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PHYLOGEOGRAPHY AND EVOLUTION OF THE SPECIES GROUP *POECILIMON JONICUS* AND SUBGENUS *HAMATOPOECILIMON* (INSECTA: ORTHOPTERA) IN THE AEGEAN REGION

ABSTRACT

of PHD Thesis

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БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

ИНСТИТУТ ПО БИОРАЗНООБРАЗИЕ И ЕКОСИСТЕМНИ ИЗСЛЕДВАНИЯ

Симеон Бориславов Борисов

ФИЛОГЕОГРАФИЯ И ЕВОЛЮЦИЯ НА ВИДОВАТА ГРУПА *POECILIMON JONICUS* И ПОДРОД *HAMATOPOECILIMON* (INSECTA: ORTHOPTERA) В ЕГЕЙСКИЯ РЕГИОН

АВТОРЕФЕРАТ

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INTRODUCTION

Phylogeography is a relatively new, rapidly developing science, that combines systematics and population genetics (Avise et al. 1987). This integrative field benefits from the development of the coalescent theory, advancements in DNA sequencing techniques and the rise of computational power (Avise 2000, 2009; Hickerson et al. 2009). The contribution of molecular techniques to phylogeography is remarkable. Molecular markers could be selected to obtain the best possible phylogenetic resolution on all taxonomic levels (e.g. Hwang & Kim 1999). A well-resolved phylogenetic tree could be plotted in space and time, highlighting correspondence between phylogenetic splits and past geological events, allowing to test various hypotheses about vicariance and dispersal (Parmakelis et al. 2006; Allegrucci et al. 2011, 2017; Chobanov et al. 2017; Dufresnes et al. 2018; Kornilios et al. 2019). Estimation of divergence times, even approximate, contributes to the integration of knowledge, linking nucleotide substitution rates with geotectonic events and paleontological data (Papadopoulou et al. 2010; De Baets et al. 2016; Barba-Montoya et al. 2017). Phylogeography also benefits from the advancements in ecological niche modeling (Carstens & Richards 2007; Alvarado-Serrano & Knowles 2014). Ecological niche modeling (ENM) links occurrence data with variables, characterizing environmental conditions, to model species' response to the environment (Guisan & Thuiller 2005, Elith & Leathwick 2009, Franklin 2010).

Order Orthoptera provides suitable models for phylogeographic studies. Orthopterans are widespread and show remarkable diversity in adaptations (Bidau 2014). Their acoustic communication system was a matter of thorough research (e.g., Alexander 1962; Elsner & Popov 1978; Greenfield 1997 and references therein) as complex communication is considered a powerful evolutionary force (see Greenfield 1997). In orthopterans, variation in wing length is high with some species capable of long migrations, while others being short-winged or flightless (Song et al. 2015). Loss of flight is suggested to increase diversification rates in beetles (Vogler & Timmermans 2012). Capability of flight is documented to affect evolutionary rates of mitochondrial protein-coding genes with more non-synonymous mutations accumulating in flightless orthopteran lineages (Chang et al. 2020). Western Palearctic bush-crciket lineages of the subfamily Phaneropterinae have lost flight ability at least twice, which triggered rapid speciation (Grzywacz et al. 2018).

Genus *Poecilimon* is among the most evolutionary intriguing orthopteran groups in the Palaearctic for its diversity and high endemism rate being the largest Palaearctic bush-cricket (Ensifera) genus with a Pontic-East-Mediterranean centre of diversity. It comprises half of the diversity of the hyperdiverse tribus Barbitistini which has ca. 300 taxa (Cigliano et al. 2020) concentrated in the Aegean, Anatolian and Ponto-Caspian region. The tribus Barbitistini comprises micropterous, sluggish, herbivore bush-crickets with complex acoustic communication system.

The present study focuses on two species groups of bush-crickets of the genus *Poecilimon*, having their centre of distribution in the Aegean area, as models for studying temporal, spatial, ecological and behavioral parameters as evolutionary forces acting within a geotectonically impetuous terrestrial environment. Conclusions are based on phylogenetic reconstructions, dating of phylogenetic lineages, comparisons with paleogeographic data, ecological niche modeling and analyses of acoustic signals.

AIM AND OBJECTIVES

The present study **aims** to explore the phylogeography of the *Poecilimon jonicus* species group and subgenus *Hamatopoecilimon*, distributed in the Aegean region, and to draw conclusions about their evolution at a temporal and spatial scale. We employ the following **objectives**:

1. Analysis of the phylogenetic relationships within the groups, based on mitochondrial and nuclear DNA markers.
2. Studying the bio-acoustic communication in the groups in the light of phylogenetic reconstructions.
3. Developing ecological niche models in order to reveal the effects of climate on distribution patterns within the group and test ecological differentiation at a taxonomic level.
4. Dating lineage splits to associate divergence times with climatic shifts and geotectonic processes in the region and thus the importance of climatic, geotectonic and behavioral factors for diversification of the group.
5. Defining the taxonomic composition of *Poecilimon jonicus* group and subgenus *Hamatopoecilimon* based on an integrative approach combining molecular genetics, ecological niche modeling, acoustics and morphology.

BACKGROUND

The background provides a review of taxonomic literature related to the genus *Poecilimon*, subgenus *Hamatopoecilimon* and the species group *P. jonicus* and discusses the role of bio-acoustic communication in the speciation of bush-crickets. It highlights the most important events of the geological history of the Eastern Mediterranean that possibly affected speciation in the region. Methods for phylogenetic inference and estimation of divergence times are briefly described. Finally, the background discusses the theoretical foundations of ecological niche modeling and its application in phylogeographic research.

METHODOLOGY

Fieldwork and material

Field expeditions for collecting new material were carried out between 2017-2019, attempting to sample all representatives of the selected species groups. Besides, material from the collection of Dragan Chobanov was studied. Occasionally, specimens were received from colleagues.

Morphology and bioacoustics

Morphological identification of collected specimens was based on several keys and descriptions (Willemse 1982; Willemse & Heller 1992; Heller & Reinhold 1992; Heller et al. 2011; Kaya et al. 2018). Male calling signals were recorded using Pettersson D500 external microphone connected to ZOOM H2 (96 kHz, 24-bit sampling frequency) or Tascam DR-680MKII (192 kHz, 24-bit sampling frequency) digital recorders. Own recordings were visualized with Audacity 2.1.2. (https://www.audacityteam.org/) and were compared to available recordings (Massa et al. 2012; Cigliano et al. 2020) and to detailed descriptions of calling songs (Heller 1984; Kaya et al. 2018).

Molecular procedures

Total DNA was extracted from hind femur muscles. Proteinase K was applied and samples were incubated for four hours for maximal digestion of proteins. The salt/ethanol protocol by Aljanabi & Martinez (1997) was followed. Samples were stored at –20 C to prevent DNA degradation. Polymerase chain reaction (PCR) was used to amplify target sequences. Two mitochondrial (COI, ND2) and one nuclear (ITS) markers were obtained from species of the *Poecilimon jonicus* complex, and three mitochondrial markers (COI, ND2, CR) – from representatives of subgenus *Hamatopoecilimon*. Thermal cycling was held following Chobanov et al. (2017) for COI and ND2, Weekers et al. (2001) for ITS and Zhao et al. (2011) for CR. Sequencing was performed by Macrogen Europe (Macrogen, Inc., Amsterdam, Netherlands). In order to provide comparison and better representation of some taxa from the *P. jonicus* species group DNA sequences from a 12S+16S rRNA fragment (Ullrich et al. 2010) were downloaded from Genbank and included in the analyses.

Data preparation and phylogenetic inferences

DNA sequences were visualized, trimmed and assembled with CodonCode Aligner version 8.0.2 (CodonCode, Dedham, MA, USA). Datasets were compiled with previously obtained own sequences and with publicly available data from GenBank (see Publications 1, 2 for details and accession numbers). Alignments were created, tested and prepared for phylogenetic analyses with suitable software – MEGA X (Kumar et al. 2018), DAMBE (Xia 2018), PartitionFinder (Lanfear et al. 2017). Details are given in Publications 1, 2.

Two independent methods for phylogenetic inference have been applied and compared. Maximum likelihood (ML) analyses were carried out using RAxML (Stamatakis 2006). Bayesian inference analyses were run in Mr. Bayes version 3.2.5 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2005). Molecular markers were analyzed separately and as concatenated matrices. Well-supported phylogenies were used to outline monophyletic clades and to revise the systematics of the group (Publication 1) or to test hypotheses regarding radiation and speciation (Publications 1,2). Phylogenetic trees were used as a base for molecular dating (Publication 1).

Phylogeny of the whole genus *Poecilimon* was reconstructed to provide a general overview and to confirm the monophyly of the model groups. DNA sequences of nuclear Internal transcribed spacer from Ullrich et al. (2010) were downloaded from Genbank. Own sequences were obtained, following the protocol by Ullrich et al. (2010), to provide easy alignment and to represent more taxa. The conservative 5.8S rRNA fragment between the two spacers was removed from the alignment for the analysis. Sequence alignment was carried out in Mega X (Kumar et al. 2018) and tested for saturation in DAMBE 7.0.39 (Xia 2003, 2018). The best substitution model was estimated with PartitionFinder (Lanfear et al. 2016). As an alternative to the Maximum Parsimony approach of Ullrich et al. (2010) Bayesian inference was applied. Analyses were accomplished in Mr. Bayes version 3.2.5 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2005) with four simulations of Markov chains and 4 × 106 generations sampling each 100th tree. Stationary distribution of the MCMC parameters was confirmed with Tracer ver. 1.7.1 (Rambaut et al. 2018). The first 25% of trees were excluded as burnin.

