Development and characterization of polymorphic microsatellite loci for spiny-footed lizards, *Acanthodactylus scutellatus* group (Reptilia, Lacertidae) from arid regions

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**Abstract**

**Background:** Spiny-footed lizards constitute a diverse but scarcely studied genus. Microsatellite markers would help increasing the knowledge about species boundaries, patterns of genetic diversity and structure, and gene flow dynamics. We developed a set of 22 polymorphic microsatellite loci for cross-species amplification in three taxa belonging to the *Acanthodactylus scutellatus* species group, *A. aureus*, *A. dumerili*/*A. senegalensis* and *A. longipes*, and tested the same markers in two other members of the group, *A. scutellatus* and *A. taghitensis*.

**Results:** Amplifications in *A. aureus*, *A. longipes* and *A. dumerili*/*A. senegalensis* were successful, with markers exhibiting a number of alleles varying between 1 and 19. Expected and observed heterozygosity ranged, respectively, between 0.046–0.893 and 0.048–1.000. Moreover, 17 and 16 loci were successfully amplified in *A. scutellatus* and *A. taghitensis*, respectively.

**Conclusion:** These markers are provided as reliable genetic tools to use in future evolutionary, behavioural and conservation studies involving species from the *A. scutellatus* group.

**Keywords:** Cross-species amplification, Nuclear markers, Population genetics, Sahara-Sahel

**Background**

Spiny-footed lizards, or fringe-toed lizards (genus *Acanthodactylus*), form a clade of small ground-dwelling lizards occurring mostly in arid regions [1, 2]. The genus is the most specious of the Lacertidae family and is widely distributed, occurring from the Iberian Peninsula, south of the Mediterranean Basin, across the Sahara-Sahel, Arabian Peninsula, and as far east as India [1, 2]. Being often abundant and occupying different types of open, flat habitats, these lizards are important elements of the vertebrate communities of deserts and arid ecosystems in North Africa and Arabia. Despite their diversity, knowledge about most of the species is still scarce and their taxonomy is partly unresolved [1–4]. Most authors agree on splitting *Acanthodactylus* into several species groups or complexes [1, 4]. The *A. scutellatus* species group shows one of most complex taxonomies [2, 5–8]. It includes six species according to the last global revision (*A. aureus*, *A. dumerili*, *A. longipes*, *A. scutellatus*, *A. senegalensis* and *A. taghitensis*) [3]. However, urgent systematic revision based on molecular data is needed given that: (1) eastern populations previously attributed to *A. longipes* are now considered a new species (*A. aegyptius*, [7]); and (2) species boundaries in *A. scutellatus*, *A. longipes*, *A. dumerili* and *A. senegalensis* as currently defined remain uncertain.

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tissue samples using EasySpin Kit (Qiagen), follow-
inons. The changes to the extraction protocol were as-
conditions) and then pooled in equimolar concentra-
in addition, assessment of gene flow in such areas of sym-
would be critical for a better understanding of the spe-
Microsatellite markers have been extremely useful, and afford-
numerous topics in conservation and evolutionary biology, al-
allowing, e.g., gene flow and population structure assess-
demographic inferences and genetic diversity estimation [10–12]. Yet, no microsatellite markers are available for the Acanthodactylus genus.

Here we describe a set of 22 polymorphic microsatel-
loci (tri- and tetranucleotides) characterized in four spe-
species included in the A. scutellatus species group (A. aureus, A. longipes and A. dumerili/A. senegalensis). Con-
sidering the uncertain species boundaries for A. dumer-
ili and A. senegalensis, we chose to refer to them as A. dumerili/A. senegalensis in the following sections. We fur-
ther tested cross-amplification of these markers in two other members of the species group, A. scutellatus and A. taghitensis.

