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Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current superfamily classification

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Abstract

Grapsoid and ocypodoid crabs receive a lot of attention in the literature due to their predominance and important role as primary and secondary consumers in intertidal as well as supratidal marine habitats. They are especially species-rich in the tropics, where they have been found to repeatedly invade terrestrial and freshwater habitats. However, the systematics of the crabs belonging to these two superfamilies is still not settled, despite recent steps clarifying phylogenetic relationships and introducing new taxa. In this study, a molecular phylogeny of grapsoid crabs primarily based on East African representatives is constructed based on DNA sequences of the mitochondrial small and large ribosomal subunits (12S and 16S rRNA), thus complementing previous molecular taxonomic studies that had been carried out with the American and East Asian fauna. In addition, selected representatives of all ocypodoid families and subfamilies were included. The monophyly of Grapsidae, Ocypodidae (*sensu stuctu*), Sesarmidae and Varunidae is well confirmed, if the genera *Cyclograpsus*, *Helice* are considered Varunidae and *Euchirograpsus* a Plagusiidae, as previously suggested. The monophyly of the family Gecarcinidae cannot be supported with our data. The family Plagusiidae in its present composition is polyphyletic. Special attention was given to the large family Sesarmidae, which has many endemic genera in the Indo-West Pacific. According to this study, two of the most speciose genera, *Chiromantes* and *Parasesarma*, are not monophyletic and need to be redefined. On the higher taxonomic level, it becomes evident that both superfamilies, Grapsoidea and Ocypodoidea, are not monophyletic in their current composition, as exemplified by a proposed sister group relationship of Varunidae and Macrophthalmidae. These results confirm those from previous molecular studies and we therefore propose to refrain from the traditional use of the Grapsoidea and Ocypodoidea as monophyletic superfamilies and treat the constituent families separately.

Key words: Molecular systematics – Thoracotremata – Decapoda – Brachyura – ribosomal RNA – mangrove crabs

Introduction

In recent years, several studies have addressed the taxonomy and phylogeny of grapsoid and ocypodoid crabs using adult and larval morphological as well as molecular approaches (Cuesta and Schubart 1997; Schubart and Cuesta 1998; Von Sternberg and Cumberlidge 1998; Schubart et al. 2000a, 2002; Kitaura et al. 2002). Gecarcinidae, Grapsidae and Ocypodidae have a long taxonomic history and were previously placed together in the Catometopa or Grapsoidea (see Rathbun 1918). Their separation into three distinct families goes back to Alcock (1900) and these were later adopted and redefined by Rathbun (1918), Balss (1957) and Guinot (1978). The latter also proposed the superfamilies Gecarcinoidea, 'Grapsidoidea' (today: Grapsoidea) and Ocypodoidea as next higher taxa to accommodate the three families within the newly created Thoracotremata.

Schubart et al. (2000a, 2002) followed Guinot (1978) by considering the former grapsid subfamilies Grapsinae, Plagusiinae, Sesarminae and Varuninae full families, and placed them at the same level as the closely related Gecarcinidae and the recently described Glyptograpsidae within the Grapsoidea. The superfamily Gecarcinoidea thereby lost its validity. The newest classification of crustacean families by Martin and Davis (2001) followed these suggestions and included the Mictyridae, which had for a while been considered part of the Grapsoidea (see Bowman and Abele 1982), in the superfamily Ocypodoidea. The Ocypodoidea on the other hand, according to Martin and Davis' (2001) classification, still consists of a large and diverse family Ocypodidae with the four subfamilies Dotillinae, Heloeciinae, Macrophthalminae and Ocypodinae. Only the camptandriid crabs have been placed in their own separate family. A recent study by Kitaura et al. (2002) has provided striking evidence that the Macrophthalminae has to be considered a sister taxon of the Varunidae and that Ocypodinae and Dotillinae are not closely related. Consequently, the Ocypodidae with the four subfamilies as currently used, may not represent a monophyletic taxon and the subfamilies should be raised to full family level as already proposed by Guinot (1978), Schubart (in Martin and Davis 2001), Schubart et al. (2002) and Kitaura et al. (2002). The sister-group relationship of Varunidae and Macrophthalminae, as evidenced by molecular phylogenies and the fact that both groups share the unique characteristic of a tRNA-gene rearrangement (see Kitaura et al. 2002), also makes the use of the superfamilies as currently defined frail, if we aim for taxonomic units representing monophyletic assemblages.

