

Biogeographic and evolutionary implications of a diverse paleobiota in amber from the early Eocene of India

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For nearly 100 million years, the India subcontinent drifted from Gondwana until its collision with Asia some 50 Ma, during which time the landmass presumably evolved a highly endemic biota. Recent excavations of rich outcrops of 50–52-million-year-old amber with diverse inclusions from the Cambay Shale of Gujarat, western India address this issue. Cambay amber occurs in lignitic and muddy sediments concentrated by near-shore chenier systems; its chemistry and the anatomy of associated fossil wood indicates a definitive source of Dipteroocarpaceae. The amber is very partially polymerized and readily dissolves in organic solvents, thus allowing extraction of whole insects whose cuticle retains microscopic fidelity. Fourteen orders and more than 55 families and 100 species of arthropod inclusions have been discovered thus far, which have affinities to taxa from the Eocene of northern Europe, to the Recent of Australasia, and the Miocene to Recent of tropical America. Thus, India just prior to or immediately following contact shows little biological insularity. A significant diversity of eusocial insects are fossilized, including corbiculate bees, rhinotermitid termites, and modern subfamilies of ants (Formicidae), groups that apparently radiated during the contemporaneous Early Eocene Climatic Optimum or just prior to it during the Paleocene-Eocene Thermal Maximum. Cambay amber preserves a uniquely diverse and early biota of a modern-type of broad-leaf tropical forest, revealing 50 Ma of stasis and change in biological communities of the diptero carp primary forests that dominate southeastern Asia today.

biogeography | arthropoda | eusociality | tropical forests

The dramatic geological history of the Indian subcontinent has had profound effects on regional climates and biota. The Indian plate separated from Africa along with Madagascar in the Middle Jurassic *ca.* 160 Ma, then from Madagascar in the mid-Cretaceous *ca.* 90 Ma, moving at the remarkable rate of 15–25 cm/y before colliding into Asia in the early Cenozoic, uplifting the Himalayas. Dates of initial contacts between India and Asia occurred approximately 50 Ma, with suturing completed by 49 Ma (1, 2), although other critical estimates of initial contact differ from the Paleocene, *ca.* 55 Ma (3), to as young as the Eocene–Oligocene boundary 35 Ma (4).

The present biota of India, including Sri Lanka, harbors taxa that reflect its geological history, with assorted vertebrates and plants having distributions that also encompass Africa, Madagascar, and often the Seychelles (these islands are a Cretaceous fragment of the India–Madagascar landmass). Such taxa include, for example, boid snakes of the genus *Eryx* (5); *Apocheilus* and *Pachypanchax* killifishes (6); the unusual frog *Naiskabatrachus* from the western Ghats, which is closely related to the endemic seychellian family Sooglossidae (7); and two sister genera of

cichlid fishes, *Etroplus* in southern India and *Paretroplus* in Madagascar (8). Plant taxa include two genera of trees in the Moraceae that are closely related to figs (*Ficus*) [*Mesogyne* in Africa and *Antiaris* in India (9)], as well as the family Dipteroocarpaceae, which is discussed below. This common, disjunct distribution is usually interpreted as a relict of gondwanan drift, though dispersal has also been invoked for some cases (see, for example, refs. 10–12).

Indeed, phylogenetic relationships further reveal that additional Recent taxa in India are basal to Southeast Asian lineages, suggesting immigration from the docked subcontinent, the so-called “out-of-India” hypothesis. Support for this hypothesis comes from studies of some amphibians, notably rapid frogs (13) and caecilians of the families Uraeophyllidae and Ichthyophiidae (14) as well as some plants (e.g., Crypteroniaceae; ref. 15). An example of the opposite, “out-of-Asia” direction of immigration involves *Paracrostoma* freshwater snails (16).

As India collided with Asia, there was undoubtedly significant biotic interchange between both landmasses, with India being both biotic “ferry” (17) and biotic sink. The question is how endemic was India’s paleobiota prior to contact, and did it contain numerous African and Malagasy taxa? One-hundred million years of isolation of the subcontinent (partly with Madagascar and the Seychelles) presumably spawned a highly diverse and endemic biota, analogous to the remarkable endemism presently seen in Australia, but which has been isolated for only the past 30 myr since the Oligocene.

