

# Bayesian analysis of ruminants using molecular and morphological data

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## Summary

Ruminants, a large group of terrestrial mammals with a diverse fossil record, are a difficult group to deal with for estimating a phylogeny. A simultaneous Bayesian analysis of DNA sequence data and morphological characters is the best way of incorporating as much information as possible to obtain a well supported tree. Molecular characters have influence on morphological characters, as can be seen in the placement of † *Triceromeryx pachecoi* close to the cervids in the ruminant tree. In cases where there is no signal in the molecular data, morphology can help to optimize the tree. This is the case in the analysis of Antilopinae, where a newly found skull character establishes the monophyly of parts of the “Neotragini”.

## Introduction

Ruminants are a fascinating group of large herbivores. They can be found in many habitats ranging from deserts to rainforests to tundra, and have conquered almost every continent. Their most striking features are the various cranial appendages: ossicones in giraffes, horns in bovids, antlers in cervids and forked deciduous horns in the North American pronghorn. Their diverse ecology and behaviour has made them a popular subject for evolutionary biologists and palaeontologists. Despite the fact that ruminants have been studied for a very long time, there is still no consensus on how the different subgroups of “higher” ruminants (giraffids, antilocaprids, moschids, cervids, and bovids) are related to each other, neither from morphological nor from molecular analyses (see Hernández Fernández & Vrba 2005).

Problems for ruminant phylogeny:

- The higher ruminants (= Pecora) radiated fairly quickly in the Early Miocene, leaving little time to accumulate mutations between the speciation events that gave rise to the five extant groups.
- Ruminant fossils mostly consist of teeth and postcranial elements. Living groups are defined by their cranial appendages --> difficult to place fossils
- Tooth evolution has followed similar trends in different lineages (Janis & Scott 1987), leading some to argue that dental characters are not very reliable in a phylogenetic analysis (Naylor & Adams 2001).
- a high amount of convergent evolution in living cervids and bovids

This study aims to combine all the available information from molecules and morphology into one simultaneous analysis. The sequence information will affect the character optimisation on the tree and thus influence the placement of the fossils as well (Asher *et al.* 2005). I include 20 living species from all six ruminant groups and the fossil palaeomerycid † *Triceromeryx pachecoi* from the Middle Miocene of Spain (Pickford & Morales 1994). It is known from several ossicones, mandible fragments and postcranial material (Crusafont 1952). Its outstanding feature is a bifurcated nuchal ossicone (Fig. 1). The palaeomerycids are thought to be either related to cervids, as they have a closed metatarsal gully, or to giraffids on the basis of their ossicones. This combined analysis favours the first concept.

Future analyses will follow a hierarchical approach to minimise the effect of convergent evolution in the living species:

- separate analyses of monophyletic subclades of bovids and cervids
- reconstruction of the morphology of their most recent common ancestor
- using these ancestors as terminal taxa together with the remaining living species and the fossils in the final analysis

At the moment, only the tree of Antilopinae can be presented. It shows that the neotragine (dwarf antelope) species Oribi, Beira, Steenbok and Dikdik are not paraphyletic (Gentry 1992, Matthee & Davis 2001) but monophyletic and form the sistergroup of Antilopini (gazelles and allies), based on a newly found morphological character, an additional suture in the squamosal bone above the ear (Fig. 2).

## Material and Methods

Molecular data:

- analysis of Ruminantia: two mitochondrial (12S rRNA, 16S rRNA) and four nuclear (Kappa casein exon 4, Protein kinase C iota, Beta spectrin non-erythrocytic, Lactoferrin promoter) genes (4399 characters)
- analysis of Antilopinae: four mitochondrial (cytochrome b, cytochrome oxidase III, 12S rRNA, 16S rRNA) and three nuclear (Kappa casein exon 4, Protein kinase C iota, Beta spectrin non-erythrocytic) genes (5445 characters)
- aligned manually using Mesquite (Maddison and Maddison 2009), ambiguously aligned parts excluded, indels coded as absent/present
- separate single gene analyses and simultaneous analysis using MrBayes (Ronquist & Huelsenbeck 2003)
- test for best partitioning scheme using Bayes factors
- trees are displayed using dendroscope (Huson *et al.* 2007) and rooted with *Tragulus* (or *Tragelaphus*)

