Phylogeography of a rare and endemic tree of Western Ghats reveals its ice age dynamics

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ABSTRACT:

The climatic shifts in the Quaternary Period acted as an important driving force for evolution of the world's biodiversity. In this study, responses to the ice age of a rare and endangered tree of Indian Western Ghats, *Syzygium travancoricum,* was investigated through a combination of molecular data and ecological niche modeling.

Results indicate the moderate (nrDNA) to high (cpDNA) population structure, presence of private alleles, and absence of phylogeographic structure. Spatial distribution of diversity does not significantly support southern colonisation, nor the Palghat Gap as a geographical barrier based on the genetic structure. Combined insights from Ecological Niche Modelling and population history depicts a probable colonisation of the Western Ghats in the late Miocene or later followed by a split into separate southern and northern populations from the middle to late Pleistocene. The prevalence of favourable conditions in the post-Last interglacial period stimulated rapid population expansion and a very recent decline. The absence of phylogeographic structure and overall climatic stability from Last Interglacial do not offer support to an existence of refugia in the Western Ghats. We conclude by discussing appropriate conservation measures for this rare and endangered tree based on our findings.

Keywords:Quaternary period, Western Ghats, endemic tree, *Syzygium travancoricum*, glacial refugia, Myrtaceae

Abbreviations: WG: Western Ghats; ENM: Ecological niche model; LIG: Last Interglacial period; LGM: Last Glacial Maxima, nrDNA - nuclear DNA, ptDNA - chloroplast DNA

Running title: Quaternary dynamics of a Western Ghats endemic tree

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Introduction

The Quaternary Period is seen as a key driver of evolution of the world's biota resulting in the current geographic patterns (Hewitt, 2000; 2004 and references therein). A great deal of research on the biotic elements of Euro-America and China has yielded insights into the responses to this period. For example, the identification of glacial refugia or cryptic refugia, prediction of extinction events, range contraction and expansion, colonisation of new landscapes, and divergence and speciation (Hewitt, 2004; Willis and Niklas, 2004; Provan and Bennett, 2007; Stewart et al., 2010).

In the past, a few paleo-palynological studies have recreated a preliminary vegetation and climate history of India during the Quaternary Period and have described the fate of the Western Ghats and surrounding regions (Singh et al., 1974; Sukumar et al. 1993; Rajagopalan et al., 1997; Meher-Homji and Gupta, 1999). Likewise, a number of studies have analysed pollen deposits from across various regions of India and elucidated local floral assemblages during similar timescale (e.g. Sharma et al., 2006; Chauhan et al., 2015). A recent study by Bose et al. (2016) revealed a local persistence of most of the Western Ghats endemics in the Quaternary and pre-Quaternary Periods, which they attributed to climatic stability sheathing taxa from migration and/or extinction. Altogether, it suggests a serious lack of studies researching the Quaternary history of the Western Ghats biota. Integration of the existing palynological insights with

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knowledge from various disciplines, e.g., insights from genealogical history would help us to understand the persistence of taxa under past climatic events, and may also give clues to their biogeographic evolution.

Syzygium travancoricum Gamble is a critically endangered and endemic tree of Western Ghats of family Myrtaceae,(IUCN 2015). This plant is mostly found in *Myristica*-dominated swamps or forest patches of Western Ghats of Kerala, Karnataka, and Goa states (Sasidharan, 1997; Chandran et al., 2008, 2010; Roby et al., 2013; Ray et al., 2014). Paleo niche modelling in a phylogeographic framework was undertaken to explain its response and to test the following hypotheses: i) *Syzygium travancoricum* underwent a southward colonization in the Western Ghats, ii) the Palghat Gap acted as a geographic barrier for this species, and iii) a few areas of Western Ghats acted as glacial refugia to sustain climatic upheaval.

Materials andMethods

Sampling, DNA Extraction, PCR, and Sequencing

Fresh leaf samples were collected from wild populations of *Syzygium travancoricum* between 2013-15 from various locations across its known distribution (Table 1, Figure 1a-b). We procured necessary permission from Forest Departments for sample collection. We sampled one to ten individuals per population. Fresh leaves were dried and stored at - 20°C.

For each sample, approximately 50 mg of leaves were used to extract total genomic DNA according to a modified CTAB procedure (Doyle and Doyle, 1987). We have amplified and sequenced two ptDNA (*trnH-psbA* and *rpl20-rps12*) and two nrDNA fragments (ITS and ETS) using our designed primers SjITS and SjETS respectively (Table S1). The polymerase chain reaction (PCR) was performed in total volume of 25μL, which contained 30–60ng plant DNA,

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50mM Tris-HCl, 1.5mM$MgCl_2$, 0.5mMdNTPs, 2mM of each primer and 0.75 unit of Taq polymerase. All reactions were performed using the following temperature profile: 3 min at 94°C, 35 cycles of 1min at 94°C, 1 min of annealing at 58°C, and 2 min at 72°C, with a final 10 min extension at 72°C.

Sequencing was performed using an ABI Prism BigDye Terminator Cycle V3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturers' protocols. All DNA sequences were edited, aligned with ClustalW as implemented in MEGA 5.2, double-checked manually, and insertions/deletions (indels) were excluded.

