Mammalian Biology (2022) 102:531–536
https://doi.org/10.1007/s42991-022-00246-2

SHORT COMMUNICATION

UCE sequencing-derived mitogenomes reveal the timing of mitochondrial replacement in Malagasy shrew tenrecs (Afrosoricida, Tenrecidae, *Microgale*)

Patrick Arnold[1] · **Sereina Rutschmann**[1]

Received: 1 November 2021 / Accepted: 14 March 2022 / Published online: 6 April 2022

Abstract
Malagasy shrew tenrecs (*Microgale*) have increasingly been used to study speciation genetics over the last years. A previous study recently uncovered gene flow between the Shrew-toothed shrew tenrec (*M. soricoides*) and sympatric southern population of the Pale shrew tenrec (*M. fotsifotsy*). This gene flow has been suggested to be accompanied by complete mitochondrial replacement in *M. fotsifotsy*. To explore the temporal framework of this replacement, we assembled mitogenomes from publicly available sequencing data of ultra-conserved elements. We were able to assemble complete and partial mitogenomes for 19 specimens from five species of shrew tenrecs, which represents a multifold increase in mitogenomic resources available for all tenrecs. Phylogenetic inferences and sequence simulations support the close relationship between the mitochondrial lineages of *M. soricoides* and the southern population of *M. fotsifotsy*. Based on the nuclear divergence of northern and southern populations of *M. fotsifotsy* and the mitochondrial divergence between the latter and *M. soricoides*, there was a mean time window for replacement of ~ 350,000 years. This timeframe implies that the effective size of the ancestral *M. fotsifotsy* southern population was less 70,000.

Keywords *Microgale* · Tenrecs · Gene flow · Mitochondrial replacement · Madagascar

Tenrecs (Tenrecidae) are small afrotherian mammals endemic to Madagascar. They include 31 species occupying diverse ecological niches (climbing, fossorial, semi-aquatic, hedgehog-like). Shrew tenrecs (*Microgale*) represent, with 21 currently recognized species, the most speciose genus among all tenrecs. Several species have been described/elevated in the last two decades (Goodman and Soarimalala 2004; Goodman et al. 2006; Olson et al. 2009), highlighting that this number likely underestimates current *Microgale* species diversity. In addition, previous genetic data show that speciation is ongoing even in sympatric shrew tenrecs (Everson et al. 2020). These authors uncovered that the Pale shrew tenrec (*M. fotsifotsy*) consists of two geographic and genetic populations: a northern population in the vicinity of Montagne d'Ambre (the type locality; this population is hereafter depicted with (N) after the species name) and a southern population with a broad distribution across central and southern highlands (S). The southern population is sympatric with the closely related Shrew-toothed shrew tenrec (*M. soricoides*) across its entire range. Based on ultra-conserved elements (UCE), the inferred nuclear topology supports the monophyly of *M. fotsifotsy* (Everson et al. 2020). UCEs are genomic regions of high conservatism among (even distantly related) species and have been shown to be valuable markers for phylogenetic inferences beyond protein-coding genes (Faircloth et al. 2012). The mitochondrial phylogeny based on the NADH dehydrogenase 2 (ND2) locus, however, suggested *M. fotsifotsy* (S) specimens to form the sister lineage to *M. soricoides* rather than to *M. fotsifotsy* (N). Deeper analysis led the authors to conclude that the mito-nuclear incongruence within *M. fotsifotsy* is based on limited gene flow between its southern population and *M. soricoides*. They further conclude that the mitogenomic lineage of *M. fotsifotsy* (S) has thereby completely been replaced by the introgressing *M. soricoides* (Everson et al. 2020). To further explore the temporal frame work of this mitogenome replacement in more detail, more complete

Handling editor: Allan McDevitt.

✉ Patrick Arnold
arnold.patrick.zoo@gmail.com

[1] Evolutionary Adaptive Genomics, Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany

mitogenome sequences would be needed but there is only a single complete mitogenome published for tenrecs so far (the distantly related Lesser hedgehog tenrec, *Echinops talfairi*). Fortunately, bioinformatic pipelines have recently become available that allow for assembling mitogenomes from existing UCE or other large-scale nuclear sequencing data sets (Vieira and Prosdocimi 2019; Allio et al. 2020). We here apply these pipelines to existing sequencing data of shrew tenrecs to (I) increase mitogenomic resources available for the group, and (II) to explore the mitogenome replacement in *M. fotsifotsy* (S) more deeply.

