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# One species or two? How many *Trapelus* species occur in Morocco?

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The genus *Trapelus* Cuvier, 1817 (family Agamidae) is distributed in arid and semi-arid regions across northern Africa, the Middle East and Asia (Rastegar-Pouyani, 1999; Wagner et al., 2011). Despite recent taxonomic revisions, the genus remains poorly resolved, with the number of species recognised remaining contentious (Wagner et al., 2011; Shahamat et al., 2020). Prior to the revision of Wagner et al. (2011), *Trapelus mutabilis* was considered widespread from Egypt in the East to Mauritania in the West. However, using both morphological data and partial 16S rRNA mitochondrial DNA (mtDNA) sequences, Wagner et al. (2011) reviewed the northern African species and described two new taxa, including *Trapelus boehmei* from across Morocco, Mauritania and entering Western Algeria and raised the possibility that *Agama aspera* [=*Trapelus asperus*], with a type locality in northeastern Algeria, could be considered a distinct species, although this taxonomic hypothesis was not developed further. This left *T. boehmei* as the only recognised *Trapelus* species in Morocco, a position that was followed by a recent field guide (del Mármol et al., 2019) and checklist (Bouazza et al., 2021). However, in an assessment of colonisation patterns of African agamids, Kissling et al. (2016) recognise *T. aspersus* [sic] as a full species, and suggest its distribution ranges from Tunisia across northern Algeria and reaching into northeast Morocco. Beddek (2017) also considered *T. asperus* as a full species, occurring with *T. boehmei* and *T. tournevillei* in Algeria. Bouam et al. (2025) tentatively referred populations from northeastern Algeria to *T. mutabilis*, while noting that they might equally represent *T. asperus* given the unresolved taxonomic situation. Here we provisionally follow Kissling et al. (2016) in referring to *T. asperus*, or *T.* cf. *asperus* when specimens were assigned based solely on molecular data.

The aim of this study was to utilise samples from across Morocco to identify levels of genetic diversity within *T. boehmei* across its range, using the 16S rRNA gene so that new sequences could be compared to those previously published (Wagner et al., 2011). This genetic marker should also be informative in assessing if *T. asperus* also occurs in Morocco, and potentially help delimit the range of the genetic lineages in the region.

## Materials and Methods

**Study area.** Samples were collected in the field during scientific surveys in Morocco and Tunisia between 2000–2024 (Fig. 1); a small piece of tail tissue was removed and stored in 96% ethanol. The majority of samples consisted of road-killed specimens, so detailed

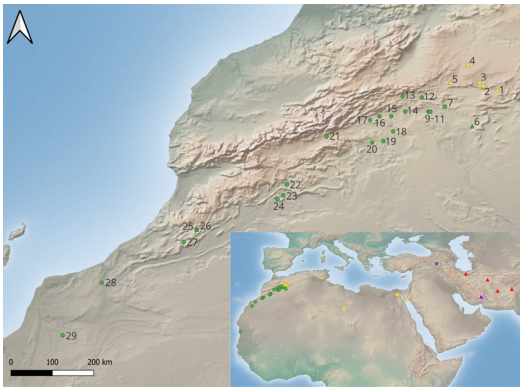


**Figure 1.** Map showing the geographic distribution of the collected samples of *Trapelus*, along with the locations of the published sequences (triangles) that were used in the phylogenetic analysis (Fig. 2). The colours on the map correspond to the different lineages identified in the phylogenetic tree (Fig. 2). Coordinates for each numbered points are listed in Table 1.

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morphological analyses could not be performed. However, previous assessments have shown that such specimens can be informative for use in defining ranges of reptile species in Morocco (e.g., Rosado et al., 2016). Two samples of *Trapelus* cf. *ruderatus* from Turkey from the CIBIO specimen collection were also sequenced and included as outgroups.

