

The forest batis, *Batis mixta*, is two species: description of a new, narrowly distributed *Batis* species in the Eastern Arc biodiversity hotspot

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Abstract The forest batis, *Batis mixta*, is a common bird of the forests of the Eastern Arc Mountains of Tanzania and in some adjacent montane and coastal forests. Through new collecting efforts in most of this range we documented a well-marked change in morphology in the middle of the range. Supplementary genetic studies of the historical population structure suggest connectivity among the south-western and northern/coastal populations, but not between these parapatric groups. It is concluded that two species are involved, and a new name *B. crypta* is proposed for the south-western populations. A marked genetic break also exists towards *B. capensis sola* in northern Malawi. The morphologically distinctive form *reichenowi* in south-eastern Tanzania is genetically nested within *B. mixta*, and for now we keep it as a subspecies of *B. mixta*.

Keywords Eastern Arc hotspot · Montane biogeography · Paraphyletic species · Phylogeography · Platysteiridae

Introduction

The many species of *Batis* (Platysteiridae, Dickinson 2003; Fuchs et al. 2004) are confusing because of the complex variation in sexual dimorphism, combinations of plumage characters and eye colour changes between different geographical populations. This also applies to the subgroup of forest-dwelling forms of eastern Africa. Opinions about how to classify these forms have varied, but they are usually (Dowsett and Dowsett-Lemaire 1993; Harris and Franklin 2000) divided into the species *Batis capensis* (Cape batis of southeastern Africa, including the form *reichenowi*, see Dowsett-Lemaire 1989, contra Lawson 1986; Traylor 1986), *B. fratrum* (Woodward's batis of Mozambique coastal forests), *B. margaritae* (Margaret's batis of Zambia and Angola), *B. diops* (Ruwenzori batis of the Albertine Rift Mts) and *B. mixta* (forest batis, mainly in Tanzanian montane forests).

The affinities of *B. mixta*, and its rank as an independent species, have been discussed by Dowsett and Dowsett-Lemaire (1980), Lawson (1986) and Dowsett-Lemaire (1989). Lawson (1986) placed the *B. mixta* populations of the Kenyan coastal forests in a separate subspecies *ultima*, diagnosed by white supralores in the male, but although he examined material from the entire range he did not recognise any differences between other populations. It was therefore a surprise when, as a result of a comprehensive new collecting effort for analysing speciation patterns of afro-montane

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forest birds (Bowie 2003; Bowie et al. 2004a, 2006; J.Fjelds , unpublished), we discovered a clear-cut morphological change in *B. mixta* in central Tanzania. This was unexpected, since this “species” appears to be fairly continuously distributed along all the Eastern Arc Mountains of Tanzania and some adjacent highlands. It is generally common, even in degraded forest, and it sometimes ventures out into the widespread *Brachystegia* (“miombo”) woodlands and (seasonally) into wooded foothill habitats. It is also known to breed in some semi-evergreen coastal forests. Because of this flexibility in habitat use and apparent seasonal movements (Burgess and Mlingwa 2000; Romdal 2001), we would expect some gene flow between populations. A detailed morphological study, supplemented by genetic data to assess the historical population structure, suggests some connectivity among populations in the south-western and northern parts of the range, but not between these two population groups.

In this paper, we present morphological and molecular evidence suggesting that *B. mixta* represents two different and incompatible species, which we will describe, along with a formal proposal of a new species name. We will also reassess the relationship with *B. capensis* with special emphasis on the form *reichenowi*, which inhabits a small area of coastal forests in south-eastern Tanzania.

Methods

Specimens used in this project were drawn from several years of collecting effort, covering a large number of montane forests in Tanzania (J. Kiure and teams from the Zoological Museum, University of Copenhagen), and the Misuku Hills and Nyika Plateau in adjacent northern Malawi (R. Bowie, with teams from the Field Museum, Chicago, including T.P. Gnoske, D.E. Willard, G.A. Voelker and P. Kaliba). Figure 1 indicates collecting sites, with reference to the locality names in Fig. 4. Altogether 124 specimens of *B. mixta* and 16 *B. capensis sola* from the Nyika Plateau were collected. In addition, we reviewed the *Batis* material in Museum Alexander Koenig in Bonn, National Museum of Kenya in Nairobi, Naturalis Museum in Leiden, Mus um National d’Histoire Naturelle Zoologie in Paris, Swedish Museum of Natural History in Stockholm, the Zoological Museum, University of Copenhagen and those in the Zoology Department, University of Dar es Salaam. Additional specimens were obtained as loans from the Natural History Museum, Tring, and the Field Museum in Chicago.

Altogether 212 *B. mixta*, 14 *B. reichenowi*, 56 *B. capensis* (including *dimorpha* and *sola*) were scored for plumage and morphometric characters, and comparisons were also made with other *Batis* species.

For molecular analyses, *B. minor*, *B. molitor*, *B. pirit* and *B. soror* were used as outgroups. To further test the monophyly of the genus *Batis*, more distant outgroups were analysed for ND2 alone: *Lanioturdus torquatus* (AY529959), *Platysteria cyanea* (AY529965) and *Dyaphorophyia chalybea* (AY52954) (Fuchs et al. 2004). All sequences collected for this study have been submitted to Genbank under accession numbers: for ND3 DQ661921–DQ662003 and for ND2 DQ662004–DQ662086.

