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Dolichopoda cave crickets from Peloponnese (Orthoptera, Rhaphidophoridae): molecular and morphological investigations reveal four new species for Greece

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Abstract

Three species belonging to the genus *Dolichopoda* (Orthoptera; Rhaphidopohoridae) are known so far from the Peloponnese, all endemic to the area. In particular, *D. matsakisi* is known from two mountains in the North, while *D. dalensi* is present in the east region. The third species, *D. unicolor*, is distributed in the southern part of the Peloponnese, inhabiting caves on Mt Taygetos and Mani Peninsula. Recently, extensive sampling work in most of the Peloponnese has led to the discovery of new taxa, morphologically differentiated by the above three known species.

To investigate the delimitation of the Peloponnesian species of *Dolichopoda*, we performed both morphological and molecular analyses. Morphological analysis was carried out by considering diagnostic characters generally used to distinguish different taxa, as the shape of epiphallus in males and the subgenital plate in females. Molecular analysis was performed by sequencing three mitochondrial genes, 12S rRNA, 16S rRNA, and COI, and one nuclear gene, 28S rRNA.

Results from both morphological and molecular analyses were used to revise the taxonomic arrangement of the Peloponnesian species. On the whole, we were able to distinguish seven lineages of Peloponnesian *Dolichopoda* species, of which *D. kofinasi* n.sp., *D.epidavrii* n.sp., *D. poseidonica* n.sp., and *D. propanti* n.sp. are described as new species.

http://www.zoobank.org/urn:lsid:zoobank.org:pub:857875CB-22B9-4CFC-9B7F-3FDDFC47A4E6

Keywords: *Barcoding, systematics, Peloponnesian* Dolichopoda *species, phylogeny*

Introduction

The Mediterranean basin is one of the world's most geographically complex regions (Blondel et al. 2010) with remarkable paleogeographic evolution and tectonic history (Cavazza & Wexel 2003).

It is also one of the global hotspots of biodiversity, showing a high proportion of both plant and animal endemic species especially in the eastern part, including the Balkans and Anatolia (Blondel et al. 2010). The Hellenic area and the Aegean islands show an intricate geological history with a series of land connection events throughout the late Tertiary (Dermitzakis & Papanikolaou 1981; Dermitzakis 1990). The area is also considered a temperate refugium and genetic diversity may have been accumulated during the several ice ages of the late Pliocene and Pleistocene epochs, leading to great lineage diversity in both terrestrial invertebrates and vertebrates (Poulakakis et al. 2015; Legakis et al. 2018).

The complex palaeogeographical history of the Mediterranean region seems to have driven the colonization and speciation processes in *Dolichopoda* Bolivar, 1880, a cavernicolous genus of Orthoptera belonging to the family Rhaphidophoridae and distributed from the eastern Pyrenees to the Caucasus Mountains and eastwards to northern Iran (Alborz Mountains). Most species of this genus are strictly dependent upon caves. However, especially in the northern part of the range, *Dolichopoda* populations

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inhabit many different habitats, including soil crevices in the forest, catacombs, Etruscan tombs, and other man-made habitats, natural caves, and large hypogean karst systems, representing a range from quasi epigean to hypogean conditions. Depending on the exploited habitat, they present variation in their semivoltine life cycle due to the different environmental conditions that change from variable climate regimes to a more constant environment (Di Russo et al. 1994).

Based on both molecular phylogenetic reconstruction and biogeographic analysis carried out on the ninety percent of known species, an eastern origin of *Dolichopoda* species could be hypothesized (Allegrucci et al. 2005, 2009, 2011). In particular, the colonization of Greece by *Dolichopoda* species followed two different routes, probably originating in Anatolia. The northern lineage currently occurring in the western Mediterranean, Thasos island, northern Greece, Ionian islands, and north-eastern Peloponnese could be the result of dispersal from the north through the Balkan peninsula. The southern lineage, including species currently inhabiting Aegean islands, Crete, south-eastern Greece, and south Peloponnese, likely arose from trans-Aegean colonization during the Messinian salinity crisis (5.96–5.33 Mya). The opening of the Mid-Aegean trench would have promoted an initial diversification within the uplift of the Anatolian Plateau, while the Messinian marine regression offered the conditions for a rapid dispersal through the whole Aegean-Hellenic region. Rather, climatic events linked to the Plio-Pleistocene are responsible for the speciation within each of the two different lineages, mainly driven by vicariance events. Also, adaptation to cave life seems to have played an important role in this process. The ancestors of *Dolichopoda* might have used caves as refugia during the unfavorable climatic conditions, beginning their adaptation to subterranean habitat. Therefore, the current distribution of *Dolichopoda* has been explained by a combination of both vicariance and dispersal events, with many processes occurring in ancestral epigean populations before the invasion of the subterranean habitat (Allegrucci et al. 2009, 2011).

Three species belonging to the genus *Dolichopoda* are known so far from the Peloponnese (Rampini et al. 2008; Di Russo et al. 2019; Figure 1), all endemic to the area. Our knowledge on the actual distribution of those species within Peloponnese became clearer recently (Di Russo et al. 2019). In particular, *D. matsakisi* Boudou-Saltet, 1972 is known from two

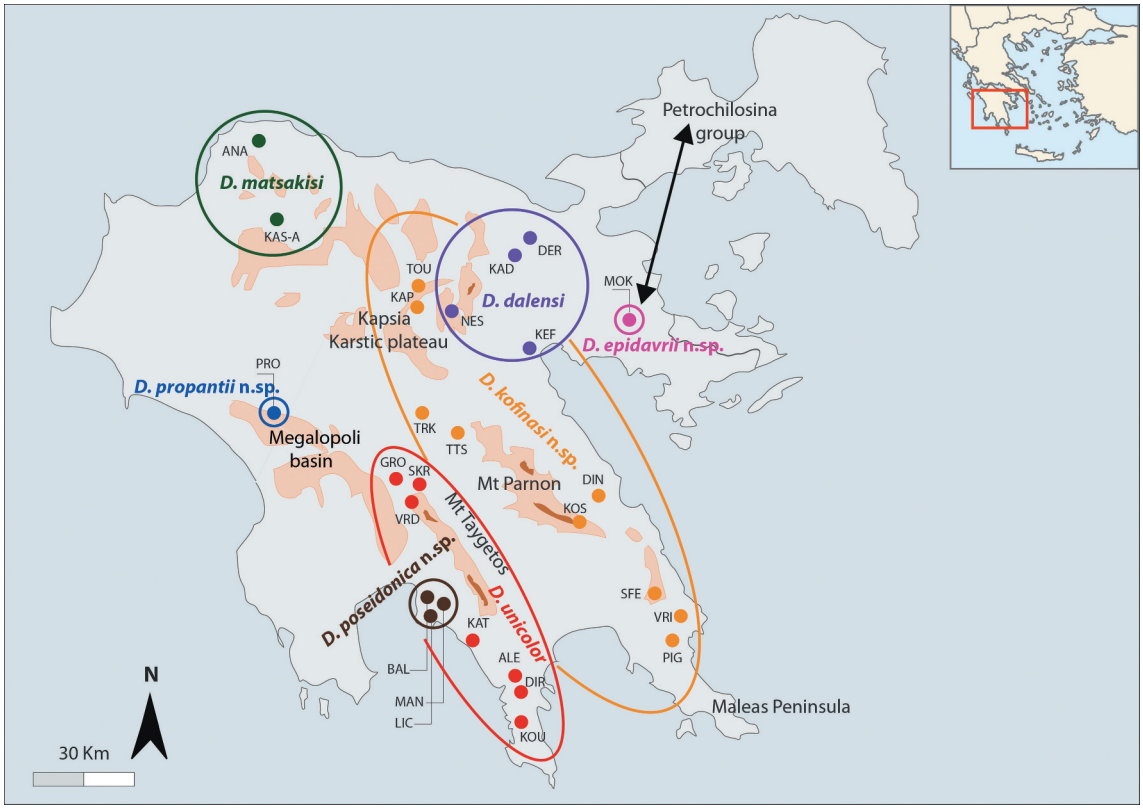

Figure 1. Geographic distribution of the Peloponnesian *Dolichopoda* populations sampled and analyzed in this study. Codes are as in Table I. Black arrow indicates the probable way of colonization of *Dolichopoda* ex-*Petrochilosina* from mainland Greece.

mountains at the northern parts of Peloponnese, namely Mt Chelmos and Mt Panachaiko, while *D. dalensi* Boudou-Saltet, 1972 is present on the east (Korinthia and Argolis). The third species, *D. unicolor* Chopard, 1964, described from specimens collected in the Katafygi cave (Selenitsa), is distributed in the southern part of Peloponnese, inhabiting caves on Mt Taygetos and Mani Peninsula.

Since 2002, we have been collecting cave cricket specimens from caves of Peloponnese. The involvement of the members of Speleological Club Poseidon during recent years, proved very fruitful, allowing for investigation of a large number of caves in the area. The preliminary results were recently published, mainly with new distributional data (Di Russo et al. 2019). Based on the diagnostic morphological characters, cave crickets collected in 19 caves could not be assigned to any known species.

The main aims of this paper were to study these populations both at a morphological and molecular level to confirm the existence of new species in this region. The new taxa were compared with the known species from Peloponnese, as well as with those from nearby areas to evaluate the hypothesis of colonization as already drawn in the previous studies (Allegrucci et al. 2009, 2011).

