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Assembling the ruminant tree: combining morphology, molecules, extant taxa, and fossils

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Abstract

A gap exists between paleontological and neontological approaches to ruminant phylogenetics, despite great increases in phylogenetic resolution through molecular work of the last three decades, and a large and growing fossil record. This gap is reflected in differing methodological approaches, with insufficient integration of the large fossil record by molecular studies on the one hand, and insufficient consideration of highly resolved genomic work by paleontological studies on the other. Both paleontological and molecular approaches seek to answer similar broad evolutionary questions, and a synthetic approach is in the interest of all. I demonstrate this by reviewing the development of each field, noting many examples in which paleontological or molecular approaches to ruminant phylogenetics are, on their own, inadequate compared to an approach which considers all sources of data together. In particular, cases such as those of *Bison, Capra,* and *Pelea* have shown that integration of genomic and anatomical data presents better resolution of relationships, and I suggest *Antilocapra* and *Moschus* may benefit from a similar approach, especially with the integration of fossil taxa into a combined (supermatrix) analysis. I present preliminary results of a new and large (in progress) morphological matrix that is intended to be used for the incorporation of anatomical data and fossil taxa into a combined analysis. The new matrix is much larger than previous morphological matrices assembled for ruminant phylogenetics, meaning it can support a larger number of fossil taxa than was previously possible. Preliminary analysis with 18 taxa recovers a highly supported tree that is mostly compatible with both traditional and molecular phylogenies, although problems of convergence remain, such as between *Antilocapra* and Bovidae. Finally, I propose standardization of ruminant clade names in order to limit miscommunication between paleontological and neontological workers. I propose phylogenetic definitions based on crown (extant) clades for the names Ruminantia and Pecora, and the use of Pan-Ruminantia and Pan-Pecora to accommodate each respective crown clade plus its stem group.

Key words: Ruminantia, phylogeny, total evidence, Bovidae.

1. Introduction

In 1968, Alan Gentry published a letter in Nature challenging the constancy of modern biogeographic boundaries into the deep past based on a study of the bovid fossil record. A few years later, Elisabeth Vrba published two papers presenting the chronology and ecology of important hominid sites South Africa, also through the bovid fossil record, and also in the pages of Nature (Vrba 1974; Vrba 1975). Though studies and descriptions of ruminants both living and fossil already had an established scientific history (e.g. Frick 1937; Lydekker 1898; Pilgrim 1947), these may have been the first and clearest demonstrations, in such a widely read journal, of the power of a diverse and widespread clade of ruminants to directly address and resolve large-scale questions of evolution, biogeography, and ecology.

Early molecular papers of the 1980s and 1990s would regularly cite these and other ecological or paleontological papers. Today, however, it seems that many molecular investigations into ruminant phylogenetics make little to no reference to the fossil record, and paleontological studies on ruminants too often make no mention whatsoever (whether to support or challenge) of the results of the many molecular phylogenetic advances of the last two decades. In that vein, the first International Conference on Ruminant Phylogenetics was held in Munich in September 2013, bringing together over fifty specialists from around the world with diverse interests in the anatomy, physiology, ecology, biogeography, phylogenetics, ontogeny, behavior,

genomics, and fossil record of ruminant species (Rössner 2013). This conference was timely, given the massive increases in phylogenetic information of the last two decades, and the need to develop an integrative approach to ruminant phylogenetics.

The goal of the current paper is to present a summary of accomplishments to date in ruminant phylogenetic work and to present some preliminary data from ongoing work. This includes outlooks for future progress, especially for combining data from extant taxa and molecular work with that from fossil taxa and morphological studies. I will also present preliminary results of a new large skeletal morphological dataset (matrix) intended for use in combined (supermatrix) phylogenetic analyses. Bovids make up some two-thirds of all ruminants, so the focus of much of the work reviewed here is based on this clade.

2.1 Molecular Phylogenetics

Domestic animals, especially goats, sheep, and cattle, form an essential economic and dietary component of human societies around the world. As a result, studies into the biology of these animals are common, from anatomical and veterinary works of centuries past, to physiological and histological investigations of the twentieth century. With the discovery of nuclear cell structures, chromosomal investigations in the 1960s and 1970s began to investigate phylogenetic relationships directly, giving some indication of the power of molecules to resolve relationships from the level of populations upwards to entire phyla (e.g. Buckland & Evans 1978; Wurster & Benirschke 1968). With the development of immunological distance techniques, Lowenstein (1986) constructed a molecular tree of Bovidae, substantiating a basal split of bovids into two major subclades. This supported (unknowingly it seems) a phylogenetic tree that Kingdon (1982) had proposed four years earlier on the basis of ecological and morphological features. In an early example of the molecular clock, Lowenstein also estimated the origins of crown Bovidae at around 25 Ma, a surprisingly good assessment for the time.

Mitochondrial DNA (mtDNA) sequencing threw open the doors for a genomic phylogenetic revolution that continues to this day. An early examination of the mtDNA of Bovini (Miyamoto et al. 1989) recovered initial molecular support for a deep split between Bovina (oxen: *Bos* spp.) and Bubalina (buffaloes: *Bubalus, Syncerus*), and the synonymy of *Bison* with *Bos* (supporting previous suggestions by van Gelder 1977, Gentry 1978, Groves 1981, and others). Broader mtDNA sampling soon established, much as Lowenstein had, the monophyly of Bovidae, its division into two major subclades, and the monophyly of most of its traditionally recognized tribes (Allard et al. 1992; Gatesy et al. 1992). Relationships among genera and species within tribes were, however, still not well resolved or supported in these early studies.

The floodgates opened in the mid to late 90s, with an explosion of molecular phylogenetic work on all aspects of life, tracking technological improvements allowing faster sequencing of ever longer portions of genomes from ever greater numbers of taxa. Ruminant phylogenetics, but especially that of bovids, greatly profited. Monophyly of Pecora, and of the five pecoran families (Antilocapridae, Bovidae, Cervidae, Giraffidae, Moschidae), was supported throughout (e.g. Cronin et al. 1996; Randi et al. 1998), though the exact relationships among these families continued to elude satisfactory resolution (and arguably still do). In fact, early on it was recognized that the poor resolution at the base of Pecora probably reflected rapid cladogenesis as a result of an adaptive radiation early in the clade's history (Hassanin & Douzery 2003; Kraus & Miyamoto 1991).

Within Cervidae, the monophyly of morphologically defined clades Cervinae (aka Plesiometacarpalia, Old World deer) and Capreolinae (aka Odocoileinae, Telemetacarpalia, or New World deer) was supported, while the antlerless water deer (*Hydropotes*) was found to be sister to roe deer (*Capreolus*) and close to Capreolinae, partly supporting the proposal of Bouvrain et al. (1989) and contradicting others based on morphological grounds (Cronin et al. 1996; Gilbert et al. 2006; Pitra et al. 2004; Randi et al. 1998; Randi et al. 2001). The molecular phylogenetic placement of *Antilocapra* as basal to all remaining pecorans (Cronin et al. 1996; Hassanin & Douzery 2003) was also something of a surprise, rather than it being more closely related to cervids or bovids as had often been proposed (Gentry & Hooker 1988; Janis & Scott 1987).

