1. Introduction

Breakup of the southern supercontinent Gondwana has been a focus of historical biogeography for decades, but studies of Gondwanan faunas rarely consider the Middle Eastern island of Socotra, located in the Indian Ocean at the triple junction of the Gondwanan plates of India, Arabia and Africa. Phylogenetic analysis of 16 complete mitochondrial genomic sequences reveals that chameleons of Socotra form an ancient lineage and were separated from relatives in North Africa and Arabia around the Oligocene, consistent with geological models of the isolation of Socotra from Arabia and Africa at this time. Phylogenetic grouping of an endemic Indian chameleon and two Arabian endemic species suggests that as the Indian Plate moved northward to Eurasia, Arabia came closer to India than formerly expected, allowing chameleons to disperse from Arabia to India. The dynamics of formation of the Red Sea and Gulf of Aden including nearby endemic faunas may be critical to understanding interactions among Gondwanan land masses as they crossed the Tethys Sea.

The faunas of land masses derived from the supercontinent Gondwana provide classic examples of cladogenesis induced by geological origins of physical changes that fragmented geographic distributions (Bossuyt et al., 2006; Briggs, 1995). Phases of Gondwanan biogeography include (1) division of the original supercontinent into fragments separated by intervening ocean leading to endemism, (2) movement of the fragments across ocean with some land masses attaining close proximity including some transitional connections, (3) accretion of some Gondwanan fragments to Laurasia and to each other to form new land masses, and (4) mountain barriers forming by tectonic plate collisions. Congruence
between geological events and cladogenesis in molecular phylogenies provides a strong foundation for understanding the historical assembly of diverse continental and island biotas.

Acrodont lizards (families Agamidae and Chamaeleonidae) illustrate all four phases of Gondwanan biogeographic history. Distinct acrodont groups are associated with the Gondwanan tectonic fragments of Afro-Arabia, India, Madagascar, Australia, and those now forming parts of Southeast Asia (Macey et al., 2000b); controversy persists regarding which of these differences trace to the original fragmentation of Gondwana (phase 1), versus dispersal between fragments in close proximity during phase 2 (Schulte et al., 2003; Hugall and Lee, 2004). Raxworthy et al. (2002) and Townsend and Larson (2002) invoke recurring dispersal of chameleons between Africa and Madagascar during phase 2. Phase 3 includes introduction of agamid lineages to Laurasia via collision of Afro-Arabia, Indian and Southeast Asian plates with Laurasia (Macey et al., 2000b), cladogenesis in the Laudakia caucasia species group on the Iranian Plateau illustrates phase 4 (Macey et al., 1998, 2000a).

The Socotra Island fauna is a largely overlooked piece of Gondwanan historical biogeography. Attached to the Afro-Arabian Plate, this island formed at the end of the Cretaceous (65 million years before present, MYBP) as part of a limestone massif (Beydoun, 1998, 2000a). The northern portion of this massif remains in Arabia in the Dhofar Mountains of southern Oman, but Eocene–Oligocene (34–41 MYBP) rifting in the Red Sea and Gulf of Aden isolated Socotra Island from Arabia (Braithwaite, 1987; Girdler and Styles, 1970). As Arabia moved northward into Eurasia, Socotra was isolated in the Indian Ocean. The first biological survey of Socotra in 1880 emphasized the uniqueness of its biota. The first explorations, led by Scottish botanist Sir Isaac Bayley Balfour and partially funded by the British Association for the Advancement of Science, discovered numerous plant taxa (Balfour, 1888) and several reptile species (Blanford, 1881; Günther, 1881). This expedition confirmed that the chameleon, Chamaeleo monachus, occurred on the island of Socotra (an erroneous locality for the type specimen in the British Museum places it in Madagascar, Gray, 1865).

Phylogenetic analysis of C. monachus and its closest relatives on other Gondwanan land masses offers insight into Socotra’s role in the first two phases of Gondwanan historical biogeography. This is the first molecular phylogenetic study of chameleons endemic to Socotra (C. monachus) and India (C. zeylanicus). We report 16 complete mitochondrial genomic sequences and phylogenetic analyses to resolve the relationships and historical biogeography of Socotran, Indian, Mediterranean, Sub-Saharan African, and Arabian species of the Chamaeleo chamaeleon species group (Fig. 1) plus outgroups from Sub-Saharan Africa and Madagascar.

2. Methods

We sequenced a complete mitochondrial genome from 13 ingroup taxa (Fig. 1) and two outgroups (C. dilepis and Kinyongia), with partial sequence for a third outgroup, Brookesia, (all but the second half of cob, trnP, trnT, presumptive Control Region, trnF, and the first half of rrsS). Laboratory protocol follows Macey et al. (2006).

