

Tree-climbing mangrove crabs: a case of convergent evolution

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ABSTRACT

Several crab species of the families Sesarmidae and Grapsidae (Crustacea: Brachyura: Grapsoidea) are known to climb mangrove trees. They show different degrees of dependence on arboreal life, with only a few of them thriving in the tree canopies and feeding on fresh leaves. Some of the sesarmid tree-dwelling crabs share a number of morphological characters and therefore have been considered to be of monophyletic origin. A phylogeny derived from 1038 base pairs of the mitochondrial DNA encoding the small and large ribosomal subunits was used to examine the evolutionary origin of tree-climbing behaviour within the Grapsoidea, and to determine whether morphological and ecological similarities are based on convergence or common ancestry. The analysis included African, American and Asian arboreal crab species plus several representatives of ground-living forms. Our results suggest that the very specialized arboreal lifestyle evolved several times independently within grapsoid mangrove crabs, providing another striking example of the likelihood of convergence in evolutionary biology and the degree of phenetic and ecological potential to be found among marine organisms.

Keywords: convergent evolution, Grapsidae, mangrove crabs, molecular phylogeny, Sesarmidae.

INTRODUCTION

Mangrove forests constitute the habitat with the richest diversity of land-dwelling crabs (see Hartnoll, 1988). These forests offer a wide variety of ecological niches for crabs that often segregate in space and time to reduce interspecific competition for food (Lee, 1998, Kathiresan and Bingham, 2001). Two of the dominant taxa of crabs, in terms of the number of species, that occur in mangroves are the grapsoid families Grapsidae and the Sesarmidae *sensu* Schubart *et al.* (2002) (Hartnoll, 1975; Jones, 1984). Most Sesarmidae rely mainly on fallen leaves for food (Robertson, 1986; Emmerson and McGwynne, 1992; Micheli, 1993; Sivasothi *et al.*, 1993; Lee, 1997, 1998; Dahdouh-Guebas

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et al., 1999; Fratini *et al.*, 2000b; Sivasothi, 2000), whereas the Grapsidae are usually algivorous and carnivorous (Sivasothi *et al.*, 1993; Fratini *et al.*, 2000a). All grapsoid mangrove crabs remain within their tidal zone in response to tidal cycles (i.e. iso-zonal response *sensu* Vannini and Chelazzi, 1985). During high tide, some crab species hide in their burrows or within the mangrove root system (see Vannini and Cannicci, 1995), while others remain out of the water by climbing up mangrove trees. Climbing the trees allows the crabs to avoid aquatic predators while making use of other feeding sites (von Hagen, 1977; Hartnoll, 1988; Vannini *et al.*, 1997; Cannicci *et al.*, 1999; Sivasothi, 2000; Erickson *et al.*, 2003). Among the tree-climbing crabs, we find different degrees of dependence on the arboreal habitat and different levels of climbing abilities. Following the classification proposed by Vannini *et al.* (1997), we recognize three main groups with increasing levels of tree-climbing abilities: non-arboreal species or only occasionally seen on roots (here termed NA species); species that mostly or exclusively live on tree trunks (TT species); and the most specialized species, which thrive in the tree canopy and often feed on fresh leaves (TC species).

Arboreal mangrove crabs can be found worldwide along the tropical coastlines of the major oceans. Most of them, and the best known examples, belong to the family Sesamidae (e.g. the genera *Aratus*, *Parasesarma*, *Episesarma*, *Selatium*), with a few also belonging to the family Grapsidae (e.g. the genus *Metopograpsus*). The sesamid species that live almost exclusively on mangrove trees (TC species) are: *Aratus pisonii*, which is restricted to *Rhizophora* mangroves of the Atlantic and Pacific tropical coasts of America (Warner, 1967; Hartnoll, 1975; von Hagen, 1977; Erickson *et al.*, 2003); the West African *Armases elegans* (see Green, 1986); and the western Indo-Pacific *Parasesarma leptosoma* (see Vannini and Ruwa, 1994; Cannicci *et al.*, 1996; Emmerson *et al.*, 2003). These three species are the only crabs known to feed directly on fresh mangrove leaves from the canopy. The Indo-Pacific species *Selatium elongatum* and *S. brockii* are also predominantly arboreal (TT species). These crabs live under the bark or in crevices of the trunk, between the upper aerial roots and the main branches, and are active out of the water at high tide (*S. elongatum*) and at night (*S. brockii*) (Vannini *et al.*, 1997; Cannicci *et al.*, 1999; Sivasothi, 2000). Other mangrove crabs with arboreal habits are the species of the genus *Episesarma*, which are very abundant in the mangroves of Asia, migrating regularly between mangrove trees (stems and canopy) and their burrows in the soft sediment surrounding the root system (TT species; Sivasothi, 2000). Several species of the genus *Metopograpsus* thrive among the roots and the lower trunk of mangrove trees (TT species; Fratini *et al.*, 2000a). However, one species, *M. latifrons*, is another specialized arboreal representative that often climbs on aerial roots and thin branches of the canopy (TC species; C.D. Schubart, unpublished observations).

At least two morphological characters are shared by all tree-climbing crabs: (1) their overall body shape (carapace) is conspicuously flattened, and (2) their walking legs have relatively long propodi (second most distal segment) and short dactyli (most distal segment) compared with ground-dwelling species (Vannini *et al.*, 1997) (Fig. 1). The shape of the dorsal carapace, however, differs greatly among arboreal mangrove crabs: species of the TT group (genera *Selatium* and *Episesarma*) have a remarkably squarish to elongate carapace, while the TC canopy climbers (*Parasesarma leptosoma*, *Aratus pisonii*, *Armases elegans*, *Metopograpsus latifrons*) are characterized by a distinctly triangular carapace with an invaginated sternum. The striking congruence in morphological characters of the three TC sesamid crabs from different parts of the world (*Aratus pisonii* from America, *Armases elegans* from West Africa and *Parasesarma leptosoma* from the western Indo-Pacific) has led to the assumption that they are closely related (Green, 1986). However, the phylogenetic basis of these shared morphological characters of arboreal mangrove crabs has never been tested.