Within Bovidae, we have seen confirmation of Kingdon's (1982) hypothesis of a basal split of this clade into Bovinae and Antilopinae (Gatesy et al. 1997; Gatesy et al. 1992; Hassanin & Douzery 1999b; Lowenstein 1986; Matthee & Robinson 1999); the polyphyletic status of 'Neotragini' and its inclusion into a larger Antilopini clade (Georgiadis et al. 1990; Rebholz & Harley 1999); a total reorganization of the clades within Caprini (formerly Caprinae) (Groves & Shields 1996; Hassanin et al. 1998; Ropiquet & Hassanin 2005); clarification of genus-level clades among Antilopini (*Nanger*, *Eudorcas, Gazella*, etc.) (Bärmann et al. 2013), Cephalophini (van Vuuren & Robinson 2001), Bovini (Hassanin et al. 2013; Miyamoto et al. 1989), Tragelaphini (Moodley et al. 2009; Willows-Munro et al. 2005), and Reduncini (Birungi & Arctander 2001; Cotterill 2005); placement of some of the most enigmatic taxa including the rhebok (*Pelea capreolus*), chiru (*Pantholops hodgsonii*), saiga (*Saiga tatarica*), impala (*Aepyceros melampus*), and the recently discovered saola (*Pseudoryx nghetinhensis*) (Decker et al. 2009; Gatesy & Arctander 2000; Hassanin & Douzery 1999a; Robinson et al. 2013); demystification of the linh duong *(Pseudonovibos spiralis*) (Hassanin et al. 2001); and sequencing of recently extinct species including the blue

antelope (*Hippotragus leucophaeus*) (Robinson et al. 1996) and the bizarre insular *Myotragus balearicus* (Lalueza-Fox et al. 2002). The impressive culmination of most of this work is exemplified by the recent analysis of the complete mtDNA genomes of most artiodactyl species by Hassanin et al. (2012).

Ruminants, making up about half of all artiodactyl species, have greatly benefitted from the phylogenomic revolution, and have been greatly restructured in terms of their phylogenetic relationships and taxonomy. There have been countless changes since the 1990s, both minor and major, to all parts and levels of the artiodactyl tree based on a better understanding of the evolutionary relationships among species. In some cases, molecular results have supported or helped choose among a number of hypotheses previously developed through anatomical data. In other cases, molecular results have proposed entirely new relationships or have discovered cryptic species. The effect of highly resolved molecular phylogenies on taxonomy is starkly illustrated by the recent ungulate taxonomy of Groves & Grubb (2011) which relies heavily on molecular phylogenies in order to greatly revise our understanding of the recognition and richness of extant ungulate species diversity. This is a commendable approach as it seeks to identify and diagnose monophyletic clades at all levels. However, the elevation by Groves & Grubb to the level of species of entities traditionally considered subspecies or populations has resulted in a vociferous debate, currently ongoing, on the numerous implications of recognizing either far too many (Heller et al. 2013; Zachos et al. 2013) or far too few (Cotterill et al. 2014) species. This debate, however, concerns disagreements on what defines a species, and not on the basic principle that systematics should be guided by the search for monophyletic clades at all levels.

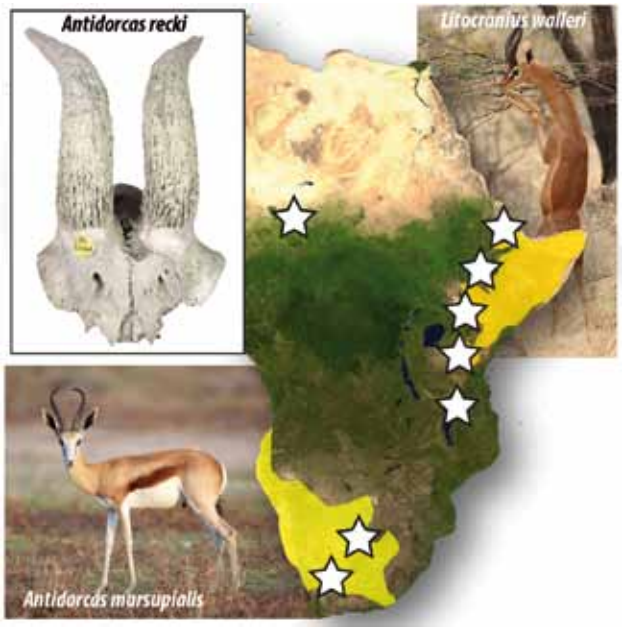

Figure 1: The living springbok (*Antidorcas marsupialis*, bottom left) and gerenuk (*Litocranius walleri,* top right) are sister taxa that reside in arid environments and have restricted and non-overlapping geographical distributions. Any attempt to reconstruct biogeographic and ecological patterns based only on the extant distribution would be incorrect, however, as fossil *Antidorcas* ranged widely across Africa during Pliocene and Pleistocene times (white stars; fossil is *Antidorcas recki* from Olduvai Gorge, Tanzania, specimen M 21462 in the Natural History Museum, London). (Image credits: springbok - Yathin S. Krishnappa, gerenuk - Steve Garvia, both from Wikimedia Commons.)

2.2 Molecular Phylogeography

The production of highly resolved molecular phylogenies has also opened doors to new and more powerful forms of evolutionary analysis. These include comparative anatomical approaches whereby investigation of structural and developmental homologies are guided by molecular phylogenies (e.g. Maier 1999; Maier 2013). Dated molecular phylogenies also produce fertile ground for testing evolutionary hypotheses of character evolution as well as speciation dynamics in deep time (Cantalapiedra et al. 2011; Cantalapiedra et al. 2014; Hernández Fernández & Vrba 2005).

Another byproduct of the highly resolved molecular phylogenies is a new and powerful phylogeographic approach for the reconstruction of species dispersal events as well as population dynamics. In the case of African ruminants, for example, phylogeographic studies on allopatric populations of impala, kudu, hartebeest, bushbuck, and giraffe, among others, have proposed, with high precision, the locations of several biodiversity refugia in western, eastern, and southern Africa (Arctander et al. 1999; Lorenzen et al. 2010; Lorenzen et al. 2012; Moodley & Bruford 2007; Nersting & Arctander 2001). The recognition of such major phylogeographic distinctions nicely showcases the degree of phylogenetic, geographic, and chronological resolution provided by molecular phylogenetics and the fertile ground it produces for both proposing and testing new evolutionary scenarios. Such phylogeographic reconstructions are also important because they reconstruct major events relevant to speciation and extinction dynamics that occur at the sub-specific level, and evidence for which might be impossible to spot independently in the morphological (including fossil) record. In the case of the bushbuck (*Tragelaphus scriptus*), for example, Moodley & Bruford (2007) and Moodley et al. (2009) mapped 23 different bushbuck haplotype groupings across 28 African biogeographic ecoregions, demonstrating the sheer diversity of this species, as well as the patterns of geographic and ecological relationships among widely dispersed populations.

Molecular phylogeography, however, complements but does not substitute for geographic range reconstruction based on the fossil record. I have previously (Bibi 2013) pointed out the case of the springbok clade *(Antidorcas* spp.*)*, restricted to southwestern Africa today but ranging widely across the

continent in the Pliocene and early Pleistocene. The fact that the living springbok *(Antidorcas marsupialis)* and the gerenuk *(Litocranius walleri)* are sister taxa on a molecular phylogenetic tree might lead to an interpretation of a long (late Miocene) separation of eastern and southwestern semi-arid regions. However, the widely distributed fossil record of *Antidorcas* spp. quickly discounts the idea of a long geographic isolation of springboks in southwestern Africa (Fig. 1). Or, for example, Reduncini, which are exclusively African today, but had significant occurrences in the Indian subcontinent in Miocene and Plio-Pleistocene times (Gentry et al. 2014; Vrba et al. in press). There is no shortage of other cases whereby past distributions of ruminant clades cannot be reconstructed from molecular evidence. The fossil record provides the most direct method to infer the biogeographic history of populations, species, and higher clades that are now totally extinct.

