Turkish Journal of Zoology

Volume 42 | Number 1 Article 5

1-1-2018

A comprehensive phylogenetic analysis of Grapsoidea crabs (Decapoda: Brachyura) based on mitochondrial cytochrome oxidase subunit 1 (CO1) genes

ZHAOZHE XIN

YU LIU

BOPING TANG

CHUNLIN ZHOU

QIUNING LIU

TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2018) 42: 46-52

doi:10.3906/zoo-1703-46

A comprehensive phylogenetic analysis of Grapsoidea crabs (Decapoda: Brachyura) based on mitochondrial cytochrome oxidase subunit 1 (*CO1*) genes

Zhaozhe XIN, Yu LIU, Boping TANG*, Chunlin ZHOU, Qiuning LIU

Jiangsu Key Laboratory for Bioresources of Saline Soils, Jiangsu Synthetic Innovation Center for Coastal Bio-agriculture, Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection, School of Ocean and Biological Engineering, Yancheng Teachers University, Yancheng, P.R. China

Received: 24.03.2017 • **Accepted/Published Online:** 06.10.2017 • **Final Version:** 10.01.2018

Abstract: The sequences of mitochondrial cytochrome c oxidase subunit I (*CO1*) genes of eight species crabs of Grapsoidea were determined and analyzed in this paper. The traditional classification is that Grapsidae contains four subfamilies comprising Grapsinae, Varuninae, Sesarminae, and Plagusiinae, which are also treated as families in some studies. There is a lack of clarity in the classification of some species, such as *Eriocheir* species. In this study, we conducted the first exploration of evolutionary relationships among species based on analysis of the molecular data of *CO1* genes of eight species (*Sesarmops sinensis*, *Clistocoeloma sinensis*, *Perisesarma bidens*, *Helice latimera*, *Helice tientsinensis*, *Helice wuana*, *Hemigrapsus sanguineus*, and *Varuna litterata*). We provide a detailed and comprehensive view of the phylogenetic relationships of the Grapsoidea species determined by maximum likelihood and Bayesian inference analyses. The results show that *S. sinensis*, *C. sinensis*, and *P. bidens* are part of Sesarmidae, Grapsoidea, while *H. latimera*, *H. tientsinensis*, *H. wuana*, *H. sanguineus*, and *V. litterata* belong to Varunidae, Grapsoidea. The results revealed that Varunidae should be monophyletic and Sesarmidae should be paraphyletic.

Key words: Phylogenetic systematics, Grapsoidea crabs, cytochrome oxidase subunit 1, Sesarmidae, Varunidae

1. Introduction

Decapoda is the most popular, economically important, and species-rich group of all crustaceans, containing approximately 18,000 living and extinct species (De Grave et al., 2009). Numerous analyses of Decapoda have been published based on complete mitochondrial genome sequences (Ma et al., 2015). However, there is still controversy regarding relationships among the major decapod taxa. Grapsidae is in the family Brachyura and the higher taxa in the classification of Decapoda (Schubart et al., 2006). Grapsidae contains four subfamilies comprising Grapsinae, Varuninae, Sesarminae, and Plagusiinae (Niem, 1993; Karasawa and Kato, 2001). However, some studies have treated these four subfamilies as families. Over the last 20 years, the topologies of the molecular trees published concerning Grapsoidea's phylogenetic relationships have been contradictory, especially for *Eriocheir* species (*Eriocheir japonica sinensis, Eriocheir japonica hepuensis*, and *Eriocheir japonica japonica*) (Chu et al., 2003; Tang et al., 2003; Wang et al., 2008).

Due to the high copy number in tissues, haploid nature, and maternal inheritance (Moritz et al., 1987), the cytochrome coxidase subunit I (*CO1*) gene has been favored as a marker in many investigations, including population genetic and systematic studies. The high variability of *CO1* makes it a useful gene for phylogenetic inference and it is often used in preference to the whole mitochondrial genome because of the availability of universal mitochondrial DNA primer sets and the potential to reduce the time required to complete the investigation.

In the present study, we attempted to elucidate the phylogenetic relationships of Grapsoidea species based on *CO1* gene sequences. Numerous Decapoda species were downloaded from GenBank. *Alpheus distinguendus* was used as an outgroup.

2. Materials and methods

2.1. Specimens and DNA extraction

Adult specimens (n = 10) of eight Grapsoidea species were collected from Yancheng, Jiangsu Province, in June 2014. The species were cultured in aerated tap water maintained at 21 ± 1 °C for 1 week before use in experiments. Total genomic DNA was isolated from single specimens of each species using an Aidlab Genomic DNA Extraction Kit (Aidlab, China) according to the manufacturer's instructions. DNA from an individual crab was used for amplification of the complete mitogenomes.

