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Cretaceous–Tertiary diversification among select Scolopendrid centipedes of South India

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ABSTRACT

Given that peninsular India was part of the Gondwanan super continent, part of its current biota has Gondwanan origin. To determine the Gondwanan component of the peninsular Indian biota, a large number of species spanning diverse taxonomic groups need to be sampled from multiple, if not all, of the former Gondwanan fragments. Such a large scale phylogenetic approach will be time consuming and resource intensive. Here, we explore the utility of a limited sampling approach, wherein sampling is confined to one of the Gondwanan fragments (peninsular India), in identifying putative Gondwanan elements. To this end, samples of *Scolopendrid centipedes* from Western Ghats region of peninsular India were subjected to molecular phylogenetic and dating analyses. The resulting phylogenetic tree supported monophyly of the family Scolopendridae which was in turn split into two clades constituting tribes Otostigmini and Scolopendrini–Asanadini. Bayesian divergence date estimates suggested that the earliest diversifications within various genera were between 86 and 73 mya, indicating that these genera might have Gondwanan origin. In particular, at least four genera of Scolopendrid centipedes, *Scolopendra, Cormocephalus, Rhysida* and *Digitipes*, might have undergone diversification on the drifting peninsular India during the Late Cretaceous. These putative Gondwanan taxa can be subjected to more extensive sampling to confirm their Gondwanan origin.

1. Introduction

The complex geological history of peninsular India and its influence on the biogeography of the Indian subcontinent has been much debated in recent times (Datta-Roy and Karanth, 2009; Karanth, 2006). Geologically, peninsular India is very distinct from the rest of the Indian subcontinent, as it was part of the Gondwanan super continent around 200 million years ago (mya) (Chatterjee and Scotese, 1999). The peninsular Indian plate along with Madagascar and Seychelles separated from the African plate (Western Gondwana) around 160 mya and from the Antarctica–Australia plate (Eastern Gondwana) around 130 mya. Subsequently, peninsular India separated from Madagascar around 88 mya and rafted across the Indian ocean before colliding with the Eurasian plate around 55 mya (Ali and Aitchison, 2008; Briggs, 2003). This complex geological history of peninsular India provides the underpinnings for the "Biotic ferry" model or the "Out-of-India" hypothesis, which proposes that rafting peninsular India carried ancient Gondwanan elements to Asia after the break-up of Gondwana super continent (Bossuyt and Milinkovitch, 2001). However, it has been suggested that the dramatic latitudinal and climatic changes that affected peninsular India during its northward drift in conjunction with massive volcanism at the Cretaceous–Tertiary (K–T) boundary caused substantial extinction of its biota (Officer et al., 1987). Nevertheless, in recent years, mounting evidence from various molecular studies has supported an Out-of-India Gondwanan origin of some tropical Asian taxa (Datta-Roy and Karanth, 2009; Karanth, 2006).

Interestingly, many of these ancient Gondwanan lineages of peninsular India are endemic to the relictual patches of wet evergreen rain forests of the Western Ghats (Biju and Bossuyt, 2003; Bossuyt and Milinkovitch, 2001; Karanth, 2006). The Western Ghats are a chain of mountains approximately 1600 km in length that run parallel to the west coast of peninsular India. However during the early Tertiary periods evergreen rain forests were extensive and covered most of peninsular India (Meher-Homji, 1983; Prasad et al., 2009). Thus, much of the Gondwanan biota of peninsular India was likely to have been adapted to wet tropical climate. Nevertheless with the fusion of peninsular India with Asia and the establishment of monsoon weather, the climate throughout much of India turned drier (Meher-Homji, 1983). Evergreen forests retreated to wetter parts of the peninsula, chiefly the Western Ghats. Thus, these relictual patches of evergreen rainforests in the Western Ghats might have served as important refugia for Gondwanan elements. The Western Ghats today exhibit a very high level of heterogeneity in vegetation both at the local and landscape level (Nagendra and Utkarsh, 2003; Subramanyam and Nayar, 1974). Additionally, it is one of the Global Biodiversity

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Hotspots which is threatened due to intense anthropogenic activity (Myers et al., 2000). In recent times, many floral and faunal surveys have been undertaken in the Western Ghats, and these studies have reported numerous new species (Ansari et al., 1970; Biju and Bossuyt, 2003; Biju et al., 2005; Giri et al., 2009). Biogeographers have noted that the biota of the Western Ghats consists of ancient Gondwanan elements, as well as recent intrusive elements from South China and Southeast Asia (Mani, 1974; Subramanyam and Nayar, 1974). However their conclusions were based on the overall similarity in flora and fauna between biogeographical realms rather than on the phylogenetic affinities of targeted taxa. Thus, one of the interesting questions pertaining to peninsular Indian biogeography that remains unanswered till date is: What component of the Western Ghats' biota is of Gondwanan origin?