Schubart et al. (2000a, 2002) have based their molecular phylogenies mainly on the mitochondrial 16S rRNA gene from grapsoid representatives from America (Atlantic and eastern Pacific), whereas Kitaura et al. (2002) built their trees with 12S and 16S rRNA, hereafter referred to simply as 12S and 16S, from thoracotreme species from East Asia (western Pacific). In this study, we provide an independent set of evidence for verification of the suggested taxonomic changes by studying the crab fauna from a third region, the Indian Ocean, with special emphasis on the East African coast. New sequences from this geographic area are complemented by others that were previously obtained from East Asian and American crabs, to maximize the number of type species representing the genera and families included in the current study. This paper also builds on the paper by Fratini et al. (2005), in which the

evolution of tree climbing in mangrove crabs was studied, showing that this ecological specialization has evolved several times independently throughout the world's mangrove forests and providing additional sequences of 12S and 16S mitochondrial DNA that have so far not been used for taxonomic inference. The aim of this study is to use this enlarged dataset to verify phylogenetic results from previous molecular studies and to provide new DNA sequences for the ongoing taxonomic revision of grapsoid and ocypodoid crabs.

Materials and Methods

Laboratory analysis

New samples for this study were collected between 1997 and 2002, mostly from East African mangroves (Table 1). The molecular studies were carried out by C.D.S. at the University of Regensburg (16S rRNA), and by S.F. at the University of Florence (12S rRNA). DNA extractions and selective amplification of portions of the mitochondrial large and small ribosomal subunits (16S and 12S respectively) were performed as reported in Fratini et al. (2005). Primers used to amplify an approximately 570 (16Sbr) to 600 (1472) basepair unit of 16S (including the primer region) were 16Sar (5′–CGCCTGTTTATCAAAAACAT–3′) or 16SL2 (5′–TGCCTGTTTATCAAAAACAT–3′) and 16Sbr (5′–CCGGTCTGAACTCAGATCACACGT–3′) or 1472 (5′–AGATAGAAACCAACCTGG–3′); primer sequences summarized in Schubart et al. (2000b). For 12S, we used 12Sai (5′–AAACTAGGATTAGATACCCTATTAT–3′), 12Sbi (5′–AAGAGCGACGGGCGATGTGT–3′) (Simon et al. 1994), 12SH2 (5′–ATGCACTTTCCAGTACATCTAC–3′) (Fratini et al. 2005) and the new primer 12SL4 (5′–GTGCCAGCMGCCGCGGTTA–3′) for approximately 440 (12Sai-bi) to 630 (12SL4-H2) basepairs of 12S. PCR conditions for the short 12S primer combination were as in Fratini et al. (2005). Amplifications of the 16S and the longer combination of 12S were carried out with 3 min denaturation at 94°C, 40 cycles with 45 s 94°C, 1 min 48°C, 1 min 72°C and 10 min final denaturation at 72°C. All the PCR products were purified with Microcon 100 filters, ExoSAP-IT (Amersham Biosciences, Freiburg, Germany) or Quick-Clean (Bioline, London, UK) and then sequenced with the ABI BigDye terminator mix followed by electrophoresis in an ABI Prism 310 Genetic Analyzer (Applied Biosystem, Foster City, CA, USA). For each sample and for both the subunits, the forward and reverse sequences were obtained. New sequence data were submitted to molecular databases (see Table 1 for accession numbers). In addition, the following sequences from GenBank were included in our analyses: *Callinectes sapidus* (AY363392), *Eriocheir sinensis* (AY274302), *Macrophthalmus banzai* (AB002132), *Mictyris brevidactylus* (AB002133), *Pseudocarcinus gigas* (AY562127).

Statistical analysis

Sequences were aligned manually with the software ESEE Version 3.2 (based on Cabot and Beckenbach 1989). Those regions in which homologous basepairs could not be defined with certainty during the alignment process (due to high variability) were excluded from the analysis. The data for 16S and 12S were first analysed as separate datasets and later combined for the phylogenetic analyses. DNA sequences of *Pseudocarcinus gigas* (Menippidae) and of the blue crab *Callinectes sapidus* (Portunidae) were included as outgroups (only *P. gigas* in the Bayesian Analysis).

A chi-square test of homogeneity of base frequencies across taxa was carried out as implemented in PAUP* (Swofford 1998), including or excluding the outgroups from the analysis. Phylogenetic congruence among 12S and 16S data partitions was also performed using the incongruence length difference (ILD) test (Farris et al. 1995) implemented in PAUP* as the partition-homogeneity test (Swofford 1998). For this test, we used random taxon addition, tree-bisection reconnection (TBR) branch swapping and heuristic searches with 1000 randomizations of the data.

The model of DNA substitution that fitted our data best was chosen using the software MODELTEST 3.6 (Posada and Crandall 1998). This approach consists of successive pairwise comparisons of alternative substitution models using hierarchical likelihood ratio tests (hLRT). Model selections were performed separately for both genes as well as for the combined dataset.

Three methods of phylogenetic inference were applied to our dataset: maximum parsimony (MP) and neighbour joining (NJ), using the software package PAUP* (Swofford 1998), and Bayesian analysis (BI) as implemented in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001).