* Correspondence: boptang@163.com

2.2. PCR and DNA sequencing

For amplification of the *CO1* genes of the eight selected Grapsoidea species, universal primers were designed based on the nucleotide sequences of known mitochondrial sequences in Brachyura (Ji et al. 2014). Primers were synthesized by Beijing Sunbiotech (China). The fragments were amplified by PCR using Aidlab Red Taq (Aidlab, China) in a total reaction volume of 50 µL volumes, containing 5 µL 10X Taq plus buffer (Mg^{2+}), 4 µL of dNTPs, 2 µL of each primer, 2 µL of DNA, 34.5 µL of ddH_2O, and 0.5 µL of Red Taq DNA polymerase. The PCR conditions were as follows: 94 °C for 3 min followed by 40 cycles of 30 s at 94 °C, annealing for 35 s at 48–56°C (depending on the primer combination), elongation for 1–3 min (depending on length of the fragments) at 72 °C, and a final extension step of 72 °C for 10 min. The PCR products were detected by 1.0% agarose gel electrophoresis and visualized with ChemiDoc. Gel extraction of clearly visible bands was performed and the PCR products were sequenced by SunBiotech (China).

2.3. Alignments

All *CO1* nucleotide sequence alignments were performed using online MAFFT software (Katoh et al., 2002; Katoh and Standley, 2003). Poorly aligned positions and divergent regions were removed using Gblocks (Castresana, 2000). In our study, the fasta sequences were converted to nex format sequences and phylip format sequences for Bayesian inference (BI) and maximum likelihood (ML) determinations. For saturation detection, DAMBE was used to detect saturated conditions of sequences (Xia and Xie, 2001).

2.4. Phylogenetic analyses

The phylogenetic trees were constructed to estimate the taxonomic status of Grapsoidea species within Decapoda. *CO1* sequences from 49 mitogenome protein coding genes were combined. Datasets were analyzed using two inference methods: BI and ML. BI was performed with MrBayes v3.2.1 (Ronquist et al., 2011). ML was calculated with raxmlGUI (Silvestro and Michalak, 2012). Substitution model selection was performed using Akaike information criteria implemented in MrModeltest v 2.3 (Cui et al., 2011; Zhao and Cui, 2012). The GTR+I+G model was the best model of *CO1* phylogenetic and molecular evolution analyses. BI and ML analyses of *CO1* nucleotide gene sequences of the eight selected Grapsoidea species were performed using the GTRCAT model.

3. Results and discussion

3.1. Cytochrome oxidase subunit 1

The *CO1* genes of eight Grapsoidea species (*S. sinensis*, *C. sinensis*, *H. latimera*, *H. tientsinensis*, *H. sanguineus*, *H. wuana*, *V. litterata*, and *P. bidens*) were sequenced in this paper. As shown in Figure 1, the lengths of sequences were from 1534 bp to 1560 bp. The *CO1* genes sequences of other 40 decapod species were downloaded from GenBank (Table 1). *A. distinguendus* was used as the outgroup.

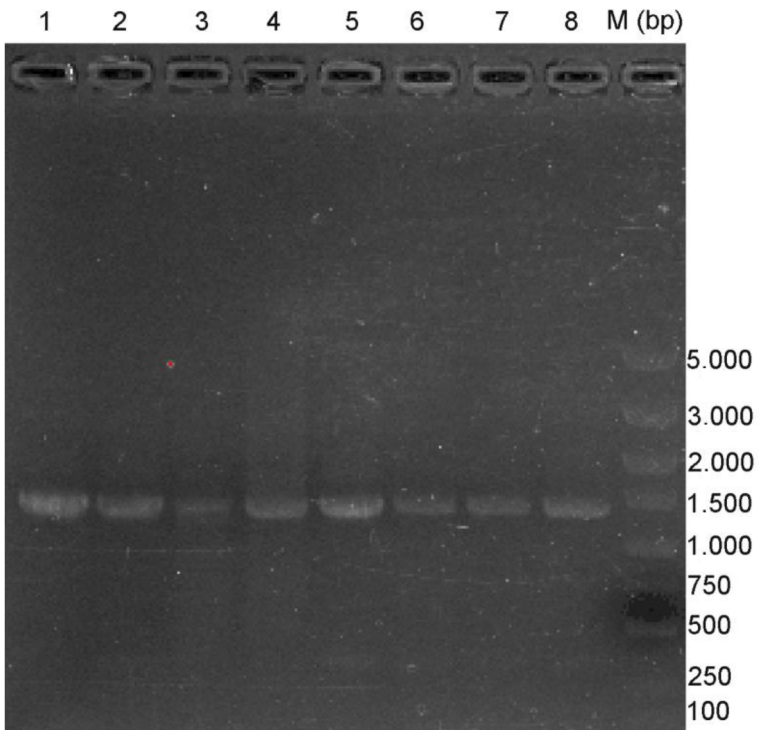

Figure 1. PCR images of eight Grapsoidea species. "1" is *S. sinensis*; "2" is *C. sinensis*; "3" is *P. bidens*; "4" is *H. latimera*; "5" is *H. tientsinensis*; "6" is *H. wuana*; "7" is *H. sanguineus*; "8" is *V. litterata*.

Table 1. Species analyzed in this paper with their GenBank accession numbers.