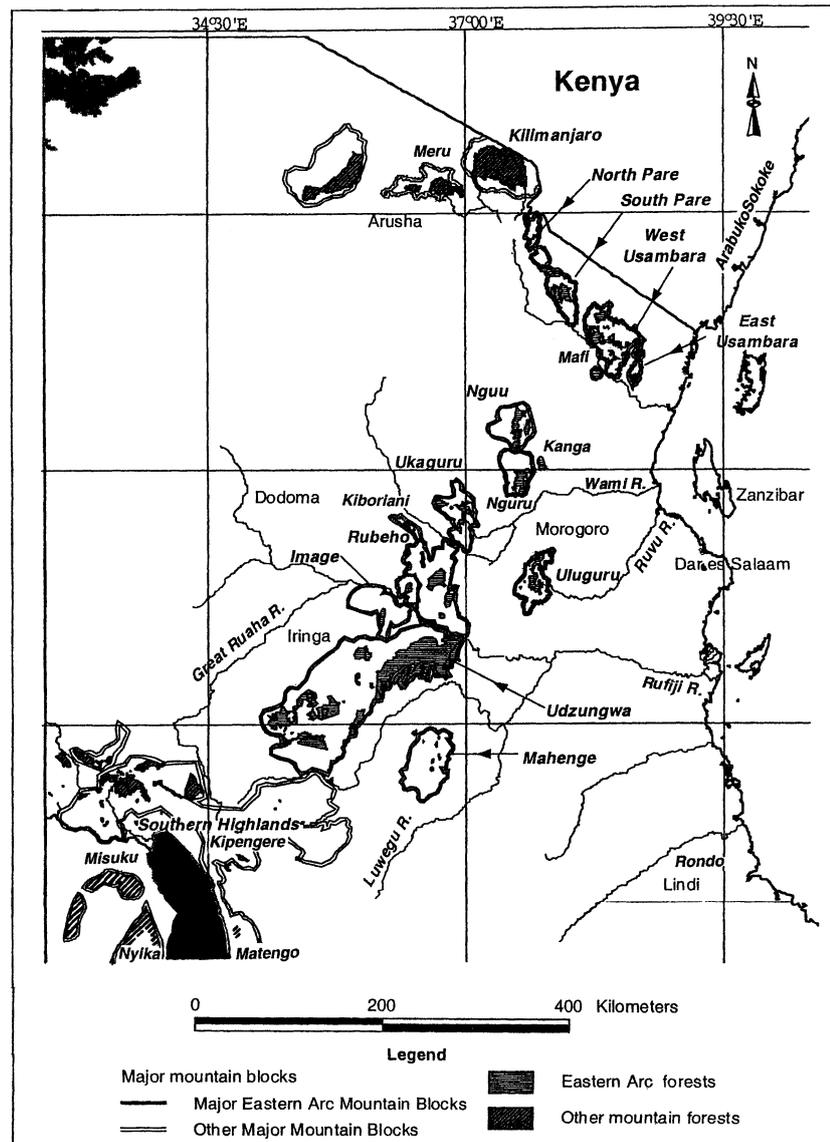
Morphological methods

For all specimens, measurements of bill (length of exposed culmen, and width of bill at the base), wing (flattened against the ruler), tarsus and tail were taken. Heavy moult in January includes more or less simultaneous tail-moult, and care had to be taken to eliminate specimens where the tail may not have been fully grown. Colour patterns and moult were described (including width, along the ventral mid-line, of the pectoral band of males).

Molecular methods

DNA was extracted from tissues using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minn., USA) following the manufacturer’s animal tissue protocol, but with an over-night proteinase K digestions at 55°C. The 5’ end of the NADH subunit 2 gene (ND2) was PCR amplified using primers L5204 (TA ACTAAGCTATCGGGCGCAT) and *mixta*-ND2H (GATGGCTATTCACCCCAGG) under standard conditions (38 cycles, denaturation at 94°C, annealing at 60°C and extension at 72°C). The NADH subunit 3 gene (ND3) with flanking tRNAs (glycine and arginine) was PCR amplified as described by Bowie et al. (2004b). PCR products were electrophoresed on 1% agarose gels, stained with ethidium bromide and visualised under UV light. Amplicons of the appropriate length were cut out of the gel and purified using the Wizard PCR-GEL clean-up kit (Promega, Madison, Wis., USA). The purified product was cycle-sequenced using Big Dye terminator chemistry (Applied Biosystems) and run on an ABI3100 automated DNA sequencer. Sequences were obtained from both strands of DNA for each identified haplotype. All sequences were checked using the program Sequencher 4.5 (Gene

Fig. 1 The Eastern Arc Mountains form a chain of isolated ancient montane fault-blocks across the savannah plains of Tanzania, flanked to the north and south by younger volcanic mountains. Names are given for all forest tracts from which samples were collected



Codes Corp) and aligned to the chicken (*Gallus gallus*) mtDNA sequence to test for the presence of any insertions, deletions or stop codons.

Analyses of the haplotypes were performed using parsimony (MP) and Bayesian Inference (BI) algorithms. Parsimony analyses, using PAUP×4.0b10 (Swofford 2002) were conducted using the heuristic search option, implementing stepwise addition with 500 random addition replicates and TBR branch swapping. Clade support for the MP analyses was estimated using 500 non-parametric bootstrap replicates, with one random addition replicate per bootstrap replicate.

MRBAYES 3.1 (Ronquist and Huelsenbeck 2003) was used to conduct a Bayesian approach to phylogenetic inference. Three independent runs of 3 million generations, each with four Metropolis-coupled

MCMC chains (one cold and three heated), were run simultaneously to optimise efforts to find peaks in tree-space. A second analysis of two independent runs of 5 million generations (also each with four MCMC chains) was conducted. A conservative approach was adopted by considering the first 10% of the sampled posterior distribution to represent the burnin. The General-Time-Reversible model of nucleotide substitution with a gamma distribution (estimated using four rate categories) and invariable sites (GTR+I+G) was used in the Bayesian analyses. A Dirichlet distribution was assumed for estimation of the base frequency parameters and an uninformative (flat) prior was used for the topology. Trees were sampled every 100 generations in the 3 million generation search and every 200 generations in the 5 million generation search.

Since the underlying assumptions of MP and model-based methods are often violated when analysing intraspecific datasets (for review see Posada and Crandall 2001), networks were used to explore phylogeographical structure in *B. mixta*. A statistical parsimony network was constructed in TCS 1.01 (Clement et al. 2000).

Initial analyses of ND2 and ND3 resulted in topologies with almost identical relationships among clades (one collapsed clade in ND3, see Results). As a consequence we report here only on the results of analyses using the combined mitochondrial DNA dataset (mtDNA) of 952 base pairs.

Results

Geographical variation in morphology

Males of *B. mixta* vary little in plumage, but we found that specimens of northern and coastal populations had some white feathers or feather-tips in the superciliary zone, unlike those collected west of the Nguru Mts (Kiboriani and Ukaguru Mts to Misuku Hills). The only exceptions are one Usambara male with no white, and one Uluguru bird with a single tiny white feather-tip in the upper lore. The amount of white superciliary feathers of northern/coastal males vary from a few white feather-tips on the border between the black lore and the grey forehead to an almost unbroken line extending to well behind the eyes in individuals from southern Kenya (see Lawson 1986), but also in a few other individuals [from South Pare Mts, Usambara Mts (Musububwe), Nguu Mts and Mt. Kanga]. Also, some *reichenowi* males have a long white superciliary stripe,

