MOLECULAR PHYLOGENETIC POSITION OF THE NEW ZEALAND SENTINEL CRAB, *MACROPHTHALMUS* (*HEMIPLAX*) *HIRTIPES* (JACQUINOT, IN HOMBRON & JACQUINOT, 1846) (DECAPODA, BRACHYURA, MACROPHTHALMIDAE)

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ABSTRACT

The systematic position of the New Zealand sentinel crab *Macrophthalmus* (*Hemiplax*) *hirtipes* (Jacquinot, in Hombron & Jacquinot, 1846) has been questioned because the zoeal morphology is incongruent with that of other species assigned to the genus. A molecular phylogeny based on the mitochondrial large subunit (16S) rRNA gene indicates *M. hirtipes* is distinct from other macrophthalmid species, and found to be more closely related to the New Zealand varunids *Austrohelice crassa*, *Hemigrapsus crenulatus*, and *Hemigrapsus sexdentatus*. This indicates that the genus *Macrophthalmus* Desmarest, 1823 needs to be revised.

RÉSUMÉ

La position systématique du crabe sentinelle *Macrophthalmus* (*Hemiplax*) *hirtipes* (Jacquinot, in Hombron & Jacquinot, 1846) a été mise en doute car la morphologie de la zoé n'est pas satisfaisante par rapport aux autres espèces de ce genre. Une phylogénie moléculaire basée sur L'ARNr de la grande sous-unité (16S) mitochondriale indique que *M. hirtipes* est distinct des autres espèces macrophtalmides, et est plus proche des varunidés de Nouvelle Zélande *Austrohelice crassa*, *Hemigrapsus crenulatus* et *Hemigrapsus sexdentatus*. Ceci indique que le genre *Macrophthalmus* Desmarest, 1823 doit être révisé.

INTRODUCTION

Macrophthalmus (*Hemiplax*) *hirtipes* (Jacquinot, in Hombron & Jacquinot, 1846) is the only species of Macrophthalmidae in New Zealand, and is currently

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assigned to the monotypic subgenus *Hemiplax* Heller, 1865. The systematic position of *M. hirtipes* has been questioned because some morphological characters of the first zoea do not conform to those of the other species of macrophthalmids (Fielder & Greenwood, 1985, 1986). First stage zoeae of *Macrophthalmus* are known from 7 subgenera (12 species), including *M. hirtipes* by Wear (1968); *M. abbreviatus* Manning & Holthuis, 1981 by Aikawa (1929) and Terada (1979); *M. brevis* (Herbst, 1804) by Rajabai (1974); *M. crinitus* Rathbun, 1913 by Hashmi (1969); *M. depressus* Rüppell, 1830 by Hashmi (1969), Terada (1979), and Pasupathi & Kannupandi (1988a); *M. erato* De Man, 1888 by Pasupathi & Kannupandi (1988b); *M. japonicus* (De Haan, 1835) by Aikawa (1929) and Terada (1979); *M. latreillei* (Desmarest, 1817) by Hashmi (1969) and Selvakumar et al. (1988); *M. pacificus* Dana, 1851 by Hashmi (1969); *M. punctulatus* Miers, 1884 by Fielder & Greenwood (1986); *M. setosus* H. Milne Edwards, 1852 by Fielder & Greenwood (1986); and *M. sulcatus* H. Milne Edwards, 1852 by Hashmi (1969). The first zoea of *M. hirtipes* differs from those of other macrophthalmids in the following characters: presence of large lateral carapace spines, absence of dorso-lateral processes on abdominal somite 3, well developed antennal exopod with two medial setae, and long carapace and telson. These zoeal characters are more typical of Varunidae H. Milne Edwards, 1853. Fielder & Greenwood (1985, 1986) suggested that larvae of *M. hirtipes* show close similarity to those of *Heloecius cordiformis* (H. Milne Edwards, 1837) (family Heloeciidae). The first zoea of *H. cordiformis* possesses dorso-lateral processes on abdominal somites 2, 3 (minute), and 4, whereas in *M. hirtipes* only somite 2 has processes.

