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Soft tissue characteristics for the reconstruction of ruminant phylogeny

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Abstract

In the attempt to derive phylogenetic relationships from the most comprehensive of character matrices, not only molecular, biochemical and osteological, but also data from less frequently used domains such as behaviour, life history, physiology and soft tissue anatomy are sought. Here, soft tissue traits that have been used in ruminant phylogenetic analyses, and that are potentially available for such analyses, are reviewed. The use of certain measures, such as the presence of an ileocaecal gland or certain skin glands, or the number of colic coils, appears unfounded. Using the presence of the gall bladder as a family trait disregards notable exceptions in the Bovidae. The largest set of potential, easily available soft tissue data appears to be related to the digestive tract, but has so far not been utilized. Generally, the paucity of recent anatomical studies is striking, which means that many available anatomical reports or drawings stem from the beginning of the 20th century. Currently no dataset exists that describes soft tissue anatomy for various organ systems in the same specimens (as is standard for osteological traits taken from whole skeletons). Suggestions are presented how data evaluation and scoring can be performed avoiding circular reasoning, and a plea is made for using data on a species, not a family level. The importance of assessing data independence and correcting for body mass-related effects is emphasized. For most organ systems, new, coordinated, systematic dissections are necessary before a reliable inclusion of soft tissue traits will become possible.

Key words: Stomach, liver, kidney, brain, intestine, skin, carotid rete, frontal sinus, lung, horn.

1. Introduction

Phylogenetic estimates are extremely important for researchers interested in morphological and physiological adaptations of organisms to ecological niches. This is because one of the most important pieces of evidence for a functional adaptation (defined as '*a phenotypic variant that results in the highest fitness among a specified set of variants in a given environment'* sensu Reeve and Sherman 1992) is convergence (Conway Morris 2003). If organisms that share a morphophysiological characteristic come from different phylogenetic backgrounds, i.e. do not share a common ancestor that had the same morphophysiological trait, then we speak of convergence or parallelism, depending on how this common morphophysiology is achieved (Futuyma 1998). Often, such convergence or parallelism occurs in connection with a similarity in a certain aspect of the animals' ecologies, such as a similar dietary niche or habitat. Strictly speaking, demonstrating convergence is no proof for any concept of function we might evoke in order to explain the adaptive value of the morphophysiological trait in this niche (Clauss et al. 2008b). It is, however, strong circumstantial evidence that such a function should exist. The function itself has to be demonstrated in different terms, such as logic and coherent narratives based on physical principles, or even better on empirical evidence of function by experiments. The omnipresence of convergence makes phylogenetic estimates so relevant for functional morphologists, physiologists, and all those interested in patterns of evolution that are based on arguments of adaptation.

Phylogenetic relationships, however, have to be reconstructed; usually, this is done with data one could call morphological on the macroscopic, microscopic or molecular level (cf. O'Leary et al. 2013 for a recent example). Ideally, these traits should not be affected by processes leading to convergence, and should be uninfluenced by immediate environmental conditions; in practice, using large numbers of traits is mostly considered adequate to overrule signals that derive from convergence. While in recent times, phylogenetic estimates are increasingly built on molecular data, macroanatomical data have been used in the past, mainly from the hard tissues in bone and teeth. Various approaches, including recent ones, have included combinations of molecular and morphophysiological data. Some of these approaches have included soft tissue traits.

In the generation of data one can use for this purpose, there probably is a basic conflict of interest: Functional anatomists and physiologists want to study those aspects of soft tissue morphology that are most likely related to what they conceive as 'function' and hence probably, at the same time, most likely subject to convergent evolution. Phylogeneticists need catalogues of unique characteristics that are best not subject to convergent evolutionary modification (and also not to immediate environmental/dietary/husbandry modification). However, it is exactly the measuring of such latter characters that functional anatomists often avoid. A typical example of this dichotomy is the volume of a certain organ of the digestive tract (which is a measure of evident functional relevance), as opposed to its detailed shape (which is a measure that does not have functional relevance but might be interesting for phylogenetic studies). Hence, peculiar macroscopic shapes, or branching patterns of vessels or ducts – structures that can deliver a function irrespective of their exact morphology – should have particular potential as soft tissue indicators of phylogeny, if they are not subject to relevant intra-specific variation.

