Dramatic niche shifts and morphological change in two insular bird species

Per Alström1,2,†, Knud A. Jønsson3,4,5,†, Jon Fjeldså3, Anders Ödeen6, Per G. P. Ericson7 and Martin Irestedt8,†

1Swedish Species Information Centre, Swedish University of Agricultural Sciences, PO Box 7007, Uppsala 75007, Sweden
2Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, People's Republic of China
3Center for Macroecology, Evolution and Climate at the Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark
4Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot SLS 7PY, UK
5Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK
6Department of Animal Ecology, Uppsala University, Norbyvägen 180, Uppsala 75236, Sweden
7Department of Zoology, and 8Department of Bioinformatics and Genetics, Swedish Museum of Natural History, PO Box 50007, Stockholm 10405, Sweden

1. Summary

Colonizations of islands are often associated with rapid morphological divergence. We present two previously unrecognized cases of dramatic morphological change and niche shifts in connection with colonization of tropical forest-covered islands. These evolutionary changes have concealed the fact that the passerine birds Madanga, Madanga ruficollis, from Buru, Indonesia, and São Tomé shorttail, Amaurocichla bocagii, from São Tomé, Gulf of Guinea, are forest-adapted members of the family Motacillidae (pipits and wagtails). We show that Madanga has diverged mainly in plumage, which may be the result of selection for improved camouflage in its new arboreal niche, while selection pressures for other morphological changes have probably been weak owing to preadaptations for the novel niche. By contrast, we suggest that Amaurocichla's niche change has led to divergence in both structure and plumage.
2. Introduction

Colonizations of islands are well-known triggers of speciation [1–4], often involving strong morphological divergence from continental relatives, sometimes resulting in spectacular radiations [5–9]. Such morphological divergence may result in erroneous assumptions about systematics as well as misinterpretations of biogeographical and ecological patterns and processes (cf. [10–16]).

We examine the phylogenetic relationships of two enigmatic forest birds of tropical islands, madanga *Madanga ruficollis* from Buru, Wallacea, Indonesia, and São Tomé shorttail *Amaurocichla bocagii* from São Tomé in the Gulf of Guinea, and demonstrate that both have undergone remarkable morphological change and niche shifts from their continental relatives, completely obscuring their systematic relationships. *Madanga ruficollis* is known from four old museum specimens [17] and a few recent observations [18–20], and has traditionally been placed in a monotypic genus in the family Zosteropidae (‘white-eyes’) [21,22]. *Amaurocichla bocagii* is placed in a monotypic genus, with presumed sylvioid relationships [23,24], although recent molecular analysis unexpectedly suggested affinities to *Motacilla* [25].

3. Material and methods

3.1. Study group

For a first preliminary molecular analysis of *Madanga*, we used mainly GenBank sequences (electronic supplementary material, table S1), representing a broad array of lineages, including most of the primary lineages within Passerida, as suggested by previous studies (e.g. [25–27]). As we obtained strong support for a close relationship between *Madanga* and Motacillidae, as had previously been suggested also for *Amaurocichla* [25], we expanded the dataset to also include representatives from all genera within Motacillidae and multiple species from the main clades found in earlier studies [28,29]. When preliminary analyses suggested a relationship between *Madanga* and a clade referred to as ‘small pipits’ by Alström & Mild [30], all the species in that clade were analysed (except *Anthus petrosus*, which has previously been treated as conspecific with *Anthus spinoletta*; cf. [30], as well as the two remaining Asian species with untested relationships, alpine pipit *Anthus gutturalis* and Nilgiri pipit *Anthus nilghiriensis*.

3.2. DNA extraction and sequencing

DNA was obtained from muscle, blood or, in a few cases, feathers or toepads (electronic supplementary material, table S1). Toepads were sampled from two *Madanga* and two *Amaurocichla* specimens. While standard laboratory procedures were used for fresh DNA samples, extractions, amplifications and sequencing procedures from archaic DNA obtained from study skin samples followed the procedures described previously [31,32]. This included extracting DNA in a dedicated ‘clean’ laboratory, and amplifying short (ca 200 bp), partly overlapping fragments. We sequenced five loci: the main part of the mitochondrial cytochrome *b* gene and part of the flanking tRNA-Thr (combined referred to as *cytb*); the mitochondrial NADH dehydrogenase subunit 2 (*ND2*); the nuclear ornithine decarboxylase (*ODC*) exon 6 (partial), intron 6, exon 7, intron 7 and exon 8 (partial); the entire nuclear myoglobin (*myo*) intron 2 and the Z-linked (*CHD1Z*) intron.