Methods
A genomic library was constructed from 12 specimens of A. aureus, collected across the species’ distribution. A tissue sample was collected from the tail tip by fol-
owing ethical guidelines for use of live reptiles (http://
www.aaalac.org/accreditation/Guidelines_for_Use_of_ Live_Amphibians_and_Reptiles.pdf). All specimens were released on site after sample collection. Field-
work was developed with permission from the Minis-
tère Délégué auprès du Premier Ministre Chargé de l’Environnement, Nouakchott (Permit: 460/MDE/PNBA) and from the Haut Commissariat aux Eaux et Forêts et l’Environnement, Nouakchott (Permit: 460/MDE/PNBA) under the Directive 2010/63/EU of the European Parliament. Genomic DNA extractions were performed from tissue samples using EasySpin Kit (Qiagen), follow-
ing an adapted protocol for tissue samples (with minor adjustments to centrifugation and incubation conditions) and then pooled in equimolar concentra-
tions. The changes to the extraction protocol were as-
follows: after adding the AB solution, we centrifuged at 3700 rpm for 4 min (instead of 4000 rpm for 2 min). After adding the Wash solution, we centrifuged at 3700 rpm for 6 min (instead of 8000 rpm for 1 min). After repeating the Wash solution step and discarding flow-through, we centrifuged at 3700 rpm for 10 min (instead of 14,000 rpm for 5 min). After adding the Elution Buffer, we incubated at 55° for 15 min (instead of 50° for 10 min). Last centrifugation was at 3700 rpm (instead of 14,000 rpm). Microsatellite isolation was developed through 454 GS-FLX Titanium pyrose-
quencing of enriched DNA libraries [13]. This process was developed by GenoScreen (http://www.pasteur-
lille.fr/fr/recherche/plateformes/tordeaux_plat.html) and included sequence data quality control, assembly and analyses, and primer design. Initially, 50 loci were selected from the library and tested for amplification using seven samples of A. aureus, A. dumerili/A. senegalensis, and A. longipes. Thirty loci amplified reliably, producing fragments of the expected size. Twenty-two were polymorphic (Table 1), and amplified with dif-
fential success in the following target species: 21 in A. aureus, 18 in A. longipes and 15 in A. dumerili/A. senegalensis. These 22 loci were therefore used for genotyping 38 samples of A. aureus, 35 of A. longipes, and 43 of A. dumerili/A. senegalensis, collected along coastal Morocco and Mauritania (Table 2; Fig. 1). Markers were multiplexed in four reactions, using M13-primer genotyping protocol with four different dye-labelled tails, and forward primer concentration of 1/10 of dye-labelled reverse primer [14] (Table 1). The transferability of the primers was tested by cross-
amplification of five specimens of A. scutellatus (from Morocco, Tunisia, Libya, Algeria and Egypt) and one specimen of A. taghitensis (Mauritania). PCR amplifi-
cations were conducted using the Multiplex PCR Kit (QIAGEN) following manufacturer’s instructions in a final 10 μl volume, always in the presence of a nega-
tive control. Touchdown PCR conditions started with an initial denaturation step of 15 min at 95 °C; first round (nine cycles) of 30 s at 95 °C, 90 s for annealing (decreasing 0.5 °C per cycle) at 58–54 °C (Multiplexes 1, 2 and 3) or 55–51 °C (Multiplex 4), and 30 s at 72 °C; second round (31 cycles) of 30 s at 95 °C, 1 min at 54 °C (Multiplexes 1, 2 and 3), or 51 °C (Multiplex 4), 30 s at 72 °C, and a final extension of 30 min at 60 °C. Amplification was performed in Biorad T100 Thermal Cyclers, and the PCR products were later separated by capillary electrophoresis on an automatic sequencer ABI3130xl Genetic Analyzer (AB Applied Biosystems). Fragments were scored against the GeneScan-500 LIZ Size Standard using the GENEMAPPER 4.1 (Applied Biosystems) and manually checked twice. Potential
evidences of null alleles, allelic dropouts and stuttering were assessed using MICRO-CHECKER v2.2.3 [15] at each locus, for each population. Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed in GENEPOP online version (http://wbiomed.curtin.edu.au/genepop/); with subsequent Bonferroni correction in both cases. Observed and expected heterozygosity were computed using GenAIEx v6.501 [16]. For some populations, samples were obtained from different localities. Consequently, analyses were based on groups of samples that are not necessarily panmitic populations, which probably accounts for deviations from Hardy–Weinberg equilibrium.

Table 1 Global characterization of the 22 microsatellite loci characterized in Acanthodactylus aureus, A. dumerili/A. senegalensis and A. longipes