The MP trees were obtained by a heuristic search with 10 replicates of random sequences addition and tree-bisection-reconnection (TBR) as branch-swapping options keeping multiple trees (MulTrees). Gaps were treated as a fifth character. For NJ, the parameters of the suggested model of evolution were implemented in PAUP. Subsequently, confidence values for the proposed groups within the inferred trees were calculated with the non-parametric bootstrap method (2000 pseudoreplicates). Only minimal trees were retained and zero-length branches were collapsed.

The BI trees were calculated using the suggested model of evolution. The Bayesian analysis was run with four MCMC (Markov chain Monte Carlo) chains for 2 000 000 generations, saving a tree every 500 generations (with a corresponding output of 4000 trees). The –ln L converged on a stable value between 5000 and 10 000 generations ('burnin phase'). The first 12 500 generations were not included in the analysis to avoid the possibility of including random and suboptimal trees. The posterior probabilities of the phylogeny were determined by constructing a 50% majority-rule consensus of the remaining trees. Consensus trees were obtained using the 'sumpt' option in MrBayes.

Results

The total alignment of the sequenced portions of 16S and 12S consisted of 1257 basepairs (bp), after removal of the primer regions. Hypervariable regions that could not be aligned with certainty (162 bp) were excluded and the remaining 1095 bp were used for the phylogenetic analysis (548 bp 16S; 547 bp 12S). The selected models of DNA substitution by hLRT and Akaike were the TVM + I + G model for the 12S and GTR + I + G model for the 16S and the combined data (Rodríguez et al. 1990) (Table 2). The TVM + I + G is a simplified GTR + I + G model, in which the frequencies of the two types of transitions (A↔C and G↔T) are equal. The GTR + I + G model was consequently used for the NJ and BI inference methods. All the sequences are A–T rich (Table 2) in agreement with observation of an A–T bias for mitochondrial DNA of arthropods (Simon et al. 1994). Table 2 also summarizes the number of variable and parsimony-informative sites, the mean number of pairwise transitions and transversions, the proportion of invariant sites and the shape parameter α of the gamma distribution as obtained from the calculated model of DNA evolution for both genes and the combined dataset. Character congruence between the 16S- and 12S-gene fragments was not rejected according to the ILD test ($p = 0.29$). Moreover, the test for homogeneity of base frequencies composition indicated that homogeneity could not be rejected across taxa (with outgroups: $\chi^2 = 170.15$, $df = 159$, $p = 0.26$; without outgroups: $\chi^2 = 152.31$, $df = 153$, $p = 0.50$).

All three phylogenetic inference methods used resulted in trees that were congruent in their overall topology with some clusters showing consistently high confidence values. The results of the three methods were therefore shown together based on the BI tree, with all confidence values ≥50 plotted on the corresponding branches (Fig. 1). The MP heuristic search yielded one shortest tree of the length 2352 with the tree scores CI = 0.388, RI = 0.485. The topology of this search

Table 1. Thoracotreme crab species used for phylogeny reconstruction, with locality of collection, museum catalogue number and genetic database (EMBL) accession numbers (16S first)