Materials and methods

Taxon sampling and laboratory procedures

Twenty cave populations from Peloponnese in Greece were analyzed in this study for a total of 31 individuals (Table I). Geographical locations of the present sampled caves are illustrated in Figure 1, where are also reported the geographic locations of all known Peloponnesian species.

Eight additional species from nearby areas previously analyzed (Allegrucci et al. 2009, 2011) were used for comparison. In particular, we considered *D. lustriae* (Rampini, di Russo, Pavesi & Cobolli, 2008) from central-western Greece, *D. vandeli* (Boudou-Saltet, 1970), *D. insignis* (Chopard, 1955), *D. petrochilosi* (Chopard, 1954), *D. cassagnaui* (Boudou-Saltet, 1980), and *D. makrykapa* (Boudou-Saltet, 1980) from central-eastern Greece, *D. parakevi* Boudou-Saltet, 1973 from Crete Island, *D. naxia* (Boudou-Saltet, 1972), *D. giulianae* Rampini & Di Russo, 2012 and *D. calidnae* Rampini & Di Russo, 2012 from the central and eastern Greek islands Naxos, Samos and Kalimnos. One species belonging to genus *Troglophilus* Krauss, 1879 within the same family was used as outgroup (*T. cavicola* (Kollar, 1833) Table I; Allegrucci et al. 2009, 2017).

DNA was isolated from the leg muscle of each individual, using a C-TAB protocol (Doyle & Doyle 1987) resuspended in 100 µl of sterile water and stored at −40°C.

The entire Cytochrome Oxidase I gene (COI, a total of 1500 bp), a 550-bp fragment of the 16S rRNA gene, and a 450-bp fragment of the 12S rRNA gene were amplified through the polymerase chain reaction (PCR) and sequenced for each individual. The large subunit of the nuclear ribosomal DNA (28S rRNA) was also sequenced. The primers used were: LCO1490, HCO2198 (Folmer et al. 1994), UEA1, UEA5, and UEA10 (Lunt et al. 1996) for the COI gene, 12Sai, 12Sbi (Kocher et al. 1989; Simon et al. 1994) for the 12S rRNA gene and 16Sar, 16Sbr (Simon et al. 1994) for the 16S gene. As regards 28S rRNA, it was partially amplified and sequenced for a fragment of 580 base pairs, belonging to domains 3–5, using primers from Friedrich and Tautz (1997). Optimal cycling parameters varied for each primer pair used. PCR products were purified using the ExoSAP digestion (Amersham Pharmacia Biotech), directly sequenced in both directions using the BigDye terminator ready-reaction kit, and resolved on ABI 3100 Genetic Analyzer (PE Applied Biosystems), following the manufacturer's protocols. Sequence data were edited and compiled using CODON-CODE ALIGNER 9.0.1. All sequences were submitted to GenBank (Accession Numbers are reported in Table I).

Each gene fragment (12S, 16S, COI, and 28S) was considered separately for the alignment. Non protein coding sequences of 16S, 12S, and 28S were aligned using CLUSTALX 2.1 (Larkin et al. 2007) with opening gap = 10 and extending gap = 0.10. These sets of parameters appear to work at best in the alignment of *Dolichopoda* sequences and have been used in all previous studies (Allegrucci et al. 2005, 2009, 2011, 2019). No heterozygous haplotypes were observed in 28S sequences. Coding sequences of COI were assembled, aligned, and translated with CODON-CODE ALIGNER.

Data analysis

Species delimitation and phylogenetic analysis

The Automatic Barcode Gap Discovery (ABGD, Puillandre et al. 2012) was employed to carry out species delimitation analysis. ABGD automatically finds the distance at which a barcode gap occurs and sorts the sequences into putative species based on this distance. The method statistically infers the barcode gap from the data and partitions the data accordingly. Populations belonging to the same

Table I. *Dolichopoda* species and outgroup taxa included in this study.

LOCALITIES			CODE	Analyzed specimens	GeneBank Accession No.
Outgroup					
Troglophilus cavicola	Covoli di Veneto Cave, Veneto, Northern Italy	[1]	**TRO**	1	12S:EF216946 16S:AY793564 COI:AY793624 28S: EF217003
Ingroups					
Genus *Dolichopoda*					
Central-Western Greece					
lustriae	Agios Andreas Cave, Valtou M., Chalkiopouli, Aitolia	[1]	**AND**	2	12S:EU887848 16S:EU887863 COI:EU887901 28S: EU887878
Central-Eastern Greece					
vandeli	Hermes Cave, Orkomenos, Dhionisos, Voiotia	[1]	**HER**	3	12S:EF216932 16S:EF216962 COI:EF217039/40 28S: EF216992
	Cave over Kopais Lake, Orkomenos, Voiotia	[1]	**GLK**	2	12S:EF216932 16S:EF216962 COI:EF217038
insignis	Panos Cave, Marathon, Athene, Attica	[1]	**PAN**	2	12S:EF216938 16S:EF216968 COI:EF217054 28S: EF217000
petrochilosi	Agia Joannis, Nea Pendeli, Athene, Attica	[1]	**JOA**	2	12S:EF216937 16S:EF216967 COI:EF217053/1/2 28S: EF216999
cassagnaui	Agia Triada Cave, Karistos, Eubea Island	[1]	**TRI**	2	12S:EF216931 16S:EF216961 COI:EF217035/6/7 28S: EF216991
makrykapa	Chiliadou beach Cave, Lamari, Eubea Island	[1]	**PKI**	2	12S:EF216941 16S:EF216971 COI:EF217942
	Paralia Pot Cave, Kato Seta, Agia Triada, Eubea Island	[1]	**KSA**	2	12S:EF216941 16S:EF216971 COI:EF217041 28S: EF216993
Central and Eastern Greek Islands					
naxia	Zeus Cave, Filotas, Naxos Island, Kyklades	[1]	**ZEU**	3	12S:EU887853 16S:EU887868 COI:EU887909/10/11 28S: EU887882
	Small Cave, Stavros, Naxos Island, Kyklades	[1]	**STA**	1	12S:EU887852 16S:EU887867 COI:EU887908 28S: EU887881
giulianae	Moni Spilianis Cave, Pithagorion, Samos Island	[1]	**SPS**	4	12S:EF216935 16S:EF216965 COI:EF217049 28S: EF216997
calidnae	Seven Virgins Cave, Kalimnos, Kalimnos Island, Dodecanese	[1]	**EPT**	4	12S:EF216933 16S:EF216963 COI:EF217047 28S: EF216995
	Skalia cave, Skalia, Kalimnos Island, Dodecanese	[1]	**SKA**	4	12S:EF216934 16S:EF216964 COI:EF217048 28S: EF216996
Northern Peloponnesus					
matsakisi	Analipsi Cave, Pititsa, Achaia	[1]	**ANA**	2	12S:EF216927 16S:EF216957 COI:EF217022/3 28S: EF216984
	Ton Limnon Cave, Kastri, Kalavrita, Achaia	[1]	**KAS-A**	2	12S:EF216927 16S:EF216957 COI:EF217024 28S: EF216985
		[2]	**KAS-AN**	1	12S:MW358500 16S:MW358531 COI:MW357333 28S:MW358606
dalensi	Kefalovrisi Cave, Argos, Argolida	[1]	**KEF**	2	12S:EF216929 16S:EF216959 COI:EF217026 28S: EF216987
	Dervenaki cave Nemea, Korinthia	[2]	**DER**	1	12S:MW358501 16S:MW358532 COI:MW357334 28S:MW358607
	Kaliakoudotripa, Nemea, Korinthia	[2]	**KAD**	1	12S:MW358503 16S:MW358534 COI:MW357336 28S:MW358609
	Arcadia: Nestani, Katavothra Nestani	[2]	**NES**	1	12S:MW358502 16S:MW358533 COI:MW357335 28S:MW358608

(*Continued*)

Table I. (Continued).