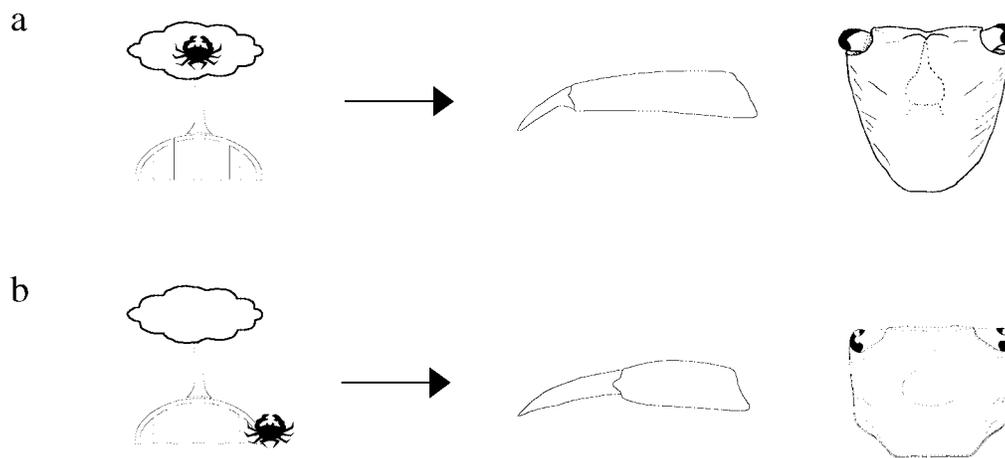


Fig. 1. Schematic drawing showing the difference in length of the distal segments of crab walking legs and in carapace shape between (a) a sesarmid tree-climbing species and (b) a mud-dwelling species.

In this study, two molecular markers (mtDNA sequences of the large and small ribosomal subunit genes) were used to examine the phylogenetic relationships among tree-climbing crabs from the mangroves of America, West and East Africa, and Southeast Asia. The analysis also included most of the ground-dwelling sesarmid species from the mangroves of East Africa. On the basis of the morphological and eco-ethological characters described here, our null hypothesis is the monophyly of all the TC tree-climbing sesarmid species. Nevertheless, here we use a taxonomy which places tree-climbing crabs from different continents in different genera (Table 1) (see Abele, 1992) and not the one suggested by Green (1986), which would include *Armases elegans* within the genus *Aratus*.

MATERIALS AND METHODS

Samples for this study were collected mostly in East African mangroves between 1997 and 2000, with additional samples obtained from the western Atlantic and Southeast Asia (Table 1). The specimen of *Armases elegans* was collected in Cameroon in 1966 and donated by H.O. von Hagen. The molecular studies were carried out by C.D.S. at the University of Regensburg (16S rRNA gene), and by S.F. at the University of Florence (12S rRNA).

Genomic DNA was extracted from muscle tissue of walking legs and chelae previously preserved in 96% ethanol by Puregene or Qiagen tissue DNA extraction kits. Selective amplification of portions of the large (16S) and small (12S) mitochondrial rDNA subunits was carried out by polymerase chain reaction (PCR) using the PCR-primers 16Sar (5'-CGCCTGTTTATCAAAAACAT-3'), 16SL2 (5'-TGCCTGTTTATCAAAAACAT-3'; a new primer), 16Sbr (5'-CCGGTCTGAACTCAGATCACACGT-3') and 1472 (5'-AGATAGAAACCAACCTGG-3') (see Palumbi *et al.*, 1991; Schubart *et al.*, 2000) for 16S, and 12Sai (5'-AAACTAGGATTAGATACCCTATTAT-3') (see Palumbi *et al.*, 1991) and 12SH2 (5'-ATGCACTTTCCAGTACATCTAC-3'; a new primer based on Taylor *et al.*, 1996) for 12S. The PCR conditions always included 5 min of initial denaturation at 94°C and 10 min of final extension at 72°C. For amplification of 16S, the following cycling programs were used: 33–40 cycles with 1 min for denaturation at 94°C, 1–1½ min for annealing at 48–50°C, and

Table 1. Grapsoid crab species used for phylogeny reconstruction, with locality of collection, museum catalogue number and genetic database (EMBL) accession number (data on arboreal lifestyle from Vannini *et al.*, 1997; Sivasothi, 2000; C.D. Schubart, unpublished observations)

Species	Lifestyle	Collection site	Catalogue #	EMBL #
SESARMIDAE Dana, 1851				
<i>Aratus pisonii</i> (H. Milne Edwards, 1837)	TC	Puerto Rico: Mayaguez	MZUF 1026	AJ784012, AJ784289
<i>Armases cinereum</i> (Bosc, 1802)	NA	USA: Mississippi	ULLZ 4392	AJ784010, AJ784288
<i>Armases elegans</i> (Herklots, 1851)	TC	Cameroon: Tico Estuary	SMF 25703	AJ784011, AJ784290
<i>Chiomantes eulinene</i> (de Man, 1895)	NA	Kenya: Mida Creek	MZUF 2501	AJ784017, AJ784291
<i>Chiomantes ortmanni</i> (Crosnier, 1965)	NA	Kenya: Gazi Bay	MZUF 2523	AJ784016, AJ784292
<i>Clistoaeloma villosum</i> (A. Milne Edwards, 1869)	NA	Kenya: Mida Creek	MZUF 2500	AJ784018, AJ784293
<i>Clistoaeloma merguense</i> (de Man, 1888)	NA	Singapore: Mandai	MZUF 2494	AJ784019, AJ784294
<i>Episesarma mederi</i> (H. Milne Edwards, 1853)	TT	Singapore: Mandai	ZRC 2000.1950	AJ784020, AJ784295
<i>Episesarma versicolor</i> (Tweedie, 1940)	TT	Singapore: Mandai	MZUF 2495	AJ784021, AJ784296
<i>Neosarmatium meinerti</i> (de Man, 1887)	NA	Kenya: Gazi Bay	MZUF 2524	AJ784013, AJ784297
<i>Neosarmatium smithii</i> (H. Milne Edwards, 1853)	NA	Kenya: Mida Creek	MZUF 2504	AJ784014, AJ784298
<i>Parasesarma catenatum</i> (Ortmann, 1897)	NA	South Africa: Mgazana	MZUF 2509	AJ784025, AJ784299
<i>Parasesarma leptosoma</i> (Hilgendorf, 1869)	TC	Kenya: Mida Creek	MZUF 2547	AJ784024, AJ784300
<i>Perisesarma guttatum</i> (A. Milne Edwards, 1869)	NA	Mozambique: Inhaca	MZUF 1023	AJ621185, AJ784301
<i>Sarmatium crassum</i> Dana, 1851	NA	Kenya: Mida Creek	MZUF 2545	AJ784015, AJ784302
<i>Selatium brockii</i> (de Man, 1887)	TT	Kenya: Mida Creek	MZUF 2546	AJ784022, AJ784303
<i>Selatium elongatum</i> (A. Milne Edwards, 1869)	TT	Kenya: Mida Creek	MZUF 2521	AJ784023, AJ784304
<i>Sesarmoides longipes</i> (Krauss, 1843)	NA	Kenya: Mida Creek	MZUF 2505	AJ784026, AJ784305
GRAPSIDAE MacLeay, 1838				
<i>Metopograpsus latifrons</i> (White, 1847)	TC	Sabah: Kota Kinabalu	ZRC 2000.1666	AJ784028, AJ784306
<i>Metopograpsus thukuhar</i> (Owen, 1839)	TT	Mozambique: Inhaca	MZUF 2508	AJ784027, AJ784307
OCYPODIDAE Dana, 1851				
<i>Uca inversa</i> (Hoffmann, 1874)	NA	Kenya: Gazi Bay	MZUF 1024	AJ784029, AJ784308

Abbreviations: NA, non-arboreal species or only occasionally seen on roots; TT, species mostly or exclusively on the tree trunks and roots; TC, species mostly or exclusively in tree canopy; MZUF, Museo Zoologico Università di Firenze; SMF, Senckenberg Museum, Frankfurt a.M.; ULLZ, University of Louisiana Zoological Collection, Lafayette; ZRC, Zoological Reference Collection, Raffles Museum at the National University of Singapore.

