



Evolution around the Red Sea: Systematics and biogeography of the agamid genus *Pseudotrapelus* (Squamata: Agamidae) from North Africa and Arabia [☆]



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ABSTRACT

Since the Oligocene, regions adjacent to the Red Sea have experienced major environmental changes, from tectonic movements and continuous geological activity to shifting climatic conditions. The effect of these events on the distribution and diversity of the regional biota is still poorly understood. Agamid members of the genus *Pseudotrapelus* are diurnal, arid-adapted lizards distributed around the Red Sea from north-eastern Africa, across the mountains and rocky plateaus of the Sinai and Arabian Peninsulas northwards to Syria. Despite recent taxonomic work and the interest in the group as a model for studying biogeographic and diversity patterns of the arid areas of North Africa and Arabia, its taxonomy is poorly understood and a comprehensive phylogeny is still lacking. In this study, we analyzed 92 *Pseudotrapelus* specimens from across the entire distribution range of the genus. We included all known species and subspecies, and sequenced them for mitochondrial (16S, ND4 and tRNAs) and nuclear (MC1R, *c-mos*) markers. This enabled us to obtain the first time-calibrated molecular phylogeny of the genus, using gene trees, species trees and coalescent-based methods for species delimitation. Our results revealed *Pseudotrapelus* as a monophyletic genus comprised of two major clades and six independently evolving lineages. These lineages correspond to the five currently recognized species and a sixth lineage relating to the synonymized *P. neumanni*. The subspecific validity of *P. sinaitus wernerii* needs further assessment as it does not form a distinct cluster relative to *P. s. sinaitus*. The onset of *Pseudotrapelus* diversification is estimated to have occurred in Arabia during the late Miocene. Radiation has likely resulted from vicariance and dispersal events due to the continued geological instability, sea level fluctuations and climatic changes within the region.

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1. Introduction

The unique biota of North Africa and Arabia inhabits a diverse array of habitats ranging from rocky plains and sandy deserts to high mountain ranges, high plateaus and low valleys, and has a complex and dynamic evolutionary history. The distinctiveness and diversity of the biota were greatly influenced by the massive

tectonic movements and climatic changes which took place during the mid-Cenozoic (Ruddiman et al., 1989; Le Houérou, 1992, 1997; Schandelmeier et al., 1997; Rögl, 1999; Bojar et al., 2002; Bosworth et al., 2005). One of the most influential geological episodes in the Saharo-Arabian region began in the Oligocene with the counter-clockwise movement of the Arabian plate. This event created the Red Sea, the Gulfs of Aden, Suez and Aqaba, and caused the uplift of the peripheral mountain ridges in western Arabia and north-eastern Africa (Girdler and Southren, 1987; Bohannon et al., 1989; Rögl, 1999; Bojar et al., 2002; Bosworth et al., 2005). The geological instability and volcanic activity around the Red Sea persist to this day (Powers et al., 1966; Bosworth et al., 2005; Edgell,

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2006). Global climate change during the Miocene and the subsequent aridification process were additional factors that greatly influenced the fauna of the Saharo–Arabian region. In particular, the expansion and contraction of desert areas in North Africa and Arabia (Hsü et al., 1977; Ruddiman et al., 1989; Flower and Kennett, 1994; Le Houérou, 1992, 1997; Zachos et al., 2001; Griffin, 2002), had a major effect on the distribution and diversification of the local fauna (e.g., Douady et al., 2003; Fonseca et al., 2009; Zhou et al., 2012).

The influence of these dramatic geological and climatic events on the biogeography and diversification of the African and Arabian herpetofauna is not well understood. Extensive studies have only recently been carried out, providing important information regarding the origin, diversity, cladogenesis and biogeography of the regional herpetofauna assemblage (e.g., Pook et al., 2009; Carranza and Arnold, 2012; Metallinou et al., 2012, 2015; Portik and Papenfuss, 2012, 2015; Šmíd et al., 2013; Kapli et al., 2015).

The agamid lizards of the genus *Pseudotrapelus* Fitzinger, 1843 are medium sized, saxicolous and heliophilous, typically active during the hottest time of day (Baha El Din, 2006). *Pseudotrapelus* range throughout the mountainous areas surrounding the Red Sea, from western Eritrea in Africa across the southern Sinai Peninsula and southern Israel to the southern and eastern coasts of the Arabian Peninsula, and northwards to southern Syria (Sindaco and Jeremčenko, 2008; Fig. 1). These lizards occupy a diverse array of arid rocky habitats in hilly and mountainous regions, including well vegetated wadis and slopes, barren rocky hillsides, and boulder-strewn plains (Arnold, 1980; Disi et al., 2001; Baha El Din, 2006; Gardner, 2013).

Systematic studies of *Pseudotrapelus* have long been hindered by the morphological similarity among African and Arabian populations. For many years *Pseudotrapelus* was thought to be comprised of a single species, *P. sinaitus*, albeit suspected to be a species complex (e.g., Baha El Din, 2006). Although identifying different morphological forms, authors conservatively classified the diversity among populations as intraspecific variation of *P. sinaitus* (Anderson, 1896, 1898, 1901; Arnold, 1980; Fritz and Schütte, 1988; Schätti and Gasperetti, 1994; Baha El Din, 2006). A recent flurry of studies on *Pseudotrapelus* has left the systematics and biogeography of the genus obscured (i.e., Melnikov et al., 2012, 2013a, 2013b, 2014, 2015; Melnikov and Pierson, 2012; Melnikov and Melnikova, 2013; Melnikova et al., 2015). Descriptions of four new species were mainly based on single specimens, thus creating much biogeographic uncertainty and taxonomic confusion. Current classifications are predominantly based on external morphology, with no comprehensive comparisons among species. Phylogenetic studies on the genus were all based on extremely low sample sizes, and were mostly based on the mitochondrial COI gene only.

To date, *Pseudotrapelus* includes five (Uetz, 2015) or six (Melnikov et al., 2015) recognized species. Before 2012 the only recognized species across the whole range was *P. sinaitus* (Heyden, 1827), described from the Sinai Peninsula (probably from close to Mt. Sinai in the southern Sinai Peninsula; Moravec, 2002; Melnikov and Pierson, 2012). The subspecies *P. sinaitus wernerii*, endemic to the basalt desert of northern Jordan and southern Syria, was described by Moravec (2002). The four recently described species, *P. aqabensis*, *P. dhofarensis*, *P. jensvindumi* and *P. chlodnickii*, are said to be differentiated by several morphological traits: body size, length of the third toe, number and position of the pre-anal pores, and the head and dorsal scalation. *Pseudotrapelus aqabensis*, described from a single specimen, was collected in the hills adjacent to the city of Aqaba, Jordan (Melnikov et al., 2012) and occurs in north-western Saudi Arabia, southern Israel and the eastern Sinai Peninsula (Melnikov et al., 2013b, 2014; Aloufi and Amr, 2015). Melnikov and Pierson (2012) described *P. dhofarensis* from the Dhofar governorate in southern Oman, although subsequent

studies have reported it as ranging from southern Oman and Yemen to Saudi Arabia (Melnikov and Melnikova, 2013). Melnikov et al. (2013a) described *P. jensvindumi* from Jebel Al Akhdar in northern Oman, based on a single specimen. It is so far known only from that particular area of eastern Arabia (Melnikov and Melnikova, 2013). Recently, Melnikov et al. (2015) described *P. chlodnickii* from a single specimen collected at Gamamiya in the Bayuda Desert, Sudan. An additional species, *P. neumanni* (Tornier, 1905), was described from the Lahej area in southern Yemen, though it was later synonymized with *P. sinaitus* by Arnold (1980) due to intermediate forms with neighboring populations (synonym accepted by Fritz and Schütte, 1988; Schätti and Gasperetti, 1994). This species was regarded as valid by Melnikov et al. (2012) and their later studies, with incomplete systematic details.

In this study we seek to clarify the systematics of *Pseudotrapelus* and to elucidate the different diversification processes affecting its evolutionary history. *Pseudotrapelus*, being mainly endemic to the mountains and rocky habitats around the Red Sea, provides an excellent model to assess the biogeographic patterns of the fauna connecting Arabia and Africa around the Red Sea. We therefore use the genus as a model to assess the influence of the dynamic geological history and climatic shifts on the origin and evolution of the regional fauna, inferring phylogenetic relationships using multilocus genetic data. We also use gene trees and species trees and species-delimitation methods based on coalescence to identify the different taxonomic units in order to compare them with the current taxonomy and to determine whether there is still undescribed diversity.

