Molecular Phylogenetics and Evolution 98 (2016) 300–313

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Peeking through the trapdoor: Historical biogeography of the Aegean endemic spider *Cyrtocarenum* Ausserer, 1871 with an estimation of mtDNA substitution rates for Mygalomorphae ☆

P. Kornilios [a,*,1], E. Thanou [a], P. Kapli [b], A. Parmakelis [c], M. Chatzaki [a,1]

[a] Department of Molecular Biology and Genetics, Democritus University of Thrace, GR-68131 Alexandroupolis, Greece
[b] The Exelixis Lab, Scientific Computing Group, Heidelberg Institute for Theoretical Studies, D-68159 Heidelberg, Germany
[c] Department of Ecology and Taxonomy, Faculty of Biology, National and Kapodistrian University of Athens, GR-15784 Athens, Greece

ARTICLE INFO

Article history:
Received 22 July 2015
Revised 23 November 2015
Accepted 31 January 2016
Available online 11 February 2016

Keywords:
Araneae
Ctenizidae
Greece
Introgression
Molecular clock
Turkey

ABSTRACT

The Aegean region, located in the Eastern Mediterranean, is an area of rich biodiversity and endemism. Its position, geographical configuration and complex geological history have shaped the diversification history of many animal taxa. Mygalomorph spiders have drawn the attention of researchers, as excellent model systems for phylogeographical investigations. However, phylogeographic studies of spiders in the Aegean region are scarce. In this study, we focused on the phylogeography of the endemic ctenizid trap-door spider *Cyrtocarenum* Ausserer, 1871. The genus includes two morphologically described species: *C. grajum* (C.L. Koch, 1836) and *C. cunicularium* (Olivier, 1811). We sampled 60 specimens from the distributions of both species and analyzed four mitochondrial and two nuclear markers. *Cyrtocarenum* served as an example to demonstrate the importance of natural history traits in the inference of phylogeographic scenarios. The mtDNA substitution rates inferred for the genus are profoundly higher compared to araneomorph spiders and other arthropods, which seems tightly associated with their biology. We evaluate published mtDNA substitution rates followed in the literature for mygalomorph spiders and discuss potential pitfalls. Following gene tree (maximum likelihood, Bayesian inference) and species tree approaches (*BEAST), we reconstructed a time-calibrated phylogeny of the genus. These results, combined with a biogeographical ancestral-area analysis, helped build a biogeographic scenario that describes how the major palaeogeographic and palaeoclimatic events of the Aegean may have affected the distribution of *Cyrtocarenum* lineages. The diversification of the genus seems to have begun in the Middle Miocene in the present west Aegean area, while major phylogenetic events occurred at the Miocene–Pliocene boundary for *C. cunicularium*, probably related to the Messinian Salinity Crisis. Our results also demonstrate the clear molecular distinction of the two morphologically described species, but possible cryptic lineages may exist within *C. cunicularium*.

1. Introduction

The broader Aegean region includes the landmasses of the southern Balkan Peninsula in the west (Greece), Anatolia in the east (Turkey) and the Aegean archipelago in between (Fig. 1). Many factors are responsible for the origin, partitioning, and maintenance of the rich biodiversity and endemism in this area, such as its geomorphology, with more than 9800 islands (Blondel et al., 2010), the refugial character of the Balkan peninsula during the repeated climatic changes that occurred since the Late Pliocene (Thanou et al., 2012 and references therein), and the presence of humans that has been recorded for more than 10,000 years (Knapp and Blake, 2005). However, the most important factors are its geographical configuration and position and its complex geological history. The Aegean region lies at the crossroad between Asia and Europe and is connected with Africa through the Middle East, resulting in an interesting mixture of faunal elements with different origins. The geological history of the area involved a series of alterations in the sea-land configurations occurring in the Cenozoic, such as repeated land connections and disconnections, formation and submergence of islands, island-area expansion or reduction, and sea-level oscillations connected to the glacial/interglacial periods (see maps in Parmakelis et al., 2006 redrawn after

☆ This paper was edited by the Associate Editor M.A. Arnedo.

* Corresponding author.
E-mail address: korniliospan@yahoo.gr (P. Kornilios).

[1] Equally contributing authors.