2.3 Molecular relationships needing further investigation

On the basis of mtDNA investigations, Moodley et al. (2009) discovered that bushbuck (*Tragelaphus scriptus*) is polyphyletic, and apparently comprised two distantly related tragelaphine species. From a morphological point of view, the fossil record indicates that the bushbuck's horn morphology is derived with respect to late Miocene and Pliocene tragelaphines, and is therefore unlikely to have evolved twice independently (Bibi 2009). The issue of bushbuck monophyly now awaits nuclear DNA (nuDNA) testing, as several cases have already been documented in which nuDNA agrees with morphological data, but contrasts with mtDNA, regarding the monophyly of clades such as *Bison* (Hassanin et al. 2013; Janecek et al. 1996; Verkaar et al. 2004) and *Capra* (Bibi et al. 2012; Pidancier et al. 2006; Ropiquet & Hassanin 2006). Such findings highlight the importance of phylogenies based on nuDNA, since mtDNA is inherited only maternally and is more strongly affected by introgression and homoplasy than is autosomal nuDNA (Funk & Omland 2003). Further nuDNA investigation of large parts of the ruminant tree will be crucial to help independently verify numerous relationships proposed on the basis of mtDNA data.

In yet other cases, the availability of both mtDNA and partial nuDNA datasets has still not provided sufficiently stable resolution of relationships. A recent example is a study by Robinson et al. (2013) on the relationship of *Pelea* to the Reduncini (*Kobus* + *Redunca*) and in which the inclusion of morphological data was necessary to produce a more credible phylogenetic placement of the rhebok as the sister taxon to the Reduncini. While we may certainly look forward to the increasing availability of whole

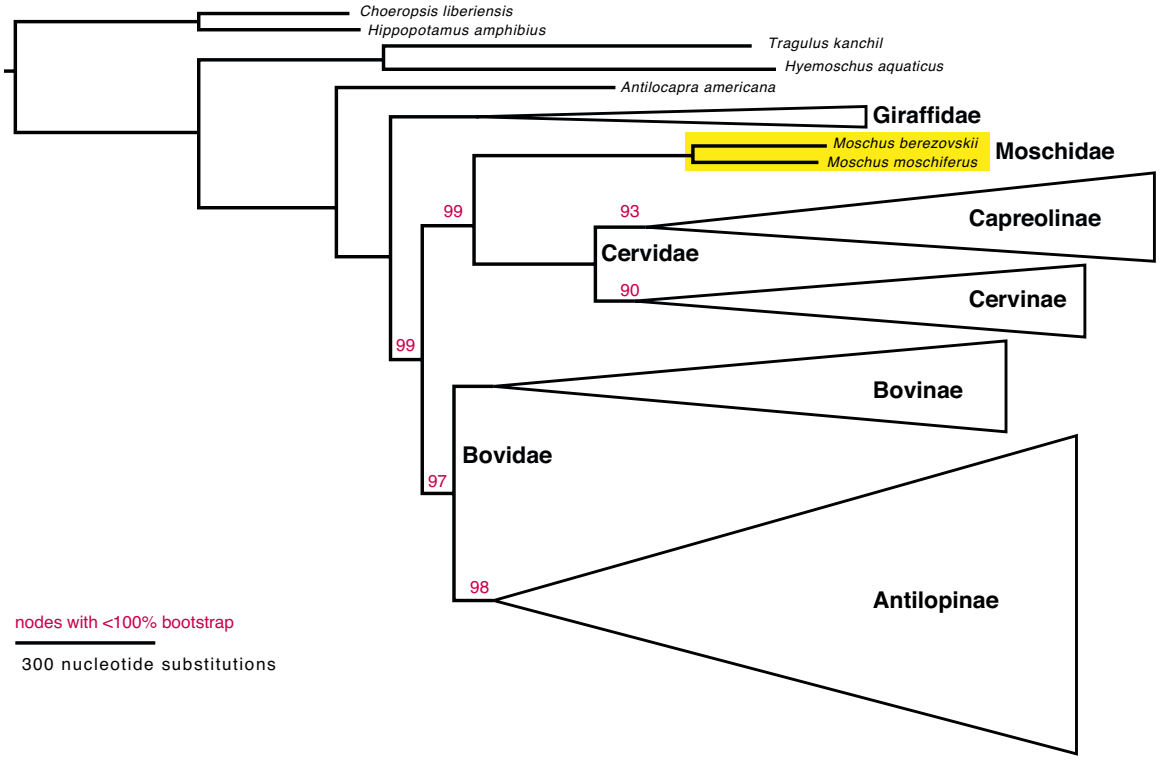

Figure 2: Consensus tree resulting from parsimony analysis of the complete mitochondrial genome of the ruminant subset (162 taxa) from the matrix of Hassanin et al. (2012). Bootstrap values for all nodes shown are 100% unless otherwise indicated. Relationships among and within families (here simplified) are not significantly different from the results of maximum likelihood or Bayesian analyses of the same data, with the exception of the placement of *Moschus* which emerges as the sister clade to Cervidae, rather than Bovidae. The reasons for this major topological switch are probably worth further investigation, particularly since morphological studies had previously determined a closer relationship of *Moschus* to Cervidae as well (Janis & Scott 1987).

genome sequences to provide further resolution of such issues, the fact is that morphological and fossil data are not being adequately incorporated into phylogenetic work on ruminants.

Two further examples where the addition of morphological (including fossil) datasets might help clarify relationships are those of the Moschidae and Antilocapridae. On the basis of morphological characters, Moschidae has been placed at the base of Pecora (Webb & Taylor 1980), close to Cervidae (Gentry & Hooker 1988; Janis & Scott 1987), or close to Bovidae (Sánchez et al. 2010). Hassanin and Douzery (2003) proposed a sister relationship of Moschidae to Bovidae, and while this has been replicated in numerous analyses, a close genomic relationship to cervids has not been totally discounted (Hassanin et al. 2012). A maximum parsimony analysis of the ruminant subset of Hassanin et al.'s (2012) complete mtDNA matrix produces a tree that is similar to these authors' Bayesian or maximum likelihood analyses, but with *Moschus* firmly allied with Cervidae (Fig. 2). Nicola Heckeberg (pers. comm.) has also found conflicting results in the placement of *Moschus* on individual mtDNA or nuclear DNA gene trees, and dos Reis et al.'s (2012) mammal-wide analysis placed *Moschus* spp. close to cervids. I have previously commented on the shifting positions of *Pelea capreolus* and *Pseudoryx nghetinhensis* on genomic phylogenies as a result of the use of different search parameters (Bibi 2013), and perhaps *Moschus* is a similar case. Could long branches leading to a very small number of surviving species be producing artifactual relationships? This probably warrants further investigation.