Species	Family	Size (bp)	Accession no.
Sesarmops sinensis	Sesarmidae	1535	KR336554
Clistocoeloma sinensis	Sesarmidae	1535	KU589292
Helice latimera	Varunidae	1534	KU589291
Helice tientsinensis	Varunidae	1534	KR336555
Hemigrapsus sanguineus	Varunidae	1534	KX456205
Helice wuana	Varunidae	1539	KX344898
Varuna litterata	Varunidae	1534	MF198252
Perisesarma bidens	Sesarmidae	1560	KY808394
Pachygrapsus crassipes	Grapsidae	1534	KC878511
Eriocheir japonica sinensis	Varunidae	1534	KM516908
Eriocheir japonica hepuensis	Varunidae	1534	FJ455506
Eriocheir japonica japonica	Varunidae	1534	FJ455505
Hemigrapsus nudus	Varunidae	1070	AF060775
Portunus pelagicus	Portunidae	1534	KM977882
Callinectes sapidus	Portunidae	1534	AY682073
Portunus trituberculatus	Portunidae	1534	AB093006
Portunus sanguinolentus	Portunidae	1534	KT438509
Charybdis japonica	Portunidae	1534	FJ460517
Scylla paramamosain	Portunidae	1535	JX457150
Scylla olivacea	Portunidae	1534	FJ827760
Scylla tranquebarica	Portunidae	1534	FJ827759
*Scylla serr*ata	Portunidae	1534	FJ827758
Charybdis feriata	Portunidae	1534	KM977705
Umalia orientalis	Raninidae	1534	KM365084
Lyreidus brevifrons	Raninidae	1534	KM983394
Ranina ranina	Raninidae	1534	KM189817
*Gandalfus yunoh*ana	Bythograeidae	1534	EU647222
Gandalfus puia	Bythograeidae	1534	KR002727
*Austinograea alays*eae	Bythograeidae	1534	JQ035660
*Austinograea rodriguezen*sis	Bythograeidae	1534	JQ035658
*Ilyoplax deschamps*i	Dotillidae	1534	JF909979
Xenograpsus testudinatus	Xenograpsidae	1534	EU727203
Homologenus malayensis	Homolidae	1534	KJ612407
Pseudocarcinus gigas	Menippidae	1534	AY562127
Damithrax spinosissimus	Mithracidae	1537	KM405516
Geothelphusa dehaani	Potamidae	1539	AB187570
Neotiwaripotamon jianfengense	Potamidae	1113	KT586040
Longpotamon siguqiaoense	Potamidae	1113	KT585964
Sinopotamon davidi	Potamidae	1113	KT585846
Sinopotamon yaanense	Potamidae	1113	KT586017
Longpotamon linhuaense	Potamidae	1113	KT585917
Sinopotamon chishuiense	Potamidae	1113	KT585828
Sinopotamon fuxingense	Potamidae	1113	KT585876
Sinopotamon emeiense	Potamidae	1113	KT585864
Longpotamon turgidum	Potamidae	1113	KT585984
Longpotamon jiangxianense	Potamidae	1113	KT585898
Longpotamon xiangxiense	Potamidae	1113	KT586005
Longpotamon huitongense	Potamidae	1113	KT585887
Alpheus distinguendus	Alpheidae	1534	NC_014883

3.2. Base composition and sequence alignment

The base compositions of the *CO1* genes of the eight selected Grapsoidea species investigated in this study are shown in Figure 2. Base contents were calculated by means of artificial statistics. The AT contents were higher than GC contents. Nucleotide and amino acid sequence alignments are shown in Figures S1 and S2. The synonymous and nonsynonymous distances for each pair of species are shown in Table 2.

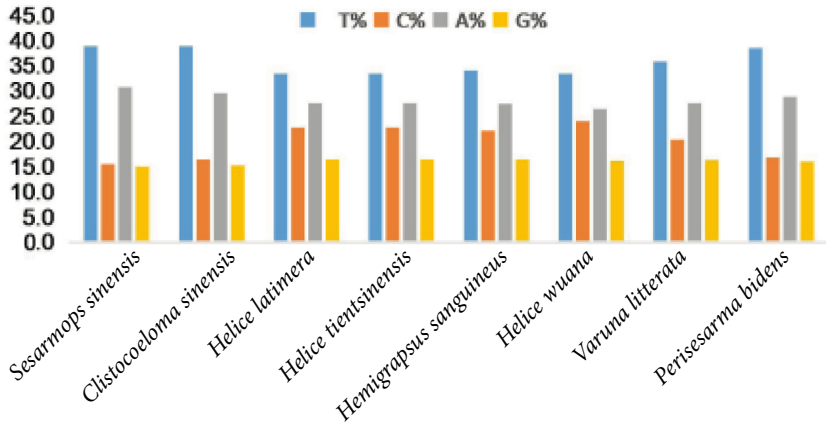

Figure 2. Base composition of the *CO1* genes of eight Grapsoidea species.

Table 2. The synonymous and nonsynonymous distances of each pair of species.

