They include the following three genera: *Emerita*, *Hippa*, and *Mastigochirus*. As reviewed by Boyko and McLaughlin (2010) and supported by recent molecular works by Roterman et al. (2013) and Bracken-Grissom et al. (2013), all morphological and molecular phylogenetic studies show that the Hippoidea is monophyletic and that the taxon is the basal taxon in the extant Anomura. Within the Hippoidea, the monophyletic status of Albuneidae, Blepharipodidae, and Hippidae is supported by their morphological (Boyko and Harvey, 2009) and molecular analyses (Bracken-Grissom et al., 2013). However, the monophyly of the above-mentioned three genera in the Hippidae is still unclear. A molecular phylogeny of the mitochondrial 16S ribosomal RNA and cytochrome *c* oxidase subunit 1 gene sequences from nine species of *Emerita* and one of *Hippa* placed *Hippa pacifica* in a clade of *Emerita* (Haye et al., 2002).

In South Africa, relatively large-scale fishing of the mole crabs *Emerita austroafricana* and *Hippa ovalis* is practiced (Kyle et al., 1997). *Emerita emeritus* has been caught for food at Phuket, Thailand (Janekorn, 1982, after Ingole et al., 1998). *Hippa adactyla*, with the local name "Ourudom", is considered a medicinal food on the Kavaratti atoll, Lakshadweep Islands, southern India (Ingole et al., 1998). Also in Japan, *H. adactyla* is commonly caught for local food in the Amami Oshima Islands under the local name "Gumyi" (N. Hisashi, personal communication) or "Kamenkua" (Fuji Television, 2006)

Accepted on September 9, 2015 * Current address:
Noto Marine Laboratory, Division of Marine Environmental Studies, Institute of Nature and Environmental Technology, Kanazawa University, Ogi, Noto-cho, Ishikawa 927-0553, Japan
E-mail: urata@staff.kanazawa-u.ac.jp

and at Nakijin Village, northern Okinawa Island, under the local name "Sunagame" (TV Asahi, 2010). Around Papua New Guinea, crustacean fishing in the South Pacific islands has been well reviewed by Dalzell et al. (1996); however, fishing of mole crabs was not mentioned in this review.

To obtain further information on phylogenetic status, genetic diversity, distribution, and food culture, we analyzed two DNA markers—nuclear 18S ribosomal RNA (18S) and mitochondrial 16S ribosomal RNA (mt16S) gene sequences—in a specimen of *H. adactyla* from Papua New Guinea. We performed interspecies and intraspecies comparisons with previously described sequences of Hippidae.

MATERIALS AND METHODS

A male specimen of *H. adactyla* was caught by a local fisherman at Karavia Bay, indenting the western part of Blanche Bay, East New Britain, Papua New Guinea (4°17′56″S, 152°9′48″E) on February 3, 2015. The specimen was fixed in 70% ethanol and held at room temperature. Genomic DNA was extracted by a standard proteinase K/sodium dodecyl sulphate (SDS)/phenol-chloroform procedure from approximately 1 mm^3 fixed muscle that was surgically picked from the left first pereopod. Polymerase chain reactions were performed with KOD FX Neo (Toyobo Co., Ltd., Osaka, Japan) according to the manufacturer's standard protocol. Two primer sets, eukaryotic primers for 18S (Moon-van der Staay et al., 2000) and 16SA/16SB (Xiong and Kocher, 1991), for the nuclear 18S ribosomal RNA gene (18S) and mitochondrial 16S ribosomal RNA gene (mt16S) sequences, respectively, were used. The three-step cycling conditions were as follows: initial denaturation for 2 min at 94°C; 30 cycles of 10 s at 98°C, 30 s at 52°C, and 90 s at 68°C; and a final extension for 5 min at 68°C. PCR products were purified with a High Pure PCR product purification kit (Roche Diagnostics GmbH, Mannheim, Germany). Fragments were sent to Macrogen Japan Corp. (Tokyo) for sequencing. Sequence data were aligned with ClustalW 2.1 (Larkin et al., 2007). Maximum likelihood approaches under a general time-reversible model were performed with MEGA v. 5.2 (Tamura et al., 2011). A comparative specimen of *H. pacifica* (*H. marmorata*) was caught at Iejima, Okinawa, Japan (26°42′53″N, 127°49′50″E) on April 26, 2015 and analyzed by the same procedure. Comparative 18S and/or mt16S sequences of *Hippa* and *Emerita* are shown in Table 1. Same superfamily two Albuneidae species, *Albunea gibbesii* (KF182440, KF182558; Bracken-Grissom et al., 2013), and *Paraleucolepidopa myops* (KF182441, KF182560; Bracken-Grissom et al., 2013) were also obtained from previous studies as outgroups.