In this study, mitogenomes of five tenrec species were newly assembled. UCE sequencing data for 18 specimens belonging to *M. fotsifotsy, M. soricoides, M. cowani* and *M. drouhardi* (Everson et al. 2020) as well as whole-genome sequencing data of *Nesogale talazaci* (Zoonomia Consortium 2020) (Table 1) were downloaded from the NCBI Sequence Read Archive (SRA) using the SRAtools 2.10 (http://ncbi.github.io/sra-tools/). Adapter sequences and low-quality reads were trimmed from the raw data using cutadapt 2.6 (Martin 2011). Mitogenome sequences were extracted from UCE sequencing data using the recently published MitoFinder pipeline (Allio et al. 2020). Contigs were assembled with MetaSPAdes (Nurk et al. 2017). The mitogenome of *Nesogale talazaci* was assembled from whole-genome sequencing data using Novoplasty2 (Dierckxsens et al. 2017). Protein-coding genes, rRNAs and tRNAs were annotated using MITOS (Bernt et al. 2013) and manually curated for gene boundaries in AliView (Larsson 2014).

Bayesian phylogenetic analyses were performed using the software BEAST v2.6.3 (Bouckaert et al. 2019). We defined the spiny tenrec *Echinops telfairi* (NC_002631.2) as the outgroup. The concatenated sequences alignment was based on the protein-coding and rRNA genes. All sequences were aligned per gene using mafft 7.407 (Katoh and Standley 2013).

The best-fit partitioning schemes and models of molecular evolution were first estimated using PartitionFinder v.2 (Lanfear et al. 2017), with linked the branch lengths and applied the greedy search algorithm. For the protein-coding genes, we defined each codon position as one possible partition. The best model was choose based on the Bayesian Information Criterion. Since the effective sample sizes (ESS) for all parameters were low and the runs did not converge under the best-fit partitioning scheme, we alternatively used the HKY substitution model with empirical base frequencies, gamma distributed rate heterogeneity and a proportion of invariant sites to reduce overparameterization. Divergence times were estimated using secondary calibrations from previous studies (Everson et al. 2016, 2020). We used normal prior distributions with standard deviation of 1 to constrain the split between *Echinops* and

Table 1 Newly assembled mitogenomes of Malagasy shrew tenrecs

#Voucher	Species (population)	SRA accession number of original UCE sequencing data (Everson et al. 2020)	Sequence length (bp)	NCBI TPA accession number for newly assembled mitogenomes (this study)
FMNH161928	*M. cowani*	SRR11305212	16,342	BK059631
FMNH166102	*M. cowani*	SRR11305211	16,342	BK059634
MVZ217026	*M. drouhardi*	SRR6053045	10,850	BK059638
FMNH154592	*M. fotsifotsy (N)*	SRR11305202	16,653	BK059354
FMNH156312	*M. fotsifotsy (N)*	SRR11305201	16,357	BK059626
FMNH172653	*M. fotsifotsy (N)*	SRR11305210	16,340	BK059642
FMNH156424	*M. fotsifotsy (S)*	SRR11305200	16,364	BK059627
FMNH156568	*M. fotsifotsy (S)*	SRR11305199	16,384	BK059628
FMNH161959	*M. fotsifotsy (S)*	SRR11305198	16,359	BK059632
FMNH166142	*M. fotsifotsy (S)*	SRR11305197	16,350	BK059636
FMNH170750	*M. fotsifotsy (S)*	SRR11305195	16,352	BK059641
FMNH156595	*M. soricoides*	SRR11305209	15,605	BK059629
FMNH159460	*M. soricoides*	SRR11305208	15,578	BK059630
FMNH162000	*M.soricoides*	SRR11305207	16,355	BK059633
FMNH166121	*M. soricoides*	SRR11305206	16,331	BK059635
FMNH166175	*M. soricoides*	SRR11305205	16,330	BK059637
FMNH166206	*M. soricoides*	SRR11305204	16,336	BK059639
FMNH167442	*M. soricoides*	SRR11305203	16,336	BK059640
US118	*N. talazaci*	SRR7704820	16,530	BK059643

Institutional abbreviations: FMNH Field Museum of Natural History Chicago, MVZ Museum of Vertebrate Zoology Berkeley, US isolate from Museum of Vertebrate Zoology Berkeley specimen for whole genome sequencing (GCA_004026705). *M. Microgale*, *N. Nesogale*