Genetic diversity and Haplotype network construction

Sequences were used to estimate the basic population genetic parameters, including the number of segregating sites (S), Watterson's parameter (θw), nucleotide diversity (π), haplotype number (h), and diversity (He) in DnaSP version 5.00.04 (Librado and Rozas, 2009). For both chloroplast and nuclear DNA, evolutionary relationships among the individuals were drawn employing median joining algorithm in NETWORK V4.5.1.6 (www.fluxus-engineering.com) (Bandelt et al., 1999).

Population and Phylogeographic Structure

In order to understand the extent of divergence, the F_{ST} value for nrDNA was estimated using an AMOVA as implemented in ARLEQUIN version 3.1.1 (Excoffier and Lischer, 2000). This was done by dividing the total distribution range, taking Palghat Gap into account, and by delimiting populations on either side of it, i.e. central and southern Western Ghats (henceforth CWG and SWG respectively) followed by a further division of CWG and SWG into smaller sub-populations (i.e. G, UK, TH, DK-NK, and CK, SK details of which is provided in table -1) thereby testing hierarchical genetic structure. This enabled us to test the hypothesis that the

Palghat Gap has been acting as biogeographic barrier for this taxon. Additionally, to test for phylogeographic structure and to identify the glacial refugia, h_T and v_T were estimated using PERMUT (Pons and Petit 1996) using the nuclear markers.

Inference and Simulation of Demographic Histories

The population history of *Syzygium travancoricum* was investigated using an Approximate Bayesian Computation (ABC) framework in DIYABC 2.0.4 based on nrDNA sequences (Cornuet et al. 2014) (Figure 2) to understand the responses of the species to Quaternary period. The dataset was divided into two groups consisted of combined CWG + CK populations and SK. The rationale of the grouping was largely based on high pair-wise F_{ST} values between DK-NK and SK coupled with the minimal effect of Palghat Gap as barrier. We have performed several iterations to test the following scenarios: i) simple divergence; ii) divergence with a bottleneck; iii) divergence with expansion; and iv) single population (data not shown), where 1×10^6 data sets for each scenario were simulated (Table S2).

The fitness of each scenario was determined stepwise by comparing with the real data sets by estimating errors and precision, best model selection, and finally quantification of the posterior largely following Shang et al. (2015): i) we selected the ‘pre-evaluation scenario-prior combinations’ option which was employed to check the similarity of the simulated data with the observed data; ii) type I and type II errors were computed to assess the confidence in choice of scenario in a standard manner as implemented in the program; iii) to compare alternative population scenarios, posterior probabilities of the scenarios were computed by employing logistic regression on the 1% of simulated data sets closest to the observed data set, and the one with the highest posterior probability was selected; iv) in order to evaluate the precision of parameter estimation, median of the absolute error was divided by the true parameter value of the 10,000 pseudo-observed data sets followed by simulation under the selected scenario; and, finally v) the models were checked to evaluate the ability of a given scenario to produce data sets similar to the real data set (Corunet et al. 2010). The posterior distributions of the parameters were estimated for the most plausible scenario that outperformed others.

Ecological Niche Modeling

Ecological Niche Modelling (ENM) was employed to predict the distribution of *Syzygium travancoricum* during four periods: (i) present time; (ii) the mid-Holocene (~6000 BP); (iii) the Last Glacial Maxima (LGM, ~ 22,000 BP); (iv) and at the Last Interglacial (LIG, ~ 120,000 – 140,000 BP). For ENM, climatic and soil moisture variables were extracted from WORLDCLIM database (www.worldclim.org) and Terrestrial water budget data archive (Willmott and Matsuura 2001). Thirty-two occurrence records were collected from the recent field data (Pascal and Ramesh, 1997; Sasidharan, 1997; Chandran et al., 2008, 2010; Ray et al., 2012, 2014; Roby et al., 2013). Of which, a total of 21 points that were at least 10 km apart (to avoid the bias among clustered occurrence records) were selected for the model development. This was done to ensure spatial independence of occurrence data. Relevant environmental variables (20; 19 bioclimatic and soil moisture) were prioritised through correlation analysis and PCA (Principal Component Analysis) to avoid model over-fitting. Firstly, the correlated variables (≥ 0.7) were grouped and from this group, the variable with the highest loading on the principle axes was selected (Slender et al. 2013). Based on selected occurrence records and environmental variables (6 bioclim + soil moisture), ensemble niche models were developed using present condition and then projected for the other three time periods (LIG, LGM, and mid-Holocene) using BIOMOD2 package of R (Thuiller et al. 2013) along with minor modifications in parameters. A total of six algorithms in BIOMOD2 [ANN (Artificial Neural Network), CTA (Classification Tree Analyses), GAM (Generalised Additive Model), GLM (Generalised Linear Model), MARS (Multivariate Adaptive Regression Splines), and RF (Random Forest)] and axEnt were used for the modelling exercise based on their earlier performances. Best performing models were selected based on: (i) the area under the relative operating characteristic curve (AUC); (ii) Cohen's kappa; (iii) the true skill statistic (TSS); and (iv) sensitivity. The binary distribution maps were generated based on ROC value of the weighted mean ensemble models. The contribution of environmental variables in model development was assessed through randomisation technique as described in BIOMOD2 to find out the variable importance in case of all models. The model-independent approach helped making a direct comparison of variable importance across the models.

