The Out-of-India hypothesis: What do molecules suggest?

Aniruddha Datta-Roy and K Praveen Karanth*
Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India
**Corresponding author (Email, karanth@ces.iisc.ernet.in)*

The remarkable geological and evolutionary history of peninsular India has generated much interest in the patterns and processes that might have shaped the current distributions of its endemic biota. In this regard the "Out-of-India" hypothesis, which proposes that rafting peninsular India carried Gondwanan forms to Asia after the break-up of Gondwana super continent, has gained prominence. Here we have reviewed molecular studies undertaken on a range of taxa of supposedly Gondwanan origin to better understand the Out-of-India scenario. This re-evaluation of published molecular studies indicates that there is mounting evidence supporting Out-of-India scenario for various Asian taxa. Nevertheless, in many studies the evidence is inconclusive due to lack of information on the age of relevant nodes. Studies also indicate that not all Gondwanan forms of peninsular India dispersed out of India. Many of these ancient lineages are confined to peninsular India and therefore are relict Gondwanan lineages. Additionally, for some taxa an "Into India" rather than "Out-of-India" scenario better explains their current distribution. To identify the "Out-of-India" component of Asian biota it is imperative that we understand the complex biogeographical history of India. To this end, we propose three oversimplified yet explicit phylogenetic predictions. These predictions can be tested through the use of molecular phylogenetic tools in conjunction with palaeontological and geological data.

[Datta-Roy A and Karanth K P 2009 The Out-of-India hypothesis: What do molecules suggest?; *J. Biosci.* **34** 687–697]
DOI 10.1007/s12038-009-0057-8

1. Introduction

Peninsular India was part of the Gondwana supercontinent about 200 million years ago (mya) (Mani 1974; Briggs 1989, 2003a; Hedges 2003). During the Jurassic period, approximately 158-160 mya, the Indo-Madgascar plate drifted away from East Africa, followed by the separation of peninsular India from Madagascar around 84-96 mya (Briggs 2003a). The geomorphological, geological, and geophysical data supporting these events have been discussed in detail by previous workers (Krishnan 1974; Chatterjee and Scotese 1999; Ali and Aitchison 2008). After the separation from Madagascar, the Indian plate supposedly underwent a period of isolation for about 30-40 million years (my), before colliding with the Eurasian plate around 40-50 mya. Given this long period of isolation experienced by the Indian plate it is expected that the fauna and flora of peninsular India would be unique and highly endemic (Kaest 1971). With the collision of the Indian and Eurasian plates there was exchange of biota between Asia and peninsular India (Mani 1974). Consequently, the "Biotic ferry model" was proposed, according to which the rafting Indian plate carried ancient Gondwanan forms to Asia (Mani 1974; McKenna 1995; Hedges 2003; Briggs 2003a; Bossuyt *et al.* 2006). Upon collision with Asia these Gondwanan forms dispersed out of India and into Asia (also called as "Out-of-India" hypothesis). However, Briggs (2003a) suggested that peninsular India was not completely isolated during its northward journey and that faunal links were maintained with Africa and Madagascar until it collided with the Eurasian plate, at least with respect to salt water resistant faunal elements. Chatterjee and Scotese (1999) also discussed fossil evidence that supports a Late Cretaceous dispersal of dinosaurs and other vertebrates from Africa and Europe. Rage (1996) even depicted an end Cretaceous arrangement of tectonic plates, in which the northern part of peninsular India was attached to the Eurasian plate and the southern part to the Madagascar plate. Thus the limited fossil data suggests that during its northward journey, the Indian plate has remained close to Africa and Madagascar even when it began to contact Eurasia (Briggs 2003a). Nevertheless, numerous recent molecular phylogenetic

Keywords. Gondwanan biogeography; India; molecular dating; molecular phylogeny

studies have supported the Out-of-India hypothesis (*see* Karanth 2006 and references therein).

As mentioned above, since the Indian plate collided with Eurasia, not only did potentially Gondwanan elements disperse out of India and into Asia, but also many Asian and African elements dispersed into India. Mani (1974) points out that these relatively young intrusive elements are largely derived from the Indo-Chinese and Malayan subregion of tropical Asia. Numerous authors have commented on the overall faunal similarity between peninsular India and Southeast Asia (Blanford 1901; Hora 1949; Jayaram 1949; Mani 1974). This out of Asia and into India scenario has been discussed in mammals based on palaeontological evidence (Clyde *et al.* 2003). Hora (1949) also discusses the Southeast Asian origin of freshwater, torrential fishes of Western Ghats and their possible dispersal routes. In case of plants, the possibility of an out of Asia and into India origin has been discussed by Lakhanpal (1970) and Bande (1992). In this regard, a recent molecular study of freshwater gastropods of supposedly Gondwanan origin is of much relevance (Köhler and Glaubrecht 2007). Results from this work indicate that the gastropods of Southern India are nested within a clade consisting of Southeast Asian taxa. It is clear that this group in fact dispersed out of Asia and into India.

Much of the evidence for the Gondwanan origin of some Asian biota and the Out-of-India hypothesis is based on fossil data and indirect evidence from species distribution ranges. As has been shown by the gastropod work (Köhler and Glaubrecht 2007), extant faunal distributions cannot be taken as evidence supporting Gondwanan origin and Out-of-India hypothesis. Another confounding factor in our attempt at understanding Gondwana biogeography is the situation wherein classification of a taxonomic group does not correspond to its evolutionary relationship, i.e, when unrelated taxa have be erroneously placed in the same taxonomic group (Karanth 2006). Thus to rigorously test these hypotheses the knowledge of phylogenetic relationships of the taxa of interest is vital.

In order to confirm that a taxonomic group is of Gondwanan origin and that vicariance due to continental drift is responsible for its present distribution, three requirements must be met. Firstly, the taxonomic group should be monophyletic and distributed predominantly in some (if not all) of the former Gondwanan fragments of South America, Africa, Madagascar, peninsular India and Australia. Secondly, in the phylogenetic trees of Gondwanan taxa, the split between taxa from various Gondwanan fragments should mirror the sequence of geophysical events that led to the breakup of the Gondwana. Thirdly, the estimated divergence dates among taxa on various Gondwanan fragments should correspond to the time when these land masses became separated from each other. In the case of Out-of-India hypothesis, the Gondwanan taxa distributed outside of India should be nested within a clade consisting of Gondwanan taxa from India. In recent times, molecular phylogenetic approaches have been increasingly used to address biogeographical problems, such as Gondwanan biogeography. In this paper we review studies undertaken on a range of taxa of supposedly Gondwanan origin, to better understand this remarkable pattern. Additionally, we discuss the utility of molecular phylogenetic approaches in the study of biogeography of the Indian subcontinent given the above mentioned requirements.