Species 1	Species 2	Ks	Ka
Sesarmops sinensis	*Clistocoeloma sinensis*	0.5328	0.0105
Sesarmops sinensis	*Helice latimera*	1.2045	0.0330
Sesarmops sinensis	*Helice tientsinensis*	1.2188	0.0339
Sesarmops sinensis	*Hemigrapsus sanguineus*	1.1454	0.0348
Sesarmops sinensis	*Helice wuana*	1.5259	0.0339
Sesarmops sinensis	*Varuna litterata*	1.0721	0.0347
Sesarmops sinensis	*Perisesarma bidens*	0.6523	0.0144
Clistocoeloma sinensis	*Helice latimera*	1.4718	0.0330
Clistocoeloma sinensis	*Helice tientsinensis*	1.4738	0.0339
Clistocoeloma sinensis	*Hemigrapsus sanguineus*	1.3194	0.0380
Clistocoeloma sinensis	*Helice wuana*	1.5554	0.0375
Clistocoeloma sinensis	*Varuna litterata*	1.1409	0.0352
Clistocoeloma sinensis	*Perisesarma bidens*	0.6135	0.0162
Helice latimera	*Helice tientsinensis*	0.0025	0.0009
Helice latimera	*Hemigrapsus sanguineus*	1.1166	0.0204
Helice latimera	*Helice wuana*	1.0013	0.0177
Helice latimera	*Varuna litterata*	1.1956	0.0240
Helice latimera	*Perisesarma bidens*	1.7926	0.0267
Helice tientsinensis	*Hemigrapsus sanguineus*	1.1291	0.0195
Helice tientsinensis	*Helice wuana*	1.0119	0.0186
Helice tientsinensis	*Varuna litterata*	1.1969	0.0249
Helice tientsinensis	*Perisesarma bidens*	1.7958	0.0276
Hemigrapsus sanguineus	*Helice wuana*	1.1184	0.0267
Hemigrapsus sanguineus	*Varuna litterata*	1.1434	0.0298
Hemigrapsus sanguineus	*Perisesarma bidens*	1.2881	0.0374
Helice wuana	*Varuna litterata*	1.2045	0.0253
Helice wuana	*Perisesarma bidens*	1.6174	0.0278
Varuna litterata	*Perisesarma bidens*	1.0745	0.0297

3.3. Phylogenetic analyses

ML and BI analyses revealed a largely congruent pattern of the *CO1* genes among Decapoda. Traditionally, Grapsidae is divided into four subfamilies: Grapsinae, Varuninae, Sesarminae, and Plagusiinae (Niem, 1993; Brossi-Garcia and Rodrigues, 1997; Cuesta and Schubart, 1999; Karasawa and Kato, 2001). However, there is controversy regarding the relationships among the subfamilies based on larval morphology (Pereyra, 1993; Schubart and Cuesta, 1998). In the present study, we investigated a limited number of Grapsoidea species, representing only the families Sesarmidae and Varunidae, which formed the focus of the subsequent investigations.

3.4. Relationships within Sesarmidae

Three species (*S. sinensis*, *C. sinensis*, and *P. bidens*) showed a clear close relationship and seemed to hold an independent position, with the closest apparent alignment with the family Sesarmidae. *S. sinensis*, *C. sinensis*, and *P. bidens* were clustered in a single branch (Figure 3) in the phylogenetic tree with high nodal support values