1–2 min for extension at 72°C. The reactions yielded a DNA-sequence of approximately 560 base pairs (bp) in length. The PCR products were purified with Microcon 100 filters and then sequenced with the ABI BigDye terminator mix in an ABI Prism 310 Genetic Analyser. The amplification of approximately 400 bp of 12S consisted of 40 cycles with 30 s at 94°C, 15 s at 46–50°C and 30 s at 72°C. The PCR products were purified by chromatography on Sepharose CL-6B and then sent for sequencing to the ENEA Plant Genome Lab (Rome), which is equipped with an automated sequencer (Perkin Elmer 373A, Applied Biosystems); the Dye Terminator method was used. For each sample and for both the subunits, the forward and reverse sequences were obtained. Sequence data were submitted to EMBL (see Table 1 for accession numbers).

Sequences were aligned manually using the software ESEE Version 3.2 (based on Cabot and Beckenbach, 1989). Those regions in which homologous base pairs 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 data sets and later combined for the phylogenetic analyses. The DNA sequence of the East African fiddler crab *Uca inversa* (Ocypodidae) was included to serve as an outgroup.

Four methods of phylogenetic inference were applied to our data set: maximum parsimony, neighbour-joining and maximum likelihood using the software package PAUP* (Swofford, 1998), and Bayesian analysis as implemented in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist, 2001).

Maximum parsimony trees were obtained by a heuristic search with 10 replicates of random sequences addition and tree-bisection-reconnection as branch swapping options keeping multiple trees (MulTrees). Otherwise, the default options of PAUP* were used. Gaps were excluded from the analysis. Subsequently, confidence values for the proposed groups within the inferred trees were calculated with the bootstrap method (2000 replicates). Only minimal trees were retained and zero length branches were collapsed.

We calculated the model of DNA substitution that fitted our data best using the software MODELTEST 3.06 (Posada and Crandall, 1998). The calculations were performed separately for both genes as well as for the combined data set. This approach consists of successive pairwise comparisons of alternative substitution models by hierarchical likelihood ratio tests. The suggested model of DNA evolution was then used for inferring the phylogenetic relationship with the maximum likelihood, the neighbour-joining and the Bayesian methods.

Maximum likelihood analysis was performed using random sequences addition and setting parameters to values calculated by MODELTEST. The heuristic search was based on branch swapping with tree-bisection-reconnection. For both methods, bootstrap analyses as a heuristic search were applied with 500 replicates for maximum likelihood and 2000 replicates for neighbour-joining.

The Bayesian analysis was run with four MCMC chains for 2 million 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 ('burn-in phase'). The first 10,000 generations were not included in the analysis to avoid the possibility of including random and sub-optimal trees. The posterior probabilities of the phylogeny were determined for the remaining trees. Consensus trees were constructed using the 'sumpt' option in MrBayes.

A Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) was applied to determine whether the difference between the log-likelihood scores ($\ln L$) of the best unconstrained maximum likelihood (ML) tree and the $\ln L$ of the best constrained maximum likelihood trees

(ML_0 , the tree in which all the tree-climbing sesarmid crabs are constrained to form a monophyletic clade; ML_1 the tree in which only the TC tree-climbing sesarmid crabs are constrained to form a monophyletic clade) was statistically significant. The maximum likelihood analysis for the constrained tree was performed setting parameters to the values of the selected model and using the same options as for the unconstrained analysis. The Shimodaira-Hasegawa test was performed as implemented in PAUP* using 1000 non-parametric bootstrap RELL approximations.

We analysed the evolution of tree-climbing in grapsoid crab by mapping ancestor states onto our phylogenetic trees using MacClade 4 (Maddison and Maddison, 2000). We did not polarize the directions of evolutionary changes and we considered our categorical trait as two states (non-arboreal NA species versus tree-climbing TC ones) and as three states (NA, TC and tree trunk species) independently.

RESULTS

The total alignment of the sequenced portions of 16S and 12S consisted of 1038 base pairs, with the primer regions removed. Hypervariable regions that could not be aligned with certainty (80 bp) were excluded, and the remaining 958 base pairs were used for the phylogenetic analysis (550 bp 16S; 408 bp 12S). These 958 base pairs included 328 variable sites, of which 214 were parsimony-informative. The pairwise comparisons of numbers and types of genetic differences between all the taxa revealed that the overall transition to transversion ratio varied from 0.7 to 6.5 (0.5 to 8.3 in 16S; 0.5 to 6 in 12S).

The maximum parsimony heuristic search yielded three shortest trees of length 762 with the following tree scores: CI = 0.575, RI = 0.541, RC = 0.311. The consensus topology of this search was identical to the topology obtained with the bootstrap method, which is shown together with the resulting bootstrap values in Fig. 2.

Application of the likelihood ratio tests revealed that the selected models of DNA substitution were the TVM + I + G model for 12S, and GTR + I + G model for 16S and the combined data (Rodríguez *et al.*, 1990). Parameter values of the models resulting from the three likelihood ratio tests are shown in Table 2. The TVM + I + G is a simplified GTR + I + G model, in which the frequencies of the two types of transitions ($A \leftrightarrow C$ and $G \leftrightarrow T$) are equal. The GTR + I + G model was consequently used for the neighbour-joining, Bayesian and maximum likelihood inference methods.