2. Flora

2.1 *Asian dipterocarps*

One of the first plant systems studied explicitly in a phylogenetic framework in order to test for the Out-of-India hypothesis were the Asian dipterocarps. Work done by Dayanandan *et al.* (1999) on this family revealed that they formed a monophyletic group with the Sarcolaenaceae, a tree family endemic to Madagascar. The most likely way by which the Dipterocarpaceae would have reached the Asian mainland is via peninsular India acting as a biotic ferry, followed by dispersal out of India. However, the split between Malagasy and Indian taxa has not been dated. Thus, more recent transoceanic dispersal cannot be ruled out as alternative explanation. Transmarine dispersal appears unlikely as these families also share similar ectomycorrhizal symbiosis, which is thought to have evolved in their common ancestor (Ducousso *et al.* 2004). Ectomycorrhizal symbiosis occurs in only 3% of angiosperms. Therefore, it is unlikely that similar mycorrhiza in the two families could have originated independently.

2.2 *Crypteroniaceae*

The family Crypteroniaceae is distributed in tropical Asia and their morphology-based taxonomy suggests affinities with African (western Gondwanan) taxa. This implies that this group may have reached Asia rafting on the drifting Indian plate. Molecular work done by Conti *et al.* (2002) confirmed their close relationship with African species and molecular date estimates by Rutschmann *et al.* (2004) suggest that Crypteroniaceae diverged from their west Gondwanan sister clade in the early to middle Cretaceous. The molecular dates are concordant with an ancient Gondwanan origin of the Crypteroniaceae, and the hypothesis that they were transported by the drifting Indian plate to Asia. Interestingly, this family has gone extinct from peninsular India, while its extant distribution includes Sri Lanka and Southeast Asia.

2.3 *Melastomataceae*

Family Melastomataceae has around 4500 species distributed among the neotropics, Asia, Africa and Madagascar. Earlier work done on this group suggested the possibility that Gondwanan fragmentation may have played a major role in the divergence and establishment of tribes within the family (Morley and Dick 2003). In this regard, another molecular phylogenetic study that uses multiple markers and calibration point to estimate divergence dates (Renner 2004) is of much relevance. This study includes the three morphologically described tribes of the family Melastomataceae namely Dissochaeteae, Melastomeae and Sonerileae. The phylogeny generated was calibrated with multiple fossils as well as tectonic events. Results indicate that the tribe Melastomeae arrived on Madagascar multiple times during the Miocene by transmarine dispersal from Africa. The Asian Melastomeae also arrived from Africa during the Miocene presumably via Middle East. Whereas the Afro-malagasy Sonerileae and Malagasy Dissochaetae are nested within Asian Dissochaetae suggesting an Asian origin of these groups. These dispersal events again were dated to the Miocene. Thus the branching order and date estimates of Melastomataceae are not consistent with the Out-of-India hypothesis.

2.4 *Rhododendron*

A molecular phylogeny of *Rhododendron* section *Vireya* from the Malesian archipelago supports contrasting scenarios (Brown *et al.* 2006). The first scenario is that vireyans are an old group of Gondwanan origin, and their current distribution in the Malesian archipelago is a result of the drifting Indian and Australian Gondwanan fragments. In other words, vireyans evolved on drifting Indian and Australian plate and later dispersed to Malesian archipelago from these landmasses presumably after the current continental positions were established. Thus Malesian vireyans constitute a combination of species that dispersed out of India as well as out of Australia. Alternately, vireyans may be a young group that dispersed out of India to Australia via Malesian islands. With respect to this scenario the authors do not discuss how vireyans might have reached India in the first place. Dating the relevant nodes of the phylogeny would be helpful in choosing between these hypotheses. Unfortunately, dating was problematic due to lack of appropriate calibration points and variable DNA substitution rates across lineages (Brown *et al.* 2006). Nevertheless it must be noted that reproductive isolation between vireyans is weak which is suggestive of a recent radiation (Williams and Rouse 2007). This recent radiation scenario in turn is not consistent with the Gondwanan origin hypothesis. The results of the study remain inconclusive with respect to the Out-of-India hypothesis. Including additional taxa from India and additional markers in conjunction with molecular dating may help in resolving this question.

3. Fauna

3.1 *Invertebrates*

3.1.1 *Freshwater gastropods:* Gastropods of the family Pachychilidae are represented in India by two genera *Brotia* and *Paracrostoma* (Köhler and Glaubrecht 2007). *Brotia* is distributed in the Ganges-Brahmaputra river basin, while *Paracrostoma* is restricted to Southern India. This family is distributed across the tropics worldwide, a pattern suggestive of a Gondwanan origin (Köhler and Glaubrecht 2003, 2006). Recent molecular work on Asian pachychilids by Köhler and Glaubrecht (2007) suggest an Out-of-Asia and into India scenario. In their phylogeny, the southern Indian *Paracrostoma* are more closely related to the Southeast Asian forms than to the Malagasy forms. Additionally, the genus *Paracrostoma* is nested within a larger clade consisting of Southeast Asian pachychilids. The most ancient members of the pachychilids from mainland Asia (*Adamietta*) are found in the easternmost regions of Southeast Asia. The other Indian genus *Brotia* is found only in certain parts of Nepal and the extreme northeast regions of India, which again is a part of the Southeast Asian biogeographic zone. The most parsimonious explanation from the phylogeny obtained by Köhler and Glaubrecht (2007) is that the ancestors of the Indian pachychilids dispersed from Southeast Asia. Here again dating of the colonization event was not possible due to the lack of appropriate fossil calibration points. Köhler and Glaubrecht (2007) also pointed out that the dispersal of these genera into India must have happened after the collision of peninsular India with Asia as their dispersal ability over marine barriers is extremely poor. It is suggested that the Southeast Asian pachychilids colonized India in middle Miocene according to fossils of *Brotia* obtained from Assam. Mani (1974) discusses the importance of Assam in being a ‘gateway’ for Southeast Asian lineages entering the peninsular Indian landmass. Moreover, the climate in Miocene was warm and humid, which would have allowed the passage of these gastropods across the currently inhospitable central Indian tracts.

