Notes from the Anatolian underground: two new mole taxa from Eastern Turkey, together with a revised phylogeny of the genus Talpa (Mammalia: Eulipotyphla: Talpidae)

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Abstract

Subterranean life is associated with strong adaptive constraints, leading to the frequent occurrence of morphologically cryptic lineages. This is true of most small mammals, including moles (Eulipotyphla: Talpidae), where a number of species have been recognised recently, particularly following the application of molecular genetics. Here, we use mitochondrial and nuclear DNA sequence data and geometric morphometrics to explore the systematics and evolution of some of the least-known Western Palaearctic moles: the *Talpa davidiana* group of Eastern Anatolia/Iran. We show that *T. davidiana* includes four taxa, two of which we describe herein: ***T. hakkariensis* sp. nov.**, *T. davidiana davidiana*, ***T. davidiana tatvanensis* ssp. nov.** and *T. streetorum* **valid species**. For the first time, we apply molecular species delimitation analyses to *Talpa*, confirming taxonomic hypotheses and suggesting the existence of further morphologically cryptic lineages. These analyses also support the recognition of *T. transcaucasica* as a **valid species** distinct from *T. levantis*. We present a revised phylogeny for Eurasian *Talpa* and increase the number of known extant taxa to 18, most of which are found in Anatolia, the global hotspot of diversity in this genus. This likely results from the isolation of suitable habitats by a combination of climatic and topographical heterogeneity.

ADDITIONAL KEYWORDS: Geometric morphometrics – molecular phylogeny – new species – species delimitation analyses – subspecies – subterranean – systematics – Talpinae.

INTRODUCTION

Life underground imposes strict selection regimes and adaptive constraints on organisms, which are manifested in multiple taxa and operate across all levels of biological organisation. A number of mammal lineages have evolved specialized subterranean lifestyles, most of these being small, burrowing taxa (e.g., Nevo, 1979; Lacey, 2000; Begall *et al*., 2007; Davies *et al*., 2015; Carotenuto *et al*., 2020; Montoya-Sanhueza *et al*., 2022). Whilst not all characters in subterranean mammals have undergone convergent evolution (e.g., Sansalone *et al*., 2020), the underground lifestyle is associated with a suite of modifications, many of which are convergent across lineages, and demonstrated at morphological, physiological and genomic levels (Lacey, 2000; Begall *et al*., 2007; Partha *et al*., 2017; Jiang *et al*., 2020). Perhaps due to strong morphological constraints, cryptic species are widespread in fossorial mammals (e.g., Peppers & Bradley, 2000; Savić *et al*., 2017; Carballo & Rossi 2018; D'Elía *et al*., 2021), including in talpid moles (e.g., He *et al*., 2017); much of this cryptic diversity being revealed recently through karyology and DNA-based studies.

Talpa Linnaeus, 1758 (Eulipotyphla: Talpidae) is a fossorial mole genus endemic to the western Palaearctic region, found primarily in Europe and western Asia; some species are widespread, but many have narrower endemic ranges, particularly in the east. Nine taxa were recognized by Hutterer (2005): the common mole *T. europaea* Linnaeus, 1758, the blind mole *T. caeca* Savi, 1822, the Roman mole *T. romana* Thomas, 1902, the Levant mole *T. levantis* Thomas, 1906, the Iberian blind mole *T. occidentalis* Cabrera, 1907, the Balkan mole *T. stankovici* Martino and Martino, 1931, Père David's mole *T. davidiana* (Milne-Edwards, 1884) and the Caucasian mole *T. caucasica* Satunin, 1908, all in the subgenus *Talpa*, and the Altai mole *T. altaica*, Nikolasky, 1883, often placed in the monospecific subgenus *Asioscalops* Stroganov, 1941 (see Bannikova *et al*., 2015). The conservative morphology of

the genus means that the number of *Talpa* lineages has been underestimated from morphological differences, prompting a series of molecular studies.

Although comprehensive genetic analyses of most *Talpa* are lacking, recent applications of DNA-based approaches have helped identify several well-defined lineages that are as evolutionarily distinct as previously recognised nominal species (Bannikova *et al*., 2015; Nicolas *et al*., 2017a,b; Kryštufek *et al*., 2018; Demirtaş *et al*., 2020), suggesting that there may be further, hidden, taxonomic diversity within the genus. Two new mole species, *T. aquitania* Nicolas, Martínez-Vargas & Hugot, 2017 (Nicolas *et al*., 2017b) from France and *T. martinorum* Kryštufek, Nedyalkov, Jonas, Astrin & Hutterer, 2018 (Kryštufek *et al*., 2018) from Bulgaria (the south-western Black Sea coast) have been described in recent years, based on molecular and morphological data. Bannikova *et al*. (2015) also recently separated two additional, genetically well-defined, lineages in the Caucasus and Anatolia, corresponding to *T. talyschensis* Vereschagin, 1945 and *T. ognevi* Stroganov, 1944. Finally, Demirtaş *et al*. (2020) demonstrated that *T. levantis* s.l. in Anatolia is divisible into divergent eastern and western lineages based on both mitochondrial and nuclear markers, and on this basis argued that the eastern lineage should be considered as a separate species (for which the available name is *T. transcaucasica* Dahl, 1945). The number of recognized species in the genus *Talpa* has therefore recently increased from nine (Hutterer, 2005) to 14 (Bannikova *et al*., 2015; Kryštufek & Motokawa, 2018; Kryštufek *et al*., 2018; Burgin *et al*., 2020; Demirtaş *et al*., 2020), a significant increase over the last seventeen years. Current understanding of the genus in a genetic and phylogenetic context remains biased towards the western part of its range in Europe, however, thereby necessitating comparative analyses with a greater emphasis on specimens from farther east, particularly in areas known to harbour high mole diversity (e.g., the Caucasus and Anatolia).

Turkey hosts six of the 14 currently recognised *Talpa* species (*T. europaea, T. davidiana, T. levantis, T. martinorum, T. ognevi,* and *T. transcaucasica* (Kryštufek & Vohralík, 2001; Bannikova *et al*., 2015; Kryštufek, *et al*., 2018; Demirtaş *et al*., 2020; Kefelioğlu *et al*., 2020). Of these, *T. davidiana* remains one of the least known, being listed as data deficient by the International Union for Conservation of Nature (IUCN), with limited information available on its distribution, population status, or possible threats. Milne-Edwards (1884) described *T. davidiana* from Akbes at the border of Syria and Asia Minor (present-day Turkey) on the basis of specimens collected by Père Armand David. He named it *Scaptochirus davidianus*, based on the dentition, which he considered to more closely resemble some eastern Asian moles than *Talpa*. Krystufek *et al*. (2001) revisited the species, showing that it clearly belongs in *Talpa* and discussed morphological variation across known localities around the southern margin of the Anatolian – Iranian high plateau in Turkey and Iran. Later, Kryštufek and Vohralik (2005) incorporated the western Iranian *T. streetorum* Lay, 1965 (as *T. streeti*) into *T. davidiana*; dividing it into two subspecies (*T. davidiana davidiana* and *T. davidiana streetorum*) and considering the latter taxon to include larger *davidiana*-type moles from Eastern Turkey.

To date, few attempts have been made to explore species boundaries in *Talpa* using formal molecular delimitation approaches, despite the difficult taxonomy of the genus (Bannikova *et al*., 2015). Rigorous species identification and delimitation are fundamental to a more robust and objective system of classification to ensure an accurate phylogeny and better-informed conservation (Pimm *et al*., 2014; Groves *et al*., 2017). Recent advances in genomic and bioinformatic tools have opened new avenues for species identification and delimitation, providing a valuable complement to the morphology-based taxonomy that remains a key framework for species identification in practice. Genomic data could even serve as the backbone of a taxonomic description in some cases, especially in taxonomically

perplexing groups whose boundaries are diffuse (e.g., species complexes and cryptic species). Many molecular species delimitation methods are currently available for the analysis of single-locus or multi-locus sequence data, which are based on either comparison of intra- and interspecific genetic distances or gene-tree approaches. The goal of these methods is to assign reliably identified samples into groups to delineate and identify species-level entities or Operational Taxonomic Units (OTUs), which are the fundamental units of biodiversity (Tautz *et al*., 2003; Vogler & Monaghan, 2007; Mallo & Posada, 2016; Rannala & Ziheng, 2020). Although they provide helpful insights, different molecular-based species delimitation approaches all have their own limitations in revealing species boundaries (e.g., Paz & Crawford, 2012; Fujisawa & Barraclough, 2013; Zhang *et al*., 2013; Tang *et al*., 2014; Dellicour & Flot, 2015; Lang *et al*., 2015; Ahrens *et al*., 2016; Arrigoni *et al*., 2016; Wang *et al*., 2016; Pentinsaari *et al*., 2017; Renner *et al*., 2017; Dellicour & Flot, 2018; Luo *et al*., 2018; Magoga *et al*., 2021). However, given their differing analytical approaches and theoretical bases, the use of these methods simultaneously offers the opportunity to delineate species boundaries in a comparative way, with congruence among methods supporting the robustness of any particular OTU (Carstens *et al*., 2013; Kekkonen & Hebert, 2014; Mutanen *et al*., 2015; Ji *et al*., 2021).

Based on newly collected, almost topotypical, material of *Talpa davidiana*, together with moles from other areas of the supposed Turkish distribution of this species and a re-examination of types, we show that *T. davidiana* sensu Kryštufek *et al*. (2001) and Sözen *et al*. (2012) includes four distinct taxa, diagnosable as phylogenetic species (sensu Cracraft 1983) using both molecules and morphology. Two of these are currently unnamed, described herein as ***Talpa hakkariensis* sp. nov.** and ***Talpa davidiana tatvanensis* ssp. nov.** respectively. We use newly generated sequences from the mitochondrial cytochrome *b* (cyt-*b*) gene and exon 11 of the breast cancer type 2 susceptibility protein (*BRCA2*) gene to place

these new taxa in a phylogeny of Eurasian *Talpa* and three molecular species delimitation analyses, together with geometric morphometrics (including the type series of *T. streetorum*) to confirm our taxonomic hypotheses.

MATERIAL AND METHODS

Sample collection

The collection localities of all specimens used in molecular and morphometric studies are given in Fig. 1 and Table 1. All capture and sacrifice protocols were approved by the Animal Experiments Local Ethics Committee at Ondokuz Mayıs University (code: 2019/28). Trapping was conducted in accordance with the guidelines approved by the American Society of Mammalogists (Sikes *et al*., 2016) under authorization of the General Directorate of Nature Conservation and National Parks, Republic of Turkey Ministry of Agriculture and Forestry (Register n. 21264211-288.04). Specimens were measured, and the skulls were removed for further study. Standard voucher specimens (skins, skulls and various tissues in ethanol) were deposited in the collections of the Department of Biology, Faculty of Sciences, Ondokuz Mayıs University), Samsun, Turkey (OMU), subcollection IG/SD (collections of first and second authors, İG and SD).

