General Palaeontology, Systematics and Evolution (Vertebrate Palaeontology)

Growth and life history of Middle Miocene deer (Mammalia, Cervidae) based on bone histology

Croissance et histoire de vie de cervidés (Mammalia, Cervidae) du Miocène moyen (Mammalia, Cervidae) : apport de l'histologie osseuse

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ABSTRACT
Our knowledge of the histology of the Cervidae (deer) was recently expanded with a work describing long bone and tooth histology of various taxa (Kolb et al., 2015a). Included in this study was the Miocene Procervulus, an early cervid whose growth rate was found to be especially low. The present study examines further “stem-cervid” bone histology in describing that of other Miocene taxa, Dicrocerus elegans and Euprox sp. With their inclusion in the dataset of Kolb et al. (2015a), we estimate the ancestral growth rates among cervids, and studied its correlation with body size. The skeletochronology of Dicrocerus suggests a relatively high growth rate for its body size differing from the condition of Procervulus and Euprox, and hence, documenting diversity in the life history traits of Miocene cervids.

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RÉSUMÉ
Notre connaissance de l'histologie des Cervidae a récemment été étendue grâce à une étude décrivant l'histologie osseuse et dentaire chez divers taxons (Kolb et al., 2015a). Cette précédente étude présentait Procervulus, un cervidé miocène dont le taux de croissance a été caractérisé comme particulièrement bas. Nous décrivons ici l'histologie osseuse de deux autres « stem-cervidés » miocènes, Dicrocerus elegans et Euprox sp. En les incorporant dans le jeu de données de Kolb et al. (2015a), nous estimons le taux de croissance ancestral au sein des cervidés, et étudions sa corrélation avec la taille corporelle. Les données squeletochronologiques chez Dicrocerus indiquent un taux de croissance relativement fort pour sa taille corporelle, contrastant avec ceux de Procervulus et Euprox, et documentant donc une diversité de traits d’histoire de vie chez les cervidés miocènes.

1. Introduction

The investigation of the palaeobiology of “stem-cervids” from the Miocene is necessary to understand the origin of the diverse life histories of deer. *Diceroscus elegans* Lartet 1837, one of these early cervids (Azanza, 1993; Gentry, 1994), was originally described from the French locality of Sansan (local Helvetian age), which corresponds to the mammalian Neogene age MN6 [Middle Miocene, Langhian, ca. 15 Ma; (Peigné and Sen, 2012)]. *Diceroscus* of a relatively intermediate body size [ca. 50 kg; (Costeur et al., 2012)], is characterized by its long pedicle and bifid “protoantler”. It is known from numerous specimens, which have allowed, for instance, to propose that the female of *Diceroscus*, like the extant *Rangifer* (Reindeer), were antlered, and that the species was marked by sexual dimorphism regarding size and shape of the antlers (Ginsburg and Azanza, 1991). However, different ontogenetic stages have been suggested as an alternative explanation (Gentry et al., 1999). The growth cycle of the “protoantler” comprises a shedding phase of the velvet-like skin and a phase with bare and dead bone tissue before casting (Azanza et al., 2012). Such a cycle appears to be an apomorphic feature groupin *Diceroscus* with later cervids. However, the protoantlers of *Diceroscus*, growing over a longer period than the annually deciduous antlers, may represent the ancestral condition for later cervids (DeMiguel et al., 2014). Although brachydont, *Diceroscus* is considered to be a mixed feeder (Solounias and Moelleken, 1994).

*Euproxx* is another cervid from the Middle Miocene of Europe (Gentry et al., 1999). Little is known about *Euproxx*, hypothesized to be sister–taxon of other Muntiacini (Azanza, 1993), albeit this is controversial (Gentry et al., 1999).

*Procervulus praecividus* (Oberfell 1957), known from the Early Miocene [MN3, Ginsburg (2011)], is regarded as being, with the rest of the Procervulinae, sister-group of all other cervids, including *Diceroscus* (Azanza, 1993). This species was recently included in a study of long bone and dental histology concerned with cervids life history evolution in order to estimate skeletal maturity and growth rate (Kolb et al., 2015a). *Procervulus* distinguished itself in featuring the lowest estimated growth rate of all the cervids sampled in that study. We here investigate if such a condition is widespread in “stem-cervids”. The bone histology and skeletalchronology of *Diceroscus* and *Euproxx* will hence be investigated here to gain insight into life history traits in early cervids, as it was previously performed regarding feeding styles (DeMiguel et al., 2008).