LOCALITIES			CODE	Analyzed specimens	GeneBank Accession No.
epidavrii	Spilaio Moni Kalamiou, Epidauros, Argolida	[2]	**MOK**	4	12S:MW358496-99 16S:MW358527-30 COI:MW357329-32 28S: MW358602-5
Southern Peloponnesus					
unicolor	Katafygi Cave, Selinitsa, Messinia	[1]	**KAT**	2	12S:EF216940 16S:EF216970 COI:EF217045/6 28S: EF216994
		[2]	**SEL**	1	12S:MW358514 16S:MW358545 COI:MW357347 28S:MW358620
	River cave of Glyfada, Dirou, Aeropolis, Laconia	[1]	**DIR**	2	12S:EF216940 16S:EF216970 COI:EF217043/4
	Alepotripa Cave,Dirou, Aeropolis, Laconia	[2]	**ALE**	1	12S:MW358515 16S:MW358546 COI:MW357348 28S:MW358621
	Koukouri cave, Kafionas, Laconia	[2]	**KOU**	1	12S:MW358517 16S:MW358548 COI:MW357350 28S:MW358623
	Cave 2, Kastorio, Taygetos, Laconia	[2]	**GRO**	1	12S:MW358516 16S:MW358547 COI:MW357349 28S:MW358622
	Skreti cave, Kastorio, Taygetos, Laconia	[2]	**SKR**	1	12S:MW358518 16S:MW358549 COI:MW357351 28S:MW358624
	Vordionatiki cave, Kasorio, Taigetos, Laconia	[2]	**VRD**	1	12S:MW358519 16S:MW358550 COI:MW357352 28S:MW358625
poseidonica	Balli Cave, Kardamili, Messinia	[2]	**BAL**	1	12S:MW358523 16S:MW358554 COI:MW357356 28S:MW358629
	Licurgo Cave, Kardamili, Messinia	[2]	**LIC**	2	12S:MW358524-5 16S:MW358555-6 COI:MW357357-8 28S: MW358630-1
	Katafigi Mantagari, Kardamili, Messinia	[2]	**MAN**	2	12S:MW358526 16S:MW358557 COI:MW357359 28S:MW358632
propantii	Spilaio Propanti, Andritsena, Ilia	[2]	**PRO**	3	12S:MW358520-22 16S:MW358551-3 COI:MW357353-55 28S: MW358626-8
kofinasi	Katavothra Tousi, Kapsia, Arcadia	[2]	**TOU**	1	12S:MW358504 16S:MW358535 COI:MW357337 28S:MW358610
	Spilaio Kapsia, Kapsia, Arcadia	[2]	**KAP**	1	12S:MW358505 16S:MW358536 COI:MW357338 28S:MW358611
	Spilaio Tyrias, Kollines Arcadia	[2]	**TTS**	2	12S:MW358506-7 16S:MW358537-8 COI:MW357339-40 28S: MW358612-3
	Spilaio Trupitses, Skortsinos Arcadia	[2]	**TRK**	1	12S:MW358508 16S:MW358539 COI:MW357341 28S:MW358614
	Barathro Sfendami, Monemvassia, Laconia	[2]	**SFE**	1	12S:MW358511 16S:MW358542 COI:MW357344 28S:MW358617
	Spilaio Pigaza, Vellies, Laconia	[2]	**PIG**	1	12S:MW358512 16S:MW358543 COI:MW357345 28S:MW358618
	Spilaio Vri, Monemvassia, Laconia	[2]	**VRI**	1	12S:MW358513 16S:MW358544 COI:MW357346 28S:MW358619
	Kosmas, Spilaio Kosma Arcadia	[2]	**KOS**	1	12S:MW358510 16S:MW358541 COI:MW357343 28S:MW358616
	Leonidio, Spilia Dionysou Arcadia	[2]	**DIN**	1	12S:MW358509 16S:MW358540 COI:MW357342 28S:MW358615
Crete Island					
paraskevi	Agia Paraskevi Cave, Skotinon, Iraklio	[1]	**PAR**	3	12S:EF216942 16S:EF216972 COI:EF217027/8
	Atzigano cave, Adrianos, Lasithi	[1]	**NIK**	2	12S:EF216942 16S:EF216972 COI:EF217030
sp. "Crete"	Diktaion Antron Cave,Psychro, Iraklio	[1]	**DHI**	2	12S:EF21693016S:EF216960 COI:217029

Notes: [1] Allegrucci et al. 2009, 2011. [2] Present paper

species therefore should be grouped in the same partition. This procedure is then recursively applied to the previously obtained groups of sequences. This analysis was carried out on the present samples and all *Dolichopoda* species previously analyzed (Table I and Allegrucci et al. 2005, 2011, Martinsen et al., 2009), considering only the COI data set and only the common base pairs consisting of 964 bp. The resulting inferences were then recursively applied to yield finer partitions (recursive partitions) until no further partitioning was possible. Genetic distance matrix (p-distance) was uploaded at https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html and ABGD was run with the default settings (*Pmin* = 0.001, *Pmax* = 0.1, Steps = 10, X (relative gap width) = 1.5, Nb bins = 20) and using p-distance.

Genetic distances between haplotypes were calculated using p-distance as implemented in MEGA 5.2 (Tamura et al. 2007). NETWORK 10.0.0.0 (Bandelt et al. 1999) was employed to calculate a median-joining network representing the genealogical relationships among mtDNA haplotypes. In this analysis, all the Peloponnesian species were considered with the addition of *D. vandeli* from Voiotia to point out the differences with *D. epidavrii* n.sp. Phylogenetic analyses were carried out on each gene fragment separately and then data were combined by constructing a concatenated matrix, partitioned by genes. All phylogenetic analyses were performed on the Peloponnesian taxa and those from nearby areas as detailed above, using Bayesian Inference (BI) analysis as implemented in the software MRBAYES 3.2.7 (Ronquist et al. 2012). J MODEL TEST 2.1.7 (Darriba et al. 2012) was used to perform a hierarchical likelihood ratio test and calculate approximate Akaike Information Criterion (AIC) values of the nucleotide substitution models for each gene fragment.

At least two simultaneous searches were conducted comprising four Markov chains (MCMC) started from a randomly chosen tree and run for 1,000,000 generations, with sampling every 100 generations. The following descriptors were assumed to indicate convergence on a common phylogenetic topology by separate Bayesian searches: similarity in log-likelihood scores at stationarity, the similarity in consensus tree topologies and PP values for supported nodes, and a final average standard deviation of split frequencies (ASDSF) for simultaneous searches approaching zero. Convergence to stationarity was indagated also using TRACER 1.7 (Rambaut et al. 2018), and the effective sample size (ESS) of all parameters showed values above 1000 (values much higher than the threshold of statistical significance) in both simultaneous searches, indicating that MCMC had converged. The first 1,000 trees were discarded as burn-in and posterior probabilities (PP) were calculated from post-burn-in trees.

To reconstruct a possible biogeographic scenario, a molecular clock analysis was performed, using BEAST 2.5.2 (Bouckaert et al. 2019) and substitution rates as previously obtained for each gene. In particular, we used rates equal to 1.6%, 1.1% and 0.7% per site/lineage/million years for COI, 12S and 16S rRNA, respectively. While a mean substitution rate of 0.06% was considered for 28S rRNA (Allegrucci et al. 2011)

In particular, we used a relaxed molecular clock, following an uncorrelated lognormal (UCLN) model of molecular evolutionary rate heterogeneity as implemented in BEAST. The UCLN model was used in BEAST to estimate the posterior density of divergence times. A Yule or "pure birth" prior process was used for the branching rate in the phylogeny. The time to the most recent common ancestor (MRCA) between each clade was estimated under the models highlighted in J MODEL TEST 2.1.7 (Darriba et al. 2012) for each partition within each gene. We did three independent runs with BEAST, each for 20 million steps. Convergence to stationarity and effective sample size (ESS) of model parameters were assessed using TRACER 1.7 (Rambaut et al. 2018), with the species tree reconstructed after a 10% burn-in using TREEANNOTATOR 2.5.2 (Bouckaert et al. 2019).

Taxonomy and morphological analysis

A total of 64 adult individuals of *Dolichopoda* from most of the same localities as those for the molecular study were used for the taxonomy and the morphological investigation. All the studied specimens were collected by hand on the wall of the caves or by pitfall traps, during several field trips conducted in the years between 2013 and 2019. Specimens were preserved in 70% ethanol and deposited in the collection of the Museum of Zoology of the University "La Sapienza" of Rome, Italy (MZUR). Permissions for collection of samples were obtained by the Ephorate of Palaeoanthropology and Speleology of the Ministry of Culture, Education, and Religious Affairs, Athens. The specimens were studied using a Leica MZ12.5 stereomicroscope. Pictures were taken using a Samsung NX mini camera. For the morphological analysis, nine external body characters were utilized: lobes of the tenth tergum; median and basal processes of the epiphallus; plica dorsalis, amount of spinulation of the hind tibia; the shape of the female subgenital plate and ovipositor; the

number of denticles on the inner valve of the ovipositor. Measures of the morphological parameters were taken using a digital caliper (0.1 mm).

Results

A total of 2970 base pairs corresponding to the entire COI gene, to 394 bp of 12S, to 547 bp of 16S, and 611 of 28S were successfully sequenced and aligned in 31 specimens of *Dolichopoda* populations from Peloponnese. Out of 2970 characters 636 sites are variable and 450 are parsimony informative.

Genetic distances and Species delimitation

The genealogical relationships among mtDNA haplotypes are shown in Figure 2, where *Dolichopoda* Peloponnesian haplotypes were organized in six main well-differentiated haplogroups. Five of them are well differentiated and correspond to the different species, *D. unicolor*, *D. kofinasi* n. sp., *D. propantii*, n.sp. *D. poseidonica* n.sp. and *D. epidavrii* n.sp. while *D. matsakisi* and *D. vandeli*, corresponding to the sixth haplogroup show a lower genetic differentiation.

To investigate this further we carried out a genetic distance analysis using the COI gene as a barcode and p distance between all studied *Dolichopoda* species (Allegrucci et al. 2005, 2009, 2011, 2014; Martinsen et al. 2009). Genetic distance values at intra- and interspecific levels are compared in Figure 3. In particular, Figure 3 shows the genetic distance values found in intra- and interspecific comparisons of all studied *Dolichopoda* species compared to the values found in comparisons between the populations from Peloponnese. Intraspecific values between all studied *Dolichopoda* species ranged from 0 to 0.025, with a mean of 0.012 (± 0.008SD), while interspecific values ranged from 0.016 to 0.126, with a mean of 0.073 (± 0.017SD). Intraspecific pairwise comparisons between the Peloponnesian populations ranged from 0.0018 to 0.0094 with a mean of 0.007 (± 0.003SD), while interspecific values ranged from 0.025 to 0.083 with a mean of 0.057 (± 0.013SD).