Results

Genetic Diversity and Haplotype Network

The overall genetic variation was moderate (chloroplast) to high (nuclear) as shown by segregating sites (S), Watterson's parameter (θw), nucleotide diversity (π), haplotype number (h) (Table 2). Genetic diversity of SWG appeared to be marginally higher than CWG except the number of nuclear haplotypes.

The combined nuclear haplotype network showed two major high frequency haplotypes connected to several others with an intermediate frequency separated by a single or multiple mutations (Figure 1c). There were a few rare ones which are represented by only one individuals. The chloroplast network was depicted by a simple single major haplotype with other low frequency ones connected to it (Figure not shown). The spatial distribution of nuclear haplotypes exhibited a predominance of H1 haplotype followed by H4 and H2. However, it has also showed the presence of several low frequency haplotypes in both the UK and SK subpopulations (Figure 1b).

Population Structure

The hierarchical genetic structure revealed moderate overall differentiation at nuclear loci (F_{ST} = 0.12, P= 0.00, Table 3) and various degree of differentiation among sub-population pairs (pairwise F_{ST} = 0.0002 - 0.16) (Table S4). The analysis indicated that the southern SWG (SK) population was distinctly different from the rest among a cluster of sub-population pairs. Also, a higher differentiation (F_{ST} = 0.42, P=0.00) between SWG and CWG (Table S5) was evident through uni-parental chloroplast marker. Similarly, the values of h_T and v_T were 0.765 and 0.0773 proved an absence of phylogeographic structure rather mixing of lineages (Petit et al., 2002).

Population history

The population history of *Syzygium travancoricum* in the Western Ghats was investigated using ABC. Among the tested models, divergence with expansion scored the highest (posterior probability = 0.968) and appeared as the best explanatory model to comprehend the evolutionary history of the tree species given current genetic diversity and its distribution (Table 4). The timing of divergence ranged from 52 - 77 Kya (Table 5, mean value), considering a generation time of 8 - 12 years (based on field investigations). Branching out of the populations gradually followed by an expansion of several degrees of magnitude (mean value: n_{1a} = 1.4 X 10^5 to n_1 = 5.52 x 10^6, n_{2a} = 2.22 X 10^5 to n_2 = 5.3 X 10^6) during late Quaternary. Both the chloroplast and nuclear datasets also yielded unimodal mismatch distributions supporting of historical expansion (Fig. S1).

Reconstruction of paleo-niche

Ensemble modelling approaches, MARS, CTA, RF, and GAM showed consistently better value for specificity (0.75-1.0) in comparison to GLM and ANN (0.4-0.9) (i.e. AUC, TSS and kappa values); with marginal difference in sensitivity. Randomization experiment with variables indicated that bioclim 7 (annual temperature range), bioclim 10 (mean temperature for warmest quarter), bioclim 16 (precipitation of wettest quarter) and soil moisture played a pivotal role in the model.

The exercise on paleo niche construction showed gradual expansion of species distribution in course of time. During the LIG period (the farthest hindcast map), the species distribution was restricted to the southern Western Ghats and a small part of the coast in central Western Ghats region (Figure 3). However, the distribution then expanded during LGM until the Holocene. Current scattered distribution could be due to human mediated fragmentation of contiguous relic forests affecting appropriate micro-habitats.

Discussion

The Western Ghats biota owes primarily to Gondwana elements and subsequent floral massing (Mani 1974) to become one of the 35 global biodiversity hotspots. A number of studies have documented the biotic diversification in Western Ghats from Cretaceous onwards (Joshi and Karanth, 2013; Prasad et al., 2009; Srivastava, 2009; Robin et al., 2015). In contrast, the Quaternary climatic oscillation has transformed the evolutionary trajectory of biota in relatively recent times (Hewitt, 2004), yet its effect on Indian biotic elements is poorly understood.

The current study has elucidated Quaternary history of an endemic and critically endangered tree of Western Ghats integrating paleo niche into the population genetics framework.

Genetic Diversity, Population Structure, and Refugia

Genetic diversity and population structure are central parameters to infer extent of population differentiation. In order to tap the molecular signal as well as to test the hypothesis of biogeographic barrier, we have investigated hierarchical genetic structure. The selected molecular markers tend to demonstrate slower mutation rate, thus it is unlikely to accumulate newer mutation in the studied time frame. However, the already existing mutations would rearrange, fix in population(s) or low frequency ones may go extinct due to genetic drift. The hierarchical genetic structure shows overall moderate (F_{ST}=0.12 in nrDNA) to high (F_{ST}=0.42 in ptDNA) F_{ST} values which denoted the two groups CWG and SWG were at moderate to high degree of differentiation. However, we did not found significant differentiation (pairwise F_{ST} at nuclear loci) between the populations at either side of the Palghat Gap (i.e., DK-NK and CK). This in turn, does not support the hypothesis that the Palghat Gap acted as biogeographic barrier for this species, contrary to the earlier findings for montane birds (Robin et al. 2012). The underlying reason may be linked to the habitat; unlike a montane species, the gene flow most likely via seed dispersal or pollen movement was not restricted by the gap and hence unable to isolate the populations across the barrier.