2. Material and methods

For *Diceroscus elegans*, eleven bones, comprising three humeri (MNHN.F.Sa7330, Sa7341, and Sa7343), three radii (MNHN.F.Sa2453, Sa2470, and Sa7063), two femora (MNHN.F.Sa6877, Sa6889), and three tibiae (MNHN.F.Sa6910, Sa6917, and Sa8163), were sampled. As indicated by epiphyseal closure, the specimens correspond to subadult to adult individuals. For *Euproxx* sp., one femur from the locality of Steinheim (Germany), with epiphyseal lines still slightly visible, was sampled (NMB Sth.1281). Thin-sections were prepared following conventional procedure (Chinsamy-Turan, 2005; Kolb et al., 2015a; Padian and Lamm, 2013). The sections were taken as close as possible from the mid-diaphyseal level (see Appendix A for estimation of relative position of each thin-section). Observations were performed with a petrographic microscope (Leica DM 2500M® under normal transmitted or cross-polarized light, in some cases with the use of a lambda compensator. Photographs were taken with a digital camera (Leica DFC 420C®) installed on the microscope. The quantification of the growth rate [annual growth rate with an estimated mean growth period of 260 days, as explained in Kolb et al. (2015a), and based on Köhler et al. (2012), was performed following published methodology (Kolb et al., 2015a), using the Leica IM 50 Image Manager® software. Only the growth rate of the femur and tibia will be discussed, because these bones were considered by Kolb et al. (2015a) as the most informative. The growth zone measurements were taken in the anterior region of the bones, in order to be consistent with the data from Kolb et al. (2015a). The histological terminology follows Francillon-Vieillot et al. (1990). Regarding the definition of the OCL, necessary for the skeletal maturity estimation, we follow Ponton et al. (2004) in acknowledging its presence when parallel-fibred/lamellar and avascular tissue forms the external-most region of the cortex.

A timetree was used to perform phylogenetically-informed statistical tests. The relationships and divergence times in extant taxa are based on Hassanin et al. (2012). Regarding relationships and divergence times of extinct taxa, the following data were used (and see above for *Diceroscus* and *Procervulus*). Megacerina sensu *Vislobokova* (2013) are here represented by the giant *Megaloceros giganteus* and *Sinomegaceros yabei*. *M. giganteus* is known from 0.4 Ma to 6.900 years BP (Stuart et al., 2004). The sampled specimen of *S. yabei* (OMNH QV-4067) comes from the Late Pleistocene (ca. 20–30,000 years) of Japan (Kolb et al., 2015b). Closely related to the Megacerina, the dwarf *Candiacervus* from Crete was also included (we consider the Megacerina to be included, along with *Candiacervus*, *Dama dama*, the Fallow deer, and *Cervus elaphus*, the Red deer, into the Cervini). Specimens of *C. rupalaphorus* are known to be at least 43,600 years old in one locality, and dated at ca. 21,500 years in another (*Vislobokova*, 2013). *Candiacervus* sp. II is also well known from the Late Pleistocene (Van Der Geer et al., 2010, p. 333). The relationships among Megacerina, *Candiacervus*, and with their closest extant relative, *Dama* (Lister et al., 2005), are not well understood (A.M. Lister, pers. comm.). We hence prefer to leave a polytomy in the topology. The divergence times within the (Megacerina + *Candiacervus* + *Dama*) clade are based on *Vislobokova* (2013). Due to the age of the Megacerina fossils, the origin of the Cervini clade was pushed back when compared to the molecular dating of Hassanin et al. (2012). Since *Euproxx* is found in MN6 (Gentry et al., 1999) and is hypothesized as sister-taxon of other Muntiacini (Azanza, 1993), the origins of Cervinae and crown-Cervidae were pushed back as well. However, this hypothesis should be treated with caution, because the phylogenetic position of *Euproxx* is regarded as controversial (Gentry et al., 1999). A nexus file containing the timetree is given as Appendix A.
A test for the presence of phylogenetic signal was performed for the growth rates and body size proxy (anteroposterior diameter of the section) using the Mesquite software (Maddison and Maddison, 2011) and a previously published procedure (Laurin, 2004; Quemeneur et al., 2013). It consists of the comparison of the squared length of the reconstructed parameter to those of 10,000 trees in which the terminal taxa were randomly reshuffled. The P-value of this test will be the number of trees (divided by 10,000) shorter than the initial tree. The ancestral character values were reconstructed using squared-change parsimony in Mesquite. The phylogenetically-informed linear regression of the growth rate against anteroposterior diameter of the section was performed using phylogenetically independent contrasts (PIC) analysis with the Mesquite software and its PDAP:PDTREE module (Midford et al., 2011).

