
Phylogeny and biogeography of the freshwater crab genus *Johora* (Crustacea: Brachyura: Potamidae) from the Malay Peninsula, and the origins of its insular fauna

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Accepted: 25 January 2007
doi:10.1111/j.1463-6409.2007.00276.x

Yeo, D. C. J., Shih, H.-T., Meier, R. & Ng, P. K. L. (2007). Phylogeny and biogeography of the freshwater crab genus *Johora* (Crustacea: Brachyura: Potamidae) from the Malay Peninsula, and the origins of its insular fauna. — *Zoologica Scripta*, 36, 255–269.

The phylogeny and biogeography of the Malayan freshwater crab genus *Johora* was studied using two mitochondrial genes, 16S rRNA (560 bp) and cytochrome oxidase subunit I (COI) (616 bp), and one nuclear gene, histone 3 (H3) (328 bp). *Johora* is shown to be monophyletic and composed of three clades that correspond with the topography of the Malay Peninsula. The three clades were estimated to be of similar age (c. 11 million years ago (mya)). The Malayan island of Pulau Tioman (with five species) was determined to have been colonised independently by two separate clades (at c. 11 and 5 mya, respectively), one of which evolved semiterrestrial habits, possibly in response to competition by the second. A partitioned Bremer support (PBS) analysis reveals that most of the support for the phylogenetic tree comes from the COI gene fragment and that the nuclear protein-encoding genes H3 is useful for reconstructing the relationships of *Johora*.

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Introduction

The freshwater crab genus *Johora* Bott, 1966; is one of only three potamid genera (the others being *Stoliczia* Bott, 1966; and *Terrapotamon* Ng, 1986) found in the Malay Peninsula. *Johora* occurs from Narathiwat Province, southern Thailand, in the north, through the length of Peninsular Malaysia to Singapore in the south (Fig. 1). Its range also includes two relatively large Malaysian islands off the east coast, Pulau Tioman and Pulau Redang (Ng 1988, 2004). Fifteen species are currently recognised, namely, *J. aipooae* (Ng 1986) [Pahang, Malaysia], *J. counsilmani* (Ng 1985) [Pulau Tioman: Pahang, Malaysia], *J. gapensis* (Bott 1966) [Selangor and Pahang, Malaysia], *J. grillator* Ng, 1988 [Pulau Tioman: Pahang, Malaysia], *J. gua* Yeo, 2001 [Pulau Tioman: Pahang, Malaysia], *J. boiseni* Ng & Takeda, 1992 [Kelantan, Malaysia], *J. intermedia* Ng, 1986 [Selangor, Pahang, Negri Sembilan and Johor, Malaysia], *J. johorensis* (Roux 1936) [Johor, Malaysia], *J. murphyi* Ng, 1986 [Johor, Malaysia], *J. punicea* (Ng 1985) [Pulau Tioman: Pahang, Malaysia], *J. singaporensis* Ng, 1986 [Singapore], *J. tabanensis* (Bott 1966) [Pahang, Selangor, Terengganu, Malaysia], *J. thiana* Leelawathanagoon, Lhekni

& Ng, 2005 [Narathiwat, Thailand], *J. thoi* Ng, 1990 [Pulau Redang: Terengganu, Malaysia] and *J. tiomanensis* (Ng & Tan 1984) [Pulau Tioman: Pahang, Malaysia].

Johora species are primarily aquatic and found in clean, clear, fast-flowing fresh waters typically associated with hill streams. This habitat preference limits their ability to disperse and has generally restricted the group to highlands, resulting in extensive speciation, with isolated highlands and drainages often having their own distinct species (Ng 1987, 1988; Ng & Yeo 2007; present study).

While much has been published about their taxonomy, a complete phylogenetic study of *Johora* has not been attempted thus far, although relationships between selected species or groups of species and origins of certain species based on morphological studies have been proposed (Ng 1987, 1988, 1990; Yeo *et al.* 1999; Yeo 2001; Leelawathanagoon *et al.* 2005). The present paper describes the molecular appraisal of the systematics and biogeography of *Johora*, including the first comprehensive phylogeny of the genus based on 16S rRNA, cytochrome oxidase subunit I (COI) and histone 3 (H3) genes. The molecular study of these freshwater crabs provides an

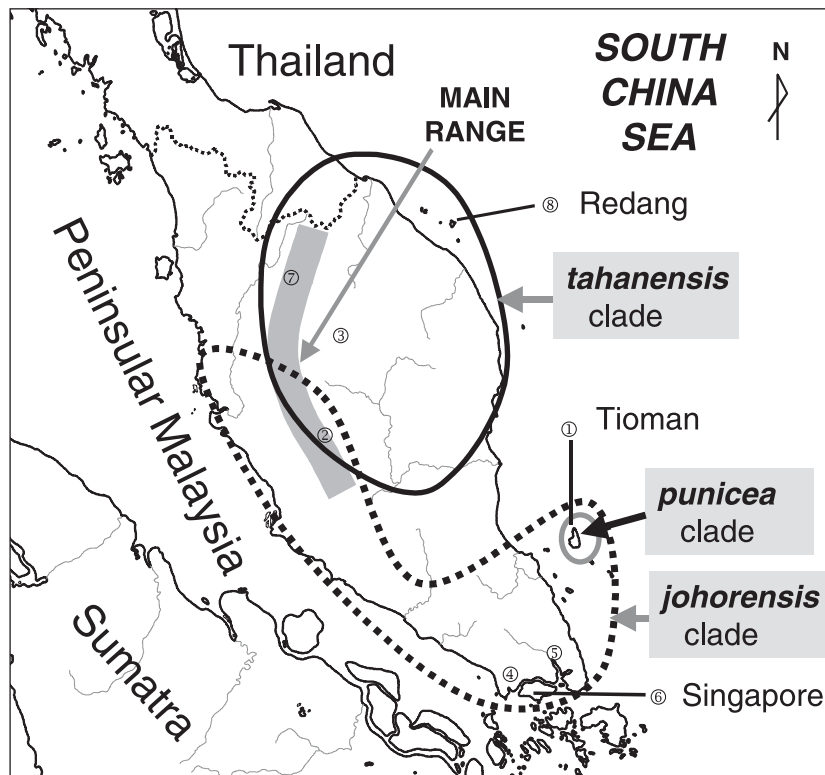


Fig. 1 Collection sites for the species of *Jobora* from Peninsular Malaysia and Singapore. The different lines indicate the possible biogeographic boundaries for each main clade based on the molecular results in our study. Locality names and the species collected: 1. Pulau Tioman, Pahang (*J. counsilmani*, *J. grillator*, *J. gua*, *J. punicea*, *J. tiomanensis*); 2. Fraser's Hill, Selangor (*J. gapensis*, *J. intermedia*); 3. Sungai Galas, Kelantan (*J. boiseni*); 4. Gunung Pulai, Johor (*J. johorensis*); 5. Kota Tinggi, Johor (*J. murphyi*); 6. Singapore (*J. singaporensis*); 7. Sungai Halong, Perak (*J. tahanensis*); 8. Pulau Redang, Terengganu (*J. thoi*).

indication of the validity of the species and populations recognised, but more significantly, it provides important insights into their systematics and regional biogeography (see also Daniels *et al.* 2002a, 2002b, 2003; Daniels 2003; Shih *et al.* 2004, 2005, 2006, 2007a, 2007b). The results of our study show that the taxonomy of the genus is largely congruent with previous morphological studies, although there are some interesting surprises. In particular, the origin of the *Jobora* species endemic to Pulau Tioman is discussed in light of the present molecular data together with the available morphological and ecological information and the island's geological history.

Materials and methods

In the present analysis, 13 out of the 15 known species of *Jobora* were included. We were unable to obtain any gene sequences from *J. aipooae* and *J. thiana* (tissues were unusable probably due to original preservation in formaldehyde); or COI and H3 sequences from *J. boiseni*. Seven genera of the family Potamidae from the eastern part of Asia continent were selected as outgroups, namely, *Stoliczia chaseni* (Roux 1936) [Malaysia], *Terrapotamon abbotti* (Rathbun 1898) [Thailand], *Potamiscus aff. yunnanense* (Kemp 1923) [China], *Candidiopotamon ratbbumae* (de Man 1914) [Taiwan], *Ryukyum yaeyamense* (Minei 1973) [Ryukyus], *Geothelphusa dehaani* (White 1847) [Japan]

and *Himalayapotamon atkinsonianum* (Wood-Mason 1871) [India]. Specimens are from the Zoological Reference Collection (ZRC) of the Raffles Museum of Biodiversity Research, National University of Singapore, and the Zoological Collections of the Department of Life Science, National Chung Hsing University (NCHUZOO; see Table 1). The following abbreviations are used: G1 for male first pleopod; cw for carapace width. Morphological terminology used essentially followed Ng (1988). All measurements are in millimetres. Malay words used in the text are 'Pulau' (island), 'Sungai' (river or stream) and 'Gunung' (mountain).

