

Tectonic calibrations in molecular dating

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Abstract Molecular dating techniques require the use of calibrations, which are usually fossil or geological vicariance-based. Fossil calibrations have been criticised because they result only in minimum age estimates. Based on a historical biogeographic perspective, I suggest that vicariance-based calibrations are more dangerous. Almost all analytical methods in historical biogeography are strongly biased towards inferring vicariance, hence vicariance identified through such methods is unreliable. Other studies, especially of groups found on Gondwanan fragments, have simply assumed vicariance. Although it was previously believed that vicariance was the predominant mode of speciation, mounting evidence now indicates that speciation by dispersal is common, dominating vicariance in several groups. Moreover, the possibility of speciation having occurred before the said geological event cannot be precluded. Thus, geological calibrations can under- or overestimate times, whereas fossil calibrations always result in minimum estimates. Another major drawback of vicariant calibrations is the problem of circular reasoning when the resulting estimates are used to infer ages of biogeographic events. I argue that fossil-based dating is a superior alternative to vicariance, primarily because the strongest assumption in the latter, that speciation was caused by the said geological process, is more often than not the most tenuous. When authors prefer to use a combination of fossil and vicariant calibrations, one suggestion is to report results both with and without inclusion of the geological constraints. Relying solely on vicariant calibrations should be strictly avoided [*Current Zoology* 57 (1): 116–124, 2011].

Key words Molecular dating, Calibrations, Fossils, Vicariance, Paleontological, Relaxed clock

1 Introduction

Methods of dating nodes on molecular phylogenies provide crucial insights into the temporal framework of macroevolutionary processes. Molecular dating has been used to investigate a broad range of biogeographic hypotheses, causes of rapid radiations, age of the last common ancestor of HIV, etc (Rutschmann, 2006). The principle behind molecular dating is that sequence divergence between clades, represented by branch lengths on a molecular phylogeny, is proportional to the time of evolution since their separation (Zuckermandl and Pauling, 1965). This makes it possible to use relative branch length information to estimate lineage divergence times. Early dating techniques assumed a constant substitution rate across time and lineages i.e. a 'strict molecular clock' (Felsenstein, 1981). The known age of a node is used as a calibration to estimate global rates of substitutions, based on which divergence times at all nodes can be extrapolated. However, it is known that most real world datasets deviate from a strict molecular clock (Britten, 1986; Welch and Bromham, 2005). Current dating methods can assume a 'relaxed clock', where rate heterogeneity is taken into account through

modelling (Aris-Brosou and Yang, 2002; Sanderson, 2002; Thorne and Kishino, 2002). Rutschmann (2006) provides an excellent synthesis of the theory behind different methods.

A dating exercise necessarily involves incorporation of one or more calibrations derived from external sources. The choice of calibration determines to a large extent the accuracy of the dating analysis. Readers are referred to Heads (2005) for a discussion of the commonly employed calibrations. Paleontological data are most common, typically as a minimum age constraint. If a taxon is represented by a fossil of known age, it must be at least as old as the fossil's age. This age can be used to specify the minimum age of origin of the taxon, i.e. the earliest time of divergence from its sister clade. The disadvantage with fossil-based dating estimates is that they are only minimum estimates and the true age remains unknown (Benton and Donoghue, 2007). They have, however, often been treated as absolute times, a practice that has been criticised strongly (Graur and Martin, 2004; Heads, 2005).

Geological information is another commonly used calibration source. A vicariant event is assumed to have resulted in a disjunct distribution pattern of a pair of

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sister taxa. The time of the most likely geological event that triggered the vicariant process is used to constrain the age of the node of divergence between the sister taxa. This procedure has been widely applied, to taxa as varied as birds, insects, amphibians, plants, fishes, mammals, etc (Table 1). I here argue that fossil calibrations should be preferred over this method, and the latter should be avoided. This is primarily because it is extremely difficult to infer with confidence that the geological process in fact resulted in vicariant speciation (Upchurch, 2008).