Institutional abbreviations:

NMB, Naturhistorisches Museum Basel, Switzerland;
MNHN, Muséum national d’Histoire naturelle, Paris,
France; OMNH, Osaka Museum of Natural History, Japan;
ZIUK, Zoologisches Institut der Universität Kiel, Germany.
Other abbreviations:

CPL, crossed polarized light; CPL-λ, crossed polarized light with the addition of a lambda compensator; NL, natural light; OCL, outer circumferential layer

3. Histological description

All sampled bones feature a free medullary cavity and a compact cortex. Except for a few minor instances, trabecular bone was not observed.

3.1. Humerus of Dicrocerus

A layer of endosteal lamellar bone of variable thickness is found on the whole circumference of the innermost cortex. Most of the inner region of the rest of the cortex is heavily remodeled in all three specimens, preventing
the description of the primary structures. In one specimen (MNHN.F.Sa7341) large resorption vacuities, most likely related to a bone drift process, can be noted (Fig. 1A). These vacuities are surrounded by lamellar bone. The middle region of the cortex is either remodeled by longitudinal, oblique, and transverse secondary osteons (MNHN.F.Sa7330, MNHN.F.Sa7341, and part of MNHN.F.Sa7343) that can describe in some places pseudo-circular patterns (Fig. 1B), or be formed by fibrolamellar tissue, and with laminar to plexiform vascularization (Fig. 1C). In the outermost cortex, the vascularization is usually reduced to a few longitudinal vascular canals, and a clear OCL is found on two of the three specimens (MNHN.F.Sa7330; MNHN.F.Sa7341). Two specimens (MNHN.F.Sa7341 and MNHN.F.Sa7343) show a peculiar pattern of remodeling, in which most of the secondary osteons are restricted and almost entirely forming an area sharply defined externally by a line of arrested growth (LAG) (Fig. 1C).

3.2. Radius of Dicrocerus

In this bone, and when present, the endosteal deposit has a particularly irregular thickness, with sometimes several cement lines ("cementing line" sensu Francillon-Vieillot et al. (1990); Fig. 2A) and few longitudinal vascular canals. The rest of the cortex, either partially or entirely remodeled, is formed by fibrolamellar bone with a plexiform or reticular vascularization (Fig. 2B), and becomes poorly vascularized outwardly. One specimen (MNHN.F.Sa7063) corresponds to a section at the level of the outer nutrient foramen, as shown by the presence of a concavity in its posterolateral region. Numerous Sharpey’s
 fibre bundles are seen in its vicinity (Fig. 2C). None of the specimens features a clear OCL.

3.3. Femur of Dicrocerus

A very narrow to absent layer of endosteal bone is observed. The middle part of the cortex is well remodeled in both available specimens. The secondary osteons are longitudinal and to a lesser extent oblique, and in some places describe pseudo-circular patterns. In the inner part of lateral region of MNHN.F.Sa6889, remnants of trabeculae are present. Where not entirely remodeled (especially in MNHN.F.Sa6889), the outermost cortex is weakly vascularized, with few, mostly longitudinal, vascular canals, and mainly consists of lamellar bone. One specimen (MNHN.F.Sa6877) shows a cement line that cannot be interpreted as a LAG, due to its irregular and wavy shape and because it is not present throughout the whole section (Fig. 3A). Based on a similar structure described by Kleveza (1996, fig. 9), we tentatively interpret it as a resorption line. Neither of the specimens features an OCL.