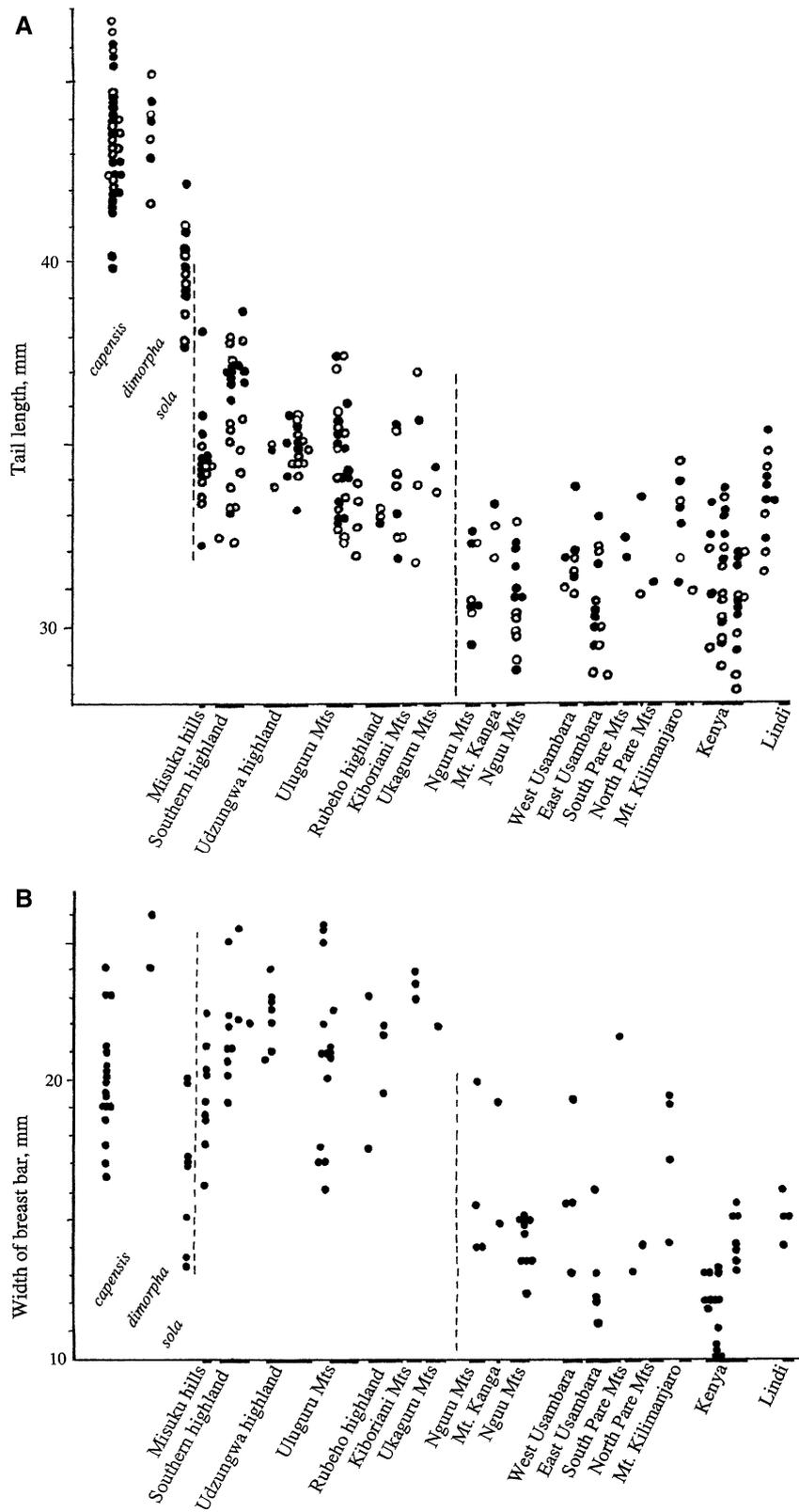
although faint and extremely thin in some individuals. We failed to see any diagnosable plumage differences between northern *mixta* males and those of *reichenowi*. Northern *mixta* males, and those of *reichenowi*, have a rather narrow black breast-bar, mostly around 15 mm wide (Fig. 3). Such narrow breast-bars are not restricted to coastal Kenya, as suggested by Lawson (1986), but dominate as far south-west as the Nguru Mts. (Fig. 3). All males from further west have rather broad breast-bars, mostly 20–25 mm, but a few males from northern Tanzania (Nguru to Kilimanjaro) also have broad breast-bars (Fig. 3; however, it should be noted that some extreme values could be a consequence of how the voucher specimen was prepared). Finally, south-western *mixta* males have a slightly darker slate-grey crown and more black on the scapulars than northern/coastal populations, and most south-western males also have many black feather-tips elsewhere on the back and in some cases quite extensively on the mid-back (Fig. 2).

In females, the white superciliary stripe is long and particularly well developed in northern/coastal populations, and these populations differ clearly from those south-west of the Nguru Mts in the pale appearance of the rufous under-parts, as these are strongly washed with white feather-tips so that the throat-patch is hardly discernable. This was described as typical of ssp. *ultima* by Lawson (1986), but in fact applies equally to all populations south to Nguru Mts. Furthermore, every female representing northern/coastal populations has a large inter-wing panel formed by rufous tips of the median coverts and broad rufous outer webs on all greater coverts. South-western populations have a tawny but not very clearly outlined throat-patch and chestnut breast, with short white feather-tips and a

Fig. 2 Two females representing the northern and southwestern populations of forest batis (Brit.Mus. 1931.12.19.103 from Amani, East Usambara Mts, and ZMUC 75.516 from Uluguru Mts, respectively). A male of the latter population is included to illustrate a typical development of *black dorsal spots*. Watercolour painting J. Fjeldså



Fig. 3 Geographical variation in tail-length (a) and width of the black pectoral bars of adult males (b). Filled symbols are for males, open symbols females. Stippled lines mark documented breaks in gene flow



restricted cinnamon wing-stripe as only the three inner greater coverts have broad rufous outer webs. These differences in the female plumage, illustrated in Fig. 2,

are diagnostic with absolutely no intergradation and are sufficient for recognition at quite some distance in the field.

Many publications describe the female of *B. mixta* as having uniform olivaceous grey upper-parts, but in fact the crown is grey contrasting with a buffy brown back in adults. Only juveniles are uniform clove-brown above, and have a light buff superciliary stripe and weakly developed “mask”.

All seven *reichenowi* females examined are grey dorsally, inclining to greyish olive on the lower back, and have a white throat and gull-grey breast, with only a few cinnamon-buff feathers on the transition towards the belly in some individuals. The sides and flanks are white with irregular buff and light grey speckles. Thus, descriptions in the literature (Grote 1911; Urban et al. 1997; Harris and Franklin 2000) suggesting rusty brown breast and sides washed with some grey are misleading. One definitively juvenile specimen (identified as such from its olive-brown crown, buffy superciliary and ill-defined “mask”; University of Dar es Salaam) had an orange-tawny breast with some grey smudges grading to orange-cinnamon flanks. Thus, the rufous breast represents the juvenile plumage and adult females have a neutral grey breast. The ochraceous inter-wing panel is extensive in immatures, as in northern *B. mixta*, but adult females have rather narrow outer margins to the outer greater coverts.

According to the label data it seems that all examined *B. mixta* populations have mahogany red or chestnut eyes with a narrow yellow ring around the pupil. However, orange eye-colour is mentioned on the labels of a few *B. mixta* specimens, and the eyes of *reichenowi* are described as red, orange or yellow (labels of T. Andersen). All populations of *B. capensis* have orange to yellow eyes.

We will not describe the plumage variation of other species of *Batis*, other than mentioning that geographically adjacent northern *B. capensis* populations (*dimorpha* of northern Mozambique to central Malawi, *sola* of Nyika Plateau in northern Malawi; see Lawson 1962, 1986) resemble southern *B. mixta*, except for the longer tail (Fig. 3), orange eyes and lack of white feather-tips to the tawny to sanford brown breast of females. Females of the form *sola* have black scapulars (unlike in *mixta*, where scapulars are coloured like the rest of the back), the tertials are fringed white (buff in *mixta*) and flanks rufous (mostly grey in *mixta*). Two females (FMNH 439410 and 439405) from the Misuku Hills have unusually dark breast (hazel to chestnut, with minimal white grizzling), and other birds have lesser wing-coverts black like *sola*, not dark brown.

The variation in measurements within *B. mixta* is rather insignificant, with considerable individual variation in some populations, but apparently with no clear geographical trends, except for the following: northern/

coastal populations have particularly short tails (Fig. 3; average 31.3 mm, against 34.9 mm further west). The wing-length does not vary in the same way and the tail/wing ratio therefore changes from 0.48 to 0.56 (average 0.52) in the northern/coastal populations to Nguru Mts, changing to between 0.51 and 0.63 (average 0.57) further west. The more or less simultaneous moult of all rectrices in all *B. mixta* populations suggests that the tail may be of little functional value, so it is unclear whether this variation has any adaptive significance. The *reichenowi* population also has a relatively short tail (Fig. 3; tail/wing ratio 0.563), and ssp. *sola* has a moderately short tail (ratio 0.59–0.68, average 0.635), whereas all other populations traditionally referred to as *B. capensis* have a markedly longer tail (ratio 0.65–0.81, average 0.74). The bill length varies little, with an average around 12 mm in most populations, slightly longer in ssp. *sola*, Kenya population of *mixta*, and *reichenowi*, and a more narrow bill shape in *reichenowi*, which in this respect resembles southern *capensis* populations.

