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Molecular Phylogeny, Taxonomy, and Evolution of Nonmarine Lineages within the American Grapsoid Crabs (Crustacea: Brachyura)

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Grapsoid crabs are best known from the marine intertidal and supratidal. However, some species also inhabit shallow subtidal and freshwater habitats. In the tropics and subtropics, their distribution even includes mountain streams and tree tops. At present, the Grapsoidea consists of the families Grapsidae, Gecarcinidae, and Mictyridae, the first being subdivided into four subfamilies (Grapsinae, Plagusiinae, Sesarminae, and Varuninae). To help resolve phylogenetic relationships among these highly adaptive crabs, portions of the mitochondrial genome corresponding to the 16S rRNA gene were sequenced for all grapsoid genera occurring in America. The resulting phylogeny confirms most of the present grapsid subfamilies but suggests reclassification of some of the genera and recognition of new taxonomic units. The two American gecarcinid genera might not represent a sister group to the Grapsidae but rather appear to have evolved within the latter. Colonization of inland habitats evolved in several lineages of the grapsoids, resulting in various forms of nonmarine life and different degrees of independence from the sea. © 2000 Academic Press

INTRODUCTION

Crabs of the superfamily Grapsoidea are presently subdivided into the families Grapsidae Macleay, 1838, Gecarcinidae Macleay, 1838, and Mictyridae Dana, 1851 (see Bowman and Abele, 1982). These families consist mostly of high intertidal to supratidal forms but also include several species that have successfully colonized limnic (e.g., *Eriocheir* spp., *Varuna* spp.) and terrestrial (e.g., *Gecarcinus* spp., *Geosesarma* spp.) habitats. Many Gecarcinidae species can be found up to several kilometers from the sea, migrating back only during the reproductive season (Bliss, 1968; Burggren and McMahon, 1988). Adaptation to inland life is even more completely achieved in several grapsid species (e.g., Jamaican and Southeast Asian Sesarminae) that have attained lifelong independence from the sea (Hartnoll, 1964; Diesel, 1989; Ng and Tan, 1995; Table 1).

The Grapsidae and Gecarcinidae have an almost worldwide distribution, being most predominant and species rich in subtropical and tropical regions. Overall, there are 57 grapsid genera with approximately 400 recognized species (Schubart and Cuesta, unpubl. data) and 6 gecarcinid genera with 18 species (Türkay, 1983; Tavares, 1991). The Mictyridae consists of a single genus and currently 4 recognized species restricted to the Indo-West Pacific (P. J. F. Davie, pers. commun., 1999). The Grapsidae commonly are subdivided into four subfamilies: Grapsinae Macleay, 1838; Plagusiinae Dana, 1851; Sesarminae Dana, 1851; and Varuninae H. Milne Edwards, 1853. This taxonomic classification has remained unmodified since Alcock (1900) and was adopted in subsequent systematic reviews and monographs of this family (e.g., Rathbun, 1918; Balss, 1957; Guinot, 1979). The classification of the grapsid genera within these four subfamilies also has remained unchanged and rarely has been questioned (but see Wilson and Gore, 1978; Cuesta and Schubart, 1997; Schubart and Cuesta, 1998; Guinot and Bouchard, 1998). Shortly after Dana's (1851) recognition of three subfamilies, H. Milne Edwards (1853) proposed an alternative taxonomy, in which he subdivided the grapsoid crabs (considered by him as a tribe) into agèles: Grapsacaea, Varunacaea, Cyclograpsacaea, Sesarmacaea, Plagusiacaea, and Gecarcinacaea. He thus placed the gecarcinid crabs at the same taxonomic level as the current grapsid subfamilies. A close relationship of the Gecarcinidae to the Grapsidae was always assumed (see Alcock, 1990; Türkay, 1983) and is reflected in the common placement of these two families within the superfamily Grapsoidea (as Grapsidoidea in Bowman and Abele, 1982).

Currently, characters used to distinguish the two families and the four grapsid subfamilies are based on adult morphology, some of them being defined ambiguously or based upon possibly plesiomorphic or conver-

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TABLE 1

Adult Habitat, Maximum Distance from the Sea, Form of Larval Development/Number of Larval Stages, and Inland Life Form Reported for American Grapsoid Crab Genera

Genus	Adult habitat	Max. distance from sea	Larval develop.	Nonmarine life form
Geograpsus	Supratidal-terrestrial	1 km	m-p/?	ad-terr
Goniopsis	Supratidal	<100 m	m-p/?	—
Grapsus	Intertidal	<10 m	m-p/?	—
Leptograpsus	Intertidal	<10 m	m-p/?	—
Pachygrapsus	Intertidal-supratidal	<100 m	m-p/6	—
Planes	Pelagic	0 m	m-p/?	—
Percnon	Shallow subtidal	0 m	m-p/6?	—
Plagusia	Intertidal-subtidal	<10 m	m-p/12?	—
Aratus	Supratidal	<100 m	m-p/4	—
Armases	Supratidal-limnic	32 km	m-p/3–4	ad-limn
Chasmagnathus	Intertidal	<100 m	m-p/4–5	—
Cyclograpsus	Intertidal	<100 m	m-p/5	—
Metopaulias	Limnic-terrestrial	40 km	f-l/2	ent-inl
Sesarma (Jamaica)	Limnic-terrestrial	40 km	f-l/2	ent-inl
Sesarma	Supratidal-limnic	30 km	m-p/2–3	ad-limn
Cyrtograpsus	Intertidal-subtidal	<10 m	m-p/5	—
Eriocheir	Limnic	1300 km	m-p/5	ad-limn
Euchirograpsus	Subtidal	0 m	m-p/5	—
Gaetice	Intertidal	<10 m	m-p/5	—
Glyptograpsus	Limnic	3.5 km	m-p/?	ad-limn
Hemigrapsus	Intertidal	<10 m	m-p/5	—
Platychirograpsus	Limnic	225 km	m-p/?	ad-limn
Tetragrapsus	Intertidal	<10 m	m-p?/?	—
Cardisoma	Terrestrial	7 km	m-p/6	ad-terr
Gecarcinus	Terrestrial	20 km	m-p/6	ad-terr

Note. Data based on Abele (1992), Cuesta and Schubart (1997), Diesel and Schuh (1998), Gilchrist (1988), Schubart and Cuesta (1998), and personal observations. Abbreviations: m-p, marine planktonic; f-l, freshwater lecithotrophic; ?, unknown or questionable; ad-terr, adults terrestrial; ad-limn, adults limnic; ent-inl, entirely inland.

gent characters (see key in Balss, 1957). Recent studies of comparative larval morphology among the Grapsidae show diagnostic characters for the Grapsinae (see Cuesta and Schubart, 1999) but variable setation patterns within the other three subfamilies (Wear, 1970; Wilson, 1980; Terada, 1982; Pereyra Lago, 1993; Cuesta and Schubart, 1997; Schubart and Cuesta, 1998). Diagnostic larval characters for the Sesarminae, Varuninae, and Plagusiinae can be defined only if some genera are reclassified (Cuesta and Schubart, 1997; Schubart and Cuesta, 1998; Cuesta *et al.*, unpublished).

