

Introduction

Neogene tectonic-climatic interactions have driven the evolution of the East Asian flora. Oaks provide useful models to investigate how these changes influence multiple evolutionary processes at both inter- and intraspecific levels.

The goal of this study is to explore the impacts of Neogene and Quaternary geological history on speciation, introgression, lineage diversification and range dynamics of *Quercus acutissima* and *Q. chenii*, two closely related oak species from Section *Cerris* and native to East Asia.

Materials and Methods

- (1) To obtain evidence of introgression, we used four methods to analyze the variation at seven nuclear microsatellite (nSSR) loci.
- (2) To estimate the times of major evolutionary events, we performed approximate Bayesian computation (ABC) of nuclear data and phylogenetic analysis of chloroplast (cp) DNA sequences.
- (3) To infer the ecologically stable areas and dispersal corridors, we employed ecological niche modelling (ENM).



Fig. 1 Acorns of *Q. acutissima* (left, bigger) and *Q. chenii* (right, smaller)



Fig. 2 Leaves of *Q. acutissima* (left, longer) and *Q. chenii* (right, shorter)

Acknowledgements

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Identification of hybrids and introgression zone

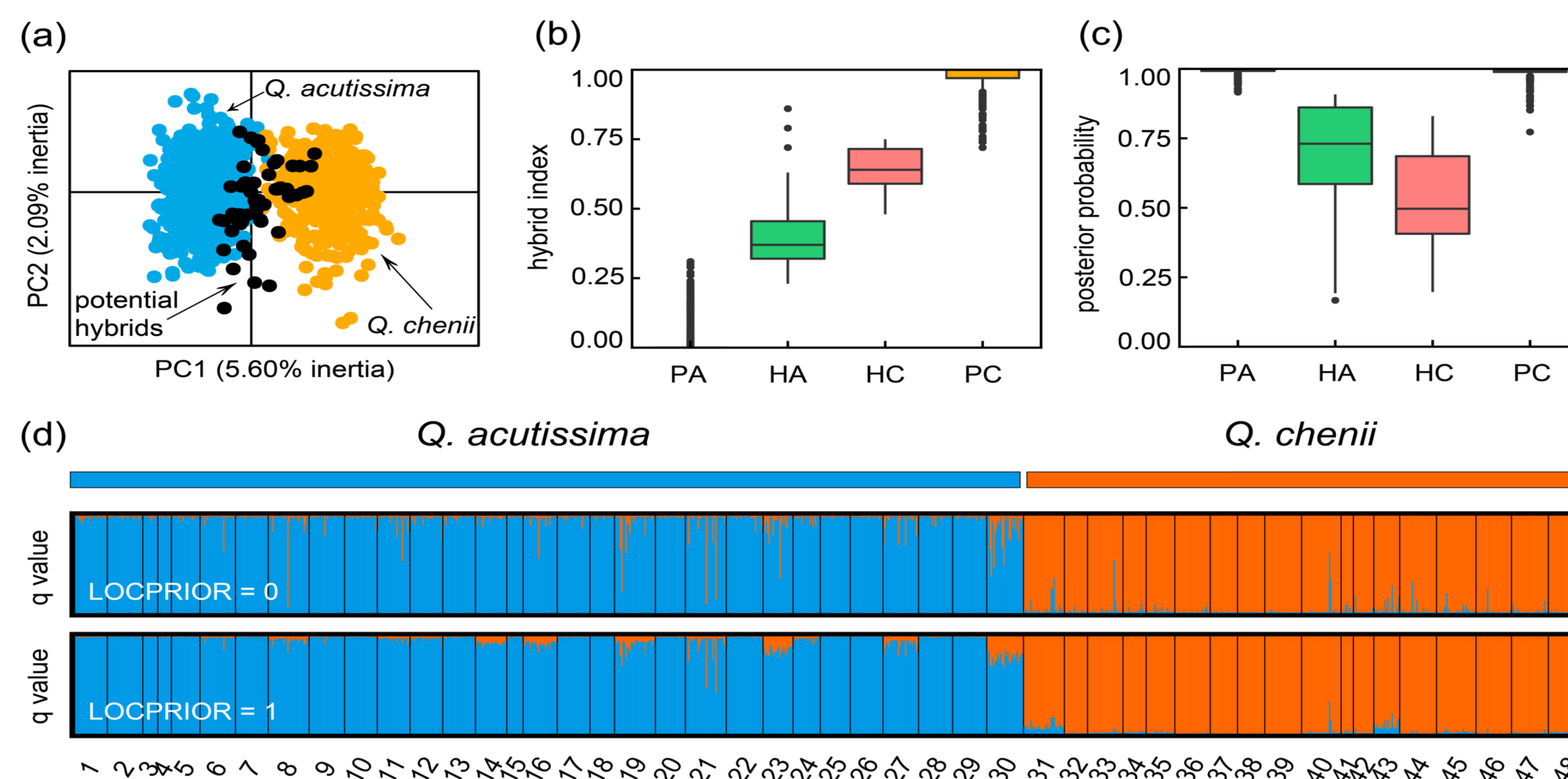


Fig. 3 A total of 51 hybrids (~4.6% of all 1111 individuals) were identified based on the variation at seven nSSR loci, which were confirmed by principal component analysis (a), maximum-likelihood hybrid index calculations (b), NEWHYBRIDS analysis (c, posterior probability to the pure groups < 0.9), and Bayesian cluster analysis (d, admixture rate  $q > 0.1$ ). PA, PC are pure groups, and HA, HC are hybrid groups corresponding to the two species.

