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A comprehensive approach towards the systematics of Cervidae

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Systematic relationships of cervids have been controversial for decades. Despite new input from molecular systematics, consensus could only be partially reached. The initial, gross (sub)classification based on morphology and comparative anatomy was mostly supported by molecular data. The rich fossil record of cervids has never been extensively tested in phylogenetic frameworks concerning potential systematic relationships of fossil cervids to extant cervids. The aim of this work was to investigate the systematic relationships of extant and fossil cervids using molecular and morphological characters and make implications about their evolutionary history based on the phylogenetic reconstructions. To achieve these objectives, molecular data were compiled consisting of five nuclear markers and the complete mitochondrial genome of 50 extant and one fossil cervid species. Several analyses using different data partitions, taxon sampling, partitioning schemes, and optimality criteria were undertaken. In addition, the most extensive morphological character matrix for such a broad cervid taxon sampling was compiled including 168 cranial and dental characters of 41 extant and 29 fossil cervid species. The morphological and molecular data were analysed in a combined approach and other comprehensive phylogenetic reconstructions. The results showed that most of the Miocene cervids were more closely related to each other than to any other cervids. They were often positioned between the outgroup and all other cervids or as the sister taxon to Muntiacini. Two Miocene cervids were frequently placed within Muntiacini. Plio- and Pleistocene cervids could often be affiliated to Cervini, Odocoileini or Capreolini. The phylogenetic analyses of this work provide new insights into the evolutionary history of cervids. Several fossil cervids could be successfully related to living representatives, confirming previously assumed affiliations based on comparative morphology and introducing new hypotheses. New systematic relationships were observed, some uncertainties persisted and resolving
systematics within certain taxa remained challenging.
A comprehensive approach towards the systematics of Cervidae

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ABSTRACT

Systematic relationships of cervids have been controversial for decades. Despite new input from molecular systematics, consensus could only be partially reached. The initial, gross (sub)classification based on morphology and comparative anatomy was mostly supported by molecular data. The rich fossil record of cervids has never been extensively tested in phylogenetic frameworks concerning potential systematic relationships of fossil cervids to extant cervids. The aim of this work was to investigate the systematic relationships of extant and fossil cervids using molecular and morphological characters and make implications about their evolutionary history based on the phylogenetic reconstructions.

To achieve these objectives, molecular data were compiled consisting of five nuclear markers and the complete mitochondrial genome of 50 extant and one fossil cervid species. Several analyses using different data partitions, taxon sampling, partitioning schemes, and optimality criteria were undertaken. In addition, the most extensive morphological character matrix for such a broad cervid taxon sampling was compiled including 168 cranial and dental characters of 41 extant and 29 fossil cervid species. The morphological and molecular data were analysed in a combined approach and other comprehensive phylogenetic reconstructions.

The results showed that most of the Miocene cervids were more closely related to each other than to any other cervids. They were often positioned between the outgroup and all other cervids or as the sister taxon to Muntiacini. Two Miocene cervids were frequently placed within Muntiacini. Plio- and Pleistocene cervids could often be affiliated to Cervini, Odocoileini or Capreolini. The phylogenetic analyses of this work provide new insights into the evolutionary history of cervids. Several fossil cervids could be successfully related to living representatives, confirming previously assumed affiliations based on comparative morphology and introducing new hypotheses. New systematic relationships were observed, some uncertainties persisted and resolving systematics within certain taxa remained challenging.

INTRODUCTION

Cervidae (deer) belong to Ruminantia together with Tragulidae (chevrotains), Antilocapridae (pronghorns), Moschidae (musk deer), Giraffidae (giraffes), and Bovidae (cattle, sheep, antelopes). Cervids are the second most diverse group of ruminants and are natively distributed in the Americas, Europe and Asia inhabiting a broad variety of habitats. Apart from the recent dispersal and radiation into South America, cervids are mainly restricted to the Northern Hemisphere (Geist, 1998; Gentry, 2000; Scott and Janis, 1987; Webb, 2000).

Despite all efforts to resolve cervid (and ruminant) systematics over the past decades, there is only partial consensus from the phylogenetic reconstructions and several problems persist. Controversial species
delimitations, unknown taxon affiliation, contradictory information from the data, and/or incomplete phylogenetic reconstruction were specified as possible reasons for these problems. To solve phylogenetic relationships of cervids (and ruminants), however, is of considerable interest, because of their important biological and economic role as wild and domestic animals (Cronin, 1991; Randi et al., 2001; Price et al., 2005).

In contrast to early systematic studies, which were often based only on a few morphological characters, there are now numerous molecular approaches and a few supertree studies reconstructing cervid systematics. However, combined or total evidence approaches are still scarce (Groves and Grubb, 1987; Groves, 2014). Although the fossil record for cervids is good, systematic relationships of fossil cervids are even more uncertain than those of extant cervids. There are numerous qualitative descriptions and comparative morphological studies for fossil cervids, but there are only very few phylogenetic approaches on fossil taxa. While these were mainly based on antler characters, Mennecart et al. (2016, 2017) presented the first phylogenetic reconstructions of Miocene cervids based on inner ear morphology.

Various hypotheses of the intra-cervid systematic relationships have been published in the last decades. While in earlier studies up to six subfamilies of Cervidae have been recognised (Ouithavon et al., 2009), the family Cervidae now is usually classified into two subfamilies, Cervinae, consisting of Muntiacini and Cervini, and Capreolinae, consisting of Alceini, Capreolini, Odocoileini, and Rangiferini (e.g., Groves and Grubb, 1990; Miyamoto et al., 1990; Cronin et al., 1996; Randi et al., 1998, 2001; Hassanin and Douzery, 2003; Kuznetsova et al., 2005; Price et al., 2005; Gilbert et al., 2006; Hughes et al., 2006; Ouithavon et al., 2009; Hassanin et al., 2012; Heckelberg et al., 2016). This classification is supported by classical morphological concepts and molecular evidence. In some studies Muntiacini is considered as a subfamily (e.g., Cronin et al., 1996; Randi et al., 1998; Kuznetsova et al., 2005; Marcot, 2007). While the systematic relationships within Muntiacini and Cervini are resolved, with very few exceptions, systematic relationships within Capreolinae are much more controversial. The position of Capreolini and Alceini is uncertain and there are many many polyphilies within Odocoileini (Heckeberg et al., 2016). The latter is the youngest clade of cervids and has a rapid diversification rate, which makes resolving the systematic relationships more difficult.

Diagnostic characters of cervids include for example the presence of two lacrimal foramina, a lacrimal fossa, a preorbital vacuity and brachyodont dentition (Fig. 1) Janis and Scott (1987, 1988); Bouvrain et al. (1989); Mickoleit (2004). The first classification based on morphological characters split Cervidae into Telemetacarpi and Plesiometacarpi, which is equivalent to the Cervinae-Capreolinae split (Brooke, 1878). This split into Capreolinae and Cervinae was also confirmed by behavioural characters (Cap et al., 2002; Groves, 2007). Further subdivision solely based on morphological features is difficult, because most cervid characters are highly conservative, partly phylogenetically uninformative and/or prone to convergence because of ecological adaptation (Groves and Grubb, 1987; Janis and Scott, 1987; Lister, 1996; Wada et al., 2007). However, there are a few morphological characters diagnosing cervid subclades (Bouvrain et al., 1989; Cronin, 1991).

With increasing molecular data outweighing morphological characters, morphology became less important in phylogenetic reconstructions (Huelsbeck and Rannala, 2000). Discrepancies between morphological and molecular studies on ruminants demonstrated the need to continue combining fossil and extant species in order to reconstruct accurate phylogenies and to understand macro-evolutionary processes, which should yield better estimates than individual analyses (Hillis and Wiens, 2000; Hernández Fernández and Vrba, 2005). Several studies show the benefit of combining molecular and morphological data of fossil and living taxa in supermatrix analyses (e.g., Asher, 2007; Geisler et al., 2011; Bibi et al., 2012; Bibi, 2014). Complete species-level taxon and extensive data sampling are required to reconstruct the ecological, biological and geographical patterns of cervid and ruminant evolutionary history (Price et al., 2005).

Here, extensive taxon and data sampling across Cervidae was undertaken for the first time. The morphological data set focused on cranial and dental characters. Five nuclear markers and the mitochondrial genome were analysed and combined with the morphological data set. Several analyses were undertaken on different partitions and the combined data sets analysing fossil and extant taxa separately and together, and under different optimality criteria. Additionally, analyses using a molecular and morphological supermatrix or a constraint topology including only one fossil at a time and the Evolutionary Placement Algorithm (EPA) approach (Berger et al., 2011) were undertaken. The total evidence approaches incorporated 79 fossil and living cervids covering their entire evolutionary history from the early Miocene until
Figure 1. Diagnostic characters of cervids. The most important anatomical features of cervids are outlined in this figure as a photograph and drawing of Blastocerus dichotomus (MNHN 1933-207). Note the brachydont dentition, the preorbital vacuity, lacrimal fossa, and lacrimal foramina. Abbreviations: pmx = premaxillary, mx = maxillary, nas = nasal, lac = lacrimal, zyg = zygomaticum, pal = palatine, pte = pterygoid, orb = orbishenoid, fro = frontal, par = parietal, ali = alisphenoid, squ = squamosal, soc = supraoccipital, ppa = paroccipital processes, bul = auditory bulla, con = condyles.

We were able to investigate the strength of morphological characters to reconstruct a cervid phylogeny, the systematic position of fossil cervids, and the influence of data partitioning and varying taxon sampling on the phylogenetic signal. The results provide new and intriguing insights into how fossil cervids are related to extant cervids.

METHODS

Data

Molecular Data
Molecular data were compiled from GenBank (ncbi.nlm.nih.gov/genbank/). Five nuclear markers and the mitochondrial genome were chosen for phylogenetic reconstructions based on their taxon sampling across cervids (n > 10). The GenBank accession numbers are in the Supplementary Material (Table S1). The molecular data set included the nuclear non-coding markers, α-lactalbumin (Lalba), protein kinase C iota (Prkci), and the sex determining region on the Y-chromosome (Sry) and the nuclear coding markers...
κ-casein (Csn) and prion protein (Prnp) and the partially coding mitochondrial genome. The coding markers were partitioned according to codon positions 1-3. Each gene was aligned in SeaView 4.2 (Gouy et al., 2010) and Mesquite v.2.75 (Maddison and Maddison, 2011); alignments were carefully checked by eye for stop codons and/or unusual codon positions by translation into amino acids, where applicable, and were manually corrected if necessary. Some regions have been excluded from the alignment, for example the first and last couple of sites, which were not available for all taxa in the alignment. The combined molecular data set included one fossil and 50 extant cervids.

**Morphological Data**

In total, 41 extant cervid species, 29 fossil cervid species, six non-cervid extant ruminants, and two non-cervid fossil ruminants were measured and character-coded into the morphological matrix. The measuring distances are in the Supplementary files, the measurements in Table S3. The extant species were studied on 232 specimens, the fossil species were studied on 504 specimens (see Table S2 for complete specimen lists). Most of the fossil cervid taxa consisted of fragments of several individuals. The fossils ranged from the Miocene to the Holocene and their temporal ranges are shown in Figure 2. The character matrices and character state lists are available on morphobank (http://morphobank.org/permalink/?P1021).

**Phylogenetic Analyses**

Figure 3 is an overview of all data sets and analyses undertaken. Tragulids were chosen as the outgroup for all analyses.

**Model Choice**

**Molecular Data.** For each alignment we used PartitionFinder (Lanfear et al., 2012) to identify the appropriate substitution model and the optimal partitioning scheme. The Hasegawa-Kishino-Yano model (HKY; Hasegawa et al., 1985), and the Generalised Time Reversible model (GTR; Tavaré, 1986) were most commonly used.

All analyses were run with a gamma distribution (Γ) without a proportion of invariant sites (I), where Γ or Γ + I was suggested, because combining Γ + I is known to cause convergence problems by creating two areas of equal probability in the tree landscape (Moyle et al., 2012). I was used when suggested as the sole analysis parameter.

After completion, the statistics of all Bayesian analyses were checked in Tracer v.1.6 (tree.bio.ed.ac.uk) and convergence between runs was checked using the visualisation tool AWTY (Wilgenbusch et al., 2004).

**Stepping Stone Analyses for Morphological Data.** The best fit of model distribution and partitioning scheme of the morphological character sets was tested using the efficient stepping stone (ss) sampling (Xie et al., 2011). The Bayes Factor (BF) was calculated as the ratio of the marginal likelihood of one model to the marginal likelihood of the competing model; BFs can then be used as the relative evidence in the data that favours one hypothesis in that respect that it predicts the observed data better than the competing hypotheses (Xie et al., 2011).

To test the combined morphological data set for the most suitable partitioning scheme, ordering scheme (unordered vs. ordered), and model distribution choice (gamma vs. not gamma), ss analyses were undertaken. First, the data set was tested for the partitioning scheme with an analysis of the unpartitioned data set, a maximally, and a minimally partitioned data set. Afterwards, the data set, applying the resulting partitioning scheme, was tested for the gamma (Γ) distribution (Yang, 1994), and for ordering characters.

Each SS analysis was run for 21.5 million generations, with a diagnostic frequency of 1000 and a sample frequency of 500 and had 40 steps in total. The general settings are the same as for a normal BI analysis with MrBayes (Ronquist et al., 2012). The initial burnin of samples and the additional burnin in each step of the ss sampling were discarded. The aforementioned importance distributions are called power posterior distributions and were sampled via the Metropolis Coupled Monte Carlo Markov Chain (MC³) run (Ronquist et al., 2012). In MrBayes this parameter is called alpha and was left as the default setting of 0.4, because in empirical studies it was found that the accuracy is maximal with an alpha value between 0.3 and 0.5 (Ronquist et al., 2012). After completion of the ss analyses the BFs of the summary of the marginal likelihoods of all 40 steps were calculated and compared with each other to decide for the favoured hypothesis.
### Figure 2. Age ranges of fossil cervids. Fossil cervids are arranged from the oldest first appearance datum (bottom) to the youngest first appearance datum (top). The stage column widths are not to scale with time. The dates were compiled from the literature (Gentry et al., 1999; Steininger, 1999; Böhme et al., 2012; Cohen et al., 2013; Croitor, 2014) and databases (NOW: www.helsinki/science/now/, PBDB: www.paleobiodb.org).

**Analyses of Morphological Data**

- **Data Sets**: All morphological data sets included 78 taxa, 41 extant cervid species, 29 fossil cervid species, 6 non-cervid extant ruminant species, and 2 fossil non-cervid ruminant species. In the data matrix, 0 indicates no species, 0.0117 species, 0.0117 species, etc.

### Cenozoic

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Figure 3. Overview of all analyses. This overview shows all analyses undertaken and the optimality criteria under which they were run. Abbreviations: BI = Bayesian inference, ML = maximum likelihood, MP = maximum parsimony.

In the cranial matrix 89 characters were coded in total; 7 characters concerning the mandible, 65 concerning the cranium, and 17 concerning antlers and pedicles. There were 17 discrete quantitative characters and 23 characters were suitable for ordering (2, 4, 5, 8–12, 14, 15 17–20, 23, 61, 74–79, 89). The combined morphological data set consisted of 168 characters, of which 19 were discrete quantitative characters and 34 were suitable for ordering (see above).
The complete mitochondrial genome (mtG) available for 33 cervid species including 39 taxa and seven non-cervid ruminants with a total of 14904 base pairs of Hassanin et al. (2012) was re-analysed. The extensive Cytb data set from Heckeberg et al. (2016) was combined with the mtG. For the combined mtG-Cytb-analyses, the original Cytb region of the mtG was replaced by the more taxon-rich Cytb
Table 1. Overview of all analyses undertaken. x indicates analyses that were not successful, * indicates topologies that are figured in the main text, # only summarising topology figured in the main text; the topologies of all other analyses can be found in the Supplemental material. Abbreviations: Dent = Dental, Cran = Cranial, Combi = Combined, UnO = unordered, O = ordered, MP = maximum parsimony, BI, MB = Bayesian inference, ML = maximum likelihood, noOut = excluding most outgroup taxa, nuc = nuclear marker, mt = mitochondrial marker, Opt. Crit.=Optimality Criterion, nchar = number of characters, ntax = number of taxa, E=Extant, F=Fossil.

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alignment. The mitochondrial combined matrix included 51 cervid species across 56 cervid taxa and six non-cervid ruminants (Table 1).