Estimation of divergence times

Divergence time estimations for lineages of *Poecilimon jonicus* s.l. aimed to test for congruence between splits and geological processes (Publication 1). Bayesian inference analyses of divergence times were carried out in BEAST (Bouckaert et al. 2019) on a concatenated mitochondrial matrix (COI+ND2). Estimations of absolute times were obtained by three different calibration strategies. First, the substitution rate of COI (0.0177 site/Ma), calibrated based on the age of the mid-Aegean trench (Papadopoulou et al. 2010), was applied, calculating the substitution rate of ND2 as 'linked'. Second, a geotectonic calibration was applied, testing two possible timeframes for the split of the Cretan lineage: (1) 10.6 Mya corresponding to a well-documented separation of Crete from the mainland (Dermitzakis and Papanikolaou 1981; Dermitzakis 1990); (2) 5.2 Mya corresponding to the last isolation of the island at the end of the Messinian (Meulenkamp 1985; Dermitzakis 1990; Anastasakis et al. 2006; Loget et al. 2006). Results outlined several alternative time estimations which were compared with previous estimates (Kaya et al. 2015; Chobanov et al. 2017). Additionally, 'timetrees' were obtained using the RelTime method (Tamura et al. 2012, 2018), applying the same geotectonic time constraints as in BEAST. Time estimations based on the 12S rRNA + 16S rRNA dataset was calibrated according to the results from the paleo-geotectonic dating of the ND2 + COI tree for a highly supported in both phylogenies branch, basal for the studied group. (See Publication 1 for details).

Ecological niche modeling and niche overlap

Ecological niche overlap was used as an additional measure of divergence between the three currently recognized Balkan subspecies of *P. jonicus* and to test if the ecological requirements of these taxa are significantly different. In addition, *P. werneri* is reported from relatively few localities (Willemse 1984) and only two specimens were found during field trips. Therefore, an ecological niche model of this species was created to shed some light on its ecological preferences. As a first step (ENMs) were created using Maxent 3.4.1 (Phillips et al. 2017). Presence data was collected from field sampling, literature and public databases (Willemse 1984; Cigliano et al. 2020; GBIF 2019). Published localities without specific geographic coordinates were visually referred to the nearest possible area with suitable habitat using Google Earth Pro (v.7.3.3.7786). Occurrence records were 'thinned' with a thinning distance of 1 km to mitigate sampling bias using the spThin R package (Aiello-Lammens et al. 2015). This keeps the maximum number of occurrence points for each species so that the minimum distance between every two points is 1 km (see also Pearson et al. 2007). The study area was chosen after examining the distribution of all taxa and covers the southwestern Balkans and the Peloponnese. The 19 bioclimatic variables from WorldClim (Hijmans et al. 2005; worldclim.org) at 30 s resolution (ca. 1 km) were downloaded and prepared for future analyses. Low effects of correlation between predictor variables on Maxent models have been reported (Feng et al. 2019). However, some authors suggest reducing correlation between variables for better performance and interpretation (e.g. Merrow et al. 2013). Pairwise correlation tests on the 19 variables have been run on ‘virtualspecies’ R package (Leroy et al. 2016), removing one variable from each pair with Pearson correlation coefficient ≥ 0.75. Maxent’s regularization multiplier and feature classes used for each model were adjusted using the R package ENMeval (Muscarella et al. 2014). Models produced by different combinations of parameters were evaluated under the AICCc criterion (Warren & Seifert 2011) to select the best settings. Maxent was run with a total of 10 000 random background points with 10 cross-validation replicates for testing.

Niche overlap was calculated for the three subspecies of *P. jonicus* with the ENMTools software (Warren et al. 2010). The resulting D index (Schoener 1968) was used to compare the levels of ecological niche difference between each two of the studied taxa. Niche identity tests were run to find whether the actual distribution of each taxon results in a significantly different model (Warren et al. 2008).

RESULTS

Position of the model groups within the genus Poecilimon

The final ITS alignment contained 151 sequences (127 from Ullrich et al. 2010 and 24 own) of 1983 bp including 1292 gaps. A total of 151 sequences were included in the analysis representing 93 taxa of the genus *Poecilimon* and 15 taxa from other genera of the tribus Barbitistini (Appendix 3). These included 493 variable sites and 325 parsimony-informative sites. The Bayesian analysis showed general congruence with the Maximum Parsimony tree by Ullrich et al. (2010), with slightly higher support for some nodes (Fig. 1). The basal position of the *P. hamatus* group within the genus was fully supported. While monophyly of most species groups of *Poecilimon* listed in Cigliano et al. (2020) was confirmed, representatives of the genera *Polysarcus* and *Phonochorion* were also arranged within *Poecilimon*, thus supporting Ullrich et al. (2010) for the paraphyletic nature of the former two taxa. The ITS tree did not resolve well the relationships within the *P. jonicus* and *P. inflatus* groups, yet, it supports that they share common ancestor. Besides, *P. bilgeri*, which was not represented in the analysis by Ullrich et al. (2010), was arranged outside *P. jonicus* sensu lato and is related to *P. pergamicus* and *P. propinquus* species group.

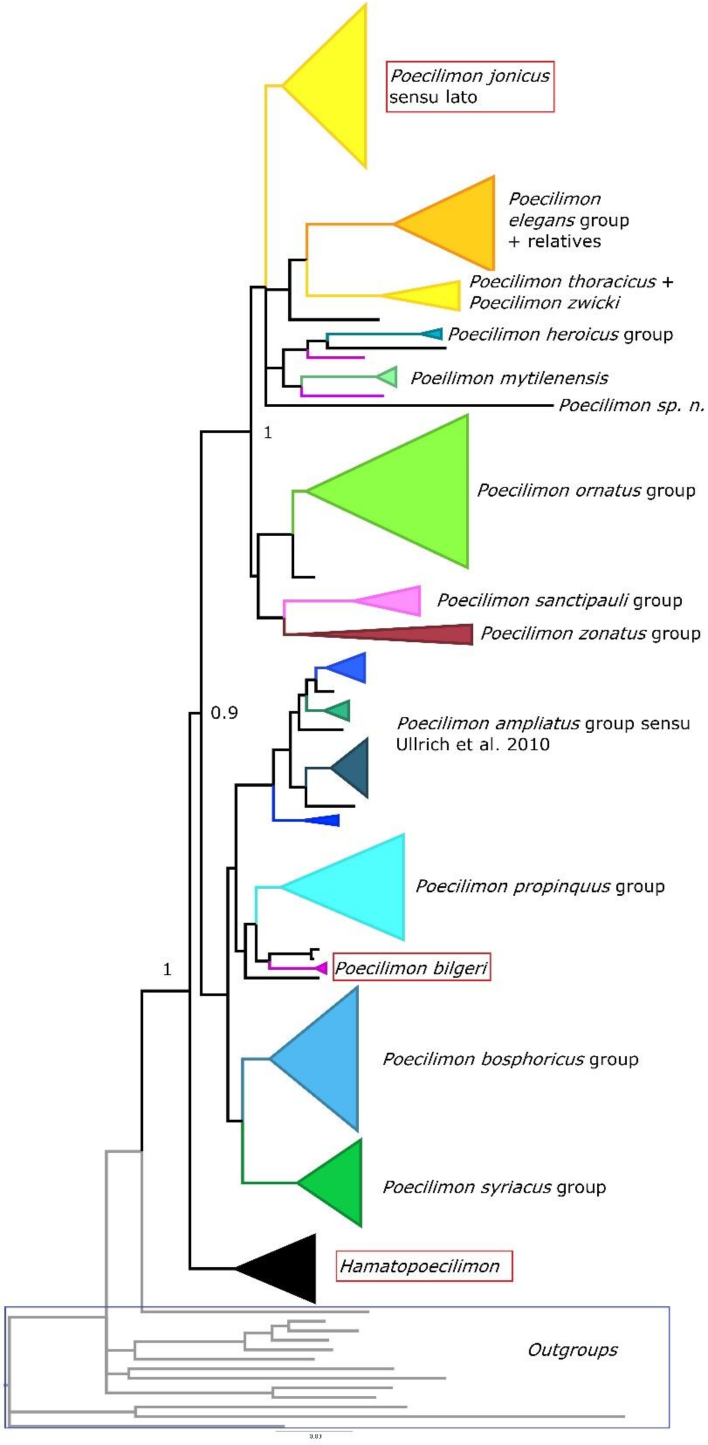

Figure 1. Bayesian inference phylogenetic tree of *Poecilimon* based on ITS sequences.

Phylogeography of the Poecilimon jonicus *species group*

Phylogenetic inferences

A total of 65 DNA sequences were obtained from the *P. jonicus* group s.l. and outgroups (COI – 23, ND2 – 25, ITS – 17). Own sequences are submitted to GenBank and accession numbers are given in Publication 1 – Supplementary Material A. The ITS dataset combined from own and published sequences consisted of 665 bp with gaps (630 bp without gaps), including 214 variable and 122 parsimony informative sites, and involved 16 ingroup and 6 outroup taxa (respectively 28 ingroup and 11 outroup haplotypes). The ITS dataset from Ullrich et al. (2010), involving all *P. jonicus* taxa sensu lato and same outgroup taxa, included 12 ingroup and 6 outgroup taxa (16 ingroup and 10 outgroup haplotypes). The latter consisted of 717 bp with gaps (679 bp without gaps), including 212 variable and 112 parsimony informative sites. The aligned concatenated mt-dataset of ND2 + COI contained 1656 bp and involved 25 unique haplotypes from 11 ingroup and six outgroup taxa. Included COI fragment had a length of 705 bp with 268 variable and 237 parsimony informative sites, and the ND2 fragment had 951 bp with 548 variable and 443 parsimony informative sites. The 16S rRNA + 12S rRNA dataset had a final length of 1835 bp, involved 25 unique haplotypes from 11 ingroup and six outgroup taxa, and included 72 indels, 666 variable sites, and 506 parsimony informative sites. Best substitution models for all datasets are given in Table 1.