DNA sequences were aligned manually. Positions encoding proteins were translated to amino acids using MacClade 4.03 (Maddison and Maddison, 2001) for confirmation of alignment. Alignments of sequences encoding tRNAs and rRNAs were constructed using secondary structural models (Kumazawa and Nishida, 1993; Macey and Verma, 1997; Gutell and Fox, 1988; Hickson et al., 1996a,b; Van de Peer et al., 1994). Regions with extensive length variation were deemed unalignable and excluded from phylogenetic analyses. In rRNA genes, 134 positions were excluded.

Fig. 1. Map of North Africa, circum Mediterranean, and Middle East depicting the distribution of ingroup taxa in the genus Chamaeleo. Numbers represent localities of taxa sampled and are cross-referenced with the phylogenetic tree presented in Fig. 2. Shading depicts clades discovered in this study. Samples and clades are: Blue circle, (1) C monachus (Socotra Island, Yemen); pink, (2) C. zeylanicus (Pakistan); red, (3) C. cyprinurus (Yemen); (4) C. arabicus (Yemen), (5) C. arabicus (Oman); green, (6) C. calcicaricairis (Somali island); (7) C. africanaus (Savana, Niger); (8) C. africanaus (Sahara, Niger); yellow, (9) C. chamaeleon (Portugal), (10) C. chamaeleon (Yemen), (11) C. chamaeleon (Cyprus), (12) C. chamaeleon (Anamur, Turkey), and (13) C. chamaeleon (Hatay, Turkey).
particularly in loop regions. In tRNA genes, 159 excluded positions from rrnS.

(ii) movement from the Mediterranean Region to Arabia. Details are discussed in

Arrows represent potential dispersal events: (I) Movement from Arabia to India; (II) movement from the Mediterranean Region to Arabia. Details are discussed in the text.

from rrmS and 276 from rrmL because of extensive length variation, particularly in loop regions. In tRNA genes, 159 excluded positions comprise: the D-loop of trnQ, I, M, W, Y, D, H and T; the D-arm replacement loop of trnC; the V (variable)-loop of trnS1; and the T-loop of trnV, I, M, A, C, Y, D, K, G, H, E, and T. For protein-coding regions a total of 257 nucleotide positions were excluded with the number of omitted aligned positions for each gene in parentheses: nad1 (27), nad2 (6), atp8 (3), atp6 (5), nad5 (135), nad5–nad6 overlap (16), nad6 (47), and cob (18). A total of 237 non-coding nucleotide positions were excluded from intergenic regions as follows: trnQ-I (10), trnW-A (10), trnA-N (17), stem-loop trnN-C (29), presumptive duplicated tRNA genes between trnV and trnY in Brookesia (132; see Townsend and Larson, 2002), trnY-cox1 (23), trnS2-D (5), nad4-trnH (4), and cob-trnT (7). Among chameleons, trnP is located in the presumptive Control Region toward trnF and not adjacent to trnT probably because of replication issues (see Macey et al., 1997a,b, 2004; Kumazawa, 2007); regions between trnT and P, and between trnP and F were excluded. Hence, a total of 1063 nucleotide positions are excluded from rrRNA, trRNA, and protein coding regions, as well as intergenic positions (not including the unaligned presumptive Control Region) leaving an alignment of 14,551 positions (3249 parsimony informative) for phylogenetic analysis. The sequence alignment is available in electronic version on the Molecular Phylogenetic and Evolution website.

Phylogenetic trees were inferred by parsimony using PAUP* beta version 4.0b8 (Swofford, 2001). Bootstrap resampling (Felsenstein, 1985) was applied to assess support for individual nodes using 1000 replicates. Decay indices (α “branch support” of Bremer, 1994) were calculated for all internal branches using searches that retained suboptimal nodes. All searches were heuristic using 100 random additions per replicate or search.

Museum voucher numbers and locality data for all newly reported sequences are in GenBank files EF222187–EF222202 (major extension of sequences refer to Macey et al., 1997, 2000b).

3. Results

Fig. 2 shows phylogenetic relationships among sampled chameleons. All branches except one are well supported by either a 99% or 100% bootstrap value and decay-index values of 27–397. Brookesia and Kinyongia root the tree, with Chamaeleo monophyletic; C. dilepis is the sister taxon to a clade comprising the remaining species. Socotran C. monachus is the sister taxon to a clade comprising Arabian (C. arabicus, C. calyptatus), Indian (C. zeylanicus), Mediterranean (C. chamaeleon) and two Sub-Saharan African (C. africamus, C. calcifer) species. Indian C. zeylanicus is the sister taxon to an Arabian clade (C. arabicus and C. calyptatus). A Sub-Saharan clade (C. africamus and C. calcifer) is the sister taxon to C. chamaeleon, whose circum-Mediterranean samples form a clade with one from Yemen. The westernmost C. chamaeleon sample from Portugal is the sister lineage to a clade comprising other conspecific samples. The isolated C. chamaeleon population from Yemen in Arabia is the sister taxon of a haplotype clade from Cyprus and Turkey. Haplotype samples from Cyprus and Turkey are nearly identical (only 11–18 nucleotide differences across 14,551 positions analyzed); Turkish haplotypes are grouped with weak support (bootstrap 79%, decay index 2).