However, the most southerly sub-population of SWG (e.g. SK) is significant because the population possessed private alleles and remained highly differentiated from the rest. This implied that the southern population is one of the centres of intra-specific diversity which is genetically distinct and could be a refugial location during the glaciation. Similarly, the central Western Ghats (i.e. north of Palghat Gap) could be the another location for refugia as indicated by high diversity and rare alleles. However, the apparent absence of phylogeographic structure (h_T<= v_T) and an indication of mixing of lineages (Petit et al., 2002) suggest the existence of refugia to be inconclusive. Likewise, climatic stability throughout Quaternary Period does not support the presence of refugia (Bose et al. 2016). Some researchers have hypothesized about Cretaceous refugia which resurrected the depauperate Western Ghats in a post-volcanic period; whereas, the apparent absence of scientific data makes the case difficult to identify ice age refugia (Prasad et al., 2009; Joshi and Karanth, 2013; Robin et al., 2015). Recently, Bose et al. (2016) ruled out the existence of ice age refugia in Western Ghats, instead they hypothesised the persistence of the local endemic biota during the last 150,000 years. Although their conclusions were drawn entirely from the reconstruction of paleo-niche the idea of biotic existence in localized refugial areas in Western Ghats appears untenable under a stable climate during Quaternary.

Population History

The spatial distribution of genetic diversity illustrates patterns but keeps silence on underlying factors. In contrast, the knowledge of a species population history can offer clues to evolutionary trajectory of the taxon. During the middle to late Miocene there was a rapid radiation of *Syzygium* genus when *Syzygium travancoricum* was split from its sister species followed by an expansion and gradual colonization of the Western Ghats (Ray et al. unpublished data). However, the genetic signatures do not provide strong evidence whether the colonization happened from north to south, as it the alternate scenario seems equally convincing. The nuclear and chloroplast genetic diversity of the SWG is marginally higher than CWG, so SWG could have acted as founder and this makes the northward colonization of the species more likely. However, the current interpretation is preliminary and is constrained by relatively low resolving power of the used markers.

The colonization could have taken place during the late Miocene to early Quaternary when paleo-climate was comparatively favorable (Gupta, 2010). Climatic perturbations caused a major shuffling, extinction, and localization of biota with the onset of Quaternary (Jackson and Overpeck, 2000; Hewitt, 2004; Willis and Niklas, 2004). This taxon might have responded to these events and its once continuous distribution became fragmented as a result of the isolation between southern and northern population which become separated during last 0.179 - 0.268 mya (i.e. during middle to late Pleistocene), perhaps due to climatic adversity (Dynesius and Jansson, 2000). However, niche models cannot track this divergence perhaps due to its relatively ancient nature (i.e. before last interglacial). The climatic conditions following this period gradually stabilised and presumably, the initiation of favourable period sparked the population expansion by several orders of magnitude 50,000-80,000 years ago. The more or less star shaped haplotype network with a high frequency central haplotype connected to low frequency haplotypes also supports an expansion, similar notion is also revealed by the unimodal distribution of pairwise differences.

The recreated paleo-niche model also depicted a narrow distribution in LIG expanding considerably during the LGM which could be attributed to the availability of favourable climate in and around central and southern Western Ghats. The paleoclimatic studies from Western Ghats have suggested a comparatively drier climate during LGM due to a weakening of the summer monsoon and the impact would be higher on montane species and species sensitive towards seasonality (Farooqui et al., 2014; Bose et al., 2016). *Syzygium travancoricum* might have taken refuge in moisture laden lowland areas due to its preference to swampy habitat usually associated with perennial water bodies (Chandran et al., 2008, 2010). Recent paleoclimatic studies reveal of continuous precipitation due to south-west and north-east monsoon with varying magnitude despite arid condition prevalent in LGM period and especially in the southern Western Ghats (Kim et al., 2008; Farooqui et al., 2014). Moreover, the vast land exposed during glacial period was covered with marshy or swamp habitat (as evidenced from fossil studies, Farooqui, 2014), a preferred condition for *S. travancoricum.* Subsequently, the strengthening of the summer monsoon and pre-monsoon rainfall in post-LGM maintained the climate appropriate for species habitat

(Farooqui et al., 2014). However, the recent patchy distribution may be due to anthropogenic disturbance causing by reduced native vegetation over the last few hundred years.

Implications for Conservation

The Western Ghats is one of the biodiversity hotspots of India. Although, the broad pattern of biodiversity has been explored to some extent the spatial dimension of genetic diversity remains unknown and these pockets of endemic diversity could be important regions for conservation of biological wealth. It becomes even more crucial in the face of global warming and consequent changes in the regional climate. The refugia which had been safer havens for the biota in the Quaternary climate can presumably be better suited to recuperate current or later climate change (Eeley, 1999) and are the ideal candidate regions for conservation priorities. Although, the analyses on refugia turns out to be inconclusive we have identified two major foci of intraspecific genetic diversity. These areas are reservoirs of rare variation or ‘hotspots’ and genetically unique compared to rest of the distribution range. Interestingly, most samples have been collected from the sacred forest patches spread over these areas (Ray et al., 2014). Many of these sacred groves conserved over centuries are also associated with swamp ecosystem dominated by Myristicaceae. They offer protection and maintain viability of the taxa perhaps by sheltering pollinators, facilitating seed dispersal, creating favourable conditions for seedling recruitment and growth. Whereas, isolated individuals are extremely vulnerable and prone to destruction (Ray et al., 2014). Therefore, conservation of the sacred eco-systems through participatory framework could help in sustaining rare and endemic taxa.

