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Phylogeny of Dorippoidea (Crustacea : Decapoda : Brachyura) inferred from three mitochondrial genes

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Abstract. The phylogenetic relationships between 10 of 13 genera of crabs from the superfamily Dorippoidea were investigated using mitochondrial 16S rRNA, 12S rRNA and cytochrome *c* oxidase subunit I gene sequences. The resultant phylogenetic trees based on the three molecular markers support the division of Dorippidae and Ethusidae as monophyletic families within the Dorippoidea. The inferred inter-generic relationships within Dorippidae concur with groupings based on the overall morphology of the carapace and structures of the male first pleopods.

Introduction

The brachyuran crabs of the superfamily Dorippoidea MacLeay, 1838 currently belong to two families, the Dorippidae MacLeay, 1838 (with nine genera and 21 species), and Ethusidae Guinot, 1977 (with four genera and 80 species) (Ng *et al.* 2008; Table 1). Dorippoids are heterotremes which have the last two pairs of the pereiopods highly modified to form appendages for carrying various objects such as bivalve shells, sea urchins, anemones, small wood pieces and leaves for camouflage (Guinot *et al.* 1995; Ng and Rahayu 2002; Castro 2005), hence their common name of 'porter crabs'.

This superfamily has attracted substantial attention from many biologists (see Holthuis and Manning 1990; Guinot *et al.* 1995), and its taxonomy is relatively stable, with both families well revised in recent years – the Dorippidae by Holthuis and Manning (1990) and the Ethusidae by Castro (2005). However, few phylogenetic studies have focused on the group. A preliminary analysis on the phylogenetic relationships of four Chinese dorippid genera (Fan *et al.* 2004), based on mitochondrial 16S rRNA sequences, suggested that *Dorippe* is basal in the subfamily and *Heikeopsis* is closely related to *Neodorippe*. The present study seeks to expand on the above work, using 10 genera, by examining phylogenetic relationships within the Dorippoidea by using DNA sequence data from three molecular markers with different mutation rates, namely mitochondrial 16S and 12S rRNA, and the mitochondrial protein-coding gene, cytochrome *c* oxidase subunit I (hereafter referred to as 16S, 12S and COI). In the present paper, we aim to establish a robust phylogenetic framework to resolve inter-generic relationships for Dorippoidea, as well as to test the affinities between the Dorippidae and Ethusidae.

Materials and methods

Taxon sampling

In all, 24 dorippoid species in 10 genera were analysed in the present study. They comprised seven dorippid and three ethusid genera, including a new ethusid genus (Naruse *et al.* 2009). Individuals from two other brachyuran families, Dromiidae De Haan, 1833 and Raninidae De Haan, 1839, were included as outgroup taxa. More than one species per genus were sequenced where possible and they are listed with relevant data in Table 2.

DNA extraction, PCR amplification and nucleotide sequencing

Genomic DNA was extracted with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and eluted in 200 µL of double distilled H_2O (ddH_2O) after extraction. The DNA extracts were evaluated by 1% agarose gel electrophoresis and ethidium bromide staining. The extracted DNA was kept at −20°C.

Polymerase chain reaction (PCR) was performed to amplify partial segments of the mitochondrial genes coding for 16S, 12S and COI. The two rRNA genes were amplified with the universal primer pairs 16Sar/16Sbr and 12Sai/12Sbi, respectively (Simon *et al.* 1994). The primers LCO1490 and HCO2198 (Folmer *et al.* 1994) were used for COI gene amplification. PCR amplifications were performed in a reaction mix containing 0.5–3.0 µL of DNA extract, 0.4 µM of each primer, 0.2 µM of dNTPs (Qiagen), 1 unit of *Taq* polymerase (Invitrogen, Carlsbad, CA), 1.5 mM of magnesium chloride (Invitrogen), 1× PCR reaction buffer and ddH_2O to a total volume of 30 µL. The PCR profile for all genes was as follows: initial denaturation for 3 min at 94°C, followed by 33–35 cycles of

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Table 1. Genera and species of Dorippoidea