The American crab fauna includes representatives of the Gecarcinidae and all four subfamilies of the Grapsidae. In the present study, phylogenetic relationships within all gecarcinid and grapsid crab genera reported from continental America were established based on DNA sequence of the mitochondrial large subunit ribosomal RNA (16S rRNA) gene. We addressed the questions whether present taxonomic relationships within the Gecarcinidae and Grapsidae are supported by molecular systematics and how often evolution to terrestrial life has occurred independently within the American Grapsoidea.

MATERIALS AND METHODS

Crabs for this study were collected between 1993 and 1999 (see Table 2 for localities and taxonomic classification). Specimens from western Mexico, Argentina, and Chile were donated. All material was preserved in 75–95% ethanol. Only one grapsid genus reported from continental America, the monotypic *Grapsodius eximius*, could not be taken into account. This species was described without illustrations (Holmes, 1900). The holotype was deposited at the California Academy of Sciences and was totally destroyed during the earthquake and fire of 1906, together with other specimens examined by Holmes. It is therefore impossible to examine Holmes' material, and no specimens have been reported thereafter that match the original description. The holotype may have been an aberrant specimen of *Pachygrapsus* or *Planes* (Mary Wicksten, pers. commun., 1996). We did include the pelagic genus *Planes*, which is occasionally washed ashore on American beaches, and the mitten crab (genus *Eriocheir*), which has been introduced to several North American habitats and is established in San Francisco Bay (Cohen and Carlton, 1997). As an outgroup sequence for this

TABLE 2

Grapsoid Crab Species Used in This Study for Phylogeny Reconstructions (Classified in Accordance with Present Taxonomy), with Locality of Collection, Museum Catalog Number, and Genetic Database (EMBL) Accession No.

Species	Collection site	Catalogue no.	EMBL Accession No.
GRAPSIDAE Macleay, 1838			
Grapsinae Macleay, 1838			
Geograpsus lividus (H. Milne Edwards, 1837)	Mexico: Veracruz	ULLZ 3711	AJ250651
Goniopsis cruentata (Latreille, 1803)	Mexico: Veracruz	ULLZ 3712	AJ250652
Grapsus grapsus (Linnaeus, 1758)	Mexico: Veracruz	ULLZ 3709	AJ250650
Leptograpsus variegatus (Fabricius, 1793)	Chile: Coquimbo	SMF 24561	AJ250654
Pachygrapsus transversus (Gibbes, 1850)	Mexico: Tamaulipas	ULLZ 3723	AJ250641
Planes minutus (Linnaeus, 1758)	Spain: off Cádiz	ULLZ 4176	AJ250653
Plagusiinae Dana, 1851			
Percnon gibbesi (H. Milne Edwards, 1853)	Puerto Rico: north coast	R 153	AJ130803
Plagusia depressa (Fabricius, 1775)	Jamaica: north coast	ULLZ 3813	AJ250649
Sesarminae Dana, 1851			
Aratus pisonii (H. Milne Edwards, 1837)	Florida: Tampa Bay	ULLZ 3838	AJ250638
Armases ricordi (H. Milne Edwards, 1853)	Mexico: Veracruz	ULLZ 3697	AJ250637
Chasmagnathus granulata Dana, 1851	Argentina: Mar Chiquita	SMF 24547	AJ250640
Cyclograpsus integer (H. Milne Edwards, 1837)	Mexico: Veracruz	ULLZ 3704	AJ250639
Metopaulias depressus Rathbun, 1896	Jamaica: Dolphin Head	SMF 24571	AJ250636
Sesarma reticulatum (Say, 1817)	Delaware: Woodland Beach	ULLZ 3835	AJ130799
Varuninae H. Milne Edwards, 1853			
Cyrtograpsus affinis (Dana 1851)	Argentina: Rio de la Plata	SMF 24545	AJ130801
Eriocheir sinensis (H. Milne Edwards, 1853)	Florida: San Francisco	ULLZ 4175	AJ250642
Euchirograpsus americanus A. Milne Edwards, 1880	Louisiana: Sackett Bank	ULLZ 3626	AJ250648
Gaetice americanus Rathbun, 1923	Mexico: Baja California	ULLZ 4106	AJ250643
Glyptograpsus impressus Smith, 1870	Panama: Farfán Beach	USNM 284160	AJ250646
Hemigrapsus oregonensis (Dana, 1851)	Washington: Friday Harbor	ULLZ 3794	AJ250644
Platychirograpsus spectabilis de Man, 1896	Florida: Tampa Bay	SMF 24567	AJ250645
Tetragrapsus jouyi (Rathbun, 1893)	Mexico: Baja California	ULLZ 4155	AJ250647
GECARCINIDAE Macleay, 1838			
Cardisoma crassum Smith, 1870	Costa Rica: Rincón	SMF 24543	AJ130805
Gecarcinus lateralis (Freminville, 1835)	Mexico: Veracruz	ULLZ 3722	AJ130804

Note. R, Collection Rudolf Diesel, Starnberg; SMF, Senckenberg Museum, Frankfurt a.M.; ULLZ, University of Louisiana at Lafayette Zoological Collection, Lafayette; USNM, Smithsonian Institution and National Museum of Natural History, Washington, D.C.

study, we used DNA of *Palicus obesus* (family Palicidae) (EMBL AJ130808). Based on the 16S rRNA gene, the Palicidae hold a basal position with respect to the Thoracotremata, a taxon to which the Grapsoidea, Ocypodoidea, and other crabs belong (Schubart *et al.*, 2000).

DNA was isolated from muscle tissue of walking legs or claws using a phenol–chloroform extraction (Kocher *et al.*, 1989). Selective amplification of a fragment of the 16S rRNA gene was carried out by polymerase chain reaction (PCR) with the primers 16Sar (5′-CGCCTGTT-TATCAAAAACAT-3′), 16Sbr (5′-CCGGTCTGAACTCA-GATCACGT-3′), 16L12 (5′-TGACCGTGCAAAGGTAG-CATAA-3′), and 16H16 (5′-TTATCRCCCCAATAAAATA-3′) (PCR conditions: 33–40 cycles of 1 min 94°C/1 min 50–55°C/2.5 min 72°C denaturing/annealing/extension temperatures). Single-stranded products were used for manual sequencing by dideoxy chain termination with S35 radioactive labeling. All sequences were later verified and extended with the reverse primer 1472 (5′-AGATAGAAACCAACCTGG-3′) (see Schubart *et al.*, 2000: Table 3). In the latter case, PCR products were purified with Microcon 100 filters prior to automatic sequencing with the ABI BigDye terminator mix in an ABI Prism 310 Genetic Analyzer.

