

Radiating in a river: systematics, molecular genetics and morphological differentiation of viviparous freshwater gastropods endemic to the Kaek River, central Thailand (Cerithioidea, Pachychilidae)

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Speciation in the context of adaptive radiation is regarded as a key process in the creation of biodiversity. While several lacustrine species flocks provide ideal models for elucidating the underlying evolutionary mechanisms, riverine radiations are both rarely known and studied. The Kaek River, a third-order tributary of the Nan River and Chao Praya drainage in central Thailand, harbours an exceptional endemic species assemblage of morphologically distinct, viviparous pachychilid gastropods. Our systematic revision, combining a morphological and molecular genetics approach, reveals the sympatric existence of at least seven species of the genus *Brotia* that is widespread in rivers of South-east Asia where usually only two species at the most coexist. At eight locations along a 100-kilometre stretch of the Kaek River, we found the syntopic occurrence of two to three species that are separated by specific habitat preferences and exhibit trophic specialization in their radula morphology. Phylogenetic analyses (using MP, NJ, ML and Bayesian inference statistics) of partial COI and 16S sequence data of 17 samples from six species occurring sympatrically and parapatrically, respectively, in the Kaek River drainage (plus the type species *B. pagodula* as out-group) indicate monophyly of all these endemic species. *Brotia soleimiana*, which also occurs in the Loei River, a tributary of the Mekong drainage system, was found to be sister to all other Kaek River pachychilids. The distinctive morphotypes, proposed here to represent biospecies, do not show high levels of genetic variation consistent with long periods of reproductive isolation. This suggests a relatively recent origin of this intrariverine radiation and rapid morphological divergence in the Kaek River *Brotia*. Recent diversification combined with ecological separation and trophic specialization parallels conditions found, albeit on a more specious level, in the lacustrine species flock of the closely related pachychilid genus *Tylomelania*, which is endemic to ancient lakes on the Indonesian island of Sulawesi. We discuss and compare the allopatric and ecological aspects of speciation in this unique riverine radiation and outline a putative historical biogeography of the Kaek River species, employing the most recent geological and palaeohydrological data for Thailand. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 82, 275–311.

ADDITIONAL KEYWORDS: adaptive variation – conchological divergence – historical biogeography – riverine species flock – speciation – trophic specialization.

INTRODUCTION

Speciation, or divergent evolution resulting in two species from an initial ancestral population, in the context of radiation is regarded as a key process in the creation of organismic diversity (e.g. Mayr, 1963, 1988, 2001; Magurran & May, 1999). Despite one and a half

centuries of research into the mechanisms since Darwin's '*Origin of species* . . .' (Darwin, 1859), speciation remains a central concern of evolutionary biologists and probably still is 'the least understood major feature of evolution' (Schluter, 2000), as revealed by an enormous and ever-growing body of studies (reviewed most recently, e.g., in Otte & Endler, 1989; Givnish & Sytsma, 1997; Grant, 1998, 2001; Howard & Berlocher, 1998; Orr & Smith, 1998; Turelli, Barton & Coyne, 2001, and other articles in *Trends in Ecology*

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and *Evolution* volume 16(7); Schilthuizen, 2001; Losos & Glor, 2003; West-Eberhard, 2003).

Modes of speciation have recently and controversially been discussed (e.g. Bush, 1975, 1994; Mayr, 1988, 2001; Schluter & Nagel, 1995; Bush & Smith, 1998; Grant, 1998; Schluter, 2001; Via, 2001; Losos & Glor, 2003). It was long held that speciation usually begins with the subdivision of populations by a geographical barrier (allopatric speciation), as the theoretical possibility of the development of reproductive isolation in geographical separation is undebated, and examples are plentiful. Small isolated populations at the margin of the distributional range of a species, i.e. peripheral isolates, are regarded especially prone to speciate. However, re-examination of several cases using phylogenetic comparison methods have led to the acceptance that the evolution of reproductive incompatibility in sympatry, i.e. speciation that does not require extrinsic barriers and geographical subdivision, is not only theoretically possible (e.g. Doebeli & Dieckmann, 2003), but is also suggested from a growing body of field studies; see, e.g., reviews in Via (2001) and Losos & Glor (2003); see also Pigeon, Chouinard & Bernatchez (1997), Schliewen *et al.* (2001) and Wilson, Noack-Kunmann & Meyer (2001) for recent case studies from limnic fishes.

In his theory of ecological speciation Schluter (2000, 2001) emphasized natural selection and suggested that reproductive isolation between populations is achieved as a by-product of adaptation caused by different environmental selection pressures. While evidence and critical tests for this model and distinction from other speciation modes is lacking thus far, the ecological speciation hypothesis makes several predictions in relation to ecologically dependent isolation that can be tested, such as, e.g., the evolution of the same phenotypic characters in closely related but independent lineages under identical extrinsic conditions (e.g. Schluter & Nagel, 1995; Rundle *et al.*, 2000), inverse correlations between morphological differentiation (as an estimator of divergent selection) and gene flow (as an estimator of reproductive isolation) (e.g. Lu & Bernatchez, 1999; Schluter, 2000), or ecological selection against hybrids (e.g. Rundle & Whitlock, 2001).

So-called 'adaptive' radiations provide perfect conditions to study the extrinsic (i.e. geographical, ecological, etc.) factors as well as the intrinsic properties and adaptation of organisms that facilitate speciation, and thus have played a fundamental role in understanding mechanisms of evolution. Our perception of adaptive radiation, however, has long been influenced by Simpson's (1953) 'beguiling metaphor' (Grant, 2001) of being the '*evolutionary process of filling the ecological barrel*'. Today it is understood mainly as the evolutionary divergence of members of a single phylogenetic lin-

eage into different niches or adaptive zones (Mayr, 1963: 633) or into a variety of different forms (e.g. Futuyma, 1998), a concept that has a long history (recently reviewed in, e.g., Schluter & McPhail, 1993; Givnish & Sytsma, 1997; Schluter, 2000; Losos & Miles, 2002). In the context of the ecological theory of adaptive radiation, it has recently been defined as the evolution of ecological (and phenotypical) diversity within a rapidly evolving lineage (Schluter, 1996, 2000, 2001). Losos & Miles (2002) argued that the term should be reserved for those clades that are exceptionally diverse in terms of the range of habitats occupied and attendant morphological adaptations; and West-Eberhard (2003: 564–597) reviewed and discussed adaptive radiation in the context of her theory of developmental plasticity to phenotypic diversification. However, others have stressed that adaptation and radiation are quite independent phenomena that may be coupled, but need not be, and that the operation of natural selection has often only been assumed rather than demonstrated (see Rose & Lauder, 1996 for a recent summary of a new, 'postspandrel' adaptationism; also Pigliucci & Kaplan, 2000; see Vogler & Goldstein, 1997, for a case study).

Nevertheless, well-known cases of adaptive radiations provide ideal settings in which to test for speciation hypotheses; currently, the factors that lead to the formation of species flocks, particularly the role of habitat specialization, are receiving much attention. Spectacular examples of those radiations comprise, for example, the classical cases studied intensively among birds such as the Hawaiian honeycreepers (Perkins, 1901, 1903; Bock, 1970; Fleischer, McIntosh & Tarr, 1998) and the Darwin finches in the Galapagos Archipelago (Lack, 1947; Grant, 1999; Petren, Grant & Grant, 1999; Sato *et al.*, 2001), reptiles like the *Anolis* lizards in the Caribbean islands (e.g. Losos, 1990; Losos *et al.*, 1998) or the *Phelsuma* day-geckos of the Seychelles (Radtkey, 1994), and cyprinid fishes in Lake Lanao (Philippines) and Lake Titicaca in South America (e.g. Kosswig & Villwock, 1965). Gobiidae in the Caribbean recently provided another striking example of a marine adaptive radiation (Rüber, van Tassell & Zardoya, 2003). Particularly well-known are the cichlid fishes in East African lakes with c. 500 species each in Lake Malawi and Lake Victoria, and c. 170 species in Lake Tanganyika (e.g. Fryer & Iles, 1972; Fryer, 1977; Echelle & Kornfield, 1984; Martens, Coulter & Goddeeris, 1994; Rossiter, 1995; Albertson *et al.*, 1999; Nagl *et al.*, 2000; Shaw *et al.*, 2000; Salzburger *et al.*, 2002; Verheyen *et al.*, 2003), as well as tilapia cichlids in crater lakes of Cameroon, which yield evidence for sympatric speciation (Schliewen, Tautz & Pääbo, 1994; Schliewen *et al.*, 2001). These diverging species flocks, rapidly evolving from a single ancestor, have been most instrumental in developing

models for speciation incorporating microgeographical effects and habitat fragmentation (e.g. Mayr, 1942, 1963; Hubendick, 1962; Kosswig & Villwock, 1965; Fryer & Iles, 1972), or nonallopatric causation (e.g. Woltereck, 1931; White, 1978) with reconciling selective forces and the nonadaptive nature contributing to the diversification (e.g. Sturmbauer, 1998; Kornfield & Smith, 2000; Danley & Kocher, 2001).

Less is known about adaptive radiations in invertebrates. Enghoff (1982) reported on a species swarm in the millipede genus *Cylindroiulus* on the Atlantic island of Madeira, and Arnedo, Oromi & Ribera (2001) described radiation of the spider genus *Dysdera* in the Canary Islands. An adaptive radiation is also known from small, flightless weevils in the genus *Miocalles* on Rapa Island in south-east Polynesia (Paulay, 1985), while Hoch & Howarth (1993, 1999) reported on the Hawaiian cave-dwelling planthopper *Oliarus*, and Rivera *et al.* (2002) on Hawaiian cave-adopted isopods, both examples that supplement knowledge about radiations of *Drosophila* fruitflies and *Laupala* crickets on Hawaii (see, e.g. Fleischer *et al.*, 1998). Among molluscs, well-known examples are the radiation of c. 40 species of achatinellid land snails on the Hawaiian archipelago (e.g. Gulick, 1905; Pilsbry & Cooke, 1912–14; Hadfield, 1986; Thacker & Hadfield, 2000) and the partulid land snails on Pacific islands (e.g. Cowie, 1992; Johnson, Murray & Clarke, 1993). In addition, there are endemic radiations described for hydrobiid snails from artesian springs in Australia (Ponder, Hershler & Jenkins, 1989; Ponder & Clark, 1990) and from the south-western USA (e.g. Hershler & Longley, 1986; Hershler & Sada, 1987; Hershler & Landye, 1988; Hershler, 1989).

As is evident from these examples, insular habitats such as oceanic islands or ancient lakes provide ideal natural laboratories to study the evolutionary processes involved in speciation and radiation of species flocks, such as the development of habitat preferences and niche formation, or predator–prey coevolution. In particular, ancient lakes, e.g. Lake Tanganyika or Lake Baikal, are known to be exceptionally rich in species numbers; alongside fish radiations they also harbour several invertebrate species flocks (Brooks, 1950; Boss, 1978; Coulter, 1991; Martens *et al.*, 1994; Fryer, 1996; Martens, 1997; Rossiter & Kawanabe, 2000). These lacustrine radiations were utilized as model systems where the constituent taxa of endemic species flocks show high degrees of inter- and intraspecific morphological differentiation and molecular divergence.

In contrast, many rivers around the world hold species-rich assemblages of molluscs; however, only rarely are there indications for a true radiation to have taken place in riverine, i.e. lotic habitats. Exceptions of streams with large endemic molluscan radia-

tions include the Pleuroceridae in the Tennessee and Coosa-Cahaba rivers of the south-eastern USA (e.g. Goodrich, 1941, 1944), but details of their phylogeny and evolution are unknown (see Lydeard *et al.*, 1997). Davis (1979, 1981) reported on an extraordinary endemic radiation of hydrobioid snails involving 11 genera and 92 species of Triculiniinae in the Mekong River. The only other cases of riverine radiations are also reported for hydrobioid snails, from streams and rivers in Tasmania and Eastern Victoria (Ponder *et al.*, 1993, 1994); however, the exact causes of radiation were unknown.

We here focus on an exceptional riverine radiation that involves endemic species of the gastropod family Pachychilidae which is widespread in South-east Asia (Köhler & Glaubrecht, 2001, 2003; Köhler, 2003). We use these freshwater gastropods of the superfamily Cerithioidea as the focal taxon because they offer many tantalizing insights into evolutionary processes correlated with the conquest of freshwater adaptive zones and the colonization of lacustrine habitats from rivers (Glaubrecht, 1996, 1998, 1999, 2000a, b, 2003b). We anticipate that a riverine case will also shed light on the intrinsic and extrinsic properties involved in radiations and the origin of diversification in general, thus helping to elucidate aspects of the evolution of intralacustrine species flocks, too. Although they make up less than 1% of the world's water, running freshwater, or lotic systems, are more permanent on both ecological and evolutionary timescales than most lake habitats (Giller & Malmqvist, 1998). At the same time, fluvial radiations in snails (see examples above) make an interesting contrast to known lacustrine radiations such as the paludomid gastropods in Lake Tanganyika (Glaubrecht, 1996, 1999; Rossiter & Kawanabe, 2000; Wilson, Glaubrecht & Meyer, 2004) and the pachychilids in the central lakes on the Indonesian island of Sulawesi (Sarasin & Sarasin, 1898; Rintelen & Glaubrecht, 1999; Rintelen & Glaubrecht 2003a, b; Rintelen, 2003; Tv. Rintelen, A. B. Wilson, A. Meyer, M. Glaubrecht, unpubl. data). This is because they allow examination of the decisive role of the environment (riverine vs. lacustrine setting) in speciation modes (allopatric vs. ecological speciation; see, e.g. Schluter, 2000) and in shaping patterns of life history traits (e.g. Stearns, 1992; Roff, 2002).

A group of pachychilid snail species endemic to the Kaek River, a small third-order stream situated in the province Phitsanulok in northern Central Thailand (Fig. 1), has been found to exhibit a remarkable degree of morphological (i.e. conchological) disparity. From a relatively restricted stretch of this river, measuring about 100 km, Brandt (1968, 1974) described a total of ten nominate species-level taxa, using primarily shell features. Two of the first species, *Brotia binodosa* and *Paracrostoma paludiformis*, were originally reported

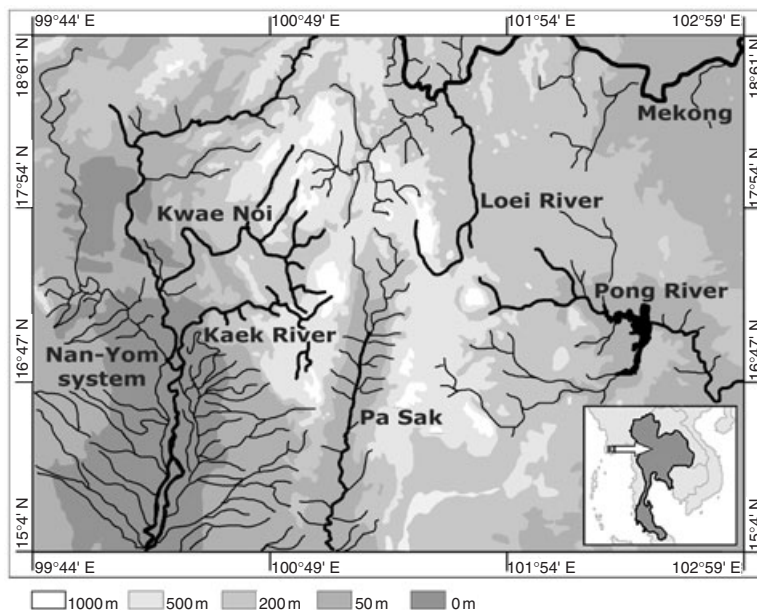


Figure 1. Physical map of Central Thailand. Relevant drainage systems adjacent to the Kaek River are highlighted.

by Solem (1966: 15–20), based on sparse material collected by B. Hansen in 1964 at the Thung Salaeng waterfall of the Kaek River. Later, Brandt (1968, 1974) systematically studied the Thai pachychilids, but failed to recognize that he was dealing with a radiation of closely related taxa. Instead he attributed five species and five subspecies from the Kaek River assemblage to two separate genera, *Brotia* and *Paracrostoma* following Solem's initial concept (see Table 3). Although this fact underscores the high level of distinctiveness with respect to shell characteristics, Brandt's procedure hampered recognition of the existence of a monophyletic riverine species flock in the sense described here for the first time.

In his review on the presumptive adaptive radiation of limnic snails in South-east Asia, Davis (1982: 392) stated correctly that 'when *Brotia* is found in rivers there is usually one species, two at the most. The exception to this is the small radiation in the Koek Noi River (erroneous for Kaek River) (north central Thailand) of the Nan-Chao Praya drainage'. Following Brandt (1974), he mentioned five species, subspecies, or phenotypically different populations along this river as the only known examples of riverine *Brotia*, in addition to the radiation of pachychilids in the lakes on Sulawesi.

In the course of an ongoing revision, integrating morphology and molecular genetics (Köhler & Glaubrecht, 2001, 2002, 2003; Köhler, 2003), the uniqueness of the Kaek River assemblage became evident. The astonishing diversity of snails inhabiting a

relatively small area, in effect a single small river, is indeed unequalled among Pachychilidae and other Cerithioidean gastropods except for *Tylomelania* in the central lakes of Sulawesi and the paludomid species flock in Lake Tanganyika (Glaubrecht, 2003b). The former has only recently been shown to actually represent a case of endemic radiation involving aspects of allopatric as well as ecological speciation (Rintelen & Glaubrecht, 1999; 2003a; Rintelen, 2003; Tv. Rintelen & M. Glaubrecht, unpubl. data).