Molecular data

Analyses of the full mtDNA data set (83 individuals; 952 base pairs; 557 from ND2 and 395 from ND3 and flanking tRNAs) recovered 54 unique sequences (haplotypes; Table 1). For the combined mtDNA dataset (952 bp) 294 (30.9%) sites were variable and 235 (24.7%) were parsimony informative. Sequence divergence among haplotypes was estimated using uncorrected pairwise distances (Table 1). The average percentage divergence among most recognised *Batis* species ranges between 8 and 15%. Within *B. capensis*, the two subspecies (*capensis* and *sola*) included in this study were closely related ($3.8 \pm 0.24\%$), as is the northern clade of *B. mixta* and *B. reichenowi* ($2.0 \pm 0.57\%$). The northern and south-western clades of *B. mixta* differ by $7.5 \pm 0.34\%$, which is considerable and in the range of divergence values between *B. diops* and *B. mixta* (Table 1).

A parsimony (MP) heuristic search using the 54 unique mtDNA sequences recovered 432 MP trees (length = 577, CI = 0.620, RI = 0.909), one of which is presented (Fig. 4). A strict consensus tree (not shown) revealed that all 432 MP trees recovered the same basal nodes and that variation among the MP trees could be attributed to changes at the tips. The MP analyses provide strong support for the montane *Batis* taxa included in this study (*B. capensis*, *B. diops*, *B. mixta*, with the lowland form *reichenowi*) forming a group (*B. capensis* complex: 100% bootstrap, 1.0 posterior probability; Fig. 4). This result holds even when

Table 1 Percentage pairwise sequence divergence (uncorrected-*p*) between *Batis* taxa included in this study

Taxa	1	2	3	4	5	6	7	8	9	10
1 <i>Batis capensis capensis</i>	0.28 ± 0.06	0.07	0.27	0.15	0.16	0.07	0.06	0.11	0.16	0.12
2 <i>B. capensis sola</i>	3.8	0.27 ± 0.05	0.24	0.18	0.20	0.12	0.14	0.09	0.11	0.14
3 <i>B. mixta</i> : north clade	11.9	11.7	1.4 ± 0.91	0.34	0.57	0.19	0.26	0.20	0.18	0.22
4 <i>B. mixta</i> : south clade	12.6	12.5	7.5	0.60 ± 0.26	0.19	0.29	0.17	0.11	0.20	0.19
5 <i>B. reichenowi</i>	12.1	12.0	2.0	7.6	0.56 ± 0.41	0.06	0.28	0.22	0.16	0.06
6 <i>B. diops</i>	13.8	12.9	8.8	10.5	9.3	–	–	–	–	–
7 <i>B. minor</i>	13.7	13.5	12.8	13.0	13.3	14.0	–	–	–	–
8 <i>B. molitor</i>	14.5	15.0	14.5	14.1	14.5	15.4	10.5	–	–	–
9 <i>B. pirit</i>	14.0	13.6	14.1	13.3	14.0	14.4	11.4	10.6	–	–
10 <i>B. soror</i>	14.7	14.6	14.7	13.7	14.8	14.0	11.3	10.6	5.6	–

Average divergence values are below the diagonal, standard deviations are above the diagonal and within taxon divergence levels are on the diagonal

more distant Platysteridae outgroups are included (*Lanioturdus torquatus*, *P. chalybea* and *P. cyanea*; ND2 analyses only), which support the monophyly of the genus *Batis*, as well as the monophyly of the montane *B. capensis* complex (topology not shown). Thus, the use of *B. soror*, *B. pirit*, *B. molitor* and *B. minor* as outgroups in this study does appear to be appropriate.

The MP topology (Fig. 4) suggests that *B. capensis* is sister to the remaining members of the complex (*B. diops*, *B. mixta* and *B. reichenowi*; bootstrap 100%, posterior probability 1.0). In addition, both subspecies of *B. capensis* included within this study are monophyletic, as expected given discrete morphological differences, and may represent distinct species. More inclusive sampling is required to confirm this.

Batis mixta forms two distinct clades (Fig. 4), a northern clade, which encompasses birds from the lowland forests of Arabuko-Sokoke to the Usambara Mts and inland to Kilimanjaro and the Nguru Mts (Fig. 1, Table 1), and a south-western clade. Interestingly, the form *reichenowi* from south-eastern Tanzania (Fig. 1) is firmly nested within the northern *B. mixta* clade (bootstrap 99%, posterior probability 0.98), although the six sampled individuals form their own clade (bootstrap 99%, posterior probability 1.0) and there is no haplotype sharing with individuals from the northern mountains.

The south-western *B. mixta* clade encompasses birds from the Ukaguru Mts to the southern highlands of Tanzania and the Misuku Hills in northern Malawi (Figs. 1, 4; Table 1). The large sequence divergence between northern and southern clades ($7.5 \pm 0.34\%$) and clear monophyly of each clade (Fig. 4) suggests that these clades do support the observed morphological differentiation (see above), and that the two clades represent distinct taxa with a break between the Nguru (north) and Ukaguru/Rubeho (south) Mts. The argument for species recognition would seem to be further

strengthened by the phylogenetic position of *B. diops* (sexually monomorphic black-and-white plumage, unlike other forms discussed here). This would render *B. mixta* polyphyletic. However, this result is only moderately supported in the MP analyses (bootstrap 55%) and is not recovered in analyses of ND3 alone (topology not shown), but is in analyses of ND2 (topology not shown).

The marginal probabilities of the BI rate matrix were, [A–C] = 0.024, [A–G] = 0.719, [A–T] = 0.022, [C–G] = 0.003, [C–T] = 0.219 and [G–T] = 0.013. The marginal probabilities of the nucleotide frequencies were A = 0.323, C = 0.309, G = 0.141 and T = 0.227, the marginal probability of the proportion of invariable sites was 0.542, and the shape of the gamma parameter was 1.89. All three independent runs of the 3 million generation BI search converged on the same topology (average deviation of split frequencies = 0.006), with each run showing a stable log-likelihood distribution post the 10% burnin. The two independent 5 million generation runs also converged on the same topology (average deviation of split frequencies = 0.0043), which was identical to that recovered from the 3 million generation run, and differed only at the tips with respect to the MP tree. As in the MP analyses, the position of *B. diops* as sister to the northern *B. mixta* clade was recovered, but with no support (posterior probability 0.72). Thus, whether *B. diops* is sister to the northern clade of *B. mixta*, or to *B. mixta* as a whole remains uncertain in the present study.