Species	Collection site	Catalogue no.	EMBL no.
Dotillidae Stimpson, 1858			
Dotilla sulcata (Forskål, 1775)	Egypt: Raz Mohamed	MZUF 2754	AM180688, DQ343274
Gecarcinidae MacLeay, 1838			
Cardisoma carnifex (Herbst, 1794)	Kenya: Mida Creek	MZUF 2851	AM180687, DQ343262
Gecarcinus lateralis (Fréminville, 1835)	Mexico: Veracuz	ULLZ 3722	AJ130804, DQ343273
Gecarcoidea lalandii H. Milne Edwards, 1837	Taiwan: Pingtung: Hengchun	ZRC 1998.423	AM180684, DQ343261
Grapsidae MacLeay, 1838			
Metopograpsus latifrons (White, 1847)	Sabah, Malaysia: Kota Kinabalu	ZRC 2000.1666	AJ784028, AJ784306
Grapsus grapsus (Linnaeus, 1758)	Mexico: Veracruz	ULLZ 3709	AJ250650, DQ343257
Pachygrapsus transversus (Gibbes, 1850)	Mexico: Veracruz	ULLZ 3723	AJ250641, DQ343256
Heloeciidae H. Milne Edwards, 1852			
Heloecius cordiformis (H. Milne Edwards, 1837)	Australia: Queensland: Tallebugdera	QM W 24043	AM180695, DQ343275
Macrophthalmidae Dana, 1851			
Macrophthalmus grandidieri A. Milne-Edwards, 1867	Kenya: Mida Creek	MZUF 2737	AM180686, DQ343268
Ocypodidae Dana, 1851			
Ocypode ryderi (Kingsley, 1881)	Kenya: Watamu	MZUF 2753	AM180689, DQ345396
Uca inversa (Hoffmann, 1874)	Kenya: Gazi Bay	MZUF 1024	AJ784029, AJ784308
Palicidae Bouvier, 1898			
Crossotonotus spinipes (de Man, 1888)	New Caledonia	MNHN-B26215	AJ130807, DQ343269
Palicus caronii (Roux, 1828)	Portugal: Madeira	RMNH D 42542	AM180692, DQ343263
Pinnotheridae de Haan, 1833			
Pinnotheres pisum (Linnaeus, 1767)	Regensburg (mussel import)	SMF 30947	AM180694, DQ343272
Plagusiidae Dana, 1851			
Euchirograpsus americanus A. Milne-Edwards, 1880	Louisiana, USA: Sackett Bank	ULLZ 3626	AJ250648, DQ343260
Percnon planissimum (Herbst, 1804)	Kenya: Watamu	MZUF 2735	AM180693, DQ343276
Plagusia depressa (Fabricius, 1775)	Jamaica: north coast	ULLZ 3813	AJ250649, DQ343259
Sesarmidae Dana, 1851			
Aratus pisonii (H. Milne Edwards, 1837)	Puerto Rico: Mayaguez	MZUF 1026	AJ784012, AJ784289
Armases cinereum (Bosc, 1802)	USA: Mississippi	ULLZ 4392	AJ784010, AJ784288
Bresedium brevipes (de Man, 1889)	Australia: Queensland: Flame Tree C.	QM W 22085	AM180685, DQ343281
Chiromantes eulimene (de Man, 1895)	Kenya: Mida Creek	MZUF 2501	AJ784017, AJ784291
Chiromantes haematocheir (de Haan, 1835)	Japan: Wakayama: Samusaura	SMF 25989	AJ308414, DQ345395
Chiromantes ortmanni (Crosnier, 1965)	Kenya: Gazi Bay	MZUF 2523	AJ784016, AJ784292
Clistocoeloma villosum (A. Milne-Edwards, 1869)	Kenya: Mida Creek	MZUF 2500	AJ784018, AJ784293
Clistocoeloma merguiense (de Man, 1888)	Singapore: Mandai	MZUF 2494	AJ784019, AJ784294
Episesarma mederi (H. Milne Edwards, 1853)	Singapore: Mandai	ZRC 2000.1950	AJ784020, AJ784295
Episesarma versicolor (Tweedie, 1940)	Singapore: Mandai	MZUF 2495	AJ784021, AJ784296
Metopaulias depressus Rathbun, 1896	Jamaica: St Ann: Alderton	ANSP 12750	AM180691, DQ343279
Neosarmatium meinerti (de Man, 1887)	Kenya: Gazi Bay	MZUF 2524	AJ784013, AJ784297
Neosarmatium smithii (H. Milne Edwards, 1853)	Kenya: Mida Creek	MZUF 2504	AJ784014, AJ784298
Neosesarma rectipectinatum (Tweedie, 1936)	Singapore: Mandai	ZRC 2000.1974	AM180681, DQ343264
Neosesarma gemmiferum (Tweedie, 1936)	Singapore: Sungei Buloh	ZRC 2000.1996	AM180682, DQ343265
Parasesarma catenatum (Ortmann, 1897)	South Africa: Mgazana	MZUF 2509	AJ784025, AJ784299
Parasesarma leptosoma (Hilgendorf, 1869)	Kenya: Mida Creek	MZUF 2547	AJ784024, AJ784300
Parasesarma plicatum (Latreille, 1806)	Thailand: Phuket	ZRC 2000.1913	AM180683, DQ343254
Perisesarma guttatum (A. Milne-Edwards, 1869)	Mozambique: Inhaca	MZUF 1023	AJ621185, AJ784301
Perisesarma bidens (de Haan, 1835)	Japan: Shirahama, Tachigatani	SMF 30167	AJ621183, DQ343278
Perisesarma eumolpe (de Man, 1985)	Singapore, Lim Chu Kang	ZRC 2000.1954	AJ621184, DQ343255
Sarmatium crassum Dana, 1851	Kenya: Mida Creek	MZUF 2545	AJ784015, AJ784302
Sarmatium striaticarpus Davie, 1992	Singapore: Mandai	ZRC 2000.2042	AM180680, DQ343277
Selatium brockii (de Man, 1887)	Kenya: Mida Creek	MZUF 2546	AJ784022, AJ784303
Selatium elongatum (A. Milne-Edwards, 1869)	Kenya: Mida Creek	MZUF 2521	AJ784023, AJ784304
Sesarma reticulatum (Say, 1817)	USA: Delaware, Woodland Beach	ULLZ 3835	AJ130799, DQ343266
Sesarmoides borneensis (Tweedie, 1950)	Malaysia: Sarawak: Bako Ntl. Park	SMF 25705	AJ225855, DQ343280
Sesarmoides longipes (Krauss, 1843)	Kenya: Mida Creek	MZUF 2505	AJ784026, AJ784305
Varunidae H. Milne Edwards, 1853			
Cyclograpsus integer (H. Milne Edwards, 1837)	Mexico: Veracruz	ULLZ 3704	AJ250639, DQ343270
Helice leachii Hess, 1865	Kenya: Mida Creek	MZUF 2502	AM180690, DQ343258
Varuna litterata (Fabricius, 1798)	Kenya: Gazi Bay	MZUF 2503	AJ308419, DQ343267