Morphological examination

Mole specimens were photographed, measured and dissected to prepare skins, skulls and mandibles for formal taxonomic study and morphometrics. Measurements of the external morphology included body weight, head and body length, tail length, and hind-foot length. The following 10 skull characters were measured following Kryštufek *et al*. (2018), using a digital caliper with an accuracy of 0.01 mm: condylobasal length of skull (CbL), length of maxillary tooth row (MxT); canine to 3rd molar), breadth of braincase, height of braincase (BcH; bullae excluded), breadth of rostrum over canines (RoC), breadth of rostrum over

molars (RoM), breadth of rostrum over canines as a percentage of condylobasal length of skull (RoC as % CbL), breadth of rostrum over molars as a percentage of condylobasal length of skull (RoM as % CbL), length of maxillary tooth row as a percentage of condylobasal length of skull (MxT as % CbL), and height of braincase as a percentage of condylobasal length of skull (BcH as % CbL). The 1st upper molar was viewed and photographed with a Leica M125 stereomicroscope with integrated Leica DF295 digital camera. The cranium and mandibles were photographed with a Nikon D7000 digital camera, in ventral and lateral views for geometric morphometric analyses.

DNA extraction, PCR amplification, sequencing, and phylogeny estimation

Total DNA was extracted from muscle tissue using DNeasy Blood & Tissue Kits (Qiagen) following the manufacturer's protocol. DNA concentration and quality were measured with a microvolume spectrophotometer (Colibri). We specifically focussed here on the '*davidiana*' group of *Talpa*; however, given that sequence data for the majority of *Talpa* species is limited, our taxonomic sampling also included *T. levantis*, *T. martinorum* and *T. ognevi* to add additional sequence data for these species to enhance understanding of the molecular phylogeny of the genus. We studied two loci, the complete mitochondrial cyt-*b* gene and a segment of the nuclear exon 11 of the *BRCA2* gene. Both of these markers have been commonly applied in mammalian phylogenetics, including moles (Bannikova *et al*., 2015; He *et al*., 2017; Demirtaş *et al*., 2020).

The cyt-*b* gene and flanking regions were amplified for 28 individuals from 15 localities with polymerase chain reaction (PCR) primers L14162 5′-GACATG AAAAATCATCGTTG-3′ (modified from L14727-SP in Jaarola & Searle, 2002) and H15917 5′-CCTGAAGTAAGAACCAGATG-3′ (modified from H16498 in Meyer *et al*., 1990). PCR amplifications were performed following Demirtaş *et al*. (2020). PCR products were electrophoresed on 1.5% agarose gel, documented with a gel visualization system and

purified with QIAquick PCR Purification Kit (Qiagen). The complete cyt-*b* gene of the *Talpa* species of interest was sequenced in both directions using one of the amplification primers (L14162) plus the following internal primers: *T. levantis*: (H15351 5′-TCTCCATTGCTGGTTTACAAGAC-3′, modified from H15915 in Irwin *et al*. (1991), L14711 (Demirtaş *et al*., 2020) and H14812_Tl 5′-TTTRTCYGTGTCKGATGAGAGTCC-3′ (this study); *T. ognevi*: H15351, L14711 and H14812_To 5′-TTTGTCTGTGTCTGATGAAAGTCC-3′ (this study); *T. martinorum*: H15351, L14711 and H14935_Tm 5′-GAATGTAGTTATCTGGGTCTCC-3′ (modified from H14935 in Demirtaş *et al*., 2020); *T. davidiana*: H15351, L14711_Td 5′-GGTRGACAAAGCCACACTCAC-3′ (modified from L14711 in Demirtaş *et al*., 2020) and H14812_Td 5′-TTTGTCTATATCTGATGRGAGTCC-3′ (this study); ***Talpa hakkariensis* sp. nov.**: H15351, L14711_Td and H14812_Th 5′-TTTGTCTGTGTCTGATGAAAGTCC-3′ (this study). The position of the 3′ end oligonucleotide of each of these newly designed internal primers is given relative to the published sequence of the *T. europaea* mitochondrial genome (GenBank Y19192, Mouchaty *et al*., 2000).

There have been many reports of nuclear mtDNA pseudogenes (NUMTs) in the genomes of a diverse range of eukaryotic species (Bensasson *et al*., 2001; Song *et al*., 2008; Dubey *et al*., 2009). To confirm that amplified sequences were of true mitochondrial origin, two different PCRs, producing overlapping fragments, were performed on all individuals of the newly described species (***Talpa hakkariensis* sp. nov.**). First, a longer fragment comprised of part of tRNA-Glu (18 bp), the entire cyt-*b* gene (1140 bp), complete tRNA-Thr (69 bp), complete tRNA-Pro (70 bp), and the 5′ end of the D-loop (460 bp) were amplified with primers L14162 and H15917. Second, a shorter fragment comprised of part of tRNA-Glu (18 bp), the entire cyt-*b* gene (1140 bp), and part of tRNA-Thr (26 bp) amplified with primers L14162 and H15351. Purified PCR products of these amplifications were sequenced

with the PCR primers plus two specific internal primers (L14711_Td and H14812_Th). Chromatograms of the sequencing runs were then aligned and overlapping sequences examined for ambiguous (i.e., double peak) sites, stop codons, or open-reading frame shifts, which might indicate contamination from NUMTs. We also assessed the accuracy and reliability of mtDNA sequences of the new species by subjecting its sequences to identification by NCBI BLAST and by comparing them against the published complete mitogenomes of four species of the genus *Talpa*: *T. aquitania*, *T. europaea*, *T. martinorum* and *T. occidentalis* (Mouchaty *et al*., 2000; Gutiérrez *et al*., 2018; Aleix-Mata *et al*., 2020; Demirtaş *et al*., 2022).

For nuclear DNA, sequences of a 927 bp portion of exon 11 of the *BRCA2* gene were generated from the same 28 moles (Table 1) using the PCR and sequencing protocols described in Demirtaş *et al*. (2020).

Sequences were visually inspected for quality and aligned in Sequencher v.4.5 (Gene Codes Corp.). Nucleotide and amino acid composition were analysed and the frequency of each haplotype estimated using DnaSP v.5 (Librado & Rozas, 2009). Genetic diversity estimates were calculated for the concatenated sequences of mitochondrial lineages of the species of interest. Nucleotide (π) and haplotype diversity (*Hd*) were calculated using Arlequin v.3.5 (Excoffier & Lischer, 2010). DNA net and raw divergences (D_a and D_{xy}; Nei, 1987) between taxa, based on both uncorrected *p*-distances and the Kimura two parameter model (K2P; Kimura, 1980), were estimated using MEGA X (Kumar *et al*., 2018). Standard errors (SE) were estimated from 10,000 bootstrap replicates.

Phylogenetic inference analyses were conducted using Maximum Parsimony (MP), Maximum-likelihood (ML) and Bayesian inference (BI). MP analysis was performed with PAUP* v.4.0b10 (Swofford, 2002) with the heuristic search approach using the TBR swapping algorithm, steepest descent option and ten random repetitions. Confidence in the

nodes of MP trees was assessed by 1,000 bootstrap replicates (Felsenstein, 1985). Three descriptive statistics for MP trees (tree length = TL, consistency index = CI and retention index = RI) were also calculated using PAUP. ML analysis was performed in RAxML v.8.2.X (Stamatakis, 2014) with 1,000 bootstrap replicates. BI analysis was performed in MrBayes v.3.2.7a (Ronquist *et al*., 2012) and involved four Markov chains of 10 (for cyt-*b* data) or five (for *BRCA2* data) million generations each, with trees being sampled every 100 generations and a burn-in of 25%. Tracer v.1.7.1 (Rambaut *et al*., 2018) was used to observe the parameters and to determine the number of trees needed to reach stationarity (burn-in). After discarding burn-in trees and evaluating convergence, remaining samples were retained for generating 50% majority rule consensus trees and calculating the posterior probabilities as the percentage of samples recovering any particular clade. Best-fit models of nucleotide substitution were estimated under AIC criterion in jModelTest2 (Darriba *et al*., 2012) with default settings. For phylogenetic analysis of cyt-*b* data, we included all newly described haplotypes and 88 complete *Talpa* cyt-*b* sequences from GenBank. Since some *BRCA2* sequences in GenBank were shorter than ours, or contained unresolved positions, a separate truncated alignment (656 bp) was prepared for phylogenetic analyses including these sequences (Table 1; Supporting Information, Table S1).

Cyt-*b* sequences of representatives of the tribe Desmanini (*Desmana moschata* (Linnaeus, 1758) and *Galemys pyrenaicus* (Geoffroy, 1811) and *BRCA2* sequences of representatives of tribe Talpini (*Euroscaptor longirostris* (Milne-Edwards, 1870), *Oreoscaptor mizura* (Günther, 1880), *Mogera robusta* (Nehring, 1891) and *Parascaptor leucura* (Blyth, 1850)) were used as outgroups in the phylogenetic analyses, following He *et al*. (2014) and Bannikova *et al*. (2015) (Supporting Information, Table S1). For the cyt-*b* data set, we used two tree topology tests (Shimodaira-Hasegawa Test (SH; Shimodaira & Hasegawa, 1999) and the Approximately Unbiased Test (AU; Shimodaira, 2002)), using IQ-

TREE (Trifinopoulos *et al*., 2016) to evaluate topological congruence between ML, BI and MP trees. These were conducted using 10,000 resamplings using the log-likelihood (RELL) method (Kishino *et al*., 1990) to evaluate congruence at *P*-values <0.05.

Species delimitation analyses

Based on our dataset of 108 *Talpa* cyt-*b* sequences (Table 1; Supporting Information, Table S1), we used three DNA-based species delimitation approaches to define species partitions, considering previously described species and our newly discovered lineages in the genus: Assemble Species by Automatic Partitioning (ASAP; Puillandre *et al*., 2021), multi-rate Poisson Tree Processes (mPTP; Kapli *et al*., 2017), and the Generalized Mixed Yule Coalescent (GMYC; Pons *et al*., 2006; Fujisawa & Barraclough, 2013). ASAP is a recently developed distance-based method, similar to the Automatic Barcode Gap Discovery (ABGD; Puillandre *et al*., 2012). It uses threshold values to distinguish between interspecific divergence and intraspecific variation, and circumscribes species partitions using a hierarchical clustering algorithm based on pairwise genetic distances from single-locus sequence alignments (e.g., barcode data sets). ASAP also provides a list of partitions (grouped by species groups), ranked to identify the best-fitting set of partitions; the lower the ASAP score, the better fitting the partition (Puillandre *et al*., 2021). ASAP analysis was performed using a multiple sequence alignment of the cyt-*b* gene, uploaded to the ASAP online portal (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html). Divergence distances were tested for all three applicable models of nucleotide evolution offered by the ASAP: Jukes-Cantor (JC69), K2P and *p*-distance. mPTP is a single-locus coalescent-based method, requiring a phylogenetic tree as input, to identify the transition between species- and population-level divergence, assuming a constant speciation rate with different intraspecific coalescent rates. This method determines the number of species that best fits the data, assuming that the branch lengths of each putative species may form different clusters (Kapli

et al., 2017). For this analysis, the Newick-formatted ML tree from RAxML was used as input on the mPTP web server (http://mptp.h-its.org/#/tree). GMYC is also a tree-based delimitation method, based on the Generalized Mixed Yule Coalescent. It requires as input an ultrametric gene tree, with branching rates based on clock-like assumptions. It then uses a likelihood approach to analyse the timing of branching events on a phylogenetic tree, searching for the most likely switch between a Yule (interspecific) and a coalescent (intraspecific) branching structure (Pons *et al*., 2006; Fujisawa & Barraclough, 2013). Empirical data suggest that GMYC provides the best results when combined with BEAST ultrametric trees (Tang *et al*., 2014). Therefore, single locus ultrametric trees were constructed using a Bayesian approach in BEAST v.2.6.6 (Bouckaert *et al*., 2014), with a lognormal relaxed clock, Yule tree prior (being used for species-level data), GTR+G+I substitution model, and all other priors set to defaults. Analyses were performed for 100 million generations, sampling trees every 1,000 generations, and BEAST log files examined in Tracer to assess convergence and adequate posterior sampling (ESS > 200). A maximum clade credibility tree was estimated in TreeAnnotator v.1.10.4 (Suchard *et al*., 2018) based on the sampled trees after discarding the first 25% generations as burn-in. The ultrametric phylogeny recovered with BEAST was then used to perform GMYC analysis, implemented in the R package *Split* (http://r-forge.r-project.org/projects/splits/) with a single threshold GMYC model (Reid & Carstens, 2012).