| Locus | GenBank assess no. | Repeat | Primer sequence (5′–3′) | Multiplex | TD | Dye |
|-------|-------------------|--------|-------------------------|-----------|----|-----|
| Ac1   | KU295182          | (ATAC)₈ | F: CTGTGGTATATCCCTTCCTCCCA R: GGTGGCTCTCCTCACAGCTATTT | 1         | 58°/54° | FAM |
| Ac4   | KU295183          | (TTC)₂₁ | F: AGAGCTTCGTGAGATTTCCCTTTTT R: CCGATCGATGTTGGCGGTT | 3         | 58°/54° | VIC |
| Ac5   | KU295184          | (AAC)₁₅ | F: GTGTGGTTCACGTGGTCCCTCC R: AGTGCTCTGAGCTCAACAGCAG | 1         | 58°/54° | VIC |
| Ac6   | KU295185          | (TTG)₁₀ | F: GATCCGCTTCAAGATTTTGCACGAG GGTTCAGGTTGCTGGG | 4         | 55°/51° | NED |
| Ac8   | KU295186          | (TTG)₁₁ | F: GAGATCGGAAAGGACTGAGGTTTA CCGATGCGAAGGAAAGGCT | 1         | 58°/54° | NED |
| Ac9   | KU295187          | (CAA)₁₅ | F: TCTCAGGCTGATTTCCTTCCAGG R: CGCAGGAGGAAGGAAAGGCT | 1         | 58°/54° | PET |
| Ac13  | KU295188          | (AAC)₁₄ | F: TCTCAGGCTGATTTCCTTCCAGG R: GTCTACACGACATCATATGC | 2         | 58°/54° | VIC |
| Ac14  | KU295189          | (CAA)₁₀ | F: TTAAGTGGGAAATGTTGGCATGT R: GCTCACGCTGTTGCTTAC | 2         | 58°/54° | VIC |
| Ac16  | KU295190          | (AGG)₁₀ | F: AGTCAATGTATCCACCTACCCCTTC R: GTCTCAGGAAAGGAAAGGCT | 2         | 58°/54° | VIC |
| Ac19  | KU295191          | (AAC)₁₄ | F: TCTCAGGCTGATTTCCTTCCAGG R: CGCAGGAGGAAGGAAAGGCT | 2         | 58°/54° | PET |
| Ac20  | KU295192          | (GTT)₁₁ | F: ATGCTAGTACGCTGAAAAAGGGGA R: GCTCACGCTGTTGCTTAC | 2         | 58°/54° | VIC |
| Ac23  | KU295193          | (CAT)₈  | F: GCGAAGCGAGCAGAGGTTTTT R: ACCCTGCTTTCTCATG | 1         | 58°/54° | FAM |
| Ac28  | KU295194          | (ACAT)₈ | F: GTTCGCAAAAGGATGAGGC R: GGAAGAGCCATGCTCTAGCA | 4         | 55°/51° | PET |
| Ac31  | KU295195          | (GTT)₁₀ | F: GAAAGTGCTACGTGAGGTTTTT R: GTCTACACGACATCATATGC | 4         | 55°/51° | FAM |
| Ac32  | KU295196          | (TTC)₁₅ | F: TAGTCCGTAACCTTCACGGTTTTT R: GCTCACGCTGTTGCTTAC | 3         | 58°/54° | VIC |
| Ac33  | KU295197          | (TGT)₁₆ | F: GGGACTGAAATGATGTTGGTTTTG R: GTCTACACGACATCATATGC | 3         | 58°/54° | VIC |
| Ac36  | KU295198          | (TGT)₉  | F: GTCACTGAGTGTTCAGTTGG T: GCCAAGTGGGAAACACATAGC | 3         | 58°/54° | VIC |
| Ac43  | KU295199          | (CAA)₁₃ | F: AGCTTGTGTAATGTTGCTTTTTG R: GAGCAGAAACACATATAGCAAGC | 4         | 55°/51° | FAM |
| Ac44  | KU295200          | (GGA)₁₃ | F: TCCTTTAGAACAGATGTTGCTTTT R: GCTCACGCTGTTGCTTAC | 4         | 55°/51° | VIC |
| Ac45  | KU295201          | (CAA)₁₀ | F: AGGCAATGGAAGCAGAGGAA R: GCTCACGCTGTTGCTTAC | 4         | 55°/51° | VIC |
| Ac47  | KU295202          | (ACA)₁₁ | F: CTGCCTCTTGGCTTCTG T: GCCAAGTGCACTCCTCTAC | 4         | 55°/51° | VIC |
| Ac49  | KU295203          | (AAC)₁₁ | F: CAAAAGATAATTGTTTGGGAGG R: GTAAAACATGAGGGAAGGAC | 4         | 55°/51° | VIC |