The mtG-Cytb combined data set contained seven partitions according to Hassanin et al. (2012). For the BI analyses two runs à four chains sampled the tree landscape at a temperature of 0.35 until the standard deviation of split frequencies was below 0.01. Trees were sampled every 5000th generation. The ML analyses for both data sets included rapid bootstrap analyses and used the same partitioning scheme as in the BI analyses.

The combined molecular matrix consisted of 17709 base pairs for 56 cervid taxa including 50 extant and 1 fossil cervid species and 6 non-cervid ruminant species (Table 1). This data set was analysed using ML and BI with the same settings as above.

Combined Molecular and Morphology Analyses
The total evidence (TE) matrix consisted of 17877 characters. The 87 taxa included two fossil and six extant non-cervid ruminant species and 29 fossil and 50 extant cervid species. This data set was run using ML, BI, and MP (Table 1).

RESULTS
Morphological Data
Figure 4 provides an overview of how well each species was sampled for morphological data. All fossil taxa are sampled for at least three partitions. The most incomplete fossil is *Eostyloceros hezhengensis* sampled from the literature with 70 % missing data followed by *Ligeromeryx praestans* with 68 % missing data. The most complete fossil cervids were *Megaloceros giganteus* with 0 % missing data and *Candidiacervus ropalophorus* with 6 % missing data. Most of the other fossil taxa have around 50 % missing data.

**Cranium**
All cervids share several anatomical features, such as two lacrimal foramina, a preorbital vacuity, and a lacrimal fossa (Fig. 1). In lateral view, the dorsal outline is convex at the braincase, concave at the fronto-nasal transition and straight at the nasals. The anterior extension of the snout is moderate depending on the overall size of the cervid species. The basicranial outline in lateral view is flexed. The preorbital vacuity varies in size and form, the lacrimal fossa can be deep and round, covering a large proportion of the facial aspect of the skull, shallow, or barely visible (particularly in females). The position of the two lacrimal foramina on the orbit rim (more internally or externally) and the position to each other is variable. A detailed description of the craniodental morphology for each cervid species investigated is in Heckeberg (2017a).

Some Miocene cervids have a sagittal crest (e.g., *Dicrocerus, Procervulus*), which is absent in all other cervids (Fig. 1). The number and size of supraorbital foramina and presence and absence of the supraorbital sulcus are variable and could potentially be features to distinguish groups of cervids; however, more specimens per species need to be investigated to confirm this. The presence of an extended vomerine septum and the division between the temporal foramina is characteristic for Capreolinae (Fig. 1). Most cervids have small, oval auditory bullae, some species have large inflated bullae (e.g., *Axis*) (Fig. 1).

Most Miocene cervids have long pedicles, the insertion point of the pedicle is directly above the orbit and the pedicle is upright (Fig. 6). Muntiacini, *Euprox* and *Eostyloceros* have long strongly inclined pedicles. In most other cervids the pedicles originate more posterior to the orbit, are inclined at 45–60° and short. *Mazama* and *Pudu* have strongly inclined and short pedicles.

**Antlers**
Even though antlers are species-specific, they have a high variability, intraspecifically and ontogenetically. No antler looks exactly the same, not even the left and the right antler of the same individual are identical. Also, antlers change from one year to the next; in addition pathologies, abnormal growth, and other phenomena occur.

While cervid genera and most species can be qualitatively distinguished based on antler morphology, translation of these distinctions into discrete characters for quantitative or phylogenetic analyses is difficult. Convergence, which can be distinguished by eye, but is sometimes too subtle to be scored differently in the character matrix is the reason for this. Three morphotypes can be distinguished in extant cervids.
**Figure 4.** Overview of the characters available for each cervid species. Extant species are arranged in alphabetical order starting from the left, fossil cervids and the two non-cervid fossils are arranged from the youngest to the oldest following the extant taxa. Morphological characters were divided into seven partitions indicated by the different colours of each bar. The y-axis represents the absolute number of present characters.
Figure 5. Details of the cervid cranial anatomy. (A) Cranium of *Dicrocerus elegans* (MNHN Sa 10308) in dorsal view. The arrow indicates the sagittal crest. (B) Basicranium of *Odocoileus hemionus* (MNHN AE724). The arrow indicates the vomerine septum typical for Capreolinae. (C) Basicranium of *Axis axis* (ZSM 1958-88). The arrows indicate the large inflated auditory bullae, rarely observed in cervids. (D) Basicranium of *Ozotoceros bezoarticus* (UMZC H.18781). The arrows indicate the small flattened auditory bullae with prominent processes.

**Morphotype 1**

This morphotype includes all cervids with single-tined or bifurcating antlers; *Mazama* and *Pudu* have single-tined antlers (*Pudu* antlers rarely develop a bifurcation). *Elaphodus cephalophus* has minute, single-tined antlers. All *Muntiacus* species have bifurcating antlers on elongated inclined pedicles. *Hippocamelus* has a bifurcating antler morphology with an open angle between the brow tine and main tine; the main tine can have additional small tines. Fossil cervids with a bifurcating antler morphology include *Procervulus*, *Dicrocerus*, *Heteroprox*, *Euprox*, and presumably *Cervus australis*.

**Morphotype 2**

This morphotype includes all cervids with antlers showing exactly three tines, e.g., *Rusa*, *Axis*, *Capreolus*, and *Ozotoceros*. The three tines are organised either in a way, where the brow tine forms a more acute angle to the main beam with the tip of the brow tine pointing posteriad (*Axis*, *Rusa*), or where it forms an open angle with the tip of the brow tine pointing more upwards or forwards (*Capreolus*, *Ozotoceros*).

Fossil cervids of the morphotype 2 include *Axis lydekkeri*, *Rusa kendengensis*, *Metacervocerus pardinensis*, ‘*Cervus* philisi’, and *Metacervocerus rhenanus* with the brow tines pointing posteriad, *Procapreolus cusanus* with the brow tines pointing upwards. *Pliocervus matheronis* antler remains are too fragmentary to infer the direction of the brow tine unambiguously. It was also suggested that this
Figure 6. Cervid antler evolution. (A) Cranium of a typical Miocene cervid showing the characteristics of early pedicles and antlers. (B) Cranium of an extant cervids showing features of the pedicles and antlers seen in modern cervids.

species had presumably four tines (Croitor, 2014); however, as this could not be observed on the studied specimens and literature, it was scored as possessing three tines.

**Morphotype 3**

This morphotype contains the more complex or palmated antlers and is present in *Alces, Blastocerus, Cervus, Dama, Elaphurus, Odocoileus, Rangifer, Rucervus. Blastocerus dichotomus, Cervus albirostris,* and *Cervus nippon* have an antler bauplan, which produces not more than four tines in adults (accessory smaller tines not included). In *Elaphurus* it is difficult to distinguish between main tines and accessory tines. Characteristic for *Cervus elaphus* are paired lower tines, called brow tine and bez tine, and trez tine (Lister et al., 2010). *Dama dama* and *Rangifer tarandus* have a ramified palmated morphology, while *Alces alces* has a palmated morphology without ramification, and thus form a subgroup within morphotype 3. The remaining eight extant cervid species develop more complex antlers with an increasing number of tines from year to year, which is erroneously widely assumed to happen in all cervids.

Fossil cervids of the morphotype 3 include *Croizetoceros ramosus, Eucladoceros ctenoides, Lagomeryx parvulus, Ligeromeryx praestans, Arvernoceros ardei, Praeelaphus perrieri, Megaloceros giganteus,* and *Palaeoplatyceros hispanicus.* The two lagomerycids, *Croizetoceros ramosus* and *Palaeoplatyceros hispanicus* represent special cases, as their antler morphology and branching pattern is unique among living and fossil cervids. Lagomerycids possess coronate antlers without a shaft, while *Palaeoplatyceros* has palmated antlers without any other tines, and *Croizetoceros ramosus* shows a serial organisation of
small tines on the main beam. *Praelaphus perrieri* has a distally trifurcating main beam with a basal brow tine, which is similar to the condition in *Arvernoceros ardei*, where the branching part of the main beam sometimes forms a palmation. The antler morphology of *Eucladoceros ctenoides* resembles that of *Cervus elaphus* with several short proximal tines, similar to the bez and trez tine. *Megaloceros giganteus* has enormous ramified palmated antlers similar to those of *Dama*. Also characteristic for Megacerini are flattened basal brow tines similar to *Rangifer* (Lister et al., 2010).

**Dentition**

Some dental characters are highly variable and thus difficult to score unambiguously. Despite convergent modifications depending on dietary requirements, a species-specific pattern underlies these adaptations in most species (pers. obs.), particularly in the lower premolars and upper molars. The difficulty is to score these species-specific patterns without scoring the convergent adaptations and the intraspecific variability.

**Upper premolars and molars.** The upper incisors and the P1 are absent in cervids. The upper premolar row is characterised by robust, compact, predominantly horseshoe-shaped teeth. P3 and P4 are less variable, P2 can have more rectangular or triangular outlines, particularly in early fossil cervids. All premolars have at least one prominent central fold, except for *Rangifer*, in which central folds are consistently missing (Fig. 7). Sometimes there are tiny additional folds, or the main central fold is serrated. A separation of the lingual cone into an antero- and posterolingual cone is relatively common (Fig. 7). In all Miocene cervids the P2 is longer than the P4, while in extant taxa the P4 is most often longer than the P2. Several fossil species have a well developed lingual cingulum (Fig. 7).

The upper molars are all two-lobed and quadrangular with only little variation in morphology. The posterior lobe of the M3 is distinctively smaller than the anterior one in most species. The entostyles are variably present. In some species the entostyle(s) has/have a λ-shaped morphology, especially in later wear (*Axis, Rusa, Rucervus* and *Elaphurus*) (Fig. 7). Metaconule folds are variably present within Cervinae and Capreolinae and are mostly small. Protocone folds are usually absent in Cervinae, while they are regularly present in Capreolinae, often well developed on all molars (Fig. 7). The same applies to fossil cervids, where tiny metaconule folds are much more common than protocone folds. Only in Miocene cervids protocone folds are common. However, in these species it often looks more like a bifurcation of the postprotocrista than a fold originating from the crista, particularly when the internal part of this bifurcation is longer than the external as on M2 in *Dicrocerus*. It is not entirely evident, whether these are two independent structures or the same structure with variable characteristics. Several species have an anterior cingulum and some fossil cervids have a lingual cingulum. The protocone and metaconule folds are variably present. In a few species the premetaconulecrista is serrated. More details are in Heckeberg (2017a).

**Lower premolars and molars.** P1 is usually absent in cervids, although it was present in individual *Lagomeryx parvulus* specimens. The P2 has a simpler morphology with fewer elements compared to p3 and p4. A strong reduction in p2 length could be observed in *Mazama* and particularly in *Ozotoceros*. In a few specimens the p2 is missing. Mesolingual cristids were variably present in p3 and p4 (absent in *Axis*, often absent in early Miocene species) (Fig. 8). P3 and p4 often show molarisation to a different extent. While p3 is molarised only in a few species and not to the same extent as p4, the p4 is molarised in many species, at least initially, and is completely molarised in *Rangifer* and *Alces* (Fig. 9). The labial incision on premolars is rarely and weakly developed in p2; it is more often developed on p3, and most often occurs on p4 (Fig. 8). P4 is the most variable tooth in cervids.

Some species show a spike like extension of the postero labial conid of the p4 towards labiad; these species are *Capreolus capreolus, Capreolus pygargus, Blastocerus dichotomus, Hippocamelus* spp., *Hydropotes inermis, Ozotoceros bezoarticus, Croizetoceros ramosus, Procapreolus cusans*, and *Cervus philisi* (Fig. 8). Whether this feature can be used as a phylogenetic character and whether it is indicative of affiliation to a certain subclade has to be investigated in the future.

All lower molars have a similar morphology; m1 and m2 are two-lobed, m3 is three-lobed. The orientation of the lingual conids and cristids may be more diagonal in some species. Ectostylids are variably present on one to three molars. never high, nevertheless they become involved in wear in aged individuals (Fig. 8). In most Miocene cervids and in *Cervus australis* external postprotocristids are present on all molars (Fig. 8). Anterior cingulids are present in several species, usually more prominent on the anterior molar position(s). In *Rucervus* and *Rusa* the anterior cingulids are particularly prominent (Fig. 8). In *Rucervus* and also to a lesser extent in *Rusa* and *Axis* the anterior and posterior labial walls of
Figure 7. Details of the upper dentition. Close ups of the upper dentition of selected cervids showing the most striking features. (A) Rucervus duvauceli (ZSM 1957-60), (B) Rangifer tarandus (ZSM 1959-211), (C) Rucervus eldii (UMZC H16194), (D) Elaphurus davidianus (UMZC H16235), (E) Odocoileus hemionus (ZSM 1971-720).

The lobes of the lower molars are indented (Fig. 8). The metastylids can be bent labiad in some species, e.g., Alces. The third lobe on m3 is variable; most often the hypoconulid and entoconulid are connected via the postento- and posthypoconulid cristids and form a crescent-shaped structure. Sometimes the third lobe is reduced to one of these elements or has an additional fold on the posthypoconulid cristid. In a few
Figure 8. Details of the lower dentition. Close ups of the lower dentition of selected cervids showing the most striking features. (A) *Rucervus djuvaucelii* (ZSM 1957-60), (B) *Rangifer tarandus* (ZSM 1959-211), (C) *Rucervus eldii* (UMZC H16194), (D) ’*Cervus’ philisi* (NMB St.V. 605), (E) *Procervulus* (MNHN LRM 114).

377 individuals the third lobe is missing entirely. More details are in Heckeberg (2017a).

Other dentition. All Miocene cervids have enlarged upper canines, which are curved posteriad. From the Pliocene onwards, the upper canines become reduced in size and are lost in some species. Extant muntiacines have enlarged upper canines, similar to those of Miocene cervids. *Hydropotes* has strongly elongated sabretooth-like upper canines, which differ in morphology from those in muntiacines and early fossil cervids. In all other extant species upper canines are reduced in size or missing entirely. Most cervines possess small upper canines. Adult capreolines rarely have upper canines, while most capreoline juveniles have deciduous upper canines.

The lower incisors, i1–i3, have a simple spatulate morphology. The crown width decreases from i1 to i3, i.e., i1 typically is distinctively broader than i2 and i3. Exceptions are *Alces, Hippocamelus,* and *Pudu,* 385
Figure 9. Variability of p4 in cervids. This sequence of the lower left p4 shows different degrees of molarisation starting with an open anterior valley on the left, development of mesolingual cristids, connection of mesolingual cristids to other tooth elements, closing of the anterior valley, and re-arrangement of tooth elements with a diagonal orientation.

where i1 is only a little broader than i2. All lower canines in Cervidae are incisiviform. More details are in Heckeberg (2017a).

Phylogenetic Analyses

Analyses of Morphological Data

Stepping Stone Analyses. In total, five stepping stone sampling analyses were undertaken; the first set of three analyses was used to determine the partitioning scheme, running one analysis with an unpartitioned, unordered data set with the Γ distribution, one with a minimal partitioning scheme, dividing the data set into a cranial and dental character set. The third data set was run with the maximal possible partitioning scheme, dividing the data set into upper post-canine dentition, lower post-canine dentition, other dentition, mandible, viscerocranium, neurocranium and antler characters. The fourth analysis was run with the unordered, unpartitioned data set, without the Γ distribution, and the fifth analysis was run with an ordered, unpartitioned data set with Γ distribution. The decision for one hypothesis is based on the Bayes Factor (BF). The results showed that the data set is best analysed unpartitioned, using the Γ distribution and with character state ordering. However, BI and MP analyses were run unordered and ordered for each character set for comparison. See Table 1 for details. Figure 10 provides a key to the colour coding of the taxonomic groups.

Figure 10. Colour code. The colour code provides the key to taxonomic groups studied here and applies to all topologies within the present work.

Standard Phylogenetic Analyses. The MP topologies of the unordered and ordered morphological data set do not contradict each other (Fig. 11). The topology based on the unordered data set is more resolved. Both topologies support monophyletic Capreolini, a sister taxon relationship of Axis axis and
the clade, the **Elaphurus-**Rusa-Elaphurus clade was always recovered in the analyses based on the genetic and combined data set, in most topologies fully resolved. It consists of the Rusa-clade, which often has Rusa-Rucervus-Elaphurus clade. The Rusa-Rucervus-Elaphurus clade and an analysis of the combined morphological data set are shown. The left topology used the unordered dataset, the topology on the right used character state ordering. Node support values are given as bootstrap support values.