Likewise, Antilocapridae has, on the basis of morphological evidence, found to be close to either cervids or bovids (Gentry & Hooker 1988; Janis & Scott 1987). Instead, molecular phylogenies unanimously place *Antilocapra* as basal within Pecora, either alone (e.g. Hassanin et al. 2012) or as a sister taxon to Giraffidae (e.g. Bininda-Emonds et al. 2007; Meredith et al. 2011). As with Moschidae, renewed investigation of potential morphological synapomorphies (especially in the fossil record), as well as of the molecular characters most influencing the topology, is needed to make better sense of these results.

2.4 Molecular Age Estimation

The nature of genetic data (nucleotide bases) means phylogenetic distances between taxa can be simply quantified by counting the number of nucleotide differences, which is a much more objective process than attempting to quantify anatomical differences. Add to this some estimate of the rate of accumulation of nucleotide differences and you have a molecular 'clock' that permits dating phylogenetic divergences far back in time. As a result, molecular phylogenetics has since its early days produced estimates of evolutionary divergence events based on the amount of genetic difference between taxa.

Such molecular age estimations abound for ruminants, but their utility has often proved limited given a large range of published age estimates from different studies, and estimates that are themselves often bracketed by very large confidence intervals. In the end, molecular age estimation requires information from the fossil record for calibration, and it is clear that not enough information has been exploited from the large fossil record of Ruminantia. To explore this idea, I re-analyzed the ruminant subset of Hassanin et al.'s (2012) complete mtDNA matrix. While Hassanin et al. had used six fossil calibrations for the entirety of Artiodactyla, I came up with 16 calibrations for the ruminant subset alone. My re-analysis using a large number of new calibrations (Bibi 2013) produced younger age estimates for nodes across the ruminant tree and smaller confidence intervals for those node age estimates.

The younger ages produced by using a larger number of fossil calibrations are closer to estimates based on the fossil record alone. This is perhaps not surprising, as a larger number of fossil calibrations would be expected to constrain the age estimates towards ages determined by the fossil record. This does bring up the question, however, as to whether a large number of discrepancies between molecular and fossil dates in countless studies in the literature (typically with molecular ages being older than allowed by the fossil record) are a result of too few fossil calibrations being used to calibrate molecular rates of change across the tree in question. All analyses permit some degree of variation and uncertainty in rates of molecular evolution across a tree. Perhaps the use of dense fossil calibration with appropriate parameters is the safest and most direct way to address this uncertainty (Bibi 2013; dos Reis et al. 2012; Parham et al. 2012).

The second main result, smaller error bars, has the benefit of providing ages that are less ambiguous to interpret and test. For example, if Pecora is estimated to have arisen anytime between 35 and 20 Ma (95% interval from Hassanin et al. 2012), the environmental events during this time period (the entire Oligocene) are many and correlations are difficult to draw and test from the large fossil record. However, using more calibrations produced the smaller age range of 23–19.4 Ma (Bibi 2013), which can be related to a narrower window of environmental events, and therefore can more easily be tested by the fossil record.

Ultimately, then, molecular dating is reliant on information from the fossil record. Molecular data can provide masses of evolutionary information unattainable through the anatomical and fossil records, but it is unlikely to ever provide a single material example of a long extinct common ancestor or any of the many lineages of organisms that have gone totally extinct. Furthermore, while a molecular tree can provide numerous hypotheses of biogeographic chan-

ge, adaptive radiation, and changes in diversification rates, the fossil record provides a direct and independent test of all such scenarios. Fossil data are therefore crucially needed to: 1) calibrate molecular clocks for dating phylogenetic trees; 2) independently and directly generate and test phylogeographic and diversification scenarios; 3) discover clades of organisms which have no living descendants, and 4) help resolve ambiguous phylogenetic relationships through combined analyses.

3. Combined analyses

Clearly then, both molecular as well as organismal-level characters are needed to reconstruct the evolutionary history and organismal biology of Ruminantia. Each approach cannot afford to ignore the other, no less than a molecular study can ignore a certain genetic locus in favor of others, or a morphological study can ignore parts of the anatomy in favor of others.

In a phylogenetic analysis, there are many ways to combine such disparate datasets as nucleotide bases (A, C, T, G) and numerical codes (0, 1, 2…). A supertree is one such representation while a supermatrix is another. Scaffold approaches are a further option. Much has been made of these approaches in the literature, and each comes with philosophical assumptions as well as different methodological requirements, but a supermatrix approach has the advantage of including both types of data and using all data at once in an analysis. Bayesian phylogenetic methods can simultaneously analyze molecular and morphological data, while also taking into account any known node ages, and ages of any included fossil taxa (tip dating) to produce a tree that includes both fossil and living taxa and that is also dated. The small size of morphological to molecular datasets is not as big a problem as it may seem, as studies show that even a few morphological characters can influence a large molecular dataset, as shown with phylogenetic analyses of *Capra* (Bibi et al., 2012) and *Pelea* (Robinson et al. 2013). However, an ideal morphological matrix should have a large enough character list to be able to resolve relationships among a large number of ruminant species. Such a morphological dataset should also be able to resolve relationships well without the aid of molecular data.

The lack of such a large and equally powerful morphological matrix is a problem for ruminant phylogenetics. Compared to the number of molecular phylogenies being regularly produced, there are relatively few studies of ruminant phylogenetics from the perspective of organismal-level characters (skeletal morphology, soft tissue morphology, and behavior). The small number is not to be underestimated, however, as many of the functional, ecological, geographical, and morphological patterns of ruminant evolution since the Eocene were discovered on the basis of such studies well before the arrival of molecular investigation.

Anatomical and paleontological investigations of ruminants (especially bovids) were already in high gear by the late 19th and early 20th Century. Among these are the works of Lydekker (1878; 1898), Frick (1937), and Pilgrim (1911, 1939). With the arrival of cladistic methods, detailed analyses of character evolution and ruminant phylogenetics were undertaken by Webb & Taylor (1980), Janis & Lister (1985),