TD touchdown temperatures
| Code | Species  | Latitude  | Longitude | Local                                      | Country          |
|------|----------|-----------|-----------|--------------------------------------------|------------------|
| 6477 | A. aureus| 20.9444   | −16.5494  | Kerekchet et Teintâne, extreme N           | Mauritania       |
| A366 | A. aureus| 21.2182   | −16.8432  | Nouâdhibou, 40 km S of                     | Mauritania       |
| A367 | A. aureus| 21.2182   | −16.8432  | Nouâdhibou, 40 km S of                     | Mauritania       |
| A368 | A. aureus| 21.2182   | −16.8432  | Nouâdhibou, 40 km S of                     | Mauritania       |
| A369 | A. aureus| 21.2182   | −16.8432  | Nouâdhibou, 40 km S of                     | Mauritania       |
| A358 | A. aureus| 21.0978   | −16.6998  | Nouâdhibou, 70 km S of                     | Mauritania       |
| A359 | A. aureus| 21.0978   | −16.6998  | Nouâdhibou, 70 km S of                     | Mauritania       |
| A360 | A. aureus| 21.0978   | −16.6998  | Nouâdhibou, 70 km S of                     | Mauritania       |
| A361 | A. aureus| 21.0978   | −16.6998  | Nouâdhibou, 70 km S of                     | Mauritania       |
| A362 | A. aureus| 21.0978   | −16.6998  | Nouâdhibou, 70 km S of                     | Mauritania       |
| A363 | A. aureus| 21.0978   | −16.6998  | Nouâdhibou, 70 km S of                     | Mauritania       |
| 5449 | A. aureus| 20.8233   | −16.5882  | PNBA: Kerekchet et Teintâne, central       | Mauritania       |
| 5458 | A. aureus| 20.8023   | −16.5718  | PNBA: Kerekchet et Teintâne, central       | Mauritania       |
| 5171 | A. aureus| 20.7190   | −16.6195  | PNBA: Kerekchet et Teintâne, central       | Mauritania       |
| 5172 | A. aureus| 20.7190   | −16.6195  | PNBA: Kerekchet et Teintâne, central       | Mauritania       |
| 5173 | A. aureus| 20.7251   | −16.6291  | PNBA: Kerekchet et Teintâne, W side       | Mauritania       |
| 5176 | A. aureus| 20.7620   | −16.6183  | PNBA: Kerekchet et Teintâne, W side       | Mauritania       |
| 6443 | A. aureus| 20.7764   | −16.6287  | PNBA: Kerekchet et Teintâne, Western face  | Mauritania       |
| 6446 | A. aureus| 20.8115   | −16.6158  | PNBA: Kerekchet et Teintâne, Western face  | Mauritania       |
| 6448 | A. aureus| 20.8115   | −16.6158  | PNBA: Kerekchet et Teintâne, Western face  | Mauritania       |
| 6435 | A. aureus| 20.7938   | −16.5462  | PNBA: Sebkhet Dbâdeb et Teintâne, W margin | Mauritania       |
| 5437 | A. aureus| 28.8731   | −10.7027  | Aoreora, 15 km E of (Plage Blanche)        | Morocco          |
| 5438 | A. aureus| 28.8731   | −10.7027  | Aoreora, 15 km E of (Plage Blanche)        | Morocco          |
| 5439 | A. aureus| 28.8731   | −10.7027  | Aoreora, 15 km E of (Plage Blanche)        | Morocco          |
| 5440 | A. aureus| 28.8731   | −10.7027  | Aoreora, 15 km E of (Plage Blanche)        | Morocco          |
| 5441 | A. aureus| 28.8731   | −10.7027  | Aoreora, 15 km E of (Plage Blanche)        | Morocco          |
| 5442 | A. aureus| 28.8731   | −10.7027  | Aoreora, 15 km E of (Plage Blanche)        | Morocco          |
| 5443 | A. aureus| 28.8731   | −10.7027  | Aoreora, 15 km E of (Plage Blanche)        | Morocco          |
| 5435 | A. aureus| 28.7447   | −10.7438  | Aoreora, 25 km S of                        | Morocco          |
| 5436 | A. aureus| 28.7447   | −10.7438  | Aoreora, 25 km S of                        | Morocco          |
| 5566 | A. aureus| 29.8511   | −9.7706   | Bou Soun                                   | Morocco          |
| 10,625| A. aureus| 28.5177   | −11.2970  | Douira, N of                               | Morocco          |
| 10,636| A. aureus| 28.3701   | −11.4387  | Douira, S of                               | Morocco          |
| 10,634| A. aureus| 28.1544   | −11.9117  | Laareig                                    | Morocco          |
| 10,636| A. aureus| 27.9291   | −12.2945  | Leirane                                    | Morocco          |
| 9048 | A. aureus| 28.9662   | −10.6000  | Plage Blanche                              | Morocco          |
| 10,635| A. aureus| 28.0875   | −12.0814  | Sidi Akhfennir                             | Morocco          |
| 10,624| A. aureus| 28.5479   | −10.9583  | Tahet Alid                                 | Morocco          |
| 6470 | A. dum./sen.| 20.9172 | −16.5418  | Kerekchet et Teintâne, extreme N           | Mauritania       |
| 6473 | A. dum./sen.| 20.9204 | −16.5415  | Kerekchet et Teintâne, extreme N           | Mauritania       |
| 6474 | A. dum./sen.| 20.9204 | −16.5415  | Kerekchet et Teintâne, extreme N           | Mauritania       |
| 3618 | A. dum./sen.| 20.0500 | −16.0582  | PNBA: Adeim el Marrâr                     | Mauritania       |
| 5111 | A. dum./sen.| 19.9733 | −16.1874  | PNBA: Agreigrat, 1 km E of                 | Mauritania       |
| 6384 | A. dum./sen.| 20.1010 | −16.1655  | PNBA: Aguilâl                              | Mauritania       |
| 5126 | A. dum./sen.| 20.1287 | −16.1581  | PNBA: Aguilâl 1                           | Mauritania       |
| 5135 | A. dum./sen.| 20.1497 | −16.1420  | PNBA: Aguilâl 4                           | Mauritania       |
| 5120 | A. dum./sen.| 20.1498 | −16.1719  | PNBA: Aguilâl, 1 km W of                   | Mauritania       |
| 5158 | A. dum./sen.| 20.7802 | −16.3944  | PNBA: Ameghououas es Sâhli                 | Mauritania       |
| 5160 | A. dum./sen.| 20.7843 | −16.4027  | PNBA: Ameghououas es Sâhli                 | Mauritania       |
| Code  | Species       | Latitude | Longitude | Local                                         | Country                      |
|-------|---------------|----------|-----------|-----------------------------------------------|------------------------------|
| 5162  | A. dum./sen.  | 20.8007  | −16.4227  | PNBA: Amgheouousas es Sâhli, 3 km NW of        | Mauritania                   |