The similarity between *M. hirtipes* and species of Varunidae has also been suggested from adult external features. Barnes (1970) recognized a strong resemblance between *M. hirtipes* and some varunids, such as *Metaplax* and *Helice*. These included some commonly displayed characteristics, such as a quadrangular carapace, the presence of transverse and oblique granular rows on each branchial region of the carapace, and the width of the sixth abdominal somite markedly exceeding the width of the base of the telson, which characters do not occur in other *Macrophthalmus* species. Furthermore, previous molecular phylogenetic studies have shown that groups of varunids formed sister group relationships with species of *Macrophthalmus* (cf. Kitaura et al., 2002; Schubart et al., 2006). In order to test the strength of Barnes' (1970) suggested link with varunids, we have sequenced selected varunids from New Zealand and included them in our analysis. Thus, we can ask whether *M. hirtipes* is linked to other macrophthalmids or linked to varunids, that have had a parallel evolutionary history on the shores of the New Zealand land mass that separated from Australia around 60 My ago.

MATERIAL AND METHODS

The species used for phylogeny reconstruction are listed, along with collection data, museum catalogue number, and genetic accession numbers, in table I. They include selected species of *Macrophthalmus* (8 species) including *M. hirtipes*, 25 species of Varunidae from the subfamilies Cyclograpsinae H. Milne Edwards, 1853, Gaeticinae Davie & N. K. Ng, 2007, Thalassograpsinae Davie & N. K. Ng, 2007, and Varuninae H. Milne Edwards, 1853, and three species of Sesarmidae as outgroups. Most of the sequences were from previous studies by Kitaura et al. (1998, 2002, 2006). The new samples for this study were collected between 1996 and 2007, mostly from Japan and New Zealand.

Total genomic DNA was extracted from muscle tissue of walking legs using the QuickGene DNA tissue kit (FUJIFILM). Selective amplification of portions of the mitochondrial large ribosomal subunits (16s rRNA) were performed as reported by Kitaura et al. (2002, 2006), using a combination of the primers L1496i, L2510C, H2492i, H2716i, and H3062. The PCR products were purified using ExoSAP-IT (USB Corporation) and then sequenced with the ABI BigDye terminator mix in an ABI Prism 310 Genetic Analyzer. All final sequences were obtained from both strands for verification.

The sequences were initially aligned using CLUSTAL W (Thompson et al., 1994) with default gap penalties, subsequently being modified slightly by eye. Positions that could not be aligned accurately were excluded from the data sets. Phylogenetic relationships were analysed with the maximum likelihood (ML) routines using the software package PAUP* ver. 4.0b8 (Swofford, 1998), and Bayesian analysis (BAY) as implemented in MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001). Sequences were first analysed with the software MODELTEST (Posada & Crandall, 1998) in order to find the evolutionary model that best fits the data. The ML analysis was performed using a heuristic search algorithm. Search options included obtaining the starting tree by step-wise additions, using the random sequence addition, and tree bisection and reconnection branch swapping. To statistically assess the consistency of nodes, bootstrap analyses as a heuristic search were applied with 100 replicates. The BAY analysis was performed by running a Markov chain Montecarlo (MCMC) algorithm for 2 000 000 generations, sampling one tree every 500 generations. The first 10% of generations were discarded (burn-in) for the tree building analysis. The posterior probabilities of the phylogeny were determined by constructing a 50% majority-rule consensus of the remaining trees.

TABLE I

List of species used for DNA analysis, with collection data, museum catalogue number, and genetic database accession numbers