4. Discussion

The deep phylogenetic divergence of Socotran C. monachus from its closest relatives in Africa, Arabia and the Mediterranean Basin confirms the prediction that this lineage formed when geological rifting in the Red Sea and Gulf of Aden isolated Socotra Island; initiated 35–41 MYBP, but may have occurred later as reflected in DNA divergence (see below). From our phylogenetic results for these chameleons, we hypothesize a historical sequence of events predominately shaped by plate tectonics but followed by unique dispersal events: (1) early vicariant isolation of a Socotra Island population from African, Mediterranean and Arabian populations, (2) vicariant separation of African and Arabian clades, (3) movement from Arabia to India prior to speciation within Arabia, (4) north–south speciation by vicariance across the Sahara, (5) east–west speciation by vicariance across the Mediterranean Basin, and (6) dispersal from the eastern Mediterranean region to southern Arabia.

Following their isolation from Socotra, Africa and Arabia were intermittently connected and disconnected during subsequent movements of these plates. Arabian and Indian chameleons form the sister taxon to those from Africa and the Mediterranean region. Sub-Saharan species (C. africamus and C. calcifer) form the sister taxon to Mediterranean populations (C. chamaeleon; although these haplotypes form a monophyletic group, their deep divergence suggests that C. chamaeleon is a complex of undescribed species). These results suggest that speciation across the Sahara followed an initial isolation of Arabia from Africa across the Red Sea Rift.

The endemic chameleon of India, C. zeylanicus, is the sister taxon to the two endemic chameleon species of Arabia (C. arabicus and C. calyptatus). Hence, the Indian population separated from Arabian populations prior to speciation within Arabia but after the Arabian populations had separated from African populations. Two scenarios could explain this result. One is that chameleons moved from Arabia into Laurasia and formed a continuous
distribution between southern Arabia and the Indian Subcontinent, followed by extinction in northern Africa, southern Iran, and western Pakistan, where suitable habitat currently exists. The second hypothesis is that as India made its final movement north across the Indian Ocean toward Asia it came closer to Arabia than previously assumed (Briggs, 2003) and possibly made contact with Arabia, permitting dispersal of chameleons from Arabia to India. Because chameleons are climbing animals they could have been swept by monsoon to mangrove swamps on the coast and potentially move between adjacent plates if the gap was not too wide, similar to what has been hypothesized for Africa and Madagascar in other chameleons (Raxworthy et al., 2002; Townsend and Larson, 2002). We favor the hypothesis of a close association of the Arabian and Indian plates over wide-scale extinction of chameleons from northern Arabia and southwestern Asia.

Among C. chamaeleon populations, the sample from Yemen in southern Arabia is the sister lineage to a clade of samples from the eastern Mediterranean (Cyprus and Turkey), and not closely related to populations in the western Mediterranean region. This phylogenetic pattern suggests dispersal of C. chamaeleon from the eastern Mediterranean to southern Arabia. Samples from Turkey and Cyprus are nearly identical and may have been transported by human activity.

Although our phylogenetic results are sound, we point to a discrepancy in our estimate in timing of divergences. Macey et al. (1998) provided a linear model for sequence evolution in the region between nad1 and cox1, with 1.3% pairwise change across a million years for agamid lizards. Applying this to our data we reach very young ages that are interpreted as an artifact of mangrove swamps being in close proximity during plate tectonic breakup; hence providing an avenue for dispersal and gene flow. For example, Socotra chameleons (13%) would have diverged from others around 10 million years ago, although we expect sequence saturation at this point and this could correspond to 15 million years. Regardless, it is unlikely that these sequence divergences and phyletic patterns are unrelated to tectonic plate movements as outlined.

The formation of the Red Sea and Gulf of Aden with their endemic faunas may provide a missing key to revealing the interactions of major Gondwanan land masses as they moved across the Tethys Sea toward Laurasia. Socotra Island, southern Arabia, and the Horn of Africa remain one of the least explored regions of the world. Our reconstructed history of the chameleon fauna endemic to these areas serves as a set of a priori hypotheses to be tested in other co-distributed taxa. Genomic sequencing of numerous biotic groups in the region will provide a diverse genetic resource for testing historical biogeographic relationships in the Middle East. It has not escaped our attention that this may be dynamic, involving plate tectonics that includes dispersal.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.08.024.

References