Genomic DNA was isolated from the muscle tissue of legs by using the Sigma mammalian genomic DNA miniprep kit. A region of approximately 550 basepairs (bp) of the 5'-end of the 16S rRNA gene was selected for amplification with polymerase chain reaction (PCR) using the primers 1471 (5'-CCTGTTTANCAAAAACAT-3') and 1472 (5'-AGAT-AGAAACCAACCTGG-3'; Crandall & Fitzpatrick 1996). A portion of the mitochondrial COI gene was amplified with PCR using the primers LCO1490 (5'-GGTCAACAAATCAT-AAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAG-GGTGACCAAAAAATCA-3'; Folmer *et al.* 1994). An internal primer designed by Roman & Palumbi (2004) for *Carcinus maenas* (5'-GCTTGAGCTGGCATAGTAGG-3') was also used. The PCR conditions for the above primers were 50 s

Table 1 Thirteen species of the genus *Jobora* and outgroups with the 16S rRNA, COI and histone 3 (H3) haplotypes from eight different localities in Peninsular Malaysia and Singapore. Numbers within brackets correspond to localities in Fig. 1.

Species	Museum catalogue no.	Localities	DDBJ Accession no. of 16S	DDBJ Accession no. of COI	DDBJ Accession no. of H3
<i>J. counsilmani</i>	ZRC 1989.3040–3048	Pulau Tioman, Pahang (1)	AB290614	AB290633	AB290652
<i>J. gapensis</i>	ZRC 1995.349	Fraser's Hill (the Gap), Selangor (2)	AB290615	AB290634	AB290653
<i>J. grallator</i>	ZRC 1996.1730	Pulau Tioman, Pahang (1)	AB290616	AB290635	AB290654
<i>J. gua</i>	ZRC 2000.2237	Pulau Tioman, Pahang (1)	AB290617	AB290636	AB290655
<i>J. hoiseni</i>	ZRC1984.6674–6755, 7683–7687	Sungai Galas, Kelantan (3)	AB290618	—	—
<i>J. intermedia</i>	ZRC 2002.0242	Fraser's Hill, Selangor (2)	AB290619	AB290637	AB290656
<i>J. johorensis</i>	ZRC 1990.575–576	Gunung Pulai, Johor (4)	AB290620	AB290638	AB290657
<i>J. murphyi</i>	ZRC 2001.2267	Kota Tinggi, Johor (5)	AB290621	AB290639	AB290658
<i>J. punicea</i>	ZRC 2002.0544	Pulau Tioman, Pahang (1)	AB290622	AB290640	AB290659
<i>J. singaporensis</i>	ZRC 2001.1130	Singapore (6)	AB290623	AB290641	AB290660
<i>J. tahanensis</i>	ZRC 1995.268	Sungai Halong, Perak (7)	AB290624	AB290642	AB290661
<i>J. thoi</i>	ZRC 2001.1167	Pulau Redang, Terengganu (8)	AB290625	AB290643	AB290662
<i>J. tiomanensis</i>	ZRC 1999.0941	Pulau Tioman, Pahang (1)	AB290626	AB290644	AB290663
Outgroups					
<i>Stoliczia chaseni</i>	ZRC 2000.1630	Gunung Brinchang, Cameron Highlands, Pahang	AB290627	AB290645	AB290664
<i>Terrapotamon abbotti</i>	ZRC 2001.0773	Southern Thailand: purchased from aquarium in Singapore	AB290628	AB290646	AB290665
<i>Potamiscus aff. yunnanense</i>	ZRC 2006.0226	purchased from Kunming market, purportedly from Jinshajiang, Yunnan, China	AB290629	AB290647	AB290666
<i>Geothelphusa dehaani</i>	NCHUZ00L 13073	Chiba, Japan	AB290630	AB290648	AB290667
<i>Candidiopotamon rathbunae</i>	NCHUZ00L 13074	Chunrih, Pingtung, Taiwan	AB208609	AB290649	AB290668
<i>Rhyukyum yaeyamense</i>	NCHUZ00L 13075	Ishigaki, Ryukyus	AB290631	AB290650	AB290669
<i>Himalayapotamon atkinsonianum</i>	ZRC 2006.0143	Mahananda River, India	AB290632	AB290651	AB290670

94 °C/70 s 45 °C/60 s 72 °C (denaturation/annealing/extension), followed by 72 °C extension for 10 min. The primers for H3 were H3AF (5'-ATGGCTCGTACCAAGCAGACVGC-3') and H3AR (5'-ATATCCTTRGGCATRATRGTCGAC-3'; Colgan *et al.* 1998) with the annealing temperature being 55 °C in PCR condition. Sequences were obtained by automated sequencing (ABI PRISM 377 Sequencer and MEGABACE DNA ANALYSIS SYSTEM 500).

The sequences were aligned with the aid of CLUSTAL W (version 1.4, Thompson *et al.* 1994) and BIOEDIT (version 5.09, T.A. Hall 2001), after verification with the complementary strand. Sequences of the different haplotypes have been deposited in the DDBJ databases (accession nos. AB208609, 290614–290670; Table 1). The matrices for the three genes were concatenated using the program SEQUENCEMATRIX that is part of the TAXONDNA software package (Meier *et al.* 2006).

The parsimony (MP) analysis was carried out using PAUP* (version 4.0b10, Swofford 2003) using heuristic search algorithms (TBR; addseq = random; nreps = 100). Branch support was established via bootstrap analysis (2000 replications). The data set was once analysed with indels coded as fifth character state and once with indels coded as missing values. For the latter data set, we also carried out a partitioned Bremer support (PBS) analysis using TREEROT (version 2, Sorenson 1999) for generating a command file that was

executed in PAUP* using the same search parameters as specified above. Minimum evolution (ME) trees were similarly constructed using PAUP* with 2000 bootstrap reiterations.

For the ME and Bayesian (BI) analysis, the best-fitting model for sequence evolution was determined for the individual genes and the combined 16S rRNA, COI and H3 dataset using MRMODELTEST (version 2.2, Nylander 2005). The model was selected by hierarchical likelihood-ratio testing (hLRT). The BI analyses were performed using MRBAYES 3.0 (Ronquist & Huelsenbeck 2003) on the concatenated data set consisting of all three genes. We used a partitioned likelihood approach (one partition for each gene), in which parameters were estimated separately for each data partition. Three different runs were executed, each for 10 million generations sampling every 10th tree. The likelihood scores revealed that the first 25% of the trees had to be discarded as burning. The posterior probabilities of each node were computed from the remaining 75% of all sampled trees.

We used DAMBE (version 4.5.32, Xia & Xie 2001) for generating saturation plots for the three genes, and for the different codon positions of the protein-encoding genes. Pairwise distances were plotted against the genetic distances as measured by K80. The relative rates of divergence in each gene were obtained by plotting the uncorrected *p*-distances of different genes against each other.

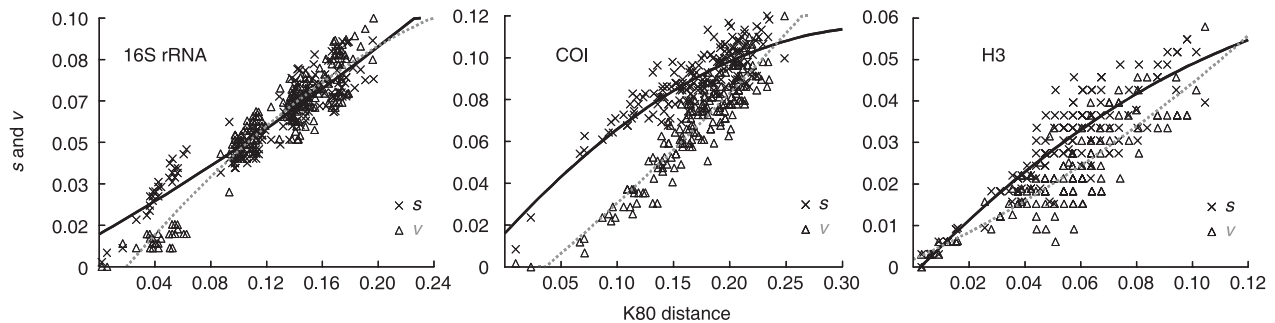


Fig. 2 Saturation plots of the number of transitions (s) and transversions (v) vs. divergence as measured by K80 for each gene (16S rRNA, COI and H3).

Because there are no known *Jobora* fossils (Ng 1988), divergence estimates were based on established divergence rates. Among the known calibrations for decapod crustaceans, the substitution rates of 0.88% and 2.33%, for 16S rRNA and COI, respectively, per million years have been established for terrestrial *Sesarma* (see Schubart *et al.* 1998). These are the only available rates for terrestrial or limnic crabs. The calibration rate of 16S rRNA for *Candidiopotamon* had previously been satisfactorily explained by the effect of orogeny of Taiwan (Shih *et al.* 2006) and has been frequently used for other decapods (see Schubart *et al.* 2000). In our study, the rate was applied to the topology of the ME tree for the combined dataset. The divergence times for the genus *Jobora* were estimated using the 0.88% per million years rate for 16S rRNA (uncorrected p -distance, see Nei & Kumar 2000) in order to avoid differential weighting between datasets. Afterwards, the relative age for each node was calculated according to the pairwise divergence of the combined dataset with the model selected. This approach was used, to obtain a linearised ME tree.