RESULTS AND DISCUSSION

Morphology

The specimen (Fig. 1A) that has a carapace with three median lobes in the frontal margin, well-developed lateral frontal lobes, submarginal row of setose pits ($N = 49$), fine transverse grooves, dactylus of pereopods II and III with anterior margin cut into right angles, and antennal flagellum with four articles, was identified as *H. adactyla* Fabricius, 1787, based on Boyko and Harvey (1999). It was an adult male, 26.1 mm long and 22.5 mm wide in the carapace. The carapace and the abdomen were generally grayish dark brown with pale orange marks in living condition. The specimen was deposited in Hiroshima University Museum as voucher HUM-C-021. The comparative specimen (Fig. 1B) that has a carapace with two median lobes in the frontal margin, about equally projecting lateral frontal lobes, submarginal row of setose pits ($N = 38$), and antennal flagellum with two articles, was identified as *H.*

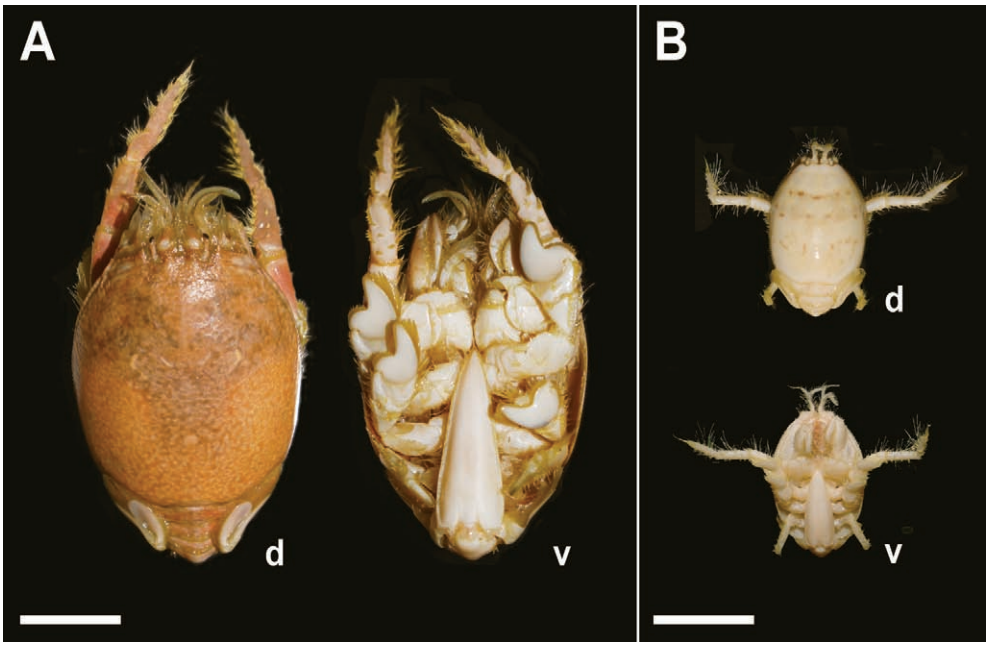

Figure 1. **(A)** An adult male specimen of *Hippa adactyla* collected in Papua New Guinea. **(B)** An adult female specimen of *H. pacifica* collected in Japan, showing dorsal (d) and ventral (v) sides. Ethanol fixed. Scale bar, 10 mm.

pacifica based on Boyko and Harvey (1999). It was an adult female, 12.0 mm long and 9.7 mm wide in the carapace. The specimen was deposited in Hiroshima University Museum as voucher HUM-C-022.

Fishing practices

The estuaries at the collection site consist of black volcanic sand, few coral reefs and mangroves. Native Tolai people caught mole crabs from the intertidal and shallow subtidal sand bottom by their foot and hand. They told me their variety of recipes for cooking the animal, for example just boiling and frying with oil or coconut milk. They said that spit roasting was the best and it could be eaten whole, shell and all. The people spoke not only English and Tok Pisin, official languages of Papua New Guinea, but also their own language, Kuanua. They called the mole crabs "Gum Gum" as a species of "Kuka", meaning crab in Kuanua and Tok Pisin. Some species of small true crabs were also caught in the same area, although these were used not for food but for fishing bait.