ANSP, Academy of Natural Sciences, Philadelphia; MNHN, Muséum national d'Histoire naturelle, Paris; MZUF, Museo Zoologico Università di Firenze; RMNH D, Naturalis Museum Leiden; SMF, Senckenberg Museum, Frankfurt a.M.; ULLZ, University of Louisiana Zoological Collection, Lafayette; QM, Queensland Museum, Brisbane; ZRC, Zoological Reference Collection, Raffles Museum at the National University of Singapore.

was identical to the consensus topology obtained after bootstrapping, shown together with resulting bootstrap values in Fig. 1.

The monophyly of each of the three grapsoid families Grapsidae, Sesarmidae and Varunidae is confirmed by all inference methods with relatively high confidence values.

Table 2. Base frequencies, total number of basepairs (bp) in alignment used for phylogeny, number of variable (var) and parsimony-informative (pi) sites, mean number of pairwise transitions (Ts), pairwise transversions (Tv) and mean transition to transversion ratios (*R*), proportion of invariant sites (Pinvar) and α-value of gamma distribution in the combined 12S + 16S dataset as well as in the constituent subsets of data

	T	C	A	G	bp	var	pi	Ts	Tv	*R*	Pinvar	α
12S + 16S	36.8	10	36.8	16.4	1095	634	478	48.08	39.64	1.44	0.307	0.658
12S	36.3	9.6	38.9	14.5	547	344	255	19.71	14.32	1.70	0.22	0.640
16S	36.8	10.3	35.1	17.9	548	290	223	28.44	25.33	1.37	0.38	0.642

The strong homogeneity of a core group within the Sesarmidae becomes more evident in all three methods after the split of *Sesarmoides*, a genus which apparently holds a basal position within the family. The next split separates the genera *Chiromantes* and *Bresedium* from the rest of the Sesarmidae. The latter consist of several species groups that cannot be solved in terms of exact branching order by any of the three inference methods. Within the family Sesarmidae, the monophyly of most genera is confirmed with high bootstrap values. Also the North American *Sesarma reticulatum* (type species of the genus *Sesarma*) and the Jamaican bromeliad crab *Metopaulias depressus* cluster together nicely, as previously suggested (Schubart et al. 2000a). Exceptions to the taxonomic congruence within the Sesarmidae are the genera *Chiromantes* and *Parasesarma*, which probably will need to be redefined. While the East African *Chiromantes eulimene* and *Chiromantes ortmanni* are strongly supported as sister taxa, the type species of the genus, *Chiromantes haematocheir*, is phylogenetically closely related to *Bresedium*, both from the Pacific. Two species of *Parasesarma* (including the type species *Parasesarma plicatum*) form a monophylum with three species of *Perisesarma*, whereas the position of *Parasesarma leptosoma* cannot be determined with reliable confidence.

A monophyly of the families Gecarcinidae and Plagusiidae (in this study represented by the genera *Cardisoma*, *Gecarcinus* and *Gecarcoidea*, and the genera *Plagusia*, *Euchirograpsus* and *Percnon*, respectively) is not supported. Two genera of the family Plagusiidae, *Plagusia* and *Euchirograpsus*, appear as sister taxa with relatively high support. However, the position of the third plagusiid genus, *Percnon*, is unresolved in most of our trees and placed as sister taxon of the Grapsidae in the Bayesian inference (0.72). The three genera of the Gecarcinidae are always found closely allied, but they appear paraphyletic, due to the inclusion of the Plagusiidae in the same clade in MP and BI.

The ocypodoid crabs which are here represented by most of the type genera of all the former subfamilies are dispersed throughout the phylogenetic tree. The genus *Heloecius* (Ocypodidae: Heloeciinae) and the clade *Ocypode* plus *Uca* (Ocypodidae: Ocypodinae) hold basal positions compared to all other 'Grapsoidea & Ocypodoidea' without being more closely related to each other. *Dotilla* (Ocypodidae: Dotillinae) and *Paracleistostoma* (Camptandriidae) form sister taxa in all three reconstruction methods and seem to be closely related to Gecarcinidae, Plagusiidae and Sesarmidae (BI = 0.96) than to other ocypodid subfamilies. *Macrophthalmus* (Ocypodidae: Macrophthalminae) is closely allied to Varunidae as already suggested by Kitaura et al. (2002). The genus *Mictyris* (Mictyridae) is another taxon with an unresolved position in most of the trees and possibly allied to Varunidae & Macrophthalmidae according to BI (0.79). The Palicidae, consisting of Palicinae and Crossotonotinae, are completely separated from all the 'Grapsoidea & Ocypodoidea' and found at the base of the tree between the menippid and portunid outgroups (both Heterotremata). This family therefore hardly should remain within the Ocypodoidea or even Thoracotremata. The pea crab *Pinnotheres* (Pinnotheroidea: Pinnotheridae) holds a basal position compared to the clade of 'Grapsoidea & Ocypodoidea', with which it forms a monophylum according to MP and BI.