Species	Collection site, date	Catalogue no.	Accession no.
Family MACROPHTHALMIDAE Dana, 1851			
Subfamily MACROPHTHALMINAE Dana, 1851			
Macrophthalmus banzai Wada & Sakai, 1989	Japan, Wakayama Pref., 1995	OMNH Ar 7663	AB002132[1]
Macrophthalmus brevis (Herbst, 1804)	Japan, Iriomote Is., 1998	OMNH Ar 5017	AB058631[2]
Macrophthalmus definitus Adams & White, 1848	Japan, Iriomote Is., 1990	OMNH Ar 7664	AB101487[3]
Macrophthalmus hirtipes (Jacquinot, in Hombron & Jacquinot, 1846)	New Zealand, Christchurch, 2007	OMNH Ar 7665	AB440189
Macrophthalmus latreillei (Desmarest, 1817)	Australia, Darwin, 1995	OMNH Ar 5019	AB058632[2]
Macrophthalmus quadratus A. Milne-Edwards, 1873	Japan, Okinawa Is., 1998	OMNH Ar 5018	AB058633[2]
Macrophthalmus sulcatus H. Milne Edwards, 1852	Vietnam, Can Gio, 2000	OMNH Ar 7667	AB101485[3]
Macrophthalmus tomentosus Souleyet, 1841	Vietnam, Giao Thuy, 2000	OMNH Ar 7668	AB101491[3]
Family VARUNIDAE H. Milne Edwards, 1853			
Subfamily CYCLOGRAPSINAE H. Milne Edwards, 1853			
Austrohelice crassa (Dana, 1851)	New Zealand, Christchurch, 2007	OMNH Ar 7669	AB440179
Chasmagnathus convexus (De Haan, 1835)	Japan, Hyogo Pref., 2007	OMNH Ar 7670	AB440180
Cyclograpsus intermedius Ortmann, 1894	Japan, Hyogo Pref., 2000	OMNH Ar 5009	AB058627[2]
Cyclograpsus lavauxi H. Milne Edwards, 1853	New Zealand, Christchurch, 2007	OMNH Ar 7671	AB440191
Helicana japonica (K. Sakai & Yatsuzuka, 1980)	Japan, Mie Pref., 2003	OMNH Ar 7672	AB440185
Helicana doerjesi K. Sakai, Türkay & Yang, 2006	Taiwan, Tanshui, 1996	OMNH Ar 7673	AB440184
Helice formosensis Rathbun, 1931	Japan, Okinawa Is., 1998	OMNH Ar 7674	AB440186
Helice tridens (De Haan, 1835)	Japan, Hyogo Pref., 1998	OMNH Ar 5010	AB058629[2]
Helice latimera Parisi, 1918	Vietnam, Tinh Gia, 2003	OMNH Ar 7675	AB440190
Helograpsus haswellianus (Whitelegge, 1899)	Australia, Tasmania, 2000	OMNH Ar 7676	AB440183
Metaplax distincta H. Milne Edwards, 1852	Thailand, Ranong, 1999	OMNH Ar 5011	AB058622[2]

TABLE I
(Continued)

Species	Collection site, date	Catalogue no.	Accession no.
Metaplax elegans De Man, 1888	Vietnam, Haiphong, 1995	OMNH Ar 5012	AB058623[2]
Metaplax takahashii Sakai, 1939	Vietnam, Haiphong, 1995	OMNH Ar 5014	AB058625[2]
Paragrapsus laevis (Dana, 1851)	Australia, Fisherman Is., 2000	OMNH Ar 7677	AB440182
Subfamily VARUNINAE H. Milne Edwards, 1853			
Eriocheir sinensis H. Milne Edwards, 1853			AY274302[4]
Hemigrapsus crenulatus (H. Milne Edwards, 1837)	New Zealand, Christchurch, 2007	OMNH Ar 7678	AB440187
Hemigrapsus penicillatus (De Haan, 1835)	Japan, Nagasaki Pref., 1996	OMNH Ar 5006	AB058628[2]
Hemigrapsus sanguineus (De Haan, 1835)	Japan, Wakayama Pref., 1998	OMNH Ar 5005	AB058630[2]
Hemigrapsus sexdentatus (H. Milne Edwards, 1837)	New Zealand, Christchurch, 2007	OMNH Ar 7679	AB440188
Parapyxidognathus deianira (De Man, 1888)	Japan, Okinawa Is., 1998	OMNH Ar 5003	AB058619[2]
Ptychognathus ishii Sakai, 1939	Japan, Okinawa Is., 1998	OMNH Ar 5002	AB058621[2]
Varuna litterata (Fabricius, 1798)	Japan, Okinawa Is., 1998	OMNH Ar 5007	AB058620[2]
Subfamily GAETICINAE Davie & N. K. Ng, 2007			
Gaetice depressus (De Haan, 1833)	Japan, Wakayama Pref., 1998	OMNH Ar 5001	AB058626[2]
Gaetice ungulatus Sakai, 1939	Japan, Iriomote Is., 1999	OMNH Ar 7680	AB440181
Subfamily THALASSOGRAPSINAE Davie & N. K. Ng, 2007			
Thalassograpsus harpax (Hilgendorf, 1892)	Japan, Iriomote Is., 1999	OMNH Ar 7681	AB440192
OUTGROUPS			
Family SESARMIDAE Dana, 1851			
Chiromantes haematocheir (De Haan, 1835)	Japan, Wakayama Pref., 1998	OMNH Ar 5008	AB057809[2]
Perisesarma bidens (De Haan, 1835)	Japan, Okinawa Is., 1998	OMNH Ar 5015	AB057810[2]
Sesarmops intermedius (De Haan, 1835)	Japan, Wakayama Pref., 1998	OMNH Ar 5016	AB057811[2]

[1] Kitaura et al. (1998); [2] Kitaura et al. (2002); [3] Kitaura et al. (2006); [4] Sun et al. (2005).