In order to address the question of riverine radiation, we examined the morphological disparity, the genetic distinctness of the morphotypes and the evolution of pachychilid snails reported from the Kaek River drainage. Our objectives in the present paper are twofold. First, to provide a systematic revision of the constituent pachychilid species of the Kaek River. Based in particular on size, shell sculpture and morphometry, we recognize seven of the ten described taxa as species. Secondly, to determine whether these represent reproductively isolated (biological) species, or if the observed diversity in shell and radula reflects ecophenotypic variation of a smaller number of taxa. Alongside the taxonomical diversity, then, we evaluate the notable morphological disparity and genetic distinctiveness of the Kaek River taxa with the aid of anatomical and molecular genetic methods, documenting shell variability, radula features (as part of possible trophic specialization), the geographical occurrence and ecological aspects of the distinguishable taxa in the Kaek River. In an attempt to compare

this riverine radiation with lacustrine radiations, it is the ultimate goal to provide the data for solving the question of how and where the Kaek taxa have evolved and by which factors their differentiation has been driven.

MATERIAL AND METHODS

ENVIRONMENTAL SETTING

Because this work aims at providing the basis for an understanding of the origin and radiation of riverine species in the Kaek River, discussion of the geology and history of relevant river drainages and basins will play a vital part. Here, some information as to the Kaek River drainage will be given, although it has to be stressed that published geological and hydrological data for this area in Thailand are rare and had to be pieced together from various sources and/or retrieved from topological and other maps, e.g. Nelles map *Thailand* (scale 1:1500 000; no. 06/19; Munich) and *Microsoft Encarta World Atlas for Windows 98*.

The Maenam Kaek (also called Klong Talo at its lower reaches near Phitsanulok; Brandt, 1968) is a tributary of the Nan River in Central Thailand running further south into the Chao Praya, which is the central drainage of Thailand, with its larger part being a large slow river flowing over a broad floodplain (Fig. 1). The Kaek River has cut into its bedrock and transects several small mountain chains composed of Jurassic sandstone and Permian limestone hills, slate and hardpan (source: National Park Division, Bangkok; see also Hutchison, 1989). It lies at 300–1028 m a.s.l., partly in a steep-sloped canyon, and flows in an east–west direction from the watershed west of Phetchabun to Phitsanulok. Parts of the Kaek River are situated within the Thung Salaeng Luang National Park.

Some *Brotia* species dealt with herein also occur in the headwater region of the Kwae Noi River that drains, north of the Kaek River, into the Nan River. To the east, the drainages of these two rivers are bordered by the southern extensions of the Luang Prabang Range, with mountainous ridges reaching (from south to north) elevations of 1035 m, 1356 m (at and near the Khao Kho), 1746 m (Phu Hin Ronkla), and 14 368 m (Phu Khat). One species relevant in the context of this paper (i.e. *Brotia solemiana*) is found at a single location outside this drainage, on the Loei River, a tributary east of the Phang Hoi Range that runs into the Mekong near the Thai–Laos border (Fig. 1).

While the latter belongs to a different drainage system (the Maenam Klong and Mekong), the Kaek and Kwae Noi Rivers are part of the Chao Praya biogeographical region established for freshwater fishes

(Yap, 2002) (for details of zoogeography see Rainboth, 1996).

The Chao Praya basin can be roughly divided into two parts. The Lower Central Plain, which is flat and low-lying, extending north as far as the province of Ang Thong (c. 15°N), represents an area of Quaternary deposits. This area was a huge bay of the South China Sea up to about 9000–10 000 y BP, when sea level stands were approximately 4 m higher than at present. In contrast, the Upper Central Plain extends north up the Chao Praya to the lower parts of the valleys of the Nan and Ping River, still in the south of the Kaek River area. This plain lies at >20 m a.s.l. and has never been subject to significant tidal flooding (Sinsakul, 1997). The upper reaches of the watershed lie at approximately 19°N, in the province of Mae Hong Son, Chiang Rai and Chiang Mai.

Limnological data on rivers and streams in tropical Asia are scarce (Dudgeon, 1995). As predicted by the river continuum concept (see, e.g. Vannote *et al.*, 1980) streams and their organismic composition and diversity are characterized by a flowing continuum, with distinct reaches not being delimited by fixed borders. However, in terms of the broadly used geomorphic or physiographical stream classification (e.g. Allan, 1995; Hauer & Lamberti, 1996; Giller & Malmqvist, 1998), we interpret the Kaek River here as a medium to large river of third order (with the Chao Praya and Nan River being mainstream rivers). According to the more useful biotic river classification scheme developed by Illies (1961) and Illies & Botosaneanu (1963), we classify the Kaek River herein to be a rhithral or middle stream section with its organismic components representing the rhithron. The rhithral is typically characterized by rather cool temperatures, high to moderate dissolved oxygen concentrations (often variable at least seasonally), with water ranging from clear to turbid and oligotrophic to mesotrophic, rather variable medium (semistable) substrates and stronger currents with a comparatively high gradient.

The water of the Kaek River is clear, relatively cold, and fast running. Mainly in its midstream the river flows swiftly over a rocky bottom with a series of several rapids and small waterfalls on its course westwards (see Fig. 2). In its upstream as well as in its downstream part, however, incline and currents are moderate. In addition, large patches of sandy or gravel bottoms can be found between the rocks in the lower course of the river, with reduced flow regimes.

Pine and bamboo forest as well as mixed species deciduous forest predominate in the area surrounding the river with grassland, lowland scrub and tropical broadleaved evergreen forest covering smaller areas. Human impact is rather limited (mostly in bathing areas, near settlements), but increases in the downstream region towards Phitsanulok.

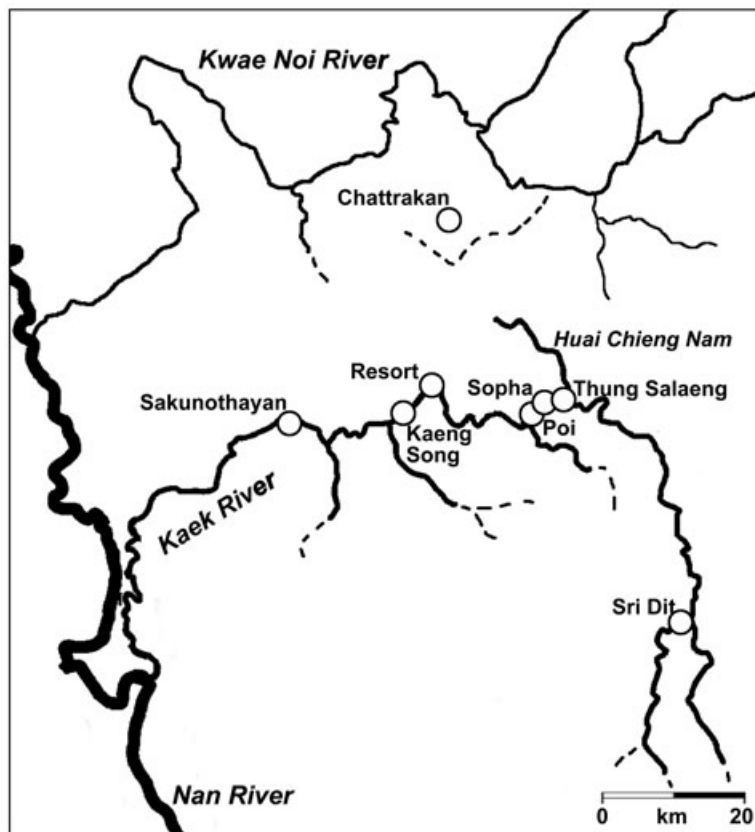


Figure 2. Physical map of the region east of Phitsanulok showing the Kaek and Kwaë Noi Rivers and their tributaries with the collection localities; for coordinates see Table 1.

MATERIAL EXAMINED AND SPECIES ASSIGNMENTS

All historical samples in museum collections have been collected either by Hansen (see in Solem, 1966) or Brandt (1968, 1974) (see Appendix for list of repositories and abbreviations used herein). Recent series of relevant taxa were collected by the second author in November 2001. Apart from the examination of type material from the various museum collections, this account is essentially based on the latter series of shells and soft bodies fixed in ethanol, of which voucher material as specified in the systematic part is deposited in the Malacozoological Collection of the Natural History Museum in Berlin (ZMB) under the respective catalogue numbers. All known collecting localities are shown in Figure 2, with details given in Table 1; see also the compilation of voucher numbers and GenBank accession numbers given in Table 2. In a few cases additional juvenile shells and radulae were extracted from dried shells, including syntype material deposited by Brandt (see details under the species). Samples were examined and assigned a priori, based on conchology, to one of the reported species as described by Solem (1966) and Brandt (1968, 1974).

In the course of the study, based on the results reported herein, the number of recognized taxa was condensed accordingly (see Table 3, and Discussion).

EXAMINATION OF THE MORPHOLOGY

Dimensions of adult and juvenile shells were measured using callipers to 0.1 mm using standard parameters (as described, e.g., in Glaubrecht, 1996; see Appendix for abbreviations of these parameters) and analysed using the SPSS software package for Windows (v. 11.0 for Windows; SPSS Inc., Chicago, 2001). Anatomy was studied using Leica stereo microscope. Extracted radulae were cleaned as described by Holznagel (1998) and mounted on stubs for SEM examination with a Jeol FSM 6300 microscope. See the Appendix for shell parameters

In viviparous freshwater Cerithioidea a distinct and abrupt sculptural transition from the larval or primary shell (= protoconch) to the adult or secondary shell (= teleoconch) is lacking, due to the abbreviation or loss of early ontogenetic stages in viviparous species, and therefore the term 'protoconch' applied to

Table 1. Locations of the collecting sites (see Fig. 2)

Collecting locality	Road km east of Phitsanulok	Geographical coordinates
Kaek River:		
Sakunothayan Falls	33	16°50.3'N, 100°31.94'E
Kaeng Song Rapids Resort	45	16°51.96'N, 100°37.83'E
Poi Falls	53	16°53'N, 100°39'E
Sopha Falls	60	16°50.75'N, 100°45.06'E
Thung Salaeng Luang NP	71	16°51.3'N, 100°46.9'E
Sri Dit Falls	80	16°52'N, 100°48'E
Huai Chieng Nam	–	16°37.63'N, 100°56.72'E
Kwae Noi River: Chattrakan near Nakhon Thai	91	not exactly known
Loei River: Tat Kok Falls near Wang Saphung	–	17°17.56'N, 100°40.81'E
		17°3.46'N, 101°31.63'E

Table 2. Origin of *Brotia* species samples used in this study, with voucher material number of the ZMB and GenBank accession numbers of sequenced specimens

Species	Inventory No.	Locality	COI	16S
<i>B. pagodula</i>	ZMB 200.208	Prov. Kamphaeng Phet, Moei River	AY 172453	AY 172443
<i>B. armata</i>	ZMB 200.193	Kaeng Song Rapids	AY 330853	AY 330810
	ZMB 200.252	Thung Salaeng Luang NP	AY 330854	AY 330809
	ZMB 200.254	Resort	AY 330834	AY 330808
	ZMB 200.265	Sakunothayan Falls	AY 330855	AY 330806
	ZMB 200.268–1	Poi Falls	AY 330837	AY 330807
	ZMB 200.268–2	Poi Falls	AY 330856	AY 330811
<i>B. binodosa</i>	ZMB 200.192	Thung Salaeng Luang NP	AY 330857	AY 330815
	ZMB 200.202	Chattrakan NP	AY 330859	AY 330819
	ZMB 200.267	Resort	AY 330860	AY 330818
	ZMB 200.269	Poi Falls	AY 330861	AY 330820
	ZMB 200.328	Kaeng Song Rapids	AY 330858	AY 330816
<i>B. microsculpta</i>	ZMB 200.191	Thung Salaeng Luang NP	AY 330836	AY 330805
	ZMB 200.200	Poi Falls	AY 330833	AY 330804
	ZMB 200.266	Resort	AY 330835	AY 330803
<i>B. pseudosulcospira</i>	ZMB 200.196	Sakunothayan Falls	AY 330862	AY 330797
<i>B. solemiana</i>	ZMB 200.174	Loei River: Tat Kok Fall	AY 330849	AY 330814
	ZMB 200.203	Sri Dit Falls	AY 330848	AY 330812

Table 3. Comparison of the Kaek River taxa recognized by Brandt (1974) and in the present study, as detailed in the species description of the systematic chapter

Taxa recognized by Brandt (1974)	Taxa recognized herein
<i>Brotia binodosa binodosa</i>	<i>Brotia binodosa</i>
<i>Brotia binodosa spiralis</i>	<i>Brotia binodosa</i>
<i>Brotia binodosa subgloriosa</i>	<i>Brotia subgloriosa</i>
<i>Brotia microsculpta</i>	<i>Brotia microsculpta</i>
<i>Paracrostoma morrisoni</i>	<i>Brotia armata</i>
<i>Paracrostoma paludiformis paludiformis</i>	<i>Brotia paludiformis</i>
<i>Paracrostoma paludiformis dubiosa</i>	<i>Brotia armata</i>
<i>Paracrostoma pseudosulcospira armata</i>	<i>Brotia armata</i>
<i>Paracrostoma pseudosulcospira pseudosulcospira</i>	<i>Brotia pseudosulcospira</i>
<i>Paracrostoma solemiana</i>	<i>Brotia solemiana</i>

oviparous forms is not comparable to the conditions found here. Consequently, we apply the more general term 'juvenile shell' for all shelled stages found in the brood pouch. Measurements of the juvenile shell follow the methods and terminology described in Glaubrecht (1996: 28, 298).

MOLECULAR GENETICS

We sequenced a total of 18 samples, including one out-group representative, namely *Brotia pagodula* Gould, 1847, the type species of the genus *Brotia* from the Moei River at Tak (Thai–Myanmar border), a tributary of the Irawaddy River.

DNA was purified from about 1–2 mm³ of foot tissue from specimens preserved in ethanol by CTAB extraction (Winnepenninckx, Bäckeljau & De Wachter, 1993). Polymerase chain reaction (PCR) was used to amplify two mitochondrial gene fragments, 646 bp of the cytochrome *c* oxidase subunit I gene (COI) and 858 bp of the 16S ribosomal gene. PCR was performed in 25 µL aliquots containing 1 × Taq buffer, 1.5 mM MgCl₂, 200 µM each dNTP, 1–2.5 U Taq polymerase, c. 100 nM DNA and ddH₂O up to volume on a Perkin Elmer GeneAmp 9600 or 2400 thermocycler. After an initial denaturation step of 3 min at 94 °C, cycling conditions were 35 cycles of 1 min each at 94 °C, 45–53 °C, and 72 °C, with a final elongation step of 5 min. Primers used were LCO 1490 5' 1490GCTCAACAAAT CATAAAGATATT-3' and HCO2198 var. 5'-TAWACT TCTGGGTGKCCAAARAAAT-3' (Folmer *et al.*, 1994; modification of HCO2198 by A.B. Wilson) for COI, and 16SF 5'-CCGCACTTAGTGATAGCTAGTTTC-3' (A.B. Wilson) and H3059-Inv 5'-CCGGTYTGAAGTCAAGAT CATGT-3' (Palumbi *et al.*, 1991) for 16S, respectively. PCR products were purified using QiaQuick PCR purification kits (Qiagen) following the standard QiaQuick PCR purification protocol. Both strands of the two genes were cycle sequenced with the original primers using ABI Prism BigDye terminator chemistry and visualized on an ABI Prism 377 automated DNA sequencer. The resulting sequence electropherograms of both strands were corrected manually for misreads and merged into one sequence file using BIOEDIT v.5.0.1 (Hall, 1999). The sequences are accessible via GenBank (accession numbers see Table 2).

SEQUENCE ANALYSIS

COI sequences were aligned manually and checked by translating the DNA sequences into amino acids using DAMBE v.4.0.75 (Xia & Xie, 2001) based on the genetic code for invertebrate mitochondrial DNA. The ribosomal DNA sequences were aligned with CLUSTAL X v.1.8.1 for Windows (Thompson *et al.*, 1997) using default settings. A combined data set was built by

concatenating the sequences. Pairwise genetic distances were calculated using PAUP* (Swofford, 1999).

Phylogenetic trees were reconstructed using Neighbour Joining (NJ) (Saitou & Nei, 1987), Maximum Parsimony (MP) and Maximum Likelihood as implemented in PAUP*. In addition, a Bayesian method of inference (BI) was employed to estimate phylogenetic relationships (see, e.g. Huelsenbeck *et al.*, 2002; Holder & Lewis, 2003) using MRBAYES v.3.0 (Huelsenbeck & Ronquist, 2001).

NJ analyses were conducted using the random initial seed option to break ties and under a general time reversible model of sequence evolution (GTR; Rodriguez *et al.*, 1990) to correct for multiple substitutions. In the MP analyses, the heuristic search algorithm was employed with ten random additions of taxa and tree bisection–reconstruction (TBR) branch swapping. Gaps were treated as a fifth base. Other settings were left on default.

Prior to ML and BI analyses, we determined which substitution model best fitted the sequence data set by running a hierarchical likelihood ratio test implemented in MRMODELTEST (Nylander, 2002). Quartet puzzling was run in PAUP* with substitution parameters adjusted according to the results of MRMODELTEST and under application of 100 000 puzzling steps. For BI analysis a Metropolis-coupled Markov chain Monte Carlo (four chains, chain temperature = 0.2) was run for 750 000 generations. A 50% majority-rule consensus tree was constructed for the last 2500 trees in order to assess the posterior clade probabilities for each node (bpp).