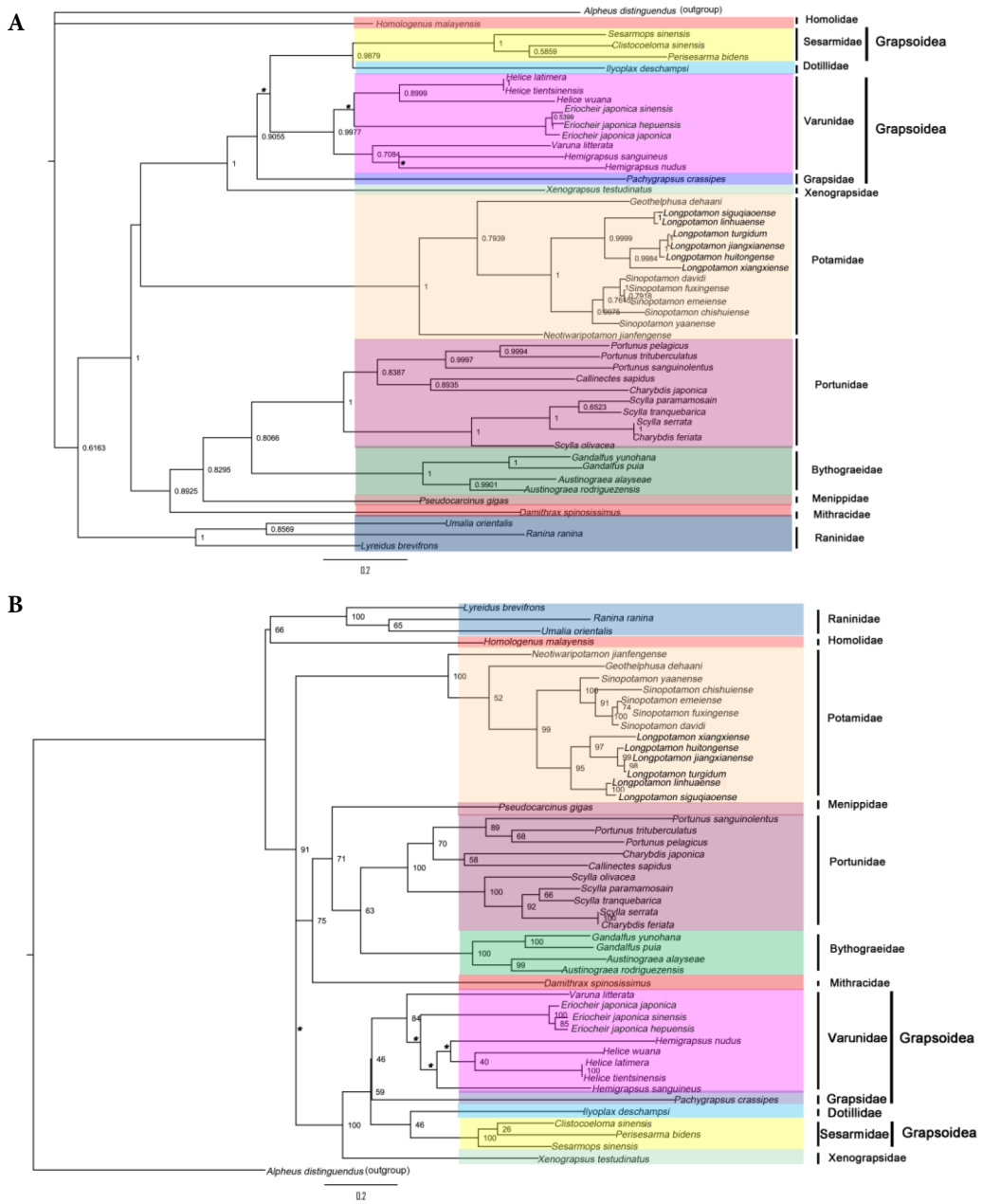

Figure 3. Inferred phylogenetic relationships based on nucleotide sequence of mitochondrial *CO1* genes using BI (A) and ML (B) analyses. *A. distinguendus* was used as the outgroup.

determined based on *CO1* genes using BI and ML analyses. Thus, *S. sinensis*, *C. sinensis*, and *P. bidens* should be attributed to Sesarmidae, Grapsoidea, despite belonging to different genera. *S. sinensis* and *P. bidens* belong to *Sesarma*, while *C. sinense* belongs to *Clistocoeloma*. *Sesarma* and *Clistocoeloma* are part of Sesarmidae, Grapsoidea. These findings are in keeping with previous reports (Von Hagen, 1978; Tang et al., 2017; Xin et al., 2017a) and confirm that the family Sesarmidae is polyphyletic.

3.5. Relationships within Varuninae

The phylogenetic tree shows that *H. latimera* and *H. tientsinensis* are the most closely related taxa, with *H. wuana* being the sister taxon. *H. sanguineus* and *Hemigrapsus nudus* are clustered in a single branch (Figure 3) with lower nodal support values determined based on *CO1* genes using BI and ML analyses. Although the node support value is low, *H. sanguineus* and *H. nudus* form a single clade, possible due to the shared gene block. Evidence clearly indicates that the clade formed by *H. sanguineus* + *H. nudus* is the sister group to the *V. litterata* clade. Furthermore, the [(*H. latimera* + *H. tientsinensis*) + *H. wuana*)] clade and the [(*H. sanguineus* + *H. nudus*) + *V. litterata*)] clades form sister groups with *E. j. sinensis*, *E. j. hepuensis*, and *E. j. japonica.* These species belong to Varunidae, Grapsoidea. *H. latimera*, *H. tientsinensis*, and *H. wuana* are part of *Helice*, Varunidae, Grapsoidea, while *V. litterata* belongs to *Varuna,* Varunidae, Grapsoidea and *H. sanguineus* belongs to *Hemigrapsus,* Varunidae, Grapsoidea.

We present evidence that both *Helice* species are significantly closer to most Varunidae genera than to the Sesarmidae based on the *CO1* genes sequences, which is in accordance with the findings reported by Guinot (1978) and Schubart et al. (1998, 2000). Preliminary results show that the genus *Varuna* clusters with the other Varunidae species based on its 16S rRNA sequence (Schubart et al., 1998, 2000). The results indicate that *Varuna* belongs to the family Varunidae, which is in accordance with the findings reported by Schubart et al. (1998, 2000). *Hemigrapsus* is most closely related to *Varuna.* Our results are consistent with those given in previous studies (McDermott, 1998; Liu et al., 2015; Xin et al., 2017b).

3.6. Other species

The phylogenetic tree constructed as part of this study shows some crab species are closely related to Grapsoidea species. *Pachygrapsus crassipes* belongs to the family Grapsidae (Cuesta and Schubart, 1999). The phylogenetic position of *Ilyoplax deschampsi* is within Grapsoidea even after eliminating the effect of LBA (Ji et al., 2014). Previous studies have shown that *Ilyoplax deschampsi* is part of Dotillidae in ambiguous classification; however, the results show that *Ilyoplax deschamps* belongs to Grapsoidea with high nodal support values determined based on nucleotide sequences of *CO1* genes. Furthermore, *Xenograpsus testudinatus*, which was originally placed in Varunidae, has been transferred to its own family (Xenograpsidae) (Ng et al., 2007). Construction of the evolutionary tree clearly shows that the orders Dotillidae and Xenograpsidae are sister groups of Grapsoidea. This finding is consistent with those given in other studies (Ng et al., 2008).