Table 1. Best substitution models under the AIC criterion for all studied datasets of the *Poecilimon jonicus* s.l. group

Dataset	**Codon position**	**BEST AIC model**
ND2	1	TPM1uf+G
	2	TVM+I+G
	3	TRN+I+G
COI	1	TrN+G
	2	HKY+G
	3	TrN+I+G
ND2+COI		TrN+I+G
ITS1+ITS2		GTR+I+G
12S+16S		GTR+G

ITS matrices well supported the monophyly of the group including the Balkan, Cretan, and Anatolian lineages, except for *Poecilimon bilgeri* that grouped with *P. pergamicus* Brunner von Wattenwyl, 1891, positioned closer to the basal branch of *Poecilimon*. BI and ML analyses showed similar topology, yet the ML tree had lower support on some branches and is not shown. BI tree for the ITS datasets is shown in Fig. 2.

Figure 2. Bayesian inference phylogeny of ITS of the *P. jonicus* s.l. species group: **a)** 665 bp alignment-tree of sequences obtained in this study and from GenBank (Ullrich et al. 2010); **b)** 717 bp alignment-tree of sequences available from Ullrich et al. (2010).

The ML and BI analyses of the ND2 + COI matrix supported the group monophyly while also providing good resolution and very high support for most branches not only at group level but also within groups (Fig. 3a). BI tree of 12S rRNA+16S rRNA (Fig. 3b) is in agreement with the published maximum parsimony tree inferred from the same data (Ullrich et al. 2010). Despite some minor differences in topology, mitochondrial phylogenies outline the following main groupings: *P. inflatus* + *P. martinae*, *P. erimanthos* + (*P. jonicus tessellatus* + *P. laevissimus*), and *P. werneri* + (*P. j. superbus* + *P. j. jonicus*).

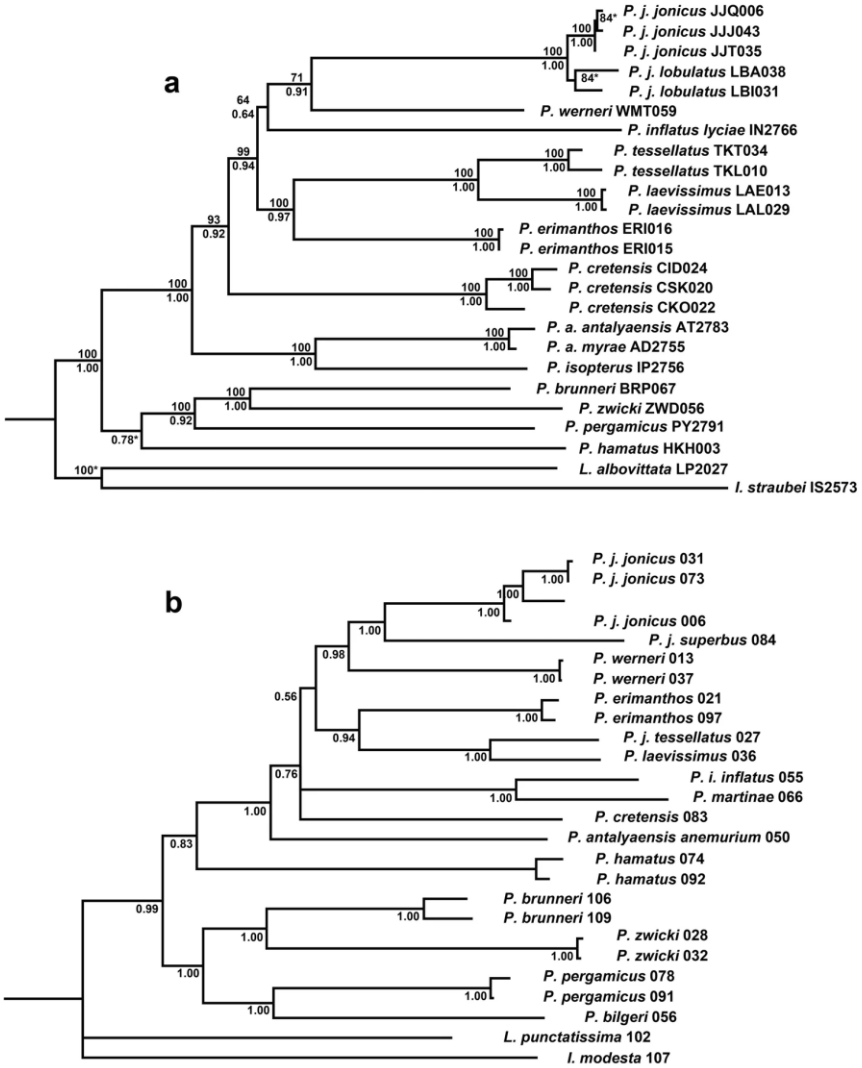

Figure 3. Mitochondrial phylogeny of *P. jonicus* s.l. Node values show node support. **a)** Combined maximum likelihood and Bayesian inference phylogenetic trees based on a 1659 bp alignment of the COI+ND2 mitochondrial dataset; **b)** Bayesian inference phylogenetic tree based on a 1835 bp alignment of the 16S rRNA+12S rRNA mitochondrial dataset (Ullrich et al. 2010).

Estimation of divergence times

The two alternative methods for estimation of divergence times (BEAST and RelTime) showed similar results. The age constraint on the Cretan lineage, reflecting the earlier isolation of Crete from the mainland 10.6 Mya, resulted in significantly old ages that do not agree with previous studies (e.g. Chobanov et al. 2017). The later isolation of Crete, corresponding to the end of the Messinian 5.2 Mya was consistent with results from Chobanov et al. (2017) and with the independent time estimation based on the substitution rate of COI (Papadopoulou et al. 2010). The BEAST chronograms of COI and ND2 calibrated by 0.0177 substitution/site/Ma suggested that *P. jonicus* group sensu lato shares the last common ancestor 7.90/7.35 (COI/ND2)Ma ago. The TMRCAs for the internal clades in *P. jonicus* group sensu lato by the same chronograms (COI/ND2) are as follows: (i) 5.57/4.00 Ma for *P. antalyaensis* + *P. isopterus*, (ii) 7.33/6.66 Ma for Cretan + Balkan clades, (iii) 5.89/* Ma (COI) for *P. inflatus* + all Balkan species (COI chronogram only), (iv) 6.63/4.47 Ma for the Peloponnese clade, (v) */6.13 Ma for *P. inflatus* + (*P. werneri* + *P. jonicus* s.str.) clade (ND2 chronogram only), and (vi) 4.96/5.11 Ma for *P. werneri* + *P. jonicus* (except *P. j. tessellatus*). The chronograms of COI/ND2 estimated the TMRCA of Barbitistini as 10.89/11.19 and that of the members of *Poecilimon* as 9.55/9.63 Ma. The BEAST and RelTime chronograms for ND2 + COI calibrated by the Cretan lineage split as 5.6 ± 0.4 Ma agreed well in node ages. The TMRCAs (BEAST/Reltime) are as follow: (i) Barbitistini 10.24/* Ma, (ii) *Leptophyes* +*Poecilimon* 9.12/*, (iii) *Poecilimon* clade 7.81/7.79 Ma, (iv) *P. jonicus* group sensu lato 6.05/6.13 Ma, (v) *P. antalyaensis* + *P. isopterus* 3.52/3.78 Ma, (vi) Cretan + Balkan clades 5.57/5.60 Ma (calibration node), (vii) Anatolian *P. inflatus* +mainland Balkan species 5.20/5.11 Ma, (viii) *P. inflatus* + (*P. werneri* +*P. jonicus*) clade 4.93/4.91 Ma, (ix) the clade including three species in Peloponnesus 4.44/4.47 Ma, and (x) *P. werneri* + *P. jonicus* 4.3/4.26 Ma, (xi) *P. laevissimus* + *P. tessellatus* ca.1.73/1.71 Ma.

For the 12S rRNA + 16S rRNA tree BEAST calculated the TMRCAs of nodes as follow (i) Barbitistini 9.34 Ma, (ii) *Poecilimon* clade 7.91Ma, (iii) *P. jonicus* group sensu lato (calibration node) 6.24Ma, (iv) *P. jonicus* group after the split of *P. antalyaensis* 5.81 Ma, (v) Anatolian *P. inflatus* +*P. martinae* + Cretan + Balkan species 5.81 Ma, (vi)*P. cretensis* + (*P. inflatus* + *P. martinae*) 5.27 Ma, (vii) the Balkan clade 5.30 Ma, (viii) the Southern Balkan clade 4.35 Ma, (ix) the northern Balkan clade 4.58Ma, (x) the divergence of Italian *P. jonicus superbus* 3.87 Ma, (xi)

P. inflatus +*P. martinae* 2.39 Ma, and (xii) *P. laevissimus* +*P. tessellatus* 2.06 Ma. Chronograms are shown in Fig. 4.