Evaluation of the limits of statistical parsimony suggests that topologies connecting haplotypes by 13 steps or fewer have a cumulative probability of greater than 95% of being connected correctly. As a consequence, TCS constructed independent networks for the northern and southern clades of *B. mixta* as these clades have diverged by more than 13 steps (Fig. 5). Within the northern/coastal *B. mixta* clade three

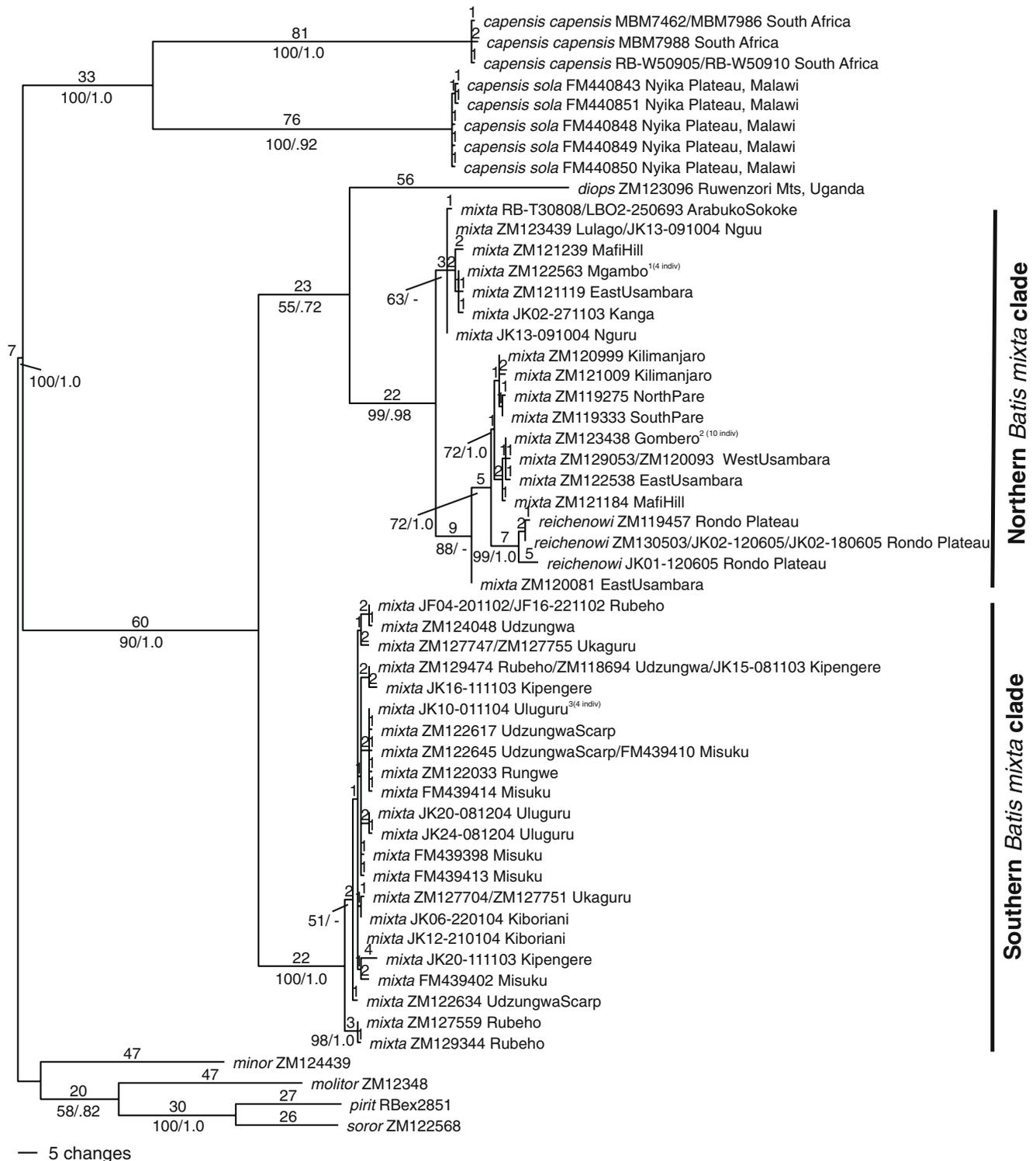


Fig. 4 Parsimony analysis of the combined mtDNA dataset (952 bp) recovered 432 MP trees (length = 577, CI = 0.620, RI = 0.909) one of which is presented here. Values above the branches represent absolute number of changes along a branch and values below are bootstrap/Bayesian support values. Individuals with identical haplotypes not listed on the figure: 1 *B.*

mixta ZM122563 Mgambo, JK05-160505, JK01-190505 and JK12-131004 Nguu. 2 *B. mixta* ZM123438 & ZM123442 Gombero, ZM132825, ZM132792 JK18-080505, JK04-190505 and JK10-190505 Nguu, ZM132696, JK05-231103 and JK09-231103 Kanga. 3 *B. mixta* ZM119882, JK10-011104 and JK14-311005 Uluguru, ZM122534 Udzungwa

divergent subnetworks were recovered, two representing a mix of individuals from montane and lowland localities (Fig. 5a, Table 1) suggesting that at some point populations may have been isolated, but that presently gene flow is obscuring any historical structure. This is further supported by the high genetic diversity within the northern *B. mixta* clade (1.4 ± 0.91). The third subnetwork consists of the six *B. reichenowi* individuals sampled, clearly illustrating the isolation of this taxon in south-eastern Tanzania, with no suggestion of recurrent gene flow with other individuals of the northern *B. mixta* clade. The southern *B. mixta* clade forms one large subnetwork with no apparent structure, suggesting that gene flow is likely taking place among montane highlands between the central and southern Eastern Arc, as well as the Kipengere Mts and Misuku Hills in Malawi (Fig. 5b, Table 1).

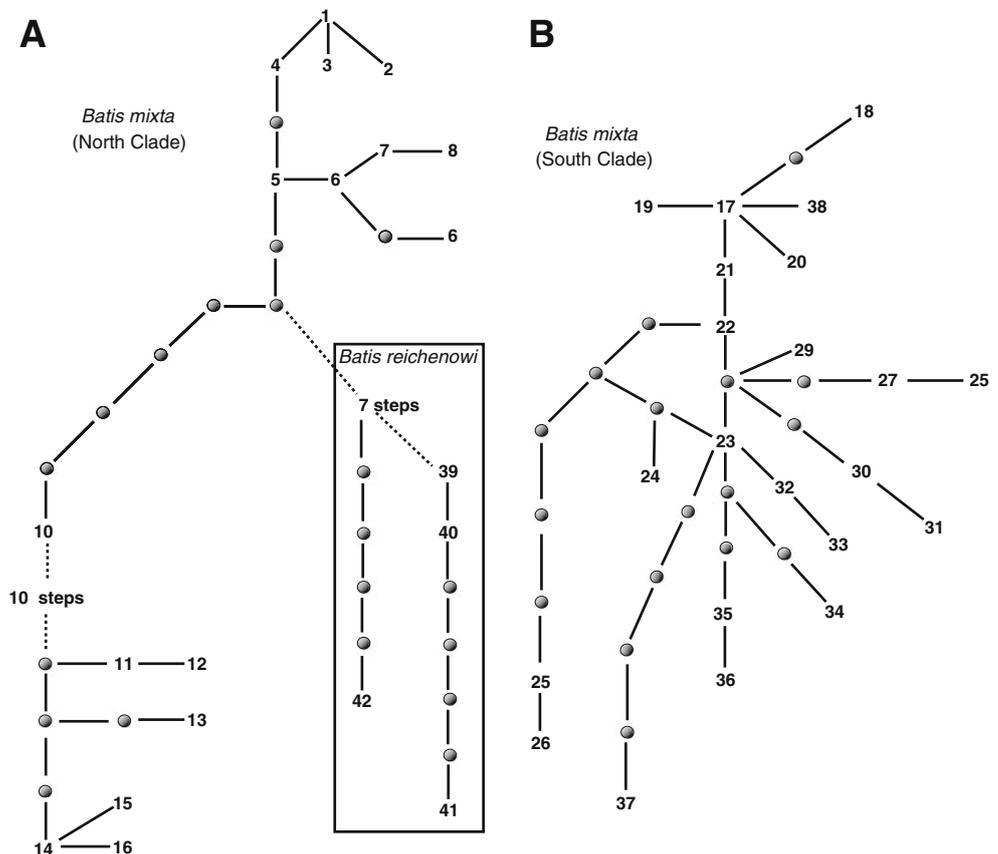
Discussion

Two population groups traditionally referred to as *B. mixta* are clearly diagnosable by morphology, notably among females. These population groups replace each other in different parts of the Tanzanian