| 6390  | A. dum./sen.  | 20.1808  | −16.1474  | PNBA: Dlô’ Matai                                | Mauritania                   |
| 6391  | A. dum./sen.  | 20.1808  | −16.1474  | PNBA: Dlô’ Matai                                | Mauritania                   |
| 6394  | A. dum./sen.  | 20.2330  | −16.1247  | PNBA: Dlô’ Matai                                | Mauritania                   |
| 2750  | A. dum./sen.  | 20.2789  | −16.1003  | PNBA: Dlô’ Matai                                | Mauritania                   |
| 3622  | A. dum./sen.  | 20.0934  | −16.0613  | PNBA: Grâret Za                                 | Mauritania                   |
| 2768  | A. dum./sen.  | 20.8070  | −16.5701  | PNBA: Kerekchet et Teintâne                    | Mauritania                   |
| 2769  | A. dum./sen.  | 20.8070  | −16.5701  | PNBA: Kerekchet et Teintâne                    | Mauritania                   |
| 6450  | A. dum./sen.  | 20.8233  | −16.5882  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6453  | A. dum./sen.  | 20.8233  | −16.5882  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6456  | A. dum./sen.  | 20.8023  | −16.5718  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6457  | A. dum./sen.  | 20.8023  | −16.5718  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6460  | A. dum./sen.  | 20.8283  | −16.5672  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6461  | A. dum./sen.  | 20.8283  | −16.5672  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6462  | A. dum./sen.  | 20.8283  | −16.5672  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6463  | A. dum./sen.  | 20.8283  | −16.5672  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6468  | A. dum./sen.  | 20.8294  | −16.5518  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 6469  | A. dum./sen.  | 20.8294  | −16.5518  | PNBA: Kerekchet et Teintâne, central           | Mauritania                   |
| 5181  | A. dum./sen.  | 20.7831  | −16.5865  | PNBA: Kerekchet et Teintâne, central 3         | Mauritania                   |
| 6445  | A. dum./sen.  | 20.8115  | −16.6158  | PNBA: Kerekchet et Teintâne, Western face      | Mauritania                   |
| 2763  | A. dum./sen.  | 20.8060  | −16.4561  | PNBA: N of Baie d’Arguin                      | Mauritania                   |
| 2743  | A. dum./sen.  | 20.0964  | −16.1798  | PNBA: NE of El Mounâne                        | Mauritania                   |
| 6377  | A. dum./sen.  | 20.1233  | −16.1266  | PNBA: Oued Nouafferd                          | Mauritania                   |
| 5139  | A. dum./sen.  | 20.1574  | −16.1037  | PNBA: Oued Nouafferd 3                        | Mauritania                   |
| 6375  | A. dum./sen.  | 20.0845  | −16.1313  | PNBA: Oued Nouafferd, 2 km S of                | Mauritania                   |
| 6376  | A. dum./sen.  | 20.0845  | −16.1313  | PNBA: Oued Nouafferd, 2 km S of                | Mauritania                   |
| 3615  | A. dum./sen.  | 20.0928  | −16.1059  | PNBA: Râs Tafarît, 16 km E of                  | Mauritania                   |
| 6433  | A. dum./sen.  | 20.8173  | −16.4858  | PNBA: Sebkhet Dbâdèb et Teintâne, 2 km E of    | Mauritania                   |
| 6431  | A. dum./sen.  | 20.7791  | −16.4602  | PNBA: Sebkhet Dbâdèb et Teintâne, 4 km E of    | Mauritania                   |
| 6426  | A. dum./sen.  | 20.7395  | −16.4150  | PNBA: Sebkhet Dbâdèb et Teintâne, 8 km SE of   | Mauritania                   |
| 6363  | A. dum./sen.  | 19.9808  | −16.1016  | PNBA: Taguílålet Jreik, 2 km W of              | Mauritania                   |
| 6364  | A. dum./sen.  | 19.9808  | −16.1016  | PNBA: Taguílålet Jreik, 2 km W of              | Mauritania                   |
| 2745  | A. longipes   | 20.0699  | −16.0896  | PNBA: 5 km E of El Mounâne                     | Mauritania                   |
| 6319  | A. longipes   | 19.6589  | −16.2639  | PNBA: Ackenjeil                                | Mauritania                   |
| 6320  | A. longipes   | 19.6589  | −16.2639  | PNBA: Ackenjeil                                | Mauritania                   |
| 6369  | A. longipes   | 20.0567  | −16.0993  | PNBA: Adeim el Marrâr, 4 km W of               | Mauritania                   |
| 6383  | A. longipes   | 20.1010  | −16.1655  | PNBA: Aguilâl                                  | Mauritania                   |
| 6386  | A. longipes   | 20.1010  | −16.1655  | PNBA: Aguilâl                                  | Mauritania                   |
| 5119  | A. longipes   | 20.1498  | −16.1719  | PNBA: Aguilâl, 1 km W of                       | Mauritania                   |
| A344  | A. longipes   | 20.0580  | −16.2380  | PNBA: Bir el Gareb, 15 km S of                 | Mauritania                   |
| A345  | A. longipes   | 20.0580  | −16.2380  | PNBA: Bir el Gareb, 15 km S of                 | Mauritania                   |
| A346  | A. longipes   | 20.0580  | −16.2380  | PNBA: Bir el Gareb, 15 km S of                 | Mauritania                   |
| 6339  | A. longipes   | 19.8079  | −16.1479  | PNBA: Elb en Nouçç, extreme S                 | Mauritania                   |
| 6340  | A. longipes   | 19.8079  | −16.1479  | PNBA: Elb en Nouçç, extreme S                 | Mauritania                   |
| 6348  | A. longipes   | 19.7819  | −16.1880  | PNBA: Grâret Agouëifa                         | Mauritania                   |
| 6349  | A. longipes   | 19.7819  | −16.1880  | PNBA: Grâret Agouëifa                         | Mauritania                   |
| 6414  | A. longipes   | 20.0546  | −16.3389  | PNBA: Îmgoûtene, 5 km NE of                    | Mauritania                   |
| 3607  | A. longipes   | 19.8232  | −16.2100  | PNBA: Iouûk, 16 km SE of                      | Mauritania                   |
Results and discussion