2. Material and methods

2.1. Taxon sampling

In order to assess the systematic status of species and populations, test biogeographic hypotheses, and investigate relationships, a comprehensive sampling from across the known distribution range of the genus was carried out. We analyzed 92 samples of all currently recognized species and subspecies of *Pseudotrapelus*, including specimens from the type localities of four species (Fig. 1; Table S1; one sequence was retrieved from GenBank). The phylogenetic position of *Pseudotrapelus* within the Agaminae subfamily has so far only been studied based on a single specimen (Joger, 1991; Macey et al., 2006; Pyron et al., 2013; Leaché et al., 2014). We therefore included specimens from several phylogenetically closely-related genera to test the monophyly of the genus. We used *Acanthocercus* and *Xenagama* specimens as close outgroups based on published evidence, and members of *Trapelus* as a distant outgroup to root the tree (Joger, 1991; Macey et al., 2006; Pyron et al., 2013; Leaché et al., 2014). Sample codes, vouchers, localities and GenBank accession numbers are given in Table S1. Sampling localities of *Pseudotrapelus* specimens are shown in Fig. 1. Samples were allocated to species on the basis of the genetic results rather than on the basis of their morphological characters, as the diagnosis available for the species is still too incomplete and distributional ranges within the genus are unclear (see Section 4.1 “taxonomic account” for details).

2.2. DNA extraction, amplification and sequence analysis

Genomic DNA was isolated from ethanol-preserved tissue samples using the SpeedTools Tissue DNA Extraction kit (Biotoools, Madrid, Spain). Individuals were sequenced for both strands of three loci. The mitochondrial dataset included two mitochondrial gene fragments, the ribosomal 16S rRNA (16S) and the protein cod-

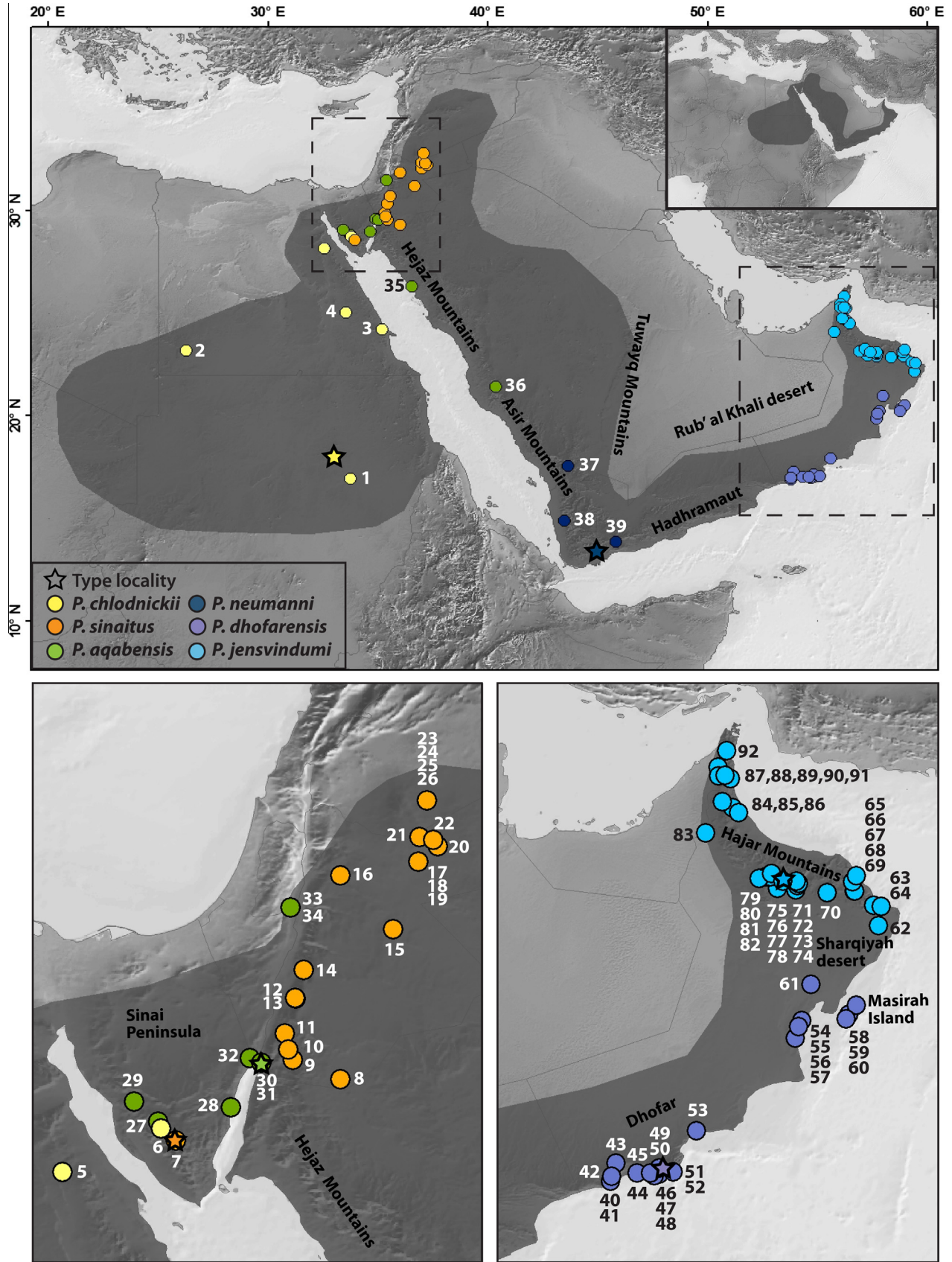


Fig. 1. Sampling localities of the *Pseudotrappelus* specimens, including type localities and the global distribution range of the genus (modified from [Sindaco and Jeremčenko \(2008\)](#)). Numbers correlate to specimens listed in [Table S1](#) and colors to specimens in [Figs. 2–4, S1 and S2](#). Taxon names correspond to changes proposed in this paper.

ing NADH dehydrogenase subunit 4 (ND4) with the adjacent histidine, serine, and leucine tRNA genes (tRNA). Two nuclear protein coding gene fragments were also amplified: the melano-cortin 1 receptor (MC1R) and the oocyte maturation factor Mos (*c-mos*). Primers, PCR conditions and source references are listed in Table S2.

Chromatographs were checked manually, assembled and edited using Geneious v.7.1.5 (Biomatter Ltd.). For the nuclear genes, MC1R and *c-mos*, heterozygous individuals were identified and coded according to the IUPAC ambiguity codes. Coding gene fragments (ND4, MC1R and *c-mos*) were trimmed so that all started at the first codon position and were translated into amino acids to ensure that there were no premature stop codons. DNA sequences were aligned, for each gene independently, using the online application of MAFFT v.7 (Katoh and Standley, 2013) with default parameters (Auto strategy, Gap opening penalty: 1.53, Offset value: 0.0). For the 16S and tRNA fragments we applied the Q-INS-i strategy, in which information on the secondary structure of the RNA is considered. In order to remove regions without specific conservation, and poorly aligned positions of 16S and tRNA, we used G-blocks (Castresana, 2000) with low stringency options (Talavera and Castresana, 2007). Inter and intra-specific uncorrected *p*-distances with pairwise deletion of the mitochondrial fragments, and the number of variable (V) and parsimony informative (Pi) sites were calculated in MEGA v.5.2 (Tamura et al., 2011).

2.3. Phylogenetic and nuclear network analyses

Phylogenetic analyses were performed for the complete datasets simultaneously using partitions by gene, and as specified by PartitionFinder v.1.1.0 (Lanfear et al., 2012) with the following parameters: linked branch length; BEAST models; BIC model selection; greedy schemes search; single partition of the complete 16S and tRNA and by codons for the other protein coding genes (ND4, MC1R and *c-mos*). We used jModeltest v.2.1.3 (Guindon and Gascuel, 2003; Durraba et al., 2012) to select the best model of nucleotide substitution for each gene partition independently. A summary of DNA partitions and relevant models is presented in Table S3.

Phylogenetic analyses for each dataset were performed using maximum likelihood (ML) and Bayesian (BI) methods. Maximum likelihood analyses were performed with RAxML v.7.4.2 (Stamatakis, 2006) using RAxMLGUI v.1.3 (Silvestro and Michalak, 2012) with a GTR+G model of evolution and parameters estimated independently for each partition. All ML analyses were performed with 100 random addition replicates and reliability of the tree was assessed by 1000 bootstrap iterations (Felsenstein, 1985). Bayesian analyses were performed with BEAST v.1.8.0 (Drummond et al., 2012) with the same dataset used in the ML analysis but without outgroups. Parameter values both for clock and substitution models were unlinked across partitions. Information on the models, priors and runs is presented in Table S3. The .xml file was manually modified to “Ambiguities = true” for the nuclear partitions to account for variability in the heterozygote positions, instead of treating them as missing data. All BEAST analyses were carried out in CIPRES science gateway (Miller et al., 2010). Posterior trace plots and effective sample size values of parameters (>200) of each run were assessed in Tracer v.1.5 (Rambaut and Drummond, 2009). LogCombiner and TreeAnnotator (both available in BEAST package) were used to infer the ultrametric tree. We treated alignment gaps as missing data, and the nuclear gene sequences were not phased. Nodes were considered strongly supported if they received ML bootstrap values $\geq 70\%$ and posterior probability (pp) support values ≥ 0.95 (Wilcox et al., 2002; Huelsenbeck and Rannala, 2004).

