The *Monticola* rock-thrushes: Phylogeny and biogeography revisited

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A B S T R A C T

We investigated the phylogenetic relationships within the *Monticola* rock-thrushes, an open-habitat genus inhabiting a large part of the Old World. Our results support one Oriental clade and one clade including African, Malagasy and Eurasian taxa. The biogeographic reconstruction obtained with the dispersal–vicariance analysis suggested Southern Africa plus Palearctic as the *Monticola* ancestral area. Our phylogenetic hypothesis suggests also some taxonomic changes. The polytypic *Monticola solitarius* includes two reciprocally monophyletic clades that should be recognized as full species, *M. solitarius s.s.* and *M. philippensis*. With the exclusion of the south-western population, *M. imerinus*, all other Malagasy rock-thrush populations should be merged in the monotypic, albeit polymorphic, *M. sharpei*. The genus *Thamnolaea* is shown to be non-monophyletic, with *T. semirufa* being part of the *Monticola* radiation, while *T. cinnamomeiventris* is related to other chat species inhabiting open-habitats. We demonstrate that a previous phylogenetic hypothesis for the rock-thrushes was flawed by the inclusion of contaminated sequences obtained from study-skins and we suggest some working guidelines to improve the reliability of the sequences obtained from old or degraded DNA.

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1. Introduction

The *Monticola* rock-thrushes are a small group of medium-sized birds distributed in the Old World. Traditionally they have been placed together with the true thrushes in the family/subfamily Turdidae/Turdinae, but analyses based on molecular data revealed that they are part of the chat radiation (Muscicapidae Saxicolinae) (Voelker and Spellman, 2004).

Outlaw et al. (2007) presented an almost complete, well-resolved phylogeny of the genus *Monticola* and discussed the biogeographic history of the clade. They sampled all species except the Malagasy *Monticola imerinus*, in most cases with multiple individuals per species, and analyzed two mitochondrial genes, cytochrome *b* (cyt *b*) and NADH dehydrogenase II (ND2). According to Outlaw et al.'s results, the genus originated in the late Miocene either in the arid regions of Northern Africa plus the Arabian Peninsula or the African savanna, or both. The origin of the Eurasian species is the result of two transcontinental dispersals from Africa, and a single further dispersal to Madagascar originated the Malagasy species.

The genus *Thamnolaea* (*sensu* Collar, 2005) includes the two African species of cliff-chats. They are medium-sized chats with a predominantly black plumage and reddish belly. *T. cinnamoeiventris* is a polytypic species distributed across most of subsaharan

Africa in savanna and sparsely vegetated habitats, while *T. semirufa* is an Ethiopian endemic restricted to rocky areas above 1500 m. While investigating the relationships of the genus *Thamnolaea*, we discovered that this genus is not monophyletic (DZ unpub.). While *T. cinnamomeiventris* is more closely related to the genus *Myrmecocichla*, *T. semirufa* is actually part of the *Monticola* radiation. To clarify its exact phylogenetic position, we added our sequences of *T. semirufa* to the published *Monticola* dataset. In our first analysis we used as outgroup a selection of Muscicapidae species different from those included by Outlaw et al., because they did not submit their outgroup sequences to GenBank.

Our results were partially different from those published. In particular, in our re-analysis *Monticola rufocinereus* constantly fell outside of and far from the core *Monticola* clade. This prompted us to sequence that species again and to re-evaluate the phylogeny of the entire genus *Monticola*.

In this paper we provide evidence that the published sequences of *Monticola rufocinereus* are in all probability the results of laboratory contamination. A re-analysis of the dataset, with correct sequences obtained from other *M. rufocinereus* individuals, provides a new, well-resolved phylogenetic hypothesis for the rock-thrushes and suggests a different biogeographic scenario for their evolution.

2. Materials and methods

We sequenced the complete ND2 and partial cyt *b* genes of one individual of *Thamnolaea semirufa*, one *T. cinnamomeiventris*, two

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Monticola rufocinereus and one each of *M. brevipes*, *M. gularis*, *M. imerinus* and *M. rufiventris*, along with six individuals of *M. solitarius*, representing all recognised subspecies. All *Monticola* and *Thamnolaea* samples except *M. gularis* are toe-pads obtained from study-skins. The toe-pad samples were extracted using the Qiagen DNA Micro Kit, following the procedure described in Zuccon (2005) and Irestedt et al. (2006). The genes were amplified in 200–300 bp, overlapping fragments using a large series of internal primers, strictly following the guidelines of Irestedt et al. (2006). The primer sequences are available from the authors.

In the dataset we included all published *Monticola* sequences used by Goodman and Weigt (2002) and by Outlaw et al. (2007). As outgroups we included a selection of Muscicapidae and Turdidae taxa (see Table 1). We obtained the sequences of the outgroup taxa from fresh samples using standard extraction protocols, primers and amplification conditions (Voelker and Spellman, 2004; Zuccon et al., 2006), with the exclusion of few sequences that we retrieved from GenBank.

We combined the *Monticola* sequences published by Outlaw et al. (2007) into four datasets, with four different outgroup selections (see Table 1 for the species included in each datasets). We then used these datasets to investigate the effect of different outgroups on the *Monticola* topology.

To generate our phylogenetic hypothesis for the rock-thrushes, we analysed a fifth dataset that includes: (1) all published *Monticola* sequences, with the exclusion of the published *M. rufocinereus*; (2) our new *Monticola* sequences; (3) one individual of *Thamnolaea semirufa*; and (4) a selection of Muscicapidae taxa, including two individuals of *Thamnolaea cinnamomeiventris*.

All datasets were analyzed under Bayesian inference using MrBayes3.1.2 (Ronquist and Huelsenbeck, 2003), implemented on the freely available Bioportal (www.bioportal.uio.no). The models best fitting the data were obtained with MrModelTest (Nylander, 2004), using the AIC criterion, in conjunction with PAUP* (Swofford, 2003). MrModelTest output suggested the GTR+Γ+I model as the best fit for all the datasets analysed. For each dataset we ran two independent runs of four incrementally heated Metropolis-coupled MCMC chains for 10 million generations, with sampling every 1000 generations, yielding 10,000 trees. We used the online version of AWTY (Wilgenbusch et al., 2004) to assess the convergence of the MCMC chains and to estimate the number of generations to discard as "burn-in".

The fifth dataset was analyzed also under the maximum likelihood criterion. Maximum likelihood searches were conducted with RAxML v. 7.0.3 (Stamatakis, 2006) using a GTR+Γ+I model and random starting tree. Nodal support was estimated using 100 bootstrap replicates.

The published sequences of *Monticola rufocinereus* were compared with other published sequences using the online BLAST search function in GenBank.

We used the dispersal–vicariance analysis (DIVA, Ronquist, 1997) for reconstructing the ancestral areas. However, Outlaw et al. (2007) used a larger number of areas (nine), that we consider overdo partition, compared with the species ranges. We re-coded the species distributions using only five areas that better fit their ranges: southern Africa, eastern Africa, Madagascar, Palearctic and Oriental region. The dispersal–vicariance analysis was performed with the software DIVA 1.2 (Ronquist, 2001).