Family Dorippidae MacLeay, 1838
  Dorippe frascone (Herbst, 1785)
  Dorippe glabra Manning, 1993
  Dorippe irrorata Manning & Holthuis, 1986
  Dorippe quadridens (Fabricius, 1793)
  Dorippe sinica Chen, 1980
  Dorippe tenuipes Chen, 1980
  Dorippe trilobata Manning, 1993
  Dorippoides facchino (Herbst, 1785)
  Dorippoides nudipes Manning & Holthuis, 1986
  Heikeopsis arachnoides (Manning & Holthuis, 1986)
  Heikeopsis japonica (von Siebold, 1824)
  Medorippe lanata (Linnaeus, 1767)
  Neodorippe callida (Fabricius, 1798)
  Neodorippe simplex Ng & Rahayu, 2002
  Nobilum histrio (Nobili, 1903)
  Paradorippe australiensis (Miers, 1884)
  Paradorippe cathayana Manning & Holthuis, 1986
  Paradorippe granulata (De Haan, 1841)
  Paradorippe polita (Alcock & Anderson, 1894)
  Philippidorippe philippinensis Chen, 1986
  Phyllodorippe armata (Miers, 1881)
Family Ethusidae Guinot, 1977
  Ethusa abbreviata Castro, 2005
  Ethusa americana A. Milne-Edwards, 1880
  Ethusa andamanica Alcock, 1894
  Ethusa barbata Castro, 2005
  Ethusa brevidentata Chen, 1993
  Ethusa ciliatifrons Faxon, 1893
  Ethusa crassipodia Castro, 2005
  Ethusa crosnieri Chen, 1993
  Ethusa curvipes Chen, 1993
  Ethusa dilatidens Chen, 1997
  Ethusa foresti Chen, 1986
  Ethusa furca Chen, 1993
  Ethusa granulosa Ihle, 1916
  Ethusa hawaiiensis Rathbun, 1906
  Ethusa hirsuta McArdle, 1900
  Ethusa indica Alcock, 1894
  Ethusa indonesiensis Chen, 1997
  Ethusa izuensis Sakai, 1937
  Ethusa lata Rathbun, 1894
  Ethusa latidactylus (Parisi, 1914)
  Ethusa longidentata Chen, 1997
  Ethusa machaera Castro, 2005
  Ethusa magnipalmata Chen, 1993
  Ethusa mascarone (Herbst, 1785)
  Ethusa microphthalma Smith, 1881
  Ethusa minuta Sakai, 1937
  Ethusa obliquedens Chen, 1993
  Ethusa orientalis Miers, 1886
  Ethusa panamensis Finnegan, 1931
  Ethusa parapygmaea Chen, 1993
  Ethusa philippinensis Sakai, 1983
  Ethusa pygmaea Alcock, 1894
  Ethusa quadrata Sakai, 1937
  Ethusa rosacea A. Milne-Edwards & Bouvier, 1897
  Ethusa rugulosa A. Milne-Edwards & Bouvier, 1897
  Ethusa sexdentata (Stimpson, 1858)
  Ethusa sinespina Kensley, 1969
  Ethusa steyaerti Hendrickx, 1989
  Ethusa tenuipes Rathbun, 1897
  Ethusa thieli Spiridonov & Türkay, 2007
  Ethusa truncata A. Milne-Edwards & Bouvier, 1899
  Ethusa vossi Manning & Holthuis, 1981
  Ethusa zurstrasseni Doflein, 1904
  Ethusina abyssicola Smith, 1884
  Ethusina alba (Filhol, 1884)
  Ethusina beninia Manning & Holthuis, 1981
  Ethusina bicornuta Chen, 1997
  Ethusina brevidentata Chen, 1993
  Ethusina castro Ahyong, 2008
  Ethusina challengeri (Miers, 1886)
  Ethusina chenae Ng and Ho, 2003
  Ethusina ciliacirrata Castro, 2005
  Ethusina coronata Castro, 2005
  Ethusina crenulata Castro, 2005
  Ethusina desciscens Alcock, 1896
  Ethusina dilobotus Chen, 1993
  Ethusina dofleini Ihle, 1916
  Ethusina exophthalma Castro, 2005
  Ethusina faxonii Rathbun, 1933
  Ethusina gracilipes (Miers, 1886)
  Ethusina huilianae Castro, 2005
  Ethusina insolita Ng & Ho, 2003
  Ethusina isolata Castro, 2005
  Ethusina longipes Chen, 1987
  Ethusina macrospina Ng & Ho, 2003
  Ethusina microspina Chen, 2000
  Ethusina ocellata Castro, 2005
  Ethusina paralongipes Chen, 1993
  Ethusina pubescens Chen, 1993
  Ethusina robusta (Miers, 1886)
  Ethusina rowdeni Ahyong, 2008
  Ethusina smithiana (Faxon, 1893)
  Ethusina somalica (Doflein, 1904)
  Ethusina stenommata Castro, 2005
  Ethusina taiwanensis Ng & Ho, 2003
  Ethusina talismani A. Milne-Edwards & Bouvier, 1897
  Ethusina vanuatuensis Chen, 2000
  Parethusa glabra Chen, 1997
  Parethusa hylophora Castro, 2005
  Serpenthusa brucei Naruse *et al.*, 2009

30 s at 94°C, 30 s at annealing temperature 47–53°C, depending on individual samples, and 30 s at 72°C, with a final extension at 72°C for 3 min.