RESULTS I: SYSTEMATIC REVISION

All pachychilid species endemic to the Kaek River are herein assigned to the genus *Brotia*. This systematic decision is based on a number of morphological characters. In addition, phylogenetic analyses of sequence data as shown below support our conclusion (see Köhler, 2003) that those taxa Brandt (1968; 1974) attributed to *Paracrostoma* Cossman 1900 together with species attributed to *Brotia* form a monophyletic group. All *Brotia* species share an identical morphology of the reproductive system (pallial oviduct, gonads, brood pouch, gonochory) and of juvenile shells. These features, among them most conspicuously some characteristics of the protoconch and the pallial oviduct, have been shown to represent synapomorphies of *Brotia* which has its distribution in mainland Southeast Asia including Sundaland, but not in the Philippines and Sulawesi (cf. Köhler & Glaubrecht, 2001, 2003). In contrast, according to recent investigations *Paracrostoma* is restricted to Southern India, and it exhibits a distinct juvenile shell and pallial oviduct

morphology (Köhler, 2003). A revision of *Paracrostoma* will be published elsewhere.

BROTIA H. ADAMS, 1866

Brotia H. Adams, 1866: 150 (type species: *Melania pagodula* Gould, 1847, by monotypy).

Antimelania Fischer & Crosse, 1892: 313 (type species: *Melania variabilis* Benson, 1836, by subsequent designation in Pilsbry & Bequaert, 1927: 300).

Wanga Chen, 1943: 20–21 (type species: *Melania henriettae* Gray 1834, by original designation).

Type species: Melania pagodula Gould, 1847.

Nomenclature and systematics: In contrast to the long-held assumption by numerous former authors, this genus is a member of the family Pachychilidae Troschel, 1857, but not of the Thiariidae. For details see Glaubrecht (1996, 1999) and Lydeard *et al.* (2002). Köhler & Glaubrecht (2001, 2002) have outlined its taxonomic history in detail.

Solem (1966) re-iterated assumptions of, e.g. Rensch (1934) and Benthem Jutting, 1956) that *Brotia* is parthenogenetic. When Solem (1966) described a new species from Thailand, *Paracrostoma paludiformis*, he noticed the close similarity to *Brotia*. However, because he identified males and detected some conchological similarities with *Paracrostoma huegeli* (Philippi, 1843) from India, he suggested that *P. paludiformis* is not treated as a member of the (presumptively parthenogenetic) genus *Brotia*, but instead of *Paracrostoma* Cossmann, 1900. Brandt (1968, 1974) followed this view; when describing new species from Thailand, those cases he was able to identify as males were assigned to *Paracrostoma* and all other samples and taxa were assigned to *Brotia*. Furthermore, he stated that *Paracrostoma* could be discriminated by a thicker and more conical shell and an operculum comprising up to 3.5 whorls, whereas *Brotia* species were more elongate, with a thinner shell and an operculum comprising 4–6 whorls. To complicate matters, *Paracrostoma* was first treated by Brandt (1968) as a subgenus of *Brotia*, but later he considered *Paracrostoma* as a genus independent from *Brotia* (cf. Brandt, 1974).

The properties of *Brotia* have been extensively described by Köhler & Glaubrecht (2001). On the basis of this description several features emerged that are peculiar to a group of species comprising also *Brotia pagodula*, type species of the genus. This group has been preliminarily designated the '*Brotia-pagodula* group' in Köhler & Glaubrecht (2001) and is considered to represent the genus *Brotia s.s.* Accordingly, in the following, several features characteristic to *Brotia* are described. One significant finding is that *Brotia* species are generally gonochoristic, which conflicts

with the statements of Solem (1966) and Brandt (1968, 1974). Solem (1966) was the first reporting on the peculiar ontogenetic development including a so-called 'soft apex' in *B. binodosa*, which is now considered a synapomorphy of the genus *Brotia* (see Köhler & Glaubrecht, 2001; Köhler, 2003).

Diagnostic characteristics: SHELL: Globular or broadly to elongately conical with a more or less turreted spire and a generally eroded apex; moderately thick. Sculpture variable, axial ribs, sometimes even with nodules, and spiral ridges at the base are frequent and characteristic elements. Body whorl comparatively large, aperture oval, well rounded or angled below and pointed above. JUVENILE SHELL: Juveniles kept in the brood pouch attain a height of up to 4 mm comprising up to four whorls. The apical whorl exhibits a distinct sculpture and shape. The apex of juvenile shells is irregularly wrinkled. This initial shell with its wrinkled texture is clearly delimited from subsequent whorls exhibiting a more or less smooth sculpture with only regular growth lines and spiral elements. In earlier ontogenetic stages the apex of the juvenile shell is not calcified and soft tissue protrudes from the apex of the shell. This tissue originates from the egg capsule nourishing the embryo. It was named 'yolk sac' by Solem (1966) and is attached to the tip of the juvenile shell. OPERCULUM: Either round with up to eight whorls and a central nucleus, or oval for the last whorls increasing more rapidly in diameter, then comprising up to six whorls. RADULA: Taenioglossate; ribbon is relatively long and robust comprising up to 30 mm in length which corresponds to about half of the shell height. Posteriorly, the radula is embedded in connective tissue ('radular sac') and coiled behind the buccal mass in the radular sac. In general, the rachidian tooth is squarish with a pronounced, more or less pointed central denticle flanked by up to three accessory denticles that taper in size. A glabella always is present. The anterior margin of the rachidian is concave or straight, the lower rim concave by the posteriorly extending glabella. The lateral teeth have a rounded swelling on their face (glabella) as well and the major denticle is flanked by two or three smaller cusps on each side. The inner marginal teeth are hooked and generally have two denticles. Both marginals possess a simple flange or ledge at their outer margin. This feature is more pronounced in the outer marginal teeth.

BROTIA ARMATA (BRANDT, 1968)

Brotia (Paracrostoma) pseudosulcospira armata Brandt, 1968: 275, pl. 10, fig. 62 ('Maenam Kaek in Phitsanulok Prov. at Gaeng Song rapids, 45 km E Phitsanulok' = Thailand, Prov. Phitsanulok, Kaek River at Kaeng Song rapids, c. 60 km east of Phitsanulok).

Paracrostoma pseudosulcospira armata – Brandt, 1974: 186, pl. 13, fig. 43; Köhler & Glaubrecht, 2002: 144.

Paracrostoma morrisoni Brandt, 1974: 188–189, pl. 14, fig. 47 ('Maenam Kaek at Sopa Falls, 71 km E of Phitsanulok' = Thailand, Prov. Phitsanulok, Kaek River at Sopa Falls, 71 km east of Phitsanulok); Köhler & Glaubrecht, 2002: 141–142.

Paracrostoma paludiformis dubiosa – Brandt, 1974: 188, pl. 14, fig. 46 ('Kaek River, 80 km E Phitsanulok' = Thailand, Prov. Phitsanulok, Kaek River, 80 km east of Phitsanulok); Köhler & Glaubrecht, 2002: 142.

Type material examined: Thailand: Prov. Phitsanulok: Holotype of *P. pseudosulcospira armata* SMF 197380 (Kaek River, Kaeng Song, 60 km east of Phitsanulok); 35 paratypes ZMH. Holotype of *P. morrisoni* SMF 215966 (Kaek River, Sopa Falls, 71 km east of Phitsanulok); six paratypes SMF 215967; five paratypes RMNH 55283/5 (Kaek River, Kaeng Song, 60 km east of Phitsanulok); 12 paratypes SMF 271191; 38 paratypes SMF 193587; 11 paratypes BMNH 1976119; 14 paratypes RMNH 55135/14. Holotype of *P. paludiformis dubiosa* SMF 215964 (Kaek River, 80 km east of Phitsanulok); six paratypes SMF 215964; five paratypes RMNH 55284/5.

Other material examined: Thailand: Provinz Phitsanulok: Kaek River: Sakunothayan Falls, 33 km east of Phitsanulok (ZMB 200.265; ZMH); Kaeng Song rapids, 45 km east of Phitsanulok (SMF 193587; ZMB 200.193); resort, 53 km east of Phitsanulok (ZMB 200.254); Poi Falls, 60 km east of Phitsanulok, 16°50.75'N, 100°45.06'E (ZMB 200.268); Thung Salaeng Luang National Park, 90 km east of Phitsanulok, 16°52'N, 100°38'E (USNM 794081; ZMB 200.252, 200.265).

Nomenclature: *Brotia armata* has been described as a subspecies of *P. pseudosulcospira* by Brandt (1968, 1974), distinguished from the (typical) nominate subspecies by its marked shell sculpture. Examination of all material available shows that both taxa, *armata* and *pseudosulcospira*, occur in sympatry at the Sakunothayan Falls. Consequently, both forms do not

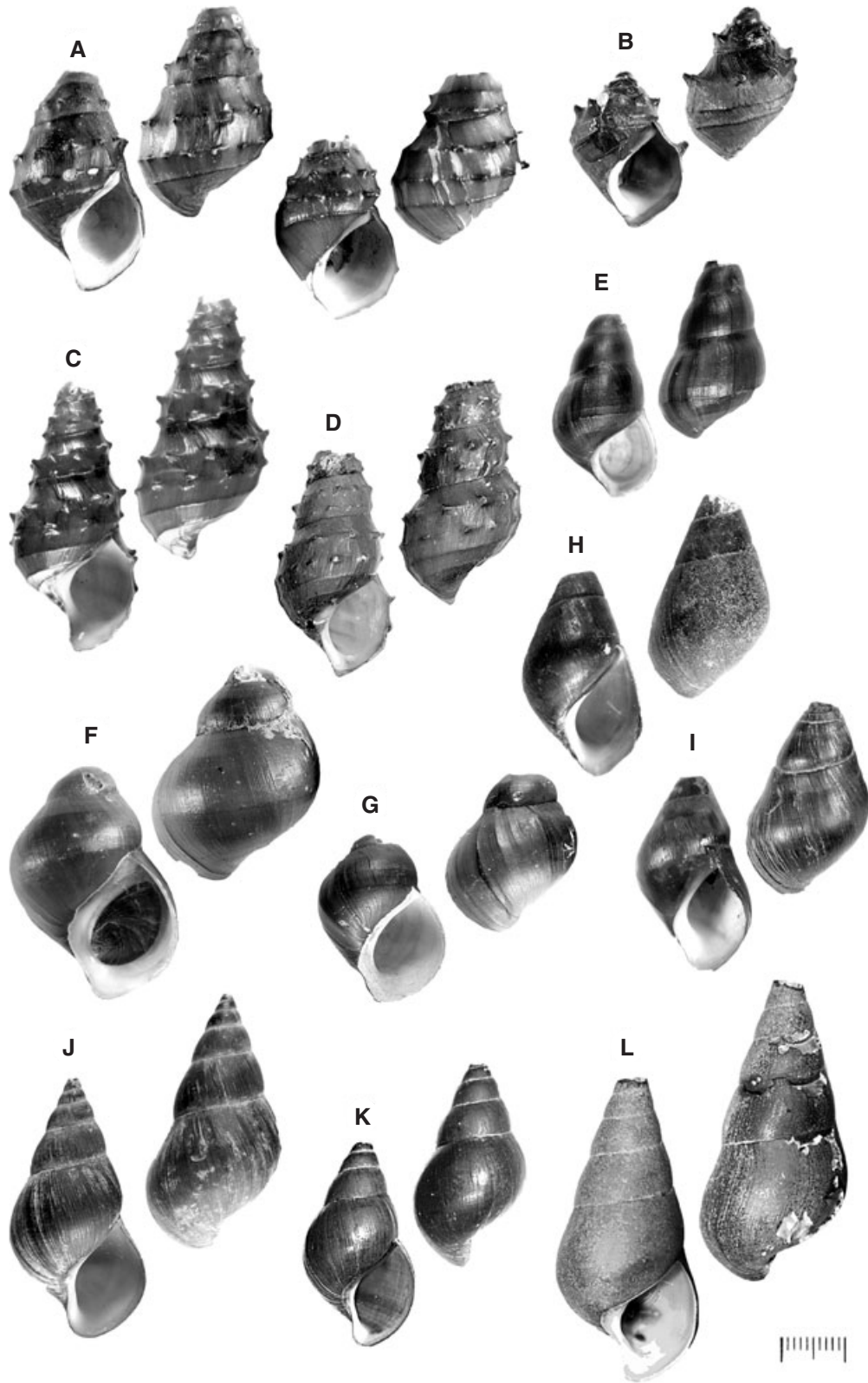
represent geographical representatives (i.e. subspecies) of the same species. Instead, *B. armata* is considered here as a species on its own, which can be distinguished from *B. pseudosulcospira* by shell features.

Brandt (1974) also described two other taxa from the Kaek River, *P. morrisoni* and *P. paludiformis dubiosa*, with an identical shell morphology. The type specimens of both taxa represent the only known material, although in the Kaek River extensive collections have been made by Brandt & Köhler. Brandt (1974) stated that *P. morrisoni* can be distinguished from *P. paludiformis dubiosa* in exhibiting two instead of only one spiral cord. This feature, however, is not considered suitable for the discrimination of the two taxa, as among the *armata* populations studied both forms are found. In addition, the type specimens of both taxa cannot be discriminated from shells of *B. armata* by analysis of morphometrical parameters (see below; Fig. 5). Consequently, *P. morrisoni* and *P. paludiformis dubiosa* are treated as synonyms of *B. armata*.

Diagnostic characteristics: Shell relatively small, conical to oval, with up to three rather flattened whorls; sculpture consists of one or three spiral cords that support a spiral row of spiny nodules.

Description: SHELL (Fig. 3A–B): Relatively small, oval to conical, spire generally eroded with up to three flattened to slightly convex whorls. Sculpture consists of one to three spiral cords, especially the upper ones supporting spiral rows of spiny nodules. On the body whorl often an additional cord is visible. Some specimens, however, are almost smooth. Aperture broadly ovate, large compared to the shell, basal margin produced. Size: H = 26–38 mm, B = 18–24 mm. JUVENILE SHELL (Fig. 4A–D): Shell smooth except for axial growth lines, apical whorl irregularly folded with a sharp transition after about half of the first whorl. Juveniles kept in the brood pouch attain a size of about 2.0–2.5 mm comprising 2.5 whorls. OPERCULUM: Oval, with up to four whorls increasing in diameter, and a subcentral nucleus. RADULA (Fig. 7A): Length of ribbon: M = 18.4 mm (SD = 4.4 mm; N = 15) with up to 180 rows of teeth. Central tooth with elongated

Figure 3. Shells of *Brotia* species endemic to the Kaek River. A, Two paratypes of *Brotia* '*pseudosulcospira armata*' (Brandt, 1968) (Kaek River, Kaeng Song; SMF 193587). B, *Brotia armata* (Kaek River, Poi Fall; ZMB 200.254). C, *Brotia binodosa* (Kaek River, Sopa Falls; ZSM 19983219). D, *Brotia binodosa* (Kaek River, Thung Salaeng Luang NP; ZMB 200.192). E, Holotype of *B. microsculpta* (Kaek River, Thung Salaeng Luang NP; SMF 197378). F, *B. paludiformis* (Kaek River, Sopa Falls; ZMH). G, *B. paludiformis* (Kaek River, Sopa Falls; SMF 215963). H, Paratype of *B. 'pseudosulcospira pseudosulcospira'* (Brandt, 1968) (Kaek River; SMF 193586). I, *B. pseudosulcospira* (Kaek River, Sakunothayan Falls; ZMB 200.299). J, Paratype of *Paracrostoma soleimiana* (Loei Province, Pong River; SMF 193585). K, *Brotia soleimiana* (Kaek River, Sri Dit Falls; ZMB 200.203). L, Paratype of *B. 'binodosa subgloriosa'* (Brandt, 1968) (Huai Chieng Nam, tributary of the Kaek River; ZSM 19983213). Scale bar = 10 mm.



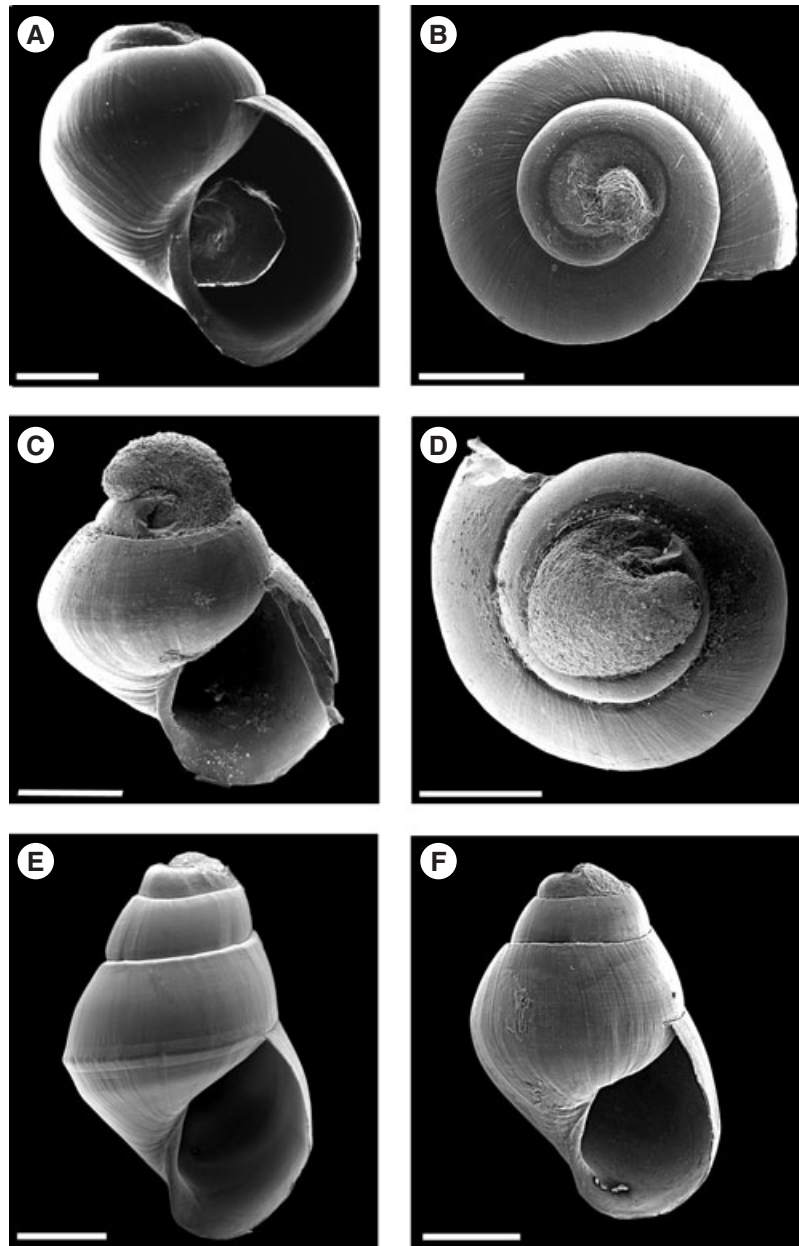


Figure 4. Juvenile shell morphology of Kaek River species. A–D, SEM images of juvenile shells removed from the brood pouch of *B. armata*. A, Front view (paratype BMNH 1976111). B, Apical view (paratype BMNH 1976111). C, Front view; note nutritional tissue at the apex (ZMB 200.252; Thung Salaeng Luang NP). D, Detail of the apex (ZMB 200.252; Thung Salaeng Luang NP). E, *B. binodosa*, front view (ZSM 19983219, Sopha Falls). F, *B. subgloriosa*, front view (paratype ZSM 19983213, Huai Chieng Nam). Scale bars = 500 μ m.

main cusp and two or three much smaller accessory cusps on each side that taper in size; glabella narrow with straight lateral margins and a rounded posterior rim that does not reach the basal rim of the central tooth. Laterals with a broad main cusp flanked by one to two accessory cusps on each side. Inner and outer marginals each with an outer, large and broad main denticle and an inner spiny accessory denticle.

