

Systematic position of the Socorro mockingbird *Mimodes graysoni*

Brian R. Barber, Juan E. Martínez-Gómez and A. Townsend Peterson

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Recently acquired tissue makes possible a first molecular assessment of the taxonomic position of the Socorro Island endemic mimid *Mimodes graysoni*. Mitochondrial DNA sequence data from the ND-2 gene was analyzed using parsimony, maximum likelihood and bayesian methods of inference. These methods all placed *Mimodes graysoni* within a strongly supported clade containing four representatives of the genus *Mimus*. Retention of the monotypic *Mimodes* would make *Mimus* paraphyletic, therefore *Mimodes graysoni* is more properly placed in the genus *Mimus*.

Brian R. Barber (correspondence), James Ford Bell Museum, and Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, USA. E-mail: barb0127@umn.edu. J. E. Martínez-Gómez, Department of Biology UM – Saint Louis, 8001 Natural Bridge Road, Saint Louis, MO 63121. A. Townsend Peterson, Natural History Museum, University of Kansas, Lawrence, KS 66045, USA.

The Socorro mockingbird *Mimodes graysoni* is a monotypic mimid genus endemic to Socorro Island, 675 km west of Manzanillo, Mexico. Originally described in the genus *Harporhynchus* (= *Toxostoma*), its systematic position has long been the subject of debate or neglect. Its anatomical structure suggested to some that *Mimodes* is a relict form of an ancestral link between mockingbirds and thrashers (Jehl and Parkes 1983). Externally, *Mimodes* resembles a thrasher, with upperparts plain brown, underparts dull brownish, and wings and tail without white. Field ornithologists nevertheless noted that it behaves much more like a mockingbird (genus *Mimus*; Grayson 1871, Jehl and Parkes 1982, 1983). Ridgway (1907) placed the Socorro mockingbird in its own genus, *Mimodes*, likely recognizing the ambiguity between anatomical and behavioral traits. This treatment has been followed almost universally since then, including Gulledge (1975), who considered *Mimodes* as part of a trichotomy with *Toxostoma* and *Mimus*.

Recently acquired tissue of *Mimodes graysoni* provided the opportunity to apply molecular tools to this question. In particular, we infer the systematic position of *Mimodes graysoni* within the Mimidae based on sequences of the ND-2 mitochondrial gene. We sequenced several taxa, obtained published data for additional major mimid clades, and analyzed the resulting data with three phylogenetic approaches.

Methods

We obtained sequence data from Hunt et al. (2001), from GenBank, for the following taxa: *Mimus gundlachi* (AF140896), *M. gilvus* (AF140895), *M. polyglottos* (AF140897), *Melanotis cearulescens* (AF140894), *Melanoptila glabrirostris* (AF140893), *Dumetella carolinensis* (AF140890), *Margarops fuscatus* (AF140891), *M. fuscus* (AF140892), *Ramphocinclus brachyurus* (AF140898), *Cinlocerthia gutturalis* (AF140887), and *C. ruficauda*

(AF140889 and AF140988). In addition, we obtained sequence data from *Sturnus vulgaris* (AF407048) for use as an outgroup (Sorenson and Payne 2001). To these data, we added 5 new sequences (museum acronyms and catalog numbers in parentheses): *Mimus saturninus* (KU tissue #2799), *Mimodes graysoni* (CNAV 023233), *Oreoscoptes montanus* (KU tissue #3445), *Toxostoma rufum* (KU tissue #1753), and *T. curvirostre* (BMM #TH60PA). The combined data set consisted of 1041 base pairs, the complete ND-2 gene, for 17 taxa. This data set represents roughly 44% (4 of 9 species) of the diversity in the genus *Mimus* (AOU 1998, Ridgely and Tudor 1989).

Genomic DNA was obtained from muscle tissue (except the *Mimodes* sample, which was obtained from liver tissue) using Qiamp extraction kits following manufacturer's protocols (Qiagen). Sequences were obtained via polymerase chain reactions for the entire ND2 gene using the primers L5215 (5'-TATCGGGCCCA-TACCCCGAATAT-3') developed by Hackett (1996) and H1064 (5'-CTTTGAAGGCCTTCGGTTTA-3') by S. Drovetski (H1064 is referenced from the ND2 gene). Amplification was carried out for 35 cycles under the following profile: initial 94°C hotstart for 150 s, 94°C denaturing for 30 s, 55°C annealing for 30 s, extension at 72°C for 70 s, and terminal extension at 72°C for 10 min. PCR products were purified using QIAquick PCR purification kits following manufacturers protocols (Qiagen). Purified PCR products were amplified asymmetrically using the PCR primers, and an additional internal primer, L347 (5'-CCATTCCACTTCT-GATTCCC-3') by S. Drovetski. Sequencing was done on a ABI-3700 automated sequencer, following manufacturer's protocols. Sequences were aligned and edited with Sequencher 3.1.1 Software (GeneCodes, Ann Arbor, MI). We translated all sequences to check for internal stop codons and examined distributions of mutation by codon position using Sequencher 3.1.1.