Geometric morphometric analysis

Geometric morphometric analysis was performed to test the morphological distinctiveness of the new taxa proposed in this paper: *Talpa davidiana davidiana*, ***T. d. tatvanensis* ssp. nov.** and ***T. hakkariensis* sp. nov.**, together with *T. streetorum* from Iran. The animals comprising these taxa were previously classified as a single morphologically variable species, *T. davidiana*, by Kryštufek *et al*. (2001), which was later subdivided into

two subspecies *T. d. davidiana* and *T. d. streetorum* (Kryštufek & Vohralik 2005). Geometric morphometric analysis was performed on the skull (ventral view) and mandible (lateral view) of the newly collected material and the type series of *T. streetorum*, housed in the Field Museum of Natural History, Chicago (FMNH; Fig. 1; Table 1). Fifteen landmarks were placed on images of the skulls to capture shape variation in the dental arcade, palatal, and basicranial areas and 15 placed on images of the mandible to capture the shape of the dental arcade, mandibular body, and the coronoid, condylar, and angular processes (Supporting Information, Fig. S1; Table S2). Only fully adult animals as determined by tooth eruption and cranial bone fusion were included in the analysis. One sample (*T. streetorum*) was heavily biased toward males, so a permutation-based MANOVA was performed on the largest samples, *T. davidiana davidiana* (cranium: M=4, F=6; mandible: M=5, F=6), to test for sexual dimorphism in the shape of the cranium and mandible.

Landmark coordinates were collected using ImageJ (Rasband, 2022) scaled in millimetres and are available in TPS format in Supporting Information Files S1 and S2. Following standard geometric morphometric practice (Bookstein, 1991; Dryden & Mardia, 1998), landmarks were superimposed using generalized Procrustes analysis (Rohlf & Slice, 1990) to remove the effects of size, translation, and rotation. Superimposed landmarks were then converted to shape coordinates by transforming them to scores with principal components analysis (PCA), retaining only the axes with non-zero eigenvalues (Dryden & Mardia, 1998). The data sets in this study are not sample limited, so the number of axes is determined by degrees of freedom from Procrustes superimposition, $2k - 4$, where k is the number of landmarks, two is the number of dimensions in each landmark, and four is the degrees of freedom lost to translation in the x and y dimensions, scaling, and rotation around the centroid (Zelditch *et al*., 2012), which is 26 for both cranium and mandible.

A multivariate morphospace was constructed using PCA based on these analyses. The significance of morphological differences between taxa was tested with a permutation-based MANOVA. All geometric morphometric analysis was performed with the packages *Geometric Morphometrics for Mathematica* v.12.4 (Polly, 2022) and *Phylogenetics for Mathematica* v.6.5 (Polly, 2019).

Sexual dimorphism is rare in moles, but was nonetheless explored in the relatively large ***T. d. tatvanensis*** ssp. nov. sample. For the cranial data set, there were 4 males and 6 females, and the permutation-based MANOVA revealed no shape differences (df: 1,8,9; F=0.39; P=0.94). Similarly, there were no differences in the mandibular data set comprising 5 males and 6 females (df: 1,9,10; F=0.70, P=0.85). Based on these results, sexes were combined in all subsequent analyses.

Table 1. Geographic origin of *Talpa* sampled in this and previous studies, with details on the distribution of haplotypes. The frequencies of haplotypes retrieved from GenBank are stated in parentheses. Cyt-*b* haplotypes (Hap.1-20, GenBank accession numbers: OQ695509– OQ695528) and *BRCA2* haplotypes (Hap.1-8, GenBank accession numbers: OQ695501– OQ695508) were new to this study. * When we aligned our *BRCA2* haplotypes with the shorter (656 bp) published haplotypes, our *T. davidiana* haplotypes (Hap.4–6) and that (KP717132) in Bannikova *et al*. (2015) became identical, giving us a single *BRCA2* haplotype in *T. davidiana* in the final dataset used for phylogenetic analyses. Locality codes correspond to Fig. 1. See Supporting Information, Table S1 for sequences from other taxa used in phylogenetic analyses. Abbreviations used for collections: OMU-IG/SD, personal collection of İ. Gündüz and S. Demirtaş, Department of Biology, Faculty of Sciences, Ondokuz Mayıs University, Samsun, Turkey; FMNH, Field Museum of Natural History, Chicago, USA. † The last four (OMU-IG/SD collection) or five/six (FMNH collection) digits of the museum collection numbers were used to identify individual specimens in geometric morphometric analysis (e.g., see Figs 7-10, 13, S1-S3; Table S3; Files S1-S2).

					Molecular		Geometric morphometric		
Taxon	**Locality**	**Locality code**	***n***	**Museum collection no†**	**Haplotype (Cyt-*b*)**	**Haplotype* (*BRCA2*)**	**Skull**	**Mandible**	**Reference**
	TURKEY								
Talpa davidiana davidiana	**Gaziantep**								
	Karagöz yaylası, Islahiye	1	1	OMU-IG/SD-1564 (Topotype)	Hap.1	Hap.4	+	+	
	Hatay								
	Çardak yaylası, Hassa	2	1	OMU-IG/SD-1565 (Topotype)	Hap.1	Hap.4	+	+	
	Kahramanmaraş								
	Andırın	3	1	OMU-IG/SD-1566	Hap.2	Hap.6	+	+	
	Osmaniye								
	Bağdaş yaylası, Sumbas	4	1	OMU-IG/SD-1448	Hap.3	Hap.4	+	+	
	Afşar mevki, Esenli, Sumbas	5	2	OMU-IG/SD-1449	Hap.4	Hap.4	+	+	
				OMU-IG/SD-1450	Hap.4	Hap.4	+	+	

	Esenli, Sumbas	6	3	OMU-IG/SD-1451	Hap.5	Hap.4	-	-	
				OMU-IG/SD-1482	Hap.5	Hap.4	-	+	
				OMU-IG/SD-1483	Hap.6	Hap.5	+	+	
	Uzunyazı yaylası, Kadirli	7	1	OMU-IG/SD-1484	Hap.7	Hap.4	+	+	
	Yirce yaylası, Sumbas	8	2	OMU-IG/SD-1485	Hap.7	Hap.4	+	+	
				OMU-IG/SD-1486	Hap.8	Hap.4	+	+	
***Talpa davidiana tatvanensis* ssp. nov.**	**Bitlis**								
	Uslu, Tatvan	9	3	OMU-IG/SD-1491	Hap.9	Hap.4	+	-	
				OMU-IG/SD-1492	Hap.10	Hap.4	+	+	
				OMU-IG/SD-1530 (Holotype)	Hap.10	Hap.4	+	+	
	Kurtikan, Tatvan	10	2	FMNH 82136	-	-	+	+	Lay, 1965
				FMNH 82137	-	-	+	+	Lay, 1965
	Çataklı Köyü, Tatvan	11	5	-	KP717363 (3)	KP717132	-	-	Bannikova *et al*., 2015
				-	KP717364 (2)		-	-	Bannikova *et al*., 2015
***Talpa hakkariensis* sp. nov.**	**Hakkari**								
	Bilmizit yaylası	12	1	OMU-IG/SD-1487	Hap.11	Hap.8	+	+	
	Cemşililan yaylası	13	6	OMU-IG/SD-1488	Hap.11	Hap.8	+	+	
				OMU-IG/SD-1532	Hap.11	Hap.8	+	+	
				OMU-IG/SD-1489	Hap.12	Hap.8	+	+	
				OMU-IG/SD-1490	Hap.13	Hap.8	+	+	
				OMU-IG/SD-1531	Hap.14	Hap.7	+	+	
				OMU-IG/SD-1533 (Holotype)	Hap.15	Hap.7	+	+	
Talpa ognevi	**Artvin**								
	Şavşat	14	1	OMU-IG/SD-1539	Hap.20	Hap.3			
Talpa levantis	**Trabzon**								
	Taşköprü, Altındere, Maçka	15	3	OMU-IG/SD-1504	Hap.16	Hap.1	-	-	
				OMU-IG/SD-1506	Hap.16	Hap.1	-	-	
				OMU-IG/SD-1505	Hap.17	Hap.1	-	-	
Talpa martinorum	**İstanbul**								
	Kağıthane	16	1	OMU-IG/SD-1357	Hap.18	Hap.2	-	-	
	Avcılar	17	1	OMU-IG/SD-1440	Hap.19	Hap.2	-	-	
	IRAN								

Talpa streetorum	**Hezar Darreh**	18	4	FMNH 96421	-	-	+	+	Lay, 1965
				FMNH 96423	-	-	+	+	Lay, 1965
				FMNH 96424 (Holotype)	-	-	+	+	Lay, 1965
				FMNH 96425	-	-	+	+	Lay, 1965
	Divandarreh	19	1	FMNH 111007	-	-	+	+	Lay, 1965

RESULTS

Phylogenetic analysis of the cyt-*b* data

The entire cyt-*b* gene (1140 bp) was sequenced from 28 moles from 15 Turkish localities, revealing 20 unique haplotypes for the following *Talpa* species: *T. davidiana* (15 individuals from nine localities, 10 haplotypes, Hap.1–10); ***T. hakkariensis* sp. nov.** (seven individuals from two localities, 5 haplotypes, Hap.11–15); *T. levantis* (three individuals from one locality, 2 haplotypes, Hap.16–17); *T. martinorum* (two individuals from two localities, 2 haplotypes, Hap.18–19) and *T. ognevi* (one individual from one locality, 1 haplotype, Hap.20) (Fig. 1; Table 1). The full cyt-*b* data set used for phylogenetic analyses contained 20 new and 88 published *Talpa* haplotypes, plus four outgroup haplotypes (Table 1; Supporting Information, Table S1). The alignment of ingroup haplotypes revealed 432 variable sites, of which 399 were parsimony informative, with 291 synonymous and 27 non-synonymous changes across all taxa. For the cyt-*b* data set, the best-fit model selected by jModelTest under AIC was GTR, with gamma correction and proportion of invariable sites (GTR+G+I), and this was employed in subsequent phylogenetic analyses (BI and ML).

