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Phylogeny, biogeography and a new taxonomy for the Gecarcinucoidea Rathbun, 1904 (Decapoda: Brachyura)

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Abstract

Phylogenetic relationships of gecarcinucoid freshwater crabs were investigated, based on morphology of the male second gonopod. In addition, a comparison of sequences from the mitochondrial large subunit rRNA gene helped to resolve the phylogeny of this group and relationships to other Old World freshwater crabs. As a result, we recognise two sister groups within the Gecarcinucoidea, the African Deckeniidae and the Asian Gecarcinucidae. Deckeniidae includes three monophyletic clades, the Deckeniinae in East Africa and on the Seychelles, the West African Globonautinae and the Malagasy Hydrothelphusinae. Gecarcinucidae comprises two sister groups, the Gecarcinucinae with representatives in Sri Lanka, India and southeast Asia, and the Parathelphusinae in India, southeast Asia, the Sundaic Islands and Australia. Interpretation of our phylogenetic results leads us to propose a new biogeographic hypothesis for the Gecarcinucoidea. Most likely, the gecarcinucoid freshwater crabs have an African origin; their distribution can be explained by successive events of dispersal. This model can be correlated with palaeogeographical and palaeoclimatological data for the Cenozoic, suggesting a gecarcinucoid dispersal to Asia via the "Lemurian Stepping-Stones", a chain of islands in the West-Indian Ocean that were emergent in times of low sea levels during the Oligocene.
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Introduction

Recently, biogeographic hypotheses for taxa with a range disjunct between Africa and Asia have arisen as a general theme in biogeography (Vences et al. 2001, 2003; Raxworthy et al. 2002; Biju and Bossuyt 2003; Morley 2003; Yoder et al. 2003; Yuan et al. 2005) and have fuelled a fundamental discussion between advocates of either vicariance or dispersal hypotheses (Heads 2005; McGlone 2005).

The question regarding biogeographic relationships between Africa and Asia concerns various faunal and floral taxa with a Circum-West Pacific distribution. Among them are the true, direct-developing freshwater crabs, one of the most diverse groups of the Brachyura. However, as long as the relationship among different freshwater crab taxa has remained uncertain,

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biogeographic work was mainly restricted to descriptive accounts of the distribution of taxa. Questions of historical biogeography concerning the origin and mode of distribution of the freshwater crabs were reviewed only by few authors (e.g. Bott 1969a, 1972; Rodríguez 1986; Ng et al. 1995, von Sternberg et al. 1999).

The phylogeny and classification of the freshwater crabs from Africa, Asia and Madagascar has been a controversial topic for the last two centuries. A major contribution to classification was the introduction of superfamilies by Bott (1970a). For the Old World he established two superfamilies, the Potamoidea Ortmann, 1896 and the Parathelphusoidea Colosi, 1920; the latter taxon name was later changed to Gecarcinucoidea Rathbun, 1904 (Holthuis 1974). The Gecarcinucoidea sensu Bott display an interesting, disjunct distribution pattern with members in West Africa, on Madagascar, and in South and southeast Asia. But especially the taxonomy of the Gecarcinucoidea remained unstable, as reflected by frequent taxonomic rearrangements during the last decades (Ng and Takeda 1994; Ng 1995; Ng et al. 1995; Cumberlidge 1999; Cumberlidge and von Sternberg 2002).

Our new biogeographic hypothesis for the Gecarcinucoidea is based on the phylogeny as reconstructed from a detailed analysis of the morphology and function of the copulatory apparatus of freshwater crabs and from molecular data (mitochondrial large subunit rRNA sequences). In addition to our phylogenetic results, a set of palaeontological, palaeogeographical, palaeoclimatological and geological data leads us to propose a plausible explanation for the relationship between African and Asian members of this brachyuran superfamily. Hereby we also want to contribute to the general biogeographic discussion of African–Asian faunal relationships, being convinced that for every group it should be carefully evaluated which biogeographic model applies with the highest probability.

Material and methods

Morphological analysis

The crabs used for the morphological study were taken from the collections of the Senckenberg Museum, Frankfurt and the Muséum National d'Histoire Naturelle, Paris (Table 1). The copulatory organs of adult males studied by histological sectioning techniques had been preserved in 5% formalin. The second gonopods were decalcified in 5% trichlorine acetic acid, embedded in Spurr's resin, and sectioned at 2 μm on an ultramicrotome (Reichert-Jung). The sections were stained with Richardson's blue. Photographs were taken with an Axioplan microscope (Zeiss).

Molecular analysis

Samples for this study were collected by the authors between 1999 and 2003 (Table 2) or obtained from different museum collections. Some of the museum specimens, which included type material, were more than 100 years old; thus, amplification of longer DNA sequences was impossible. Genomic DNA was extracted from the muscle tissue of walking legs using the Puregene kit (Gentra Systems). Selective amplification of an approximately 550 basepair fragment (excluding primers) from the large ribosomal subunit (16S) was carried out by polymerase chain reaction (PCR) under the following conditions: 40 cycles, with 45 s denaturing at 94 °C, 1 min annealing at 48 °C, and 1 min extension at 72 °C (with 4 min initial denaturation and 10 min final extension times). Primers used were 16L29 (5′–YGCCTGTTTATCAAAAACAT–3′) and 16H37 (5′–CCGGTYTGAACTCAAATCATGT–3′) (both modifications of 16Sar and 16Sbr from Palumbi et al. 1991) or 1472 (5′–AGATAGAAACCAACCTGG–3′). The amplification of older museum samples required the use of specific internal primers (see Schubart et al. 2000). PCR products were purified with Microcon 100 filters and then sequenced with the ABI BigDye terminator mix in an ABI Prism 310 Genetic Analyzer (Applied Biosystem, Foster City, USA). New sequence data have been submitted to the European genetic database EMBL (see Table 2 for accession numbers).

In addition to the sequences generated in this study, our phylogenetic analyses included previously published sequences corresponding to the same 16S gene region from GenBank, originating from the studies by Schubart et al. (2000) (2 sequences) and Bossuyt et al. (2004) (14 sequences). The sequence from *Globonautes macropus* was kindly made available by Trisha Spears of Florida State University. Sequences were aligned manually. Those regions in which pairings of homologous basepairs could not be ascertained during the alignment process (due to the high frequency of indels in hypervariable areas) were excluded from the analysis. DNA sequence from the crab *Carpilius corallinus* (Carpiliidae) from GenBank (AF503462) was included to serve as outgroup.

A χ^2-test for homogeneity of base frequencies across taxa was carried out as implemented in PAUP[*] (Swofford 1998), including or excluding the outgroup from the analysis. The model of DNA substitution that fitted our data best was chosen using the software MODELTEST 3.6 (Posada and Crandall 1998). Three methods of phylogenetic inference were applied to our dataset: maximum parsimony (MP) using the software package PAUP[*] (Swofford 1998), Bayesian analysis (BI) as implemented in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001), and minimum evolution (ME) with the program MEGA2 (Kumar et al. 2001). MP trees were

Table 1. Male crab specimens examined for histology of the second gonopod: scientific names and taxonomic authorities, museum catalogue numbers, and geographic origins

Species	Catalogue no.	Provenance
Afrithelphusa gerhildae Bott, 1969	SMF 12 145	Guinea, Kindia, NE of Conakry
Calappa granulata (Linnaeus, 1758)	SMF 12 497	Unknown
Ceylonthelphusa rugosa (Kingsley, 1880)	SMF 4378	Sri Lanka, Bellhull-Oya
Ceylonthelphusa soror (Zehntner, 1894)	SMF 4394	Sri Lanka, Batticaloa
Deckenia imitatrix Hilgendorf, 1869	SMF 2877	East Africa
Eriphia verrucosa (Forskal, 1775)	Uncatalogued	Croatia, Rovinj
Gecarcinucus jacquemontii H. Milne-Edwards, 1844	SMF 1763	India, Bombay
Gubernatoriana escheri (Roux, 1931)	SMF 5140	India, Shembaganur
Holthuisana angustifrons (Milne Edwards, 1869)	SMF 4272	Australia, Kimberley Res. Stat.
Holthuisana biroi (Nobili, 1905)	SMF 7373	New Guinea, Borowai, Lake Sentani (2°36′S, 140°23′E)
Hydrothelphusa agilis (A. Milne-Edwards, 1872)	SMF 2616	Madagascar, Tamalaoe
Hydrothelphusa goudoti (A. Milne-Edwards, 1853)	Uncatalogued	Madagascar
Liberonautes latidactylus (De Man, 1903)	SMF 6597	Liberia, Bong range, Farmington River (6°20′N, 10°18′W)
Madagapotamon ankaraharae (Nobili, 1906)	MNHN 677	Madagascar, Antsiranana
Malagasya antongilensis (Rathbun, 1905)	SMF 2615	Madagascar
Menippe mercenaria (Say, 1818)	SMF 3997	Gulf of Mexico, Florida
Oziothelphusa senex (Fabricius, 1798)	SMF 4368	Sri Lanka, Kanniyat, near Trincomalee
Parathelphusa bogorensis Bott, 1970b	SMF 2753	Java, Bogor
Parathelphusa celebensis (De Man, 1892)	SMF 1790	Southeast Sulawesi
Parathelphusa maculata de Man, 1879	SMF 2757	Singapore, Mardai Road
Perbrinckia enodis (Kingsley, 1880)	SMF 4391	Sri Lanka, Kandy
Platythelphusa armata A. Milne-Edwards, 1887	SMF 6882	Tanzania, Lake Tanganjika, Gombe National Park
Potamon setiger Rathbun, 1904	SMF 24197	Syria, Orontes River
Potamonautes perlatus (H. Milne-Edwards, 1837)	SMF 23 255	South Africa
Potamonautes obesus (A. Milne-Edwards, 1868)	SMF 7821	Zimbabwe, Salisburg
Salangathelphusa brevicarinata (Hilgendorf, 1882)	SMF 12019	Thailand
Sartoriana spinigera (Wood-Mason, 1871)	SMF 26 057	India, Nagaland, market in Dimapur
Seychellum alluaudi (A. Milne-Edwards & Bouvier, 1893)	SMF 12 926	Seychelles, La Digue (4°20′S, 55°50′E)
Spiralothelphusa hydrodroma (Herbst, 1794)	SMF 2823	Sri Lanka, Lake Mundale
Spiralothelphusa wuellerstorfi (Heller, 1862)	SMF 4406	Nicobar islands
Sundathelphusa cassiope (De Man, 1902)	SMF 1802	Moluccas, Batjan
Terrathelphusa kuhlii (De Man, 1883)	SMF 5088	Java, Cibodas
Travancoriana sp. Bott, 1969	SMF 24 914	India, Kerala, Mavoor/Mapram

