

Evolution of Agamidae

Agamidae is a family of squamates, including 53 genera and over 300 extant species spanning Asia, Africa, and Australia. Archeological specimens and other data hypothesize the ancestor of Agamidae in eastern Asia (Greer, 1989). Evolution of the family resulted in vast morphological and ecological differences seen on the three continents. Phylogenetic comparison of the family has been done with tRNA secondary structure analysis as well as mitochondrial DNA analysis. Relationships between taxa and dates of divergence into geographic regions can be estimated using the phylogenetic data.

The Agamidae family is considered most closely related to the Chamaeleonidae as a sister clade (Figure 1). Chameleons share acrodont (fused to the jaw) teeth with the agamids and the two families together are referred to as the Acrodonta. The acrodonts are related by a weakly supported sister clade to the Iguanids; a family of American, Madagascan, and Fijian lizards.

Parsimony analysis of nucleotide sequences identifies eight major clades in the Acrodonta (Macey et al., 2000a). Most agamid lizards are placed into three distinct clades (Figure 2). One clade is composed of all taxa occurring in Australia and New Guinea. *Physignathus cocincinus* (water dragon) from Southeast Asia is the sister taxon to the Australia-New Guinea clade. A second major clade is composed of taxa occurring from the Indian Subcontinent east through South and East Asia. A third clade is composed of taxa occurring from Africa east through Arabia and West Asia to the Indian

Subcontinent. These clades contain all agamid lizards except *Uromastyx*, *Leiolepis*, and *Hydrosaurus*, which represent three additional clades of the Agamidae. The Chamaeleonidae forms a clade weakly supported as the sister taxon to the Agamidae.

All eight branches of the Acrodonta contain members mainly found on land masses derived from Gondwanaland. Agamid lizard migration with Gondwanan plates is examined statistically by Macey et al. (2000a). Their hypothesis suggests that the African-West Asian clade is of African or Indian origin, and the South Asian clade is either of Indian or Southeast Asian origin. The resulting shortest tree suggests a likely African origin for the African-West Asian clade and an Indian origin for the South Asian clade. The Australia-New Guinea clade rafted with the Australia-New Guinea plate and forms the sister group to the Southeast Asian taxon *Physignathus cocincinus* that occurs on plates that broke from northern Australia-New Guinea. Other acrodont taxa are inferred to be associated with the plates of Madagascar (Chamaeleonidae), India (*Uromastyx*), or Southeast Asia (*Hydrosaurus* and *Leiolepis*).

Macey et al. (2000a) theorized three historical events which may be responsible for the sharp faunal barrier between Southeast Asia and Australia-New Guinea, known as Wallace's line (Figure 3). These events include primary vicariance caused by plate separations, secondary contact of Southeast Asian plates with Eurasia, and the dispersal of the Indian fauna (after collision of the Indian subcontinent) to Southeast Asia. Acrodont lizards show the first and third of these biogeographic patterns. Modern faunal diversity is influenced primarily by events such as tectonic collisions and land bridge connections, which promote episodic turnover of continental faunas by introducing new fauna into a given area. Repeated tectonic collisions are an important phenomena

promoting continental biodiversity and has been investigated using phylogenetics. The current diversity of the agamids in the Australian region is considered to have increased through multiple colonizations from Asia on the basis of DNA sequence data (Fuller et al., 1998).

Wallace's Line, separating the terrestrial faunas of South East Asia from the Australia–New Guinea region, is the most prominent and well-studied biogeographic division in the world (Schulte et al., 2003). Phylogenetically distinct subgroups of major animal and plant groups have been documented on either side of Wallace's Line since it was first proposed by Wallace himself in 1859. The earliest agamids are dated to the late Jurassic and the two most primitive living taxa, *Leiolepis* and *Uromastyx*, are northern (Greer, 1989). The time scale of the invasion of southern and western continents has been debated, with an early invasion hypothesized during the Mesozoic across Wallace's Line and a different, more recent invasion hypothesized from Southeast Asia. Australian agamid specimens have been dated to no earlier than the Middle Miocene. As a result of this data, Greer (1989) hypothesized a relatively recent invasion by agamids of southern and western continents, including Australia only relatively late in their history. Using molecular phylogenetics and dating techniques, Schulte et al. (2003) determined that the split between taxa in the South East Asian and the Australian–New Guinean geological regions occurred during the Late Jurassic to Early Cretaceous in two independent lizard clades. This estimate is compatible with the hypothesis of rifting Gondwanan continental fragments during the Mesozoic and strongly rejects the hypothetical origin of various members of the Australian–New Guinean lizards as relatively recent invasions from

South East Asia. This result suggests an ancient fragmentation of lizard taxa on either side of Wallace's Line.

Looking closer at the Australia-New Guinea clade (Figure 4), a monophyletic grouping of *Caimanops*, *Diporiphora*, *Pogona*, and *Trympanocryptis* receives weak support (Macey et al., 2000a). Sister taxa include the pairings of *Amphiboluras* and *Chlamydosaurus*, and *Ctenophorous* and *Rankina*. Taxa from New Guinea, including *Aura* and *Hypsilurus*, do not appear to form a monophyletic group with respect to Australian taxa. This might suggest multiple invasions of herpatofauna from Australia into New Guinea.

