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Another record or a new taxon? A candidate species of *Chalcides* LAURENTI, 1768, in North Africa
(Squamata: Sauria: Scincidae)

Weiterer Nachweis oder neues Taxon? Ein Kandidat für eine bisher unbenannte nordafrikanische Art der Gattung *Chalcides* LAURENTI, 1768
(Squamata: Sauria: Scincidae)

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KURZFASSUNG

Die Gattung *Chalcides* umfaßt etwa 30 hauptsächlich nordafrikanische Skinkarten. Die Beurteilung ihres taxonomischen Status und ihrer Verbreitung war in der Literatur der vergangenen Jahre beträchtlichen Veränderungen unterworfen. Im Mai 2014, fanden die Autoren einen Skink vom *Chalcides*-Typ im Théniet El Had Nationalpark (Algerien) und klassifizierten ihn als *Chalcides mertensi* KLAUSEWITZ, 1954, aufgrund des Aussehens und der Verbreitung. Vom mitochondrialen Gen Cytochrom b wurde ein Abschnitt von 396 bp als Referenzwert gegenüber der vorhandenen Phylogenie der Gattung *Chalcides* sequenziert. Überraschenderweise stand der untersuchte Skink genetisch Exemplaren von *Chalcides minutus* CAPUTO, 1993 am nächsten, die in 300 km Entfernung gefunden worden waren. Der morphologische Vergleich des neuen Fundes mit der Originalbeschreibung zeigte, daß der Skink wahrscheinlich eine unbeschriebene *Chalcides*-Art darstellt und daß eine umfassende Revision der algerischen Skinke erforderlich sein wird, um die Phylogenie des *C. minutus-mertensi* Artenkomplexes zu entwirren.

ABSTRACT

The genus *Chalcides* comprises about 30 species of scincid lizards mainly distributed across North Africa, its taxonomic status and distribution as described in the literature has fluctuated in recent years. In May 2014, the authors found a skink of the *Chalcides* type in Théniet El Had National Park (Algeria) initially classified as *Chalcides mertensi* KLAUSEWITZ, 1954, based on its morphological similarity and distribution. A region of 396-bp of the cytochrome b mitochondrial gene was sequenced as a reference against a preexisting phylogeny of the genus *Chalcides*. Surprisingly, this skink was genetically closely related with specimens of *Chalcides minutus* CAPUTO, 1993, found 300 km away. Comparison of the morphology between the new record and the original descriptions showed that this skink is likely to represent a new species of *Chalcides*, and that a major revision of Algerian skinks is needed to unravel the phylogeny of the *C. minutus-mertensi* species complex.

KEY WORDS

Reptilia: Squamata: Scincidae: *Chalcides*; *Chalcides minutus*, *Chalcides mertensi*, candidate species, mitochondrial DNA, phylogeny, new country record, Théniet El Had National Park, Algeria

INTRODUCTION

Skinks (Sauropsida; Scincidae) are one of the most diverse families of lizards including about 1,600 species, i.e., one-quarter of all lizard species (HEDGES et al. 2014). Seven subfamilies of scincid lizards are currently recognized, with Scincinae being one of the largest (279 species - HEDGES 2014). This subfamily mostly includes members distributed in Africa and its arid regions, such as *Chalcides* (LAURENTI, 1768), *Scincus* (LAURENTI, 1768) or *Scincopus* (PETERS, 1864). The skinks of the genus *Chalcides* are distributed across northern Africa down to Somalia and Kenya, through the Levant to Turkey, Arabia, Iraq, Iran and Pakistan, as well as in southern Europe including Spain, Portugal, France, Italy and Greece (CARRANZA et al. 2008; GIOVANNOTTI et al 2007; KORNILIOS et al. 2010). The genus is represented by about 30 species, some of them very elongated and with different degrees of limb reduction (CAPUTO et al. 1995). One of these species, the Small Three-toed Skink *Chalcides minutus* CAPUTO, 1993, is only