Interestingly, the Cretaceous and Early Cenozoic vertebrate fossil record of India thus far reveals little endemism. Fossil mammals from the Early Eocene of western India, for example, have affinities with contemporaneous taxa from Europe (18, 19), and models have been proposed of broad land–bridge connections between drifting India with Africa, Madagascar, and/or island arcs from the Asian landmass (4, 20), which explains the existence of widespread groups in Paleogene India. Abundant evidence is presented here of recently excavated, diverse arthropod fossils preserved in extensive amber deposits from the Cambay Shale [early Ypresian (50–52 Ma)] of western India, which directly

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addresses conflicting models of the biotic and geological history of India and southern Asia.

Cambay amber was deposited not only during a tectonically critical period, but also at the peak of the Early Eocene Climatic Optimum (EECO), a time when tropical and subtropical biomes spread globally (21, 22). Radiations of disparate, ecological keystone insects that are hypothesized to have taken place during the EECO include highly social lineages, pollinators, parasitoids, and diverse phytophages (23, 24). These specifically are the corbiculate bees, including the eusocial stingless bees and honey bees, which are competitively superior pollinators; ants of the presently dominant subfamilies Formicinae, Dolichoderinae, and Myrmecinae; the “higher” termites (family Termitidae, comprising 80% of all Recent termite species); the highly diverse schizophoran flies and chalcidoid wasps; as well as the phytophagous lineages of neococcoid scale insects and the macrolepidopterans. Although diverse arthropods are preserved as compressions in Eocene shales from western North America and Europe, it is the vast mid-Eocene (Lutetian) Baltic amber deposits that have contributed the most to understanding Early Cenozoic insects (23). Because amber preserves minute, delicate organisms with lifelike, microscopic fidelity, this preservation greatly refines inference on relationships of extinct taxa. Baltic amber and the contemporaneous Rovno amber from the Ukraine, as well as the latest Paleocene-earliest Eocene Oise amber from France, were formed in a warm temperate/“subtropical summerwet” paleoenvironment (25), although the first two of these were formed by conifers and the Oise amber had an angiosperm origin (26, 27). The Cambay Shale amber deposits, in contrast, were formed in a broad-leaved, tropical, everwet paleoenvironment at the paleoequator (vs. 47–52°N for Baltic and 42–47°N for Oise amber). Besides the Cambay amber, the only other major deposits of fossiliferous amber produced in a fully tropical paleoenvironment are from the Miocene of Chiapas, Mexico (28) and Hispaniola (29), whose paleobiotas are very similar to Recent ones of Central America and the Caribbean. The Eocene Cambay amber and its diverse arthropod inclusions thus also provide unique data on the early evolution of tropical forest ecosystems.

Results and Discussion

Cambay Amber and Its Geological Context. Approximately 150 kg of amber were collected within deep lignitic deposits of the Older Cambay Shale in Gujarat state, western India, from the Vastan (N 21° 25.239, E 073° 07.249) and Tadhkeshwar (N 21° 21.400, E 073° 04.532) lignite mines (Fig. 1A). These strata have been assigned an age of mid- to early-Ypresian (50–52 Ma) based on shark teeth (30), the index foraminiferan *Nummulites burdigalensis burdigalensis* (31), and dinoflagellates (32). Amber was concentrated in near-shore sediments by low energy, brackish water and marine incursions (33) into seams 5–10-cm thick of an “amber conglomerate,” with most pieces ranging in size from sand grains to several centimeters, and scattered pieces up to 20 cm in length (Figs. S1 and S2). Chemical composition is distinctive, being a Class II or dammar-type resin (34, 35), which is a cadinene-based polymer produced mostly by trees in the Dipterocarpaceae (36, 37). Fossil wood from the Vastan mine (Fig. S1 C and D) containing amber and resin canals has an anatomy attributable to that of the Dipterocarpaceae (Fig. S3), thus confirming the botanical source of this amber.

Cambay amber is poorly crosslinked and polymerized, as freshly exposed surfaces are soft and often tacky, and pieces dissolve completely in toluene and chloroform. This unique property allows extraction of entire inclusions, although they are extremely fragile and readily fragment. Extracted insects examined with scanning electron microscopy have cuticle with ultrastructural preservation (Fig. 1 B and C), which allows submicron resolution of morphological characters and, therefore, far improved phylogenetic inference.