Discussion

Phylogenetic relationships among grapsoid and ocypodoid crabs based on morphological and molecular results have been proposed in a number of recent studies (Von Sternberg and Cumberlidge 1998; Schubart et al. 2000a, 2002; Kitaura et al. 2002). Here an attempt shall be undertaken to summarize all of the respective results and to discuss and clarify those relationships where conflicting results have been published.

The grapsoid families were redefined by Schubart et al. (2000a, 2002) to reflect monophyletic units. This required: (i) removing several genera (i.e. *Chasmagnathus*, *Cyclograpsus*, *Helice*-group, *Helograpsus*, *Paragrapsus* and *Metaplax*) from the Sesarmidae and including them in the Varunidae (Schubart and Cuesta 1998; Schubart et al. 2000a, 2002; Kitaura et al. 2002); (ii) further homogenizing the Varunidae by placing *Euchirograpsus* in the Plagusiidae (Schubart et al. 2000a,b, 2002); (iii) establishing a new family (Glyptograpsidae) for *Glyptograpsus* and *Platychirograpsus* (Schubart et al. 2002); and (iv) according the family Gecarcinidae the same rank as the other grapsoid families (Schubart et al. 2000a).

In the current molecular phylogeny, three of the above-mentioned grapsoid families (Grapsidae, Sesarmidae, Varunidae) are monophyletic, whereas the Gecarcinidae and Plagusiidae in their current composition may not be. Because the present study uses a different and larger set of taxa from previous studies that triggered the proposed taxonomic changes, it represents an adequate and largely independent approach to test the revised taxonomy of Schubart et al. (2000a, 2002).

The Grapsidae had been postulated to be monophyletic based on six American genera (Schubart et al. 2000a). However, Kitaura et al. (2002) obtained phylogenetic results in which the Grapsidae (represented by the three species *Grapsus albolineatus*, *Pachygrapsus minutus* and *Metopograpsus thukuhar*) were paraphyletic and basal to the Sesarmidae, Varunidae, Camptandriidae and Ocypodidae (composed of three subfamilies). In the present study, we included the non-American genus *Metopograpsus* (*Metopograpsus latifrons*) in addition to the type species of the genera *Pachygrapsus* (*Pachygrapsus transversus*) and *Grapsus* (*Grapsus grapsus*), thus including the taxonomically defining species of the family. We obtained confirmation for a monophyletic Grapsidae in all of the phylogenetic analyses (74–85% and 99 confidence levels), thereby corroborating morphological results from

