Brooding in *Corbicula madagascariensis* (Bivalvia, Corbiculidae) and the repeated evolution of viviparity in corbiculids

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The limnic bivalve genus *Corbicula* Megerle von Mühlfeld, 1811 is a hyper-invasive neozoon in North and South America as well as in Europe, where currently some taxa are rapidly extending their range. In addition to its extraordinarily invasive potential, the 'Asiatic clam' is remarkable for its recently discovered wide spectrum of reproductive strategies comprising oviparity, ovoviviparity and euviviparity. It renders *Corbicula* an ideal model for studying evolutionary transformations of reproductive features, in particular with respect to intrabranchial incubation (brooding) of embryos and shelled larvae in freshwater lineages. Based on rare material from Madagascar we here present evidence for prolonged incubation interpreted as being indicative of euviviparous reproduction in *C. madagascariensis* Smith, 1882. This mode is not only novel for corbiculids from the Ethiopian biogeographical region, but suggests — in combination with a mtDNA phylogeny — a more complicated pattern of the evolution of reproductive modes in corbiculids than previously assumed. We find an independent origin of viviparity and even euviviparity in the South American *Neocorbicula* Fischer, 1887 and the Afro-Asian *Corbicula*, representing a remarkable example of parallel evolution in New and Old World corbiculids.

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Introduction

The limnic bivalve *Corbicula* Megerle von Mühlfeld, 1811, native to an area extending from the the Middle East, Africa and Madagascar through Asia to Australia, is a hyper-invasive neozoon in Europe as well as North and South America, where it was unknown prior to 1924 and the 1970s, respectively. Currently, some taxa are rapidly extending their range, rendering the genus of special ecological importance since the bicontinental spread of clonal lineages in the New World is currently viewed as a portent of things to come (e.g. Britton & Morton 1979; McMahon 1983; Vaate & Greijdanus-Klaas 1990; Kinzelbach 1991; Araujo *et al*. 1993; Ituarte 1994; Renard *et al*. 2000; Siripattrawan *et al*. 2000; Beasley *et al*. 2003; Lee *et al*. 2005; Beran 2006).

In addition to their extraordinarily great invasive potential the 'Asian clams' are also interesting for their recently discovered wide spectrum of reproductive strategies. These involve sexually reproducing species with both sexes or hermaphrodites and several other unusual reproductive features, ranging from oviparity and ovoviviparity to euviviparity (Ituarte 1994; Byrne *et al*. 2000; Glaubrecht *et al*. 2003; Korniushin & Glaubrecht 2003), as well as a variety of genetic structures, i.e. polyploidy, androgenesis and clonality, with mechanistically diverse genetic interaction among clones of *Corbicula* (Komaru *et al*. 1997, 1998; Komaru & Konishi 1999; Qiu *et al*. 2001; Pfenninger *et al*. 2002; Glaubrecht *et al*. 2003; Lee *et al*. 2005).

Although these particular properties of their genetic structure potentially obscure phylogenetic relationships of some (i.e the clonal) freshwater lineages, they nonetheless render *Corbicula* an ideal model group for studying evolutionary transformations of reproductive features, in particular with respect to intrabranchial incubation (brooding) of embryos and shelled larvae in freshwater lineages, as proposed and

shown by Glaubrecht *et al.* (2003) and Korniushin & Glaubrecht (2003). For many years only the South American *Neocorbicula* Fischer, 1887 was known to be euviviparous (Ituarte 1994). Recent studies of Southeast Asian taxa (in particular from the Indonesian island of Sulawesi) have revealed novel reproductive modes in *Corbicula*, hinting at euviviparity having developed independently among different lineages of corbiculids (Korniushin & Glaubrecht 2003). This is further supported by findings reported here.

Currently, African corbiculids are mainly subsumed under two species-level taxa, viz. *C. fluminalis* (Müller, 1774) and *C. astartina* Martens, 1860, comprising — particularly in the case of the former — a plethora of subspecific differentiations. A third species, *C. madagascariensis* Smith, 1882, occurs on Madagascar (Mandahl-Barth 1988; Daget 1998; Korniushin 2004). However, the systematics of corbiculids has not been resolved satisfactorily, since morphological characters alone have proved to be equivocal (e.g. Glaubrecht *et al.* 2006). The state of corbiculid taxonomy may be taken as another prime example of the general dissent in the delineation of species (for a review of this unresolved 'species problem' in malacology see e.g. Glaubrecht 2004). For example, for Asian corbiculids the 'splitters' have distinguished several taxa, with, for example, Prashad (1924, 1929, 1930) listing up to 70 species and Brandt (1974) listing 26 species for Thailand alone (of which 20 proved to be synonyms of *C. fluminea* based on allozyme studies; Kijviriya *et al.* 1991). For Indonesia see Djajasamita (1975, 1977). In contrast, 'lumpers' accept only two conchologically highly variable, polymorphic taxa (e.g. Morton 1979, 1986).

This confusion in species identity and taxonomy in corbiculids becomes evident from the comparison of, for example, Mandahl-Barth (1988) and Daget (1998) for Africa, Morton (1986) and Harada & Nishino (1995) for Japan, Brandt (1974) and Woodruff *et al.* (1993) for Thailand, or Djajasamita (1975, 1977) and Glaubrecht *et al.* (2003) for Indonesian and particularly Sulawesi taxa. For the particular case of the cementing (i.e. sessil) corbiculid named 'Posostrea' from Lake Poso in Sulawesi, compare Bogan & Bouchet (1998) and Rintelen & Glaubrecht (2006). Accordingly, the confusing taxonomy and unresolved systematics of *Corbicula* have long hampered both fundamental and applied studies on these important limnic bivalves.

Recently, a number of studies based on mitochondrial sequence analyses have attempted to provide an at least preliminary insight into the phylogenetic relationships of corbiculids. Siripattrawan *et al.* (2000) and Park & Kim (2003) both found the New World corbiculids *Polymesoda* and *Neocorbicula* to be basal to all other corbiculids, with the estuarine (i.e. brackish-water) *C. japonica* Prime, 1864 from East Asia and the freshwater *C. madagascariensis* in basal position to all other taxa within *Corbicula*. The latter was

utilized as an outgroup in the study by Lee *et al.* (2005), which had a more restricted focus on invasive populations in the New World. A common feature of all phylogenies available so far is that the examined Asian, European and American *Corbicula* morphotypes form a cluster with poorly resolved relationships, with morphological and molecular characters sometimes in contradiction (e.g. Siripattrawan *et al.* 2000; Pfenninger *et al.* 2002; Glaubrecht *et al.* 2003; Park & Kim 2003; Lee *et al.* 2005; Rintelen & Glaubrecht 2006).

