Moroccan herpetofauna distribution updates including a DNA barcoding approach

The herpetofauna of the Kingdom of Morocco is among the richest and most diverse in the Maghreb and Western Europe. This fact is clearly related with the considerable topological variation of the region, with the Rif and Atlas Mountains dividing the country into climatically different zones. The first step towards any ecological, conservation or modeling approaches concerning this rich diversity is to develop accurate distribution datasets. Bons & Geniez (1996) presented a detailed assessment of the known diversity at that time. However, various researchers have since then presented data indicating extension of known ranges for many species (e.g., Guzman et al. 2007; Harris et al. 2008, 2010; Barnestein et al. 2010; Barata et al. 2011; Beukema et al. 2013; Damas-Moreira et al. 2014), and it is clear that current records are still limited, especially in the eastern region (Barata et al. 2011; Beukema et al. 2013). At the same time, modeling approaches have been employed, which may help to highlight regions in need of further prospection (de Pous et al. 2010). In particular, in an extensive review of the distribution and biogeography of Moroccan amphibians (Beukema et al. 2013), models indicating regions of high probability of occurrence were presented for all species along with greatly improved distribution maps. New records for amphibians can therefore both be compared to these models and can be informative in determining whether distribution maps are stabilizing for such well-studied groups. Additionally, they can also be used to draw a parallel scenario in groups known to be much harder to locate, such as fossorial species or snakes.

The accuracy of distribution maps relies on species being correctly identified. This is not always simple – many forms of the Moroccan herpetofauna have recently been identified as species complexes (e.g., Rato et al. 2012), and several of these are "cryptic" implying that identification is made using molecular markers. This includes forms of the *Podarcis vaucheri* (Boulenger, 1905) complex (Pinho et al. 2007), or lineages within various geckos including *Quedenfeldtia* (Barata et al. 2012), *Ptyodactylus* (Perera & Harris 2010) and *Stenodactylus* (Metallinou et al. 2012). Other species can be difficult to identify when only juveniles or tadpoles are collected. Additionally, many groups such as snakes are often widely sampled as road-killed animals, and in some cases, identifying remains to the species level is again difficult. In these cases the use of a DNA "barcoding" approach (Hebert & Gregory 2005) can be useful.

In the present study, the authors compiled records of two expeditions to Morocco over a combined five week period in spring 2013 and 2014. Sampling covered a wide range of southern and eastern Morocco, and was done in complement to a recent survey of northern and central regions (Damas-Moreira et al. 2014). In total, 138 localities were sampled and 53 species recorded. GPS coordinates and a detailed list of species per locality are given in Table 1. Photos of most animals are available on request from the authors.

Distribution data was compared to published records and interesting new localities discussed in the text that follows. In cases where a species diagnosis based on morphological characters could not be made with certainty, a DNA barcoding approach was used. DNA was extracted using standard high-salt methods (Sambrook et al. 1989). PCR was used to amplify a region of the 12S rRNA, using published protocols (Harris et al. 1998). This gene was chosen rather than the classic barcoding COI region since comparative published data for 12S was already available for most of the presumed species. For example, 12S rRNA sequences are available for most toad species from Morocco (e.g., Harris & Perera 2009; de Pous et al. 2013), so this