In the case of the Western Ghats, given the large number of species spanning diverse taxonomic groups, addressing the aforementioned question will require enormous amounts of time and resources. This is because, for a robust test of the Gondwanan origin hypothesis, these taxa will have to be sampled from multiple, if not all, Gondwanan fragments (Datta-Roy and Karanth, 2009). Nevertheless, only a subset of the Western Ghats' biota that have putative Gondwanan origin need to be targeted, but *a priori* how do we choose these taxa? One approach is to choose taxa that have Gondwanan distribution, i.e., are currently distributed in multiple Gondwanan fragments. However, molecular studies suggest that the current "Gondwanan distributions" in some taxa can be better explained by recent dispersal events that occurred after the breakup of the Gondwana (Datta-Roy and Karanth, 2009). Thus taxa distribution alone does not provide us with adequate information in choosing putative Gondwanan elements. Therefore, an alternative, more robust approach, that does not rely solely on taxa distributions, needs to be developed to identify putative Gondwanan elements of the Western Ghats. Here, we explore the feasibility of identifying putative Gondwanan elements of the Western Ghats biota primarily based on divergence dates estimates among taxa sampled from this region. Given that peninsular India separated from Madagascar around 80–90 mya and supposedly underwent a prolonged period of isolation before collision with Eurasia (Bossuyt and Milinkovitch, 2001), Gondwanan elements on this landmass are predicted to have undergone ancient diversification that would predate the collision event (around 55 mya).

As mentioned earlier numerous molecular studies have been undertaken on the Gondwanan elements of peninsular India, but much of these studies are based on vertebrate taxa and a few plant species (see (Datta-Roy and Karanth, 2009) and the references therein). Till date, no study has been undertaken on terrestrial invertebrates. Interestingly, the Late Cretaceous fossil assemblage of peninsular India also consists predominantly of vertebrate taxa with a few crustaceans forming the only invertebrate component (Khajuria and Prasad, 1998). These fossils suggest both Gondwanan, as well as Eurasian affinity for the Cretaceous biota of peninsular India (Bajpai and Kapur, 2008; Prasad and Rage, 1995).

In this regard, terrestrial flightless invertebrates have immense potential as a suitable model system to study Gondwana biogeography. This is because, they usually have limited dispersal ability (Beavis and Rowell, 2006; Contreras-Diaz et al., 2003; Eason, 1964; Edgecombe and Giribet, 2007) and therefore are more likely to be tied-down to the landmass they occur in. Secondly, many of the terrestrial invertebrates represent some of the oldest faunal groups on the planet, encompassing deep evolutionary history (Giribet et al., 2001; Wheeler et al., 2004). Centipedes (Class: Chilopoda), for instance, are one of the most ancient (Upper Silurian), terrestrial arthropods and are distributed in all the continents except Antarctica (Edgecombe and Giribet, 2007). They are nocturnal, predatory animals with low dispersal ability (Edgecombe, 2007) and form a substantial component of soil fauna (Kalisz and Powell, 2000). Centipedes of the family Scolopendridae (Scolopendromorpha) exhibit high levels of diversity (23 spp.) and endemicity (13 spp.) in the Western Ghats. Worldwide this family includes of 458 species across 19 genera belonging to four tribes – Otostigmini, Asanadini, Arrhabdotini and Scolopendrini. Three of these tribes are represented in the Western Ghats, but Otostigmini and Scolopendrini exhibit higher levels of diversity than Asanadini. Additionally, Otostigmini and Scolopendrini also have contrasting distributional patterns. Otostigmini is found in tropical climates and is mostly distributed in the former Gondwanan landmasses of South America, Africa, India, and Australia, while Scolopendrini is also found in temperate climates and has a worldwide distribution. Thus, these two tribes provide us with an interesting comparison to test contrasting biogeographical scenarios. Given the Gondwanan distribution of Otostigmini, it is plausible that the members of this tribe in the Western Ghats have an ancient Gondwanan origin and underwent diversification on drifting peninsular India. In contrast, Scolopendrini given its cosmopolitan habit and global distribution might have arrived on the Indian plate after its collision with Asia. Thus, diversification among Indian Otostigmini is predicted to fall in Late Cretaceous to Early Paleocene when peninsular India was drifting towards Asia. On the other hand, diversification within Indian Scolopendrini is predicted to have had occurred post collision in the Eocene (less than 55 mya).

To test these hypotheses, the molecular phylogeny of family Scolopendridae, sampled predominantly from Western Ghats, was constructed using two mitochondrial DNA markers, 16S ribosomal DNA (16S rDNA) and cytochrome oxidase I (COI), and one nuclear 28S ribosomal DNA (28S rDNA) gene. Additionally, divergence dates within tribes Otostigmini and Scolopendrini were estimated through a Bayesian approach with multiple fossil calibrations. The resulting phylogeny was also compared with morphology based taxonomy of the Scolopendrids.

2. Materials and methods

2.1. Taxon sampling

Scolopendridae is represented in the Indian subcontinent by three tribes Scolopendrini, Asanadini and Otostigmini. Scolopendrini consists of three genera, *Scolopendra, Cormocephalus* and *Arthrorhabdus*. Asanadini is monogeneric with genus *Asanada* in India. Otostigmini is represented by four genera *Rhysida*, *Ethmostigmus*, *Digitipes and Otostigmus* in India. As our focus was on the molecular phylogeny of family Scolopendridae, taxon sampling was done at the generic level. Accordingly sampling was done across the Western Ghats such that six out of the eight genera representing all three tribes were sampled. These include *Ethmostigmus*, *Rhysida*, *Digitipes*, *Scolopendra*, *Asanada*, and *Cormocephalus*. We have also included sequences of the genus *Alipes* mainly distributed in South Africa and Madagascar as well as three genera from the closest families Cryptoptidae and Scolopocryptopidae namely *Cryptops, Theatops* and *Scolocryptops*. Additionally, five different genera of Geophilomorpha and two species of Craterostigmomorpha were used as outgroup in the analysis (Table 1). All the species of family Scolopendridae were identified using key provided by Jangi and Dass (1984). In addition, Chilopoda database (http://chilobase.bio.unipd.it/) was referred for the recent advances in systematics of Scolopendrids.