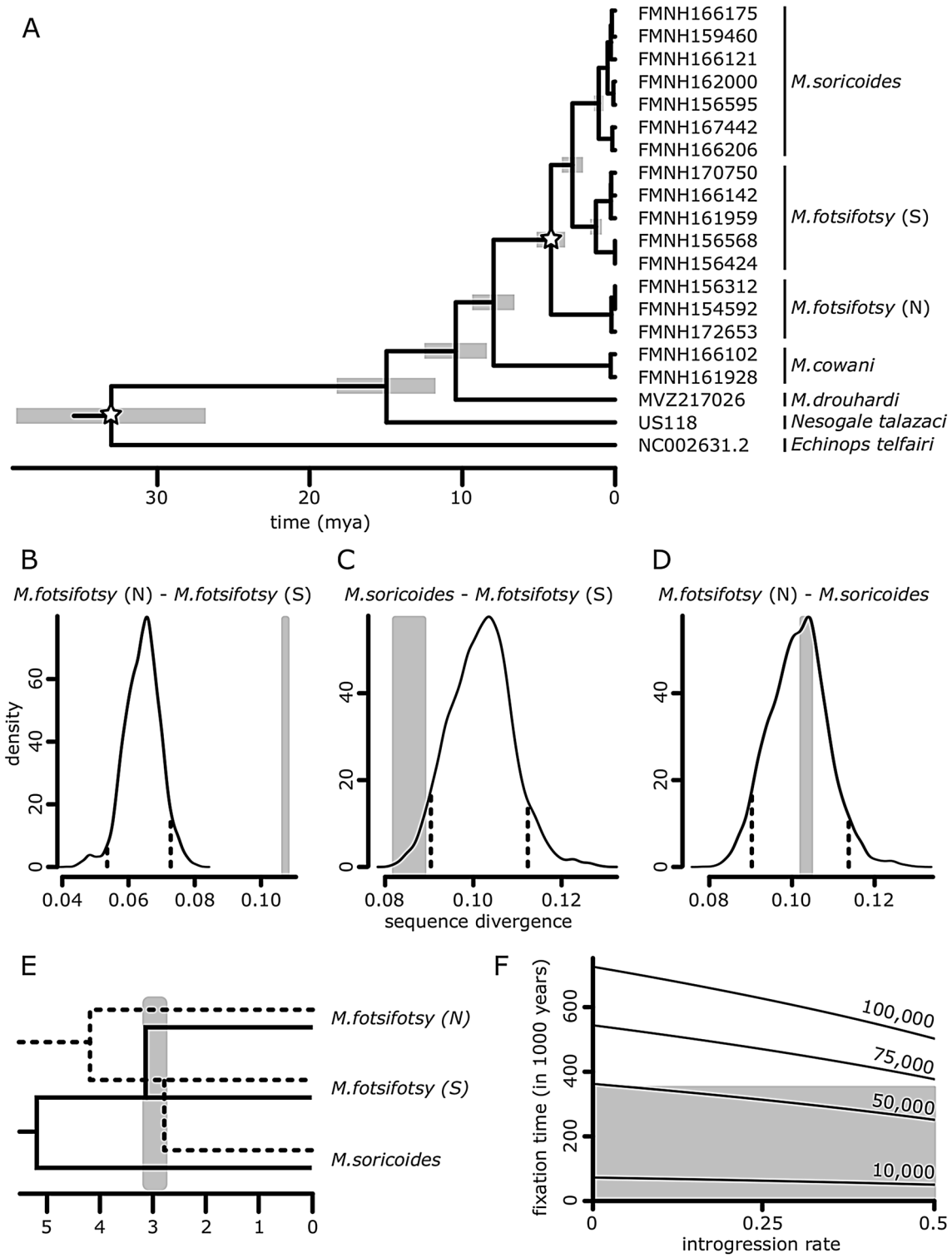
the ingroup (35.59 Ma). Similarly, we used normal prior distributions to constrain the divergence between *M. fotsifotsy* (N) and *M. soricoides* (6.0 Ma). Divergence dates were estimated using a relaxed clock as tree prior with a lognormal distribution (Drummond et al. 2006). We ran two independent analyses and performed for each 10^8 generations, sampling every 10^4 generations. The results of the two analyses were combined using LogCombiner v.2.6.3 (Bouckaert et al. 2019) removing the first 10% as burn-in. We used Tracer v.1.7.1 (Rambaut et al. 2018) to check the convergence within and between analyses and to ensure that the ESS for all parameters reached > $3*10^3$. We pruned from the trees 10% as burn-in and generated maximum-clade-credibility trees with node heights set to mean age estimates using TreeAnnotator v.2.6.3 (Heled and Bouckaert 2013).