Distribution: Thailand: Endemic to the Kaek River; known from the middle course between the Sakunothayan Falls (33 km east of Phitsanulok) and the Thung Salaeng Luang NP (90 km east of Phitsanulok); see Table 4.

Ecology: Observations by MG and FK (authors of this paper) showed that *B. armata* is a hard-substrate

Table 4. Known presence of *Brotia* species at the collecting sites along the Kaek River; cf. Figure 1. The species are sorted by their substrate preference. Note that the habitat of *B. subgloriosa* is somewhat doubtful (see Systematic Revision, above)

	Sakunothayan	Kaeng Song	Resort	Poi Falls	Sopha Falls	Thung Salaeng Luang	Huai Chieng Nam	Sri Dit Falls
Road km east of Phitsanulok	33	45	53	60	71	80	91	–
On rock								
<i>B. armata</i>	+	+	+	+	+	+		
<i>B. binodosa</i>		+	+	+	+	+		
<i>B. paludiformis</i>					+			
<i>B. pseudosulcospira</i>	+							
On gravel/boulders								
<i>B. solemiana</i>								+
On sand								
<i>B. microsculpta</i>			+	+	+	+	+	
<i>B. subgloriosa</i>					+		+	

dweller; specimens were found attached exclusively to boulders or rocks in waters with swift current in a section between 5 cm above and 20 cm below water surface.

Synonymous taxa: Neither the types of *P. morrisoni* nor those of *P. paludiformis dubiosa* can be discriminated with certainty from other shells of *B. armata* by means of their morphology. In addition, statistical analyses of shell parameters do not yield support for the distinction of any of the aforementioned taxa as is shown, for example, in a comparison of shell shape (H/B index) and aperture form (Fig. 5). The minor discrepancies in the shell sculpture of the type specimens of *P. morrisoni* and *P. paludiformis dubiosa*, consequently, are believed to represent intraspecific variability within *B. armata*.

Similar species: A sculpture similar to *B. armata* is exhibited by *B. binodosa*, which is generally more

turreted and slender, though, and possesses more whorls. Both species can be discriminated in particular by the shell parameter index H/B (see Fig. 6). Another species, *B. pseudosulcospira* (see below), lacks spines, its shell is often even, smooth and much thicker, with darker colour, and it attains a larger size.

BROTIA BINODOSA (BLANFORD, 1903)

Melania binodosa Blanford, 1903: 282–283, pl. 8, fig. 2 ('Siam, in fluminibus majoribus' = in large rivers, Thailand; restricted to Sopha Falls, at the Kaek River near Phitsanulok by Brandt, 1974: 175).

Brotia binodosa – Solem, 1966: 15, fig. 1(a),(b).

Brotia (Brotia) binodosa binodosa – Brandt, 1974: 174–175, pl. 12, fig. 26.

Brotia spinata – Köhler & Glaubrecht, 2002: 148 (partim).

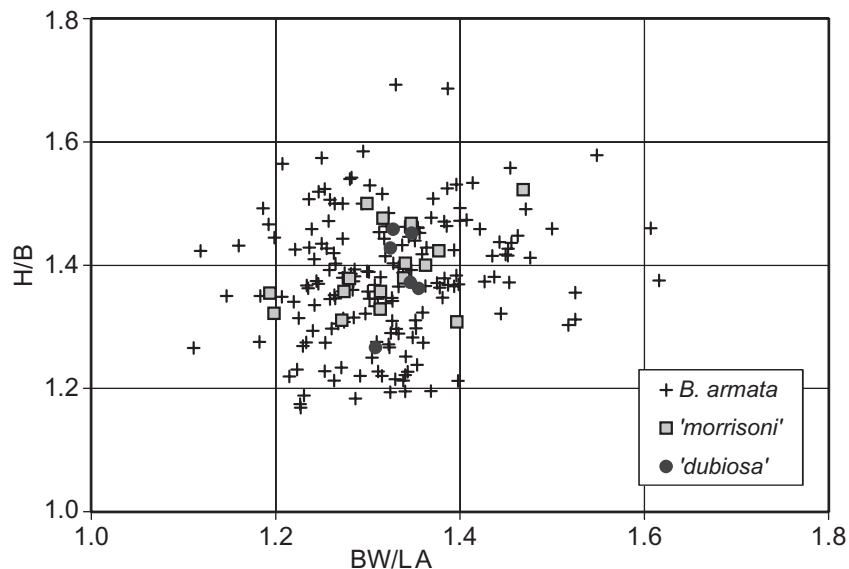


Figure 5. Morphometric comparison of the type specimens of '*Paracrostoma morrisoni*' and '*Paracrostoma paludiformis dubiosa*' with shells of *Brotia armata* using parameters H/B and BW/LA.

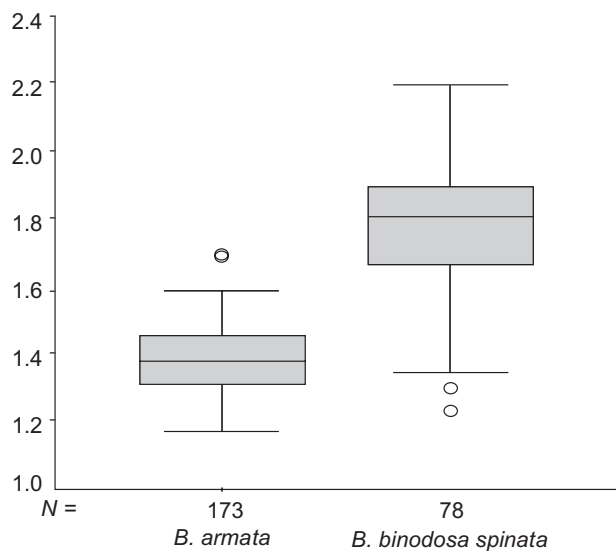


Figure 6. Comparison of *B. armata* and *B. binodosa* by means of the shell parameter H/B. Box plot diagram showing the median, the 25%- and 75%-percentile and largest nonextremes (less than 1.5 times of box height).

Brotia (Brotia) binodosa spiralis Brandt, 1974: 176, pl. 12, fig. 27 ('Thailand: Kaek River, 38,5 km E Phitsanulok' = Thailand, Prov. Phitsanulok, Kaek River 38.5 km east of Phitsanulok).

Brotia spinata spiralis – Köhler & Glaubrecht, 2002: 130.

Type material examined: Thailand: Holotype of *M. binodosa* BMNH 1903.2.28.2 ('Siam, leg. Blanford')

and paratype BMNH 1903.2.28.3. Holotype of *B. binodosa spiralis* SMF 220340 ('Kaek River, 38,5 km E Phitsanulok').

Other material examined: Thailand: Prov. Phitsanulok, Chattrakhan Fall, Kwae Noi river in the Chattrakhan NP, north of Nakhon Thai (ZMB 200.202); Kaek River (SMF 193577; RMNH 55288); Kaeng Song Falls (SMF 193874); resort, 53 km east of Phitsanulok (ZMB 200.267); Poi Falls (ZMB 200.269; SMF 205137); Sopha Falls (ZSM 19983214, 6, 8; RMNH 55288/6; SMF 193575, 220339; AMS 146761); Thung Salaeng Luang NP (ZMB 200.192; ZSM 19983217; SMF 193578; BMNH; AMS 146760); Tap Tami Falls (ZSM 19983215; SMF 193576; ZHM).

Nomenclature: The shell of *B. binodosa* is very similar to that of '*Melania*' (= *Brotia*) *spinata* Godwin-Austen, 1872 from the Kopili River (Jaintia-Khâsi hills north of Silchar; Meghalaya, India). Blanford (1903) also noticed this striking similarity but stated that *M. binodosa* is more slender and the columella more curved. It has been suggested by Köhler & Glaubrecht (2002) that both taxa might be conspecific. However, in the absence of any *spinata* material from northern India we have no evidence to base a formal taxonomic decision on and therefore here prefer to keep the name employed by Brandt (1968, 1974) for the Kaek River populations of this species, which is *B. binodosa*.

Blanford (1903) gave the location for a small collection of this species collected by W. Mahon Daly only unprecisely as 'common in large rivers', although stating in his introduction that Daly's material mainly originated from Phitsanulok (see also Brandt, 1974:

2); accordingly we follow Brandt (1974) in restricting the type locality. Brandt (1968, 1974) described two subspecies, namely *B. binodosa subgloriosa* and *B. binodosa spiralis*, both from the Kaek River. While *B. subgloriosa* is regarded herein as separate species closely related to *B. binodosa*, we consider *B. binodosa spiralis* as a synonym of *B. binodosa*; instead of representing a vicariant subspecies it is merely a somewhat aberrant form with less developed sculpture.

Diagnostic characteristics: Highly turreted shell with two spiral rows of spiny nodules supported by more or less prominent spiral cords. Aperture angled and produced below.

Description: SHELL (Fig. 3C–D): Medium-sized, with an elongately turreted spire with three to four whorls and an eroded tip. Whorls convex with a subsutural depression, separated by a narrow and inconspicuous suture. Sculpture consisting of more or less developed spiral ridges that are most prominent at the base, and two spiral rows of pointed nodules or tiny spines, each supported by a spiral cord. Shell thin but solid, colour brown to red-brown with a glossy surface. Basal whorl relatively large. Aperture oval, angled and produced below, inside white. Shell size: H = 25–35 mm. B = 14–18 mm. JUVENILE SHELL (Fig. 4E): Conical, comprising up to 3.5 whorls. Sculpture smooth only with faint growth lines except for the wrinkled apical whorl. From the third whorl on a remarkable spiral keel is visible at about the centre of the whorl. In some specimens also two spiral rows of smooth knobs are present from this point on. OPERCULUM: Oval, with up to five whorls gradually increasing in diameter, and a nearly central nucleus. RADULA (Fig. 7B): Length of ribbon: M = 20 mm (SD = 1; N = 3), with up to 190 rows of teeth. Very similar to *B. armata*, rhachis tends to be more squarish in size.

Distribution: Material of the species as delimited herein is known only from the Kaek River and the closely adjacent Kwae Noi River (see Fig. 1). Blanford (1903) stated that *M. binodosa* is common in large rivers of Thailand. However, based on all known localities of relevant material we suggest here that its occurrence be restricted as stated above. This is supported by Brandt (1974), who noted that *B. binodosa* is restricted to these two tributaries of the Nan River.

Ecology: Brandt (1974) found this species attached to stones and boulders in a swift flowing and clear stream grazing on algae; this was confirmed by our own observations in the field.

Similar species: Among the Kaek River species assemblage *B. pseudosulcospira* is somewhat similar in exhibiting a sculpture that always lacks spiny nodules. Also, the shell of the latter is much stronger and

comprises only up to three whorls. Another similar species is *B. armata* which, however, has a generally much more conical shell with fewer whorls. As shown in Figure 6, both species can be discriminated by their different H/B ratios in adult shells, with the median in *B. binodosa* = 1.4, in *B. armata* = 1.8).

BROTIA MICROSCULPTA BRANDT, 1968

Brotia microsculpta Brandt, 1968: 272, pl. 10, fig. 59 ('Thailand: Maenam Kaek, in Thung Salaeng Luang Botanical Garden, 80 km E of Phitsanulok' = Kaek River, Thung Salaeng Luang NP, Prov. Phitsanulok; Holotype SMF 197378, 10 paratypes SMF 205356/10); Köhler & Glaubrecht, 2002: 141.

Brotia (Brotia) microsculpta – Brandt, 1974: 180, pl. 13, fig. 36.

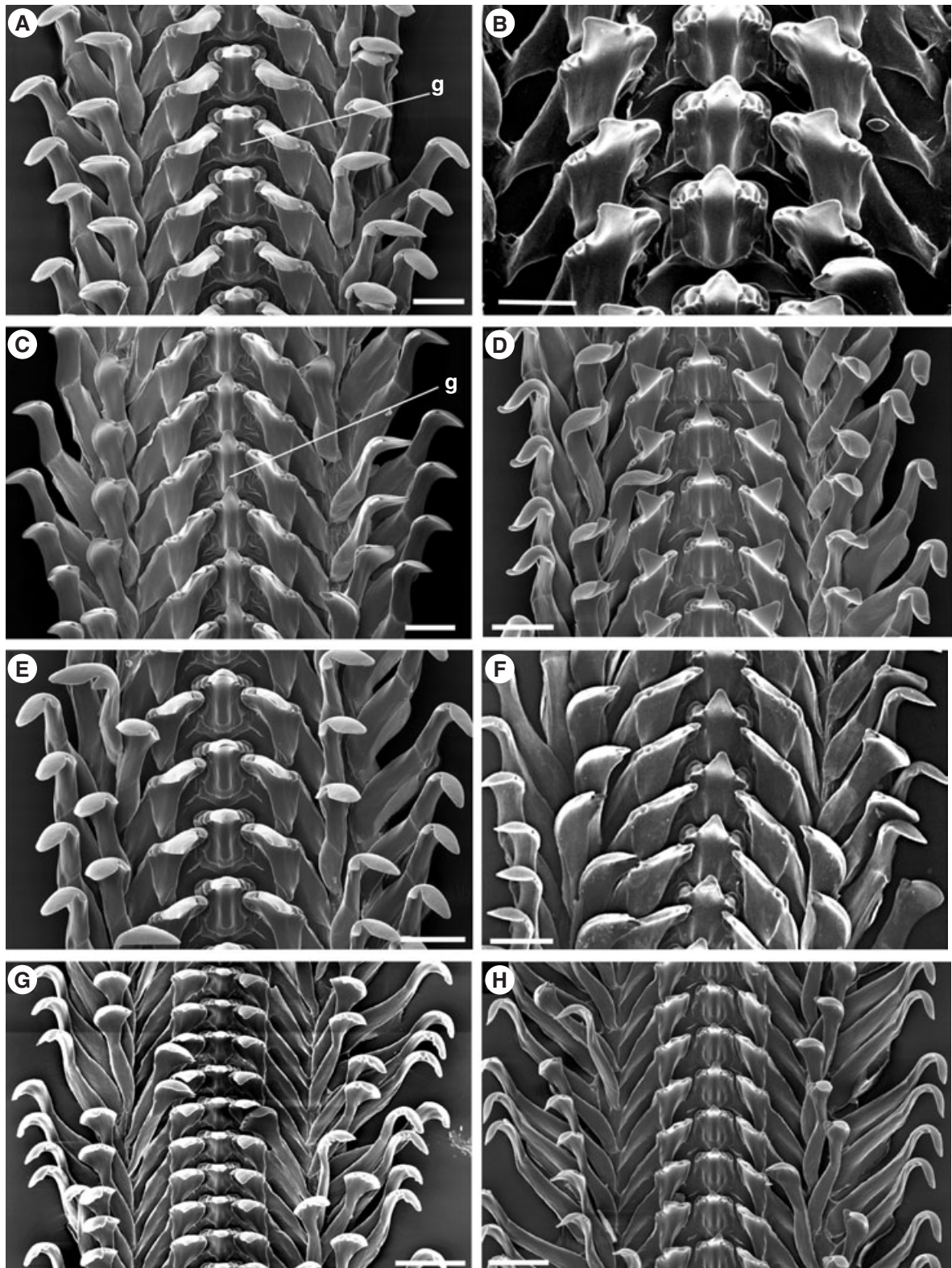
Type material examined: Thailand: Holotype SMF 197378/1 ('Thung Salaeng Luang, leg. 17.12.67' = Kaek River, Thung Salaeng Luang NP), ten paratypes SMF 205356/10.

Other material examined: Thailand: Prov. Phitsanulok, Kaek River: Resort 53 km east of Phitsanulok (ZMB 200.266); Poi Falls (ZMB 200.200); Sopha Falls, 71 km east of Phitsanulok (ZSM 19983240); Thung Salaeng Luang NP (ZMB 200.191).

Nomenclature: This species has been described by Brandt (1968) based on its small size and distinct microstructure. The paratypes (ZMH) look dissimilar to the holotype and likely represent juvenile stages; thus, it remains to be solved if they actually are members of the same species.