In this contribution, I aim at both collating studies that have used soft tissue traits, and to collate information on soft tissue traits that could potentially be used, in reconstructing the phylogeny for ruminants. The definition of 'soft tissue' will not be completely adhered to, as some osseous or cartilagenous structures, and some physiological traits, will also be mentioned. Although I strove for completeness (omitting the placenta and foetal membranes, but see Klisch & Mess 2007, 2013), this review is far from being exhaustive. In presenting what I found, I will mention several issues to consider in these approaches, but will not perform a phylogenetic analysis myself.

2. Materials and methods

This contribution represents a literature review, for which publications on ruminant phylogeny were screened for the presence of soft tissue characters, and for which comparative studies on ruminant soft tissue anatomy were targeted. As search engines, 'Google scholar' as well as 'Pubmed' were used, and the reference list of the acquired publications. Illustrations of various organ systems mentioned in this contribution can be inspected in the 'Soft tissue biology' presentation for the International Conference on Ruminant Physiology 2013 that can be downloaded at http://www.zooklinik.uzh.ch/teaching-en/invitedlec/2-Day-Seminar-1.html.

3. Results

3.1 Studies that reconstruct phylogeny by (also) using soft tissue traits

The supplementary material of O'Leary et al. (2013) allows the tracing of various studies in mammals that included soft tissue anatomy in a phylogenetic reconstruction, and provides examples how different traits of organ systems can be coded. Langer (2001) used the morphology of the digestive tract of ungulate and cetacean families to explore the hypothetical phylogeny derived from that organ system alone. The following ruminant-specific phylogenetic studies were identified that contained soft tissue traits from more than one organ system.

Garrod (1877b) listed a large variety of anatomical features, including the presence of a gall bladder, the shape of liver lobes, intestine lengths, shape of rumen papillae and of the reticular cells, the number of omasal leaf classes, the morphology of the glans penis and the placenta. To what degree these different datasets influenced this author's concluding proposition for phylogenetic relationships between ruminants does not become evident; no mathematical or pre-defined approach appears to have been applied.

Janis & Scott (1987) argued with the presence of a gall bladder, the presence of an ileocaecal gland, the number of coils in the spiral colon (2.5 vs. 3.5), as well as placenta morphology on a family level to differentiate Cervidae from other families. They also mention the presence of an omasum separating Pecora from Tragulidae, and suggest – based on the evaluation of anatomical drawings from Hofmann (1973) and Langer (1974) – that the relative position of the entrance of the oesophagus into the rumen might be more ventral in giraffe as a 'primitive' or a derived condition, and recommend more work on this feature.

Groves & Grubb (1987) used the pelage spot pattern as well as interdigital, preorbital, metatarsal, tarsal and other skin glands, amongst other traits, to reconstruct Cervidae phylogeny.

Hassanin & Douzery (2003), taking information mainly from Janis & Scott (1987), used the presence of an abdominal musk gland, the presence of an omasum, the position of the cardia in relation to the forestomach (dorsal or 'more ventral'), the presence of a gall bladder, the presence of an ileocaecal gland, an 'extensible' tongue, and the number of mammary glands (2 vs. 4), as well as placenta morphology, on a ruminant family level.

3.2 Systematic or sporadic investigations of soft tissue traits

In the following paragraphs, examples for organ(system)s are given that could be, or have been used, for phylogenetic reconstructions. A list of the individual morphological measurements that could be or have been taken from these organ(system)s is not given; the reader is referred to the cited publications.

3.2.1 Respiratory system

3.2.1.1 Cranial sinuses:

Arguably, the cranial sinuses could also be considered part of osseous anatomy, but their relationship to the respiratory system may justify its inclusion here. As part of a larger study on all mammalian groups, Paulli (1900) gives a detailed description of the morphology of the nasal turbinates and associated sinuses in tragulids, several cervids, and domestic ruminants, including detailed reports on intraspecific variation. Farke (2010) demonstrated phylogenetic relationships in frontal sinus morphology of 62 Bovidae species.