Eastern Arc Mts, but the gap between them, consisting of 45 km of lowland between the Nguru and Ukaguru Mts, is densely dotted by small wooded hills (1,000–1,300 m) with mosaics of semi-deciduous forest, a habitat that is visited during the dry season according to observations from the Nguru side. Thus, the break in gene flow in this area is in marked contrast to the pattern of shared haplotypes and lack of morphological differences across the 60-km gap of lowland savannah between the Rubeho/Udzungwa and Uluguru Mts, and across the 115 km of lowland plains separating the Nguru/Kanga and Usambara Mts (Bowie et al. 2004a, 2005). It therefore seems that the complete replacement between the Nguru and Ukaguru Mountains must reflect that the two populations are sufficiently divergent to be unable to establish themselves within each other’s respective geographical territories.

To the south, the Misuku Hills (with *B. mixta*) are separated from the Nyika Plateau (with *B. capensis sola*) by mid-altitude rangeland and palm savannah, but at one point the gap between the respective forest zones is only 10 km. A few individuals from the Misuku area had morphological traits approaching those of *sola* (see above), but these individuals are nested with birds from south-western Tanzania in the molecular

Fig. 5 Statistical parsimony networks for the two clades of *Batis mixta*: **a** northern clade and **b** southern clade. Haplotype numbers correspond to individuals and localities listed in Table 1. Solid circles represent intermediate hypothetical haplotypes that are necessary to link all observed haplotypes together in the network



phylogeny (Fig. 4). Thus, if at any time in the past intergradation has taken place, this is at least not reflected in the current variation in mitochondrial DNA.

The isolated population now referred to as *B. capensis reichenowi* (or as an independent species by Sinclair and Ryan 2003) would represent a phylogenetic species (Cracraft 1997) based on the distinctive appearance of the female plumage, and possibly also by eye-colour. However, it is not a terminal taxon genetically, and its divergence in Fig. 5a is smaller than that between some haplotypes obtained from different individuals of the same local *mixta* populations (from Mafi Hill and East Usambara Mts). Its position in Figs. 4 and 5 suggests that this is a small satellite population of the northern *mixta*, maybe a relict population left after a humid period with more extensive forest cover in the coastal zone, in which genetic drift caused a rapid change in alleles, has affected the expression of melanin (possibly the MC1R gene; see Mundy et al. 2004).

Vocal differences between *B. capensis* and *mixta* are described by Dowsett-Lemaire (1989; for Nyika Plateau and the Misuku Hills, northern Malawi), Urban et al. (1997) and Harris and Arnott (1988). Furthermore, the wing-beats of *B. mixta* produce a very distinctive wing-whirring sound, which may be related to a distinctive mode of flight (with the stubby tail being virtually non-functional). We can confirm that most populations referred to as *B. mixta* give calls corresponding to those described in these publications. However, the vocal repertoire is incompletely understood and we do not have tape recordings from the northern populations, or from *reichenowi*, which could tell whether these are vocally distinct from the south-western populations.

One may wonder why the differences between south-western and northern *B. mixta* populations have been overlooked so far. One reason could be that few museums have specimens from many parts of the range. However, others have compared material from different populations, so in this case it seems that they were not very careful, and just attributed the variation to differences between individuals. A contributing factor could be that, because of the rather fluffy plumage of *Batis* spp., it is difficult to make nice skins with clearly discernable plumage patterns. However, the difference in the development of the wing-stripe of females (Fig. 2) is easy to see in most museum specimens. Looking at published illustrations, it is clear that Plate 31 in Urban et al. 1997, Plate p. 210 in Stevenson and Fanshawe (2002) and Plate p. 565 in Sinclair and Ryan (2003) illustrate northern *mixta* populations, and

Plate 31 in Harris and Franklin (2000) is the south-western population (but with wrong colour on the flanks). All published illustrations of *reichenowi* are misleading (with more or less chestnut breast, instead of the correct grey coloration). We see the latter errors as unfortunate cases of citation inbreeding since Grote's (1911) original description, emphasising the need to consult specimens when preparing artwork.

To conclude, what has hitherto been considered one species, *B. mixta*, is in fact two morphologically and genetically distinct species replacing each other in different parts of the combined range. The Eastern Arc Mountains and adjacent coastal zone are under a constant climatic influence from the Indian Ocean (Lovett and Wasser 1993) and seem to be a centre of diversification, or "species pump", as populations of different phylogenetic ages could persist in suitable places, and could eventually disperse and enrich the regional biota (Fjeldså and Lovett 1997; Jetz et al. 2004). Based on patterns of endemism, the main centres for population persistence may be in the Usambara/Coastal, Uluguru and Udzungwa forests (Burgess et al. 2006). The divergence of populations inhabiting such local, predictable places in the Tanzanian forest mosaic would seem to be a case of non-adaptive divergence or ecological stasis (Ricklefs and Latham 1993). The *reichenowi* population is best regarded as an aberrant and marginal isolate of the northern/coastal *B. mixta* populations, and therefore should be ranked as a subspecies until demonstrated otherwise.

Since no scientific name exists that would apply to the populations from the Ukaguru Mts to Misuku Hills we will here formally name and describe this form, and re-define other taxa in this complex.

Batis crypta, sp. nov.

Holotype: ZMUC 95.264, ad. male leg. 11. November 2003, Mdandu forest in the Kipengere range W of Njombe, Ludewa District, Iringa Region, Tanzania.

Topotype: Female ZMUC 95.263, ad. female leg. 12. November 2003, same place.

Paratypes: ZMUC 75.501-75.516, 94.097-94.099, 95.257-95.285; Field Museum numbers 439395-439411, Leiden numbers 37532 and 37533, University of Dar es Salaam numbers B 276, 408, 609 and 636.

Description of the holotype: forehead and crown slate in colour grading to dark gull grey on lower back, with large black feather-tips on the mid-back and diffuse white feather-centres, especially on the lower back; scapulars and upper-tail coverts black. Sides of head with broad black mask extending to sides of neck.

Wings black, median coverts broadly tipped white, outer web of inner greater covert white, as is the outer edge of the inner secondary; otherwise all remiges have pale grey outer edges and whitish inner edges. Tail black, all rectrices with small white tips, and outer rectrix with narrow white outer edge. Under-parts from chin to vent white, except for broad (22 mm) black pectoral band, some grey smudges along the sides and tibiae black.

Measurements of the type: wing 65.6 mm, tail 37.0 mm, tarsus 19.7 mm, culmen 13.7 mm, mass 12.5 g.