Diagnostic characteristics: Shell small, conical to elongated, with mostly three remaining, slightly rounded whorls; sculpture smooth. Aperture round, not produced at the base. Operculum round, not oval as in all other Kaek river *Brotia*. Radula with prolonged marginal teeth.

Description: SHELL (Fig. 3E): Shell relatively small, conical to elongately conical, not thick but solid. Spire truncated with mostly three remaining convex whorls; whorls rounded in diameter. Sculpture smooth, with fine axial growth lines and faint spiral lines, only. Aperture almost round, relatively small compared to the shell height, basely rounded but not produced. Shell size: H = 10–25 mm. B = 8–15 mm. JUVENILE SHELL unknown, because only very limited material and no gravid females found. OPERCULUM: Round to only slightly oval, with 5–6 regular whorls. RADULA (Fig. 7G–H): Length of the ribbon M = 11.8 mm (SD = 1.7 mm; N = 3) with about 190 rows of teeth. Teeth smaller and rows closer to each other than in *B. armata*. Rachidian comparatively small; cutting edge with three accessory cusps. Marginal teeth very



long, curved possessing one to three inner accessory cusps.

Distribution: Thailand: Endemic to the Kaek River and, according to Brandt (1974), its northern tributary, the Huai Chieng Nam (see Fig. 2; Table 4).

Ecology: Lives on or buried into sandy soft substrate, according to our own observations at four collection localities from 53 to 80 km from Phitsanulok along the Kaek River; see Table 4. Although not known yet from there, we anticipate that *B. microsculpta* also occurs further downstream where sandy substrate is also available. It is, next to *B. subgloriosa*, the only of the Kaek species to be found exclusively on sand. Species density appears to be much smaller, likely because animals buried in sand were hard to find even by digging in the substrate.

Similar species: Easily recognizable by its smaller shell, round operculum, and its occurrence in soft substrate habitat only. Somewhat similar is *B. pseudosulcospira*, which has a more conical shape, thicker shell and flatter whorls.

BROTIA PALUDIFORMIS (SOLEM, 1966)

Paracrostoma paludiformis Solem, 1966: 17, pl. 1, fig. H–J, textfig. 2 (‘Thailand, Provinz Phitsanulok: Kaek River at the Thung Salaeng Luang Falls’) (not *Semisulcospira paludiformis* Yen, 1939).

Paracrostoma paludiformis paludiformis – Brandt, 1974: 187, pl. 14, fig. 45.

Paracrostoma paludiformis – Köhler & Glaubrecht, 2002: 121–156.

Type material: Non-vidi; Solem (1966: 1) referred to material collected in February 1964 by Birgit D. Hansen, who was employed on the 1st. Thai–Danish Expedition to Thailand (in 1958–59); the material is apparently housed with the Universitetets Zoologiske Museum, Copenhagen, Denmark.

Material examined: Thailand: Prov. Phitsanulok: Kaek River: Sopha Falls, 71 km east of Phitsanulok (ZMH; BMNH; SMF 215963).

Nomenclature: Solem (1966) employed the name *P. paludiformis* for this species from the Kaek River in Thailand since he considered the shell being conchologically very similar to *Semisulcospira paludiformis*

Yen, 1939 from ‘Lu-ho-wan, Inner Hainan’ (Yen, 1939: 55, pl. 4, fig. 73). The type material (SMF) of *S. paludiformis* Yen, 1939 has been lost and only the original figure is available. However, it is highly questionable if the Thai specimens referred to by Solem (1966) is conspecific with Yen’s species from Hainan. Solem (1966) described the development of the juvenile shells with features typical for *Brotia* (i.e. with a ‘soft apex’). Based on this description, it is evident that the Thai *B. paludiformis* (*sensu* Solem, 1966) is truly a representative of *Brotia*. Pachychilid species known from Hainan, though, generally lack this peculiar feature of ontogenetic development. Thus, certainly both taxa are not conspecific (if the type locality of Yen’s species has not been confused). Consequently, Solem’s description has to be considered the diagnosis of a then unknown new species. Its name *paludiformis* does not need to be changed because both Solem’s and Yen’s taxon are members of different genera (a case of secondary homonymy; ICZN, 1999: Art. 59.1).

Brandt (1974) described a new subspecies, *P. paludiformis dubiosa*, in spite of its exhibiting a shell sculpture quite distinct from the nominate form. This subspecies is considered herein as a synonym of *B. armata* based on the similar shell morphology.

Diagnostic characteristics: Shell relatively large, ovate, with two or three convexly rounded whorls, body whorl conspicuously inflated. Sculpture entirely smooth except for the growth lines, aperture broadly oval.

Description: SHELL (Fig. 3F–G): Medium-sized to large, broadly ovate with two or three well rounded and convex whorls. Spire eroded. Body whorl large and inflated. Sculpture smooth consisting only of faint growth lines and only rarely with a spiral row of small and rounded nodules. Colour chestnut brown. Aperture wide and oval, well rounded below. Shell size: H = 24–30 mm. B = 18–22 mm. OPERCULUM: Oval to slightly elongated, with up to three whorls increasing fast in diameter, and a subcentral nucleus. RADULA (Fig. 7C): Length of the ribbon: M = 23.4 mm (SD = 1.3 mm; N = 3) with about 190 rows of teeth. Denticle morphology corresponding to *B. armata*. SOFT BODY ANATOMY AND JUVENILE SHELLS: unknown.

Distribution and ecology: Endemic to the Kaek River in Thailand, where it is known from the Sopha Falls only; see Table 4. Owing to material lacking from our

Figure 7. Radular morphology of the endemic Kaek River species. SEM images of radula segments viewed from above. Arrows mark well-developed and narrow glabella, respectively. A, *B. armata* (ZMB 200.254). B, *B. binodosa*, detail of rachidian and laterals (ZSM 19981932). C, *B. paludiformis* (SMF 215963). D, *B. pseudosulcospira* (paratype, ZMH). E, *B. solemiana* (ZMB 200.203). F, *B. subgloriosa* (ZSM 199883213). G, *B. microsculpta* (ZMB 200.266; Resort). H, *B. microsculpta* (ZMB 200.200; Poi Falls). Scale bars = 100 µm.

own collections, we do not have any indication of the preferred substrate of this species.

BROTIA PSEUDOSULCOSPIRA (BRANDT, 1968)

Brotia (Paracrostoma) pseudosulcospira Brandt, 1968: 274–275, pl. 10, fig. 61, textfig. 40 ('Maenam Kaek in Phitsanulok Prov., at Wang Nok Nang Aen, Wang Tong District, Thailand' = Thailand, Provinz Phitsanulok, Wang Tong District, Kaek River at Wang Nok Nang Aen).

Paracrostoma pseudosulcospira pseudosulcospira – Brandt, 1974: 185, Taf. 13, Abb. 42.

Paracrostoma pseudosulcospira – Köhler & Glaubrecht, 2002: 144.

Type material examined: Thailand: Prov. Phitsanulok, Kaek River: Holotype SMF 197379; 23 paratypes SMF 193586; five paratypes SMF 194061; 11 paratypes BMNH 1976120; 12 paratypes ZMH (dry); 11 paratypes ZMH (alc.).

Other material examined: Thailand: Prov. Phitsanulok, Kaek River: Sakunothayan Falls, 33 km east of Phitsanulok (ZMB 200.196, 200.299).

Nomenclature: Besides the distinct nominate form of *B. pseudosulcospira*, Brandt (1968) described a second subspecies *P. pseudosulcospira armata*, which is treated here as an independent species, because both taxa occur in sympatry (see above).

Diagnostic characteristics: Conical shell with up to three flattened whorls, sculpture rather smooth with only growth lines and occasionally spiral cords that are not present at the base of the shell. Aperture widely ovate, well rounded.

Description: SHELL (Fig. 3H–I). Medium-sized, conical, robust, frequently with only two flattened whorls and eroded spire. Sculpture smooth except for growth lines, occasionally more or less developed, regularly spaced spiral cords may be present. If spiral cords are present, than they are lacking at the base of the shell. Aperture widely ovate, well rounded and slightly produced below. Size: H = 26–40 mm, B = 18–24 mm. JUVENILE SHELL: Smooth with faint growth lines only, apical whorl with wrinkled structure and a sharp transition at about half of the first whorl. Juveniles removed from the brood pouch attain a size of 2.0–2.5 mm with 2.5 whorls. OPERCULUM: Oval, with up to four whorls increasing fast in diameter, and a subcentral nucleus. RADULA (Fig. 7D): Length of ribbon: M = 25 mm (SD = 2.5 mm; N = 3) with up to 180 rows of teeth. Central tooth comparatively broad, glabella very narrow; otherwise corresponding to the radula of *B. armata*.

Distribution: Endemic to the Kaek River, but restricted to its western most portion. It is known only

from Brandt's (1968) sampling at Wang Nok Nang Aen, east of Wang Tong (see Fig. 2) and our own sampling from the Sakunothayan Falls close by; see Table 4.

Similar species: The shell of *B. pseudosulcospira* is characteristic on its own. Although *B. paludiformis* also has a smooth shell, it shows convexly rounded whorls and an inflated body whorl. In addition, the latter lacks spiral lirae as can be observed in at least some specimens of *B. pseudosulcospira*. *Brotia microsculpta* and *B. subgloriosa* have a more turreted shell with a more slender shape, and *B. armata* has spiny nodules.

BROTIA SOLEMIANA (BRANDT, 1968)

Brotia (Paracrostoma) solemiana Brandt, 1968: 273, pl. 10, fig. 60 ('Maenam Pong at Ban Pa Nok Kao, Loei Prov.' = Thailand, Prov. Loei, Pong River bei Ban Nok Kao).

Paracrostoma solemiana – Brandt, 1974: 186, pl. 13, fig. 44; Köhler & Glaubrecht, 2002: 147.

Type material examined: Thailand: Prov. Loei, Pong River: Holotype SMF 197377; seven paratypes SMF 193583; six paratypes SMF 193585; two paratypes RMNH 55233/2.

Other material examined: Thailand: Prov. Loei, Loei River: Tat Kok Falls at road 2216 near Wang Saphung (ZMB 200.174); Prov. Phitsanulok, upper course of the Kaek River at Sri Dit Falls (ZMB 200.203).

Nomenclature: Brandt (1968, 1974) mentioned the slender shape of the shell, the flattened whorls and the elongated aperture to be characteristic for this species. Furthermore, he stated that the species is endemic to the Pong River, between the provinces of Loei and Kon Kaen, which is east of the Kaek drainage and an affluent of the Mekong River. Here material from outside the Pong River system (ZMB 200.174, 200.203) is attributed to this species also mainly as a result of a corresponding shell morphology.

Diagnostic characteristics: Conical shell with two or three flattened whorls; sculpture smooth except for growth lines and occasionally fine spiral ridges, spiral lirae lack at the base of the shell; aperture widely ovate, acute or produced below.

Description: SHELL (Fig. 3J–K): Medium-sized, conical, robust, with two or three flattened whorls and an eroded tip. Sculpture smooth except for growth lines, in some specimens inconspicuous spiral ridges may be present; spiral lirae lack at the base of the shell; aperture widely ovate, acute or produced below. Colour yellowish to greenish brown. Size of the shell: H = 26–40 mm, B = 18–24 mm. OPERCULUM: Oval, with up to

four whorls increasing in diameter, and a subcentral nucleus. RADULA (Fig. 7E): Length of the ribbon: $M = 16.0$ mm (SD = 3.4 mm; $N = 4$) and 150–160 rows of teeth, thus being smaller than other Kaek species. Rachidian relatively narrow, otherwise in general corresponding to *B. armata*.

Distribution: With wider distribution in Thailand than all other species dealt with here. Next to the type locality in the Pong River system and from the Loei River (Tat Kok Falls near Wang Saphung), both in the Loei province (Central Thailand), we here attribute material found at Sri Dit Falls in the western most headwater region of the Kaek River (see Figs 1 and 2; Table 4) to this species.

Ecology: The Sri Dit locality provides a somewhat distinct habitat for *Brotia solemiana*, which lives on gravel bottoms attached to stones and smaller rocks and in river sections with swift current (F. Köhler, pers. observ.).

Similar species: This is a distinctive species. Of the slightly similar taxons, *B. pseudosulcospira* has more flattened whorls and a more conical shell, *B. subgloriosa* is more turreted and generally larger, and *B. microsculpta* possesses a smaller body whorl, a rounded aperture, and a circular operculum.

BROTIA SUBGLORIOSA (BRANDT, 1968)

Brotia binodosa subgloriosa Brandt, 1968: 269, pl. 10, fig. 56, textfig. 38 ('Thailand: Huai Chieng Nam, tributary of the Kaek River, about 92 km east of Phitsanulok at the bridge of the Friendship Highway').

Brotia (Brotia) binodosa subgloriosa – Brandt, 1974: 175–176, pl. 13, fig. 28.

Brotia spinata subgloriosa – Köhler & Glaubrecht, 2002: 129.

Type material examined: Thailand: Holotype of *B. binodosa subgloriosa* SMF 19737 ('Prov. Phitsanulok, Huai Chieng Nam, tributary of the Kaek River, 91 km E of Phitsanulok at the bridge of the Friendship Highway'), 20 paratypes SMF 193572, paratype ZSM 19983213, six paratypes ZSM 19983219, 11 paratypes ZMH.

Nomenclature: Brandt (1968) described this taxon as a subspecies of *B. binodosa* and stated that both are connected to each other by intermediate morphs. However, as such intermediate morphs have not been identified among the material examined, it remains dubious whether or not they really exist. Following the statement of Brandt (1968, 1974), *B. subgloriosa* and *B. binodosa* occur sympatrically in sections of the Kaek River, which renders a relation of both as geographical representatives unlikely. For this reason,

B. subgloriosa is considered as a separate species closely related to *B. binodosa*. Additional paratypes are housed with the USNM according to Brandt (1968).

Diagnostic characteristics: Shell elongately turreted, entirely smooth, aperture elongately produced and relatively narrow.

Description: SHELL (Fig. 3L): Medium sized; elongately turreted with a solid shell. Spire with up to five convex and rounded whorls and a truncated tip. Sculpture consisting of thin growth lines only. Colour olive-brown, often covered with dark mineral deposits. Basal whorl relatively large. Aperture wide, elongately produced below. Shell size: $H = 25$ – 45 mm, $B = 16$ – 24 mm. JUVENILE SHELL (Fig. 4F): Conical, comprising up to 3.5 whorls. Sculpture smooth only with faint growth lines except for the wrinkled apical whorl. OPERCULUM: Oval, with up to five whorls gradually increasing in diameter, and a nearly central nucleus. RADULA (Fig. 7F): Length of ribbon: 18 mm ($N = 1$) with 220 rows of teeth. Glabella broad with a straight lower rim. Corresponding to *B. armata*.

Distribution and ecology: Endemic to the Kaek River (Thailand), between 65 km (at Sopha Falls) and 92 km east of Phitsanulok, and its tributary, the Huai Chieng Nam, as reported by Brandt (1968: 270). He also mentioned this taxon to be found 'on sandy ground of the river at comparatively quiet parts, sometimes even buries in the sand... but at Ban Palo it is also found in rapids'. Unfortunately, the exact substrate remains somewhat doubtful, as any other observations are lacking.

Similar species: *Brotia subgloriosa* is superficially similar to other Thai species with smooth shells. However, for example, *B. microsculpta* is much smaller and has a comparatively smaller and round aperture with round operculum; *B. pseudosulcospira* is much more conical in shape and has flattened whorls instead, and *B. solemiana* is more compact with a comparatively broader but shorter shell.

RESULTS II: MOLECULAR GENETICS

We used 17 samples from six species occurring sympatrically and parapatrically, respectively, in the Kaek River system, including one sample of *B. binodosa* from the nearby Kwae Noi River (Chattrakan, ZMB 200.202). Since only dry shell material of *B. paludiformis* and *B. subgloriosa* was available, these species lack from our molecular analyses. The combination of two fragments of the COI and 16S rRNA gene yielded an alignment of 1504 nucleotides. Of these 1224 bp were constant, 173 bp variable but parsimony-uninformative, and 107 bp polymorphic.

MRMODELTEST revealed the general time reversible model (GTR) with six rate classes and a gamma-distributed rate heterogeneity (GTR+ Γ +I) as the best-fit model of DNA evolution. For ML and BI analysis the parameters were adjusted according to the results of MRMODELTEST (gamma factor $\alpha = -0.6816$; proportion of invariable sites = 0.5378).

Maximum parsimony analysis yielded two most parsimonious trees with a length of 377; the strict consensus tree is shown in Figure 8A. The phylogenetic tree reconstructions resulting from neighbour-joining, maximum likelihood and Bayesian inference analyses reveal more or less identical topologies (Fig. 8B–D), with minor differences as to the position of a few populations within the Kaek River assemblage. However, most notably, all trees indicate the existence of a monophyletic assemblage of *Brotia* taxa endemic to the Kaek River, with *B. solemiana* from the Loei River drainage (ZMB 200.174) sister to this species flock. Although the second *B. solemiana* sample (ZMB 200.203) from the Kaek River headwaters at Sri Dit holds a position at or near the basis of the Kaek River flock, it was not found in any of the analyses to cluster together with its conspecific from Loei.

Under the assumption that the respective taxa represent individual (bio-) species with separate gene pools, populations of each taxon recognized a priori by its shell morphology should group together in molecular phylogenetic analyses. However, our phylogenetic reconstruction of mitochondrial genes revealed that samples assigned to individual morphotypes do not cluster together, thus indicating a mismatch between gene tree and species tree. In addition, the neighbour-joining tree (Fig. 8B) shows a very shallow topology indicating that only a few mutations and base substitutions having occurred.