All phylogenetic trees obtained from the four different inference methods (maximum parsimony, neighbour-joining, maximum likelihood, Bayesian analysis) showed the same general topology (Figs. 2–5), the minor differences not affecting our main question. Two areas of the trees remain unresolved with some (neighbour-joining, maximum parsimony) of the tree construction methods – that is, the exact branching order of five species groups within the Sesarmidae and the branching order among the genera *Clistocoeloma*, *Episesarma* and *Selatium*, which is fully resolved with Bayesian analysis and maximum likelihood. Most other groupings were supported with 90–100% bootstrap values in one or more trees, providing strong support to the respective sister group relationships. The phylogenies presented in Figs. 2–5 show that tree-climbing crabs can be found in three different lineages within the Sesarmidae (*Episesarma* and *Selatium*, *Armases elegans* and *Aratus pisonii*, *Parasesarma leptosoma*) and at least once within the Grapsidae (*Metopograpsus*). The monophyly of congeneric species was confirmed for the genera *Episesarma*, *Selatium*, *Clistocoeloma*, *Armases*, *Neosarmatium* and *Chiromantes*, as well as

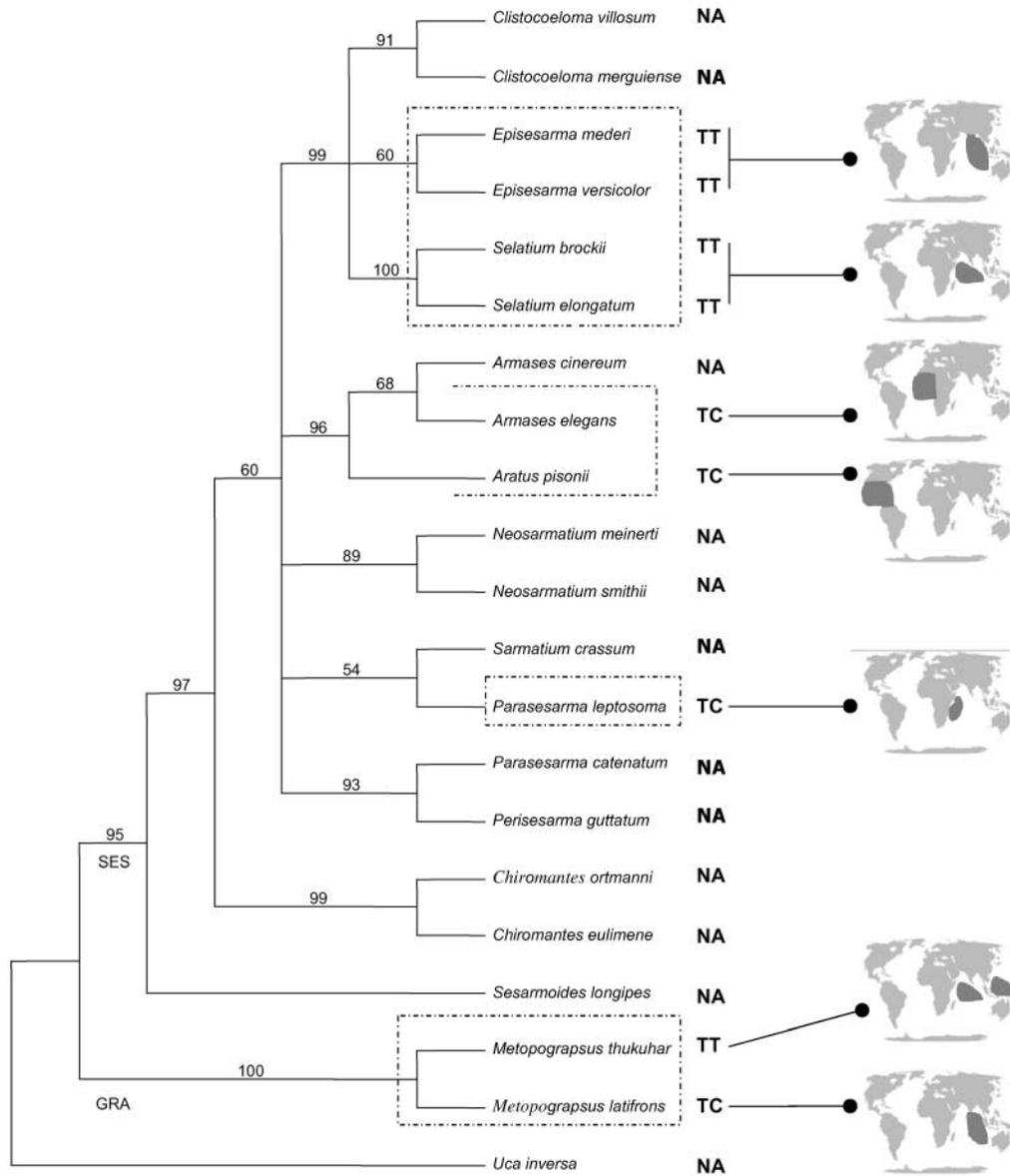


Fig. 2. Consensus tree from maximum parsimony (heuristic search, random addition, 2000 bootstrap replicates) analysis representing origin of the tree-climbing behaviour within the Grapsoidea as inferred from 958 base pairs of the 16S and 12S mitochondrial rRNA genes. The ocypodid *Uca inversa* was used as an outgroup. Only confidence values higher than 50% are shown in the tree. GRA, branch leading to species of Grapsidae; SES, branch leading to species of Sesarmidae. Abbreviations correspond to the degree of tree climbing (see Table 1). The geographic distribution of the tree-climbing species is also reported.

Table 2. Comparison of base frequencies (freqX), nucleotide substitution rates ($X \leftrightarrow Y$), proportion of invariable sites (I) and gamma distribution shape parameters (G) of the 12S rRNA gene, the 16S rRNA gene, and the combination of both of these genes under the selected substitution models

	12S rRNA	16S rRNA	12S + 16S rRNA
Substitution model	TVM + I + G	GTR + I + G	GTR + I + G
freqA	0.40	0.36	0.37
freqC	0.09	0.10	0.10
freqG	0.15	0.17	0.16
freqT	0.36	0.37	0.37
A \leftrightarrow C	3.31	0.31	0.99
A \leftrightarrow G	27.34	17.69	22.98
A \leftrightarrow T	5.34	2.44	3.47
C \leftrightarrow G	1.15	0.0	0.37
C \leftrightarrow T	27.34	8.12	12.7
G \leftrightarrow T	1.0	1.0	1.0
I	0.43	0.45	0.46
G	0.68	0.55	0.68

for the families Sesarmidae and Grapsidae. However, our phylogenetic results do not confirm the current taxonomy of the genus *Parasesarma*.

The plausibility of two null hypotheses (H_{0-1} , the monophyly of all the sesarmid tree-climbing crabs; and H_{0-2} , the monophyly of the TC sesarmid species) was tested by comparing the likelihood score of the unconstrained maximum likelihood tree ($-\ln L = 4770.19$) with the likelihood scores of the constrained maximum likelihood trees ($-\ln L_{0-1} = 4860.93$, $-\ln L_{0-2} = 4800.22$). The Shimodaira-Hasegawa test revealed that both null hypotheses can be rejected, because of highly significant ($P < 0.001$) differences to the unconstrained tree in the log-likelihood scores ($\Delta \ln L_{0-1} = 90.74$ and $\Delta \ln L_{0-2} = 30.05$).