The size and quality of PCR products were assessed in 1% agarose gel electrophoresis. Before sequencing, amplification products were purified with a gel purification kit (Qiagen), following the manufacturer's instructions. The 16S, 12S and COI gene segments were sequenced with the same forward and reverse primers for PCR amplification. Sequences were obtained by dye terminator cycle sequencing with ABI Prism dRodoamine terminator (Applied Biosystems, Foster City, CA) and read on an ABI 3100 Genetic Analyzer (Applied Biosystems). Sequences were analysed with ABI SeqEd version 1.0. and aligned with Clustal W (Thompson *et al.*

Table 2. Classification, sampling localities and voucher ID of the species and GenBank accession number of the gene sequences in the present study
Sequences with an asterisk indicate those not obtained by the authors. MBMCAS, Marine Biological Museum of Chinese Academy of Sciences; NTOU, National Taiwan Ocean University, Keelung, Taiwan; ZRC, Zoological Reference Collection, Raffles Museum at the National University of Singapore

Superfamily/ family	Species	Sampling locality	Voucher no.	GenBank accession numbers		
				16S	12S	COI
Dorippoidea						
Dorippidae	*Dorippe quadridens* (Fabricius, 1793)	Japan: Okinawa Island, Ishikawa, fish port	ZRC 2008.0064	EU636946, EU636947	EU636962, EU636963	EU636978, EU636979
	Dorippe sinica Chen, 1980	China: Guangdong, Nanao Island, Qianjing, fish port	ZRC 1999.0470	EU636948	EU636964	—
	Dorippe tenuipes Chen, 1980	South China Sea	MBMCAS Q39B-1	AY452771*,	—	—
		South China Sea	MBMCAS K82B-17	AY452772*	—	—
	Dorippoides nudipes Manning & Holthuis, 1986	Thailand: Phuket, Pichai fish port	ZRC 2001.1057	EU636949	EU636965	EU636980
	Medorippe lanata (Linnaeus, 1767)	Israel	ZRC	EU636950	EU636966	EU636981
	Philippidorippe philippinensis Chen, 1986	Philippines: Visayas, Bohol, north coast of Panglao	ZRC 2008.0075	EU636945	EU636961	EU636977
	Paradorippe granulata (De Haan, 1841)	Japan: off Hota Uchibo coast of Boso Peninsula by gill-net	ZRC 1999.0082,	EU636941, EU636942,	EU636958, —	EU636974, —
		East China Sea	MBMCAS D64B-1	AY452773*, AY452774*	— —	— —
	Paradorippe polita (Alcock & Anderson, 1984)	East China Sea	MBMCAS C37B-9	AY452776*, AY452777*	— —	— —
	Neodorippe simplex Ng & Rahayu, 2002	Australia: Ludmilla Creek, mangroves, Darwin, NT	ZRC 2003.0335	EU636943	EU636959	EU636975
	Neodorippe callida (Fabricius, 1798)	China: Hainan, Yinggehai	MBMCAS 00085	AY452775*	—	—
	Heikeopsis arachnoides (Manning & Holthuis, 1986)	Taiwan: Pingtung, Tungkang fish port	ZRC 1997.392	EU636944	EU636960	EU636976
	Heikeopsis japonica (von Siebold, 1824)	East China Sea	MBMCAS V310B-6,	AY452769*,	—	—
		East China Sea	MBMCAS D7B-10	AY452770*	—	—
Ethusidae	*Ethusa sexdentata* (Stimpson, 1858)	Philippines: PANGLAO 2005, Station CP2358, 569–583 m, 8°52.1′N, 123°37.1′E	ZRC	EU636951, EU636952, EU636953	EU636967, EU636968, EU636969	EU636982, EU636983, EU636984
	Ethusina macrospina Ng & Ho, 2003	Taiwan: TAIWAN 2001, Station 141	ZRC 2003.0229	EU636954	EU636970	—
	Serpenthusa brucei Naruse *et al*., 2009	Vanuatu: west of Malo island, 15°39.5′S, 167°01.5′E, 272–286 m	ZRC 2008.0025	EU636955	EU636971	EU636985
Outgroups						
Raninidae	*Ranina ranina* (Linnaeus, 1758)	Taiwan: Taipei Keng Fang	NTOU B00012	EU636957	EU636973	EU636987
Dromiidae	*Lauridromia dehaani* (Rathbun, 1923)	Hong Kong: fish market	Unvouchered	EU636956	EU636972	EU636986

1994) with default gap weighting parameters and subsequently checked and adjusted manually. In addition, nine 16S sequences generated by Fan *et al*. (2004) were downloaded from GenBank and incorporated in our analyses.

Phylogenetic analyses

To infer phylogenetic relationships, three tree-search methods were utilised. Maximum parsimony (MP) and maximum likelihood (ML) were performed in PAUP* 4.0b10 (Swofford 2002) whereas Bayesian inference (BI) was carried out with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Single-locus searches were conducted for each gene (16S, 26 terminal taxa; 12S, 16 terminal taxa; COI, 14 terminal taxa) and taxa with complete datasets were included for combined data analyses (14 terminal taxa). Before analysing the combined dataset, 1000 replicates of incongruence length difference (ILD) test (Farris *et al.* 1995) was carried out in PAUP* 4.0b10 (Swofford 2002) to examine possible incongruence between loci. There was no evidence of phylogenetic conflict between 12S, 16S and COI partitions ($P = 0.052$), justifying a combined data approach.