MNHN = Muséum National d'Histoire Naturelle, Paris; SMF = Senckenberg Museum, Frankfurt a. M.

obtained by a heuristic search with 20 replicates, using random sequence addition and tree-bisection-reconnection as branch-swapping options, and keeping multiple trees (MulTrees). Transversions were weighted as three times transitions; gaps were excluded from the analysis. Otherwise, the default options of PAUP* were used. For ME, the gamma shape distribution as calculated by Modeltest was implemented, and distances calculated under a Tamura–Nei model. Subsequently, confidence values for the proposed groups within the inferred trees were calculated with the bootstrap method (2000 pseudoreplicates). Only minimal trees were retained; zero length branches were collapsed. The BI trees were calculated using the suggested model of evolution. The Bayesian analysis was run with four MCMC chains for two million generations, saving a tree every 500

generations (with a corresponding output of 4000 trees). The −lnL converged on a stable value between 5000 and 8000 generations ("burn-in phase"). The first 10,000 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 for the remaining trees. Consensus trees were constructed using the 'sumpt' option in MrBayes.

Results

Morphological results

Male copulatory organs of freshwater crabs

The male copulatory apparatus of all examined freshwater crabs consists of the two anteriormost,

Table 2. Freshwater crab specimens used for DNA sequencing and subsequent phylogeny reconstruction: scientific names and taxonomic authorities, museum catalogue numbers, geographic origins, and gene database (EMBL) accession numbers

Species	Catalogue no.	Provenance	EMBL acc. no.
Afrithelphusa gerhildae Bott, 1969; paratype	SMF 12145	Guinea, Kindia, NE of Conakry	AM234633
Cylindrotelphusa steniops (Alcock, 1909); syntype	NHML 1909.10.13.6	South India	AM234634
Cylindrotelphusa sp.	SMF 2754	South India, Malabar, Puddapaddy	AM234635
Deckenia imitatrix Hilgendorf, 1869	SMF 2877	East Africa	AM234636
Gecarcinucus jacquemontii H. Milne-Edwards, 1844	NHML 1895.11.8	India, Bombay, Kaman River	AM234637
Hydrothelphusa madagascariensis (A. Milne-Edwards, 1872)	NHMW 13371	Madagascar	AM234638
Hydrothelphusa sp.	SMF 30957	Madagascar, Antsampandrano nr Antsirabe	AM234639
Irmengardia johnsoni Ng & Yang, 1985	SMF 30158	Singapore, Nee Soon Swamp Forest	AM234640
Madagapotamon humberti Bott, 1965	MNHN B 25562	Madagascar	AM234641
Nautilothelphusa zimmeri (Balss, 1933)	ZRC 2000.1711	Sulawesi, Lake Matano	AM234642
Parathelphusa matannensis (Schenkel, 1902)	ZRC 2000.1685	Sulawesi, Lake Matano	AM234643
Parathelphusa sarasinorum Schenkel, 1902	ZRC 2000.1697	Sulawesi, Lake Poso	AM234644
Potamon ibericum Bieberstein, 1809	SMF 30960	Turkey, Ihlara, near Tüz Gölü	AM234645
Potamon fluviatile (Herbst, 1785)	SMF 30166	Greece, Paramythia (39°28′N, 20°43′E)	AM234632
Potamonautes obesus (A. Milne-Edwards, 1868)	MZUF 2734	Kenya, Arabuko Sokoke Forest	AM234646
Potamonautes perlatus (H. Milne-Edwards, 1837)	SMF 30155	South Africa, Eerste River, Stellenbosch	AM234647
Salangathelphusa brevicarinata (Hilgendorf, 1882)	ZRC 2000.1918	Thailand, Phuket, Ton Sai Falls	AM234648
Sartoriana spinigera (Wood-Mason, 1871)	SMF 29344	West Bengal, India	AM234649
Siamthelphusa holthuisi Nayanetr & Ng, 1990	ZRC 2000.1923	Thailand, Krathing Falls	AM234650
Sundathelphusa minahassae (Schenkel, 1902)	ZRC 2000.1681	Sulawesi, Tomohon	AM234651
Seychellum alluaudi (A. Milne-Edwards & Bouvier, 1893) 1	SMF 12925	Seychelles, La Digue (04°20′S, 55°50′E)	AM234652
Seychellum alluaudi (A. Milne-Edwards & Bouvier, 1893) 2	SMF 30157	Seychelles, La Digue	AM234653

MNHN = Muséum National d'Histoire Naturelle, Paris; MZUF = Museo Zoologico, Università di Firenze; NHML = Natural History Museum, London; NHMW = Naturhistorisches Museum Wien; SMF = Senckenberg Museum, Frankfurt a.M.; ZRC = Zoological Reference Collection, Raffles Museum, National University of Singapore.

strongly modified pairs of pleopods, referred to as gonopods. The first gonopod (G1) is three-segmented; the lateral edges of the terminal segment overlap, thus forming a tube. In most species, the terminal segment is divided into a terminal and subterminal joint which are connected by a flexible zone consisting of a dorsal membrane-like cuticle (Fig. 1A). This division is lacking in few species. The second gonopod (G2) of the investigated freshwater crabs is thin, in most taxa with the distal part elongated and forming an open groove or closed tube. The distal and proximal parts are separated by a spoon-like protrusion situated dorsally or surrounding the second gonopod (Fig. 1B).

The terminology used for the gonopod descriptions follows Magalhães and Türkay (1996) and Brandis et al. (1999). The part of the gonopod directed towards the sides of the body is referred to as "lateral", the side

facing the sternum as "dorsal", and the side facing the abdomen as "ventral" (Fig. 1C).

Different types of second-gonopod morphology

We could identify two different second-gonopod configurations occurring among the Old World freshwater crabs:

(A) Second gonopods in which the spoon-like protrusion is situated dorsally, covering only the dorsal part of the gonopod stem (Fig. 2B). The gonopod's distal part forms a closed tube by a dorsal invagination of the surface and by the overlapping lateral margins (Fig. 3B). This type of G2 was observed in *Liberonautes latydactylus*, *Platythelphusa armata*, *Potamonautes perlatus*, and *P. obesus* (all Potamonautidae), and had been described for the
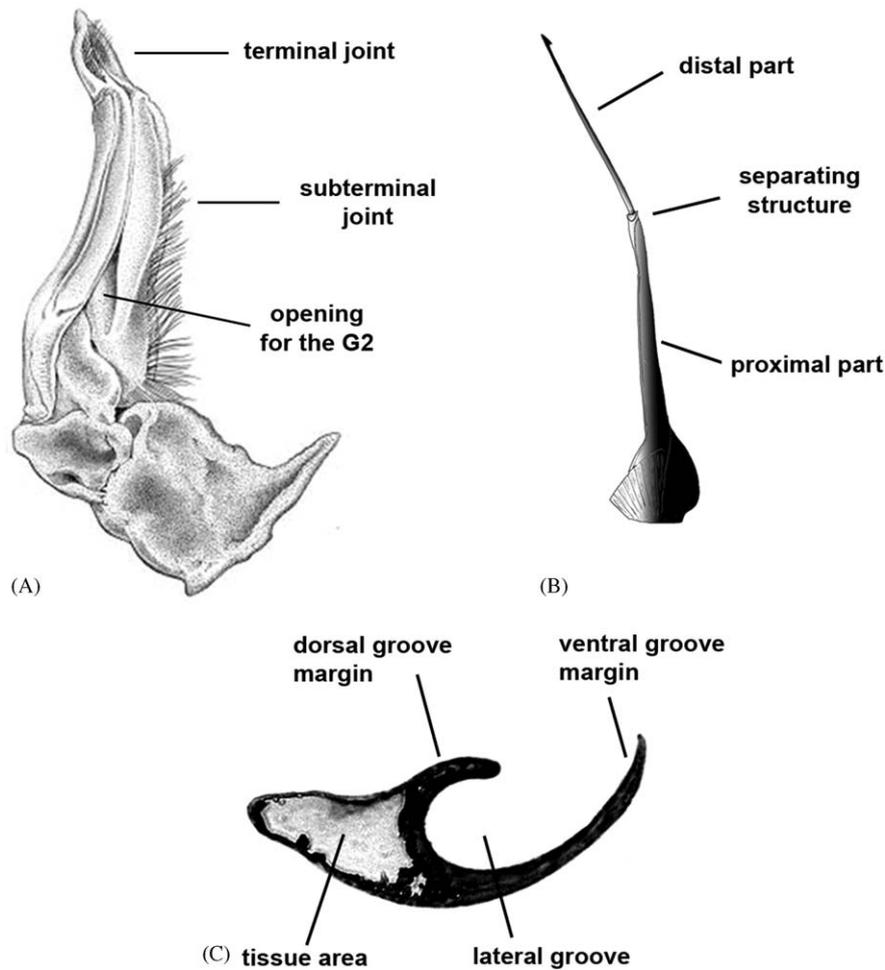
Fig. 1. Terminology of freshwater crab male gonopod morphology. (A) first gonopod; (B) second gonopod = G2; (C) cross-section through distal part of G2, lateral groove with both ventral and dorsal margins broadened.