3. Results and discussion

3.1. Re-analysis of Outlaw et al.'s dataset

The first dataset recovers a tree perfectly congruent with that published by Outlaw et al., both in topology and supported nodes

(Fig. 1A). Only one of our outgroup species is shared with Outlaw et al. (*Cercomela familiaris*), while the other three are congeneric (*Myrmecocichla nigra*, *Thamnolaea cinnamomeiventris* and *Oenanthe deserti* used by us *vs. Myrmecocichla formicivora*, *Thamnolaea arnotti* and *Oenanthe monticola*), but apparently this had no influence in the analysis output. We conclude that the *Monticola* sequences deposited in GenBank are those used in the published analysis and no submission mistakes occurred.

The other three datasets differ in the outgroups: the second and third datasets include more Muscicapidae taxa (Fig. 1B and C) while in the fourth we added also three Turdidae (Fig. 1D). The trees were rooted with the Muscicapini or the Turdidae taxa, depending on the dataset. The three recovered trees share the same core *Monticola* clade (shaded in grey in Fig. 1), perfectly congruent both in topology and supported nodes. In contrast, the position of the two individuals of *Monticola rufocinereus* is instable, falling in a rather basal position, far away from the core *Monticola* clade and without support (thick branches in Fig. 1). We conclude that the *Monticola* monophyly recovered by Outlaw et al. (2007) is not supported by their data, and is a spurious result due to the outgroup and rooting choices.

The results coming from the increased outgroup analyses are highly suspicious because *M. rufocinereus* has always been placed within the genus *Monticola*, both on morphological and behavioural grounds (Urban et al., 1997).

Another unexpected point is the ~5% genetic divergence between the two individuals. Helbig et al. (1995) compared the genetic distances of several subspecies and sister species of birds using cyt *b* data. They observed that the intraspecific divergences were in the range 0–2.6%. At 5.2%, the cyt *b* divergence between the two *M. rufocinereus* individuals is much higher than the maximum observed intraspecific divergence.

A comparison of the two published *M. rufocinereus* sequences revealed that their divergence is not uniform across the entire sequence length. For both the ND2 and cyt *b* genes, the first half of the two sequences are almost identical (*p*-distances < 1%, both genes), while they are highly divergent in the second half (*p*-distances 4.4% and 10.5%, for ND2 and cyt *b*, respectively, Fig. 2). In contrast, the sequence divergence remains uniform across the entire gene length in all intraspecific comparisons of the other *Monticola* species.

We obtained complete ND2 (1041 bp) and partial cyt *b* (998 bp) sequences from two *M. rufocinereus* study-skins. When we compared our sequences to those published, it become evident that they are totally different (Fig. 3) and cannot belong to the same species (*p*-distances > 10%, both genes). Moreover, our sequences closely resemble each other, with an intraspecific *p*-distance < 0.5% (both genes), in line with the intraspecific divergences observed in the other *Monticola* species.

Using the BLAST search function in GenBank we discovered that both the putative ND2 and cyt *b* published *M. rufocinereus* sequences share almost identical portions with other published sequences (grey shading in Fig. 3). Both ND2 published sequences share 439 bp with *Ficedula crypta* (GenBank accession number DQ674473) and one of them shares 190 bp with *Ficedula strophiata* (GenBank accession number DQ674475). Both cyt *b* published sequences share 377 bp with *Tarsiger chrysaeus* (GenBank accession number DQ285439). The almost complete identity of the published putative *M. rufocinereus* sequences with those of birds belonging to different genera is highly suspicious for genes that are so variable even at the intraspecific level. The two *Ficedula* sequences were included in a molecular phylogeny of the *Ficedula* flycatchers (Outlaw and Voelker, 2006), published by the same research group just before the *Monticola* phylogeny. Also a *Tarsiger chrysaeus* sequences was included in the *Ficedula* phylogeny as outgroup, although that sequence was not submitted to GenBank.

Table 1
Samples and sequences included in this study, with museum accession numbers and collection localities. Sequences published previously are listed together with their GenBank accession numbers and references. Museum acronyms: AMNH: American Museum of Natural History; BD: Witwatersrand, P. Bloomer; FMNH: Field Museum of Natural History; MBM: Marjorie Barrick Museum of Natural History; MM: Moscow Museum; NMBV: National Museum, Bloemfontein; NRM: Swedish Museum of Natural History; USNM: Unites States National Museum, Smithsonian Institution; UWBM: Burke Museum, University of Seattle. [1]: Outlaw et al. (2007); [2]: Goodman and Weigt (2002); [3]: Voelker and Spellman (2004); [4]: Fuchs et al. (2006); [5]: Zuccon et al. (2006). *: vouchered samples. DS1–5 indicate which sequences were included in the datasets 1–5.