The best-fit model of nucleotide substitution used for ML and BI analyses was determined in Modelltest version 3.5 (Posada and Crandall 1998) by using the hierarchical likelihood ratio test (hLRT; Huelsenbeck and Crandall 1997). Heuristic MP and ML searches were executed using tree bisection reconnection (TBR) branch swapping with random addition sequence of 10 replicates, with the starting tree for branch-swapping obtained by stepwise addition. Gaps were treated as missing data. Topological robustness was assessed by bootstrap analysis, on the basis of full heuristic search of 1000 and 200 (500 for combined dataset) pseudoreplicates for MP and ML tree searches. For BI analyses, each locus was partitioned in the combined dataset. There was no difference in topologies from analysis using a single model. A Markov chain Monte Carlo (MCMC) search was initiated with random trees and run for 2 000 000 generations, with a sampling frequency of every 100 generations. Convergence was checked by plotting likelihood scores against generation and 6000 initial trees were discarded as 'burn-in'. Two separate analyses and four independent chains were executed to check for convergence of topology.

Results

A total of 17, 16 and 14 sequences were obtained for 16S, 12S and COI, respectively, for 11 of 13 species. Although we had tissue samples of species from two other genera, *Nobilum histrio* (Nobili, 1903) and *Parethusa glabra* Chen, 1997, they could not be amplified successfully. The former was represented by many specimens that were probably originally preserved in formalin, whereas the latter was represented only by very small specimens. All sequences were deposited into the GenBank database (see Table 2 for accession numbers).

Of the 484 base pairs (bp) aligned for 16S (total length from 451 to 469 bp), 257 were variable and 215 were parsimony informative. With an aligned length of 369 bp (total length from 341 to 356 bp) in 12S, 210 were variable and 155 were parsimony informative. The aligned length for COI was 658 bp, with 265 variable and 232 parsimony-informative sites. There were few segments with alignment ambiguity, and excluding them from phylogenetic analysis did not change tree topologies or statistical support. The average sequence divergences in 16S, 12S and COI were 18.3%, 16.9% and 18.9%, respectively, within the Dorippidae and 14.8%, 19.9% and 25.7%, respectively, within the Ethusidae. The interfamilial sequence divergences between Dorippidae and Ethusidae were 32.5%, 37.1% and 25.7% in 16S, 12S and COI, respectively.

Results of a homogeneity test supported a combined analysis of the three gene segments ($P = 0.052$). For the complete