Alimohammadian et al. earlier reported inclusions in Eocene India amber (38), but from the Vastan mine only, and that report was based on only five arthropod inclusions unidentified to family. Thus far, we have found more than 700 arthropod inclusions, belonging to 14 orders and more than 55 families (Table 1), as well as numerous plant and fungal remains such as spores, mycelia, stems, pollen, portions of leaves, and even several small flowers.

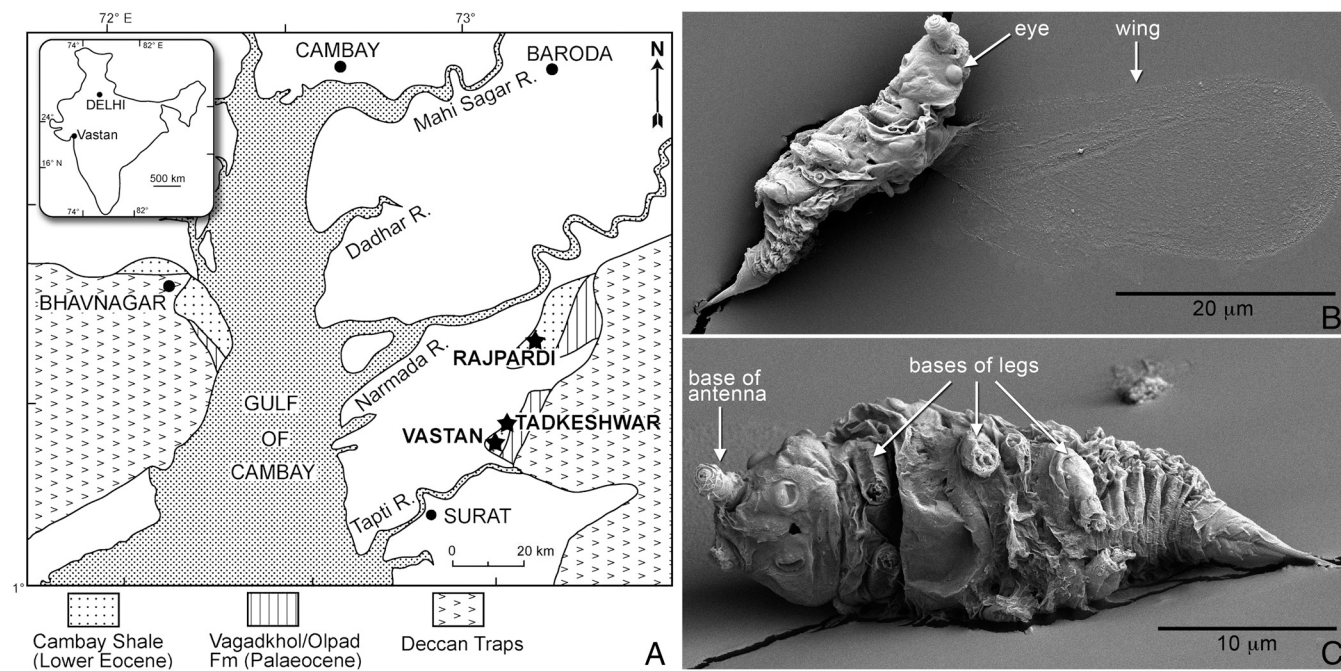


Fig. 1. (A) Map showing location of mines and distribution of formations yielding Early Eocene Cambay amber from Gujarat, India. (B and C) Scanning electron micrographs of male scale insect (Neococcoidea), fully extracted from Cambay amber using solvents; (B) oblique dorsal view with wing outstretched, (C) ventral view.