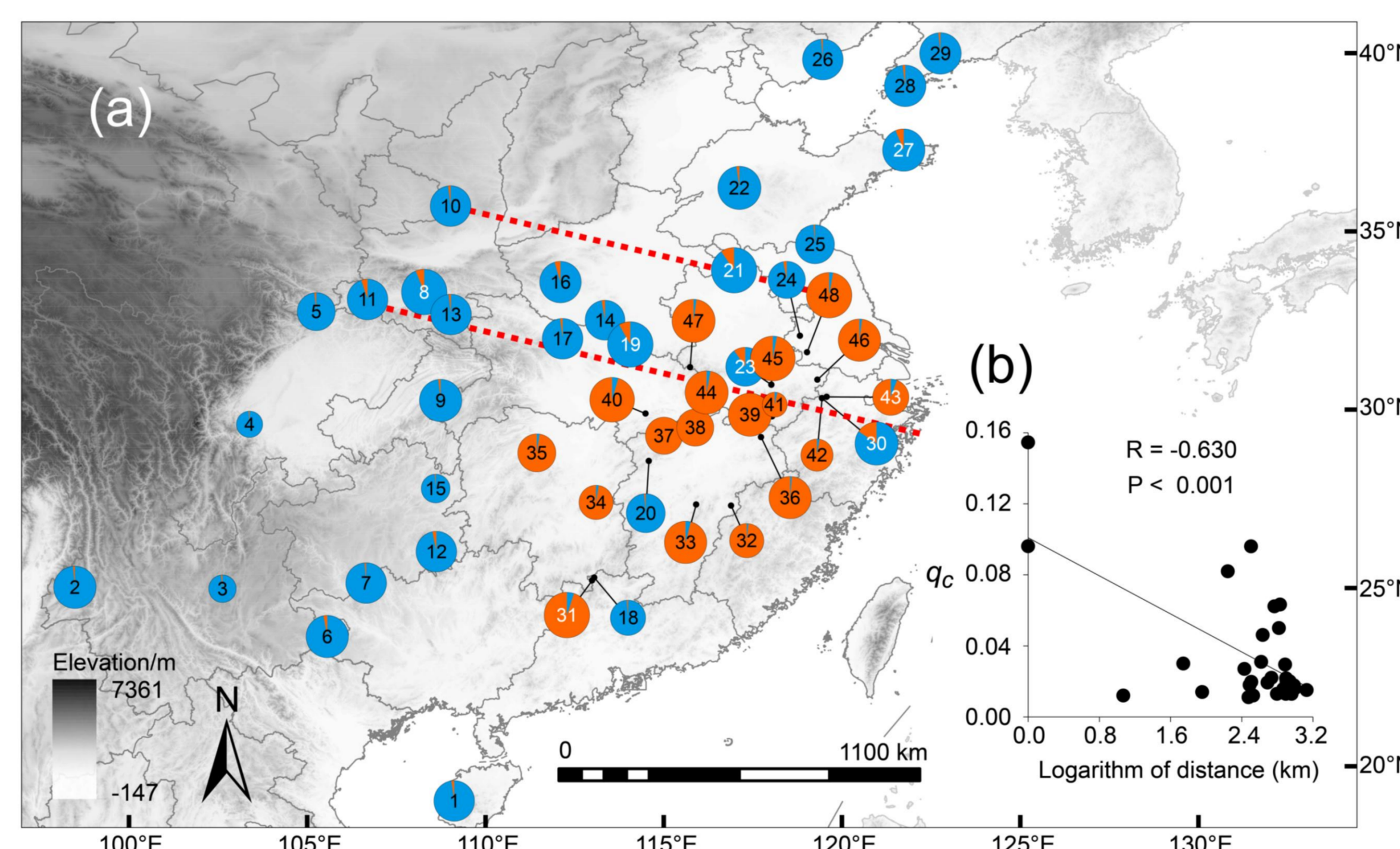


Fig. 4 (a) Geographical distribution of two ancestral groups assigned to *Q. acutissima* (blue, 1-30) and *Q. chenii* (orange, 31-48) as inferred by STRUCTURE. More than 80% of hybrids were found in a putative introgression zone (between red dashed lines). (b) Introgression rate in each *Q. acutissima* population ( $q_c$ ) decreased with increasing distance to nearest *Q. chenii* population.