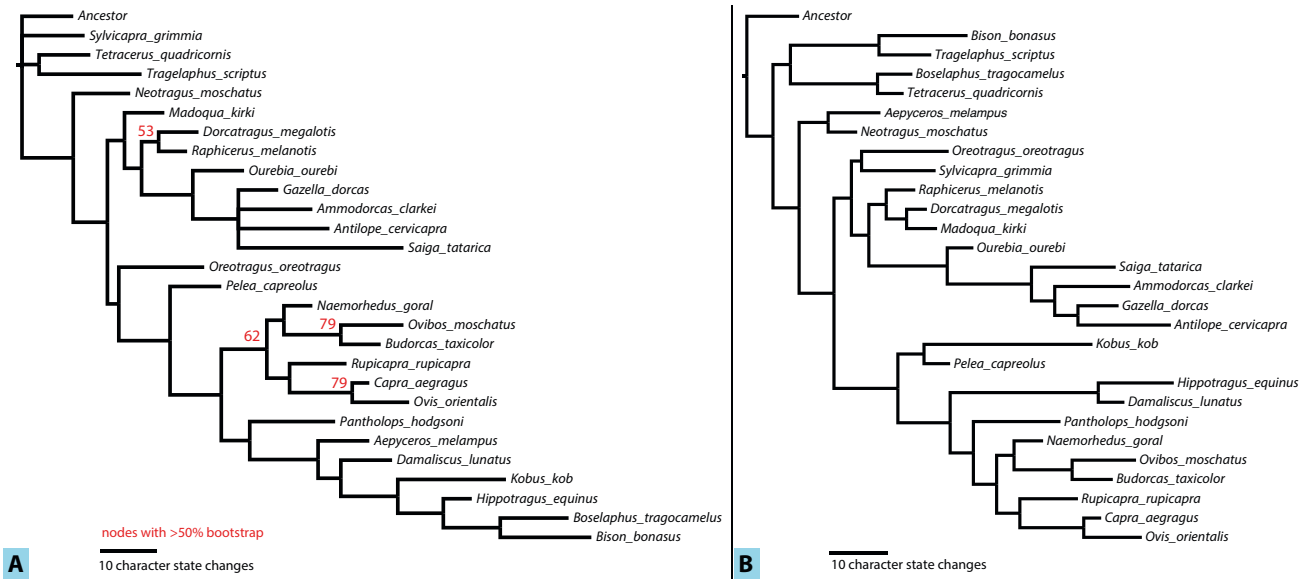

Figure 3: (a) Parsimony analysis of the morphological character matrix of Gentry (1992). The results are not significantly different from those presented in the original study. Branch lengths are proportional to the number of character state changes. Only four clades are found to have bootstrap support of greater than 50%. **(b)** A handmade modification of the cladogram in A to reflect currently accepted phylogenetic relationships (mainly as a result of molecular studies). Branch lengths are proportional to the number of character state changes as optimized using Gentry's matrix. This allows for morphological characters to be mapped onto a tree determined through other (molecular) means, and synapomorphies to be optimized despite high homoplasy in the dataset.

Janis & Scott (1987), Gentry & Hooker (1988), and Scott & Janis (1993), followed later by Métais & Vislobokova (2007), Sánchez et al. (2010), and Mazza (2013) among others. Analyses focusing just on bovids (the majority of ruminant species) include Vrba (1979, 1997), Groves (1981), Gentry (1992), Geraads (1992), Vrba et al. (1994), Vrba & Gatesy (1994), Vrba & Schaller (2000), as well as Bibi (2009), and Bärmann (2012).

However, unless they dealt with limited taxonomic scope, or a limited set of characters, all these studies have lacked the power to confidently resolve relationships at both deep and shallow nodes of the ruminant tree armed only with a matrix of morphological characters. This has been reflected over the years, for example, in the continuously uncertain placement of *Antilocapra* and *Moschus* among Pecora, as well as of enigmatic taxa such as *Hydropotes* among Cervids, and *Pelea*, *Saiga*, *Pantholops*, and *Aepyceros* among bovids, not to mention the relationships of numerous fossil taxa.

Perhaps the most significant large morphological character matrix of ruminant species constructed to date comes from Gentry's (1992) study on Bovidae, which includes 112 characters from across the skeleton, and which has been reused many times in further studies (e.g. Thomas 1994) including in combination with molecular datasets (Gatesy et al. 1997; Gatesy & Arctander 2000; Hassanin & Douzery 2003). However, as was apparent from Gentry's own results, this morphological matrix provides only a coarse level of resolution within Bovidae. A re-analysis of Gentry's matrix recovers four most parsimonious trees of 537 steps, and a strict consensus of 546 steps (Fig. 3a). This tree is not significantly different from that of the original study, and recovers monophyletic groupings of certain Antilopini and also Caprini. Otherwise, this tree bears little resemblance to either traditional or molecular taxonomies of bovids. Even a monophyletic Bovidae relative to the hypothetical outgroup taxon is not recovered, and only four nodes on the tree have bootstrap values >50%.

In order to assess the distribution of the characters on a 'true tree', I manually modified the consensus tree to match the results of most molecular analyses (Fig. 3b). This handmade tree requires 594 steps, i.e. it is about 10% longer than the most parsimonious trees. When branch lengths are assigned using Gentry's matrix, the new tree shows relatively longer internal branch lengths, meaning many clades are found to have numerous morphological character state changes supporting them. Such synapomorphies can be considered 'hidden morphological support' (e.g. Gatesy & Arctander, 2000) in that they provide diagnostic support for clades *post hoc*, but are too affected by homoplasy to adequately resolve a parsimony analysis.

Gentry's matrix therefore serves well for the exploration of character state changes on a tree determined through other (molecular) means. However, it would be useful to develop a morphological matrix that provides a highly resolved species tree with good support even when analyzed alone. Such a morphological character matrix requires that the number of informative characters (or character states) be large enough to permit the analysis of many taxa. A low number of characters is another problem affecting many morphological analyses of ruminants. With molecular data, the number of variable nucleotide bases (informative characters, essentially) can be very high, allowing for the analysis of hundreds or thousands of species together. With morphological data, this can be a problem, especially when high levels of homoplasy mean that many individual characters cannot provide unambiguous support for just a single clade. For example, a bovid analysis that I previously undertook (Bibi 2009) attempted to resolve relationships among 76 living and fossil bovid species using 147 skull characters, a character to taxon ratio of just 2:1. It is therefore no surprise that the analysis generated over 3000 most parsimonious trees and a poorly resolved consensus tree with only four nodes with bootstrap values over 50%. A larger matrix is necessary for the phylogenetic resolution of any significant number of ruminant taxa, especially considering the hundreds of living and fossil species of ruminants that could be included in a morphological phylogenetic analysis.

4. A new morphological matrix

To this end, I have recently compiled a large number of skeletal characters for a range of living and fossil ruminant taxa with the aim of producing a matrix that will allow for combined analyses of morphology, molecules, fossil, and living taxa. So far the number of characters stands at 409, and while a good proportion of these are taken or modified from previous studies (cited above), many are also new. Almost 3/4 of the characters are from the skull. Fig. 4 shows the distribution of the character set on the skeleton. The number of scored taxa is still low, at 18, but these already include some crucial fossils such as *Archaeomeryx optatus*, *Parablastomeryx gregorii*, as well as an un-named stem pecoran from the Oligocene Hsanda Gol Formation in Mongolia (McKenna expedition material at the American Museum of Natural History, New York, USA). The entire study, however, is still in the process of being compiled and completed, and here I only provide preliminary results that give some sense of the quality and potential of the character matrix.

I ran a heuristic search in PAUP* (Swofford 2002), using tree-bisection-reconnection with 1000 replicates, random addition sequence, and using *Archaeomeryx optatus*, *Hyemoschus aquaticus,* and *Tragulus napu* as outgroups. Bootstrap was run at 100 replicates of 100 search replicates each. The search produced three most parsimonious trees of 809

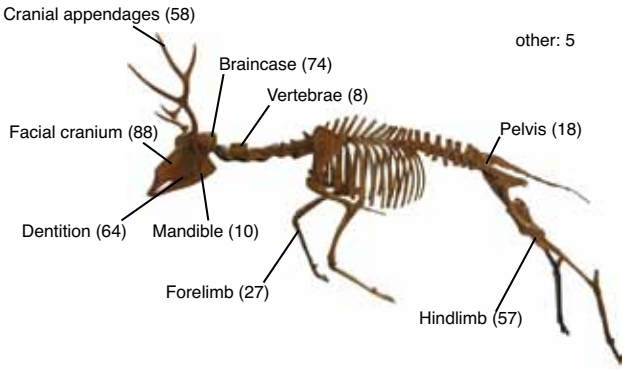

Figure 4: Distribution of 409 skeletal characters used in the new (in progress) morphological analysis. Skeleton of *Ramoceros osborni* from the American Museum of Natural History (credit: Ryan Somma, Wikimedia Commons).