Table 1. Taxa of arthropods in Cambay Amber

MYRIAPODA			
Diplopoda		Sciarioidea	Ceratopogonidae Psychodidae <i>Phlebotiella eoindianensis</i>
Polydesmoidea			Lygistorrhinidae: <i>Palaeognoriste</i> Mycetophilidae: Manotinae Keroplastidae
ARACHNIDA		Brachycera	
Acarina			Rhagionidae Dolichopodidae Medeterinae indet. ?Neurogoninae indet. Sympycninae indet.
Families undet.			Phoridae Metopininae indet. <i>Rhopica</i> sp. Phorinae indet.
Araneae	Mimetidae Pholcidae Thomisidae Uloboridae		
Scorpiones	Buthidae?		
Pseudoscorpiones			
HEXAPODA		Schizophora	Campichoetidae: <i>Pareuthychaeta</i> sp. Milichioidea
Collembola	Families undet.		
Orthoptera	Family undet.		
Dictyoptera			
Mantodea	Families undet. Chaeteessidae		
Isoptera	Rhinotermitidae		
Blattodea	Families undet.		
Psocodea	Lepidopsocidae Families undet.		
Thysanoptera	Families undet.		
Hemiptera			
Sternorrhyncha	Coccoidea		
Auchenorrhyncha	Families undet. Cicadellidae		
Heteroptera	Families undet. Dipsocoromorpha Miridae Leptopodidae: <i>Leptosalda</i>		
Coleoptera	Families undet. Staphylinidae Chrysomelidae: Alticinae		
Hymenoptera			
Chalcidoidea	Aphelinidae, nr. <i>Euryischia</i> Mymaridae Family undet.		
Proctotrupeoidea	Diapriidae Scelionidae		
Aculeata	Bethylidae (?) Formicidae Dolichoderinae Formicinae Myrmecinae Ponerinae Pseudomyrmecinae Apidae Electrapini Melikertini		
Diptera	Limoniidae Cecidomyiidae Chironomidae		

(continued)

Amber Paleobiota and Biogeography. Some insect taxa in Cambay amber have phylogenetic affinities with taxa from the Eocene of northern Europe, particularly the Baltic amber (younger by some 8–10 myr). These include the following: A genus of small bees near *Melikertes* (Apidae: Melikertini) (Fig. 2A) and a much larger species of bee in or near the extinct genus *Protobombus* (Electrapini) (a group also preserved as compressions in the oil shales of Messel/Eckfeld, Germany) (39, 40). In flies (Diptera), Baltic connections include a fungus gnat of the genus *Palaeognoriste* (family Lygistorrhinidae) (41) (Fig. 2C); the acalyptate fly genus *Pareuthychaeta* (Campichoetidae), and a genus of medeterine Dolichopodidae. Affinities specifically to the Eocene of northern Europe are perhaps biased by the fact that Baltic amber is the most prolific and best studied amber Lagerstätte in the world, containing the most diverse insect fauna of all fossil deposits (23).

Despite the biased comparisons to the Baltic amber paleobiota, phylogenetic affinities of the identified insects in Cambay amber also lie with Recent taxa from southeastern Asia and Australasia. These include a scuttlefly (Phoridae) of the genus *Rhopica*, known only as eight Recent described species from Australia to southeastern China (Hainan Island) (Fig. 2D) (42), and another medeterine dolichopodid closely related to an undescribed genus from Australasia. In ants, a highly sculptured, distinctive myrmecine (Fig. 2B) is closely related to a small group of Recent southeast Asian and Australian genera, *Lordomyrma*, *Meyriella*, and *Austromorium*. Perhaps the most interesting Australasian ant connection of the Cambay amber concerns the dolichoderine genus *Leptomymex*, known in the Recent of New Caledonia, Australia, and New Guinea and, remarkably, in Miocene amber from the Dominican Republic.

An unexpected biogeographic connection of insects within Cambay amber concerns two rare groups that presently exist in the Neotropical Region and also occur in Miocene amber from Mexico and/or the Dominican Republic. One of these species is a leptosaldine heteropteran (Fig. 2E), known otherwise as one Recent species from Colombia (*Saldolepta kistnerorum*), and as two species in Miocene amber from nuclear America (*Leptosalda chiapensis*, in Mexican amber, and an undescribed species of the same genus from the Dominican Republic). The other presently Neotropical group is represented by a late-instar nymph (Fig. 2F) of the distinctive, basal family of mantises, Chaeteessidae, of which there are eight described Recent species throughout the New World tropics and several undescribed species in Dominican amber.

The Cambay amber fauna thus shows widespread, even global, affinities, with only minor indication of isolation or insularity (43, 44), and surprisingly no African or Malagasy connections

yet found. This evidence supports the nonexclusive hypotheses that there were at the very least archipelagic connections between precontact India and Asia (4, 20, 31), or that India and Asia collided earlier in the Paleocene (e.g., ref. 3).