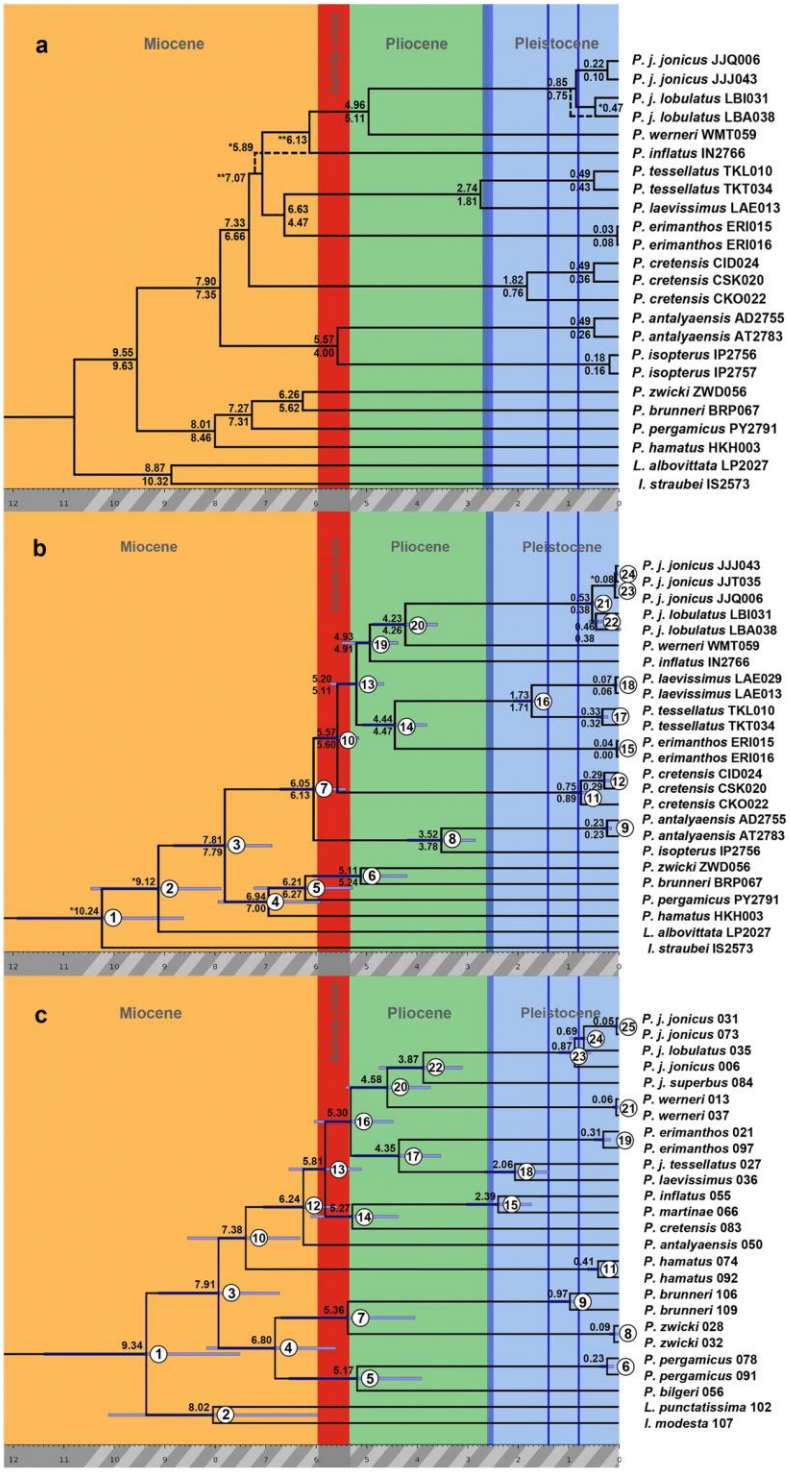

Figure 4. Divergence time estimations for the *P. jonicus* species group. Node labels show time in millions of years. **a)** BEAST maximum clade credibility tree inferred from COI and ND2 partitions calibrated for the substitution rate of COI.

b) BEAST chronogram and RelTime timetree inferred from the COI+ND2 phylogeny calibrated for the last separation of Crete (values above nodes BEAST, values below nodes RelTime.

c) BEAST chronogram inferred from the 16S rRNA+12S rRNA phylogeny calibrated for the TMRCA (interval) of *P. jonicus* group (node 12) as suggested from theCOI+ND2 chronogram. Different topologies are marked with “*”. Blue lines indicate major climatic shifts during the Pleistocene.

Bio-acoustic communication in the *P. jonicus group*

Own song recordings from all sampled taxa confirmed published results for the majority of taxa, suggesting that evolution of calling song was reinforced in sympatric taxa, while remaining comparatively uniform in closely related allopatric taxa (compare Heller 2006). Comparison between own and published bio-acoustic data suggests a complex stable song pattern with a short main syllable and two afterparts characteristic for *P. j. tessellatus* which differs from the pattern of the other subspecies of *P. jonicus* (see Heller 1988 and Publication 1). The subspecies *P. j. superbus* from Italy has a song pattern very similar to that of *P. j. jonicus* (Massa et al. 2012 and own data). Structure of male calling song of some taxa is compared in fig. 5.

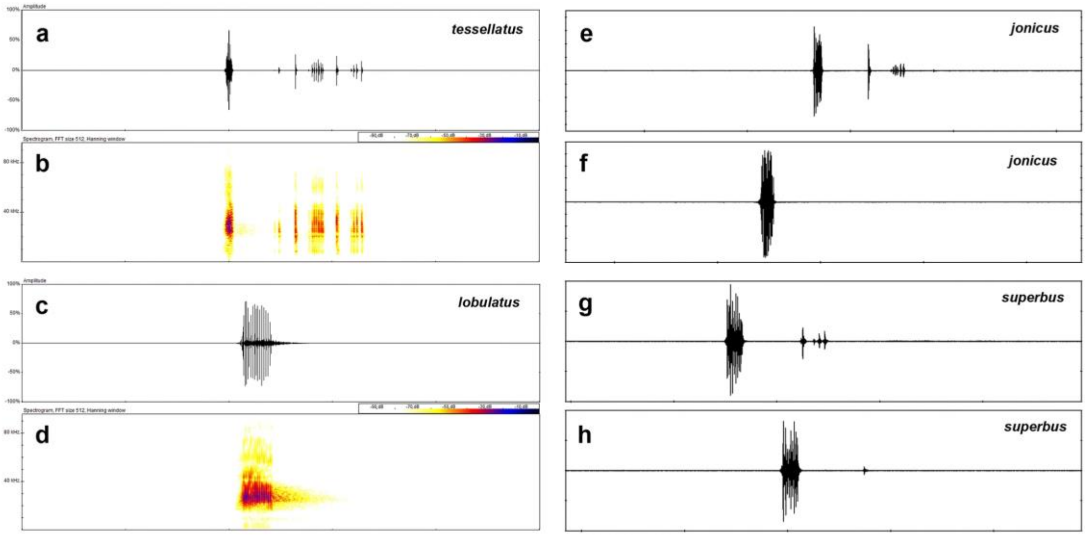

Figure 5. Examples of the male calling song presented in a time frame of 500 ms with oscillograms (a, c, e, f, g, h) and spectrograms (b, d) of taxa currently considered subspecies of Poecilimon jonicus. **a)** P. j. tessellatus, Greece, Peloponnesos, Kallithea, 22.4°C, daily recording, own data; **b)** same; **c)** *P. j. lobulatus*, mainland Greece, Amphilochia, 21°C, daily recording, own data; **d)** same; **e)** *P. j. jonicus*, Albania, Kolonje district, Qafa e Qarrit pass, 1.5 km W of Pepellash, 1200 m a.s.l, lab recording, 30°C, ZOOM-H4 digital recorder, sample rate 96 kHz,G. Puskas, source: OSF online; **f)** *P. j. jonicus*, Albania, S of Vlorë, N of Qeparo vill., ca. 25°C, daily recording, own data; **g**) *P. j. superbus*, Italy, Liguria, Orsomarso, Valle Fiume Argentino, 25°C, source: Massa et al. 2012; and **h)** same, different part of the recording.

Phylogeny of Hamatopoecilimon

A total of 37 new DNA sequences were obtained from subgenus *Hamatopoecilimon* and outgroups (COI – 12, ND2 – 11, CR – 14). All six species were represented. Localities with geographic coordinates and The final alignment of ND2 consisted of 957 bp of which 375 variable and 331 parsimony informative sites. The COI fragment consisted of 1008 bp of which 287 variable and 258 parsimony informative sites. The final alignment of the control region was 976 bp including 15 indels, 295 variable sites and 284 parsimony informative sites. A total of eight trees were inferred, using different markers and methods. BI and ML methods showed agreement on the tree topology although ML analyses show generally lower bootstrap support. All single gene trees and the concatenated dataset strongly support the early split of *P. unispinosus* + *P. ikariensis* branch. All trees support the monophyly of the other four species in *Hamatopoecilimon*. COI (Fig. 6a) and CR (Fig. 6b), separately, support (*P. hamatus*+*P.deplanatus*)+(*P. paros*+*P. klausgerhardi*) topology, which is in concordance with the results from the concatenated matrix (Fig. 6d). All trees support the *P. paros*+*P. klausgerhardi* clade. Most gene trees received poor support for a *P. hamatus*+*P. deplanatus* clade except for the BI analysis of the CR matrix – Bayesian posterior probability close to 1 (Fig 6b). The concatenated mitochondrial dataset (NAD+COI+CR) (fig. 6d) showed improved support for most nodes. The BI tree, inferred from the concatenated dataset, obtained full support of all nodes presenting the topology (*P. unispinosus*+*P. ikariensis*) + ((*P.hamatus*+*P.deplanatus*)+(*P. paros*+*P. klausgerhardi*)). The ND2 tree (Fig. 6c) is the only one showing different topology. ND2 suggests *P. deplanatus* being a sister clade to *P. hamatus*+(*P.paros*+*P. klausgerhardi*). See Fig. 6.