3.4. Femur of Euprox

Although rather thin, the layer of endosteal bone in the sampled specimen of Euprox sp. (NMB Sth.1281) features two cement lines (Fig. 3B). The cortex is mostly formed by plexiform fibrolamellar bone with a high amount of fibrous tissue. Externally the vascularization becomes reticular and is less present. Secondary osteons are extremely scarce. The external-most cortex is mostly formed by lamellar bone and does not show any vascularization (Fig. 3B). However, this concerns a very thin layer of bone, which prevents from securely recognizing an OCL.

3.5. Tibia of Dicrocerus

An endosteal lamellar layer of irregular thickness is found in all specimens. Most of the cortex is formed by fibrolamellar plexiform bone. Reticular vascularization is also observed, sometimes forming lenticular areas between laminae of the plexiform vascularization (Fig. 4). The outermost cortex is formed by avascular lamellar bone. The secondary osteons have a longitudinal, oblique, or radial
orientation. The available specimens are weakly remodeled, with secondary osteons restricted to the inner half of the cortex. One specimen (MNHN.F.Sa6917) features an external deposit of avascular lamellar bone, but it is too thin to securely recognize it as an OCL.

4. Skeletochronology

When not obscured by secondary remodeling (especially the case in the sections farther away from the midshaft), all sampled bones feature at least one identifiable LAG. The LAG counts for each specimen (of which the count is not prevented by remodeling) are listed in Table 1. In Dicrocerus, for one specimen (MNHN.F.Sa7330; humerus) it was possible to conduct an estimation of skeletal maturity, thanks to its preservation of LAGs that are not obscured by secondary remodeling and of a clear OCL (Fig. 5). No LAG is lost (resorbed) during ontogeny in the femur and tibia of cervids (Kolb et al., 2015a). Based on the generally similar histomorphological pattern, it is also likely the case in the humerus and radius. With a count of five LAGs before the OCL, we hence hypothesize that the skeletal maturity in Dicrocerus was reached after five years. More specimens are required to make such an assertion for Euprox sp., because it was not possible to securely recognize an OCL in the available specimen.

The thickness of all zones and associated growth rates are given in Table 1. In Dicrocerus, and for the femur, only the second growth zone (between the first two LAGs) of one specimen could be measured (Fig. 3A). Being 0.98 mm thick, it corresponds to an annual growth rate of 3.77 μm per day. For the tibia, we were able to measure the thickness of the second growth zone on two specimens; in one of those specimens, we were also able to measure that of the second and third zones. The mean value of annual growth rate for the first three zones in the tibia is of 2.15 μm per day.

![Fig. 5.](Color online). Seven lines of arrested growth (LAGs; indicated by arrows) and the OCL (outer circumferential layer; indicated by bracket) in the medial region of a humerus of Dicrocerus elegans (MNHN.F.Sa7330). Euprox, the sampled femur yielded the first two LAGs defining a second growth zone that is 0.47 mm thick (Fig. 3B). This corresponds to an annual growth rate of 1.81 μm per day.

5. Discussion and conclusions

The free medullary cavity and compact cortex is a feature common to all cervids and more generally to most terrestrial amniotes (Laurin et al., 2011). The bone histology of Dicrocerus is on the whole consistent with that of other small to medium-sized cervids (Kolb et al., 2015a). Indeed, the primary bone tissue is mostly fibrolamellar and highly vascularized in the inner and middle cortex, with a mostly plexiform pattern, and the outer cortex is weakly
vascularized and formed by lamellar bone. The skeletal maturity, estimated in Dicrocerus to be reached after five years (during the sixth year of life), is reminiscent of that of Cervus elaphus (5–6 years old), Megaloceros (5–6 years old), and Candiacerpus (5–7 years old; Kolb et al., 2015a).