Taxon	Accession number	ND2	cyt b	Locality	DS 1	DS 2	DS 3	DS 4	DS 5
Catharus ustulatus	NRM 20016340*	GU237101	GU237074	USA				×	
Cercomela familiaris	NRM 680265*	GU237102	GU237075	Francistown, Botswana	×	×	×	×	×
Copsychus albospecularis	FMNH 352903	AY329418 [3]	AY329455 [3]	Toliara, Madagascar			×	×	
Cossypha dichroa	MBM 7428	AY329419 [3]	AY329456 [3]			×	×	×	×
Ficedula hypoleuca	NRM 976132*	DQ146345 [4]	GU237076	Sweden			×	×	
Luscinia calliope	NRM 20056739	GU237103	GU237077	Vu Quang National Park, Vietnam			×	×	
Melaenornis edolioides	NRM 20076771	GU237104	GU237078	Nigeria		×			×
Monticola angolensis	MBM 10867	EF434535 [1]	EF434508 [1]	Southern Region, Malawi	×	×	×	×	×
Monticola angolensis	MBM 11185	EF434536 [1]	EF434509 [1]	Southern Region, Malawi	×	×	×	×	×
Monticola bensoni	FMNH 380005	AF276796 [2]	AF276786 [2]	Western side of Andringitra, Madagascar					×
Monticola bensoni	FMNH 380005	EF434537 [1]	EF434510 [1]	Western side of Andringitra, Madagascar	×	×	×	×	
Monticola bensoni	FMNH 380007	AF276795 [2]	AF276785 [2]	Vohimena Forest, Madagascar					×
Monticola bensoni	FMNH 380010	AF276790 [2]	AF276780 [2]	Vohimena Forest, Madagascar					×
Monticola bensoni	FMNH 396194	EF434538 [1]	EF434511 [1]	Namaza River, Madagascar	×	×	×	×	×
Monticola brevipes	MBM 7416	EF434539 [1]	EF434512 [1]	North Cape Province, South Africa	×	×	×	×	×
Monticola brevipes	MBM 7417	EF434540 [1]	EF434513 [1]	North Cape Province, South Africa	×	×	×	×	×
Monticola brevipes	NRM 570036*	GU237105	GU237079	Damaraland, Namibia					×
Monticola brevipes	UWBM 53090	EF434541 [1]	EF434514 [1]	Orange Free State, South Africa	×	×	×	×	×
Monticola cinclorhynchus	AMNH 831315	EF434542 [1]	EF434515 [1]	Kipsung, Nepal	×	×	×	×	×
Monticola cinclorhynchus	FMNH 347868	AF276788 [2]	AF276777 [2]	North-western Pakistan	×	×	×	×	×
Monticola explorator	UWBM 53127	EF434543 [1]	EF434516 [1]	Orange Free State, South Africa	×	×	×	×	×
Monticola gularis	NRM 20036789	GU237106	GU237080	Kon Tum Province, Vietnam					×
Monticola gularis	UWBM 47213	EF434545 [1]	EF434518 [1]	Khabarovskiy Kray, Russia	×	×	×	×	×
Monticola gularis	UWBM 59864	EF434544 [1]	EF434517 [1]	Dornod Aymag, Mongolia	×	×	×	×	×
Monticola imerinus	NRM 570039*	GU237107	GU237081	Tsimanampetsoa Lake, Madagascar					×
Monticola pretoriae	BD 83317	EF434546 [1]	EF434519 [1]	Gauteng Province, South Africa	×	×	×	×	×
Monticola pretoriae	BD 83318	EF434547 [1]	EF434520 [1]	Gauteng Province, South Africa	×	×	×	×	×
Monticola pretoriae	BD 83328	EF434550 [1]	EF434523 [1]	Gauteng Province, South Africa	×	×	×	×	×
Monticola pretoriae	BD 83329	EF434549 [1]	EF434522 [1]	Gauteng Province, South Africa	×	×	×	×	×
Monticola pretoriae	BD 83330	EF434548 [1]	EF434521 [1]	Gauteng Province, South Africa	×	×	×	×	×
Monticola rufiventris	AMNH 23243	EF434551 [1]	EF434524 [1]	Gonga, Nepal	×	×	×	×	×
Monticola rufiventris	NRM 570035*	GU237108	GU237082	Tonkin, Vietnam					×
Monticola rufocinereus	FMNH 190270	EF434552 [1]	EF434525 [1]	Limuru, Kenya	×	×	×	×	
Monticola rufocinereus	NRM 570037*	GU237109	GU237083	Eritrea, Gheleb					×
Monticola rufocinereus	NRM 570038*	GU237110	GU237084	Eritrea, Asmara					×
Monticola rufocinereus	USNM 522520	EF434553 [1]	EF434526 [1]	Balaghes Valley, Ethiopia	×	×	×	×	
Monticola rupestris	MBM 7884	EF434554 [1]	EF434527 [1]	South Africa	×	×	×	×	×
Monticola saxatilis	MM P97	EF434555 [1]	EF434528 [1]	Tyva, Russia	×	×	×	×	×
Monticola saxatilis	MM P98	EF434556 [1]	EF434529 [1]	Tyva, Russia	×	×	×	×	×
Monticola saxatilis	UWBM 46533	EF434558 [1]	EF434531 [1]	Almaty Oblysy, Kazakhstan	×	×	×	×	×
Monticola saxatilis	UWBM 59738	EF434557 [1]	EF434530 [1]	Dornod Aymag, Mongolia	×	×	×	×	×
Monticola sharpei erythronotus	FMNH 384798	AF276789 [2]	AF276778 [2]	Montagne d'Ambre, Madagascar					×
Monticola sharpei salomonseni	FMNH 384724	AF276791 [2]	AF276781 [2]	Ankaratra Massif, Madagascar					×
Monticola sharpei salomonseni	FMNH 384724	EF434560 [1]	EF434533 [1]	Ankaratra Massif, Madagascar	×	×	×	×	
Monticola sharpei sharpei	FMNH 363837	EF434559 [1]	EF434532 [1]	Eastern Andringitra, Madagascar	×	×	×	×	
Monticola sharpei sharpei	FMNH 363838	AF276792 [2]	AF276782 [2]	Eastern side of Andringitra, Madagascar					×
Monticola sharpei sharpei	FMNH 384786	AF276794 [2]	AF276784 [2]	Andohahela, Madagascar					×
Monticola solitarius longirostris	NRM 570032*	GU237111	GU237085	Israel					×
Monticola solitarius longirostris	NRM 570043*	GU237112	GU237086	Iran					×
Monticola solitarius madoci	NRM 570042*	GU237113	GU237087	Malaysia					×
Monticola solitarius pandoo	NRM 570033*	GU237114	GU237088	Lao Bao, Vietnam					×
Monticola solitarius philippensis	FMNH 358375	EF434561 [1]	EF434534 [1]	Sibuyan Island, Philippines	×	×	×	×	×
Monticola solitarius philippensis	NRM 570034*	GU237115	GU237089	Shansi, China					×
Monticola solitarius solitarius	NRM 570031*	GU237116	GU237090	Algeria					×
Muscicapa striata	NRM 20046170*	GU237117	GU237091	Sweden		×	×	×	×
Myadestes occidentalis	NRM 20066505	GU237118	GU237092	El Salvador				×	
Myrmecocichla nigra	NRM 570041*	GU237119	GU237093	Dembos, Angola	×	×	×		×
Niltava sundara	NRM 947301	GU237120	GU237094	Vinh Phu, Vietnam			×	×	
Oenanthe deserti	NRM 20046660	GU237121	GU237095	Iran	×	×	×	×	×
Phoenicurus phoenicurus	NRM 20016219*	GU237122	GU237096	Sweden		×	×	×	×
Saxicola rubetra	NRM 20016186*	GU237123	GU237097	Sweden		×	×	×	×
Tarsiger cyanurus	UWBM 51572	AY329438 [3]	AY329475 [3]	Russia		×	×	×	×
Thamnolaea cinnamomeiventris	MBM 11043	AY329439 [3]	AY329476 [3]						×
Thamnolaea cinnamomeiventris	NMBV 04832*	GU237124	GU237098	South Africa					×
Thamnolaea semirufa	NRM 570040*	GU237125	GU237099	Sidamo, Ethiopia					×
Turdus philomelos	NRM 20036737*	DQ466886 [5]	GU237100	Sweden				×	

The evidence presented here shows that the published *M. rufocinereus* sequences are highly anomalous and should be discarded. In our opinion there is a high chance that those sequences were contaminated by other samples during the lab procedures.