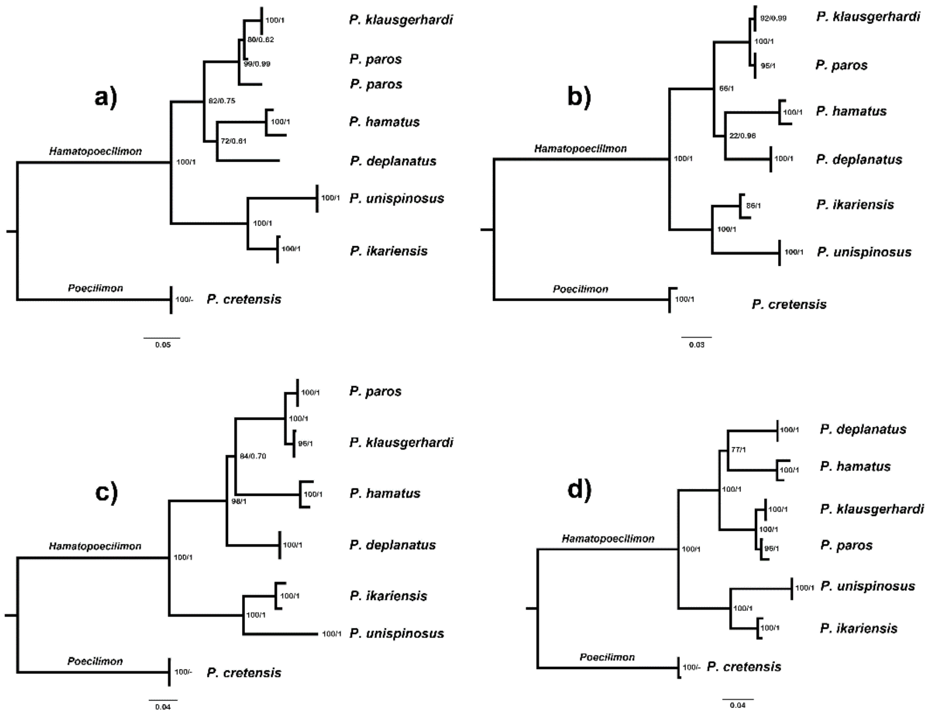

Figure 6. Mitochondrial phylogeny of subgenus *Hamatopoecilimon* **a)** 1008 bp of COI; **b)** 976 bp of the control region (CR); **c)** 957 bp of ND2; **d)** concatenated dataset (ND2+COI+CR) with 2941 bp. Branch numbers show branch support (ML bootstrap/BI Bayesian posterior probability.

Ecological niche modelling

After excluding highly correlated variables (|r| > 0.75), the following nine bioclimatic variables were retained for modeling: **Bio2** – Mean Diurnal Range; **Bio3** – Isothermality; **Bio6** – Min (minimum) Temperature of Coldest Month; **Bio7** – Temperature Annual Range; **Bio8** – Mean Temperature of Wettest Quarter; **Bio9** – Mean Temperature of Driest Quarter; **Bio10** – Mean Temperature of Warmest Quarter; **Bio13** – Precipitation of Wettest Month; **Bio14** – Precipitation of Driest Month. The jackknife test for both *P. jonicus jonicus* and *P. jonicus lobulatus* showed that the most important predictor variable was Bio13 – Precipitation of Wettest Month, which has the greatest training gain when used as a single variable and seriously decreases training gain when omitted. Additionally, Bio3 – Isothermality had similar importance for *P. jonicus lobulatus*. The niche identity test for *P. j. jonicus* and *P. j. lobulatus* did not reject the hypothesis of niche identity (Fig. 5). For *P. tessellatus* the most important predictor variable was Bio14 – Precipitation of Driest Month, together with Bio3 – Isothermality. The model of *P. werneri* was more complex with Bio6

– Min Temperature of Coldest Month, Bio9 – Mean Temperature of Driest Quarter, Bio10 – Mean Temperature of Warmest Quarter, Bio14 – Precipitation of Driest Month being among the most important predictors (see Fig. 7). Selected response curves are shown in Fig. 8. Fig. 9 presents models in geographic space. Summary of the niche overlap tests (D index) is provided in Table 2. The test of niche identity (Warren et al. 2008) for sister taxa *P. j. jonicus* and *P. j. lobulatus* did not reject the null hypothesis of identical niche (Fig. 10)

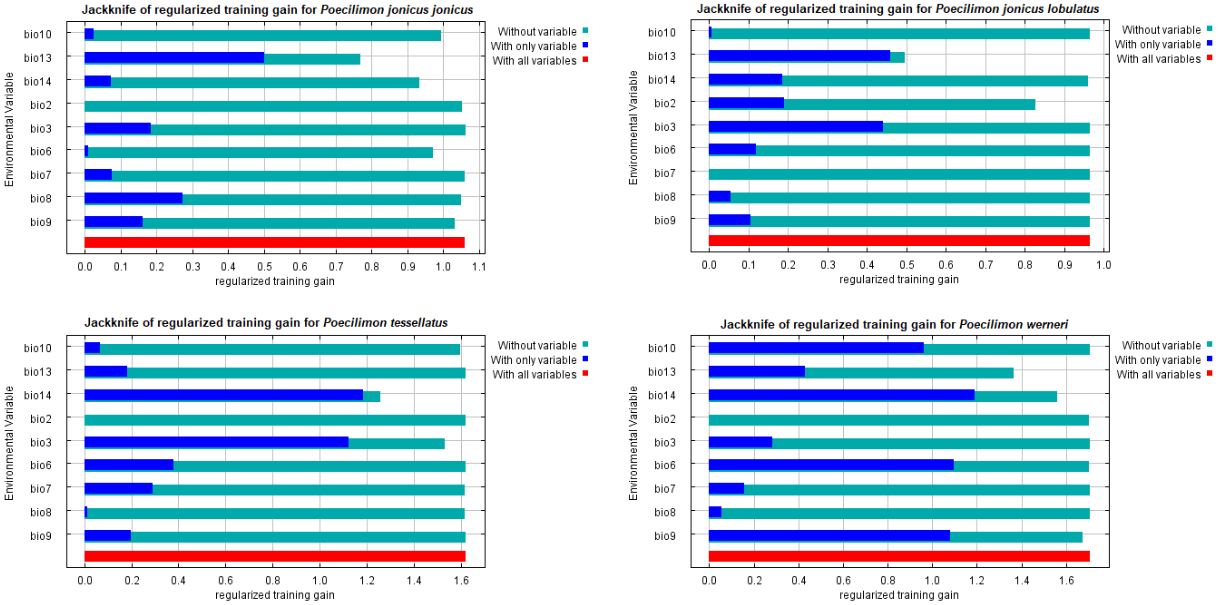

Figure 7. Jackknife of regularized training gain for: **a)** *P. j. jonicus*; **b)** *P. j. lobulatus*; **c)** *P. tessellatus*; **d)** *P. werneri*.

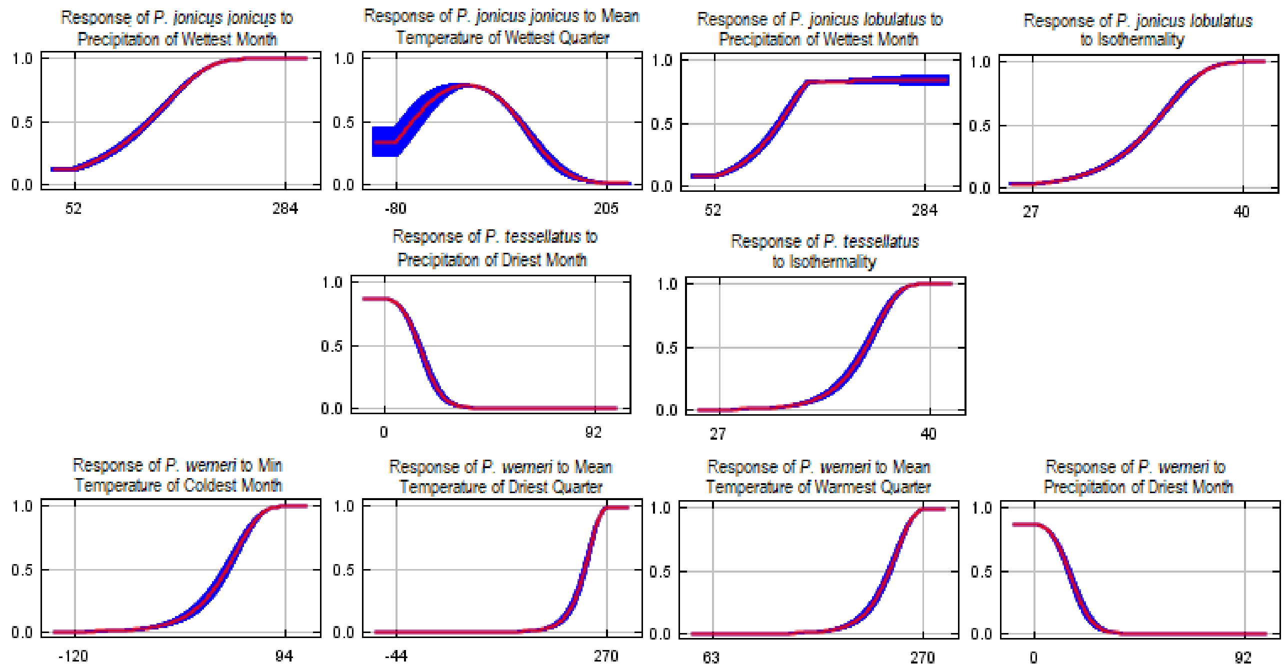

Figure 8. Response curves of the studied species to some of the most important predictors: **a**) response of *P. jonicus jonicus* to Precipitation of Wettest Month; **b)** response of *P. jonicus jonicus* to Mean Temperature of Wettest Quarter; **c**) response of *P. jonicus lobulatus* to Precipitation of Wettest Month; **d**) response of *P. jonicus lobulatus* to Isothermality; **e)** response of *P. tessellatus* to Precipitation of Driest Month; **f**) response of *P. tessellatus* to Isothermality; **g**) response of *P. werneri* to Min Temperature of Coldest Month; **h)** response of *P. werneri* to Mean Temperature of Driest Quarter; **i)** response of *P. werneri* to Mean Temperature of Warmest Quarter; **j**) response of *P. werneri* to Precipitation of Driest Month.