Description of the topotype: forehead and crown dark gull grey, with narrow white border towards black lore; back, including scapulars, olive brown with diffuse white feather-centres on lower back, upper-tail coverts black. Sides of head with broad black mask extending to sides of nape. Wings bone brown with feathers edged olive-brown, or buffy brown on secondaries, olive-buff on primaries; median coverts broadly tipped tawny, outer web of three inner greater coverts ochraceous-tawny, as is the outer edge of the inner secondary. Tail black, all rectrices with small (worn) white tips, and outer rectrix with narrow white outer edge. Throat ochraceous-tawny, palest on chin and towards the black mask, and separated by diffuse white patch from tawny to hazel brown breast with distal part of most feathers (outer 1 mm) whitish; sides mostly grey, with some ochraceous spots on transition to the white belly; tibiae blackish brown.

Measurements of topotype: wing 56.8 mm, tail 33.9 mm, tarsus 19.5 mm, culmen 11.7 mm, mass 10 g.

Diagnosis: males with dark grey crown and never with any white in the superciliary zone (like *B. capensis*); black humerals and usually this spreads on to the middle back, which may be quite extensively spotted with black feather-tips (Fig. 2); breast-bar mostly broader than in *B. mixta*, 15–27 mm, average 21.0 mm. Females diagnostically different from *B. mixta* by the narrow inter-wing panel, where only the three inner greater coverts have broad cinnamon outer vanes, while the remaining greater coverts have thin rusty edges that may wear away so that there is only a restricted cinnamon stripe in the closed wing (unlike broad cinnamon patch in *mixta* females, see Fig. 2). Prominent tawny throat patch more or less clearly separated by white lower throat from chestnut pectoral bar, with white terminal filaments on the feathers giving a grizzled appearance, but much less so than in *B. mixta*. Thus, throat patch and breast bar appear intermediate between the vague pattern of *B. mixta* and the solid chestnut of *B. capensis*.

Individual variation: some males are almost indistinguishable from those of *B. mixta* with broadest

breast-bars and smallest white spots in the upper lore. In some females the throat-patch is more golden than the breast-bar, but in other cases the colour is the same, and always much darker than in *B. mixta*.

Measurements ($n = 51$ males, 62 females): flattened wing 56.1–66.0 mm (average 62.8 for males, 60.3 for females), tail 31.9–38.2 mm (average 34.9 for males, 34.8 for females); exposed culmen 10.9–14.5 mm (average 11.9 for males, 11.5 for females); tarsus 16.2–20.8 mm (average 19.0 for males, 18.9 for females); mass 10.0–15.1 g (average 12.8).

Geographical range: distributed from Ukaguru Mts on the Morogoro-Dodoma border in the north-east and Kiboriani and Wota Mts (Dodoma district) in the north, through the Rubeho and Iringa highlands (Iringa), and southern highlands near Lake Malawi (Njombe, Kipengere and Livingstone Mts, Mt. Rungwe), to the Matengo highland W of Songea and Misuku Hills in the extreme NW Malawi highlands, and in the isolated Uluguru Mts (Morogoro). Elevational range is 540–2,160 m, with peak abundance in evergreen montane forest around 1,500 m (Romdal 2001).

Etymology: the scientific name emphasises that this form was overlooked in earlier revisions of the group; for instance, Lawson (1986) examined 77 specimens from its geographical range. Vernacular name: the name forest batis has been used for the taxonomic unit, which we now divide into two species, and in order to minimise confusion we prefer to start afresh with the names dark batis for the new species and short-tailed batis for the re-defined *B. mixta*.

Taxonomic notes on other forms

Batis mixta mixta (Shelley, Proc. Zool. Soc. London 1889: 259, pl. 40; Mt. Kilimanjaro. Lawson (1986).

Diagnosis: adult males always have some white feathers on the border between black lore and grey forehead, and this may or may not continue as a more or less continuous and conspicuous white superciliary stripe; dorsally dark gull grey, generally lighter than in *B. crypta*, and rarely with black spots other than on the scapulars; breast-bar mostly 10–17 mm wide, rarely broader. Adult females with feathers of throat and breast broadly tipped white, causing a hoary appearance and almost obliterating the salmon-orange throat-patch; orange-cinnamon inter-wing panel comprising broad outer webs of all greater coverts and thus forming a large, bright patch in the closed wing. Tail generally shorter than in *B. crypta*, but not sufficiently for safe identification. Measurements ($n = 44$ males, 47 females): flattened wing 56.4–65 mm, average 61.4 for males, 60.4 for females), tail 28.6–33.5 mm (average

31.3 for males, 31.3 for females); exposed culmen 10.9–14.5 mm (average 12.4 for males, 12.2 for females); tarsus 16.2–18.8 mm (average 18.5 for males, 18.5 for females); mass 9.0–15.0 g (average 12.1).

Distribution: coastal SE Kenya (see below) and in north eastern Tanzania from Mt Kilimanjaro and Arusha NP, with sighting from Lake Manyara (Britton 1980), and S to Usambara, Nguu and Nguru Mts. We examined birds from Usambara Mts (22), Pare Mts (7), Kilimanjaro (6), Mt. Meru (1), Nguu Mts (13), Nguru Mts (8), Mt Kanga (3), Vumari (1) and southern Kenya (37). Distributed from sea level to 2,300 m (on Mt. Kilimanjaro).

Batis mixta ultima (Lawson 1962, see also Lawson 1986) of coastal SE Kenya (Ganda, Rabai, Shimba Hills, Arabuko-Sokoke Forest, Shimoni), cannot be maintained, as the claimed diagnostic features of male individuals, extensive white in the supercilium, is also found in some individuals elsewhere in the species' range. The other claimed characteristics, a narrow breast-bar (of males) and extensive white feather-tips on the throat and breast (of females), are shared throughout all populations of our *B. m. mixta*. Furthermore, the Kenyan populations do not form a monophyletic clade in our gene tree. We suggest that *B. mixta ultima* be synonymised with *B. m. mixta*.

Batis mixta reichenowi Grote 1911, Orn. Monatsber. 19:162–163; Mikindani.

Initially published as a species but placed with *B. mixta* by Lawson (1986) and Traylor (1986), and then moved to *B. capensis* by Dowsett-Lemaire (1989) and Dowsett and Dowsett-Lemaire (1993). Although it is morphologically distinctive, our genetic data suggest that it represents an outlier (relict) population of the genetically quite variable *B. mixta*.

Diagnosis: Adult males may not differ diagnosably from *B. m. mixta* except by orange or yellow eyes (and even this is insufficiently documented). Adult females are diagnosably distinct as the crown and upper back are neutral grey inclining to greyish olive on lower back; throat white, eventually with a hint of cinnamon, breast-bar neutral grey, sometimes with traces of pinkish cinnamon mainly on the transition towards the white belly (specimens with more cinnamon or buff below being juveniles and immatures); ochraceous inter-wing panel broad (as in *B. m. mixta*) in juveniles, but adults have narrower pale edges on the outer greater coverts.

Measurements ($n = 7$ males, 7 females): flattened wing 57.5–62 mm (average 59.6 for males, 59.8 for females), tail 31.5–35.2 mm (average 33.8 for males, 33.1 for females), exposed culmen 11.8–13.9 mm (average 13.2 for males, 12.6 for females, generally less

wide than in *B. m. mixta*); tarsus 17–19.3 mm (18.2 for males, 18.6 for females).