As mentioned in the Introduction, the eating culture and fishing of mole crabs are known in the India and Japan. Our result suggests a wide geographical distribution of them in the Indo-Pacific. To what extent and how the culture diffused will be future research questions.

Molecular analysis

PCR fragments, 1895 bp for 18S and 561 bp for mt16S, were well amplified from the specimen of *H. adactyla*, and the analyzed sequences, 1800 bp for 18S and 521 bp for mt16S, were deposited in GenBank under the accession IDs LC052326 and LC052327, respectively. Those of analyzed *H. pacifica* were LC055145 and LC055144.

These sequences did not completely match others in GenBank by BLAST search. However, the phylogenetic results from both 18S and mt16S, 1683bp and 291 bp showed close relationships with known sequences of *Hippa* species (Fig. 2). The topology of the 18S tree showed that the two genera *Emerita* and *Hippa* split into two clades with high bootstrap support. The mt16S tree also showed a clade of *Hippa* with relatively high bootstrap support, but a clade of *Emerita* was obscure. These results are not inconsistent with the morphological classification based on the monophyly of each genus, unlike the results of Haye et al. (2002). However, sufficient comparable sequences of *Hippa* species have not yet been deposited enough in GenBank (which now contains sequences of only two species), and no sequence of *Mastigochirus* has been analyzed yet. Future molecular studies of *Hippa* and *Mastigochirus* species may clarify the genetic monophyly of these genera.

The mt16S sequence of *H. adactyla* from Papua New Guinea was identical to that of *H. adactyla* KJ132557 (Tsang et al., 2014) collected at Nan'ao Township, Yilan County, Taiwan (24°26′24.64″ N, 121°48′24.40″E, by Taiwan National Museum of Natural Science) except for a 66-base deletion in the latter sequence; but differed by 52-base substitutions from *H. adactyla* KF051307 (Roterman et al., 2013) collected at Durban, South Africa (30°03′00″ S, 30°53′16″E), intertidal zone on a sandy beach, September 16, 2003 (C.N. Roterman, personal communication). This *H. adactyla* KF051307 was close to *H. pacifica*, as shown in Figure 2B. The 18S sequence of *H. adactyla* from Papua New Guinea also differed from that of *H. adactyla* collected at Durban (KF051278; Roterman et al., 2013) by three base

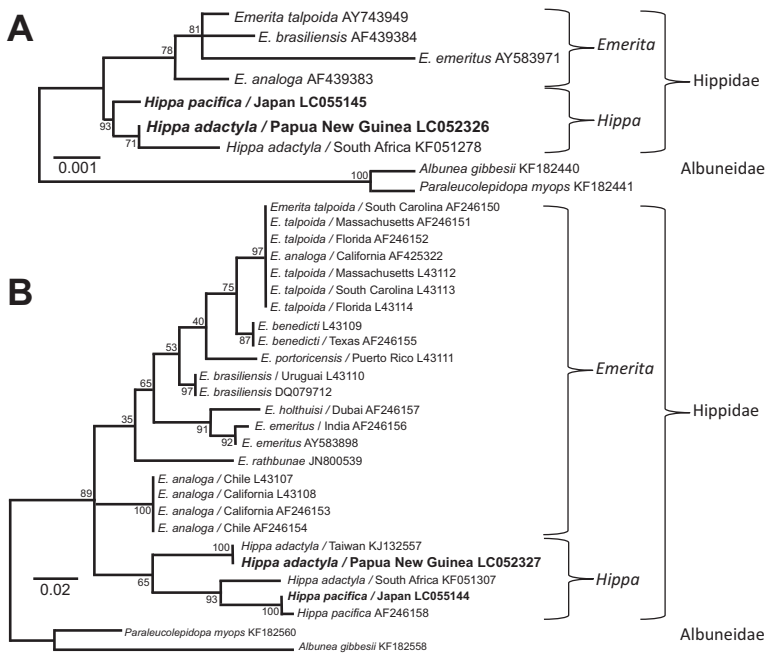

Figure 2. Molecule-based phylogenetic trees of *Hippa adactyla* and Hippidae species. **(A)** Based on partial 18S sequence (1683 bp). **(B)** Based on partial mt16S sequence (291 bp). Two Albuneidae species, *Albunea gibbesii* and *Paraleucolepidopa myops*, are included as outgroups. Bold lettering indicates new data of this article. The topology and branch length of cladograms were produced by maximum likelihood. Numbers above branches denote bootstrap support. Codes after species names are GenBank accession IDs.