Sequences were aligned manually with the multisequence editing program ESEE (Cabot and Beckenbach, 1989) taking into account the secondary structure of the gene (Schneider-Broussard and Neigel, 1997; Appendix 1). Variable regions that could not be aligned with complete certainty, as well as positions with compensatory mutations in stem regions, were excluded from phylogenetic analyses (see Appendix 1). We used Kimura two-parameter distances (s + v) and neighbor-joining (NJ) to analyze distance matrices of sequence divergence with the program MEGA (Kumar *et al.*, 1993). Statistical significance of groups within inferred trees was evaluated by the interior branch method (Rzhetsky and Nei, 1992). A maximum-parsimony (MP) tree was constructed using the program

PAUP (Swofford, 1993) and 2000 bootstrap replicates of a heuristic search with random sequence addition and tree bisection and reconnection as branch swapping option. We repeated the MP several times with different weightings of transitions versus transversions (s/v = 1/2, 1/3, 1/5, 0/1) and compared the respective results. Gaps were always treated as missing.

RESULTS

The total alignment of the sequenced 16S mtDNA region consisted of 589 positions, not including the primer regions (Appendix 1). After deletion of variable positions and compensatory mutations, 531 homologous bp were available for phylogenetic analyses of the 25 species. They included 216 variable and 153 parsimony-informative positions.

Pairwise comparison of number and type of genetic differences revealed that the transition to transversion ratio (s/v) comprised a range between 5 (sister genera) and 0.5 (distantly related genera). This can be explained by the fact that higher rates of change in transitions and multiple hits become more significant as pairwise distances increase. The Kimura two-parameter distance separates the two types of changes and accounts for the scaling of multiple hits by correcting with distance. For MP, several analyses were run, each time assigning different weights to transversions. In all cases, the overall topology of the single consensus tree was the same and only bootstrap values and consistency indices varied. For the single bootstrap consensus tree presented in Fig. 1 (length = 1304, CI = 0.361, RI = 0.454), transversions were weighted three times greater than transitions.

Neighbor-joining and maximum-parsimony methods rendered similar tree topologies that differed only in a higher number of unresolved nodes in MP. The tree presented in Fig. 1 therefore represents the consensus topology for the two methods and shows confidence values of the interior branch method of the NJ analysis and bootstrap values of the MP analysis. Only values above 50% and their corresponding nodes are shown. The following groups were supported with 90–100% confidence levels in at least one of the two methods: (1) all genera belonging to the subfamily Grapsinae (NJ: 99/MP: 74); (2) all Sesarminae genera except *Cyclograpsus* and *Chasmagnathus* (99/99); (3) within the Sesarminae, the genera *Sesarma* and the monotypic *Metopaulias* (99/100); (4) the genera *Armases* and *Aratus* (96/96); (5) most of the genera attributed to the subfamily Varuninae in addition to *Cyclograpsus* and *Chasmagnathus* (99/98); (6) the varunine genera *Hemigrapsus, Tetragrapsus*, and *Gaetice* from the American west coast (97/87); (7) *Glyptograpsus* and *Platychirograpsus* (99/100); (8) the genera *Plagusia* (Plagusiinae) and *Euchirograpsus* (Varuninae) (98/76); and (9) the two gecarcinid genera *Cardisoma* and *Gecarcinus* (96/50). Both methods furthermore suggest a basal position of the genus *Percnon* and, at the next level (NJ only), of the subfamily Grapsinae in comparison to the remaining Grapsoidea.

DISCUSSION

The present molecular phylogeny based on the 16S rRNA gene confirms many aspects of current systematic classification of the Grapsoidea. Among the American grapsid genera, three multigenus groups that roughly correspond to traditional subfamilies were strongly supported by the molecular data. The first of these groups comprises the six American genera of the Grapsinae, thus suggesting a monophyly of this taxonomic unit and corroborating results from adult and larval morphological systematics (Dana, 1851; Alcock, 1900; Rice, 1980; Cuesta and Schubart, 1999).

The second multigenus group consists of the sesarmine genera *Sesarma, Metopaulias, Armases*, and *Aratus*. The close relationship of these genera is not surprising, given that the monotypic *Metopaulias depressus* evolved within the Jamaican species of *Sesarma* (see Schubart *et al.*, 1998), *Armases* was until recently included within the genus *Sesarma* (see Abele, 1992), and *Aratus* was shown to belong to the *Armases* complex (see Niem, 1996). These four genera and additional ones from the Indo-West Pacific formerly included in *Sesarma sensu lato* appear to represent the core of what should be considered the subfamily Sesarminae. In a previous study, Schubart *et al.* (1998) showed that the American *Sesarma* (including the monotypic genus from Jamaica, *Metopaulias*) are monophyletic and that the separation from the genus *Armases* finds strong support from molecular data.

Two other grapsid genera occurring in America traditionally have been placed within the Sesarminae: *Cyclograpsus* and *Chasmagnathus*. We here present evidence that both of these genera are significantly closer to most Varuninae genera than to the above-mentioned four Sesarminae genera, based on their 16S mtDNA. These findings corroborate results from a recent study which, on the basis of zoeal morphology, suggested that *Cyclograpsus* and *Chasmagnathus* should be transferred to the Varuninae (Schubart and Cuesta, 1998). Morphological characters of the megalopal stage also argue against placement of *Cyclograpsus* and *Chasmagnathus* within the Sesarminae (Cuesta *et al.*, unpublished). On the basis of adult morphology, H. Milne Edwards (1853) included both of these genera in a tribe termed "Cyclograpsacaea" and not his "Sesarmacaea." Guinot (1979: pp. 207–209) noted that the location of the male genital openings in *Metaplax, Cyclograpsus*,