3.2.1.2 Larynx and Hyoid:

Anatomical drawings from textbooks on domestic ruminant anatomy (Frewein et al. 2004), as well as numerous studies on the laryngeal anatomy of individual ruminant species (Burne 1917; Lander 1919; Frey & Gebler 2003; Frey et al. 2006; 2007; 2012; Erdoğan & Pérez 2013) suggest that morphological variation of this organ system has potential as a phylogenetic signal. In a similar manner, difference in the hyoid anatomy between ruminant species have been described (Saber & Hofmann 1984) and could potentially be used in phylogenetic reconstructions.

3.2.1.3 Lung:

Gunnell & Simmons (2005) provide an example how lung morphology could be used in general mammal phylogeny reconstruction. For Rodentia, Wallau et al. (2000) provided an excellent example how detailed morphological studies of the lung can be used for this purpose. A similar study for ruminants is lacking. Reports and illustrations of the respiratory tract of individual ruminant species (Hofmann 1962; Nakakuki 1983; Frewein et al. 2004; Liao et al. 2009) suggest that this organ system has potential as a phylogenetic signal. Additionally, in an investigation of 23 ruminant and 33 other land mammals, Bovidae but not non-bovid ruminants had higher resting breathing frequencies than the mammalian average (Mortolaa & Lanthier 2005), suggesting that respiration physiology may also contain a phylogenetic signal.

3.2.2 Cardiovascular system

3.2.2.1 Rete mirabile:

The intracranial carotid rete has been described in various artiodactyls, including ruminants (Lawrence & Rewell 1948; Daniel et al. 1953; Carlton & McKean 1977; Blix et al. 2011; Zdun et al. 2013), but is notably absent in the Tragulidae species investigated so far (Fukuta et al. 2007). Whether this current state represents a primary absence of the rete in Tragulidae (Mitchell & Lust 2008) or a secondary loss (Janis 2009) remains debated.

3.2.2.2 Vascular branching patterns:

General domestic animal anatomy (Frewein et al. 2004) as well as publications on individual ruminant species (Fukuta et al. 2003; Ahn et al. 2008; Pérez & Erdoğan 2014) suggest that branching patterns of the vascular system could have potential as phylogenetic signals. Wible (1986) provides an excellent example how the course of blood vessels – which can be reflected in ‘imprint’ shapes on osseous structures – might be used for phylogenetic reconstructions in mammals. Langer (1996) shows that the gastric arteries of cetaceans contain a strong phylogenetic signal.

3.2.3 Urogenital system

3.2.3.1 Kidney:

The most comprehensive description of mammalian kidneys is probably that of Sperber (1944). A similar, much shorter comparative approach was reported by Yi et al. (1987). The most remarkable finding of both studies is that, whereas ruminant kidneys usually have a smooth coherent surface, that of cattle is lobated, which corresponds to several internal renculi, as described in detail for domestic cattle (Frewein et al. 2004) but also found in other representatives of cattle-type ruminants such as *Bos grunniens*, *Bubalus bubalis* and *Bison bison* (Sperber 1944; Liumsiricharoen et al. 1997); *Bubalus depressicornis*, interestingly, shows a similar internal pattern of renculi but a smooth (Yi et al. 1987) or only very slightly lobated (Sperber 1944) surface. The taxonomic affiliation of *Ovibos moschatus* with sheep appears reflected in their common, simple kidney anatomy (Sperber 1944). Today, in contrast to Sperber (1944), we would probably consider the *Bubalus depressicornis* condition a secondary reduction in surface lobation associated with a secondary reduction in body size.

3.2.3.2 Male reproductive tract:

Garrod (1877b) suggested that the morphology of the glans penis could be used to evaluate phylogenetic relationships in ruminants. This impression is supported when inspecting the graphical documentation of this (Garrod 1877a; Garrod 1877b) and other authors (Lönnberg 1900; Pocock 1918b-f; Frewein et al. 2004).