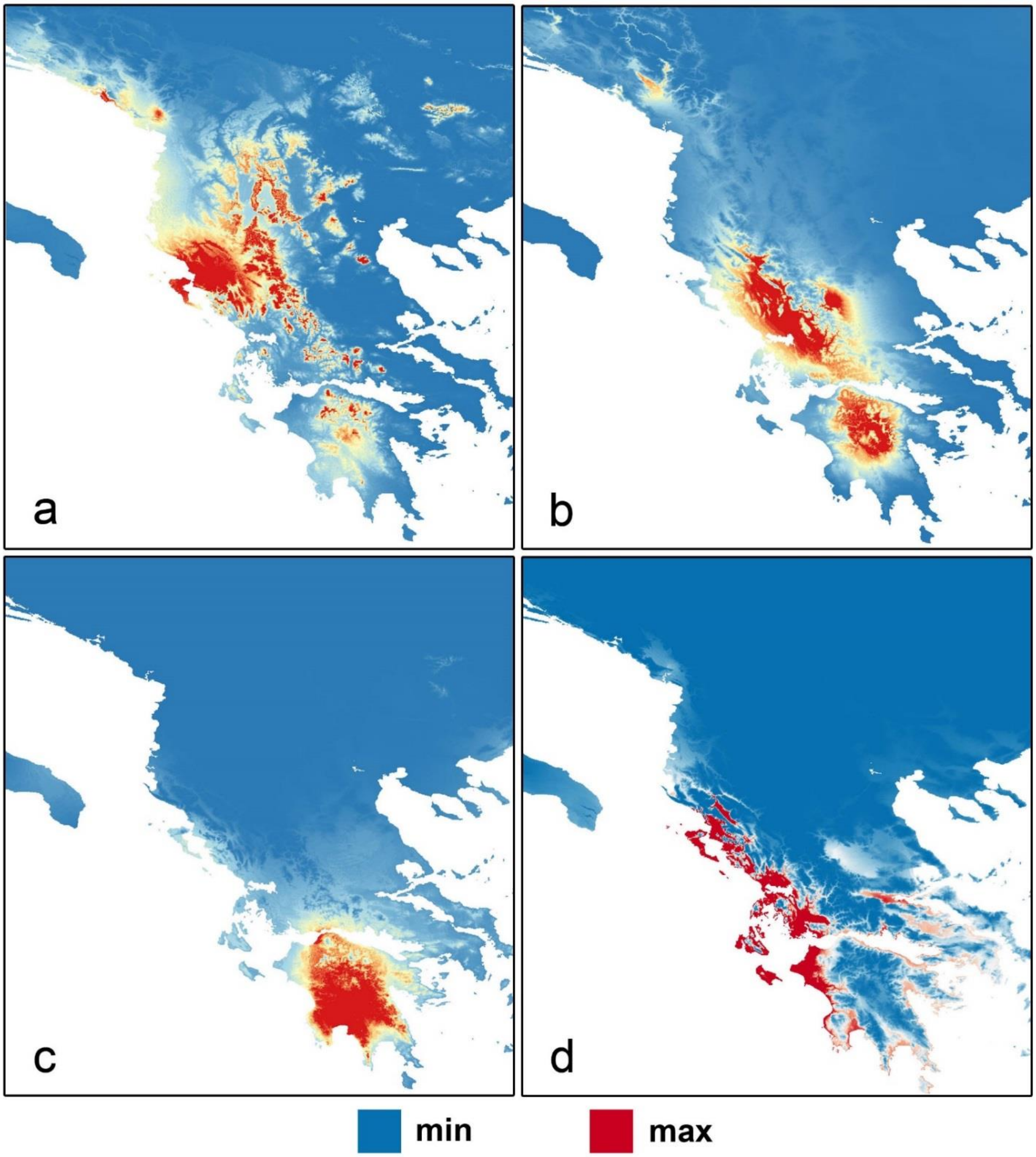

Figure 9. Geographic representation of ENM of: **a**) *P. j. jonicus*; **b)** *P. j. lobulatus*; **c**) *P. tessellatus*; **d**) *P. werneri*.

Table 2. Schoener's D index of niche overlap

SPECIES	*P. werneri*	*P. jonicus lobulatus*	*P. jonicus jonicus*	*P. tessellatus*
P. werneri	1,00	0,32	0,23	0,42
P. jonicus lobulatus	-	1,00	0,48	0,42
P. jonicus jonicus	-	-	1,00	0,21
P. tessellatus	-	-	-	1,00

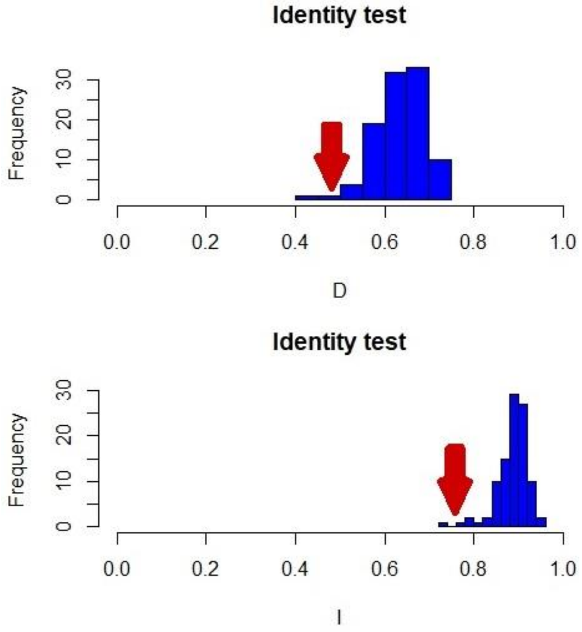

Figure 10. Niche identity test for *P. j. jonicus* and *P. j. lobulatus*. Histograms show frequency distributions of D and I index of 100 randomized models. The red arrows point the actual D and I of the two subspecies. Null hypothesis of identical ecological niches cannot be rejected (see Warren et al. 2008 for details).

DISCUSSION

Phylogeny of Poecilimon

The ITS tree from this study as well as the one from Ullrich et al. (2010) did not provide good resolution for all groups, especially for younger splits closer to the tip of the tree. However, most of the species groups defined by morphological and bio-acoustic traits (listed in Cigliano et al. 2020) were proved monophyletic. Representatives of *Poecilimon* shared last common ancestor ca. 9.6–7.8 Ma (Publication 1) which is related to the geotectonic activity during the Tortonian and possibly to the formation of the Mid-Aegean trench (see Poulakakis et al. 2014). The basal position of *Hamatopoecilimon* allows drawing some conclusions about the origin of the genus *Poecilimon*. As discussed by Ciplak et al. (2010) and Heller et al. (2011) genus *Poecilimon* shares similar evolutionary history with the bush-cricket genus *Eupholidoptera* Maran, 1953. The ancestral stock of *Poecilimon* most probably originated in southwestern Anatolia and was subjected to series of radiation events northwards and multiple allopatric speciation. Our results are in agreement with previous studies that point the major role of the Pleistocene cyclic glaciations for the radiation and diversification of the genus (La Greca 1999; Kaya et al. 2015).

Origin and divergence of the P. jonicus *group*

Received phylogenetic trees and TMRCAs allow us to make robust statements about the age of the ancestor, the place of origin, and thehistorical factors which triggered the evolution of Barbitistini.Members of *Poecilimon* shared last common ancestor ca. 9.6–7.8Ma, which corresponds to the first transgression of the Aegean area in Tortonian. These results are in congruence with the estimated TMRCA for the related genus *Isophya* as 8.3–8.8 Ma (Chobanov et al. 2017). Mitochondrial chronograms show that members of the *P. jonicus* group sensu lato share a common ancestor 8–6 Ma ago, which corresponds to the Messinian period. As the Anatolian clade (*P. antalyaensis* +*P. isopterus*) constitutes the basal branch and the Cretan (*P. cretensis*) the subsequent one, we conclude that the Anatolian mainland is the most plausible place of origin of ancestors of the group. Hence, it can be assumed that desiccation in the Mediterranean during the Messinian salinity crisis (MSC) (5.96–5.33 Ma) provided corridors for dispersal of an ancestral stock over the present distribution range of the group.

The following post-Messinian transgression of the Mediterranean and Aegean area ca. 5.3–5.2 Ma (Jolivet et al. 2006) acted as a vicariant event resulting in rapid splits of four geographically isolated stocks: i) ancestral of *P. inflatus* + *P. martinae* in Anatolia; ii) *P. cretensis* on Crete, iii)

Northern Balkan group in the southern Balkan mainland; iv) Southern Balkan group on the Peloponnese peninsula. During the Pliocene, the land-sea configuration in the region further complicated with the development of many islands and peninsulas, as well as vast inland freshwater areas (marshes and lakes) (Popov et al. 2004). This caused vicariant events that contributed to further divergence in the group. (splits of *P. werneri*, *P. jonicus superbus*, *P. erimanthos*, *P. antalyaensis* and *P. isopterus*). The Pleistocene is marked with the onset of the northern hemisphere glaciations which are reported to have triggered speciation processes within Barbitistini (Kaya et al. 2015; Chobanov et al. 2017). Recent divergence in the group correlates with major climatic shifts during the Pleistocene (*P. j. jonicus, P. j. lobulatus,* the significant interspecific divergence within *P. cretensis*).

Phylogeographic implications for Hamatopoecilimon

The basal position of subgenus *Hamatopoecilimon* allows drawing conclusions about the origin of *Poecilimon*. Since there are no older lineages on Crete or Peloponnese, *Poecilimon* possibly originated on the east side of the Aegean trench. The Aegean islands may thus have been colonized later after the appearance of suitable land bridges during the MSC. Though mitochondrial phylogeny supports simultaneous divergence of *P. deplanatus, P. hamatus* and *P. paros*/*P. klausgerhardi*, morphological traits clearly place *P. deplanatus* in its own lineage. Early divergence of *P. deplanatus* (before Mid Pliocene) combined with polytomy at the tree root of the “southern group” may be linked with gene exchange between those lineages prior to the isolation of Karpathos. Based on genetic distances and phylogeny dispersal of *P. paros*+*P. klausgerhardi* lineage to the Cyclades may have thus also occurred during the Pliocene as suggested for a few lineages (compare Poulakakis et al. 2014, Kornilios et al. 2018). This may explain the lack of *Hamatopoecilimon* on the Balkan mainland and on Crete. Therefore, we have a case of dispersal of the “southern group” followed by isolation contrary to the possible vicariance scenario in the case of *P. ikariensis* and *P. unispinosus, P. hamatus* and *P. deplanatus*. Split between *P. paros* and *P. klausgerhardi* may be quite recent as suggested by low genetic distances, morphological similarity and lack of acoustic differentiation (Heller et al. 2011). The latter might have happened during Pleistocene as a result of repeated shifts in the sea level and thus the connection-disconnection of the Southern and Northern Cyclades.