3.2 *Vertebrates*

3.2.1 *Fishes:* Freshwater fish are an ideal model system to study Gondwanan biogeography as they are tied to the various drifting landmasses due to their inability to tolerate salt water. The Asian osteoglossiform fish arowana (*Scleropages formosus)* is particularly interesting. Osteoglossiformes are primary freshwater fishes and are distributed in tropical and subtropical regions of the world. Molecular work done by Kumazawa and Nishida (2000) suggest that the Asian arowana is more closely related to Australasian species than to South American species, suggesting that they might have dispersed into Asia from Australia. However, molecular dating suggests that the split between Asian and Australian arowana has occurred early, about 138 ± 18 mya. Based on molecular and geological evidence, Kumazawa and Nishida (2000) proposed a model according to which the Asian arowana vicariantly diverged from Australasian ancestors in the eastern margins of Gondwana and migrated into Eurasia from the Indian subcontinent. It is interesting to note that arowanas have not been reported from India, even though they may have carried this lineage over to Asia. It is plausible to assume that the arowanas went extinct in India due to aridification and drastic climate change that occurred in India upon collision with Eurasia (Karanth 2003).

Similar to the Asian arowanas, the phylogeny and distribution of apocheiloid fishes strongly indicates the role of vicariance (in this case, of the Indian and the Malagasy plate) for their diversification (Murphy and Collier 1997). In their phylogeny, the Indo-Malayan and Malagasy taxa branch together to the exclusion of a monophyletic South American-African clade. This topology indicates that the ancestors of the apocheiloid fishes were present on the drifting Indian landmass, where they diverged into species that we presently find in Asia. In addition, there has been a separate, independent radiation in Madagascar. It is to be noted that apocheiloid fishes are secondary freshwater fishes and therefore can tolerate brackish waters (Myers 1949; Briggs 2003b). Thus a transoceanic dispersal can be ruled out. The node representing the split between Indo-Malayan and Malagasy taxa needs to be dated to rigorously test the Out-of-India hypothesis.

Cichlid fishes of the order Persiformes have also been used as a model system to test the Out-of-India hypothesis and in general to study Gondwanan biogeography. The current distribution of these freshwater fishes is essentially Gondwanan. Various molecular phylogenies of cichlids support four distinct groups each corresponding to the various Gondwanan fragments i.e. South America, Africa, Madagascar, and South Asia. Additionally the South America- Africa forms branch together and Indo-Malagasy taxa form another clade (Chakrabarty 2004; Sparks 2004; Sparks and Smith 2004). Thus, these molecular phylogenies suggest that this group might have originated in the early Cretaceous and that their current distribution is attributable to the break-up of the Gondwana super continent (Chakrabarty 2004). Fossil data however, suggests that they are much younger, and may have originated in the Eocene or in Early Tertiary (Murray 2001). Additionally, molecular dating supports Cenozoic dispersal of cichlids rather than Mesozoic divergence (Vences *et al.* 2001).

3.2.2 *Amphibians:* A major chunk of studies pertaining to the Out-of-India hypothesis have dealt with amphibians. Bossuyt and Milinkovitch (2001) proposed that some amphibian lineages in tropical Asia are of Gondwanan origin, and were carried to Asia by drifting peninsular India and subsequently dispersed out of India. They produced a molecular phylogeny of Ranidae, and dated their nodes on the basis of India's separation from Madagascar. Amphibians generally have very low dispersal abilities (Bossuyt and Milinkovitch 2001) and their complete inability to disperse over salt water (Duellman and Trueb 1986; Bossuyt and Milinkovitch 2001, but *see* Vences 2003) makes them a good system for testing the Out-of-India hypothesis. The phylogenetic analysis and date estimates suggest that around six ranid lineages diverged on the drifting peninsular Indian landmass during late Cretaceous. This scenario is further supported by fossil evidence of ranids from peninsular India which date back to the late Cretaceous and early Eocene (Prasad and Rage 2004; Bajpai and Kapur 2008). When drifting Indian landmass established contact with Asia around 50 mya (Briggs 2003a) at least three of these lineages dispersed out of the Indian plate, (Bossuyt and Milinkovitch 2001; Bossuyt *et al.* 2006). These lineages include rhacophorine treefrogs, Raninae, and Dicroglossinae. The earliest fossil evidences of Raninae in the Eurasian landmass date back to 23-34 mya (Duellman and Trueb 1986) which is consistent with the dispersal of this group out of India post contact with Asia. Interestingly, Bossuyt and Milinkovitch (2001) also showed that at least three other frog lineages that are endemic to Western Ghats, namely Nyctibatrachinae, Micrixalinae, and Ranixalinae, are also of Gondwanan origin. These lineages did not disperse out of peninsular India and, thus, are ancient Gondwanan relicts.

The new frog genus *Nasikabatrachus* reported from the humid forests of the Western Ghats is yet another case of a Gondwanan relict that provides support for the biotic ferry model (Biju and Bossuyt 2003; Dutta *et al.* 2004). Molecular and anatomical studies suggested that this genus is related to members of the family Sooglossidae which are endemic to the Seychelles (Biju and Bossuyt 2003). *Nasikabatrachus* is currently placed in the Seychelle family Sooglossiade, and is the only known mainland member of this family (Frost *et al.* 2006). Molecular dating suggests that this genus has a very ancient evolutionary history that extends into the early Cretaceous. These molecular as well as morphological data support a scenario wherein members of the family Sooglossidae were present in the India-Madagascar-Seychelles landmass and were carried northwards after the separation of Madagascar and India/Seychelles. The Seychelles became subsequently separated from peninsular India resulting in the vicariant divergence between *Nasikabatrachus* and the rest of the sooglossids.