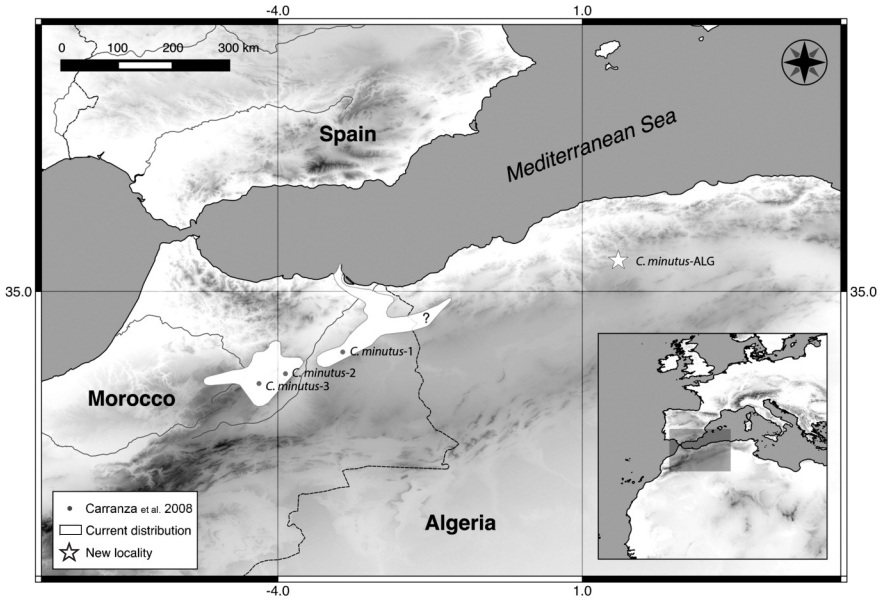

Fig. 1: Map showing the current distribution of *Chalcides minutus* Caputo, 1993, in Morocco and the new locality record at the Théniet El Had National Park in Algeria (star). Spatial data was retrieved from Mateo Miras et al. (2016) at < http://maps.iucnredlist.org/map.html?id=61481 >. Despite the distributional model predicting the presence of *C. minutus* in Algeria, the question mark symbol was added to highlight the absence of documented citations.

Abb. 1: Karte der Verbreitung von *Chalcides minutus* Caputo, 1993 in Marokko sowie der neue Fundort im Théniet El Had Nationalpark in Algerien (Stern). Die Verbreitungsangaben stammen von Mateo Miras et al. (2016) auf < http://maps.iucnredlist.org/map.html?id=61481 >. Obwohl das Verbreitungsmodell das Vorkommen von *C. minutus* in Algerien vorhersagt, wurde das Fragezeichen gesetzt, um das Fehlen von Nachweisen deutlich zu machen.

found in two isolated areas in northeastern Morocco and northwestern Algeria, including Melilla (Spain) that are separated by the Moulouya River valley (Fig. 1). Despite it is thought to be locally common in those areas, *C. minutus* is cataloged as a vulnerable species (VU) (Mateo Miras et al. 2016). Moreover, the taxonomic status of *C. minutus* is still not clear. As currently known, *C. minutus* is a composite of species, with individuals from the type locality forming a long independent lineage and the remaining most closely related to *Chalcides mertensi* Klausewitz, 1954 (Carranza et al. 2008).

MATERIALS AND METHODS

Study area.- Sampling was performed in Théniet El Had National Park (Algeria). On May 26, 2014, at approximately 13:00 h (GMT+1) with 17 °C, a skink of the *Chalcides* type (Fig. 2) was found sunbathing on a rock (WGS84 datum; 35.510710° N, 1.590127° E, 1,330 m a.s.l.). The specimen was detected in a meadow of a cleared forest of cedars (*Cedrus atlantica*) shaped by *Lolium multiflorum*, *Hordeum murinum* and *Anisantha rubens*. At first sight, the animal was identified as *C. mertensi* due to its morphological features and distribution, since this species appears to be present in the whole of northern Algeria (Mateo Miras et al. 2006) likely

Fig. 2: Image of the studied *Chalcides* specimen found in the Théniet El Had National Park, Algeria. Photo: J. Ferrer.