Conclusions

Molecular phylogeography with paleo niche modelling has provided vital insights into ice age dynamics of a rare and endemic tree of Western Ghats, a region known for its exceptional biodiversity. The findings have unraveled a probable northward colonisation of the Western Ghats followed by a population split and expansion in response to ice age. We have found two foci of intra-specific genetic diversity, relatively stable climate and a lack of phylogeographic structure but these do not conform to the existence of refugia in the region. Similarly, the role of the Palghat Gap as barrier is not supported by a lack of structure across the gap. To summarise, this study provides useful information on the dynamics of a rare endemic tree, while spawning a range of new questions. For example, in the absence of refugia, what are the probable causes of the mixing of lineages over time? What are the current dynamics in terms of gene flow? What is the inheritance of the genetically distinct southern SWG region? There is a potential for answering these evolutionary questions with highly polymorphic markers like microsatellites and SNPs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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PERMISSION TO CARRY OUT FIELDWORK

We have received required permission for collecting specimens in few cases. However, in most cases specimens collected from fringe areas outside the sanctuaries or national parks.

AUTHORS' CONTRIBUTIONS

AR and RR conceived of the study, drafted the project design, collected field specimens, and carried out the molecular lab work and analyses. AR, RR, and TVR participated in drafting the manuscript. All authors gave final approval for publication.

DATA ARCHIVING

Data could be archived upon acceptance of the manuscript.

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Table 1: Sampling information of *Syzygium travancoricum* for chloroplast DNA and nuclear DNA analyses (N(ptDNA) = number of individuals sequenced for chloroplast DNA, N(nrDNA) = number of individuals sequenced for nuclear DNA).

Region	Area/ location	Site/ population	voucher number	Collectors	Latitude (°N)	Longitude (°E)	N(ptDNA)	N(nrDNA)
CWG (Central Western Ghats)	Goa (G)	Brahmakamal Sacred grove, Goa	HJCB1313	Rajasri Ray, Vishnu Mukhri	15.58	74.17	4	5
	Uttar Kanada (UK)	Katlekan	HJCB1304	Rajasri Ray, Vishnu Mukhri	14.27	74.75	5	6
		Joginmane	HJCB1306	Rajasri Ray, Vishnu Mukhri	14.41	74.71	2	4
		Mattigar	HJCB1301	Rajasri Ray, Vishnu Mukhri	14.31	74.87	12	12
		Murkundi Nala	HJCB1305	Rajasri Ray, Vishnu Mukhri	14.29	74.8	1	1
		Duginmane	HJCB1303	Rajasri Ray, Vishnu Mukhri	14.31	74.86	3	4
		Aralihonda	HJCB1302	Rajasri Ray, Vishnu Mukhri	14.29	74.89	6	6
		Hulkodu	HJCB1320	Rajasri Ray, Vishnu Mukhri	14.14	74.96	0	2
	Thirthalli	Thirthalli	HJCB1310	Rajasri Ray, Vishnu Mukhri	13.69	75.31	5	5
	Dakshin Kanada and north Kerala (DK-NK)	Coorg	HJCB1311	Rajasri Ray, Vishnu Mukhri	12.14	75.77	1	2
		Subramanya	HJCB1312	Rajasri Ray, Vishnu Mukhri	12.69	75.61	1	2
		Palieri Kavu, Kerala	HJCB1309	Avik Ray, Rajasri Ray, Vishnu Mukhri	12.19	75.19	4	3

		Andaloor, Kerala	HJCB1308	Avik Ray, Rajasri Ray, Vishnu Mukhri	11.79	75.48	9	14
SWG (Southern Western Ghats)	Central Kerala (CK)	Kalasamala, Kerala	HJCB1307	Avik Ray, Rajasri Ray, Vishnu Mukhri	10.67	76.09	11	13
	Southern Kerala (SK)	Thenmala	HJCB1314	Avik Ray, Rajasri Ray, Vishnu Mukhri	8.97	77.05	5	6
		Onnam Mile-S	HJCB1316	Avik Ray, Rajasri Ray, Vishnu Mukhri	8.92	77.11	6	7
		Onnam Mile-N	HJCB1315	Avik Ray, Rajasri Ray, Vishnu Mukhri	8.91	77.11	8	7
		K.valavu	HJCB1319	Avik Ray, Rajasri Ray, Vishnu Mukhri	8.89	77.13	1	2
		Poovanathu Mode	HJCB1318	Avik Ray, Rajasri Ray, Vishnu Mukhri	8.86	77.08	4	4
		Plavu Chal	HJCB1317	Avik Ray, Rajasri Ray, Vishnu Mukhri	8.87	77.09	7	6

Table 2: Genetic diversity and phylogeographic structure of *Syzygium travancoricum* for chloroplast DNA and nuclear DNA markers (S= number of segregating sites, h=number of haplotypes, Hd= haplotype or gene diversity, π = Nucleotide diversity, θw = Watterson's parameter).