The likelihood-ratio test was calculated by PAUP* in order to test whether the clock was rejected by the combined dataset. A maximum log-likelihood value under an unconstrained best model (selected by MRMODELTEST) was calculated, then a log-likelihood value was used for the constraint (clock enforced) tree. Doubling the difference between the two values is assumed to be chi-square distributed (with the degrees of freedom = number of taxa - 2). If the probability of chi-square is significant (e.g., $P < 0.05$), then the hypothesis of constant rate across all branches of the tree is rejected.

Results

For the genus *Jobora*, a 546 bp segment (excluding the primer regions) of the 16S mtDNA gene was amplified and aligned; 118 positions were variable and 84 parsimony informative. Among the total number of sequences, 13 different haplotypes were distinguished (Table 1). The studied segment of 16S sequences was AT rich (71.9%) (T: 36.1%, A: 35.8%,

G: 17.7%, C: 10.3%). For the COI gene, a 616 bp segment was compared, resulting in 12 different haplotypes. The studied segment of COI sequences was also AT rich (65.1%) (T: 36.8%, A: 28.3%, G: 15.3%, C: 19.7%). In this gene, 186 positions were variable and 135 parsimony informative. A 328 bp segment of the H3 gene was compared, and 12 different haplotypes was obtained. The segment of H3 sequences was GC rich (60.8%) (T: 17.1%, A: 22.1%, G: 26.1%, C: 34.7%), with 33 positions were variable and 18 parsimony informative. The pairwise nucleotide divergences for 16S rRNA, COI and H3 with each model selected, and the number of basepair differences are shown in Table 2 (indels are considered substitution). For the 16S rRNA, the basepair difference within the genus ranges from 1 to 64 (mean is 45.9) and divergence ranges from 0.18% to 18.59% (mean is 11.56%). The basepair differences (and divergences) within the *joborensis*, *tabanensis* and *punicea* clades are 24.4 (5.59%), 23.3 (5.20%) and 14.0 (3.1%), respectively. The average basepair difference and divergence between the three main clades of *Jobora* are 58.3 (from 56.2 to 60.0) and 15.16% (from 14.88% to 15.64%), respectively (Table 2).

Saturation plots suggest that the three genes were overall not saturated for either transitions or transversions (Fig. 2). However, COI is starting to saturate for third positions in distantly related taxa. The third positions of H3 are not saturated, but the first positions are almost and the second positions completely invariable (data not shown). COI was faster to diverge than 16S rRNA, and H3 was much slower to diverge than both COI and 16S rRNA (Fig. 3). The average divergences of the genus *Jobora* for 16S rRNA, COI and H3 are 11.56, 34.63 and 4.64, respectively (Table 2). The relative divergence rates among 16S rRNA, COI and H3 is 2.5 : 7.5 : 1.

The phylogenetic tree constructed from the MP analyses and the respective confidence values from the MP, ME and BI analyses, are shown in Fig. 4. For both MP analyses (indel = fifth state and indel = missing), a single tree was recovered with a tree length of 1502 (fifth state) or 1449 steps

Table 2 Percentage of the pairwise nucleotide divergence matrix (lower-left) and the bp differences (upper-right) based on the 16S rRNA (560 bp), COI (616 bp) and H3 (328 bp) genes among different haplotypes of the genus *Jobora* and outgroups (Table 1). Nucleotide divergences for preferred models: 16S rRNA and COI: GTR + I + G, and H3: HKY + G. Indels are considered as substitutions in calculating the distances.

	<i>johorensis</i> clade							<i>tahanensis</i> clade			<i>punicea</i> clade			Outgroups						
	<i>J. tiomanensis</i>	<i>J. counsilmani</i>	<i>J. murphyi</i>	<i>J. johorensis</i>	<i>J. singaporensis</i>	<i>J. gapensis</i>	<i>J. intermedia</i>	<i>J. tahanensis</i>	<i>J. thoi</i>	<i>J. hoiseni</i>	<i>J. grillator</i>	<i>J. gua</i>	<i>J. punicea</i>	<i>Stoliczia chaseni</i>	<i>Terrapota abboti</i>	<i>Potamiscus aff. yunnanense</i>	<i>Geothelphusa dehaani</i>	<i>Candidopotamon rathbunae</i>	<i>Ryukyum yaeyamense</i>	<i>Himalayapotamon</i>
<i>johorensis</i> clade	24.4,59.4,2.6/4.92,21.22,0.88*							56.2,90.3,12.4†			58.7,80.9,14.9†									
<i>J. tiomanensis</i>	—	1,	23,	22,	30,	33,	29,	58,	52,	61	64,	58,	60,	85,	76,	80,	88,	80,	72,	93,
		14,	69,	65,	73,	65,	62,	99,	95,		84,	86,	86,	114,	91,	112,	109,	121,	111,	116,
		0	2	2	3	1	2	13	10		13	11	19	17	25	19	18	18	20	27
<i>J. counsilmani</i>	0.18,	—	22,	21,	31,	32,	28,	57,	51,	60	63,	57,	59,	84,	77,	79,	89,	79,	73,	92,
	2.5,		70,	67,	75,	62,	64,	99,	96,		84,	87,	87,	113,	92,	111,	111,	123,	111,	119,
	0		2	2	3	1	2	13	10		13	11	19	17	25	19	18	18	20	27
<i>J. murphyi</i>	4.21,	3.99,	—	15,	29,	30,	24,	59,	53,	61	61,	54,	56,	83,	75,	79,	88,	77,	79,	88,
	26.11,	27.58,		41,	55,	64,	63,	82,	87,		86,	76,	79,	96,	103,	113,	105,	101,	106,	117,
	0.65	0.65		0	5	3	4	15	12		15	13	21	19	23	19	18	18	20	29
<i>J. johorensis</i>	4.14,	3.93,	2.77,	—	23,	23,	22,	55,	51,	58	60,	55,	57,	84,	71,	77,	87,	74,	76,	89,
	23.83,	25.6,	10.12,		60,	54,	53,	89,	89,		80,	72,	74,	90,	94,	107,	105,	101,	103,	114,
	0.65	0.65	0		5	3	4	15	12		15	13	21	19	23	19	18	18	20	29
<i>J. singaporensis</i>	5.97,	6.22,	6,	4.75,	—	31,	21,	60,	54,	61	62,	60,	62,	88,	71,	85,	91,	79,	78,	92,
	26.98,	28.8,	18.72,	20.4,		66,	56,	78,	90,		82,	78,	83,	99,	102,	120,	108,	112,	105,	117,
	1	1	1.78	1.78		4	5	12	9		10	8	16	14	22	18	15	17	17	27
<i>J. gapensis</i>	7.22,	6.98,	6.22,	4.65,	6.42,	—	22,	56,	52,	57	62,	55,	55,	87,	80,	82,	86,	80,	76,	91,
	25.15,	24.72,	21.35,	16.16,	23.59,		50,	90,	96,		76,	82,	84,	96,	91,	112,	98,	108,	108,	117,
	0.32	0.32	1.03	1.03	1.4		1	14	11		14	12	20	18	26	20	19	19	21	28
<i>J. intermedia</i>	5.86,	5.63,	4.95,	4.66,	4.11,	4.4,	—	56,	48,	60	61,	55,	57,	85,	79,	84,	88,	80,	83,	91,
	22.8,	26.58,	23.35,	17.15,	19.15,	15.07,		81,	93,		78,	77,	78,	102,	92,	107,	100,	109,	104,	115,
	0.65	0.65	1.4	1.4	1.78	0.31		15	12		15	13	21	19	25	21	20	20	22	29
<i>tahanensis</i> clade	14.95,50.49,5.67†							23.3,74.4/4.58,31.67,1.39*			60.0,87.2,14.8†									
<i>J. tahanensis</i>	16.34,	16.02,	15.51,	14.18,	15.94,	14.4,	14.48,	—	28,	10	62,	57,	59,	80,	77,	84,	102,	81,	87,	93,
	52.81,	54.79,	45.94,	50.03,	44.05,	51.14,	48.6,	74,	74,		81,	84,	86,	97,	104,	111,	107,	105,	110,	115,
	6.06	6.06	7.48	7.48	5.33	6.84	7.49	4	4		15	12	19	18	24	20	19	16	18	28
<i>J. thoi</i>	14.19,	13.88,	13.46,	12.73,	13.7,	12.92,	11.81,	5.58,	—	32	63,	58,	59,	82,	83,	87,	104,	83,	90,	92,
	49.45,	51.69,	47.01,	50.98,	48.12,	59.35,	52.84,	31.67,			91,	90,	91,	106,	118,	123,	122,	118,	119,	130,
	4.16	4.16	5.34	5.34	3.57	4.79	5.34	1.39			14	11	18	17	23	17	18	15	17	25
<i>J. hoiseni</i>	17.61	17.28	17.18	15.33	16.22	14.88	15.9	1.74	6.42	—	62	59	61	84	78	86	104	83	86	93