MrBayes settings

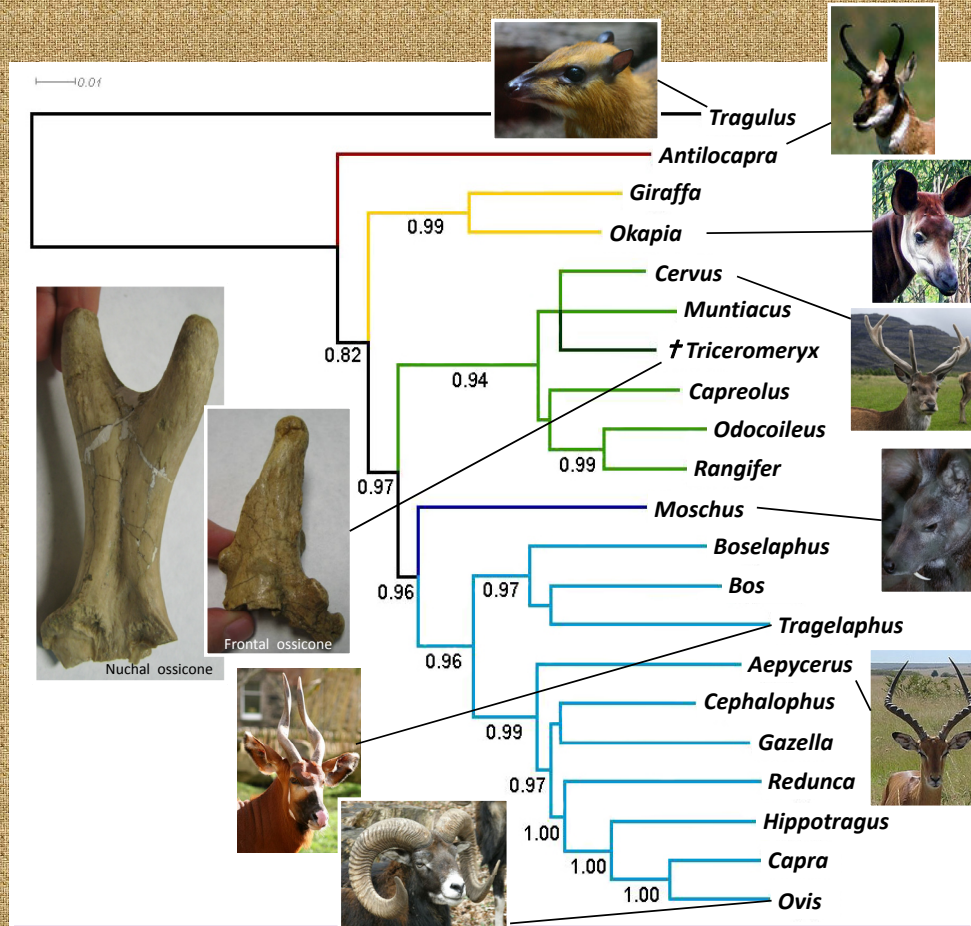
- data partitions according to codon positions and noncoding regions, rRNA and indels (standard data)
- separate GTR+I model for every DNA partition
- two independent runs with one cold and three heated chains each, terminated when split-frequency value dropped under 0.01
- burn-in fraction set to 25%

Morphology:

Ruminantia: informative characters for ruminants taken from O’Leary & Gatesy (2008). The matrix was completed whenever possible from own observations of museum specimens and scored for *Okapia*, *Muntiacus*, *Sylvicapra*, *Nanger* and † *Triceromeryx*.

Antilopinae: selected characters from Gentry (1992), Grooves (2000), Vrba & Schaller (2000), and own observations (98 characters).

The morphological data sets were added to the molecular data as a separate partition of standard data.