	Nuclear					Chloroplast				
	S	h	Hd	π	θw	S	h	Hd	π	θw
CWG	11	16	0.763	0.0016	0.003	7	7	0.603	0.0012	0.0018
SWG	17	15	0.807	0.0025	0.005	14	11	0.477	0.0012	0.0036
Total	26	29	0.789	0.0021	0.0065	19	16	0.697	0.0018	0.0042

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Table 3 - Results of Analysis of Molecular Variance (AMOVA, nDNA) of *Syzygium travancoricum*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	5.461	0.06810	7.70
Among populations within groups	4	5.419	0.03955	4.47
Within populations	105	81.517	0.77635	87.82
Total	110	92.396	0.88400	

F_{ST} : 0.12178

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P-value = 0.0000

Table 4. Posterior probability of each scenario and its 95% confidence interval based on the logistic estimate by DIYABC.

Scenarios	Posterior Probablity	95% Confidence interval (lower–upper)
Divergence	0.0314	0.0284 - 0.0343
Divergence with expansion	0.9681	0.9652 - 0.9711
Divergence with bottleneck	0.0005	0.0004 - 0.0006

Table - 5: Parameter estimation in DIYABC, n_1 and n_2 are effective population size at time t=0, n_{1a} and n_{2a} are effective population sizes at time t2 and t3 before present, and t1 time before present the two populations diverged from each other (time in terms of generation time).

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Parameters	Mean	Mode	95% Confidence interval (lower–upper)
n_1	5.52×10^6	9.8×10^6	7.79×10^5 - 9.81×10^6
n_2	5.3×10^6	8.52×10^6	6.92×10^5 - 9.76×10^6
n_{1a}	1.4×10^5	4.52 X 10^4	9.32×10^3 - 6.33×10^5
n_{2a}	2.22×10^5	6.1×10^4	2.07×10^4 - 7.76×10^5
t_1	6.48×10^3	9.56×10^3	8.98×10^2 - 9.87×10^3
t_2	6.34×10^3	9.45×10^3	6.85×10^2 - 9.87×10^3
t_3	2.24×10^4	1.41×10^4	5.55×10^3 - 4.65×10^4

Figure 1: a) A broader view of the geographic distribution ofthe taxa; b) six major areas / localities of *Syzygium travancoricum* populations where sampling carried out, and the distributions of nuclear DNA haplotypes; c) nuclear haplotype network.

Figure 2: The three scenarios of population demography examined in DIYABC. t is time scale measured in generations and n is effective population size of the corresponding populations (e.g. n_1 = effective population size of CWG at t=0) during the time period (e.g., 0–t_1, 0–t_2, 0–t_3). The population divergence has taken place t_1 time before present followed by i) no change in population size, ii) an expansion (at t_2 and t_3 time before present when n_{1a} changed to n_1 and n_{2a} changed

to n_2 respectively) or iii) a bottleneck in both the populations (at t_2 andt_3 time before present when n_{1a} changed to n_1 and n_{2a} changed to n_2 respectively).

Figure 3: Potential distribution of *Syzygium travancoricum* Gamble in Western coast of India at different time periods: (a) the Last Inter Glacial Period (LIG); (b) the Last Glacial Maxima (LGM), green border indicates extended land mass during LGM; (c) the Holocene and (d) Current distribution pattern

Supplementary information legends

Table S1: Primer pairs used in the study

Table S2 - The details of the prior distributions of demographic parameters (n_1 and n_2 are effective population size at time t=0, n_{1a} and n_{2a} are effective population sizes at time t2 and t3 before present, and t1 time before present the two populations diverged from each other).

Table S3a-b: Genbank accession numbers of the individuals included in the current study: a) nuclear DNA, b) chloroplast DNA

Table S4: Population Pairwise Fst of nuclear data [Goa (G), Uttar Kanada (UK), Thirthalli (TH), Dakshin Kanada and North Kerala (DK-NK),Central Kerala (CK), and Southern Kerala (SK)]

Table S5 - Results of Analysis of Molecular Variance (AMOVA, cpDNA) of *Syzygium travancoricum*

Figure S1 (a–b): Mismatch distributions based on nuclear and chloroplast sequences.