Corbicula japonica has received relatively more attention (Miyazaki 1936; Maru 1981; Harada & Nishino 1995) and ecological as well as reproductive biological features seem to support its genetic distinctness, among them its brackish-water habitat, oviparity and monoflagellate haploid spermatozoa (Park & Kim 2003; Korniushin 2004). In contrast, due to the scarcity of available material from Madagascar, the reproductive biology of *C. madagascariensis* remains unknown.

Studying the corbiculid type material in the Mollusca collection of the Natural History Museum of the Humboldt University in Berlin (formerly Zoological Museum Berlin, ZMB) in the course of revisionary work on this family (see details in Glaubrecht *et al.* 2006), evidence for an euviviparous mode in *C. madagascariensis* was found upon closer inspection of one group of specimens from Madagascar, as described below. This is not only novel for corbiculids from the Ethiopian biogeographical region, but it sets this taxon apart from other oviparous and ovoviviparous taxa, in particular all known morphotypes of *C. fluminalis* (Müller, 1774), *africana* (Krauss, 1848) and *fluminea* (Müller, 1774), as delineated recently by Korniushin (2004). Finally, based on a revised and improved molecular phylogeny using mitochondrial gene sequence data for Corbiculidae, we discuss the evolution of distinct reproductive modes in these bivalves, suggesting the independent origin of euviviparity and, presumably, matrotrophy.

Materials and methods

The Mollusca collection of the Natural History Museum of the Humboldt University in Berlin holds one lot of specimens of *C. madagascariensis* Smith, 1882 from Madagascar (ZMB Moll. 45.446), with one desiccated animal preserved within the closed valves of one of the two extant specimens. The sample, with the original label '*ZMB Moll. 45446: Zwei Schalen von Corbicula sikorae Ancey n. sp.; Madagascar, Annanarivo, F. Sikora*', was originally identified as *Corbicula sikorae* Ancey, 1890. This species name is treated as a junior synonym of *C. madagascariensis* in the most recent catalogue of African bivalves (Daget 1998).

The molecular phylogeny is based on a *c*. 710 bp gene fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene, which has proven suitable for this approach in earlier studies by the present and other authors. This gene

was also chosen because a large number of corbiculid COI sequences is already accessible in GenBank and laboratory methods are well established; in contrast, the same comprehensive data set for nuclear genes for Corbiculidae is missing.

In addition to two sequences of *Batissa violacea* from Sulawesi (see Table 1), published sequences (Siripattrawan *et al.* 2000; Glaubrecht *et al.* 2003; Rintelen & Glaubrecht 2006) were taken from GenBank. Laboratory protocols as described by Rintelen & Glaubrecht (2006), and universal primers (LCO1490 and HCO2198; Folmer *et al.* 1994) were used. *Rungia cuneata* and *Mercenaria mercenaria* were chosen as outgroups following Park & Kim (2003).

For the phylogenetic analysis we used the COI sequence of *C. madagascariensis* for a specimen (UMMZ 255293) from Madagascar, first published by Siripattrawan *et al.* (2000) and deposited in GenBank as AF 196275 (see Table 1). It should be noted that there was some confusion regarding the species identity of this taxon, since different names are in use for mtDNA sequence data (COI and 16S) deposited with GenBank. Recently Korniushin (2004) has argued and discussed in detail that the so-called '*C. africana*' (Krauss, 1848) of some molecular analyses (Pfenninger *et al.* 2002) actually represents *C. madagascariensis* from Madagascar, as correctly assigned by Siripattrawan *et al.* (2000). While the former should be considered a synonym (see Glaubrecht *et al.* 2006), the latter is accepted as representing a distinct species (see e.g. Daget 1998; Korniushin 2004). Thus, we regard this sequence from an animal originating from Madagascar used here to be representative of this taxon.

The sequence alignment of 614 bp was created by eye with BioEdit 5.09 (Hall 1999). The phylogeny was produced using three methods: maximum parsimony (MP) with PAUP 4.0b010 (Swofford 2003), maximum likelihood (ML) with Treefinder (Jobb 2005) and Bayesian inference (BI) with MrBayes 3.1 (Ronquist & Huelsenbeck 2003). The MP analysis was done using a full heuristic search with random addition (10 replicates) and TBR, while the simple addition option and TBR were used in a MP bootstrap analysis (100 replicates). For the ML and BI analyses an appropriate model of sequence evolution was selected using MrModeltest 2.2 (Nylander 2004); based on the Aikaike Information Criterion, the GTR + I + Γ model was employed. The ML analysis was done with the Treefinder default settings, and 1000 RELL bootstrap replicates. For the BI analysis posterior probabilities of phylogenetic trees were estimated by a 2 000 000 generation Metropolis-coupled Markov Chain Monte Carlo algorithm (four chains, chain temperature = 0.2), with parameters estimated from the data set. A 50% majority-rule consensus tree was constructed following a 15 001 trees burn-in to allow likelihood values to reach stationarity. Modes of reproduction were reconstructed on the tree using MacClade 4.08 (Maddison & Maddison 2005).

Morphological voucher material of all corbiculids studied herein is deposited with the malacological collection of ZMB, if not stated otherwise (Table 1); all molecular sequences given in Table 1 with their respective accession and collection numbers are available from GenBank.

Results

On the species identity

Conchological examination of the two specimens (ZMB 45.446) and comparison with Ancey's (1890) and Smith's (1882) original descriptions support the proposed synonymization of *Corbicula sikorae* Ancey, 1890 with *C. madagascariensis* Smith, 1882.

According to the inventory record in ZMB, this lot was bought directly from the Austrian collector Franz Sikora (?–1902), who was based in Réunion. For seven years he also collected specimens in Madagascar for various European museums. The specimens of *C. madagascariensis* in question were inventoried in ZMB in 1892. The material came from the type locality, given as 'Fleuve Mangoro, dans l'intérieur de Madagascar, de Tananarive à la côte orientale, à une altitude de 700 mètres au-dessus du niveau de la mer (Sikora)' (Ancey 1890: 345), with 'Annanarivo' an apparent misspelling of Antananarivo (French: Tananarive), which is near the type locality of Smith's *C. madagascariensis* (cf. Smith 1882: 388).

The whereabouts of Ancey's type material is unknown (Daget 1998; Counts 1991). However, since we have no further information relating to any potential relationship between Ancey, Sikora and the then curator of ZMB, Eduard von Martens (1832–1904), we refrain from assuming that this material actually represents types. Moreover, the size parameters given in Ancey's original description (L: 11.5, H: 8.5, W: 5 mm) do not correspond with those of either of the two ZMB specimens. Instead, we regard the ZMB material as representing topotypical material collected by Sikora, together with Ancey's later type material. It should be noted, however, that if Ancey's types are lost, as appears to be the case, the ZMB material qualifies for the designation as neotypes.