The analysis of ancestral character states based on a parsimony approach clearly showed the independent origin of tree-climbing ability within the families Grapsidae and Sesarmidae based on a non-arboreal ancestor. This analysis also revealed that within the family Sesarmidae, tree-climbing ability was gained or lost more than once. However, it did not allow us to identify the exact number of independent evolutionary events that led to arboreal life (from two to four times depending on the tree on which we mapped the trait), due to unsolved basal nodes within the sesarmid phylogeny (see Figs. 2–5).

DISCUSSION

Our data suggest that tree-climbing lifestyles and the associated morphology have evolved convergently in several lineages of mangrove crabs of the families Grapsidae and Sesarmidae. The alternative explanation, that arboreal habits are plesiomorphic in these brachyuran taxa and were later lost in some of the lineages and species, is highly unlikely considering the fact that tree-climbing mangrove crabs can only be found in the families Sesarmidae and Grapsidae and that the vast majority of crabs belonging to these families (>250 species) are bottom dwellers. The massive ecological and morphological

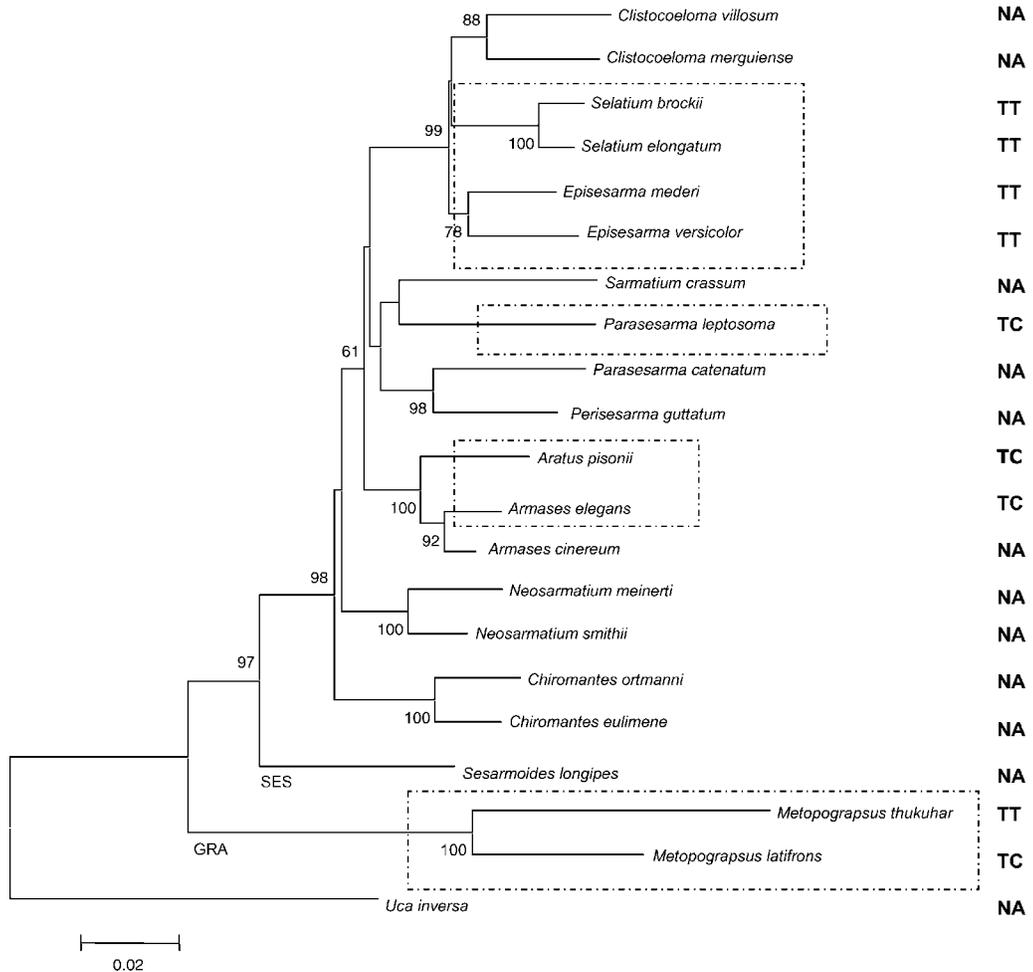


Fig. 3. Maximum likelihood tree based on GTR + I + G distance model (heuristic search, random addition, 500 bootstrap replicates) representing origin of the tree-climbing behaviour within the Grapsoidea as inferred from 958 base pairs of the 16S and 12S mitochondrial rRNA genes. The ocypodid *Uca inversa* was used as an outgroup. Only confidence values higher than 50% are shown in the tree. GRA, branch leading to species of Grapsidae; SES, branch leading to species of Sesarmidae. Abbreviations correspond to the degree of tree climbing (see Table 1).

radiation and speciation of the Sesarmidae is most likely related to the evolution of mangroves in Southeast Asia (Jones, 1984) and must have been initiated by marine or brackish water ancestors. The likelihood of finding convergent evolution in sesarmid crabs has already been highlighted in a previous study, which showed that long legs and flattened body forms – which are characteristic of land crabs of the family Sesarmidae in Jamaica (genus *Sesarma*) and Southeast Asia (genus *Sesarmoides*) – are the result of convergent evolution and not common ancestry (Schubart *et al.*, 1998).

Three sesarmid crab species from mangrove canopies (TC species) have a striking morphological similarity: *Aratus pisonii* from America, *Armases elegans* from West Africa