Caecilians are another group of amphibians that are ideally suited to test the Out-of-India hypothesis. In India, caecilians are represented by three families; Ichthyophiidae, Caeciliidae, and Uraeotyphlidae. Uraeotyphlidae are endemic to Western Ghats of South India whereas Ichthyophiidae are also distributed in Sri Lanka and Southeast Asia. Members of the Caeciliidae are represented by two genera in India, *Indotyphlus* from the Western Ghats and *Gegeneophis* from Western Ghats and Northeast India. Caeciliidae are widely distributed in other Gondwanan fragments, including South America, Africa and the Seychelles. Molecular phylogenetic studies on the caecilians of India by Wilkinson *et al.* (2002) suggest that the endemic Indian caeciliid *Gegeneophis ramaswami* is the sister taxon of all Seychelle caeciliids. This relationship between Indian and Seychelle caeciliids is consistent with the biotic ferry model as an explanation for the origin of the endemic Indian caeciliids. Additionally, in their phylogeny the families Ichthyophiidae and Uraeotyphlidae are sister taxa. This relationship is further supported by studies based on complete mitochondrial genome data (Mauro *et al.* 2004). Molecular dating places the split between these two families at around 94 mya also suggesting Gondwanan origin (Wilkinson *et al.* 2002). Thus among the three caecilian families in India two are Gondwanan relicts (Caeciliidae and Uraeotyphlidae) while the third family Ichthyophiidae has a Gondwanan origin and has dispersed out of India. This out of India dispersal of ichthyophiids is also supported by results of Gower *et al.* (2002) based on a more extensive sampling of this group throughout tropical Asia. In their phylogeny the Indian ichthyophiid lineages are basal to those from Southeast Asia.

3.2.3 *Reptiles:* Unlike amphibians, most reptiles are relatively resistant to salt water and have consequently been associated with numerous transoceanic dispersals (Raxworthy *et al.* 2002; Smith *et al.* 2007; Raselimanana *et al.* 2009). Chameleons (family Chamaeleonidae) are among the few extant vertebrates that have a distribution which appears congruent with the Gondwanan break-up of Madagascar and Africa. Molecular phylogenetic studies on 52 species of chameleons from Africa and Madagascar suggested that they originated in Madagascar after the fragmentation of Gondwana, and are thus are not a relict from the Gondwanan period (Raxworthy *et al.* 2002). In fact, the most parsimonious biogeographic explanation, which requires minimum dispersal and extinction events, supports a post-Gondwanan origin of chameleons in Madagacar. The study suggests ancient dispersals from Madagascar to Africa and a single more recent dispersal to India, bringing the only member of this group, *Chamaeleo zeylanicus* to India. Recently, Macey *et al.* (2008) proposed that the Indian chameleon dispersed into India from Africa via Arabia. Thus with respect to chameleons, molecular phylogeny supports a recent into India scenario, unlike that of the amphibians discussed above.

Another group of reptiles, the family Agamidae exhibit a contrastingly different biogeographical pattern (*see* Macey *et al.* 2000). Agamids are the sister group of the chameleons and occur mainly on land masses of Gondwanan origin. Work done by Macey *et al.* (2000) on agamids revealed three distinct clades, which largely correspond to the major Gondwanan landmasses of Australia-New Guinea, Indian subcontinent and Africa-West Asia. Many Southeast Asian agamids are nested within the Indian clade, suggesting an Indian origin (Out-of-India). Thus the tree topology does lend support to the Out-of-India hypothesis nevertheless alternative scenarios cannot be ruled out (Macey *et al.* 2000). Here again, dating of crucial nodes of this phylogeny might be helpful in choosing between various hypotheses.

3.2.4 *Birds:* Perhaps one of the most intriguing examples for the Out-of-India hypothesis comes from the ratites, a group of flightless birds. Living and recently extinct ratites were distributed in the southern continents and Gondwanan fragments including South America, Africa, Madagascar, Australasia and New Zealand. Cooper *et al.* (2001) confirmed the monophyly of ratites and the molecular estimates of their divergence supported Late Cretaceous vicariance of living and extinct ratites. Interestingly, their work suggested that ratites arrived in Indo-Madagascar through the Kerguelen plateau from Australia in the late Cretaceous, after which they dispersed out of India into Africa via Eurasia before going extinct in Asia. Molecular phylogenetic evidence, thus suggests an out of India and into Africa (and Asia) scenario, rather than out of Africa and into Asia. But this scenario needs to be viewed with caution as there is mounting evidence that suggest that the Kerguelen plateau was separated from other landmasses by wide seas and therefore might not have served as a dispersal route (Ali and Aitchison 2008). In this regard, Briggs (2003b) provides an alternative out of Africa and into India scenario to explain the presence of ostrich fossils in India.

4 Discussion

Our literature survey yielded around 18 publications spanning 21 taxonomic groups in which the biotic ferry model has been tested or suggested based on molecular phylogenetic approaches (table 1). At least 15 of these groups provide support for this model and for the Gondwanan origin of these taxa. It must be noted that in a few cases the support is ambiguous due to lack of information on the age of certain nodes. There are three cases where molecular data supports a more recent into India scenario rather than out of India. In three other groups the biotic ferry model could not be conclusively proved due to various reasons (table 1). Among the taxa with a Gondwanan origin, around nine lineages have dispersed out of India (Out-of-India hypothesis) and the remaining six lineages are confined to peninsular India

Table 1. Molecular studies done on Asian taxa of putative Gondwanan (Gond.) origin and their status with respect to "Out-of-India" hypothesis

Taxa	Status	Cat.	References
Plants			
Asian dipterocarps	Out-of-India[2]	*	Dayanandan *et al.* 1999
Cypteroniaceae[1]	Out-of-India	B	Conti et al. 2002; Rutschmann *et al.* 2004
Melastomataceae	Into India[3]	C	Renner 2004
Rhododendron	Out-of-India[2,3]	*	Brown *et al.* 2006
Freshwater Gastropod			
Pachychilidae	Into India	C	Köhler and Glaubrecht 2007
Fishes			
Asian arowanas[1]	Out-of-India	A	Kumazawa *et al.* 2000
Apocheiloid fishes	Out-of-India[2]	*	Murphy et al. 1997
Cichlid fishes	Out-of-India[4]	B	Sparks et al 2004, Chakrabarty 2004
Amphibians (frogs)			
Rhacophorine	Out-of-India	A	Bossuyt and Milinkovitch 2001
Raninae	Out-of-India	A	Bossuyt and Milinkovitch 2001
Dicroglossinae	Out-of-India	A	Bossuyt and Milinkovitch 2001
Nyctibatrachinae	Gond. relict	A	Bossuyt and Milinkovitch 2001
Micrixalinae	Gond. relict	A	Bossuyt and Milinkovitch 2001
Ranixalinae	Gond. relict	A	Bossuyt and Milinkovitch 2001
Nasikabatrachidae	Gond. relict	A	Biju and Bossuyt 2003; Dutta *et al.* 2004
Amphibians (caecilians)			
Ichthyopiidae	Out-of-India	A	Gower et al. 2002; Wilkinson *et al.* 2002
Caeciliidae	Gond. relict	A	Wilkinson *et al.* 2002
Uraeotyphlidae	Gond. relict	A	Wilkinson *et al.* 2002
Reptiles			
Chameleon	Into India	C	Raxworthy *et al.* 2002; Macey *et al.* 2008
Agamid lizards	Out-of-India[2,5]	*	Macey *et al.* 2000
Birds			
Ratites (Ostrich)[1]	Out-of-India	B?	Cooper *et al.* 2001

For details on category (Cat.) assignment see text. Star indicates insufficient information for category assignment. ?, Category assignment doubtful.
[1]Taxa currently extinct in India.
[2]Molecular dating not available.
[3]Insufficient taxon sampling.
[4]Molecular dating suggest recent origin.
[5]Alternate explanations equally likely.