Haplotype networks were constructed for the nuclear genes MC1R and *c-mos* (only full length sequences included). To resolve the multiple heterozygous single nucleotide polymorphisms, the on-line web tool SeqPHASE (Flot, 2010) was used to convert the input files, and the software PHASE v.2.1.1 to resolve phased haplotypes (Stephens et al., 2001; Stephens and Scheet, 2005). Default settings of PHASE were used, except for phase probabilities, which were set as 0.9 for *c-mos* and 0.5 for MC1R. The phased nuclear sequences were used to generate median-joining networks using NETWORKS v.4.6.1.3 (Bandelt et al., 1999).

2.4. Species delimitation approaches and coalescent-based species tree

To evaluate the relationships and species boundaries within *Pseudotrapelus*, we used different species delimitation approaches, including a Bayesian coalescence approach (species tree; Edwards, 2009) and two delimitation methods.

We first used the Generalized Mixed Yule-coalescent analysis (GMYC; Pons et al., 2006) for estimating species boundaries. As this method relies on single locus data, we used a Bayesian concatenated mitochondrial phylogenetic tree including haplotypes only, reconstructed with BEAST v.1.8.0 (Drummond et al., 2012). Information on the models, priors and runs is presented in Table S3, and parameters applied were as above. We performed the GMYC function implemented in R (R development Core Team, 2013) using the “splits” package (Species Limits by Threshold Statistics; Ezard et al., 2009). We applied a single threshold algorithm and compared to the null model (i.e., all individuals belong to a single species) using a log-likelihood ratio test as implemented in the GMYC package.

Multilocus coalescence-based Bayesian species trees for *Pseudotrapelus* were estimated using BEAST (Heled and Drummond, 2010). The first tree was based on the results obtained from the GMYC analysis to define the lineages to be used as putative species and the second species tree was based on the BP&P ‘species’ (see below). Outgroups were excluded and only GMYC and BP&P lineages with a full set of genes were included. Analyses were run with phased nuclear genes, unlinked parameter values for clock, substitution models and trees (linked trees for the mtDNA partitions). The Yule process was used as the species tree prior with a random starting tree. Information on the models, priors and runs is presented in Table S3.

Multilocus Bayesian coalescent species delimitation analyses were conducted with Bayesian Phylogenetics and Phylogeography (BP&P v.2.2; Rannala and Yang, 2003; Yang and Rannala, 2010) using nuclear the loci (MC1R and *c-mos*) only. We used the first species tree recovered from BEAST (based on the GMYC analysis) as our guide tree. Both algorithms 0 and 1 were used, assigning each species delimitation model equal prior probability. As prior distributions on the ancestral population size (θ) and root age (τ) can affect the posterior probabilities for models (Yang and Rannala, 2010), and since no empirical data were available for *Pseudotrapelus*, we tested four different combinations of priors (Leaché and Fujita, 2010; see Table S3). We ran each of the rjMCMC analysis twice to confirm consistency between runs (with sampling intervals of five). We considered speciation probability values ≥ 0.95 as strong evidence of a speciation event.

2.5. Estimation of divergence times

Lineage divergence times were estimated in BEAST v.1.8.0 (Drummond et al., 2012) with one representative of each independent GMYC lineage (based on gene partitions; the nuclear genes unphased; see Table S1). For these analyses we included outgroups and combined the ND4 and tRNA datasets together in order to be able to implement evolutionary rates for the same mitochondrial

region available from the literature (see below). We used two dating approaches, in both of which the .xml file was manually modified to “Ambiguities = true” for the nuclear genes (MC1R and *c-mos*). Information on the models, priors and runs for each calibration approach is presented in Table S3.

The first dating analysis was based on the average sequence evolution rates estimated for the agamid genus *Phrynocephalus* (Macey et al., 2006; Pyron et al., 2013) according to Pang et al. (2003): 0.0073–0.0132 substitutions/site/million years for 16S and 0.0113–0.0204 substitutions/site/million years for ND4 + tRNA. The ucl.d.mean priors of 16S (initial 0.0073; lower 0.0073; upper 0.0132) and ND4 + tRNA (initial 0.0113; lower 0.0113; upper 0.0204) were Uniform. The clock.rate priors for MC1R and *c-mos* were Uniform (initial 0.001; lower 0; upper 0.0204).

For the second dating analysis we used the posteriors of selected nodes from Leaché et al. (2014): (a) the split between *Xenagama taylori* and *X. batillifera* (Normal distribution, mean 0.3, stdev 0.15); (b) the split of *X. zonura* (Normal distribution, mean 2.2, stdev 1) from other *Xenagama* species; (c) the root, split of *Trapelus* (Normal distribution, mean 38.8, stdev 5.5). The ucl.d.mean priors of 16S and ND4 + tRNA and clock.rates priors for MC1R and *c-mos* were Uniform (initial 0.001; lower 0; upper 1).

Divergence times for the ingroup only (*Pseudotrapelus*) were also estimated using a coalescent species tree approach in BEAST applying the rates of Pang et al. (2003), which gave very similar results to the other calibration strategy (see results; Table 1). “Species” were defined based on the results of the BP&P analyses. See Section 2.4 and Table S3 for the models, priors and parameter specifications.

2.6. Ancestral area reconstruction

To infer the phylogeographic history and estimate the ancestral range of *Pseudotrapelus*, we used the Bayesian Stochastic Search Variable Selection (BSSVS; Lemey et al., 2009) of the discrete phylogeographic model implemented in BEAST. We analyzed the data (including those of the closely related outgroups, *Acanthocercus* and *Xenagama*), assigning three discrete biogeographic areas corresponding to the mountain ranges in the current distribution range of *Pseudotrapelus*: (1) eastern Arabia – including the Hajar Mountains in northern Oman and the United Arab Emirates (UAE); (2) southern and western Arabia – including southern Oman, Yemen, Saudi Arabia, Israel, Jordan and the Sinai Peninsula; (3) Africa – including Egypt and Sudan, and the Horn of Africa region.

We used the same dataset (GMYC representatives), models and prior settings as in the dating analysis, and for a temporal frame we applied the dating of evolution rates (rates based on Pang et al.,

2003; Table S3). Additional specifications were: symmetric discrete trait substitution model; strict clock model for the location trait; exponential prior for the discrete location state rate (locations.clock.rate) with mean of 1.0 and offset of 0.

3. Results

3.1. Taxon sampling and sequence data

Our dataset comprised 92 *Pseudotrapelus* specimens sampled from localities across the distribution range of the genus, including type localities (Fig. 1; Table S1). Sequences of 19 individuals belonging to other genera, sampled and retrieved from GenBank, were used as outgroups (Fig. 2; Table S1). The dataset included mitochondrial gene fragments of 16S (492 bp; $V = 71$; $\text{Pi} = 66$), ND4 (681 bp; $V = 265$; $\text{Pi} = 245$) and tRNA (153 bp; $V = 47$; $\text{Pi} = 45$), and nuclear gene fragments of MC1R (663 bp; $V = 31$; $\text{Pi} = 25$) and *c-mos* (372 bp; $V = 6$; $\text{Pi} = 4$) totaling 2361 bp. The concatenated mitochondrial dataset revealed 67 unique haplotypes. Nuclear markers included 47 haplotypes for MC1R and 55 for *c-mos* with a 0.5 and 0.9 probability phasing threshold, respectively. Uncorrected genetic variation (p -distance) between and within species for the 16S and the ND4 gene fragments is presented in Table S4.

3.2. Phylogenetic analyses and nuclear networks

The results of the phylogenetic analyses indicate that *Pseudotrapelus* is monophyletic (Fig. 2). The African genus *Xenagama*, from which we analyzed three of four recognized species, is also monophyletic. However, the genus *Acanthocercus* is polyphyletic, as two African *Acanthocercus* species (*A. annectens* and *A. atricollis*) form a clade with *Xenagama*, whereas the two Arabian species form a clade with *Pseudotrapelus*. Moreover, the African *Acanthocercus cyanogaster* is the sister taxon to all these lineages.

Pseudotrapelus is divided into two major clades, Eastern and Western (Fig. 2). The two clades are composed of six clearly distinct and well-supported lineages that mostly correspond to current taxonomic classifications (Figs. 2 and S1). The six lineages are well differentiated from each other in both mitochondrial and concatenated gene trees, in the BEAST species tree, the species delimitation analyses (GMYC, BP&P) and nuclear haplotype networks (Figs. 2–4, S1 and S2). Bayesian and ML analyses yielded almost identical topologies for both partition approaches (PartitionFinder and independent genes; see material and methods and Table S3) with high Bayesian posterior probabilities and bootstrap values (Figs. 2 and S1). Genetic distances (p -distance) appear

Table 1

Results for each of the three calibration approaches used in this study (mean and the HPD 95% confidence interval): (i) rates of 16S and ND4 + tRNA based on Pang et al. (2003); (ii) calibration points based on the posteriors of Leaché et al. (2014); (iii) BEAST analysis based on the rates of Pang et al. (2003).