Table 2 Continued.

	johorensis clade					tahanensis clade					punicea clade			Outgroups							
	<i>J. tiomanensis</i>	<i>J. counsilmani</i>	<i>J. murphyi</i>	<i>J. johorensis</i>	<i>J. singaporensis</i>	<i>J. gapensis</i>	<i>J. intermedia</i>	<i>J. tahanensis</i>	<i>J. thoi</i>	<i>J. hoiseni</i>	<i>J. grillator</i>	<i>J. gua</i>	<i>J. punicea</i>	<i>Stoliczia chaseni</i>	<i>Terrapotamon abbotti</i>	<i>Potamiscus aff. yunnanense</i>	<i>Geothelphusa dehaani</i>	<i>Candidiopotamon rathbunae</i>	<i>Ryukyum yaeyamense</i>	<i>Himalayapotamon</i>	
punicea clade	15.64,38.68,7.42†					14.88,44.65,6.90†					14.4,28.7,7.7 2.74,7.07,3.20*										
<i>J. grillator</i>	18.59, 42.26, 5.92	18.25, 43.98, 5.92	17.68, 40.33, 7.3	16.79, 36.39, 7.3	17.46, 34.19, 4.16	17.36, 32.65, 6.68	17.1, 37.12, 7.31	16.22, 40.16, 6.73	16.48, 47.1, 6.16	16.83	—	20, 39, 5	19, 41, 16	88, 96, 19	80, 96, 22	79, 111, 21	103, 107, 14	90, 103, 17	93, 110, 18	92, 115, 30	
<i>J. gua</i>	15.22, 44.64, 5.04	14.91, 47.11, 5.04	13.94, 33.8, 6.37	13.85, 32.02, 6.37	15.69, 32.88, 3.35	13.89, 37.78, 5.76	13.85, 36.77, 6.38	13.35, 43.18, 5.43	13.61, 44.87, 4.76	14.61	3.95, 9.6, 1.81	—	3, 6, 2	82, 91, 13	82, 90, 16	74, 109, 17	104, 103, 12	86, 99, 15	90, 103, 14	91, 111, 25	
<i>J. punicea</i>	15.67, 44.79, 9.81	15.35, 47.26, 9.81	14.38, 36.42, 11.62	14.36, 35.59, 11.62	16.14, 37.52, 7.53	13.7, 40.11, 10.82	14.28, 38.74, 11.63	13.84, 45.77, 9.75	13.85, 46.79, 8.6	15.11	3.71, 10.58, 7.14	0.54, 1.02, 0.66	—	84, 93, 16	84, 92, 22	75, 109, 23	106, 103, 20	87, 100, 21	92, 105, 20	93, 110, 30	
Outgroups																					
<i>Stoliczia chaseni</i>	23.55, 74.31, 8.67	23.2, 76.6, 8.67	21.18, 61.42, 10.38	21.54, 56.1, 10.38	23.98, 70.99, 6.51	23.07, 54.65, 9.62	22.06, 73.27, 10.39	18.19, 60.98, 9.83	20.97, 78.22, 8.63	20.67	23.01, 55.23, 10.35	19.39, 53.3, 6.54	20.05, 56.18, 8.15	—	81, 79, 20	81, 100, 20	93, 87, 18	80, 81, 19	80, 97, 16	91, 87, 29	
<i>Terrapotamon abbotti</i>	21.69, 49.77, 18.72	22.08, 52.09, 18.72	21.73, 59.57, 16.03	20.1, 51.45, 16.03	20.63, 58.96, 14.98	23.42, 48.82, 20.43	23.51, 54.65, 19.22	21.18, 64.32, 16.58	24.48, 73.39, 15.55	21.56	23.88, 53.08, 14.19	23.16, 45.14, 9.27	23.66, 45.81, 14.91	20.33, 37.33, 12.7	—	78, 98, 23	82, 94, 21	72, 94, 23	75, 91, 20	99, 104, 32	
<i>Potamiscus aff. yunnanense</i>	25.88, 68.09, 10.62	25.52, 67.81, 10.62	24.53, 69.44, 10.34	23.97, 62.36, 10.34	27.32, 80.08, 9.57	26.87, 72.18, 11.72	27.25, 74.94, 12.59	24.94, 76.5, 11.37	27.16, 85.63, 8.6	25.83	24.27, 74.22, 11.91	20.78, 68.42, 9.28	21.01, 68.57, 13.6	20.64, 62.02, 12.04	21.47, 58.65, 16.4	—	109, 103, 17	87, 113, 18	89, 102, 16	101, 113, 25	
<i>Geothelphusa dehaani</i>	22.49, 68.62, 9.58	22.88, 74.26, 9.58	22.37, 63.13, 9.33	22.7, 58.09, 9.33	23.64, 64.14, 7.28	21.22, 58.64, 10.6	22.7, 58.58, 11.42	29.22, 65.6, 9.78	32.08, 83.69, 9.07	30.42	31.36, 55.46, 6.31	30.58, 54.23, 5.59	31.02, 56.26, 10.77	23.32, 42.53, 10.1	21.62, 46.44, 13.23	34.14, 61.26, 8.84	—	77, 100, 12	68, 106, 12	104, 97, 31	
<i>Candidiopotamon rathbunae</i>	23.79, 91.47, 10.1	23.44, 98.56, 10.1	23.43, 71.35, 9.83	21.39, 69.94, 9.83	24.04, 87.07, 9.08	23.38, 72.8, 11.18	23.68, 80.94, 12.03	22.06, 71.47, 7.7	23.22, 90.4, 7.08	23.53	29.01, 68.68, 8.61	25.43, 65.05, 7.82	25.65, 65.54, 12.18	20.43, 43.57, 11.49	19.94, 57.46, 15.96	28.74, 75.14, 10.07	18.05, 51.97, 5.46	—	54, 97, 10	94, 109, 27	
<i>Ryukyum yaeyamense</i>	20.61, 69.04, 11.73	20.99, 71.54, 11.73	21.56, 61.32, 11.43	20.78, 57.14, 11.43	20.97, 58.14, 9.09	20.74, 59.44, 12.92	22.75, 62.05, 13.86	25.03, 66.69, 9.07	28.5, 83.02, 8.38	24.59	28.85, 62.52, 9.32	26.2, 57.99, 7.18	26.62, 60.91, 11.36	20.43, 55.27, 9.1	19.73, 48.95, 13.01	28.67, 67.67, 8.15	14.97, 52.83, 5.47	11.22, 42.28, 4.14	—	95, 111, 24	
<i>Himalayapotamon atkinsonianum</i>	33.81, 95.64, 19.73	33.39, 103.48, 19.73	28.35, 100.04, 22.82	29.37, 95.14, 22.82	31.13, 98.2, 20.23	31.94, 90.07, 21.49	30.68, 99.72, 22.84	31.59, 95.5, 20.85	31.38, 124.61, 16.48	30.94	31.6, 83.54, 24.12	29.27, 84.68, 18.72	29.81, 81.21, 24.09	33.44, 56.69, 24.69	34.67, 75.72, 32.35	40.78, 83.79, 17.36	36.49, 68.11, 26.9	31.89, 86.11, 20.15	38.73, 85.12, 15.87	—	

*The basepair and divergence differences within each clade; †The basepair difference between clades; ‡The divergence difference between clades.

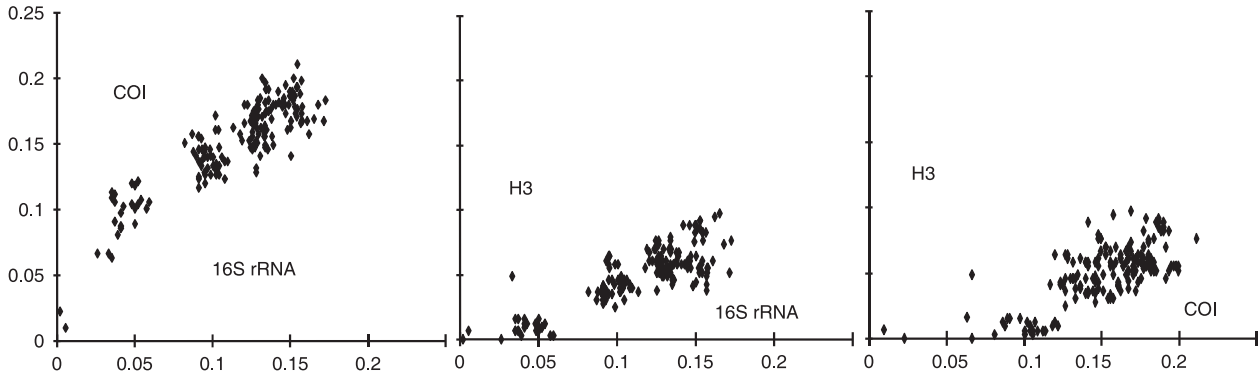


Fig. 3 Correlation between the uncorrected *p*-distances for each gene (16S rRNA, COI and H3); scales of the X- and Y-axes were adjusted to the same to show the relative rates of divergence in each gene.