The South Asian clade (Figure 4) contains the unique genus *Draco*, a group of wide-ranging lizards that have evolved the ability of gliding flight (made possible by elongate ribs which support a flight skin). This genus is the sister taxon to the Himalayan *Japalura variegata* and the *Japalura tricarinata* of the Indian Subcontinent (Macey et al., 2000a). The sister taxon to the clade containing *Japalura* and *Draco* contains all remaining South Asian species (McGuire et al., 2001). The most parsimonious tree results in a grouping of *Otocryptis* and *Sitana* as a sister taxon to all remaining taxa except *Japalura* and *Draco*. A monophyletic group of Indian *Calotes* species is well supported and forms a sister taxon to a clade composed of the wide-ranging *C. versicolor*, *C. mystaceus*, and *C. emma*. *Salea* is a sister taxon to a weakly supported clade of an Indian group (*Ceratophora*, *Cophotis*, and *Lyriocephalus*) and a Southeast Asian group (*Aphamiotis*, *Gonocephalus*, and *Bronchocela*). Monophyly of the Southeast Asian *Acanthosaura*, *Japalura*, and *Pseudocalotes* is strongly supported. With Indian taxa spanning the root of the tree and Southeast Asian taxa being nested within the Indian

taxa, the phylogenetic distribution of taxa suggests an Indian origin for this clade, although a Southeast origin is possible.

Within the African-West Asian clade, taxa are distributed on the Gondwanan plates of Arabia and the Indian Subcontinent (Figure 6). The African genus *Agama* forms a monophyletic clade (Macey et al., 2000a). A clade consisting of the Arabian *Pseudotrapelus* and *Trapelus* forms the sister taxon to *Laudakia* and *Phrynocephalus* species. A clade of *Laudakia*, potentially of Indian origin, forms the sister taxon to the other *Laudakia* species and *Phrynocephalus*. Within the Indian clade of *Laudakia*, *L. sacra* of Tibet forms the sister taxon to a clade containing the wide-ranging *L. nupta*. African and Arabian taxa are basal in comparison to the West Asian taxa which are in a nested position of the tree.

The phylogenetic results by Macey et al. (2000a) provide an area cladogram for taxa occurring on Gondwanan plates (Figure 7). Phylogenetic analysis results support an association of *Leiolepis* species with Southeast Asian plates of Gondwanan origin. *Hydrosaurus* also occurs on the Southeast Asian plates of Gondwanan origin. A vicariant split between the Southeast Asian *Physignathus cocincinus* and the Australian-New Guinean clade is shown in the cladogram. The South Asian clade appears to be associated with the Indian-Southeast Asian region. The taxa in the Africa-West Asian clade are associated with either the Afro-Arabian or Indian plate. The genus *Uromastix* is associated with the Indian plate and *Chameleo*, a genus of the Chamealeonidae (and a sister taxa of Agamidae) is of African-Madagascan origin.

Phylogenetic relationships within the *Laudakia* (Africa-West Asian taxa) on the Iranian Plateau were investigated by Macey et al. (1997) using 1708 aligned bases of

mitochondrial DNA sequence from the genes encoding several tRNA molecules and subunits of NADH dehydrogenase and cytochrome C oxidase. Three hypotheses for historical fragmentation of *Laudakia* populations on the Iranian Plateau were tested. The shortest tree suggests fragmentation resulted from a north-south split caused by uplifting of the Zagros Mountains in the late Miocene or early Pliocene [5-10 MYBP (million years before present)]. Statistical tests reject the other hypotheses that fragmentation of populations proceeded along continuous mountain belts that surround the Iranian Plateau. Geologic history provides dates for most branches of the tree. A plot of DNA substitutions against dates from geologic history (Figure 8) refines the date for the north-south split across the Iranian Plateau to 9 MYBP (late Miocene). A hypothesis of area relationships for the biota of the Iranian Plateau is generated from the phylogenetic tree. The resulting area cladogram for *Laudakia* features a north-south split and may be a general template for other fauna of the Iranian Plateau.

Mitochondrial DNA analysis reveals the South Asian genus *Ceratophora* has a middle Miocene origin with the most recent branching of species happening at the Pliocene/Pleistocene boundary (Schulte et al., 2002). Phylogenetic analysis reveals the unique characteristic of rostral horns having multiple origins and possible losses over the evolutionary history of *Ceratophora*.

Analysis of mitochondrial DNA sequence data reveals phylogeographical relationships between the African genus *Agama* (Mathee, 2002). Three distinct geographical regions can be distinguished among the lizard populations. The first in southern Namibia, the second is restricted to the western dry arid regions of South Africa, and the third is distributed throughout the less arid southern and eastern parts of the

subcontinent. The geographically structured differences among populations within *Agama* clades are probably a result of dispersal and historic isolations among populations. Significant genetic differences between the *Agama* suggest vicariance, probably as a response to natural climatic changes during the past three million years.