Accordingly, the genetic distances between Kaek River taxa were found to be rather low. The uncorrected pairwise distances amongst all endemic Kaek taxa are on average 7.1% for COI and 3.4% for 16S. This corresponds to the genetic distance observed within $N = 39$ species of *Tylomelania* endemic to Sulawesi, with values on average of 6.3% (ranging from 0.3 to 11.8%) for COI and 3.4% (ranging from 0.2 to 6.5%) for 16S, respectively (Rintelen, 2003), and to *Pseudopotamis* from the Torres Strait Islands with values on average of 6.5% (ranging from 0.3 to 12.6%) for COI and 4.1% (ranging from 0.6 to 7.6%) for 16S (Glaubrecht & Rintelen, 2003). On the other hand, the

amount of intrageneric genetic distance contrasts with that observed in other pachychilid genera. For example, we found 13% sequence divergence for COI and 13.9% for 16S in *Jagora* from the Philippine Islands (Köhler & Glaubrecht, 2003), whereas in other species of *Brotia* from outside the Kaek River, e.g. within *B. pagodula*, pairwise distance was on average 10.1% for COI and 9.4% for 16S (Köhler, 2003).

When plotted as a taxon-area cladogram based on the BI consensus tree (Fig. 9), the individual samples from Kaek River reveal no correlation between their position in the phylogenetic reconstruction and their geographical occurrence along the river, with the notable exception only of the outgroup *B. pagodula* and the sister taxon *B. solemiana*, as mentioned above.

In conclusion, neither the a priori assigned morphotypes nor the distinct morphotypes from the same locality cluster together.

RESULTS III: ECOLOGICAL OBSERVATIONS

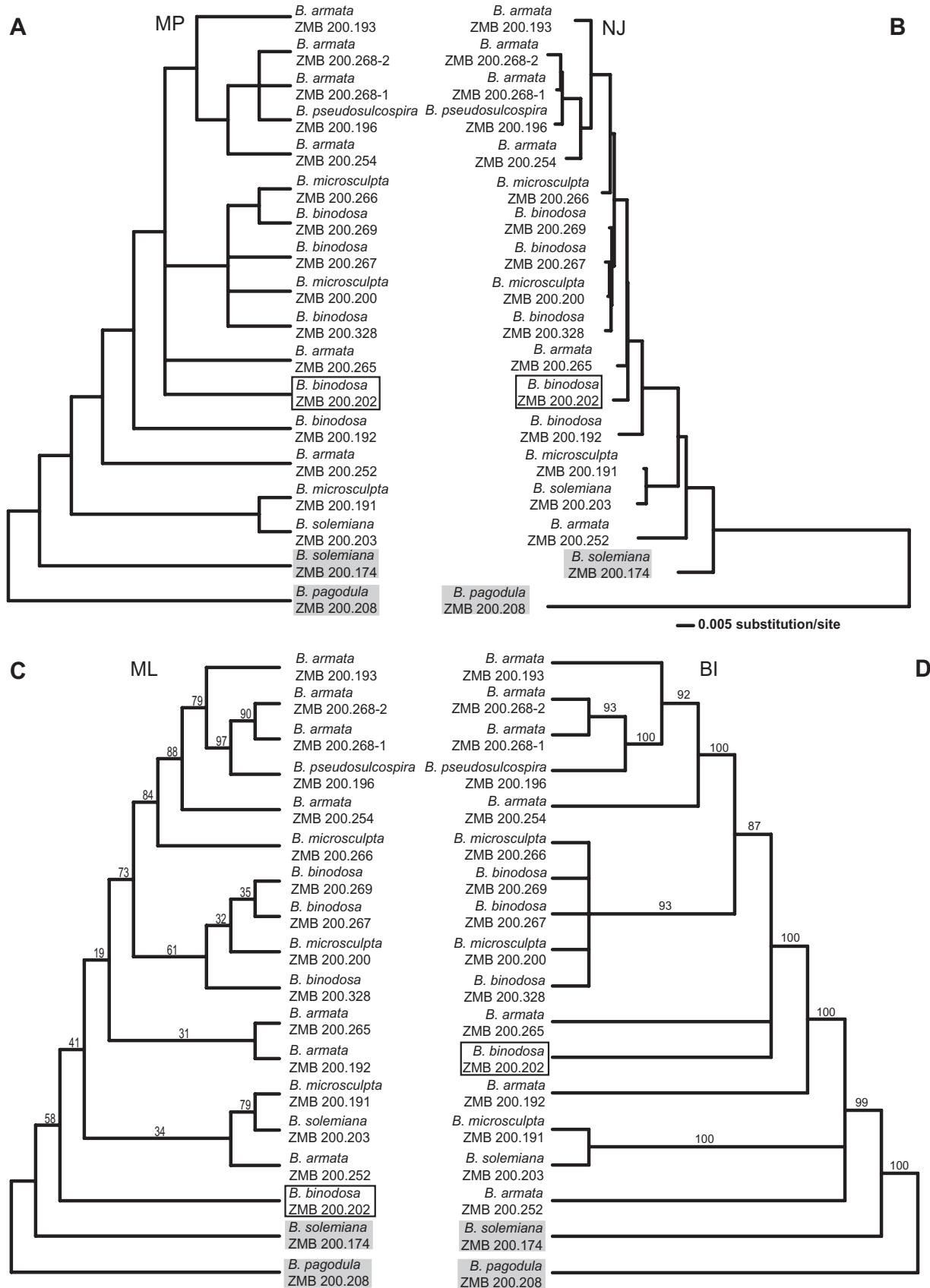
SPECIES–SUBSTRATE CORRELATION

In general, we found a strong species–substrate correlation in the *Brotia* species endemic to the Kaek River. As is evident from the compilation given in Table 4, based on the data described in the Systematic Revision, *B. armata* and *B. binodosa* are found exclusively on hard substrate (i.e. rocks and boulders), whereas *B. microsculpta* and perhaps the more restricted *B. subgloriosa* (but see above under the species) are soft-substrate dwellers. Thus, at each locality along the Kaek River, two to three taxa are found together, and where sandy habitat is present, *B. microsculpta* joins the two aforementioned hard-substrate dwellers.

Interestingly, among the Kaek species flock only *B. microsculpta* is a purely soft-substrate dweller. It was found buried in the sand, whereas all other species (most notably *B. armata* and *B. binodosa*) live attached to rocks in midstream and downstream of the river where the current is swift. Small specimens (mainly *B. armata*) inhabit the upper sector of rocks from 0 to 20 cm below water surface, while large specimens of *B. binodosa* and *B. pseudosulcospira* are found closer to the bottom, at depths of 50 cm or more. These species are absent from areas with moderate currents (in the upstream region) and from sandy areas.

While *B. pseudosulcospira* occurs only at one (westernmost) location, and *B. solemiana* only at the east-

Figure 8. Phylogenetic relationships of *Brotia* species endemic to the Kaek River, Central Thailand, indicated by analyses of the concatenated sequence data of the partial COI and 16S gene fragments; outgroup: *B. pagodula*. A, Strict consensus cladogram of two maximum parsimony trees. B, Neighbour-joining phylogram. C, Maximum likelihood tree. D, Bayesian inference consensus tree with posterior clade probabilities (bpp). Highlighted in grey: taxa from localities outside the Kaek River; frame indicates a *B. binodosa* sample from the Kwae Noi River (loc. Chattrakhan).



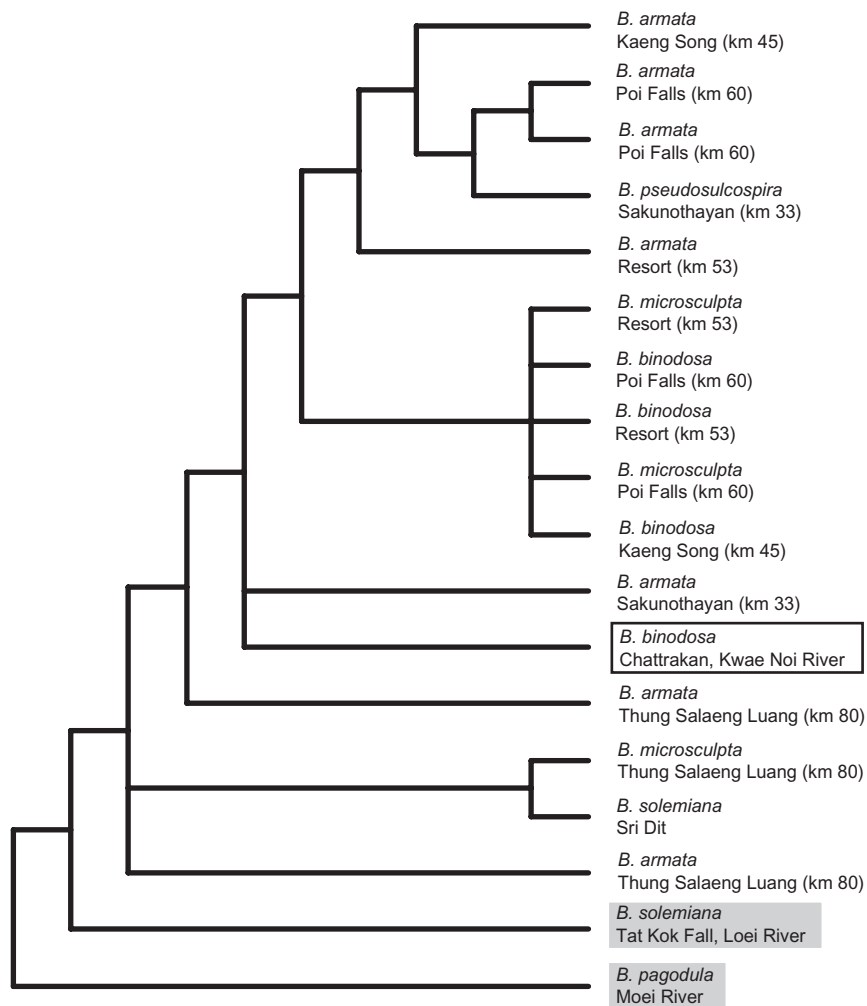


Figure 9. Area cladogram of the Kaek River taxa, based on the BI consensus tree (see Figure 8), revealing lack of a strict geographical context within the Kaek River populations, but indicating an origin of the riverine radiation from *B. solemiana* from the Mekong drainage (Loei River).

ernmost site of Sri Dit, a total of five species live sympatrically at Sopha Falls. *Brotia solemiana* was also found outside the Kaek River, where it inhabits hard substrate (gravel and boulders) at the Tat Kok Falls, Loei River.

In the endemic Kaek River *Brotia* species we generally observe that shell shape, thickness and sculpture are correlated with different substrate type. Smooth, elongated, and rather thin shells are found in species dwelling on stones and/or in quiet waters, whereas shells are more conical, sculptured and/or thick in species living on boulders and rocks exposed to the current. However, no correlation was found between shell sculpture and locality. Species with smooth shells occur both in the upstream area with a moderate current flowing over a stony bottom (*B. solemiana*) and in sandy downstream localities (*B. microsculpta*). The former appears to be restricted to the gravel substrate

in the swift current headwater region of the Kaek River (ZMB 200.203) where it lived attached to stones and smaller rocks, as well as in similar stream habitats of the Loei River (ZMB 200.174) outside the Kaek drainage.

RADULA DIFFERENTIATION AND SUBSTRATE

As is evident from the data in the Systematic Revision (see above), there are at least three types of radula morphology in the *Brotia* species in the Kaek River that correlate with habitat (i.e. substrate). *Brotia armata* and *B. binodosa* possess the 'normal' pachychilid type with a radula of 18–20 mm and about 180–200 rows, a squarish rachidian with well-developed glabella and large main denticles, laterals with short and strong basal extensions and somewhat kneed marginals with only a single smaller side cusp

(Fig. 7A–B). This radula pattern, also found in many congeneric species from other rivers in South-east Asia, appears to be typical for grazers on hard substrates. It is also shared by *B. subgloriosa* (Fig. 7F) for which, however, the exact substrate remains somewhat doubtful (see under the species).

The radula in *B. paludiformis*, *B. pseudosulcospira* and also *B. solemiana* is quite distinct. This second morphology exhibits a rachidian with very narrow glabella instead (see Fig. 7C–E). While the radula of the first two species in this group is longer, with 24–25 mm in length and 180–190 rows, the radula of *B. solemiana* is the smallest among the Kaek River taxa, with only 16 mm length, 150–160 rows, and a glabella that is more or less intermediate between the two groups. Typically this latter species, occurring in the Kaek River as well as outside the region in streams of the Mekong drainage and appearing basal to the rest of the Kaek radiation, is restricted to gravel bottoms and, thus, also represents a hard substrate-dweller, albeit of slightly different character.

The third, most distinct, radula morphology, however, is seen only in the typical soft-substrate dweller *B. microsculpta* (Fig. 7G–H). This radula type has about 190 rows and is only 12 mm long (the animals are also much smaller, though). The denticles are lined up much more closely and are also considerably smaller than in other Kaek River taxa. In addition, the laterals, which appear to have a more rounded, flaring shape distally, possess considerably longer basal extensions than those seen in any of the other Kaek species. The marginals are very long and curved at the distal end, with mostly two cusps flanking the broad major denticle. The entire impression is that of a ‘thiarid’ type radula for it reveals features otherwise characteristic for Thiaridae that typically inhabit soft substrates where these detritus-feeders ‘brush’ food particles (e.g. diatoms) from sandy to muddy substrates (Glaubrecht, 1996).

In summary, *B. microsculpta* from soft, sandy substrate exhibits a clearly distinct pachychilid radula type that sets it apart from the other hard-substrate dwellers among the Kaek River species flock. Within the latter group, two radula types can be distinguished by the possession of a rachidian tooth with well-developed glabella in *B. armata*, *B. binodosa* and *B. subgloriosa*, on the one hand, and a narrow glabella in *B. paludiformis*, *B. pseudosulcospira* and *B. solemiana* on the other.

DISCUSSION

SPECIES AND SPECIATION

Contributing to the controversy surrounding different modes of speciation and the mechanisms involved in adaptive radiation is undoubtedly the lack of agree-

ment as to what constitutes a species, a concept fraught with uncertainty, ambiguity and an annoying ontology (e.g. Otte & Endler, 1989; Haffer, 1992; Bush, 1994; Mayden, 1997; Howard & Berlocher, 1998; Wilson, 1999; Wheeler & Meier, 2000; Hey, 2001). However, as is evident from many studies, the combination of genetic and phenotypic analyses provides a powerful approach for designating species limits. In combination with information on geographical occurrence, it allows biological species to be distinguished not only as perceivable units, but also as natural entities if we make the careful distinction between *species taxon* (with identifying characteristics) and *species entity* (as a group of coevolving populations). (For further discussion see, e.g., Hey, 2001, and Glaubrecht, 2003a, 2004; see the latter also for a review of case studies from malacology.) Thus, within this framework of species as natural entities in space and time an identifiable species taxon can serve as a hypothesis of a species entity. Our treatment herein of the Kaek River taxa as biological species should be understood explicitly along these lines.

In freshwater gastropods high levels of morphological *disparity* and taxonomic *diversity* are frequently correlated, but often only because traditionally disparity was equated with diversity, as has been exemplified for limnic Cerithioidea such as the Mediterranean melanopsid snail *Melanopsis* (cf. Glaubrecht, 1993, 1996, 2003a, 2004). In particular this taxon has led palaeontologists to speculate on speciation and radiation, on transformation in evolutionary *formenreihen*, punctuated equilibrium and heterochronicity (e.g. Willmann, 1981; Geary, 1988, 1990; Geary *et al.*, 2002). The same holds true for another ‘classical’ case study from Cerithioidean freshwater gastropods, namely the thiarid *Melanoidea* from Lake Turkana (e.g. Williamson, 1981). Although these examples have frequently been used as model case or reference (e.g. Gould, 2002), the available neontological data from exactly these groups were left largely untapped, leading to the underestimation of the phenotypical plasticity and ecophenotypical variability in the *Melanopsis praemorsa* superspecies and/or other relevant biological features, such as parthenogenesis in *Melanoidea*, as discussed in Glaubrecht (1993, 1996, 2003a, 2004; see also references therein).

The same traditional and typological perception as to conchological variability in freshwater snails has resulted in a plethora of synonyms in case of those forms assigned to the genus *Brotia* in South-east Asia. In the course of the systematic revision of pachychilids, based on reconciling morphological and molecular genetic data (Köhler & Glaubrecht, 2001, 2002, 2003; Glaubrecht & Rintelen, 2003; Köhler, 2003), not only was the number of species in general reduced, but also the basis was provided for an

evaluation of phenotypical (i.e. conchological) plasticity in these gastropods. Owing to the earlier typological approach that resulted in the traditional overestimation of taxonomical diversity due to conchological disparity, but also in the context of the genetically apparently closely related but morphologically highly distinct *Brotia* taxa found to be endemic in the Kaek River, we have to ask whether we are indeed dealing with a species flock rather than a single, highly polymorphic species with maybe several sympatric morphs exhibiting different ecophenotypical adaptations in shell and radula in response to a variable environment.

NO CASE OF A SINGLE-SPECIES INTERPRETATION

It was Davis (1982) who first noted that there are normally only two sympatric *Brotia* species per river in South-east Asia, and who contrasted this fact with the ten described (partly supra-)specific taxa in the Kaek River. Davis also raised the question of conchological plasticity as a result of ecophenotypic adaptation within a species vs. indication for a true radiation.

When Brandt (1968, 1974) ranked some of the Kaek taxa as subspecies due to their close similarity in shell (see Table 3), it reflected his perception of a morphological species concept. Following the biological species concept, however, taxa are considered as subspecies under the condition that they represent not only morphologically distinguishable but also geographically separated (i.e. vicariant) representatives of a single species (for the historical development of the concept of species and subspecies see, e.g., Stresemann, 1927; Mayr, 1942, 1963: 205–213; Haffer, 1992).