**Genetic analyses.** In the laboratory, DNA was extracted from the collected tissue samples using standard High Salt methods (Sambrook et al., 1989), and then a PCR was employed to amplify a section of the 16S rRNA gene, using the primers 16SL and 16SH from Palumbi et al. (1996). Amplification conditions consisted of 95 ºC for 7 minutes followed by 30 cycles of 30 seconds at 95 ºC, 45 seconds at 55 ºC and 1 minute at 72 ºC, followed by a single cycle at 72 ºC for 10 minutes. Quality of the reaction was assessed by visual examination after electrophoresis in a 2% agarose gel, and positive PCR products were then cleaned and sequenced at a commercial facility (Stabvida, Caparica, Portugal). All new sequences were submitted to GenBank (Table 1).

**Phylogenetic analysis.** Sequences were aligned in Geneious Prime 2025.2.1 (Kearse et al., 2012) using ClustalW (Thompson et al., 2003), with sequences from the same genus for the same region present in GenBank (16 sequences). The most appropriate model of molecular evolution (HKY+I) was identified using MEGA 12.0.9 (Kumar et al., 2018). A maximum likelihood (ML) tree was then determined, and bootstrap support estimated with 2000 replicates. We also used Bayesian inference (BI) to estimate the phylogeny using Mr. Bayes v.3.2.7 (Huelsenbeck and Ronquist, 2001), using the model of evolution chosen previously. The analysis was run for one million generations, saving one tree every 1000 generations.

**Table 1.** List of partial 16S rRNA sequences produced for this study.

| Code | Latitude | Longitude | Map number | Accession number |
|---|---|---|---|---|
| DB3279 | 32.4866 | -1.5819 | 1 | PX959918 |
| DB14196 | 31.9766 | -3.2690 | 2 | PX959923 |
| DB14197 | 31.9812 | -3.3142 | 3 | PX959924 |
| DB14222 | 30.2097 | -6.8694 | 4 | PX959925 |
| DB1603 | 29.1101 | -9.1369 | 5 | PX959914 |
| DB14702 | 31.9773 | -3.2919 | 6 | PX959927 |
| DB14691 | 32.1017 | -2.8983 | 7 | PX959926 |
| DB14739 | 31.9734 | -3.2795 | 8 | PX959928 |
| DB24022 | 32.6846 | -2.0309 | 9 | PX959934 |
| DB24068 | 31.8685 | -4.2509 | 10 | PX959935 |
| DB24091 | 31.9819 | -3.8995 | 11 | PX959936 |
| DB24159 | 32.5971 | -1.9429 | 12 | PX959937 |
| DB24162 | 31.7740 | -4.7809 | 13 | PX959938 |
| DB24172 | 31.8630 | -4.5462 | 14 | PX959939 |
| DB24208 | 33.1103 | -2.3284 | 15 | PX959940 |
| DB24214 | 32.6645 | -2.7607 | 16 | PX959941 |
| DB19966 | 26.5412 | -12.5061 | 17 | PX959931 |
| DB20010 | 27.8196 | -11.5215 | 18 | PX959932 |
| DB20034 | 28.8093 | -9.4638 | 19 | PX959933 |
| DB25342 | 31.2311 | -4.7324 | 20 | PX959942 |
| DB25354 | 31.2639 | -4.4480 | 21 | PX959943 |
| DB27596 | 29.8452 | -7.1078 | 22 | PX959944 |
| DB16418 | 38.9440 | 43.1140 | 23 | PX959929 |
| DB16437 | 38.9440 | 43.1140 | 24 | PX959930 |
| DB5244 | 34.7115 | 8.5169 | 25 | PX959922 |
| DB2386 | 32.3510 | -3.9661 | 26 | PX959915 |
| DB2387 | 32.3220 | -3.4796 | 27 | PX959916 |
| DB2388 | 32.3220 | -3.4796 | 28 | PX959917 |
| DB3368 | 29.9460 | -6.9662 | 29 | PX959919 |
| DB3960 | 31.5002 | -4.2018 | 30 | PX959920 |
| DB3975 | 31.3735 | -5.8760 | 31 | PX959921 |