Parsimony analyses were conducted with the branch-and-bound option using TBR branch-swapping, with all positions and transversion/transitions weighted equally in PAUP* (Swofford 1998). As differentiation among taxa was relatively subtle, most observed substitutions were transitions, so this weighting scheme is appropriate. Support for each node was determined via heuristic bootstraps (1000 replicates; Felsenstein 1985).

For maximum likelihood (ML) and Bayesian analyses, Modeltest 3.06 (Posada and Crandall 1998) was used to determine the molecular model of evolution that best explained our data. The ML analysis was conducted in PAUP* (Swofford 1998), using a heuristic search. Nodal support was determined via 100 bootstrap replicates using the heuristic search option. Both analyses used the TBR branch-swapping algorithm.

Bayesian analyses were implemented in MRBAYES 2.01 (Huelsenbeck and Ronquist 2001). The gamma shape parameter and base frequencies were not defined *a priori*, rather, these parameters were treated as unknowns to be estimated during the analysis. Markov chain Monte Carlo (MCMC) searches via four chains were run for 1,000,000 iterations, and the resulting Markov chains were sampled every 100 generations to produce a trimmed data set of 10,001 sample points. Random swapping between chains decreases the likelihood that an individual chain will be trapped in a local optima. To further decrease the likelihood of being trapped in a local optima, we ran three individual analyses with the same parameters, each initialized with a different random tree. If all three analyses stabilized on the same likelihood values and recovered the same topology, we assumed that local optima had been overcome. Plots of likelihood values against numbers of iterations were used to determine when the likelihood values stabilized. The burn-in trees, those trees obtained before the log likelihood values reach stationarity, were discarded before posterior probabilities were calculated. Posterior probabilities (proportion of sampled trees in which a given node appears) of nodes and tree parameters were estimated from the remaining trees.

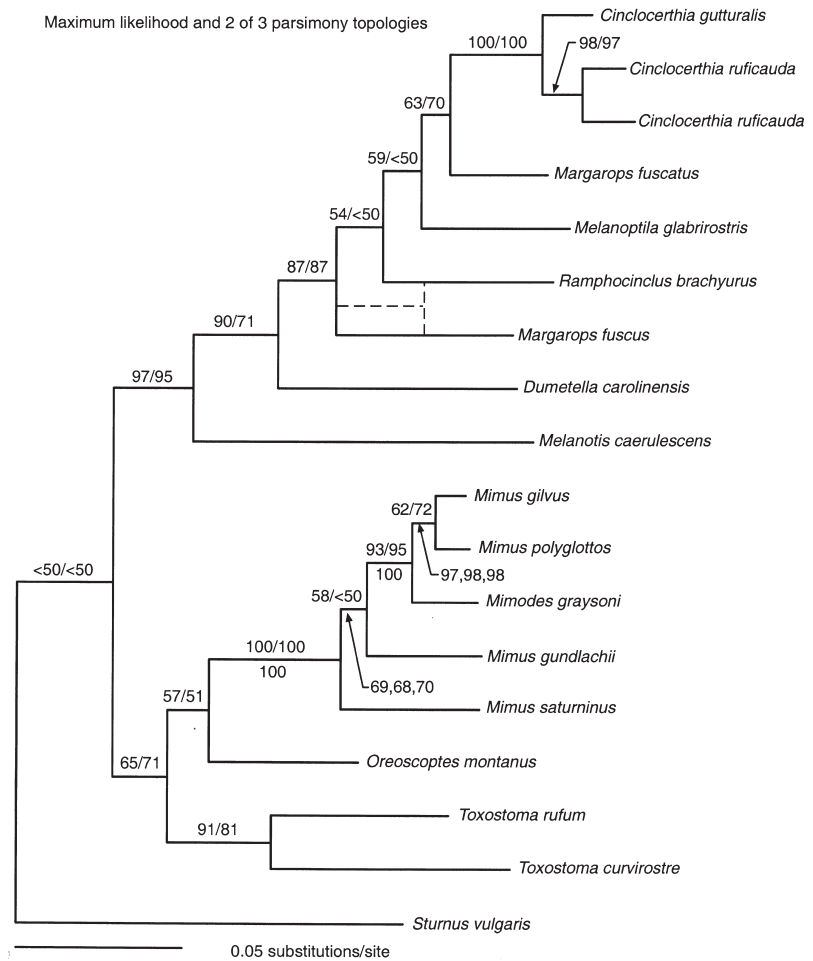
Alternative topologies were examined by comparing two constrained trees to the ML topology, *Mimodes graysoni* basal to the *Mimus* clade and *Mimodes* basal to the two *Toxostoma* taxa. A Shimodaira-Hasegawa (1999) test was done to compare these topologies to the ML tree.