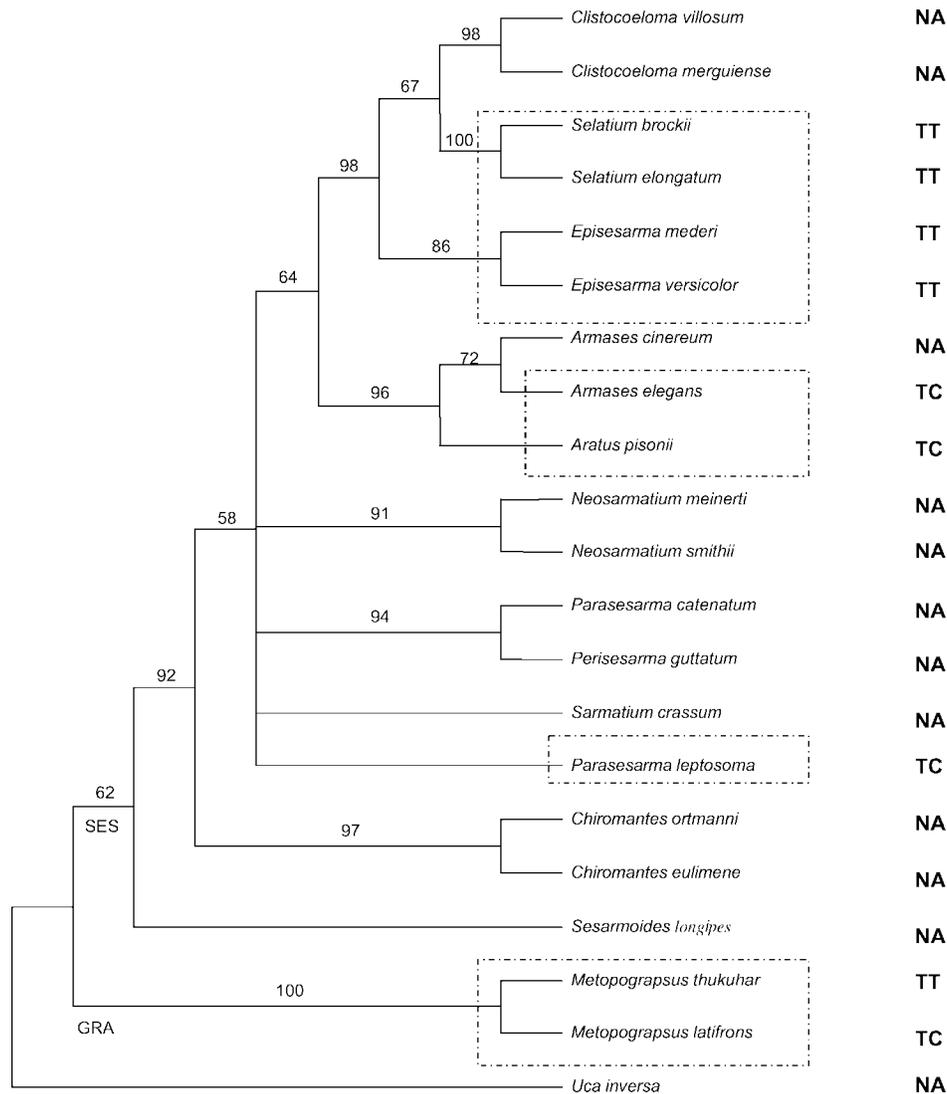


Fig. 4. Neighbour-joining tree based on GTR + I + G distance model (bootstrap method with 2000 replicates) representing origin of the tree-climbing behaviour within the Grapsoidea as inferred from 958 base pairs of the 16S and 12S mitochondrial rRNA genes. The ocypodid *Uca inversa* was used as an outgroup. Only confidence values higher than 50% are shown in the tree. GRA, branch leading to species of Grapsidae; SES, branch leading to species of Sesarmidae. Abbreviations correspond to the degree of tree climbing (see Table 1).

and *Parasesarma leptosoma* from the western Indo-Pacific. These crabs are characterized by flat bodies, long propodi and short dactyli, as well as triangular carapaces. Based on these similarities, it had been suggested that the African species *Armases elegans* should be included in the American genus *Aratus* (see Green, 1986). Our phylogenetic data show that the two Atlantic species, *Aratus pisonii* and *Armases elegans*, are phylogenetically close and

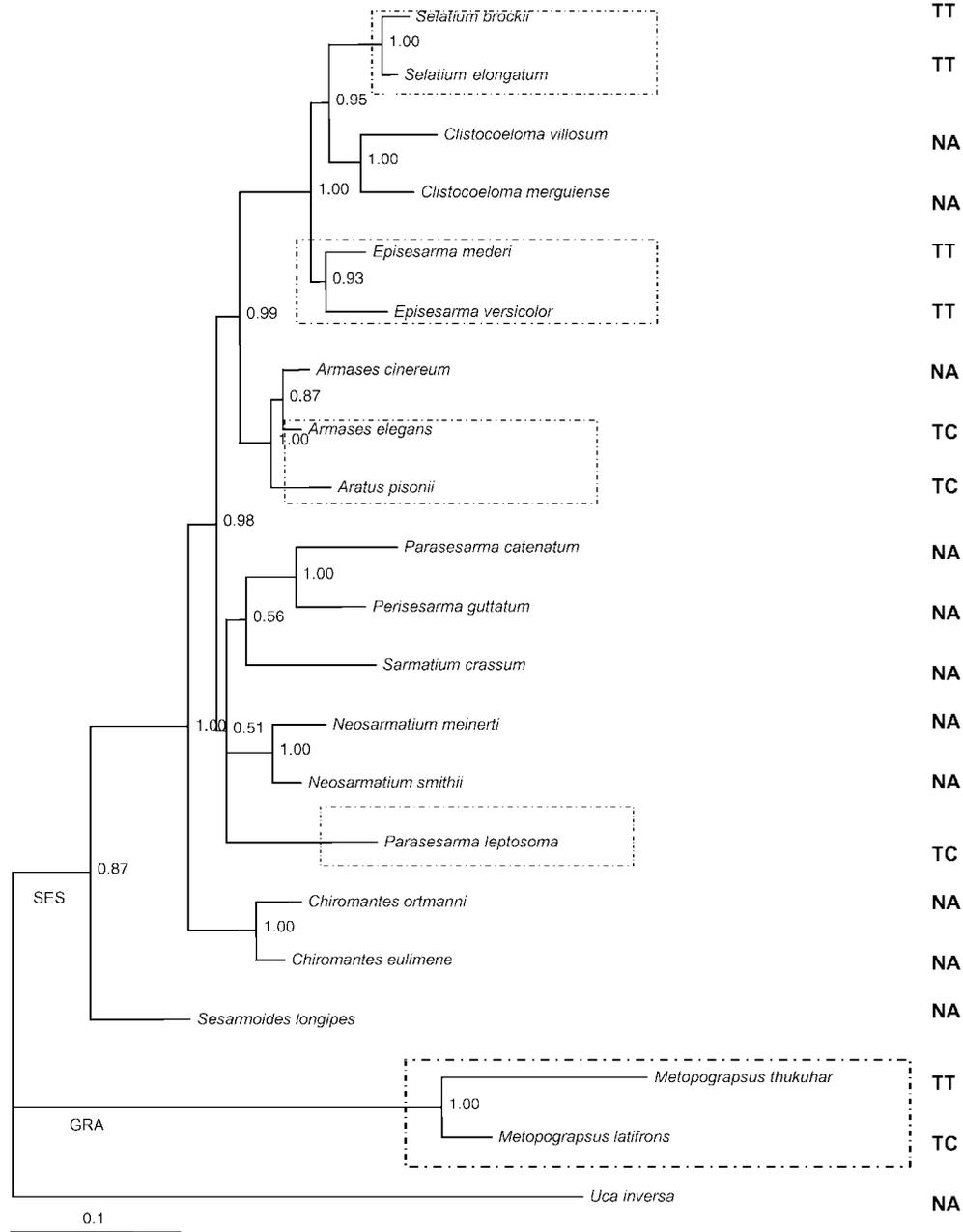


Fig. 5. Bayesian tree based on GTR + I + G distance model representing origin of the tree-climbing behaviour within the Grapsoidae as inferred from 958 base pairs of the 16S and 12S mitochondrial rRNA genes. The ocypodid *Uca inversa* was used as an outgroup. Only posterior probabilities higher than 50% are shown in the tree. GRA, branch leading to species of Grapsidae; SES, branch leading to species of Sesarmidae. Abbreviations correspond to the degree of tree climbing (see Table 1).