Figure 11. Topologies from the morphological analyses.
Some extant clades were recovered, e.g., Muntiacini, Odocoileina, Capreolini. Eight Miocene cervids were constraint as monophyletic polytomous to each other. In each of the 93 analyses only one fossil Dremotherium feignouxi, based on the correct

The BI and ML topologies of the combined nuclear and mitochondrial Combined Molecular Analyses.

Combined Mitochondrial Genes. The BI topology of the combined mitochondrial analysis showed higher support values for the majority of nodes than the Cytb only topology, but lower support values for some nodes than for the mtG analysis. The ML topology differed in generally lower support values for most nodes, but was otherwise largely congruent (Fig. 13). The placement of non-cervid ruminants differed in both topologies. The main difference concerning cervid taxa is the position of Capreolus in both topologies. The placement of non-cervid ruminants differed some nodes than for the mtG analysis. The ML topology differed in generally lower support values for most

Analyses of Molecular Data

Nuclear Genes. Although interpretations of the systematic relationships on genus and species level was difficult in the single gene topologies due to low taxon sampling and/or lack of resolution, the combined nuclear topology was well resolved and supports the higher hierarchical clades. The BI and the ML topologies were largely congruent (Fig. 13). There was no split into Odocoileina and Blastocerina as observed in the topologies based on the mitochondrial markers. The unexpected placement of Capreolus capreolus in this topology may be caused by the possibly contaminated Sry sequence of this species.

Combined Mitochondrial Genes. The BI topology of the combined mitochondrial analysis showed higher support values for the majority of nodes than the Cytb only topology, but lower support values for some nodes than for the mtG analysis. The ML topology differed in generally lower support values for most nodes, but was otherwise largely congruent (Fig. 13). The placement of non-cervid ruminants differed in both topologies. The main difference concerning cervid taxa is the position of Pudu mephistophiles (based on the correct Cytb sequence (Heckeberg et al., 2016)), which was the sister taxon to Blastocerina in the BI topology and the sister taxon to Rangifer and Odocoileini in the ML topology. This combined topology includes the polyphilies for Rucervus, Hippocamelus, Odocoileus, Mazama, and Pudu.

Combined Molecular Analyses. The BI and ML topologies of the combined nuclear and mitochondrial analyses were largely congruent, the support values were partly lower, particularly in the ML topology, in comparison to the topologies based on the mitochondrial markers (Fig. 13). Both topologies differed in the position of non-cervid ruminants, and the positions of Alces alces and Pudu mephistophiles, which remain uncertain. The split of Odocoileini into Blastocerina and Odocoileini was supported.

Combined Molecular and Morphological Analyses

Bayesian Inference. The BI combined topology was largely unresolved (Fig. 14). Most extant cervids formed clades; the three Axis species and two Rucervus species formed a well supported clade. There was also an supported clade including eight Miocene cervids.

Maximum Likelihood In the ML combined topology the nodes were poorly or not at all supported (Fig. 14). Some extant clades were recovered, e.g., Muntiacini, Odocoileina, Capreolini. Eight Miocene cervids formed a clade.
sets support the respective nodes. Certain exceptions to the original hypothesis came from clades: All Miocene Muntiacini, and Odocoileini form unsupported clades. Capreolini is a supported clade. All Miocene forms a clade.}

Figure 15 qualitatively summarises the topologies from all analyses undertaken. The topology was not generated by an analysis but was drawn to show the consensus of all topologies and which character
Figure 13. Topologies resulting from the molecular data sets. The topologies of the Bayesian inference analyses of the combined nuclear data set, the combined mitochondrial data set and the combined molecular data set (i.e., nuc + mt) are shown. Node support values are given as Bayesian posterior probabilities.
Figure 14. Topologies from the combined molecular and morphological analyses. The topologies were obtained with maximum posterior probability (B) and bootstrap support values (ML).
Figure 15. The qualitative summary topology of all analyses is shown. (A) represents the overview of the systematic relationships of higher cervid taxa, including the positions of some fossil cervids. (B) shows the systematic relationships of several Plio- and Pleistocene cervids. (C) shows the systematic relationships of Miocene cervids.

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DISCUSSION

For the first time, fossil and extant cervid species were combined in the so far most extensive data set including molecular and morphological data. Various data sets and partitions were analysed under different optimality criteria. In addition, the alternative approaches SFA and EPA were undertaken to investigate the systematic positions of fossils. The results provided new insight into the systematic relationships of fossil cervids and extant cervids. Many hypotheses about the systematic relationships of extant taxa could be confirmed; however, known controversies persisted, but could be specified in more detail. For most fossil cervids, we were able to find an affiliation to extant relatives, which has not been quantitatively tested previously.

Combining different data types helped to investigate the systematic relationships in detail and to reconstruct the evolutionary history of cervids. The initial separate analyses of the different data sets provided insights into the phylogenetic signal of the respective data. Some areas of the morphological topologies were congruent with the molecular topologies, some were not. However, the support of the morphological topologies did not contradict the molecular hypotheses.

Genotypic data partitions usually contain proportionally more characters than osteological data, which is assumed to be crucial for accuracy. On the other hand, osteological data partitions can be sampled for many more taxa, which partly cannot be sampled for molecular data (fossils) (O’Leary, 1999). Thus, morphological characters still have relevance in times of genomic analyses and serve as an independent test for molecular data, because of the relative distance between phenotype and genotype and different evolutionary dynamics of both types of data. Because selection targets on the phenotype, the resulting topology could potentially provide information on the selective history of taxa (Lee and Camens, 2009; Groves, 2014). If the same topology is supported by different data sources and reflects biological evidence at all scales (principle of consilience), it is more likely that the topology is ‘correct’ (Bibi et al., 2012).

The challenges of the data sets here were the high levels of homoplasy (particularly in the morphology) and the rapid radiations of ruminant tribes. Consensus might be difficult to achieve, because short branch lengths and/or lack of resolution potentially represent a genuine rapid diversification of clades, which may not be further solved just by increasing the sequence length or the taxon sampling. Markers that are less influenced by convergent evolution, such as rare genomic changes or cytogenomics may be useful additions in the future (Rokas and Holland, 2000; Price et al., 2005; Hernández Fernández and Vrba, 2005).

Models of evolution. So far, there is no appropriate evolutionary model for morphological characters in model-based approaches such as BI and ML (O’Reilly et al., 2016). The only model of morphological evolution, which is widely used in model-based phylogenetic algorithms (BI, ML), is the Markov k (Mk) model by Lewis (2001). It is not fully understood how the standard models of molecular evolution (e.g., HKY, GTR) translate variable rate frequencies and substitution rates to morphological data (Spencer and Wilberg, 2013). Although topologies from model-based approaches, particularly ML, are typically better resolved than strict consensus topologies from parsimony analysis, the better resolution is not necessarily meaningful. The apparent better resolution may simply be a result of an incorrect model of morphological evolution (Spencer and Wilberg, 2013).

Comparison of mitochondrial vs. nuclear vs. total evidence topologies. Previous studies demonstrated that combining mitochondrial and nuclear markers increases robustness of higher hierarchical cervid clades (Randi et al., 1998). The topologies resulting from nuclear markers often agree with morphology, but often contradict topologies resulting from mitochondrial markers (Bibi, 2014). There are few phylogenetic reconstructions for cervids based on nuclear markers (Cronin et al., 1996; Gilbert et al., 2006). Analyses of nuclear markers have the potential to characterise the distribution of genetic variation (Balakrishnan et al., 2003). Combining and interpreting nuclear and mitochondrial markers can help to uncover recent hybridisation events, as in Elaphurus davidianus, which takes up different positions when analysed with mitochondrial markers compared to nuclear markers (Fig. 13).

The nuclear topologies here, were largely congruent to those published previously. Incorporating more nuclear DNA is crucial to test relationships in ruminant systematics based on mitochondrial DNA and should be sequenced for a broader range of taxa than is available to date.
Miocene Cervids

The earliest cervids are from the mid early Miocene (MN3) represented by *Procervulus*, *Ligeromeryx*, and *Acteocemas* and became more numerous and widely distributed during the Miocene. In the late early and early middle Miocene *Stephanocemas*, *Heteroprox*, *Lagomeryx*, and *Dicrocerus* appeared (Ginsburg and Azanza, 1991; Dong, 1993). A low cervid diversity is assumed during the late Miocene and all typical Miocene cervids became extinct before the late Miocene (Ginsburg and Azanza, 1991; Böhme et al., 2012).

It was suggested to put *Lagomeryx*, *Procervulus*, *Heteroprox*, *Euprox*, *Dicrocerus*, *Stephanocemas* into a subfamily as a ‘primitive’ clade within Cervidae (Azanza, 1993b; Ginsburg, 1985; Rössner, 1995). Miocene cervids were usually considered to be distant from crown cervids representing a distinct group of stem cervids. They were subdivided into Lagomercyinae(-dae), Procervulinae (-dae) and Dicrocerinae (-ini). All of them were regarded as sister clades to Cervidae (Mennecart et al., 2016). It was suggested that *Lagomeryx*, *Ligeromeryx*, and *Paradicrocerus* form the lagomerycids, *Heteroprox* and *Procervulus* form the procervulines, and *Acteocemas*, *Stehlinoceros*, and *Dicrocerus* form the dicrocerines (Gentry et al., 1999). In none of the analyses here this split into three groups was distinctive. So far, not many attempts to reconstruct the phylogeny of Miocene cervids have been made (Azanza Asensio, 2000).

Recently, Mennecart et al. (2016, 2017) presented the first phylogenetic analyses based on inner ear characters for several fossil cervids.

In the phylogenetic analyses here, Miocene cervids were most often placed either between the outgroup and all other cervids, mostly unresolved; sometimes a few taxa formed a clade. The placement between the outgroup and other cervids was expected from their temporal distribution and their shared higher proportion of plesiomorphic characters. The systematic relationships within early Miocene cervids have been and still are controversial. (Rössner, 1995; Azanza et al., 2013).

*Lagomeryx parvulus* and *Ligeromeryx praestans*. Qualitative morphological comparisons, especially on antler morphology, suggest that *Lagomeryx parvulus* and *Ligeromeryx praestans* are closely related to each other. Only one analysis (cranial data set) here supports the sister taxon relationship of these two taxa. Therefore, a subfamily Lagomercyinae would be justified based on morphological qualitative comparisons, but is not supported in the topologies. Data completeness or presence of specific characters that are absent in the other taxon could be the reasons. Also, whether lagomerycids form a family as the sister taxon to Cervidae could not be entirely ruled out, but the tendency of *Ligeromeryx*, *Lagomeryx*, and *Palaeoplatyceros* to form a clade within a clade consisting of Miocene taxa indicated that lagomerycids potentially form a subfamily of Cervidae in a stem position.

The systematic position of lagomerycids, has always been controversial. They have been considered as a family between Giraffidae and Cervidae (Teilhard de Chardin, 1939), as part of the superfamily Cervoidea (Romer, 1966; Viret, 1961; Young, 1964), as a separate subfamily within Cervidae (Vislobokova et al., 1989), as a family of aberrant giraffoids, as a junior synonym of Palaeomerycidae (Pilgrim, 1941; Simpson, 1945; Young, 1964), as junior synonym of Muntiacini/-ae (Chow and Shih, 1978), as more closely related to Antilocapridae (Ginsburg, 1985; Solounias, 1988), or as representing an entirely independent clade (Bubenik and Bubenik, 1986; Azanza, 1993b; Azanza and Ginsburg, 1997).

The discussions on the taxon in the literature and the new insights resulting from the analyses here clearly show that the systematic position of Lagomerycidae represents one of the most controversial of ruminant families, so far without unambiguous consensus; however, cranial and postcranial morphology support the affiliation as stem Cervidae (Chow and Shih, 1978; Leinders and Heintz, 1980; Vislobokova et al., 1989; Azanza and Ginsburg, 1997; Mennecart et al., 2017).

*Procervulus dichotomus* and *Procervulus praelucidus*. In most analyses here, *Procervulus* was placed in a stem position and *Procervulus* and *Dicrocerus* were more closely related to each other than to other cervids. A sister taxon relationship of *Procervulus* and *Heteroprox* was not observed. In the combined morphological and TE analyses, a close relationship of *Procervulus dichotomus* and *Procervulus praelucidus* to *Dicrocerus elegans* was confirmed.

*Procervulus* was assumed to be the Miocene descendant of *Amphitragulus* and *Dremotherium* (Gentry, 1994; Rössner, 1995). Presumably, transitional forms existed, which were not documented in the fossil record (Rössner, 1995). *Procervulus* has often been hypothesised to be the sister taxon to all other cervids (Janis and Scott, 1987; Groves, 2007). In previous studies, *Procervulus* was placed as the sister taxon to *Heteroprox* Azanza Asensio (2000); Mennecart et al. (2016) and both were the sister taxon to the clade...
containing *Dicrocerus elegans*. In Mennecart et al. (2017) *Procervulus dichotomus* was the sister taxon to *Heteroprox larteti* and *Procervulus praelucidus* the sister taxon to both of them; this clade was placed between *Lagomeryx parvulus* and all other cervids, which is similar to our results.

**Heteroprox larteti.** In the analyses here, *Heteroprox larteti* was most often placed in an unresolved position, between the outgroup and cervids, as the sister taxon to *Euprox furcatus* or *Dicrocerus elegans*, or in a clade with other Miocene taxa (morphology, TE). Some topologies indicated a potential closer relationship to Muntiacini based on apomorphic characters, similar to *Euprox furcatus*.

*Heteroprox* was assumed to be the descendant of *Procervulus* (Rössner, 1995). In Azanza Asensio (2000) *Heteroprox* was most often placed as the sister taxon to *Procervulus* or as an (unresolved) stem lineage. Similarly, in Mennecart et al. (2017) *Heteroprox larteti* was the sister taxon to *Procervulus dichotomus*.

**Dicrocerus elegans.** In the analyses here, *Dicrocerus elegans* was most often placed closely related to *Procervulus*, sometimes as the sister taxon to *Heteroprox larteti*, or between the outgroup and cervids. Based on our results and discussions in the literature, *Dicrocerus* is most certainly a stem cervid with affinities primarily to *Procervulus* and secondarily to other Miocene cervids. In a few analyses a potentially closer relationship to Muntiacini was observed.

Azanza et al. (2011) suggested that *Dicrocerus* is a transitional form between the Procervulinae and crown Cervidae, which had also been hypothesised by Vislobokova (1990). In Azanza Asensio (2000) *Dicrocerus elegans* was placed as the sister taxon to *Acteoceras* and *Stehlinoceras (=Paradicrocerus)* and this clade was the sister taxon to all burr-bearing antlered cervids. In Mennecart et al. (2017) *Dicrocerus elegans* was the sister taxon to *Eostyloceros hezhengensis* in a sister taxon position to the crown cervids.

**Euprox furcatus.** In the TE analyses here, *Euprox furcatus* was most often placed in an unresolved position or as the sister taxon to *Heteroprox larteti*; in the TE analyses it was placed in a clade with other Miocene cervids. The results indicate that *Euprox furcatus* shares characters with other Miocene cervids, but also already had apomorphic characters, which imply a closer relationship to extant Muntiacini than to other crown cervids.

It was suggested that modern *Muntiacus* and fossil muntiacines such as *Eostyloceros*, *Metacervulus*, and *Paracervulus* diverged from *Euprox* (Vislobokova, 1990; Croitor, 2014). *Euprox* was the first cervid with burr-bearing antlers and a pedicle inclination similar to that of muntjacs. Therefore, it has been suggested in several studies that *Euprox* may be the earliest representative of crown cervids (Azanza, 1993b; Gentry et al., 1999; Dong, 2007; Azanza et al., 2013; Mennecart et al., 2016, 2017). It was often considered as a member of Muntiacini, which would imply that Muntiacini is the sister taxon to all other cervids. In Azanza Asensio (2000), *Euprox* is variably placed closely related to *Amphiprox*, to extant *Muntiacus* and *Elaphodus*, to *Eostyloceros*, or to *Metacervulus*, or as the sister taxon to a clade containing all five of the above species or a subset thereof. In Mennecart et al. (2016), *Euprox furcatus* was placed as the sister taxon to *Cervus elaphus*. They further stated that *Dicrocerus elegans*, *Euprox furcatus*, and *Cervus elaphus* differ from the other Miocene cervids, i.e., Procervulinae, in certain inner ear characters; *Euprox furcatus* had the most derived characters among them. In Mennecart et al. (2017) *Euprox furcatus* was placed as the sister taxon to all crown cervids.