The newly measured growth rates of Dicrocerus and Euprox were added to the dataset of Kolb et al. (2015a) in order to discuss them in the broader context of the Cervidae. A significant phylogenetic signal was found for the anteroposterior diameter of the section (P-value = 0.0163). For the second annual growth rate, a P-value of 0.0746 was obtained. Even though this is higher than the usual 5% threshold, we consider it as indicative of a pattern of biological significance. It should be noted that this value is at least partly explained by the fact that a high growth rate was independently acquired in Alces, the Elk, and the Megacerina clade (Fig. 6A). As a matter of fact, pruning Alces from the tree brings the P-value of this test to 0.0286. Furthermore, a phylogenetically-informed linear regression was performed. After trying all combination of
log-transformation for each variable, the untransformed growth rate against log-transformed anteroposterior bone diameter obtained the greatest correlation coefficient ($r = 0.87$). Since a phylogenetic signal is also significantly present in the log-transformed anteroposterior bone diameter ($P$-value $< 0.01$), we used it for the rest of the analysis. The absence of significant correlation for both the growth rate and the log-transformed anteroposterior bone diameter in all diagnostic checks (absolute values of standardized contrasts against their standard deviations, or against their estimated nodal values, or against the heights of their base nodes, or the estimated nodal values against heights of the base nodes of the contrasts) allowed us to perform the PIC analysis (Midford et al., 2011). With a $R^2$ of 0.75 and a $P$-value lower than 0.0004, we find that the growth rate in cervids is strongly correlated with body size (Fig. 6B), confirming the results of Kolb et al. (2015a).

The growth rate corresponding to the femoral second zone is, in Dicrocerus (3.77 μm/day), close to that of Dama and Candiacervus sp. II (both at 3.34 μm/day), much lower than the large-sized Megaloceros (7.69 μm/day), Sinomegaceros (9.88 μm/day), and Alces (9.53 μm/day). However, Dicrocerus features a much greater second annual growth rate thanProcervulus (0.81 μm/day) and Euprox sp. (1.81 μm/day). Dicrocerus falls slightly above the 95% confidence interval of the phylogenetically correct linear regression (Fig. 6B), indicating a relatively high growth rate for its size. The ancestral value reconstructed for all cervids is 1.63 μm/day (Fig. 6A). The ancestral state for the Dicrocerus plus crown-cervids clade is 2.24 μm/day, which is already much higher than that of the Early Miocene Procervulus and closer to that of Dicrocerus, and which will be roughly found as the ancestral value for the crown-cervids, the Muntiacini (here represented by Muniaicus and the Middle Miocene Euprox), and the Cervinae as well. A first departure from this value is found at the origin of the Capreolinae, here represented by Alces and Capreolus capreolus (Roe deer). But this result is likely an artefact, as Alces, much larger than Capreolus, was shown to lower the significance of the statistical test (see above). The increase of growth rate is more probably acquired in a more restricted taxon, and sampling extinct Alcini would clarify this question. A more documented increase of growth rate is found for the Cervini, with an ancestral growth rate of 4.63 μm/day. While the relationships between Dama, Candiacervus, and the large-sized Megaceros were left as unresolved, it can be emphasized that a reversion to a lower growth rate is found in Candiacervus (ancstral value for the genus of 2.85 μm/day), and a steep increase is found as the ancestral value for Megaceros, with a growth rate of 7.64 μm/day.

The bone histology of Dicrocerus is reminiscent of those of other small to medium-sized deer. The skeletal-tochronological data suggest that Dicrocerus featured a higher growth rate than the other “stem-cervid” Procervulus and than the Middle Miocene Euprox. While growth rate was shown to be strongly correlated to body size, that of Dicrocerus was found as particularly high (slightly above the confidence interval of the phylogenetically-informed regression), documenting diversity in the early evolution of life history traits of the Cervidae.

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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.crvp.2015.07.001.

References


Ginsburg, L., 2011. The Early Burdigalian (MN3; Miocene) large mammals from Estrepouy (Aquitaine basin, France); an updated faunal list. Estud. Geol. 67, 411–417.


Kolb, C., Scheyer, T.M., Weitschege, K., Forasiepi, A.M., Amson, E., van der Geer, A., Hayashi, S., van den Hoek Ostende, L.W., Sánchez-Villagra,