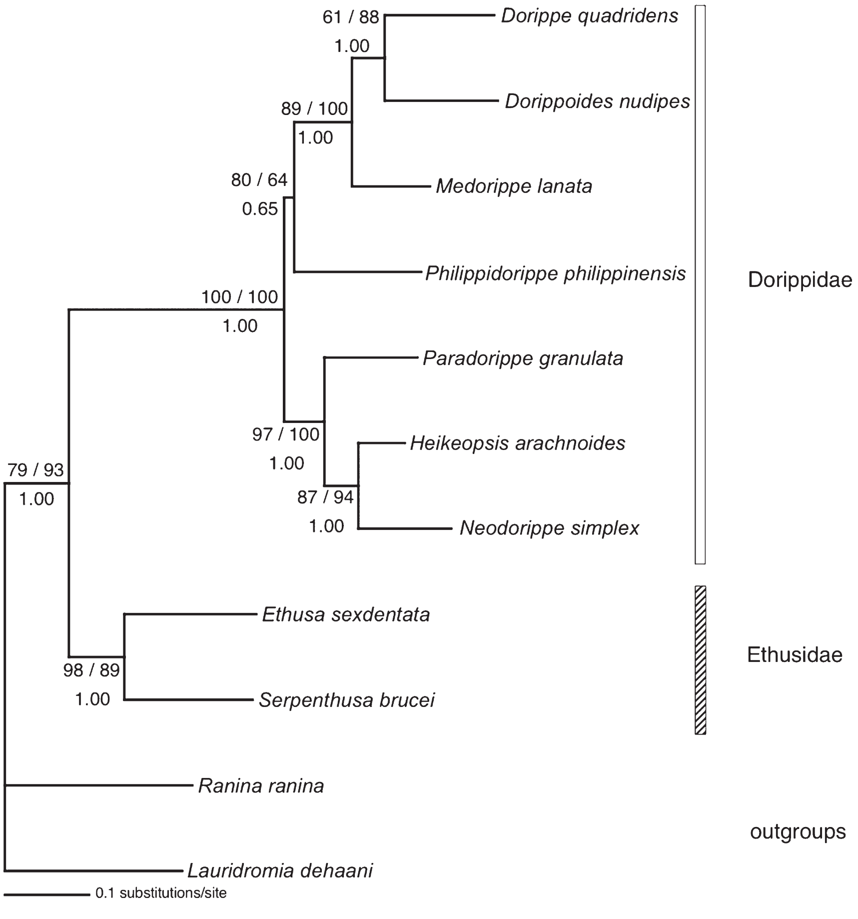

Fig. 1. Maximun likelihood tree of combined dataset. Numbers above branches are maximum parsimony (MP)/maximum likelihood (ML) bootstrap values; those below branches are Bayesian posterior probabilities.

dataset, the best-fit DNA substitution model was a general time-reversible model (GTR+I+G; $\alpha = 1.293$; $I = 0.430$; Rodríguez *et al.* 1990). MP analysis yielded three most parsimonious trees with 1752 steps, with consistency (*CI*) and retention indices (*RI*) of 0.598 and 0.631, respectively. Moreover, all search methods yielded trees that were largely compatible (Fig. 1). Tree searches using 16S sequences alone (substitution model: TVM+G; $\alpha = 0.382$) also yielded trees that were similar to that recovered for combined data analyses, the only exception being the position of *Philippidorippe philippinensis* (three most parsimonious trees of 672 steps, $CI = 0.592$ and $RI = 0.781$) (Fig. 2).

Sequence data of the 16S gene from 24 ingroup individuals revealed that each genus formed a distinct and highly supported clade (intrageneric species divergence 0–12.7% in 16S). Although more than one individual were sequenced for a species in some cases, we chose to present one example for simplicity because the low intraspecific divergence resulted in species-specific terminal clades (16S: 0–0.9%; 12S: 0–0.3%; COI: 0–1.5%). The COI and 12S gene trees are not shown here as they are concordant with the 16S tree. Both the Dorippidae and Ethusidae were well supported in trees on the basis of both the 16S and combined datasets (100% bootstrap (BP) support in MP and ML analyses; Bayesian posterior probabilities (BPP) = 1.0 for Dorippidae, BP values ≥98% in MP and ≥89% in ML analyses and BPP = 1.0 for Ethusidae).

In Dorippidae, the genera *Dorippe*, *Dorippoides* and *Medorippe* clustered together (16S: BP values 96% in MP and 97% in ML analyses; BPP = 1.0, combined: BP values 89% in MP and 100% in ML analyses; BPP = 1.0), whereas *Neodorippe*, *Heikeopsis* and *Paradorippe* formed another distinct clade (16S: BP values 88% in MP and 68% in ML analyses; BPP = 1.0, combined: BP values 97% in MP and 100% in ML analyses; BPP = 1.0). *Philippidorippe philippinensis* clustered with the *Dorippe*–*Dorippoides*–*Medorippe* clade in the combined dataset, with weak to moderate support (BP values 80% in MP and 64% in ML analyses; BPP = 0.65), and its position could not be clearly determined in the 16S gene tree. The three ethusid genera were distinct from each another and together formed a strongly supported clade (16S: BP values 99% in both MP and ML analyses; BPP = 1.0, combined: BP values 98% in MP and 89% in ML analyses; BPP = 1.0). On the basis of the 16S data, *Ethusina* and *Serpenthusa brucei* Naruse *et al.*, 2009 appear to be sister taxa with weak support (BP values 60% in ML analysis;