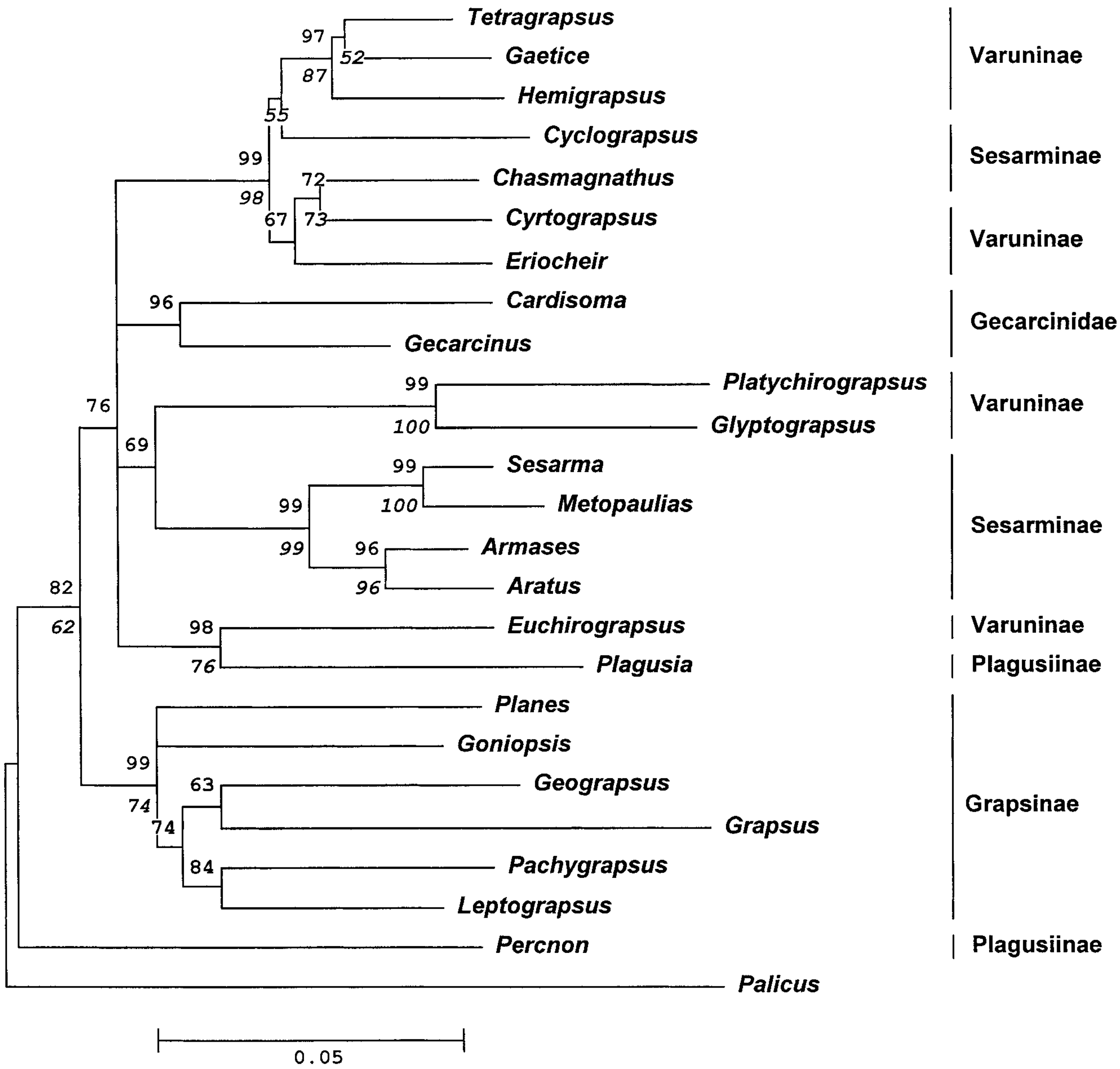

FIG. 1. Phylogenetic relationships within all American grapsoid genera as inferred from 531 conserved bp of the 16S rRNA gene. Consensus tree of neighbor-joining (Kimura two-parameter distances, internal node test) and maximum-parsimony (2000 bootstraps of heuristic search, transversions weighted three times transitions) analyses, showing only nodes supported by confidence values higher than 50% (NJ above node, MP below node and in italics).

and *Helice* (the latter genus being closely related to *Chasmagnathus*) corresponded to the sternal position as found in the Varuninae. At this point, we therefore feel that there is strong enough evidence for transfer of *Cyclograpsus* and *Chasmagnathus* from the Sesarminae to the Varuninae. Former inclusion of these genera within the Sesarminae was based on the presence of a hairy crest on the third maxilliped (see Alcock, 1900; Rathbun, 1918; Balss, 1957). The validity of this character for higher order taxonomy needs to be reevaluated (confirmed by M. Türkay, pers. commun., 1999). According to preliminary morphological and molecular data, several of the Indo-West Pacific sesarmine genera (*Helice*-group, *Helograpsus*, *Paragrapsus*, and *Metaplax*) are also better classified within the Varuninae (Cuesta *et al.*, unpublished; Schubart *et al.*, unpublished).

The third strongly supported multigenus group is composed exclusively of genera that belong to the subfamily Varuninae and the aforementioned *Cyclograpsus* and *Chasmagnathus*. This renders considerable support to the existence of this taxonomic unit, which is regarded as controversial by some specialists (Jamieson *et al.*, 1996; R.v. Sternberg, pers. commun., 1998). However, on the basis of our DNA-based study, the Varuninae (in the traditional sense, as well as after inclusion of *Cyclograpsus, Chasmagnathus,* and possibly other ex-sesarmine genera) cannot be considered a monophyletic group, as long as at least three genera currently placed within this subfamily are not removed. All three of these genera (*Glyptograpsus, Platychirograpsus,* and *Euchirograpsus*) stand clearly outside the major varunine grouping in our 16S mtDNA phylogeny and in larval morphological comparisons (Cuesta and Schubart, 1997).

Two of these genera, *Glyptograpsus* and *Platychirograpsus,* are evidently closely related and seem to hold an independent position within the Grapsoidea, maybe closest to the Sesarminae. Their inclusion within the Varuninae is herewith refuted. This was also concluded after studying larval morphology of *G. impressus* (see Cuesta and Schubart, 1997) and *P. spectabilis* (see Cuesta *et al.*, unpublished). An ongoing study of adult morphology is revealing several characters that are unique to these two genera and favor their placement in a new taxonomic group (Schubart and Cuesta, unpublished).

The third genus that is currently considered a member of the Varuninae but clearly stands outside the main grouping is *Euchirograpsus.* Guinot and Bouchard (1998) noted that the morphology of the abdominal holding system of this genus suggests that it is misplaced in the Varuninae. Of particular interest in the present study is the positioning of this subtidal crab genus as sister group to the genus *Plagusia.* A close connection of *Euchirograpsus* to the Plagusiinae has been postulated previously on the basis of striking similarities in larval morphology (Wilson and Gore, 1978; Cuesta and Schubart, 1997). The present status of the Plagusiinae is therefore put into question, especially since in the present study the phylogenetic position of the second genus traditionally placed within this subfamily, *Percnon,* is basal with respect to all other Grapsoidea. *Plagusia* and *Percnon* were formerly placed together and distinguished from other grapsids on the basis of a single morphological character, a carapacial front with antennal clefts (see keys in Rathbun, 1918; Balss, 1957). The Plagusiinae, next to the type genus *Plagusia,* would now also include *Euchirograpsus* (see also d'Udekem d'Acoz, 1999) and the taxonomic position of *Percnon* awaits future comparisons (i.e., *incertae sedes*).