(Gondwanan relicts). Interestingly, in three cases taxa have gone extinct in India after dispersing out of India.

The Indian subcontinent is placed in the Oriental biogeographic realm along with Southeast Asia (Wallace 1876), though it contains elements from various other biogeographic realms (Mani 1974) that have colonized the subcontinent at various times. The taxa that were already present on the Indian plate during Mid to Late Cretaceous period are of Gondwanan origin. During the northern drift of Indian plate, after the separation from Madagascar and before the collision with Eurasia, it probably underwent biotic exchange with Africa, Asian and other landmasses. This scenario is supported by late Mesozoic fossil record of lizards, frogs and mammals from India that show affinity with fossils from Africa, South America and Asia (Chatterjee and Scotese 1999). To accommodate this scenario authors have either invoked close proximity (~ 500 kms) of northward rafting peninsular India to the east coast of Africa (e.g. Briggs 2003a) or intermittent land bridge connections between peninsular India and other landmasses before collision with Eurasia (Hedges 2003; Chatterjee and Scotese 1999). There is also some molecular evidence for this relaxed continental drift model (Bocxlaer *et al.* 2006). Thus, during its northward movement, peninsular India not only carried with it ancient Gondwanan elements but also acquired other elements while drifting close to other land masses. Some of these forms dispersed out of India when peninsular India made contact with the Asian plate (Out-of-India hypothesis), while others have remained on peninsular India. The component of the ancient Gondwanan elements that are confined to peninsular India represent the Gondwanan relicts (table 1). Still other Gondwanan forms dispersed out of India but subsequently went extinct in India. A subset of lineages that had differentiated in Africa, Eurasia and tropical Asia dispersed into peninsular India when contact between India and Asian was established. Given this complex biogeographical history, the biota of India can be divided into three broad categories: pre-contact ancient Gondwanan elements with origin from Mid to Late Cretaceous, groups representing post break-up and pre-contact dispersers from Africa-Madagascar/Eurasia/Australia, and post-contact recent dispersers from Africa and Asia (Mani 1974; Karanth 2006).

In order to better understand the Out-of-India component of Asian biota, it is imperative that we understand the biogeographical history of India keeping in mind the three broad categories discussed above. Figure 1 presents an oversimplification of these three categories in a phylogenetic framework. Category A, represent the ancient Gondwanan elements of India whose origin can be traced back to the time when the Indian plate was still attached to one or more Gondwanan fragments (Madagascar/Australia/Africa). This scenario would predict a sister group relationship between Indian taxa and those from one of the Gondwanan fragments. Additionally the divergence date between these sister clades would correspond to the time when the two landmasses became separated, for example, an Indo-Malagasy split would date to around 80 mya (time point 2 and node w in figure 1A). Asian elements that have dispersed out of India after contact with Asia would be nested within a larger Indian clade and this dispersal event would have dated back to a period of no more than 50 mya (time point 3 and node y in figure 1A). Category B, includes taxa that have dispersed from Africa-Madagascar/Eurasia/Australia into drifting peninsular India subsequent to the separation from Madagascar, but prior to the collision with Asia. Here the Indian clade would branch with taxa from Africa-Madagascar/Eurasia/Australia and the divergence date between these two clades is predicted to be between 80-50 my (between times point 2 and 3, node x in figure 1B). Elements that have dispersed out of India, post-contact would be nested within a larger Indian clade. This dispersal event is predicted to have occurred around 50 mya or less (time point 3 and node y in figure 1B). Category C represents taxa that have dispersed into India after peninsular India collided with Asia. Indian taxa are nested within a clade of taxa from tropical Asia. The age of this dispersal event would be less than 50 million years (time point 3 and node z in figure 1C). These explicit phylogenetic predictions could be used to identify Gondwanan components of the Indian/Asian biota. Extant taxa of putative Gondwanan origin must be subjected to these phylogenetic analyses to establish their pre-contact origin (i.e. to exclude the possibility of scenario C in figure 1). Once the pre-contact biota are identified, the next step would be to determine whether these forms are confined to peninsular Indian (Gondwanan relicts) or have dispersed out of India. Based on the information available 17 out of the 21 taxonomic groups could be assigned to various categories (table 1). Amphibians and freshwater fishes are usually intolerant to salt water and are often tied down to the various land masses. Thus they are predicted to exhibit the pattern illustrated in category A as is apparent from table 1. Where as in the case of plants post break-up dispersal into Indian plate (category B) has been inferred. In four cases categories could not be assigned due to lack of information on divergence date and incomplete taxon sampling. Chameleons, gastropods and some species of the plant family Melastomataceae fall under category C. These represent recent post contact into India component of the Indian biota.