However, we neither found indications of clinal variation in the Kaek River populations nor distinguished geographical features that separate these populations from each other. Instead, two taxon pairs that were earlier regarded as subspecies, *B. armata*/*B. pseudosulcospira* and *B. binodosa*/*B. subgloriosa*, actually occur in sympatry and syntopy at Sakunothayan and Sopha Falls, respectively (see Table 4). Similarly, we have no indication that other taxa living at different locations along the Kaek River could conceivably represent only subspecific populations. The morphometric differentiation between most Kaek taxa in concert with their different radular morphology provide sufficient indication for their reproductive isolation. Because they maintain distinct populations even in sympatry, we regard them as representing separate biological species.

The lack of any substantial genetic differentiation resulting in the notable mismatch of gene and species trees in Kaek River *Brotia* does not contrast with the situation among other limnic gastropod species widely accepted as valid. Most remarkably, the situation in

the Kaek taxa is similar to the closely related congeneric *Tylomelania* endemic to lakes on Sulawesi that reveal genetic differences in COI and 16S sequences on a comparable level and are accepted as representing good species (Rintelen, 2003; Tv. Rintelen, A. B. Wilson, A. Meyer, M. Glaubrecht, unpubl. data). Among the only other Cerithioidean gastropods for which data on genetic distance are available to date, namely the North American Pleuroceridae, accepted species often exhibit pairwise distances ranging from 1.5 to 17% (Lydeard *et al.*, 1997; Holznagel & Lydeard, 2000).

Thus, based on the biological species concept with its implicit test of sympatry, and in agreement with the conchological, ecological and molecular genetic data, we do not see any justification for subsuming the populations in the Kaek River within one single species only. Instead, seven distinct taxa are accepted herein as representing true biospecies.

PHENOTYPIC PLASTICITY

The shells of limnic gastropods are notoriously phenotypically plastic and variable environmental conditions can produce substantial modifications (e.g. Dillon, 2000), as is for instance discussed for *Melanopsis* and other cases in Glaubrecht (1993, 1996, 2003a, b). Thus, even marked differences in shell shape and sculpture do not necessarily indicate the presence of more than one species. Strictly speaking, phenotypical plasticity is understood as the ability to express different phenotypes depending on the biotic or abiotic environment *within* one species (e.g. Agrawal, 2001 for a review). As discussed above, though, the evidence points at the coexistence of distinct natural entities with different morphologies.

The morphological disparity in taxa from the Kaek River studied herein, i.e. the variation of shell (see Fig. 3) and radula (Fig. 7), is astonishing even in light of the known plasticity of the genus in entire South-east Asia (see Köhler, 2003). As schematically illustrated in Figure 10, the conchological differentiation of the seven *Brotia* species from this single small Thai river varies along several axes of morphological space, from the spinous/conical *Brotia armata* at one end to the smooth/elongate *B. microsculpta* at the other. Although some correlation with the preferred habitat and substrate are deducible from the ecological observations given above, an assessment of the significance of these distinct phenotypic traits is lacking, as is an understanding of the genetic basis of phenotypical variation in general for gastropods. For the limnic pomatiopsid *Oncomelania hupensis*, Davis & Ruff (1973) were able to show that apparently a single mutation in only one gene is sufficient for producing axially ribbed shells in a smooth-shelled population, suggesting that a relatively simple underlying genetic

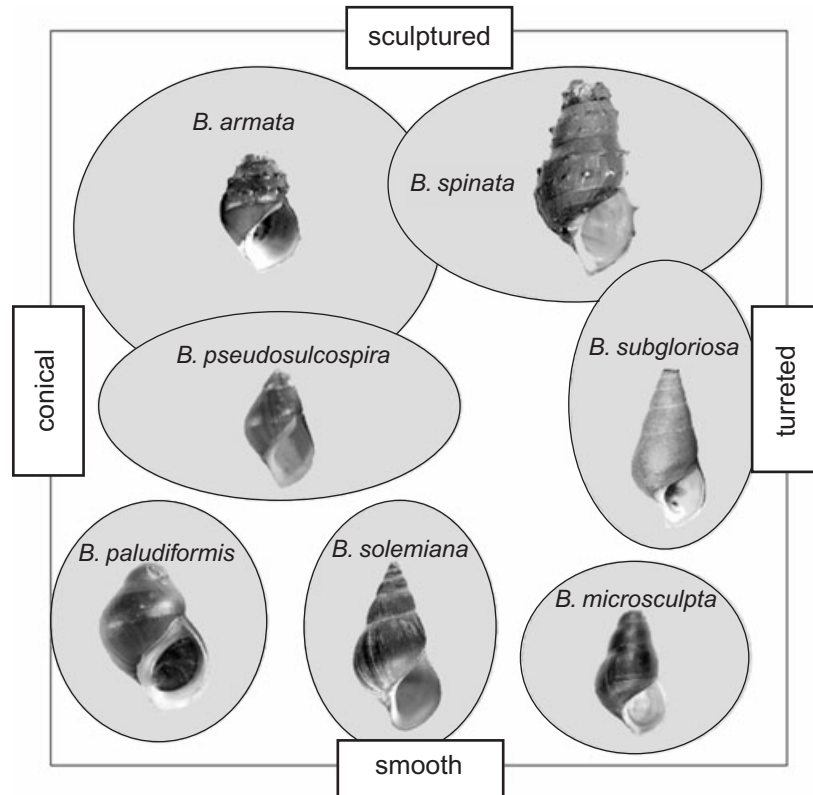


Figure 10. Schematic comparison by means of shell sculpture and shape of the Kaek River species as recognized in the present paper.

mechanisms (likely controlled by a few genes) might be responsible for gastropod shell traits. In a natural experimental situation in *Oncomelania* from the Miao River in the Yangtze floodplain in China, Davis *et al.* (1999) found that ribbing is indeed genetically controlled by a single gene with multiple alleles and suggested this to be an adaptation for dealing with annual flooding and survival by water transport. However, understanding the mechanisms that generate phenotypic variation such as shell sculpture and shape and being evolutionary relevant (i.e. inherited and selected with an adaptive value) still remains a fundamental challenge for contemporary evolutionary biology.

ADAPTIVE RADIATION AND ECOLOGICAL SPECIATION

Current discussions on adaptive radiation hypotheses centre around the differential contribution of history vs. ecology. The ecological theory of speciation differs most significantly from the idea of geological speciation in that differentiation is not only a by-product of separation but also triggered and/or facilitated through ecological interactions. With natural selection

playing a fundamental role in these theories it is possible to test its role under different ecological conditions.

The hypothesis of ecological speciation has been employed by Schluter (2000) to explain adaptive radiation as the rapid diversification of a lineage in combination with niche formation. It maintains that adaptive radiation or the existence of a species flock is the outcome of divergent natural selection resulting from different environments or habitat use and resource competition. Schluter proposed four criteria: (i) monophyly; (ii) rapid speciation; (iii) phenotype–environment correlation; and (iv) trait utility. While the first two criteria, that are essentially phylogenetic, have only recently been tested even for the ‘classical’ case studies of adaptive radiation (see Schluter, 2001; Losos & Miles, 2002), the third and fourth criteria are most frequently neglected in existing studies. To demonstrate ecological differentiation, not only utilization of different environments, but also a fitness benefit, through the efficiency to exploit other resources, need to be shown. Applying Schluter’s criteria here for the present example of the endemic pachychilid taxa in the Kaek River, we will subsequently discuss if it actu-

ally provides an, albeit rare, incidence of adaptive riverine radiation.

IS THERE A MONOPHYLETIC AND RAPID RADIATION OF *BROTIA* SPECIES IN THE KAEK RIVER?

Based on morphological as well as molecular genetic data, it is evident that all species described from the Kaek River form a monophyletic group of *Brotia*. To assign some of these taxa to *Paracrostoma*, as suggested by Solem (1966) and Brandt (1968, 1974), was erroneous, and is also not supported from our revision of South-east Asian Pachychilidae in general (e.g. Köhler, 2003; F. Köhler & M. Glaubrecht, unpubl. data).

Although the mitochondrial gene tree does not exactly reflect relationships *within* the Kaek River species, the molecular phylogeny (Fig. 8) strongly hints at a common ancestry with *B. solemiana*, which is basal to the Kaek River clade. While it was assumed earlier that *B. binodosa* inhabits other streams than the Kaek River, its occurrence is actually restricted to this drainage as detailed under the respective species. In contrast, as verified in the present and earlier studies, *B. solemiana* is found also at localities in the Loei and Pong Rivers, i.e. streams belonging to the Mekong drainage in the east of the Kaek River (Fig. 1), suggesting a possible origin in this area and subsequent speciation (see below).

The lack of significant resolution in our molecular phylogenies, i.e. the mismatch of gene and species trees, and the comparatively shallow topology of the Kaek River gastropods imply a relatively recent origin of this intrariverine radiation and, consequently, rapid morphological divergence. Several cases are known, e.g. cichlid fishes in East African lakes (e.g. Rossiter, 1995), where morphologically distinct (sympatric) species have evolved without marked changes in the mitochondrial genome. However, there are alternative explanations employed in view of lacking resolution and flat topology that can only be falsified by inference of additional molecular genetic data.

First, it can be hypothesized that speciation had occurred only relatively recently, so there was not sufficient time for substantial changes in this particular gene fragments. Our study does not overcome this problem of incomplete lineage sorting, i.e. the maintenance of an ancestral allele polymorphism across population or species boundaries with the coalescence process not completed for the respective gene. Consequently, the real relationships between populations or taxa are obscured by the fact that the locus examined traces ancestral information through different genetic routes, resulting in gene history that may not fit to the history of the populations (Page & Holmes, 1998; Nichols, 2001). Secondly, the short branches and unre-

solved topology in the mtDNA trees may reflect hybridization, and introgression may be the reason why the species are not correctly recognized in a phylogenetic tree.

Mitochondrial DNA may be particularly prone to this phenomenon which was also observed in *Tylomelania* on Sulawesi (Tv. Rintelen, A. B. Wilson, A. Meyer, M. Glaubrecht, unpubl. data; Rintelen, 2003). To resolve this situation, the use of other molecular markers (of the nuclear genome) and the application of other techniques, in particular amplified fragment length polymorphism (AFLP) analysis or the use of microsatellites may help to clarify the actual relationships by investigating the gene flow between single populations (see, e.g. Albertson *et al.*, 1999; Richard & Thorpe, 2001; overview in Savekoul *et al.*, 1999).

TROPHIC SPECIALIZATION AND THE POSSIBLE ROLE OF COMPETITION

In general, genetically based phenotypic variation is assumed to reflect ecological adaptation. Due to their being closely linked to speciation, not only the environment–phenotype correlation but also evidence of ecologically dependent differentiation and utilization of specific traits is of paramount importance for the assessment of adaptive radiation.

The substrate preference and radula differentiation seen in Kaek River species parallels those known from the endemic species flock of the confamilial *Tylomelania* on Sulawesi (Rintelen & Glaubrecht, 2003a,b; Rintelen, 2003; Tv. Rintelen, A. B. Wilson, A. Meyer, M. Glaubrecht, unpubl. data) and, thus, follows an expectation based on the evolutionary diversification of these snails when occurring sympatrically. It provides evidence for the adaptive dynamics of radula features and lends support to the general argument that the environment, in the present case the habitat (i.e. substrate type) and maybe also resource utilization on a finer scale, determine the evolution of morphology.

As we know from studying ontogenetical series in *Tylomelania* and observations of radulae of juvenile *Brotia* from South-east Asian rivers (cf. M. Glaubrecht, F. Köhler & Tv. Rintelen, unpubl. data), the particular radula pattern described above is not just a case of ecophenotypical adaptation, but is actually an inherited species-specific morphology based on genetic divergence. Therefore, we interpret the hard-substrate and soft-substrate radula differentiation seen in the species with respective habitat preferences as trophic specialization that parallels other cases, for example in sympatric lake whitefish ecotypes (e.g. Lu & Bernatchez, 1999), further supporting the ecological speciation hypothesis.

However, further data are certainly needed not only on differences in diets, but also on the details of

exploitation of biofilms (composed, e.g. of diatoms, algae, bacteria, etc.) by microphagous snails. Hawkins *et al.* (1989) have shown that different types of grazers can coexist as a consequence not only of different feeding mechanisms but also of using different items in biofilms. Although it was long assumed that limnic gastropods are indiscriminant browsers on the periphytic layers, recent studies imply that these snails actually do show a preference for specific parts of the periphyton and that food preference is coupled to microhabitat selection (review in Brönmark, 1989). Accordingly, next to physiological divergence and differences, e.g. in gizzard musculature, specializations particularly in the details of radula morphology are held to be most important in resource partitioning and thus niche separation among closely related freshwater snail species (see also Kesler *et al.*, 1986).

In the case of the Kaek River species it remains to be studied whether the differences in radula morphology correspond with different feeding preferences and food utilizations. However, we anticipate this to provide a mechanism that would allow partitioning of resources and consequently the continuing coexistence of more than two riverine species of *Brotia* usually found in South-east Asian rivers.

In this context, competition and, closely associated with this, character displacement, have been topics of much debate and regarded of fundamental importance not only in influencing community structure, but also in providing a mechanism of speciation and radiation (see Schluter, 1994, on experimental evidence for divergence). Only few studies on competition have addressed this for invertebrates in stream ecosystems, and then when focusing mostly on taxonomically dissimilar species. However, commonly congeneric pairs of grazers coexist not only in South-east Asian pachychilids (as discussed above), but also among other cerithioidean groups. Thus, competition seems most likely among these closely related taxa considering their similar resource requirements.

Recently, Cross & Benke (2002) provided experimental evidence for intra- and interspecific competition among coexisting lotic snails from the Cerithioidean family Pleuroceridae. They reported on reduced growth rates in two species of *Elimia* in a second-order spring-fed stream by increased density of snails. Because minimal differences for either species were found, implying a lack of competitive dominance, it was suggested that the two are functionally redundant species with density-dependent responses in growth rate resulting in similar grazing pressure across a density gradient. These few observations point to future avenues of comparative research and experimental studies testing the importance of competition between species of coexisting lotic and lentic

snails, in order to evaluate the mechanisms of trophic specialization and annidation in course of a truly adaptive radiation.

COMPARISON WITH LACUSTRINE RADIATIONS

Although lotic systems are generally considered harsh environments (as determined by physical factors and stochastic events reducing densities), they are more permanent on both ecological and evolutionary timescales than most lake habitats; the only notable exception are ancient lakes, which provide ample opportunity for many different organisms to speciate (see Introduction), which is quite in contrast to the riverine setting where environmental changes are mostly gradual and do not prevent gene flow.

Interestingly, only rarely have true riverine radiations been recognized, with closely related species occurring in sympatry or having adjacent ranges. The unique situation described here for seven coexisting *Brotia* species, suggesting a recent origin of this radiation and rapid morphological divergence, parallels those of recent diversification in confamilial species flocks in the ancient lakes on Sulawesi. A high degree of both inter- and intraspecific morphological differentiation and molecular divergence was found in the lacustrine (but not the riverine) species of the endemic *Tylomelania* (see Rintelen & Glaubrecht, 1999, 2003a, 2003b; Rintelen, 2003; Tv. Rintelen, A. B. Wilson, A. Meyer, M. Glaubrecht, unpubl. data). A mtDNA phylogeny of about 47 taxa (38 among them lacustrine) revealed that four parallel and independent lineages have colonized the two lake systems within the last 1–2 million years. Within each of these clades a burst of very recent radiation resulted in a partial mismatch of species and gene trees, as apparently the rates of molecular and morphological evolution have been highly divergent in lake species as opposed to widespread riverine taxa from creeks and rivers around the lake systems and across Sulawesi. Increased shell strength and thickness associated with the transition to a lacustrine environment is interpreted as indication of strong selective pressure due to molluscivorous crabs and, thus, of coevolution with this predator within these lentic systems. While there is evidence for trophic specialization linked with differential habitat use within the lake indicating ecological speciation in *Tylomelania*, allopatric mechanisms might also have been involved.

In contrast, the lotic system in the Kaek River with its sympatric, and partly syntopic, species flock reveals conditions where promoting speciation through allopatric isolation seems implausible. This strongly hints at spatial segregation by trophic substrate specialization correlated with adaptation in radula morphology to different microphagous graz-

ing niches as the ultimate trigger of speciation. Several recent studies have tried to detect the geographical pattern of speciation (e.g. Barraclough & Vogler, 2000; Losos & Glor, 2003) and developed models to look into the link between geographical patterns and ecological processes of speciation by studying evolutionary branching in spatially structured populations (e.g. Doebeli & Dieckmann, 2003), both establishing the eminent role of the spatial factor in evolution and highlighting the importance of local processes of adaptive divergence for geographical patterns of speciation. However, on a finer scale and exemplified by adequate case studies, future comparative work of the Sulawesi lakes with their relatively large species flocks contrasted with the less species-rich Kaek River group might help in elucidating these aspects of radiation and origin of diversification.

EVOLUTION OF AN ENDEMIC RIVERINE RADIATION

With respect to biological diversity it has been predicted (albeit not found, e.g., for molluscs in streams of Western North America; see Frest & Johannes, 2002), that generally gamma diversity (i.e. species richness across a range of related habitats; see, e.g. Rosenzweig, 1995) is greatest in the mid-portion or rhitral and declines both towards the crenon (headwater) and potamon (lowland stream) section. The pachychilid species flock of the Kaek River is certainly an interesting exception for various reasons. First, it is among the rare fluvial radiations, and the only known flock both for the family Pachychilidae and limnic Cerithioidea (e.g. Glaubrecht, 1996; Köhler, 2003), with the exception of the essentially unexplored case of North American pleurocerids from the rivers in the south-eastern USA. Secondly, studies have usually looked only into the faunal composition of tropical rivers across taxonomic groups (e.g. Starmühlner, 1976, 1983, 1993; Bandel & Riedel, 1998; note, however, that in the latter case many species were regularly misidentified), and not even attempted to evaluate species composition or causation for radiation in an evolutionary biology context.