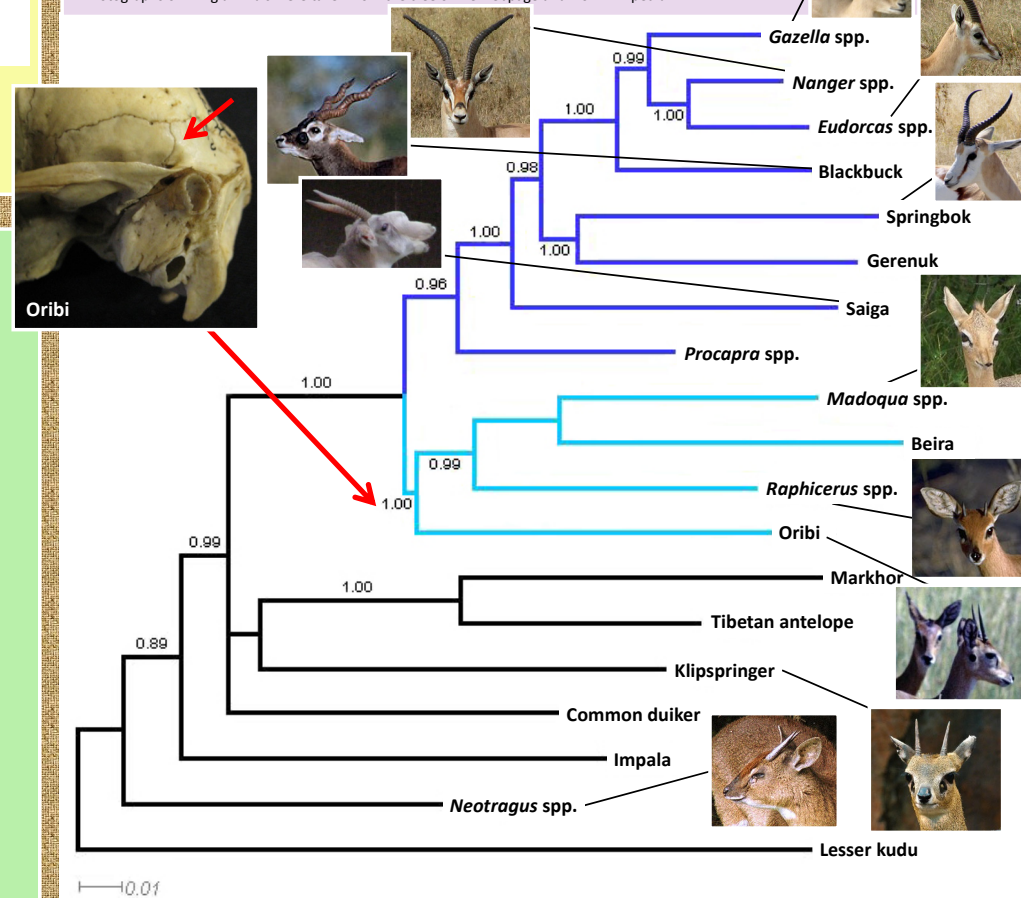


## Legend

Fig. 1 (above) Ruminant tree from simultaneous Bayesian analysis of molecular and morphological data.

Fig. 2 (below) Tree of Antilopinae from simultaneous Bayesian analysis of molecular and morphological data. Dark blue lines indicate members of Antilopini, light blue lines members of Raphicerini. The latter were set as a constraint for the analysis based on a newly discovered apomorphic character (an additional suture in the squamosal bone, see red arrows).

Branch labels = posterior probabilities (percentage of sampled trees showing that branch). Only posterior probabilities higher than 0.8 are displayed. Photographs of living animals were taken from the tree of life webpage and from wikipedia.



## The Tree of Ruminantia

Although the topologies of the single gene analyses differed quite a lot, the combined molecular analysis of the six genes favours one tree with a clade containing cervids, moschids and bovids, the latter two being sister taxa (Fig. 1), as proposed by Hassanin & Douzery (2003). The posterior probabilities of the branches are very high for some clades, giving statistical support for the aforementioned relationships. The deepest node however is not statistically significant, although a topology with *Antilocapra* as sister taxon to the remaining Pecora is favoured.

Adding the morphology to the analysis didn’t change the topology and the branch support. † *Triceromeryx* is placed amongst the cervids, but this is due to the fact that only *Cervus*, *Muntiacus* and *Odocoileus* are scored for the morphological character set and a lot of information is missing for † *Triceromeryx*. If this interpretation of † *Triceromeryx* being the sister taxon of cervids is correct, it would imply that ossicones either arose twice (in palaeomerycids and giraffes) or that they are homologous in the two groups and were transformed into other types of cranial appendages (antlers and horns) in the course of evolution of Pecora.

## The Tree of Antilopinae

Antilopini are a monophyletic group of bovids that consists of gazelles (*Gazella* spp., *Eudorcas* spp., *Nanger* spp.), the Indian blackbuck, the Springbok, the Gerenuk, the Saiga, and the Tibetan and Mongolian gazelles (*Procprapa* spp.). The “Neotragini” or “dwarf antelopes” are considered to be a polyphylum of small species with short, straight horns. Two of the members, the Suni (*Neotragus*) and the Klipspringer, are rather distantly related to the other four genera: the Oribi, Beira, Steenboks and Grysbocks (*Raphicerus* spp.), and Dikdiks (*Madoqua* spp.). These species (Kuznetsova & Kholodova (2003) proposed the name Raphicerini although they could not show that they are monophyletic) were often thought to form a paraphylum at the base of Antilopini (e.g., Gentry 1992, Matthee & Davis 2001). Others found the Oribi (Gatesy *et al.* 1997) or the Dikdiks (Kuznetsova & Kholodova 2002) to be nested within Antilopini.

This is the most complete study of the phylogenetic relationships of Antilopini and “Neotragini”, together referred to as Antilopinae (29 ingroup species plus 6 outgroup representatives). For the combined analysis I used molecular markers and morphological characters (see methods), and chromosome rearrangement data for gazelles (Vassart *et al.* 1995, 60 characters). The results show that the Raphicerini are monophyletic and form the sistergroup of Antilopini, based on a newly found morphological character of Raphicerini (Fig. 2). Gazelles are monophyletic. Although there was no sequence data available for three of the species (Beira, Salt’s dikdik, and *Neotragus pygmaeus*), they can be confidently placed in the tree.

## Discussion

Combining morphological data with molecular data in a simultaneous analysis is highly beneficial for phylogenetic reconstruction. It drastically increases the number of characters and therefore the statistical support of the tree. It also increases the number of taxa that can be analysed together, helping to understand the evolution of the groups. Missing data *per se* does not have a negative impact.

By using a hierarchical approach, different character sets can be applied at different analytical levels. This is important for fast evolving genes like cytochrome b, that was very useful in resolving antelope phylogeny, but completely useless in ruminant phylogeny.

In future analyses I want to incorporate more DNA sequences as well as score the morphological characters for many more species, living and extinct. My aim is to obtain a well resolved tree that makes it possible to retrace the evolution of the striking ruminant head ornamentation and the behaviour that is associated with it.

## Literature

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