Secondary structure analysis of tRNAs (Macey et al., 2000b) results in two proposed phylogenetic trees of the Acrodonta (Figure 9). One tree plots the majority of Australia-New Guinea taxa and several other taxa as independent monophyletic groups which diverged earlier in their history. The second tree plots several more closely related sister clades, similar to the results seen in several other studies. The first tree (A) is conservative in that evolution of the stem and loop structures in tRNA genes required a minimum of three evolutionary events. This contrasts with the second tree (B) in which seven evolutionary events would be required. This tree is favored because parallelisms of stem and loop evolution is not likely to happen in the exact same place they were lost. This phylogenetic tree (Figure 10) is comparable to the results (Figures 4-6) seen in analysis of mitochondrial DNA (Macey et al., 2000a). In analysis of the tRNA structures, seven deviations from the standard structure are revealed. Four of these characters are autapomorphies and three of the characters are synapomorphies. Various gains and losses of bases in the tRNA molecules are a result of autapomorphies and are diagrammed in Figure 10. Monophyly of the acrodonta is supported by the absence of a portion of the sequence encoding tRNA^{Cys}. tRNA analysis again confirms a clade containing all taxa sampled from Australia and New Guinea. Monophyly of *Acanthosaura* is supported by a truncated T-stem in the sequence encoding tRNA^{Tyr}. The tRNA analysis and resulting phylogenetic tree (Figure 10) sampled several more taxa

than the mitochondria DNA study (Figures 4-6), yet all of the sister clades supported by DNA analysis are confirmed by tRNA secondary structure analysis.

A phylogenetic tree (Figure 11) from mitochondrial DNA analysis provides similar results to Macey et al. 2000a among the Australian and New Guinean taxa (Schulte et al., 2003). The Southeast Asian taxon *Leiolepis* again is seen forming a sister clade to Australian and New Guinean taxa. This tree differs from the previously described trees in that *Moloch horridus* does not form a monophyletic group with *Hypsilurus dilophus* but forms an independent sister clade to the remaining Australian and New Guinean taxa. Several *Hypsilurus* species form a monophyletic group, including taxa solely from New Guinea. Schulte et al. (2003) again verifies the pairings of *Pogona* and *Tympanocryptis* and *Caimanops* and *Diporiphora* as sister taxa. Numerous *Ctenophorus* taxa were sampled and form a monophyletic group. The majority of the Australian species are grouped further down the tree than the basal Southeast Asia and New Guinea groups. Schulte et al., (2003) uses this data as support of the previously discussed ancient divergence of taxa across Wallace's line.

Honda et al. (2000) further confirm the monophyly of the Agamidae family from mitochondrial DNA sequences. The sister relationship of *Uromastyx* and *Leiolepis* was also confirmed. *Uromastyx* has occasionally been referred to as an independent subfamily or family because it has a few highly specialized morphological features (including herbivory and enlarged scales covering the tail). Analysis of mitochondrial genes does not support the designation of the taxon as a family or subfamily. Although gene sequences do not support the validity of the family Uromastycidae, the taxon does show a sister relationship with all remaining agamids (Honda et al., 2000).

Species richness varies greatly across agamid lineages and is shown in Figure 12. Stuart-Fox et al. (2003) found that the distribution of species richness among agamid genera (Figure 13) is not consistent with a random model and that certain characteristics may provide a deterministic explanation for variation in species richness. Body size, life history, sexual selection, ecological generalism, range size and latitude were all tested as variables. Of all the variables tested, only sexual size dimorphism and sexual dichromatism predicted species richness among agamids.

Several unique morphological characters are found within agamid genera (Greer, 1989). Some species such as *Physignathus cocincinus* have developed aquatic behavior. Several *Ctenophorus* species are associated with rocks. The best known agamid taxon is likely the *Chlamydosaurus* which has a large frill around the neck that is raised when the organism is alarmed. *Moloch* is very unique and is covered with hard spiny scales which may prevent them from being swallowed by snakes. These, along with several other morphological and ecological differences can be seen throughout the Agamidae family. The differences are likely a result of evolution in response to environmental and predatory conditions.

Evolution of the Agamidae results in the vast differences in species richness and morphology currently spanning the three continents of Asia, Africa, and Australia. Different means of geographical and ecological isolation happened on each of the three continents and can be seen in phylogenetic data for various genera. Three zones of isolation include Wallace's line, the Iranian mountains, and various African ecological zones. The resulting taxa at different geographical areas can be phylogenetically analyzed using techniques including mitochondrial DNA analysis and tRNA secondary

structure analysis. In addition to possible locations of divergence, the cladograms can be used to describe relationships between the taxa and estimated times of divergence. Eight clades are recognized within the Agamidae with three major continental groups.