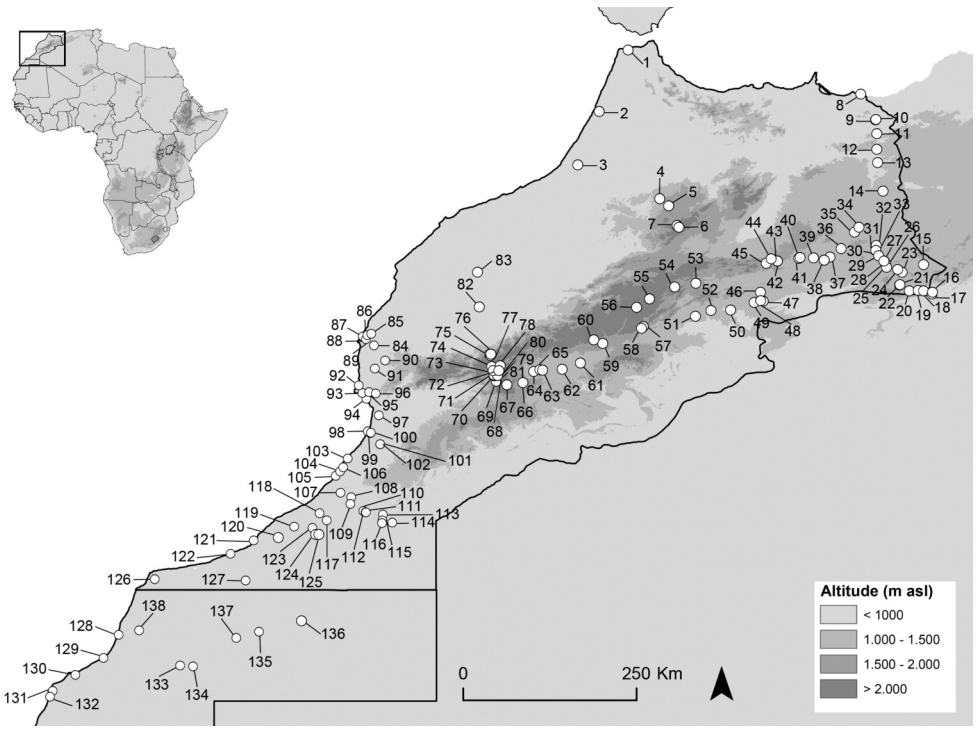

Fig. 1: Sampling points of the authors' expeditions to Morocco in spring 2013 and 2014. For locality coordinates and specimens observed see Table 1.

gene could be used to confirm the species diagnosis of tadpoles of this group. All species for which this approach was used are highlighted in Table 1, and are discussed in the text when relevant. New sequences from 16 specimens (marked by asterisks in Table 1) are published in GenBank (accession numbers KT005785 to KT005799 and KT205305).

Overall, the present findings indicate that even for better-known groups the current distribution maps are imprecise. Several new distribution points and extensions of the known range were found in the eastern region, where models predicated that more species are to be expected (BEUKEMA et al. 2013), or coincided with high probability areas proposed for certain species. This further demonstrates the value of these models, which can be used to guide future prospection in Morocco and other regions.

Bufonidae – *Amietophrynus mauritanicus* (SCHLEGEL, 1841), a specimen from locality 13 was superficially very similar to *Bufo spinosus* DAUDIN, 1803, but the aridity of the habitat was unusual for this latter species. DNA barcoding confirmed the specimen as *A. mauritanicus*. Other bufonids from this region need to be checked carefully, as misidentifications are possible. *Bufo spinosus* (Localities 13, 60, 67). Despite the considerable recent advances in mapping the amphibian distribution in Morocco (BEUKEMA et al. 2013), one new record (Locality 13, Fig. 2A) represents a considerable range extension in northeastern Morocco. Also for *Barbarophryne brongersmai* (HOOGMOED, 1972) (Localities 90 and 107), locality 90 represents a new locality in a region where distribution models predicted the occurrence of the species with high probability (BEUKEMA et al. 2013). These samples were