2 Vicariance Identified through Analytical Methods in Historical Biogeography

Vicariant speciation events are often inferred using analytical methodology in historical biogeography. The principles behind most methods are rooted in the school of vicariance biogeography, where vicariance is assumed to be the null-hypothesis for an explanation of disjunct distributions (Wiley, 1988). All such methods are inherently biased towards vicariance (Kodandara-

maiah, 2009a). When a geological change leads to vicariance between two areas, it is reasonable to expect that several groups of organisms are similarly affected. GAC (General Area Cladogram) based methods assume that a particular disjunct distribution pattern repeated across more than two taxa is the result of vicariance (Kodandaramaiah, 2009a). However, this assumption is often invalidated (Lieberman, 2003; Cook and Crisp, 2005). Concordant dispersal between two areas (also called ‘geo-dispersal’ in the case of dispersal between two terrestrial areas) leads to the same pattern of disjunctions as vicariance. Moreover, such methods are susceptible to ‘pseudo-congruence’ (Donoghue and Moore, 2003), where multiple events occurring at different points in time lead to the same observed extant pattern. It follows that a vicariance-based calibration reliant on such methods can result in spurious timing results.

The most popular analytical method has been DIVA (Dispersal Vicariance Analysis; Ronquist, 1997), which uses data from a single taxon to reconstruct the most likely ancestral distributions over the nodes in the phylogeny. This reconstruction is used by the user to infer

Table 1 Examples of different types of vicariance-based calibrations employed in molecular dating analyses

Calibration source	Publication	Taxon
New Zealand-Australia rifting	Ericson et al. 2002	Birds
	Roelants and Bossuyt 2005	Amphibians
	Cooper et al. 2001	Birds
	Pellmyr and Leebens-Mack 1999	Insects
	Renner et al. 2000	Plants
Madagascar-India rifting	Bossuyt and Milinkovitch 2001	Amphibians
	Braby et al. 2005	Insects
Africa-South America rifting	Renner et al. 2000	Plants
	Roelants et al. 2007	Amphibians
	Pellmyr and Leebens-Mack 1999	Insects
Separation of continental North American from Baja California	Becerra 2003	Plants
North America-Eurasia rifting	Zakharov et al. 2004	Insects
Separation of New Caledonia from Australia	Crisp et al. 2004	Plants
Isolation of Crete (Messinian Salinity Crisis)	Nazari et al. 2007	Insects
Isolation of Balearic Islands (Messinian Salinity Crisis)	Lalueza-Fox et al. 2005	Mammals
Separation of the Sardinia-Corsica microplate	Caccone and Sbordoni 2001	Insects
	Caccone et al. 1994	Amphibians
	Ketmaier et al. 2003	Snails
Changes in drainage pattern of rivers	Waters et al. 2007	Freshwater fish
	Stillman and Reeb 2001	Crustaceans
Rise of the Panamanian Isthmus	Robles et al. 2007	Crustaceans
	Teskey and Beheregaray 2009	Marine teleosts
	Birmingham and Lessios 1993	Echinoderms
	McCartney 2000	Echinoderms
	Lee and Foighil 2005	Molluscs
	Duda and Kohn 2005	Molluscs
	Craig et al. 2004	Marine fish (serranids)
	Duncan et al. 2006	Marine fish (sharks)

dispersal and vicariance events at particular nodes. The reconstruction algorithm used by DIVA seeks to minimize the number of dispersals, which renders the method partial towards inference of vicariant scenarios (Kodandaramaiah, 2009b). Furthermore, unless lineage divergence times are known, one cannot distinguish between vicariance and dispersal at most nodes as both scenarios are represented by the same ancestral area reconstruction (Kodandaramaiah, 2009b). Hence, vicariant events identified using results from a DIVA analysis are unreliable and not suitable for use in dating analyses (e.g., Braby, et al. 2005; Bocxlaer et al., 2006).

3 Vicariance Assumed without Analytical Methodology.

Vicariance is presumed to be the most likely (or the only) explanation for a given distribution pattern, best exemplified by putative Gondwanan groups. Taxa are assumed to be incapable of crossing geological barriers such as oceans, regardless of their vagility potential. In the case of groups found on landmasses of Gondwanan origin, speciation is presumed to be the result of plate tectonic movements rather than post-breakup dispersal. While there was some consensus in the latter part of the 20th Century about the predominance of vicariance, it is now becoming increasingly clear that trans-oceanic dispersals are much more common than previously assumed (de Queiroz, 2005). This has been demonstrated even in groups such as amphibians that have traditionally been considered to be highly sedentary and incapable of dispersing across marine barriers (Evans et al., 2003; Vences et al., 2003). The assumption of vicariant speciation where the true mode of speciation was a more recent dispersal leads to spuriously older timing estimates. At the same time, one cannot preclude the possibility that speciation proceeded allopatrically *before* the presumed geological event. In effect, vicariance-based dating analyses can only provide minimum estimates, similar to fossil-based dating. This is rarely appreciated in literature; timing estimates are most often regarded as absolute ages.