substitutions. These facts suggest the following two points: (1) *H. adactyla* has some genetic diversification in 18S and mt16S; and (2) the population of *H. adactyla* in Taiwan, located in the northern hemisphere, has a close genetic relationship with the specimen collected in Papua New Guinea, approximately 4,600 km from Taiwan. The latter may be consistent with wide distribution as a single population. However, human-mediated introduction can also be considered as an explanation, given that our collection site is not far from Rabaul, an important port for foreign fishing vessels where Taiwanese ships frequently come into. Future studies of genetic polymorphism among other Indo-Pacific populations may clarify the human disturbance of this species.

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Table 1. 18S and mt16S sequences of *Hippa* and *Emerita* included in phylogenetic analyses.

	Locality	GenBank accession IDs		Publication
		18S	mt16S	
Hippa adactyla	South Africa	KF051278	KF051307	Roterman et al., 2013
	Taiwan	-	KJ132557	Tsang et al., 2014
	Papua New Guinea	LC052326	LC052327	This paper
H. pacifica	-	-	AF246158	Haye et al., 2002
	Japan	LC055145	LC055144	This paper
Emerita analoga	-	AF439383	-	Perez-Losada et al., 2002
	Chile	-	L43107	Tam et al., 1996
	California	-	L43108	Tam et al., 1996
	California	-	AF246153	Haye et al., 2002
	Chile	-	AF246154	Haye et al., 2002
	California	-	AF425322	Zaklan and Cunningham, unpublished
E. benedicti	-	-	L43109	Tam et al., 1996
	Texas	-	AF246155	Haye et al., 2002
E. brasiliensis	-	AF439384	-	Perez-Losada et al., 2002
	Uruguai	-	L43110	Tam et al., 1996
	-	-	DQ079712	Porter et al., 2005
E. emeritus	-	AY583971	AY583898	Ahyong and O'Meally, 2004
	India	-	AF246156	Haye et al., 2002
E. holthuisi	Dubai	-	AF246157	Haye et al., 2002
E. portoricensis	Puerto Rico	-	L43111	Tam et al., 1996
E. rathbunae	-	-	JN800539	Bybee et al., 2011
E. talpoida	-	AY743949	-	Babbitt and Patel, unpublished
	Massachusetts	-	L43112	Tam et al., 1996
	South Carolina	-	L43113	Tam et al., 1996
	Florida	-	L43114	Tam et al., 1996
	South Carolina	-	AF246150	Haye et al., 2002
	Massachusetts	-	AF246151	Haye et al., 2002
	Florida	-	AF246152	Haye et al., 2002

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パプアニューギニアで食用とされるミナミスナホリガニ *Hippa adactyla* の分子的同定

浦田　慎，田中隼人，大塚　攻

広島大学大学院生物圏科学研究科，
附属瀬戸内圏フィールド科学教育研究センター竹原ステーション，
〒725-0024 竹原市港町5-8-1

要　旨　パプアニューギニアのラバウル近郊で現地人により採取されたスナホリガニの一種ミナミスナホリガニ *Hippa adactyla* Fabricius, 1787について，核18S 及びミトコンドリア16S リボソーム RNA 遺伝子配列を解析し，近縁種間及び同種内の関係について分子的に比較した。その結果，インド—太平洋域に広く分布するとされる本種において，パプアニューギニア産個体はインド洋産個体と分子的に異なる一方，台湾産個体とは極めて近い関係にあることが示された。あわせて，本種を「グムグム（Gum Gum）」と呼んで食用とする現地の習慣を，他の地域の食習慣とともに紹介した。本稿は，南太平洋域のスナホリガニ類の分子的情報と，同域での食用採取について，初めて報告するものである。

キーワード：異尾下目，スナホリガニ科，食用，ミナミスナホリガニ，18S，ミトコンドリア16S