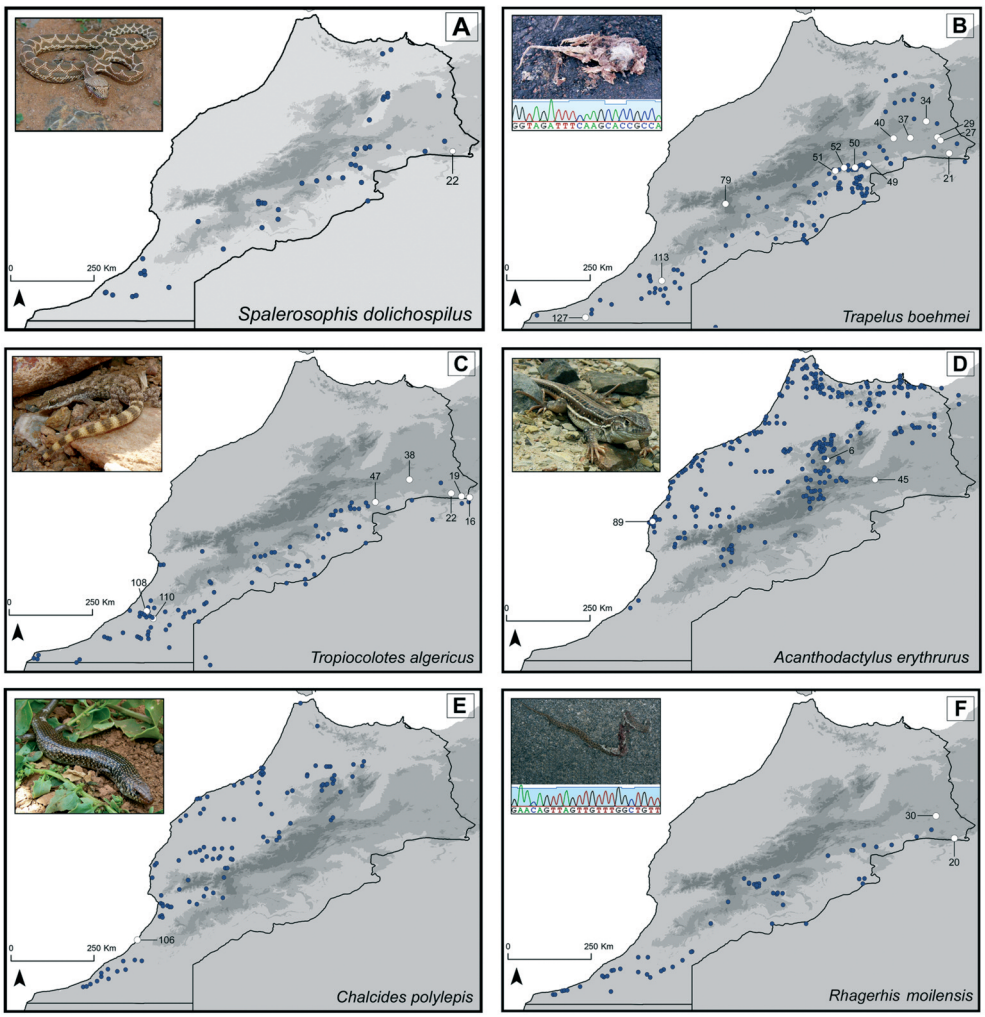

Fig. 2: Maps of species occurrence showing the most significant extensions of known ranges reported here. Blue dots represent published data records and white dots represent new distribution points detected in this study. The pictures of *Rhagerhis moilensis* (REUSS, 1834) and *Trapelus boehmei* WAGNER, MELVILLE, WILMS & SCHMITZ, 2011, include a part of the 12S chromatograms used to confirm genetically the species. All photographs were taken by Daniele Salvi.

tadpoles, and their identity was confirmed by DNA sequencing.

Testudinidae – *Testudo graeca* LINNAEUS, 1758 (Localities 35, 87, 88, 101). Locality 35 is a new record in the high plateau region of northeastern Morocco, where only a few isolated records of this species are known.

Agamidae – *Trapelus boehmei* WAGNER, MELVILLE, WILMS & SCHMITZ, 2011 (Localities 21, 27, 29, 34, 37, 40, 49, 50, 51, 52, 79, 113, 127, 133). Many new records were identified in the northeastern high plateau region (Localities 21, 27, 29, 34, 37 and 40). This is a region where models suggest that more surveys are needed to

complete distribution maps (Beukema et al. 2013), and this large number of new records for a fairly common and conspicuous species confirms it. Probably *T. boehmei* actually occurs throughout this whole region. One other locality, at a point north of Jebel Sirwah (Locality 79), is extremely interesting as it is both a new altitude record for the species (2,273 m a.s.l.; 1,500 previously known maximum altitude following Bons & Geniez 1996), and also extends the species distribution considerably into this region between the High and Anti Atlas Mountains. This sample was collected from a roadkill (Fig. 2B), and species identity was confirmed using DNA sequencing.