Next to subfamilial relationships of the Grapsidae, another major question of this study centered on relationships between the Grapsidae and the Gecarcinidae. As mentioned before, H. Milne Edwards (1853) placed the Gecarcinidae at the same taxonomic level as five other "agèles" of grapsoid crabs. Alcock (1900: p. 280) recognized the Gecarcinidae as a distinct family "but with some hesitation, for Milne-Edward's estimation of the group as a subfamily of Grapsidae has much to recommend it." After Alcock, the Gecarcinidae have always been treated as a distinct family or even superfamily (Guinot, 1978), based only on the carapacial shape (Rathbun, 1918; Balss, 1957) and despite striking similarities of spermatozoa (Jamieson *et al.*, 1996), larvae (Cuesta *et al.*, unpublished), and adult morphological characters (Türkay, 1983; P. K. L. Ng, pers. commun., 1999) to those of grapsids. In the cladistic analysis of Sternberg *et al.* (1999), the Gecarcinidae form a sister group to Grapsinae and Sesarminae and the varunines and *Euchirograpsus* occupy a more basal position on their tree. The present molecular study, as well as a previous one (Fig. 1 in Schubart *et al.*, 2000), gives additional evidence for the close relationship between grapsid and gecarcinid crabs and suggests that the Gecarcinidae evolved within the Grapsidae.

In her brachyuran classification, Guinot (1978) suggested elevation of several currently recognized families to superfamilies, e.g., Majoidea, Xanthoidea, Grapsoidea, Gecarcinoidea, Ocypodoidea, and Mictyroidea. In the case of the species-rich and highly variable Xanthoidea and Majoidea, this suggestion has found increasing acceptance (Martin and Davis, unpublished), but this has not been the case for most of the other families. Results of the present study suggest that the Gecarcinidae and the grapsid subfamilies should be placed at the same taxonomic level to make taxonomy and phylogeny congruent. Instead of classifying the Gecarcinidae as an additional grapsid subfamily, we propose to raise all grapsid subfamilies to a family level. Marked morphological (and ecological) differences found among the subfamilies would justify such a step. This will also facilitate future taxonomic work and the comparison with possibly closely related ocypodoid families. We would thereby follow Guinot (1978) by accepting the familial status of Grapsidae (restricted), Plagusiidae, Sesarmidae, and Varunidae. However, we do not follow Guinot (1978) in that we retain family status for the Gecarcinidae and include it among the families of the Grapsoidea.

A possible limitation of this study is that non-American grapsoid genera could not be analyzed. Inclusion of additional Indo-West Pacific genera will most likely increase the resolution of the tree and hopefully shed some light on the relationship among the newly established grapsoid families. Preliminary results show that the genus *Varuna* is placed together with the other Varunidae based on its 16S rRNA sequence (Schubart *et al.*, unpublished). In the case of the Gecarcinidae, the inclusion of the genus *Epigrapsus* seems important, since it might represent a link between *Cardisoma* and *Gecarcinus* (see Türkay, 1983). Other possible limitations of our results, such as accidental sequencing of pseudogenes, have been generally discussed in Schubart *et al.* (2000).

The degree to which nonmarine life has been achieved within the different genera treated in this study is listed in Table 1. Three different forms of inland life can be distinguished. The first, "adults limnic," is composed of those crabs that spend most of their lives in or near freshwater but migrate back to the sea or shed the larvae into the river current, so that larval development takes place in the ocean. This life style is typical of the genera *Eriocheir*, *Glyptograpsus*, and *Platychirograpsus*, some species of *Armases*, and other Indo-Pacific sesarmid and varunid genera (discussed in Diesel and Schuh, 1998). The second group, "adults terrestrial," is characterized by the same evolutionary constraint, namely the need to undergo larval development in the sea, for which reason land-dwelling crabs, such as *Cardisoma*, *Gecarcinus*, *Gecarcoidea*, and *Geograpsus*, or hermit crabs, such as *Coenobita*, have to return to the sea to spawn (Bliss, 1968; Hartnoll, 1988). Finally, there is a third group of grapsoid crabs, "entirely inland," which became independent from the sea by abbreviating larval development. Next to the Jamaican *Metopaulias* and endemic *Sesarma* that breed in inland waters (Hartnoll, 1964; Diesel, 1989), a similar life cycle has been reported for the Southeast Asian *Geosesarma* (see Soh, 1969) with the recent discovery of several species with direct development (Ng and Tan, 1995; Schubart and Ng, unpublished). All freshwater crab families included by Bowman and Abele (1982) in the "Potamoidea" also belong in this group.

From this listing it becomes evident that the first two forms of nonmarine life, in which only adults thrive in inland habitats, appear in different taxonomic units and do not seem to have a common phylogenetic basis (except a possible predisposition for terrestrial habits in the Thoracotremata, as suggested by Hartnoll (1988)). It must be assumed that, several times independently, adult crabs have extended their foraging ranges from the marine intertidal into more terrestrial habitats, especially in the humid tropics. However, as long as marine larval development is required, evolution toward inland life remains a "cul-de-sac." All "entirely inland" forms among the Grapsoidea are species found within the Sesarmidae. It is only in this family that marked reduction of larval stages is found, most often coupled with the production of large yolk-rich eggs (Hartnoll, 1964, 1988; Diesel *et al.*, 2000). We thus observe a phylogenetic basis for the shared ability to produce limnic lecithotrophic larvae. This ontogenetic trait is probably the most important factor for attaining a complete independence from the sea, as demonstrated by similar life forms in the genus *Geosesarma* of Southeast Asia and the Jamaican endemics of *Sesarma* and *Metopaulias*.

APPENDIX 1

Alignment of 589 bp of the 16S rRNA Gene in 24 Genera of American Grapsoid Crabs and the Outgroup *Palicus*