To evaluate whether the conflicting signal between the nuclear and mitogenome-based phylogenies is supported by significant mitochondrial sequence divergence between *M. fotsifotsy* (N) and *M. fotsifotsy* (S), a null distribution of expected mitogenomic sequence divergence was generated in R (R Core Team 2020). For this, mitogenomic sequence divergence was simulated under the nuclear, UCE-based tree topology from Everson et al. (2020) for *M. cowani* (as outgroup), *M. soricoides*, *M. fotsifotsy* (N) and *M. fotsifotsy* (S). To account for temporal uncertainties of nodes, 100 time-trees were generated with node ages drawn from normally-distributed priors based on 95% confidence intervals in the previously published time tree (Everson et al. 2020) using the compute.brtime function from the ape package (Paradis and Schliep 2019). Sequence evolution was then simulated 100 times for each time tree using the simSeq function of the phangorn package (Schliep 2011), resulting in 10,000 sequences for every species. Sequence lengths in the simulations equaled the length of the empirical, concatenated dataset. Mutation rate was estimated from the uncorrected p-distance of *M. cowani* and *Echinops* sequences and their divergence time (Everson et al. 2016). Sequence divergence among *M. soricoides*, *M. fotsifotsy* (N) and *M. fotsifotsy* (S) were calculated in every simulation round using dist.dna function (model = K80, i.e., K2P) from ape package. Finally, observed mitogenomic sequence divergence in the empirical data set was compared to the simulated null distribution. The mean time period necessary for the introgressing mitogenome lineage to reach fixation was computed using the formula from Kimura and Ohta (1969): $T(p) = -2\ N*(1-p)*\ln(1-p)/p$, where N is the *M. fotsifotsy* (S) effective population size and p is the proportion of the introgressing *M. soricoides* mitogenome lineage (readapted from 4N for autosomes to 2N for mitochondrial DNA following Pagani et al. (2016), Posth et al. (2017)). The fixation time was calculated for different combinations of introgression rate and *M. fotsifotsy* (S) effective population sizes. Generation time was assumed as 3.62 years (according the COMBINE database; Soria et al. 2021).

Mitogenomes could be reconstructed from single contigs for all 19 specimens (Table 1). Complete mitogenomes with full annotation were obtained for 18 specimens. For *M. drouhardi* (MVZ217026), only a partial mitogenome could be reconstructed and only ten of the protein-coding genes could be annotated. All mitochondrial genomes produced here were submitted to GenBank under the Third Party Annotation (TPA) database (Table 1). Our results increase the number of tenrec mitogenomes from 1 to 20 and mitogenomic resources are now available for six tenrecid species. As the southern population of *M. fotsifotsy* likely represents a separate species (Everson et al. 2020), complete mitogenomic data are already available for this new candidate.

Our mitogenome phylogeny supports previous findings from a single mitochondrial locus. *M. fotsifotsy* is paraphyletic as its southern population is sister to *M. soricoides*, suggesting deep mitochondrial divergence between the northern and southern population of *M. fotsifotsy* (Fig. 1A). Bayesian dating analysis reveals that the mitochondrial lineages of *M. fotsifotsy* (N) and the *M. fotsifotsy* (S)—*M. soricoides* clade split 4.19 mya (95% CI: 3.29–5.17). *M. fotsifotsy* (S) and *M. soricoides* split 2.79 mya (95% CI: 2.13–3.53). Although using the similar age priors as in Everson et al. (2020), node ages to and among outgroups are generally younger than inferred from nuclear or combined data (Everson et al. 2016, 2020). The divergence between *M. fotsifotsy* (N) and *M. soricoides* is nevertheless similar (Fig. 1A; 95% CIs greatly overlap). Simulating sequence evolution further supports deep mitochondrial divergence between the northern and southern population of *M. fotsifotsy* (Fig. 1B). Empirical sequence divergence is significantly higher than expected under the simulated evolution according a nuclear-based topology (> 95% percentile). In addition, empirical sequence divergence among specimens of *M. fotsifotsy* (S) and *M. soricoides* is significantly smaller than expected (< 5% percentile) and implies a closer evolutionary history than suggested by the simulations based on nuclear-based topology (Fig. 1C). To validate our simple model of sequence divergence, we also compared *M. fotsifotsy* (N) and *M. soricoides*, whose phylogenetic relationship is unaffected using either nuclear or mitogenomic data. The empirical divergence of their mitochondrial sequences falls close to the mean of the null distribution of expected divergence according the nuclear-based topology (Fig. 1D). It is also in a similar magnitude as the empirical divergence in *M. fotsifotsy* (N)—*M. fotsifotsy* (S) (Fig. 1B). This comparison thus serves as a positive control.

