

TINAMOU (TINAMIDAE) SYSTEMATICS: A PRELIMINARY COMBINED ANALYSIS OF MORPHOLOGY AND MOLECULES

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Resumen. – Sistemática de tinámidos (Tinamidae): un análisis combinado preliminar de morfología y moléculas. – El interés en la evolución de las perdices (Palaeognathiformes: Tinamidae) deriva de que son consideradas primitivas entre las aves actuales. Pese a esto, el conocimiento acerca de la filogenia de este grupo Neotropical es muy escaso. Dos nuevos estudios produjeron filogenias de la familia usando morfología externa e interna (237 caracteres) y secuencias de ADN mitocondrial (1143 pb) y nuclear (1145 pb), respectivamente. Las dos hipótesis presentan algunas similitudes pero también varias incongruencias: ambas apoyan la monofilia del grupo de las perdices de áreas abiertas pero son incongruentes en la monofilia de las perdices de selva y las relaciones filogenéticas dentro de estos dos grupos. Aquí presentamos un resumen de los dos estudios anteriores y los datos moleculares y morfológicos en un análisis combinado. El análisis bajo pesos implicados divide la familia en dos grupos monofiléticos, uno compuesto por las especies de selva y el otro por las especies de áreas abiertas. Estos resultados son también una de las dos hipótesis alternativas obtenidas en los análisis con pesos iguales. Además, *Eudromia* y *Tinamotis* ocupan una posición basal dentro de las perdices de áreas abiertas, y todos los géneros tradicionales son redelimitados como monofiléticos.

Abstract. – Interest in the evolution of the tinamous (Palaeognathiformes: Tinamidae) derives from the fact that they are primitive among extant birds. In spite of this, very little is known about the phylogeny of this Neotropical group. Two new studies produced phylogenies for the family using external and internal morphology (237 characters) and mitochondrial (1143 bp) and nuclear (1145 bp) DNA sequences, respectively. The two hypotheses had some similarities but also several disagreements: both recovered a clade of aridland tinamous but they disagreed on the monophyly of the forest-dwelling tinamous, as well as on the relationships within these two groups. Here we summarize the previous two studies and combine the morphological and molecular dataset into a single analysis. In the weighted combined hypothesis, the family is divided into two monophyletic groups, one composed of forest taxa, and one of aridland taxa. This is also one of the two alternative hypotheses recovered in unweighted analyses. The combined tree also places *Eudromia* and *Tinamotis* as basal within the aridland tinamous, and recovers all traditional genera as monophyletic. *Accepted 29 December 2003.*

Key words: Tinamidae, tinamous, systematics, molecular, morphology, combined analysis.

INTRODUCTION

Ratites and tinamous (Infraclass Palaeognathae) together are the sister group to all other living birds (Cracraft & Clarke 2001,

Groth & Barrowclough 1999). The family Tinamidae comprises 47 described species that have been traditionally classified into nine genera (Blake 1977, Sibley & Monroe 1990), but remarkably few systematic studies