Phyllodactylidae – *Tarentola deserti* Boulenger, 1891 (Localities 15, 17, 24, 26, 32, 38). This species' distribution is restricted to the south of the Oriental and Meknes-Tafilalet provinces. Combined with other new records in the region (e.g., Damas-Moreira et al. 2014), these additional localities show a fairly continuous distribution near the Algerian border for *T. deserti*.

Gekkonidae – *Tropiocolotes algericus* Loveridge, 1947 (Localities 16, 19, 22, 38, 47, 108, 110, 132, 134, 135). Again, several new records for this underexplored northeastern region were found (Fig. 2C), in particular locality 38 represents a new northern record for the species in Morocco, and is quite isolated from currently known localities.

Lacertidae – *Acanthodactylus erythrurus* (Schinz, 1833) (Localities 6, 45, 89). Locality 45 constitutes a considerable extension of the known range for this species towards the eastern side of the Atlas Mountains (Fig. 2D). Interestingly, other more "Mediterranean" species are also known from this region, including *P. vaucheri* and *Scelarcis perspicillata perspicillata* (Duméril & Bibron, 1839) (Bons & Geniez 1996). The new localities are quite isolated from the known populations of the species and may thus be interesting from a phylogeographic viewpoint. Further prospection in the region for other typical Mediterranean species is clearly warranted. *Atlantolacerta andreanskyi* (Werner, 1929) (Localities 55, 56, 68, 80) may actually represent a species complex (Barata et al. 2012), with genetically distinct lineages occurring in populations separated by less than 50 km. Therefore these localities, although not far from other known localities in the Middle and High Atlas, are extremely important as further assessment may help to delimit taxonomic units within the complex. *Scelarcis perspicillata* (Localities 44, 60, 67, 78). Locality 44 confirms the presence of an isolated population in this eastern region. Locality 60 had the *S. perspicillata perspicillata* morphotype, but was in an area where *S. p. chabanaudi* (Werner, 1931) is distributed. Regarding *Mesalina olivieri* (Audouin, 1829) (Localities 13 and 45), locality 45 is a considerable extension of its known range, further highlighting the lack of records for this area.

Scincidae – *Chalcides montanus* Werner, 1931 (Localities 57, 76). With the confirmation of *Chalcides lanzai* Pasteur, 1967, as a full species (Carranza et al. 2008), there are very few records of *C. montanus* left in the Middle and High Atlas. Locality 57 is a new record locality along with only five or six more known from the Middle Atlas. *Chalcides polylepis* Boulenger, 1890 (Locality 106, Fig. 2E). The known distribution of this species had a considerable gap of over 100 km between the northern populations and those south of the Anti-Atlas. Locality 106, a sandy coastal area, lies almost in the middle of this gap and may therefore indicate that the distribution is actually continuous.

Colubridae/Lamprophiidae – For *Coronella girondica* (Daudin, 1803) and *Malpolon insignitus* (Geoffroy de St-Hilaire, 1809) (Locality 45) small extensions of their known ranges are reported here, again representing examples of "Mediterranean" species found in the area along with *A. erythrurus*. New records of *Psammophis schokari* (Forskål, 1775) (Localities 26, 31, 37, 39, 44, 97, 114, 120) from the northeastern region (Localities 31, 37, 39, 44) confirm that this species is widespread in the region. Damas-Moreira et al. (2014) reported new localities of *Spalerosophis dolichospilus* (Werner, 1923) in the northeastern region, where it had not previously been known (Bons & Geniez 1996). The additional record here (Locality 22) fur-

Table 1 (this page and two following pages): Details of sampled localities, including latitude, longitude and species found. The sixteen specimens that were identified using DNA barcoding approach are indicated with *.