P. ikariensis is present only on Ikaria, while *P. unispinosus* has wider distribution on the Islands of Chios, Lesvos and the neighboring mainland Turkey. As Ikaria was recently connected to the mainland, its separation may have allowed the "last stand" for *P. ikariensis* escaping from possible competition with *P. unispinosus* on the mainland. Hamatopoecilimon represents a remnant from an ancient lineage that split comparatively early in the geological history of the Aegean area. Data from our study would be useful for testing further hypotheses regarding the origin and evolution of Poecilimon and other related genera in connection with the paleogeography of the Aegean region.

SYNTHESIS OF THE RESULTS

Phylogeny

Genus *Poecilimon*. Nuclear phylogeny of *Poecilimon* was obtained based on the ITS dataset of Ullrich et al. (2010), compiled with own sequences. The Bayesian inference tree shows general agreement with the published maximum parsimony tree (Ullrich et al. 2010). Our analysis confirms the monophyly of *Poecilimon* and most described species groups, while suggesting polyphyly of others. The paraphyletic genera *Polysarcus* and *Phonochorion* group within *Poecilimon* with high support. All representatives of the species groups *P. jonicus* and *P. inflatus* form a monophyletic clade, excluding *P. bilgeri*. The monophyly of subgenus *Hamatopoecilimon* and its basal position within *Poecilimon* are confirmed.

***Poecilimon jonicus* group**. The phylogenetic relationships within the *P. jonicus* group s.l. were inferred using three mitochondrial (COI, ND2, 12S+16S) and two nuclear (ITS1, ITS2) markers. The combined COI+ND2 matrix supports the monophyly of *P. jonicus* group s.l. including all the Balkan, Cretan and Anatolian lineages. The Anatolian taxa *P. antalyaensis* and *P. isopterus* form a monophyletic clade that branches out first, while the Anatolian *P. inflatus* is arranged within the Balkan clade. Divergence of *P. cretensis* prior to the Balkan lineages is suggested. On the Balkans two lineages can be outlined: 1) northern clade — *P. werneri* + (*P. j. superbus* + (*P. j. jonicus* + *P. j. lobulatus*)) and 2) southern clade — *P. erimanthos* + (*P. j. tessellatus* + *P. laevissimus*). The 12S rRNA+16S rRNA dataset of sequences obtained from Genbank (Ullrich et al. 2010) shows similar results also supporting the exclusion of *P. bilgeri* from the *P. jonicus* species group.

Subgenus *Hamatopoecilimon*. The monophyly of *Hamatopoecilimon* was confirmed by all three mitochondrial markers used (COI, ND2, CR). The BI tree of the concatenated mitochondrial matrix (COI+ND2+CR) shows full support for the following topology: (*P. unispinosus* + *P. ikariensis*) + ((*P.hamatus* + *P.deplanatus* + (*P. paros* + *P. klausgerhardi*)). The only disagreement is the separate analysis of ND2 which suggests *P. deplanatus* being a sister clade to *P. hamatus* + (*P.paros* + *P. klausgerhardi*) with the highest support in comparison to the other single matrices. All selected markers showed strong phylogenetic performance. In all cases ND2 had the highest percentage of variable and parsimony-informative sites and demonstrated strong phylogenetic signal. Similar results have been reported for the genus *Isophya* (Chobanov et al. 2017) and for order Odonata (Cheng et al. 2018).

Acoustic communication

Own song recordings from all sampled taxa confirmed published results for the majority of taxa, suggesting that evolution of calling song was reinforced in sympatric taxa, while remaining comparatively uniform in closely related allopatric taxa (compare Heller 2006). Comparison between own and published bio-acoustic data suggests a complex stable song pattern with a short main syllable and two afterparts characteristic for *P. j. tessellatus* which differs from the pattern of the other subspecies of *P. jonicus* (see Heller 1988 and Publication 1). The subspecies *P. j. superbus* from Italy has a song pattern very similar to that of *P. j. jonicus* (Massa et al. 2012 and own data). Own recordings of songs from representatives of *Hamatopoecilimon* did not show any differences from previous results (Heller et al. 2011).

Ecological niche modelling

Ecological niche models were created for *P. j. jonicus*, *P. j. lobulatus*, *P. j. tessellatus* and *P. werneri* using the 19 bioclimatic variables (Hijmans et al. 2005). Jackknife test on the set of environmental variables for *P. j. jonicus* and *P. j. lobulatus* showed that the most important predictor variable for both taxa was Bio13 – Precipitation of Wettest Month. For *P. tessellatus* the most important predictor variable was Bio14 – Precipitation of Driest Month, together with Bio3 – Isothermality. The model of *P. werneri* was more complex with Bio6 – Min Temperature of Coldest Month, Bio9 – Mean Temperature of Driest Quarter, Bio10 – Mean Temperature of Warmest Quarter, Bio14 – Precipitation of Driest Month being among the most important predictors. Measurement of the ecological niche overlap (Schoener's D index) showed that *P. werneri* solidly differs in ecological requirements from the other closely related taxa. Low niche overlap was detected between *P. j. tessellatus* and the other taxa (Table 1). Despite the relatively low niche overlap between *P. j. jonicus* and *P. j. lobulatus* the hypothesis of niche identity could not be rejected based on the analyzed variables.

Estimation of divergence times

The two alternative methods for estimation of divergence times — BEAST (Bouckaert et al. 2019) and RelTime (Tamura et al. 2012, 2018) showed similar results. The age constraint on the Cretan lineage, reflecting the earlier isolation of Crete from the mainland 10.6 Mya, resulted in significantly old ages that do not agree with previous studies (e.g. Chobanov et al. 2017). The later isolation of Crete, corresponding to the end of the Messinian 5.2 Mya was consistent with results from Chobanov et al. (2017) and with the independent time estimation based on the substitution rate of COI (Papadopoulou et al. 2010; Publication 1).

Our estimates suggest that *Poecilimon* shares the last common ancestor 9.5–7 Mya and the *P. jonicus* group sensu lato — 7.9–6 Ma ago. The Anatolian lineage including *P. antalyaensis* + *P. isopterus* evolved 8–6 Mya, while *P. inflatus* + *P. martinae* separated from lineages currently occurring in the Balkans 5.9–4.9 Mya. The split between the northern and the southern Balkan clades was estimated at 7–5.2 Mya. The lineage of *P. j. superbus* diverged from the Balkan lineage ca 3.9 Mya. Based on those estimates we infer Anatolian origin of the group with Messinian age dispersals to the Balkans and Crete and subsequent vicariant diversification ruled by the Pleistocene climatic cycles.

Taxonomy

The current study combines evidence from different fields to revise the systematics and to shed light on the evolutionary history of the selected groups. The former *P. jonicus* and *P. inflatus* species groups represent a monophyletic lineage, which rapidly diversified during the late Miocene. Therefore, a total of 16 taxa (11 species) are referred as belonging to the *Poecilimon jonicus* species group. Phylogenetic analyses showed that *P. bilgeri*, formerly included in the *P. inflatus* group, is related to other taxa outside *P. jonicus* lineage and thus it is excluded from the group. The Anatolian *P. inflatus*+*P. martinae* appear as an internal branch of the Balkan lineages with high support. Molecular phylogeny strongly supports that *P. jonicus tessellatus* belongs to the southern Balkan clade together with *P. laevissimus* and *P. erimanthos*. Differences in calling song between *P. j. tessellatus* and the other subspecies support the above observations and the species status of *P. tessellatus*. On the contrary, the calling song of *P. j. superbus* from Italy is similar to that of *P. j. jonicus*, yet, phylogeny and molecular dating suggest that *P. superbus* evolved in isolation for a very long time (ca. 4 Mya). Considering that closely related allopatric bush-crickets tend to retain ancestral song patterns (Heller 2006), we infer that *P. j. superbus* should obtain species status. To reflect the results of the present study, two taxonomic changes are made — *P. tessellatus* (Fischer, 1853), stat. rev., and *P. superbus* (Fischer, 1853), stat. rev.

CONCLUSIONS

Phylogeny

The confirmed basal position of subgenus *Hamatopoecilimon* within the genus *Poecilimon* and comparison with the evolution of the bush-cricket genus *Eupholidoptera* allows hypothesizing that the ancestral stock of *Poecilimon* most probably originated in southwestern Anatolia and was subjected to series of radiation events north- and east-wards and multiple allopatric speciation events. Our results support earlier views on the major role of the Pleistocene cyclic glaciations for the radiation and diversification of the genus. Phylogenetic reconstructions based on mitochondrial and nuclear markers revealed robust phylogenies that corroborated phylogeographic hypotheses and systematic decisions. Both *Poecilimon jonicus* group and subgenus *Hamatopoecilimon* are monophyletic groups that most probably originated on the east side of the Mid-Aegean trench and dispersed in a west direction during periods of low sea level.

Acoustic communication

Our results confirm diversity and complexity of calling songs within the studied groups that characterizes taxa on a species level. The reinforcement of song evolution in sympatric taxa contrasts the uniformity of songs in allopatry at the top of the phylogenetic trees. Therefore, song evolution, being subjected to a variety of environmental factors (both biotic and abiotic), needs to be carefully addressed concerning evolutionary hypotheses, as it may not follow phylogenetic patterns. Yet, comparative acoustics may firmly contribute to solving taxonomic and evolutionary issues in *Poecilimon*.

Ecological niche modelling

Using detailed layers of climatic variables and a complete set of locality data we show the importance of constructing ecological niche models and performing niche comparisons to outline main ecological factors for the studied lineages, reveal the possible actual range of taxa, hypothesize about ecological and geographic barriers between lineages, reveal contact zones of different lineages, and provide grounds for taxonomic changes in the studied group. The importance of precipitation on the distribution of model taxa and the response to specific environmental variables reflecting the thermophilous nature of *P. werneri* outlined sharp borders of the ecological requirements between species, while low niche overlap suggested partial ecological differentiation between subspecies of *Poecilimon jonicus* and pointed to the need of a more detailed research in the field in future.