Description of maternal animal and juveniles

ZMB 45.446 comprises two adult specimens with originally closed valves with the following size measurements for the larger and smaller specimens, respectively: L: 15.5 and 12.9 mm, H: 12.1 and 9.8 mm, W: 6.9 and 5.8 mm. Most of the conchological features (e.g. shell shape and sculpture) are perfectly in accord with the original descriptions, except that the colour of the inner valves is blueish-white in *sikorae* according to Ancey, while that of *madagascariensis* is, according to Smith's original description, 'lilac with two or three rather distant concentric purple zones, stained with dark purple down the posterior side, and with smaller stain of the same colour at the anterior side'. The ZMB specimens, which

Table 1 Sample and sequence provenance, GenBank accession numbers, and museum accession numbers for ZMB samples of Corbiculidae.

| Taxon | Locality data | Accession numbers | | Reference |
		Museum	GenBank	
Mercenaria mercenaria (Linnaeus, 1758)	USA		U47648	Baldwin *et al.* (1996)
Rangia cuneata (Sowerby I, 1831)	USA, New Jersey		U47652	Baldwin *et al.* (1996)
Batissa violacea Lamarck, 1806	Sulawesi	ZMB 103030	DQ837726	This study
	Sulawesi	ZMB 103031	DQ837727	This study
Polymesoda caroliniana (Bosc, 1801)	USA, Florida	UMMZ 265499	AF196276	Siripattrawan *et al.* (2000)
Neocorbicula limosa (Maton, 1809)	Argentina	UMMZ 266500	AF196277	Siripattrawan *et al.* (2000)
Corbicula anomioides	Lake Poso	ZMB 190808	DQ285604	Rintelen & Glaubrecht (2006)
(Bogan & Bouchet, 1998)	Lake Poso	ZMB 190826	DQ285605	Rintelen & Glaubrecht (2006)
C. australis (Lamarck, 1818)	Australia, NSW	UMMZ 266662	AF196274	Siripattrawan *et al.* (2000)
C. fluminalis (O.F. Müller, 1774)	China		AF457996	Park & Kim (2003)
	China		AF457997	Park & Kim (2003)
	China		AF457998	Park & Kim (2003)
C. fluminea (Müller, 1774)	Thailand	ZMB 200169	DQ285577	Rintelen & Glaubrecht (2006)
	Thailand	UMMZ 266691	AF196270	Siripattrawan *et al.* (2000)
	Korea	UMMZ 266690	AF196269	Siripattrawan *et al.* (2000)
	USA (introduced)		U47647	Baldwin *et al.* (1996)
C. japonica (Prime, 1864)	Japan	UMMZ 266688	AF196271	Siripattrawan *et al.* (2000)
	Korea		AF367440	Park & Kim (2003)
	Korea		AF357441	Park & Kim (2003)
C. javanica (Mousson, 1849)	Java	ZMB 106449	AY275668	Glaubrecht *et al.* (2003)
	Java		AF269096	Renard *et al.* (2000)
	Java		AF457993	Park & Kim (2003)
C. lamarckiana Prime, 1864	Thailand	ZMB 200214	DQ285578	Rintelen & Glaubrecht (2006)
C. leana Prime, 1864	Japan	UMMZ 266668	AF196268	Siripattrawan *et al.* (2000)
C. linduensis Bollinger, 1914	Sulawesi	ZMB 190842	DQ285579	Rintelen & Glaubrecht (2006)
C. loehensis Kruimel, 1913	Lake Mahalona	ZMB 190582	DQ285580	Rintelen & Glaubrecht (2006)
	Lake Lontoa	ZMB 190768	DQ285581	Rintelen & Glaubrecht (2006)
	Lake Masapi	ZMB 103011	AY275666	Glaubrecht *et al.* (2003)
*C. madagascariensis** Smith, 1882	Madagascar	UMMZ 255293	AF196275	Siripattrawan *et al.* (2000)
C. matannensis Sarasin & Sarasin, 1898	Lake Matano	ZMB 190553	DQ285582	Rintelen & Glaubrecht (2006)
	Lake Matano	ZMB 103052	DQ285583	Rintelen & Glaubrecht (2006)
	Lake Matano	ZMB 190580	DQ285584	Rintelen & Glaubrecht (2006)
	Lake Matano	ZMB 103003	AY275664	Glaubrecht *et al.* (2003)
	Lake Matano	ZMB 190556	DQ285585	Rintelen & Glaubrecht (2006)
	Lake Matano	ZMB 103002	AY275663	Glaubrecht *et al.* (2003)
	Lake Mahalona	ZMB 103009	AY275665	Glaubrecht *et al.* (2003)
	Lake Mahalona	ZMB 190564	DQ285586	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 191044	DQ285587	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 191042	DQ285588	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 190773	DQ285589	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 190772	DQ285590	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 190771	DQ285591	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 190779	DQ285592	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 190569	DQ285593	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 190777	DQ285594	Rintelen & Glaubrecht (2006)
	Lake Towuti	ZMB 190568	DQ285595	Rintelen & Glaubrecht (2006)
C. moltkiana Prime, 1878	Sumatra	ZMB 103024	AY275660	Glaubrecht *et al.* (2003)
	Sumatra	ZMB 103025	AY275657	Glaubrecht *et al.* (2003)
	Sumatra	ZMB 103032	AY275658	Glaubrecht *et al.* (2003)
	Sumatra	ZMB 103034	AY275659	Glaubrecht *et al.* (2003)
C. possoensis Sarasin & Sarasin, 1898	Lake Poso	ZMB 190019	DQ285596	Rintelen & Glaubrecht (2006)
	Lake Poso	ZMB 190024	AY275661	Glaubrecht *et al.* (2003)
	Lake Poso	ZMB 190823	DQ285597	Rintelen & Glaubrecht (2006)
	Lake Poso	ZMB 190870	DQ285598	Rintelen & Glaubrecht (2006)
	Lake Poso	ZMB 190997	DQ285599	Rintelen & Glaubrecht (2006)
	Lake Poso	ZMB 103028	AY275662	Glaubrecht *et al.* (2003)

Table 1 *Continued*

| Taxon | Locality data | Accession numbers | | Reference |
		Museum	GenBank	
C. sandai Reinhardt, 1878	Japan	UMMZ 266689	AF196272	Siripattrawan *et al.* (2000)
		UMMZ 266689	AF196273	Siripattrawan *et al.* (2000)
C. spp.	China		AF457989	Park & Kim (2003)
	China		AF457990	Park & Kim (2003)
	China		AF457994	Park & Kim (2003)
	China		AF457995	Park & Kim (2003)
	China		AF457999	Park & Kim (2003)
	Israel		AY097298	Pfenninger *et al.* (2002)
	Israel		AY097299	Pfenninger *et al.* (2002)
	Korea		AF457992	Park & Kim (2003)
	Taiwan		AF457991	Park & Kim (2003)
	Vietnam		AF468017	Park & Kim (2003)
	Vietnam		AF468018	Park & Kim (2003)
C. subplanata Martens, 1897	Sulawesi	ZMB 190843	DQ285600	Rintelen & Glaubrecht (2006)
	Sulawesi	ZMB 190852	DQ285601	Rintelen & Glaubrecht (2006)
	Sulawesi	ZMB 190785	DQ285602	Rintelen & Glaubrecht (2006)
	Sulawesi	ZMB 190581	DQ285603	Rintelen & Glaubrecht (2006)

*in GenBank as *C. africana*; see Korniushin (2004) and text for details.

exhibit a white interior with a purplish stain due to the periostracum's colour showing through the white porcellaneous layer, agree with the *sikorae* description and differ from the *madagascariensis* description only in this point of presumably minor importance.