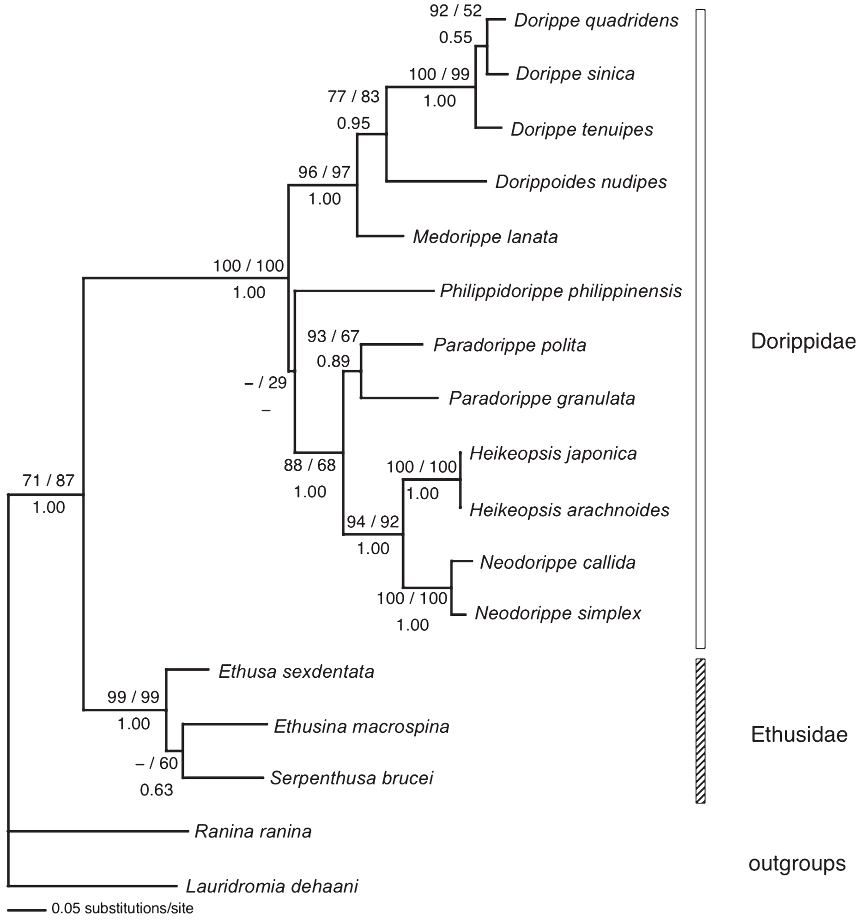

Fig. 2. Maximum likelihood tree of 16S rRNA. Numbers above branches are maximum parsimony (MP)/maximum likelihood (ML) bootstrap values; those below branches are Bayesian posterior probabilities. '–' indicates node not recovered in the corresponding analysis.

BPP = 0.63). However, this could not be corroborated with additional data because we were unable to obtain the complete 12S and COI sequences for *E. macrospina*.

Discussion

The present analyses based on the three mitochondrial gene sequences clearly demonstrate that the Dorippidae and Ethusidae are distinct lineages. Sequence divergences between the two families are very high, indicating a deep separation of the two families in their evolutionary history. The datasets support the hypotheses that both taxa should be recognised as distinct families on the basis of their numerous morphological differences (see discussion in Ng *et al.* 2008). The Ethusidae has long been part of the Dorippidae until Guinot (1977) established a new subfamily for *Ethusa* and *Ethusina* on the basis of marked differences in the position of the afferent branchial orifices and the structure of the last two pairs of legs and the condition of male gonopores (Holthuis and Manning 1990; Castro 2005). Ng *et al*. (2008) presented arguments as to why both taxa should be recognised as separate families. There is also some ecological segregation, with most dorippids being shallow water taxa whereas most ethusids live in waters deeper than 200 m (Chen 1986; Holthuis and Manning 1990; Castro 2005; Ng *et al.* 2008).

Within each family, the genera analysed are also genetically distinct, suggesting that the generic divisions proposed in the last major revision of the Dorippidae by Holthuis and Manning (1990) are warranted. Several cases merit discussion. Holthuis and Manning (1990) recognised *Dorippe sensu stricto* and *Dorippoides* as separate genera, whereas Serène and Romimohtarto (1969) treated them as subgenera. Our genetic analyses support their generic status, with the three *Dorippe* species tested forming a strongly supported clade that is clearly divergent from *Dorippoides* (average sequence divergences 17.5%, 18.4% and 18.3% in 16S, 12S and COI, respectively). Holthuis and Manning (1990) also established a new genus, *Medorippe*, for one species (*M. lanata*) that was traditionally placed in *Dorippe*; this decision is corroborated by the results from the present study (average sequence divergences 15.5%, 18.5% and 14.9% in 16S, 12S and COI respectively).

Holthuis and Manning (1990) also recognised *Neodorippe* and *Nobilum* as separate genera (Serène and Romimohtarto (1969) regarded both as subgenera). In addition, they argued that *Dorippe japonica* von Siebold, 1824 and a new species, *H. arachnoides*, should be referred to a new genus, *Heikea*. The latter name was changed to *Heikeopsis* by Ng *et al*. (2008) as Holthuis and Manning's (1990) name was a junior homonym. The present genetic data supports the acceptance of *Neodorippe* and *Heikeopsis* as separate genera. *H. japonica* forms a distinct