	1 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890	4444444445 1234567890	5555555556 1234567890	6666666667 1234567890	7777777778 1234567890	8888888889 1234567890
	1	1		2			2		
Sesarma	GTCTGTTTGT	AGATATAAAA	AGTCTAGCCT	GCCCACTGAT	-AAATAA---	---TTTTAAT	GGCCGCGGTA	TTTCTGACTG	TGCAAAGGTA
Metopaulias	....	....	......A...	....	..G.......	...-......	......A...	....	....
Armases	....A....A	....	....	.........-	..GT..-...	.........G	....	.....A....	....
Aratus	.........A	.........G	....	....	...G..-...	.........G	....	.....A....	....
Cyclograpsus	.........G	....	...T......	....	TT.-..-...	TAAA..A...	....	..-T....C.	....
Chasmagnathus	....A....A	..T......	...T..A...	....	.....TG...	TAAA..G...	....	.C-T....C.	....
Cyrtograpsus	.........A	..T.......	...T......	....	.......AAA	TAA...G...	....	..-T....C.	....
Eriocheir	.........A	..T.......	...T..A...	....	TG.-..G...	TA.-..A...	....	..-T....C.	....
Gaetice	....A....A	....	..T..A...	....	T..G...AA.	...-..A...	....	.C-T....C.	....
Tetragrapsus	....A....G	....	...T..A...	....	T..-..A..	TA.-..A...	....	..-T....C.	....
Hemigrapsus	....A....G	....	..T..A...	....	T..TAG....	TA.-..A...	....	..-T....C.	....
Platychirograpsus	....A....A	.T.A.....T	...T..A...	..T.....-	.....T....	T....A..G	A.....A...	.C-T......	....
Glyptograpsus	....A....A	.T.A.....T	...T..A...	..T......-	...G.--...	......A..G	A.....A...	..-.......	....
Euchirograpsus	....	..T......G	....G.A...	.....T....	.-...--...	......A..A	....	..-T....C.	....
Plagusia	....	..T.G..G.G	...T..A...	....	....A--...	......-..A	....	A.-T....C.	....
Percnon	....AC...G	GTG.....GT	....	....	......-..G	......A..G	....	C.-.....C.	....
Grapsus	......C...	....G..G.C	....	....	...---..G	T.T..A..G	....	..-.....C.	....
Goniopsis	.........A	....G....C	....	....	.-.T.T-..G	...-..A..G	....	..-.....C.	....
Geograpsus	.........A	..G......C	....	....	.--G.--..G	TA.-..A..G	....	C.-T....C.	....
Planes	....	..T......C	....	....	.--G.--..G	TA.-..A..G	....	..-.....C.	....
Pachygrapsus	.........G	C	....	....	...GC.-...	...-..A..G	....	..-.....C.	....
Leptograpsus	.........A	....G....C	....	....	...G..-..	...-.A..G	....	..-.....C.	....
Cardisoma	....	.........G	...T..A...	....	.----..AA.	......-..G	....	..-T....C.	....
Gecarcinus	.........G	.........G	...T..A...	.........-	..G.-..AGA	TA.-..A..G	....	.A-T....C.	....
Palicus	....T.A..A	....T..T..	.....G....	.....G..G-	....G-...	....C.A..A	....	..-T....C.	....
		X		X	XXXXXXXXXX	XXXX			

APPENDIX 1—*Continued*

	1	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111
	9999999990	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
Sesarma	GCATAATAGT	TAGTTTCTTA	ATTGGAATCT	TGTATGAATG	GTTTGACAAG	AAAAAATCTG	TCTC-ACAAT	TATT-TATTG	AATTTAACTT
Metopaulias	........A.	....	...A......	....	....	....	...T......	..A.......	....
Armases	.......C..	....	....	....	....	......C...	...TT.T...	....	....
Aratus	.......C..	....	...A......	....	....	.....TC...	...TT.T...	....	....
Cyclograpsus	.......C..	......T...	....A.....	....	...G.....A	.G.G.GC...	...T.T-..	....T.G...	....
Chasmagnathus	.......C..	......T...	....	....	...G.....A	.G........	...T.T-.C	....	..C.......
Cyrtograpsus	.......C..	......T...	....	....	.C.G.....A	.G........	...T.T-..	....	....
Eriocheir	.......C..	......T...	....	....	...G.....A	.G..G.....	...T.T-..	.GC.......	.....G....
Gaetice	.......C..	......T...	....	....	..G.....A	.G........	....TGT-G.	..A.......	....
Tetragrapsus	.......C..	......T...	....	....	...G.....A	.G........	...T.T-..	.GA.......	..C.......
Hemigrapsus	.......C..	......T...	....	....	...G.....A	.G........	....T.T-..	........C.	....
Platychirograpsus	.......C..	......T...	....A.....	....	...G.....A	......AT..	....TGT-..	..A.......	....
Glyptograpsus	.......CA.	......T...	....A.....	....	...G.....A	.....GAT..	....T.T-..	..A....G..	....
Euchirograpsus	.......C..	....	...A......	....	...G.....A	....	...TT.T-..	.TA..AT...	....
Plagusia	.....G..A.	....	.....G....	....	...G......	....	...TT.T-..	.TA.....G.	....
Percnon	........A.	...G.CT...	.....G....	....	...G.....A	.G.G..A...	...TTGTGG.	ATA-.A....	....
Grapsus	.......C..	....	.....G....	....	.C.G.....A	.G.GGG....	...TTGT.G.	..A-.G..A.	..G.......
Goniopsis	.......C..	.T........	....G....	....	...G.....A	.G...GA...	....T.T...	.TA-......	....
Geograpsus	.......C..	....	.....G....	....	...G.....A	.G.G.G....	....T.TG..	..A-......	..C.......
Planes	.......C..	....	....	....	...G.....A	.G....C...	....T.T...	..G-......	....
Pachygrapsus	.......C..	....	.....G....	....	...G.....A	.G........	...TTGT.G.	CG.-......	....
Leptograpsus	.......C..	....	.....G....	....	...G.....A	.G........	....T.T.G.	.GG-..C...	....
Cardisoma	.......C..	......T...	....	....	...G.....A	GG........	....T.TGGC	...A.A.G..	....
Gecarcinus	....	......T...	....	....	...G.....A	.G........	...T.T-G.	.....A....	....
Palicus	........A.	...G..T...	....	..A.....A.	...AA....A	.G...G....	...ATTA-..	ATA.AA....	....
	1111111111	1111111112	2222222222	2222222222	2222222222	2222222222	2222222222	2222222222	2222222222
	8888888889	9999999990	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
	3	3							
Sesarma	TTAAGTGAAA	AGGCTTAAAT	AAATTAAAAA	GACGATAAGA	CCCTATAAAG	CTT-AATATT	ATT-TTATTA	TTTAATAG--	--AATTTT--
Metopaulias	....	....	....	....	....	....	...T......	.......A..	........TT
Armases	....	....	....	....	....	....	.AAT......	.......A..	....
Aratus	....	....	G.G.......	....	....	....	.AGT..T...	....	....
Cyclograpsus	....	....	........GG	....	....	....	..AA..-...	....G.T...	....
Chasmagnathus	....	....	GT......GG	....	.....G....	....G.....	.AAA..-...	....G.TA..	....
Cyrtograpsus	....	....	.C......GG	....	.....G....	....	.AGA.A-...	....G.TA..	...T....T.
Eriocheir	....	....	GT......GG	....	....	....	.AA...-...	.A..G.CA..	....
Gaetice	....	....	........GG	....	....	.........A	TGA..A-...	C...G.TA..	....
Tetragrapsus	....	....	........GG	....	....	....	.AA..G-...	C...G.TA..	....
Hemigrapsus	....	....	....	....	....	....	.A-..A..A.	C...G.TA..	....
Platychirograpsus	....	........G.	GT........	....	....	....T....A	..A...C.A.	C.A..CT...	....
Glyptograpsus	....	....	G.T.......	....	....	....T....A	.A....T.A.	..A...T...	......--..
Euchirograpsus	....	....	......G.GG	....	.........A	....T.....	.AA..AG...	.A.TTCT...	....
Plagusia	....	....	G.......GG	....	.........A	....T.....	G....A-...	.A..GCTA..	........TT
Percnon	....	....	.TT.......	....	....	...A.....A	..A..-..G	G.A.T.T.TT	TT......T.
Grapsus	........G.	....	G.G.....GG	....	....	....T....G	..G.C.-G..	G.A.TCT...	........T.
Goniopsis	....	....	GT...G..GG	....	....	....G.....	T.A..A-A..	G.AG..TT..	....
Geograpsus	....	....	G.......GG	....	....	....	C...AA-GC.	C.A...T...	......--..
Planes	....	....	.T......GG	....	....	....G.....	C.C..A-AC.	.CA...TA..	........T.
Pachygrapsus	..G.......	.....C....	........GG	....	....	....G.....	.GG.AG-A..	..A.CCTT..	....
Leptograpsus	....	....	........GG	....	....	....G.....	.CA..G-G..	..AG.CT...	......G...
Cardisoma	....	....	G.......GG	....	....	....G....G	TAAG..-...	....G.T...	........T.
Gecarcinus	....	....	..T.......	....	....	....	.AAA.A-...	..GG..T...	....
Palicus	....	....	.T.C.GGTGG	....	.........A	....T.....	.A.....AAT	..A.T.TA..	......--..
	X								