Studies reviewed here illustrate the importance of molecular phylogenetic approach in conjunction with molecular dating in addressing questions pertaining to Gondwana biogeography. In this regard it must be pointed that molecular dating of relevant nodes is fraught with numerous issues. Firstly finding suitable fossils to calibrate the molecular clock might be problematic. Secondly, assigning the fossil to the appropriate node might be difficult due to ambiguous classification of the fossil. This in turn might result in either over or under estimation of various divergence dates. Additionally properties inherent to molecular data such as rate heterogeneity among branches and among site rate variation might introduce error in the divergence date estimates (Yoder and Yang 2000; Arbogast *et al.* 2002; Near *et al.* 2005; Drummond *et al.* 2006). To address these issues the use of multiple markers along with multiple calibration points have been recommended (Near *et al.* 2005; Drummond *et al.* 2006). Given these issues pertaining to molecular dating our confidence in a particular hypothesis can be enhance further by incorporating information on the life history trait and biology of the species group under study. For example if the phylogeny and divergence date estimates of a taxonomic group are consistent with category B (figure 1) then transoceanic dispersal is invoked to explain the occurrence of that group in peninsular India. This conclusion can be strengthened further if it is established

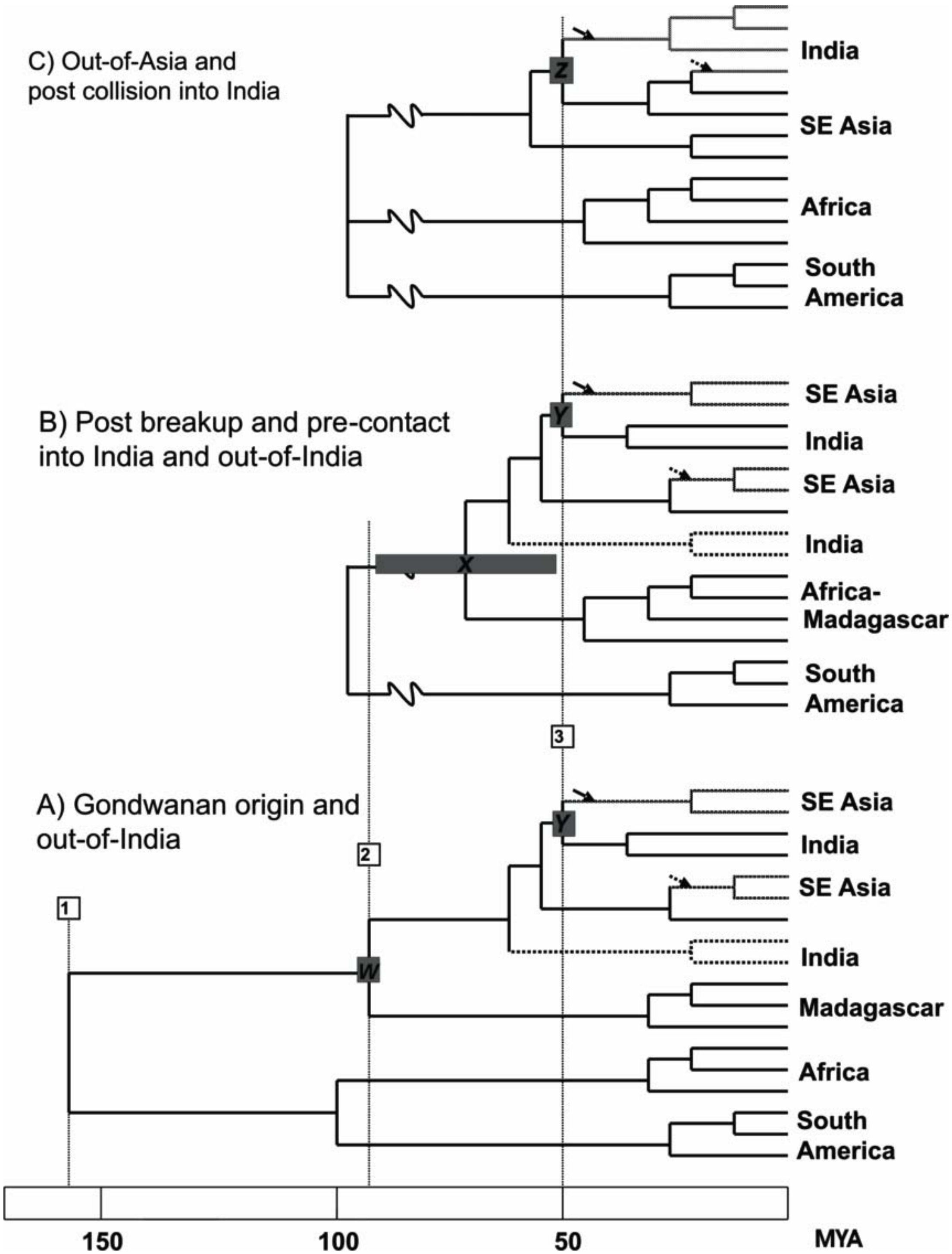

Figure 1. Phylogenetic predictions for various biogeographical scenarios discussed in the paper. Numbers in box correspond to approximate dates for various geological events during Gondwana break up: **(1)** separation of Indo-Malagasy from Africa (160 mya); **(2)** separation of India from Madagascar (80 mya); **(3)** collision of Indian plate with Asia (50 mya). Grey bars and shaded squares represent the expected age of important nodes w, x, y and z under different biogeographical scenarios. Arrows indicate recent (dotted) and older (bold) dispersal events, either out of India **(A** and **B)** or into India **(C)**. Dotted branches represent Gondwanan relicts, these taxa do not have counterparts in Asia. Grey branches represent lineages that have either dispersed out of India into Asia or into India from Asia. MYA, Million Years Ago.

that the taxonomic group in question can withstand salt water such as in the case of some reptiles (Raxworthy *et al.* 2002; Smith *et al.* 2007; Raselimanana *et al.* 2009). Alternately the species might exhibit other biological features that could help in long distance dispersal such as wind or migratory bird dispersal of plant seed (Renner 2004). In the case of species group that exhibit the scenario outlined in category A one would predict these species to have very low dispersal ability and/or exhibit other biological constraint such as salt water intolerance. Information on such life history traits of a species can easily be collected from extant species.

It is also apparent that for a coherent understanding of this fascinating pattern a multidisciplinary approach is needed wherein information from diverse fields such as palaeontology, geology, taxonomy, biogeography, molecular phylogeny and ecology are used. Clearly, collaboration between scientists from these fields is vital for such an endeavor. For example, palaeontological data is an important component in phylogenetic analysis. Firstly, fossil data from Jurassic-Cretaceous period will help us shortlist extant candidate species for phylogenetic analysis that could have Gondwanan origin. Secondly, fossil data will also help date important nodes on the phylogenetic trees of extant species. It is important that the calibration of the molecular clock be independent of the various geological events corresponding to break-up of Gondwana to avoid circularity in dating estimates. Furthermore for complete taxon sampling of putative Gondwanan species international collaborations have to be established (Beheregaray 2008).

The review presented here is based on few studies and cover a small number of taxa, nevertheless it is apparent from these studies that molecular tools in conjunction with geological and palaeontological data are valuable in studies pertaining to Gondwana biogeography.