In conclusion, the *CO1* genes of eight Grapsoidea species (*S. sinensis*, *C. sinensis*, *P. bidens*, *H. latimera*, *H. tientsinensis*, *H. wuana*, *H. sanguineus*, and *V. litterata*) were sequenced and analyzed in this study. The sequences of these *CO1* genes exhibited a highly A + T bias. The phylogenetic analyses indicate that *S. sinensis*, *C. sinensis*, and *P. bidens* should be attributed to the family Sesarmidae. *H. latimera*, *H. tientsinensis*, *H. wuana*, *V. litterata*, and *H. sanguineus* belong to the family Varunidae. Sesarmidae and Varunidae should belong to Grapsoidea.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31672267 and 31640074), the Natural Science Foundation of Jiangsu Province (BK20160444), the Natural Science Research General Program of Jiangsu Provincial Higher Education Institutions (15KJB240002, 12KJA180009 and 16KJA180008), Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection (JLCBE14006), the Special Guide Fund Project of Agricultural Science and Technology Innovation of Yancheng city (YKN2014022), and the Jiangsu Provincial Key Laboratory for Bioresources of Saline Soils (JKLBS2014013 and JKLBS2015004).

References

Brossi-Garcia AL, Rodrigues MD (1997). Zoeal morphology of *Pachygrapsus transversus* (Gibbes) (Decapoda, Grapsidae) reared in the laboratory. Rev Bras Zool 14: 803-819.

Castresana J (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17: 540-552.

Chu KH, Ho HY, Li CP, Chan TY (2003). Molecular phylogenetics of the mitten crab species in *Eriocheir*, sensu lato (Brachyura: Grapsidae). J Crustacean Biol 23: 738-746.

Cuesta JA, Schubart CD (1999). First zoeal stages of *Geograpsus lividus* and *Goniopsis pulchra* from Panama confirm consistent larval characters for the subfamily Grapsinae (Crustacea: Brachyura: Grapsidae). Ophelia 51: 163-176.

Cui BK, Tang LP, Dai YC (2011). Morphological and molecular evidences for a new species of Lignosus (Polyporales, Basidiomycota) from tropical China. Mycol Prog 10: 267-271.

De Grave S, Pentcheff ND, Ahyong ST, Chan TY, Crandall KA, Dworschak PC, Felder DL, Feldmann RM, Fransen CHJM, Goulding LYD et al. (2009). A classification of living and fossil genera of decapod crustaceans. Raffles B Zool 21: 1-109.

Guinot D (1978). Principes d'une classification évolutive des Crustacés Décapodes Brachyoures. Bulletin Biologique de la France et de la Belgique 112: 211-292.

Ji YK, Wang A, Lu XL, Song DH, Jin YH, Lu JJ, Sun HY (2014). Mitochondrial genomes of two brachyuran crabs (Crustacea: Decapoda) and phylogenetic analysis. J Crustacean Biol 34: 494-503.

Karasawa H, Kato H (2001). The systematic status of the genus *Miosesarma* Karasawa, 1989 with a phylogenetic analysis within the family Grapsidae and a review of fossil records (Crustacea: Decapoda: Brachyura). Paleontol Res 5: 259-275.

Katoh K, Misawa K, Kuma K, Miyata T (2002). MAFFT a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30: 3059-3066.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30: 772-780.

Liu QN, Zhang HB, Jiang SH, Xuan FJ, Li CF, Zhang DZ, Zhou CL, Tang BP (2015). The complete mitochondrial genome of *Eriocheir japonica sinensis* (Decapoda Varunidae) and its phylogenetic analysis. Biochem Syst Ecol 62: 241-248.

Ma HY, Ma CY, Li CH, Lu JX, Zou X, Gong YY, Wang W, Chen W, Ma LB, Xia LJ (2015). First mitochondrial genome for the red crab (*Charybdis feriata*) with implication of phylogenomics and population genetics. Sci Rep 5: 11524.

McDermott JJ (1998). The western Pacific brachyuran *Hemigrapsus sanguineus* (Grapsidae) in its new habitat along the Atlantic coast of the United States reproduction. J Crustacean Biol 18: 308-316.

Moritz C, Dowling TE, Brown WM (1987). Evolution of animal mitochondrial DNA relevance for population biology and systematics. Annu Rev Ecol Syst 18: 269-292.

Ng NK, Davie PJF, Schubart CD (2007). Xenograpsidae, a new family of crabs (Crustacea: Brachyura) associated with shallow water hydrothermal vents. Raffles B Zool Supplement 16: 233-256.

Ng PKL, Guinot D, Davie PJF (2008). Systema Brachyurorum Part I. An annotated checklist of extant brachyuran crabs of the world. Raffles B Zool 17: 1-286.

Niem VH (1993). Phylogenetic and systematic position of *Sesarma rubripes* Rathbun, 1897 (Brachyura: Grapsidae). Zool Med Leiden 67: 185-195.

Pereyra Lago R (1993). The zoeal development of *Sesarma eulimene* de Man (Decapoda, Brachyura, Grapsidae), and identification of larvae of the genus *Sesarma* in South African waters. S Afr J Zool 28: 173-181.

Ronquist F, Huelsenbeck JP, Teslenko M (2011). Draft MrBayes version 3.2 Manual. Tutorials and Model Summaries.