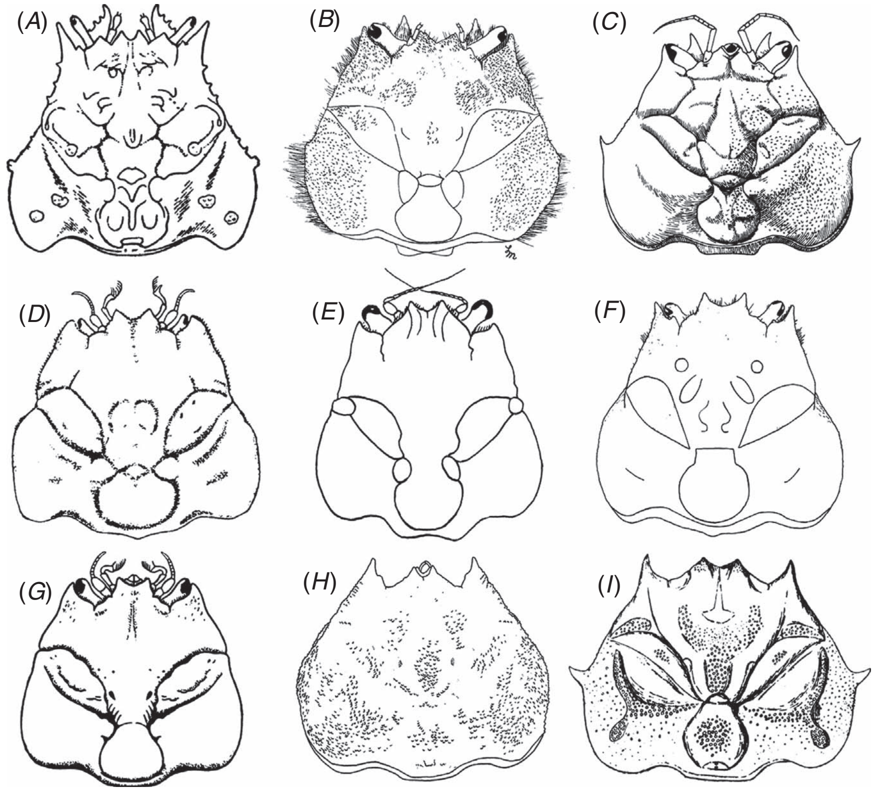

Fig. 3. Carapace outlines of all nine dorippid genera. (*A*) *Dorippe quadridens*, (*B*) *Dorippoides facchino*, (*C*) *Medorippe lanata*, (*D*) *Heikeopsis japonica*, (*E*) *Neodorippe callida*, (*F*) *Nobilum histrio*, (*G*) *Paradorippe cathayana*, (*H*) *Philippidorippe philippinensis* and (*I*) *Phyllodorippe armata*. (*A–H*, after Holthuis and Manning 1990; *I*, after Manning and Holthuis 1981.)

clade with *H. arachnoides* that is clearly divergent from the *Neodorippe* clade (average sequence divergences 12.4% in 16S). A similar result was reported by Fan *et al.* (2004). Unfortunately, no genetic material could be extracted from the material of *Nobilum* on hand.

The third genus established by Serène and Romimohtarto (1969) was *Paradorippe* with four extant species, of which two are included in the present study. *P. granulata* and *P. polita* form a distinct clade with high bootstrap and posterior probability support, with intrageneric divergence being 12.5% in 16S. This is the highest intrageneric percentage divergence recorded in the present study.

On the basis of morphology, there are three distinct groups in the Indo-West Pacific Dorippidae. These groups are as follows: (1) *Dorippe*, *Dorippoides* and *Medorippe*: carapace hexagonal, with antero- and posterolateral margins distinct, separated by an angle or spine; frontal margin quadridentate, with lateral tooth sometimes small but still distinct; male first pleopod relatively simple, the basal part possessing a distinct lobe, with the distal part relatively short, gently curved and cylindrical, the tip being simple, tapering (Figs 3*A–C*, 4*A–C*); (2) *Heikeopsis*, *Neodorippe* and *Nobilum*: carapace trapezoidal, antero- and posterolateral margins not demarcated, appearing contiguous, no anterolateral angle or spine discernible; frontal margin bidentate to vaguely quadridentate, the lateral teeth being small; male first pleopod slender, the basal part without a lobe, with the distal part very elongate, slender and strongly bent, the tip possessing folds and/or processes (Figs 3*D–F*, 4*D–F*); (3) *Paradorippe* and *Philippidorippe*: carapace trapezoidal, antero- and posterolateral margins not demarcated, no anterolateral angle or spine discernible; frontal margin bidentate or weakly quadridentate, with lateral teeth very small; male first pleopod relatively simple, the basal part without a lobe, with the distal part relatively short, gently curved and cylindrical, the tip possessing folds (Figs 3*G*, *H*, 4*G*, *H*).

The morphology of the only wholly Atlantic genus, *Phyllodorippe*, is ambiguous. It has a carapace that is distinctly hexagonal in shape, with the antero- and posterolateral margins clearly demarcated and possessing a distinct anterolateral spine (like *Dorippe* and *Medorippe*), whereas the frontal margin is distinctly bidentate (like *Neodorippe*, *Heikeopsis* and *Nobilum*) (Fig. 3*I*). The male first pleopod of *P. armata* (Miers, 1881) has a unique structure (Manning and Holthuis 1981); the base is relatively narrow and subrectangular, the distal section is spiraled, somewhat S-shaped and just before the tip, there is a prominent lobe (Fig. 4*I*). Unfortunately, we were unable to get tissue for this species so its affinities are uncertain.