The perils of this paleogeographic calibration approach are well illustrated by examples relating to the putative vicariance of New Zealand (NZ) biota. NZ has remained isolated since ca. 80 my (million years), following its split from Australia (McLoughlin, 2001). NZ's biota was long thought to be composed of Gondwanan relict groups and hence presented a strong case for the use of the 80 my time as a calibration point. This has been enforced in several analyses (Cooper et al.,

2001; Ericson et al., 2002; Roelants and Bossuyt, 2005). Waters and Craw (2006) reviewed evidence for vicariant origins of numerous NZ taxa and concluded that there was little evidence for Gondwanan vicariance. A large proportion of NZ's biota is now thought to have evolved as a result of dispersal in the Oligocene (Worthy and Holdaway, 2002). Even the plant genus *Nothofagus* (Southern Beeches), previously a paradigmatic example of Gondwanan vicariance, is considered to have reached NZ through dispersal (Knapp et al., 2005). Consequently, the use of the 80 my calibration can lead to erroneously old lineage divergence times over the phylogeny (de Jong, 2007). It is worth noting that the results from a recent paper (Phillips et al., 2010) indicate that the split between the Australian and New Zealand clades of ratite birds occurred about 60 mya (million years ago), which is inconsistent with assumption of vicariance used in Cooper et al. (2001). Contrastingly, some NZ groups (e.g. tuataras, frogs) are in fact *older* than the 80 mya age as evidenced by paleontological data (Waters and Craw, 2006). In such cases, the calibration would work reasonably well, but *only if* the resulting age estimates are treated as minimum ages.

The most compelling examples of vicariance are from Gondwanan groups, and hypothetical tectonic vicariant events have naturally been the most popular paleogeographic calibrations. As in the case of NZ's biota, it is becoming increasingly apparent that many groups hitherto believed to be Gondwanan in origin evolved much later (McDowall, 2002; Raxworthy et al., 2002; de Queiroz, 2005; Knapp et al., 2005; Barker et al. 2007; Trénel et al., 2007). Other putative causal events such as the Messinian Salinity Crisis, the rise of the Panamanian Isthmus, separation of microplates, etc have also been applied (Table 1). In essence, calibrations reliant on vicariance assume that the taxon in question speciated passively and is completely (or almost completely) incapable of crossing the said geological barrier. Given the overwhelming data on the prevalence of dispersal in nature, how confident can we be in this assumption? The chances of the true speciation event having occurred before or after the presumed geological event are unacceptably high.

The presumed vicariance between Pacific and Atlantic marine taxa caused by the rise of the Isthmus of Panama (ca. 3.1 to 3.5 mya) has been hugely popular. Lessios (2008) states 'as of January 2008, there have been 251 studies that used a calibration obtained from the isthmian schism to date phylogenetic events that have occurred elsewhere'. Numerous authors believe that at

least some geminate clades distributed disjunctly on either side of the Isthmus diverged much before the closure of the Isthmus, possibly through isolation-by-distance (de Weerd, 1990; Jackson et al., 1993; Knowlton and Weigt, 1998; Banford et al., 1999; Muss et al., 2001; Marko, 2002; Heads, 2005; Lessios, 2008). The use of this calibration source provides a classic example where divergence may have preceded the hypothetical vicariant event.

4 Circular Reasoning in Biogeography

Timing estimates from molecular dating are commonly used to infer ages of historical biogeographic events within a taxon. Using ages derived from vicariant calibrations to support or reject biogeographic hypotheses amounts to circular reasoning (Yoder and Nowak, 2006; de Jong, 2007) in so far as vicariance is in itself a biogeographic hypothesis. It can thus be argued that vicariant calibrations are to be avoided completely if one wants to infer ages of biogeographic events without entering into the realm of circularity. Examples of studies that wrongly used geological events in such an exercise include Caccone et al. (1994), Caccone and Sbordoni (2001), Ketmaier et al. (2003) and Braby et al. (2005).