3.2.3.3 Female reproductive tract:

The relevance of the placenta for ruminant phylogeny is described elsewhere (Klisch & Mess 2007; 2013). Differences in the shape of the cervix between domestic ruminant species (Frewein et al. 2004) suggest that this organ could also contain phylogenetic information.

3.2.4 Integumentary system

3.2.4.1 Pelage coloration:

Comparative analyses of ruminant pelage coloration have been presented by Stoner et al. (2003) and Caro & Stankowich (2010); such data is comparatively easy to come by in terms of photographs or drawings from textbooks and can be used for phylogenetic analyses.

3.2.4.2 Skin glands:

Pocock (1910; 1918a-f; 1944) provided a detailed list of ruminant skin gland occurrence, including mammary gland number, and also including a review of the comprehensive work on this topic published before his time. Subsequent work did not contribute much to these insights (Krölling 1930). A more recent collation of ruminant gland information, including original observations, was given by Sokolov (1982) and Groves & Grubb (1987). Skin glands appear to set Tragulidae apart from the crown Pecora (Dubost 1975; Geist 1999). Comparing sweat gland activity in eight Bovidae species, Robertshaw & Taylor (1969) concluded that this measure does not imply a phylogenetic signal. Work on *Odocoileus virginianus* demonstrated that the presence of skin glands can vary within a single species (Dubost 1975).

Because a 'musk gland' has been suggested to set Moschidae apart from other ruminants (Hassanin & Douzery 2003), this organ might deserve special attention. This gland is a preputial gland (e.g. Green 1987; Sokolov et al. 1987). According to Groves & Grubb (1987), preputial glands are also described for *Dama dama* (Kennaugh et al. 1977) and several small ruminants (Ansell 1969); additionally, they have been described in Tragulidae (Dubost 1975), *Capricornis crispus* (Atoji et al. 1989), *Ovibos moschatus* (Flood et al. 1989), *Antilocapra americana*, *Antilope cervicapra* and various Cervidae (Odend'hal et al. 1996). Although the preputial gland of Moschidae, and most definitely those of *Dama dama* (Odend'hal et al. 1996), might differ from that of other species, to my knowledge no comparative evaluation has been published that definitely allows distinguishing Moschidae based on gland presence or morphology alone. Evidently, this could possibly be achieved by coding for the chemical composition of the gland's secretion, but even in this respect, a comparative evaluation is missing.

3.2.4.3 Horns:

While antlers undoubtedly qualify as osseous structures, the horns of bovids include both osseous and non-osseous structures. Horn occurrence and shape has been included in various comparative studies (Caro et al. 2003; Stankowich & Caro 2009). It should be noted that certain aspects of horn (and antler) shape correlate with body size (e.g. Lemaître et al. 2014).

3.2.5 Nervous system (brain)

Examples of how traits of brain anatomy can be coded and used for phylogeny reconstructions can be found in Johnson et al. (1994), but ruminants only played a marginal role in these studies.

The appearance of the cerebral cortex has been described in a large number of African ruminants with the explicit intention to deduct phylogenetic relationships (Oboussier 1966; von Tyszka 1966; Oboussier 1967; 1970; Ronnefeld 1970; Oboussier 1971; Oboussier & Möller 1971; Oboussier 1972; 1976; 1978); however, the findings are not presented in table form. These publications are summarized by Hackethal (1981), who gives some examples where similarities in the cerebral furrowing patterns mirror known phylogenetic relationships; most interesting in this respect is perhaps the finding that *Syncerus caffer* (the only member of the Bovini in these studies) was found to group with the Tragelaphini, a grouping that the author – apparently unaware of the sister tribe status of the two (e.g. Hassanin & Douzery 2003) – uses as an example why cerebral cortex patterns alone cannot resolve phylogeny. Whether the extensive graphic documentation in these publications, or the textual descriptions, can be of future use for phylogenetic studies, and to what extent investigations of other research groups (e.g. Lander 1919; www.brainmuseum.org) can be added to this collection, remains to be demonstrated.

Pérez-Barbería & Gordon (2005) detected a phylogenetic signal in the body mass – brain mass allometry in ruminants, which suggests that relative brain mass could also be used as a trait for phylogeny reconstruction.