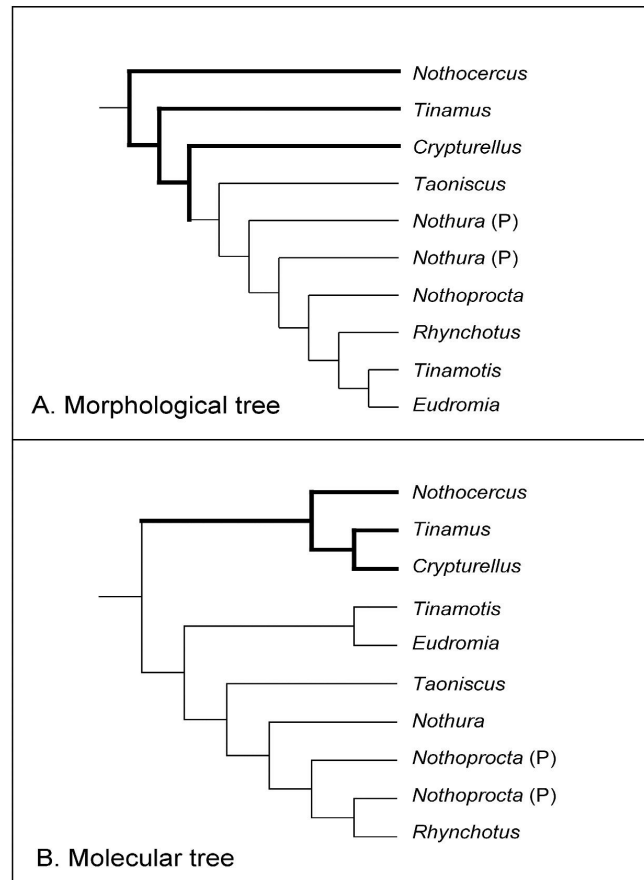


FIG. 1. A) Intergeneric relationships obtained in a previous cladistic analysis of morphological data (modified from Bertelli 2002). B) Intergeneric relationships obtained in previous maximum parsimony and maximum likelihood analyses of morphological data (modified from Porzecanski 2003).

have evaluated their generic monophyly and interrelationships. Building on the work of Salvadori (1895) and von Boetticher (1934), Miranda-Ribeiro (1938) divided the tinamou species into two general categories, and proposed a subfamily for each of these: one harboring the forest tinamous, Tinaminae, and the other composed of open-area or aridland tinamous, Nothurinae. Subsequent studies focused on subsets of the genera and particular aspects of the morphology (Jehl 1971, Hudson *et al.* 1972, Elzanowski 1982), but it

was not until very recently that comprehensive and modern phylogenetic analyses of the family were performed.

Bertelli (2002) and Bertelli *et al.* (2002) presented cladistic analyses of the family based on morphological data (see also Bertelli 2004). The most comprehensive database included characters of several types: osteological (108 characters), integumentary (80), myological (40), and behavioral/reproductive (11) (Bertelli 2002). The resulting morphological hypothesis does not recover a monophyl-

etic assemblage of forest tinamous (Fig. 1A). The forest genera are placed at the base of the tree and are related sequentially to a more derived, and monophyletic, assemblage of aridland tinamous. All genera are recovered as monophyletic except *Nothura*. Finally, *Eudromia* and *Tinamotis* are sister groups, and form the most derived clade within the aridland tinamous (Fig. 1A).

Simultaneously, Porzecanski (2003) carried out a molecular study of the family, using mitochondrial cytochrome *b* and nuclear RAG-2 gene sequences. The mitochondrial and nuclear trees conflicted with respect to the basal relationships within the family, but basal nodes lacked significant support in both cases. The trees most congruent with other sources of data, such as plumage characters, resulted from analyses of the nuclear sequences alone, as well as of both loci together. Unlike the morphological analyses, those hypotheses divided the family into two main clades corresponding to the aridland and forest tinamous (Fig. 1B). In addition, all molecular trees recovered *Nothura* as a monophyletic genus, but *Nothoprocta* as paraphyletic. Finally, *Eudromia* and *Tinamotis* formed a clade, but this clade was placed in a basal position within the aridland tinamous.

Given the multiple areas of conflict among the morphological and molecular tinamou phylogenies, it was our aim to explore the results of a combined, total-evidence analysis.

METHODS

We combined all of the existing morphological and molecular data into a single matrix. This matrix was composed of 58 terminal taxa and 2525 characters. Taxa included all described species of Tinamidae (with subspecies in some cases) and what appears to be a new form of *Nothura* (Bertelli *et al.* in prep.). In addition, six ratite genera were used as out-

groups, and a basal neognath, *Megapodius*, as the root of the tree. When a particular type of character was not available for a given species those characters were coded as missing. All genera, however, had a least one species for which all types of characters were available. The matrix was analyzed using the program SPA (Goloboff 1996) given that it allows the use of cost matrices for complex morphological character state transformations (generalized Sankoff parsimony). For comparative purposes, we carried out an equal weight analysis using NONA (Goloboff 1993a), and an analysis under implied weights was carried out using Pee-wee (Goloboff 1993b). In all cases, searches were performed using 20 random addition sequences with 20 TBR-ratchet iterations each, for a total of 400 iterations. Branch support was estimated by calculating absolute and relative Bremer indices using the program TNT (Goloboff *et al.* 2000).