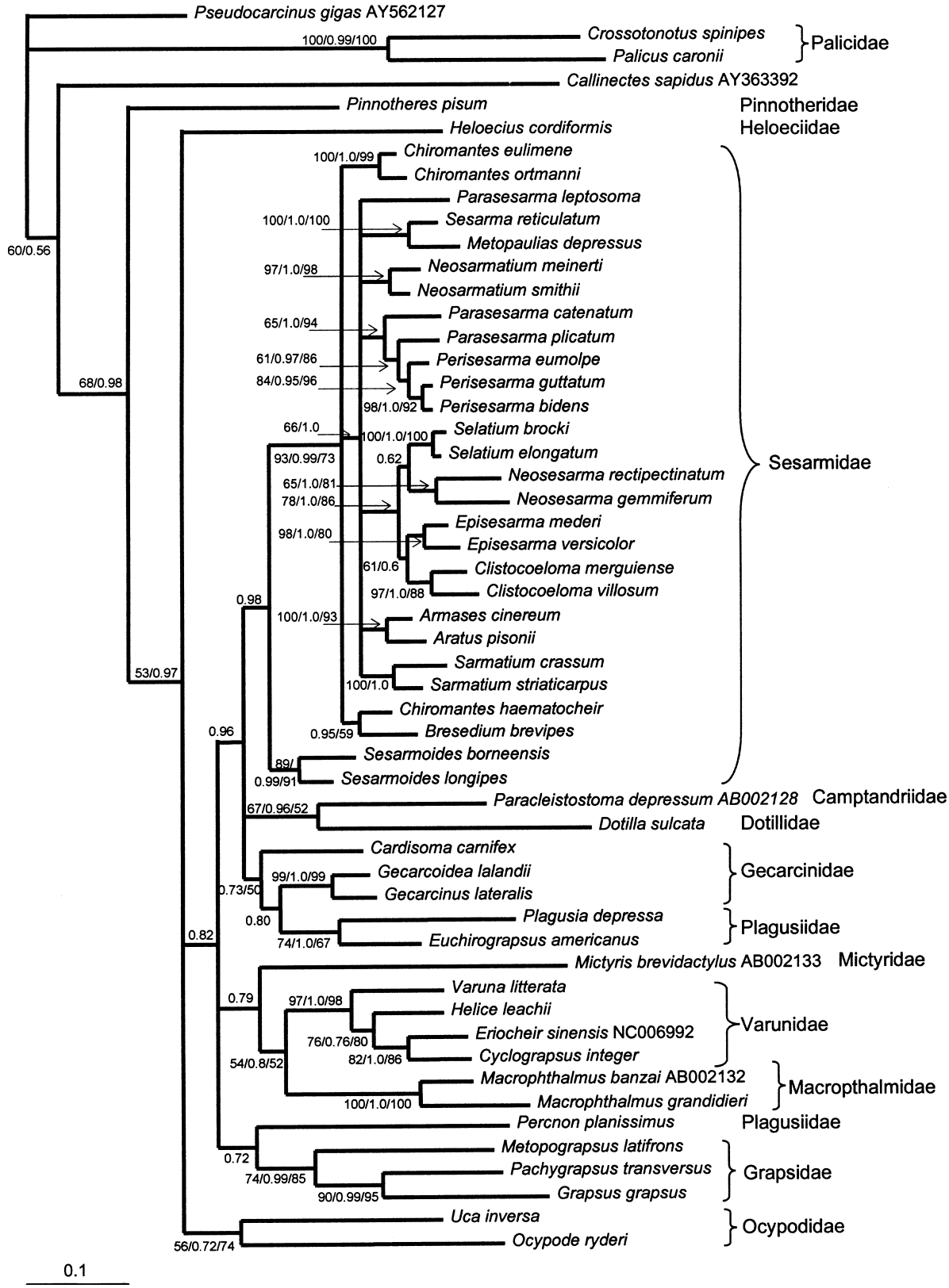

Fig. 1. Phylogenetic consensus tree of 54 brachyuran crabs (50 Thoracotremata) constructed with the maximum parsimony (MP), Bayesian inference (BI) and neighbour-joining (NJ) methods. Significance values are listed in the order MP (bootstrap values), BI (posterior probabilities), NJ (bootstrap values). Two thousand bootstrap pseudoreplicates were carried out with a heuristic search and random addition. *Pseudocarcinus gigas* and *Callinectes sapidus* were used as outgroups. Only confidence values higher than 50% are shown in the tree

Von Sternberg and Cumberlidge (1998) and the molecular insights from Schubart et al. (2000a, 2002).

The revised Sesarmidae represent a solid monophyletic clade, especially after the split of the genus *Sesarmoides*, confirming the basal position of this genus within the family. Moreover, the monophyly of most of the constituent genera of the Sesarmidae is supported by our dataset, with the exception of *Chiromantes* and *Parasesarma*, two of the more speciose genera that apparently need to be redefined. When doing so, it will be important to consider the phylogenetic position of the type species of the respective genera. Currently it appears that several members of the genus *Perisesarma* (with an anterolateral carapace tooth) are placed within the genus *Parasesarma* (without anterolateral carapace tooth, type genus *P. plicatum*). The mangrove tree-climbing *P. leptosoma* does not cluster with its congenerics, which would favour inclusion in a separate genus (see also Fratini et al. 2005). The type species of the genus *Chiromantes*, *C. haematocheir*, is more closely related to *Bresedium brevipes* than to the two African members of the genus, *C. ortmanni* and *C. eulimene*, suggesting paraphyly of *Chiromantes*. This could be taxonomically solved by placing the two African forms in a new genus, in the process of the ongoing revision of the genus *Chiromantes* (Ng and Schubart, in preparation).

Four genera belonging to the revised Varunidae are strongly supported as a monophyletic group with 97–100% confidence values. A broader representation of varunid taxa has been used recently to demonstrate monophyly of this family and separation of *Glyptograpsus–Platychirograpsus* (Schubart et al. 2002). Therefore, only a small subset of this family including the type species of the type genus, *Varuna litterata*, has been included to represent the position of this group and to allow inclusion of more taxa from the other, less clearly resolved, groups.

The family of large land-dwelling crabs, Gecarcinidae, is represented by three genera in this study. This is more than any other molecular study had included. According to the phylogenetic position of these three genera, the family Gecarcinidae may have to be considered paraphyletic, because their clade also comprises two plagusiid genera, including the type genus *Plagusia*. Further studies with all gecarcinid taxa and additional molecular markers are necessary to confirm their phylogenetic relationship and their position within the Thoracotremata.