http://dx.doi.org/10.1016/j.ympev.2016.01.021
1055-7903/

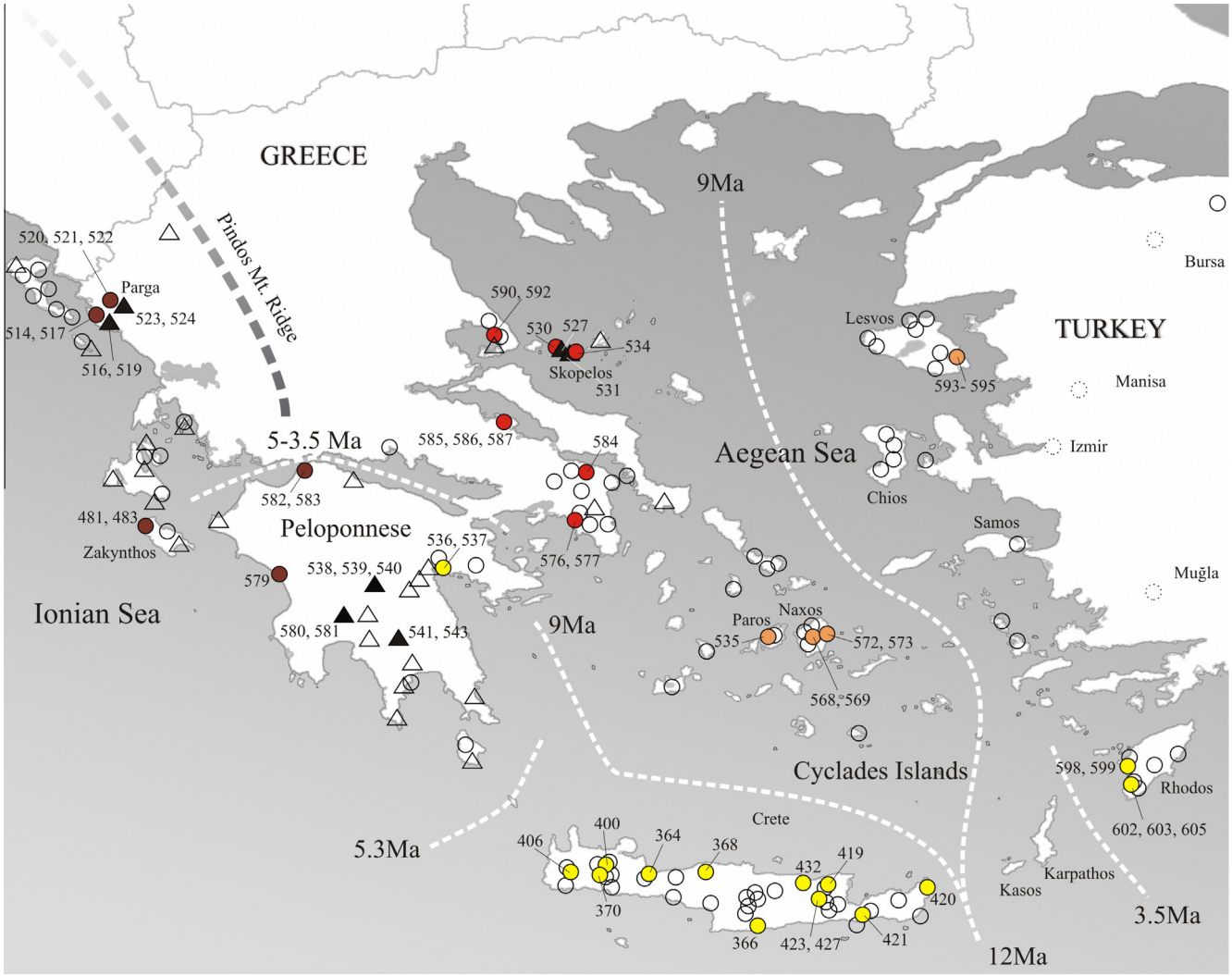

Fig. 1. Map of the Aegean region showing: (a) Confirmed presence-data of *Cyrtocarenum* species as open circles (*C. cunicularium*) and open triangles (*C. grajum*). Published (Decae, 1983, 1996) and unpublished records (Decae, personal communication; our data) are shown. Dashed circles represent *C. cunicularium* locations (provinces) in Turkey. (b) The sampling localities of this study and the distribution of main phylogenetic groups (*C. grajum*: black triangles; *C. cunicularium* C1 clade: brown circles; C2 clade: red circles; C3 clade: orange circles; C4 clade: yellow circles). Numbers refer to specimen codes given in the Appendix A. (c) Names of important geographic areas and islands, main palaeogeographic barriers (white dashed lines) and their respective ages, the Pindos Mt, ridge (black dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Creutzburg, 1963; Dermitzakis and Papanikolaou, 1981; Dermitzakis, 1990).

Molecular phylogenetic and phylogeographical studies have been conducted for many animal taxa of this region, but for the more speciose invertebrates, very few studies exist. Spiders (order Araneae) are one of the most under-represented groups, despite their high diversity (Bosmans and Chatzaki, 2005), including some 1175 valid species (Chatzaki et al., unpublished data). The phylogeography of only two spider species distributed in Greece has been investigated so far, namely *Eresus walckenaeri* (Johannesen et al., 2005) and *Loxosceles rufescens* (Planas et al., 2014; Luo and Li, 2015). We may also add the study of Opatova and Arnedo (2014a) referring to the phylogenetic position of the Cretan endemic *Macrothele cretica* within the genus *Macrothele*, which led to some phylogeographic conclusions regarding the entire genus.

In this study we focus on Mygalomorph spiders, one of the three major evolutionary lineages within the order Araneae (i.e. Mesothelae, Mygalomorphae and Araneomorphae). Mygalomorphs include tarantulas, funnel web spiders and trapdoor spiders, and possess traits that differ markedly from araneomorph spiders and other arthropods (Hamilton et al., 2011). They are sedentary habitat-specialists with long life spans (especially the females) and delayed sexual maturity (Bond et al., 2006; Hamilton et al., 2011). Most importantly, most taxa have very limited dispersal abilities which, together with their aforementioned characteristics, often leads to clustering of individuals in demes and extreme genetic structuring of populations (e.g. Bond et al., 2001; Arnedo and Ferrández, 2007; Bond and Stockman, 2008). In this sense, mygalomorphs can serve as excellent model systems for phylogeographical investigations (Bond et al., 2006; Starrett and Hedin, 2007; Hamilton et al., 2011).

Only five mygalomorph genera (approx. 10–12 described species) occur in the Aegean region. *Cyrtocarenum* is the only endemic genus, while the remaining four have much wider geographic distributions beyond the focal area. It belongs to true trapdoor spiders (Ctenizidae), a group of ground dwelling spiders that construct underground, silk-lined burrows that open to the surface with a trapdoor (Opatova and Arnedo, 2014b). Two *Cyrtocarenum* species have been described on the basis of morphological characters (Decae, 1996), *C. grajum* (C.L. Koch, 1836) and *C. cunicularium*