Genetic analysis (Figs 1, 2) generally supports the first and second morphological groupings for the Indo-West Pacific Dorippidae. The genetic data is more ambiguous for the affinities of *Paradorippe* and *Philippidorippe*. In the ML tree with only the 16S gene, *Paradorippe* and *Philippidorippe* cluster together as a sister group to *Neodorippe* and *Heikeopsis*, although the support is very poor (BP values <30% in ML analysis; Fig. 2). For the combined analyses using the three genes, however, *Philippidorippe* is sister to the clade with *Medorippe*, *Dorippoides* and *Dorippe* (BP values 80% and 64% in MP and ML analyses, respectively; BPP = 0.65), whereas *Paradorippe* is sister to *Neodorippe* and *Heikeopsis* (BP values 97% in MP and 100% in ML analyses; BPP = 1.00; Fig. 1). Thus, *Philippidorippe* appears to be a basal genus in the *Medorippe*–*Dorippoides*–*Dorippe* clade when all three genes are used for analyses. *Philippidorippe* is an unusual taxon in the Dorippidae because it occurs in much deeper waters, whereas all the other genera are shallow-water taxa (Chen 1986; Holthuis and Manning 1990). On the basis of the available genetic data, it is nevertheless clear that Fan *et al.*'s (2004) assertion that *Dorippe* is basal in the family may not be true.

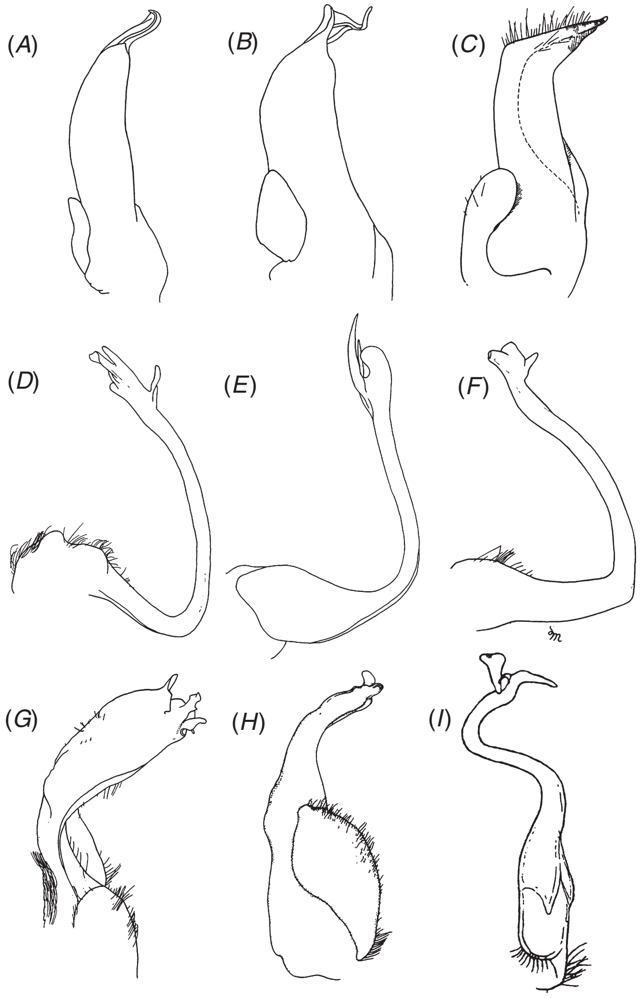

Fig. 4. Male first pleopod structure from all nine dorippid genera. (*A*) *Dorippe quadridens*, (*B*) *Dorippoides facchino*, (*C*) *Medorippe lanata*, (*D*) *Heikeopsis japonica*, (*E*) *Neodorippe callida*, (*F*) *Nobilum histrio*, (*G*) *Paradorippe cathayana*, (*H*) *Philippidorippe philippinensis* and (*I*) *Phyllodorippe armata*. (*A–H*, after Holthuis and Manning 1990; *I*, after Manning and Holthuis 1981.)

As has been discussed, the phylogenetic trees generally match the morphological groupings recognised in the Dorippidae, and matching genetic and morphological patterns is useful (see also Mitsuhashi *et al.* 2007). The structures of the male first pleopods merit a more detailed discussion, especially because they are usually species- and genus-specific. Holthuis and Manning (1990) highlighted the importance of the presence or absence of a basal lobe on the male first pleopod in the systematics of the Dorippidae. The presence of basal lobe characterises the clade that contains *Medorippe*, *Dorippoides* and *Dorippe*. Only the genus *Philippidorippe*, which lacks a basal lobe, was ambiguous appearing with either group (Figs 1, 2). Although *Nobilum* could not be included in the present genetic study, the close similarity in the carapace and pleopodal morphology between this genus and *Heikeopsis* and *Neodorippe* (Holthuis and Manning 1990) suggests a close relationship.

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