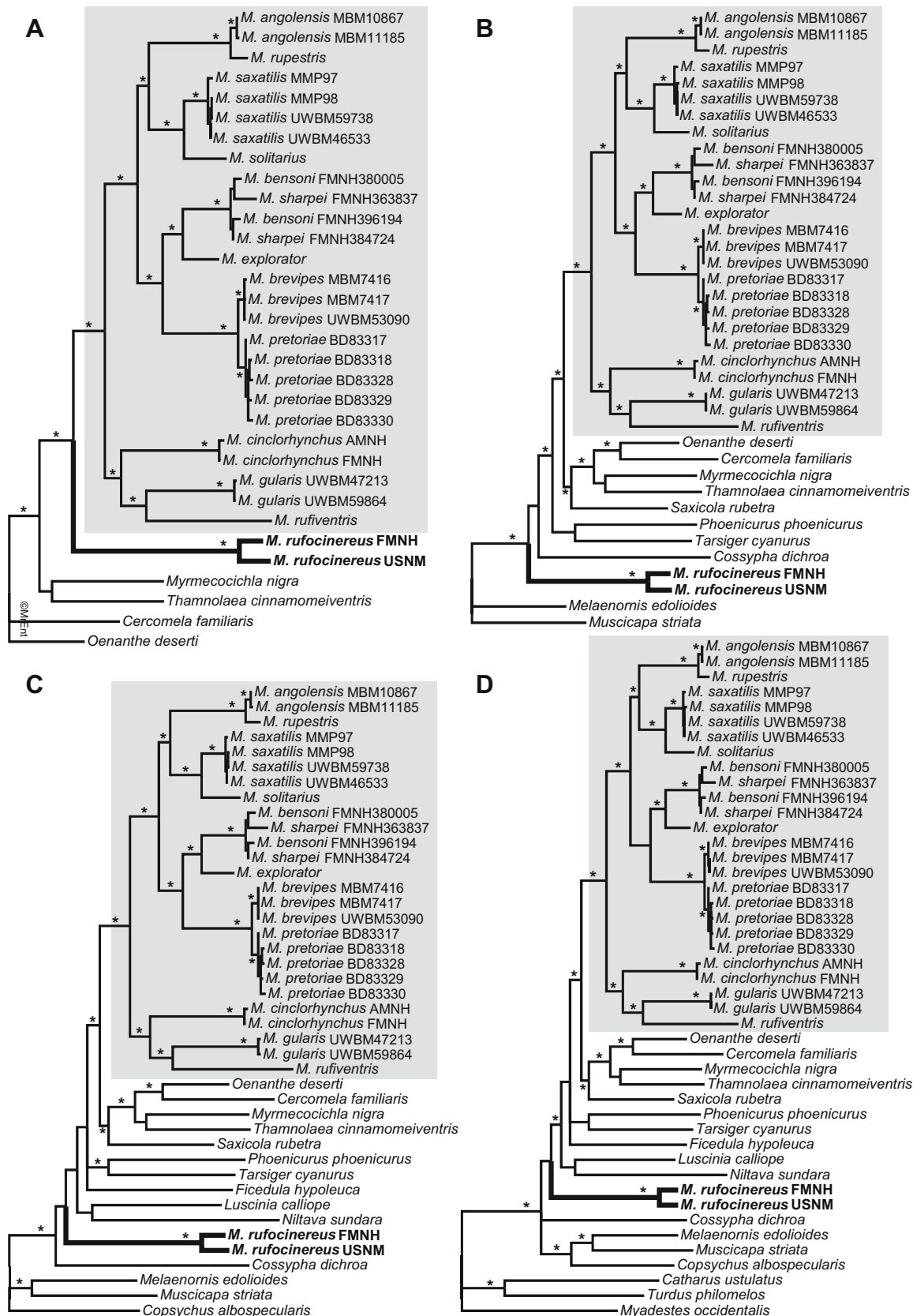**Fig. 1.** The majority rule consensus trees obtained from the re-analysis of Outlaw et al.'s (2007) *Monticola* datasets by Bayesian inference, using four different outgroup taxa sets. Posterior probabilities equal or higher than 0.95 are indicated with an asterisk at the node. The core *Monticola* clade, identical in all four trees, is highlighted in grey; whereas the putative *M. rufocinereus* clade is in bold.
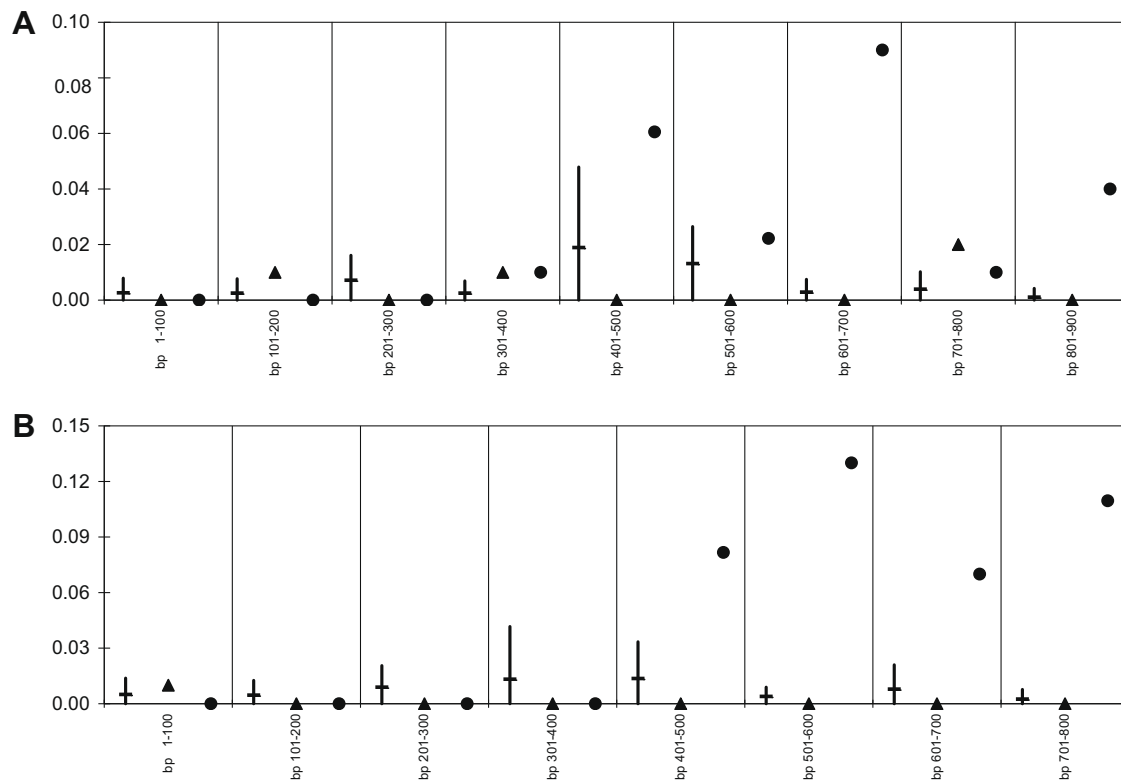
Fig. 2. Intraspecific sequence divergences within the published *Monticola rufocinereus* sequences (●), the new *M. rufocinereus* sequences obtained in this study (▲) and the remaining *Monticola* species (mean and range of variation). The *p*-distance values have been calculated in 100 bp long windows, corresponding to the positions in the alignment in Fig. 3. (A) NADH dehydrogenase II and (B) cytochrome *b*.

Although our *M. rufocinereous* sequences were obtained from study-skins, we consider that they are correct for three reasons. First, the two samples were extracted and sequenced several weeks apart, but nonetheless the two individuals proved to be almost identical in both genes. We initially processed one individual, only taking a second as a control when we discovered that our sequences were so different from those published. Second, the sequences were amplified as short, overlapping fragments, and no mismatches were observed in the overlapping regions. Third, each fragment was analysed separately. The trees recovered from each dataset were poorly resolved, but nonetheless in all cases the position of *M. rufocinereous* in all trees was the same.

We had access to only a single individual each of *M. imerinus* and *Thamnolaea semirufa*, and thus we were not able to control our sequences against a second individual. Nevertheless, we consider these sequences correct: (1) the overlapping regions of adjacent fragments presented no mismatches; (2) the fragment by fragment analysis provided congruent results; (3) all fragments proved to be different from all other passerines amplified in our laboratory; and (4) we re-amplified and re-sequenced both samples and the second set of amplification provided identical sequences. For the remaining toe-pad samples, more that one individual was available and the modest divergence among conspecific individuals is a clear indication of the reliability of our sequences.