Schubart CD, Cannicci S, Vannini M, Fratini S (2006). Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current superfamily classification. J Zool Syst Evol Res 44: 193-199.

Schubart CD, Cuesta JA (1998). First zoeal stages of four *Sesarma* species from Panama, with identification keys and remarks on the American Sesarminae (Crustacea: Brachyura: Grapsidae). J Plankton Res 20: 61-84.

Schubart CD, Diesel R, Hedges SB (1998). Rapid evolution to terrestrial life in Jamaican crabs. Nature 393: 363-365.

Schubart CD, Neigel JE, Felder DL (2000). Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. Crustacean Issues 12: 817-830.

Silvestro D, Michalak I (2012). raxmlGUI a graphical front-end for RAxML. Org Divers Evol 12: 335-337.

Tang BP, Xin ZZ, Liu Y, Zhang DZ, Wang ZF, Zhang HB, Chai XY, Zhou CL, Liu QN (2017). The complete mitochondrial genome of *Sesarmops sinensis* reveals gene rearrangements and phylogenetic relationships in Brachyura. PLOS ONE 12: e0179800.

Tang BP, Zhou KY, Song DX, Yang G, Dai AY (2003). Molecular systematics of the Asian mitten crabs, genus *Eriocheir* (Crustacea; Brachyura). Mol Phylogenet Evol 29: 309-316.

Von Hagen HO (1978). The systematic position of *Sesarma* (*Sesarma*) *rectum* Randall and a new definition of the subgenus *Sesarma* (Brachyura, Grapsidae). Crustaceana 34: 4-54.

Wang CH, Li CH, Li SF (2008). Mitochondrial DNA-inferred population structure and demographic history of the mitten crab (*Eriocheir sensu stricto*) found along the coast of mainland China. Mol Ecol 17: 3515-3527.

Xia X, Xie Z (2001). DAMBE software package for data analysis in molecular biology and evolution. J Hered 92: 371-373.

Xin ZZ, Liu Y, Zhang DZ, Chai XY, Wang ZF, Zhang HB, Zhou CL, Tang BP, Liu QN (2017a). Complete mitochondrial genome of *Clistocoeloma sinensis* (Brachyura: Grapsoidea): gene rearrangements and higher-level phylogeny of the Brachyura. Sci Rep 7: 4128.

Xin ZZ, Liu Y, Zhang HB, Zhang DZ, Zhou CL, Tang BP, Liu QN (2017b). Mitochondrial genome of *Helice tientsinensis* (Brachyura: Grapsoidea: Varunidae): gene rearrangements and higher-level phylogeny of the Brachyura. Gene 627: 307-314.

Zhao CL, Cui BK (2012). A new species of Perenniporia (Polyporales, Basidiomycota) described from southern China based on morphological and molecular characters. Mycol Prog 11: 555-560.