2.2. DNA isolation and purification

Genomic DNA extraction was carried out using ethanol preserved tissue samples. In most cases, leg tissues were used, but in few cases segment tissue were also used. The extraction was

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Table 1
List of specimens used in the current analysis. Asterisks indicate taxa for which sequences were downloaded from GenBank.

	Name		CES No.	COI	16S rDNA	28S rDNA	Latitude	Longitude
Scolopendromorpha Scolopendridae Scolopendrini	*Cormocepahlus monthanii			DQ201430	AF370861	AF173280		
	C. nigrificatus		CES07129	JN003997	JN003886	JN003942	11.202	76.439
	C. nigrificatus		CES07136	JN003998	JN003887	JN003943	11.201	76.438
	C. nigrificatus		CES07139	JN003999	JN003888	JN003944	11.204	76.439
	C. spl		CES07200	JN004000	JN003889	JN003945	8.756	77.114
	C. nudipes		CES07205	JN004001	JN003890	JN003946	15.409	74.476
	C. sp2		CES07220	JN004002	JN003891	JN003947	15.273	74.960
	C. westwoodi		CES07256	JN004003	JN003892	JN003948	14.624	75.668
	C. westwoodi		CES07258	JN004004	JN003893	JN003949	14.624	75.668
	C. nudipes		CES07265	JN004005	JN003894	JN003950	13.038	77.200
	Scolopendra cf. morsitans	1	CES07106	JN004006	JN003895	JN003951	15.409	74.476
	S. cf. morsitans	2	CES07107	JN004007	JN003896	JN003952	15.409	74.476
	S. cf. morsitans		CES07203	JN004008	JN003897	JN003953	13.038	77.200
	S. cf. morsitans		CES07204	JN004009	JN003898	JN003954	13.038	77.200
	S. cf. morsitans		CES07212	JN004010	JN003899	JN003955	18.419	73.907
	S. cf.amazonica		CES07235	JN004011	JN003900	JN003956	14.436	74.426
	S. cf.amazonica		CES07252	JN004012	JN003901	JN003957	14.436	74.426
Asanadini	Asanada aghakari		CES07225	JN004013	JN003902	JN003958	14.990	74.378
	A. brevicornis		CES08951	JN004014	JN003903	JN003959	15.962	74.002
Otostigmini	Rhysida lithobiodis		CES07102	JN004015	JN003904	JN003960	15.409	74.476
	R. cf. imarginata		CES07148	JN004016	JN003905	JN003961	13.038	77.200
	R. sp. l		CES07165	JN004017	JN003906	JN003962	8.680	77.159
	R. longipes		CES07172	JN004018	JN003907	JN003963	8.659	77.178
	R. longipes		CES07180	JN004019	JN003908	JN003964	8.719	77.125
	R. longipes		CES07187	JN004020	JN003909	JN003965	8.731	77.124
	R. cf. imarginata		CES07193	JN004021	JN003910	JN003966	8.730	77.124
	R. cf. imarginata		CES07202	JN004022	JN003911	JN003967	13.038	77.200
	R. cf. imarginata		CES07224	JN004023	JN003912	JN003968	14.988	74.375
	R. cf. imarginata		CES07238	JN004024	JN003913	JN003969	14.436	74.426
	R. longipes		CES07262	JN004025	JN003914	JN003970	14.922	75.248
	R. longipes		CES07263	JN004026	JN003915	JN003971	14.922	75.248
	R. cf. imarginata		CES07271	JN004027	JN003916	JN003972	16.292	73.908
	R. cf. imarginata		CES07275	JN004028	JN003917	JN003973	16.292	73.908
	R. cf. imarginata		CES07276	JN004029	JN003918	JN003974	16.292	73.908
	Ethmostigmus sp.l		CES07279	JN004030	JN003919	JN003975	15.962	74.002
	E. sp. 2		CES07286	JN004031	JN003920	-	12.389	75.490
	Digitipes cf. coonoorensis		CES07125	JN004032	JN003921	JN003976	11.202	76.439
	D. cf. coonoorensis		CES07126	JN004033	JN003922	JN003977	11.201	76.438
	D. sp. l		CES07127	JN004034	JN003923	JN003978	11.178	76.415
	D. cf. coonoorensis		CES07132	JN004035	JN003924	JN003979	11.204	76.439
	D. sp. l		CES07133	JN004036	JN003925	JN003980	11.205	76.442
	D. cf. coonoorensis		CES07134	JN004037	JN003926	JN003981	11.209	76.441
	D. cf. coonoorensis		CES07137	JN004038	JN003927	JN003982	11.209	76.441
	D. cf. barnabasi		CES07157	JN004039	JN003928	JN003983	8.678	77.160
	D. cf. barnabasi		CES07160	JN004040	JN003929	JN003984	8.680	77.159
	D. cf. barnabasi		CES07161	JN004041	JN003930	JN003985	8.680	77.159
	D. cf. barnabasi		CES07162	JN004042	JN003931	JN003986	8.682	77.163
	D. cf. barnabasi		CES07166	JN004043	JN003932	JN003987	8.666	77.171
	D. cf. barnabasi		CES07169	JN004044	JN003933	JN003988	8.664	77.179
	D. cf. barnabasi		CES07171	JN004045	JN003934	JN003989	8.659	77.178
	D. cf. barnabasi		CES07173	JN004046	JN003935	JN003990	8.663	77.171
	D. cf. barnabasi		CES07174	JN004047	JN003936	JN003991	8.664	77.169
	D. sp. 2		CES07219	JN004048	JN003937	JN003992	15.273	74.960
	D. sp. 2		CES07223	JN004049	JN003938	JN003993	14.988	74.375
	D. sp. 2		CES07226	JN004050	JN003939	JN003994	14.990	74.378
	D. sp. 2		CES07230	JN004051	JN003940	JN003995	14.989	74.371
	D. sp. 2		CES07233	JN004052	JN003941	JN003996	14.516	74.541
	*Alipes crotalus			AY288742	AY288720	AY288707		
	*Rhysida nuda			DQ201432	AY288722	AF173282		
	*Ethmostigmus rubripes			AY288721	AF370836	AF173281		