The availability of tissue samples of rare or extinct taxa in phylogenetic analyses is a major limitation in molecular studies. Study-skins are becoming a more and more important alternative DNA source, but caution must be observed in using them in phylogenetic studies.

Using multiple individuals to confirm the sequences is probably the best strategy when using ancient DNA samples, but for rare species specimen availability may constrain this approach. As an alternative, re-sequencing the same sample represents a valid option although, as the amount of template extracted from study-skins is generally limited, this requires finding a trade-off between the template amount needed to obtain complete sequences and that depleted for a second set of amplifications.

In any case, we strongly recommend using highly complementary primers to amplify the target loci in short, overlapping fragments, with the overlapping regions long enough to guarantee a high probability of picking up mismatches between fragments. Finally, each fragment should be analysed independently. Although short datasets usually provide poor resolutions at the base of the tree, they are usually enough to identify correctly the more terminal clades and any incongruent placement of contaminated fragments.

3.2. A new phylogenetic hypothesis for the Monticola rock-thrushes

Bayesian analysis of the dataset including the correct sequences recovers a well-resolved tree (Fig. 4). The three Asian species form the most basal branch, followed by a clade including the southern African *M. brevipes*, *M. pretoriae*, *M. explorator* and the Malagasy taxa. In the remaining clade, the Palearctic-Oriental species *M. solitarius* and *M. saxatilis* are basal to *M. rufocinereus*, *Thamnolaea semirufa* and the pair *M. angolensis*–*M. rupestris*. The maximum likelihood analysis recovered a topology almost identical to the Bayesian inference. They differ only in one node: while in the Bayesian tree *M. saxatilis*, *M. s. solitarius* and *M. s. philippensis* form a tricotomy, in the maximum likelihood that node is resolved placing *M. s. philippensis* basal to the other two clades, although without support. Although our topology is similar to Outlaw et al.'s tree, there are a few significant differences that require detailed discussion.

In our tree, *M. rufocinereus* is not the most basal lineage, but an internal branch. It has a rather standard plumage pattern that

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A: NADH dehydrogenase II

M. rufocinereus EF434552   -----------------------------------------------------------TACAATCTCGAGCAACCATTGAATCATGGCCTGAACCGGACTTGAAATCAACACCCTGGCAATTCTACCCCTAA
M. rufocinereus EF434553   ---AACCCCCAAGCAAAACTAATCTTTTCCACCAGCTTAGTCCTAGGAACCACTAT...................................................................
F. crypta DQ674473         ------....C................A....G..C..C............................................................................
F. strophiata DQ674475     GTG...............G....A...T..T...C........C.C......A.......C........A.....GG.T.....C..........A.T..C..C.....
M. rufocinereus GU237109   ....................CA..T....C..C.T........G.C......A........C........A..T..GG.....C....T......A..C....C.....
M. rufocinereus GU237110   ....................CA..T....C..C.T........G.C......A........A..T..GG.....C....T......A..C....C.....

M. rufocinereus EF434552   TCTCAAAATCCCACCACCCACGAGCTATTGAAGCCGCAACTAAATACTTCCTAGTCCAAGCAGCCGCCTCTGCCCTGATCTTGTTCTCCAGCATGACCAATGCCTGACAAACCGGACAATGAGATATTAC
M. rufocinereus EF434553   ..............................................................................................................
F. crypta DQ674473         ..............................................................................................................
F. strophiata DQ674475     ...................C......T.........T..T....A........A.T..A...CA.........A.......A.G...........G.......C..
M. rufocinereus GU237109   ...........T........C.............C..G........G.A.......T.....A....A.TC.A..TG.......A....C..A........T.........CG.
M. rufocinereus GU237110   ...........T........C...............G.......G.A.....T.....A....A.TC.A..TG.....A....C..A........T.........CG.

M. rufocinereus EF434552   CCAGTTGACCTGCCCAGCATCATGCCTAGTCCTAACCACAGCCATTGCAATAAAACTAGGCCTAGCCCCATTCCACTTCTGATTCCCCGAAGTACTCCAAGGCACCTCCCTAACCACTGGCCTACTTTTA
M. rufocinereus EF434553   ............................................................................................................T.....
F. crypta DQ674473         ..............................................................................................................
F. strophiata DQ674475     ..T.C.A.........CA......C.........T..............G.............T...........A........A..C.....T...C..
M. rufocinereus GU237109   A..AC.A...CA...CA.C.........A....G.............C..........A...TT..C............T...C..........T..TT....T..C........CC.G
M. rufocinereus GU237110   A..AC.A...CA...CA.C.........A....G.............C..........A...TT..C............T...C..........T..TT....T..C........CC..

M. rufocinereus EF434552   TCCACAGCCATAAAACTACCCCCAATAACACTATTTTTCATAACCTCCCAAGCACTAAACCCAACCCTCCTCACCATCATGGCTATTCTCTCTGCCGCCCTGGGAGGATGAATAGGACTAAACCAAACAC
M. rufocinereus EF434553   ...................................................................A............A.......TNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
F. crypta DQ674473         ...................................................................A............A.................................G........
F. strophiata DQ674475     .........G...T.C..T........C...C.C.........T..T.........TT.A..AG.T..T.......C.....CA.T...A...........
M. rufocinereus GU237109   ..T............T.C........T......C.C..........T.G..........A...C......C..C..T.....A..G..T..G..G...............G....
M. rufocinereus GU237110   ..T............T.C........T......C.C..........T.G..........A...C......C..C..T.....A..G..T..G..G...............G....

M. rufocinereus EF434552   AAATCCGAAAAATCTTAGNNNNNNNNNNNNNNNNNNNNNNNNNTTGAATAGCCATCATCATTGCCTACAGCCCCAAACTAGCCTTATTAAACTTCTACCTATACGCCCTAATCACCGCAGCTGTATTCCT
M. rufocinereus EF434553   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN..............................T.....T..C............A...........
F. crypta DQ674473         .G.C...........G.CTTTCTCATCCATTTCCCACTTAGGA......G..T.........CT......A.......C..C............A...G.....T....C......
F. strophiata DQ674475     ...C..NNNNNNNNNNNNNNNNNNNNNNNNNN....................C.........T......T..G....TA......
M. rufocinereus GU237109   ...CA.........C...CTTTTTCATCCATTTCCCATCTAGG......G...........TG..T.....T..T....C.............G.........GGTTA.C..C.......
M. rufocinereus GU237110   ...CA.........C...CTTTTTCATCCATTTCCCATCTAGG......G...........TG..T.....T..T....C.............G.........GGTTA.C..C.......

M. rufocinereus EF434552   CACCCTAAACTCCATAAACACCCTAAACCTATCCACACTCATAACCGCGTGAACTAAAACCCCAGCACTAAGCGCAACCCTGATGCTAACCCTACTCTCCCTTGCAGGCCTTCCTCCCCTAACAGGCTTC
M. rufocinereus EF434553   ...........A..C..A...........G........A.....A.A..................A...................A.................................
F. crypta DQ674473         G.........T..A..C........A..........T......A.....A..........A.........T..........A...G..C.......C..C..T..G.......
F. strophiata DQ674475     ...T........A..C.................A..................A.....
M. rufocinereus GU237109   A...........A..T.........T.....T......T....A.A......A........T..C..T......A..A..C......C.......C......A..A..C.......C......
M. rufocinereus GU237110   A...........A..T.........T.....T......T....A.A......A........T..C..T......A..A..C......C.......C......A..A..C..T......T......