Clade/taxon	Calibration analysis (Mya)		
	Pang et al. (2003)	Leaché et al. (2014)	BEAST
Root	31.8 (25.4, 38.9)	32.1 (21.9, 42.3)	–
<i>Xenagama</i> – <i>Acanthocercus</i> – <i>Pseudotrapelus</i>	18.3 (15, 21.7)	17.7 (11.2, 24.4)	–
<i>Acanthocercus cyanogaster</i>	16.1 (12.9, 19.5)	15.5 (9.8, 21.7)	–
<i>Acanthocercus atricollis</i> – <i>Xenagama</i>	7.6 (5.9, 9.5)	7.3 (4.5, 10.3)	–
<i>Xenagama zonura</i>	2.8 (1.9, 3.8)	2.5 (1.6, 3.7)	–
<i>Xenagama taylori</i> – <i>X. bitillifera</i>	0.28 (0.1, 0.5)	0.25 (0.1, 0.4)	–
<i>Acanthocercus</i> – <i>Pseudotrapelus</i>	15.9 (12.6, 19.3)	15.3 (9.7, 21.4)	–
<i>Acanthocercus adramitanus</i> – <i>A. yemensis</i>	6.7 (5.1, 8.4)	6.4 (3.9, 9.1)	–
<i>Pseudotrapelus</i>	8.1 (6.6, 9.8)	7.8 (4.9, 10.9)	8.1 (5.6, 10.8)
<i>Pseudotrapelus chlodnickii</i> – <i>P. sinaitus</i>	5.1 (3.8, 6.6)	4.9 (2.9, 7.2)	5.2 (3.2, 7.4)
<i>Pseudotrapelus jensvindumi</i>	5.6 (4.4, 6.8)	5.3 (3.2, 7.4)	5.2 (3.3, 7.2)
<i>Pseudotrapelus dhofarensis</i>	4.2 (3.3, 5.2)	3.9 (2.4, 5.6)	4.1 (2.5, 5.7)
<i>Pseudotrapelus aqabensis</i> – <i>P. neumanni</i>	3.6 (2.7, 4.5)	3.3 (2, 4.8)	3.4 (2, 4.8)

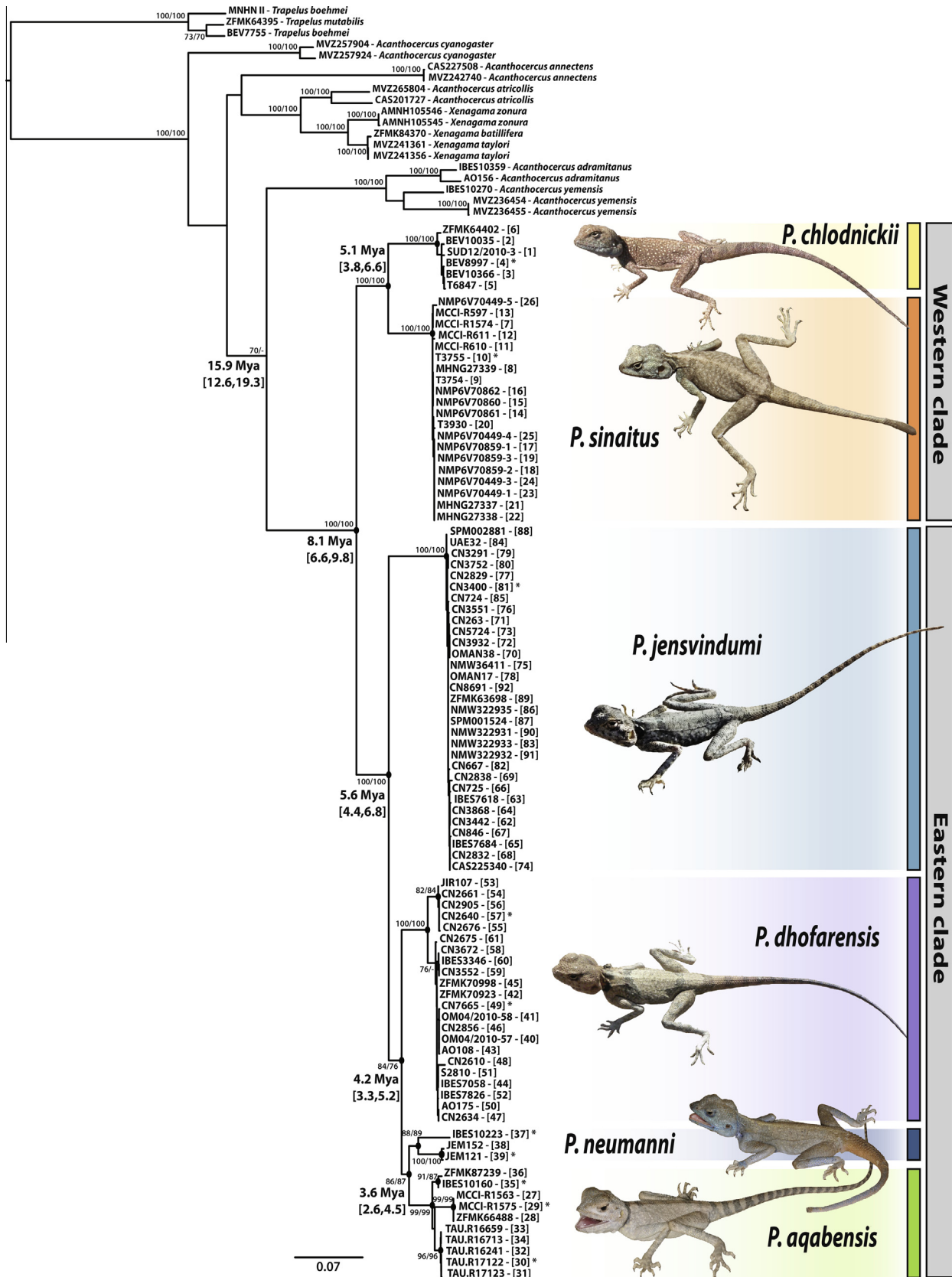


Fig. 2. Maximum likelihood (ML) gene tree of *Pseudotrapelus* inferred from 2361 bp of mitochondrial (16S, ND4-tRNA) and nuclear (MC1R, c-mos) gene fragments. Black dots on the nodes indicate posterior probability in the Bayesian analysis (values ≥ 0.95 , for both gene partitions and partitions by PartitionFinder [PF]; see Section 2.3 of Materials and Methods), and the ML bootstrap support values are indicated near the nodes (values $\geq 70\%$; ML, ML-PF). Age estimates based on the rates of Pang et al. (2003) are indicated near the relevant nodes and include the mean and, between brackets, the HPD 95% confidence interval. Asterisks indicate representatives used in the GMYC analysis (see Fig. S2). Taxon names correspond to changes proposed in this paper. Sample codes and colors correlate to specimens in Table S1 and in Figs. 1–5, S1 and S2.

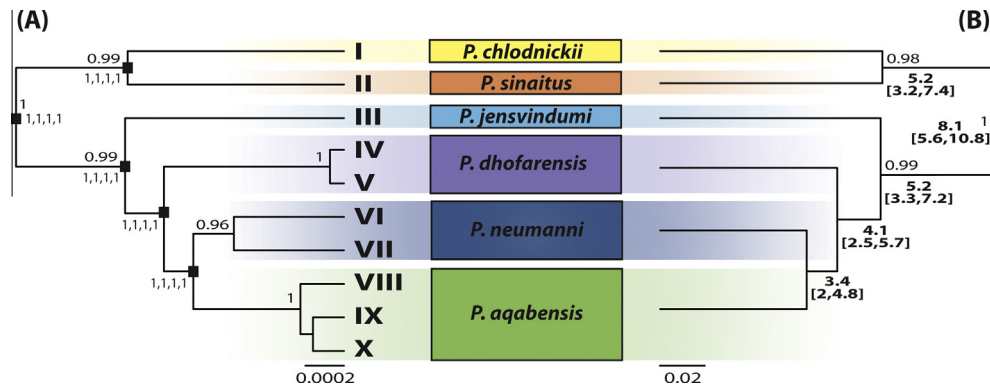


Fig. 3. Species trees inferred in [†]BEAST. Posterior probabilities are indicated above the nodes (values ≥ 0.95 shown). (A) Specimens assigned to putative species based on the GMYC species delimitation result (see Fig. S2). Black rectangles on the nodes indicate taxa recognized by the species delimitation analyses inferred by BP&P (nuclear genes only; posterior values unite for all analyses are indicated below the nodes; see details in Table S3 and Section 2.4 in Material and Methods). (B) Specimens recognized as putative species by BP&P with time estimates based on the rates of Pang et al. (2003) (see Section 2.5 in Material and Methods). Taxon names correspond to changes proposed in this paper. Colors correspond to species in Figs. 1–5, S1 and S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to be low within each lineage (16S: 0–1.1%; ND4: 0.3–7.4%; Table S4), especially within *P. chlodnickii*, *P. sinaitus* and *P. jensvindumi* (Table S4).