RESULTS

The total alignment of the sequenced mitochondrial 16S ribosomal DNA region consisted of 1257 nucleotide sites. Of the aligned sequences, the regions that could not be aligned unambiguously were discarded from the analysis resulting in a total of 962 bp (including gaps). The selected model of DNA substitution by hLRT was the TVM + I + G model. Parameter values under the selected substitution model are as follows: base frequencies, freqA = 0.4142, freqC = 0.0555, freqG = 0.1076, freqT = 0.4227; nucleotide substitution rates A↔C 0.3997, A↔G 9.4887, A↔T 0.6938, C↔G 0.5993, C↔T 9.4887, G↔T 1.0000; proportion of invariant sites (I) = 0.36; variable site followed a gamma distribution with shape parameter = 0.52.

Both ML and BAY analyses produced the same topology (fig. 1). *Macrophthalmus hirtipes* clustered together with species of Varunidae, rather than with those of Macrophthalmidae. Within the Varunidae clade, two major evolutionary groups were found, one clusters the species endemic to New Zealand or Australia, and the other clade joined the species found throughout East Asia. *Macrophthalmus hirtipes* is found in the New Zealand or Australia clade, and strongly supported as a sister taxon of *Austrohelice crassa* (Dana, 1851) (98/1.0 in ML/BAY). These two sister species form a subgroup with *Hemigrapsus sexdentatus* (H. Milne Edwards, 1837) and *H. crenulatus* (H. Milne Edwards, 1837) with high confidence value, all four species endemic to New Zealand.

The present study includes many varunid species belonging to the subfamilies Cyclograpsinae, Gaeticinae, Thalassograpsinae, and Varuninae. The resultant trees do not support a monophyly of the subfamilies, Cyclograpsinae and Varuninae (see fig. 1). New Zealand species of *Hemigrapsus* (*H. sexdentatus*, *H. crenulatus*) and *Cyclograpsus* (*C. lavauxi* H. Milne Edwards, 1853) form a lineage distinct from those of the East Asian species (*H. sanguineus* (De Haan, 1835), *H. penicillatus* (De Haan, 1835), and *C. intermedius* Ortmann, 1894).

DISCUSSION

The resultant trees (fig. 1) indicate that the New Zealand sentinel crab, *Macrophthalmus hirtipes*, is distinct from other macrophthalmids and is more closely related to the Varunidae. This result agrees well with the larval evidence from the literature. At present, there are little known larval data for *Macrophthalmus* species, but several characters that are common within the species so far described allow *M. hirtipes* to be separated from other macrophthalmids, as suggested by Fielder & Greenwood (1985, 1986) and Pasupathi & Kannupandi (1988a, b). Table II summarizes the first zoea characters of the species of *Macrophthalmus* so far described.