## Results and Discussion

Our analysis included 28 new sequences from samples of *Trapelus* from across Morocco and one specimen from Tunisia, that we aligned with 16 sequences from GenBank. In total, the aligned dataset consisted of 46 samples, with 430 bp. This is a shorter region than the length sequenced in this study (478 bp) but allowed a complete full-length comparison with available data from GenBank. Both ML and BI analyses resulted in the same overall estimate of relationships (Fig. 2). Most of the samples from across Morocco grouped with published sequences of *T. boehmei*, including specimens from Mauritania and Algeria, with little variation across the range. A separate lineage consisted of *T. m. pallidus, T. m. poppeki*, *T. mutabilis* and a clade comprising all the samples from northeast Morocco and the sample from Tunisia, which we consider as *T.* cf. *asperus*. A published sequence (KU097510, considered as *T. boehmei*) from Morocco also groups with our samples from northeast Morocco, although no locality data other than country was given for this specimen (Tamar et al., 2016).

This new data supports the hypothesis that two genetically distinct lineages of *Trapelus* occur in Morocco, and following Kissling et al. (2016) these would correspond to *T. boehmei* across most of Morocco, with *Trapelus asperus* in the northeast. Clearly, our analysis is based on a single mtDNA marker, which can be misleading in certain situations such as when there is mitochondrial introgression