Maximum parsimony analysis yielded a single most parsimonious tree (TL = 1724, CI = 0.3588, RI = 0.8726). ML analysis and Bayesian analysis resulted in a similar topology to MP analysis (*P*-value > 0.05 for both SH and AU tests), with minor differences among trees in the positioning of specific terminal branches within taxa. Most putative species identified through species delimitation analyses, including the newly described ***T. hakkariensis* sp. nov.**, were recovered as monophyletic, with strong nodal support in BI, ML and MP trees; posterior values (BI) and bootstrap support (ML and MP) obtained for each node were 1.0 and 100% for all main clades (i.e., defined species). Branches leading to *T. altaica* and *T. caucasica* + *T. ognevi* were placed as the first two dichotomies within the genus, although only highly supported in BI analysis (Fig. 2). In all trees, ***T. hakkariensis* sp. nov.** is

recovered as sister to a clade comprising *T. davidiana* plus *T. talyschensis* within the '*davidiana*' group (Bannikova *et al*., 2015) with high posterior probability (1.0) but with moderate bootstrap support (ML, 74%).

Our phylogenetic analyses revealed two distinct sub-clades within *T. davidiana* for the first time. The first, composed of Hap.1–8, including one haplotype (Hap.1) found in two almost topotypical specimens of *Talpa davidiana* from Gaziantep and Hatay, corresponds to the nominotypical subspecies *Talpa davidiana davidiana*, whereas the second sub-clade, composed of Hap. 9–10 and KP717363–KP717364 identified in specimens collected in Tatvan-Bitlis (Fig. 1; Table 1), corresponds to a new subspecies, ***Talpa davidiana tatvanensis* ssp. nov.**, which is described below. ***T. hakkariensis* sp. nov** showed a haplotype diversity (H_d) of 0.8571 +/- 0.1371 and nucleotide diversity (π) of 0.2598% +/- 0.1767% while *T. davidiana* showed a haplotype diversity (H_d) of 0.9526 +/- 0.10252 and nucleotide diversity (π) of 1.4394% +/- 0.7477%. Of the two subspecies of *T. davidiana*, *T. d. davidiana* exhibited a haplotype diversity (H_d) of 0.9333 +/- 0.0620 and nucleotide diversity (π) of 0.2444% +/- 0.1594%, whereas ***T. d. tatvanensis* ssp. nov.** exhibited a haplotype diversity (H_d) of 0.8667 +/- 0.0714 and nucleotide diversity (π) of 1.1396 % +/- 0.6349%.

In line with previous studies (Kryštufek *et al*., 2018; Demirtaş *et al*., 2020; Kefelioğlu *et al*., 2020; Demirtaş *et al*., 2022), *T. martinorum* was clustered with *T. aquitania*, *T. europaea* and *T. occidentalis* in the same clade with high posterior probability (0.92) but with low bootstrap support. Consistent with the results of our species delimitation analysis (see below), a clear split between *T. levantis* and *T. transcaucasica* was detected in the phylogeny. *T. transcaucasica* is much more divergent on cyt-*b* sequences from the two subspecies of *T. levantis* than either is from each other (net and raw K2P distances between *T. transcaucasica* (haplotypes KP717334–KP717335, KP717337, FN640570, FN640574) and *T. levantis levantis* (haplotypes MN868440–MN868453, KP717339–KP717340, FN640572, Hap.16–17)

are 5.88% and 7.32%, respectively; between *T. transcaucasica* and *T. levantis dogramacii* Kefelioğlu, Kryštufek, Selçuk, Hutterer & Astrin, 2020 (haplotypes KP717336, KP717338) 5.39% and 6.12%; between *T. levantis levantis* and *T. levantis dogramacii* 2.99% and 3.79%) (see Fig. 2).

Phylogenetic analysis of the *BRCA2* data

A 927 bp portion of exon 11 of *BRCA2* was successfully amplified and sequenced in 28 moles from 16 localities, resulting in eight unique haplotypes designated as Hap.1–8, one of which (Hap.1) was found in three individuals of *T. levantis*, one (Hap.2) in two individuals of *T. martinorum*, one (Hap.3) in one individual of *T. ognevi*, three (Hap.4–6) in 15 individuals of *T. davidiana*, and two (Hap.7–8) in seven individuals of ***T. hakkariensis* sp. nov.** (Table 1). Our sequences were assessed against 27 *Talpa BRCA2* sequences retrieved from GenBank. Alignment of our eight *BRCA2* haplotypes with the shorter (656 bp) published haplotypes (see Materials and methods) resulted in two redundant *T. davidiana* haplotypes (Hap.5–6), giving a total of 33 haplotypes in the final dataset (Table 1; Supporting Information, Table S1). These haplotypes had 47 variable nucleotides over 656 bp, 26 of which were parsimony informative. For the *BRCA2* data set, the best-fit model selected by jModelTest under AIC was HKY. The trees obtained in BI, ML and MP analyses yielded very similar topologies (Fig. 3). As in the cyt-*b* phylogeny, *T. davidiana*, *T. talyschensis* and ***T. hakkariensis* sp. nov.** (i.e., the '*davidiana*' group) were more closely related to each other than any were to other species in the genus. The posterior values (BI) and bootstrap support (ML and MP) obtained for the node that unites these three species were 1.0 and 94%. In line with our species delimitation (see below) and phylogenetic analyses based on cyt-*b* data, a clear split between *T. levantis* and *T. transcaucasica* was also detected in the *BRCA2* phylogeny (Fig. 3).

Molecular species delimitation analysis

Results from the three species delimitation models using the cyt-*b* gene are summarized in Fig. 2. In general, the results of the multiple molecular methods were in mutual agreement and identified 15–18 putative species, largely coinciding with recognized phylogenetic lineages inferred from BI, ML and MP topologies, with few exceptions, namely the delimitation of three species into several molecular operational taxonomic units MOTUs (i.e., *T. caeca*, *T. levantis* and *T. transcaucasica*). Despite several instances of incongruence between the results of species delimitation methods, all consistently separated the new species ***T. hakkariensis* sp. nov.** as a distinct entity (Fig. 2). The ASAP analysis retrieved identical MOTU results on each JC69, K2P, and *p*-distance substitution model, which all yielded 15 MOTUs with ASAP scores of 6.00, 5.00, and 5.00, respectively. These 15 MOTUs corresponded well to known species, at the threshold distance of 4.41% (K2P), 4.43% (JC69) and 4.98% (*p*-distance) with available molecular data: nine species recognized in the most recent revision of the genus (*T. europaea*, *T. altaica*, *T. caeca*, *T. caucasica*, *T. davidiana*, *T. levantis*, *T. romana*, *T. occidentalis* and *T. stankovici*), two recently described species (*T. aquitania* and *T. martinorum*), three recently split species (*T. talyschensis*, *T. transcaucasica* and *T. ognevi*) and the new species we describe below (***T. hakkariensis* sp. nov.**). In contrast to the ASAP results, the GMYC analysis grouped *Talpa* cyt-*b* sequences into 16 MOTUs, retrieving one more MOTU that corresponds to a recently described sublineage of *T. levantis* (i.e., haplotypes KP717336 and KP717338), corresponding to *T. l. dogramacii* from western Anatolia (Kefelioğlu *et al*., 2020) (Fig. 2). Relative to ASAP and GMYC methods, the mPTP model was the least conservative and resulted in 18 MOTUs, fragmenting some species into several individual clusters. The three additional MOTUs observed with the mPTP method resulted from highly divergent haplotypes in *T. caeca* (haplotype FN640559), *T. levantis* (haplotypes KP717336 and KP717338), and *T.*

transcaucasica (haplotype FN640570) (Fig. 2). None of the analyses suggested that the two subspecies of *T. davidiana* recognised here were separate species.

Geometric morphometric analysis

All four taxa were completely distinct in mandibular shape, with no overlap amongst groups and also having relatively distinct cranial shapes, albeit with some overlap, particularly between ***T. hakkariensis* sp. nov.** and ***T. d. tatvanensis* ssp. nov.** (Fig. 4; Supporting Information, Figs S2, S3). Separation amongst groups in cranial shape was strongest on PC1 (23.4% variance), which describes a narrower rostrum and more posteriorly positioned braincase (which can also be geometrically interpreted as a longer rostrum) in the two *T. davidiana* taxa relative to *T. streetorum* and ***T. hakkariensis* sp. nov.** (Supporting Information, Fig. S4). PC1 of mandibular shape explains 28.2% of the variance in that data set and separates *T. d. davidiana* from *T. streetorum* in having more anteriorly and dorsally positioned mandibular foramina, a broader angular process, and a more anteriorly inclined coronoid process. PC2 of mandibular shape (18.9% variance) separates ***T. hakkariensis* sp. nov.** from the other three taxa in having mandibular foramina that are closer to each other and narrower angular and coronoid processes (Supporting Information, Fig. S4, S6B).

Based on the MANOVA with permutation tests, skull shape was different overall amongst groups (P=0.00, R^2 = 26.9), and all taxa were different from each other except ***T. d. tatvanensis* ssp. nov.**, which failed significance tests with all other taxa due to both morphological overlap and smaller sample size (Supporting Information, Fig. S5A). Tests for mandibular shape were also different overall (P=0.00, R^2=38.4), as were the pairwise between-taxa tests except for *T. d. davidiana* and ***T. d. tatvanensis* ssp. nov.**, which failed largely due to small sample size rather than a lack of morphometric distinctiveness (Fig. 4; Supporting Information, Fig. S5B). Taxa are also distinctively different in the size of crania and mandibles, as measured with geometric morphometric centroid size (Supporting

Information, Fig. S5C, D). For both data sets, the size ordering is (smallest to largest): *T. d. davidiana*, ***T. d. tatvanensis* ssp. nov.**, ***T. hakkariensis* sp. nov.** and *T. streetorum* (although in mandibles, the latter two species are co-equal in size).

In terms of the shape of the cranium, there is a gradient (PC 1) in which the palate becomes more elongated and the lateral projection of the braincase becomes more posteriorly deflected from *T. streetorum* to ***T. hakkariensis* sp. nov.**, ***T. d. tatvanensis* ssp. nov.**, and ***T. d. davidiana*** (Supporting Information, Figs. 4, S4, S6A). The shape of the mandible of ***T. hakkariensis* sp. nov.** differs from *T. streetorum* in having the posteriormost mandibular foramen positioned more anteriorly and having a coronoid process that is narrower in anterior-posterior breadth and inclined more anteriorly (Supporting Information, Fig. S6B). Both of these morphometric differences and the MANOVA tests indicate that these two taxa are distinctively different from each other morphologically.

Taxonomy

Here we describe two new mole taxa, and provide redescriptions or diagnoses for two others (*Talpa davidiana davidiana* and *T. streetorum*). Exact label data are cited for specimens; double slashes (//) indicating separate lines.

***Talpa hakkariensis* sp. nov.**

Zoobank registration: urn:lsid:zoobank.org:act:58C51143-81D5-45C3-BC16-381429ADCE74

Type locality: Turkey, Hakkari Province, Cemşililan yaylası ca. 8 km NNE of Durankaya, 37˚38’N 43˚37’E, 2,700-2,980 m (Figs 1, 5A, Table 1).

Holotype. Adult female, specimen no. 1533, stuffed skin labelled “13/08/21-1533 ♀ IG/SD// *Talpa hakkariensis* sp. nov.// Cemşililan yaylası, Hakkari// 160-31-21 = 73.84 g.// 13, August, 2021” Body frozen at -50˚C, internal organs in ethanol, skull extracted and labelled

with specimen number (Fig. 6A). Collected by İ. Gündüz and S. Demirtaş. Sequences available from GenBank: (cyt-*b* haplotype: Hap.15, Accession number OQ695523; *BRCA2* haplotype: Hap.7, Accession number OQ695507); standard voucher specimens (skin, frozen body, skull, and various tissues in ethanol) are deposited in the Department of Biology, Faculty of Sciences, Ondokuz Mayıs University (OMU-IG/SD), Samsun, Turkey.