carried out using standard phenol chloroform method (Sambrook and Russell, 2001). Two mitochondrial DNA markers, 16S rDNA (~450 bp), COI (~600 bp) and one nuclear 28S rDNA gene (~350 bp) were PCR amplified using primers used by Edgecombe et al. (2002).

PCR amplification was carried out in a 50 μl volume reaction, with 1.25 units of Taq DNA polymerase (Banglore genei, Bangalore, India), 0.20 mM of dNTPs (Eppendorf), 2.5 mM of $MgCl_2$, 0.1 μM of each primer (Sigma) and 2 μl of template DNA (1 μg/μl). The standard PCR profile consisted of first denaturing step at 94 °C for 2 min, followed by 35 amplification cycles (94 °C for 15 s, primer specific annealing temp. for 40 s, and 72 °C for 15 s) and a final step at 72 °C for 10 min in Eppendorf thermocycler. The annealing temperature for COI ranged from 45 to 47 °C, for 16S rDNA was 49 °C and for 28S rDNA was 52 °C. The PCR products were purified using Eppendorf or Qiagen PCR purification kit and were sequenced at MWG biotech Pvt. Ltd., Bangalore, India.

2.3. Phylogenetic analysis

The chromatogram files were checked manually using the program Chromas lite 2.01 (http://www.technelysium.com.au/

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chromas_lite.html) and then aligned in ClustalW with default settings (Thompson et al., 1994). Each gene was aligned separately and individual genes for each taxa were concatenated to derive the combined dataset. To check for homogeneity in the data set, an Incongruence Length Difference (ILD) test was carried out in WINCLADA (Farris et al., 1995; Nixon, 1999). Heterogeneity in base composition was examined in PAUP for each individual gene data set.

Three different trees building methods, maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis were used. These methods were implemented on combined data set where gaps were treated as missing data. Parsimony and likelihood analyses were carried out in PAUP (Swofford, 2002) through heuristic searches with TBR branch swapping, 10 random-addition replicates, and a random starting tree. Parsimony bootstrap supports for the branch nodes were determined through bootstraping with 1000 replicates and 10 random-addition option.

The program Modeltest 3.7 (Posada and Crandall, 1998) was used on each individual gene dataset to select a model of sequence evolution through Akaike Information Criteria (AIC). The chosen model (GTR + I + Γ) and the estimated parameters were used in PAUP to derive the ML tree.

Partitioned Bayesian analysis was performed using MrBayes 3.2 (Huelsenbeck and Ronquist, 2001). A total of three partitions according to genes were made, namely 16S, COI and 28S. We used the GTR + I + Γ substitution model with uniform priors for all the three partitions. Base frequencies, rates for the GTR model, and the shape parameter of the gamma distribution were all estimated from the data in the program MrBayes for each partition separately. The program was run for four million generations wherein sampling was done for every 100 generations. To determine whether this run length was adequate we evaluate the standard deviation of the split frequencies which was below 0.01 after one million generations. Accordingly the first 10,000 trees (corresponding to one million generations) were discarded as burn-in. A consensus tree was built using the remaining trees.