MICRO-CHECKER revealed no evidence of allelic dropout or stuttering, and no heterozygote excess was observed. In addition, no loci appeared to be in linkage disequilibrium. Table 3 summarizes occurrence of heterozygote deficiency and suspected null alleles for all loci in all populations in the three target species. While the occurrence of null alleles would limit the use of some of these markers in the affected species, other departures from Hardy–Weinberg equilibrium probably result from pooling several sampling localities in the same “populations” (see above). Additionally, even markers showing such evidences might be adequate to apply in other populations and they are applicable in at least one of these species.

All loci genotyped for each species were polymorphic (Table 4), except for Ac44 that amplified only for A. longipes. The Ac36 was also monomorphic in A. dumerili/A. senegalensis tested populations but polymorphism was observed in inland samples of this species (own unpublished data, Lopes, Velo-Antón, Crochet, Brito). The number of alleles per locus varied between 5 and 19 in A. aureus, and between 1 and 9 in A. dumerili/A. senegalensis and A. longipes. Expected and observed heterozygosity varied, respectively, between 0.594–0.893/0.188–1.000 in A. aureus, 0.223–0.829/0.154–0.826 in A. dumerili/A. senegalensis (ignoring Ac36), and 0.046–0.862/0.048–0.905 in A. longipes (ignoring Ac44). Most markers amplified in both A. scutellatus (17 loci) and A. taghitensis (16 loci).

Although the applicability of each marker may depend on the species considered, the information provided in our work allows a selection of good markers for future use on assessments of genetic structure, genetic diversity, gene flow, and demographic inferences, expanding the
Fig. 1 Distribution of genotyped samples used for Acanthodactylus aureus, A. dumerili/senegalensis and A. longipes. White circles correspond to Pop1, while grey circles correspond to Pop2. Circles are proportional to sample size. The rectangle in the map of A. aureus represents the area depicted in the maps of A. dumerili/senegalensis and A. longipes. The samples sizes of the populations are the following: Pop1 = 21 and Pop2 = 17 in A. aureus; Pop1 = 24 and Pop2 = 19 in A. dumerili/A. senegalensis; and Pop1 = 14 and Pop2 = 19 in A. longipes.

Table 3 Observations of heterozygote deficiency and null alleles

|         | A. aureus | A. longipes | A. dumerili/senegalensis |
|---------|-----------|-------------|---------------------------|
|         | Pop1      | Pop2        | Pop1                      | Pop2                      | Pop1                     | Pop2                     |
|         | Het. Def. | Null alleles| Het. Def.                 | Null alleles              | Het. Def.                | Null alleles             |
| Ac4     |            |             |                          |                          |                          |                          |
| Ac5     |            |             |                          |                          |                          |                          |
| Ac6     | *          | *           | *                        | *                        | *                        | *                        |
| Ac13    |            |             |                          |                          |                          |                          |
| Ac16    | *          | *           |                          | *                        | *                        | *                        |
| Ac19    |            |             |                          |                          |                          |                          |
| Ac23    |            |             |                          |                          |                          |                          |
| Ac31    | *          | *           |                          |                          | *                        | *                        |
| Ac32    |            |             |                          |                          |                          |                          |
| Ac33    | *          | *           | *                        | *                        | *                        | *                        |
| Ac43    |            |             |                          |                          |                          |                          |
| Ac45    |            |             |                          |                          |                          |                          |

Results are presented for Acanthodactylus aureus, A. dumerili/senegalensis and A. longipes. Significant values after Bonferroni correction are marked with an asterisk. Since the heterozygote deficiency was estimated in GENEPOP while null alleles were assessed in MICROCHECKER, differences in the estimation methods may explain the observed lack of concordance between heterozygote deficiency and null alleles in some cases.