genera *Demanietta*, *Dromothelphusa*, *Flabellamon*, *Isolapotamon*, *Nanhaipotamon*, *Sinopotamon* (see Brandis 2002), *Potamiscus* (see Brandis 2000), *Himalayapotamon*, *Potamon* (see Brandis 2001), and *Socotrapotamon* (see Apel and Brandis 2000) (all Potamidae). We refer to this type of second gonopod as the ''potamoid G2'':

(B) Second gonopods in which the spoon-like protrusion surrounds the stem, covering its entire dorsal and lateral sides (Fig. 2A). The distal part forms an open groove by a dorso-lateral invagination of the surface. We refer to this type of second gonopod as the ''gecarcinucoid G2''. Within this configuration, four different groove morphologies can be identified:

(1) The West African type (Fig. 4A). In the species *Afrithelphusa gerhildae* the groove is a shallow dorso-lateral invagination of the gonopod surface, with the ventro-lateral margin slightly broadened.

(2) The Malagasy-East African type (Fig. 4A). A deep groove is formed by a dorso-lateral invagination with a broadened ventrolateral groove margin. This type we found in *Deckenia imitatrix*, *Hydrothelphusa agilis*, *H. goudoti*, *Madagapotamon ankaraharae*, *Malagasya antongilensis*, and *Seychellum alluaudi*.

(3) The South Indian/Sri Lankan type (Fig. 4B). This type of groove is generally shallow, covers the entire lateral side, and only has the ventral margin broadened whereas the dorsal margin remains smooth and indistinct. In the genus *Ceylonthelphusa* the groove is nearly absent, its ventral margin and the gonopod itself forming a continuous, leaf-like distal part. This type is present in *Ceylonthelphusa rugosa*, *C. soror*, *Gecarcinucus jacquemontii*, *Oziothelphusa senex*, *Perbrinckia enodis*, *Salangathelphusa brevicarinata*, *Spiralothelphusa hydrodroma*, *S. wuellerstorfi*, and *Travancoriana* sp.

(4) The Indian – Southeast Asian type (Fig. 4C). The groove is covering the entire lateral side of the second gonopod, with both ventral and dorsal
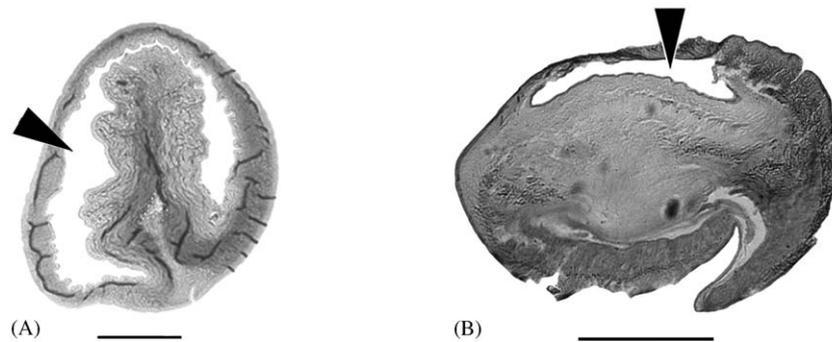
Fig. 2. Cross-sections through separating structure of second gonopod. (A) *Sartoriana spinigera* (Gecarcinucoidea), "gecarcinucoid G2" with spoon-like protrusion surrounding stem; (B) *Potamon setiger* (Potamoidea), "potamoid G2" with spoon-like protrusion forming a dorsal, cup-like structure (see Brandis et al., 1999). Arrowheads point to enclosed lumina. Scale bars: $A = 50\,\mu m$, $B = 200\,\mu m$.
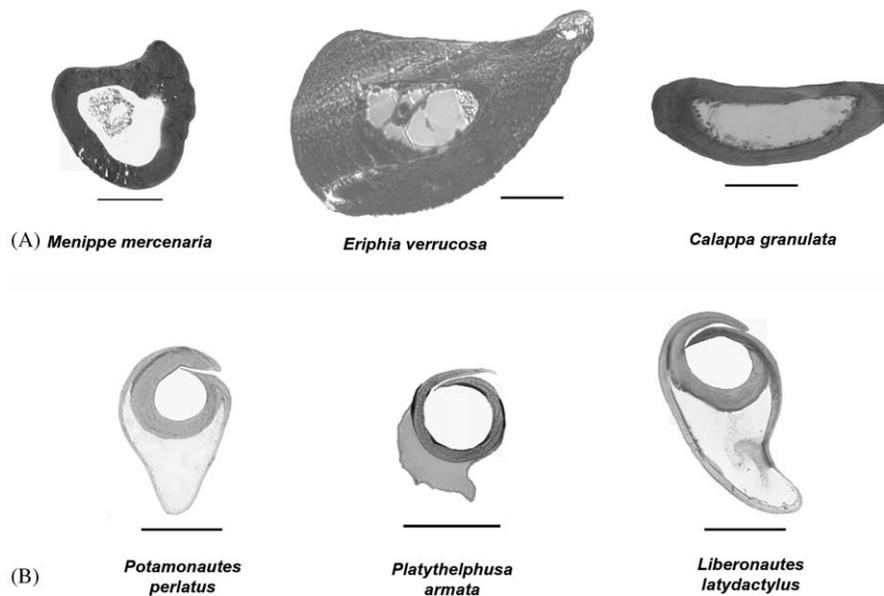

(A) **Menippe mercenaria**   **Eriphia verrucosa**   **Calappa granulata**

(B)   **Potamonautes perlatus**   **Platythelphusa armata**   **Liberonautes latydactylus**

Fig. 3. Cross-sections through distal part of second gonopod. (A) marine brachyurans; (B) Potamonautidae. Scale bars: *Menippe mercenaria* = 200 μm; *Eriphia verrucosa* = 100 μm; all others = 50 μm.

groove margins broadened. At the distal tip of the G2, the ventral margin is much broader than the dorsal one. The cuticle enclosing the tissue of the gonopod's distal part is reduced to a thin membrane, whereas the groove itself is formed by a thick cuticle. *Gubernatoriana escheri*, *Holthuisana angustifrons, H. biroi, Parathelphusa bogorensis, P. celebensis*, *P. incerta*, *Sartoriana spinigera* (see Brandis and Sharma 2005), *Sundathelphusa cassiope*, and *Terrathelphusa kuhlii* show this type of gonopod structure.

In several genera such as *Barythelphusa, Cylindrothelphusa, Phricotelphusa, Sendleria, Siamthelphusa* and *Somanniathelphusa*, the distal part of the G2 is reduced

in length; therefore these gonopods cannot be assigned to one of the four groove types. In all these cases it is possible to identify a small appendix on the G2, which we interpret as a relic of an originally elongated distal part.

Phylogeny of the Gecarcinucoidea based on second-gonopod morphology

Outgroups: *Calappa granulata* (Linnaeus, 1758), *Eriphia verrucosa* (Forskal, 1775), *Menippe mercenaria* (Say, 1818). These three marine species possess a G2 functionally similar to those of freshwater crabs. The gonopod is strongly elongated, its distal part is flexible and flagellum-like, with the dorsal surface forming a very shallow groove by invagination (Fig. 3A). The
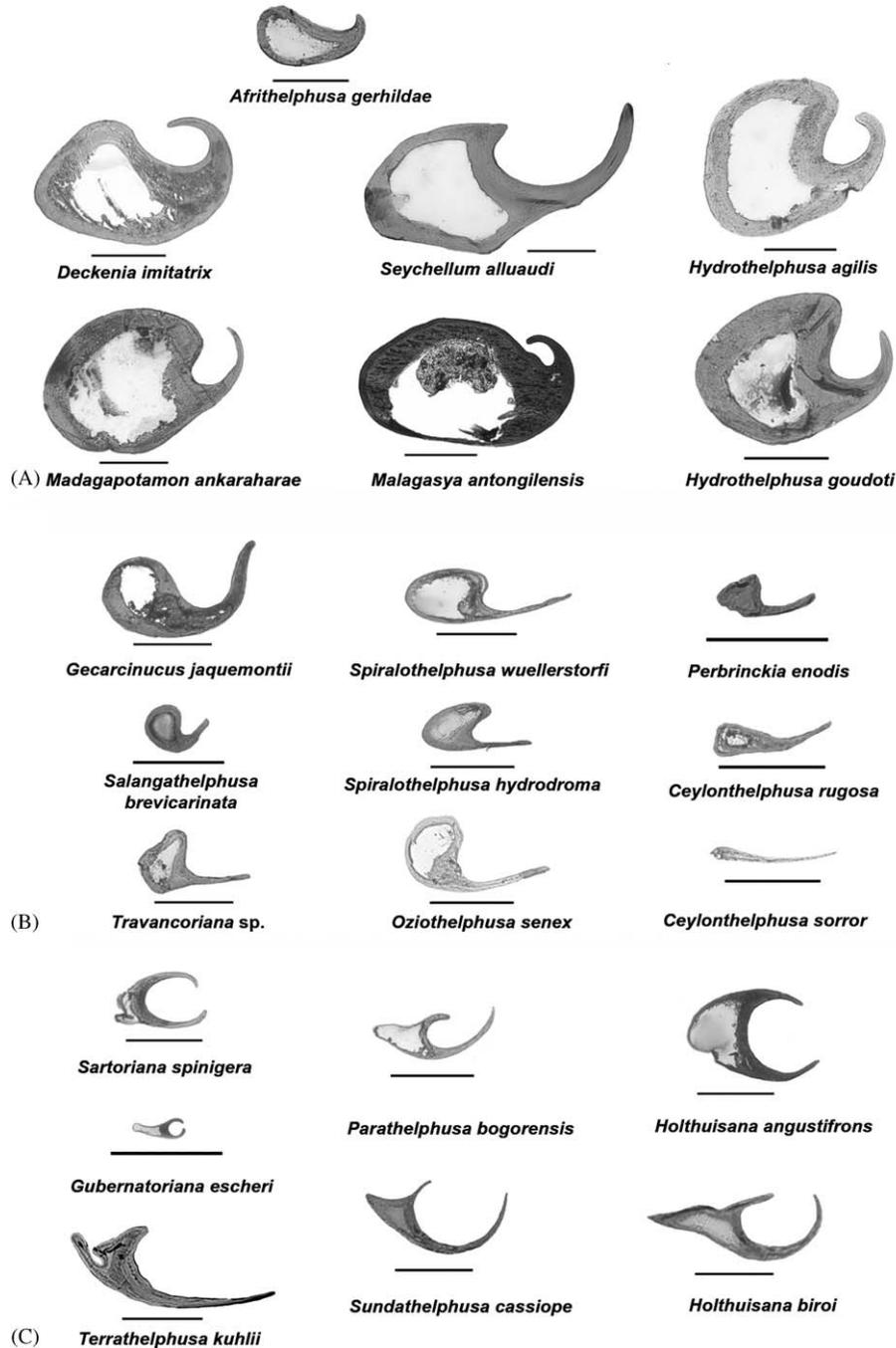
Fig. 4. Cross-sections through distal part of second gonopod in Gecarcinucoidea. (A) Deckeniidae; (B) Gecarcinucinae, right-hand column shows Sri Lankan freshwater crabs; (C) Parathelphusinae. Scale bars: 50 μm.