Overall, mitogenomic data assembled here and in Everson et al. (2020) imply complete mitochondrial replacement in the southern population of *M. fotsifotsy* by the *M. soricoides* mitogenomic lineage. This is in concordance with limited



but significant nuclear gene flow reported before (Everson et al. 2020). The replacement must have taken place after the nuclear split between northern and southern population (3.14 mya; Everson et al. 2020) but before the split between the mitochondrial lineages of *M. fotsifotsy* (S) and *M. soricoides* (2.79 mya; this study), resulting in a mean time for replacement of ~ 350,000 years (Fig. 1E). This seemingly small temporal window nevertheless covers roughly 100,000 generations in shrew tenrecs. Although empirical data on ancestral population sizes are completely lacking for Malagasy shrew tenrecs, testing different introgression rates with the formula above provides maximum limits for effective

◂**Fig. 1** Detailed history of mitogenome replacement in the southern population (S) of *M. fotsifotsy*. **A** Dated phylogeny based on newly assembled mitogenomes. Grey bars indicate 95% confidence intervals of node ages. *M*: *Microgale*. Nodes used for calibration are indicated with a white star. All nodes have posterior probability of 1 except for the node between the sister specimens FMNH166142 and FMNH170750 (PP < 0.5). **B**–**D** Simulated distribution of mitochondrial sequence divergence under a nuclear-based topology among different shew tenrec species/populations. Dotted lines indicate 95% (**B**) and 5% (**C**) percentiles, respectively. Grey areas indicate range of empirically estimated sequence divergences among specimens. **E** Summary phylogeny visualizing differences in nuclear (solid line) and mitochondrial (dotted line) topology and divergence dates. Grey area indicates temporal window for mitogenome replacement. **F** Time to fixation of introgressing *M. soricoides* mitochondrial lineage in relation to introgression rate under varying *M. fotsifotsy* (S) effective population sizes (numbers above lines). Grey area indicates fixations times within the 350,000 years window for mitogenome replacement

population size of *M. fotsifotsy* (S). Results show that in a population of effective size less than 50,000 the mitogenome is rapidly replaced even under small introgression rates (Fig. 1F). If there have been higher introgression rates, 350,000 years would still be sufficient to fix the introgressing *M. soricoides* mitogenome lineage under *M. fotsifotsy* (S)'s effective population size of up to ~ 70,000 (Fig. 1F).

Mitochondrial replacement has frequently been observed in closely related mammal species, for instance in hares, roe deer and chipmunks (Good et al. 2008; Melo-Ferreira et al. 2012; Matosiuk et al. 2014). These studies highlighted the importance of divergence with gene flow in mammals, i.e. when lineage divergence occurs on a shorter timescale than does the completion of reproductive isolation. This phenomenon is most-extensively investigated in chipmunks, which show widespread and multiple mitochondrial introgression among species (Sullivan et al. 2014). It was recently shown that mitogenome introgression and replacement in this group occurred with only little or no nuclear introgression and that species boundaries are largely impermeable to nuclear gene flow (Sarver et al. in press). Gene flow among *Microgale* species here mirrors the situation in chipmunks as nuclear gene flow was relatively limited despite complete mitochondrial replacement (Everson et al. 2020). Thus, *M. fotsifotsy* and *M. soricoides* likely diverged under gene flow permeable for mitochondrial introgression. Signatures of positive directional (adaptive) selection are rarely detectable in cases of mitochondrial replacement (Sarver et al. 2017). More likely, the replacement in *M. fotsifotsy* (S) arose from a combination of demographic effects associated with range expansion, mate choice and the selection against hybrids (Bonnet et al. 2017). In conclusion, we were able to further elucidate the temporal framework of gene flow among *Microgale* species that have led to complete mitochondrial replacement. In addition, we highlight the usefulness of existing sequencing data to retrieve other genetic information.

Funding Open Access funding enabled and organized by Projekt DEAL.

Availability of data All sequence read data and newly assembled mitogenomes are publicly available from NCBI GenBank. Accession numbers are listed in Table 1.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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