5 Vagility of the Taxon in Relation to the Probability of Vicariance

The vagility potential of a taxon in relation to a given barrier may be related to its propensity for vicariant speciation *vis-a-vis* speciation by dispersal (Bouchard and Brooks, 2004; Kodandaramaiah, 2009a). As an example, one can expect that the formation of a narrow marine barrier has a higher probability of resulting in vicariance in amphibians compared to more vagile taxa such as birds or butterflies (Kodandaramaiah, 2009a). This is because flying animals are more likely to be able to maintain geneflow across the marine barrier compared to amphibians that are widely considered intolerant towards salinity (Brown and Guttman, 2002; Bocxlaer et al., 2006). This raises the question of whether historical vicariant processes can be inferred with reasonable confidence in some cases where dispersal is extremely unlikely. It is true that there is strong theoretical backing for the idea that the proportions of speciation by dispersal and vicariance vary across clades, although there is little empirical support (Bouchard and Brooks, 2004). However, more importantly, dispersal across marine barriers has been reported even in the case of groups such as amphibians and freshwater

fishes that have long been considered incapable of crossing such barriers (McDowall, 2002; Evans et al., 2003; Vences et al., 2003). In summary, it appears difficult to conclude that vicariance was the mediator of speciation even when strong *a priori* reasons, physiological or otherwise, indicate that dispersal is improbable. Nevertheless, vicariant calibrations may be more justified in certain groups compared to others.

6 Imprecision of the Calibration Source

Neither fossils nor tectonic events are suitable for use as point calibrations, although both have often been wrongly so used (Graur and Martin, 2004). In the case of tectonic events, a major source of uncertainty is the timing of the geological change; this is especially true of ancient rifting events. For instance, the split between India and Antarctica is controversial, and estimates range from 80 mya to 135 mya (Briggs, 2003; Ali and Aitchison, 2008). Moreover, tectonic changes are 'episodes' rather than 'events'. Initial rifting has often been used, when in fact it might take millions of years for a marine barrier to form between the landmasses after the onset of flooding. Even the timing of the rise of the Panamanian Isthmus, which is probably the best dated tectonic event (Lessios, 2008), is far from precise. Multiple lines of evidence indicate that it disrupted marine biota along a broad temporal axis (Knowlton and Weigt, 1998; Marko, 2002). Nonetheless, more recent tectonic events like the Messinian Salinity Crisis and the rise of the Panamanian Isthmus are likely to possess narrower error bars.

Fossil-based dating has similar problems when the age of the fossil is treated as a point estimate. Paleontological data do not necessarily appear as taxa, but might merely represent apomorphies that diagnose clades (Estabrook et al., 2007). As such, there may be uncertainty in assigning a fossil to a particular clade, and some fossils will be more reliable than others (Reisz and Müller, 2004; Rutschmann et al., 2007).

Problems with the use of point calibrations and suggestions to minimize errors have been discussed extensively (Thorne and Kishino, 2002; Graur and Martin, 2004; Reisz and Müller, 2004; van Tuinen and Hadly, 2004; Benton and Donoghue, 2007; Drummond and Rambaut, 2007; Pulquério and Nichols, 2007; Rutschmann et al., 2007; Marshall, 2008). A welcome trend in recent years has been a shift away from simple point calibrations, with the latest techniques allowing users to place upper and lower bounds on calibrations, and to incorporate calibrations in the form of parametric dis-

tributions (Thorne et al., 1998; Sanderson, 2002; Drummond et al., 2006; Britton et al., 2007). Readers are referred to Ho and Phillips (2009) for a comprehensive review of such techniques. These techniques can be applied both to paleogeographic and paleontological calibrations, considerably alleviating problems associated with treating calibrations as point estimates. Groups represented by a good paleontological record have an advantage in that fossils usually occur with sample distributions over time that allow estimation of statistical confidence limits on calibration (Marshall, 1997, 2008).

Assuming that the problems with the use of point calibrations are suitably addressed by the above methods, fossil-based dating is a far superior alternative to vicariance-based dating. The multiple sources of uncertainty that vicariant calibrations introduce mean that we can have very little confidence in the resulting estimates, and the probability of obtaining misleading results is extremely high. The strongest assumption, that speciation was in fact caused by the said geological process, is more often than not the most tenuous. The fossil-based approach almost always results in minimum ages whereas the paleogeographic method often leads to older ages, but can also result in considerably younger estimates; one can never know when it does either. Fossils are unavailable for several taxa and researchers have understandably resorted to vicariance-based ones to analyze them. When fossils are unavailable, secondary calibrations (i.e. derived from other dating analyses) which are in turn based on fossils can be employed. Although secondary calibrations have their own suite of problems (Shaul and Graur, 2002; Graur and Martin, 2004) they are a better alternative to vicariance-based calibration, as long as they are applied cautiously.