M. rufocinereus EF434552   CTACCCAAATGATTAATCATCCAAGAGCTAACCAAACAAGAAATAGCCCCAATAGCAACAATCATTGCCCTCCTCTCCTTGCTAAGCCTGTTCTTCTATGTACGTCTCGCATACTGCGCA
M. rufocinereus EF434553   .....................G........T................................A.................................
F. crypta DQ674473         .....T......C....................G..T...GC.......GC..........C.A.............CC...A..T..G.....T..G
F. strophiata DQ674475     .....................G........T.......T..C..............A.................----------------
M. rufocinereus GU237109   ...........C....T.......A.................GC.........C.....T..T...C.A...........CT....C.........G
M. rufocinereus GU237110   ...........C....T.......A.................GC.........C.....T..T...C.A...........CT....C.........G


B: cytochrome b

M. rufocinereus EF434525   --------------------------CACCGCCCTAGCCTTCAACTCCGTCGCCCACATATGCCGAAACGTACAATTTGGCTGATTAATCCGAAACCTACACGCAAACGGAGCCTCATTCTTCTTCATC
M. rufocinereus EF434526   ----TTAGCCACACACTACACAGCAGA....A...........T....................................................................
T. chrysaeus DQ285439      GTTAC....................A.............T......................................................................
M. rufocinereus GU237083   CCTAC.........................A......T..........G....T..C....T.......C...................................
M. rufocinereus GU237084   CCTAC.........................A......T..........G....T..C....T.......C.................T.................

M. rufocinereus EF434525   TGCATCTACCTGCACATCGGCCGAGGGTTCTACTACGGCTCCTACCTAAACAAAGAAACCTGAAACGTAGGGGTCATCCTCCTCCTAATTCTAATAGCAACTGCCTTCGTAGGCTACGTTCTCCCCTGAG
M. rufocinereus EF434526   ..............................................................................................................
T. chrysaeus DQ285439      ..............................................................................................................
M. rufocinereus GU237083   .........T.A...........C........T..............C..A..A.....T......T.....................C..T.....
M. rufocinereus GU237084   .........T.A...........C........T..............C..A..A.....T......T.....................C..T.....

M. rufocinereus EF434525   GACAAATATCATTCTGAGGAGCTACAGTAATCACCAACCTATTCTCAGCAATCCCATACATCGGCCAAACACTAGTAGAATGAGCTTGAGGAGGCTTCTCANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
M. rufocinereus EF434526   ....................................................................................GTAGACAACCCTACACTANNNNNNNNNNNN
T. chrysaeus DQ285439      ....................................................................................GTAGACAACCCCACACTAACTCGATTCTT
M. rufocinereus GU237083   ....G..G......................T...............G....................C.......A....GTAGACAATCCCACACTAACCCGATTCTT
M. rufocinereus GU237084   ....G..G......................T...............G....................C.......A....GTAGACAATCCCACACTAACCCGATTCTT

M. rufocinereus EF434525   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTACACGAAACAGGATCAAACAACCCACTAGGCATCCCTGCAGACTGCGACAAAATCCCTTTCCACCCC
M. rufocinereus EF434526   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNT.....................A.....C.........T...........C........
T. chrysaeus DQ285439      CGCTCTCCACTTCCTCCTCCCATTCGTCATCGTAGGCCTTACACTAGTTCACCTCACATTCC............C....................................A......A
M. rufocinereus GU237083   CGCCCTCCACTTCCTACTACCATTCCTCATTGTAGGGCTCACACTAGTCCATCTTACATTCC............C....................T...........A......A
M. rufocinereus GU237084   CGCCCTCCACTTCCTACTACCATTCCTCATTGTAGGGCTCACACTAGTCCATCTTACATTCC............C....................T...........A......A

M. rufocinereus EF434525   TACTACTCCACTAAAGACATCCTAGGATTCGCACTAATACTCGTTCTGCTCGTCTCACTAGCCCTATTCTCCCCTAACCTACTAGGGGACCCAGAAAACTTCACCCCAGCCAACCCACTAGCCACACCAC
M. rufocinereus EF434526   ..T.....T..A..........G........G...A.C.A........C........G........C...GC......A............T..T..G................
T. chrysaeus DQ285439      .........T..A........T.....G.........C......CC...T.A....C......T......A....C..A.................A.......T..........C.
M. rufocinereus GU237083   ...........A..............C.....C..G..AA.C..C....A.TG.T......T..........T........A.........A......
M. rufocinereus GU237084   ...........A..............C.....C..G..AA.C..C....A.TG.T......T..........T........A.........A......

M. rufocinereus EF434525   CACACATCAAACCCGAATGATACTTCCTATTCGCATACGCCATCCTTCGATCCATCCCAAACAAACTAGGAGGTGTACTGGCCCTAGCAGCCTCCGTCCTAGTCCTATTCCTCACCCCACTTCTCCATAC
M. rufocinereus EF434526   .......T..........................G.T.................A.....A..A.........A....................T.........C..G..C.A
T. chrysaeus DQ285439      .T..T......................................A..C.T..............C..A....C..T..............AGT...C..A.....C..
M. rufocinereus GU237083   .C..T.....G....G..............A....A..C..........C...A......T..A.....A.........G..T.....A.....C..
M. rufocinereus GU237084   .C..T.....G....G..............A....A..C..........C...A......T..A.....A.........G..T.....A.....C..

M. rufocinereus EF434525   ATCAAAACTACGATCAATAA
M. rufocinereus EF434526   ........A...........
T. chrysaeus DQ285439      ...T....A...C...C...
M. rufocinereus GU237083   ...C.........C..TC...
M. rufocinereus GU237084   ...C.........C..TC...
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Fig. 3. Alignments of the published *Monticola rufocinereus* sequences, the putative contaminant species and the new *M. rufocinereus* sequences obtained in this study. The regions identical between the published *M. rufocinereus* sequences and the putative contaminant species are highlighted in grey. (A) NADH dehydrogenase II and (B) cytochrome *b*.

agrees well with those of the other African *Monticola*. This species shift in the tree topology has a deep influence on the biogeographic reconstruction, as discussed below.