RESULTS

NONA and SPA results were incompatible only with respect to three nodes, all internal nodes with the genus *Nothoprocta*; the NONA tree is shown here with the conflicting nodes marked by asterisks (Fig. 2). This combined hypothesis agrees with the molecular tree and the morphological tree that aridland tinamous form a monophyletic group. The relationships among the forest tinamous, however, are not resolved: the genus *Nothocercus* is placed as basal to all other tinamous in some optimal trees, but the forest tinamous are monophyletic in other optimal trees.

The combined tree also agrees with the molecular hypothesis in placing *Eudromia* and *Tinamotis* as basal within the aridland tinamous. In contrast, and in agreement with the morphological results, the combined tree recovers *Nothura* as paraphyletic and *Nothoprocta* as monophyletic. Of the internal nodes in the tree, 74% are relatively well-supported

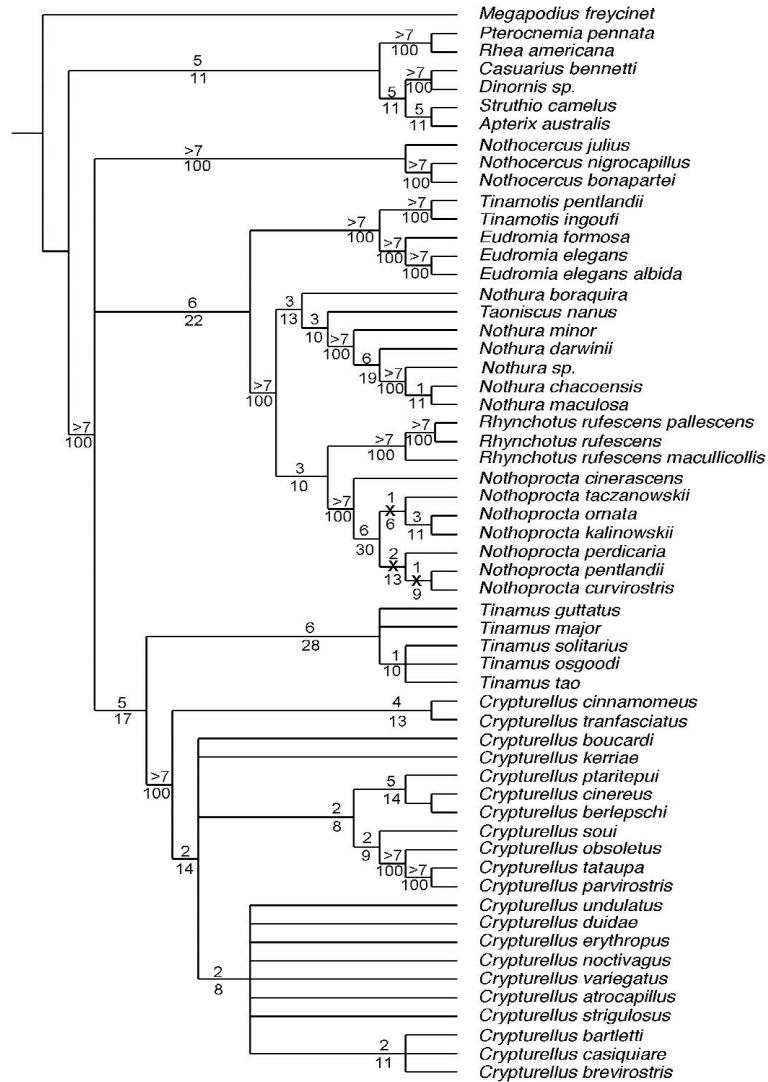


FIG. 2. Strict consensus tree of the 44 most parsimonious trees obtained under equal weights analysis, length = 979 steps, CI = 0.29. Values above and below branches show the absolute and relative Bremer support values (see Methods). The branches that are not congruent with the consensus obtained under Sankoff parsimony analysis are marked with a “x”.

(absolute Bremer support ≥ 3), and 42% of the nodes have relative Bremer support ≥ 100 . Except for *Nothura*, which is paraphyletic, and for *Tinamus*, the monophyly of all remaining polytypic genera is well-supported.