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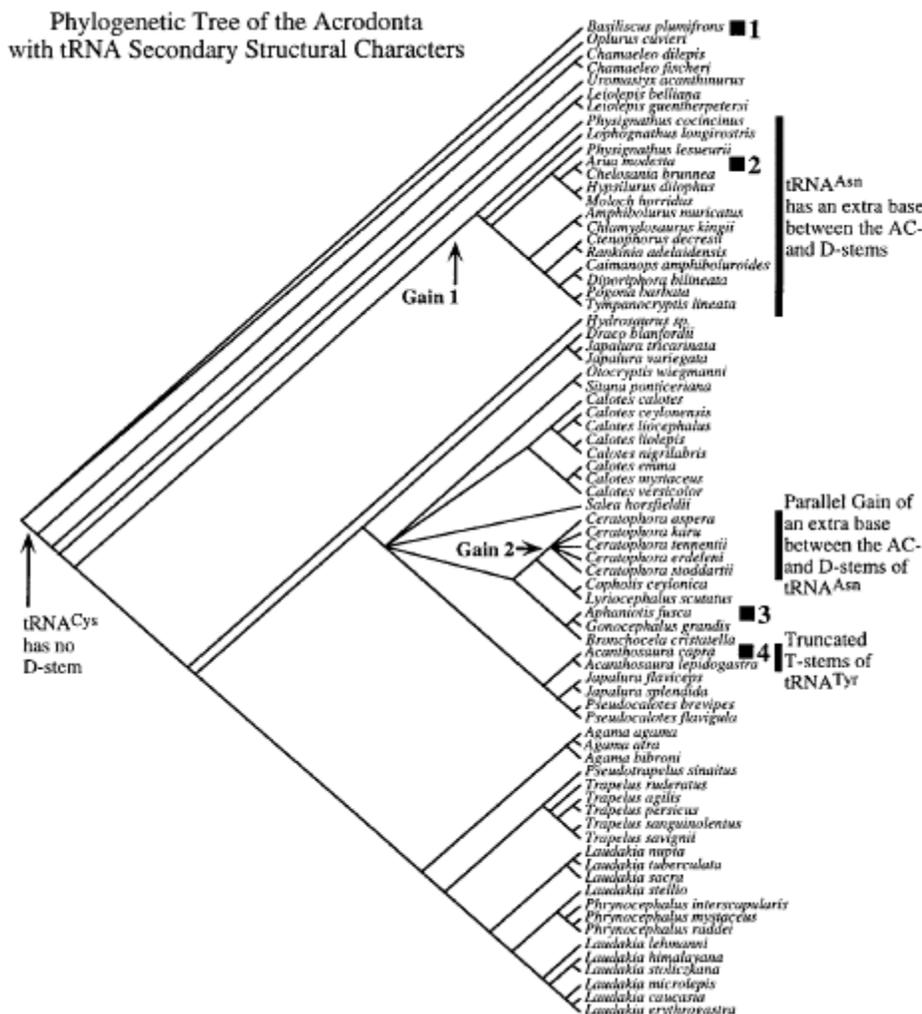


Figure 10 Phylogenetic tree of the Acrodonta showing changes in secondary structure of tRNAs. Seven deviations from the standard secondary structure of tRNAs are shown. Four of these characters are autoapomorphies, labeled with a square and numbered: (1) enlargement of the variable loop beyond five bases, (2) an extra base between the AA- and D- stems in the sequence encoding tRNA^{Asn}, (3) loss of a base pair from the middle of the AA-stem in the sequence encoding tRNA^{Gln}, and (4) loss of a base between the AA- and D- stems in the sequence encoding tRNA^{Asn}. Three characters are synapomorphies and are indicated with arrows: (1) monophyly of the Acrodonta is supported by the absence of a D-stem in the sequence encoding tRNA^{Cys}, (2) monophyly of *Acanthosaura* is supported by a truncated T-stem in the sequence encoding tRNA^{Tyr}, (3) a clade containing all taxa sampled from Australia and New Guinea is supported by the presence of an extra base between the AC- and D-stems in the sequence coding tRNA^{Asn}. Synapomorphies are shown at different levels of phylogenetic divergence: the Acrodonta, the Southeast Asian clade *Acanthosaura*, a South Asian clade, and a clade from Southeast Asia, Australia, and New Guinea. (Macey et al., 2000b)

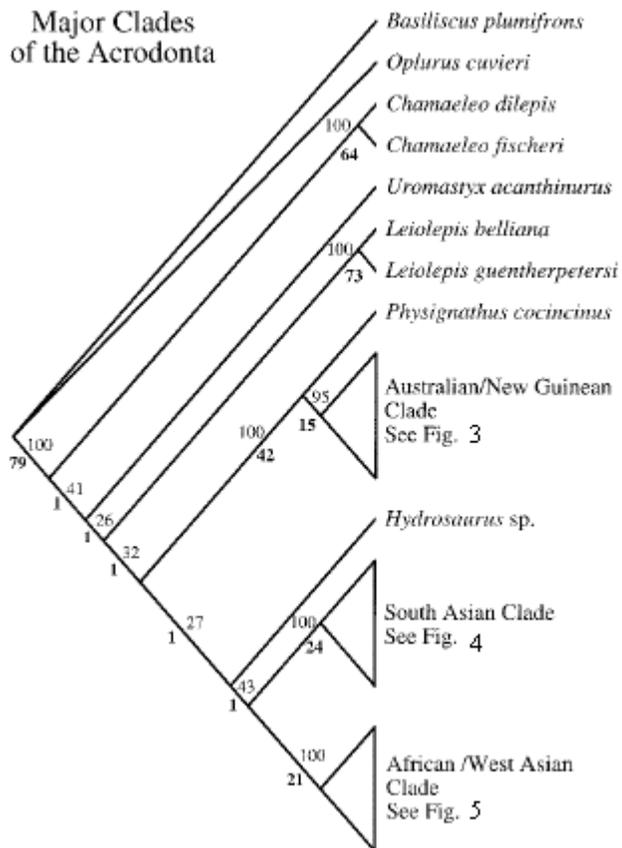


Figure 2 Phylogenetic arrangements of the Acrodonta derived from analysis of 1,041 DNA sequence characters. Eight major branches can be recognized: (1) *Chamaeleo*, (2) *Uromastyx*, (3) *Leiolepis*, (4) *Physignathus cocincinus*, (5) an Australian-New Guinean clade, (6) *Hydrosaurus*, (7) a South Asian clade, and (8) an African-West Asian clade. Bootstrap values are presented above branches, and decay indices are shown in bold below branches. (Macey et al., 2000a)