Abb. 2: Das untersuchte *Chalcides*-Exemplar aus dem Théniet El Had Nationalpark, Algerien. Photo: J. Ferrer.

including the Théniet El Had National Park (Dahmana et al. 2014). The animal was manually captured with all corresponding permits and deposited as voucher (reference code: CN9327) in the reptile collection of the Institute of Evolutionary Biology (IBE-CSIC), Barcelona, Spain.

Molecular analyses.- To better identify the specimen, tissue samples were taken for later analysis of mitochondrial DNA. Genomic DNA was extracted using the Speedtools Tissue DNA Extraction Kit (Biotools B&M Labs S. A.) following the manufacturer's instructions. Amplification for a region of 396-bp of the cytochrome b (cyt*b*) mitochondrial gene was conducted using the universal primers Cytb1 5´-CCA TCC AAC ATC TCA GCA TGA TGA AA-3´ and Cytb2 5´-CCC TCA GAA TGA TAT TTG TCC TCA-3' (Kocher et al. 1989) following conditions described elsewhere (e.g., Šmíd et al. 2013). Purification and sequencing of the PCR product was carried out by Macrogen Inc., Amsterdam. Chromatograms were checked manually, assembled and edited using Geneious Pro v.9.0.2 (Biomatters Ltd.).

To accurately determine the species to which the sampled skink specimen belongs, the authors downloaded from GenBank sequences of several representatives of the genus *Chalcides*, especially from the 'grass-swimming clade' (*sensu* Carranza et al. 2008) and aligned them with the specimen's sequence of interest using the online version of MAFFT v.7 (Katoh & Standley 2013) with default parameters. The focus was put on the 'grass-swimming clade' because of its similar morphological characters compared to the present specimen from Algeria. A combined dataset comprising 25 unique haplotypes (22 from Carranza et al. 2008; 2 from Giovannotti et al. 2013; 1 from the present study) was used for phylogenetic analyses (Table 1).

Both Maximum-likelihood (ML) and Bayesian Inference (BI) approaches were used for phylogenetic reconstruction. The jModelTest v. 2.1.4 (Darriba et al. 2012) was used to select the most appropriate model of nucleotide substitution under the Akaike Information Criterion (AIC). The ML trees were generated in RaxML ver. 7.0.3 (Stamatakis 2006) using the GTR+G model with a heuristic search with 100 random addition replicates. The reliability of node supports was validated using 1,000 bootstrap replicates. The BI analyses were performed with BEAST v1.8.0 (Drummond et al. 2012) using the HKY+I model. Two independent runs of 5 x 10^7 generations were carried out, sampling at intervals of 10,000 generations, producing 5,000 trees each. Convergence was confirmed with Tracer v.1.6.0 (Rambaut & Drummond 2013) checking that the corresponding values of effective sampling size (ESS) were higher than 200. The results of the two replicates were combined with LogCombiner applying a 10 % burn-in. The maximum clade credibility tree was generated from the combined distribution of topologies using Tree Annotator (both programs are included in BEAST package). Additionally, genetic distances were measured using MEGA6 (Tamura et al. 2013).

Morphological analysis.- Morphological measurements were taken using

Table 1: Details of materials and sequences used in this study.
Tab. 1: Das Material und die Sequenzen, die in der Untersuchung verwendet wurden.