3.3. Phylogenetic analyses

In addition to the sequences obtained specifically for this study, we also used sequences from GenBank (electronic supplementary material, table S1). *Cytb* and *ND2* were analysed for all species and two to three nuclear loci for most species. For *Anthus spragueii* only *Cytb* was available, and for *Anthus brachyurus* only *cytb* and *ND2*. See electronic supplementary material, table S1 for details. All new sequences have been deposited in GenBank (electronic supplementary material, table S1).

Sequences were aligned using Muscle [33] in *Seaview* v. 4.3.4 [34,35]; some manual adjustment was carried out for the non-coding sequences. For the nuclear loci, heterozygous sites were coded as ambiguous. Trees were estimated by Bayesian inference (BI) using *MrBayes* v. 3.2 [36,37] as follows. (i) For the Motacillidae dataset, using *Emberiza* and *Passer* as outgroups, all loci and all sequences, including multiple individuals of the same species when available, were analysed separately (single-locus analyses). (ii) For the same dataset, as well as the one comprising multiple oscine lineages, using the suboscines *Tyrannus* and *Manacus* as outgroups, sequences from one individual per species were
3.4. Morphological analyses

We examined all four known specimens of Madanga and four specimens of Amaurocichla in the American Museum of Natural History, New York (AMNH) and one additional specimen (holotype) of Amaurocichla in The Natural History Museum, Tring, UK (NHM). These were compared with large series of pipits (Anthus) and wagtails (Motacilla) and other relevant species. All the species in clade A (see Results) were measured: wing length (flattened and stretched); tail length (ruler inserted under tail); bill length (to skull); bill depth and width (at distal edges of nostrils); tarsus length (to last complete scutum before toes); hind-claw length (to thin skin at base). Only internally sexed specimens were measured, with the aim to measure 10 of each sex when possible. Measurements were taken in the AMNH, Swedish Museum of Natural History, Stockholm (NRM) and National Zoological Museum of China, Beijing (NZMC). A principal component analysis (PCA) was performed in SPSS STATISTICS v. 20 (IBM Corp.). In addition, X-rays of all four Madanga specimens were examined and compared with skeletons of other relevant taxa.

4. Results

4.1. Molecular markers

Madanga was nested within Motacillidae, among the primarily Eurasian ‘small pipits’ Anthus (sensu [30]) (figure 1, clade A; electronic supplementary material, figures S1–S3). Madanga and the New Guinean A. gutturalis formed a strongly supported clade (D), sister to another well supported clade (C) comprising the northern Palaearctic breeders A. trivialis and A. hodgsoni and South Indian A. nilghiriensis. Amaurocichla was nested within Motacilla, as sister to the Afrotropical M. clara and M. capensis, in a clade (G) also containing the Malagasy M. flaviventris; these clades were strongly supported. Clade D was also supported by a unique 19 bp deletion in the ODC alignment (electronic supplementary
Figure 1. Chronogram of Motacillidae, based on cytb and a molecular clock (2.1%/Myr), with the topology constrained to fit the multilocus tree (electronic supplementary material, figure S2). Values at branches are posterior probabilities (PP)/maximum-likelihood bootstrap (MLBS) percentages; * indicates PP 1.00 or MLBS 100%. Letters A–G denote clades discussed in the text. The 'pipit' and 'wagtail' clades have been highlighted by differently coloured branches; A.—Anthus, M.—Motacilla. Boxes at tips of branches represent, from left to right, habitat (black—forest; white—open; grey—forest or more open) and foraging niche (black—arboreal; white—terrestrial; grey—terrestrial and arboreal). Illustrations by P.A. (from [30]), Ren Hathway (3, 9–11, 14, 16–19; from [56]) and J.F. (4, 15).
material, figure S2). In the single-locus analyses (electronic supplementary material, figure S3), there was conflict between mitochondrial and nuclear markers regarding the exact position of Madanga, with the mitochondrial tree inferring Madanga to be sister to the four other species in clade B. There was also some conflict among single-locus analyses regarding the precise position of Amaurocichla (electronic supplementary material, figure S3). Although not the focus of this study, Macronyx croceus and Tmetothylacus tenellus were inferred to be sisters (clade F), deeply nested within Anthus, and Dendronanthus indicus was found to be sister to Motacilla; all these relationships received strong support.