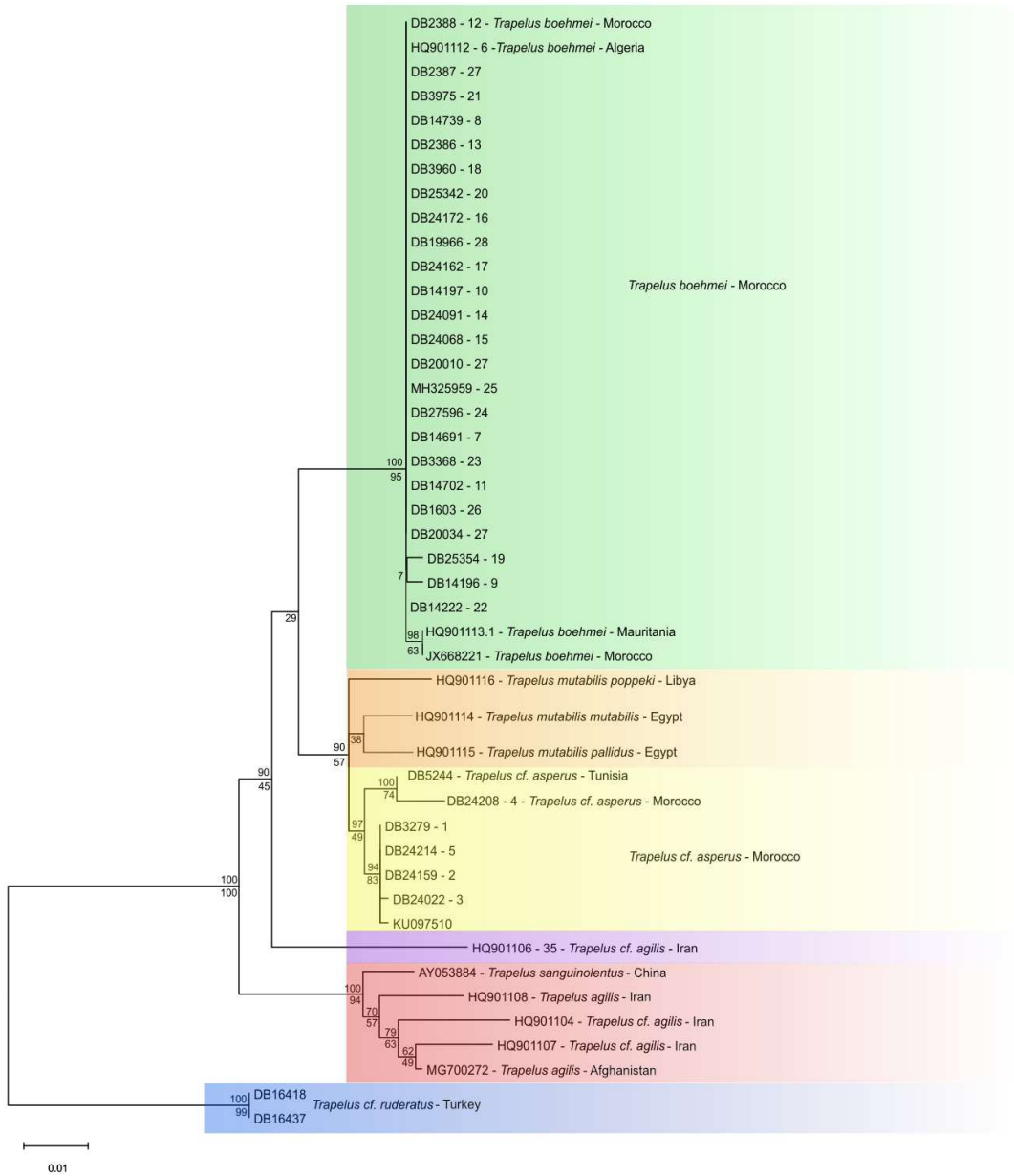


**Figure 2.** Maximum likelihood phylogenetic tree of *Trapelus*. Values below the nodes are maximum likelihood bootstrap replicates, values above the nodes are Bayesian posterior probabilities. Colours reflect localities shown in Figure 1.

(Ballard and Whitlock, 2004). However, in many cases in Morocco, preliminary studies have identified deep divergences between reptile populations using mtDNA markers, and these have subsequently been confirmed as full species, for example within *Acanthodactylus erythrurus* (Harris et al., 2024). Our analysis also indicates that across most of the Moroccan range of *T. boehmei* there is minimal variation in the 16S rRNA gene, with only seven haplotypes identified and with no clear structure. While our data needs confirmation, it seems to indicate that *T. boehmei* (sites 6–29 in Fig. 1) and *T.* cf. *asperus* (sites 1–5 in Fig. 1) are parapatric, separated across the Moulouya river basin, which is a well-known phylogeographic barrier (Salvi et al., 2018;

Beddek et al., 2018). Wagner et al. (2011) suggested that morphologically *T. boehmei* differed from *T. asperus* in "possessing no spiny scales and a heterogeneous dorsal scalation". Assessment of specimens from across northeast Morocco would be useful to confirm this. The sample of *T.* cf. *asperus* from Tunisia is very similar to specimens from northeast Morocco (0.7% minimum uncorrected genetic distance), a pattern similar to that observed in *Ophisops occidentalis* (Harris et al., 2025). Further field sampling would also be needed to confirm this. Taxonomic consideration of the *T. mutabilis/T. asperus* clade falls outside the framework of this manuscript.

Wagner et al. (2011) considered *T. asperus* "a probably valid taxon", while Kissling et al. (2016) treated *T. asperus* as a full species, but neither gave a detailed explanation regarding how they reached these conclusions. Uncorrected genetic distances of 2.34% (this study) between *T.* cf. *asperus* and *T. mutabilis poppeki* are notably less than that found between most species of *Trapelus*, but not considerably lower than the 2.63% between *T. boehmei* and *T. mutabilis pallidus* (Wagner et al., 2011). Clearly, a detailed assessment of morphological characteristics and additional nuclear DNA markers are needed to appraise the hypothesis that *T. asperus* should be considered a full species, or whether it would be more appropriate to treat it as a subspecies of *T. mutabilis*. Regardless, our data clearly indicates that two genetically distinct lineages of *Trapelus* occur in Morocco, *T. boehmei* and *T.* cf. *asperus*, as well as the approximate distribution of these.

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