There is a large temporal gap in the early putative fossil muntjac-like cervid lineage between the first representatives, *Euprox*, and the presumed direct ancestors of muntiacines, e.g., *Eostyloceros* (Azanza Asensio and Menendez, 1989; Azanza, 1993b), and additionally an even larger gap between those early fossils and the first members of extant *Muntiacus*, which appear in the Pleistocene. For more certainty of the systematic relationships it would be crucial to find more fossil material that would link the early presumed muntiacines with the crown muntiacines.

**Palaeoplatyceros hispanicus.** In most analyses here *Palaeoplatyceros hispanicus* was placed between the outgroup and cervids, as the sister taxon to *Lagomeryx parvulus* or as the sister taxon to most other Miocene taxa. *Palaeoplatyceros* is highly incomplete and has a combination of plesiomorphic traits and apomorphic traits, such as ‘presence of a burr’.

*Palaeoplatyceros hispanicus* can be distinguished from all other contemporaneous cervid species based on the palmination of antlers; however, its systematic position is problematic (Azanza Asensio, 2000). In Azanza Asensio (2000), *Palaeoplatyceros* was mostly placed as the sister taxon to all other cervids with burr-bearing antlers. Unless more material becomes available, its systematic position will remain uncertain.
controversial. Based on the analyses here, Palaeoplatycteros is likely a stem cervid with burr-bearing antlers.

**Pliocervus matheronis.** Pliocervus matheronis is known from the Messinian (upper Turolian, MN13). In the analyses here, *Pliocervus matheronis* was most often placed in an unresolved position, mostly between the outgroup and cervids and sometimes related to other Miocene taxa.

Although Simpson (1945) included Pliocervinae, comprising Cervocerus, Cervavitus, Procervus, and *Pliocervus*, which were regarded as the immediate crown Cervini precursors (Gentry, 1994; Groves, 2007), in Cervinae, others could not find any phylogenetic relationship of *Pliocervus* with Cervini/Cervinae (Petronio et al., 2007). Gentry et al. (1999) placed *Cervavitus* and *Pliocervus* among Cervidae, whereas Azanza and Montoya (1995) and Azanza Asensio (2000) classified *Pliocervus* as Cervinae. It was suggested to be closely related to the holometacarpal *Cervavitus* within Pliocervini, which was included in Cervinae (Czyżewska, 1968; Vislobokova, 1990; Azanza Asensio, 2000).

The high morphological similarity of *Pliocervus matheronis* to the late Miocene Pavlodaria orlovi implies that these two genera could be closely related or possibly even synonymous. It was suggested that the subfamily Pliocervinae Symeonidis 1974, containing *Pliocervus* and *Pavlodaria* is a synonym of Capreolinae. In Azanza Asensio (2000) *Pliocervus matheronis* was variably placed and seems to have the highest proportion of apomorphic characters compared to other Miocene cervids. In most recent studies *Pliocervus* was regarded as incertae sedis (Croitor, 2014).

A definite morphological characterisation of *Pliocervus* is still missing and its systematic position remains controversial (Godina et al., 1962; Czyżewska, 1968; Korotkevich, 1970; Azanza Asensio, 2000; Petronio et al., 2007; Croitor, 2014). More and new morphological and biometric data are needed to solve the systematic relationships of *pliocervines* (Di Stefano and Petronio, 2002).

**Eostyloceros hezhengensis.** Eostyloceros hezhengensis from the late Miocene of China was used for scoring characters (Deng et al., 2014). In the analyses here, *Eostyloceros hezhengensis* was most often placed in an unresolved position or within Muntiacini, suggesting that it is probably more closely related to muntjacs than to other cervids, which would support results from comparative morphology. Thus, *Eostyloceros hezhengensis* can be considered as a direct ancestor of muntjacs.

*Euprox* is considered as the direct ancestor of *Eostyloceros, Metacervulus*, and *Paracervulus*; after a change from subtropical to more temperate climate and *Euprox*-like cervids were replaced by representatives of *Eostyloceros* (Azanza Asensio and Menendez, 1989; Azanza, 1993b; Pitra et al., 2004). This lineage is assumed to lead to extant *Muntiacus* (Vislobokova, 1990; Croitor, 2014). In Azanza Asensio (2000), *Eostyloceros* was always closely related to *Muntiacus* and *Metacervulus*, while in Menecart et al. (2017) *Eostyloceros hezhengensis* was not placed within Muntiacini but was the sister taxon to *Diceros elegans*.

**Pliocene and Plio-Pleistocene Cervids**

There is no generally accepted classification of Plio- and Plio-Pleistocene cervids Pfeiffer (1999); however, for Villafranchian cervids (MN16) the following classifications were suggested: Croizetoceros ramosus, Metacervus parvadinensis, *Cervus* philisi, *Cervus* perolensis, Eucladoceros crassidentes were considered as Cervini, Arvernoceros ardei as Megacerini, and Libralces gallicus (not included here) and Procapreolus cusanus were considered as Capreolinae.

In most morphological topologies here, Plio- and Pleistocene cervids were placed within crown cervids, sometimes forming subclades. Some Plio- and Pleistocene cervids were placed more closely related to extant Cervini. Most of them were nested in a clad together with Pleistocene cervids. In a few topologies the majority of Pliocene cervids were in an unresolved sister taxon position to all other Cervidae.

**Cervus australis.** In the phylogenetic analyses here, *Cervus australis* was most often placed in an unresolved position, sometimes closer to Muntiacini than to other cervids; it was also placed between the outgroup and cervids, as the sister taxon to *Eostyloceros hezhengensis* and *Praeelaphus etueriarum*, to *Hippocamelus bisulcus*, or *Muntiacus muntjak*. Based on qualitative morphological comparisons it is most likely a stem cervid, potentially closer to Muntiacini.

This species was originally described by De Serres (1832) and all known specimens are from Montpellier, France (Gervais, 1852; Czyżewska, 1959). Little further information is available in the literature concerning this species. Many entries point to muntiacines, e.g., *Paracervulus australis* (Gentry,
Arvernoceros ardei. In our analyses Arvernoceros ardei was placed in an unresolved position, often close to or within Cervini. In some topologies it was placed as the sister taxon to Metacervocerus pardinensis, Praelaphus perrieri, and Metacervocerus rhenanus. It was placed as the sister taxon to Dama dama in several topologies.

Arvernoceros was part of the first radiation of Cervinae/i together with Metacervocerus, Praelaphus, Axis, and Rucervus (Croitor, 2014). The systematic position of Arvernoceros ardei has been subject to speculation for decades, its definition is still incomplete and affinities to other cervids unclear. Depéret (1884) found similarity to Axis, but no affiliation to Dama; it was suggested that it is most similar to Megacerini (Heintz, 1970; Vislobokova, 1990, 2012). Arvernoceros ardei was considered to be closely related to modern Elaphurus (Teilhard de Chardin and Piveteau, 1930), declared as incertae sedis genus by (Lister, 1987), closely related to Axis Di Stefano and Petronio (2002), closely related to Rucervus (Croitor, 2009, 2018). Despite some uncertainties in the morphological analyses, a closer relationship to Dama dama than to other cervids was suggested here.

Croizetoceros ramosus. In most of the analyses here, Croizetoceros ramosus was placed in an unresolved position; it was sometimes the sister taxon to Procapreolus cusanus, Alces alces, Ozotoceros bezoarticus, or Odocoileus. Our results suggest a placement within Capreolinae and most likely within Odocoileini.

The antler morphology of Croizetoceros ramosus does not share similarities with any extant cervid species or with other cervid species from the Villafranchian (Heintz, 1970). Unfortunately, there is not much known about its skull morphology (Croitor, 2014). In Mennecart et al. (2017) Croizetoceros was placed as the sister taxon to Capreolinae.

‘Cervus’ perolensis. In the analyses here, ‘Cervus’ perolensis was placed in an unresolved position and as the sister taxon to several cervine taxa. Repeated placements within Cervini suggest that ‘Cervus’ perolensis almost certainly belongs to Cervini and is likely closely related to and/or an ancestor of Cervus.

‘Cervus’ perolensis, Metacervocerus rhenanus, and ‘Cervus’ philisi were found to be similar to each other and ‘Cervus’ perolensis and Metacervocerus pardinensis were classified as Pseudodama Azzaroli (1953); Azzaroli and Mazza (1992a). Later, ‘Cervus’ perolensis was considered as a descendant of ‘Cervus’ philisi by Stefaniak and Stefaniak (1995). Spaan (1992), however, concluded that ‘Cervus’ philisi and ‘Cervus’ perolensis are junior synonyms of Metacervocerus rhenanus and should be renamed as such, which was supported by Pfeiffer (1999). If this were true, ‘Cervus’ philisi and ‘Cervus’ perolensis should come out in a similar systematic position as Metacervocerus rhenanus.

Procapreolus cusanus. In the analyses here, Procapreolus cusanus was placed between the outgroup and cervids, within Capreolinae, sometimes within Odocoileini, and as the sister taxon to both Capreolus. Thus, Procapreolus cusanus most likely belongs to Capreolinae and the previously suggested close relationship to Capreolus was confirmed in some analyses.

Despite the widely accepted assumption that Procapreolus cusanus is closely related to or even a direct ancestor of Capreolus, the origin of Capreolus within Procapreolus is still under debate (Lechner-Doll et al., 2001). Some authors hypothesise that it may be assigned to Capreolus rather than Procapreolus (Valli, 2010). Others place it in an intermediate position between lower Pliocene and Pleistocene Procapreolus species and extant Capreolus (Czyżewska, 1968; Heintz, 1970; Lechner-Doll et al., 2001).

Metacervocerus pardinensis. In the analyses here, Metacervocerus pardinensis was most often closely related to or within Cervini, which suggests that Metacervocerus pardinensis is a member of Cervini and probably a close relative and/or ancestor of Cervus.

The temporal distribution of Metacervocerus pardinensis suggests that it could be an ancestor of ‘Cervus’ philisi. Metacervocerus pardinensis and Metacervocerus rhenanus have enough morphological differences to justify two different species (Spaan, 1992). Dietrich (1938) proposed that Metacervocerus pardinensis is synonymous with etueriarum, perrieri, issiodorensis, and rhenanus. Based on similarities to Rusa deer, the genus Metacervocerus was erected to represent European rusine deer (Croitor, 2006a). However, their systematic position remained controversial. Metacervocerus pardinensis was classified as Pseudodama by Azzaroli and Mazza (1992a), while De Vos and Reumer (1995) assigned Metacervocerus
Dama and Metacervocerus rhenanus to Cervus, Pfeiffer (1999) to Dama, and Di Stefano and Petronio (2002) to Rusa. Differences in the skull morphology suggest that Metacervocerus does not belong to the Cervus-Rusa evolutionary lineage, which needs stronger evidence from the fossil record. Croitor (2014) suggested it is more likely that Metacervocerus pardinensis represents an ancestor of Dama.

**Praelapheus perrieri.** In the analyses here, Praelapheus perrieri was placed close to or within Cervini, which suggests that Praelapheus perrieri is a member of Cervini and probably closely related to and/or the ancestor of Cervus.

The teeth and postcranial material from Praelapheus perrieri and Eucladoceros are indistinguishable; however, Praelapheus perrieri and Eucladoceros cteneoides do not coexist in any of the known localities, although they occupy the same niches. The systematic relationships remained uncertain (Croitor, 2014). Already Portis (1920) proposed a new subgenus Praelapheus for ‘Cervus’ perrieri, as well as for C. avernensis, C. etueriarum from the early Villafranchian (Croitor, 2014). Praelapheus perrieri was considered as the earliest representative of Cervus in Europe by Di Stefano and Petronio (2002), however, even though it is an early cervine, there is no clear evidence that it is directly related to Cervus and it more likely represents an extinct lineage within the early cervine evolution (Croitor, 2014).

**Praelapheus etueriarum.** In the analyses here, Praelapheus etueriarum was placed between Eostyloceros and Eucladoceros cteneoides, as the sister taxon to Metacervocerus rhenanus, Eostyloceros, or Eucladoceros cteneoides. Placements as the sister taxon to the Cervus-clade and within Muntiacini suggest that Praelapheus etueriarum belongs to Cervinae and most likely to Cervini.

There is consensus that Praelapheus is a member of the early radiation of Cervini and Perrieri, warthae, and lyra may be synonyms as they represent similar and contemporaneous cervids (see above) (Croitor, 2014). Heintz (1970) suggested that Praelapheus etueriarum was established based on a juvenile Praelapheus perrieri, which is yet to be proven.

**Eucladoceros cteneoides.** Here, Eucladoceros cteneoides was most often placed within Cervinae and/or Cervini, which also indicate a potentially close relationship to Cervus.

Most of the previously defined Eucladoceros species were synonymised with Eucladoceros cteneoides (Azzaroli and Mazza, 1992a; De Vos and Reumer, 1995; Pfeiffer, 1999; Croitor and Bonifay, 2001; Valli and Palombo, 2005). ‘E. senecensis’ has been suggested to be an ancestor of Megaceroides or Megaceros giganteus in particular (Azzaroli and Mazza, 1992a,b; Kuehn et al., 2005). Pfeiffer (2002) proposed that Eucladoceros, Megaceros, and Cervus form a group. Flerov (1952) suggested that Eucladoceros is an ancestor of Alces, which is not supported by others (Heintz, 1970; Croitor, 2014). The comb-shaped antler morphology is unique and more similar to Cervus elaphus or Cervus albirostris than to any other living cervid (pers. obs.). Because upper canines in Eucladoceros cteneoides are absent it was interpreted that the genus most likely does not belong to the Cervus-Rusa-lineage (Croitor, 2014); instead, Eucladoceros cteneoides was hypothesised as a descendant of an early three-tined ancestor of Axis or Metacervocerus (Croitor, 2014). In Mennecart et al. (2017) Eucladoceros cteneoides was placed as the sister taxon to the Cervus-Rusa-clade, which confirms the results from our analyses.

**Metacervocerus rhenanus.** In the analyses here, Metacervocerus rhenanus was mostly placed as the sister taxon to Cervini and/or within Cervinae, which suggests that Metacervocerus rhenanus is a member of Cervini and potentially is either a close relative and/or ancestor of Cervus or Axis.

The genus Metacervocerus was established by Dubois (1904) as Cervus (Axis) rhenanus for the small sized deer from Tegelen. Spaan (1992) synonymised ‘Cervus’ philisi from Senéze with ‘C’. rhenanus based on dentition and antler morphology. Croitor and Bonifay (2001) assigned it to the genus Metacervocerus. Several three-tined cervids were described from the early Pleistocene of Europe (De Vos and Reumer, 1995); Metacervocerus rhenanus was considered to include ‘C’. philisi, ‘C’. perolensis, C. ischnoceros, and Pseudodama lyra and ‘Cervus’ philisi was suggested to be a junior synonym of Metacervocerus rhenanus (Azzaroli et al., 1988; Spaan, 1992). Metacervocerus rhenanus was hypothesised to be an ancestor of Dama dama (Pfeiffer, 1999; Di Stefano and Petronio, 2002); however, this hypothesis was ruled out by the coexistence of both genera in the early Pleistocene (Croitor, 2014).
From the analyses based on the present data sets, the synonymy of ‘Cervus’ philisi and ‘Cervus’ perolensis with Metacervocerus rhenanus could not be confirmed. All analyses placed the three taxa differently and not closely related to each other. This may be caused by the differing availability of characters for each taxon and should be tested based on exclusively overlapping characters.

**Pleistocene Cervids**

In the early Pleistocene, Pliocene forms were successively replaced by more modern cervids. By the middle Pleistocene, most Pliocene and some early Pleistocene cervids became extinct, while extant representatives appeared (Dong, 1993).

Pleistocene cervids are more similar to extant forms. In the morphological topologies, similarly to the Plio- and Plio-/Pleistocene cervids, the Pleistocene cervids were distributed across crown group clades, sometimes forming subclades. The majority of Pleistocene cervids were placed within Cervini.

**‘Cervus’ philisi.** In the analyses here, ‘Cervus’ philisi was most often placed within Cervinae or Cervini sometimes within the extant Cervus-clade, which suggests that ‘Cervus’ philisi belongs to Cervini with a potentially closer relationship to Cervus. The results further support previous findings that ‘Cervus’ philisi cannot be assigned to any extant cervid (except maybe Cervus nippon). ‘Cervus’ philisi together with Praealaphus perrieri potentially represents an extinct clade leading to Cervus. The suggested synonymy of Metacervocerus rhenanus, ‘Cervus’ philisi, and ‘Cervus’ perolensis could not be supported in the analyses.