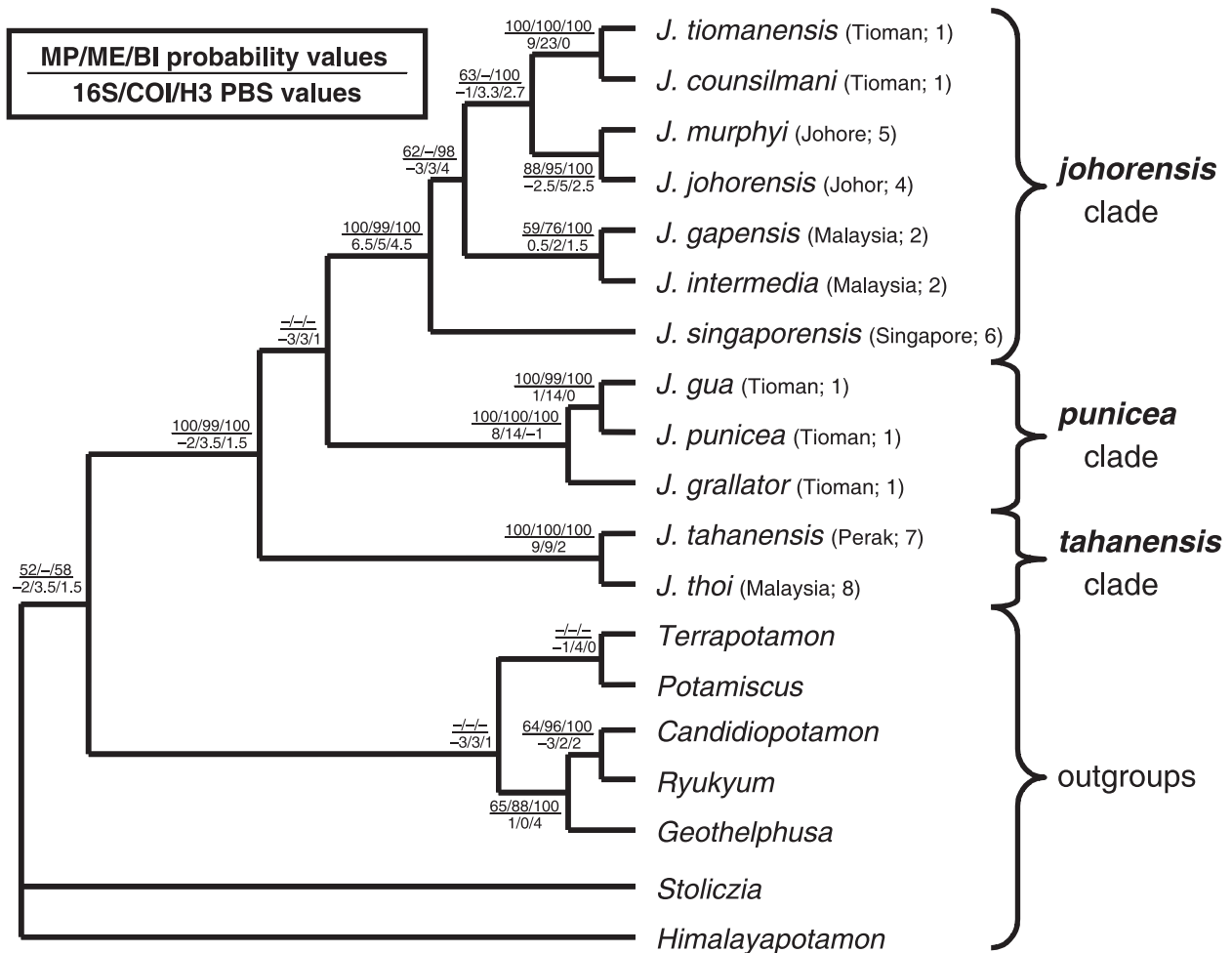


Fig. 4 A parsimony tree (MP) of the species of *Johora* from Peninsular Malaysia and Singapore, and the outgroups, based on 1502 bp of the combined 16S rRNA, COI and H3 genes. First row values at the branches represent MP bootstrap/minimum evolution (ME) bootstrap/Bayesian posterior (BI) probabilities (—=node not supported). Second row numbers are partitioned Bremer support (PBS) values for 16S rRNA, COI and H3.

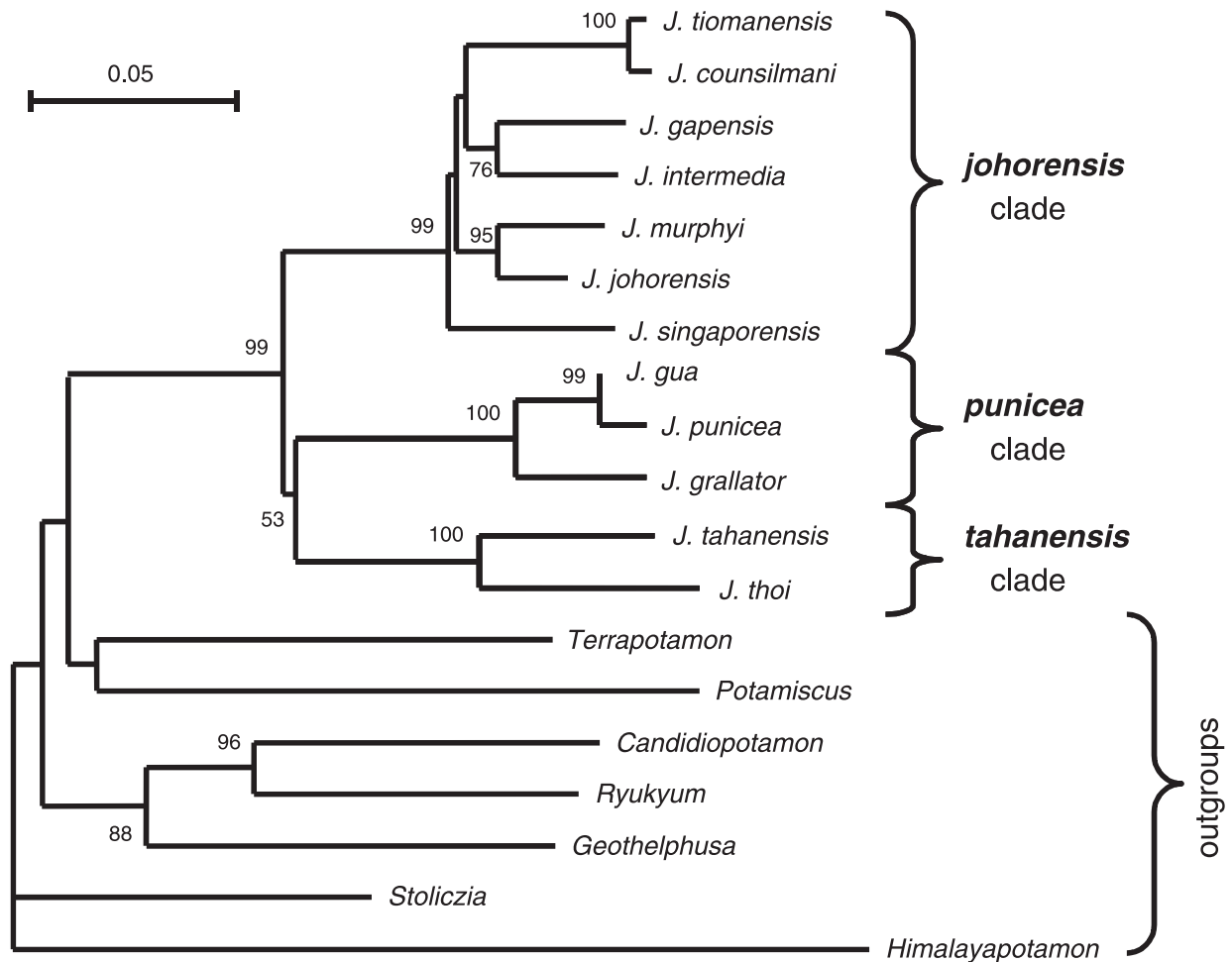


Fig. 5 A minimum evolution tree of the species of *Jobora* from Peninsular Malaysia and Singapore, and the outgroups, based on 1502 bp of the combined 16S rRNA, COI and H3 genes. Probability values at the nodes represent confidence after 2000 bootstrap reiterations.