When homoplastic characters are down-weighted (i.e., in the Pee-Wee analysis), the family is divided into two groups: a forest clade and an aridland clade (Fig. 3). In these weighted analyses a new arrangement within

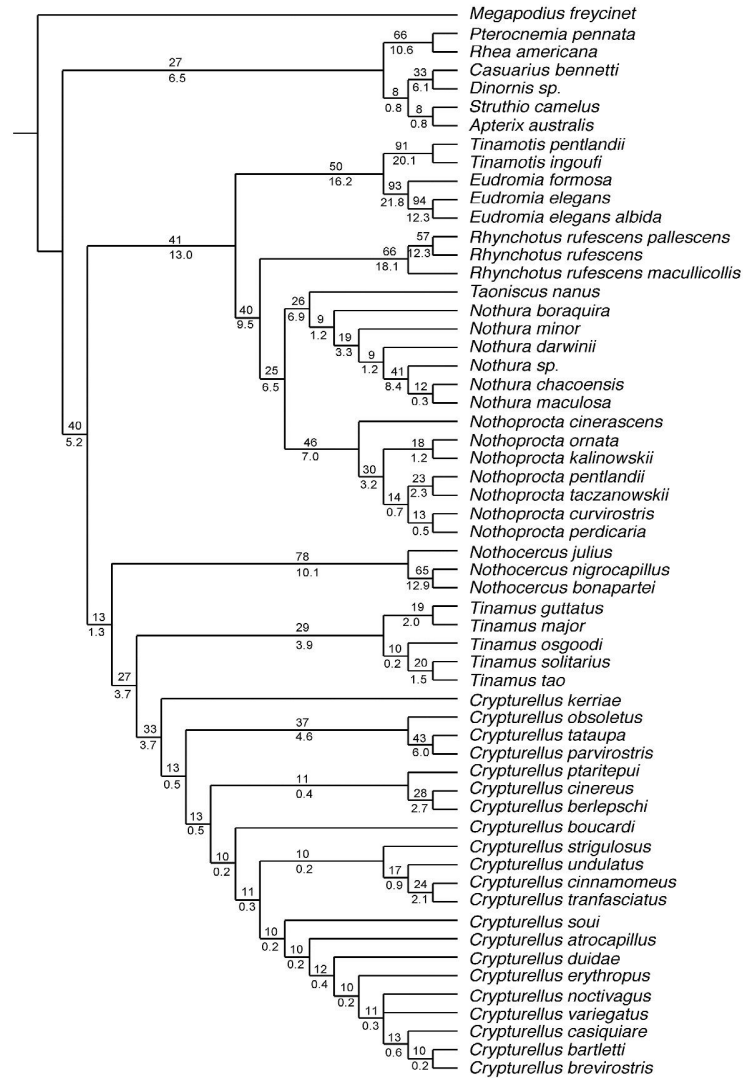


FIG. 3. Single most optimal tree obtained under implied weight analysis. Support indicated as in Figure 2.

the aridland tinamous is recovered (see Fig. 3). In this case, *Rhynchotus* is the sister group to a clade composed of *Taoniscus*, *Nothura*, and *Nothoprocta*.

DISCUSSION

The low level of conflict found among the

analyses with and without step-matrices is an indication that the interaction among the characters is maintained regardless of whether some of the character-state transformations are penalized or not. These unweighted analyses result in two alternative hypotheses that conflict with respect to the basal relationships in the family. The weighted analysis resolves

this conflict in favor of the least homoplastic characters, which support one of the two alternatives: a division of the family into a forest clade and an aridland clade. These preliminary results lend support to Miranda-Ribeiro's (1938) division of the family into two subfamilies and would suggest an early divergence between forest and aridland taxa, followed by little plasticity in broad habitat preferences over the rest of the group's evolution. In addition, all of the traditional genera are recovered as monophyletic in the weighted hypothesis.

Cytochrome *b* data are considered to be saturated at the distances found among tinamous (Porzecanski 2003). Nevertheless, we find that they provide a similar number of synapomorphies in the basal nodes as the morphology. Thus, all types of characters are contributing evidence at the different hierarchical levels of the tree. Nodal support varies throughout the trees, with areas of conflict among the different types of characters visible as areas of particularly low branch support. These areas of the tree will require further study, preferably with expanded molecular and morphological sampling.

The molecular data were previously used (Porzecanski 2003) to shed light on the timing of diversification within the family. Given that some of the same nodes are recovered in the combined weighted analysis, those estimates apply here as well. Based on alternative calibrations of the nuclear distances, the earliest divergences within Tinamidae are postulated to have occurred in the early Eocene, while most of the subsequent diversification within the family is estimated to have occurred between the Oligocene and Miocene.

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