2.4. Divergence time estimation

One of the oldest centipede fossils is *Devonobius delta* known from middle Devonian (385–397 mya) sediments near Gilboa, New York, USA (Shear and Bonamo, 1988). On the basis of completely different structure of maxilliped coxosternae, this species has been placed in a new family Devonobiidae and new order Devonobiomorpha which shows sister relationship to Epimorpha (Scolopendromorpha + Geophylomorpha) (Shear and Bonamo, 1988). Accordingly the stem group of Epimorpha has been assigned a mean age of around 390 mya with a standard deviation of 5 million years (my) to incorporate the middle Devonian epoch. Another fossil *Cratoraricrus oberlii* described from Lower Cretaceous (Aptian) of Brazil is one of the oldest fossil available for the family Scolopendridae (Wilson, 2003). This dates back to 112–125 mya according to International Stratigraphic Chart made by International Commission on Stratigraphy in 2008. This species has been classified as Scolopendrid based on the possession of bisegmented tarsi and paramedian longitudinal grooves on the sternites. Additionally, one more fossil description was available for an individual belonging to order Geophilomorpha which dates back to 150–155 mya (Schweigert and Dietl, 1997). These three fossil records were used to calibrate the molecular clock. The program BEAST (v1.4.8) (Drummond and Rambaut, 2007) which uses Markov chain Monte Carlo (MCMC) method, was implemented on the combined dataset to estimate node ages within family Scolopendridae. BEAST uses Bayesian approach to evaluate tree topology and divergence time simultaneously along with branch lengths incorporating uncertainty in both. The tree was rooted using two species from the order Craterostigmomorpha which is extant sister taxa to Epimorpha. The models and priors set for analysis were as follows. Combined dataset was partitioned according to individual genes and GTR + I + Γ model of sequence evolution with uniform priors was applied to each partition. The same model was chosen by Modeltest for each individual gene dataset. Given that a strict clock model of molecular evolution is purported to be biologically unrealistic (Drummond et al., 2006) a relaxed molecular clock model with uncorrelated lognormal distribution was used. A normal prior of 390 my with a standard deviation (±) of 5 was applied to the time to most common recent ancestor (tMRCA) for the in-group comprised of all Epimorpha and 150 my ± 5 was applied to the tMRCA of Geophilomorpha. The Lower Cretaceous Scolopendrid (112–125 my) fossil, whose exact phylogenetic position is unknown, was used to set the age of family Scolopendridae. Here a lognormal distribution with the log mean of 3.6 ± 1 was used such that the minimum age constrain for this node was 110 my. The Yule process for speciation, which is appropriate for species level phylogenies (Drummond and Rambaut, 2007), was used as tree prior for MCMC analysis. The program was run for 100 million generations and convergence of the chains to the stationary distribution was determined using the program TRACER (v1.4.1) (Rambaut and Drummond, 2003). The tree was visualized in the program FigTree (v1.2.2) (Drummond and Rambaut, 2007). Apart from Yule process, Coalescent process was also explored with other parameters but data set was best explained through the above mentioned combination. See Fig. 1 in Supplementary section for information on various nodes used to calibrate the molecular clock.

3. Results

3.1. Phylogenetic analysis

The dataset consisted of two mitochondrial genes COI (584 bp), 16S rDNA (509 bp) and one nuclear 28S rDNA (359 bp) marker from 71 specimens representing three orders of centipedes. Out of these 56 specimens belonging to 19 species (including *cf.*), seven genera, and three tribes of the family Scolopendridae were sequenced for the aforementioned three genes in the current study (Table 1). Remaining sequences were downloaded from GenBank. They belonged to the family Cryptoptidae and Scolopocryptopidae (Order Scolopendromorpha) and the outgroup taxa from Order Geophilomorpha and Craterostigmomorpha.

As is typical for invertebrate mitochondrial genes (Zaldivar-Riveron et al., 2008), our mitochondrial sequences were also A + T rich (68%) when compared to the nuclear ribosomal gene (43%). Significant heterogeneity in base composition was also detected in the mitochondrial genes and not in the nuclear ribosomal gene through the χ^2 test in PAUP (Swofford, 2002).

Incongruence Length Difference (ILD) test was carried out to detect heterogeneity among these data sets. Since the test was not significant ($p = 0.1667$) all subsequent analyses were carried out on the combined data set i.e. two mitochondrial and one nuclear ribosomal genes (1452 bp including indels). The maximum parsimony (MP), maximum likelihood (ML) and partitioned Bayesian tree building methods retrieved very similar trees. Since the topological congruence among these trees was also supported through Shimodaira–Hasegawa (SH) test which was not significant ($p = 0.164$), only the partitioned Bayesian tree is shown, along with the Bayesian posterior probabilities and parsimony bootstrap support for various nodes (Fig. 1).

In the Bayesian phylogram, family Scolopendridae was monophyletic with 99% bootstrap support (BS) and posterior probability (PP) of 1 (Fig. 1). Within Scolopendridae monophyly of tribe Otostigmini was strongly supported by high PP and 72% BS, while tribe Asanadini is nested within tribe Scolopendrini, rendering Scolopendrini paraphyletic. This Asanadini–Scolopendrini clade received high PP

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of 1 and low BS. Within this clade the tribe Asanadini showed sister relationship to the genus *Scolopendra* with high PP and BS.

The Otostigmini clade consisted of two distinct lineages, one representing the sole *Rhysida nuda* individual from Australia and the other constituting the remaining genera, including Indian *Rhysida*. Thus, our results do not support the monophyly of genus *Rhysida*. The clade constituting the genera *Ethmostigmus*, *Alipes* and Indian *Digitipes* and *Rhysida* was supported by PP of 1 and 52% BS. Among these genera monophyly of *Digitipes* and Indian *Rhysida* had received high PP and BS supports. Though, the relationship among them was not resolved except for the sister relationship between *Ethmostigmus* and *Alipes* that received low PP and BS but this relationship is based on only one sample of *Alipes*. Within *Ethmostigmus* clade, the Australian *Ethmostigmus* branched with the Indian individuals with a posterior probability of 1 and 82% BS.

Members of the tribe Scolopendrini fell into two distinct clades each with high PP and BS; one clade corresponded to *Scolopendra–Asanada* and the other consisted of genus *Cormocephalus*. In the *Scolopendra–Asanada* clade, individual genera were monophyletic with high support. Within genus *Cormocephalus*, the Australian species *Cormocephalus monteithi* was sister to the species from India.

3.2. Estimation of divergence times

Molecular dates based on the three fossil calibration points are shown in Fig. 2. This analysis was carried out using Bayesian inference in the program BEAST and is independent of the phylogenetic analysis carried out in the program MrBayes which also uses Bayesian approach. Additional runs wherein the oldest fossil was dropped from the analysis produced similar date estimates for various nodes. The effective sample sizes (ESS) were over 200 for all the nodes discussed below. These age estimates suggest that Epimorpha stem group was 418 my old with a credible interval (CI) of 460–380 my (not shown) and family Scolopendridae arose in the Upper Triassic around 227 (CI 299–156) mya (Fig 2). A recent study which uses likelihood framework to estimate divergence dates in centipedes reported similar divergence dates for these two nodes (Murienne et al., 2010).