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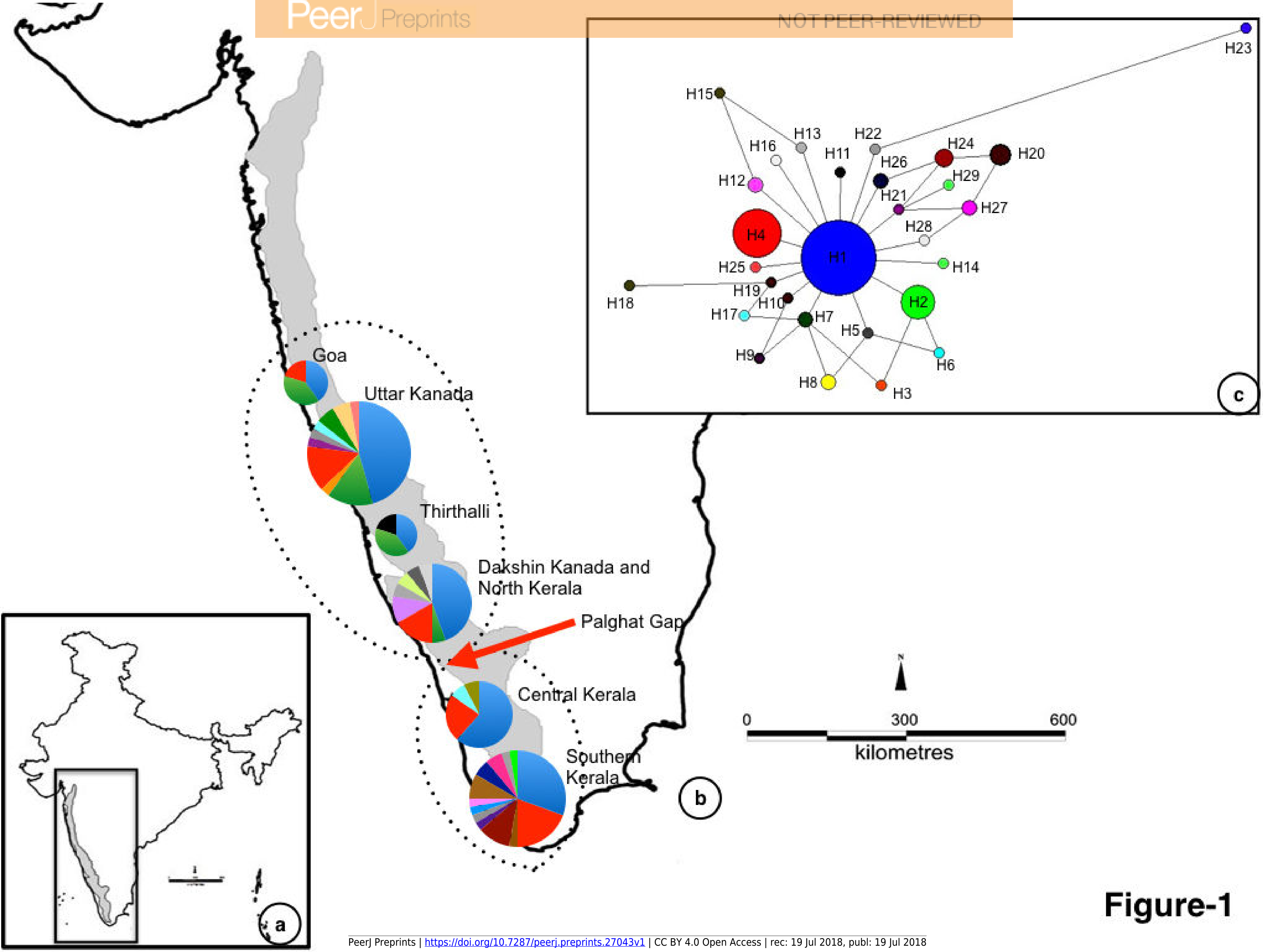
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H5
H9
H6
H8
H3
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Uttar Kanada
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Figure-1