The Malagasy taxa formerly in the genus *Pseudocossyphus* have been shown to be part of the *Monticola* clade (Goodman and Weigt, 2002). Three morphologically diagnosable forms with allopatric distributions have been granted species status as *M. sharpei* in the montane rainforests of eastern Madagascar, *M. bensoni* in the central Madagascar and *M. erythronotus* in the Amber Mountain and Masoala Peninsula in the north of the island (Morris and
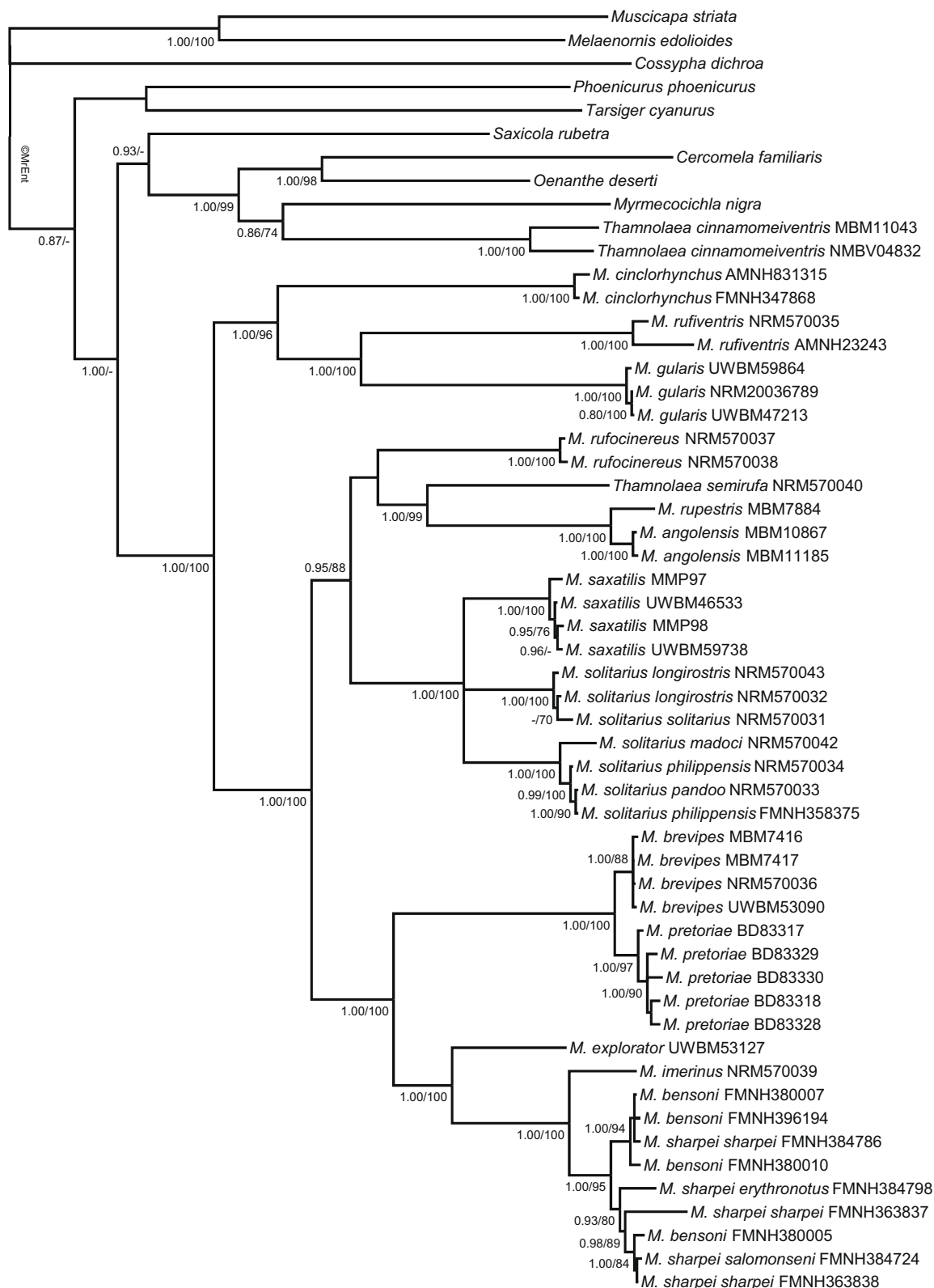
Fig. 4. The majority rule consensus tree obtained from the Bayesian analysis of the new *Monticola* dataset. Posterior probabilities equal or higher than 0.80 are indicated at the node, followed by bootstrap support values equal or higher than 0.70 obtained from the maximum likelihood analysis.

Hawking, 1998; Sinclair and Langrand, 1998; Collar, 2005). Despite their morphological differences and disjunct ranges, Goodman and Weigt (2002) observed only modest genetic divergences between phenotypically *M. sharpei* and *M. bensoni*, with no match between morphological types and haplotypes. They suggested that the two forms should be merged in a single monotypic species. The single individual of *M. erythronotus* showed a little more divergence from the other forms, and Goodman and Weigt (2002) suggested retain-

ing it as a valid species. In our analysis, all three putative forms are genetically indistinct and the haplotypes do not match with either morphotype or geographic origin. The single *M. erythronotus* haplotype is well nested among the others and this form does not warrant species status.

A fourth, morphologically more distinctive species, *M. imerinus*, is restricted to the southern coast of Madagascar. For this last taxon Goodman and Weigt (2002) were able to include only a short cyt *b* sequence (307 bp), but predicted that it should be specifically distinct. On the basis of complete sequences, we are able to confirm their prediction. *M. imerinus* appears to be a valid species, clearly distinct from *M. sharpei* (combined ND2-cyt *b* p-distances 3.6%).

The non-monophyly of the two cliff-chats of the genus *Thamnolaea* is totally unexpected. In our tree, *T. semirufa* is recovered nested within the *Monticola* clade, sister to the pair *M. rupestris-M. angolensis*, while *T. cinnamomeiventris* clusters in a clade outside *Monticola* together with *Myrmecocichla*, *Oenanthe* and *Cercomela*, three saxicolid representatives of other open-habitats genera. Morphologically the two cliff-chats are rather similar: almost all their plumage is dark grey-blackish, with a dark reddish belly in both sexes. Only males have white areas on the wings, but in *T. semirufa* the white patch is formed by the basal portion of the primaries, while the median and lesser coverts are responsible for the white patch in *T. cinnamomeiventris*. The plumage similarity appears to be a case of convergence, because the two species differ in other morphological and behavioural traits that support the inclusion of *T. semirufa* within *Monticola*. All *Monticola* species are sexually dimorphic, and the same applies to the two *Thamnolaea*. However, while in all *Monticola* and in *T. semirufa* the juvenile plumage in both sexes is similar to the female plumage, in *T. cinnamomeiventris* sexual dimorphism is already present in the juveniles. As their common name suggests, the *Thamnolaea* cliff-chats inhabit open areas, like cliffs and ravines, a habitat preference shared with all African *Monticola*. However, *T. semirufa* builds its own nest in rock crevices, like most *Monticola*, whereas *T. cinnamomeiventris* occupies swallows' mud nests, usually of *Hirundo abyssinica* (Keith et al., 1992).