Taxon ID	Country / Land	GenBank cyt*b*	Reference
C. chalcides chalcides-1	Italy	EU278212	Carranza et al. (2008)
C. chalcides vittatus-1	Tunisia	EU278216	Carranza et al. (2008)
C. chalcides vittatus-2	Italy	EU278215	Carranza et al. (2008)
C. guentheri	Israel	EU278233	Carranza et al. (2008)
C. lanzai-1	Morocco	EU278149	Carranza et al. (2008)
C. mauritanicus-1	Morocco	EU278203	Carranza et al. (2008)
C. mauritanicus-2	Morocco	EU278201	Carranza et al. (2008)
C. mauritanicus-3	Morocco	EU278200	Carranza et al. (2008)
C. mauritanicus-4	Morocco	EU278202	Carranza et al. (2008)
C. mertensi-1	Tunisia	EU278209	Carranza et al. (2008)
C. mertensi-CME2	Tunisia	KF057000	Giovannotti et al. (2013)
C. mertensi-CME3	Tunisia	KF057001	Giovannotti et al. (2013)
C. minutus-1	Morocco	EU278204	Carranza et al. (2008)
C. minutus-2	Morocco	EU278205	Carranza et al. (2008)
C. minutus-3	Morocco	EU278206	Carranza et al. (2008)
C. minutus-ALG	Algeria	KX963337	This study / Diese Arbeit
C. parallelus	Morocco	EU278153	Carranza et al. (2008)
C. pseudostriatus-1	Morocco	EU278217	Carranza et al. (2008)
C. pseudostriatus-2	Morocco	EU278218	Carranza et al. (2008)
C. striatus-1	Spain	EU278232	Carranza et al. (2008)
C. striatus-2	Spain	EU278231	Carranza et al. (2008)
C. striatus-3	Spain	EU278230	Carranza et al. (2008)
C. striatus-9	Spain	EU278228	Carranza et al. (2008)
C. striatus-11	Spain	EU278227	Carranza et al. (2008)
C. striatus-12	Spain	EU278226	Carranza et al. (2008)

a digital calliper. The following continuous characters, according to Caputo (1993), were measured: body length from snout tip to vent; tail length; distance between axilla and groin; distance between snout tip and cranial insertion of forelimb; forelimb length; hindlimb length; head length from snout tip to the ear opening; maximum head with; head depth at the angle of the jaw; frontal scale length; internasal width. Each measurement was made three times and the average was recorded.

RESULTS AND DISCUSSION

Phylogenetic comparison.- Both ML and BI analyses resulted in trees with exactly the same topology that only differed in node support. The authors therefore present the ML tree (Fig. 3), but also provide information about posterior probabilities values $\geq$ 0.95 using white dots. The tree is similar in topology to Fig. 2 in Carranza et al. (2008), with *C. minutus* being a polyphyletic species. Unlike the initial assessment, that is that the studied specimen belonged to *C. mertensi*, the molecular analyses revealed that it is more closely related to *C. minutus*-2 and *C. minutus*-3 from Azrou and Jbel Bou Iblane in the Middle Atlas, than to any other *Chalcides* along the phylogeny of the 'grass-swimming clade' *sensu* Carranza et al. (2008). The uncorrected genetic distances (p-distances, pairwise deletion) between the studied individual and *C. minutus*-2 and *C. minutus*-3 are 7.16 ± 1.38 % and 6.84 ± 1.35 %, respectively. The specimen diverges by more than 13 % (13.55 ± 1.85 %) with respect to *C. minutus*-1 from Debdou, Morocco, which is the type locality of the species. Given this wide genetic variability, it is clear that *C. minutus* as currently considered is a species complex (Carranza et al. 2008).