distal part is separated from the proximal one by a field of setae or by a cuticular projection covered with setae. This morphology represents the plesiomorphic state relative to the more complex tube or groove systems in freshwater crabs.

A cladogram was constructed based on the morphology of the second gonopods (Fig. 5). The corresponding clades are described in the following section.

Clade 1.0: Gecarcinucoidea. The Gecarcinucoidea share two apomorphies of the second gonopod:

(1) The spoon-like structure separating the proximal and distal parts of the elongated G2 surrounds the second gonopod and is bare of setae. In the Potamoidea, this structure is situated dorsally on the gonopod stem, never surrounding it.

(2) The possession of an open groove formed by a dorso-lateral or lateral invagination of the distal part of the second gonopod with at least its ventral margin broadened. In *Potamon*, *Potamonautes* and related genera we always find a closed tube, formed by a dorsal invagination of the G2 surface and the overlapping of the broadened lateral margins.

These apomorphies are also shared by the East African freshwater crabs of the genus *Deckenia*, by all Malagasy freshwater crabs so far assigned to the Potamonautidae (see Cumberlidge 1999; Cumberlidge and Sternberg 2002; Cumberlidge et al. 2004), and by the genera *Afrithelphusa* from West Africa and *Seychellum* from the Seychelles.

Subclade 1.1: Deckeniidae. Apomorphies: G2 large, with a thick cuticle; G2 groove formed by a dorso-lateral projection; ventral groove margin not wider than diameter of G2, its rim directed to dorsal side (Fig. 4A).

Subclade 1.2: Gecarcinucidae. Apomorphies: G2 groove covers entire lateral side of gonopod, its ventral margin contiguous with ventral side of G2; ventral margin of G2 groove at least as broad as diameter of G2 itself; second gonopods generally small in diameter, their cuticle reduced in thickness.

Subclade 1.2.1: Gecarcinucinae. Apomorphies: Groove of G2 shallow with ventral margin strongly broadened (Fig. 4B). The G2 of all investigated Sri Lankan species are smaller in diameter than those of Indian Gecarcinucinae (Fig. 4B, right-hand column). The grooveless G2 in the genus *Ceylonthelphusa* can be derived from the Gecarcinucinae-type G2, as there are species with a rudimental groove (*Ceylonthelphusa rugosa*).
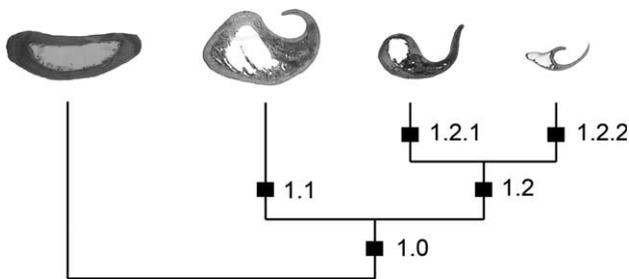

Fig. 5. Cladogram for Gecarcinucoidea based on morphology of male second gonopod; terminal cross-sections shown to scale. Outgroup = *Calappa granulata*; clade 1.0 = Gecarcinucoidea; subclade 1.1 = Deckeniidae (*Deckenia imitatrix*); 1.2 = Gecarcinucidae; 1.2.1 = Gecarcinucinae (*Gecarcinucus jacquemontii*); 1.2.2 = Parathelphusinae (*Parathelphusa bogorensis*).

Subclade 1.2.2: Parathelphusinae. Apomorphies: Both ventral and dorsal groove margins broadened; tissue area of distal part approximately triangular; tissue enclosed by very thin cuticle only (Fig. 4C).

Results of the molecular analysis

The total alignment of the sequenced portions of the 16S rDNA, after removal of the primer regions, consisted of 585 basepairs (bp). Hypervariable regions that could not be aligned with certainty (49 bp) were excluded, and the remaining 536 bp were used for phylogenetic analyses. Of those, 270 positions were variable and 220 parsimony-informative. Application of the likelihood ratio tests revealed that the selected model of DNA substitution by hLRT and Akaike was the GTR + I + G model (Rodríguez et al. 1990). All the sequences are A-T rich. A test for homogeneity of base frequency composition across taxa did not reject homogeneity.

The three phylogenetic inference methods resulted in trees that showed important deviations in topology, indicating a high level of homoplasy in this dataset and that many phylogenetic relationships remain unsolved even after this analysis. High bootstrap values are mostly restricted to terminal taxa, such as congeneric species within *Potamon*, *Potamonautes* and *Hydrothelphusa*, or closely related genera (e.g. *Deckenia* – *Seychellum* or *Globonautes* – *Afrithelphusa*). Many higher order relationships (e.g. monophyly of the Gecarcinucoidea and Potamoidea) cannot be determined with certainty (Figs. 6–8). The MP heuristic search yielded one shortest tree of the length 2465, with the tree scores CI = 0.31, RI = 0.508, and RC = 0.158. These values corroborate the high level of homoplasy within this dataset. 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. 6. Results from the Bayesian analysis with the corresponding posterior probabilities are shown in Fig. 7, whereas a ME distance tree with bootstrap values is presented in Fig. 8.