3.2.6 Digestive system

The digestive system of ruminants has received prominent attention in the scientific literature (e.g. Hofmann 1973; Langer 1973b; Hofmann 1988). The main focus of this attention was the apparent convergent evolution of morphophysiological traits among the different feeding types – browsers, intermediate feeders, grazers (Hofmann 1989; Clauss et al. 2008b). However, such convergence usually contains sufficient residual variability to suggest that these traits also contain phylogenetic signals. Importantly, it appears that while there is a strict morphophysiological separation between strict browsers and other feeding types, differences between intermediate feeders and grazers may be less systematic and leave much room for phylogenetic differences (Codron & Clauss 2010). The Bovini are an interesting example in this respect, because they present, in many details of the digestive tract, the most extreme opposite morphophysiological adaptations compared to browsers, yet many Bovini species appear to be less strict grazers than other Bovidae (Clauss & Hofmann 2014).

3.2.6.1 Salivary glands:

Salivary glands differ in size between ruminant feeding types, but the variation within feeding types suggests that other signals like phylogeny are also contained in the relative salivary gland mass (Hofmann et al. 2008). When evaluating salivary gland mass, it is important to note that different studies might produce different results due to different dissection techniques (Hofmann et al. 2008). The shape of salivary glands has not been studied in a comparative way, but could potentially also contain phylogenetic signal.

3.2.6.2 Palate:

The anatomy of the hard palate (Hofmann 1988) potentially provides a valuable phylogenetic signal. The number of palatal ridges was used as a trait by O'Leary et al. (2013); it should be noted that this trait will vary systematically with body size. With respect to the soft palate, Maier (2013) showed that the arrangement of the levator palatini muscle in ruminants differs systematically between species and hence also probably contains phylogenetic signal.

3.2.6.3: Tongue:

Reported details suggest that tongue anatomy may provide a valuable phylogenetic signal (Sonntag 1922; Sonntag 1925; Hofmann 1988). Given that not only Giraffidae, but also cattle (M. Clauss, pers. obs.; cf. the many photographs documenting cattle reaching with their tongue deep into their own nostrils, and the typical feeding behaviour of cattle that includes grass sward comprehension with the tongue), tragulids (G. Rössner, pers. comm.; cf. photographs documenting tragulids reaching with their tongue above their own eyes) or *Rangifer tarandus* (Hofmann 2000) can extend their tongues far out of their mouth, I do not think that characterising the Giraffidae by an 'extensible tongue', in contrast to other groups, is correct. *Rangifer tarandus* appears to be peculiar in having a bilobular tongue tip (Hofmann 2000).

3.2.6.4 Chewing muscles:

Most likely, the detailed anatomy of the chewing muscles could be used as a phylogenetic signal in ruminants (e.g. Axmacher & Hofmann 1988; Sasaki et al. 2001; Endo et al. 2002), although a comparative evaluation that goes beyond the mass of the masseter (Clauss et al. 2008a) is lacking so far. Chewing muscles have been used, for example, to discuss phylogenetic relationships in rodents (Hautier & Saksiri 2009).

3.2.6.5 Forestomach composition:

Whereas Pecora have an omasum, this forestomach compartment is not present in Tragulidae (reviewed in Clauss & Rössner 2014). It should be noted that this has no influence on the process of rumination, and tragulids ruminate as well (Hendrichs 1965).

3.2.6.6 Rumen shape:

In my personal view, the potentially most promising set of existing, 'ready-to-use' soft tissue information is the standardized depiction of the ruminant forestomach by Hofmann (Hofmann 1969; 1984; 1985; Hofmann & Nygren 1992; Hofmann et al. 1995). This collection of shape information can be easily expanded by standardised drawings or photographs (Pytel 1969; Stafford & Stafford 1991; Agungpriyono et al. 1992; Stafford 1995; Yamamoto et al. 1998; Clauss et al. 2006c; 2009a; Pérez & Jerbi 2012; Pérez & Ungerfeld 2012; Jerbi & Pérez 2013; Pérez et al. 2014). Two aspects of rumen shape lend themselves to easy coding, in my view, using this information.