ABGD analysis proposed several partitions that varied according to the different a priori thresholds. Apart from the two extreme a priori threshold values (P = 0.001 and P = 0.035), for which an aberrant number of species hypotheses were obtained (almost every haplotype was considered as a different species hypothesis for P = 0.001 and, conversely, all the haplotypes were combined in a single species hypothesis for P = 0.035), all the tested a priori thresholds lead to the same splitting with all the groups corresponding to the nominal species. The Peloponnesian taxa were subdivided into seven different groups: one group consisted of populations from eastern Messinia and south-western Lakonia and belonging to *D. unicolor*, the second group included populations from Central Arkadia up to eastern Lakonia and belonging to *D. kofinasi*, n. sp. The third group comprised populations from south-western Messinia limited to the Kambos and Kitries bay area and belonging to *D. poseidonica*, n. sp., the fourth group included taxa from a single cave (Propanti cave) in north-central Messinia and belonging to *D. propantii*, n. sp. A fifth group included taxa from a single cave in western Argolida, belonging to *D. epidavrii* n. sp. while a sixth group identified taxa from

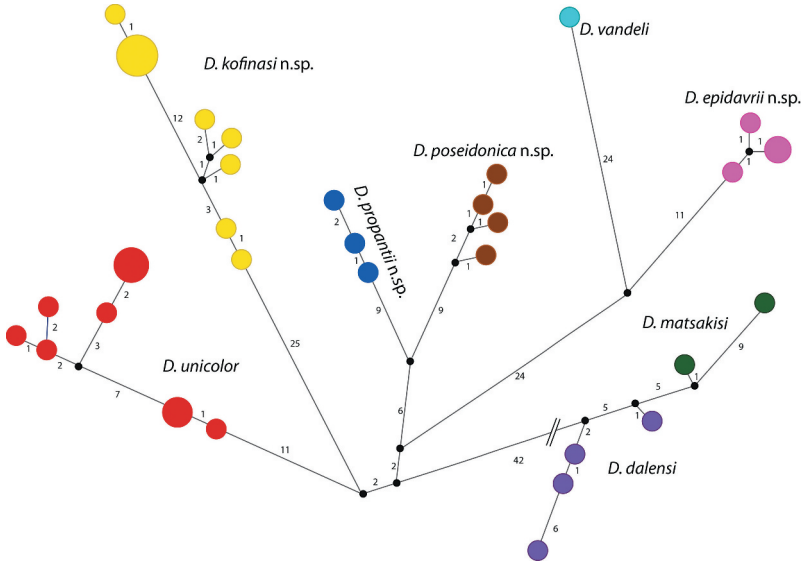

Figure 2. Median-joining network analysis in the populations of Peloponnesian *Dolichopoda* considered in this study: circled areas are proportional to the number of individuals sharing the same haplotype; numbers, along connections, indicate the number of nucleotide substitutions.

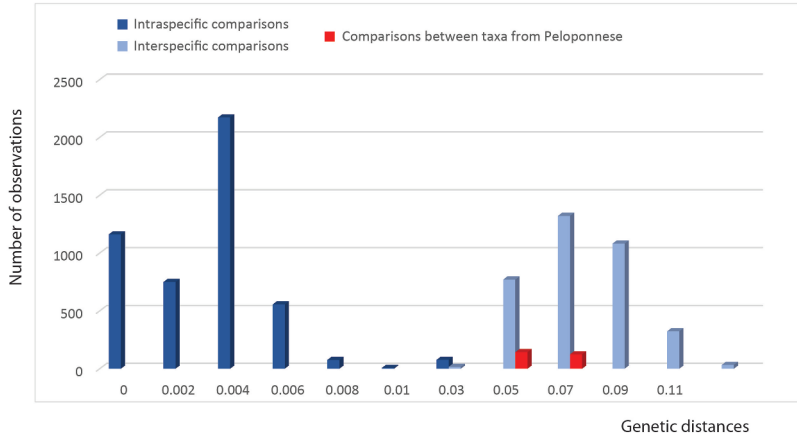

Figure 3. Distribution of genetic distance values (p-distances) at different taxonomic levels. Pairwise comparisons at the intra- and inter-specific level in all studied *Dolichopoda* species are reported. Comparisons between taxa from Peloponnesus are evidenced in red.

North Peloponnese, belonging to *D. matsakisi* and the seventh group included taxa belonging to *D. dalensi* and coming from the eastern Peloponnese (Figure 1).

Phylogenetic analysis

J MODEL TEST (Darriba et al. 2012) indicated GTR + I + G (Lanave et al. 1984; Gu et al. 1995) as the best model of DNA substitution for the COI and 16S genes, while the best models were GTR+G and GTR for 12S and 28S, respectively.

The phylogeny based on the combined data sets is highly supported (Figure 4) The studied Greek *Dolichopoda* species are separated into four main clusters with the Aegean species (*D. calidnae, D. giulianae, D. naxia*, and *D. paraskevi*) being sister to all the other clusters, as expected. Phylogenetic analysis also emphasized the presence of new species. In particular, *D. epidavrii* n. sp. is sister to *D. vandeli* while *D. kofinasi* n. sp., *D. propantii* n. sp., and *D. poseidonica* n. sp., are sisters to *D. unicolor*.

Although the deep nodes of the phylogeny are not resolved, the tree topology of each studied gene is rather similar to the topology obtained by combining all data sets (Figure 4). Nuclear ribosomal gene (28S rRNA) produced a topology distinguishing only some clades, and most of the taxa relationships were unresolved. This is due to the low level of polymorphism revealed in this gene. However, its presence in the combined dataset increases the resolution of the deep nodes in phylogeny.The chronogram in Figure 5 shows that the four new species from Peloponnese have been separated during the Plio-Pleistocene era.

Systematics and morphological analysis

In this section, we report the description of the new four species identified for the Peloponnese.

Family **RHAPHIDOPHORIDAE** Walker, 1871Genus ***Dolichopoda*** Bolivar,1880

Dolichopoda epidavrii

Di Russo & Rampini n. sp.

(Figure 6)

Type material. –**Holotype** ♂: Greece, Peloponnese, Argolida, Epidavros, Spilaio Monis Kalamiou, 697 m, 08.12.2019, Di Russo & Kofinas leg. (MZUR)

Other material examined. –1♂, 3♀, 2 nymphs, same locality, date, and collector as the holotype;

Type locality. The Spilaio (cave) Monis Kalamiou is located on a small mountain called Psili Rachi. The entrance is facing south and is located close to the monastery (Moni-μονή) of Kalamiou.The presence of cave crickets in the cave was communicated to us by Giannis Farsarakis.

Etymology. The name of the new taxon refers to the archaeological site of Epidavros, not far from the cave locality.

Diagnosis. This taxon presents a unique characteristic for the cave crickets of Peloponnese: the median process of the epiphallus is bifurcate at the apex, contrary to the pointed median process present in all the other species of Peloponnese. The male shows affinities to male *D. vandeli*, differing mainly in the trapezoidal ninth tergum, the more elongated lobes of the tenth