In the past, ‘Cervus’ philisi was considered to be related to Axis (Depéret and Mayet, 1911), to Rusa (Stehlin, 1923; Viret, 1954), and to Cervus nippon (Schaub, 1941). Heintz (1970) suggested an evolutionary Metacervocerus pardinensis-‘Cervus’ philisi-‘Cervus’ perolensis-lineage. However, the temporal occurrence of these species in the fossil record contradicts this hypothesis. It was suggested that ‘Cervus’ perolensis is the descendant of ‘Cervus’ philisi (Stefaniak and Stefaniak, 1995; Croitor, 2006a, 2014) and that Metacervocerus rhenanus from Tegelen and ‘Cervus’ philisi from Senéze are synonymous and that ‘Cervus’ philisi and ‘Cervus’ perolensis are junior synonyms of Metacervocerus rhenanus (Spaan, 1992). Later, ‘Cervus’ philisi was included in the genus Metacervocerus (Croitor and Bonifay, 2001; Croitor, 2006a). In Mennecart et al. (2017) ‘Cervus’ philisi was placed closely related to Axis and Rucervus duvaucelii.

**‘Cervus’ sivalensis.** The remains of ‘Cervus’ sivalensis resemble Rucervus duvaucelii in morphology and size and Rucervus eldi in antler morphology (Azzaroli, 1954). Here, ‘Cervus’ sivalensis was placed as the sister taxon to Megaloceros giganteus to a clade consisting of Axis lydekkeri, Rusa kendengensis, and Metacervocerus pardinensis to Metacervocerus pardinensis, to the Elaphurus-Rucervus-Rusa-clade, or in a polytomy with Metacervocerus pardinensis and Cervus canadensis within the Cervus-clade. The placements within Cervini and close to the Cervus-clade show that ‘Cervus’ sivalensis belongs to Cervini and is most likely closely related to Cervus, Rusa, and/or Rucervus. Together with Axis lydekkeri it could belong to the ancestral group of cervids that leads to Axis, Cervus, Rusa, and Rucervus. Although the tooth morphology of ‘Cervus’ sivalensis resembles that of Rucervus (pers. obs.), a placement closely related to Rucervus could not be found. There is still a lot of confusion concerning the taxonomy and systematics of this taxon and a revision is needed (Lydekker, 1884; Azzaroli, 1954; Arif et al., 1991; Samiullah and Akhtar, 2007).

**Axis lydekkeri.** Even though Axis lydekkeri is a fairly complete fossil and despite the morphological similarities to Axis, Axis lydekkeri was not placed as closely related to extant Axis in our analyses. Here, Axis lydekkeri was mostly placed as the sister taxon to or within Cervini, or within the Cervus-clade, which shows that Axis lydekkeri belongs to Cervini.

Axis lydekkeri was suggested to be more closely related to the smaller Axis species of today (‘Hyelaphus’) than to Axis axis, but a clear systematic relationship to any of them could not yet be confirmed (Zaim et al., 2003; Meijaard and Groves, 2004).

**Rusa kendengensis.** In the analyses here, Rusa kendengensis was most often placed within Cervini and sometimes as the sister taxon to the Cervus-clade, which shows that Rusa kendengensis belongs to Cervini. Even though based on comparative anatomy it is more similar to Rusa, the analyses placed it more closely to Cervus. Rusa kendengensis potentially belongs to an extinct group of ancestors including also Axis lydekkeri and ‘Cervus’ sivalensis, which gave rise to modern Axis, Cervus, and Rusa.
There is little information about *Rusa kendengensis* in the literature; the only study on this species reported that it belongs to *Rusa* and not to *Cervus* as previously assumed for most Pleistocene cervids from Java (Zaim et al., 2003). More material of this species is needed to further investigate its systematic relationships.

**Candiacervus ropalophorus.** In the analyses here, *Candiacervus ropalophorus* was often placed close to several fossil cervine taxa and/or within Cervinae; in the SFA it was placed within Odocoileini. The investigated *Candiacervus ropalophorus* specimens were fairly complete; therefore, it was unexpected that this taxon was difficult to place. Frequent placements as the sister taxon to Cervini or within Cervini indicated that *Candiacervus ropalophorus* belongs to Cervini. The often hypothesised close relationship to megacerine/damine deer could only be found in one topology.

For *Candiacervus ropalophorus*, up to six different size groups representing six taxonomic units, sometimes even eight morphotypes have been suggested, but with differing views on the actual taxonomic affiliations Simonelli (1907, 1908); Kuss (1975); Kotsakis and Palombo (1979); De Vos (1979, 1984, 2000); Van der Geer et al. (2006). *Candiacervus ropalophorus* is the smallest species of the eight morphotypes. Since no cranial material can be unambiguously assigned to *Candiacervus cretensis* or *Candiacervus rettynensis*, only *Candiacervus ropalophorus* can be considered as clearly recognisable species based on cranial and postcranial elements (De Vos, 1984).

The systematic position of *Candiacervus* is controversial; a close relationship to *Megaceros*, Praemegaceros, Eucladoceros, *Cervus*, or *Croizetoceros*, as has been suggested before (Kuss, 1975; De Vos, 1984). It remains difficult to determine the ancestor of the Greek island deer, and data are still insufficient to establish robust phylogenetic relationships of Cretan deer (Van der Geer et al., 2006).

**Megaloceros giganteus.** In the morphological analyses here, *Megaloceros giganteus* was placed in varying positions, within Cervinae, as the sister taxon to *Dama dama*, and often closely related to *Rangifer tarandus* (presumably due to similarities in antler morphology). A close relationship to *Dama*, as strongly suggested by molecular analyses (Lister et al., 2005), is also supported in the TE BI and ML topologies. Together with the evidence from comparative morphology a close relationship of *Megaloceros giganteus* to *Dama* is almost certain.

There is a broad consensus today that *Megaloceros* consists of only one species, *Megaloceros giganteus* (Vislobokova, 1990, 2012, 2013; Azzaroli and Mazza, 1993; Croitor et al., 2006; Croitor and Bonifay, 2001; Croitor, 2014). All recent phylogenetic analyses consistently placed *Megaloceros giganteus* within Cervinae (Lister et al., 2005; Hughes et al., 2006; Vislobokova, 2009). In some studies *Megaloceros giganteus* was placed closely related to *Cervus elaphus* based on molecular data (Kuehn et al., 2005) and morphological data (Geist, 1998; Pfeiffer, 1999, 2002; Vislobokova, 2009). Lönnberg (1906) put it close to *Rangifer* because of a completely ossified vomer and palmated brow tines; however, it was found that the division of the nasal cavity is only ossified in the anterodorsal part of the vomerine septum, which is different from the condition in Capreolinae and presumably is a side effect of the cranial pachyostosis (Lister, 1994; Croitor, 2006b, 2014). Already Lydekker (1898) suggested an affiliation of *Megaloceros giganteus* to the damine group, which was supported in several subsequent studies using morphological, molecular or both types of data (Gould, 1974; Kitchener, 1987; Lister, 1994; Lister et al., 2005; Vislobokova, 2009). In the topology of Marcot (2007) *Megaloceros giganteus* was the sister taxon to all cervine taxa, and in Pfeiffer (2002) it was the sister taxon to two extant *Cervus*. In Mennecart et al. (2017) *Megaloceros giganteus* was the sister taxon to *Dama*.

**Odocoileus.** In the analyses here, both fossil *Odocoileus* specimens were most often placed as the sister taxon to odocoileine taxa, within Blastocerina, and sometimes to the other fossil *Odocoileus*.

The results for both fossil *Odocoileus* suggest that they are included within Capreolinae and within Odocoileini. However, only a few analyses placed them as sister taxa or closely related to their presumed living descendants *Odocoileus virginianus* and *Odocoileus hemionus*. Particularly the BSPG specimen was more often placed closely related to *Mazama* species. In Mennecart et al. (2017) the fossil *Odocoileus* BSPG specimen was placed in a trichotomy with the extant *Odocoileus* species.

**Muntiacus.** The fossil *Muntiacus muntjak* was often placed within Muntiacini, mostly as the sister taxon to *Muntiacus atherodes*. The results show that the fossil *Muntiacus* is certainly a member of Muntiacini.
**Extant Cervidae**

Until recently, there were no comprehensive studies investigating the phylogenetic relationships of extant cervids based on morphology. Due to the highly conservative craniodental features of cervids, implications from the topologies based on morphology alone were limited. In the molecular topologies here, the systematic relationships of most clades above genus level were consistently recovered and well supported by different data sets. Many systematic relationships at genus- and/or species-level were also stable and were consistently placed on the same positions in topologies based on various molecular data sets. However, even though molecular data contributed to delimiting cervid clades and helped understanding the morphological evolution, some nodes remain unresolved or unstable. In the molecular and combined topologies, apart from a very few exceptions, Cervidae, Capreolinae, and Cervinae were monophyletic; Cervini, Muntiacini, Odocoileini including Rangifer most often were monophyletic, too. The unstable position of Capreolini and Alceini questioned the monophyly of Capreolinae.

**Cervi**

The phylogenetic relationships of Cervini here, were similar to the results of recent molecular studies including Cervini; (Randi et al., 1998, 2001; Meijaard and Groves, 2004; Pitra et al., 2004; Hernández Fernández and Vrba, 2005; Gilbert et al., 2006; Hughes et al., 2006; Marcot, 2007; Ouithavon et al., 2009; Hassanin et al., 2012; Heckeberg et al., 2016). The relationships within the subclades vary slightly depending on the taxon and character sampling.

There has been a long ongoing discussion about the genus and subgenus status of cervine taxa. In this study and in most of the recent literature (e.g., IUCN, 2016; Mattioli, 2011) six genera were distinguished: Axis, Cervus, Dama, Elaphurus, Rucervus, and Rusa. Przewalski’s horse was often listed as a seventh separate genus; however, extensive morphological investigation did not find enough difference for a separate genus status (pers. obs.). Elaphurus, Rucervus, and Rusa are often considered as subgenera (Meijaard and Groves, 2004; Pitra et al., 2004; Gilbert et al., 2006; Hassanin et al., 2012), but have many morphological distinctive features that justify separate genera (pers. obs.).

**Axis.** The study of Meijaard and Groves (2004) was so far the only one to include the three species, Axis axis, Axis porcinus and Axis kuhli, for which molecular data was available. In the supertree analysis of Hernández Fernández and Vrba (2005) all four Axis species were included. Axis was not monophyletic in some studies (Pitra et al., 2004; Marcot, 2007; Agnarsson and May-Collado, 2008). This is most likely caused by re-analysing the same misidentified sequences (see discussion in Gilbert et al. (2006)).

In the analyses here Axis formed a well supported clade. Axis axis was always the sister taxon to the other two Axis species. Based on craniometrics and morphological similarities Axis calaminensis, Axis kuhli, Axis porcinus were considered to be closely related to each other and distinct from Axis axis (Meijaard and Groves, 2004). This was confirmed by our molecular and combined topologies. In most of the topologies here Axis was closely related to Rucervus, which differs from the results in Pitra et al. (2004) and the supertree analysis in Hernández Fernández and Vrba (2005).

**Cervus.** The morphological analyses here, resulted in varying positions for the four Cervus species. All of them have a very similar cranial and dental morphology (pers. obs.). In the nuclear analyses, Cervus elaphus, Cervus canadensis, and Cervus nippon were more closely related to each other than to Cervus albirostris. In the mtG analyses Cervus albirostris and Cervus nippon formed a clade and Cervus elaphus was the sister taxon to them; if Cervus canadensis was included it was the sister taxon to Cervus nippon (and Cervus albirostris, if it was a trichotomy) and Cervus elaphus was the sister taxon to all of them. This was also the case in the combined molecular and TE analyses. This difference between mitochondrial and nuclear genes may indicate an ancient hybridisation event.

In previous studies, Cervus elaphus was the sister taxon to Cervus nippon (Lister, 1984; Randi et al., 1998). or Cervus nippon was the sister taxon to Cervus canadensis, with Cervus elaphus and Rusa as the sister taxa to them (Randi et al., 2001; Pitra et al., 2004; Hughes et al., 2006). Cervus canadensis was the sister taxon to Cervus nippon with Cervus albirostris and Cervus elaphus as the sister taxon to all of them in Kuwayama and Ozawa (2000); Groves (2006); Zachos et al. (2014). This contradicts results from traditional morphology, where Cervus elaphus and Cervus canadensis were usually sister taxa (Kuwayama and Ozawa, 2000). However, Polzien and Strobeck (2002) stated that the divergence of mtDNA noted for Cervus nippon, Cervus canadensis, and Cervus elaphus is congruent with geographical, morphological, and behavioural distinctions.
In some studies, *Cervus albirostris* was the sister taxon to the other *Cervus* species (Hernández and Vrba, 2005); it was the sister taxon to *Cervus nippon*, with *Cervus canadensis* as the sister taxon to both and *Cervus elaphus* the sister taxon to all of them (Marcot, 2007), as in Hassanin et al. (2012), excluding *Cervus canadensis*. In Agnarsson and May-Collado (2008) *Cervus albirostris* was the sister taxon to *Cervus elaphus*, and *Cervus nippon* to both of them. *Cervus albirostris* was the sister taxon to *Cervus canadensis* and *Cervus nippon* in some studies or to *Cervus nippon* (Polziehn and Strobeck, 2002; Liu et al., 2002; Groves, 2006), which is also confirmed in the analyses here. In contrast to this, Flerov (1952) suggested that *Cervus albirostris* diverged from *Rusa* in the late Pliocene and Koizumi et al. (1993) considered it more closely related to *Rucervus*. However, all recent molecular studies placed it closer to the *Cervus* species (Leslie, 2010). *Cervus albirostris* almost certainly evolved in temperate northern Eurasia; *Epirusa hilzheimeri* or *Eucladoceros* may have been its Pleistocene ancestors (Di Stefano and Petronio, 2002; Flerov, 1952; Zdansky, 1925; Geist, 1998; Grubb, 1990; Leslie, 2010).

It is known that hybridisation between *Cervus nippon* and *Cervus elaphus* (mainly *Cervus elaphus* females and *Cervus nippon* males) occurs and that hybrids are fertile. Hybridisation may lead to extensive introgression (Zachos and Hartl, 2011). Studies on population genetics and subspecies of red deer exclusively used mtDNA, which may suggest relationships that are not reproducible when using paternal genes. Hybridisation could have occurred frequently in *Cervus*. The topologies here suggested varying sister taxon relationships across the four *Cervus* species.

**Dama.** In the analyses here, *Dama dama* and *Dama mesopotamica* were always sister taxa to each other and in most cases placed as the sister taxon to a clade consisting of *Cervus, Rusa, Elaphurus davidianus,* and *Rucervus eldii*. In previous studies, both *Dama* species were also sister taxa to each other (Randi et al., 2001; Lister et al., 2005; Hughes et al., 2006; Hassanin et al., 2012).

**Elaphurus.** In the nuclear analyses here, *Elaphurus davidianus* was mostly placed close to *Cervus*, while it was consistently placed as the sister taxon to *Rucervus eldii* in all mitochondrial, molecular combined, and TE analyses. In the morphological analyses it was placed closer to *Cervus* based on cranial characters and closer to *Rucervus* and *Rusa*, particularly *Rucervus schomburgki*, based on the dentition and the morphological combined data set.

The oldest known fossils of the *Elaphurus davidianus* lineage are known from the late Pliocene or slightly earlier (Taru and Hasegawa, 2002) and the first certain *Elaphurus davidianus* fossils date from the mid Pleistocene (Ji, 1985). The speciation of *Elaphurus* has been discussed as an ancient (late Pliocene or earlier) hybridisation event (Meijaard and Groves, 2004). *Cervus canadensis* or a closely related ancestor supposedly was the male parent and *Rucervus eldii* or a very close ancestral relative the female parent (Taru and Hasegawa, 2002; Meijaard and Groves, 2004; Pitra et al., 2004; Groves, 2006). The unique antler morphology and the overall phenotype of *Elaphurus davidianus* is distinct from all other cervids (Lydekker, 1898; Emerson and Tate, 1993; Meijaard and Groves, 2004; Pitra et al., 2004). Although some similarities to *Rucervus eldii* were stated (e.g., Meijaard and Groves, 2004), morphological scrutiny does not necessarily support that. The morphology of *Elaphurus* contains apomorphic character states and is not intermediate between its two parent taxa (Groves, 2014; ); own observations). This phenomenon is called transgressive segregation and the new phenotypes may be favoured in the new hybridogenetic population (Rieseberg et al., 1999; Groves, 2014).