Acknowledgements

We would like to thank members of the Karanth laboratory and two reviewers for their intellectual input and critical comments. This work was partly funded by grants from Ministry of Environment and Forest, New Delhi.

References

Ali J R and Aitchison J C 2008 Gondwana to Asia: Plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166–35 Ma); *Earth Sci. Rev.* **88** 145–166

Arbogast B S, Edwards S V, John W and Slowinski J B 2002 Estimating divergence times from molecular data on phylogenetic and population genetic timescales; *Annu. Rev. Ecol. System.* **33** 707–740

Bajpai S and Kapur V V 2008 Earliest cenozoic frogs from the Indian subcontinent: Implications for out-of-India hypothesis; *J. Palaeontol. Soc. India* **53** 65–71

Bande M B 1992 The Palaeogene vegetation of peninsular India (megafossil evidence); *Palaeobotanist* **40** 275–284

Beheregaray L B 2008 Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere; *Mol. Ecol.* **17** 3754–3774

Biju S D and Bossuyt F 2003 New frog family from India reveals an ancient biogeographical link with the Seychelles; *Nature (London)* **425** 711–714

Blanford W T 1901 The distribution of vertebrate animals in India, Ceylon, and Burma; *Philos. Trans. R. Soc. London Series B (Containing Papers of a Biological Character)* **194** 335–436

Bocxlaer I V, Roelants K, Biju S D, Nagaraju J and Bossuyt F 2006 Late Cretaceous vicariance in Gondwanan amphibians; *Plos One* **74** 1–6

Bossuyt F, Brown R M, Hillis D M, Cannatella D C and Milinkovitch M C 2006 Phylogeny and biogeography of a cosmopolitan frog radiation: Late Cretaceous diversification resulted in continent-scale endemism in the family Ranidae; *System. Biol.* **55** 579–594

Bossuyt F and Milinkovitch M C 2001 Amphibians as Indicators of Early Tertiary "Out-of-India" dispersal of vertebrates; *Science* **292** 93–95

Briggs J C 2003a The biogeographic and tectonic history of India; *J. Biogeogr.* **30** 381–388

Briggs J C 2003b Fishes and Birds: Gondwana Life Rafts Reconsidered; *System. Biol.* **52** 548–553

Briggs J C 1989 The historic biogeography of India: Isolation or contact?; *System. Zool.* **3** 322–332

Brown G K, Nelson G and Ladiges P Y 2006 Historical biogeography of *Rhododendron* section *Vireya* and the Malesian Archipelago; *J. Biogeogr.* **33** 1929–1944

Chakrabarty P 2004 Cichlid biogeography: comment and review; *Fish Fisheries* **5** 97–119

Chatterjee S and Scotese C 1999 The breakup of Gondwana and the evolution and biogeography of the Indian Plate; *Proc. Indian Natl. Sci. Acad.* **A65** 397–425

Clyde W C, Khan I H and Gingerich P D 2003 Stratigraphic response and mammalian dispersal during initial India-Asia collision: Evidence from the Ghazij Formation, Balochistan, Pakistan; *Geology* **31** 1097–1100

Conti E, Eriksson T, Schönenberger J, Sytsma K J and Baum D A 2002 Early Tertiary Out-of-India Dispersal of Crypteroniaceae: Evidence from phylogeny and molecular dating; *Evolution* **56** 1931–1942

Cooper A, Lalueza-Fox C, Anderson S, Rambaut A, Austin J and Ward R 2001 Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution; *Nature (London)* **409** 704–707

Dayanandan S, Aston P S, Williams S M and Primack R B 1999 Phylogeny of the tropical tree family Dipterocarpaceae based on nucleotide sequences of the chloroplast rbcL gene; *Am. J. Bot.* **86** 1182–1190

Drummond A J, Ho S Y W, Phillips M J and Rambaut A 2006 Relaxed phylogenetics and dating with confidence; *PLoS Biol.* **4** 699–710

Ducousso M, Béna G, Bourgeois C, Buyck B, Eyssartier G, Vincelette M, Rabevohitra R, Randrihasipara L, Dreyfus B and Prin Y 2004 The last common ancestor of Sarcolaenaceae and Asian dipterocarp trees was ectomycorrhizal before the India–Madagascar separation, about 88 million years ago; *Mol. Ecol.* **13** 231–236

Duellman W E and Trueb L1986 *Biology of Amphibians* (Baltimore: Johns Hopkins University Press) pp 477–492

Dutta S K, Vasudevan K, Chaitra M S, Shanker K and Aggarwal R K 2004 Jurassic frogs and the evolution of amphibian endemism in the Western Ghats; *Curr. Sci.* **86** 211–216

Frost D R, Grant T, Faivovich J, Bain R H, Haas A, Haddad C F, RafaelO D S, Channing A, Wilkinson M, Donnellan S C, Raxworthy C J, Campbell J A, Blotto B L, Moler P, Drewes R C, Nussbaum R A, Lynch J D, Green D M and Wheeler W C 2006 The Amphibian tree of life; *Bull. Am. Mus. Nat. Hist.* **297** 8–370

Gower D J, Kupfer A, Oommen O V, Himstedt W, Nussbaum R A, Loader S P, Presswell B, Müller H, Krishna S B, Boistel R and Wilkinson M 2002 A molecular phylogeny of ichthyophiid caecilians(Amphibia: Gymnophiona: Ichthyophiidae): Out of India or out of South East Asia?; *Proc. R. Soc. London B Biol. Sci.* **269** 1563–1569

Hedges S B 2003 The coelacanth of frogs; *Nature (London)* **425** 669–670

Hora S L 1949 Satpura hypothesis of the distribution of Malayan fauna and flora of peninsular India; *Proc. Natl. Inst. Sci. India* **15** 309–314

Jayaram K C 1949 Distribution of Lizards of peninsular India with Malayan affinities; *Proc. Natl. Inst. Sci. India* **15** 403–409

Karanth K P 2006 Out-of-India Gondwanan origin of some tropical Asian biota; *Curr. Sci.* **90** 789–792

Karanth K P 2003 Evolution of disjunct distributions among wet-zone species of the Indian subcontinent: Testing various hypotheses using a phylogenetic approach; *Curr. Sci.* **85** 1276–1283

Keast A 1971 Continental drift and the evolution of the biota on Southern Continents; *Q. Rev. Biol.* **46** 335–378

Krishnan M 1974 Geology; in *Ecology and biogeography in India* (ed.) M S Mani (The Hague: Dr W Junk Publisher) pp 60–98