7 Example

I discuss issues with the two calibration approaches using a published example and also take this opportunity to critique some of my own work. The butterfly genus *Junonia* (Nymphalidae: Junoniini) comprises 30 species occurring in North and South America, Africa, tropical parts of Asia and Australasia. We studied the historical biogeography of this group based on a three gene phylogeny (Kodandaramaiah and Wahlberg, 2007). There are no reported fossils for this soft-bodied group of insects. We were hence confronted with the choice of either a secondarily derived source or a vicariance-based one. We eventually constrained the age of divergence of

Junoniini (which includes *Junonia* and five other genera) at 35.5 my based on results from Wahlberg (2006) which in turn relied on fossils. Although we mentioned that the confidence intervals on our age estimates were likely to be quite wide, we did not stress strongly enough in the discussion and abstract on the issue of the ages being minimal estimates. An ultrametric tree depicting the ages of divergences among *Junonia* species derived from Fig. 5 in Kodandaramaiah and Wahlberg (2007) is presented here (Fig. 1A). From these results, we concluded, among others, that there was an initial colonization of Asia from Africa across the Arabian Peninsula ca. 20 mya, followed by a back dispersal into Africa ca. 5 mya and another dispersal into the New World ca. 3 mya. The approximate age of *Junonia* was later corroborated in another study (Wahlberg et al., 2009) that used 7 minimum and 6 maximum age constraints. Furthermore, other related butterfly groups, dated independently, have reaffirmed that the Arabian Peninsula has been an important corridor of geo-dispersal between Africa and Asia about 20 mya, coincident with the collision of the northwards drifting African plate with Eurasia (Aduse-Poku et al., 2009; Kodandaramaiah et al., 2010; Wahlberg, 2006, but this study used the same calibration). These results reinforce the scenario of out-of-Africa origin and dispersal-mediated diversification of *Junonia*.

Now consider the effect of a tectonic calibration source. For the area cladogram in Fig 1, node 1 is suitable for application of a vicariant calibration because an allopatric speciation event between Africa and Asia can be unambiguously inferred at this node. Allopatric speciation between specific areas cannot be reliably identified over other nodes (see biogeographic analysis in Kodandaramaiah and Wahlberg, 2007). Vicariance at node 1 could have happened either when the Indian subcontinent (ca. 80–135 mya: Briggs, 2003; Ali and Aitchison 2008) or Africa (ca. 105 mya: McLoughlin, 2001) rifted from Gondwana. Fig. 1B depicts the chronogram based on the 105my calibration, analyzed using the same software (r8s; Sanderson, 2004) and protocols as in Kodandaramaiah and Wahlberg (2007). The minimum age of *Junonia* is estimated at an implausible ca. 153 my. The oldest butterfly fossils are dated at ca. 50 my, and even the most ancient butterflies are unlikely to have lived >100 million years ago (Vane-Wright, 2004; de Jong, 2007; Wahlberg et al., 2009). In the light of this, a minimum age of 153 my for *Junonia* is untenable. More importantly, even if one assumes that this age is true, the divergence of the African and South