Paratypes (6). 1 male, specimen no. 1487, stuffed skin labelled "10/06/21-1487 ♂ IG/SD// *Talpa hakkariensis* sp. nov.// Bilmizit yaylası-Hakkari, 37˚36'N 43˚38'E // 164-28-20 = 73.05g.// 10, June, 2021"; 1 male, specimen no. 1488, stuffed skin labelled "10/06/21-1488 ♂ IG/SD// *Talpa hakkariensis* sp. nov.// Cemşililan yaylası-Hakkari// 166-30-20 = 77.38g.// 10, June, 2021"; 1 male, specimen no. 1489, stuffed skin labelled "10/06/21-1489 ♂ IG/SD// *Talpa hakkariensis* sp. nov.// Cemşililan yaylası-Hakkari// 170-31-21 = 71.25g.// 10, June, 2021"; 1 male, specimen no. 1490, stuffed skin labelled "10/6/21-1490 ♂ IG/SD// *Talpa hakkariensis* sp. nov.// Cemşililan yaylası-Hakkari// 165-29-21 = 67.96g.// 10, June, 2021; 1 female, specimen no. 1531, stuffed skin labelled "11/08/21-1531 ♀ IG/SD// *Talpa hakkariensis* sp. nov.// Cemşililan yaylası-Hakkari// 147-33-21 = 60.34g.// 11, August, 2021"; 1 female, specimen no. 1532, stuffed skin labelled "12/08/21-1532 ♀ IG/SD// *Talpa hakkariensis* sp. nov.// Cemşililan yaylası-Hakkari// 154-30-21 = 67.39g.// 12. August, 2021". Bodies frozen at -50˚C, internal organs in ethanol, skulls extracted and labelled with specimen numbers. Sequences available from GenBank: (cyt-*b* haplotypes: Hap.11–14, Accession numbers OQ695519– OQ695522; *BRCA2* haplotypes: Hap.7–8, Accession numbers OQ695507– OQ695508); standard voucher specimens (skins, frozen body, skulls, and various tissues in ethanol) are deposited in the Department of Biology, Faculty of Sciences, Ondokuz Mayıs University (OMU-IG/SD), Samsun, Turkey.

Measurements of holotype. Body mass 73.84 g, head and body length 160 mm, tail length 31 mm, hind-foot length 21 mm, condylobasal length of skull 33.40 mm, maxillary tooth row

14.93 mm, breadth of braincase 16.90 mm, height of braincase 9.29 mm, breadth of rostrum over canines 5.02 mm, breadth of rostrum over molars 9.30 mm (Supporting Information, Table S3).

Measurements of paratypes. Means±standard deviation (minimum–maximum). Linear measurements are in mm; body mass in grams. Sample size = 6 for all measurements. Body mass 69.56±5.30 (60.34–77.38), head and body length 161.00±7.920 (147.0–170.0), tail length 30.17±1.570 (28.0–33.0), hind foot 20.67±0.471 (20.0–21.0), condylobasal length of skull 32.88±0.421 (32.10–33.24), maxillary tooth row 14.15±0.142 (13.88–14.31), breadth of braincase 16.30±0.348 (15.8–16.88), height of braincase 9.31±0.102 (9.20–9.48), breadth of rostrum over canines 5.24±0.279 (4.88–5.59), breadth of rostrum over molars 9.86±0.442 (9.24–10.4) (Supporting Information, Table S3).

Diagnosis. Very large mole; skull large with a stout, broad rostrum; dental formula of C3/3 I1/1 P4/4 M3/3. A member of the subgenus *Talpa*, closest to *T. davidiana* and *T. streetorum* morphologically and *T. davidiana* and *T. talyschensis* genetically (Figs 2-3, 6). Distinguished from both *T. davidiana* and *T. talyschensis* by the larger body size (***T. hakkariensis* sp. nov.** body mass 60.34-77.38 g, head and body length 147-170 mm; *T. davidiana* body mass 34.12-52.87 g, head and body length 128-153 mm; *T. talyschensis* body mass 31-49 g, head and body length 104-114 mm; (Fig. 6A)). Condylobasal length of skull in ***T. hakkariensis* sp. nov.** also consistently greater, 32.10-33.40 mm vs. 29.04-31.27 in *T. davidiana* studied here, and a mean of 31.10 mm in *T. talyschensis* (Zaitsev *et al*., 2014). The new species also differs from *T. davidiana davidiana* on: the broader, blunter rostrum (9.24-10.40 mm over the molars in ***T. hakkariensis* sp. nov.** vs. 8.35-9.87 mm in *T. davidiana*; Figs 7-10); typically four rather than three premolars in each maxilla (Figs 7-10 – only one of the 9 *T. davidiana davidiana* specimens we examined had four premolars – see also Kryštufek *et al*., 2001); 1st upper molar with small, reduced parastyle, always much more prominent in *T. davidiana*

davidiana (Fig. 11A); larger hands and feet (see Fig. 12); and longer (28-33 vs. 16-18 mm) and paler tail, with most tail hairs of more uniform length (compare Fig. 12D-E). The broad snout of the new species resembles that of ***T. davidiana tatvanensis* ssp. nov.**, but this taxon is much smaller than ***T. hakkariensis* sp. nov.** (see below) and has a relatively prominent parastyle on the first upper molar, as in *T. davidiana davidiana*. Distinguished from *T. streetorum* by the latter's larger cranium (see above), in having the posteriormost mandibular foramen positioned more anteriorly and having a coronoid process that is narrower in anterior-posterior breadth and inclined more anteriorly (Supporting Information, Fig. S6B). The net *p* distance in cyt-*b* sequence between *T. davidiana* and ***T. hakkariensis* sp. nov.** is 10.50%; raw distance 11.33%. The net and raw K2P distances of 11.79% and 12.64%, respectively, between these taxa is in the range observed between other species of moles (e.g., within *Talpa*, interspecific net and raw K2P distances average ca. 11.47% and 12.61%, respectively). In fact, the net genetic distance in cyt-*b* sequence *T. davidiana* and ***T. hakkariensis* sp. nov.** is much higher than the raw (total) genetic distance between several recognized sister species in *Talpa* (e.g., *T. aquitania* vs *T. occidentalis, T. caucasica* vs *T. ognevi* and *T. levantis* vs *T. transcaucasica*) (Supporting Information, Table S4, S5).

Description. A relatively large, heavy mole (Figs. 5B, 6A), with a medium tail; 17.07-22.45% of head and body length. Tail (Fig. 12D) parallel-sided, with sparse, stiff, sub-erect, overlapping bristles; individual bristles mostly pale greyish, almost translucent, up to 25% blackish or with black cores; bristles relatively even in length; tail tip tapered and rounded, visible when bristles are moved. Overall appearance of tail paler than body. Dorsal and ventral pelage greyish brown throughout, with marked silvery sheen; individual hairs typically brownish at tips, greyish below. Body elongated. Palm of manus (Fig. 12A) large, broad, relatively flat; claws at end of all five phalanges, relatively broad and flattened dorsoventrally, with convex upper and concave lower margins, flattened oval in cross-

section. Feet (Fig. 12D) large, approximately two-thirds tail length. Muzzle (Figs 5B, 6A) elongated, with hairs, including longer sensory bristles, paler than on head. Snout pinkish with close, fine, small pale hairs.

Skull (Fig. 7) large, with a stout, broad rostrum. Width across canines 15.0-17.05%, and width across molars 27.84-31.92% condylobasal length. Maxillary tooth row equals 42.36-44.7% of the condylobasal length. The braincase is broad and deep, flattened above; height of neurocranium 27.71-29.12% of condylobasal length. The posterior margin of palatine is usually slightly posterior to the imaginary line connecting the posterior alveolar margins of 3rd upper molars, and the anterior border of the infraorbital foramen is above the 2nd upper molar.

Upper incisors of decreasing size; 3rd incisor approximately ½ the size of the 1st. Upper molars robust, decreasing in size 1-3. 4th upper premolar largest; 1st intermediate and 2nd and 3rd small (Fig. 7). 1st upper molar with very small, almost imperceptible parastyle (Fig. 11A), visible in holotype and some paratypes, but lost in specimens with substantial tooth wear; protocone much larger than paracone; mesostyle relatively flattened at apex, with clear mesal furrow. 2nd upper molar with well-developed parastyle, somewhat bilobed at apex in holotype and most paratypes; protocone only slightly larger than paracone; mesostyle well developed and clearly bilobed at apex. 3rd upper molar with well-developed parastyle, slightly more prominent than mesostyle; mesostyle clearly bilobed at apex; protocone only slightly larger than paracone. Mandible Fig. 13A. Lower incisors subequal. 1st lower premolar large, with prominent distal cusp; 4th lower premolar subequal to 1st; 2nd and 3rd lower premolars much smaller. 1st and 2nd lower molars large; 3rd smaller; all with prominent parastyles.

Etymology. The new species is named in reference to Hakkari Province, Turkey where the type locality is situated (adjective).

Distribution and ecology. Known from a handful of geographically close localities in the mountains of Hakkari province, Turkey. Moles were collected from moist soils beside a mountain stream at Cemşililan yaylası (Fig. 5A), in Eastern Anatolian Montane Steppe subject to nomadic grazing, but otherwise with little human influence. Most of the precipitation in this region falls as winter snows. At Bilmizit yaylası, specimen was collected in a similar habitat, but in this case without surface water.

Comment. This new mole likely corresponds to the specimens from Hakkari referred to *T. davidiana* by Kryštufek *et al*. (2001), *T. streetorum* by Doğramacı (1989) and *T. d. streetorum* by Kryštufek & Vohralik (2005). Our genetic, morphological, and morphometric data indicate that Hakkari populations represent a distinct species of broad-rostrum mole rather than a subspecies of *T. davidiana*, and our morphometric analysis indicates that it is also distinct from *T. streetorum* (see above). At the time of writing, we have been unable to sequence the type material of *T. streetorum*, or obtain new material from Iran, but consider the morphometric results from our comparison of skulls conclusive. Kryštufek & Vohralik (2005) noted the similarities between the moles from Hakkari and a fossil mole from the Late Pleistocene of Israel, *T. chthonia* Bate, 1937, which they speculated could be conspecific. Because we consider ***T. hakkariensis* sp. nov.** and *T. streetorum* to be distinct, it is not clear without further analysis which if either would be associated with this fossil but, if conspecific with either, the earlier name *T. chthonia* would have priority.

***Talpa davidiana davidiana* (Milne-Edwards, 1884)**

Scaptochirus davidianus Milne-Edwards, 1884

Type locality: Listed as “aux environs d'Akbès, sur les confins de la Syrie et de l'Asie Mineure” - environs of Akbes at the border between Syria and Asia Minor - by Milne-Edwards (1884). Most likely present-day Akbez, in Hatay Province, Turkey, close to the Syrian border. This seems more likely than Meidan Ekbis (often listed as Meydanakbes or

Meydan Akbes) in adjacent Syria (as suggested by Kryštufek *et al*. (2001)), as the Turkish locality is surrounded by mountains and mesic habitats suitable for moles, rather than (semi-) desert. In any event, the two localities are geographically very close, separated by approximately 7 km. The holotype is deposited in the Muséum National d'Histoire Naturelle, Paris, comprising a stuffed skin and dry preserved skull and mandibles.