The Western clade includes two lineages (Fig. 2) taxonomically recognized as *P. chlodnickii* and *P. sinaitus*. The lineages are clearly distinct from one another, with high genetic distances (16S: 5%; ND4: 14.1%; Table S4). *Pseudotrappelus chlodnickii* includes specimens from north-eastern Africa (Egypt and Sudan) and the western Sinai Peninsula (Fig. 1). *Pseudotrappelus sinaitus* includes specimens from Jordan, Syria and the Sinai Peninsula, ascribed to both subspecies of *P. sinaitus* – *P. s. sinaitus* and *P. s. wernerii*. This lineage includes samples from the proposed type locality of the species from the Sinai Peninsula (Mt. Sinai in the southern Peninsula).

The Eastern clade includes the four remaining lineages. A northern Arabian lineage corresponding to *P. jensvindumi* (including the holotype and specimens from the type locality at Jebel Al Akhdar; Fig. 1) is sister to the other three, and ranges throughout the Hajar Mountains in northern Oman and the UAE. The three remaining lineages are from central and southern Oman; Yemen and southern Saudi Arabia; and northern Saudi Arabia to the Sinai Peninsula. The Omani lineage is sister to the other two lineages and is recognized as *P. dhofarensis* (specimens sampled from and around the type locality at Jebel Samhan in Dhofar; Fig. 1). It is comprised of samples from the Al-Wusta and Dhofar Governorates in central and southern Oman, respectively, including the population from Masirah Island. The samples from the Sinai Peninsula are phylogenetically closely related to samples collected from southern Israel, Aqaba in Jordan and western Saudi Arabia, recognized as *P. aqabensis* (including samples from the type locality in Aqaba, Jordan; Fig. 1). The Yemeni and southern Saudi Arabian lineage corresponds to *P. neumanni*, previously recognized as a synonym of *P. sinaitus* (see Section 4.1 for the taxonomic account). The separation between *P. aqabensis* and *P. neumanni* is strongly supported in the concatenated tree (Fig. 2), but weakly supported in the mitochondrial analyses (ML: 70%, 64%; BI: 0.94, 0.97; Fig. S1).

The haplotype networks, constructed for the phased, full length nuclear markers MC1R and *c-mos*, are presented in Fig. 4. The MC1R network shows similar patterns and closely agrees with the phylogenetic trees, as most of the observed polymorphism contributes to the differentiation of specimens assigned to six lineages/species. Within this network, clear haplotype differentiation is evident as no derived alleles are shared between species, including sympatric species, which are clearly distinct from each other. The subspecies of *P. sinaitus*, however, do share

alleles. The *c-mos* network shows private alleles for the two sympatric species from the Western clade (*P. sinaitus* and *P. chlodnickii*), whereas, again, the two subspecies of *P. sinaitus* share alleles. Ancestral alleles are shared among the four Arabian species of the Eastern clade, suggesting incomplete lineage sorting.

3.3. Species delimitation and species trees

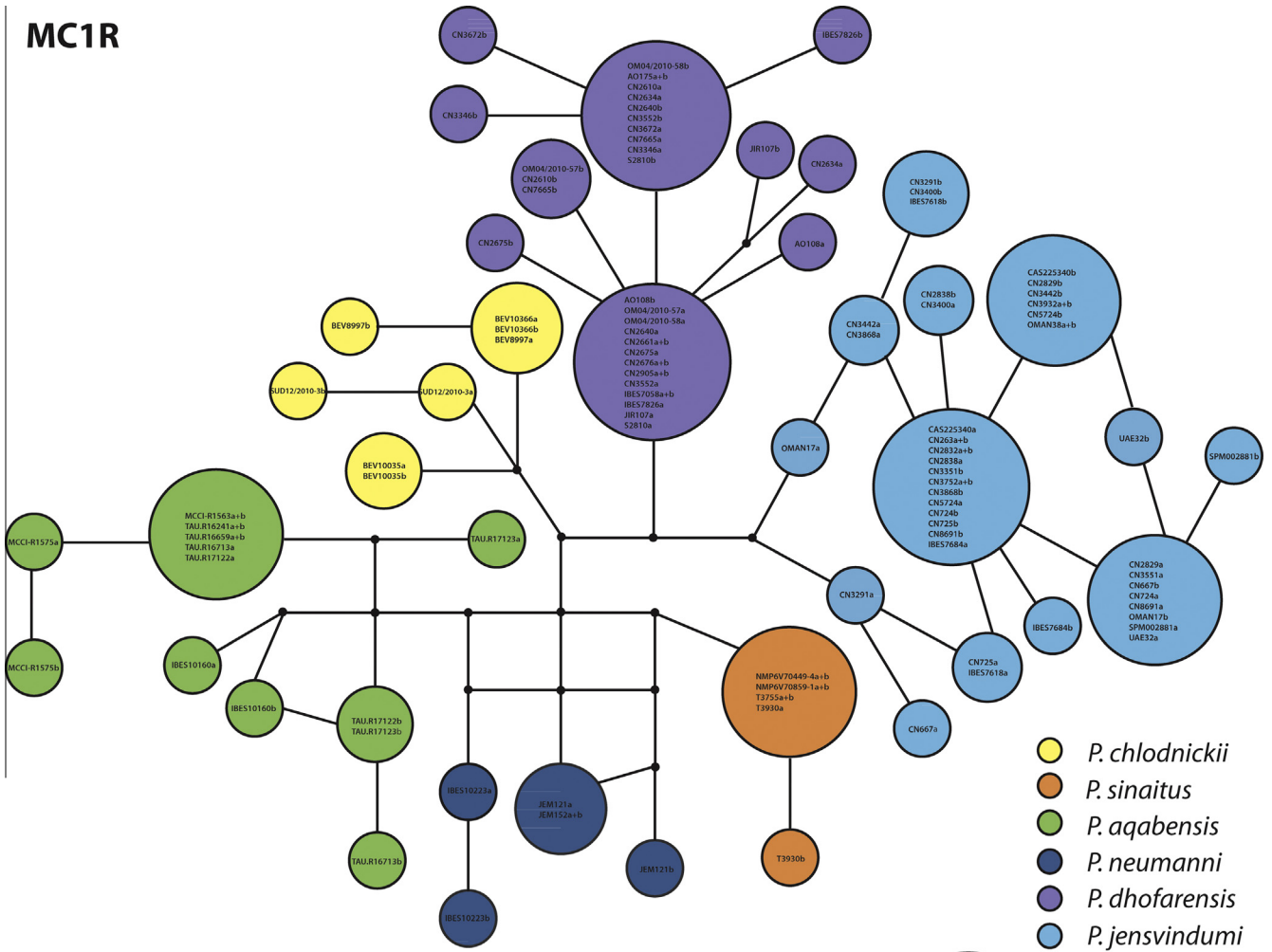
The level of genetic variability within *Pseudotrappelus* is not high, as reflected in both the genetic distances (*p*-distance; Table S4) and the results of the GMYC analysis with the single threshold approach (Fig. S2; based on the concatenated mitochondrial haplotype dataset). The latter analysis recovered two clades and 10 effective putative species for both partition approaches ($\log L_{null} = 501.521, 499.842$; $\log L_{GMYC} = 512.195, 511.434$; LR = 21.35, 23.183; $p < 0.001$; based on gene partition and PartitionFinder, respectively; Fig. S2). The result of the likelihood ratio test was significant for both partition approaches, indicating that the null model (i.e., a single population) could be rejected.

The Bayesian coalescent approach, using [†]BEAST, was performed by treating each of the 10 GMYC entities as a separate putative species (Fig. 3A). Several GMYC “species” unite together (IV and V; VI and VII; VIII, IX and X), resulting in a similar topology to that of the ML and BI concatenated and mitochondrial phylogenetic trees (Figs. 2 and S1). Besides the posterior probability for the grouping of VI and VII (0.96; Fig. 3A), the posterior probability of the other relationships is 0.99–1, implying that essentially all species trees in the posterior distribution had each lineage as monophyletic. The relationships within the Eastern clade are not supported (i.e., posterior probability values of 0.88 for the separation of *P. dhofarensis*, and 0.92 for the separation between *P. aqabensis* and *P. neumanni*).

The results of the coalescent species delimitation analyses (BP&P; nuclear data only), using the [†]BEAST tree inferred with the 10 GMYC “species” as the guide tree, yielded a six putative species model, with mostly consistent results regardless of the rjMCMC algorithm, (θ) and (τ) priors, and starting tree used (Fig. 3A).

The [†]BEAST tree, based on the BP&P six species model guide tree (Fig. 3B), supports the separation into two clades, and the distinctiveness of three species – *P. chlodnickii*, *P. sinaitus* and *P. jensvindumi*. The tree, however, did not support the relationships among three lineages within the Eastern clade, which should be regarded as distinct species according to the previous BP&P analysis.