Distribution: recorded in several patches of coastal forest on the Rondo and Makonde Plateaus west of Mikindani in the Lindi district, and in the coastal forests of the Kilwa district, south-eastern Tanzania (Baker and Baker 2002). It may occur in other forest patches north towards the Rufiji River and presumably in the extensive forests of the plateau in north-eastern Mozambique, just south of the border.

Zusammenfassung

Die Kurzschwanzbatis *Batis mixta* besteht aus zwei Arten: Beschreibung einer neuen, nahverbreiteten *Batis* Art in Ostafrika

Die Kurzschwanzbatis *Batis mixta* ist in den Wäldern der Eastern Arc Mountains von Tanzania und in einigen benachbarten Berg- und Küstenwäldern weit verbreitet. Neue Aufsammlungen ergaben deutliche morphologische Unterschiede im mittleren Bereich der Verbreitung. Ergänzende genetische Studien der historischen Struktur deuten auf enge Verbindungen innerhalb der südwestlichen Populationen und der nördlichen Küstenvorkommen hin, jedoch nicht zwischen diesen parapatrischen Gruppen. Es wird geschlossen, dass es sich um zwei Arten handelt und für die südwestliche Form wird der neue Name *Batis crypta* vorgeschlagen. Ein deutlicher genetischer Unterschied besteht auch zu *Batis capensis sola* im nördlichen Malawi. Die morphologisch distinkte *reichenowi* im südöstlichen Tanzania ist genetisch bei *B. mixta* eingemischt und als Unterart von *B. mixta* zu sehen.

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References

- Baker N, Baker E (2002) Important Bird Areas in Tanzania. Wildlife Conservation Society of Tanzania, Dar es Salaam
- Bowie RCK (2003) Birds, molecules and evolutionary patterns among Africa's islands in the sky. Unpublished PhD thesis, University of Cape Town
- Bowie RCK, Fjelds  J, Hackett SJ, Crowe TM (2004a) Systematics and biogeography of Double-Collared Sunbirds from the Eastern Arc Mountains, Tanzania. *Auk* 121:660–681
- Bowie RCK, Fjelds  J, Hackett SJ, Crowe TM (2004b) Molecular evolution in space and through time: mtDNA phylogeography of the Olive Sunbird (*Nectarinia olivacea/obscura*) throughout continental Africa. *Mol Phylogenet Evol* 33:56–76
- Bowie RCK, Voelker G, Fjelds  J, Lens L, Hackett SJ, Crowe TM (2005) Systematics of the Olive Thrush *Turdus olivaceus* species complex with reference to the taxonomic status of the endangered Taita Thrush *T. helleri*. *J Avian Biol* 36:391–404
- Bowie RCK, Fjelds  J, Hackett SJ, Bates JM, Crowe TM (2006) Coalescent models reveal the relative roles of ancestral polymorphism, vicariance and dispersal in shaping phylogeographical structure of an African montane forest robin. *Mol. Phylogenet. Evol* 38:171–188
- Britton PL (1980) Birds of East Africa. East African Natural History Society, Nairobi
- Burgess ND, Mlingwa COF (2000) Evidence for altitudinal migration of forest birds between montane Eastern Arc and lowland forests in East Africa. *Ostrich* 71:184–190
- Burgess ND, Cordeiro N, Daggart N, Fjelds  J, Hansen LA, Howell K, Kilahama F, Lovett JC, Menegon M, Moyer D, Nashanda E, Perkin A, Stanley W, Stuart S (2006) The biological importance of the Eastern Arc Mountains of Tanzania. *Biol Conserv* (in press)
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Mol Ecol* 9:1657–1660
- Cracraft J (1997) Species concepts in systematics and conservation biology: an ornithological viewpoint. In: Claridge MF, Daway HA, Wilson MR (eds) *Species: the units of biodiversity*. Chapman & Hall, London, pp 325–339
- Dickinson EC (2003) The Howard and Moore complete checklist of the birds of the world, 3rd edn. Princeton University Press, Princeton
- Dowsett RJ, Dowsett-Lemaire F (1980) The systematic status of some Zambian birds. *Gerfaut* 70:151–199
- Dowsett RJ, Dowsett-Lemaire F (1993) A contribution to the distribution and taxonomy of Afrotropical and Malagasy birds. Tauraco Press, Liège
- Dowsett-Lemaire F (1989) Ecological and biogeographical aspects of forest bird communities in Malawi. *Scopus* 13:1–80
- Fjelds  J, Lovett J (1997) Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centers. *Biodiver Conserv* 6:325–347
- Fuchs J, Bowie RCK, Fjelds  J, Pasquet E (2004) Phylogenetic relationships of the African bush-shrikes and helmet-shrikes (Passeriformes: Malaconotidae). *Mol Phylogenet Evol* 33:428–439
- Grote H (1911) Neue Vogelarten aus Ostafrika. *Orn Monatsber* 19:162–163
- Harris T, Arnott G (1988) The shrikes of southern Africa. Struik, Cape Town
- Harris T, Franklin K (2000) Shrikes and bush-shrikes. Including wood-shrikes, helmet-shrikes, flycatcher-shrikes, philentomas, batises and wattle-eyes. Helm, London
- Jetz W, Rahbek C, Colwell RK (2004) The coincidence of rarity and richness and the potential signature of history in centers of endemism. *Ecol Lett* 7:1180–1191
- Lawson WJ (1962) Geographic variation in Woodward's Batis *Batis fratrum* (Shelley). *Durban Mus Novit* 6:220–224
- Lawson WJ (1986) Speciation in the forest-dwelling populations of the avian genus *Batis*. *Durban Mus Novit* 13:285–304
- Lovett JC, Wasser SK (1993) Biogeography and ecology of the rain forests of Eastern Africa. Cambridge University Press, Cambridge
- Mundy NI, Badcock NS, Hart T, Scribner K, Janssen K, Nadeau NJ (2004) Conserved genetic basis of a quantitative plumage trait involved in mate choice. *Science* 303:1870–1873
- Posada D, Crandall KA (2001) Intraspecific gene genealogies: tree grafting into cladograms. *Trends Ecol Evol* 16:37–45
- Ricklefs RE, Latham RE (1993) Global patterns of diversity in mangrove floras. In: Ricklefs RE, Schluter D (eds) *Species diversity in ecological communities. Historical and geographical perspectives*. University of Chicago Press, Chicago, pp 215–229
- Romdal TS (2001) Altitudinal distribution and abundance patterns of bird species in the Eastern Arc Mountains, Tanzania. *Scopus* 21:35–54
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574
- Sinclair I, Ryan P (2003) Birds of Africa south of the Sahara. A comprehensive illustrated field guide. Struik, Cape Town
- Stevenson T, Fanshawe J (2002) Field guide to the birds of East Africa. Poyser, London
- Swofford DL (2002) PAUP*: phylogeny analysis using parsimony (*and other methods), version 4.0b 10. Sinauer, Sutherland
- Traylor MA (1986) The Platysteiridae, Puffback Flycatchers. In: Mayr E, Cottrell GW (eds) *Check-list of birds of the world*, vol 11. Mus. Compar. Zool, Cambridge, pp 376–386
- Urban EK, Fry CH, Keith S (1997) Birds of Africa: thrushes to puffback flycatchers, vol 5. Academic, London