The most ecologically significant aspect of the Cambay amber fauna is the diverse social insects. Besides early corbiculate bees, the fauna also includes the earliest definitive Rhinotermitidae termites (Fig. 2*G*), which is a recently derived, cosmopolitan family of 335 living species whose oldest definitive fossils until now were from the Miocene (24). Cambay amber also preserves one of the earliest diverse paleofaunas of ants (Formicidae), the only other of comparable age is preserved in amber from Oise, France. The oldest ants are from the Albian (Early Cretaceous) (23), and all Cretaceous species are primitive and rare, always comprising much less than 1% of all insect inclusions in any one deposit. Baltic amber contains nearly 150 species of ants in 9 subfamilies (45, 46), a fauna that has accrued after more than a century of study upon hundreds of thousands of inclusions. Ants in Baltic amber comprise 8% of all arthropod inclusions; the 15 species of ants in Cambay amber comprise 6% of all arthropods, including early records for the Recent subfamilies Pseudomyrmecinae and the very diverse Myrmecinae, as well as species in the subfamilies Dolichoderinae, Formicinae, and Ponerinae. Peak diversity of Recent ants occurs in primary, lowland tropical forests, which further reflects the paleoenvironment of the Cambay amber.

This amber preserves a very diverse paleofauna associated with an ancient forest of Dipterocarpaceae, a family that virtually defines forests of tropical southeastern Asia today, and which comprises up to 80% of the canopy-emergent trees in this region (47). Borneo is the center of extant diversity, with some 287 species in 9 genera; India is on the western edge of the Asian distribution. Synthesis of large amounts of resin occurs primarily in five genera in the subfamily Dipterocarpoideae, an Asian monophyletic group of the greatest species diversity (470 spp.). Resin production in these trees can be so voluminous that it is even the source for some Neogene oil deposits from Southeast Asia (48). Despite earlier views that dipterocarp forests of Southeast Asia developed in the Miocene (49, 50)—indeed, the oldest dipterocarp pollen from India is Miocene—the large volume of dammar-type resin and anatomy of the wood in the Cambay Shale reveals an age of Dipterocarpaceae more than twice that commonly held. Molecular phylogeny of the family indicates that subfamilies Pakarimoidea (1 species, South America) and Montoideae (1 species from Colombia, 34 species from Africa and Madagascar) are sister groups to the Dipterocarpoideae (51), although reanalysis of those data indicates that the Malagasy family Sarcolaenaceae is actually most closely related to this last

subfamily (52). Either way, a gondwanan origin of Asian dipterocarps is strongly supported (also indicative of a pre-Neogene age of the family), but the Laurasian affinities of arthropods in Cambay amber attest to a disparate biogeographic heritage of this dominant ecosystem.

Molecular estimates of the origins of broad-leaved (angiospermous) tropical forests range from the Early to mid-Cretaceous (53), which conflicts with direct macrofossil evidence such as leaves and wood, that such forests developed in the early Cenozoic, generally the Eocene to Paleocene (54–58), with the earliest records being the Early to Late Paleocene of the Western Hemisphere (55, 58). One reason for the discrepancy is that molecular estimates are typically much older than divergence times based on morphology and fossils (59, 60). Second, molecular estimates and pollen records cannot account for the physiognomy that distinguishes rain forest, such as arborescence, leaf shape, and the presence of epiphytes and lianas (54). Third, many groups existed in the fossil record far before they became ecologically dominant, such as ants and termites (23, 24), so it is likewise unrealistic to assume that the times of estimated taxon divergence or radiation is the same as the spread of a dominant ecosystem. Based on direct fossil evidence, the Cambay Shale Formation provides one of the earliest unequivocal Asian records of a diverse, broad-leaved tropical forest and its diverse arthropod fauna.

Methods

Specimen preparation and photography: Amber was screened for inclusions, and pieces with inclusions were trimmed with a water-fed diamond saw, embedded in epoxy (Buehler) according to the protocols in ref. 61, and ground and polished with a water-fed flat lap wheel using emory papers of 400–4,000 grit sizes. Embedding stabilizes pieces for very close, accurate trimming. Selected inclusions were extracted by dissolving in toluene for several hours, then soaking in 70% ethanol. Photomicrography used a fiber optic flash illumination system (MicrOptics®) coupled to a Nikon digital camera and an Infinity®K-2 lens.

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