i. the number of ruminal blindsacs (one, two or three): while investigated Tragulidae species have only the ventral ruminal blindsac, the rumen of all other investigated species of non-Cervidae has two blindsacs; the same applies to all investigated species of the Capreolinae. Most investigated Cervinae, however – *Elaphurus*, *Cervus*, *Rusa* and *Muntiacus* spp., - have three ruminal blindsacs. The same is true for *Dama dama*, although the third blindsac is rudimentary in this species (Geiger et al. 1977). In contrast, the rumen of *Axis axis* clearly displays only two blindsacs (Pérez et al. 2014).

ii. the position of the dorsal rumen and blindsac in relation to the ventral counterpart (shorter, or of equal posterior extension): The dorsal rumen is of equal posterior extension in the investigated species of Giraffidae, Bovini and Tragelaphini, those Cervinae that have three blindsacs, in Alcelaphinae, Hippotraginae, and in *Kobus ellipsiprymnus* and *K. leche* (but not *K. kob*). In all other investigated species, the dorsal rumen is shorter.

Other aspects of rumen shape, including a putative variation in the position of the cardia (oesophageal entrance into the rumen), cannot be derived from this information.

3.2.6.7 Rumen papillation:

Although attempts have been made to describe rumen papillae shape on a species level (e.g. Garrod 1877b), the intra-individual and intra-specific variation in papillae size and shape (reviewed in Clauss et al. 2009b) appears too large to make this approach promising. Apparently in contrast to all other ruminant species investigated so far, *Rangifer tarandus* has edged ruminal papillae (depicted in Josefsen et al. 1996). The distribution pattern of papillae in the rumen – from a homogenous papillation to a clear stratification with few papillae in the dorsal and ventral, and large papillae in the middle regions (Clauss et al. 2009b) – could be used as a phylogenetic signal.

3.2.6.8 Rumen pillars:

Differences in rumen pillar thickness that apparently correlate with feeding type have been demonstrated (Clauss et al. 2003b) and could be used as phylogenetic signal. Rumen pillar anatomy might have potential for phylogenetic reconstructions but has not been described for a larger number of species in a comparative approach. The usefulness of this trait can be seen in the peculiarity of an accessory rumen pillar documented in *Bubalus bubalis* (Hemmoda & Berg 1980) that is also found in *Bubalus depressicornis* (Clauss et al. 2009c).

3.2.6.9 Reticular crests:

Ruminants differ distinctively in the morphology of their reticular crests, which can range from very shallow to extremely prominent (Garrod 1877b; Neuville & Derscheid 1929; Hofmann 1988; Clauss et al. 2010a), and most likely contain a useful phylogenetic signal.

3.2.6.10 Omasum:

The graphics cited in 3.2.6.6 indicate that the size of the omasum varies enormously among ruminants, and there are corresponding differences in the number of omasal laminae, in their size and shape, and in their total surface (Garrod 1877b; Hofmann 1988; Clauss et al. 2006a). Bovini are distinct in terms of an exceptionally large omasum size (but with a convergence in *Ovibos moschatus*) (Clauss & Hofmann 2014); a comparative evaluation of a larger species set is missing so far.

3.2.6.11 Stomach muscle architecture:

Differences in the arrangement of (smooth) muscle layers have been used to discuss questions of homology between the forestomachs of various mammalian herbivores (Langer 1973ab, 1988), and are potentially useful also at a lower taxonomic level.

3.2.6.12 Rumen physiology:

Maybe the most distinctive difference in rumen physiology between different ruminant species is the degree by which fluid passes through the rumen faster than particles (Clauss & Lechner-Doll 2001; Clauss et al. 2006b; Müller et al. 2011). 'Moose-type' ruminants have a comparatively low fluid throughput through the rumen, whereas 'cattle-type' ruminants have a high throughput (Clauss et al. 2010b). Additionally, there apparently is a phylogenetic signal in the digestive efficiency achieved by different ruminant species (Pérez-Barbería et al. 2004), but the dependence of this physiological measure on the diet used in the experimental measuring may make it less useful as a trait for phylogenetic reconstructions.