Morphological comparison.- Body measures (in mm) of the specimen

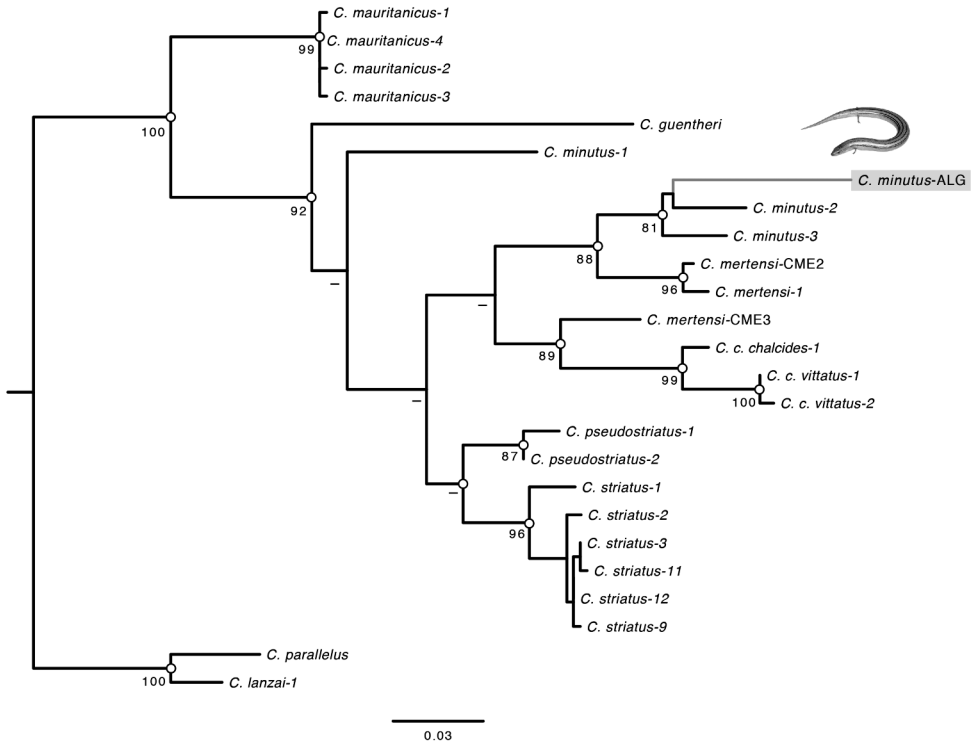

Fig. 3: Maximum-likelihood tree of the *Chalcides* 'grass-swimming clade' (*sensu* CARRANZA et al. 2008) including the new specimen from the Théniet El Had National Park, Algeria. Bootstrap values ≥ 70 % of the ML analysis are shown next to the nodes. White dots indicate Bayesian posterior probability values ≥ 0.95.

Abb. 3: Maximum-likelihood Baum des *Chalcides* 'grass-swimming clade' (*sensu* CARRANZA et al. 2008), einschließlich des vorliegenden Exemplars aus dem Théniet El Had Nationalpark in Algerien. Bootstrap-Werte ≥ 70 % der ML-Analyse stehen neben den Knoten; weiße Ringe an den Knoten zeigen Bayessche A-posteriori-Wahrscheinlichkeiten ≥ 0,95 an.

were approximately within the range of values (except tail that was regenerated) of *C. minutus* recorded by CAPUTO (1993) (interval values for each character in parentheses): body length from snout tip to vent = 125.00 (70.50 – 114.84); tail length = 47.00 (90.57 – 98.58); distance between axilla and groin = 105.00 (52.76 – 91.25); distance between snout tip and cranial insertion of forelimb = 21.45 (13.00 – 19.18); forelimb length = 5.53 (3.75 – 5.80); hindlimb length = 7.81 (5.33 – 8.69); head length from snout tip to the ear opening = 11.47 (7.13 – 9.96); maximum head with = 6.44 (4.40 – 6.79); head depth at the angle of the jaw = 4.89 (3.00 – 5.06); frontal scale length = 4.79 (3.30 – 4.09); internasal width = 2.23 (1.74 – 2.28). However, the specimen displayed the 'striped' dorsal pattern (Fig. 2) present in the species *C. mertensi* (see CAPUTO 1993). Moreover, it was considerably larger and wider than any sample from the original description. Although the above measurements are based upon a single individual, it is likely that these differences are the consequence of the large divergence time between *C. minutus* s. str. CAPUTO, 1993, and the studied sample specimen from Algeria (Fig. 3). Further studies including more individuals will be necessary to confirm these observations.