APPENDIX 1—*Continued*

	2222222222	2222222222	2222222223	3333333333	3333333333	3333333333	3333333333	3333333333	3333333333
	7777777778	8888888889	9999999990	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
					4 4				
Sesarma	---AAA--TA	TAAGTATTTG	-GTA-AT-AA	TTTATATTTT	ATTGGGGTGA	TAATAATAAA	ATGATTATTA	ACTG-TT-AA	TTAATTAATA
Metopaulias	T.........	...A.G....	....	....	....	....G.....	....	.........G	...-....A.
Armases	......TG..	...A......	.......T..	...T......	.......C..	....GG..T.	.A........	....	..TT....A.
Aratus	......AA..	...A.....A	....G.T.C	C..T......	.......C..	....GG..T.	.A........	........G.	..TT.A....
Cyclograpsus	.....GT...	...AA.C..A	.......TG.	A-.T......	.......C..	...G.G....	..TT...G..	....C.....	G.T-.A....
Chasmagnathus	...--.TT..	...AA.C..A	...G...TG.	A-.T......	.......C..	...AGG....	....	....C.....	..T-.A.G..
Cyrtograpsus	...---....	...AA.CA.A	.......TG.	A-.T......	.......C..	..A.G....	......TC..	....C.....	.CT-.A....
Eriocheir	....GGT...	...AG....T	....A..TG.	A-.T......	.......C..	...G.G....	....	....C.....	CAT-.A.G..
Gaetice	......T...	...AA.C..A	...G...C..	G-.T......	.......C..	...A.G....	..TG...C..	....C.....	A.T-..T...
Tetragrapsus	......T...	...AA....T	......CT..	A-.T......	.......C..	...A.G....	..T.C..C..	....C.....	..T-C.T...
Hemigrapsus	..CGTC....	...AA.C..A	...T...T..	A-.T......	.......C..	...A.G....	..T.C..A..	....C.....	..T-......
Platychirograpsus	..G.G...-.	...AA.G..T	A......T.T	A-.T......	G.........	...AG.....	...T......	...ATA-...	..T.....A.
Glyptograpsus	..A.....-.	...AG....A	.A.....T.T	A-.T......	G......C..	C..GC.....	...T......	...ATAAA..	A.T-....-.
Euchirograpsus	......T...	...AAG..C.	......CT.T	A-.T....A.	....	...A.G....	..T.......	....C.....	.GT-.A....
Plagusia	TT.--.....	...AGGC..A	.....G...T	AA.T....A.	....	...AGG....	..T.GGA..	....T.....	..T-..T.A
Percnon	......T...	...AAT...A	.A.T...A.T	....	.......C..	...A.G....	..T.C.....	....C..T..	---.G.GC..
Grapsus	..A..GTA-.	...AGGG..A	.T..C.AA.G	.-CT....GC	.......C..	.G.A.G....	....A..G..	....C..T.-	..T-..-GG.
Goniopsis	..AT..T...	...AA....A	.C.GTTAA..	G-.T......	.......C..	..A.G....	...C...A..	....C..T.G	ACC-.A-CG.
Geograpsus	.AT...TT..	...AA....A	.A.GTGAATT	.-.T....G.	.......C..	...A.G....	...G..GG..	....C..T..	.GG-.C-.GC
Planes	.....TTC..	...AA..C..	.T.GTTAA..	G-.T....G.	.......C..	..GA.G....	.G.....C..	....C..T.G	..T--A-.G.
Pachygrapsus	..A.G.T...	...AGG...A	...GTG.AGT	.-.T....A.	.......C..	..GA.G....	......GG..	....C..C--	..T-..T.G.
Leptograpsus	.....GTT..	...AGG...A	....TCGA..	.-.T....A.	.......C..	..A.G....	.......G..	....C..T.-	..T-.-..G.
Cardisoma	..ATG.....	...AGG...A	...GAT.T.G	AAG-....G.	.......C..	...GGG....	......G...	....C....-	..G-..T.A.
Gecarcinus	.GA.T.T...	...AA....A	....A-.A..	A-.T....A.	.......A..	.....G....	......G...	....C....-	..TT.A....
Palicus	...--.TT..	...AA-...A	.AA.TTAA.T	A-.T......	G......C..	C..G.G..T.	..TTC.-...	....C....G	A..-.A..AT
	XXXXXXXXX			X				XX	XXXXXXXXXX