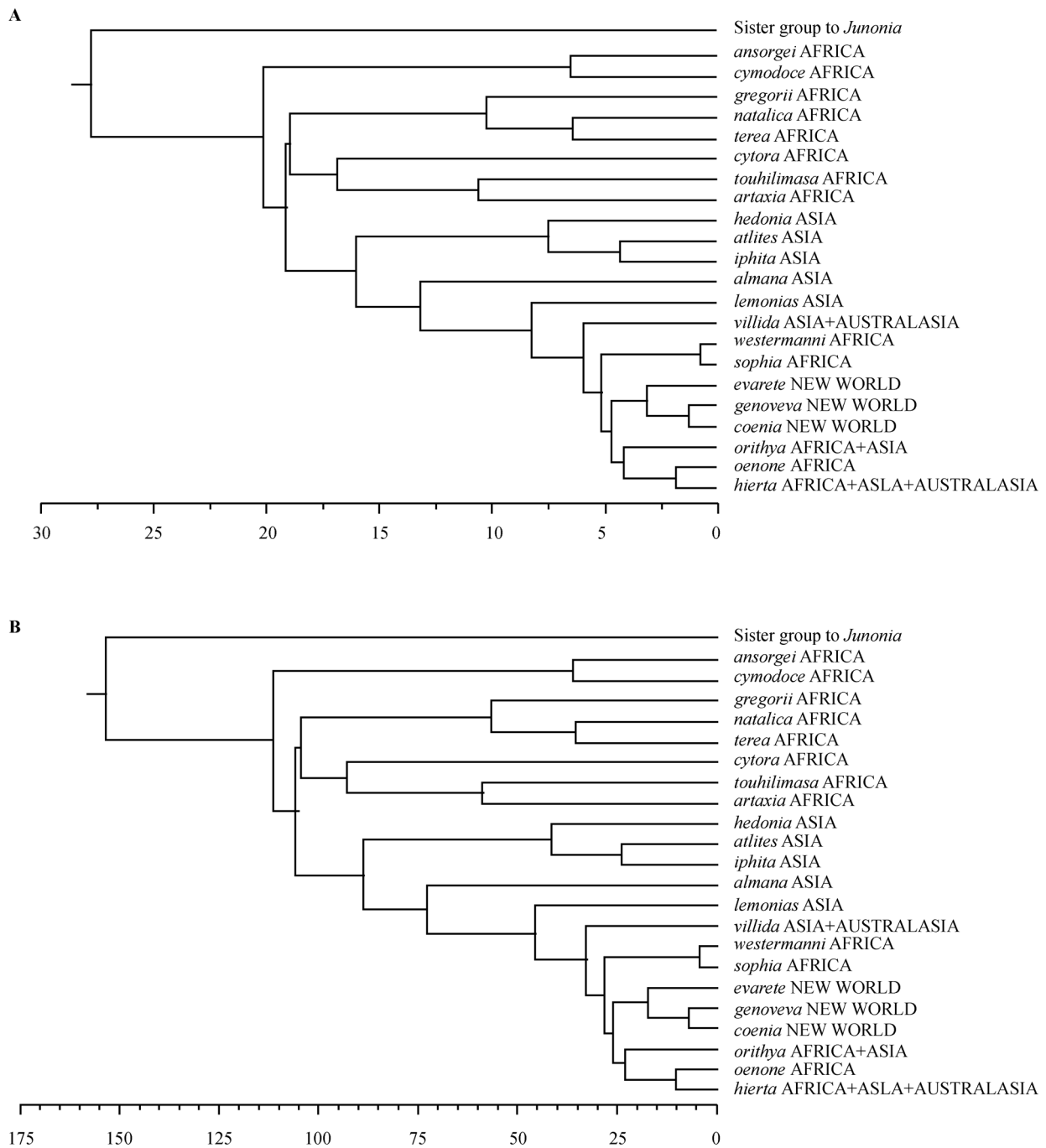


Fig. 1 Molecular dating of divergences within *Junonia* using fossil- and vicariance-based calibrations

A. Ultrametric tree of *Junonia* species derived Fig. 5 in Kodandaramaiah and Wahlberg (2007). The tree represents minimum ages of divergences among *Junonia* species. The calibration enforced (not shown in figure) was the minimum age of divergence of Junoniini at 35 my. The horizontal axis corresponds to time in millions of years. **B.** Ultrametric tree resulting from reanalysis of the *Junonia* dataset using a vicariance-based calibration where the divergence at node 1 was fixed at 105 my, corresponding to the rifting of Africa from Gondwana.

American clades, at nodes 2 & 3 respectively, must have occurred through dispersals, reinforcing the notion that butterflies can and do disperse across continents given a span of millions of years. If so, on what basis can we assume vicariance at node 1 apart from the fact that no fossils are known for this group? This example is ad-

mittedly simplistic and the differences between paleontological and paleogeographic calibrations may not be so dramatic in other groups. Nevertheless, it serves to illustrate the point that vicariant calibrations are not justified merely on the grounds of fossils being unavailable; judiciously enforced secondary calibrations

are arguably better than purely vicariance-based ones.

Both types of constraints, paleontological and paleogeographic, have frequently been used together (e.g., Renner et al., 2000; Duda and Kohn, 2005; Bocxlaer et al., 2006; Roelants et al., 2007), sometimes using a process of reciprocal illumination between fossil-based and vicariance-based calibrations (Conti et al., 2002). When authors prefer to use both available fossils and vicariance-based sources, one suggestion is to report results from the combination of both as well as from fossils only. Another approach is to examine the relative contribution of each calibration (Renner et al., 2000), for example by testing the robustness of inferred ages to the exclusion of individual calibrations (cf. Bocxlaer et al., 2006; Roelants et al., 2007). Calibrations reliant solely on vicariance are, however, unjustified and should be strictly avoided.

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