Demographic history inferred by ABC

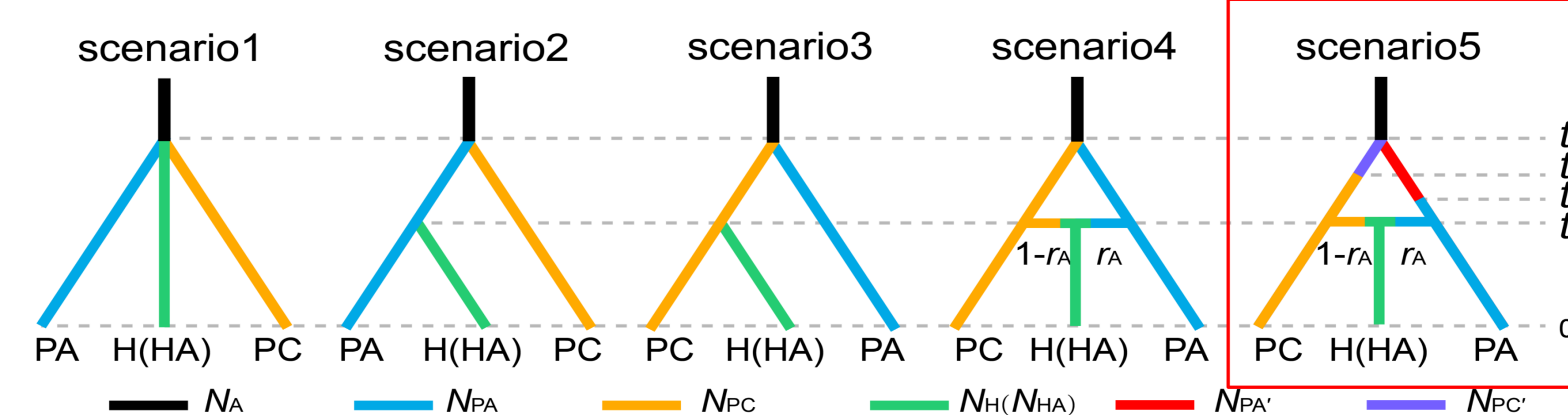


Fig. 5 ABC favoured scenario 5. Posterior parameter estimations suggested that the speciation between *Q. acutissima* (PA) and *Q. chenii* (PC) ( $t_2$ ) was dated to the early Miocene (22.66 Ma). The two species may have experienced demographic expansions ( $t_A$ ,  $t_B$ ) in the late Miocene (10.23–9.66 Ma). The admixture event ( $t_1$ ) was estimated to have occurred during the Pleistocene (1.61 Ma)