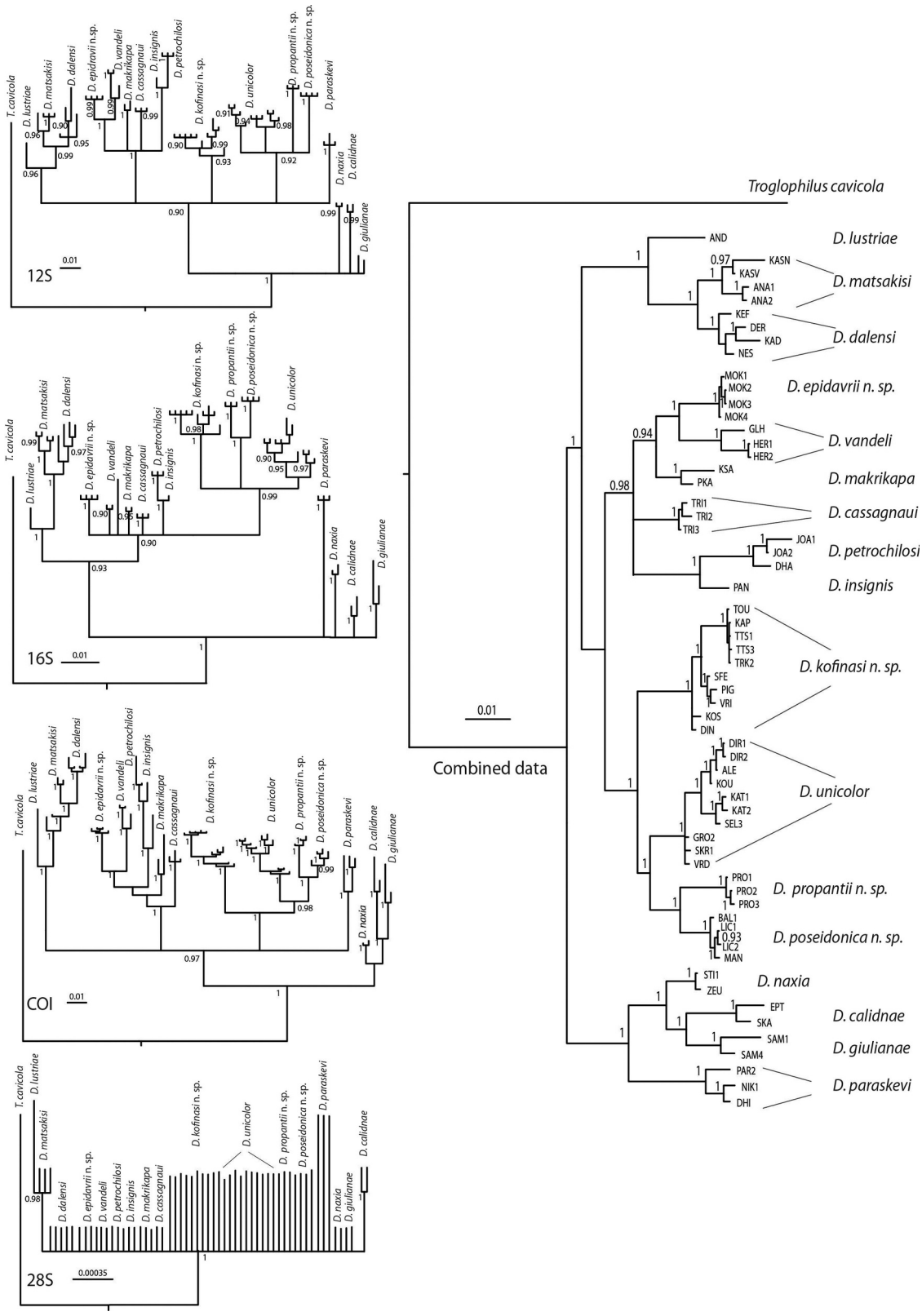

Figure 4. Bayesian Inference analysis for each gene separately and for the combined data set carried out on the Peloponnesian species of *Dolichopoda*. The geographically closest species were also considered. Values above branches indicate posterior probabilities derived from BI analysis. Scale bars: 0.00035–0.01 substitutions per site. Only posterior probability (PP) values ≥ 0.90 are shown.

Figure 5. Divergence times among the analyzed *Dolichopoda* species inferred by Bayesian analysis using relaxed molecular clocks. Bars at the nodes represent the 95% highest posterior density (HPD) credibility interval.

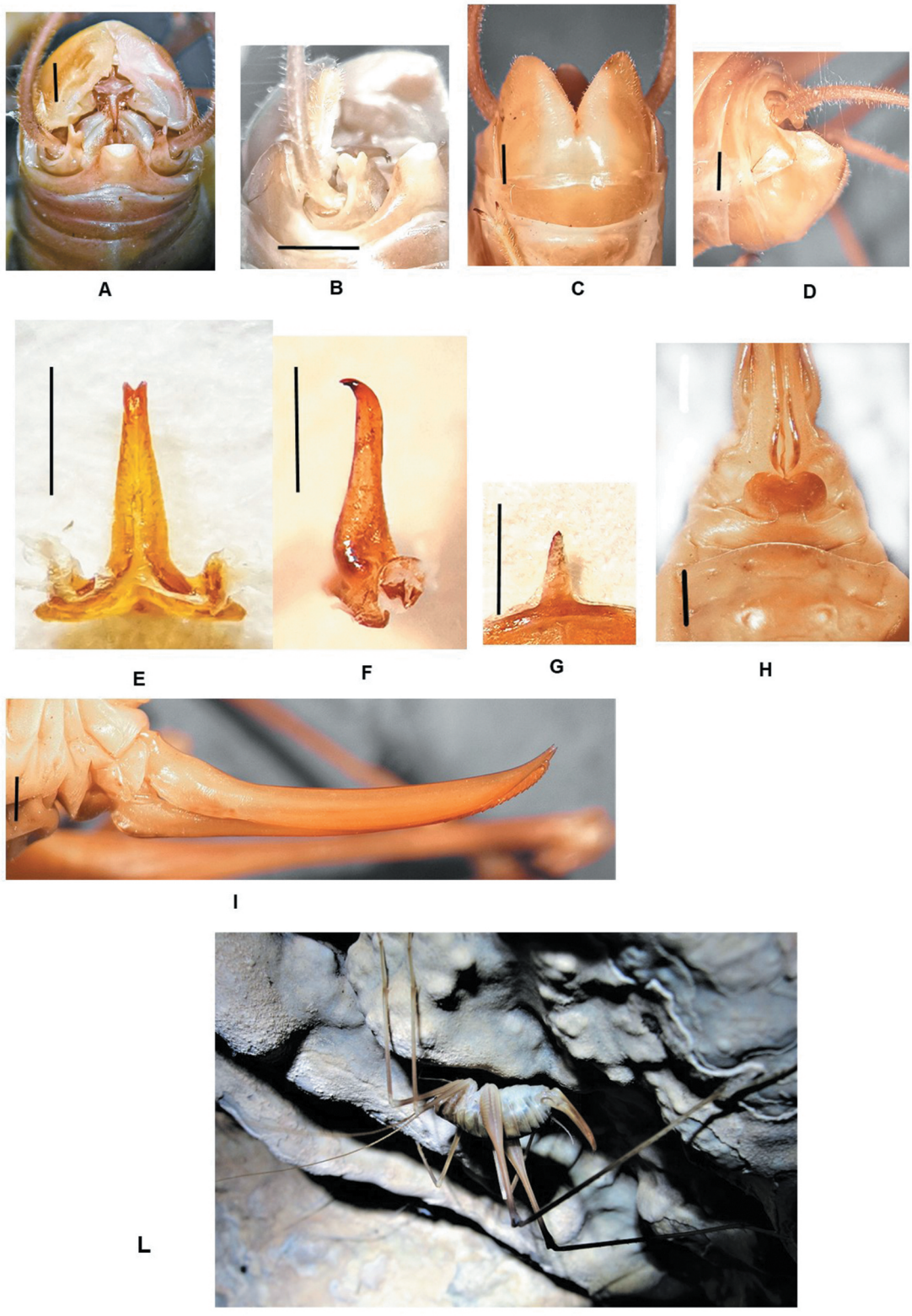

Figure 6. *Dolichopoda epidavrii* n. sp: (a) male tenth tergum; (b) tenth tergum lateral view; (c) male subgenital plate (ventral view); (d) male subgenital plate (lateral view); (e) median process of epiphallus (dorsal view); (f) median process of epiphallus (lateral view); (g) plica dorsalis; (h) female subgenital plate; (i) ovipositor (lateral view); (L) Female habitus *D.epidavrii*. Scale bars: 1 mm.

tergum, and the finger-like plica dorsalis. The female has a subgenital plate similar to those of *D. petrochilosi*, being hearth-shaped.

Thus it belongs to the species complex widespread in the eastern central Greece (Attica and Voiotia) and W Aegean Islands (Evvoia, Skyros) (ex - *Petrochilosina* subgenus).

Description

Male

Relatively big in size; body-color not uniform, thorax and abdomen brownish dorsally while paler ventrally. Legs long, uniformly yellowish with the posterior edge darker. Femora unarmed. Fore tibia armed with 4–5 spines on sides of the lower edge, 3/3 spines on the upper edge. Mid tibia with 5/7 short spines on both sides of the upper edge, 3/4 spines on the lower edge. The hind tibia is longer, with 16/18 spines of varying length on both sides of the upper edge and 2 homogeneous spines on the lower external edge. ninth abdominal tergite prominent trapezoidal with the posterior edge slightly rounded (Figure 6(a)); tenth tergum with two narrow and elongated lateral lobes, rectangular in shape, with the terminal edge strongly sinuous (Figure 6(b)). The lobes are separated by a large median depression. Sub genital plate globular, deeply incised in the middle, lateral lobes are triangular with two very short styli (Figure 6(c, d)). Median process of the epiphallus almost slender, triangular, bifurcate at the apex, in the lateral view slightly curved. Narrow basal process with the anterior lobes more developed than the posterior ones (Figure 6(e, f)). Plica dorsalis sclerotized fingerlike in shape (Figure 6(g)).

Length (mm): body16.1; pronotum 3.9; fore femur16.0; mid femur 15.5; hind femur 24.0; fore tibia18.0; mid tibia 18.0; hind tibia 32.5; hind tarsum11.0; 1st article of hind tarsus 6.0.

Female

Relatively bigger than male (mm 16.2–18.8). VII, VIII, and IX sternites are well developed showing posterior edge prominent. Subgenital plate, sclerotized, heart-shaped (Figure 6(h)). Ovipositor uniformly curved (mm 12–14) with apex strongly curved upward. The inferior valves with the base strongly squared have 15–16 denticles (Figure 6(i))

Dolichopoda kofinasi Di Russo & Rampini n. sp.

(Figure 7)

Type material.– Holotype ♂: Greece, Peloponnese, Lakonia, Mt Koulochera, Sfendami cave, 19.09.2015, Kofinas leg., 1025 m (MZUR).

Other material examined.– 8♂. 5♀, same locality, date, and collector as the holotype;

Lakonia: Monemvasia, Vri cave, 2♂, 2♀, 05.11.2014, Kofinas leg., 37 m; Demonia, cave Aghion Anargiron, 3♂, 2♀, 24.01.2015, Kofinas leg., 177 m; Vellies, cave Pigaza, 2♀, 1 nymph, 24.01.2015, Kofinas leg., 115 m; Arkadia, Kosmas, cave Kosma, 21.03.2013, F. Ballarin leg., 3♂, 2♀, 04.12.2016, Di Russo leg., 1144 m; Leonidio, cave Dionysou 04.12.2016, Di Russo leg., 542 m; Kapsia, cave Kapsia, 5♀, 5 nymphs 21.02.2016, Di Russo leg.; 2♀, 27.08.2019, Rousiotis leg., 630 m; Kapsia, Katavothra Tousi, 3♀, 4 nymphs 08.08.2016, Kofinas leg., 653 m; Kollines, Spilaio Tyrias, 7 nymphs, 27.07.2019, Kofinas leg., 592 m; Skortsinos, cave Troupitses, 11 nymphs, 27.07.2019, Kofinas leg., 474 m.