Monticola solitarius is a polytypic species that occurs over a wide area of the Palearctic and Oriental regions. A cline of decreasing dimensions and colour saturation runs from the Mediterranean basin to central Afghanistan. Nominate *solitarius* on the west of the cline and *longirostris* on the east intergrade over a large belt across Turkey and the Levant. A second cline of increasing dimensions runs from eastern Afghanistan to China and Japan. Also here two forms are involved: *pandoo* in the west and *philippensis* in the east. A disjunct population, *madoci*, with smaller and darker birds, occurs in the Peninsular Malaya and Sumatra. Contrary to all others, the subspecies *philippensis* is markedly distinct: the males have a rich rufous-chestnut breast and belly instead of dark blue. Despite their striking difference in plumage, the blue-breasted *pandoo* and the rufous-breasted *philippensis* interbreed over a wide area of south-western and southern China. The morphologically distinct *philippensis* was occasionally granted a separate species status (e.g. Sharpe, 1903), but the presence of many intermediates usually led to the recognition of a single species (e.g. Hartert, 1910; Vaurie, 1959; Cramp, 1994; Collar, 2005). Our results indicate that *Monticola solitarius* forms two clearly distinct clades in an unresolved tricotomy with *M. saxatilis*. However, the limit between the two species occurs in eastern Afghanistan, at the meeting of the western and eastern clines, and not between the blue- and rufous-breasted forms, as might have been expected. The two reciprocally monophyletic *M. solitarius* clades are as divergent from each other as either is from *M. saxatilis* (combined ND2-cyt *b* p-distances 4.8–5.1%). The existence of two dimensional clines that meet in eastern Afghanistan and the free interbreeding between *pandoo* and *philippensis* are consistent with the genetic data and further support

the recognition of two species within *solitarius* as currently recognised. However, in eastern Afghanistan and north-western Pakistan Clement and Hathway (2000) report of the presence of a small area of intergradation between the subspecies *longirostris* and *pandoo* requiring further study.

3.3. The biogeographic history of the Monticola rock-thrushes revisited

The DIVA analysis requires a fully bifurcated tree. We resolved the single tricotomy involving *Monticola saxatilis*, *M. s. solitarius* and *M. s. philippensis* by placing *M. saxatilis* as the most basal clade, on the basis of the greater morphological divergence of *M. saxatilis* from the other two taxa.

The unconstrained DIVA analysis performed on the pruned topology restricted to the *Monticola* clade suggested that the ancestral area of the genus encompasses either southern Africa plus Palearctic, or Africa plus Palearctic, or Africa, Palearctic and Madagascar. In other words, the unconstrained DIVA analysis fails to identify a restricted ancestral area beyond suggesting a generic Old World origin for the clade. This is not surprising. As pointed out by Ronquist (1996), ancestral area optimization in DIVA becomes less reliable as we approach the root node. The tendency for the root distribution to be large and include all the area units is in part a consequence of the cost assignment rules that favour vicariant speciation (Ronquist, 1997), and in part due to the fact that global optimal states at each node are dependent on the optimizations of the node below.

The phylogeny of the Muscicapidae is not known, nor is the sister clade of *Monticola*. This represents a major limit in a proper DIVA analysis. All *Monticola* species distributions are limited to one or, in few cases, a maximum of two of the area units that we defined. In order to attempt to alleviate the lack of information on the phylogenetic relationships outside the *Monticola* clade, we therefore ran a constrained DIVA analysis, limiting the number of possible ancestral areas to two, i.e. assuming that the ancestor had a distribution similar to the extant species. The constrained DIVA analysis identified the southern Africa plus the Palearctic as the ancestral area (Fig. 5).

DIVA is a cladistic methods based on reversible parsimony. It uses a three-dimensional matrix with different weighting for vicariance, dispersal and extinction (Ronquist, 1997). By minimizing dispersal and extinction events, DIVA analyses invariably reconstruct large ancestral areas and strongly favour vicariance scenarios. In agreement with it models, DIVA reconstruct an early vicariance between Africa and Eurasia (the two major clades are Eurasian and mostly African, respectively). However, we consider the result of the constrained DIVA analysis biologically unrealistic, because not a single passerine bird species has a current distribution that both encompasses and is restricted to the southern portion of Africa plus the Palearctic. The DIVA output is computationally correct given the available data, but it is the full applicability of the DIVA analysis per se in this specific case that is questionable. As discussed above, the DIVA output is influenced by the topology below the focal node. For a reliable DIVA analysis it is important to know the phylogenetic relationships within the target clade, plus a complete topology of the closely related taxa. Information on Muscicapidae relationships is scanty (e.g. Voelker and Spellman, 2004) and not even the *Monticola* sister-group is known. The few taxa that we included as outgroups are obviously inadequate to answer the question. The DIVA analysis suggests that the ancestral areas for the first two nodes above the *Monticola* root are the Palearctic for the three exclusively Asian species, and southern Africa for the remaining taxa. A DIVA analysis including the *Monticola* rock-thrushes and all their related taxa might be able to choose between an African *vs.* an Asian origin for the genus.
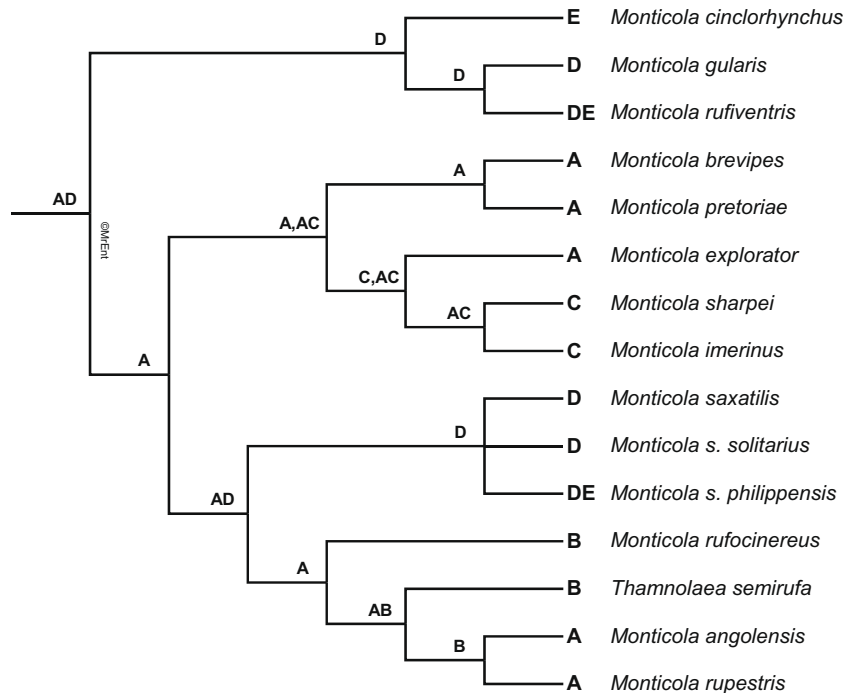
Fig. 5. Ancestral area reconstruction for the *Monticola* rock-thrush clade. The area distributions of the extant taxa are indicated in front of the taxon names. Ancestral areas estimated by DIVA are shown at the node, respectively. Area coding: A, southern Africa; B, eastern Africa; C, Madagascar; D, Palearctic; E, Oriental region.

3.4. Taxonomic recommendations

On the basis of the results presented here, we recommend the following:

(1) the species *Thamnolaea semirufa* should be transferred to the genus *Monticola* in the new combination *Monticola semirufus* (Rüppell, 1837);

(2) the species *Monticola solitarius* should be split into two polytypic species: *M. solitarius s.s.* with the subspecies *solitarius* and *longirostris* for the western taxon, and *Monticola philippensis* (Statius Muller, 1776) with the subspecies *philippensis*, *pandoo* and *madoci* for the eastern taxon;

(3) only two Malagasy taxa should be recognised: *Monticola imerinus* for the population of the southern and south-western coast of Madagascar, and *M. sharpei* for the remaining populations. Pending further studies, *M. sharpei* should be treated as a monotypic species.

Acknowledgments

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