Point	Latitude	Longitude	Species sampled
1	35.771	-5.787	*Discoglossus scovazzi*
2	34.853	-6.224	*Tarentola mauritanica*
3	34.046	-6.548	*Psammodromus algirus*
4	33.544	-5.318	*Psammodromus algirus, Tarentola mauritanica*
5	33.434	-5.180	*Bufotes boulengeri, Tarentola mauritanica*
6	33.112	-5.028	*Acanthodactylus erythrurus, Podarcis vaucheri, Timon tangitanus*
7	33.142	-5.051	*Podarcis vaucheri*
8	35.111	-2.300	*Acanthodactylus boskianus*
9	34.726	-2.078	*Chalcides ocellatus*
10	34.732	-2.075	*Agama impalearis, Tarentola mauritanica*
11	34.520	-2.058	*Agama impalearis, Chalcides ocellatus*
12	34.286	-2.057	*Acanthodactylus boskianus, Agama impalearis, Psammodromus algirus, Trogonophis wiegmanni*
13	34.080	-2.047	*Amietophrynus mauritanicus*, Bufo spinosus, Mesalina olivieri*
14	33.654	-1.963	*Stenodactylus mauritanicus*
15	32.552	-1.358	*Agama impalearis, Ptyodactylus oudrii, Tarentola deserti, Uromastyx nigriventris*
16	32.135	-1.223	*Acanthodactylus scutellatus, Tropiocolotes algericus*
17	32.107	-1.226	*Tarentola deserti**
18	32.159	-1.357	*Uromastyx nigriventris*
19	32.165	-1.438	*Cerastes cerastes, Tropiocolotes algericus*
20	32.158	-1.572	*Rhagerhis moilensis**
21	32.248	-1.714	*Trapelus boehmei*
22	32.241	-1.717	*Agama impalearis, Spalerosophis dolichospilus, Stenodactylus mauritanicus, Tropiocolotes algericus*
23	32.436	-1.679	*Uromastyx nigriventris*
24	32.479	-1.748	*Tarentola deserti*
25	32.510	-1.913	*Uromastyx nigriventris*
26	32.566	-1.924	*Agama impalearis, Psammophis schokari, Tarentola deserti*
27	32.597	-1.943	*Trapelus boehmei*
28	32.607	-1.955	*Acanthodactylus boskianus*
29	32.685	-2.031	*Trapelus boehmei*
30	32.759	-2.071	*Rhagerhis moilensis**
31	32.760	-2.070	*Psammophis schokari*
32	32.763	-2.070	*Bufotes boulengeri, Chalcides ocellatus, Tarentola deserti**
33	32.838	-2.066	*Acanthodactylus boskianus, Chalcides ocellatus, Stenodactylus mauritanicus*
34	33.110	-2.328	*Acanthodactylus boskianus, Trapelus boehmei*
35	33.038	-2.392	*Acanthodactylus boskianus, Testudo graeca*
36	32.795	-2.594	*Acanthodactylus boskianus, Mesalina guttulata**
37	32.665	-2.761	*Psammophis schokari, Trapelus boehmei*
38	32.615	-2.846	*Tarentola deserti, Tropiocolotes algericus, Uromastyx nigriventris*
39	32.653	-3.008	*Psammophis schokari*
40	32.656	-3.211	*Ptyodactylus oudrii, Trapelus boehmei*
41	32.645	-3.227	*Ptyodactylus oudrii, Uromastyx nigriventris*
42	32.602	-3.551	*Amietophrynus mauritanicus*
43	32.602	-3.553	*Pelophylax saharicus, Ptyodactylus oudrii*
44	32.636	-3.641	*Agama impalearis, Psammophis schokari, Ptyodactylus oudrii, Scelarcis perspicillata, Timon tangitanus*
45	32.569	-3.719	*Acanthodactylus erythrurus, Coronella girondica, Malpolon insignitus, Mesalina olivieri*
46	32.133	-3.801	*Amietophrynus mauritanicus, Mauremys leprosa*
47	32.005	-3.765	*Acanthodactylus boskianus, Mesalina guttulata, Stenodactylus mauritanicus, Tropiocolotes algericus*
48	31.997	-3.801	*Psammophis schokari**
49	31.982	-3.900	*Trapelus boehmei*
50	31.869	-4.251	*Trapelus boehmei*
51	31.774	-4.781	*Trapelus boehmei*
52	31.863	-4.546	*Trapelus boehmei*
53	32.267	-4.777	*Acanthodactylus boskianus, Agama impalearis*
54	32.210	-5.092	*Timon tangitanus*
55	32.036	-5.466	*Atlantolacerta andreanskyi, Discoglossus scovazzi, Podarcis vaucheri*
56	31.912	-5.663	*Atlantolacerta andreanskyi*