MC1R



c-mos

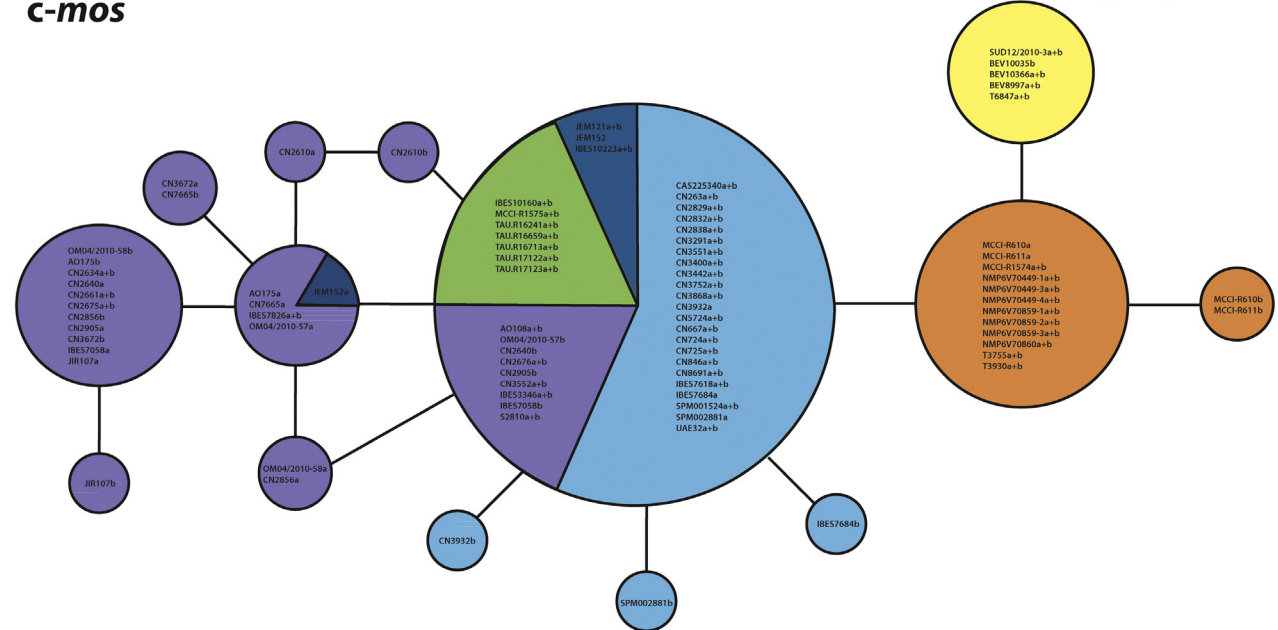


Fig. 4. Unrooted haplotype networks of MC1R and *c-mos* nuclear markers. Circle size is proportional to the number of alleles, with colors corresponding to species in Figs. 1–3, S1 and S2. Codes correlate to the two alleles (i.e., a and b) of specimens listed in Table S1. Taxon names correspond to changes proposed in this paper. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Divergence time estimates

High effective sample sizes were observed for all parameters in the BEAST analyses for each dating approach. The dates from each approach are presented in Table 1. As the two approaches resulted in almost identical dates, we continued with the dating rates published in Pang et al. (2003). The results of the dating analyses using both the gene-tree and species-tree were almost identical (Table 1; Figs. 2 and 3B), with younger dates for the species tree.

Our results, based on the concatenated dataset (Table 1; Fig. 2), indicate that *Pseudotrappelus* split from Arabian *Acanthocercus* around 15.9 million years ago (Mya; 95% HPD: 19.3–12.6 Mya). Based on the species tree analysis (Table 1; Fig. 3B) the genus started diverging through the late-Miocene ca. 8.1 Mya (95% HPD: 10.8–5.6 Mya), mostly radiating during the late-Miocene and early- mid-Pliocene. Speciation within the Western clade into *P. chlodnickii* and *P. sinaitus* appears to have occurred approximately 5.2 Mya (95% HPD: 7.4–3.2 Mya). The split of *P. jensvindumi* from the Eastern clade occurred at a similar time, around 5.2 Mya (95% HPD: 7.2–3.3 Mya). The divergence of *P. dhofarensis* is estimated to have occurred during the Pliocene at ca. 4.1 Mya (95% HPD: 5.7–2.5 Mya) and cladogenesis of *P. neumanni* and *P. aqabensis* at ca. 3.4 Mya (95% HPD: 4.8–2 Mya).

3.5. Biogeographic reconstructions

Results of the discrete phylogeographic analyses within a temporal framework are summarized in Fig. 5. Node ages were similar to the dating analysis with the same dataset (Table 1; Fig. 2). *Pseudotrappelus* most likely originated in western Arabia (78% probability; Fig. 5) at the same time as its Arabian relatives, *Acanthocercus yemensis* and *A. adramitanus*. Subsequent splits within the genus separated *P. chlodnickii* (to Africa) and *P. jensvindumi* (to north-eastern Arabia).

4. Discussion

This study provides the first, robust, time-calibrated phylogenetic reconstruction of the relationships and diversity of the genus *Pseudotrappelus*. Furthermore, we evaluate the evolution and biogeography of *Pseudotrappelus*, including representatives of all recognized populations and species from across the entire distributional range of the genus (Fig. 1). All molecular analyses in this study present high levels of nodal support (Figs. 2–5, S1 and S2). The divergence time estimates derived from two calibrations, between the gene tree and species tree approaches, resulted in almost identical dates, thus strengthening our confidence in these results (Table 1).

4.1. Taxonomic accounts within *Pseudotrappelus*

The molecular results of this study reveal *Pseudotrappelus* as a diverse genus. Species delimitation analyses revealed six distinct lineages that warrant species status (Figs. 2–4, S1 and S2), supported also by morphological differences (Melnikov et al., 2015; Photographic material, data not shown). Regarding nomenclature, we consider the species' names ascribed by Melnikov et al. (2012, 2013a, 2015) and Melnikov and Pierson (2012) to be consistent with the distinct lineages we identified (as we sampled them at or close to the type localities). We advocate that these names remain valid according to the rules of zoological nomenclature.

The three species occurring in the Sinai Peninsula are morphologically different from each other, have distinct mitochondrial assignments, no shared nuclear alleles in any analysis and no heterozygote specimens were detected (Figs. 2–4, S1 and S2). *Pseudotrappelus sinaitus* was described from “Sinai” with no further data (Heyden, 1827). According to Moravec (2002), followed by Melnikov and Pierson (2012), the type locality should be regarded as Mt. Sinai. Our samples from this location and from Jordan are therefore assigned to *P. sinaitus*. The lack of sampling of *P. sinaitus*