Diversification of three cpDNA lineages

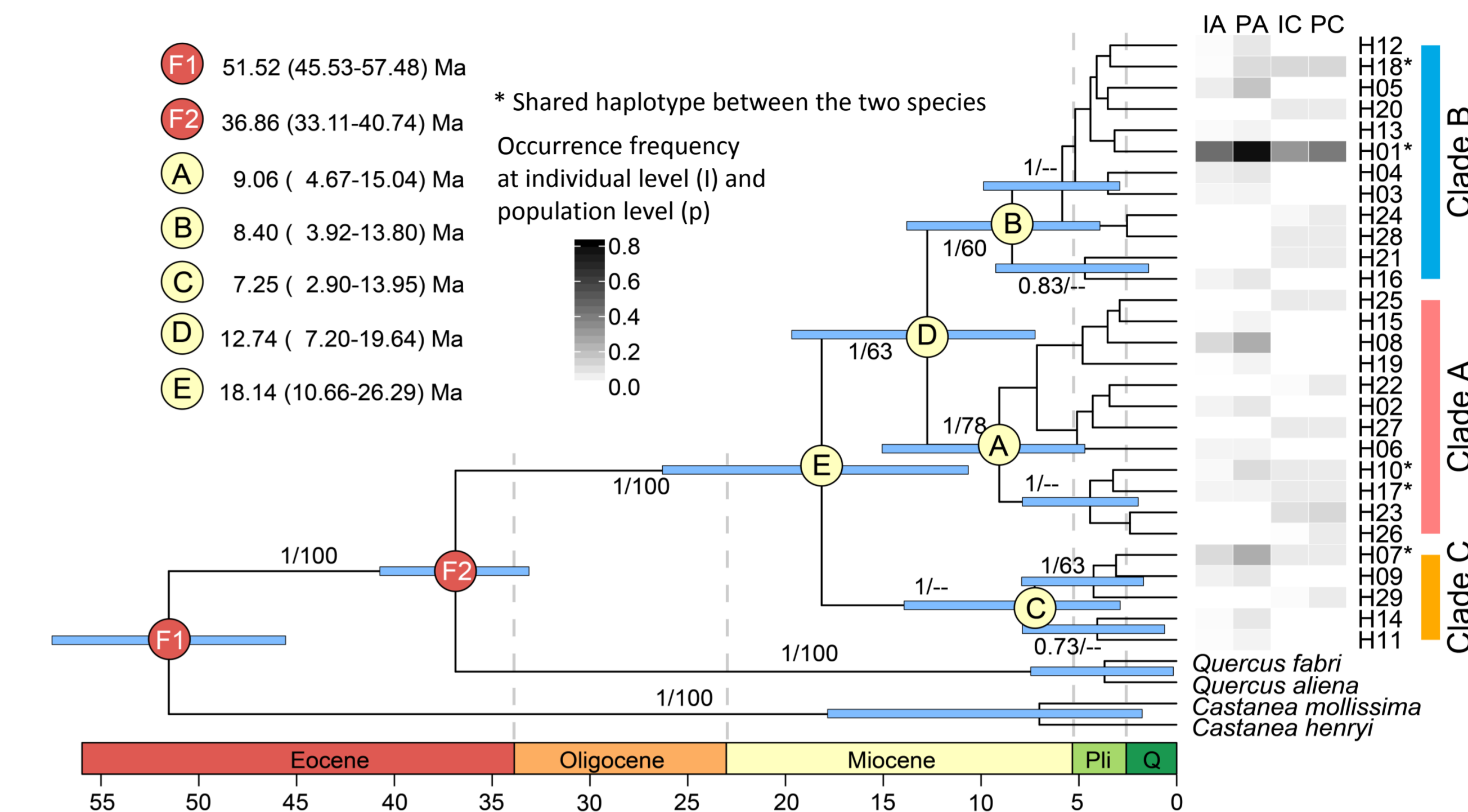


Fig. 6 The time to the most recent common ancestor of all the 29 haplotypes was dated to the early Miocene (18.14 Ma). Major clades (node A–D) diverged during the middle to the late Miocene. Diversification rate increased dramatically in the Pliocene (4.0 Ma) and then decreased in the early Pleistocene (2.2 Ma).