Table 1 (part 2):

Point	Latitude	Longitude	Species sampled
57	31.621	-5.560	*Chalcides montanus, Mesalina guttulata, Ptyodactylus oudrii, Quedenfeldtia moerens, Saurodactylus brosseti, Tarentola mauritanica*
58	31.595	-5.593	*Tarentola mauritanica*
59	31.365	-6.172	*Agama impalearis, Amietophrynus mauritanicus, Pelophylax saharicus*
60	31.421	-6.304	*Bufo spinosus, Pelophylax saharicus, Scelarcis perspicillata*
61	31.075	-6.505	*Acanthodactylus boskianus, Uromastyx nigriventris*
62	30.978	-6.779	*Uromastyx nigriventris*
63	30.969	-7.071	*Uromastyx nigriventris*
64	30.944	-7.210	*Acanthodactylus pardalis, Amietophrynus mauritanicus, Mauremys leprosa, Mesalina guttulata, Pelophylax saharicus*
65	30.972	-7.111	*Uromastyx nigriventris*
66	30.779	-7.371	*Acanthodactylus boskianus, Agama impalearis*, Lytorhynchus diadema, Ptyodactylus oudrii*
67	30.744	-7.610	*Acanthodactylus boskianus, Amietophrynus mauritanicus, Bufo spinosus, Hyla meridionalis, Natrix maura, Podarcis vaucheri, Psammodromus algirus, Quedenfeldtia trachyblepharus, Scelarcis perspicillata, Timon tangitanus*
68	30.885	-7.748	*Atlantolacerta andreanskyi, Quedenfeldtia trachyblepharus**
69	30.802	-7.767	*Agama impalearis, Pelophylax saharicus, Psammodromus algirus, Timon tangitanus,*
70	30.877	-7.802	*Acanthodactylus boskianus*
71	30.891	-7.802	*Acanthodactylus boskianus, Agama impalearis, Ptyodactylus oudrii, Quedenfeldtia moerens, Timon tangitanus*
72	30.960	-7.728	*Timon tangitanus*
73	30.956	-7.817	*Hemorrhois hippocrepis*
74	31.017	-7.837	*Chamaeleo chamaeleon*
75	31.200	-7.870	*Quedenfeldtia moerens*
76	31.208	-7.851	*Chalcides montanus, Natrix maura, Quedenfeldtia moerens*
77	31.190	-7.854	*Podarcis vaucheri*
78	31.035	-7.709	*Acanthodactylus boskianus, Podarcis vaucheri, Quedenfeldtia trachyblepharus, Scelarcis perspicillata, Timon tangitanus*
79	30.886	-7.746	*Trapelus boehmei**
80	30.886	-7.746	*Atlantolacerta andreanskyi*
81	30.944	-7.741	*Quedenfeldtia trachyblepharus, Timon tangitanus*
82	31.919	-8.025	*Tarentola mauritanica*
83	32.441	-8.049	*Amietophrynus mauritanicus, Natrix maura*
84	31.345	-9.594	*Tarentola mauritanica*
85	31.520	-9.634	*Eumeces algeriensis, Tarentola mauritanica*
86	31.502	-9.697	*Chalcides mionecton*
87	31.481	-9.760	*Saurodactylus brosseti, Testudo graeca*
88	31.466	-9.759	*Chalcides mionecton, Testudo graeca*
89	31.442	-9.718	*Acanthodactylus erythrurus, Chalcides mionecton, Trogonophis wiegmanni*
90	31.122	-9.430	*Barbarophryne brongersmai*, Saurodactylus brosseti*
91	30.998	-9.583	*Saurodactylus brosseti*
92	30.746	-9.824	*Agama impalearis*
93	30.633	-9.768	*Tarentola mauritanica*
94	30.544	-9.706	*Saurodactylus brosseti*
95	30.646	-9.671	*Agama impalearis, Tarentola mauritanica*
96	30.625	-9.565	*Timon tangitanus*
97	30.299	-9.521	*Psammophis schokari, Saurodactylus brosseti*
98	30.057	-9.687	*Acanthodactylus aureus*
99	30.031	-9.645	*Amietophrynus mauritanicus*, Mauremys leprosa*
100	30.038	-9.639	*Eumeces algeriensis, Saurodactylus brosseti*
101	29.865	-9.499	*Testudo graeca*
102	29.865	-9.500	*Saurodactylus brosseti*
103	29.651	-9.990	*Saurodactylus brosseti*
104	29.514	-10.058	*Agama impalearis*
105	29.454	-10.104	*Tarentola mauritanica*
106	29.387	-10.172	*Chalcides mionecton, Chalcides polylepis, Saurodactylus brosseti*
107	29.137	-10.098	*Barbarophryne brongersmai*, Natrix maura, Pelophylax saharicus*
108	29.064	-9.933	*Saurodactylus brosseti, Tropiocolotes algericus*
109	28.968	-9.952	*Acanthodactylus boskianus*
110	28.863	-9.755	*Tarentola mauritanica, Tropiocolotes algericus*