Type locality. Sfendami cave is a pit hole located close to the peak of Mt Koulochera. The entrance is facing west, towards the valley of Molai and Mt Taygetos. Next to the entrance is standing one of the few maple trees (*Acer* sp., sfendami) of the area. The pit hole was explored for the first time two decades ago by Giannis Kofinas-Kallergis, who observed the cave crickets.

Etymology. The new taxon is dedicated to our friend Giannis Kofinas-Kallergis, founder and active member of the "Poseidon" Speleological Club, who explored and collected for the first time *Dolichopoda* specimens from the type locality.

Diagnosis. The new taxon is similar to *D. unicolor*, differing mainly on leg spinulation, the sub-rectangular apex of the lobes of the tenth tergum and the wide triangular median process of the epiphallus. The inferior valves of the ovipositor have 12–14 denticles.

Description

Male

Size relatively short with legs slender and elongated; color brownish darker dorsally.

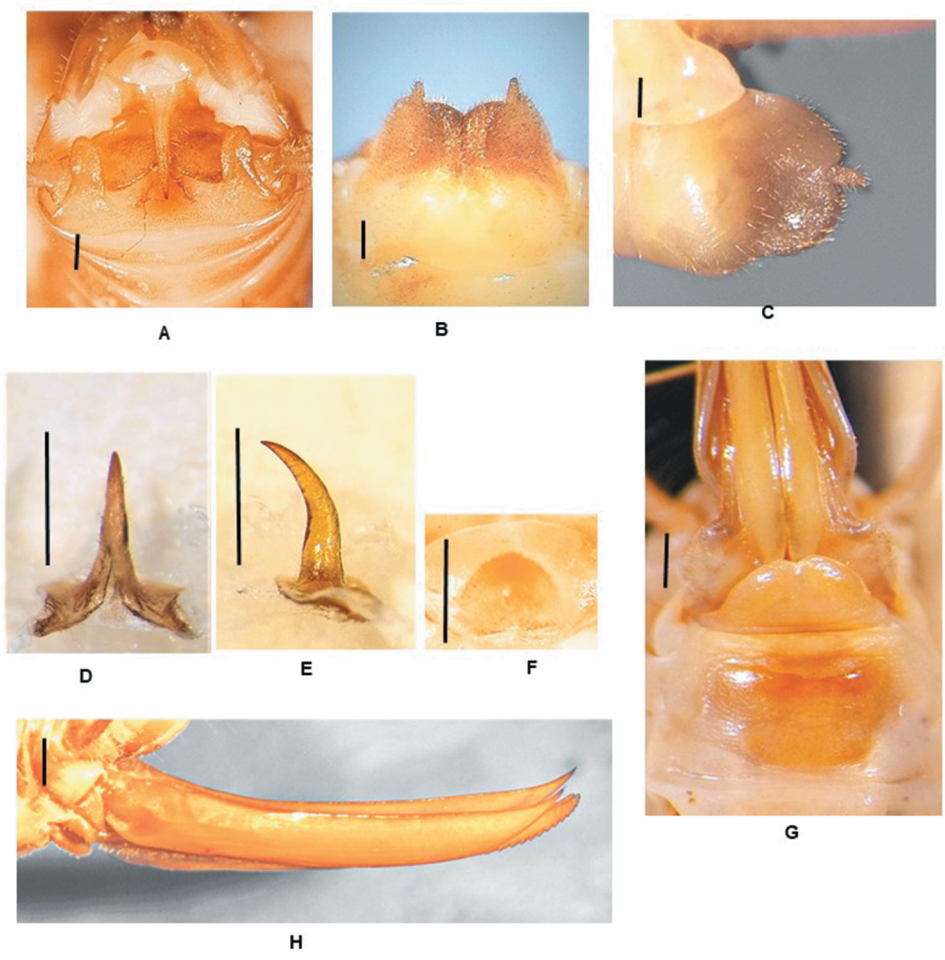

Figure 7. *Dolichopoda kofinasi* n. sp.: (a) male tenth tergum; (b) male subgenital plate (ventral view); (c) male subgenital plate (lateral view); (d) median process of epiphallus (dorsal view); (e) median process of epiphallus (lateral view); (f) plica dorsalis; (g) female subgenital plate; (h) ovipositor (lateral view). Scale bars: 1 mm.

Legs long, slender, uniformly yellowish. Femora unarmed. Fore tibia armed with 4–5 spines on sides of the lower edge, 2/0 spines on the upper edge. Mid tibia with 3/4 short spines on both sides of the upper edge, 4/5 spines on the lower edge. Hind tibia longer, with 14/16 (3/3) spines of varying length on both sides of the upper edge and 2 homogeneous spines on the lower external edge.

X tergum with the lateral lobes subrectangular and rounded at the apex; the two lobes are separated by a large concave strongly haired margin (Figure 7(a)). Sub genital plate globular, deeply incised in the middle. The wide lateral lobes are trapezoidal and haired, with the posterior edges almost rounded, showing two large cylindrical styli (Figure 7(b, c)).

Median process of the epiphallus triangular in shape, uniformly curved enlarged at the base, and acute at the apex (Figure 7(d, e)). The basal process is wide with the posterior edge curved, while the anterior one is bilobate and deeply incised. Plica dorsalis short, membranous, domed in shape (Figure 7(f)).

Length (mm): body 12.3; pronotum 3.4; fore femur 13.9; fore tibia 16.0; mind femur 14.0; mind tibia 16.5; hind femur 20.5; hind tibia 27.3; hind tarsus 9.00; 1st article of hind tarsus 5.0.

Female

The length of the body ranges between 11–15 mm (ovipositor excluded) and the general form is similar to the male. VIII sternite transverse and sclerificate. Sub genital plate triangular, sclerified, slightly incised in the middle (Figure 7(g)). Ovipositor relatively elongated (10–11 mm), curved distally; the inferior valves, slightly curved at the base have 12–14 denticles (Fig. H).

Dolichopoda propantii
Di Russo & Rampini n. sp.
(Figure 8)

Type material.– Holotype ♂: Greece, Peloponnese, Ilia, Andritsena, Propanti cave, 966 m., 18.08.2019, C. Di Russo leg. (MZUR)

Other material examined. –2♂, 2♀, 18.08.2019, same locality, date, and collector as the holotype; 1♀, 5 nymphs, 20.08.2017, Di Russo leg.; 2♂, 1♀, 3 nymphs, 21.05.2018, Di Russo leg.

Type locality. Propanti cave is located NW of the town of Andritsena. The cave was investigated for the first time by the pioneer speleologist Anna Petrochilou in 1966 (Petrochilou 1969). She was the first to report on the presence of cave crickets in the cave, as "D. petrochilosi".

Etymology.– The new species name refers to the name of the type locality.

Diagnosis.– Closely resembling *D. unicolor*, differing mainly in the triangular, relatively short and almost entirely flattened epiphallus, the plica dorsalis having a median finger-like protuberance and the different number of the leg spinulation.

Description

Male

Size relatively small. Body-color is uniformly brownish. Legs very long, slender, and brownish with the femora unarmed. Fore tibia armed with 3–5 spines on sides of the inferior edge, 2/2 spines on the upper edge, a pair of spurs of equal length on the apex. Mid tibia with 5/7 short spines on both sides of the upper edge, 4/4 spines on the lower edge, and two

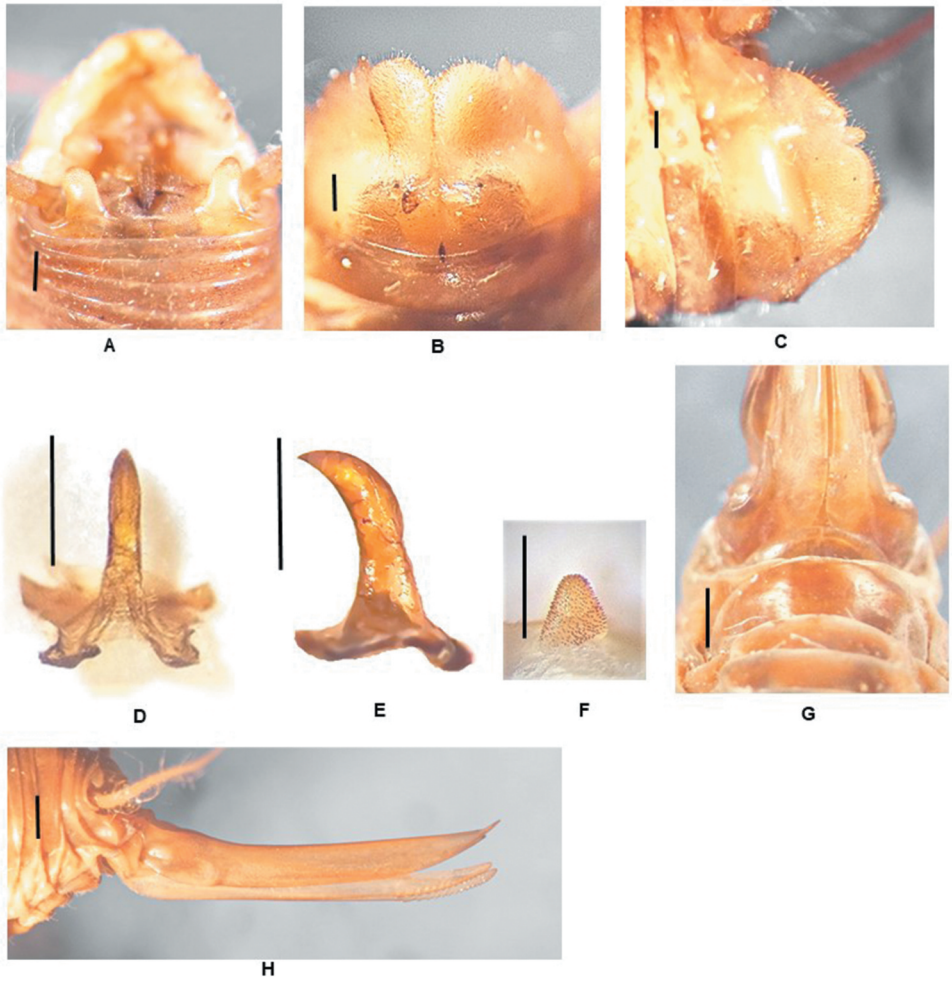

Figure 8. *Dolichopoda propantii* n. sp.: (a) male tenth tergum; (b) male subgenital plate (ventral view); (c) male subgenital plate (lateral view); (d) median process of epiphallus (dorsal view); (e) median process of epiphallus (lateral view); (f) plica dorsalis; (g) female subgenital plate; (h) ovipositor (lateral view). Scale bars: 1 mm.