Kumazawa Y and Nishida M 2000 Molecular phylogeny of osteoglossoids: A new model for Gondwanian origin and plate tectonic transportation of the Asian arowana; *Mol. Biol. Evol.* **17** 1869–1878

Köhler F and Glaubrecht M 2003 Morphology, reproductive biology and molecular genetics of ovoviviparous freshwater gastropods (Cerithioidea, Pachychilidae) from the Philippines, with description of a new genus *Jagora*.; *Zool. Scripta* **33** 33–59

Köhler F and Glaubrecht M 2006 A systematic revision of the Southeast Asian freshwater gastropod *Brotia* (Cerithioidea: Pachychilidae); *Malacologia* **48** 159–251

Köhler F and Glaubrecht M 2007 Out of Asia and into India: on the molecular phylogeny and biogeography of the endemic freshwater gastropod *Paracrostoma* Cossmann, 1900 (Caenogastropoda: Pachychilidae); *Biol. J. Linn. Soc.* **91** 621–657

Lakhanpal R N 1970 Tertiary Floras of India and their bearing on the historical geology of the region; *Taxon* **19** 675–694

Macey J R, Kuehlc J V, Larsond A, Robinsone M D, Ugurtasf I H, Ananjevag N B, Rahmanh H, Javedi H I *et al.* 2008 Socotra Island the forgotten fragment of Gondwana: Unmasking chameleon lizard history with complete mitochondrial genomic data; *Mol. Phylog. Evol.* **49** 1015–1018

Macey J R, Schulte J A, Larson A, Ananjeva N B, Wang Y Z, Pethiyagoda R, Rastegar-Pouyani N and Papenfuss T J 2000 Evaluating trans-tethys migration: An example using acrodont lizard phylogenetics; *System. Biol.* **49** 233–256

Mani M S (ed.) 1974 Biogeography of Peninsula; in *Ecology and biogeography in India* (The Hague: Dr W Junk Publishers) pp 614–646

Mauro D S, Gower D J, Oommen O V, Wilkinson M and Zardoya R 2004 Phylogeny of caecilian amphibians (Gymnophiona) based on complete mitochondrial genomes and nuclear RAG1; *Mol. Phylog. Evol.* **33** 413–427

McKenna M C 1995 The Mobile Indian Raft: A reply to Rage and Jaeger; *System. Biol.* **44** 265–271

Morley R J and Dick C W 2003 Missing fossils, molecular clocks and the origin of the Melastomataceae; *Am. J. Bot.* **90** 1638–1645

Murphy W J and Collier G E 1997 A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): The role of vicariance and the origins of annualism; *Mol. Biol. Evol.* **14** 790–799

Murray A M 2001 The fossil record and biogeography of the Cichlidae (Actinopterygii: Labroidei); *Biol. J. Linn. Soc.* **74** 517–532

Myers G 1949 Salt tolerance of freshwater fish groups in relation to zoogeographic problems; *Bijdr. Dierkd.* **28** 315–322

Near T J, Meylan P A and Shaffer H B 2005 Assessing concordance of fossil calibration points in molecular clock studies: An example using turtles; *Am. Nat.* **165** 137–146

Prasad G V R and Rage J-C 2004 Fossil frogs (Amphibia: Anura) from the Upper Cretaceous intertrappean beds of Naskal, Andhra Pradesh; *Rev. Paléobiol.* **23** 99–116

Rage J C 1996 Le peuplement animal de Madagascar: unecomposante venue de Laurasie est-elle envisageable?; in *Biogéographie de Madagascar* (ed.) W R Lourenco (Paris: ORSTOM) pp 27–35

Raselimanana A P, Noonan B, Karanth K P, Gauthier J and Yoder A D 2009 Phylogeny and evolution of Malagasy plated lizards; *Mol. Phylog. Evol.* **50** 336–344

Raxworthy C J, Forstner M R J and Nussbaum R A 2002 Chameleon radiation by oceanic dispersal; *Nature (London)* **415** 784–787

Renner S S 2004 Multiple Miocene Melastomataceae Dispersal between Madagascar, Africa and India; *Philosophical Transactions: Biol. Sci.* **359** 1485–1494

Rutschmann F, Eriksson T, Schonenberger J and Conti E 2004 Did Crypteroniaceae really disperse out of India? Molecular dating evidence from rbcl, ndhf, and rpl16 intron sequences; *Int. J. Plant Sci.* **165** S69–S83

Smith S A, Sadlier R A, Bauer A M, Austin C C and Jackman T 2007 Molecular phylogeny of the scincid lizards of New Caledoniaand adjacent areas: Evidence for a single origin of the endemic skinks of Tasmantis; *Mol. Phylog. Evol.* **43** 1151–1166

Sparks J S 2004 Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae); *Mol. Phylog. Evol.* **30** 599–614

Sparks J S and Smith W L 2004 Phylogeny and biogeography of the Malagasy and Australasian rainbowfishes (Teleostei: Melanotaenioidei): Gondwanan vicariance and evolution in freshwater; *Mol*. *Phylog*. *Evol*. **33** 719–734

Vences M, Freyhof J, Sonnenberg R, Kosuch J and Veith M 2001 Reconciling fossils and molecules: Cenozoic divergence of cichlid fishes and the biogeography of Madagascar; *J. Biogeogr*. **28** 1091–1099

Vences M, Vieites D R, Diesmos A, Glaw N A F, Brinkmann H, Kosuch J, Veith M and Meyer A 2003 Multiple overseas dispersal in amphibians; *Proc*. *R. Soc*. *London* **B270** 2435–2442

Wallace A R 1876 *The geographical distribution of animals* (London: Harper and Brothers)

Wilkinson M, Sheps J A, Oommen O V and Cohen B L 2002 Phylogenetic relationships of Indian caecilians (Amphibia: Gymnophiona) inferred from mitochondrial rRNA gene sequences; *Mol*. *Phylog*. *Evol*. **23** 401–407

Williams E G and Rouse J L 1997 Evolutionary history and speciation in section *Vireya*.; in *Proceedings of the 1994 Pacific Region International Rhododendron Conference*, Burnie, Tasmania, Australia, (ed.) N Jordan, pp 52–60

Yoder A D and Yang Z 2000 Estimation of primate speciation dates using local molecular clocks; *Mol*. *Biol*. *Evol*. **17** 1081–1090

*e*Publication: 26 October 2009