After collection, the shell muscles of the dying animal apparently contracted long enough to keep the valves closed when stored by Sikora and later deposited in the ZMB collection. After we opened the valves of the larger specimen the remains of a mummified body became visible. This desiccated specimen reveals a membranous structure in the upper half of the shell and a cluster of 24 shelled offspring (Fig. 1). These juveniles all possess mineralized shells with distinct sculpture and are of different sizes. They are embedded in the remnants of the desiccated soft body, most likely the maternal ctenidial lamellae (Fig. 1A–C). Opening the valves has resulted in parts of the dried tissue being broken off, with three additional juveniles dislodged (Fig. 1D).

The shell length of the juveniles ranges from 2 to 3 mm in the four largest specimens to less than 0.5 mm in the smallest. No size classes are evident, but instead the clutch represents a continuous ontogenetic growth series. Apparently, these juveniles, which all have a more or less developed umbo (Fig. 1C,D) did not develop as a single cohort, but in an asynchronous mode.

Due to the mummified state of the body, the original location of brooding (i.e. inner and/or outer demibranchs) is not discernible. Nevertheless, our findings suggest that this *Corbicula* from Madagascar is euviviparous, based on the fact

of long incubating extremely large shelled juveniles and therefore indirectly hinting at matrotrophy (see Discussion).

Molecular phylogeny

The revised molecular phylogeny (Fig. 2) reveals a sister-group relationship of the SE Asian *Batissa violacea* and all other corbiculids. The two Neotropical *Polymesoda* and *Neocorbicula* are sister to all *Corbicula*. Within the latter, a basal split between an estuarine (*japonica*, including '*fluminalis*' from China) and a freshwater clade is found. Within the latter *C. madagascariensis* is sister to the rest of all freshwater *Corbicula* taxa.

However, levels of support vary considerably for parts of the topology. While most of the clades described above are extremely well supported (bootstrap values between 85 and 100 in all methods), the sister-group relationship between the American *Polymesoda* and *Neocorbicula* seems more doubtful.

There is also no strong support for the branching order depicted in Figs 2 and 3 within the freshwater clade of *Corbicula*, with the exception of the basal position of *C. madagascariensis*.

These phylogenetic uncertainities, however, do not affect the reconstruction of the evolution of viviparity in corbiculids. Plotting the occurrence of distinct reproductive strategies (listed in Table 2) on the phylogenetic tree using MacClade (Fig. 2) reveals the origin of viviparity in general in two independent lineages or clades, respectively: the South American *Neocorbicula* and the Afro-Asian *Corbicula*. In case of *Neocorbicula*, this coincides with one of three separate euviviparous
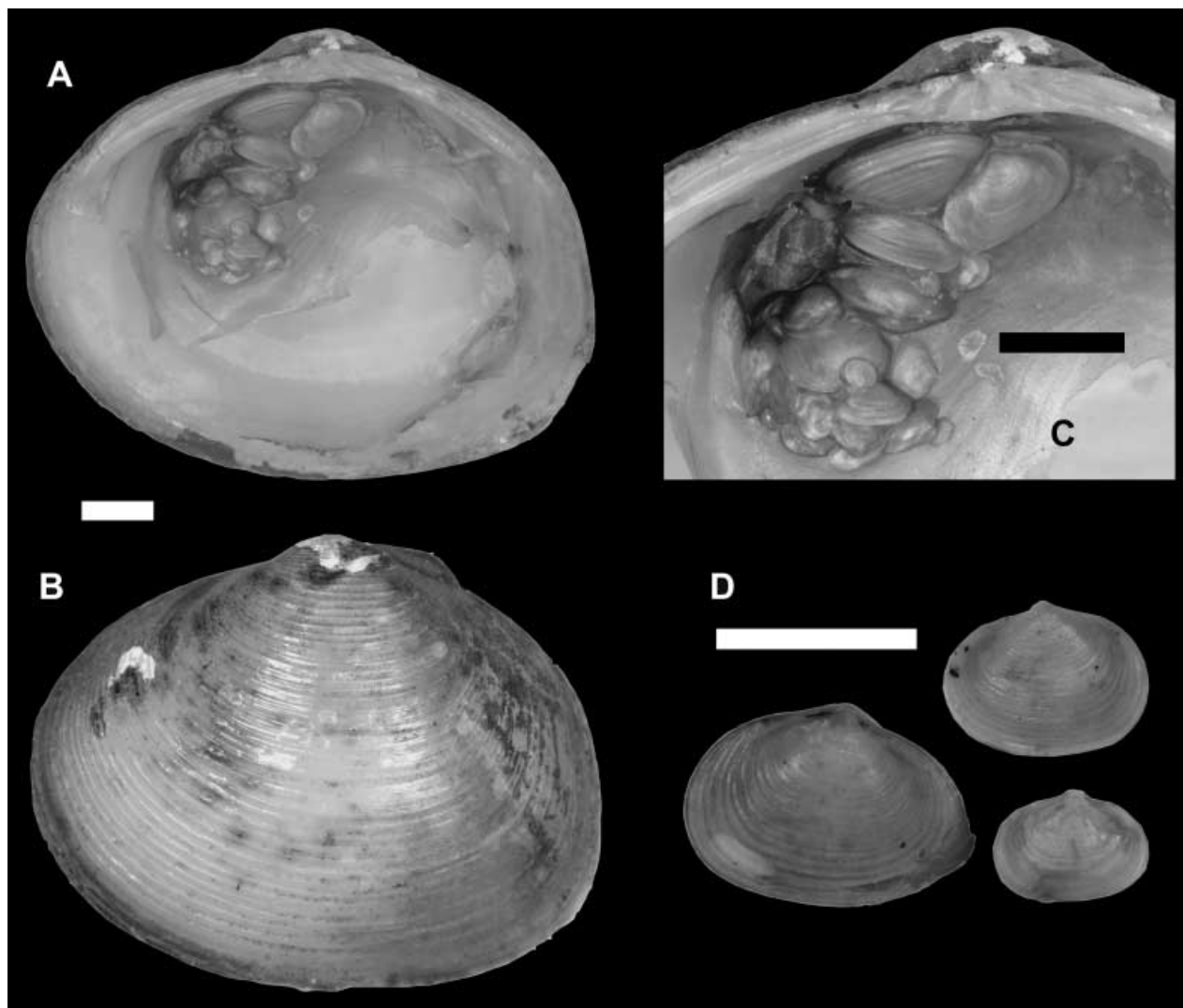
Fig. 1 A–D. Brooding in *Corbicula madagascariensis*. Topotypical specimen of '*C. sikorae*' (ZMB Moll. 45.446); see text for details. —A. Right valve with a clump of juveniles surrounded by the mummified body. —B. Left valve of the same specimen. —C. Clump of juveniles. —D. Dislodged juveniles. Scale bars, 2 mm.