Estimation of divergence times

Active geotectonic processes in the Aegean strongly affected the speciation and radiation of the flightless, poorly mobile lineages of *Poecilimon*. Molecular dating analyses allowed correlation of lineage splits in the studied groups with well-known geological events. Our study confirmed the major role of the Messinian salinity crisis in the area for faunal exchange between the two sides of the Mid-Aegean Trench and the marine corridor between the Mediterranean and Pontic Sea, thus boosting speciation and diversification. The post-Messinian flooding served as a main force for vicariant speciation at the deep nodes of the phylogenetic tree of *Poecilimon*, while Pleistocene climatic cycles affected the terminal nodes and impelled intraspecific diversification. The phylogeographic reconstructions of the evolution of *Hamatopoecilimon* revealed late trans-Aegean dispersal that indicates the existence of post-Messinian land bridges between Anatolia and the Cyclades, a discovery of great interest requiring deeper investigation. The evolution of *Poecilimon jonicus* group and subgenus *Hamatopoecilimon* was ruled by a combination of geotectonic and climatic events resulting in an explosive diversification since the end of the Miocene and reflected the overall evolutionary success of the genus *Poecilimon*.

Taxonomy

By employing an interdisciplinary approach, we unraveled the relationships and diversity within two intriguing groups of bush-crickets characteristic with highly fragmented distribution, spatially complex phenotype and supposedly long evolutionary history. Studying phylogenetic relationships of the groups on a spatial and temporal scale, comparing and mapping their ecological preferences (based on climate variables) and behavioral characteristics (song patterns), we were able to define their taxonomic composition and propose revision of the systematics and taxonomy of the *Poecilimon jonicus* group and subgenus *Hamatopoecilimon*.

CONTRIBUTIONS OF THE STUDY

Contributions on phylogeny

1. DNA sequence data of ITS1 and ITS2 available from GenBank was used to reevaluate the phylogeny of the genus *Poecilimon* employing Bayesian inference and Maximum likelihood analyses. Nuclear phylogeny supports the monophyly of *Poecilimon*, yet the genera *Polysarcus* and *Phonochorion* join within *Poecilimon*, which calls for their paraphyly. This underlines the need of further research on the systematics of the genus and the evolutionary processes that resulted in high diversity and morpho-acoustic specialization.
2. Three mitochondrial (COI, ND2 and CR) and two nuclear (ITS1, ITS2) molecular markers were used for phylogenetic reconstructions of the relationships within *Poecilimon jonicus* species group (COI, ND2, ITS1 and ITS2) and subgenus *Hamatopoecilimon* (COI, ND2 and CR). The ND2 performed best as a single marker at the species-group level, while a strong phylogenetic signal of the mitochondrial control region on a species level in subgenus *Hamatopoecilimon* was revealed.

Contributions on acoustic communication

3. New recordings of calling songs of the studied groups were obtained and compared with available data. Evidence from bio-acoustic analyses was used to support taxonomic changes of *Poecilimon tessellatus* (Fischer, 1853), stat. rev. and *P. superbus* (Fischer, 1853), stat. rev.

Contributions on ecology

4. Ecological niche models shed light on the ecology of the rare species *Poecilimon werneri* and revealed new possible areas of occurrence of the latter.
5. Variation in ecological requirements of closely related taxa was measured to reveal ecological niche limits and range boundaries and thus elucidate distinct taxonomic units.

Contributions on phylogeography

6. Different methods (BEAST, Reltime) for molecular dating were tested on the model groups and consistent results were reported.
7. Two molecular clock calibration strategies – mutation rate and geologic events – were compared and critically evaluated based on the evolutionary history of the studied groups.

8. Correlations of lineage splits with major geotectonic and climatic events in the East Mediterranean are reported. Phylogenies and divergence time estimations confirm that sea level drop during the Messinian allowed terrestrial lineages to cross the MAT. The intensive flooding that marked the beginning of the Pliocene resulted in isolation of landmasses and created conditions for vicariant speciation in the Aegean region. The present study provides evidence that post-Messinian land bridges possibly connected western Anatolia and the Cyclades.

Contributions on taxonomy

9. Based on combined nuclear and mitochondrial datasets the presented study outlines monophyletic groups within genus *Poecilimon* and defines evolutionary the taxonomic units *Poecilimon jonicus* species group and subgenus *Hamatopoecilimon*.
10. Based on behavioral, ecological, phylogenetic and paleogeographic data the present interdisciplinary study allows for clear delimitation of taxonomic units within *P. jonicus* species group. Monophyly of the *Poecilimon jonicus* species group sensu lato and rapid diversification at the base of the trees does not allow for clear differentiation of species complexes and hence it unites all taxa from the formerly considered species groups *P. jonicus* group sensu stricto and *P. inflatus* group, except for *P. bilgeri*, which is related to lineages outside *P. jonicus* group and therefore is excluded based on polyphyly. Therefore, *P. jonicus* group comprises 16 taxa (11 species) (Publication 1).
11. The study confirms the monophyly and basal position of the subgenus *Hamatopoecilimon* within *Poecilimon*, and all six species considered in this subgenus retain their status.

LIST OF RESEARCH ARTICLES RELATED TO THIS PHD THESIS

Publication 1

Borissov, S. B., Bobeva, A., Çıplak, B., & Chobanov, D. (2020). Evolution of *Poecilimon jonicus* group (Orthoptera: Tettigoniidae): a history linked to the Aegean Neogene paleogeography. Organisms Diversity & Evolution, 1-17.

Publication 2

Borissov, S., & Chobanov, D. (in press). Mitochondrial phylogeny of subgenus *Hamatopoecilimon*. Articulata, 35.

LIST OF PUBLISHED ABSTRACTS FROM CONGRESSES RELATED TO THIS PHD THESIS

Borisov, S., & Chobanov, D. Comparison between environmental niche modelling and molecular marker analysis of the *Poecilimon jonicus* group in southern Greece and Crete. European Congress on Orthoptera Conservation Smolenice, Slovakia. Oral presentation. 19-21 September 2018

Borissov, S., & Chobanov, D. Phylogenetic reconstructions of closely related species groups of the genus Poecilimon (Insecta: Orthoptera) from the Balkans, Anatolia and Crete. International Zoological Congress of “Grigore Antipa” Museum Bucharest, Romania. Oral presentation. 21 - 24 November 2018

Chobanov, D., Bobeva, A.B., & **Borisov, S.B.** Phylogeography and conservation status of bushcrickets within a complex archipelago-mainland system: target group *Poecilimon jonicus* and *P. hamatus* species complexes. 13th International Congress of Orthopterology Agadir, Morroco. Oral presentation. 24-28 March 2019.

ФИЛОГЕОГРАФИЯ И ЕВОЛЮЦИЯ НА ВИДОВАТА ГРУПА *POECILIMON JONICUS* И ПОДРОД *HAMATOPOECILIMON* (INSECTA: ORTHOPTERA) В ЕГЕЙСКИЯ РЕГИОН

Симеон Борисов

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Егейският район (южните Балкани, Мала Азия и множеството острови в Егейско море) се характеризира със засилена геотектонска активност, следствие от сблъсъка на три геотектонски плочи, и голямо климатично разнообразие под влиянието на географското положение, сложният релеф и прилежащите водни маси. Районът е ключов за двупосочното разселване на сухоземна фауна между Европа и Азия.

Представеното изследване се концентрира върху две моделни видови групи микроптерни, растителноядни скакалци от род *Poecilimon* Fischer, 1853, най-богатият на видове род дългопипални скакалци в Палеарктика. За представителите на рода е характерна двустранна, видово специфична биоакустична комуникация, която осигурява вътревидовото разпознаване и локализирането на половия партньор и в повечето случаи представлява репродуктивна бариера. Обект на изследване са видовата група *P. jonicus* и подрод *Hamatopoecilimon* Heller, 2011, които имат разпокъсано разпространение западно и източно от Средно-егейската падина, на остров Крит и на архипелазите Циклади и Южни Спориди.

Цел на изследването е да се проучи филогеографията на скакалците от избраните групи и да се направят изводи за техните темпове на еволюция и еволюционни стратегии, както и пътищата и времето на разселване на филогенетичните линии. Приложен е интегративен подход, включващ молекулярно-генетични, акустични методи и моделиране на екологичните ниши. Предложена е таксономична ревизия на групата *P. jonicus* на основата на молекулярна филогения и биоакустика. Представени са добре подкрепени филогенетични реконструкции, базирани на митохондриални и ядрени маркери. Приложени и сравнени са различни методи за датиране на филогенетичните линии и стратегии за калибриране. Изготвени са модели на климатичните ниши на избрани видове и са извършени тестове за припокриване на нишите между близкородствени таксони.

Резултатите утвърждават видовия състав на видовата група *Poecilimon jonicus* sensu lato, като в нея е включена групата *P. inflatus* (Kaya et al. 2018) на основата на монофилия. От състава на групата *P. jonicus* s.l. е изключен видът *P. bilgeri* Karabag, 1953, чието родство с други групи е потвърдено чрез молекулярни изследвания. Два подвида на вида *P. jonicus* (Fieber, 1853) придобиват статус на вид – *P. tessellatus* (Fischer, 1853), stat. rev., и *P. superbus* (Fischer, 1853), stat. rev. Изследването потвърждава монофилията и базовото положение на подрод *Hamatopoecilimon* в род *Poecilimon*. Анализирана е времевата рамка за еволюционните процеси в моделните групи и са изказани филогеографски хипотези относно видообразуването и разселването в Егейския регион. Потвърдена е ролята на миоценските сухоземни връзки за разпространението на сухоземната фауна и са представени доказателства за наличието на по-късни сухоземни коридори в южната част на Егейския басейн.