steps, these differing only in the placement of *Cephalophus natalensis* (as either basal within Bovidae, basal within Bovinae, or basal within Antilopinae). The resulting consensus tree is well resolved, with greater than 70% bootstrap support for all nodes, and several at 100% (Fig. 5). Clades such as Tragulidae, Pecora, *Moschus* + *Parablastomeryx,* Cervidae, Alcelaphini, and Reduncini are well defined. While skull characters make the largest contribution to the dataset and the analysis, the postcranial characters are crucial for resolving several important nodes, among these Tragulidae and Pecora. Among Bovidae, the Bovinae have strong support, as do most of the Antilopinae, but bovid monophyly fails with the placement of *Antilocapra americana* among the antelopes. This appears due to convergence with Reduncini and Alcelaphini in features related to cursoriality and living in grassland habitats (long face, hypsodont dentition, long slender limbs) in addition to the similarities of the bony horn cores of *Antilocapra* and bovids. Perhaps the addition of fossil antilocaprid taxa could help recover monophyletic antilocaprid and bovid clades. Besides *Antilocapra*, the placement of *Cephalophus natalensis* is uncertain in the analysis, this being at least in part due to convergent or plesiomorphic similarities with *Tetracerus quadricornis*. Also *Pelea capreolus* (rhebok) is placed close to both Alcelaphini and Reduncini, while it should more likely be sister to Reduncini (Robinson et al. 2013; Vrba et al. 1994). The addition of further reduncin taxa, as well as hippotragins and caprins, could help better resolve this part of the tree.

Interestingly, the analysis cannot resolve the relationships of *Archaeomeryx* relative to crown ruminants. This reflects the difficulty of optimizing many of the morphological features of *Archaeomeryx*. While retaining numerous primitive non-ruminant features (such as upper incisors), *Archaeomeryx* also shares many characters in common with pecorans to the exclusion of tragulids (Tab. 1). This has no doubt contributed to the varied placement of *Archaeomeryx* with respect to Pecora in the literature. *Archaeomeryx* has previously been assigned to the Leptomerycidae and placed closer to pecorans

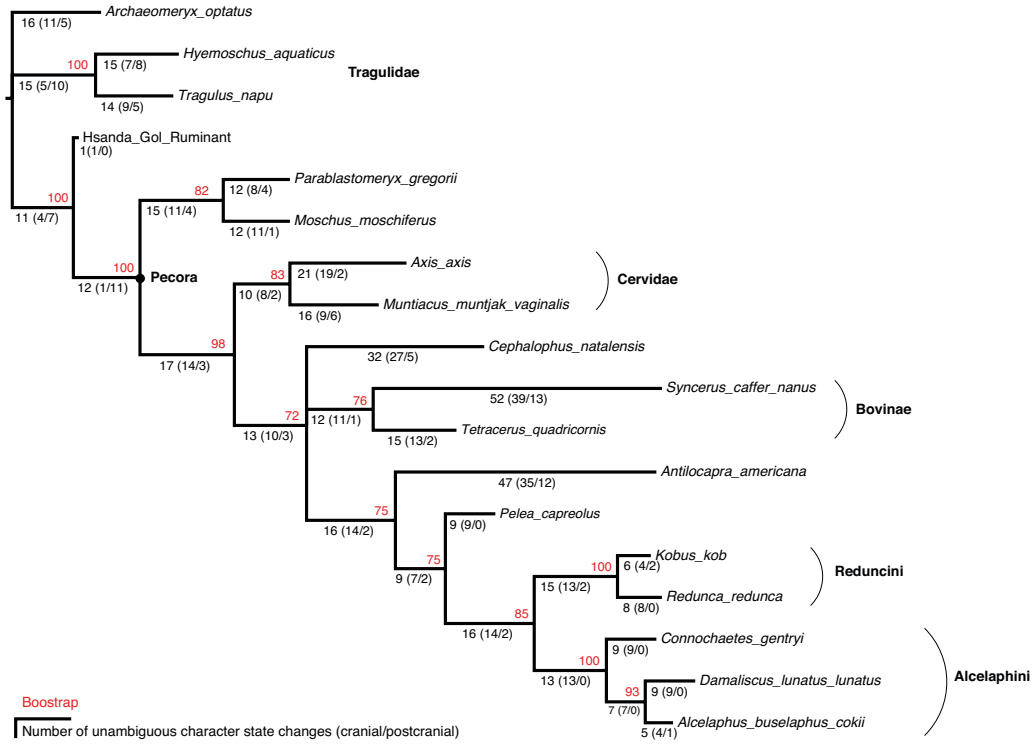

Figure 5: Consensus tree of three most parsimonious trees resulting from the analysis of the new (in progress) morphological matrix of 409 characters. Branch lengths are proportional to character state changes and these are indicated below branches. Bootstrap values above branches. The analysis recovers many of the established relationships among ruminants, through a few problems due to convergence still remain *(Antilocapra, Cephalophus),* and further outgroups are required to resolve the relationship of *Archaeomeryx* relative to Pecora.

Table 1: Characters of *Archaeomeryx optatus* relevant to its phylogenetic relationship to Ruminantia. Based on specimens AMNH 20311, 20311-8, and information in Matthew & Granger (1925) and Colbert (1941). The polarity of the tragulid and pecoran-like characters requires further outgroup assessment. AMNH = American Museum of Natural History, New York (USA)

Non-ruminant characters	Ruminant characters	Tragulid-like characters	Pecoran-like characters	Unique characters
Upper incisors present	Trapezoid and magnum fused (trapezo-magnum)	Maxillary palatal ridges very wide apart	Upper and lower premolar rows slightly reduced in length	Distal radius with a shallow fossa and no trochlea
Metatarsals 3 & 4 totally unfused	Lower canine incisiform	Protocone absent in P2 and poorly developed in P3	Tibial medial malleolus present	Proximal humerus with sharp crest along posterior surface
Cubonavicular thick proximo-distally	P1 absent	Styles on upper premolars and molars very low relative to cusps	p4 with relatively well-developed metaconid	Calcaneum with large and deep fovea on lateral surface
Tail is long		p2 is long, as long as p3	Calcaneum dorsal tubercle present, long and slender heel, less curled cnv facet	Astragalus with large antero-posteriorly oriented ridge along ventral surface
		Metacarpal much shorter than metatarsal	Astragalus with crest on medial surface	
			Lateral tubercle on dorsal surface of astragalus is small	
		Metacarpals 3 & 4 unfused (though weakly fused in *Tragulus*)	Posteromedial process on cubonavicular is strongly projecting	
		Metacarpals 2 & 5 fully formed, with phalanges		
		Metapodial distal keels not extending to anterior surface		
		Short metpodials that are about half the length of the femur (with convergence in *Muntiacus*, *Syncerus*, Caprini)		
		Flexed astragalus with long and narrow shape		
		Axis with small peg-like dens and widely separated atlas articular facets		

than to tragulids (Janis 1987; Webb & Taylor 1980), or found to lie basal to tragulids and thereby outside the crown ruminant clade (Gentry & Hooker 1988; Mazza 2013; Métais & Vislobokova 2007). The addition of *Archaeomeryx* into the current analysis casts doubt on many of the supposedly 'primitive' features of tragulids. For example, the calcaneum in *Archaeomeryx* bears a large tubercle on the fibular facet, a feature shared with pecorans, while in tragulids the facet is flat. Is the presence of the tubercle a derived feature uniting *Archaeomeryx* and pecorans? Or is the presence of a tubercle in fact the primitive condition among ruminants? Better resolution at the base of the ruminant tree can be achieved by the inclusion of further Eocene ruminants in addition to extant artiodactyls such as hippotamids and suids.