lineages, with the other two found in *C. madagascariensis* and *C. linduensis*; see Discussion for details.

Discussion

While most marine bivalves are oviparous, some exceptions to this are found, for example in Ostreidae, Carditidae, Galeommatidae and Teredinidae, which incubate larvae in or between the gill filaments (e.g. Mackie 1984; Ponder 1998). On the other hand, the various reproductive morphologies and strategies found in limnic bivalves have aroused much interest recently, in particular the Unionoidea, (which additionally have different parasitic larval stages; Mackie 1984;

Graf & Ó Foighil 2000; Schwartz & Dimock 2001), and also the Sphaeriidae (Mansur & Meier-Brook 2000; Cooley & Ó Foighil 2000; Korniushin & Glaubrecht 2002). A particular point of interest has been the specialized incubatory structure, termed brood pouch or marsupium, which in some limnic bivalves may also have a nutritive function (i.e. matrotrophy); see, e.g. Tankersley & Dimock (1992), Hetzel (1993), Mansur & Meier-Brook (2000), Cooley & Ó Foighil (2000), Graf & Ó Foighil (2000), Hoeh *et al*. (2001) and Korniushin & Glaubrecht (2003).

Viviparous strategies in these bivalves have been related to the colonization of, and speciation in, freshwater habitats
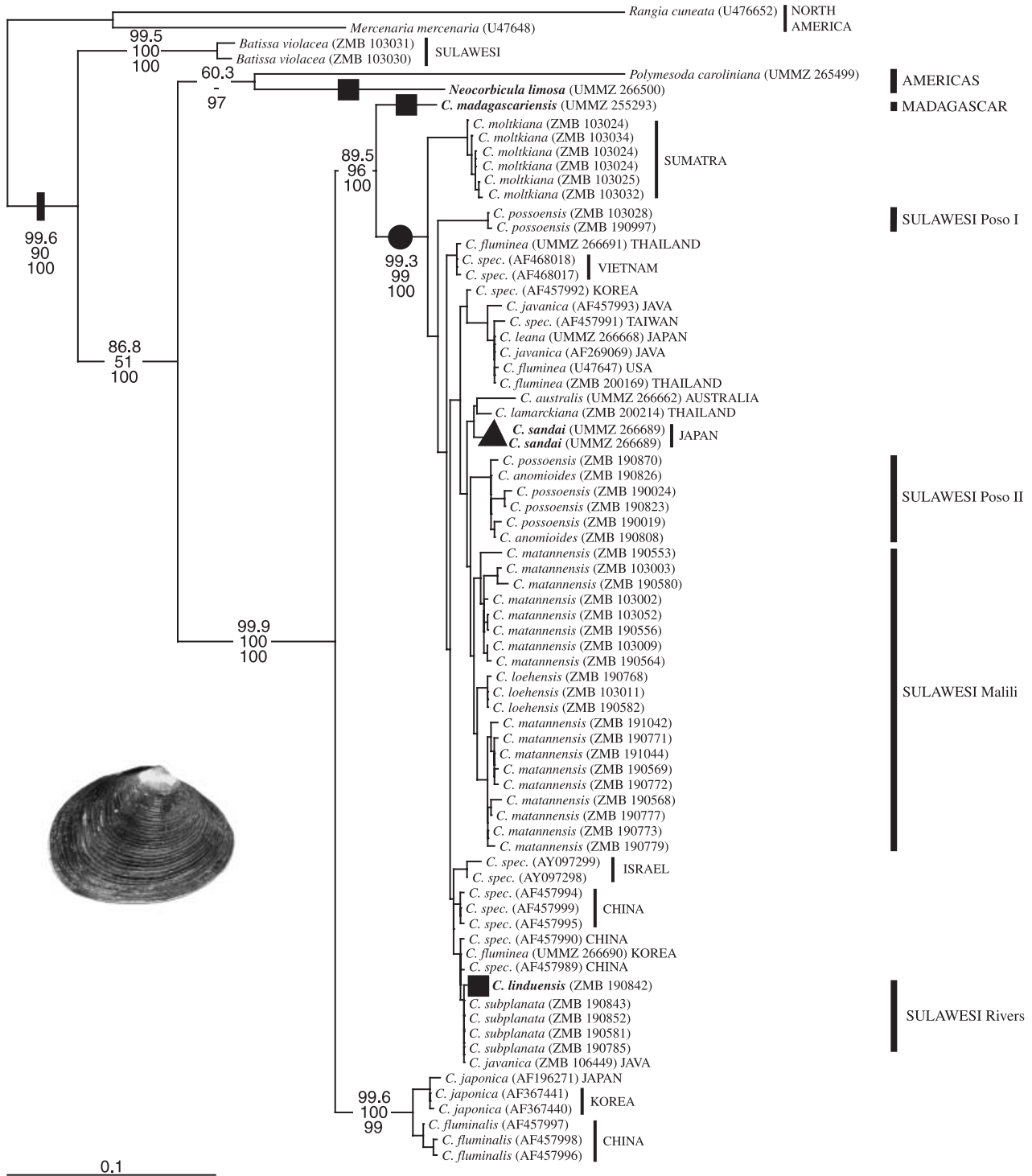
Fig. 2 Phylogenetic relationships among Corbiculidae, based on mtDNA (COI); maximum likelihood phylogram. The numbers on branches are, from top, maximum likelihood (RELL) and maximum parsimony bootstrap values, and Bayesian inference posterior probabilities; the scale bar indicates the number of substitutions per site. Symbols indicate reproductive modes: bar, basal ovipary; triangle, derived ovipary; circle, ovovivipary; square, euvivipary.
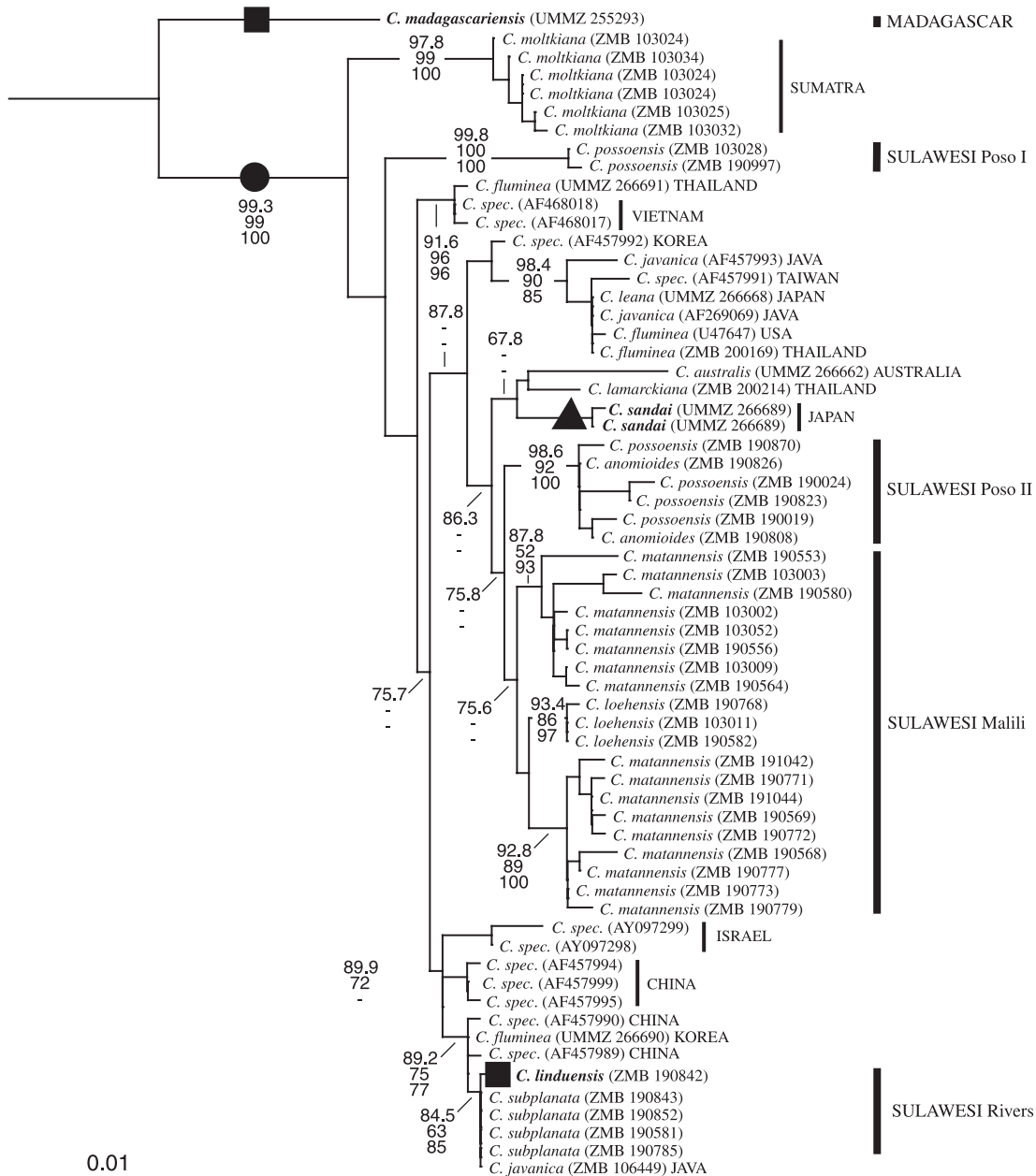
Fig. 3 Phylogenetic relationships among the freshwater species of *Corbicula* based on mtDNA (COI); maximum likelihood phylogram as in Fig. 2, partly enlarged and graphically modified. The numbers on branches are, from top, maximum likelihood (RELL)- and maximum parsimony bootstrap values, and Bayesian inference posterior probabilities; the scale bar indicates the number of substitutions per site. Symbols indicating reproductive modes as in Fig. 2.