the two corresponding genera appear to be sister taxa (see also Niem, 1996). However, the position of the American marsh crab *Armases cinereum* (type species of the genus) revealed that the tree-living *Armases elegans* is more closely related to its congeneric marsh crab than to the arboreal *Aratus pisonii* and therefore should remain within the genus *Armases* (and not *Aratus* as used by Green, 1986). The genus *Armases* consists of 11 species, which are typically ground-dwelling forms in marshes and mangroves with squarish carapaces (Abele, 1992). The inclusion of more members of this genus in the phylogenetic analysis might reveal whether tree climbing and triangular carapaces have evolved independently in *Armases elegans* and *Aratus pisonii* or whether arboreal life is plesiomorphic within this group and was lost in the other species of *Armases*. Occasionally, other species of *Armases* have been reported to climb trees, bromeliads or walls (Abele, 1973; von Hagen, 1977; Gray and Christy, 2000), but they are never found in the canopies of mangrove trees feeding on green leaves.

The western Indo-Pacific *Parasesarma leptosoma* differs morphologically from *Aratus* and *Armases* primarily by two transverse pectinated ridges on the propodus of the male cheliped. Otherwise, it has a very similar morphology to the other two canopy dwellers. Although our data do not resolve the exact phylogenetic position of *Parasesarma leptosoma* within the Sesarmidae (weakly supported affinity to the genus *Sarmatium*), we can infer that there is no sister-group relationship between *Parasesarma leptosoma* and the group including *Aratus* and *Armases*, and thus all morphological, ecological and behavioural similarities must be due to convergent evolution. The current taxonomy of the genus *Parasesarma* is put into question by our finding that *Parasesarma catenatum* has a much closer phylogenetic relationship to the genus *Perisesarma* (represented by *Perisesarma guttatum*) than to *Parasesarma leptosoma*, suggesting that this genus is not monophyletic.

On the other hand, our data place the TT tree-climbing species of the Sesarmidae (genera *Selatium* and *Episesarma*) within a single monophyletic group, together with the ground-dwelling genus *Clistocoeloma*. This grouping is in accordance with the morphology of these crabs, as all of them have a single longitudinal pectinated crest on the cheliped propodus and squarish to elongate carapaces. The inclusion of more species and genera (e.g. *Neosesarma*) that also belong to this group, based on their morphology, should clarify whether tree climbing in *Episesarma* and *Selatium* is convergent or plesiomorphic. The current generic classification of the species of *Episesarma* and *Selatium* is congruent with the results presented here.

Overall, we conclude that there are at least three independent lineages with pronounced adaptations to arboreal life in the crab family Sesarmidae: (1) *Aratus pisonii* and *Armases elegans*, (2) *Parasesarma leptosoma*, and (3) the genera *Episesarma* and *Selatium*. The Sesarmidae is generally recognized as a group primarily adapted to a sheltered soft-sediment coastal environment, often digging more or less extensive burrows (Hartnoll, 1975). Assuming that this lifestyle is plesiomorphic, climbing in this family probably evolved independently from the clinging and climbing ability shown by many Grapsidae (cliff- and reef-dwellers on exposed shores such as *Grapsus*, *Leptograpsus* and *Pachygrapsus*, or rock- and tree-dwellers in sheltered environments such as *Metopograpsus* spp.) and Plagusidae (also belonging to the superfamily Grapsoidea; Schubart *et al.*, 2002), such as *Plagusia* and *Percnon*. This is also suggested by our phylogeny, in which the branch leading to tree-climbing crabs of the genus *Metopograpsus* (family Grapsidae) is rooted outside of the family Sesarmidae. These crabs therefore adapted to arboreal life convergently to all Sesarmidae. One grapsid species, *M. latifrons*, shows a highly adapted tree-climbing behaviour and spends most of its time in the canopies of mangrove trees. The fact that

specialization to life in mangrove canopies in this species is coupled with the same morphological characters as described for *Parasesarma leptosoma*, *Armases elegans* and *Aratus pisonii* (triangular and flat carapace, long propodi and short dactyli) is an additional demonstration of the likelihood that morphological, ecological and behavioural patterns can evolve convergently under similar selection pressures.

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REFERENCES

- Abele, L.G. 1973. A note on the Brazilian bromeliad crabs (Crustacea, Grapsidae). *Arquivos de Ciências do Mar*, **12**: 123–126.
- Abele, L.G. 1992. A review of the grapsid crab genus *Sesarma* (Crustacea: Decapoda: Grapsidae) in America, with the description of a new genus. *Smithsonian Contrib. Zool.*, **527**: 1–60.
- Cabot, E.L. and Beckenbach, A.T. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Comp. Appl. Biosci.*, **5**: 233–234.
- Cannicci, S., Ruwa, R.K., Ritossa, S. and Vannini, M. 1996. Branch-fidelity in the tree crab *Sesarma leptosoma* (Decapoda, Grapsidae). *J. Zool. (Lond.)*, **238**: 795–801.
- Cannicci, S., Fratini, S. and Vannini, M. 1999. Use of time and food resources in the mangrove climbing crab *Selatium elongatum* (Grapsidae: Sesarminae). *Mar. Biol.*, **135**: 335–339.
- Dahdouh-Guebas, F., Giuggioli, M., Oluoch, A., Vannini, M. and Cannicci, S. 1999. A note on feeding habits of non-ocypodid mangrove crabs from Kenya. *Bull. Mar. Sci.*, **64**: 291–297.
- Emmerson, W.D. and McGwynne, L.E. 1992. Feeding and assimilation of mangrove leaves by the crab *Sesarma meinerti* de Man in relation to leaf-litter production in Mgazana, a warm-temperate southern African mangrove swamp. *J. Exp. Mar. Biol. Ecol.*, **157**: 41–53.
- Emmerson, W., Cannicci, S. and Porri, F. 2003. New records for *Parasesarma leptosoma* (Hilgendorf, 1869) (Crustacea: Decapoda: Brachyura: Sesarminidae) from mangroves in Mozambique and South Africa. *Afr. Zool.*, **38**: 351–355.
- Erickson, A.A., Saltis, M., Bell, S.S. and Dawes, C.J. 2003. Herbivore feeding preferences as measured by leaf damage and stomatal ingestion: a mangrove crab example. *J. Exp. Mar. Biol. Ecol.*, **289**: 123–138.
- Fratini, S., Cannicci, S., Abincha, L.M. and Vannini M. 2000a. Feeding, temporal and spatial preferences of *Metopograpsus thukuhar* (Decapoda; Grapsidae): an opportunistic mangrove dweller. *J. Crust. Biol.*, **20**: 326–333.
- Fratini, S., Cannicci, S. and Vannini, M. 2000b. Competition and interaction between *Neosarmatium smithii* (Crustacea, Grapsidae) and *Terebralia palustris* (Mollusca, Gastropoda) in a Kenyan mangrove. *Mar. Biol.*, **137**: 309–316.

