Phylogenetic Relationships Within the Class Mammalia: A Study Using Mitochondrial 12S RNA Sequences

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The interrelationships of the three mammalian groups, Monotremata, Marsupialia, and Eutheria, have been studied using DNA sequences from the mitochondrial 12S ribosomal RNA gene. The results suggest that the monotremes diverged from the living therians only shortly before eutherians and marsupials separated from each other, although there is some evidence for a slowdown in rate of base change in the monotreme lineage. Within the Monotremata, the two extant species of tachyglossids show a very close genetic relationship and the data suggest a very recent divergence. We have also confirmed that the Patagonian Monito del Monte, Dromiciops australis, is more closely related to the australidephian marsupials than it is to other South American species.

KEY WORDS: 12S rRNA; marsupials; molecular evolution; monotremes; phylogeny.

INTRODUCTION

The Monotremata, today represented by only three species in two families, is a Gondwanan group of animals now found only in Australia and New Guinea, though a recent fossil from South America (Pascual et al., 1992) shows that they were previously more widespread. The poor fossil record and complete loss of adult teeth in extant species have long been a major problem in our understanding not only of the precise taxonomic relationship of the two monotreme families (Ornithorhynchidae and Tachyglossidae) to one another, but also of their relationship to the other two extant mammal groups, the Marsupialia and Eutheria, which together constitute the subclass Theria (see Pascual et al., 1992; Archer et al., 1992).

In most taxonomic works the platypus (Ornithorhynchus anatinus) and echidnas (Tachyglossus aculeatus and Zaglossus bruijni) are regarded as being very distinct mammals and are placed in a separate subclass, Prototheria. The Prototheria are said to have diverged from therian mammals at some time in the Mesozoic, well before the marsupials and eutherians diverged from one another in early Cretaceous times (see Clemens, 1989). Increasingly, this scenario is being questioned and the suggestion made that the

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Monotremata is a close sister group to the living Theria (see Rowe, 1988; Kemp, 1988; Clemens, 1989; Czelusniak et al., 1990; Wible, 1991; Westerman and Edwards, 1992). It would thus seem less and less possible to accept a simple dichotomy of early mammals into therians and nontherians (Kemp, 1988), although this is still a favored hypothesis for some (see Archer et al., 1993).

A number of molecular studies have focused on the relationships of platypus and echidnas both to one another and to other mammals. These have included genetic, biochemical, and DNA–DNA hybridization studies (for references see Westerman and Edwards, 1992), although few of them have included both living species of echidna. All have pointed to a very early divergence between the Ornithorhynchidae and the Tachyglossidae, with the former family probably more closely approximating the form of the common ancestor (see also Pascual et al., 1992; Archer et al., 1992). The DNA–DNA hybridization studies of Westerman and Edwards (1992) suggested that the initial divergence between the two monotreme families took place very early (late Cretaceous–early Tertiary) but that the divergence between the two living tachyglossids (Tachyglossus aculeatus and Zaglossus bruijni) may be a very recent event.

In the present study we have sought to use sequence data from the mitochondrial 12S RNA gene to investigate further the phylogenetic relationships of the three extant mammal groups Monotremata, Marsupialia, and Eutheria, as well as to confirm (or otherwise) the recent divergence of the two extant echidnas. The 12S RNA gene was chosen for this analysis, as it has previously proven useful over a broad taxonomic range (see Kocher et al., 1989; Ballard et al., 1992). Although our primary objective was to understand better the phylogenetic relationships of the three groups of living mammals, there continues to be a good deal of controversy as to the precise phylogenetic relationships within the Marsupialia. While most workers now accept two major groupings, the cohorts Australidelphia and Ameridelphia (Aplin and Archer, 1987; Marshall, 1988), there is still a good deal of discussion over the placement of the South American species Dromiciops australis, the only living microbiotheriid. On the basis of tarsal morphology Szalay (1982) suggested the placement of Dromiciops with Australian marsupials in the cohort Australidelphia. The weight of evidence, including DNA–DNA hybridization data (Kirsch et al., 1991; Westerman and Edwards, 1991), microcomplement fixation data [Sarich (personal communication) cited by Aplin and Archer (1987) and Marshall et al. (1990)], and sperm morphology (Temple-Smith, 1987), favors this association, though reinterpretations of the tarsal data do not (Hershkovitz, 1992). By including a number of marsupial species in our study, we hoped to be able to resolve the relationship of the family Microbiotheriidae vis-à-vis other marsupials using DNA sequence data.

**MATERIALS AND METHODS**

**Tissues and Sequences**

Tissues for DNA extraction were obtained from the following sources: *Ornithorhynchus anatinus* liver (Dr. Ian McDonald, University of Melbourne), *Tachyglossus aculeatus* liver (Dr. Peggy Rismiller, University of Adelaide), *Zaglossus bruijni* muscle (Dr. Tim Flannery, Australian Museum, Sydney), *Sminthopsis crassicaudata* liver (Dr. Ian McDonald, University of Melbourne).