3.2.6.13 Abomasum:

There are notable differences in abomasal anatomy between ruminant species, such as the number of abomasal folds (Hofmann 1973) or the thickness of the abomasal mucosa (Hofmann 1988) that have potential phylogenetic relevance.

3.2.6.14 Intestine:

There is some indication that the length of the intestines could represent a phylogenetic signal (Hofmann 1988; Pérez et al. 2008). A major limitation is that the majority of the (limited) available data so far is reported without the body mass of the corresponding specimens (Garrod 1877b). Because there is an evident relationship between body mass and intestine length, length data has to be corrected for body mass (Weckerly 1989). Also, intestine length is subject to intra-specific variation depending on (seasonal) resource availability (Weckerly 1989). The differences in small intestine length between domestic ruminants and domestic horses (Frewein et al. 2004) could suggest that a very long small intestine is typical for ruminants in general, but this hypothesis awaits testing.

3.2.6.15 Ileocaecal gland:

Glandular tissue at the ileocaecal junction has been described in domestic ruminants (Frewein et al. 2004) and also in a variety of nondomestic species, including Moschidae, *Dama dama*, *Alces alces*, *Odocoileus virginianus*, *Tragelaphus scriptus* and *Oryx beisa* (Garrod 1877a), *Okapia johnstoni* and

Giraffa camelopardalis (Burne 1917; Neuville 1922; Derscheid & Neuville 1924); its absence has been reported in a *Muntiacus* spp. and a *Boselaphus tragocamelus* (Derscheid & Neuville 1924). A structure that corresponds to the ileocaecal gland, without being named as such, was described by Mitchell (1905, p. 518) for 'several antelopes', with *Gazella marica* as a specific example. Given these findings and the paucity of species in which an absence has been reported so far, it does not appear justified to use presence of this structure as a trait in phylogeny reconstructions.

3.2.6.16 Spiral colon:

The ruminant spiral colon shows remarkable variation between species both in terms of its length and in the resulting number of coils (Home 1814; Garrod 1877b; Mitchell 1905; Westerling 1975; Hofmann 1988; Woodall & Skinner 1993; Pérez et al. 2008; Pérez et al. 2009); to my knowledge, findings so far do not allow to ascribe any specific number of coils to a ruminant family, but coding would have to be made on a species level. As with most quantitative anatomical measures, there is a clear relationship between body mass and the length of the colon and hence also the number of coils, already stated by Garrod (1877a). This relationship is most likely modified by adaptations to habitat (Woodall & Skinner 1993; cf. Clauss et al. 2003a; Clauss et al. 2004) but may well contain a phylogenetic signal, again exemplified by Bovini with their particularly short colon (Clauss & Hofmann 2014). Westerling (1975) documented substantial intra-specific variability in the arrangement of colic coils, and Mitchell (1916, p. 218-219) provides a good example why counts made by different investigators may not be comparable.

3.2.6.17 Supra-Meckelian fold:

The part of the colon that follows, in sequence, the spiral colon, before the final part of the large intestine that is drained by the posterior mesenteric vein, is sometimes called the 'supra-Meckelian fold' (Mitchell 1905); this term is best avoided today. The arrangement of this part of the colon is variable between (but also within) species (Mitchell 1905; Westerling 1975; Pérez et al. 2008; Pérez et al. 2009) and might be useful as a phylogenetic character, but has not been documented in a large number of species.

3.2.18: Mesenteries:

Differences in the arrangement of mesenteries have been used to discuss questions of homology between the forestomachs of various mammalian herbivores (Langer 1973ab, 1988, 2001). Within ruminants, individual particuliarities regarding mesenteries have been described sporadically (e.g. Pérez et al. 2008). Whether such information can provide a reasonable phylogenetic signal within ruminants that is not subject to enormous intra-specific variation remains to be tested.