(indel = missing) and with a consistency index of 0.510 (fifth state) or 0.506 (indel = missing) and a retention index of 0.513 (fifth state) or 0.511 (indel = missing). The two trees only differ with regard to the placement of a clade consisting of the outgroups *T. abbotti* and *Potamiscus* aff. *yunnanense*. For the ME and BI analyses, the best model selected by MRMODELTEST was the GTR + I + G model for the 16S rRNA and COI genes as well as the concatenated data set consisting of 16S rRNA, COI and H3 segments (model parameters for full data set: proportion of invariable sites = 0.5360, gamma distribution shape parameter = 0.8255), and the best model for H3 gene is HKY + G model. Both the ME and BI analyses similarly recover trees that are very similar to the parsimony trees (see Fig. 4). The phylogram of ME analysis with branch lengths was shown in Fig. 5.

In all analyses the genus *Jobora* is very well supported as monophyletic. Three main clades can be discerned within the

genus, here referred to as the *johorensis*, *tahanensis* and *punicea* clades for convenience. These three clades agree with the biogeographical distributions of the taxa (Fig. 1). The *johorensis* clade includes seven species occurring in the western and southern highlands of Peninsular Malaysia (*J. johorensis*, *J. murphyi*, *J. gapensis* and *J. intermedia*), Singapore (*J. singaporensis*), and Pulau Tioman (*J. tiomanensis* and *J. counsilmani*); the *tahanensis* clade includes three species in the eastern and northern parts of Peninsular Malaysia (*J. tahanensis*, *J. boiseni* and *J. thoi*; the position of *J. boiseni* was not shown in trees); and the *punicea* clade includes three species endemic to Pulau Tioman (*J. punicea*, *J. grallator* and *J. gua*). The relationship among the three clades is not clear, because the *tahanensis* and *punicea* clades are sister groups only in ME tree with weak support (Fig. 5).

The PBS analysis (see Fig. 4) reveals that most of the support in the parsimony analysis comes from the COI gene

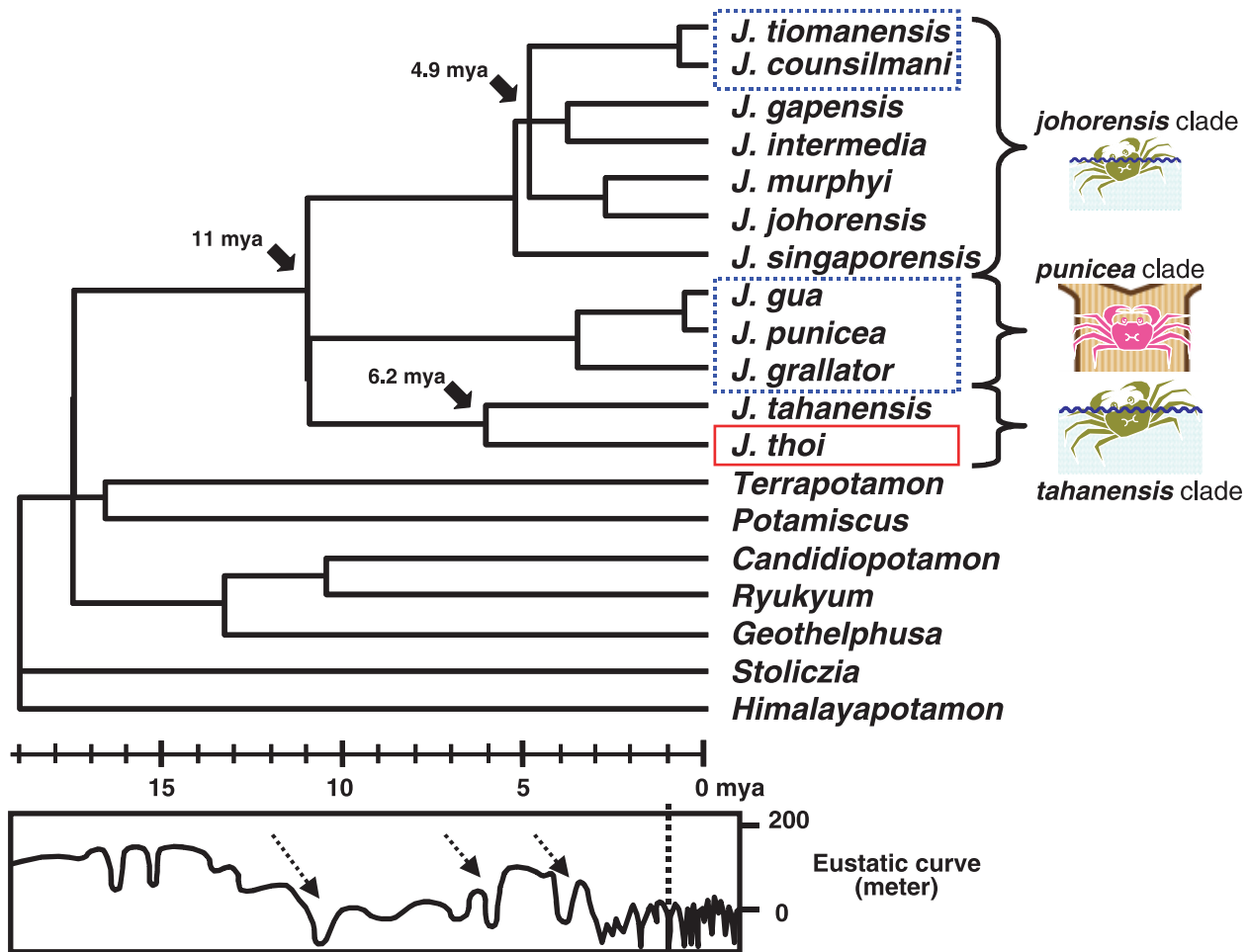


Fig. 6 A linearised minimum evolution tree for the species of *Jobora* from Peninsular Malaysia and Singapore, and outgroups, based on 1502 bp of the combined 16S rRNA, COI and H3 genes, with the estimated times of divergence. The dotted and black lined blocks mean the species are distributed in Pulau Tioman and Pulau Redang, respectively (Fig. 1). The icons below the clades illustrated their aquatic and semiterrestrial/terrestrial habitats, and the general relative size of carapace. The eustatic changes in global sea level are shown below (modified from Woodruff 2003). The dotted arrows mean the abrupt decline and rise of the sea levels which might correspond to the separation indicated by black arrows at the nodes in tree.

fragment (total support = 101.8) while the H3 (26.7) and 16S fragment (19.5) only make minor contributions. However, on a per parsimony-informative character basis, H3 is most informative (1.48 per character), with COI (0.75) and 16S (0.23) trailing behind. Of the 10 nodes with negative PBS values, nine are caused by 16S rRNA.

Using the log-likelihood ratio test, the assumption of a molecular clock for the combined dataset is not tenable ($P < 0.01$). The divergence time for the genus *Jobora* was estimated as 11 million years ago (mya; divergence is 9.73% for p -distance) from the substitution rate of 0.88% per million years for 16S rRNA (Schubart *et al.* 1998). The relative position and time of divergence for each node was shown as a linearised tree (Fig. 6). The separation time of the Tioman

subclade (*J. tiomanensis* and *J. counsilmani*), and the sister subclades (*J. gapensis*, *J. intermedia*, *J. murphyi* and *J. johorensis*), was estimated as 4.9 mya. The colonisation time of *J. thoi* on Pulau Redang was estimated as 6.2 mya.

Discussion

Phylogeny

The monophyly of the genus *Jobora* is confirmed in the present study, supporting Ng's (1987) decision to elevate the group from subgenus status (originally under *Stoliczia*) to a full genus. This also validates his use of the long, well-developed flagellum on the exopod of the third maxilliped as the primary synapomorphy for *Jobora*. The molecular evidence here also suggests that *Jobora* can be divided into three distinct and

well supported clades: the *joborensis*, *tabanensis*, and *punicea* clades (Figs 4 and 5; see Results).

The *joborensis* clade consists of mostly small- to medium-sized species (usually up to about 20 mm cw; largest recorded: 24.6 mm cw for *J. murphyi*), except for *J. tiomanensis* and *J. counsilmani* in Pulau Tioman which grow relatively larger (up to about 43.0 mm cw for the smaller species, *J. counsilmani*; largest recorded: 51.5 mm cw for *J. tiomanensis*). Members of the *joborensis* clade usually possess low epigastric and post-orbital carapace cristae with a tapered and curved or hooked G1 terminal segment (Ng 1987, 1988; Yeo et al. 1999). *Jobora joborensis*, *J. murphyi*, *J. gapensis* and *J. intermedia* were originally regarded as subspecies of *J. joborensis* (see Bott 1970; Ng 1986, 1987, 1988), but were subsequently recognised as full species by Ng & Takeda (1992) based primarily on G1 differences, their disjunct distributions, and the absence of sympatry between the geographically close taxa. The genetic distances between these taxa are consistent with this decision (Table 2). The present study does, however, cast some doubt on the specific status of *J. counsilmani* and *J. tiomanensis*. Yeo et al. (1999) had argued that *J. counsilmani* should be regarded as a full species rather than a subspecies of *J. tiomanensis* due to the absence of potential hybrids with intermediate G1 characteristics between the two taxa despite intensive and prolonged sampling of Pulau Tioman where these taxa occur. In addition, these two taxa appear to be restricted to separate drainages on the small island although a single specimen clearly referable to *J. tiomanensis* was collected from Sungai Keliling (eastern Tioman) where *J. counsilmani* was found (Yeo et al. 1999). Nevertheless, Yeo et al. (1999) did comment that the two species are very close. In our study, there is only a 1 bp difference of the 16S rRNA and only a 14 bp difference (2.27%) of the COI between the two species (Table 2). Such a small difference is often a sign for a lack of species-level differentiation, as has been discussed for some species from East Asia (Shih et al. 2004, 2005, 2006, 2007a, 2007b). However, currently, the two species can still be separated by discrete gonopodal differences (Yeo et al. 1999; Ng 2004) and as such, should be regarded as separate taxa for the moment. Additional analyses using discrete morphological and morphometric characters, using perhaps a larger series of specimens collected from more areas and at different times of the year may be necessary to resolve this question (see also Harrison & Hanley 2005).