All analyses agree in supporting the Old World freshwater crabs as a monophyletic unit with respect to the New World representatives from the Pseudothelphusidae, *Epilobocera* and *Eudaniela* (BI: 1.0, ME: 85%, MP: 48%). One exception to the overall low resolution at higher order relationships within Old World freshwater crabs is the family Gecarcinucidae with its constituent subfamilies Gecarcinucinae and Parathelphusinae. This taxon is supported by the three phylogenetic methods with mostly high confidence values (BI: 0.92, ME: 99%, MP: 44%), thereby separating the Asian fauna from the African one (the latter also including representatives from Madagascar and the Seychelles).
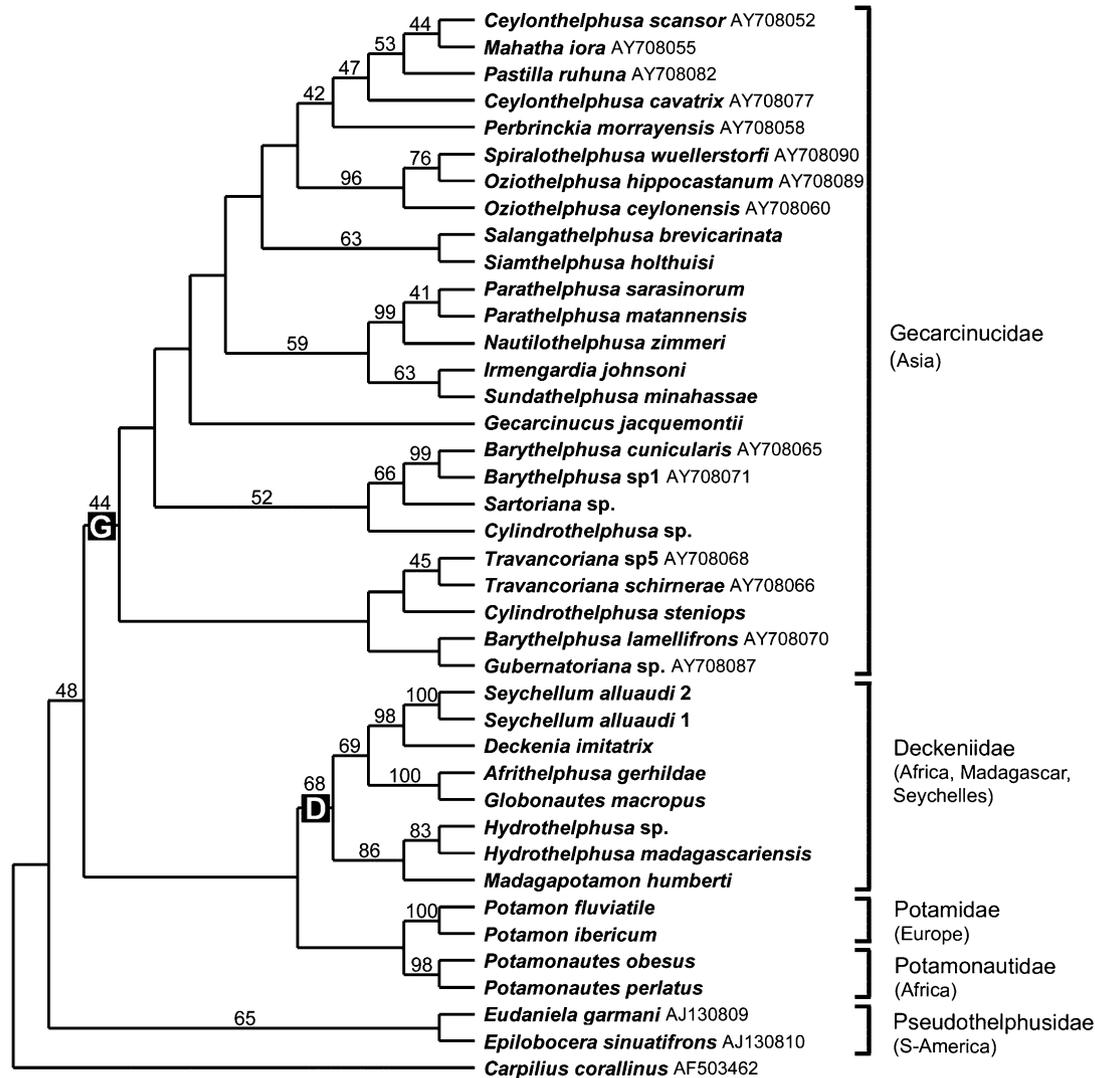
Fig. 6. Consensus tree of Old World freshwater crabs based on maximum parsimony (heuristic search, random addition, 2000 bootstrap pseudoreplicates) with *Carpilius corallinus* as outgroup; transversions weighted as three times transitions; tree length 2465, CI = 0.31, RI = 0.508, RC = 0.158. Only confidence values higher than 40% are shown. Squares D and G represent character states of second gonopod, equivalent to subclades 1.1 and 1.2 from Fig. 5, respectively.

Within Africa, two phylogenetic groups can be distinguished easily. One includes the two representatives from the genus *Potamonautes*, that are very closely linked (BI: 1.0, ME: 99%, MP: 98%). The second group, with relatively high bootstrap values (BI: 1.0, ME: 87%, MP: 68%), comprises members of freshwater crabs from the extreme west and east of the African continent, that were previously considered to belong to three distinct families (Deckeniidae, Gecarcinucidae and Potamonautidae). This probably is the most important finding of the present genetic analysis, because together with the morphological results it offers a solution to the longstanding discussion of the origin and taxonomic classification of the freshwater crabs from Madagascar and the Seychelles. The strong homogeneity of this group is even more surprising when considering the large geographic distances between western and eastern Africa. Within this group, the apparently monophyletic Malagasy freshwater crabs (BI: 1.0, ME: 98%, MP: 86%) branch off first, while the West African Globonautinae and the East African *Deckenia* and *Seychellum* from the Seychelles form another strongly supported monophylum (BI: 1.0, ME: 99%, MP: 98%).

It is much more difficult to depict consistent patterns of phylogenetic structure within the Asian freshwater crabs. Apart from the relatively clearcut separation from all African representatives, the subdivision into the traditionally used families Parathelphusidae (including Sundathelphusidae) and Gecarcinucidae is indicated only slightly and depends on the respective definition
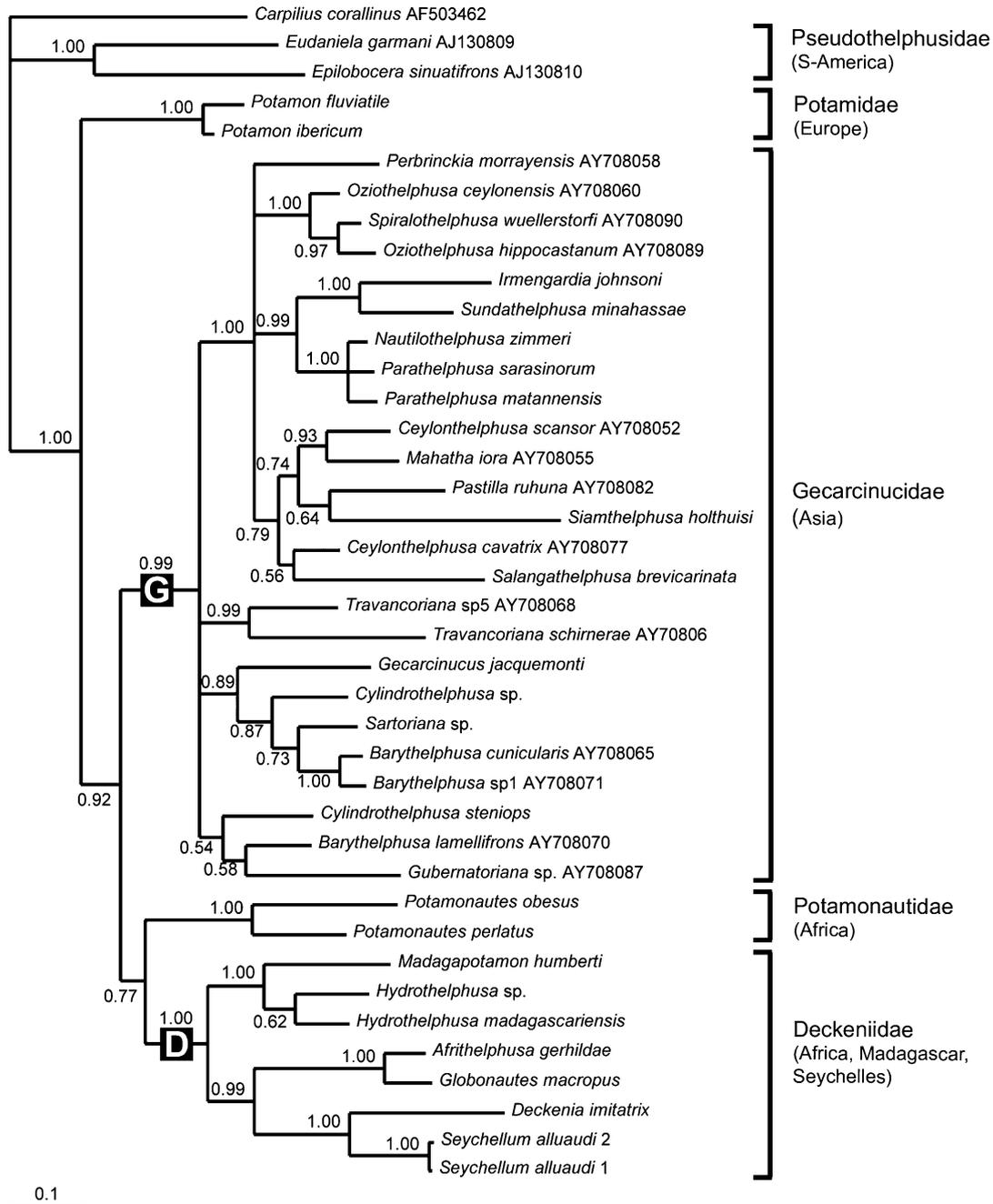
Fig. 7. Bayesian tree (GTR + I + G distance model) of Old World freshwater crabs with *Carpilius corallinus* as outgroup; 2 million generations; only posterior probabilities higher than 0.5 are shown. Squares D and G represent character states of second gonopod, equivalent to subclades 1.1 and 1.2 from Fig. 5, respectively.

of these families. The core of Parathelphusinae around *Parathelphusa* includes the genera *Nautilothelphusa*, *Sundathelphusa* and *Irmengardia*, all from Malaysia and Indonesia (BI: 0.99, ME: 93%, MP: 59%). On the other hand, the core of Gecarcinucinae around *Gecarcinucus* is found on the Indian subcontinent, with certain affinities to the genera *Cylindrothelphusa*, *Sartoriana*, *Barythelphusa* and, to a lesser extent, to *Travancoriana*