- Gray, H.M. and Christy, J.H. 2000. Predation by the grapsid crab, *Armases angustum* (Smith, 1870), on tadpoles of the green poison frog, *Dendrobates auratus* Girard, 1855. *Crustaceana*, **73**: 1023–1025.
- Green, J.L. 1986. Biology of *Aratus elegans*. MSc thesis, University of North Wales.
- Hagen, H.O., von. 1977. The tree climbing crabs of Trinidad. *Studies on the Fauna of Curaçao and other Caribbean Islands*, **54**: 25–59.
- Hartnoll, R.G. 1975. The Grapsidae and Ocypodidae (Decapoda: Brachyura) of Tanzania. *J. Zool. (Lond.)*, **177**: 305–328.
- Hartnoll, R.G. 1988. Evolution, systematics and, geographical distribution. In *Biology of the Land Crabs* (W.W. Burggren and B.R. McMahon, eds.), pp. 6–54. Cambridge: Cambridge University Press.
- Huelsenbeck, J.P. and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754–755.
- Jones, D.A. 1984. Crabs of the mangal ecosystem. In *Hydrobiology of the Mangal* (F.D. Por and I. Dor, eds.), pp. 89–109. The Hague: Dr. W. Junk.
- Kathiresan, K. and Bingham, B.L. 2001. Biology of mangroves and mangroves ecosystems. *Adv. Mar. Biol.*, **40**: 84–251.
- Lee, S.Y. 1997. Potential trophic importance of the faecal material of the mangrove sesarmin crab *Sesarma messa*. *Mar. Ecol. Progr. Ser.*, **159**: 275–284
- Lee, S.Y. 1998. Ecological role of grapsid crabs in mangrove ecosystems: a review. *Mar. Freshwater Res.*, **49**: 335–343.
- Maddison, D.R. and Maddison, W.P. 2000. *MacClade 4: Analysis of Phylogeny and Character Evolution*, Version 4.0. Sunderland, MA: Sinauer Associates.
- Micheli, F. 1993. Feeding ecology of mangrove crabs in North Eastern Australia: mangrove litter consumption by *Sesarma messa* and *Sesarma smithii*. *J. Exp. Mar. Biol. Ecol.*, **171**: 165–186.
- Niem, V.H. 1996. Phylogenetic relationships among American species of *Sesarma* (subgenus *Armases*) (Brachyura, Grapsidae). *Crustaceana*, **69**: 330–348.
- Palumbi, S., Martin, A., Romano, S. et al. 1991. *The simple fool's guide to PCR*. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.
- Posada, D. and Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**: 817–818.
- Robertson, A.I. 1986. Leaf-burying crabs: their influence on energy flow and export from mixed mangrove forests (*Rhizophora* spp.) in northeastern Australia. *J. Exp. Mar. Biol. Ecol.*, **102**: 237–248.
- Rodríguez, F., Oliver, J.F., Marín, A. and Medina, J.R. 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.*, **142**: 485–501.
- Schubart, C.D., Diesel, R. and Hedges, S.B. 1998. Rapid evolution to terrestrial life in Jamaican crabs. *Nature*, **393**: 363–365.
- Schubart, C.D., Neigel, J.E. and Felder, D.L. 2000. Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues*, **12**: 817–830.
- Schubart, C.D., Cuesta, J.A. and Felder, D.L. 2002. Glyptograpsidae, a new brachyuran family from Central America: larval and adult morphology, and a molecular phylogeny of the Grapsoidea. *J. Crust. Biol.*, **22**: 28–44.
- Shimodaira, K. and Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.*, **16**: 1114–1116.
- Sivasothi, N. 2000. Niche preferences of tree-climbing crabs in Singapore mangroves. *Crustaceana*, **73**: 25–38.
- Sivasothi, N., Murphy, D.H. and Ng, P.K.L. 1993. Tree climbing and herbivory of crabs in the Singapore mangroves. In *Mangrove Fisheries and Connections*. Proceedings of the ASEAN-Australian Marine Science Project: Living Coastal Resources Workshop1 (A. Sasekumar, ed.), pp. 220–237. Ipoh, Malaysia.

- Swofford, D.L. 1998. *PAUP* – Phylogenetic Analysis Using Parsimony (* and Other Methods)*, Version 4. Sunderland, MA: Sinauer Associates.
- Taylor, D.J., Hebert, P.D.N. and Colbourne, J.K. 1996. Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12S rDNA sequence and allozyme variation. *Mol. Phyl. Evol.*, **5**: 495–510.
- Vannini, M. and Cannicci, S. 1995. Homing behaviour and possible cognitive maps in crustacean decapods. *J. Exp. Mar. Biol. Ecol.*, **193**: 67–91.
- Vannini, M. and Chelazzi, G. 1985. Adattamenti comportamentali alla vita intertidale tropicale. *Oebelia NS*, **11**: 23–37.
- Vannini, M. and Ruwa, R.K. 1994. Vertical migrations in the tree crab *Sesarma leptosoma* (Decapoda, Grapsidae). *Mar. Biol.*, **118**: 271–278.
- Vannini, M., Oluoch, A. and Ruwa, R.K. 1997. The tree-climbing crabs of Kenyan mangroves. In *Mangrove Ecosystems Studies in Latin America and Africa* (B. Kjerfve, B.L. De Lacerda and E.S. Diop, eds.), pp. 325–338. UNESCO Technical Papers in Marine Sciences. New York: UNESCO.
- Warner, G.F. 1967. The life history of the mangrove tree crab, *Aratus pisonii*. *J. Zool. (Lond.)*, **153**: 321–335.