Madanga and A. gutturalis were estimated to have diverged from a most recent common ancestor 3.98 (95% HPD 2.65–5.33) million years ago (Ma), Amaurocicha and M. capensis + M. clara 3.34 (95% HPD 2.17–4.64) Ma (figure 1; electronic supplementary material, figure S4).

4.2. Morphology

Madanga shows hardly any plumage similarity with Anthus (e.g. completely lacks dark streaking; shows unpatterned head, with no pale supercilium or dark moustachial and malar stripes; rather uniformly green wings without contrastingly pale wing-bars or tertial edgings; vivid green upperparts, and grey head and underparts with a well-demarcated pale rufous throat patch; figure 1). The only decidedly Anthus-like plumage trait is the pale markings on the inner webs of the outermost pair of rectrices, although these are less contrasting than in Anthus. By contrast, Madanga displays typical Anthus structure, although it has less contrast than other Holarctic/Oriental Anthus, with less elongated tertials; on regression factor 1, which reflects overall size, Madanga is completely separated from the other ‘small pipits’ (figure 2). It most resembles A. hodgsoni and A. trivialis, especially in bill and hind-claw structure (figure 2; electronic supplementary material, tables S2–S3). By contrast, Madanga’s sister, A. gutturalis, is the largest species in clade A, although it resembles Madanga in bill and hind-claw structure (figure 2; electronic supplementary material, tables S2–S3).

Madanga differs from all species in Zosteropidae by its rufous throat and in multiple structural aspects (e.g. proportionately longer toes and claws, and the presence of a slight hump on the culmen of the upper mandible over the elliptical nostrils; by contrast, zosteropids have an evenly curved culmen to the upper mandible and slit-like nostrils with more pronounced operculum).

The plumage of Amaurocichla bears no resemblance to any Motacilla, being uniformly dark brown above and on the wings and tail and paler brown below with slightly paler upper throat and belly (figure 1). Moreover, the blackish bill often has diffusely set-off paler tip, unlike all wagtails. Also the structure is strongly divergent from all wagtails, having proportionately longer bill, much shorter, more rounded wings and much shorter tertials and tail, with 10 rectrices (12 in Motacilla) with projecting shafts (figure 1). Amaurocichla differs markedly in structure from the ‘warbler’ genus Macrosphenus, with which it has been associated [24], by e.g. its vestigial 10th primary, slight hump at the base of the culmen of the upper mandible, no hook at the tip of the upper mandible, less prominent rictal bristles, and proportionately longer and slimmer tarsi, toes and claws, the latter also less strongly decurved.

5. Discussion

5.1. Island colonizations, niche shifts and morphological divergence

We provide evidence that two motacillid species have successfully colonized tropical islands, Buru and São Tomé, although they have diverged so much in morphology, habitat choice and behaviour (cf. figure 1) that their systematic affinities have been gravely misinterpreted. Despite the genus Anthus, with more than 40 extant species [22], having existed for some 8–9 Myr (electronic supplementary material, figure S4), the plumage divergence within this genus has been minimal compared to most other similar sized groups of birds, and different pipit species are renowned for being difficult to separate morphologically [30,56,57]. By stark contrast, Madanga’s plumage does not resemble that of any other pipit. Likewise, Amaurocichla bocagii is very different from other wagtails in plumage and has also diverged dramatically in bill, wing and tail structure.