and *Gubernatoriana* (relationships vary with reconstruction method). The phylogenetic position and genetic homogeneity of two genera from Thailand (*Siamthelphusa* and *Salangathelphusa*), as well as of all representatives from Sri Lanka, is solved unsatisfactorily. Also the validity of several gecarcinucid genera needs to be questioned, as for example in the case of *Barythelphusa* and *Cylindrothelphusa*.
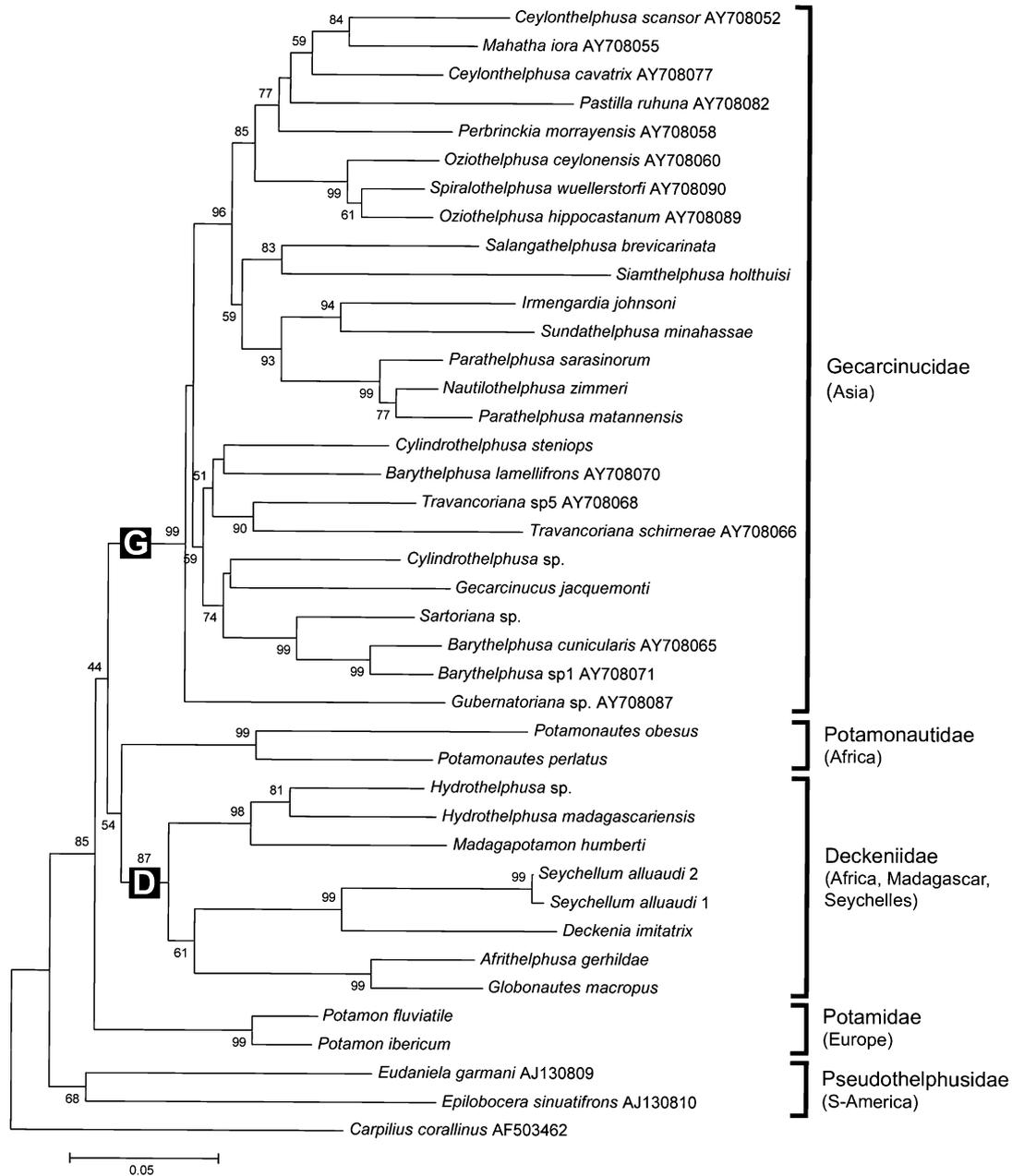
Fig. 8. Minimum evolution tree (Tamura–Nei distances, gamma correction according to calculated model) of Old World freshwater crabs with *Carpilius corallinus* as outgroup; 2000 bootstrap pseudoreplicates; only confidence values higher than 40% are shown. Squares D and G represent character states of second gonopods, equivalent to subclades 1.1 and 1.2 from Fig. 5, respectively.

Discussion

The phylogenetic significance of gonopod morphology

So far, the use of freshwater crab gonopods for phylogenetic analyses was restricted strongly by false assumptions on their morphology. The G2 were described for all African freshwater crabs as "always solid, similar on both their ventral and dorsal sides" (Cumberlidge 1999). Consequently, they were regarded

as similar to the second gonopods of the archaeobrachyuran Dromiidae and Dynomenidae and of the Menippidae, and thus as representing the plesiomorphic state in freshwater crabs (Cumberlidge 1999). In contrast, the present investigation shows that the G2 of African freshwater crabs is never solid, but in all taxa forms a tube or a groove as described above. It cannot be directly compared to the gonopods of dromiids as the latter show a completely different morphology (see Guinot 1979; Hartnoll 1968). In certain taxa (e.g. Oziidae, Eriphiidae, Calappidae) the distal part of the

elongated second gonopod is also flexible and flagellum-like with the dorsal surface slightly invaginated, forming a very shallow groove (Fig. 3A). In this respect, this type of second gonopod has a morphology similar to that in freshwater crabs, suggesting a similar function. We assume that this latter type of G2 is the plesiomorphic state with respect to the freshwater crabs' G2, as the original morphology of a locomotory appendage is less modified compared to more complex second gonopods (Bauer 1986).

The trend to a more complex type of second gonopod in freshwater crabs as compared to marine brachyurans is probably correlated with a more precisely directed sperm transfer (Bauer 1986). The specific location of the sperm in the female genital apparatus possibly prevents polyspermy, and thereby appears to be an adaptation to direct development and lecithotrophy, where females have only few but large eggs (Guinot et al. 1997). This explains the occurrence of a similar sperm transfer system in the Potamoidea, where the distal part of the G2 forms a tube and the females possess complex spermathecae (Brandis et al. 1999). Although homology of the gecarcinucoid grooves and the potamoid tubes of the G2 cannot be ruled out, the two types show basic differences. The sperm transferring structure of the G2 develops dorsally in the Potamoidea but laterally in the Gecarcinucoidea, and the respective contact zones between distal and proximal parts of the second gonopod differ morphologically.

We propose that in the genera with a G2 lacking the distal part, a functional change in sperm transfer has taken place. In these species, the sperm is possibly less specifically placed in the female genital apparatus. It is likely that this happened several times convergently, because in several genera (e.g. *Gubernatoriana* and *Sundathelphusa*) both elongated and reduced distal parts of the second gonopod occur.

Phylogenetic conclusions

The results of the morphological and molecular analyses display a high degree of congruence. This argues for gonopod structure as an adequate character for the analysis of the phylogenetic relationships in freshwater crabs at higher taxonomic levels. Both analyses support the existence of two monophyletic families within the Gecarcinucoidea: the African Deckeniidae and the Asian Gecarcinucidae. Since a G2 groove is completely absent in the marine outgroups and the Deckeniidae display a relatively simple groove type, an alternative possibility would be that the deckeniid G2 groove is plesiomorphic, and Deckeniidae not monophyletic. However, this possibility is ruled out by the molecular analysis showing high support for the monophyly of Deckeniidae.

Within Deckeniidae, at least two monophyla are supported by morphological and molecular data: Globonautinae in West Africa and Deckeniinae from East Africa and the Seychelles. The present 16S rRNA sequence data, and those of Spears et al. (2004) and Daniels et al. (2006), argue strongly for the monophyly of the freshwater crabs from Madagascar. The morphology of the G2 does not allow a complete resolution of deckeniid phylogeny. Nevertheless, we recognise three subfamilies within the Deckeniidae: Globonautinae from West Africa, Deckeniinae from the East African coast and the Seychelles, and Hydrothelphusinae from Madagascar. Von Sternberg and Cumberlidge (1999) assumed, based on cladistic analyses, that Deckeniinae is the sister taxon of the genus *Platythelphusa* (= Platythelphusidae according to Cumberlidge 1999). However, the G2 in *Platythelphusa* shows a dorsally invaginated tube with overlapping margins, as in all Potamonautidae investigated (Fig. 3B). Therefore, *Platythelphusa* can be ruled out as the sister taxon of Deckeniinae, and is placed in Potamonautidae instead.

Within Gecarcinucidae, two subfamilies (Gecarcinucinae and Parathelphusinae) are morphologically unambiguously defined. Molecularly, the Gecarcinucinae of Sri Lanka and the genera *Oziothelphusa* and *Spiralothelphusa* form a monophylum, while all investigated Parathelphusinae of southeast Asia appear to form another. This confirms that the reduction of the G2 groove in the Sri Lankan freshwater crabs of the genus *Ceylonthelphusa* must have occurred secondarily.

The situation on the Indian mainland seems to be more complex. The phylogenetic position of the genera *Barythelphusa*, *Cylindrothelphusa*, *Gecarcinucus*, *Gubernatoriana*, *Sartoriana* and *Travancoriana* is unstable with respect to the gene trees built with different methods, and does not reflect the morphology of the G2. This indicates a fast radiation of the Gecarcinucidae on the Indian subcontinent, preventing the 16S rRNA from accumulating sufficient phylogenetic information between splits. In contrast, the G2 favours the assignment of *Sartoriana* and *Gubernatoriana* to Parathelphusinae, and argues for the recognition of a separate clade for *Gecarcinucus*, *Travancoriana*, *Salangathelphusa* and all the Sri Lankan gecarcinucoids, i.e. Gecarcinucinae. The complexity of the Parathelphusinae-type G2 strongly reduces the likelihood of convergent character development. *Barythelphusa* and *Cylindrothelphusa* have reduced second gonopods, and the molecular analysis points to polyphyly of one or both of these genera. The genus *Siamthelphusa* also has a reduced G2. However, the molecular data point to a close relationship with *Salangathelphusa*.