Taxonomic status of *Chalcides minutus*.- The authors consider that *C. minutus* consists of at least two different

species. One of these includes *C. minutus*-1 along with individuals from the type locality of *C. minutus* Caputo, 1993, and therefore should retain its taxonomic name. The new candidate species would have a wider distribution and include individuals previously assigned to *C. minutus* (i.e., *C. minutus*-2 and *C. minutus*-3 from Middle Atlas) along with the present, newly discovered specimen from Algeria. This separate species was also noticed in Carranza et al. (2008), but to date, no more specimens were found belonging to this diverging clade. On the other hand, the above results are based on a single gene phylogeny that may not necessarily represent the species tree. Taxonomic revisions may be necessary in the future after a more comprehensive study including several molecular markers performed.

A new candidate species of *Chalcides*.- The results show that the skink found in the Théniet El Had National Park (Algeria) is likely to represent a new species of *Chalcides*, different from *C. minutus* s. str. Caputo, 1993, and its sister taxon *C. mertensi* s. str. Klausewitz, 1954 (Fig. 3). In fact, the single genotype obtained is valuable owing to the scarce information on three-toed skink populations from Algeria, also considering the difficulty in obtaining samples from an area that is going through a difficult political situation. This discovery would constitute the first locality record for the new species in Algeria, and also a new species of vertebrates catalogued for the Théniet El Had National Park. This locality is quite far from the nearest populations previously assigned to *C. minutus* from the Middle Atlas, located more than 300 km to the west (Fig. 1).

Chalcides taxonomy, still unsolved.- The discovery of another specimen of a new candidate species of *Chalcides* in Algeria reveals that the taxonomy of skinks of the genus *Chalcides*, and especially from the 'grass-swimming clade', is still incomplete and needs to be revised. The authors emphasized the presence of a different evolutionary unit within the Small Three-toed Skink *C. minutus*, that should be named differently. Similarly, Giovannotti et al. (2013) discovered a new evolutionary unit within northern Tunisian populations of the Algerian Three-toed Skink *C. mertensi*. Altogether, these findings stress the importance of including individuals from Algeria on future projects aimed to explore the biodiversity of North African skinks. Next studies should clarify the taxonomic status of both *C. minutus* and *C. mertensi*, assigning specific names to distinct evolutionary linages. In this way, a major revision of Algerian skinks is needed to refine the phylogeny of the *C. minutus-mertensi* species complex.

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REFERENCES

Caputo, V. (1993): Taxonomy and evolution of the *Chalcides chalcides* complex (Reptilia, Scincidae) with the description of two new species.- Bolletino del Museo Regionale di Scienze Naturali di Torino, Torino; 1: 47-120.

Caputo, V. & Lanza, B. & Palmieri, R. (1995): Body elongation and limb reduction in the genus *Chalcides* Laurenti, 1768 (Squamata Scincidae): a comparative study.- Tropical Zoology, London; 8: 95-152.

Carranza, S. & Arnold, E. N. & Geniez, P. & Roca, J. & Mateo Miras, J. A. (2008): Radiation, multiple dispersal and parallelism in the skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert.- Molecular Phylogenetics and Evolution, San Diego; 46: 1071-1094.

Dahmana, A. & Merabet, K. (2014): Observations herpetologiques dans le Parc National de Theniet el Had. Rapport de mission de terrain effectuée le 21 mai 2014.- Atlantica, Alger; 2: 4-6.

Darriba, D. & Taboada, G. L. & Doallo, R. & Posada, D. (2012): jModelTest 2: more models, new heuristics and parallel computing.- Nature Methods, New York; 9: 772-772.

Drummond, A. J. & Suchard, M. A. & Xie, D. & Rambaut, A. (2012): Bayesian phylogenetics with BEAUti and the BEAST 1.7.- Molecular Biology and Evolution, Oxford; 29: 1969-1973.