(e.g. Byrne *et al.* 2000; Graf & Ó Foighil 2000; Hoeh *et al.* 2001). This discussion parallels that relating to gastropods, e.g. several lineages of limnic and viviparous Cerithioidea (Glaubrecht 1996, 1999, 2006; Strong & Glaubrecht 2002, 2007; Köhler *et al.* 2004; Rintelen & Glaubrecht 2005). As much as corbiculids have turned out in this context to be useful for studying reproductive and life-history strategies in bivalves in general and limnic taxa in particular, the complex phenomena found in these molluscs have revealed the limitations of commonly utilized terminology; see Korniushin & Glaubrecht (2003) for more detail and clarification.

Three reproductive modes — oviparity, ovoviviparity and euviviparity — are exhibited among Corbiculidae. These differences reflect distinct biological properties, as summarized in

Table 2 Survey of reproductive strategies in Corbiculidae. *Corbicula* species are listed roughly by the geography of their occurrence in the Oriental and Australian provinces.

Taxon	Reproduction	Brooding site	Reference
Neocorbicula limosa	incubation (20–45 embryos) euviviparity	within inner demibranchs (endobranchous)	Ituarte (1994)
Corbicula japonica	oviparous	—	Byun & Chung (2001)
C. madagascariensis	incubation (*c.* 27 embryos) euviviparity	brood pouch within demibranchs?	this study
C. fluminea	incubation (thousands) ovoviviparity	endobranchous	Kraemer & Galloway (1986) Morton (1986)
C. fluminalis	incubation (several hundred) ovoviviparity	endobranchous	Korniushin (2004)
C. largillierti	incubation (> 10 000), ovoviviparity	within water tubes inner demibranchs	Ituarte (1994)
C. leana	incubation (numerous) ovoviviparity	endobranchous	Miyazaki (1936) Morton (1986)
C. sandai	oviparous	–	Hurukawa & Mizumoto (1953)
C. moltkiana	incubation (several hundred) ovoviviparity	endobranchous	Glaubrecht *et al.* (2003) Korniushin & Glaubrecht (2003)
C. javanica	incubation (several hundred) ovoviviparity	endobranchous	Glaubrecht *et al.* (2003) Korniushin & Glaubrecht (2003)
C. matannensis	incubation (several hundred) ovoviviparity	endobranchous	Glaubrecht *et al.* (2003) Korniushin & Glaubrecht (2003)
C. loehensis	incubation (several hundred) ovoviviparity	endobranchous	Glaubrecht *et al.* (2003) Korniushin & Glaubrecht (2003)
C. anomioides	incubation (numerous) ovoviviparity	endobranchous	Bogan & Bouchet (1998)
C. possoensis	incubation (numerous) ovoviviparity	inner + outer demibranchs (tetragenous)	Glaubrecht *et al.* (2003) Korniushin & Glaubrecht (2003)
C. linduensis	prolonged incubation (10–35 embryos) euviviparity	endobranchous	Glaubrecht *et al.* (2003) Korniushin & Glaubrecht (2003)
C. australis	incubation (500–3000 embryos) ovoviviparity	endobranchous	Byrne *et al.* (2000)