Material examined (Turkey; specimen numbers in parenthesis): Two specimens, almost topotypical to Milne-Edwards' material: 1 female (1565), Hatay Province, Hassa, Çardak yaylası, 36° 50′ N, 36° 26′ E, 1,457 m (Figs 1, 5C, Table 1); 1 male (1564), Gaziantep Province, İslahiye, Karagöz yaylası, 36° 58′ N, 36° 27′ E, 1,442 m. Nine additional specimens, with cyt-*b* sequences clustering closely with the above, as follows: 1 male (1566), Kahramanmaraş Province, Andırın, 37° 35′ N, 36° 21′ E, 1,104 m; 1 male (1450), 1 female (1449), Osmaniye Province, Sumbas, Esenli, Afşar mevki, 37° 37′ N, 36° 05′ E, 870 m; 1 male (1483), 1 female (1482), Osmaniye Province, Sumbas, Esenli, 37° 37′ N, 36° 05′ E, 870 m; 1 female (1484), Osmaniye Province, Kadirli, Uzunyazı yaylası, 37° 42′ N, 36° 12′ E, 1,300 m; 2 females (1485 & 1486), Osmaniye Province, Sumbas,Yirce yaylası, 37° 40′ N, 36° 08′ E, 1,710 m; 1 male (1448), Osmaniye Province, Sumbas, Bağdaş yaylası, 37° 40′ N, 36° 10′ E, 1,255 m. Specimens 1482 and 1483, external measurements only, lost to farm dog; all seven remaining with stuffed skins preserved dry, bodies frozen at -50˚C, internal organs in ethanol, skulls extracted and labelled with specimen numbers.

Diagnosis. Medium-sized mole; skull relatively large with a stout, broad, rostrum. A member of the subgenus *Talpa*, closest to ***T. davidiana tatvanensis* ssp. nov.**, morphologically and genetically (Figs 2-3, 6). For morphological characters distinguishing the two subspecies, see ***T. davidiana tatvanensis* ssp. nov.** below. For characters separating it from ***T. hakkariensis* sp. nov.** see under that species. Differs from *T. streetorum* on its smaller size, narrower rostrum and more posteriorly positioned braincase. The net and raw cyt-*b* K2P distances of

1.52% and 2.19%, respectively, between *T. davidiana davidiana* and ***T. davidiana tatvanensis* ssp. nov.** is relatively low, leading us to consider these taxa as geographically isolated subspecies, rather than species.

Redescription. A medium-sized mole (Figs 5D, 6B), body mass 34.12-52.87 g and head and body length 128-145 mm in specimens examined. With a relatively short tail; 11.27-13.24% of head and body length (Supporting Information, Table S3). Tail (Fig. 12E) parallel-sided, with sparse, stiff, sub-erect, overlapping bristles; individual bristles mostly blackish or with black cores, ca. 20-25% greyish brown, translucent; bristles considerably variable in length, giving a bushy appearance; tail tip tapering and rounded, visible when bristles are moved. Overall appearance of tail similar colour to body. Dorsal and ventral pelage greyish brown throughout, with noticeable silvery sheen, particularly laterally and ventrally; individual hairs typically brownish at tips, greyish below. Body elongated. Palm of manus (Fig. 12B) broad, relatively flat; claws at end of all five phalanges, flattened dorsoventrally, with convex upper and slightly concave lower margins, flattened oval in cross-section. Feet (Fig. 12E) approx. equal to tail length. Muzzle (Figs 5D, 6B) elongated, with hairs, including longer sensory bristles, much paler than on head. Snout pinkish with close, fine, small pale hairs.

Skull (Fig. 8) moderate, with a relatively stout, broad rostrum. Width across canines 13.56-14.71%, and width across molars 28.12-29.54% condylobasal length. Maxillary tooth row equals 40.66-42.97% of the condylobasal length. The braincase is broad and deep, flattened above; height of neurocranium 28.50-30.79% of condylobasal length. The posterior margin of palatine is usually slightly posterior to the imaginary line connecting the posterior alveolar margins of 3rd upper molars, and the anterior border of the infraorbital foramen is above the 2nd upper molar.

Upper incisors of decreasing size; 3rd incisor slightly less than ½ the size of the 1st. Usually with 3, rarely 4, upper premolars. 3rd upper premolar largest; 1st intermediate and 2nd smallest (Fig. 8). Upper molars robust, decreasing in size 1-3. 1st upper molar with well-developed parastyle (Fig. 11B), smaller in specimens with substantial tooth wear; protocone much larger than paracone; mesostyle relatively flattened at apex, slightly bifurcated. 2nd upper molar with well-developed parastyle; protocone larger than paracone; mesostyle well developed and clearly bilobed at apex. 3rd upper molar with well-developed parastyle, slightly more prominent than mesostyle; mesostyle bilobed at apex; protocone larger than paracone. Mandible Fig. 13B. Lower incisors 1, 2 & 4 subequal; 3 smaller. 1st lower premolar large, with prominent distal cusp; 3rd lower premolar subequal to 1st; 2nd lower premolar much smaller. 1st and 2nd lower molars large; 3rd smaller; all with prominent parastyles.

Distribution and ecology. Known with certainty only from localities in Hatay, Gaziantep, Kahramanmaraş and Osmaniye Provinces, in the extreme southeast of Turkey. Rather than being found in the Syrian Desert, the type material was also probably collected in Hatay Province (see above). Records from Bitlis (Kryštufek *et al*., 2001; Bannikova *et al*., 2015) and Hakkari (Kryštufek *et al*., 2001) Provinces likely refer to ***T. davidiana tatvanensis* ssp. nov.** and ***T. hakkariensis* sp. nov.**, respectively. Moles were trapped in agricultural areas, including an orchard, village grazing lands and small residual marshes.

Comment. Whilst we did not directly examine the type specimen, our Hatay and Gaziantep animals were collected within a maximum of 10 and 17 km of Milne-Edwards' type locality, respectively, on the same side of the Amanos Mountains and are therefore effectively topotypical. Their crania and mandibles closely resemble high-resolution digital photos of the holotype available online at https://science.mnhn.fr/taxon/species/talpa/davidiana#milne-edwards%2C_1884; albeit that specimen exhibiting substantial tooth wear. Ours are the first specimens of *T. davidiana davidiana* to be DNA sequenced.

***Talpa davidiana tatvanensis* ssp. nov.**

Zoobank registration: urn:lsid:zoobank.org:act:9DAEF970-8067-4BC9-A3FF-40A1BC6EAA68

Type locality: Turkey, Bitlis Province, Uslu, Tatvan ca. 1 km E of Kaynarca, 38° 23′ N, 42° 37′ E, 1,735 m (Figs 1, 5E, Table 1).

Holotype. Adult male, specimen no. 1530, stuffed skin labelled "10/08/21-1530 ♂ IG/SD// *Talpa davidiana tatvanensis* ssp. nov.// Uslu, Tatvan, Bitlis// 137-17-19 = 45.25 g.// 10, August, 2021" Body frozen at -50˚C, internal organs in ethanol, skull extracted and labelled with specimen number (Fig. 6C). Collected by İ. Gündüz and S. Demirtaş. Sequences available from GenBank: (cyt-*b* haplotype: Hap.10, Accession number OQ695518; *BRCA2* haplotype: Hap.4, Accession number OQ695504); Standard voucher specimens (skin, frozen body, skull, and various tissues in ethanol) are deposited in the Department of Biology, Faculty of Sciences, Ondokuz Mayıs University (OMU-IG/SD), Samsun, Turkey

Paratypes (2). 1 female, specimen no. 1491, stuffed skin labelled "11/06/21-1491 ♀ IG/SD// *Talpa davidiana tatvanensis* ssp. nov.// Uslu, Tatvan, Bitlis // 148-18-17 = 46.95g.// 11, June, 2021"; 1 female, specimen no. 1492, stuffed skin labelled "11/06/21-1492 ♀ IG/SD// *Talpa davidiana tatvanensis* ssp. nov.// Uslu, Tatvan, Bitlis // 153-19-18 = 48.01g.// 11, June, 2021. Bodies frozen at -50˚C, internal organs in ethanol, skulls extracted and labelled with specimen numbers. Sequences available from GenBank: (cyt-*b* haplotypes: Hap.9–10, Accession numbers OQ695517– OQ695518; *BRCA2* haplotype: Hap.4, Accession number OQ695504); standard voucher specimens (skins, frozen body, skulls, and various tissues in ethanol) are deposited in the Department of Biology, Faculty of Sciences, Ondokuz Mayıs University (OMU-IG/SD), Samsun, Turkey.

Measurements of holotype. Body mass 45.25 g, head and body length 137 mm, tail length 17 mm, hind-foot length 19 mm, condylobasal length of cranium 31.19 mm, maxillary tooth row 12.94 mm, breadth of braincase 15.20 mm, height of braincase 9.58 mm, breadth of rostrum over canines 4.76 mm, breadth of rostrum over molars 9.72 mm (Supporting Information, Table S3).

Measurements of paratypes. Linear measurements are in mm and body mass in grams. Body mass 46.95–48.01, head and body length 148.0–153.0, tail length 18.0–19.0, hind foot 17.0–18.0, condylobasal length of cranium 30.16–31.03, maxillary tooth row 12.71–13.14, breadth of braincase 14.96–15.16, height of braincase 9.07–9.16, breadth of rostrum over canines 4.69–4.75, breadth of rostrum over molars 9.59–9.87 (Supporting Information, Table S3 for measurements including additional FMNH specimens 82136 and 82137).

Diagnosis. Medium-sized mole; skull relatively large with a stout, broad, rostrum, particularly broad and robust over molars. A member of the subgenus *Talpa,* closest to *T. davidiana davidiana*, morphologically and genetically (Figs 2-3, 6). Differs from ***T. hakkariensis* sp. nov.** on its smaller size and characters discussed under ***T. hakkariensis* sp. nov.** Differs from *T. streetorum* on its smaller size, narrower rostrum and more posteriorly positioned braincase. Distinguished from *T. davidiana davidiana* primarily by the broader, more strongly arcuate rostral base over the molars (compare Figs 8 & 9). Rostral breadth over molars 8.35–8.94 mm in nine *T. davidiana davidiana* examined (including two almost topotypical specimens from Hatay Province), vs. 9.59–9.87 mm in ***T. davidiana tatvanensis* ssp. nov.**; rostrum also broader over canines, but differences less obvious (4.69–4.76 mm in *T. davidiana tatvansensis* **ssp. nov.** vs. 4.09–4.45 in *T. davidiana davidiana*). ***T. davidiana tatvanensis* ssp. nov.** also has a somewhat paler tail than *T. davidiana davidiana,* with most tail hairs greyish brown (compare Fig. 12E & F). The head and body pelage of the new subspecies is also slightly paler overall than in specimens of *T. davidiana davidiana*

examined (see Fig. 6B & C). The net cyt-*b p* distance between these subspecies is 1.52%; raw distance 2.15%. The net and raw cyt-*b* K2P distances of 1.52% and 2.19%, respectively, between *T. davidiana davidiana* and ***T. davidiana tatvanensis* ssp. nov.** is relatively low, leading us to consider this taxon as a geographically isolated subspecies rather than species, at present, despite the consistent morphological differences observed.