	3333333333	3333333333	3333333333	3333333334	4444444444	4444444444	4444444444	4444444444	4444444444
	6666666667	7777777778	8888888889	9999999990	0000000001	1111111112	2222222223	3333333334	4444444445
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
Sesarma	CAAAAA-TAA	ATGAATATAA	AATGACTTAG	TAAATGATCC	TATATTAGAG	ATTAAAAGTT	TAAGTTACTT	TAGGGATAAC	AGCGTTATTT
Metopaulias	....	G.........	G..TTTA--A	....G.....	.G........	....	....	....	....
Armases	....	.........T	G--T.T.A.T	....	.T.-...A..	....	....	....	....
Aratus	....	........T.	T.GT.T...T	....	.T.-...A..	....	....	....	....
Cyclograpsus	..TTT...GG	T...G..-..	...------T	..G.......	..G-...A..	....	....	....	.....C....
Chasmagnathus	..TT.....G	....T..---	...TT----T	....	..G-...A..	....	....	....	....
Cyrtograpsus	..TTG.....	....T.GAG-	--.TT----T	....	..A-...T..	....	....	....	....
Eriocheir	..CTT....T	G...T..--.	...T-----T	....	..A-...A..	....	....	....	....
Gaetice	..CTT.....	...TC..C.-	-G.T-----T	....	..A-A..A..	....	....	....	....
Tetragrapsus	..CTT.....	...TT..C.-	--.TT----T	....	..A-A..A..	....	....	....	....
Hemigrapsus	..CTT....G	G..TC...--	-G.TT----T	....	..ATA..A..	....	....	....	....
Platychirograpsus	....G.....	G...G.T.--	...T-----.	...TG.....	.GA-...A..	...T......	....C.....	....	....
Glyptograpsus	...CG...G.	G.....T.--	...------T	...T......	..A-A..A..	...TT.....	C.........	....	....
Euchirograpsus	....	.......A--	...T-----.	....A.....	.GA-A..A..	....	....	....	....
Plagusia	.........G	.....GTG--	---------T	A.G.......	.TG-...A..	....	....	....	....
Percnon	....	G.......G.-	-G.T.----T	..G.......	.TA-A.....	........A.	....	....	....
Grapsus	....G.A...	G...T..G.G	G.GT.T---T	....A.....	.C.-..G.G	...TT...A.	C.........	....	....
Goniopsis	....T.....	T...C..ACG	C-.C.TA..T	...TG.....	..G-A..A..	........A.	....	....	....
Geograpsus	....TTA..T	T..TT..AT.	..-------T	....	.TA-G..A..	....T...A.	....	....	....
Planes	.....C...T	....T..AT.	---------A	.TT.A.....	..A-A..A..	...T....A.	....	....	.....A....
Pachygrapsus	....	G...G..AC.	GGAA-----.	....G.....	.T.-A..A..	...T....AA	....	....	....
Leptograpsus	.....T....	G...T.AG-	---T.TA--A	C...A.....	..G-A..A..	........A.	....	....	....
Cardisoma	....GG...G	G...T.--G.	...T-----.	....G.....	..G-..TG.	........A.	....	....	....
Gecarcinus	....T.....	.....---G.	.-.T-----T	....	..G-...A..	....	....	....	....
Palicus	..T.G.....	.....-----	---------A	...-.G...	.T.-A..A..	....GG..A.	....	....	.....A....
		XXXX	XXXXXXXXXX						

APPENDIX 1—*Continued*

	4444444444	4444444444	4444444444	4444444444	4444444445	5555555555	5555555555	5555555555	5555555555
	5555555556	6666666667	7777777778	8888888889	9999999990	0000000001	1111111112	2222222223	3333333334
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
	5		5			6	7	7	8
Sesarma	TTTTTGAGAG	TTCTTATCGA	AAAAAAAGTT	TGCGACCTCG	ATGTTGAATT	AAAATAT-CT	ATATAATTGC	AGTAGTTATA	TAAGAAGGTC
Metopaulias	.....A....	....	....	....	....	....	.........T	....	....
Armases	....	....	G.........	....	....	....	G........T	..C.......	....
Aratus	....	....	....	....	....	....	.........T	....	....
Cyclograpsus	..C..A....	.A........	..G.......	....	....	.....G....	T....G....	..C..C....	A...T.....
Chasmagnathus	....	.A........	....	....	....	....	T.........	..C.......	G...T.....
Cytograpsus	.....A....	.A........	....	....	....	......C...	T.........	..C.......	A...T.....
Eriocheir	..C..A....	.A........	..G.......	....	....	....	T........T	..C.......	....T.....
Gaetice	..C..A....	.A........	..G.......	....	....	.....G....	T.........	..C.......	A...T.....
Tetragrapsus	..C.......	.A........	..G.......	....	....	.....G....	T........T	..C.......	A...T.....
Hemigrapsus	..C..A....	.A........	..G.......	....	....	......C...	T........T	..C.......	C...T.....
Platychirograpsus	....	....	.....G....	....	....	......GT..	G..C......	..C...G..	......A...
Glyptograpsus	....	...C......	....	....	....	.......T..	...C......	..A...G..	.T....A...
Euchirograpsus	....	....	....	....	....	....	....	..A.......	.G........
Plagusia	.C........	.C.C......	...G......	....	....	.....T....	T........T	....	....
Percnon	....	.C.A......	....	....	....	.GG.......	T.G.G.....	..C.....C.	AT........
Grapsus	.C........	....	...G......	....	....	....GT....	T.........	..A.......	AG........
Goniopsis	....	....	....	....	....	.....T....	T........T	..C.......	A.........
Geograpsus	....	....	.......G...	....	....	.....T....	T..C......	..G....G.T	.T........
Planes	....	.C........	....	....	....	....	T..C.....T	..G....G..	A.........
Pachygrapsus	....	....	....	....	....	....AT....	T..C......	..C....G..	AG........
Leptograpsus	....	....	....	....	....	.....T....	CC.C......	..G....G.G	G.........
Cardisoma	....	.C.A......	G.........	....	....	....	G..C......	.......G..	.G........
Gecarcinus	....	.C........	....	....	C	....	G..C......	.......G..	....
Palicus	.....A....	.......T..	....	....	....	.T........	.......-..	...C......	......A...
	XX					X	XX X		X

	5555555555	5555555555	5555555555	5555555555	555555555
	4444444445	5555555556	6666666667	7777777778	888888888
	1234567890	1234567890	1234567890	1234567890	123456789
	8	6			
Sesarma	TGTTCGACCT	TTAAATTTTT	ACATGATTTG	AGTTCAAACC	GGT-TTAAG
Metopaulias	....	....	....	....	...A.....
Armases	....	....	....	....	..C.G.G..
Aratus	....	....	....	....	..C.G....
Cyclograpsus	....	.....A....	....	....	..C.G.G..
Chasmagnathus	....	.....A....	..G.......	....	..C.G.G..
Cyrtograpsus	....	..G..A....	....	....	..C.G....
Eriocheir	....	.....A....	....	....	..C.G....
Gaetice	....	.....A....	....	....	..C.G.G..
Tetragrapsus	....	.....A....	....	....	..C.G.G..
Hemigrapsus	....	.....A....	....	....	..C.G....
Platychirograpsus	........T.	.....G....	....	..C...G...	..C.G.G..
Glyptograpsus	........T.	....	....	....	..C.G....
Euchirograpsus	....	....	....	....	..C.G.G..
Plagusia	....	....	....	....	..C.G.G..
Percnon	....	.......C..	....	......G...	..C.G.G..
Grapsus	....	....	....	......G...	..C.G....
Goniopsis	....	.....A....	....	......G...	..C.A.G..
Geograpsus	....	....	....	......G...	..C.G.G..
Planes	....	....	....	......G...	..C.G.G..
Pachygrapsus	....	.....A....	....	......G...	..C.GGG..
Leptograpsus	....	....	....	......G...	..C.G.G..
Cardisoma	....	....	....	......G...	..C.G.G..
Gecarcinus	....	....	..G.......	......G...	..C.G.G..
Palicus	........T.	....T.....	....	......G...	....G.G..

Note. Positions marked with an "X" were not considered for the phylogenetic analysis because of uncertainties in the alignment or because of representing compensatory mutations. Paired regions in secondary structure for which compensatory mutations were found are indicated by lines above the alignment and marked with corresponding numbers.

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