Despite a number of clear issues, however, the tree is well resolved and well supported. These preliminary results indicate that the new morphological matrix has the power to resolve relationships among ruminant species with a high degree of confidence, and the relatively large number of characters should permit for the analysis of a large number of taxa. Such a morphological matrix could then be combined with a molecular matrix for a broad analysis of both living and extinct ruminant taxa together.

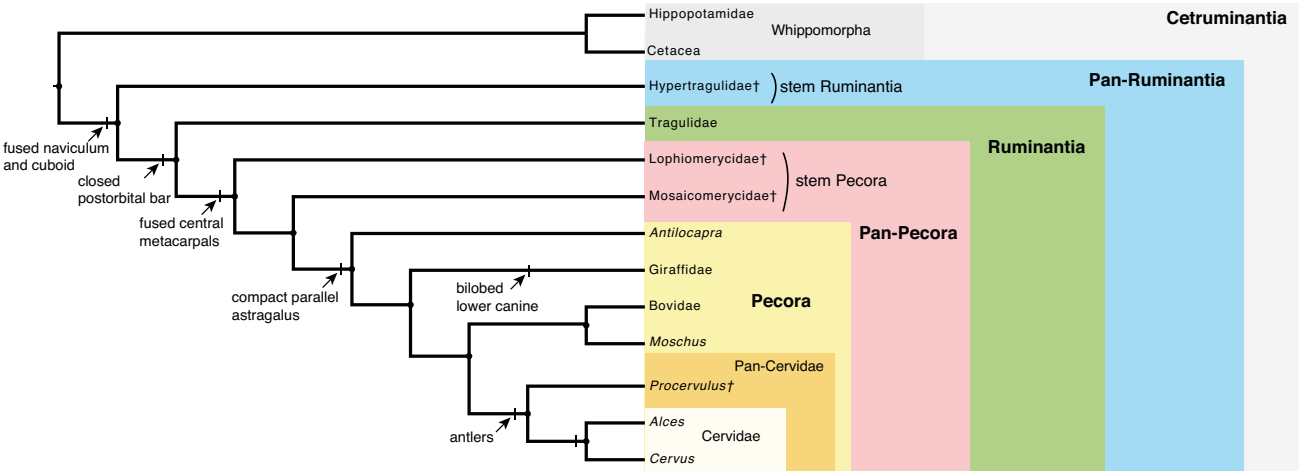

Figure 6: Representative phylogenetic tree indicating the composition of clade names defined in the text according to phylogenetic nomenclature. Commonly used clade names (Ruminantia, Pecora, Cervidae) are restricted to crown (extant) clades. Total clades, those comprising a crown clade plus the stem leading to it, are identified with the prefix 'Pan-' (Pan-Ruminantia, Pan-Pecora, Pan-Cervidae). Note that clade names are defined on the basis of crown clades, not apomorphies. Use of a standardized phylogenetic nomenclature will help reduce miscommunication between paleontologists and neontologists.

5. Taxonomic Nomenclature

Taxonomic nomenclature should reflect phylogenetic relationships, and should be standardized to minimize confusion among biologists from different specialities. Paleontologists often define clades on the basis of shared anatomical features, while neontologists (particularly molecular phylogenetics) define clades on the basis of phylogenetic composition. Therefore, clade names used by paleontologists will often refer to different and much larger entities than the same names as used by neontologists. For example, to a paleontologist, Ruminantia may be the clade defined by the presence of fused navicular and cuboid tarsal bones and an incisiform lower canine (e.g. Janis & Scott 1987). Hypertragulids, then, would be considered ruminants (Ruminantia). To a neontologist, however, Ruminantia refers to a crown (extant) clade defined by the most recent common ancestor of all its living members. Lacking several synapomorphies of living ruminants places hypertragulids outside the crown clade, which means, from a phylogenetic perspective, that hypertragulids cannot be included in Ruminantia. The age and composition of the clade labelled 'Ruminantia' on the tree of Hassanin et al. (2012: fig. 1) is only a subset of that labelled 'Ruminantia' on the tree of Gentry & Hooker (1988: fig. 9.8). Besides the obvious confusion that this can cause, concrete problems can arise from such miscommunication. For example, in the paleonotological literature, a molecular phylogeneticist reads that the earliest ruminants arose in the Middle Eocene. The researcher then uses this information to date the node corresponding to 'Ruminantia' on a tree of extant species to a Middle Eocene age. This results in drastically inflated age estimates for the entire tree. The same problem applies to Pecora, variably defined either on the basis of morphological features (e.g., a compact, parallel-sided astragalus) or as the crown clade encompassing giraffes, bovids, deer, *Moschus* and *Antilocapra*. Likewise a fossil may be attributed to Cervidae if it possesses antlers (e.g., *Procervulus*), while the node labelled Cervidae on a molecular tree defines only a subset of the antlered clade. In all three cases, clades defined on the basis of morphological features are older and larger than those defined on the basis of extant taxa. This is because fusion of the cuboid and navicular occurred prior to the appearance of the most recent common ancestor of living ruminants, a short compact astragalus evolved prior to the most recent common ancestor of all living pecorans, and antlers appeared well before the most recent common ancestor of all living deer.

I here propose that we standardize our usage of ruminant clade names by using phylogenetically based clade definitions (rather than apomorphically-defined clade definitions). This affects paleontologists in particular, as neontologists are generally restricted to dealing with crown clades anyway. Following recommendations for phylogenetic nomenclature (de Queiroz & Gauthier 1994; de Queiroz 2007; Cantino & de Queiroz 2010), I here present phylogenetic definitions and diagnoses for the clades Ruminantia and Pecora based on crown clades. Total clades, which are made up of a crown clade plus taxa along its stem branch, are named by attaching the prefix 'Pan-' to the crown clade name (Lauterbach 1989; Meier & Richter 1992; Joyce et al. 2004). As noted above, the following phylogenetically based clade definitions for Ruminantia and Pecora result in different (smaller) clades than those based on anatomical features (e.g. Janis & Scott 1987; Webb & Taylor 1980). Determining whether a fossil taxon belongs within Ruminantia or Pecora naturally relies on the identification of diagnostic apomorphies, which are

also provided. However, the highly functional (locomotory, dietary) role of many of these features means the probability of convergent evolution is very high, and in many cases certain (Janis & Theodor in press; Janis & Scott 1987). This results in a grim but not insurmountable challenge to phylogenetics, one that I hope can be overcome by the combined analytical approach advocated above.

Ruminantia Scopoli, 1777 converted clade name
Pan-Ruminantia new clade name

Definitions: Ruminantia refers to the clade originating from the most recent common ancestor of *Tragulus javanicus* (Osbeck, 1765) and *Bos taurus* Linnaeus, 1758. This definition is node-based and designates a crown clade.