-, markers that failed to amplify in a certain species.
### Table 4 Characterization of the 22 microsatellite loci

|        | A. aureus |        | A. longipes |        | A. dumerili/senegalensis |        | A. scutellatus |        | A. taghitensis |        |
|--------|-----------|--------|-------------|--------|--------------------------|--------|---------------|--------|---------------|--------|
|        | N         | He     | Ho          | N      | He                        | Ho     | N             | He    | Ho            | N      |
| Ac1    | 20        | 0.63   | 0.70        | 17     | 0.70                      | 0.59   | 5             |        |               |        |
|        | 251–267   | 14     | 0.25        | 0.14   | 21                        | 0.25   | 0.24          | 3      |               |        |
| Ac4    | 21        | 0.88   | 0.76        | 16     | 0.89                      | 1.00   | 16            | 0.67  | 0.00          | 5      |
|        | 230–275   | 6      | 0.67        | 0.00   | 5                         | 0.74   | 0.20          | 5      |               |        |
| Ac5    | 21        | 0.82   | 0.90        | 17     | 0.89                      | 0.94   | 13            | 162–198| 14            | 0.60  |
|        | 278–290   | 5      | 0.67        | 0.00   | 6                         | 0.64   | 0.71          | 6      |               |        |
| Ac6    | 17        | 0.72   | 0.35        | 16     | 0.76                      | 0.19   | 9             | 121–145| –             | –     |
|        | 201–231   | 14     | 0.68        | 0.71   | 21                        | 0.77   | 0.71          | 7      |               |        |
| Ac8    | 21        | 0.82   | 0.76        | 5      | 0.78                      | 1.00   | 10            | 201–231| 14            | 0.68  |
|        | 204–234   | 7      | 0.68        | 0.71   | 21                        | 0.77   | 0.71          | 4      |               |        |
| Ac9    | 21        | 0.87   | 0.86        | 17     | 0.85                      | 0.76   | 17            | 190–244| –             | –     |
|        | 208–244   | 10     | 0.54        | 0.40   | 15                        | 0.72   | 0.33          | 6      |               |        |
| Ac13   | 21        | 0.83   | 0.95        | 17     | 0.74                      | 0.71   | 9             | 140–179| 14            | 0.72  |
|        | 201–231   | 14     | 0.72        | 0.64   | 21                        | 0.69   | 0.67          | 8      |               |        |
| Ac14   | 21        | 0.59   | 0.52        | 17     | 0.66                      | 0.53   | 12            | 221–266| 14            | 0.24  |
|        | 211–243   | 14     | 0.24        | 0.29   | 21                        | 0.41   | 0.38          | 2      |
| Ac16   | 16        | 0.75   | 0.31        | 17     | 0.79                      | 0.53   | 8             | 101–125| 14            | 0.45  |
|        | 92–113    | 10     | 0.54        | 0.40   | 15                        | 0.72   | 0.33          | 6      |               |        |
| Ac19   | 19        | 0.87   | 0.79        | 6      | 0.79                      | 0.67   | 13            | 208–244| 10            | 0.54  |
|        | 211–226   | 10     | 0.54        | 0.40   | 15                        | 0.72   | 0.33          | 6      |               |        |
| Ac20   | 17        | 0.87   | 0.82        | 12     | 0.85                      | 0.67   | 14            | 150–201| 14            | 0.65  |
|        | 168–180   | 14     | 0.65        | 0.79   | 21                        | 0.71   | 0.81          | 5      |               |        |
| Ac23   | 21        | 0.67   | 0.57        | 17     | 0.82                      | 0.82   | 10            | 116–146| 14            | 0.76  |
|        | 114–138   | 12     | 0.67        | 0.58   | 18                        | 0.79   | 0.59          | 2      |
| Ac31   | 16        | 0.80   | 0.69        | 17     | 0.76                      | 0.76   | 14            | 306–366| 12            | 0.67  |
|        | 312–333   | 13     | 0.77        | 0.77   | 21                        | 0.86   | 0.90          | 9      |               |        |
| Ac32   | 21        | 0.85   | 0.81        | 15     | 0.86                      | 0.87   | 13            | 232–277| 14            | 0.70  |
|        | 245–269   | 13     | 0.77        | 0.77   | 21                        | 0.86   | 0.90          | 9      |               |        |
| Ac33   | 21        | 0.83   | 0.52        | 16     | 0.86                      | 0.63   | 15            | 120–165| 14            | 0.70  |
|        | 129–153   | 10     | 0.50        | 0.50   | 16                        | 0.63   | 0.56          | 5      |               |        |
| Ac36   | 21        | 0.84   | 0.86        | 16     | 0.84                      | 0.81   | 12            | 110–152| 14            | 0.50  |
|        | 113–116   | 10     | 0.50        | 0.50   | 21                        | 0.36   | 0.38          | 2      |               |        |
| Ac43   | 18        | 0.81   | 0.78        | 17     | 0.83                      | 0.71   | 10            | 94–124        | 9      |
|        | 106–130   | 9      | 0.61        | 0.58   | 21                        | 0.80   | 0.76          | 8      |               |        |
| Ac44   | –         | –      | –           | –      | –                         | –      | –             | –      |               | –      |
Table 4 continued