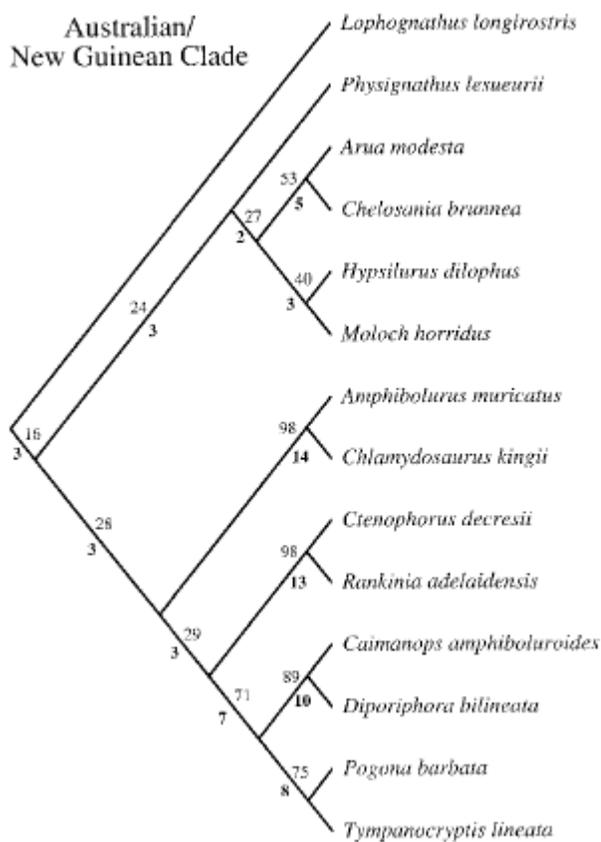


Figure 4 Phylogenetic relationships within the Australian-New Guinean clade of the Acrodonta. Taxa occurring in New Guinea (*Arua* and *Hyspilurus*) appear not to form a monophyletic group with respect to Australian taxa. Bootstrap values are presented above branches, and decay indices are shown in bold below branches. (Macey et al., 2000a)

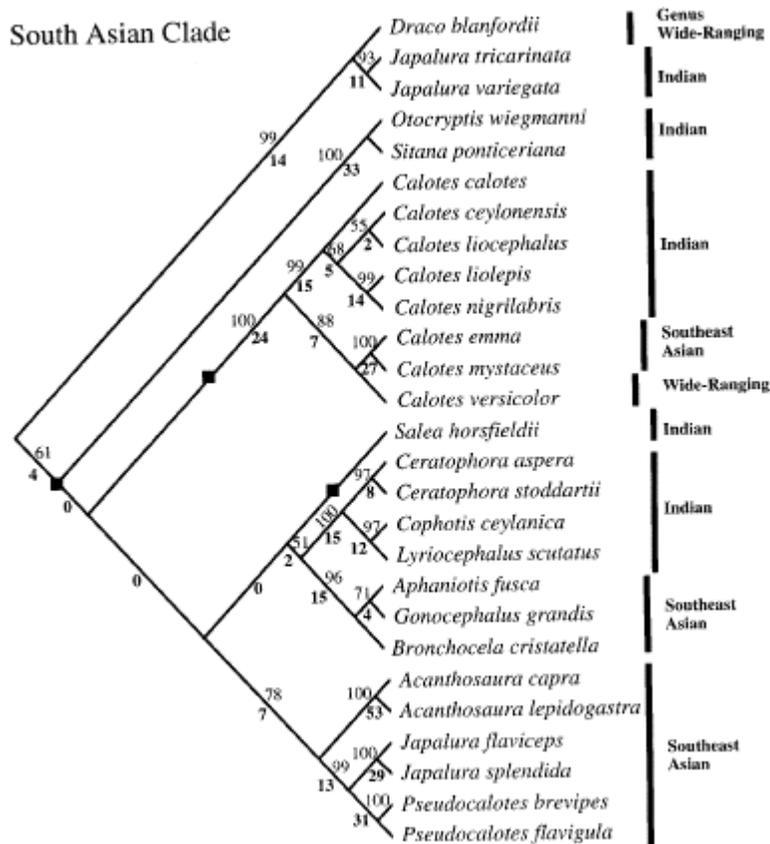


Figure 5 Phylogenetic relationships within the agamid South Asian clade derived from analysis of 1,041 DNA sequence characters. Indian taxa span the root of the tree, and Southeast Asian taxa are nested within Indian taxa. Bootstrap values are presented above branches, and decay indices are shown in bold below branches. (Macey et al., 2000a)