Within Otostigmini, the divergence between genera *Rhysida, Digitipes* and *Ethmostigmus* occurred in the Early Cretaceous between 146 and 132 mya. Whereas, within Scolopendrini, the divergence between genera *Cormocephalus, Scolopendra and Asanada* was even older, in the late Jurassic 193–126 mya. The earliest diversifications within each of the above genera fell between 86 and 73 mya. The earliest split among the endemic Indian lineage of *Digitipes* was in the late Cretaceous around 86 mya. The basal split among Indian *Rhysida* species was around 78 mya, also in the late Cretaceous. Indian *Ethmostigmus* species diverged from Australian *E. rubriceps* around 80 mya, though Indian species have diverged more recently from each other around 38 mya, in the Eocene. In Scolopendrini, the Indian members of the globally distributed genus, *Scolopendra* turned out to be an ancient lineage wherein the estimated earliest divergence date was around 85 mya. Interestingly, another cosmopolitan genus *Cormocephalus* also represented old radiation showing diversification in the late Cretaceous. Here, the Australian *C. monteithi* diverged from Indian species around 85 mya and among Indian species the earliest split was around 72 mya.

4. Discussion and conclusions

4.1. Molecular systematics of family Scolopendridae

Attems' (1930) classification scheme divides Scolopendromorpha into the families Cryptoptidae and Scolopendridae. Shelley (2002) divides Cryptopidae further into Cryptopidae and Scolopocryptopidae whereas Lewis (2006) has reassigned one genus from the family Cryptoptidae into a new family Mimopidae. Our molecular analysis based on limited sampling is concordant with Attem's classification. In addition, monophyly of the family Scolopendridae as reported by Edgecombe and Giribet (2004) based on both morphology and molecular data, is also supported. Our combined as well as individual gene analysis always retrieved Scolopendridae as monophyletic with strong support in all tree building methods.

Molecular phylogeny of the family Scolopendridae reported here differs from the morphology base topology of Edgecombe and Koch (2008) with respect to the phylogenetic position of tribe Asanadini. According to Edgecombe and Koch (2008) Asanadini and Scolopendrini are sister tribes, whereas in the phylogeny derived from our dataset Asanadini is nested within Scolopendrini. Within Scolopendrini, analyzed specimens of the genus *Scolopendra* appeared to be either *S. morsitans* or *S. amazonica* based on morphological characters but showed very high levels of sequence divergence between them. It is likely that the Indian "*S. morsitans/amazonica*" specimens might constitute a species complex with many cryptic species. Clearly more of taxonomy work based on both morphology and molecules needs to be undertaken on the Indian *Scolopendra*.

Within Otostigmini, two groups are recognized based on the spiracle numbers – *Rhysida–Ethmostigmus* and *Otostigmus–Digitipes* (Jangi and Dass, 1984). The parsimony tree (not shown) retrieved two distinct lineages among Otostigmini, one leading to *Ethmostigmus–Rhysida–Alipes* group and the other comprising of the genus *Digitipes* thus showing some congruence with the morphology based classification. Addition of the genus *Otostigmus*, missing from the current analysis, will clarify the current results.

The Indian members of the genus *Digitipes* form a distinct clade with high support, but this result is based on samples from four out of six endemic species from peninsular India. Furthermore, our phylogeny does not have African species of this genus, which we suspect might be a separate lineage. Including the remaining Indian and African members of this genus will provide us with insights into the evolution and diversification of genus *Digitipes* in peninsular India. Interestingly, species in the genus *Rhysida* sampled from India form a separate clade distinct from Australian *Rhysida nuda* which had a basal position in Otostigmini. Thus, *Rhysida* as currently constituted is not monoplyletic and might have to split into multiple genera. Inclusion of endemic genera and species from Southeast Asia, Australia, Africa and Neotropics might help us resolve some of these issues.

4.2. Cretaceous–Tertiary diversification among Indian Scolopendrids

Our estimates of divergence dates for the earliest splits within each genus unveiled the intricate biogeographic history of the Scolopendrids of peninsular India. The prediction of a late Cretaceous diversification of Indian members of the Tribe Otostigmini (*Rhysida*, *Ethmostigmus* and *Digitipes*) holds true only in the case of *Digitipes* and *Rhysida.* The earliest diversification in the *Digitipes* clade started near late Cretaceous (86 mya, CI 135–40), when Peninsular India was moving towards Asia and was isolated from all other landmasses. Genus *Digitipes* has six endemic species in the Western Ghats and three in central Africa. In the current molecular phylogenetic and dating analyses only the Western Ghats endemic species were included thereby suggesting that diversification among them might have happened on the drifting peninsular Indian landmass before its collision with Asia around 55 mya. Thus, this group represents a putative Gondwanan relict, as it has not dispersed out of India (as per Datta-Roy and Karanth, 2009). To confirm this scenario the African *Digitipes* and remaining Indian members of this genus need to be included in the analysis. Divergence estimate within Indian *Rhysida* species was also in late