|                | A. aureus | A. longipes | A. dumerili/senegalensis | A. scutellatus | A. taghitensis |
|----------------|-----------|-------------|--------------------------|---------------|---------------|
|                | Pop1      | Pop2        | Pop1                     | Pop2          |               |
| N              | N He Ho N alleles | N He Ho N alleles | N He Ho N alleles | N He Ho N alleles | N He Ho N alleles |
| Ac45           | 19        | 0.85 0.95   | 17 0.68 0.47            | 12            | 133–172       |
| Ac47           | 16        | 0.83 0.75   | 17 0.80 0.71            | 19            | 187–262       |
| Ac49           | 17        | 0.61 0.65   | 17 0.79 0.71            | 14            | 186–225       |
| Ac28           | 16        | 0.72 0.81   | 17 0.80 0.65            | 9             | 127–166       |
| Mean           | 20        | 0.82 0.76   | 17 0.80 0.71            | 12            | 14 0.61 0.5   |

Sample size (N), number of alleles, allelic size range (expressed in base pairs), expected heterozygosity (He), and observed heterozygosity (Ho) are indicated for *Acanthodactylus aureus*, *A. dumerili/A. senegalensis* and *A. longipes*. Sample size, number of alleles, and allelic range are also presented for *A. scutellatus* and *A. taghitensis*

- -, markers that failed to amplify in a certain species
possible themes for evolutionary, behavioural and conservation studies in this species group.

Authors’ contributions
SCL carried out the laboratory tasks, performed the molecular analyses, and drafted the manuscript. PP and SL participated in the microsatellite marker optimization and validation. GVA, PAC and RG contributed to the molecular analyses. JCB designed and supervised the study. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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References
1. Salvador A. A revision of the lizards of the genus Acanthodactylus (Sauria Lacertidae). Bon Zool Monog. 1982;16:1–167.
2. Arnold EN. Osteology, genitalia and the relationships of Acanthodactylus (Reptilia: Lacertidae). Bull Brit Mus Nat Hist (Zoology). 1983;44:291–339.
3. Crochet PA, Geniez P, Ineich I. A multivariate analysis of the fringe-toed lizards of the Acanthodactylus scutellatus group (Squamata: Lacertidae): systematic and biogeographical implications. Zool J Linnean Soc. 2003;137:117–55.
4. Harris D, Arnold E. Elucidation of the relationships of spiny-footed lizards, Acanthodactylus spp. (Reptilia: Lacertidae) using mitochondrial DNA sequence, with comments on their biogeography and evolution. J Zool. 2000;252:351–62.
5. Bons J. Les lacentiliens du Sud-Ouest Marocain: systématique, répartition geographique, étologie, écologie. Trav Inst Sci Chérifien. 1959;18:1–130.
6. Mellado J, Olmedo G. El género Acanthodactylus en Marruecos: problemas de identificación en los grupos de especies A. pardalis y A. scutellatus. Amphib Reptilia. 1990;11:131–46.
7. Baha El Din SM. A contribution to the herpetology of Sinai. Brit Herp Soc Bul. 1994;48:18–27.
8. Baha El Din SM. A new lizard of the Acanthodactylus scutellatus group (Squamata: Lacertidae) from Egypt. Zool Middle East. 2007;40:21–32.
9. Sindaco R, Jeremcenko VK. The reptiles of the Western Palearctic: annotated checklist and distributional atlas of the turtles, crocodiles, amphibians and lizards of Europe, North Africa, Middle East and Central Asia. Latina: Edizioni Belvedere, Monografie della Societas Herpetologica Italica, 2008.
10. Schlotterer C. The evolution of molecular markers: just a matter of fashion? Nat Rev Genet. 2004;5:63–9.
11. Wan QH, Wu H, Fujihara T, Fang SG. Which genetic marker for which conservation genetics issue? Electrophoresis. 2004;25:2165–76.
12. Selkoe KA, Toonen RJ. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett. 2006;9:615–29.
13. Malasa T, Gilles A, Meglez F, Blanquart H, Dutory S, Coste-Daot C, et al. High-throughput microsatellite isolation through 454 GS-FLX titanium pyrosequencing of enriched DNA libraries. Mol Ecol Res. 2011;11:638–44.
14. Schuelke M. An economic method for the fluorescent labelling of PCR fragments. Nat Biotech. 2000;18:233–4.
15. Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004;4:535–8.
16. Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics. 2012;28:2537–9.