Description. A medium-sized mole (Figs 5F, 6C), with a relatively short tail; 12.16-12.42% of head and body length. Tail (Fig. 12F) parallel-sided, with sparse, stiff, sub-erect, overlapping bristles; individual bristles mostly pale greyish, almost translucent, up to 20% blackish or with black cores; bristles considerably variable in in length, giving a bushy appearance; tail tip tapered and rounded, visible when bristles are moved. Overall appearance of tail paler than body. Dorsal and ventral pelage greyish brown throughout, with noticeable silvery sheen, particularly laterally and ventrally; individual hairs typically brownish at tips, greyish below. Body elongated. Palm of manus (Fig. 12C) broad, relatively flat; claws at end of all five phalanges, flattened dorsoventrally, with convex upper and slightly concave lower margins, flattened oval in cross-section. Feet (Fig. 12F) approx. equal to tail length. Muzzle elongated, with hairs, including longer sensory bristles, much paler than on head. Snout pinkish with close, fine, small pale hairs.

Skull (Fig. 9) moderate, with a very stout, broad rostrum. Width across canines 15.11-15.75%, and width across molars 30.91-32.73% condylobasal length (13.56-14.71 and 28.12-29.54 in *T. davidiana davidiana* examined). Maxillary tooth row equals 41.49-42.35% of the condylobasal length. The braincase is broad and deep, flattened above; height of neurocranium 29.52-30.71% of condylobasal length. The posterior margin of palatine is usually slightly posterior to the imaginary line connecting the posterior alveolar margins of 3rd upper molars, and the anterior border of the infraorbital foramen is above the 2nd upper molar.

Upper incisors of decreasing size; 3rd incisor slightly less than ½ the size of the 1st. With 3 upper premolars in all known specimens. 3rd upper premolar largest; 1st intermediate and 2nd smallest (Fig. 9) Upper molars robust; 3rd much smaller than 1st and 2nd. 1st upper molar with small but distinct parastyle (Fig. 11C) in all specimens; protocone much larger than paracone; mesostyle relatively flattened at apex, slightly bifurcated. 2nd upper molar with well-developed parastyle; protocone larger than paracone; mesocone well developed and clearly bilobed at apex. 3rd upper molar with well-developed parastyle, slightly more prominent than mesostyle; mesostyle bilobed at apex; protocone larger than paracone. Mandible Figure 13C. Lower incisors (3) subequal. 1st lower premolar large, with prominent distal cusp; 3rd lower premolar subequal to 1st; 2nd lower premolar much smaller. 1st and 2nd lower molars large; 3rd smaller; all with prominent parastyles.

Etymology. The new subspecies is named after Tatvan, Bitlis Province, Turkey, where the type locality is situated (adjective).

Distribution and Ecology. Known from only the vicinity of Tatvan, Bitlis Province, Turkey, close to Van Gölü. Moles were trapped in grazed, high-altitude grassland, close to agricultural areas (Fig. 5E).

Comment. ***Talpa davidiana tatvanensis*** **ssp. nov.** appears to correspond to the moles from Tatvan referred to *T. davidiana* by Kryštufek *et al*. (2001). There are approximately 560 km between known localities of the new subspecies and the nearest known *T. davidiana davidiana* in Turkey. Whilst ***T. davidiana tatvanensis*** **ssp. nov.** is distinguishable from the nominotypical species on both molecules and morphology, differences are smaller than those between these taxa and ***T. hakkariensis*** **sp. nov.** and *T. streetorum*, and branch support values are lower. For these reasons, and the fact that our species delimitation analyses do not

separate *davidiana* and ***tatvanensis***, we consider these taxa as geographically separated, divergent populations of subspecies.

***Talpa streetorum* Lay, 1965**

Talpa streeti Lay, 1965 incorrect original spelling.

Type locality: Iran, Kurdistan Province, Hezar Darreh, 35° 25′ N, 47° 07′ E, 2,180 m (Fig. 1, Table 1).

Material examined: We examined the dry-preserved skulls of five specimens from the type series, housed in the Field Museum of Natural History, Chicago, with specimen numbers 96421-96425. Of these, the holotype is 96424. In addition, we examined a further dry-preserved skull of *T. streetorum* from Divandarreh in Iranian Kurdistan, close to the type locality, labelled 111007. Our study is restricted to skull characters, as skins and other body parts were preserved in alcohol and so are unlikely to retain their original colouration and dimensions well. Collection of topotypical specimens in the future would allow these features to be better documented, as they are also missing from the original description (Lay, 1965).

Measurements of holotype. Condylobasal length of skull 32.51 mm, maxillary tooth row 14.33 mm, breadth of braincase 16.42 mm, height of braincase 10.13 mm, breadth of rostrum over canines 5.07 mm, breadth of rostrum over molars 10.53 mm (Supporting Information, Table S3).

Measurements of paratypes. Means±standard deviation (minimum–maximum). Linear measurements are in mm. Sample size = 4 for all measurements. Condylobasal length of skull 32.20±0.600 (31.55–32.96), maxillary tooth row 14.23±0.160 (14.08–14.44), breadth of braincase 16.75±0.180 (16.59–17.00), height of braincase 9.76±0.320 (9.34–10.01), breadth of rostrum over canines 5.00±0.140 (4.79–5.10), breadth of rostrum over molars 10.64±0.230

(10.44–10.97) (Supporting Information, Table S3, for measurements including additional FMNH specimen 111007).

Diagnosis. Very large mole; skull large with a stout, broad, rostrum, closest in overall appearance to ***T. hakkariensis* sp. nov.** (compare Figs 7 & 10). Upper premolars 1-3 and lower premolars 2-3 peglike, and 1st upper molar with at most trace of parastyle cusp, as in ***T. hakkariensis* sp. nov.** (see Fig. 11). Differs from ***T. hakkariensis* sp. nov.** in having multiple mandibular foramina and a broader base to the coronoid process (Fig. 13). Our measurements show that condylobasal length and breadth of the anterior rostrum are about the same in the two species (***T. hakkariensis* sp. nov.** means 32.88 mm and 5.24 mm; *T. streetorum* means 32.51 mm and 5.07 mm, respectively), but *T. streetorum* is larger in terms of palatal and braincase widths (***T. hakkariensis* sp. nov.** means 9.86 mm and 16.30 mm; *T. streetorum* means 10.70 mm and 16.88 mm, respectively). The skull as a whole as measured by centroid size is larger in *T. streetorum* than in ***T. hakkariensis* sp. nov.**, as is the mandible (Fig. S2).

Comment. Note that the correct name for this taxon is *T. streetorum*, as corrected by Lay (1967), because it was named after William S. and Janice K. Street, the genitive singular being used in error in the original description. *T. streetorum* was used by a number of workers (e.g., Niethammer, 1969; Ziegler, 1971), but appears to have been dropped since Corbet (1978) considered it an unjustified emendation. Following Article 31.1.2 of the Code, however, the correct name is clearly *T. streetorum*. Despite the lack of genetic data, our morphometric analyses show that *T. streetorum* differs significantly from ***T. hakkariensis* sp. nov.** and *T. davidiana* s. lat. on skull characters. On this basis, we reinstate *T. streetorum* as a **valid species**, apparently endemic to Iran. Further work in the future, including DNA sequence data, would be useful to confirm this hypothesis. It was not possible to obtain tissues for molecular analyses due to restrictions outside our control.

DISCUSSION

Using a combination of mitochondrial and nuclear gene sequences, we show that the diversity of Anatolian moles is even greater than previously realised. Detecting species boundaries in cryptic subterranean taxa such as *Talpa* is intrinsically difficult, and for the first time, we apply rigorous molecular species delimitation models to test taxonomic hypotheses in the genus. In the case of our newly defined taxa, we take an essentially phylogenetic approach to defining species (see Cracraft, 1983), supporting our DNA-based species hypotheses with morphological and morphometric analyses to accurately define taxa (e.g., Vogler & Monaghan, 2007; Oates *et al*., 2022). Our analyses confirm the distinctness of all previously recognised *Talpa* taxa, including the recently segregated *T. transcaucasica* (Demirtaş *et al*., 2020), and support the recognition of the new species and subspecies described herein.

On the basis of genetics, morphology and morphometrics, we define *T. davidiana* in a narrower sense than Kryštufek *et al*. (2001) and Kryštufek & Vohralík (2005). Large moles from Hakkari Province, Turkey represent a new species, ***T. hakkariensis* sp. nov.**, which is the largest mole thus far known in Anatolia, and amongst the largest *Talpa* described to date. The large Hakkari specimens included in Kryštufek *et al*.'s (2001) study likely correspond to this species. Although there are no generally accepted criteria for delineating species based on genetic distance, interspecific cyt-*b* distances observed in mammals range from 2.50% to 19.23% (Bradley & Baker, 2001). Baker & Bradley (2006) estimated the number of mammalian phylogroups using cyt-*b* data from sister species recognized in classical morphological studies and hypothesized that there may be more than 2,000 unrecognized mammal species. They also concluded that if mammalian phylogroups are separated by a genetic distance of >5% in the cyt-*b* gene, this strongly suggests the presence of distinct species. In light of this, the net K2P distance of 11.79% reported here between *T. davidiana*

and ***T. hakkariensis* sp. nov.** falls well within the range of sister species cyt-*b* divergence observed in mammals. It is also considerably higher than distances observed between most currently recognized *Talpa* species, which range from 5.66% (*T. levantis* and *T. transcaucasica*) to 15.55% (*T. martinorum* and *T. talyschensis*) (Table S5). On this basis, together with the results of molecular species delimitation analyses, descriptive morphology and morphometrics, *T. davidiana* and *T. **hakkariensis* sp. nov.** each possess a combination of apomorphic traits and can be considered as separate species under a phylogenetic species concept (Cracraft, 1983; Bradley & Baker, 2001; Baker & Bradley, 2006).

On the basis of almost topotypical and other specimens, we show that the true *T. davidiana* is a distinct, smaller-bodied taxon, divisible into two geographically isolated populations, which can be readily distinguished, both genetically and morphologically (see above). Whilst the precise distributional ranges of the two subspecies remain unclear, it seems these taxa may be entirely allo- or parapatric. Despite these molecular and morphological differences, formal molecular species delimitation analyses fail to separate these populations as distinct species-level taxa, which is why, for the moment, we adopt the rank of subspecies here. Whilst the use of subspecies has sometimes been criticized (e.g., Burbrink *et al*., 2022), we feel that this rank provides an appropriate compromise here. Clearly, further study of *T. davidiana* sensu lato, including a wider range of individuals and populations for both molecules and morphology, would be instructive.