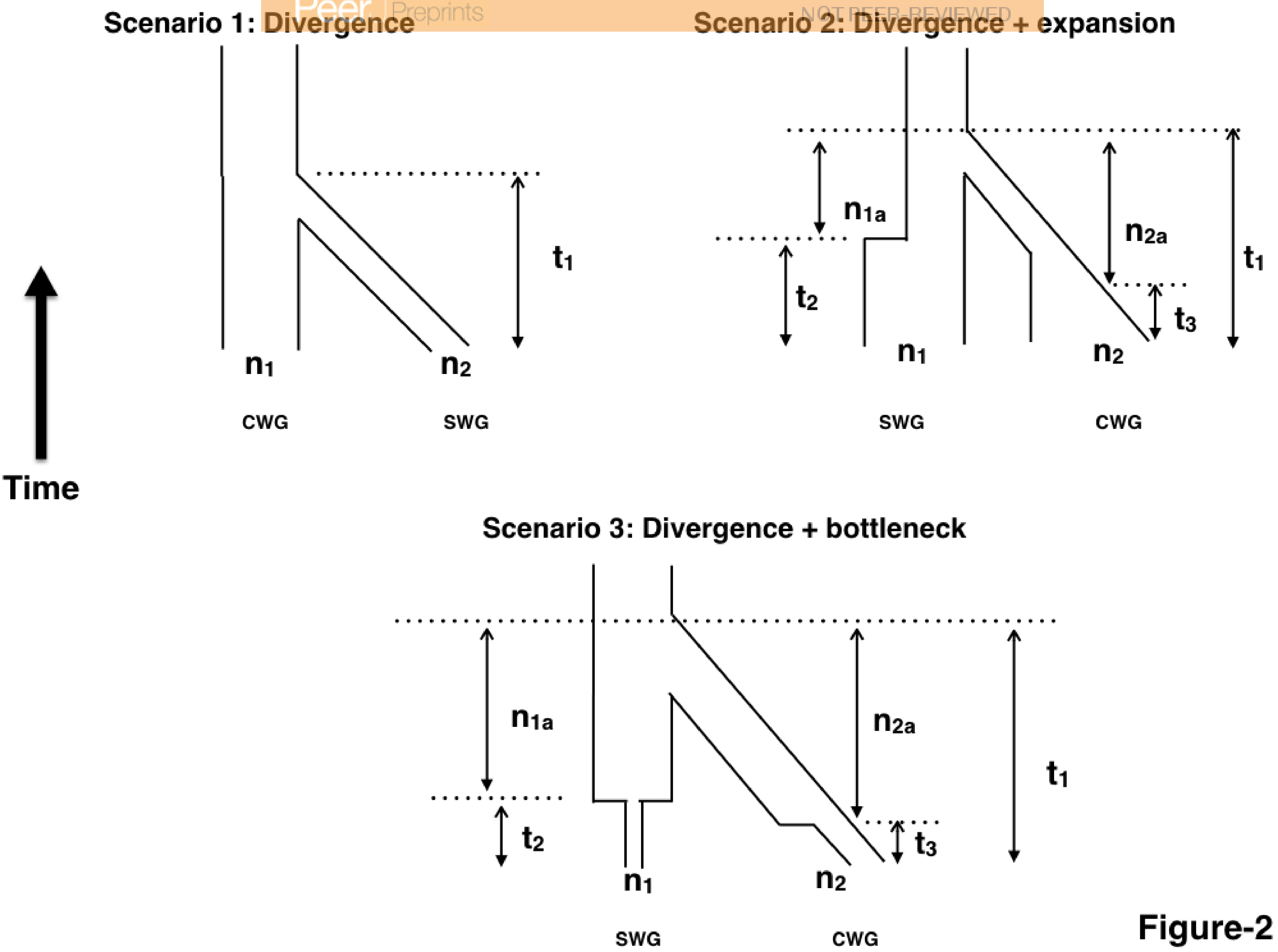

Figure-2

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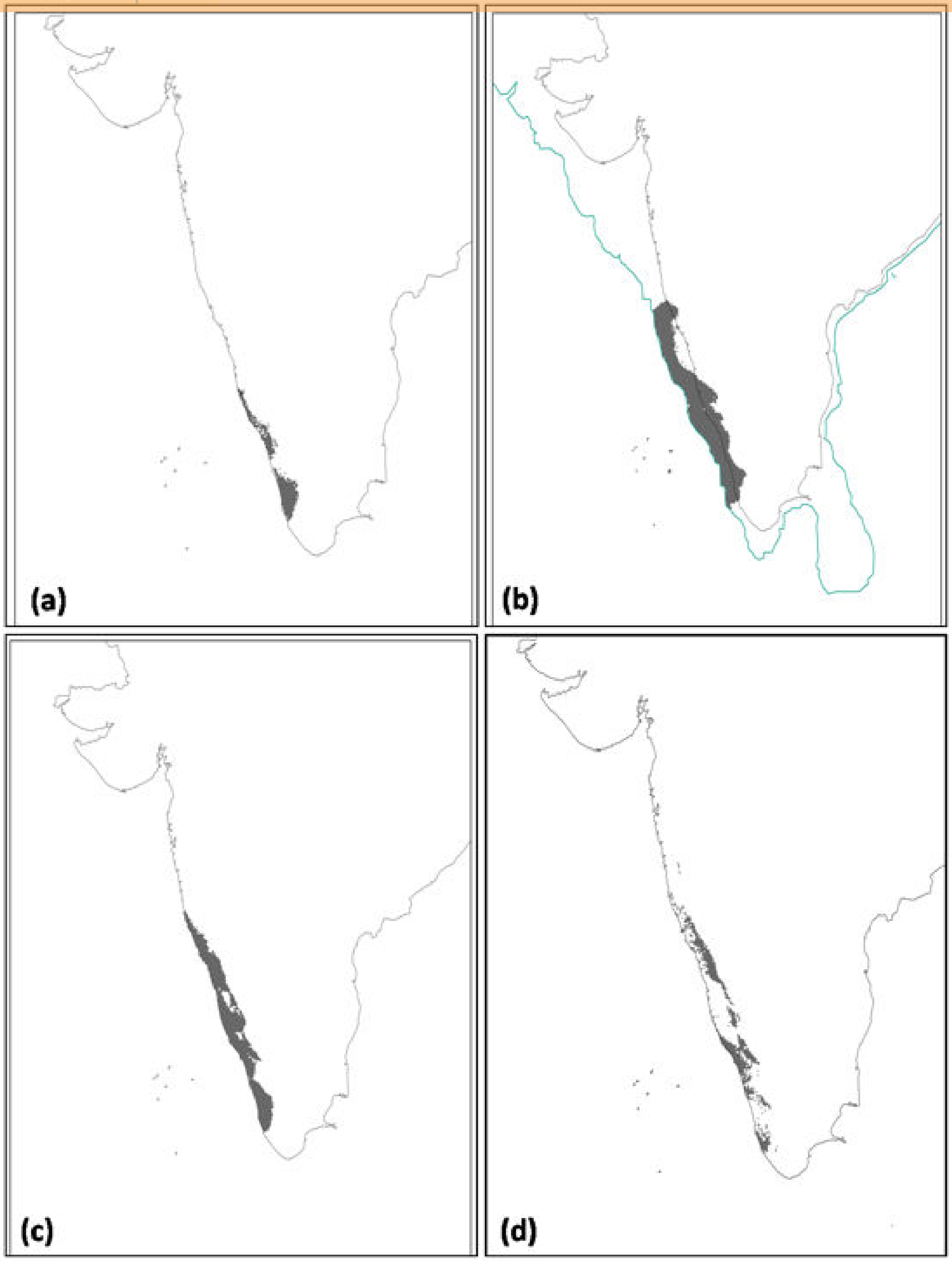

Figure-3