Table 2, and should also be clearly distinguished semantically. Thus, we here differentiate for corbiculids: (1) an ovoviviparous strategy, i.e. the short-termed incubation of larva in maternal gills (pediveliger stage to juveniles with shells of *c.* 0.25–0.4 mm) as discussed, for example, for *C. australis* (cf. Byrne *et al.* 2000), *C. fluminalis* (cf. Korniushin 2004), and for most Indonesian *Corbicula* (cf. Korniushin & Glaubrecht 2003), vs. (2) a truly euviviparous strategy, which involves prolonged incubation and the development of relatively large shelled offspring (> 1–5 mm).

In general, nourishment of the embryos and juveniles can come from yolk or possibly even from maternal tissue, i.e. the epithelium lining the marsupium; only in the latter case do we use the term matrotrophy. Maternal nourishment in corbiculids can be inferred from the prolonged incubation and extremely large shelled juveniles as suggested for *Neocorbicula* by Ituarte (1994). It was also explicitly suggested for *C. australis* by Byrne *et al.* (2000: 195), based on: (1) the mucous cell lining in the epithelium of the interlamellar junction of the ctenidial marsupium, indicative of absorptive epithelium in the velum of the larvae, and (2) the considerable difference between fully grown eggs (125 μm) and the released juveniles (250 μm). Byrne *et al.* hypothesized this to indicate that 'embryonic development may be supported by exogenous nutrients provided by the parent'. This extraembryonic nutrition would qualify for euviviparity according to the terminology and definition in Korniushin & Glaubrecht (2003).

Viviparity in Corbiculidae

Forming a pattern analogous to that of another limnic bivalve family, viz. Sphaeriidae (see e.g. Korniushin & Glaubrecht 2002), corbiculid taxa occurring in estuarine and brackish-water habitats (e.g. Neotropical *Polymesoda* and Oriental *Batissa*, as well as *Corbicula japonica* in Japan, Korea and China) are generally nonbrooding but possess free-swimming veliger larvae. In contrast, the purely freshwater inhabitants among both limnic bivalve groups (sphaeriids and corbiculids) generally incubate their offspring in marsupial structures within the ctenidium, releasing shelled juveniles.

Within *Corbicula* therefore with the exception of the estuarine marsh clam *Corbicula japonica* which is dioecious, nonbrooding and characterized by the development of free-swimming veligers (see details in Morton 1986; Byun & Chung 2001), almost all other freshwater *Corbicula* are hermaphrodites and ovoviviparous with incubation in the maternal gill (Korniushin & Glaubrecht 2003; Glaubrecht *et al.* 2003; Korniushin 2004).

Curiously, only *C. sandai*, which is endemic to Lake Biwa in Japan and has some other unusual features (e.g. being

diploid, diocious with uniflagellate sperm), was found in an earlier study (Hurukawa & Mizumoto 1953) to be nonbrooding with its larvae being nonswimming, transforming into benthic juveniles immediately after leaving the egg capsules at a size of 0.18 mm (see Table 2). This has resulted in some speculation as to the lake's fauna being a marine relic (see Harada & Nishino 1995: 404, and references therein). Similar speculations have long been upheld in the case of the thalassoid malacofauna of Lake Tanganyika in East Africa (see review in Glaubrecht 1996). The lacustrine *C. sandai* being oviparous is noteworthy insofar as many limnic organisms including molluscs, in particular from so-called 'ancient' lakes, are known to exhibit viviparity even when closely related nonlacustrine forms are oviparous, but most rarely vice versa. Therefore, it would be interesting to establish, based on the study of extensive series, whether *sandai* is indeed nonbrooding, in order to exclude the theoretical possibility that the originally studied material for whatever reason lacked larvae and marsupial gills, a point also raised earlier by Morton (1979).

Hitherto, prolonged incubation of large embryos with well-developed shells, here termed euviviparity (see Table 2), was thought to not only characterize native South American corbiculids, but also believed to be an important feature for elevating the rank and placing them in a seperate genus *Neocorbicula* Fischer, 1887 (Parodiz & Hennings 1965). The latter genus not only differs substantially in shell morphology but also in characteristics of branchial incubation of embryos, which is carried out in different ways (Ituarte 1994). Generally, *Corbicula* (with the exception of *C. japonica*) incubate embryos in large numbers (> 10 000) within the water tubes of the two inner demibranchs (endobranchous) which are unmodified; late veligers or pediveligers (*c.* 200–240 µm in diameter) are released after a short incubation period (e.g. Britton & Morton 1979). In contrast, *Neocorbicula* develops brood pouches by cellular proliferation of the interlamellar junction epithelium of the inner demibranchs, with a single embryo within each pouch and a total of about 20–30 (maximum 45) embryos of different cohorts; the offspring are released as fully developed juveniles with sizes of *c.* 1.1 mm, with some remaining within the pouch until their shells have reached 4–5 mm in length (Ituarte 1994). However, detailed studies as to the existence of specialized tissue with a nutritive function, as done for sphaeriids (e.g. Hetzel 1993), and thus providing direct evidence for matrotrophy, are lacking not only for *Neocorbicula* but also for other corbiculids.

Modes of incubation

Recently, in Indonesian corbiculids, particularly in populations from Sulawesi and Sumatra (which possess uniflagellate spermatozoa, thus being meiotic, i.e. nonclonal), a greater diversity of brooding characteristics was documented than witnessed previously for the rest of the collective Old World range of *Corbicula* (Glaubrecht *et al.* 2003; Korniushin & Glaubrecht 2003). All *Corbicula* species endemic to Sulawesi and *C. moltkiana* from Sumatra were found to be endobranchous, i.e. to incubate their young in their inner demibranchs at least until the stage of juveniles with straight-hinged shells (D-shaped), thus being ovoviviparous.