Because of this hybridisation molecular phylogenetic analyses result in conflicting systematic positions as clearly shown here, but also in earlier studies. Analyses of mitochondrial data placed *Elaphurus davidianus* as the sister taxon to *Rucervus eldii* (Randi et al., 2001; Pitra et al., 2004), while Electrophoretic patterns of 22 proteins and k-casein DNA, and the karyotype placed *Elaphurus* closer to *Cervus* (Emerson and Tate, 1993; Cronin et al., 1996; Meijaard and Groves, 2004).

**Rucervus.** *Rucervus* species have a unique antler morphology and their teeth are uniquely folded indicating a specialisation for graminivory (Grubb, 1990; Meijaard and Groves, 2004); both provide useful morphological characters. The hypothesis that *Rucervus* is more closely related to *Rusa* than to *Cervus* was partly supported in the nuclear analyses and the morphological analyses here, while in the mitochondrial, molecular combined, and TE analyses *Rucervus* was polyphyletic with *Rucervus eldii* more closely related to *Elaphurus davidianus* and the other two species more closely related to *Axis*, Based on this it was suggested that *Rucervus eldii* may represent a different evolutionary lineage than the other two *Rucervus* species (Meijaard and Groves, 2004) and was sometimes put into a separate genus *Panolia* (Pocock, 1943; Groves, 2006). It is now widely regarded as *Rucervus eldii* (Wilson and Reeder,
The placement of *Rucervus eldii* separate from its two congeners in molecular topologies (especially mtDNA) is most likely artificially caused by the hybridisation of *Rucervus eldii* and *Cervus canadensis* in the past.

*Rucervus duvaucelii* and *Rucervus schomburgki* were sister taxa to each other in the analyses here and were mostly the sister taxon to *Axis*. The last specimen of *Rucervus schomburgki* became extinct in 1938. The first accounts on the species were by Blyth (1863), who noted the distinctive antler pattern.

According to Gührer (1936), the geographical distribution of *Rucervus eldii* was monophyletic except for the BI analyses. In the MP analyses, Muntiacini were placed more closely related to *Rucervus duvaucelii* and potentially interbreeding with *Rucervus eldii* in its natural habitat. The earliest fossils of *Rucervus* date back to 2.9 mya (Azzaroli et al., 1988; Meijaard and Groves, 2004).

**Rusa.** In the morphological analyses here, *Rusa* was more closely related to *Rucervus* (rarely to *Axis*). In the nuclear analyses, it was close to *Rucervus* or within Cervini, while it was more closely related to *Cervus* in the mitochondrial, combined molecular, and TE analyses. When all four *Rusa* were included, *Rusa timorensis* and *Rusa unicolor* were sister taxa and *Rusa marianna* and *Rusa alfredi* were sister taxa.

Despite some new insights into the systematic relationships of *Rusa*, uncertainties remain (Heckelberg et al., 2016). The Philippine *Rusa alfredi* and *Rusa marianna* share morphological similarities, and are distinct from the other two *Rusa* because of the overall smaller size. *Rusa unicolor* and *Rusa timorensis* from the mainland and Indonesia were considered to be more derived (Groves and Grubb, 2011), which is in contrast to the assumption that based on the high similarity of *Rusa unicolor* to pliocervines, an extinct lineage of Pliocene cervids, it is the most ancestral of the four extant rusine deer (Petronio et al., 2007; Leslie, 2011).

Although the monophyly of *Rusa* has been controversial based on morphological and molecular evidence (Meijaard and Groves, 2004; Hernández Fernández and Vrba, 2005; Randi et al., 2001; Leslie, 2011), in our analyses *Rusa* is more often supported to be monophyletic than not.

The first appearance of *R. unicolor* was recorded from the middle Pleistocene (Zong, 1987; Dong, 1993; Meijaard and Groves, 2004). The oldest *R. timorensis* is reported from the late Pleistocene (Van Mourik and Stelmasiak, 1986; Dong, 1993) and suggested to have then dispersed south-eastwards to Taiwan and Java (Meijaard and Groves, 2004).

**Muntiacini**

In the recent literature, muntiacines have been included in phylogenetic reconstructions to a different extent (Randi et al., 1998; Wang and Lan, 2000; Randi et al., 2001; Pitra et al., 2004; Hernández Fernández and Vrba, 2005; Gilbert et al., 2006; Hughes et al., 2006; Marcot, 2007; Ouithavon et al., 2009; Hassanin et al., 2012). The systematic relationships within Muntiacini vary mostly depending on the taxon sampling, but do not contradict each other. The monophyly of Muntiacini uniting *Muntiacus* and *Elaphodus* has never been questioned Gilbert et al. (2006) and is supported by our analyses.

**Elaphodus.** *Elaphodus cephalophus* was always the sister taxon to the other muntiacine species in all molecular and TE analyses presented here, which is also widely supported in the literature (e.g., Wang and Lan, 2000; Hernández Fernández and Vrba, 2005; Agnarsson and May-Collado, 2008; Hassanin et al., 2012). In contrast, in Marcot (2007) *Elaphodus cephalophus* is the sister taxon to all cervids.

*Elaphodus cephalophus* has the smallest known antlers, which are completely covered by tufts (Leslie et al., 2013). Groves and Grubb (1990) considered *Elaphodus cephalophus* as the most primitive representative of living muntiacines. However, this is in contrast to the absence of fossils with such diminutive antlers. The first *Elaphodus* fossils are known from the Pleistocene of China, which were larger than *Elaphodus cephalophus*; therefore, the decrease in size can be considered as evolutionary trend in this species (Leslie et al., 2013).

**Muntiacus.** All muntjacs have long pedicles, facial crests, and bifurcating antlers (pers. obs.; e.g., Ma et al., 1991). In the morphological analyses here, muntiacine taxa were placed as the sister taxon to most other cervids or in an unresolved position. In most of the combined morphological analyses Muntiacini was monophyletic except for the BI analyses. In the MP analyses, Muntiacini were placed more closely related to other small cervids, such as *Mazama* and *Pudu*.
The earliest fossil of the *Muntiacus* lineage is *Muntiacus leilaoensis* from Yunnan, China and was dated to the late Miocene 9–7 mya (Dong et al., 2004). All *Muntiacus* species consistently formed a clade as the sister taxon to Cervini in the mitochondrial, molecular combined, and TE analyses here. A clade consisting of *Muntiacus crinifrons*, *Muntiacus feae*, and *Muntiacus muntjak* and a clade consisting of *Muntiacus putaoensis*, *Muntiacus truongsonensis*, *Muntiacus rooseveltorum*, *Muntiacus vaquangensis*, and *Muntiacus reevesi* were recovered in the mitochondrial and combined molecular analyses. *Muntiacus atherodes* was placed in a polytomy with these clades. In the TE analyses *Muntiacus reevesi* was placed between *Elaphodus cephalophus* and the other muntjacs and *Muntiacus atherodes* was the sister taxon to *Muntiacus feae*.

Several new muntjacine species have been discovered in the 1990s; subsequently, five to possibly six new muntjac species were established, *Muntiacus gongshanensis*, *Muntiacus crinifrons*, *Muntiacus feae*, *Muntiacus reevesi*, *Muntiacus muntjak* (Lan et al., 1995). Ma et al. (1986b,a) stated that *Muntiacus crinifrons* and *Muntiacus rooseveltorum* derived from *Muntiacus reevesi*, whereas *Muntiacus feae* and *Muntiacus muntjak* derived from a different lineage. The species status of *Muntiacus rooseveltorum* has been controversial for decades (Amato et al., 1999b); for example, Groves and Grubb (1990) suggested that *Muntiacus rooseveltorum* is the synonym of *Muntiacus feae* and *Muntiacus feae* is the sister taxon to *Muntiacus muntjak* and *Muntiacus crinifrons*. This is supported by most molecular studies and the topologies of this work. Sometimes, *Muntiacus crinifrons* and *Muntiacus gongshanensis* are considered as a single species (Amato et al., 1999b). It was proposed that *Muntiacus atherodes* should be included in *Muntiacus muntjak* based on morphological evidence, because the holotype of *Muntiacus atherodes* is a subadult male with single-tined antlers (Ma et al., 1986b). The two specimens investigated here were indeed subadult individuals with not yet fully developed antlers (pers. obs.). However, molecular topologies here and in the literature indicate a separate species status for *Muntiacus atherodes* (Heckeberg et al., 2016). The genus status of *Megamuntiacus* is not justified demonstrated by the sequence divergence estimated for the mitochondrial variation and by morphological comparisons; therefore, it is referred to as *Muntiacus* (Schaller, 1996; Giao et al., 1998; Amato et al., 1999a; Rabinowitz et al., 1999; Wang and Lan, 2000). Apart from the larger size, there are no morphological features that would justify a separate genus (pers. obs.).

**Alceini**

**Alces.** *Alces* has a highly derived skull morphology with an elongated viscerocranial proportion and antlers that protrude horizontally. The dentition shows similar modifications as in *Rangifer*. In the morphological analyses here, *Alces alces* was in an unresolved position or placed as the sister taxon to *Odocoileus hemionus, Mazama chunyi, Ozotoceros bezoarticus* or *Cervus canadensis*. In the mitochondrial, combined molecular and TE analyses *Alces alces* was consistently placed as the sister taxon to Capreolini, except for the BI combined molecular topology, where it was placed between Capreolini and Odocoileini plus *Rangifer*.

The first *Alces alces* is known from the Riss glaciation 200-100 kya; those late Pleistocene moose were larger than their extant representatives (Franzmann, 1981). In most recent studies, *Alces* was placed as the sister to Capreolini (Randi et al., 1998; Pitra et al., 2004; Hughes et al., 2006; Agnarsson and May-Collado, 2008; Hassanin et al., 2012) or as the sister taxon to *Capreolus* (Hernández Fernández and Vrba, 2005). In Marcot (2007) *Alces* was the sister taxon to Capreolini and Odocoileini and Rangiferini, while it was in a polytomy with Odocoileini plus *Rangifer* and Capreolini or the sister taxon to Odocoileini plus *Rangifer* in Gilbert et al. (2006). More controversial positions included *Alces* as the sister taxon to Cervini or *Dama dama* in Kuehn et al. (2005) and the sister taxon position to *Rangifer* in Pfeiffer (2002). *Alces* was in a polytomy with Odocoileini and Rangiferini in Lister (1984) and took up variable positions in previous studies as summarised in Lister (1998). Thus, the systematic position of *Alces* remains unresolved.

**Capreolini**

Most analyses based on the combined morphological data set supported monophyletic Capreolini. However, the systematic position of Capreolini varied and could not be determined with certainty using morphological data only. In the molecular analyses here, Capreolini was always monophyletic and mostly placed closely related to or in most cases as the sister taxon to Odocoileini plus *Rangifer*.

Miyamoto et al. (1990) suggested that Capreolini probably originated in the late Miocene in the Old World. The assumption of a late Miocene Old World origin of Capreolinae is in congruence with our
findings considering the placement of *Procapreolus*. Cronin (1991) hypothesised that *Alces* and *Rangifer* split earlier than the *Capreolus* lineage, but after the separation of Cervinae and Capreolinae.

**Capreolus.** In the morphological, molecular, and TE topologies *Capreolus capreolus* and *Capreolus pygargus* both species were consistently placed as sister taxa. In the mitochondrial, molecular combined and TE topologies, *Capreolus* was always the sister taxon to *Hydropotes* with strong support. Molecular studies of the past decades support the consistent placement of *Hydropotes* as the sister taxon to *Capreolus* forming monophyletic Capreolini (Douzery and Randi, 1997; Randi et al., 1998; Hassanin and Douzery, 2003; Pitra et al., 2004; Hughes et al., 2006; Gilbert et al., 2006; Marcot, 2007; Agnarsson and May-Collado, 2008; Hassanin et al., 2012; Heckeberg et al., 2016).

**Hydropotes.** Here, *Hydropotes* and *Capreolus* were sister taxa in the morphological combined, mt, molecular combined and TE analyses. In the past, *Hydropotes* was considered as a separate subfamily Hydropotinae as the sister taxon of all other cervids (e.g., Groves and Grubb, 1987; Janis and Scott, 1987; Hernández Fernández and Vrba, 2005; Kuznetsova et al., 2005). Already Bouvrain et al. (1989) favoured the hypothesis that *Hydropotes* and Capreolini are sister taxa. The first molecular studies indicated that *Hydropotes* is included in monophyletic Cervidae (Kraus and Miyamoto, 1991). From this follows that *Hydropotes* lost the antlers secondarily and developed enlarged upper canines as compensation (Douzery and Randi, 1997; Randi et al., 1998; Hassanin and Douzery, 2003).

Randi et al. (1998) demonstrated that the two *Capreolus* species and *Hydropotes* share a G at position 525 of *Cytb*, which occurs only rarely in other mammal species and stated that ‘this replacement represents a nearly exclusive synapomorphy for the *Hydropotes-Capreolus*-clade. Further, the telemetacarpal condition and a large medial opening of the temporal canal are morphological features that *Hydropotes* shares with other Capreolineae (Bouvrain et al., 1989; Douzery and Randi, 1997; Randi et al., 1998). Behavioural characters also suggested that *Hydropotes inermis* is closely related to *Capreolus* (Cap et al., 2002).

In contrast to the opinion stated in the extensive review of *Hydropotes inermis* (Schilling and Rössner, 2017) more and more evidence (mitochondrial and nuclear DNA, morphology, behaviour) point to a sister taxon relationship of *Hydropotes* and *Capreolus*.

**Rangiferini**

**Rangifer.** The systematic position of *Rangifer* was variable in the morphological analyses here. *Rangifer* has some apomorphic characters, not shared by other cervids, which is likely the cause of the difficulties to place the taxon based on morphology only. In the molecular and TE topologies *Rangifer tarandus* was consistently placed as the sister taxon to Odocoileini. This is supported by the most recent literature, (Randi et al., 1998; Hassanin and Douzery, 2003; Pitra et al., 2004; Hernández Fernández and Vrba, 2005; Gilbert et al., 2006; Hughes et al., 2006; Agnarsson and May-Collado, 2008; Duarte et al., 2008; Hassanin et al., 2012). *Rangifer* was in a polytomy with Odocoileini and Alceini in Lister (1984). Pfeiffer (2002) found that *Rangifer* is the sister taxon to *Alces* based on morphological characters.

*Rangifer* appeared in the fossil record in the Pleistocene; based on its arctic specialisations it is hypothesised that it dispersed to America during the Pleistocene contemporaneously with *Alces* (Gilbert et al., 2006).

**Odocoileini**

In the morphological topologies here most odocoileine taxa were in unresolved and/or variable positions. In several topologies the small odocoileine cervids were in a clade with muntiacine taxa. In the nuclear topologies, systematic relationships within Odocoileini were partly or entirely unresolved. In the mitochondrial, combined molecular, and TE topologies here, Odocoileini split into the two subclades Blastocerina and Odocoileina (Heckeberg et al., 2016).

In previous phylogenetic studies, the taxon sampling for Odocoileini varied greatly, therefore, it is difficult to compare the topologies (Douzery and Randi, 1997; Randi et al., 1998; Pitra et al., 2004; Hernández Fernández and Vrba, 2005; Hughes et al., 2006; Gilbert et al., 2006; Marcot, 2007; Agnarsson and May-Collado, 2008; Duarte et al., 2008; Hassanin et al., 2012). In these studies, Odocoileini usually formed a monophyletic group with Rangiferini as the sister taxon to them. *Blastocerus dichotomus*, *Ozotoceros bezoarticus*, and *Pudu puda* were particularly unstable across studies with comparable taxon sampling. In the topologies here, they were sensitive to changes in the analysis parameters. Odocoileina and Blastocerina were sister taxa in several recent studies (Pitra et al., 2004; Hughes et al., 2006; Gilbert
1184 et al., 2006; Marcot, 2007; Agnarsson and May-Collado, 2008; Hassanin et al., 2012; Heckeberg et al., 2016). This is also the case in Duarte et al. (2008), but Pudu puda was in a polytomy to those clades. In addition, the results here and those of previous studies showed polyphyly for three odocoileine genera Hippocamelus, Mazama, and Pudu and for both species of Odocoileus (Pitka et al., 2004; Gilbert et al., 2006; Agnarsson and May-Collado, 2008; Duarte et al., 2008; Hassanin et al., 2012; Heckeberg et al., 2016). It remains uncertain, whether Pudu is monophyletic, polyphyletic within Blastocerina or polyphyletic with one species in Blastocerina and one species in Odocoileina. More morphological and molecular, particularly nuclear markers, and cytogenetic data are needed to reconstruct the complex evolutionary history of Odocoileini (Duarte et al., 2008; Hassanin et al., 2012).