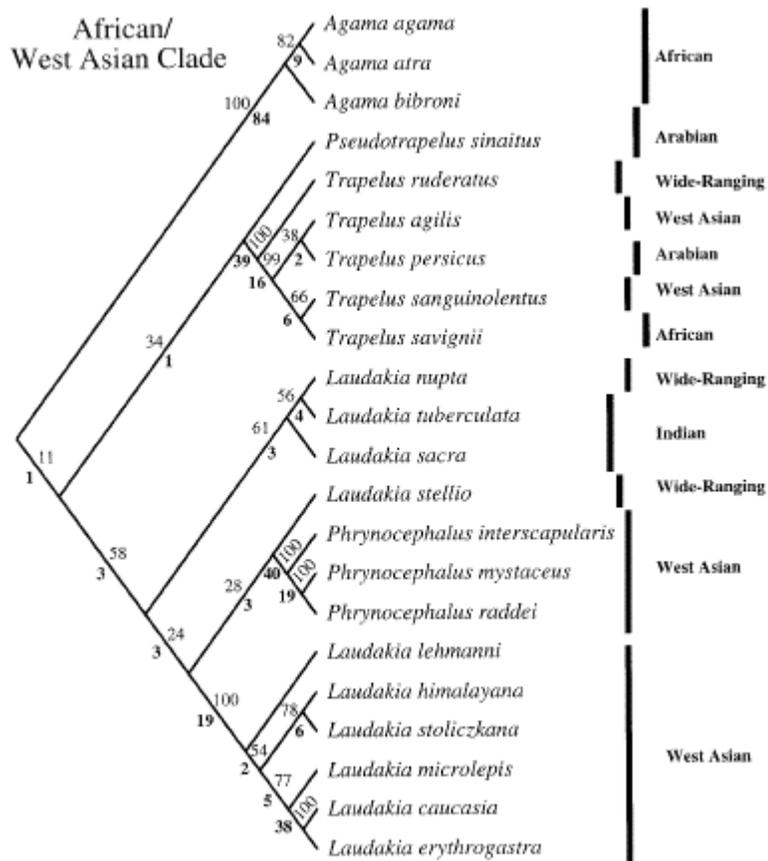


Figure 6 Phylogenetic relationships among the agamid African-West Asian clade derived from analysis of 1,041 DNA sequence characters. Taxa occurring on Africa and Arabia are in a basal position with taxa occurring on the periphery of the Indian Subcontinent (West Asia) being in a nested position on the tree. Bootstrap values are presented above branches, and decay indices are shown in bold below branches. (Macey et al., 2000a)

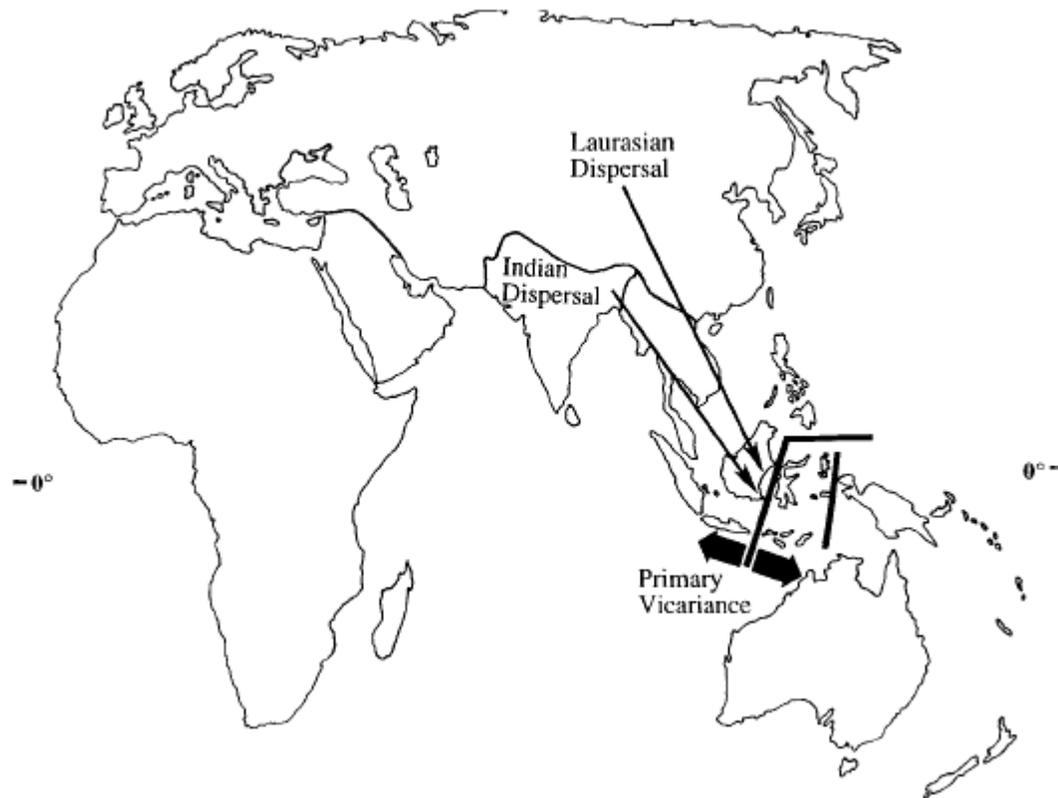


Figure 3 Several major events have been described for the sharp faunal division now observed across Wallace's line. The tectonic split of Southeast Asian blocks from the northern margin of the Australian-New Guinean plate caused a primary division between the faunas. (Macey et al., 2000a)