Pan-Ruminantia refers to the total clade composed of the crown clade Ruminantia and all extinct organisms or species that share a more recent common ancestor with Ruminantia than with any extant organism or species that is not a member of Ruminantia. In other words, Pan-Ruminantia includes *Bos taurus* Linnaeus, 1758 and all organisms or species that share a more recent common ancestor with *Bos taurus* Linnaeus, 1758 than with *Hippopotamus amphibius* Linnaeus, 1758, *Balaena mysticetus* Linnaeus, 1758, *Camelus dromedarius* Linnaeus, 1758, or *Sus scrofa* Linnaeus, 1758. This is a branch-based definition and designates a crown clade plus a stem group.

Reference phylogenies: Fig. 6 here and fig. 1 in Hassanin et al. (2012).

Composition: Ruminantia contains around 212 living species (Wilson & Reeder 2005) or many more following the taxonomy of Groves & Grubb (2011). Extant species are divided among two subclades, Tragulidae (equivalent to Tragulina) and Pecora. There are countless extinct fossil ruminant taxa that are members of Pan-Ruminantia, but not Ruminantia. Hypertragulids, lacking several diagnostic apomorphies of crown ruminants, are not included in Ruminantia. Possession of a fused navicular and cuboid, however, does place them on the stem branch leading to ruminants. If *Archaeomeryx optatus* is closer to pecorans than are tragulids, then *A. optatus* belongs in Ruminantia. If *A. optatus* is basal to the clade defined by tragulids and pecorans, then it belongs outside the crown clade and among the stem ruminants.

Diagnostic apomorphies: Members of Ruminantia are differentiated from other living artiodactyls by the development of foregut fermentation that takes place through a three or four-chambered stomach that includes a rumen, reticulum, abomasum, and in pecorans an omasum (camelids have independently evolved a three-chambered stomach); the absence of upper incisors and upper first premolars; an incisiform lower canine; a closed post-orbital bar (though this may be independently derived in tragulids and pecorans); fused magnum and trapezoid carpals; fused cuboid and navicular tarsals; and a fibula reduced to a malleolar bone (Janis & Scott 1987). Numerous other characters, including many molecular characters, also diagnose Ruminantia. These diagnostic characters are common to the ancestry of all living ruminants and therefore must have evolved some time prior to the origin of the clade. Therefore, to be determined as belonging within Ruminantia, an extinct taxon must possess these characters (to the degree determinable) plus at least one character allying it to a crown clade taxon.

Pecora Linnaeus, 1758 converted clade name
Pan-Pecora new clade name

Synonym of Pecora: Eupecora Webb & Taylor, 1980

Definition: Pecora refers to the crown clade originating from the most recent common ancestor of *Antilocapra americana* Ord, 1815; *Moschus moschiferus* (Linnaeus, 1758); *Giraffa camelopardalis* (Linnaeus, 1758); *Cervus elaphus* Linnaeus, 1758; and *Bos taurus* Linnaeus, 1758. This definition is node-based and designates a crown clade.

Pan-Pecora refers to the total clade composed of the crown clade Pecora and all extinct organisms or species that share a more recent common ancestor with Pecora than with any extant organism or species that is not a member of Pecora. In other words, Pan-Pecora includes *Bos taurus* Linnaeus, 1758 and all organisms or species that share a more recent common ancestor with *Bos taurus* Linnaeus, 1758 than with *Tragulus javanicus* (Osbeck, 1765). This is a branch-based definition and designates a crown clade plus a stem group.

Reference phylogenies: Fig. 6 here and fig. 1 in Hassanin et al. (2012).

Composition: Pecora includes around 204 living species (Wilson & Reeder 2005), or many more following the taxonomy of Groves & Grubb (2011). Extant species are divided among five subclades, traditionally given family rank. In order of increasing species richness, these are: Antilocapridae, Moschidae, Giraffidae, Cervidae, and Bovidae. All members of Pecora are also by definition members of the more inclusive clade Pan-Pecora. Ruminant clades such as Lophiomerycidae and Bachitheriidae possess some of the diagnostic apomorphies of pecorans and are therefore members of Pan-Pecora (i.e., they are closer to Pecora than to Tragulidae). However, these also lack characters diagnostic of crown pecorans, placing them on the stem branch leading to Pecora, and not within Pecora itself. Such clades are

therefore members of Pan-Pecora but not Pecora. Previous authors' use of the terms 'Pecora' to include the total clade and 'Eupecora' for the crown clade should be updated to 'Pan-Pecora' and 'Pecora', respectively. This preserves the same meaning of Pecora for all biologists, whether studying living or fossil taxa, and limits the proliferation of new clade names.

Diagnostic apomorphies: Pecora may be diagnosed by many anatomical characters. A main feature is the presence of a four-chambered stomach (including omasum). Osteological characters include premolars with well developed lingual cusps, a spout-like odontoid process of the axis, metapodial II-III shafts fused along their entire length, metapodial distal keels continuing onto anterior surface, and a compact and parallel-sided astragalus. To be referred to Pecora, an extinct taxon must possess these characters (to the degree determinable) plus at least one character allying it to a crown clade taxon. Pecorans are also well supported by numerous molecular characters. Most pecoran species may further be diagnosed by the presence of cranial appendages in male individuals (horns, antlers, ossicones), but some species are without cranial appendages, perhaps secondarily, and developmental evidence suggests cranial appendages may be independently derived and therefore not homologous among the different pecoran families (see Davis et al. 2011).

6. Conclusions

Until a few decades ago, the principal means to investigate evolutionary history was through study of the anatomy of organisms both living and extinct. In particular, processes of species origination and extinction, and the development of ecological and biogeographic patterns was almost exclusively the domain of paleontology and proceeded largely through the discovery and analysis of the fossil record. The rise of molecular biology, and the ever-decreasing labor and costs associated with sequencing genomes has produced a massive and powerful new bioinformatics industry that can, quite independently of the fossil record, examine past patterns and processes of evolutionary change. The degree of independence of morphological and molecular approaches is ultimately limited, however, as these approaches investigate similar issues and ask similar questions, meaning a synthetic and complementary approach between the two fields is not just possible, but also necessary. Combined molecular and organismal approaches have been successfully applied to many vertebrate and invertebrate clades over the last decade, and can also be brought to investigations of ruminant phylogenetics.

I have briefly reviewed the state of the field in ruminant phylogenetics and outlined some ways forward, focusing especially on the challenge of bridging molecular and morphological approaches to the study of this clade. Molecular phylogenetics has in just two decades produced very large and strongly supported phylogenies that include almost every single extant ruminant species. A major task for the near future then is the integration of organismal-level data and the large ruminant fossil record with the existing molecular phylogenetic picture. This will require collaboration between genomic and 'phenomic' workers in order to develop, grow, maintain, combine, and analyze disparate sources of data that will allow for the creation of such an all-inclusive and dated phylogenetic tree. Such a collaborative spirit also requires a common taxonomic language and I have proposed standardizing the use of clade names to refer exclusively to crown (extant) clades, and I have given examples of phylogenetically-based definitions for Ruminantia and Pecora. The sheer size of the ruminant fossil record also means larger collaborations aimed specifically at reconstructing phylogenetic relationships among fossil taxa are needed among paleontologists. To this end, I have compiled a large morphological character list to aid the integration of fossil taxa from across the ruminant tree into a combined molecular and morphological phylogenetic framework. Preliminary results from the analysis of this matrix, as yet being compiled, recover many of the monophyletic clades expected by both anatomical and molecular phylogenetic studies, and bode well for coming efforts to reconstruct the phylogeny of both living and fossil ruminants through analysis of both molecular and morphological data.

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