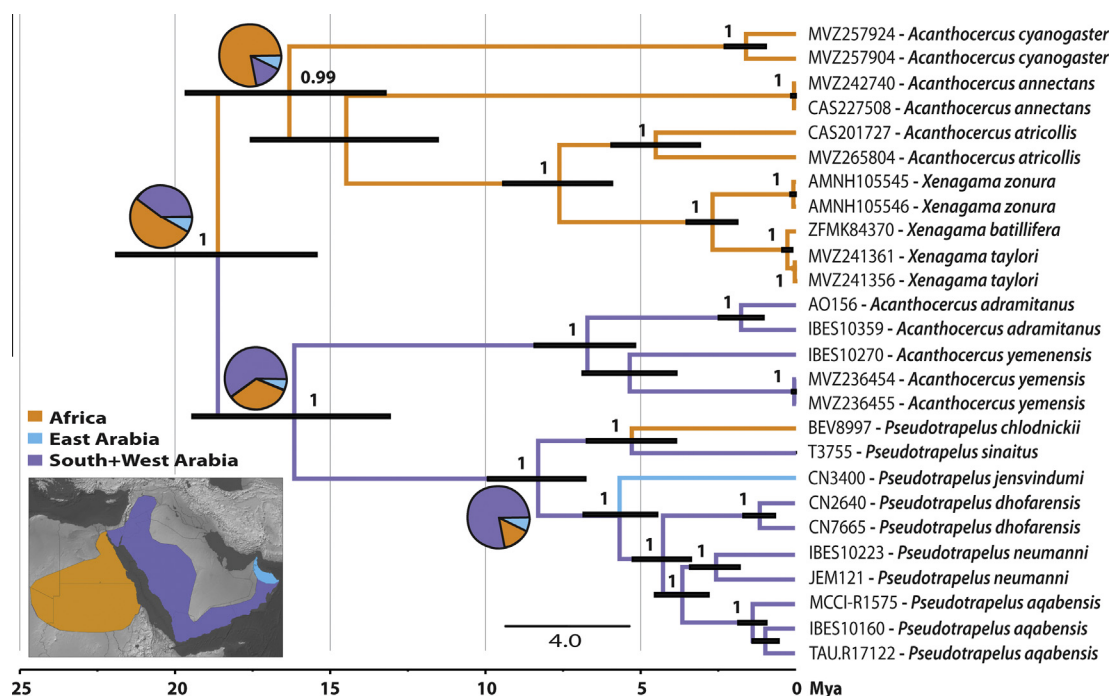


Fig. 5. The BEAST consensus tree using the BSSVS method of ancestral area reconstruction with a temporal framework based on the rates of Pang et al. (2003) (Mya; HPD 95% confidence interval bars at each node). Branch color indicates inferred ancestral range (ranges for *Pseudotrappelus* visualized in the lower left map), with posterior probabilities of ancestral range above the nodes (values ≥ 0.95 are shown). A pie chart describing the probability of each inferred area is presented near the major nodes. Taxon names correspond to changes proposed in this paper. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in north-eastern Africa precludes us from assessing its presence in this area, though Melnikov et al. (2013a, 2013b, 2015) reported its presence there based on a single sequence retrieved from GenBank. The lineage sampled at the type locality of *P. aqabensis* in Aqaba, Jordan, is classified as *Pseudotrapelus aqabensis*, also distributed in the Sinai Peninsula, Israel, Jordan and northern Saudi Arabia (Fig. 1; similar to Melnikov et al., 2014). The lineage from north-eastern Africa, including the Sinai Peninsula, sampled close to the type locality of *P. chlodnickii* in Sudan, is thus assigned to *Pseudotrapelus chlodnickii*, with a broader distribution than previously thought in Melnikov et al. (2015) (Fig. 1). The Sinai *P. chlodnickii* specimen (ZFMK64402; location code 6 in Fig. 1) has not been successfully sequenced for nuclear loci, but exhibits a *P. chlodnickii* phenotype, thus excluding the possibility that the occurrence of *P. chlodnickii* mitochondrial data in the Sinai Peninsula is due to introgression with *P. sinaitus*. The two Omani species, *P. dhofarensis* and *P. jensvindumi*, were sampled from and around their type locality, including the holotype of the latter, and the genetic analyses presented in this study (Figs. 2–4, S1 and S2; Table S4) support their specific distinctiveness. *Pseudotrapelus jensvindumi* is restricted to the Al Hajar Mountains in northern Oman and the UAE (Fig. 1), though Melnikov and Melnikova (2013) assigned its range only to the western and central ridges of these mountains. *Pseudotrapelus dhofarensis* is distributed in central and southern Oman, though we cannot confirm its occurrence in the Hadhramaut area in south-eastern Yemen, as reported in the morphological study of Melnikov and Melnikova (2013).

Our results show distinctiveness of a lineage from Yemen and southern Saudi Arabia (Figs. 2–4, S1 and S2; Table S4), and it should thus be assigned a different name. Tornier (1905) described *Pseudotrapelus neumanni* from the Lahej area in southern Yemen based on the large dorsal scales on the head and body, their direction of imbrication, direction of the nostrils, four preanal pores and equal length of the third and fourth toes (Tornier, 1905). Authors over the years have recognized the populations from southern and eastern Yemen as distinct morphological forms. However, due to the conservative approach taken by these authors, these populations were classified as intraspecific variations of *P. sinaitus*, as intermediate forms connect the specimens from Lahej to the population in the surrounding areas (Anderson, 1896, 1898, 1901; Arnold, 1980; Fritz and Schütte, 1988; Schätti and Gasperetti, 1994). Based on the genetic results we resurrect the specific status of *Pseudotrapelus neumanni* (Tornier, 1905).

Species boundaries between *P. dhofarensis* and *P. aqabensis* relative to *P. neumanni* are less satisfactory and a meticulous assessment of their morphological and genetic distinctiveness is required, especially in the connecting areas of Asir and Hadhramaut (Fig. 1). We provisionally recognize six valid species of *Pseudotrapelus*, but recommend further studies to fully examine the relationship of *P. aqabensis* and *P. dhofarensis* to *P. neumanni*. In addition, the paucity of samples from the mountain ridges of Saudi Arabia such as the Tuwayq Mountains around Riad and the coastal Hejas and Asir mountain ridges, as well as the mountainous area between Jordan and Saudi Arabia (Fig. 1), leaves open the possibility that additional lineages may be revealed.

The current systematics within *P. sinaitus* is interesting, with its range extending from north-eastern Africa, throughout the Sinai Peninsula, into southern Israel, Jordan, north-western Saudi Arabia and southern Syria (Sindaco and Jeremčenko, 2008; Melnikov and Melnikova, 2013; Fig. 1). The subspecies *P. s. werneri* Moravec, 2002 from the Basalt desert of Jordan and southern Syria is phylogenetically extremely closely related to the nominate subspecies. Specimens collected from the type localities of the two subspecies cluster together (Figs. 2–4, S1 and S2). In addition, the genetic distance within *P. sinaitus* is especially low for both 16S and ND4 (0.1% and 0.3%, respectively; Table S4). Thus, we suggest that the

taxonomy of *P. sinaitus* needs re-evaluation, considering morphological and genetic variation, as well as additional ecological data.

4.2. Phylogenetic relationships within *Pseudotrapelus* and its Agaminae relatives

The close phylogenetic relationships among *Pseudotrapelus*, *Acanthocercus* and *Xenagama*, within the subfamily Agaminae, were established in several studies based on a single *Pseudotrapelus* specimen (Joger, 1991; Macey et al., 2006; Pyron et al., 2013; Leaché et al., 2014). The broad sampling in our study supports *Pseudotrapelus* monophyly. The monophyletic African genus *Xenagama* comprises four recognized species, all endemic to the Horn of Africa (Wagner et al., 2013; Leaché et al., 2014; Fig. 2). The genus *Acanthocercus* is polyphyletic (also in Leaché et al., 2014; Figs. 2 and 5) and is currently the subject of an ongoing study (Wagner et al., unpubl. data).

Our results reveal that *Pseudotrapelus* is genetically diverse, comprised of two clades divided into six well-defined lineages. This study thus provides support for the specific status of *P. aqabensis*; *P. chlodnickii*; *P. dhofarensis*; *P. jensvindumi*; *P. neumanni* and *P. sinaitus* (Figs. 2–5, S1 and S2). The gene trees, species trees, species delimitation analyses and nuclear network of MC1R support the six lineages as discrete (Figs. 2–4, S1 and S2). Incomplete allele sorting is present in the nuclear marker *c-mos* for the four Arabian species, most probably as a result of their relatively recent divergence and shared ancestral evolutionary history (Fig. 4). The absence of allele sharing in the nuclear gene fragments (Fig. 4) in the Sinai Peninsula, where three species co-occur (Fig. 1), suggests restricted gene flow and reproductive isolation.

The coexistence of three sympatric agamid species, with similar body-sizes, activity times, habitat and dietary preferences, in the southern area in the Sinai Peninsula, presents an interesting avenue for further research. Furthermore, evaluating gene flow between species/populations will provide additional information regarding species boundaries. Environmental heterogeneity, e.g., altitudinal clines along mountains or different types of rocky habitats, is important in facilitating niche divergence and thus speciation or enabling species coexistence (Pianka, 1969; Keller et al., 2009). Studying how sympatric species utilize their environment will undoubtedly help to elucidate the mechanisms enabling their coexistence. This is particularly the case for species with relatively similar morphology, ecology and habitats such as *Pseudotrapelus*. Arnold (1980) noted that morphological variation and differentiation along an elevational gradient between the two Arabian *Acanthocercus* species (*A. adramitanus*, 0–2000 m a.s.l. and *A. yemensis*, 2000–3000 m a.s.l.) were the potential traits enabling their coexistence. *Pseudotrapelus*, in the sympatric range with *Acanthocercus*, prefers drier habitats and lower elevations (Arnold, 1980). In the Sinai Peninsula, Norfolk et al. (2010) compared habitat use and behavioral patterns of two morphologically distinct, sympatric agamids (*Stellagama stellio* and *Pseudotrapelus sinaitus*) in order to understand how they utilize different niches to minimize interspecific competition. The authors observed that the species had distinct ecological niches with different microhabitat use, color patterns, thermoregulatory activity times and territorial social signaling. These studies highlight the potential mechanisms driving ecological character differentiation in sympatric *Pseudotrapelus* species.

Genetic divergence among *Pseudotrapelus* species is similar to that found within the genus *Agama* (e.g., 16S: 3.9%; ND4: 9.1% between *Agama impalearis*-*A. boueti*; Geniez et al., 2011; Gonçalves et al., 2012; respectively). Relatively low interspecific divergence is apparent between the south-eastern Arabian species *P. dhofarensis*, *P. neumanni* and *P. aqabensis* (16S: 2.2–2.3%; ND4: 11.2–12.9%; Table S4). The degree of intraspecific genetic diversity

is remarkably high within *P. neumanni* (16S: 1.1%; ND4: 7.4%; Table S4). As *P. neumanni* was not thoroughly sampled (Table S1), we cannot rule out that this taxon is composed of several independent lineages. We therefore recommend a more comprehensive analysis of this species, including additional samples from currently un-sampled areas.

4.3. Biogeography of *Pseudotrapelus* and its closely-related agamid relatives

The results of the ancestral area reconstruction clearly show distinct geographical groups extending along the opposite sides of the Red Sea and the Gulf of Aden (Fig. 5). These include an African (*Acanthocercus* and *Xenagama*) and an Arabian (*Acanthocercus* and *Pseudotrapelus*) groups, which started diverging during the early-Miocene approximately ca. 18 Mya (Table 1; Fig. 5). The ancestral area probability of the split between these groups is almost equal for both African and Arabian origins (52% and 40%, respectively) as opposed to a split originating in eastern Arabia (8%). This inconclusive result may stem from the lack of biogeographical information regarding the geographical origin of the genus *Trapelus*. Wagner et al. (2011) suggested an Asian origin for *Trapelus*, thus indicating the probable Asian/Arabian origin of these three Afro-Arabian genera. The separation of the Arabian lineage of *Acanthocercus* (*A. yemensis* and *A. adramitanus*) from *Pseudotrapelus* is suggested to have occurred during the mid-Miocene, around 16 Mya (Table 1; Figs. 2 and 5).