Results

Of 1041 bp, 612 were constant, 134 were autapomorphic, and 295 were potentially parsimony informative. Of informative positions, 208 were third codon position, 24 second position, and 63 first position. The sequences did not contain internal stop codons, and visual inspection of each sequence indicated that the great majority of mutations for each were in the third position. The parsimony analysis resulted in three equally parsimonious topologies (length = 1024, CI = 0.542, RI = 0.579, RC = 0.314, and HI = 0.458). All three topologies included a monophyletic *Mimus*+*Mimodes* clade: two trees included the topology shown in Fig. 1, whereas the third topology showed *Mimus gundlachii*+*M. saturninus* as a clade sister to the rest of *Mimus* and *Mimodes*. Monophyly of *Mimus* resulted in all 1000 bootstrap replicates. The node supporting *Mimodes* as sister to *Mimus polyglottos*+*M. gilvus* was well-supported, occurring in 95% of bootstrap replicate trees.

The hierarchical likelihood ratio test chose the following model $-\ln L = 245.8168$, substitution with all rates equal, base frequencies A = 0.4043, C = 0.1639,

Fig. 1. The results of maximum likelihood, parsimony, and Bayesian inference approaches to estimating a phylogeny from sequence data for the Mimidae. Conflicting nodes in results from Bayesian analyses are shown by dashed lines. Branch lengths are from maximum likelihood analysis. Numbers above lines are nodal support for Maximum likelihood and parsimony analyses (ML/P). Numbers below lines are 3 estimates of Bayesian posterior probabilities for nodes of interest for this study.



$G = 0.1654$, $T = 0.2665$, gamma distribution shape parameter = 1.6296, portion of invariable sites = 0. These parameters correspond to the F81+G model of evolution. The maximum likelihood analyses under the F81+G model recovered the same tree ($-\ln L = 6693.58$) as Fig. 1. Nodal support assessed via 100 bootstrap replicates indicated 100% support for monophyly of *Mimus*+*Mimodes*, and 93% support for placement of *Mimodes* as sister to *Mimus polyglottos*+*M. gilvus*.

Three Bayesian analyses initiated with random starting trees reached stationarity at approximately 5000 iterations of the Markov chains, and converged at similar likelihood values (plots not shown). The burn-in was removed before posterior probabilities and consensus trees were calculated, leaving 9951 data points for analysis. Mean likelihood values after burn-in were -6469.75 , -6469.70 , and -6469.63 . The three analyses resulted in the same topology (Fig. 1), varying only slightly in posterior probabilities. Of interest is the

support for monophyly of *Mimus*+*Mimodes* and *Mimodes*+*Mimus polyglottos*+*M. gilvus*: in all three analyses, posterior probabilities for these nodes were 1.0. The Bayesian analysis recovered the same overall topology as the parsimony and maximum likelihood analyses (Fig. 1), except for recovering a *Margarops fuscus*+*Ramphocinclus brachyurus* clade (dashed line in Fig. 1).

The Shimodaira-Hasegawa (1999) test rejected both alternative topologies (*Mimodes* basal to *Mimus*, $P < 0.03$ and *Mimodes* basal to *Toxostoma* $P < 0.001$).

Discussion

Under parsimony, maximum likelihood, and Bayesian approaches, the monophyly of *Mimus* including *Mimodes* was recovered with strong nodal support, 100% for parsimony and ML, and 1.00 Bayesian posterior

probability. Likewise, the sister relationship of *Mimodes* to *Mimus polyglottos*+*M. gilvus* was recovered in all analyses, with 1.00 posterior Bayesian probability and 93 and 95% nodal support under ML and parsimony, respectively. Hence, all three phylogenetic approaches placed *Mimodes* in a clade containing four representatives of *Mimus*. Retention of the monotypic *Mimodes* (Ridgway 1907) is not supported by our data, as it would make *Mimus* paraphyletic. A more appropriate generic placement for the Socorro mockingbird would thus be within *Mimus*.

Traditional systematics often rely on phenotypic characters for determining phylogenetic relationships and taxonomy. However, many of these characters are under selection and therefore can be inappropriate or misleading. Island forms may experience more rapid evolution via heightened genetic drift or natural selection as a consequence of small population sizes, absence of interspecific competition, exploitation of novel niche dimensions, and other evolutionary opportunities opened by insular ecosystems (e.g. Carlquist 1965, 1974, Lack 1976). These scenarios have been postulated to have led to the diversification and specialization of groups such as Darwin's finches, Hawaiian honeycreepers and birds of paradise, and might explain why many island species are frequently challenging taxonomically.

Mimodes graysoni exhibits morphological features similar to those observed in other island forms, such as longer tarsi and bill, and greater overall body size (e.g. Carlquist 1974, Grant 1968). It also exhibits dull plumage, as has been documented in other insular forms (Grant 1965). These morphological changes during the species' evolution on Socorro Island yielded the present form, which is intermediate in appearance between *Mimus* and *Toxostoma*, and which caused years of confusion regarding the systematic placement of the species. Neutral molecular markers provide a powerful tool for understanding phylogenetic relationships of this species with its mainland relatives. The phylogenetic hypothesis presented in this paper provides a framework in which the morphological evolution of this unique form, and island forms in general, can be studied (Harvey and Pagel 1991).

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