The members of the *tabanensis* clade are generally larger in size (up to about 25 mm cw in the smallest species, *J. boiseni*; largest recorded: 41.2 mm cw for *J. thoi*) than those of the *joborensis* clade. They are usually characterised by relatively strongly developed epigastric and postorbital cristae, densely hirsute third maxillipeds, and long, more or less straight, sabre-shaped and usually hirsute G1 terminal segment (Ng 1987, 1988, 1990; Ng & Takeda 1992). The *tabanensis* clade

is congruent with all previous morphological studies, which have postulated close relationships between its taxa (Ng 1990; Ng & Takeda 1992). *Jobora thiana*, which is missing from the present analysis, is morphologically closely related to these species (Leelawathanagoon et al. 2005), and would most likely nest within this clade when its genetic data becomes available.

Within the *punicea* clade, *J. punicea* and *J. gua* are relatively small- to medium-sized species (up to 24.7 mm cw in *J. punicea*), while *J. grallator* is somewhat larger (up to 33 mm cw; Ng & Lim 1989; Yeo et al. 1999; Yeo 2001). The *punicea* clade also supports morphological studies that have hypothesised close relationships between its three species (Ng & Lim 1989; Yeo 2001). Based on the 3 bp difference of the 16S rRNA; and 6 bp difference (0.97%) of the COI between *J. punicea* and *J. gua* (Table 2), the two species may even be conspecific (see Shih et al. 2004, 2005, 2006, 2007a, 2007b). Again, as there are discrete morphological differences between *J. punicea* and *J. gua* (Yeo 2001; Ng 2004), it may be best to regard them as separate species for the moment. More significantly, our study supports Ng's (1987) comment that the resemblance of the G1 structures of *J. punicea* and *J. singaporensis* (*joborensis* clade) is likely to be due to convergence. The occurrence of *J. tiomanensis* and *J. counsilmani* (two fully aquatic species) on Pulau Tioman had previously led to speculation of possible affinities and a possibly close relationship with *J. punicea*, especially since the latter species has more terrestrial habits (Ng 1985; Yeo et al. 1999), but the present study shows otherwise. The dataset strongly suggests that the five known Pulau Tioman potamids are actually a paraphyletic grouping (see later). The *punicea* clade is more closely related to the *tabanensis* clade on the basis of the ME tree for the combined dataset, although the support value for this clade is not high (Fig. 5).

In our study, the relative divergence rate of *Jobora* among 16S rRNA, COI and H3 is 2.5 : 7.5 : 1. The ratio of divergences between COI and 16S rRNA is about 3 which is faster than the 2.5 known from other studies (Schubart et al. 1998; Tong et al. 2000; Shih et al. 2007b). The divergence rate for H3 is much slower which is typical for most nuclear genes. The PBS analysis reveals that H3 is a useful gene for reconstructing the relationships of *Jobora*. The overall contribution from this fragment is lower than the contribution by COI, but on a per character basis, H3 performs better than the other genes. Surprisingly, it is the widely used 16S rRNA that provides the least support and the most widespread conflict on the parsimony tree.

Biogeography

The two largely peninsular groups (*joborensis* and *tabanensis* clades) appear to have fairly distinct distributions on either side of the Main Range (where several mountains are more than 2000 m high) that runs longitudinally in a NW to SE

direction down the centre of much of Peninsular Malaysia (Fig. 1). There is only some possible overlap in distribution in a small area between Fraser's Hill (no. 2 in Fig. 1) and Bentong, west of Gunung Benom for *J. intermedia* (*joborensis* clade) and *J. tabanensis* (unpublished data), but even there, the two species have not been collected together in the same stream. The area is hydrologically complex, with tributaries of different river systems from both sides of the Main Range draining it, with the two species occurring in different drainages. Despite the 'overlap', one distributional pattern is apparent: the spine of the Main Range together with the extensive lowlands in the south and south-eastern parts of the peninsula (most of Johor and southern Pahang) appear to separate the *joborensis* clade of the western and isolated southern highlands from the *tabanensis* clade of the eastern and northern highlands. This pattern also suggests that, along the Main Range, the species of the *tabanensis* clade (*J. tabanensis* and *J. boiseni*) are usually found in drainages flowing eastwards towards the South China Sea; while species of the *joborensis* clade (*J. gapensis* and *J. intermedia*) are usually present in drainages flowing westwards towards the Straits of Malacca and Andaman Sea. Genetic isolation therefore need not necessarily only be caused by a major topographical barrier such as a mountain range, but also by the intervening land between drainages that remain separate even during wet season flooding (especially in the case of primarily aquatic groups like *Jobora*), with the montane habitats *de facto* islands (see Ng 1987).

Origins of the island fauna: Pulau Tioman and Pulau Redang

The true freshwater crab fauna (i.e., species that are independent of the marine environment for completing their life cycles) of Pulau Tioman, the largest offshore island on the east coast (133.6 km²), has been well studied, with eight endemic species from three families currently recognised: two species of *Geosesarma* (Sesarmidae), one species of *Parathelphusa* (Parathelphusidae) and the remaining five being *Jobora* species: *J. tiomanensis*, *J. counsilmani*, *J. punicea*, *J. gua* and *J. grallator* (Ng 1985, 1987, 1988; Ng & Lim 1989; Yeo *et al.* 1999; Yeo 2001). This is a very high degree of endemism for such a small island (Yeo *et al.* 1999; Yeo 2001). Pulau Redang, some 380 km north of Pulau Tioman and about 25 km from the peninsula, is the second largest offshore island on the east coast (25 km²; Medway 1966; Mohamed & Bujang 1992), has only two endemic species, one species of *Geithusa* (Parathelphusidae) and one *Jobora* species, *J. thoi* (Ng 1989a; 1990).

With regards to the Pulau Tioman fauna, Ng (1987) has previously proposed that *J. tiomanensis* and *J. counsilmani* may have evolved from a common ancestor shared with *J. murphyi* from the Kota Tinggi area of south-east Johor during periods of low sea level (glacials) when Pulau Tioman was connected

to the peninsula. The present study, which places these three species in the same clade (*joborensis* clade) supports this hypothesis. Implicit in all previous studies of *Jobora* species of Pulau Tioman (Ng 1985, 1987, 1988; Yeo *et al.* 1999; Yeo 2001) had been the belief that they form a monophyletic group of closely related species that diversified from a common ancestor, that is, there was a single invasion/colonisation event by *Jobora*. The present study based on molecular evidence, however, shows that the *Jobora* species of Pulau Tioman are not monophyletic, but instead belong to two separate clades, that is, the *joborensis* clade (with *J. tiomanensis* and *J. counsilmani*) and the *punicea* clade (with *J. punicea*, *J. gua* and *J. grallator*; Figs 4 and 5). *Jobora tiomanensis* and *J. counsilmani* are unusually large species for the *joborensis* clade (the largest known *Jobora* specimen is an individual of *J. tiomanensis* measuring 51.5 mm cw), but this can perhaps be explained by island gigantism, a common evolutionary phenomenon among insular fauna (Ng 1987; see also Foster 1964; Carlquist 1980; Whittaker 1998; Hormiga 2002; Drewes & Stoelting 2004; Keogh *et al.* 2005). The genetic datasets now suggest that there have been at least two invasion/colonisation events of Pulau Tioman by *Jobora* species (once by ancestors of the *punicea* clade species, and again by ancestors of *J. tiomanensis* and *J. counsilmani*). The timings of these invasions can be read from the linearised tree (Fig. 6). We believe that the first invasion was by members of the *punicea* clade (at c. 11 mya) followed later by those of the *joborensis* clade (at c. 4.9 mya). The calibration rate (0.88% per mya) of molecular dating was based on the terrestrial crabs of the genus *Sesarma* (Sesarmidae) in Jamaica (see Schubart *et al.* 1998). Although this rate was regarded as reasonable for the phylogeny of Taiwanese freshwater crabs (Potamidae: *Geothelphusa* and *Candidiopotamon*) and can be correlated with geological events (Shih *et al.* 2004, 2006, 2007b), applying the same rates for *Jobora* in Peninsular Malaysia needs discussion, especially since the hypothesis of a constant rate is rejected.