Giovannotti, M. & Nisi Cerioni, P. & Kalboussi, M. & Aprea, G. & Caputo, V. (2007): Phylogeographic inferences from the mtDNA variation of the three-toed skink, *Chalcides chalcides* (Reptilia: Scincidae).- Journal of Experimental Zoology, New York, Hoboken; (B) 308 (3): 297-307.

Giovannotti, M. & Nisi Cerioni, P. & Splendiani, A. & Kalboussi, M. & Ruggeri, P. & Caputo, V. (2013): Mitochondrial DNA reveals high genetic divergence between populations of *Chalcides mertensi* Klausewitz, 1954 (Reptilia: Scincidae) from Tunisia.- Amphibia-Reptilia, Leiden; 34: 389-399.

Hedges, S. B. (2014): The high-level classification of skinks (Reptilia, Squamata, Scincomorpha).- Zootaxa, Auckland; 3765: 317-338.

Hedges, S. B. & Marion, A. B. & Lipp, K. M. & Marin, J. & Vidal, N. (2014): A taxonomic framework for typhlopid snakes from the Caribbean and other regions (Reptilia, Squamata).- Caribbean Herpetology [OA, online only, < http://www.caribbeanherpetology.org/ >]; 49: 1-61.

Katoh, K. & Standley, D. M. (2013): MAFFT multiple sequence alignment software version 7: improvements in performance and usability.- Molecular Biology and Evolution, Oxford; 30: 772-780.

Kocher, T. D. & Thomas, W. K. & Meyer, A. & Edwards, S. V. & Paabo, S. & Villablanca, F. X. & Wilson, A. C. (1989): Dynamics of mitocondrial DNA evolution in animals: amplification and sequencing with conserved primers.- Proceedings of the National Academy of Sciences of the USA, Washington; 86: 6196-6200.

Kornilios, P. & Kyriazi, P. & Poulakakis, N. & Kumlutaş, Y. & Mylonas, M. & Lymberakis, P. (2010): Phylogeography of the ocellated skink *Chalcides ocellatus* (Squamata, Scincidae), with the use of mtDNA sequences: A hitch-hiker's guide to the Mediterranean.- Molecular Phylogenetics and Evolution, San Diego; 54: 445-456.

Mateo Miras, J. A. & Joger, U. & Pleguezuelos, J. & Slimani, T. & El Mouden, H. & Geniez, P. (2006): *Chalcides mertensi*.- The IUCN Red List of Threatened Species 2006: e.T61480A12476313. WWW document available at < http://www.iucnredlist.org/details/61480/0 > [last accessed: November 2, 2016].

Mateo Miras, J. A. & Joger, U. & Pleguezuelos, J. & Slimani, T. & El Mouden, E. H. & Geniez, P. & Martínez-Solano, I. (2016): *Chalcides minutus*.- The IUCN Red List of Threatened Species 2016: e.T61481A12476589. WWW document available at < http://www.iucnredlist.org/details/61481/0 > [last accessed: October 27, 2016].

Rambaut, A. & Drummond, A. J. (2013): Tracer V1.6.- WWW document and software available at < http://beast.bio.ed.ac.uk/Tracer > [last accessed: October 27, 2016].

Šmíd, J. & Carranza, S. & Kratochvíl, L. & Gvoždik, V. & Nasher, A. K. & Moravec, J. (2013): Out of Arabia: a complex biogeographic history of multiple vicariance and dispersal events in the gecko genus *Hemidactylus* (Reptilia: Gekkonidae).- PloS one, Lawrence; 8 (5): e64018.

Stamatakis ,A. (2006): RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models.- Bioinformatics, Oxford; 22: 2688-2690.

Tamura, K. & Stecher, G. & Peterson, D. & Filipski, A. & Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.- Molecular Biology and Evolution, Oxford; 30: 2725-2729.

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