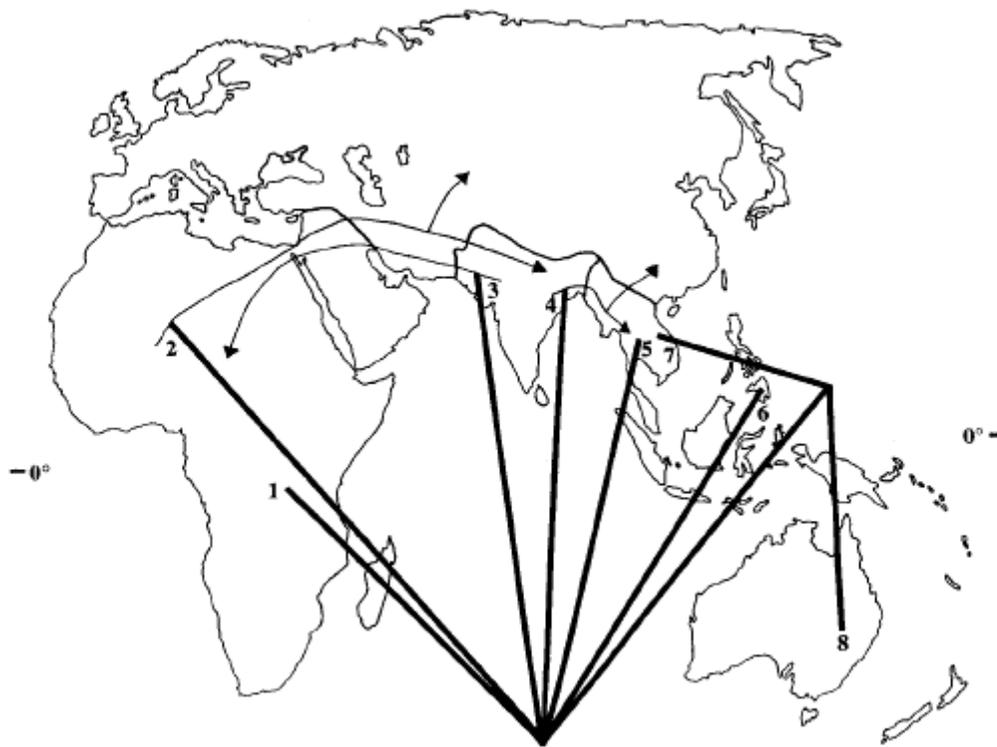


Figure 7 Acrodont distribution shown on an area cladogram. Numbers on branches refer to clades defined in the phylogenetic analysis: 1= *Chameleo*; 2= African-West Asian clade; 3= *Uromastyx*; 4= South Asian clade; 5= *Leiolepis*; 6= *Hydrosaurus*; 7= *Physignathus cocincinus*; and 8= Australian-New Guinean clade. (Macey et al., 2000a)

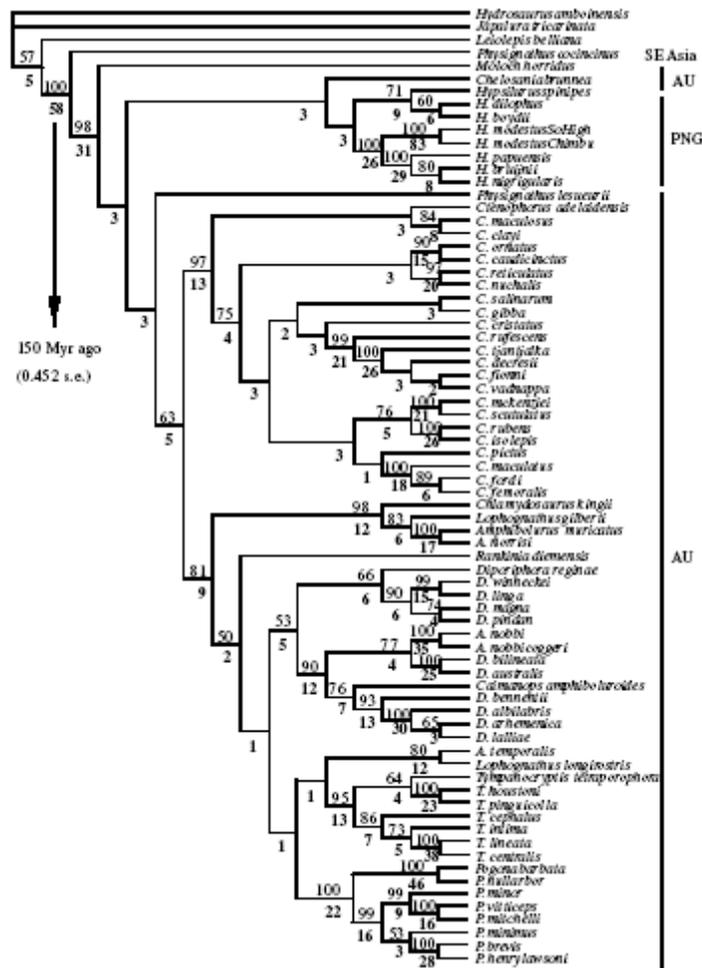


Figure 11 Phylogenetic relationships among agamid lizards are displayed by the single most parsimonious tree resulting from analysis of 1611 aligned base positions of mitochondrial DNA sequence data. Bootstrap values are indicated above the branches and decay indices are denoted below the branches. (Schulte et al., 2002)