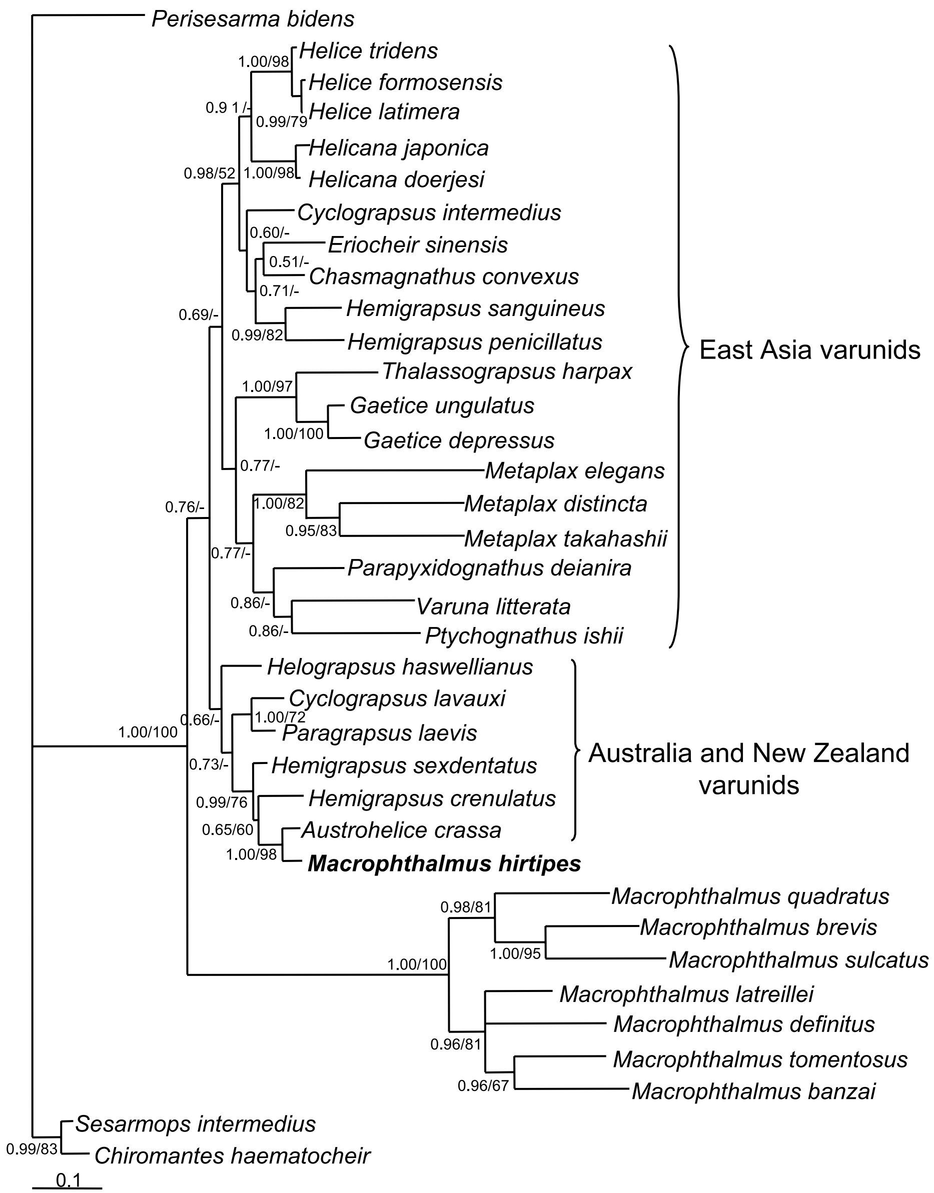

Fig. 1. Phylogenetic consensus tree of some species of the Varunidae and Macrophthalmidae constructed with Bayesian inference. Confidence values reflecting posterior probabilities for each internal branch are shown around branches, and bootstrap values from maximum likelihood analysis (100 replicates) are also shown after the posterior probabilities. Confidence values higher than 50% are shown in the tree.

TABLE II

Selected first zoeal characters of the species of *Macrophthalmus* so far described

	Carapace					Antenna	Maxilliped1	Abdomen	Telson
	Lateral spine (mm)	Dorsal spine (mm)	Rostral spine (mm)	Carapace length (mm)	Total length (mm)	Exopod medial setae	Basal setation	Lateral knobs	
M. hirtipes (cf. Wear, 1968)	+**(0.25)**	+**(0.46)**	+**(0.29)**	**0.45**	**1.25**	**2**	**3,3,3,3 (12)**	**2**	**fork > body**
M. abbreviatus (cf. Aikawa, 1929, 1937; Terada, 1979)	–	+(0.12/?)	+(0.16/?)	0.38/0.33	0.9/?	0	2,2,2,2 (8)/2,2,3,2 (9)	2-3	fork < body*
M. sulcatus (cf. Hashmi, 1969)	–	+(0.183)	+(0.088)	0.34	1.102	0	(8)	2-3	fork < body
M. brevis (cf. Rajabai, 1974)	–	+(0.105)	+(0.157)	?	0.78	0	(6)	2-3	fork < body*
M. japonicus (cf. Aikawa, 1929, 1937; Terada, 1979)	–	+(?)	+(?)	?/0.33	?	0	2,2,2,2 (8)/2,2,3,2 (9)	2-3	fork < body*
M. depressus (cf. Hashmi, 1969; Rajabai, 1974; Pasupathi & Kannupandi, 1988b)	–	+(0.161/ 0.13/0.2)	+(0.132/ 0.127/0.18)	?/?/0.38	0.845/0.94/?	0	(8)/1,1,1,1 (4)/(10)	2-3	fork < body
M. crinitus (cf. Hashmi, 1969)	+(0.051)	+(0.124)	+(0.139)	0.29	0.771	1	(8)	2-3	fork < body
M. pacificus (cf. Hashmi, 1969)	–	+(0.22)	+(0.147)	0.38	1.102	1	(7)	2-3	fork < body