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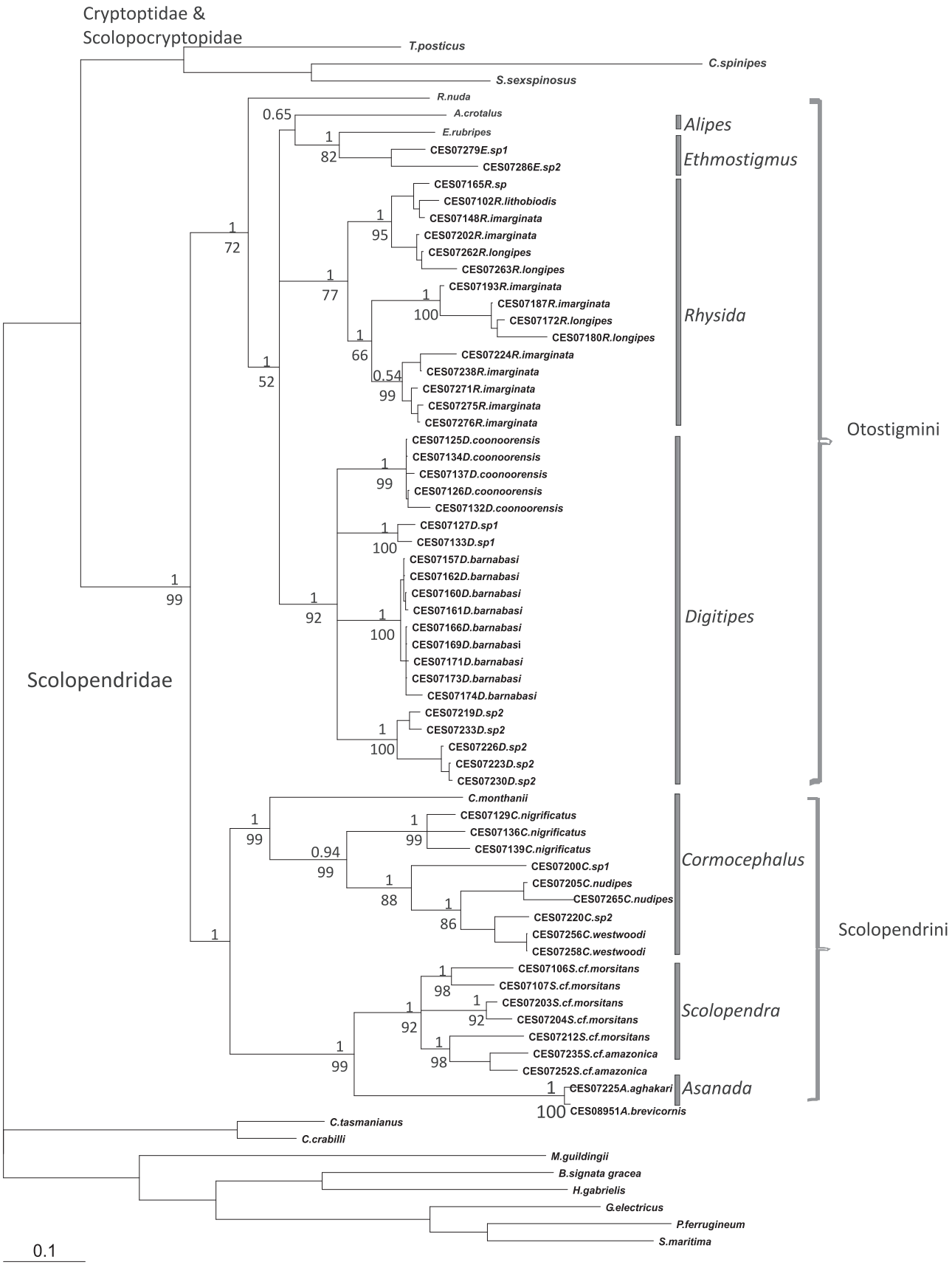

Fig. 1. Bayesian tree of the family Scolopendridae based on three genes along with posterior probability and parsimony bootstrap values. Bootstrap supports and posterior probability less than 50% and 0.5% respectively are not shown.