Blastocerus. In the analyses here, Blastocerus dichotomus was positioned in an unresolved position based on morphological data and consistently placed within Blastocerina in the molecular and TE analyses. Most often it was positioned between Pudu puda (sometimes also Mazama nemorivaga) and the other Blastocerina. In previous studies Blastocerus took up variable positions, most likely depending on the taxon sampling, for example, as the sister taxon to Hippocamelus bisulcus plus Mazama gouazoubira (Duarte et al., 2008), as the sister taxon to Mazama gouazoubira (Agnarsson and May-Collado, 2008), in a polytomy with Mazama gouazoubira, Pudu puda, Hippocamelus antisensis (Gilbert et al., 2006), as the sister taxon to Pudu puda (Hughes et al., 2006), and as sister taxon to Mazama nemorivaga (Hassanin et al., 2012). Studies with a more extensive taxon sampling (Heckeberg et al., 2016) and the analyses of this work indicated a systematic position of Blastocerus as the sister taxon to most blastocerine species, with Mazama nemorivaga as the sister taxon to them and Pudu puda as the sister taxon to all other Blastocerina. A few analyses placed Blastocerus as the sister taxon to all other Blastocerina. These differing placements of Blastocerus most likely resulted from a differing taxon sampling. The first Blastocerus fossils are known from the Pleistocene of Brazil and Paraguay. The populations in central Brazil most likely expanded between 28–25 kya and it was assumed that there were no geographical barriers until about 300 years ago (Merino and Rossi, 2010).

Hippocamelus. In several of the morphological topologies, both Hippocamelus species were monophyletic, sometimes with Ozotoceros as the sister taxon. Two of the four sequences for Hippocamelus antisensis formed a clade with Hippocamelus bisulcus, while the other two formed a clade with Ozotoceros bezoarticus (Heckeberg et al., 2016). This makes it almost certain that two of the four sequences are misidentified or mislabelled; a less likely possibility is that this polyphyly represents a valid split within the genus. Without knowing the exact provenance of the samples it cannot be determined which sequences are truly Hippocamelus antisensis. In the molecular combined and TE analyses here, we included those Hippocamelus antisensis mt-sequence(s), with which the genus is monophyletic (Heckeberg et al., 2016). Hippocamelus was the sister taxon to Mazama gouazoubira (plus Mazama chuni, if included).

Duarte et al. (2008) stated that it is surprising that members of morphologically cohesive genera such as Hippocamelus, Mazama, or Pudu were not monophyletic based on molecular data. Hippocamelus antisensis and Hippocamelus bisulcus were found to be osteologically nearly indistinguishable (Flueck and Smith-Flueck, 2011, pers. obs.). Based on this, a monophyly for Hippocamelus is more likely than a polyphyly as suggested by some of the molecular data. Thus, the potential polyphyly within Hippocamelus cannot be confirmed or ruled out yet; new sequences and more investigations are needed to clarify which of the available sequences genuinely belong to H. antisensis.

The first Hippocamelus bisulcus is known from the late Pleistocene of Chile, Argentina, and Bolivia (Canto et al., 2010; Merino and Rossi, 2010). Odocoileus lucasi is considered to be the ancestor of Hippocamelus bisulcus.

Mazama. In the morphological analyses here most Mazama species were placed as closely related to each other most likely because of their small size and because they are morphologically almost indistinguishable (González et al., 2009; own observations). In Gutiérrez et al. (2015), the suggested potential morphological difference of Mazama bricenii and Mazama rufina referring to the degree of concavity of the dorsal outline in lateral view is controversial, as both individuals seem to differ greatly in age based on the tooth crown height. The second character, the lacrimal fossa, can generally be highly variable among species. In the specimens scrutinised here, all Mazama bricenii skulls show a weak concavity in the dorsal outline, not as deep as in the figure of Gutiérrez et al. (2015). One of the two Mazama rufina specimens (NHMW 528) has a more clearly concave outline, the other one (ZSM 1927/41) has a straight outline. In the most recent studies (Gutiérrez et al., 2015; Heckeberg et al., 2016) and the
molecular analyses here, *Mazama bricenii* consistently was the sister taxon to *Mazama rufina*. Gutiérrez et al. (2015) suggested that *Mazama bricenii* is not a valid taxon, but a junior synonym of *Mazama rufina*. The systematic relationships of *Mazama* were discussed in detail in Heckeberg et al. (2016) and polyphyly persists throughout different molecular and TE data sets. The complex taxonomy of *Mazama* needs a thorough revision.

While the monophyly of *Mazama* has never been questioned based on morphological characters, molecular studies repeatedly suggested polyphyletic relationships (Gilbert et al., 2006; Duarte et al., 2008; Gutiérrez et al., 2015; Escobedo-Morales et al., 2016; Heckeberg et al., 2016). Duarte et al. (2008) suggested that *Mazama gouazoubira* and *Mazama nemorivaga* should be assigned to a different genus. The low morphological diversity among *Mazama* is not correlated with the genotypic diversification, which leads to the problematic taxonomy; thus, a varying number of species were established based on different types of data (Groves and Grubb, 1987, 1990; Duarte and Merino, 1997; Duarte et al., 2008).

Only little is known about rare *Mazama* species (and neotropical cervids in general), which represent the least studied organisms and many aspects of their life history are poorly understood (Duarte et al., 2012e.d,b,a.f; Lizcano et al., 2010; Gutiérrez et al., 2015).

Previous molecular studies and the topologies here showed polyphyly of *Mazama americana*, which suggested that it comprises several evolutionary units. The genetic distance between the two *Mazama americana*-clades was higher than the genetic distance of *Mazama bororo* and *Mazama nana*. Therefore, at least two species were assumed to be within the *Mazama americana*-complex, with a separate evolution of the two clades starting 1 mya and 2 mya, respectively (Duarte et al., 2008; Abril et al., 2010). The first fossil *Mazama* are known from the Pleistocene of Argentina, Ecuador, Peru, and Brasil (Merino and Rossi, 2010).

**Odocoileus.** In the morphological analyses based on the combined data set here *Odocoileus hemionus* is the sister taxon to *Alces alces*, and in several topologies *Odocoileus virginianus* is the sister taxon to them. In all other morphological topologies, odocoileine taxa are placed in unresolved or varying positions. In the analyses including mitochondrial markers and a broad taxon sampling, both species were polyphyletic. In the analyses based on the nuclear markers, polyphyly of the species were not observed.

Despite all the research undertaken on the genus, the taxonomy remains difficult. There are numerous subspecies (8–10 for *O. hemionus*, 37–38 for *O. virginianus*; Wilson and Reeder (2005); Mattioli (2011)), which possibly, at least partly, represent separate species (Groves and Grubb, 2011).

Latch et al. (2009) demonstrated that there are two different morphotypes of *O. hemionus*, the mule deer and black-tailed deer, which is supported by a strong genetic discontinuity across the spatial distribution. Early investigations of mtDNA data demonstrated that *O. hemionus* is polyphyletic because the sequences of the mule deer (*O. hemionus*) and *O. virginianus* are more similar than the DNA of the black-tailed deer (*O. hemionus columbianus*) is to both of them (5–7% different) (Carr et al., 1986; Cronin et al., 1988, 1996; Latch et al., 2009).

Similarly, the genetic divergence within *O. virginianus* is remarkably high, even higher than the genetic distance between other subspecies and between *O. virginianus* and mule deer. This led to the classification of white tailed deer into two distinct groups, the cariacou-division and the virginianus-division (Wilson et al., 1977; Smith et al., 1986; Groves and Grubb, 1987; Grubb, 1990). Some topologies here (Figs 13, 14) and the literature (Heckeberg et al., 2016) most likely show the two distinct genetic groups in each of the *Odocoileus* species. *Odocoileus virginianus* is a highly plastic species occupying a great variety of geographically and ecologically extensive habitats between Canada and Peru, however, extreme habitat differences do not necessarily lead to large morphological divergence (Smith et al., 1986; Moscarella et al., 2003; Merino and Rossi, 2010; Duarte et al., 2012c). Introggression seems to be the likely explanation because natural hybridisation and interbreeding between both species of *Odocoileus* have been documented (Groves and Grubb, 2011; Hassinan et al., 2012).

The first *Odocoileus* is from the early Pliocene (3.5 mya) of North America, where they were the most common cervids until the Pleistocene. *Odocoileus virginianus* appeared 2 mya presumably as the descendant of *O. brachydontus*, which originated in Central America and dispersed to higher latitudes only recently (Hershkovitz, 1972; Smith, 1991; Merino and Rossi, 2010). It has been assumed that *Odocoileus virginianus* evolved in North America; it was further suggested that all South American cervid fossils belong to *Odocoileus* and that *Mazama* later diverged as a consequence of isolation within South America (Smith et al., 1986; Moscarella et al., 2003). This is in contrast with the most recent molecular topologies (e.g., Escobedo-Morales et al., 2016; Heckeberg et al., 2016) and this work (Figs 13, from...
which it appears that *Odocoileus* originated from the odocoileine *Mazama*-clade.

**Ozotoceros.** Similar to *Blastocerus*, the systematic position of *Ozotoceros* varied with the taxon sampling. With an extensive taxon sampling *Ozotoceros bezoarticus* was relatively consistently placed as the sister taxon to *Hippocamelus, Mazama gouazoubira* and *Mazama chunyi* (if included) in the analyses here.

The origin of *Ozotoceros bezoarticus* possibly dates back to 2.5 mya coinciding with a substantial cooling event; fossils are known from the late Pleistocene and Holocene of Brazil, the late Pleistocene of Uruguay, and the Holocene of Argentina (Gonzalez et al., 1998; Merino and Rossi, 2010).

**Pudu.** Both *Pudu* species are almost indistinguishable based on morphology, but do not evidently form a monophyletic group based on molecular data (Heckeberg et al., 2016). *Pudu puda* was placed as the sister taxon to all Blastocerina in almost all of the analyses here and in previous studies with a sufficient taxon sampling (Hassanin et al., 2012). The systematic position of its congener, unfortunately, is much less certain. *Pudu mephistophiles* was most often placed as the sister taxon to all Odocoileini plus *Rangifer* or to Odocoileini. Only in one topology there *Pudu mephistophiles* was included within Blastocerina.

The spatial and chronological origin of *Pudu* is unknown. *Pudu* most likely diverged from an odocoileine lineage, which existed in America since the Miocene-Pliocene-boundary (Merino and Rossi, 2010; Gonzalez et al., 2014). *Pudu* was probably restricted to South America since the Pliocene (Escamilo et al., 2010).

**Aspects of the Evolution of Cervidae**

**Morphological Evolution**

**Cranium** The cranial morphology of cervids is highly conservative (Lister, 1996; Merino and Rossi, 2010). Also, some morphological characters in ruminants likely are the results of convergent evolution and thus are homoplasic, which may cause difficulties in reconstructing phylogenetic relationships (Bouvrain et al., 1989; Douzery and Randi, 1997). Despite the homoplasy, some clades were well defined and re-occurring across different data sets in the topologies here.

Differences in the size of the praeorbital vacuity are primarily species specific, but have also an ontogenetic component, since they are often smaller in aged individuals. Similarly, the lacrimal fossa varies in size and depth in different species, presumably depending on the presence, size, and usage of the lacrimal gland. Also, there is a difference between males and females. The position of the lacrimal foramina to each other and on the orbit rim can potentially be used to distinguish groups of cervids. The consistent presence of two lacrimal foramina is typical for cervids, but is also present in some bovid species. In *Dremotherium feignouxi* sometimes only one lacrimal foramen is present (Costeur, 2011). The contact of the lacrimal and the frontal at the orbit rim without interlocking sutures was first observed in Rössner (1995). This trait is most likely an intraspecific variability and could be an effect of ageing.

Evolutionary trends observed in Pliocene cervids include an increase of the overall body size, a decrease of the pedicle length relative to the antler length and an associated increase of the antler length (Heintz, 1970). The degree of inclination of the pedicles changes through time and is presumably a result adapting to rich vegetation. With the stronger inclination the insertion point of the pedicle on the skull moved posteriad. The pedicle in early Miocene cervids is entirely above the supraorbital process and not in contact with the braincase; the pedicles are vertical in lateral view, parallel or converging in frontal view. The shortening of the pedicles could be related to the increasing size of antlers, because a longer and heavier set of antlers would put a biomechanically unfavourable leverage on the pedicles.

Basicranial and ear region characters were not yet widely used when inferring morphological phylogenies, but were assumed to have strong potential to provide characters, which are less prone to convergent evolution caused by climatic change (Janis and Theodor, 2014). Recently, it has been shown that traits of the inner ear provide useful characters with phylogenetic signal (Mennecart et al., 2016, 2017).

**Antlers** There is broad consensus that antlers originated only once (Loomis, 1928; Azanza and Morales, 1989; Azanza, 1993a,b; Azanza et al., 2011; Heckeberg, 2017b). The antlers of most Miocene cervids have a simple bifurcating pattern, sometimes with an additional tine, or are coronate (Azanza et al., 2011). These antlers are relatively short, do not have a shaft and the bifurcation originates directly from a broad antler base. From the late Miocene onwards, more complex branching patterns developed, the length of antlers increased and antlers developed a shaft below the first bifurcation. Evolution of size and
complexity of antlers is associated with reduction or loss of upper canines (Scott, 1937; Beninde, 1937; Geist, 1966; Brokx, 1972).

In extant cervids, short and simple antlers and long and more complex or palmed antlers are present. Many extant cervids develop exactly three tines (Heckeberg, 2017b). The three antler morphotypes have previously been associated with ecological habitats: simple antlers for the tropics, a three-tined antler plan for woodland areas typical in East Eurasia or India, and the large and complex display organs in temperate regions (Pitra et al., 2004). The simple antlers in Mazama and Pudu are considered as a secondary adaptation to dense vegetation.

There is a lot of inter- and intraspecific variation in antlers (Goss, 1983; Heckeberg, 2017b). The high variability of antlers is a problem particularly in fossil taxa, where the entire intraspecific variation cannot always be observed due to the lack of a sufficient number of specimens or the incompleteness of ontogenetic stages. The taxonomy of fossil cervids is often based on antler morphology, because antlers are easy to identify and numerous in the fossil record antler morphology having more distinctive, features than other anatomical characters (Kurtén, 1968; Fry and Gustafson, 1974; Lister et al., 2010; Merino and Rossi, 2010). Thus, the validity of some fossil cervid taxa is doubtful. To base classifications just on antler morphology is problematic for the given reasons.

In contrast to Loomis (1928), Gentry et al. (1999) stated that cranial appendage morphology proved to be more suitable than tooth morphology to distinguish species. This applies in general to Pecora and specifically to Cervidae. It is true that different cervid species can be easily identified based on their antler morphology (branching pattern, orientation, size). Antler characters were often used to solve intra-subfamily relationships, but they are problematic because of convergent development and subsequent homoplasy in antler characters (Pitra et al., 2004).

Since Cervidae is diagnosed by the presence of antlers (Janis and Scott, 1987; Pitra et al., 2004), the reason for the absence of antlers in Hydropotes inermis species was controversially discussed; a primitive condition and secondary loss have been suggested. To solve this issue, thorough research on the process(es), which trigger the growth of the first set of antlers in antler-bearing species and when and why these processes/prerequisites are absent in Hydropotes inermis needs to be undertaken. The more widely accepted hypothesis that Hydropotes inermis secondarily lost its antlers was applied here and the presence of antlers is the synapomorphy of Cervidae.

**Dentition** Variations of accessory dental elements in combination with the degree of molarisation of premolars can be used to identify genera or species. Widely accepted evolutionary trends in cervids concerning the dentition are increasing hypsodonty, the reduction of the premolar row length and the reduction or loss of upper canines (Heintz, 1970; Dong et al., 2004). However, the hypsodonty index, although widely used in ruminant phylogeny, has been considered to be a misleading character due to its ambiguous definition and convergent evolution among all large herbivorous mammals (Janis and Scott, 1987; Hassanin and Douzery, 2003).