Although high levels of species inventories and endemisms have been reported for other freshwater gastropods from lotic systems, among them, for example, the North American Pleuroceridae and some Australian Hydrobiidae, speciation mechanisms (including testing allopatric vs. ecological speciation models) have not been considered or investigated and often not even discussed. Among the many diverse species assemblages that have recently been described for fluvial hydrobiids (see Introduction), only Davis (1979, 1981) explicitly discussed an endemic radiation in the Mekong River, yielding

three tribes, 11 genera and 92 species in a period of about 12 Myr, apparently driven by extrinsic processes correlated with the massive tectonics caused by the Himalayan orogeny that led to the formation of the major river systems of South-east Asia and western China. Davis (1979) demonstrated a diverse array of size, shape, sculptural pattern of the shell, radula morphology and reproductive anatomy of triculine snails of the hydrobioid family Pomatopsidae. These snails occupy a wide range of environments similar to those of the gastropods from other unrelated groups on which their shells converge, with basic habitats defined by substrates, depth of water and current, and have radiated into new environments and geographical regions after colonization of South-east Asia via the northward moving Indian plate. This species assemblage is most diverse in the middle Mekong region at the entrance of the Mun River in the east of Thailand. There the Mekong is most diverse in riverine environments, with the river progressively widening and an increasing number of islands and habitats created by the complexity and sheltering of large bedrock outcroppings including innumerable types of rapids and waterfalls. The triculine snails have filled up a wide array of habitats, with sympatric species partitioning niches through use of different food as reflected through different radula morphologies, and by use of the same area by different species at different times of the year. Further diversification has occurred by different sets of species occupying different sections of the Mekong River or its tributaries.

With the exception of an early attempt by Brandt (1974), who in a brief section suggested the distinction of the malacofaunas from various regions in Thailand according to separate drainage systems, we are not aware of any more general biogeographical account on limnic animals in Thailand, with two notable exceptions. First, in his zoogeographical treatment on cyprinid fishes from South-east Asian rivers, Rainboth (1996) also gave a profound introduction into the geography and geological history of the region, on which the following section is largely based. Second, in his biogeographical analyses of freshwater fishes from a total of 19 biogeographical zones in South-east Asia defined by the main river systems and their underlying geology, Yap (2002) found support for a sister-area connection of the Chao Praya and the Mekong, most likely resulting from the capture of lower-order streams than from the capture of the principal rivers. In the following we discuss the possible geographical origin of the Kaek River radiation by outlining a preliminary scenario for this riverine species flock among Pachychilidae, presenting it as a potentially falsifiable hypothesis, with the explicit caveat that inferring past speciation pro-

cesses from present biogeographical patterns always has its pitfalls.

PALAEOGEOGRAPHICAL RECONSTRUCTION

In order to present the basis for understanding the zoogeographical implications of the phylogeny obtained for the Thai *Brotia* taxa, it is necessary to look into the geography and history of river systems in the region. However, geological and hydrological data are still rare and/or scattered. The only historical summary of river configurations has been that of Rainboth (1996), but due to the mosaic nature of available information a coherent synthesis of the hydrology is still lacking.

Using data presented by Gregory (1925), who showed first that modern river systems have very different appearances than those early in the Cenozoic, Rainboth (1996) presented a comprehensive account of the historical development of drainage basins in mainland South-east Asia with a summary of the complex late Cenozoic river-basin histories of this fascinating region. River basins worldwide have been changed in general by two major palaeogeographical forces, (i) contraction/expansion caused by sea-level changes, in the case of South-east Asia the advances and retreat of the South China Sea, and (ii) realignments caused by tectonic uplift and erosion.

Undoubtedly, sea-level retreat with the development of extended land areas and Pleistocene river basins as a manifestation of global climatic changes had profound effects on the distribution and evolution of South-east Asian biota. In a recent analysis and interpretation of the palaeogeography and biogeography of the Thai–Malay Peninsula, Woodruff (2003) has argued that sea-level high stands both during the Miocene (of +150–220 m, at 24–13 Mya) and Pliocene (of +100 m, at 5.5–4.5 Mya) resulted in marine transgressions. From topographic maps it is evident that under the assumption that this reconstruction is correct, a sea level at +100 m and more would have resulted in a northward extension of the Gulf of Siam and flooding of the Chao Praya river basin. In this case the shoreline would have been perpendicular to the Kaek River. It is not yet fully clear to what extent the region of interest here would have been affected, but we propose that due to elevation and uplift as described below the effects of sea-level changes were less important for the pachychilid fauna inhabiting mountainous streams in the north-east of these basins.

In contrast, we think that changes in drainage configuration that have been most extensive during the Quaternary were critical for the evolution of these gastropods under study, when stream captures of various magnitudes have changed river alignments as a result of local tectonic or hydrological processes. As described

in Rainboth (1996: 147–162) during the Post-Oligocene age the course of major rivers in Central and Northern Thailand has changed dramatically with, for example, the Salween and upper Mekong Rivers then connected to separate tributaries of the Chao Praya. To understand the main lines of argument we present here a brief summary of the palaeogeography.

The Indochinese Peninsula and Central to Northern Thailand has terranes of Gondwanan affinity, with major uplifts beginning in the Triassic and subsequent additions in the entire region formed through the collision of tectonic microplates (terranes) during the lower Mesozoic period, and has seen high tectonic activity during the Cenozoic (e.g. Hutchison, 1989; Hall & Blundell, 1996; Hall & Holloway, 1998; Hall, 1998; Metcalfe, 1998; Hall, 2000; Metcalfe *et al.*, 2001). Central and Northern Thailand is the eastern margin of a continental lithospheric block called Sinoburma-lyaya which was separated throughout the Palaeozoic by the Palaeotethys from a similar one named Indosinia (now the Indochinese Peninsula). The margin of the former Precambrian cratonic block runs along a line extending from Uttaradit in Thailand to Luang Prabang in Laos (see Fig. 1), where an up-lift area in today's mountain range is witness of the collision of Sinoburma-lyaya with Indosinia during the early Mesozoic. This Indosinian Orogeny also created an ancient suture zone running along the Uttaradit–Luang Prabang line. This zone of crustal weakness resulted in subsequent block faulting and mountain building throughout Northern Thailand and the margin of the Khorat Plateau when under pressure from the Himalayan Orogeny (see Rainboth, 1996). Northward movements of former Gondwanan plates such as Africa and India and subtending of the Asian continent during the Oligocene (beginning 38 Mya) and Miocene resulted in marked changes in Asian tectonics through massive mountain-building ranging from the Mediterranean to Yunnan, China. The Himalayan Orogeny was coupled with the uplift of the Tibetan Plateau, a huge platform of former ocean floor, initiating drainage patterns that were to become the main rivers of Asia.

Mountains in the elevated area of Sinoburma-lyaya run on a north–south axis, and valleys separating them are drained in the north by the Mekong River and in the south by four large rivers that converge to form the Chao Praya. The Mekong that today drains extreme northern Thailand and the Khorat Plateau of north-east Thailand, is the principal river system of the Indochinese Peninsula, a role it only gained recently. The Mekong has a complex Pleistocene and Recent history with extensive changes in the region currently drained and an important sequence of stream captures resulting in its modern configuration. Rainboth (1996: 154–155) suggested that the upper Mekong previously passed further south, as a linear

continuation of its present course in Laos and parallel to the four valleys in Thailand, to run perhaps through the valleys of the current Pa Sak or Loei rivers of Thailand, before resuming its sharp turn to the east at 18°N flowing through the area of uplift in the Khorat Plateau. This plateau today is a large, generally flat plain, but actually represents an elevated sedimentary basin and not a real plateau according to Hutchison (1989). It comprises three major sedimentary basins that were originally formed by Mesozoic continental–lagoonal deposits laid down during the change of land from shallow marine habitats to freshwater (see Rainboth, 1996: 158). Quaternary tectonic activity in the Khorat Plateau, accompanied by great lava flows particularly during the lower to middle Pleistocene, has contributed to the alternation of river-drainage patterns, as internal parts of the Indochinese Peninsula buckled and its southernmost tip sank. Thus, the flat appearance of this plateau is now considered a recent feature in an area that had a pronounced relief prior to and during the early Pleistocene. For example, deeply cut channels that are now filled with Quaternary sediments may not be evident from current drainage patterns, and previous major drainages of the Khorat Plateau with outlets maybe to the upper Gulf of Thailand are obscured, as supposed by Rainboth (1996: 159–160).

Available data suggest that the Chao Praya lost its headwaters to the growing Mekong in the middle and upper Pleistocene. Most notable in this context is that in the area east of the Kaek River the headwaters of the southward-flowing Pa Sak River (a tributary of the Chao Praya) and the Loei River (running north into the Mekong) are today in close conjunction, separated by hills of relatively Recent (Cenozoic) igneous origin (Hutchison, 1989). Uplift in this region of the Phang Hoi Range, maybe related to late Tertiary–Quaternary tilting of the Khorat Plateau (that changed its elevation and probably inclined during the Pleistocene; see above), as well as subsidence of the Chao Praya basin in central Thailand (with considerable prior sedimentation time in the ancient subduction zone between Sinoburmalaya and Indosinia) might have separated a once continuous drainage into today's two separate basins. This might also have affected the area of the headwaters of the Kaek Rivers where today a mountain ridge of 1300 to >1700 m a.s.l. elevation separates the drainages of the Kaek from the Pa Sak River. Prior to the uplift, these systems of the Loie, Pa Sak and Kaek rivers might have been in contact directly, or linked through stream capture events.

HISTORICAL BIOGEOGRAPHY

Based on this palaeogeographical reconstruction, derived from known geological data and indicating

changed drainage systems and stream captures caused by major tectonic disruptions, the following scenario on the origin and evolution of *Brotia* in the Kaek River can be developed. Central to this is the fact that our molecular phylogeny found *B. solemiana* from the Loei River to be basal to the entire Kaek River clade, hence suggesting that a common ancestor of *B. solemiana* and the Kaek assemblage first colonized the Kaek River and gave rise to its radiation. This indicates a faunistic connection of taxa from today's Chao Praya drainage (to which the Nan River and its tributaries, the Kaek and Kwae Noi Rivers, belong) with those in the Mekong River, as is reported for freshwater fishes (see Yap, 2002). It is not clear at which time the colonization of the Kaek River has happened, but the recent disjunction of *B. solemiana* might be the result of a river capture by the Chao Praya drainage in the course of up-folding of the Luang Prabang Range that separated the Mekong system and its tributaries, e.g. the Pong and Loei Rivers.

Accordingly, we hypothesize here that with other faunas pachychilid snails could have been captured and added to rivers from the Mekong via the Loei to the Kaek and thus Chao Praya. This would explain the origin of the Kaek River flock in a taxon from the Mekong drainage. Likewise, it is possible to speculate (as did Davis, 1979: 56 for the triline snails), that during the complex geological history with its wide spectrum of tectonic changes affecting the major drainage systems, rivers or at least some of their sections could have stranded as new lakes, isolating faunas for a million of years or more, thus offering ample opportunity also for lacustrine radiations.

Based on the palaeohydrological scenario of Pleistocene stream capture that is in accordance with the alignments of post-Himalayan river systems originally suggested in Gregory (1925), Rainboth (1996) discussed the zoogeographical affinities of closely related freshwater cyprinid fishes of the genus *Hypsibarbus*. In an attempt to determine former river connections and possible stream captures during the Pleistocene producing the current distribution of species from a clade of *Hypsibarbus* occurring along the eastern margin of the Sinoburmalay craton region, he hypothesized an earlier pathway linking the middle Mekong with the Chao Praya of central Thailand through the Loei–Pa Sak valley (Rainboth, 1996: 167–169). In addition, it was noted earlier in several freshwater fishes that while upland species are found in smaller streams of the middle Mekong, their congeners are more likely to be found in the upper Chao Praya than the lower Mekong. Thus, an adjacent river system (i.e. the Chao Praya) had greater faunistic similarity to both the upper and middle Mekong than each had to the other (Rainboth, 1996: 163).

This assessment of the relationships of river-basin faunas among fishes, concluding that the middle Mekong's fauna most closely resembles that of the Chao Praya (and Mekong of central Thailand), parallels our finding from pachychilid gastropods that with *Brotia solemiana*, occurring in the Loei and Pong rivers in the east of the Uttaradit–Lualang Prabang mountain range and being basal to the Kaek River species flock, the middle Mekong drainage reveals closer malacofaunistic affinities to the Chao Praya than the latter to other Thai rivers. This underscores the opportunity to examine limnic gastropod phylogeny for the evaluation of historical relationships of drainage basins during the Cenozoic, as was suggested also for freshwater fishes. In this context, Rainboth (1996: 164) stressed the suitability of the genus *Hypsibarbus* whose species are typically found in smaller upland streams where they prefer areas that have coarse substrates, as does *Brotia*.

Interestingly, the monophyletic triculine snail radiation with 11 genera and 92 species that Davis (1979) considered began in the late Tertiary is basically restricted to a region of today's middle Mekong, with greatest diversity found along a 450-km stretch of the river in southern Laos and northern Cambodia, situated between Khemarat in Thailand and Kratie in Cambodia, and including the lower Mun River of easternmost Thailand at its entrance to the Mekong, where islands of bedrock form stable obstacles about which the arrangement of sand bars, muddy deposition area, etc. is constantly in flux due to annual floods. Davis (1979: 51) proposed a correlation of this local endemism and tectonic activity. Although not giving more details, he hypothesized that tectonic changes (in the course of the Himalayan upthrust, starting after the colonization of the Mekong River system by triculine snails in middle to late Miocene) created new streams and isolated peripheral colonies in new aquatic systems only to subject these later to new stream captures and new lake formations, receiving fauna from previously isolated systems. As new lakes and river systems developed, shifted or became extinct, with continuous habitat repeatedly separated and joined, selective pressure shifted and there were numerous possibilities for snails to enter many new adaptive zones in virgin territory devoid of potentially competing taxa lacking in the newly formed upper Mekong drainage where the triculine stock first entered. Davis (1979: 56) also proposed that over time, as the river cut into its bedrock and wore away suitable substrates upstream, the richest snail diversity migrated downstream, forcing the snail fauna slowly but inexorably downstream where it opened up new areas.

Unfortunately, the age of the pachychilid radiation in the Kaek River is not known and it is impossible to

determine exactly the sequence of events that led to the phylogeny and species distribution of *Brotia*. However, our study of the phylogenetic relationships of South-east Asian Pachychilidae provides preliminary indication of a relatively recent genetic differentiation of the Kaek species flock. In conjunction with the scenario outlined above, this would coincide with a Pleistocene event, about 1–2 Mya. We anticipate that sophisticated molecular genetic studies will be able to test the hypothesis as to the timing of isolation of the Kaek River taxa from other *Brotia* species in South-east Asia, as soon as data from additional populations of pachychilids become available from adjacent regions in Thailand. Historical and ecological hypotheses may then verify whether, for example, Quaternary tectonic events gave impetus to speciation, creating barriers to dispersal. In the course of an approach known as the heuristic process of reciprocal illumination linking phylogeny, palaeogeography and zoogeography, it will thus be possible to test the idea proposed here that river catchments of tributaries played the initial role for the Kaek radiation, and to produce an estimate of the importance of river pathways that no longer exist, but which have left an indelible mark on the evolution and distribution of limnic taxa.

CONCLUSION

In the Kaek River, a small third-order tributary to the Nan-Chao Praya river system, the endemic species assemblage comprising at least seven sympatric and partly syntopic species stand out among other pachychilid gastropods of mainland South-east Asia, in revealing a combination of conchological disparity, ecological differentiation (substrate specificity) and trophic (i.e. radula) specialization. In representing a riverine radiation of a monophyletic species flock with presumably relatively recent diversification, their origin from a colonizer deriving from the Mekong drainage system adjacent to the east can be hypothesized, with river capture providing a possible hydrological explanation. However, the causation for an *in situ* speciation along the river and the contribution of geographical (i.e. historical) vs. ecological factors remain unresolved. Future investigations may elucidate this situation by looking into the factors that could account for considerable morphological divergence, but not genetical differentiation (on mitochondrial sequence level) encountered within a single drainage system. For this, it is necessary to include also material of *B. paludiformis*, *B. subgloriosa* and samples of *B. solemiana* from further localities outside the Kaek River. A better resolved species-level phylogeny, e.g. using AFLPs, will be necessary to assess the level of genetic variation and gene flow among populations; in

conjunction with field studies determining the level of environment–phenotype correlations and other factors relevant for coexistence, it may be possible to solve the origin and causation of this unusual riverine species flock and to further illuminate the role of intrinsic vs. extrinsic factors in the course of allopatric and/or ecological speciation.

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APPENDIX

ABBREVIATIONS

Repositories

- AMS, Australian Museum, Sydney (Australia)
 BMNH, The Natural History Museum, London (UK)
 MCZ, Museum of Comparative Zoology, Cambridge, MA (USA)
 MHNG, Muséum d'Histoire Naturelle, Genève (Switzerland)
 MNHN, Muséum National d'Histoire Naturelle, Paris (France)
 SMF, Senckenbergmuseum, Frankfurt/Main (Germany)
 USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC (USA)
 ZMB, Natural History Museum, Humboldt University Berlin (Germany) (formerly Zoological Museum Berlin)
 ZMH, Zoological Museum and Zoological Institute, University of Hamburg (Germany)
 ZSM, Zoologische Staatssammlung, München (Germany)

Shell parameters

- BW, height of body whorl
 H, shell height
 LA, length of aperture
 N, number of whorls
 B, shell breadth
 WA, width of aperture