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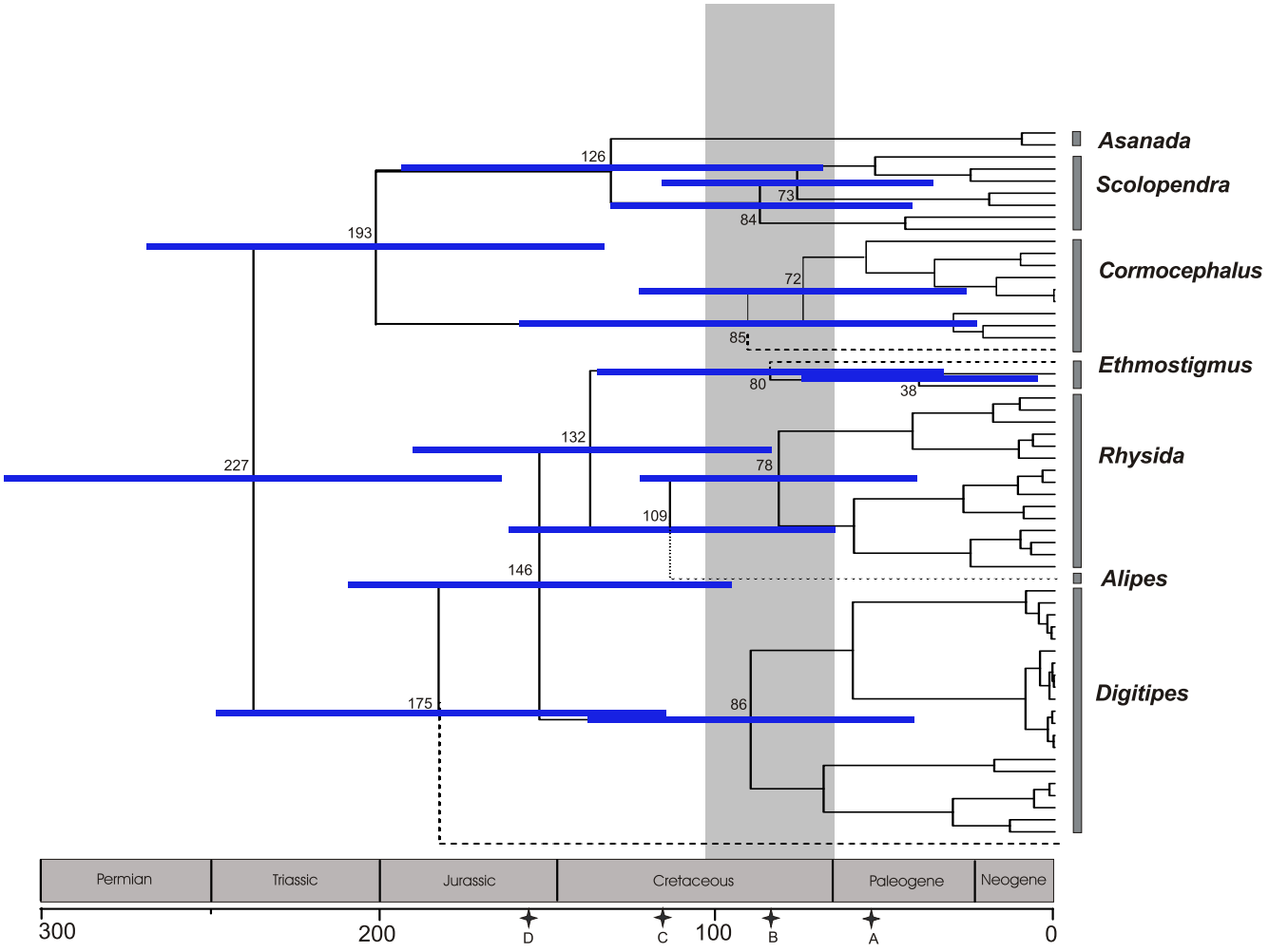

Fig. 2. Chronogram for Scolopendridae obtained through Bayesian estimation of divergence dates. Dash and dotted lines in the phylogeny indicate lineages from Australia and Africa respectively. Blue bars with the mean divergence time (for all nodes which are >35 mya) indicate the credible intervals. Late Cretaceous period is shown as grey column and stars indicating the key Gondwana geological events that are relevant to the peninsular Indian Plate. (A) Collision of peninsular India with Eurasian plate (55 mya). (B) Separation of India from Madagascar (88 mya). (C) Separation of India–Madagascar from Antarctica–Australia (130 mya). (D) Separation of India–Madagascar from the African plate (160 mya). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Cretaceous (78, CI 40–115 mya). Apart from Indian subcontinent, Neotropics and Southeast Asia also holds a number of species in the genus *Rhysida*. To confirm its Gondwanan affinity, we need to include members of *Rhysida* from these regions.

Interestingly, contrary to our expectation, at least two genera in the tribe Scolopendrini appear to have undergone diversification in the Late Cretaceous. Members of genus *Scolopendra* from peninsular India represent one of the oldest generic level radiations among the Scolopendrids sampled here. The earliest nodes in this clade again fall in the Late Cretaceous around 84 mya (CI 126–40). Thus, the sampled *Scolopendra* appear to have diverged from each other during the time when peninsular India was drifting through Indian ocean and some members of this lineage might have dispersed out of India. To rigorously test this scenario, the relationships between *Scolopendra* from India and other parts of the world needs to be ascertained. The earliest diversification among the members of the genus *Cormocephalus* was also in the Late Cretaceous around 85 mya (CI 150–70) suggesting that this lineage also has a Gondwanan history. The divergence estimate within Indian *Cormocephalus* was around 73 mya (CI 92–42), which suggests that this Indian lineage might have dispersed out of India after collision of peninsular India with Asia around 55 mya. Additional sampling of *Cormocephalus* from other Gondawanan fragments as well as from Southeast Asia will clarify this scenario. In the case of the genus *Asanada*, on the other hand, the divergence date between the two specimens collected from India suggest their post-collision origin, though further sampling is needed to confirm this evolutionary scenario.

Thus, our phylogenetic analysis and date estimates suggest that at least four genera of *Scolopendrid centipedes* might have undergone diversification on the drifting peninsular India during Late Cretaceous and thus represent putative Gondwanan elements. Three of them, *Scolopendra*, *Cormocephalus* and *Rhysida* probably dispersed out of India, while the other, *Digitipes*, might be a Gondwanan relict. Additionally, results indicate that certain taxa specific traits such as global distribution and cosmopolitan habit cannot be taken as surrogate data to infer recent dispersal. Furthermore, the limited sampling approach implemented here is useful in identifying the peninsular Indian taxa that might have Gondwanan origin. These taxa can now be targeted for further study wherein samples from other landmasses are included for a robust test of the hypotheses generated here.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.04.024.

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