apical spurs similar to those of the fore tibia. Hind tibia longer, with 21/24 spines of varying length on both sides of the upper edge and 1/2 homogeneous spines on the lower edge.

Posterior edge of the tenth tergum with two prominent rounded lobes (Figure 8(a)). Epiproctum triangular Subgenital plate globular, deeply incised in the middle, with two enlarged lateral lobes, the styli are short (Figure 8(b, c)). Epiphallus sclerotized with relatively short median process almost entirely flattened and triangular in shape; from the side, the median process is thickened and strongly curved at apex; the posterior basal processes are quite well developed while the anterior ones are reduced Figure 8(d, e)). Plica dorsalis little sclerotized at the base, with a cylindrical fingerlike protuberance, covered with bristles in the middle of the apical part (Figure 8(f)).

Length (mm): body 15.00; pronotum 3.0; fore femur 15,00; fore tibia 18; mid femur 14.5; mid tibia 19; hind femur 23; hind tibia 29; hind tarsus 11.00; 1st article of hind tarsus 6.0.

Female

The length of the body ranges between 13,5 and 14.5 .mm (ovipositor excluded) and the general form is similar to the male. Sub genital plate transverse with a rounded posterior edge slightly incised in the middle (Figure 8(g)). Ovipositor almost straight, with an average length of 10 mm, showing a pointed apex hooked; the inferior valves are curved at the base and have 15–16 denticles (Figure 8(h)).

Dolichopoda poseidonica
Di Russo & Rampini n. sp.
(Figure 9)

Type material.– Holotype ♂: Greece, Peloponnese, Messinia, western Mani, Kardamili: cave Katafygi Mantagari, 15.02.2015, G. Kofinas leg. (MZUR)

Other material examined.– 2♂, 3♀ same locality, date and collector as the holotype; Greece, Peloponnese, Messinia, western Mani, Kardamili: Spilaio (cave) Balli, 3♂, 3♀, 10 nymphs, 27.01.2018, G. Kofinas leg.; pit-hole Lykourgou, 3♂, 2♀, 4 nymphs, 27.01.2018, G. Kofinas leg.

Type locality.– cave Katafygi Mantagari is located NW of Kardamili. It is a horizontal cave, was investigated for the first time by members of "Poseidon" Speleological Club.

Etymology.– The name of the new taxon is dedicated to the "Poseidon" Speleological Club (PSC), Kalamata, members of which collected for the first time specimens from the type locality and more than that greatly improved our research on the cave crickets of Peloponnese. The specimens were collected during the 13th Christmas pie cutting event and a seminar held by PSC at the type locality.

Diagnosis.– Closely resembling *D. unicolor*, differing mainly in the tenth tergum which has two prominent elongations, laterally folded, the different number of the leg spinulation and the median process of the epiphallus being less elongated, more thickened and slightly curved.

Description

Male

Size relatively small. Body-color is uniformly yellow-brown. Legs very long, slender, and brownish with the femora unarmed. Fore tibia armed with 3–4 spines on sides of the inferior edge, 2/4 spines on the upper edge, a pair of spurs of equal length on the apex. Mid tibia with 5/5 short spines on both sides of the upper edge, 4/5 spines on the lower edge, and two apical spurs similar to those of the fore tibia. Hind tibia longer, with 17/19 spines of varying length on both sides of the upper edge and 0/2 homogeneous spines on the lower edge. Posterior edge of the tenth tergum with two prominent elongations, laterally folded (Figure 9(a)).

Subgenital plate globular at the bottom, with two symmetrical lateral lobes and rounded posterior edges, the styli are cylindrical and short (Figure 9(b, c)). Epiphallus sclerotized with a relatively short median process, triangular in shape; from the side, the median process is thickened at the base and slightly curved; the posterior basal processes are rather well developed while the anterior ones are reduced (Figure 9(d, e)). Plica dorsalis little sclerotized, the basal lobes with 2–3 strong bristles and a cylindrical domelike protuberance covered with bristles occurs in the middle of the apical part. (Figure 9(f)).

Length (mm): body 13.5; pronotum 3.5; fore femora 13.8; middle femora 13.0; hind femora 21.4; fore tibia 14.4; middle tibia 14.6; hind tibia 24.8; hind tarsus 9.7; 1st article of hind tarsus 5.7.

Female

The length of the body ranges between 12–13 mm (ovipositor excluded) and the general form is similar to the male. VII–IX sternites carinate, with a

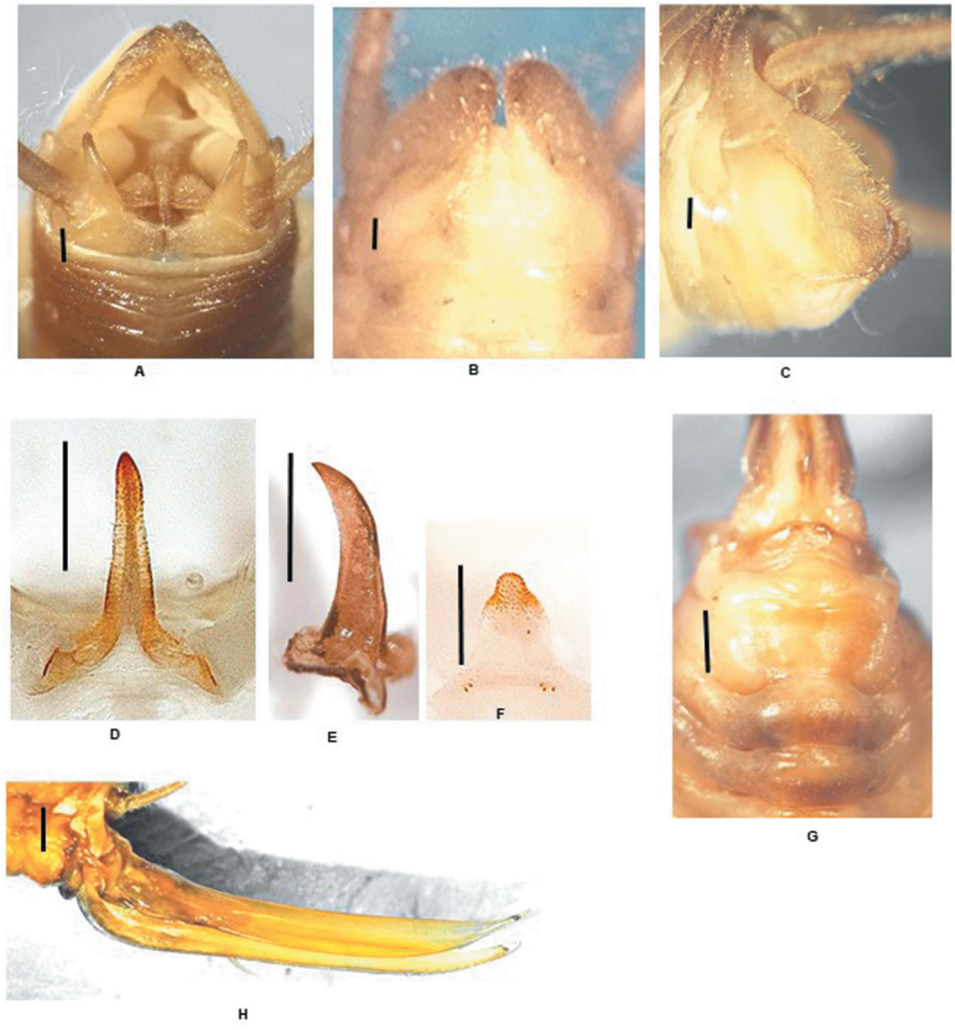

Figure 9. *Dolichopoda poseidonica* n. sp.: (a) male tenth tergum; (b) male subgenital plate (ventral view); (c) male subgenital plate (lateral view); (d) median process of epiphallus (dorsal view); (e) median process of epiphallus (lateral view); (f) plica dorsalis; (g) female subgenital plate; (h) ovipositor (lateral view). Scale bars: 1 mm.

protuberance in the middle. Subgenital plate triangular (Figure 9(g)) with the rounded distal part more thickened.

Ovipositor almost straight with an average length of 12.5 mm, and a pointed apex curved upwards. Inferior valves slightly curved at the base with 15–16 denticles (Figure 9(h)).