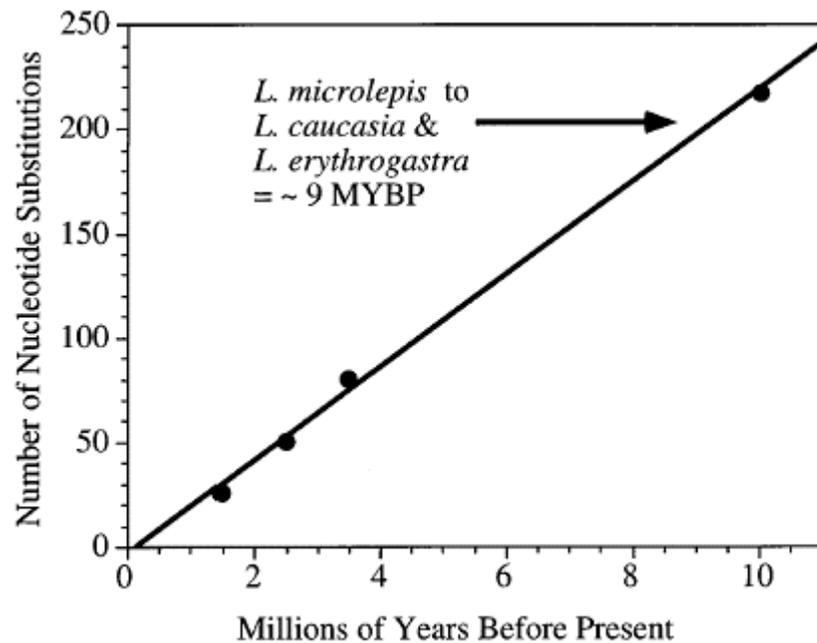


Figure 8 Plot of nucleotide substitutions against time derived from geologic history. The rate of evolution for this segment of mtDNA is 0.65% change per lineage per million years. Divergence of *L. microlepis* to *L. caucasia* and *L. erythrogastra* is calculated to about 9 MYBP (Macey et al., 1997).

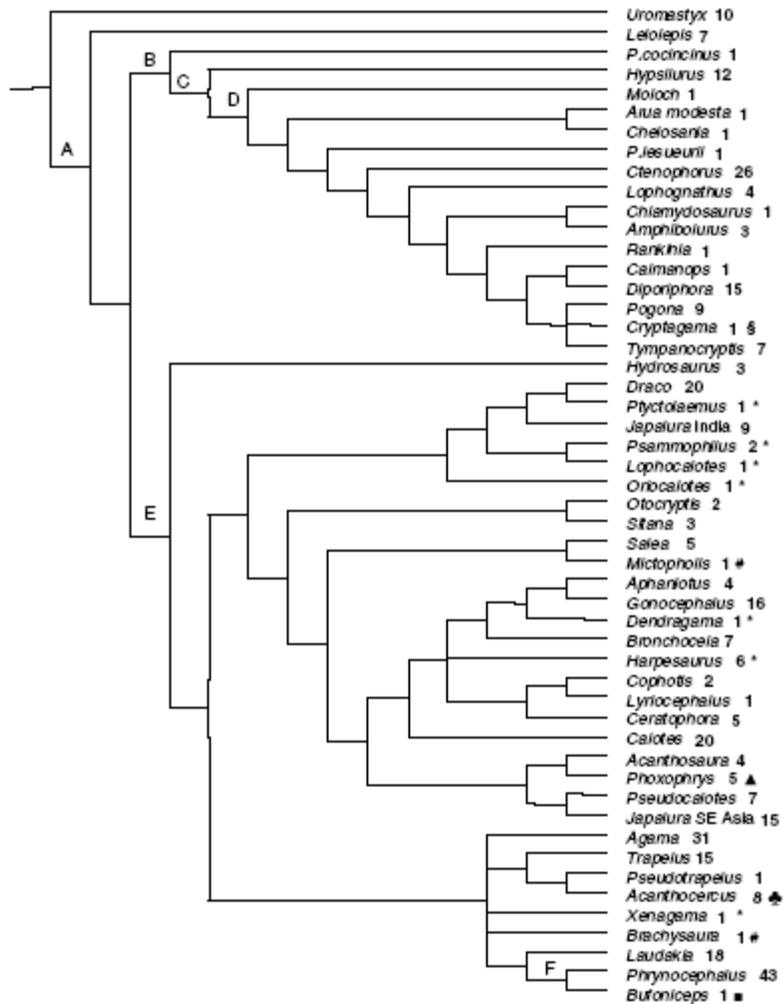


Figure 12 Complete phylogeny of agamid genera used for comparative analysis of correlates of species richness. The tree is based on the phylogeny of Macey et al. (2000a). Species richness for each genus is given in bold next to each taxon name. Symbols indicate genera not included on Macey et al.'s (2000a) original phylogeny. (Stuart-Fox, 2003)

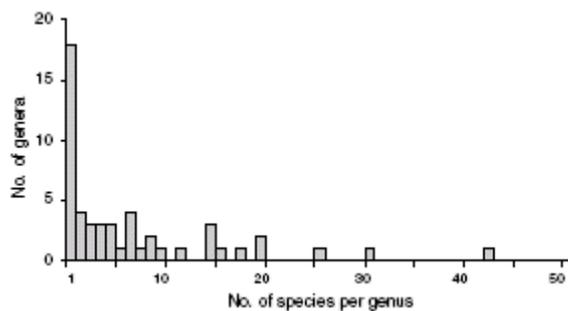


Figure 13 Frequency histogram of species per genus within the Agamidae. (Stuart-Fox, 2003)

Figure 1 Abbreviated phylogenetic tree including Agamidae and several other selected families. (Greer, 1989)