Colonization of islands requires the capacity to cross open water and may be further facilitated by sociality, which increases the potential for simultaneous arrival of multiple individuals [4]. The family Motacillidae is globally distributed, and includes several long-distance migrants with loose flocking behaviour [28,30,56,58], making them suitable candidates for island colonization. Indeed, endemic pipits are found on several oceanic islands, and one species complex has colonized multiple islands from Sri Lanka to New Zealand [56].
Figure 2. Principal component analysis of seven structural variables of the ‘small pipits’ [30]. Arrows indicate Madanga ruficollis and its sister Anthus gutturalis. Photos of M. ruficollis (top) and two pipits (Anthus hodgsoni yunnanensis, bottom left; Anthus nilghiriensis) in their typical habitats. Photos Rob Hutchinson, Aurélien Audevard and Arka Sarkar, respectively.

Madanga occurs in stunted montane forest [19,59], where it has been noted to feed like a nuthatch (Sitta) on epiphyte-covered branches and tree trunks, sometimes following mixed-species feeding flocks [59] (R. Hutchinson, F. Rheindt, P.-H. Fabre 2013, personal communication). Amaurocichla is found in primary forest, where descriptions in the literature suggest diverse foraging strategies, including feeding on the ground in riparian habitats as well as on tree trunks and branches [24,60,61]. In contrast to both Madanga and Amaurocichla, nearly all other motacillids occur in open habitats with short grass, such as savannah, steppe, meadows and tundra, and along rivers and lake sides, from sea level to above the tree limit [30,56]. Moreover, unlike Madanga and Amaurocichla, all other motacillids forage exclusively on the ground [30,56] (the statement that Dendronanthus indicus ‘is equally likely to seek food in trees [as on the ground]’ [56] is incorrect [30]; personal observation).

The radical niche shifts in Madanga and Amaurocichla were most likely instigated by their colonization of tropical islands that, before human settlement, were probably completely forest-covered. In the case of Madanga, the conditions for a niche shift were probably favourable, as its ancestor, by chance, settled in an area with strongly epiphyte-covered, i.e. unusually ‘ground-like’, trees (by contrast, its sister species, A. gutturalis, colonized an area with open grasslands [56], i.e. a common pipit habitat). We hypothesize that Madanga’s niche shift originated by opportunistic feeding in trees used for shelter, and that it was morphologically and behaviourally suitably preadapted for this new niche. For example, short, decurved hind-claws, as in all species in clade B, are typical of arboreal birds (cf. the longer, straighter hind-claws of the species in clade E [30]); two of Madanga’s closest relatives, A. trivialis and A. hodgsoni, are atypical among pipits in breeding mainly in wooded habitats, and in frequently taking cover in trees when
flushed off the ground [30,56], and Madanga’s poorly known sister species, A. gutturalis, is also said to often fly into bushes and trees when alarmed [56]; the species in clade C, which is sister to the Madanga/ A. gutturalis clade, forage by creeping around in rather dense vegetation of grasses and forbs [30], a habitat rather similar to epiphyte-covered branches; and the species in clade C are all capable of walking rather freely on branches [30]. Novel feeding behaviours leading to niche shifts might spread quickly in a small founder population, first culturally and later obtaining a genetic basis (cf. [4, pp. 128, 133]).

Amaurocichla’s niche shift is less marked than in the case of Madanga, as it is less arboreal. Its close relative M. flaviventris sometimes breeds in forest clearings, open secondary forest and Eucalyptus plantations, and the even more closely related M. clara occurs along streams and rivers in forested country (although not in closed forest) [56,62].

In general, birds show a strong correlation between morphology and ecology (review [63]). Niche shifts can result in feeding-related morphological differentiation, as in the Hawaiian honeycreepers [8,64], Malagasy vangas [9,65], Darwin’s finches (e.g. [6]) and ground tit Pseudopodoces humilis [66]. This seems to be true also for Amaurocichla, which has diverged markedly in structure from other motacillids, which are all structurally basically similar. Amaurocichla’s notable bill elongation is almost certainly feeding-related, whereas the dramatically shortened tail and wings and change in plumage are likely adaptations to its closed forest habitat. Shortening of the tail and wings and cryptic plumage colouration would be advantageous in closed forest, and short tails and wings and mostly brown plumage are common in various distantly related groups of passerines inhabiting dense undergrowth (e.g. many Cettiidae, Pellorneidae, Pnoezygidae, Troglodytidae and Rhinocryptidae) (e.g. [67,68]).