Table 1 (part 3):

Point	Latitude	Longitude	Species sampled
111	28.839	-9.712	*Uromastyx nigriventris*
112	28.838	-9.721	*Uromastyx nigriventris*
113	28.809	-9.464	*Trapelus boehmei*
114	28.688	-9.318	*Psammophis schokari, Uromastyx nigriventris*
115	28.718	-9.466	*Uromastyx nigriventris*
116	28.677	-9.472	*Uromastyx nigriventris*
117	28.719	-10.302	*Saurodactylus brosseti, Tarentola mauritanica*
118	28.829	-10.412	*Tarentola mauritanica*
119	28.628	-10.791	*Saurodactylus brosseti*
120	28.455	-11.038	*Psammophis schokari**
121	28.416	-11.398	*Saurodactylus brosseti*
122	28.221	-11.750	*Saurodactylus brosseti*
123	28.607	-10.519	*Saurodactylus brosseti*
124	28.498	-10.478	*Ptyodactylus oudrii, Saurodactylus brosseti, Tarentola mauritanica*
125	28.499	-10.428	*Saurodactylus brosseti*
126	27.835	-12.884	*Hemorrhois algirus*
127	27.820	-11.522	*Trapelus boehmei*
128	27.004	-13.428	*Tarentola chazaliae*
129	26.652	-13.652	*Tarentola chazaliae*
130	26.400	-14.075	*Acanthodactylus aureus**, *Tarentola chazaliae*
131	26.155	-14.418	*Acanthodactylus busacki, Saurodactylus brosseti*
132	26.075	-14.457	*Acanthodactylus busacki, Saurodactylus brosseti, Tropiocolotes algericus*
133	26.541	-12.506	*Trapelus boehmei*
134	26.529	-12.307	*Sphenops sphenopsiformis, Tropiocolotes algericus*
135	27.051	-11.322	*Stenodactylus mauritanicus, Tropiocolotes algericus*
136	27.199	-10.694	*Acanthodactylus boskianus*
137	26.955	-11.664	*Tarentola annularis*
138	27.067	-13.118	*Rhagerhis moilensis**

ther increases the known range close to the Algerian border in this region. Bons & Geniez (1996) suggested *Rhagerhis moilensis* (Reuss, 1834) (Localities 20, 30, 138) to be "represented in all regions with a Saharan climate except for a zone between Boudenib and Figuig". Locality 20 is exactly in this region (Fig. 2F), while locality 30 not only extends the range in the region but is the most northern record for the species in Morocco. Roadkilled samples were confirmed through DNA sequencing.

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