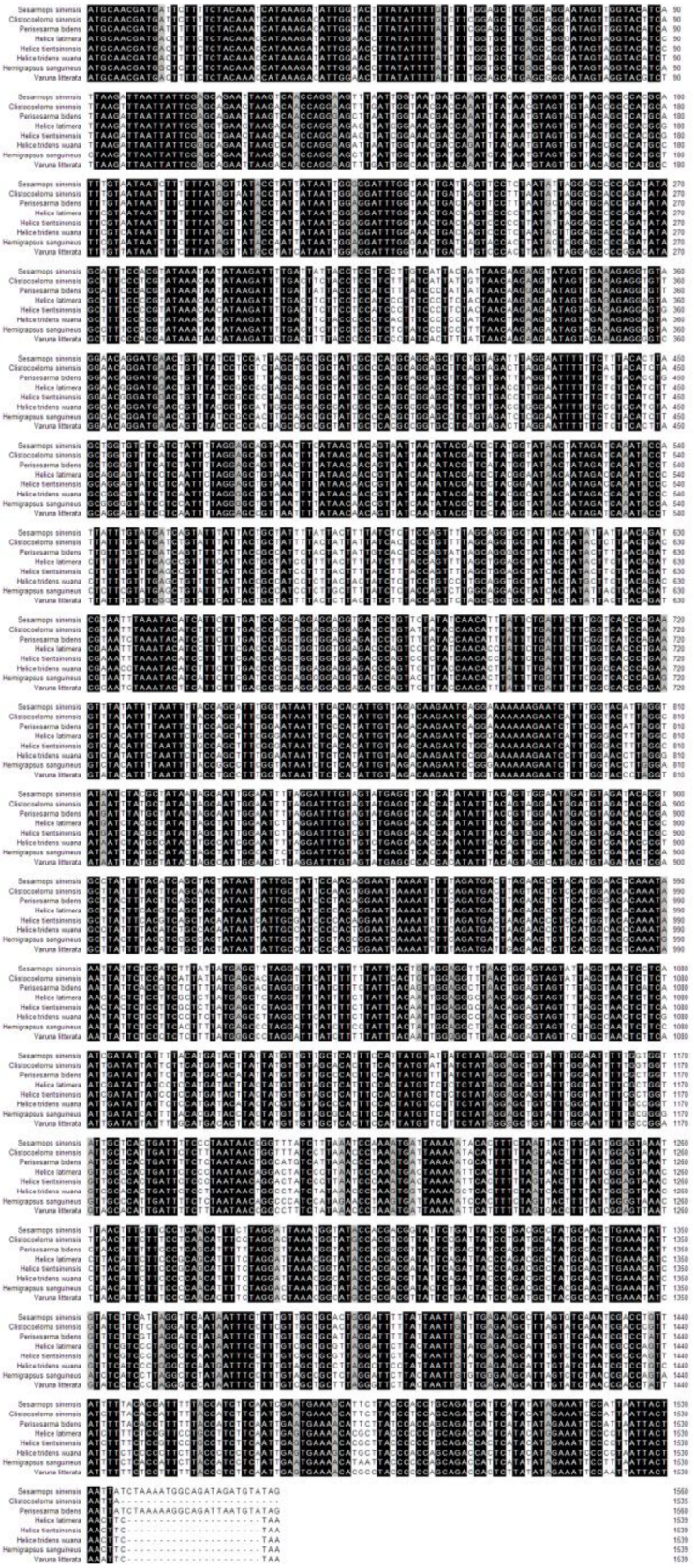

Figure S1. Nucleotide sequences alignment information of the *CO1* genes of eight Grapsoidea species.

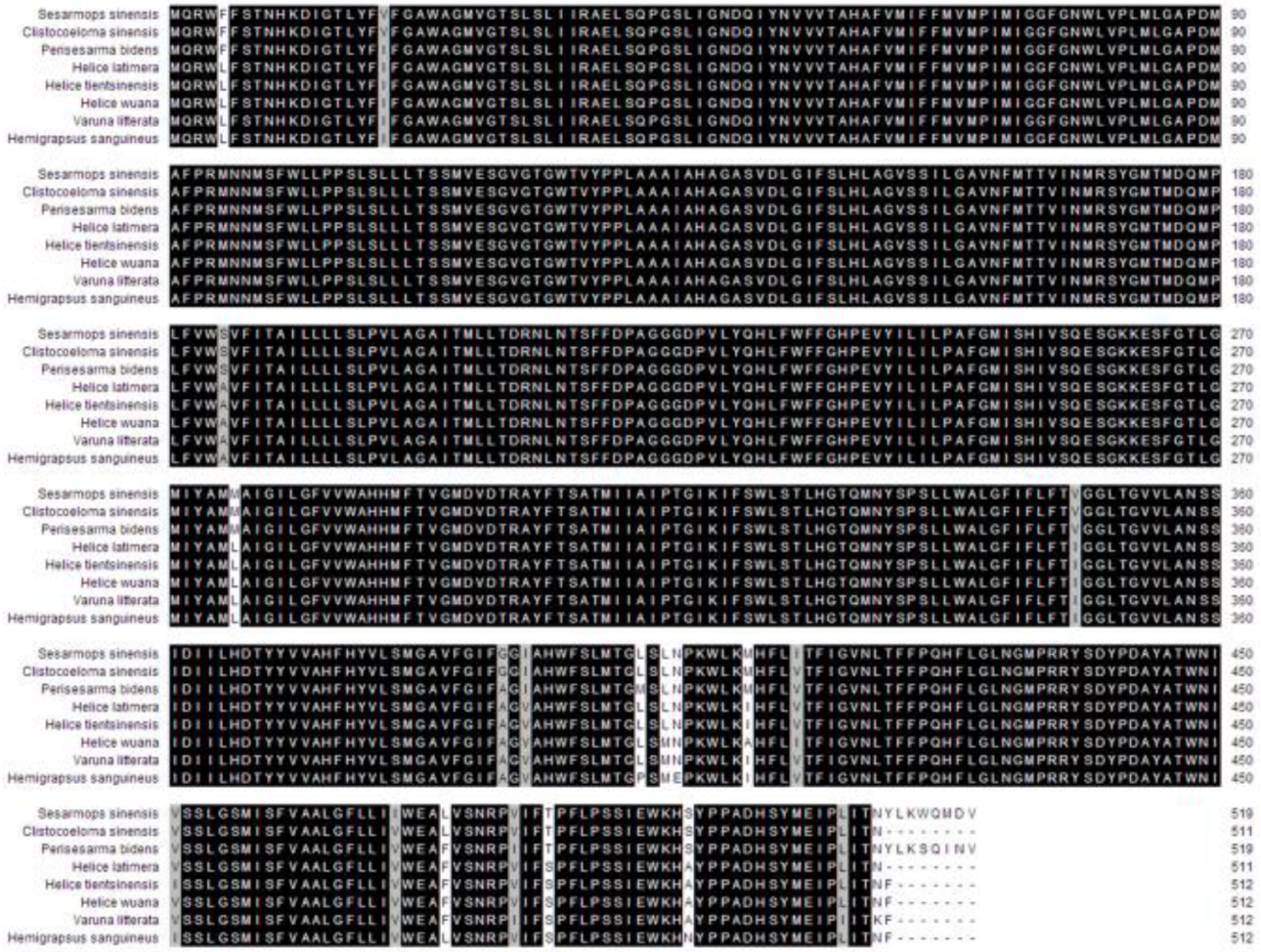

Figure S2. Amino acid sequences alignment information of the *CO1* genes of eight Grapsoidea species.