In contrast, two other strategies were found among the endemic Sulawesi taxa that were hitherto unknown in any other congeners. *Corbicula possoensis* Sarasin & Sarasin, 1898 from Lake Poso, which was found as adelphotaxon to the cementing (i.e. sessile) *C. anomioides* (see details in Rintelen & Glaubrecht 2006) from the same lake, exhibits tetragenous brooding (i.e. incubation in both demibranchs). However, as in most other *Corbicula* species, it incubates relatively small, straight-hinged juveniles, whereas in another species from Sulawesi, viz. *C. linduensis* Bollinger, 1914, prolonged incubation within the maternal gill was found, with juvenile shells reaching up to 1.3 mm in length and with well-developed hinges. In addition, this latter euviviparous taxon is reported to have sequential brooding, releasing umbonal larvae of about 1.5 mm (Korniushin & Glaubrecht 2003; Glaubrecht *et al.* 2003). While in all other corbiculid studies so far brooding is synchronous, the only other known exception to this was found in *Neocorbicula limosa*, where incubation is also sequential (Ituarte 1994).

Thus, next to *Neocorbicula* in South America, only *C. linduensis* from Sulawesi is truly representative of a euviviparous corbiculid from Southeast Asia. The evidence provided here, even when indirect, now adds *C. madagascariensis* from Madagascar. The position and size of the shelled juveniles found in the adult body of the latter taxon are comparable to those encountered only in the former. The size variation within the juveniles further suggests that the brood originates from more than one preceding spawning event. Consequently, we anticipate here that the type of brooding in *C. madagascariensis* also follows a sequential mode as found in *C. linduensis*. Following a similar argument put forward for sphaeriids by Cooley & Ó Foighil (2000), this can be hypothesized as a derived condition in both cases among corbiculids, while synchronous brooding is the plesiomorphic feature.

In contrast, other African corbiculids such as *C. fluminalis*, ranging from North Africa and the Middle East to Central Asia, have ovoviviparous brooding with short-termed incubation of many larvae in gills (Korniushin 2004). They were both found to incubate a large number of small larvae of similar size of around 0.2 mm (with the largest larvae 217 µm), lying densely packed within (and filling almost all the available) interlamellar spaces of the ctenidium. These larvae were D-shaped, weakly calcified and fragile, the hinge edge straight and no hinge structures seen, and of varying sizes. This finding of a distinct reproductive mode serves as further

argument against the synonymization of *C. africana* and *C. madagascariensis*.

Evolution of viviparity and historical biogeography

Finding another euviviparous corbiculid from Madagascar that apparently incubates and spawns asynchronously, a phylogenetic hypothesis can be suggested concerning the evolution in corbiculids of viviparous modes in general and euviviparity in particular. The tree topology resulting from our molecular genetic analyses (Figs 2 and 3) not only clearly rejects a single-origin hypothesis of brooding and euviviparity in corbiculids, but suggests, by the occurrence of euviviparity in *Neocorbicula*, *C. madagascariensis* and *C. linduensis*, that this mode of reproduction evolved three times independently. Interestingly, the ovipary of *C. sandai* from Lake Biwa, if confirmed (see above), is a secondarily derived condition in an otherwise (ovo)-viviparous clade.

As a note of caution, however, it should be pointed out that the crucial clade containing *Polymesoda* and *Neocorbicula* is the least supported of all the major clades. Therefore, our data do not unequivocally reject the possibility that these two taxa might be paraphyletic with respect to *Corbicula*. While this would be irrelevant for our interpretation if *Neocorbicula* were sister to a clade comprising *Polymesoda* and *Corbicula*, a sister-group relationship of *Neocorbicula* and *Corbicula* would in contrast make a single origin of viviparity in corbiculids equally parsimonious (three evolutionary steps) as the two-fold independent evolution of brooding suggested here. The same is essentially true for the threefold independent origin of euviviparity assumed for *N. limosa*, *C. madagascariensis* and *C. linduensis*. The comparatively poor support for the branching pattern within Asian freshwater *Corbicula* (see Fig. 3) does not completely rule out a more basal position for *C. linduensis*.

An alternative hypothesis to the repeated independent evolution of both brooding and euviviparity within Corbiculidae is thus the assumption of euviviparity as a synapomorphic feature of potential Gondwanan lineages in South America (*Neocorbicula*), Madagascar (*C. madagascariensis*) and Sulawesi (*C. linduensis*). Nevertheless, we consider a single origin of brooding and euviviparity in corbiculids highly unlikely, on geographical and biological grounds. The latter alternative not only requires that ovoviviparity is the derived condition in *Corbicula* (which is possible but not very likely), it also forces us to conclude that the brackish-water *C. japonica* secondarily lost incubation (which is even less likely). Accordingly, we here favour the hypothesis presented above, viz. that brooding in general and a prolonged incubation in ctenidial brood pouches in particular (i.e. euviviparity) has developed repeatedly in different evolutionary lineages among corbiculids.

This conclusion accords with some differences in the anatomical fine structures of the intrabranchial marsupia

in *Neocorbicula* and *Corbicula linduensis*, as discussed in Korniushin & Glaubrecht (2003). We further propose based on the available data that within the genus *Corbicula* ctenidial incubation has evolved only once, accompanied by the development of brood pouches within water tubes of the inner demibranchs and again, as an apomorphic feature, only once involving both demibranchs in the tetragenous *C. possoensis* from Lake Poso in Sulawesi.

A comparison of branch lengths representing genetic distances in our molecular phylogeny indicates that among the euviviparous corbiculids, in particular *N. limosa* but also *C. madagascariensis*, are considerably older than *C. linduensis*, prompting us to suggest the recent and rapid evolution of euviviparity in *C. linduensis*. This supports a previous study where we have shown that even a most striking morphological adaptation in *Corbicula*, such as sessility (i.e. the cementation of one valve onto the substrate) in *C. anomioides*, evolved rather rapidly (Rintelen & Glaubrecht 2006).

Given the known geological history of Gondwana since the Mesozoic, involving long separation of South America, Asia and the two islands Madagascar and Sulawesi, we anticipate that *Neocorbicula*, *C. madagascariensis* and at least two species on Sulawesi (viz. *C. linduensis* and *C. possoensis*) each independently developed slightly different brooding modes with varying degrees of viviparity. The shallow and partly unresolved polytomy in Indonesian *Corbicula* suggests a fairly recent origin of the endemic species on Sulawesi (Fig. 3), apparently preceded by older differentiation in riverine taxa all over Southeast Asia. The timing of these events remains largely unknown. However, at least for Sulawesi the known Miocene age of its individual terranes estimated at *c*. 15–5 Mya (see details in Wilson & Moss 1999; Hall 2002) and the origin of some ancient lake faunal elements estimated at *c*. 1–2 Mya (e.g. *Tylomelania* gastropods; see Rintelen & Glaubrecht 2005) make a Plio-Pleistocene origin and evolution of these endemic corbiculids likely. In conclusion, the origin of euviviparity at different times and independently in three New and Old World corbiculids can be considered a most remarkable example of parallel evolution.

Acknowledgements

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