One group of grapsoid families remains even less satisfactorily resolved. There is a clear indication that the Plagusiidae, as currently defined, must be considered a polyphyletic taxon. While *Euchirograpsus* represents the sister taxon of *Plagusia* in all our analyses, thus confirming the inclusion of this genus in the Plagusiidae (Cuesta and Schubart 1997; Schubart et al. 2000a), the genus *Percnon* never clusters with the rest of the Plagusiidae and its position is also impossible to finally resolve with the current dataset: it appears basal to other grapsoid groups included in this analysis with certain affinities to the Grapsidae (MP). Similar to the conclusions reached by Schubart et al. (2000a) using the American grapsoid dataset and *Percnon gibbesi*, it can also be concluded from this study that *Percnon planissimum* probably does not belong to the Plagusiidae and currently cannot be placed anywhere else. Current efforts are concentrated to sequence other species of the genus and to include nuclear markers to confirm the isolated phylogenetic position of this genus. Traditionally, *Percnon* had been included in the Plagusiidae, because of two antennal pouches in its carapace front, a character that it only shares with the genus *Plagusia*.

Finally, the lack of clear separation between members of the Ocypodoidea from all the Grapsoidea in all the three tree-building methods confirms previous results by Schubart et al. (2000b) and Kitaura et al. (2002) that the superfamilies Ocypodoidea and Grapsoidea with their constituent families (or subfamilies) cannot be as clearly separated, as the taxonomic subdivision would suggest. The phylogenetic clustering of Macrophthalminae with Varunidae (see also Kitaura et al. 2002) on one hand, and of Dotillinae and Camptandriidae with Sesarmidae and Gecarcinidae plus Plagusiidae on the other hand, are good demonstrations that the current taxonomic system does not seem to reflect natural relationships and may hinder the unbiased consideration of evolutionary units in future taxonomic classifications. We therefore suggest again (see also Schubart in Martin and Davis 2001; Kitaura et al. 2002) and begin to implement the raise of all ocypodid subfamilies to family level (see Table 1, Fig. 1) and to refrain from the traditional use of the two superfamilies Grapsoidea and Ocypodoidea. New names for the higher taxon including all the relevant families should be Thoracotremata or the wide use of the family Grapsoidea (*sensu lato*) as the older of the two names, with a similar composition as already used by Rathbun (1918). This will allow a new unbiased phylogenetic comparison and classification of all the families currently included in the Thoracotremata and a systematic classification that will more precisely reflect the tree of life.

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Riassunto

Filogenesi molecolare di granchi Grapsoidea dell'Africa Orientale basata su due geni mitocondriali e ridiscussione dello status sistematico di tale superfamiglia.

I granchi appartenenti alle superfamiglie Grapsoidea e Ocypodoidea da sempre sono oggetto di notevole interesse scientifico a causa del loro importante ruolo ecologico negli ambienti intertidali o sopratidali. Le due superfamiglie sono particolarmente rappresentate, in termini di numero di specie e d'abbondanza relativa, nelle zone tropicali e subtropicali, dove hanno invaso ripetutamente anche ambienti dulcacquicoli e terrestri. La sistematica delle specie appartenenti a queste due superfamiglie è ancora lontana dall'essere completamente risolta, nonostante studi molecolari recenti abbiano chiarito specifiche relazioni filogenetiche e definito nuovi taxa. Questo studio ha ricostruito la filogenesi di alcune specie di Grapsoidea dell'Africa Orientale sequenziando una porzione delle due subunità ribosomali del DNA mitocondriale (12S e 16S rRNA), confermando e completando precedenti studi molecolari condotti su specie americane e asiatiche. In questo studio sono stati inclusi anche rappresentanti di tutte le

famiglie e sottofamiglie di ocipodidi. I nostri risultati confermano la monofilia della famiglia Grapsidae, Ocypodidae (*sensu stuctu*) Sesarmidae e Varunidae a condizione che, secondo quanto recentemente suggerito, i generi *Cyclograpsus* e *Helice* siano rimossi dalla famiglia Sesarmidae ed attribuiti ai Varunidae, ed *Euchirograpsus* dalla famiglia Varunidae ai Plagusiidae. Invece, i nostri dati supportano solo debolmente o non supportano per niente la monofilia della famiglia Gecarcinidae. La famiglia Plagusiidae è probabilmente polifiletica. Questo studio pone inoltre particolare attenzione alle relazioni interne alla famiglia Sesarmidae che include molti generi endemici nell'area Indo-Pacifica occidentale. Sulla base dei nostri dati, i generi *Chiromantes*, *Parasesarma* e *Perisesarma* sono polifiletici e necessitano di essere ridefiniti. Infine, i risultati di questo studio mostrano chiaramente che la superfamiglia Grapsoidea e Ocypodidea non sono monofiletiche così come attualmente definite, come evidenziato dalla relazione di sister group tra Varunidae e Macropthalmidae. Questo conferma i risultati di precedenti studi molecolari e pertanto proponiamo di non attenersi al tradizionale uso delle superfamiglie Grapsoidea ed Ocypodoidea.

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