Discussion

Barcode and morphological analyses confirm the existence of four new species in the Peloponnese. From the genetic point of view, the median-joining network analysis (Figure 2), carried out on all Peloponnesian species, revealed six distinct haplogroups with five of them corresponding to the nominal species *D. unicolor*, *D. kofinasi* n. sp., *D. propantii* n. sp., *D. poseidonica* n. sp., and *D. epidavrii* n. sp. The sixth haplogroup corresponds to *D. matsakisi* and *D. dalensi*, that resulted closely related from a genetic point of view, but morphologically well differentiated (Di Russo et al. 2014), suggesting that they separated from each other recently and complete lineage sorting has not yet been achieved. All the haplogroups are geographically distributed with a resulting scenario rather heterogeneous. Pairwise comparisons between the Peloponnesian species range between 2.5% and 8.3%, falling in the range of interspecific comparisons observed in all the other studied *Dolichopoda* species (Figure 3, Allegrucci et al., 2014) and consistent with divergence values found in other insect species (see for

example, Hernández-Triana et al. 2019; Lencioni et al. 2021). ABGD analysis confirms these results, grouping the Peloponnesian taxa into seven different groups. Morphological analysis is in agreement with the molecular one, indicating that, at least two or three combinations of characters, as the shape of the tenth tergum, epiphallus, and plica dorsalis, discriminate these new four taxa in Peloponnese. In particular, the two western Peloponnesian species *D. poseidonica* n. sp. and *D. propantii* n. sp. are well-differentiated from *D. unicolor*, showing the tenth tergum's lobes elongated and folded and the epiphallus short and almost flattened, respectively. *D. kofinasi* n. sp. can be distinguished from *D. unicolor*, mainly for the wide triangular median process of epiphallus and the domelike plica dorsalis. Finally, *D. epidavrii* n. sp., confined in the Northeast Peloponnese (Argolis) is strongly related to the "Petrochilosina" group of endemic species to Attica and Voiotia, Evvoia and Skyros island. In particular, it appears closely related to *D. vandeli* from Voiotia for the shape of the ninth tergum, and to *D. petrochilosii* from Attica, for the heart-shaped subgenital plate in the female. Moreover, *D. epidavrii* shares the synapomorphy, represented by the bifurcate apex of the epiphallus, with *D. christosnifoni* Di Russo & Rampini, 2018 from the western Cyclades, *D. lycia* (Galvagni, 2006) and *D. fortuita* Gorochov & Ünal, 2015 from south Turkey. On the contrary in all the other Peloponnesian species the pointed apex of the epiphallus may represent a plesiomorphic character, being shared by the majority of the *Dolichopoda* known both in the western and in the eastern part of the geographic distribution of the genus. Only in the Transcaucasian species, the epiphallus shows another different shape being truncated at the apex.

The Peloponnese is known for its remarkable and endemic fauna (Sfenthourakis & Legakis 2001) both within invertebrates and vertebrates, and new species are continuously discovered. For example, endemic new species of Coleoptera Cerambycidae (Vartanis & Borek 2019), Diptera Hybotidae (Grootaert & Alexiou 2020) have been recently described. The phylogeny illustrated in Figure 4 based on the combined data sets support the major phylogenetic relationships previously demonstrated (Allegrucci et al. 2009, 2011) highlighting that the Peloponnesian populations represent differentiated clades. In particular, *D. epidavrii* n. sp. links to *D. vandeli* as expected also from geographical and morphological points of view. *D. kofinasi* n. sp., *D. propantii* n. sp., and *D. poseidonica* n. sp. link to *D. unicolor*, being *D. kofinasi* n. sp. the most differentiated one. Interestingly, *D. kofinasi* n. sp. is one of the Greek species with the largest spatial range (Figure 1). Greece is characterized by a dry and warm climate with the vegetation represented mainly by Mediterranean bush present with low shrubs. Forests can be found only at elevated altitudes. Mediterranean bush with low shrubs represents an environment fundamentally inhospitable to *Dolichopoda*; most species of this genus are strictly dependent upon caves, although individuals are often observed outside in moist or mesic woods, especially in the northern part of the genus's range. Here, the favorable environmental conditions permit the gene flow between the different populations (Sbordoni et al. 1985; Allegrucci et al. 1997) making the range of each species rather large. The different bioclimatic and vegetational conditions found in the south-eastern part of the genus's range make that the highest *Dolichopoda* species diversity is found in the Hellenic area, where we might expect that gene flow among cave populations could be more limited. Indeed, the spatial range of each species is restricted and often limited to only one cave (Boudou-Saltet 1980). *Dolichopoda kofinasi* n. sp. extends its range from the Arcadian plateau in the north, up to the northern foothills of Mt Taygetos and, through the Mt Parnon, it arrives at the extremities of the Maleas Peninsula. The inhabited area is very diverse, being wet and cold in the north, and very dry and hot in the south. This geographic diversity is also evident from the phylogeny in Figure 4, where a phylogeographic pattern can be observed with the northern populations well differentiated from the southern ones.

D. kofinasi n. sp. is sister to *D. propantii* n. sp., *D. poseidonica* n. sp., and *D. unicolor* (Figure 4), being the most differentiated one. Divergence times suggested that it originated 3.2 Ma (HPD 2.33–4.12 Ma; Figure 5) in the Pliocene. In that period and starting from the Upper Miocene, Mount Parnon has undergone a series of settlements due to the extensional faults causing its displacement and deformation (Skourtsos & Lekkas 2011). These settlements could have determined the formation of geographic barriers between the ancestor of *D. kofinasi* n. sp. and the other Peloponnesian species, causing its isolation and subsequent speciation.

D. propantii n. sp. and *D. poseidonica* n. sp. separated from each other about 1.7 Ma (HPD 1.03–

2.36 Ma; Figure 5), in the Pleistocene. Their separation from the sister taxon *D. unicolor* happened 2.7 Ma (HPD 1.91–3.5 Ma; Figure 5), during the Pliocene. *D. propantii* n. sp. seems to be isolated on a small mountain, connected by a low ridge with the southern populations of *D. unicolor* and isolated from the eastern populations of *D. kofinasi* through the basin of Alfios river, the largest Peloponnesian river, originating from the northern foothills of Mt Taygetos. The presence of large paleo-lakes in the Megalopolis basin, since Late Pliocene (2.8 Mya), placed between the northern slope of Mt Taygetos and Arcadia plateau (Dermitzakis & Papanikolaou 1981; Fountoulis et al. 2014), may have affected dispersion, and allowed speciation on this small mountain. *D. poseidonica* n. sp. inhabits caves situated in a small area of land inside the Messinian Gulf. Only a shallow narrow basin isolates this area from the overhanging Mt Taygetos and the populations of *D. unicolor*. In particular, the Kambos and Kitries bay area is characterized by a depression that separates it from the rest of Mt Taygetos by at least 1.8–2 Mya (Fountoulis et al. 2014). This depression has been probably filled with shallow brackish water for a long time, constituting an efficient barrier to the gene flow with the close populations of *D. unicolor*.

Our data suggest that the present-day distribution of Peloponnesian cave crickets seems to be in alignment with the palaeogeographic history of the area. Several studies demonstrated that the genetic variation and differentiation of different species of both invertebrates and vertebrates has been influenced by the complex palaeogeographic and climate history of the Aegean region (Poulakakis et al. 2015 and references therein). In the present case, dating estimates (Figure 5) confirm the previous hypothesis that the dispersion toward Peloponnese occurred two times, independently. In particular, colonization of North Peloponnese hosting *D. matsakisi* and *D. dalensi*, proceeded from North-western Greece, while the central southern Peloponnese, hosting *D. unicolor, D. kofinasi* n. sp., *D. propantii* n. sp. and *D. poseidonica* n. sp. was colonized starting from eastern Greece (Allegrucci et al. 2009, 2011). This is also confirmed by the discovery of *D. epidavrii* n. sp. in the North Eastern Peloponnese (Argolis). This species belongs to a species group distributed in the Central – Eastern mainland Greece and Western Aegean. The group was once considered as a separate subgenus, *Petrochilosina* Boudou-Saltet 1980, but the taxonomic value and the use of the subgenus category in the genus *Dolichopoda* has been abandoned (Boudou-Saltet 1983; Alexiou et al. 2015). The finding of a member of this group in Argolis suggests a past land connection. In fact, during most of the Pleistocene, Peloponnese became reconnected with the north mainland, most probably causing the invasion and establishment of "ex-*Petrochilosina*" populations in the area of Argolis. It is clear that the shallow west Saronic Gulf, connecting Argolis Peninsula with east-central Greece, was land during most of the Pleistocene (Dermitzakis 1989), allowing colonization of new areas through dispersal. This hypothesis seems to agree with the separation of *D. epidavrii* n. sp. from the rest of the group, being the divergence time estimate equal to 1.8 Ma (HPD1.11, 2.5; Figure 5).

In conclusion, present results, in alignment with previous studies (Allegrucci et al. 2009, 2011), suggest that the climatic and tectonic events that occurred in the Plio-Pleistocene are important factors driving the isolation and consequently speciation in the *Dolichopoda* cave crickets. The silvicolous ancestors of *Dolichopoda* might have used caves as refugia during the unfavorable climatic conditions, beginning their adaptation to subterranean habitat. Therefore, the current distribution of *Dolichopoda* can be explained by a combination of both vicariance and dispersal events, with many processes occurring in ancestral epigean populations before the invasion of the subterranean environment.

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