The divergence time estimated here for the genus *Jobora* appears to be much earlier than those for the potamids from East Asian islands, for example, *Candidiopotamon* (Shih *et al.* 2006) and *Geothelphusa* (Shih *et al.* 2004, 2007b). A late Miocene divergence was also estimated for the genus *Potamonautes* (family Potamonautidae) in South Africa (Daniel *et al.* 2002b, 2003, 2006). We therefore hypothesize that the genus *Jobora* is a very old one that has split from its ancestor in the middle Miocene and the divergence scenarios were possibly related to global eustatic changes.

Although the Sundaland platform was tectonically relatively stable since the late Eocene and above sea level during most of the Neogene (R. Hall 1998, 2001, 2002; Woodruff 2003), the eustatic changes in global sea level due to glaciations (Haq *et al.* 1987) probably changed the area of land exposed and formed new marine barriers which would obstruct the

dispersal of terrestrial organisms, and encourage divergence and speciation (see Woodruff 2003). Based on the global sea level fluctuation models (Woodruff 2003; also see the bottom of Fig. 6), sea levels were 100–150 m above that of the present day 24–13 mya, and the Isthmus of Kra would have been submerged. This would have effectively separated the Malay Peninsula from Indochina (Woodruff 2003). The separation of *Jobora* from the outgroups at *c.* 17.5 mya (Fig. 6) can possibly be explained by the submergence of the Isthmus of Kra. At *c.* 10–11 mya, an abrupt decline of the sea levels to –90 m (below the present day) occurred and the *punicea* clade probably colonised Pulau Tioman by the land bridge linking it to the mainland. Voris (2000) had commented that a sea level drop of 30–40 m would easily have allowed for the formation of such a bridge. When the sea levels returned to *c.* 0 about 10 mya, the island would again have been cut off by sea and stopped any gene flow between Pulau Tioman and the peninsula. The separation time estimated, 11 mya, was reflected in the molecular dating of the three main clades. The simultaneous separation of the other two clades (*joborensis* and *tabanensis* clades) is suggested to be due to orogeny (of the Main Range), which is also regarded as a key factor in catalysing the speciation of freshwater crabs in Taiwan (Shih *et al.* 2004, 2006). The *joborensis* clade invaded Pulau Tioman next at 4.9 mya (*J. tiomanensis* and *J. counsilmani*) which may be linked to either the period when the sea levels fell to –50 m at *c.* 6 mya then rose to +100 m at *c.* 5.5–4.5 mya, or when it fell to –20 m at *c.* 3.5 mya then rose to +70 m soon after.

It is possible that there have been extended periods when the island was directly connected to the Peninsular Malaysia during periods of low sea levels (glacials), up to as recently as 10 000–11 000 years bp during the late Pleistocene (Voris 2000; Meijaard 2003). However, the exposed broad and flat lowland area between Peninsular Malaysia and Pulau Tioman would probably have been swampy (Hendrickson 1966a; Inger & Voris 2001). This would function no less effectively than sea water as a barrier to dispersal in *Jobora*, which is a highland group typically found in clear, fast-flowing streams. Likewise, it has also been proposed that most amphibians on Pulau Tioman, which are stenotopic inhabitants of fast-flowing streams, would not likely disperse over ‘flat, low-lying landscape’ (Hendrickson 1966b; Inger & Voris 2001; Grismer *et al.* 2004) during these glacial periods. If this is true, then the two clades which colonised Pulau Tioman could have evolved separately from the peninsular fauna through vicariance. Vicariance has also been proposed for other groups on Pulau Tioman, for example, reptiles (Hendrickson 1966a; Grismer *et al.* 2003), forest terrestrial arthropods (Bullock 1966), freshwater fishes (Alfred 1966), mammals (Medway 1966) and aquatic beetles (Ciampor 2004).

The distance between Pulau Tioman and the peninsula is relatively short (*c.* 32 km and 45 km from Pulau Tioman

south-west to Mersing, Johor and west to Pahang, respectively), and as such, dispersal by rafting is also possible, especially during the annual north-east monsoon that brings heavy rainfall and high discharge of freshwater (and reduced salinities in estuarine areas) and flotsam from rivers in nearby eastern Johor into the South China Sea. This mechanism has also been suggested to explain colonisation of small islands off southeastern Taiwan by freshwater crabs of the genus *Geothelphusa* (Shih *et al.* 2004). As discussed earlier, this dispersal/recruitment hypothesis (either overseas or overland) has also been proposed for other groups on Pulau Tioman, for example, forest terrestrial arthropods (Bullock 1966), mammals (Medway 1966) and frogs (*Fejavyana cancrivora*; Hendrickson 1966b). However, such overseas dispersal of *Jobora* is not well supported by the present molecular results. The large molecular distances suggest that the island species have been isolated from the peninsula for a relatively long time (Fig. 6).

The *joborensis* clade includes seven primarily aquatic species occurring in the western and southern highlands of Peninsular Malaysia (*J. joborensis*, *J. murphyi*, *J. gapensis* and *J. intermedia*), Singapore (*J. singaporensis*) and Pulau Tioman (*J. tiomanensis* and *J. counsilmani*); the *tabanensis* clade includes three primarily aquatic species in the eastern and northern parts of Peninsular Malaysia (*J. tabanensis*, *J. boiseni* and *J. thoi*); and the *punicea* clade includes three species endemic to Pulau Tioman that have semiterrestrial to terrestrial habits (*J. punicea*, *J. gua* and *J. grillator*; Ng 1985, 1986, 1987, 1988, 1990, 2004; Ng & Lim 1989; Ng & Takeda 1992; Yeo *et al.* 1999; Yeo 2001). Mapping the ecological habits (primarily aquatic or semiterrestrial/terrestrial) of the *Jobora* species onto the phylogeny (Fig. 6), it appears that the semiterrestrial habit has evolved once, that is, in the *punicea* clade, with *J. punicea* and *J. grillator* known to be semiterrestrial to terrestrial, dwelling in and around drier parts of stream banks (Ng & Lim 1989; Yeo *et al.* 1999; Ng & Yeo 2007). *Jobora gua* was obtained from a shallow underground stream in a cave, but it would not be unrealistic to suspect that it might have semiterrestrial habits within the cave, which has extremely moist walls and floor (Yeo 2001; H. H. Tan, personal communication) and underground stream systems. This would be similar to the behavioural pattern observed in another better known cavernicolous crab, *Stygotelphusa bidiensis* (Parathelphusidae) from Sarawak, East Malaysia, Borneo (Ng 1989b; Ng & Yusoff 1990). As all other *Jobora* species in Peninsular Malaysia have primarily aquatic habits, the semiterrestrial habit is likely to have evolved in the ancestors of the *punicea* clade only after they invaded/colonised Pulau Tioman. It might be hypothesised then that the evolution of the semiterrestrial habit could have been in response to one or both of the following: (1) interspecific competition from the ancestors of *J. tiomanensis* and *J. counsilmani* that arrived in the second wave of invasion/colonisation of Pulau

Tioman, with the proposed gigantism of the *joborensis* clade in Pulau Tioman possibly resulting in their dominating competition with the smaller-sized *punicea* clade for resources and habitat; and (2) drought as the climate and sea levels around Pulau Tioman fluctuated with glacial/interglacial periods (with raised sea levels forcing fauna up into the well-drained and relatively dry mountain tops; Bullock 1966; Hendrickson 1966a, 1966b; Khoo 1977). From what is known of Malayan freshwater crab biology (see Ng & Yeo 2007), the latter hypothesis seems more likely.

With regards to the single island species of *Jobora* on Pulau Redang, *J. thoi* (*tabanensis* clade), the genetic analysis leaves no doubt that it is derived from a common ancestor shared with *J. tabanensis*. Its colonisation on the island is estimated to have occurred at 6.2 mya and is more likely to be caused by the sea levels falling to -50 m at c. 6 mya before rising to +100 m at c. 5.5–4.5 mya (Fig. 6). The lower crab diversity of Pulau Redang compared to Pulau Tioman is probably due to the smaller area (Medway 1966; Ricklefs & Lovette 1999), low number of freshwater drainages, most of which are relatively short (Medway 1966; Ng 1989a; 1990), and may also be associated with a potentially shorter period of isolation from Peninsular Malaysia (see Voris 2000: Fig. 1) as a result of the island's closer proximity and shallower intervening seas when compared to that of Pulau Tioman.

Acknowledgements

This study was supported by a grant from the National Science Council, ROC (Taiwan) (NSC 92-2621-B-178-003-) to the corresponding author, HT Shih, and from the National University of Singapore (R-154-000-222-112) to DCJ Yeo and PKL Ng. Additional funding was also received from the Raffles Museum of Biodiversity Research, National University of Singapore. We thank H. H. Tan for information on the habitat of *J. gua*. Two anonymous reviewers, who greatly improved this manuscript, are also acknowledged.

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