One important aspect in which our molecular and morphological analyses differ is the monophyly versus paraphyly of the superfamily Gecarcinucoidea. All phylogenetic models based on the 16S rRNA propose

a possible relationship of Deckeniidae with the pota-monautids and potamids. However, the corresponding branching points are not well supported, and it seems that the molecular marker used is unable to definitely resolve this part of the phylogeny. On the other hand, the morphological characters of the G2 defining the Gecarcinucoidea appear to have a high probability of homology. They should therefore replace the character of the mandibular palp (Bott 1969b, 1970a, b), the doubtful phylogenetic significance of which has been discussed by von Sternberg et al. (1999) and Cumber-lidge and von Sternberg (2002). Nevertheless, we cannot definitely exclude that the gecarcinucoid characters of the G2 represent the plesiomorphic state for all Old World freshwater crabs, and that therefore the pota-moid G2 morphology is possibly derived from deck-eniid-like ancestors. This would argue for Potamoidea to branch between Deckeniidae and Gecarcinucidae. In

Table 3. Classification within Gecarcinucoidea based on phylogenetic results, including taxonomic authorship, years of first publication, and type genera

Gecarcinucoidea Rathbun, 1904

Deckeniidae Ortmann, 1897
  Deckenia Hilgendorf, 1868
    Globonautinae Bott, 1969
      Globonautes Bott, 1959
    Deckeniinae Ortmann, 1897
      Deckenia Hilgendorf, 1868
    Hydrothelphusinae Bott, 1955
      Hydrothelphusa A. Milne-Edwards, 1872
Gecarcinucidae Rathbun, 1904
  Gecarcinucus H. Milne-Edwards, 1844
    Gecarcinucinae Rathbun, 1904
      Gecarcinucus H. Milne-Edwards, 1844
    Parathelphusinae Colosi, 1920
      Parathelphusa H. Milne-Edwards, 1853

that case, we would propose a single superfamily for all Old World freshwater crabs, the Potamoidea. The monophyly of all Old World freshwater crabs seems to be probable, but further molecular and morphological analyses are neccessary to shed light on their phylogeny and to identify the closest marine relatives.

The taxonomic implications of our phylogeny are summarised in Table 3. The distribution of the gecarcinucoid families and subfamilies is illustrated in Fig. 9.

Biogeographic conclusions

Biogeographic models for the Gecarcinucoidea

Currently, there are three main scenarios available explaining the present freshwater crab distribution. Two of them argue for multiple transitions of brachyurans from marine to freshwater; one is a vicariance model based on an assumed Gondwanan distribution of the freshwater crab ancestors.

Biogeographic hypotheses based on multiple transitions into the freshwater

The hypothesis dating back to Bott (1969b, 1970b, 1972) and Pretzmann (1973) was recently referred to as the "polyphyletic hypothesis" (Sternberg et al. 1999); it proposes several independent invasions into the fresh-water environment by unspecified ancestral marine stem groups. Each immigration is assumed to have given rise to a new freshwater crab taxon on the family- or even genus level. The Gecarcinucoidea are supposed to originate from the southern Atlantic, their marine stem group invading Africa, India and southeast Asia independently in the Paleogene (Bott 1972). This
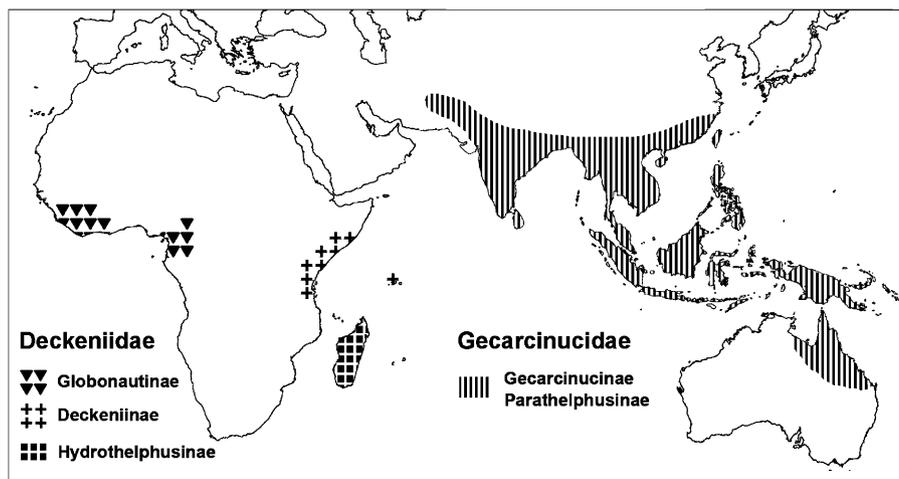

Fig. 9. Distribution map for Gecarcinucoidea and their subgroups as proposed in the present study.

hypothesis was rightfully criticised for being highly speculative (von Sternberg et al. 1999).

However, we think that the same is true for the "monophyletic hypothesis" of von Sternberg et al. (1999) which assumes a monophyletic origin of all freshwater crab families (except for the South American Trichodactylidae) as a sister group of all thoracotreme crabs. The ancestral group to thoracotremes and non-trichodactylid freshwater crabs supposedly had a pancoastal distribution along the southern Tethys during the Cretaceous. As in Bott's scenario, the freshwater crab stem group would have invaded limnic or brackish habitats several times independently, acquiring adaptions to freshwater life in convergence. This hypothesis implicitly assumes that the marine ancestor populations of the present freshwater crab families either completely transformed into freshwater-adapted brachyurans or became extinct in their marine habitat. Otherwise, the monophyly of non-trichodactylid freshwater crabs could hardly be claimed. Like Bott's "polyphyletic hypothesis", this assumption is less parsimonious compared to that of a single freshwater-colonisation event. Therefore we assume, at least for the Gecarcinucoidea, a single transition into freshwater.

Both of the above-mentioned hypotheses are in conflict with the present phylogeny of the Gecarcinucoidea, and can hardly explain a closer relationship of the East African Deckeniinae with the West African Globonautinae than with the Malagasy Hydrothelphusinae. Moreover, the African gecarcinucoids do not appear to be closely related to the South American freshwater crabs, as would be expected if their common ancestor ranged along the southern Tethys.

The vicariance hypothesis

The third hypothesis of freshwater crab biogeography was referred to by von Sternberg et al. (1999) as the "archaic population hypothesis". We will refer to it as the "vicariance hypothesis", because its distinction from the other hypotheses primarily is the proposed explanation of gecarcinucoid distribution (by means of vicariance) and not its assumption of a monophyletic origin of freshwater crabs. The vicariance hypothesis was proposed by Rodríguez (1986), Ng and Rodríguez (1995) and Ng et al. (1995). These authors assumed that the ancestors of a monophyletic clade [New World Pseudothelphusidae + Old World Gecarcinucoidea] once had a Gondwanan distribution, and that the independent evolution of the present families resulted from the successive tectonic splitting of the Gondwana continent. Every tectonic break-up should coincide with a further separation of freshwater crab groups, while active dispersal is left out of consideration.

However, our phylogenetic analysis rejects a closer relationship of the Gecarcinucoidea with the neotropical Pseudothelphusidae. In addition, the timing of the Gondwana break-up argues against a vicariant distribution of freshwater crabs in general. The independent history of the eastern part of Gondwana began with the separation of a continental block including India, Madagascar, the Seychelles, Antarctica and Australia from the African continent in the Jurassic 165–150 million years ago (Mya) (Rabinowitz et al. 1983; Storey et al. 1995), followed by the splitting-off of India and Antarctica-Australia in the early Cretaceous (about 120 Mya; Powell et al. 1988). Late Cretaceous seafloor spreading in the Mascarene Basin resulted in the separation of Madagascar and Greater India, the latter continuously moving northward (McKenzie and Sclater 1973; Storey et al. 1995; Morley 2000). The micro-continent of the Seychelles separated from India in the early Paleocene (Davies 1968; McKenzie and Sclater 1973) and 54–45 Mya India finally collided with the Asian plate (Patriat and Achache 1984; Dewey et al. 1989). These estimates of tectonic separation times indicate the problematic argumentation of the vicariance hypothesis. As already criticised by von Sternberg et al. (1999), this hypothesis assumes divergence of freshwater crab taxa at times from which no eubrachyuran fossils have been identified. The earliest fossil record of freshwater crabs dates from the Miocene (Glaessner 1930, 1969; Szombathy 1916), and the earliest Eubrachyura (Calappidae and Dorippidae) occurred in the Early Cretaceous (Glaessner 1969), thus 130 and 50 million years, respectively, after the separation of eastern Gondwana from Africa.

Dispersal of Gecarcinucoidea with Africa as centre of origin

In the following, we present a hypothesis that explains the present gecarcinucoid distribution by means of dispersal, in agreement with our phylogenetic re-evaluation of the gecarcinucoid families. Our biogeographic model fits well with geological, palaeoclimatological and palaeogeographical developments during the Paleogene and Neogene periods.

Today, the African gecarcinucoids occur only in small areas of West Africa (Globonautinae) and along the East African coast (genus *Deckenia*). The isolated distribution of primitive gecarcinucoids on the African continent probably coincides with Pleistocene rainforest refuges to which the tropical gecarcinucoid crabs were restricted when arid savannas dominated the African vegetation (Morley 2000). When rainforests spread again after the end of the last ice age, most African habitats might have been occupied by Potamonautidae already, which are more resistant to drier climate than the Deckeniidae. A fast and relatively recent spreading of potamonautids is plausible because morphology of the G2 tube is very constant among all African

potamonautids investigated, indicating a recent radiation. Also the hydrography of the African freshwaters with their huge drainage areas and only shallow watersheds allows fast invasion into different fluviatile systems (Beadle 1981).

The morphology-based phylogenetic analysis indicates that the genus *Afrithelphusa* displays the least-derived G2 character states. According to the theoretical considerations of Hennig (1966), the less-derived character state of deckeniids suggests an African centre of origin for Gecarcinucoidea. This is also in accordance with the biogeographic fact that more primitive members of a group mostly occur on the periphery of the geographic range (Briggs 1984), as the centre of radiation and highest species diversity of Gecarcinucoidea are not found in Africa but in India and southeast Asia.