Kryštufek *et al*. (2001) synonymised *T. streetorum* with *T. davidiana* and later Kryštufek & Vohralík (2005), in a discussion focussing on *T. chthonia* from the Pleistocene of Israel, made *streetorum* a subspecies of *T. davidiana,* including Iranian type material, large *davidiana*-type Hakkari moles, and *davidiana* from the environs of Lake Van. They speculated that this subspecific taxon could be the same as the fossil *T. chthonia* but could not draw firm conclusions. Hakkari animals, sequenced here for the first time, are genetically

very divergent from *T. davidiana* and differ significantly from *T. streetorum* on skull characters, and are best recognised as a separate species, as is done here. *T. streetorum* differs from ***T. hakkariensis* sp. nov.** in having multiple mandibular foramina and a broader base to the coronoid process – significant morphological differences in talpids. In addition, geometric morphometric tests show that the two are significantly different in both mandibular and cranial shape, and centroid size shows that *T. streetorum* is larger in both structures than ***T. hakkariensis* sp. nov.** Given the distinctiveness in morphology of *T. streetorum* and ***T. hakkariensis* sp. nov.**, together with the geographical distance between known populations, we think the most plausible taxonomic hypothesis is that these moles are different species. As demonstrated by a number of recent studies, including ours, mole taxa typically have small geographical ranges, particularly in semi-arid areas such as southern and eastern Turkey and Iran. Moles from the Lake Van area are quite different from ***T. hakkariensis* sp. nov.** and appear referable to *T. davidiana*, but are morphologically and genetically distinct from topotypical material and so are described as ***T. davidiana tatvanensis* ssp. nov.**

Our species delimitation and phylogenetic analyses strongly suggest that *T. transcaucasica* should be considered an independent species, following Demirtaş *et al*. (2020), rather than a subspecies of *T. levantis*, as suggested by Kefelioğlu *et al*. (2020). *T. transcaucasica* is much more divergent on cyt-*b* sequences from the two remaining subspecies of *T. levantis* than either is from each other (see results). *T. transcaucasica* also differs from *T. levantis* **sspp.** on *BRCA2* sequences (Demirtaş *et al*., 2020). In fact, the nucleotide difference between the *BRCA2* sequences of *T. transcaucasica* and *T. levantis* **sspp.** is much higher than between several well-established related species of *Talpa*. The *T. transcaucasica BRCA2* haplotype (KP717115) differs from all *T. levantis* **sspp.** *BRCA2* haplotypes at three unique positions over the 656 bp used in our phylogeny, whereas the *T. occidentalis BRCA2* haplotype (KP717106), for example, differs from all *T. europaea*

BRCA2 haplotypes at just one unique position (Fig. 3). In light of these factors, we reinstate *T. transcaucasica* as a **valid species** rather than a subspecies of *T. levantis*.

In summary, our work substantially improves our understanding of Eurasian moles and increases the number of extant taxa recognised in the genus *Talpa* to 18 (16 species, plus two subspecies); a revised checklist of the genus is provided below. Since Hutterer (2005), the number of recognised taxa has therefore doubled, and we have no doubt that further investigations will reveal additional diversity in this genus, particularly in Western Asia, where a combination of climatic and topographical heterogeneity (as discussed in Demirtaş *et al*., 2020) appears to have resulted in extensive diversification in these animals.

Revised checklist of global *Talpa* species

Talpa Linnaeus, 1758

altaica Nikolasky, 1883

aquitania Nicolas, Martínez-Vargas & Hugot, 2017

caeca Savi, 1822

caucasica Satunin, 1908

†*chthonia* Bate, 1937

davidiana (Milne-Edwards, 1884)

davidiana davidiana (Milne-Edwards, 1884)

***davidiana tatvanensis* ssp. nov.**

europaea Linnaeus, 1758

***hakkariensis* sp. nov.**

levantis Thomas, 1906

levantis levantis Thomas, 1906

levantis dogramacii Kefelioğlu, Kryštufek, Selçuk, Hutterer & Astrin, 2020

martinorum Kryštufek, Nedyalkov, Jonas, Astrin & Hutterer, 2018

occidentalis Cabrera, 1907

ognevi Stroganov, 1944

romana Thomas, 1902

stankovici Martino and Martino, 1931

streetorum Lay, 1965

talyschensis Vereschagin, 1945

transcaucasica Dahl, 1945

†*tyrrhenica* Bate, 1945

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Declarations

Conflicts of Interest: The authors declare that they have no conflicts of interest in relation to this work.

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SUPPORTING INFORMATION

Table S1 Additional GenBank accession numbers (not listed in Table 1) for sequences of Talpidae species used in phylogenetic analyses.

Table S2 Anatomical descriptions of the landmarks used in geometric morphometric analysis.

Table S3 Tables of caliper measurements for the specimens examined in this study.

Table S4 Net (D_a, above the diagonal) and raw (D_{xy}, below the diagonal) *p* distances between *Talpa* species based on cyt-*b*, with associated standard errors (SE) based on 10,000 bootstrap replicates. Species names are abbreviated as follows: *T. europaea* (T.eur.), *T. aquitania* (T.aqu.), *T. occidentalis* (T.occ.), *T. martinorum* (T.mar.), *T. romana* (T.rom.), *T. caeca* (T.cae.), *T. stankovici* (T.sta.), *T. talyschensis* (T.tal.), *T. levantis* (T.lev.), *T. transcaucasica* (T.tra.), *T. caucasica* (T.cau.), *T. ognevi* (T.ogn.), *T. altaica* (T.alt.), *T. davidiana* (T.dav.) and ***T. hakkariensis* sp. nov.** (T.hak.).

Table S5 Net (D_a, above the diagonal) and raw (D_{xy}, below the diagonal) K2P distance distances between *Talpa* species based on cyt-*b*, with associated standard errors (SE) based on 10,000 bootstrap replicates. Species names are abbreviated as follows: *T. europaea* (T.eur.), *T. aquitania* (T.aqu.), *T. occidentalis* (T.occ.), *T. martinorum* (T.mar.), *T. romana* (T.rom.), *T. caeca* (T.cae.), *T. stankovici* (T.sta.), *T. talyschensis* (T.tal.), *T. levantis* (T.lev.), *T. transcaucasica* (T.tra.), *T. caucasica* (T.cau.), *T. ognevi* (T.ogn.), *T. altaica* (T.alt.), *T. davidiana* (T.dav.) and ***T. hakkariensis* sp. nov.** (T.hak.).

Figure S1 Geometric morphometric landmark schemes for the cranium and mandible.

Figure S2 Principal components (PC) morphospace for the cranium geometric morphometric data set showing specimen numbers and geographic origin of each specimen.

Figure S3 Principal components (PC) morphospace for the mandible geometric morphometric data set showing specimen numbers and geographic origin of each specimen.

Figure S4 Thin plate spline models showing the difference in shape between the negative and positive ends of the first three PC axes for the cranium (A-C) and mandible (D-F) data sets.

Figure S5 Results of MANOVA tests for differences in cranium (A) and mandible (B) shape and plots of differences in centroid size of crania (D) and mandibles (E).

Figure S6 Thin plate spline plots showing the difference in cranium (A) and mandible (B) shape between *T. streetorum* and ***T. hakkariensis* sp. nov.**

File S1. Mandible landmark coordinates used in this study in TPS format. This configuration has 15 landmarks.

File S2. Skull landmark coordinates used in this study in TPS format. This configuration has 15 landmarks.

Figure legends

Figure 1. Map of locations (see Table 1) of *Talpa* moles from Turkey and Iran studied here.

Figure 2. Results of BI, ML, and MP analyses combined on a BI tree based on the complete cyt-*b* gene sequences of *Talpa* species. ML and MP bootstrap values greater than 70% and posterior probabilities (PPs) greater than 0.80 are shown at nodes. Haplotypes Hap.1–20 are from this study. Species assignments from one distance-based (ASAP) and two tree-based (GMYC, mPTP) delimitation methods are represented by vertical bars on the right side of the phylogeny. The black colour on the bars signifies that there is agreement across delimitation approaches, whereas grey colour indicates disagreement. For geographical origins of the published sequences, see Table 1 and Table S1.

Figure 3. Results of BI, ML, and MP analyses combined on a ML tree based on *BRCA2* sequences of *Talpa* and outgroup species. ML and MP bootstrap values greater than 70% and posterior probabilities greater than 0.80 are shown at nodes. Haplotypes Hap.1–8 are from this study. For the geographical origins of the published sequences, see Table 1 and Table S1.

Figure 4. Geometric morphometric results. Principal components (PC) morphospaces for the cranial landmarks (A-B) and for the mandibular landmarks (C-D) and scree plots for the variance explained by the individual PCs for the cranium (E) and mandible (F). See Fig. S1 for the landmarking schemes, Figs. S2-S3 for morphospace plots with individual specimens labelled, and Fig. S4 for thin plate spline models of the shape variation along each PC axis.

Figure 5. Freshly trapped Anatolian *Talpa in situ*, with overviews of habitats at collecting localities. A) ***T. hakkariensis* sp. nov.**, type locality, Turkey, Hakkari Province, Cemşililan yaylası ca. 8 km NNE of Durankaya; B) ***T. hakkariensis* sp. nov.**, Holotype, specimen 1533; C) *T. davidiana davidiana*, topotypical locality, Turkey, Hatay Province, Hassa, Çardak yaylası; D) *T. davidiana davidiana*, specimen 1565; E) ***T. davidiana tatvanensis* ssp. nov.**,

type locality, Turkey, Bitlis Province, Uslu, Tatvan ca. 1 km E of Kaynarca; F) ***T. davidiana tatvanensis* ssp. nov.** Holotype, specimen 1530.

Figure 6. Dorsal view of Anatolian *Talpa* skins. A) ***T. hakkariensis* sp. nov.**, Holotype, specimen 1533; B) topotypical *T. davidiana davidiana*, specimen 1565; C) ***T. davidiana tatvanensis* ssp. nov.** Holotype, specimen 1530. Scale bar = 50 mm.

Figure 7. *Talpa hakkariensis* sp. nov., Holotype, specimen 1533, cranium in A) lateral; B) ventral and C) dorsal views. Scale bar = 10 mm.

Figure 8. Topotypical *Talpa davidiana davidiana*, specimen 1565, cranium in A) lateral; B) ventral and C) dorsal views. Scale bar = 10 mm.

Figure 9. *Talpa davidiana tatvanensis* ssp. nov. Holotype, specimen 1530, cranium in A) lateral; B) ventral and C) dorsal views. Scale bar = 10 mm.

Figure 10. *Talpa streetorum* Holotype, Field Museum of Natural History, Chicago, specimen 96424, cranium in A) lateral; B) ventral and C) dorsal views. Scale bar = 10 mm.

Figure 11. Lateral view of left upper molar in Anatolian-Iranian *Talpa*, anterior to the left. A) ***T. hakkariensis* sp. nov.**, Holotype, specimen 1533; B) topotypical *T. davidiana davidiana*, specimen 1565; C) ***T. davidiana tatvanensis* ssp. nov.** Holotype, specimen 1530; D) *T. streetorum* Holotype, Field Museum of Natural History, Chicago, specimen 96424. Arrows indicate parastyle.

Figure 12. Feet and tails of Anatolian *Talpa*. A&D) ***T. hakkariensis* sp. nov.**, Holotype, specimen 1533; B&E) topotypical *T. davidiana davidiana*, specimen 1565; C&F) ***T. davidiana tatvanensis* ssp. nov.** Holotype, specimen 1530. Scale bar = A–C = 25 mm; D–F = 20 mm.

Figure 13. Mandibles of Anatolian-Iranian *Talpa*. A) ***T. hakkariensis* sp. nov.**, Holotype, specimen 1533; B) topotypical *T. davidiana davidiana*, specimen 1565; C) ***T. davidiana tatvanensis* ssp. nov.** Holotype, specimen 1530; D) *T. streetorum* Holotype, Field Museum of Natural History, Chicago, specimen 96424. Scale bar = 2 mm.