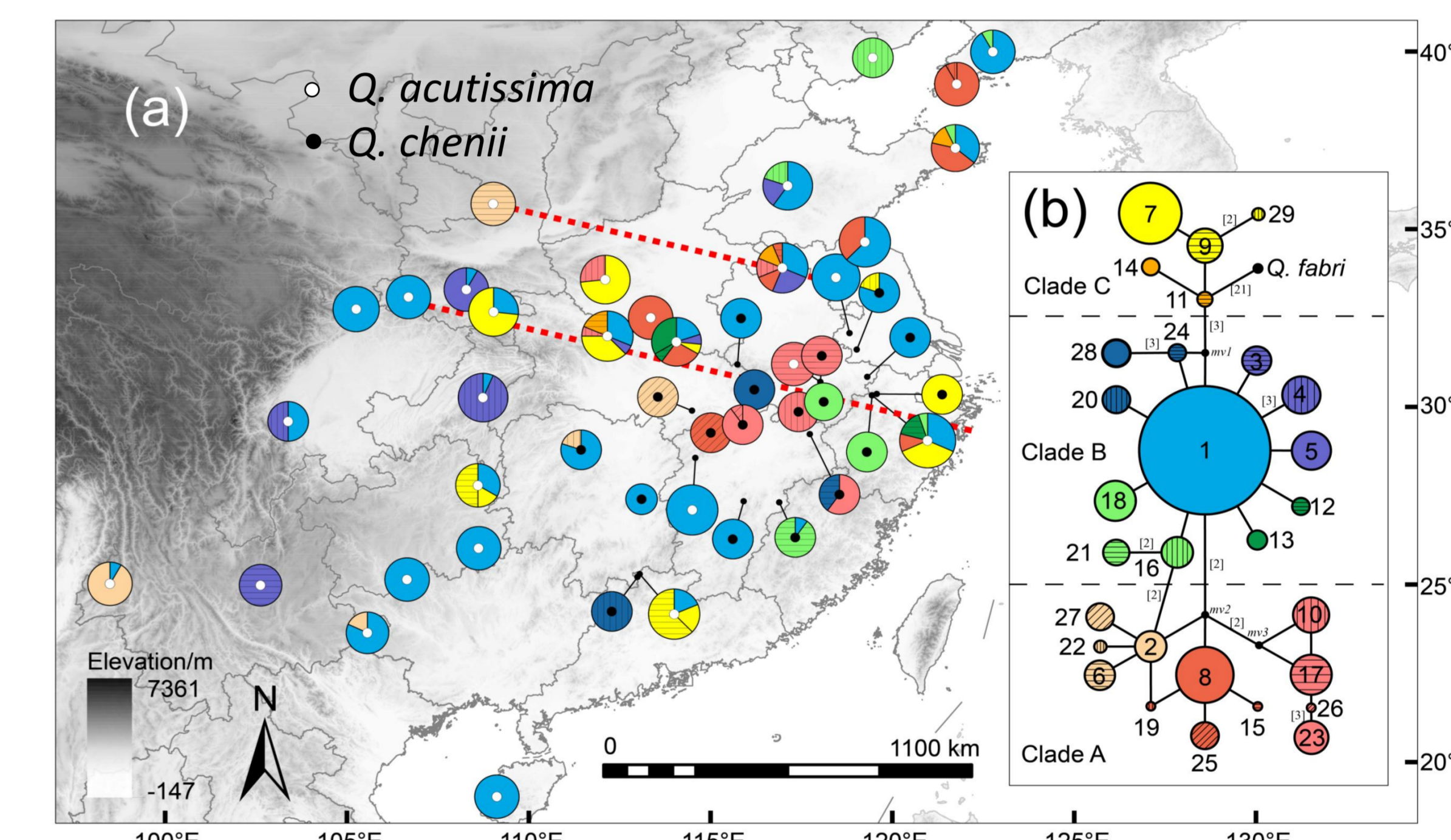


Fig. 7 Geographical distribution (a) and median-joining network (b) of 29 cpDNA haplotypes. More than 90% of individuals with regionally shared haplotypes (H7, H10, H17 and H18) were concentrated in the putative introgression zone (between red dashed lines) inferred by nSSR markers.

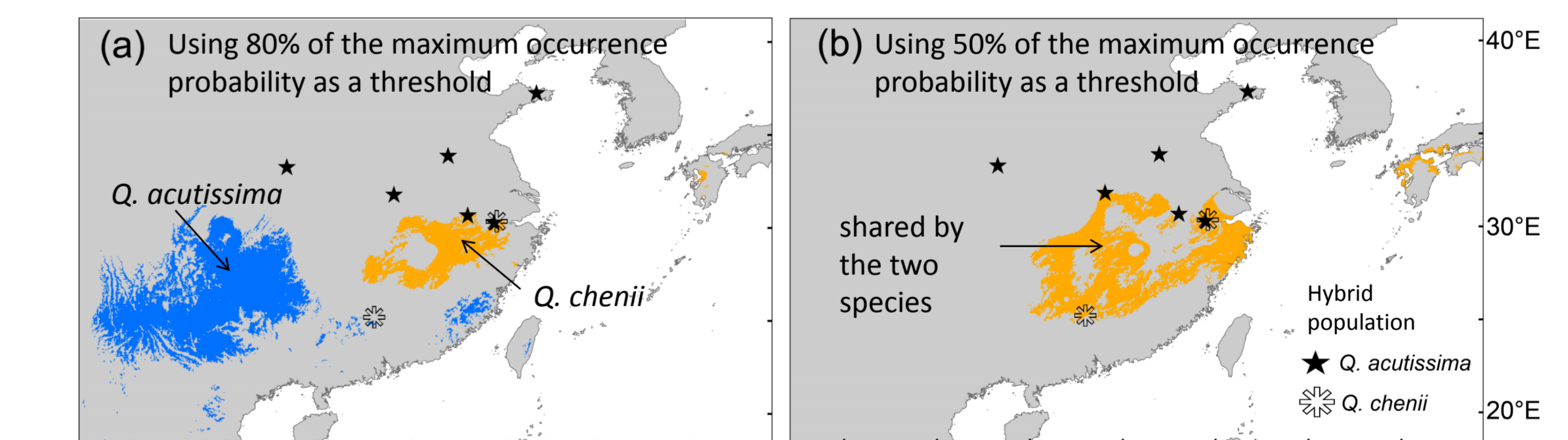


Fig. 8 Ecologically stable area inferred from ENM suggested the scenario of secondary contact underlying Quaternary climatic fluctuations.

Summary

- Speciation of *Q. acutissima* and *Q. chenii* was probably triggered by the rapid uplift of the Qinghai-Tibetan Plateau (QTP), together with the onset of Asian monsoons during the early Miocene.
- Quaternary climatic fluctuations may contribute to the historical introgression between the two species.
- This study illustrates how the combination of geological formations and associated climate changes shape the opportunities for speciation and hybridization.