TABLE II
(Continued)

	Carapace					Antenna	Maxilliped1	Abdomen	Telson
	Lateral spine (mm)	Dorsal spine (mm)	Rostral spine (mm)	Carapace length (mm)	Total length (mm)	Exopod medial setae	Basal setation	Lateral knobs	
M. setosus (cf. Fielder & Greenwood, 1986)	–	+(0.23)	+(0.22)	0.32	?	0	2,2,3,2 (9)	2-3	fork = body
M. punctulatus (cf. Fielder & Greenwood, 1986)	–	–	+(0.05)	0.31	?	1	2,2,2,3 (9)	2-3	fork < body
M. erato (cf. Pasupathi & Kannupandi, 1988a)	+(0.11)	+(0.29)	+(0.24)	0.35	?	1	2,2,2,2 (8)	2-3	fork > body*
M. latreillei (cf. Hashmi, 1969)	–	+(0.161)	+(0.117)	0.3	0.955	0	(9)	2-3	fork = body

* Data from figures; +, presence; –, absence; ?, no description.

The first zoea of the species other than *M. hirtipes* are small (0.29-0.38 mm in carapace length), and have dorso-lateral processes on abdominal somites 2 + 3, none or one medial seta on the antennal exopod, long furcal rami, and no lateral carapace spines. The first stage zoea of *M. hirtipes* is larger (carapace length: 0.45 mm) than other known *Macrophthalmus* zoeae and has long telson furcal rami, large lateral and dorsal carapace spines, and a well-developed exopod of the antenna with two medial setae, and it also lacks dorso-lateral processes on abdominal somite 3. These zoeal characteristics of *M. hirtipes* conform closely to those of the Varunidae as summarized by Cuesta et al. (2000).

Behavioural aspects also support the close relationship between *M. hirtipes* and varunid species. Allocleaning behaviour, in which a crab is foraging on the carapace or walking legs of other conspecific individuals, occurs widely throughout *Macrophthalmus* species (Kitaura et al., 2004), whereas in genera other than *Macrophthalmus*, the reports of allocleaning are limited to some species of *Uca* (cf. Crane, 1958, 1975; Hagen, 1962; Yamaguchi, 1971; Murai, 1992) and of Sesarmidae (cf. Seiple & Salmon, 1982; Abele et al., 1986). Furthermore, a waving display is commonly observed in species of *Macrophthalmus*, but in the Varunidae, occurrence of a waving display is limited to some species of *Metaplax* (cf. Pretzmann, 1971; Beinlich & Polivka, 1989; Kitaura et al., 2002), *Hemigrapsus* (cf. Lindberg, 1980), and *Helicana* (cf. Nara et al., 2006). *Macrophthalmus hirtipes* has never been observed to perform allocleaning or waving display behaviours, and therefore these characteristics are in accordance with varunids, and not with macrophthalmids.

Separately we have proposed that the sub-genus ***Hemiplax*** Heller, 1865, containing a single species originally known as *Cleistostoma hirtipes* Hombron & Jacquinot, 1846, should be removed from *Macrophthalmus* Desmarest, 1823 and elevated to full generic status (McLay et al., 2010).

The present study includes 25 varunid species belonging to the subfamilies Cyclograpsinae, Gaeticinae, Thalassograpsinae, and Varuninae. The resultant trees do not support a monophyly of the subfamilies, Cyclograpsinae and Varuninae, and shows that the Australia and New Zealand varunids appear in the same lineage, being a sister group to those of East Asia (see fig. 1). This finding suggests the geological divergence of the varunids. However, there are still many varunid species for which no sequences have yet been obtained. Future studies including more varunids should allow us to test the robustness of the present phylogeny.

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