Our divergence time estimates and those of two published studies on agamid lizards, suggest the origin of *Pseudotrapelus* occurred around 15–19 Mya (Macey et al., 2006; Leaché et al., 2014; Table 1). Our calibrations support the suggested role of the Gomphotherium land bridge between Eurasia–Arabia–Africa (~18 Mya; Tchernov, 1992; Rögl, 1999) in the evolutionary diversification of Agamidae, as suggested by previous studies. Joger (1991) suggested this as a potential dispersal route for agamids from Asia into Arabia and later into Africa during the Miocene; whereas Macey et al. (2006) suggested an Afro-Arabian origin and the role of this land bridge as the later of two possible dispersal routes into Asia.

The early-Miocene separation at 18 Mya (Table 1; Fig. 5) between the Arabian and African groups may correspond to the expansion of the Red Sea. The tectonic breakage of the Arabian plate from Africa, estimated to have occurred from the Oligocene onwards, ultimately opened up the Red Sea, starting at the southern end around the Gulf of Aden, expanding at the northern end by the early-Miocene (Menzies et al., 1992; Bosworth et al., 2005). Although the estimated time of tectonic divergence predates the inferred dates in our phylogeny, the continuous rifting of the Red Sea during the Miocene (Girdler et al., 1980; Girdler, 1991; Bosworth et al., 2005; Edgell, 2006; Autin et al., 2010) may have acted as a vicariance event, playing a significant role in the divergence between these Afro-Arabian agamid groups. The dynamic environment may have also contributed to the separation within the Arabian group, between *Acanthocercus* and *Pseudotrapelus*, as members of both genera prefer rocky habitats and are distributed in the mountainous areas of the Arabian shield (Arnold, 1980; Schätti and Gasperetti, 1994). Several other routes were previously hypothesized to enable the dispersal of reptiles from Africa to Arabia and vice versa (i.e., the Sinai Peninsula; a temporary land bridge of halite deposits in the Red Sea ~14–10 Mya; Bab el Mandeb Strait existing ca. 10–5.3 Mya; Bosworth et al., 2005) (e.g., Amer and Kumazawa, 2005; Pook et al., 2009; Portik and Papenfuss, 2012; Šmíd et al., 2013). The origin of *Pseudotrapelus* or the divergence of the Afro-Arabian agamid groups in our dating predates the two latter routes (Figs. 2 and 5). Although the northern land bridge of the Sinai Peninsula was established during the Miocene,

dispersal via this route for both *Acanthocercus* and *Xenagama* is unlikely, as both genera are not currently found in this area. In order for this to have been the dispersal route, both genera must have dispersed through this region and subsequently become extinct.

4.4. General biogeography of *Pseudotrapelus*

Pseudotrapelus diversification began during the late-Miocene, around 8.1 Mya (Table 1; Fig. 3B) probably in western Arabia (Fig. 5), with later expansions into Africa (i.e., *P. chlodnickii*) and eastern Arabia (i.e., *P. jensvindumi*). According to our results, cladogenesis within the genus continued during the Pliocene (Table 1; Fig. 3B). These divergence time estimations contrast with the Oligocene date suggested for *Pseudotrapelus* diversification in the morphological study by Melnikov and Melnikova (2013; 23–28 Mya). The radiation within *Pseudotrapelus* and its current distribution may have been shaped by a combination of several environmental conditions around the Red Sea from the mid-Miocene onwards.

The mid-Miocene climate change, especially the aridification process, triggered the expansion of arid areas in North Africa and Arabia (Ruddiman et al., 1989; Flower and Kennett, 1994; Le Houérou, 1997; Griffin, 2002; Edgell, 2006). The heliphilous nature of *Pseudotrapelus* and their affinity to hot arid regions is likely to have enabled their dispersal, range expansion and subsequent diversification within these areas. This environmental process has also been hypothesized to have triggered diversification within the agamid genus *Uromastix* (Wilms, 2001; Amer and Kumazawa, 2005). The progressive aridification and fluctuating climate increased sand areas in both Arabia and North Africa, and are likely to have promoted vicariance and isolation within montane or hard-substrate taxa, such as *Pseudotrapelus* (Fig. 1). Similar patterns were also suggested for the agamid genus *Agama* (Gonçalves et al., 2012), the rock-dwelling *Ptyodactylus* geckos (Metallinou et al., 2015) and snakes of the genus *Echis* (Arnold et al., 2009; Pook et al., 2009). Sedimentary basins later forming the Rub' al Khali and Sharqiyah (formerly Wahiba) sand deserts (Powers et al., 1966; Edgell, 2006; Preusser, 2009), characterize the interior of the Arabian Peninsula during the late-Miocene. These areas are likely to have restricted saxicolous species within the Arabian Peninsula to the mountainous areas, resulting in their currently localized range patterns (Arnold, 1986; Schätti and Gasperetti, 1994; Gardner, 2013; Fig. 1). In Africa, the Sahara desert restricts *Pseudotrapelus* to the mountains of north-eastern Africa (Schleich et al., 1996; Baha El Din, 2006).

The late-Miocene (ca. 8.1 Mya) divergence between the Western and Eastern *Pseudotrapelus* clades is hypothesized to have resulted from habitat fragmentation caused by the dynamic environment around the Red Sea (Figs. 3B and 5). The continuous mid- late-Miocene tectonic motions caused geological instability in western Arabia, for example leading to the creation of the Aqaba-Levant transform and periodic volcanic activity (Bosworth et al., 2005). In addition, a temporal land connection existed between Africa and Arabia, which later became submerged with the expansion of the Red Sea (~14–10 Mya; Richardson and Arthur, 1988; Rögl, 1999; Bosworth et al., 2005). These proposed vicariance events correspond to the divergence scenarios suggested for other reptile taxa in the region (e.g., *Uromastix*, Wilms, 2001; Amer and Kumazawa, 2005; *Echis*, Pook et al., 2009; *Hemidactylus*, Šmíd et al., 2013). The continuing habitat fragmentation during the Miocene-Pliocene transition may have also been associated with the divergence between *P. chlodnickii* and *P. sinaitus* in the northern area of the Red Sea ca. 5.2 Mya (Figs. 3B and 5).

The late-Miocene to mid-Pliocene divergence of the Arabian *Pseudotrapelus* inhabiting the coastal shield mountains and northern Oman (*P. aqabensis*, *P. neumanni*, *P. dhofarensis* and *P. jensvindumi*) remain difficult to interpret due to the poorly studied climatic and geological trends within the Arabian interior. This period was characterized by geological activity in the Arabian shield and northern Oman and by the expansion of the Red Sea (Girdler, 1991; Bosworth et al., 2005; Kusky et al., 2005; Edgell, 2006). Habitat fragmentation caused by environmental instability during this period is suggested to have facilitated the divergence in the agamid *Uromastix* (Amer and Kumazawa, 2005), with five taxa occurring in south-western Yemen (Wilms and Schmitz, 2007), and within *Hemidactylus* geckos (Šmíd et al., 2013, 2015). In northern Oman, the uplift of the Hajar Mountains continued during the Miocene-Pliocene transition, and the region was separated from the southern mountainous areas of Arabia by low basins with fluvial deposits, which later formed the Rub' al Khali and Sharqiyah sand deserts (Radies et al., 2004; Preusser et al., 2005; Edgell, 2006; Preusser, 2009). These basins and sandy deserts, ranging from the coast of north-eastern Oman to the interior of the Arabian Peninsula, formed biogeographical barriers which are purported to have influenced the late-Miocene split of the snake *Echis omanensis* from *Echis coloratus* (Pook et al., 2009). These barriers may have also been responsible for the split of the northern Omani *P. jensvindumi* around the same time (5.2 Mya; Table 1; Fig. 3B). This *Pseudotrapelus* divergence is another addition to recent studies presenting phylogenetic differences between northern and southern Omani populations (*Hemidactylus*, Carranza and Arnold, 2012; *Pristurus*, Badiane et al., 2014; *Ptyodactylus haselquistii*, Metallinou et al., 2015).

This study provides a first time-calibrated perspective on the historical biogeography of the genus *Pseudotrapelus* and its phylogenetically close relatives within the Agaminae, including their inferred geographical origin and proposed diversification drivers. Our study suggests that strong environmental changes, including geological instability and aridification (Flower and Kennett, 1994; Bosworth et al., 2005; Edgell, 2006), might have affected the distribution of *Pseudotrapelus*, triggering the evolution and divergence of lineages within Africa and Arabia. The distribution of *Pseudotrapelus* between these regions is restricted to rocky habitats, suggesting that the processes that led to its current range may also relate to the biogeographical patterns of other taxa in the region.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.ympev.2015.12.021>.

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