In the case of Madanga, surprisingly little divergence has occurred in feeding-related morphology, despite the extraordinary switch in niche and substantial period (ca 4 Myr) of independent evolution (cf. Amaurocichla, which has been separated from its closest relatives for 3.3 Myr). We suggest that the selection for feeding-related morphological change has been weak because the lineage leading to Madanga was suitably preadapted to this new niche (see above). However, the smaller size, which is the main structural difference between Madanga and its relatives, might have evolved in response to the niche change, as a smaller, lighter body would be advantageous for feeding on branches and trunks. By contrast, the plumage might have been under strong selection for improved crypsis in its novel niche. At least the plain green upperparts of Madanga probably provide better camouflage in trees than the contrastingly marked upperparts of most other pipits, as indicated by the fact that uniformly green upperparts are common in arboreal passerines in many unrelated families (e.g. Pycnonotidae, Phylloscopidae, Zosteropidae, Regulidae, Chloropsieidae and Vireonidae). It is possible that Madanga’s strong plumage divergence is at least partly the result of changed sexual selection pressures, though the data are not available to test this. A similar example, with strong plumage and niche divergence but comparatively slight structural differentiation, concerns the cinnamon ibon Hypocryptadius cinnamomeus, also previously misclassified as an aberrant zosteropid but now identified as a canopy-adapted sparrow [13].

5.2. Taxonomy

A comprehensive taxonomic revision of Motacillidae is required. As a first step, Madanga should be synonymized with Anthus (type species: A. pratensis) and Amaurocichla with Motacilla. However, we advocate awaiting a more comprehensive sampling before making any further taxonomic recommendations.

5.3. Conclusion

To conclude, we suggest that the strong morphological divergences in the lineages leading to Madanga ruficolis and Amaurocichla bocagii were triggered by fundamental niche shifts following colonizations of forest-covered tropical islands.

Data accessibility. Supplementary figures and tables, DNA sequence alignments and details of samples, including GenBank numbers (KP671495–KP671599) and photos are deposited in Dryad: http://dx.doi.org/10.5061/dryad.40s3g.

Acknowledgements. We thank Peter Capainolo, Joel Cracraft, Paul Sweet, Thomas Trombone and Lydia Garetano at AMNH for photos and X-rays of Madanga specimens, DNA samples and access to collections; Town Peterson and Mark Robbins at the University of Kansas Natural History Museum for DNA sample; Aristide Andrianarimisa, Steven Piper and Christer Hemborg for DNA samples; Malcolm Wilson and Staffan Ulfstrand for field assistance; Mats Björklund for support; Marta Vila, Eevi Karvonen, Margaretta Hultqvist and Afsaneh Ahmadzadeh for laboratory
support; Ulf Johansson at NRM, and Jun Chen and Peng He at IOZ for access to collections; James Eaton, Pierre-Henri Fabre, Rob Hutchinson, Frank Lambert, Frank Rheindt, Aurélien Audevard and Arka Sarkar for information and/or photos; Josep del Hoyo and Lynx Edicions for permission to use illustrations from Handbook of the Birds of the World; Nigel Redman and A&C Black Publishers for digital copies of P.A.’s illustrations; and Nigel Collar, Lars Larsson, Urban Olsson, Trevor Price, Staffan Ulfstrand and two anonymous reviewers for valuable comments.

Author contributions. K.A.J. and M.I. conceived the idea; P.A. and J.F. collected the museum data; A.Ö. and P.A. collected some DNA samples; K.A.J. carried out initial molecular analyses; P.A. carried out the final molecular and morphological analyses; and M.I., A.Ö. and K.A.J. carried out the laboratory work. All authors contributed to the design of the study and writing of the manuscript, and gave final approval for publication.

Funding statement. Funding provided by the Swedish Research Council (grant no. 621-2010-5321 to P.G.P.E. and B-AA/BU08416-315 to Mats Björklund, to A.Ö.) and Jornvall Foundation (to P.A.); Chinese Academy of Sciences (to K.A.J.); and the Danish National Research Foundation (to the Center for Macroecology, Evolution and Climate, to K.A.J. and J.F.).

Conflict of interests. None of the authors have any competing interests.

References