The phylogeny of gecarcinucoid freshwater crabs is reflected by the successive development of the second gonopod's groove from a dorso-lateral, shallow invagination to a prominent and deep lateral groove. We propose that the historical dispersal route of the Gecarcinucoidea from Africa to Australia is also reflected by this gradient of plesiomorphic character states in the West African genus *Afrithelphusa* to more complex ones in the Asian–Australian Parathelphusinae. The Deckeniidae of East Africa, Madagascar and the Seychelles represent intermediates.

To understand how gecarcinucoid dispersal out of Africa could have taken place and how it may fit into a geological setting, it is important to estimate the time of radiation of the early gecarcinucoid freshwater crabs in Africa. The Miocene fossil record shows a completely developed freshwater crab fauna (Glaessner 1930, 1969; Szombathy 1916). Although these fossils probably belong to the Potamidae, the origin and the evolution of freshwater crabs in general has to be dated before the Miocene (von Sternberg and Cumberlidge 2001). As most gecarcinucoid crabs are closely associated with rainforests, most probably the evolution of the Gecarcinucoidea has taken place in a warm and humid climate. This argues for gecarcinucoid evolution predating the Oligocene, as in this time period the African climate became cooler, leading to an expansion of savannas and a restriction of rainforests to the equatorial region (Morley 2000). The earliest point for a first radiation of freshwater crabs must therefore be dated earlier, possibly in the Middle Eocene (approximately 40 Mya). During this period, most of Africa was supposedly covered by dense rainforests (Morley 2000).

Dispersal to Madagascar, the Seychelles and South Asia

For a plausible hypothesis of further dispersal of gecarcinucoid freshwater crabs from the African mainland to Madagascar, to the Seychelles and onwards to India it is neccessary to take into account the palaeogeographic evolution of the Indian Ocean and especially the exact distribution of land and sea. The Indian Ocean is characterised by the continental fragments of Madagascar and the Seychelles and by several scattered, elevated platforms and ridges. Of biogeographic importance for the present investigation are the Laccadive-Chagos ridge and the Mascarene plateau. They represent a system successively formed 55–60 Mya (Maledives), 48 Mya (Chagos Ridge), and 40 Mya (Mascarene Plateau) (Morgan 1972a, b, 1981). We propose that these ridges and the adjacent continental shelves served as a non-contiguous landbridge between Africa, Madagascar and India during times of low sea levels.

Rage (1996) proposed an hypothesis of a landbridge between Madagascar and India during the late Cretaceous formed by the Seychelles microcontinent. However, global sea levels during the Cretaceous were the highest in Mesozoic and Cenozoic history, approximately 260 m higher than today (Haq et al. 1987, 1988). Therefore, large parts of the continental margins were widely covered by shallow epicontinental seas, and the Seychelles at most could have been only small granitic spots in the sea, widely separated from any landmass.

The situation of high sea levels changed during the Tertiary. Especially in the Oligocene, global sea levels dropped markedly (Haq et al. 1987, 1988; Zachos et al. 1992). This regression can be dated to the Rupellian-Chattian boundary approximately 28 Mya. It led to an assumed drop to 150–200 m below present sea levels (Vail and Hardenbol 1979; Schlanger and Premoli Silva 1986). At that time, significant portions of the continental shelves as well as the Chagos/Laccadive Plateau and the Mascarene Plateau were emergent (Fig. 10). Freshwater crabs could have crossed the therefore narrow Mozambique channel between Africa and Madagascar, possibly by rafting as it is assumed for mammals, reptiles and amphibians (Tattersall 1993; Raxworthy et al. 2002; Vences et al. 2003; Yoder et al. 2003), and aided by small emergent islands as stepping stones (McCall 1997).

The Oligocene terrestrial areas also include the Seychelles Bank, which encompasses over 50,000 km², now at an average depth of 75 m. The Chagos/Laccadive Plateau and the Mascarene Plateau were nearly contiguous during the Oligocene and served as stepping-stones for the dispersal of several plant taxa between Malesia and Africa/Madagascar via India/Sri Lanka (Schatz 1996). Schatz (1996) therefore proposed that they be named the "Lemurian Stepping-Stones".

As in fact no contiguous landbridge between Africa, Madagascar and India existed, it is important to note that some freshwater crab genera, such as *Seychellum* or *Spiralothelphusa*, are reported to be insensitive to salt water, at least for shorter periods (Bott 1969b, 1970b; Haig 1984). In contrast, the Potamoidea seem to have
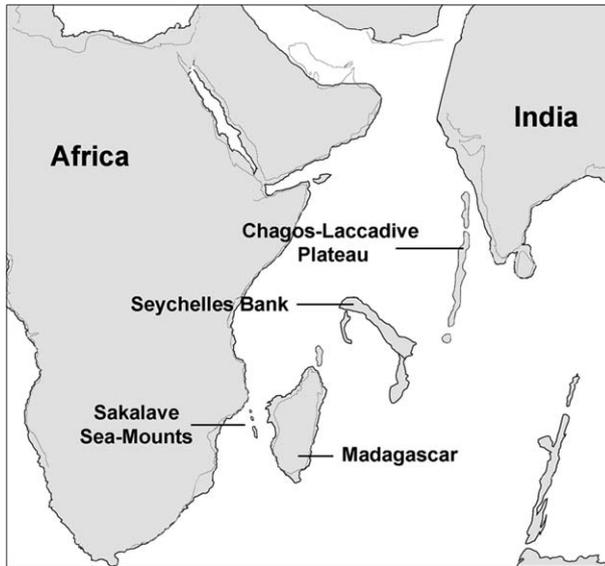
Fig. 10. Palaeogeographic reconstruction of Indian Ocean during Oligocene (approximately 30 Mya), displaying the "Lemurian Stepping-Stones". Based on http://www.odsn.de, McKenzie and Sclater (1973), McCall (1997), and Popov et al. (2004).

only little tolerance to salt water (authors' personal observations). The gecarcinucoid salt-water tolerance makes it probable that gecarcinucoid crabs could have survived shorter passages on rafts or through brackish water between the 'Lemurian Stepping-Stones'. Palaeo-climatic investigations indicate that the Mascarene and Laccadive plateau as well as India experienced warm and humid conditions at that time, so that dispersal to India was facilitated (Morley 2000). We interpret the species *Seychellum alluaudi* as a relic of such dispersal. This hypothesis is supported by the intermediate gonopod morphology in Deckeniidae, as stated above. The phylogenetic analysis proposes that the Seychelles were colonised directly from the African mainland and not via Madagascar, as the genera *Deckenia* and *Seychellum* are more closely related to each other than to the Malagasy Hydrothelphusinae.

The hypothesis of dispersal via the "Lemurian Stepping-Stones" is supported by recent insights into the biogeography of hyperoliid treefrogs (Vences et al. 2003) and cichlid fishes (Kiener and Richard-Vindard 1972; Vences et al. 2001). The studies of Vences et al. argue for a Paleogene dispersal of the two vertebrate groups from Africa/Madagascar to the Seychelles and further on to India, respectively.

At first glance, an alternative to the stepping-stone model could be that the gecarcinucoid crabs reached India via North Africa and the Middle East, when Africa connected with Eurasia in the Late Miocene (Morley 2000). However, this possibility can be excluded, as in North Africa as well as in the entire Middle East the climate was extremely arid during that time

(Morley 2000). Moreover, gecarcinucoid freshwater crabs do not occur in this region today, and the presence of gecarcinucoids on the Seychelles would remain unexplained.

Dispersal to Southeast Asia and Australia

In the Late Miocene more seasonal climate prevailed in North India, resulting in the disappearence of rainforests. Thus the gecarcinucoid dispersal to southeast Asia is likely to have occurred later. Ever-wet conditions in the Late Miocene were restricted to rainforest refuges on Sri Lanka and in the Western Ghats. In the Pliocene, the climate changed to wetter conditions, resulting in widespread rainforests with close affinities to southeast Asia (Morley 2000). Gecarcinucoid freshwater crabs entered southeast Asia most probably during this period. After the Pliocene, in the Pleistocene, the climate changed again, and large parts of India were subjected to arid conditions (Guleria 1992). This is in agreement with Bossuyt et al. (2004), who argue for a Pre-Pleistocene dispersal of the genera *Oziothelphusa* and *Spiralothelphusa* from a Sri Lankan rainforest refuge to South India.

A second remarkable regression took place in the Pliocene, when sea levels dropped to about 100 m below the present value, so that larger landmasses were emergent again (Vail and Hardenbol 1979; Haq et al. 1987). As the southeast Asian shelf fell dry, it was possible for freshwater crabs to enter the Malesian archipelago and the adjacent island groups. Dispersal to New Guinea most probably took place either via the Philippines or via Sulawesi. These Pleistocene dispersal routes were proposed by Moss and Wilson (1998) for a general faunal exchange between Australia and southeast Asia. As terrestrial connections between Australia and New Guinea occurred throughout the Neogene and Quaternary (Doutch 1972), freshwater crabs could enter the Australian mainland.

Our biogeographical approach links phylogenetic data based on morphology and genetics with palaeogeographical and palaeoclimatological settings. The combination of this colonisation model and the phylogenetic relationships of the newly defined Gecarcinucoidea could sufficiently explain the current distribution of gecarcinucoid freshwater crabs throughout Africa, Asia and Australia. This model is in agreement with the estimated age of the group according to the current fossil record of freshwater crabs and palaeoclimatological data.

Acknowledgements

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