The first deer had brachydont dentition and were considered as leaf-eaters; recent dental analyses generally support these findings, but also showed that Procervulus ginsburgi likely was a seasonal mixed feeder. Based on this a facultative leaf-grass mixed feeding strategy with preference for leaf-eating is likely the primitive dietary state in cervids and ruminants (DeMiguel et al., 2008).

Ginsburg and Heintz (1966) regarded the bifurcation of the postprotocrista into an internal and external crista as a derived cervid character based on its presence in Dicrocerus and Euprox. Amphimoscus is the only other non-cervid pecoran species that shows this trait (Janis and Scott, 1987). The bifurcated postprotocrista was regarded as an advanced cervid character in Janis and Scott (1987), while later this character is referred to as ‘primitive presence of bifurcated protocone’. In extant cervids this feature is present in Odocoileus, Blastocerus, Alces, Mazama, Pudu, and Capreolus (Janis and Scott, 1987). These observations could be confirmed here by morphological comparisons. One species of Palaeoplatycteros hispanicus (MNCN 39181) shows both a bifurcating postprotocrista and a tiny protocone fold on the prepontocrista. This indicates that both structures may in fact be developmentally independent, however, as this could only be observed in one specimen, it remains speculation.

Throughout the evolutionary history of cervids the lingual cingulum, regularly present on molars and sometimes even on premolars of fossil cervids, becomes reduced and eventually lost in extant cervids. In Rucervus, Rusa, and Axis the anterior and posterior lingual walls of the molars tend to be indented; this is also observed in Axis lydekkeri, Rusa kendengensis, and ‘Cervus’ sivalensis.
p2 is the tooth with the fewest changes in occlusal morphology throughout cervid evolution; only a
shortening is observed in most extant taxa and in a few individuals p2 is lost entirely.

The elongated upper canines in *Hydropotes inermis* are actively used in intraspecific fights. It is likely
that the presence and/or size of upper canines is somehow genetically linked with the antlers; this brings
up the question, why female deer have upper canines, too (Brokx, 1972). Even though they are often
much smaller, especially in species, where males have enlarged upper canines, they are present without
any obvious function. In other ungulates, where males use their canines in intraspecific fights, for example
in equids, upper and lower canines are lost in almost all females. Much more research is needed to find
this link and associated interactions and effects on behaviour.

**Systematics of Ruminant Families**

Despite decades of research the systematic relationships of the six ruminant families, especially among
the pecoran families have been proven to be difficult (Kraus and Miyamoto, 1991; Cronin et al., 1996;
Randi et al., 1998; Cap et al., 2002; Hassanin and Douzery, 2003; Hassanin et al., 2012). Particularly,
the position of Moschidae, Antilocapridae, and Giraffidae remained problematic. Hassanin and Douzery
(2003) and Price et al. (2005) presented an overview of the systematic relationships of ruminants dating
back to 1934.

Most recent molecular studies relatively consistently showed that the clade consisting of Moschidae
plus Bovidae was the sister taxon to Cervidae, which was the sister taxon to Giraffidae, then Antilocapridae;
Tragulidae was the sister taxon to all of them (Kuznetsova et al., 2005; Marcot, 2007; Agnarsson and
May-Collado, 2008; Hassanin et al., 2012).

In the molecular topologies here, the systematic relationships among the six ruminant families varied.
Most variation was observed in the nuclear markers; Cervidae was sometimes unresolved as the sister
taxon to Antilocapridae, Giraffidae and Bovidae, with Moschidae as the sister taxon to all of them. Most
often, however, Moschidae and Bovidae were sister taxa to each other with Cervidae as the sister taxon,
and Antilocapridae and Giraffidae as sister taxon to that clade, either unresolved or as clade.

This demonstrates that the supposed consensus about the systematic relationships among ruminant
families is an artefact of repeatedly re-analysing identical data sets with similar parameters. More
and different types of data are needed to solve this problem in a more sophisticated and consistent way,
particularly because of the potential implications for conservation in some genera (Price et al., 2005). Also,
further work is needed to investigate the impact of inclusion of fossil taxa (Agnarsson and May-Collado,
2008; O’Leary and Gatesy, 2008).

**Evolutionary History**

During the Eocene, selenodont artiodactyls diversified and ruminants were the only successful descendants
from this radiation. Subsequent rapid radiations of ruminants resulted in the most diverse group of large
mammals today (Hernández Fernández and Vrba, 2005).

Collision of the African and Indian continents with Eurasia around 40 mya caused drastic environ-
mental changes triggering artiodactyl evolution. The expansion and diversification of grasslands at the
Eocene-Oligocene-boundary (34 mya) coincided with climate changes from warm and humid to colder
and drier conditions (Prothero and Heaton, 1996; Meng and McKenna, 1998; Hassanin and Douzery,
2003). The divergence of major ruminant lineages has occurred within a very short period of time since
their origination and ruminant evolution rates were not constant through time (DeMiguel et al., 2013).
From the Oligocene to the mid Pliocene global climatic and vegetational changes led to several successive
rapid radiations within Pecora with additional short-termed diversification events within Bovidae and
Cervidae (Hernández Fernández and Vrba, 2005). This rapid cladogenesis and parallel evolution may
explain the lack of resolution or taxon instability in ruminant topologies and the plethora of convergent

From the Oligocene to the Miocene cooler and more arid climate led to the replacement of forest
habitats with open grasslands in Asia favouring the diversification and dispersal of many pecoran groups
(Meijaard and Groves, 2004; Lorenzini and Garofalo, 2015). C3 grass dominated habitats occurred around
22 mya, C4 grass expanded around 17.5 mya (DeMiguel et al., 2013). These conditions were perfect
for the origin and diversification of Cervidae and other ruminant groups. The resulting competition of
overlapping habitats of grazers and browsers must have played a crucial role in the evolution of Cervidae
(Gilbert et al., 2006). At the Oligocene-Miocene boundary, the first cervoids appeared diverging from
Oligocene taxa like *Dremotherium* or *Bedenomeryx* (Ludt et al., 2004). The antlerless *Dremotherium*
from the early Miocene of Europe has been suggested as the earliest member of cervids (Brooke, 1878; Ginsburg and Heintz, 1966; Vislobokova, 1983). *Dremotherium* was consistently found to be most similar to cervids and together with *Amphitragulus* is now widely considered to be an early cervoid (Heintz et al., 1990; Gentry et al., 1999). The exact systematics of *Dremotherium feignouxi* remain problematic as it shares morphological traits with cervids and moschids (Pomel, 1853; Costeur, 2011). In the analyses here, *Dremotherium feignouxi* was most often placed in an unresolved position, confirming its controversial affinities.

Although Central Asia/Eastern Eurasia has been long regarded as the centre of origin and evolution of Cervidae (Vislobokova, 1990; Groves, 2006), evidence from the fossil record indicated that the origin of cervids may be in Europe (Heckeberg, 2017b). Their past diversity is known from around 26 fossil genera (Dong, 1993). Gilbert et al.’s (2006) reconstruction of the ancestral cervine, which was reconstructed to have had antlers with three tines, sexual dimorphism, moderately sized upper canines (smaller than in muntjacs), and a deep lacrimal fossa, cannot be confirmed by the fossil record.

In the early Miocene geographical changes played an important role by opening migration routes in Europe, Asia, and Africa. This had an rapid increase of ungulate diversity as a consequence, which remained like that during the warm climate of the Miocene Climatic Optimum throughout the middle Miocene. During the Miocene forest habitats were replaced by grasslands, which favoured the greatest radiation of ruminants (Hassanin and Douzery, 2003). Stadler (2011) showed that there was a slight but not significant increase in the diversification rate of mammals 15.85 mya. Around 15 mya, the sea-levels fell due to cooling climate in the high latitudes and forming ice sheets in the Eastern Antarctic; the fallen dry areas became grasslands (Haq et al., 1987; Flower and Kennett, 1994; Miller et al., 1991; Ludt et al., 2004).

The climate further cooled causing colder winters and drier summers when the circulation of warm deep water between the Mediterranean and the Indo-Pacific was interrupted. Subsequently grasslands spread over Europe and Asia between 8 and 7 mya providing perfect conditions for ruminants to further diversify (Ludt et al., 2004).

The cooling climate and increased seasonality in the late Miocene likely played a crucial role in the decline of large mammal diversity and causing endemic to occur in the climate belts. The lower diversity and the endemic of today may have originated already in the late Miocene (12 mya) and may be more complex than assumed (to lay in the Quaternary Climatic Cycles) (Costeur and Legendre, 2008). In the late Miocene the temperature gradient from equator to pole was weak and higher latitudes were warmer than today (Michelena et al., 2011).

During the Late Miocene of Asia environmental changes and uplift of the Tibetan plateau (11–7.5 mya; Amano and Taira (1992)) coincided with a global increase in aridity, seasonality and subsequent spread of grassland in Asia (Flower and Kennett, 1994; Gilbert et al., 2006). A glaciation period at the Miocene/Pliocene boundary caused a drop in sea levels triggering further diversification particularly within cervids (Ludt et al., 2004). A crucial factor for South East Asian cervid evolution was the split of the Indo-Melanesian and Sundaic faunistic subregions caused by high sea levels, which cut through the Thai/Malay Peninsula during the Early Pliocene separating faunas for the duration of around 1 my (Woodruff, 2003; Meijaard and Groves, 2004). After the warm Middle Pliocene, the Pliocene-Pleistocene boundary was characterised by drastic cooling (2.4–1.8 Ma) (Meijaard and Groves, 2004).

There is broad consensus that ancestral odocoileine cervids entered America from Siberia via the Bering Strait in the early Miocene/early Pliocene (Gustafson, 1985; Webb, 2000; Merino et al., 2005). The Bering land bridge disappeared around 9000 years ago with rising sea levels and the formation of the Bering Sea ending the faunal exchange between American and North Asia (Ludt et al., 2004). It is assumed that their ancestors were Eurasian Pliocene deer with three-tined antlers, such as *Cervavitus* (Fry and Gustafson, 1974; Gustafson, 1985). The first (presumed) odocoileine taxa were *Eochoerus* from Florida and *Bretzia* from Nebraska (around 5 my old), which are similar to *Pavodaria* from Northeastern Kazakhstan (Fry and Gustafson, 1974; Vislobokova, 1980; Webb, 2000; Gilbert et al., 2006).

The split between Odocoileini and *Rangifer* was suggested to have occurred in the middle Miocene between 15.4 and 13.6 mya, although their origins and relationships are unknown; the presence of close relatives of *Rangifer* among South American odocoileine fossils from the Pleistocene has been suggested (Gros and Grubb, 1987; Douzery and Randi, 1997). Cervids migrated from North to South America via the Panamanian bridge 2.5 mya (Plio-Pleistocene boundary) (Webb, 2000; Merino et al., 2005). The split of Odocoileini into Blastocerina and Odocoileina was dated to around 3.4 mya. It was hypothesised that
there was a diversification within Odocoileini in North America 5.1 mya, which is also supported by the
test record (Vrba and Schaller, 2000; Gilbert et al., 2006; Hassanin et al., 2012). The first unambiguous
adult antler fragment of Odocoileus is from 3.8–3.4 mya (Gustafson, 1985). The polyphyletic split of the
Mazama species into the two subclades, Blastocerina and Odocoileina, led to the interpretation that South
America was colonised at least twice. First, by the ancestor of Blastocerina in the Early Pliocene (4.9–3.4
mya), although this cannot yet be confirmed by the fossil record nor by a certain presence of a connection
between North and South America. However, a much earlier closure of the Panama Isthmus between 15
and 13 mya was recently suggested (Montes et al., 2015). The second colonisation was by the ancestor
of Mazama americana and Odocoileus virginianus around the Plio-/Pleistocene boundary Gilbert et al.
(2006). Stadler (2011) reported a significant rate shift of speciation to a decreasing diversification rate at
3.35 mya, which coincides with high tectonic activity.

Hershkovitz (1982) assumed a small odocoileine ancestor living in North, Central, or South America
during the Miocene-Pliocene-boundary from which Mazama and Pudu diverged. This hypothesis sug-
gested an increase in body size over time in other odocoileines, which is in contrast to the traditional view
of secondarily dwarfed Mazama and Pudu. As a logical consequence, the existence of medium sized
forms during the late Miocene and Pliocene of Asia and North America was assumed, which would be
the ancestors of the small odocoileines. This is also supported by the fossil record (Webb, 2000). Slightly
more recently, Merino and Rossi (2010) hypothesised that the first deer entering South America were medium
sized with branched antlers; these presumably diverged into Mazama and Pudu with simpler antlers, most
likely independently from each other.

Six fossil cervid genera are known from South America; they include Agalmaceros (1.8–0.8 mya),
Chariotorcas (1.8–subrecent), Antifer (1.2–subrecent), Epieuycerus 1.2–subrecent, Morenelaphus 0.5–sub-
recent, and Paracer (0.5–0.2 mya) (Hoffstetter, 1952; Tomiati and Abbazzi, 2002; Merino et al., 2005; Merino and Rossi, 2010; Gonzalez et al., 2014). Their fossil record is scarce and thus, the validity of
some of the species is doubtful (Alcaraz and Zurita, 2004; Menegaz, 2000; Merino and Rossi, 2010). So
far, there are only few studies on extinct neotropical cervids and even fewer attempting to reconstruct the
phylogeny of fossil and extant neotropical deer.

Neotropical cervids underwent a rapid radiation after migration into South America, where they
filled niches, which are occupied by bovids on other continents, making them the most diverse group of
ungulates in South America (Gilbert et al., 2006; Merino and Rossi, 2010). The low resolution among
Odocoileini haplotypes also suggests a rapid radiation event dating to about 2.5 mya, which coincides
with the land mammal invasion from North to South America (Webb, 2000; Gilbert et al., 2006). Today’s
South American cervids are adapted to a wide range of ecological habitats (Merino et al., 2005). The
radiation most likely was influenced by the absence of other ruminant artiodactyls and appears to be
the opposite scenario as in Africa, where bovids dominated. Morphology, physiology, adaptation of the
digestive system, temporal and spatial distribution of vegetation, and physicochemical properties of plants
triggered the diversification, thus making the evolutionary patterns very complex (Merino and Rossi,
2010).

The origination of living cervids of South America was estimated to 200 kya for Hippocamelus,
Blastocerus, Ozotoceros, 65 kya for Mazama, 48 kya for Odocoileus, and 16 kya for Pudu (Merino et al.,
2005). These recent dates document the rapid radiation of South American cervids, which is probably the
reason for the difficulties in resolving their relationships. After decades of research, the taxonomy and
evolutionary history of South American cervids remains enigmatic, partly because of the scarce Plio- and
Pleistocene fossil record (Fry and Gustafson, 1974; Webb, 2000).

**CONCLUSION**

The comprehensive data collection and results from the phylogenetic analyses provided new insights
into the systematic relationships of fossil and extant cervids. These relationships were investigated using
molecular and morphological characters separately and combined.

The morphological data sets were partly informative for extant taxa and gave new insights into the
systematic relationships of fossil taxa. There were some consistent splits within the morphological
topologies, for example the Elaphurus-Rucervus-Rusa-clade, Muntiacini, and Capreolini. The SFA and
EPA approaches were particularly useful for investigating the placement of fossil taxa.

In most of the molecular and combined analyses, extant clades on subfamilial and tribal level
were monophyletic. While systematic relationships within Cervinae were relatively stable, with many
consistently recovered subclades, systematic relationships within Capreolinae were more variable. Even
the monophyly of this subfamily could not be confirmed in all topologies.

No link between particularly incomplete taxa and phylogenetic instability was observed. For the
Miocene cervids, a placement in a stem position between the outgroup and all other cervids, or in a sister
position to Muntiacini was suggested in the analyses here. Most of the Miocene cervids were more closely
related to each other than to other cervids. Plio- and Pleistocene cervids, were most often placed within or
close to extant cervids and the majority of them within Cervini, some within Capreolinae or Muntiacini.

We extensively tested the systematic positions of extant and especially fossil cervids for the first time
under a comprehensive phylogenetic approach. Inclusion of more fossil cervids, postcranial characters,
soft anatomy and life history data, and cytogenetics would be useful in future analyses. Further, rare
genomic changes, such as gene duplication and genetic code changes, intron indels, and mitochondrial
gene order changes, and SNP chips have become more popular as complementary markers and should be
included as addition to the molecular partition in cervids.

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