3.2.6.19 Liver:

Gunnell & Simmons (2005) provide an example of how liver morphology could be used in general mammal phylogeny reconstruction. The shape of the various liver lobes, and in particular that of the 'Spigelian' or caudate lobe, has been suggested as a phylogenetic character in ruminants (Garrod 1877b). Information on additional species could be gleaned from other sources (Lönnberg 1900; Lander 1919).

3.2.6.20 Gall bladder:

The presence of the gall bladder has often been used in describing ruminant families. The probably most comprehensive collection of information on this trait was presented by Gorham & Ivy (1938). This survey reports on the presence of a gall bladder in species of Tragulidae, Antilocapridae and Moschidae. It reports the gall bladder's absence in species of Cervidae - citing reports of a gall bladder present in three *Axis axis* and one *Mazama* spp. from Crisp (1862), but also citing reports of its absence in these species/ genus. It notes the gall bladder's general presence in Bovidae with the clear exception of all Cephalophinae species investigated as well as reports of an absence in Crisp (1862) for *Raphicerus melanotis*, *Pelea capreolus*, *Addax nasomaculatus*; these three species are not listed correspondingly in Gorham & Ivy (1938), but another finding of a gall bladder in *A. nasomaculatus* is mentioned. Crisp (1862) also reported the absence of a gall bladder in *Alcelaphus buselaphus* and *Damaliscus pygargus*, whereas Gorham & Ivy (1938) additionally mention a report of a gall bladder in these species. Finally, Crisp (1862) states the absence of a gall bladder in three goats, which is explicitly considered a mistake by Gorham & Ivy (1938), although Crisp (1862) specifically states that he has observed a gall bladder in other goat specimens. For *Giraffa camelopardalis*, Gorham & Ivy (1938) collated conflicting statements reports with a majority reporting the absence and a minority reporting the presence of a gall bladder. Similarly, Cave (1950) only reported gall bladders in two out of 19 giraffe specimens he dissected, but Kobara & Kamiya (1965) found a gall bladder in each of two specimens they investigated. Burne (1939) reported the absence of a gall bladder in *Okapia johnstoni*.

3.2.7 Skeletal muscles

Whidden (2000) as well as Diogo & Wood (2011, 2012) gave recent examples how muscle anatomy can be used for phylogenetic investigations. To my knowledge, similar approaches for ruminants are limited to comparisons of individual species (e.g. Bell & Flower 1876). Evidently, muscle tissue holds a great potential for phylogenetic signal.

4. Discussion

Studies that have used soft tissue measures to solve phylogenetic questions have a long-standing tradition. Such an approach was followed, rarely in a formal systematic manner, by late 19th- and early 20th-century anatomists, and has been refined since (e.g. Wallau et al. 2000, Gunnell & Simmons 2005, Diogo & Wood 2011, 2012). Remarkably, many recent studies using soft tissue anatomy rely on reports from those anatomy pioneers, which makes many claims difficult to trace. Closer inspection makes many of the traits used appear doubtful, in particular in conjunction with their use on a family level. Assumed differences in soft tissue anatomy mostly follow known taxonomic boundaries and are not used to decide cases of unsolved phylogenies on a detailed level.

4.1 General issues related to trait representation for phylogenetic reconstructions

4.1.1 Trait independence

There appears to be a general consensus that the traits that are used for phylogeny reconstruction should be independent (O'Leary et al. 2013). In other words, a repeated coding of basically the same trait by coding its various expressions individually should be avoided. Theoretically, such repeated coding would induce bias in favour of traits that are captured in a larger variety of morphologic details than traits that are only captured once. A typical example in ruminant soft tissue anatomy would be the presumed parallel convergence in many different traits of the digestive tract between feeding types in the anatomy of the salivary glands, various stomach compartments, intestines, and liver (Hofmann 1989). Although the large variation in the individual traits, and the low (though significant) correlation coefficient of individual variables with proxies of feeding type suggests that there is sufficient residual variation to preclude a strict character dependence, formal tests of this assumption would probably be a good asset. In the course of such formal tests, differences in divergence patterns through evolutionary time could be assessed and used to derive scenarios on the succession of trait evolution (sensu Streelman & Danley 2003) on a very detailed level. Other examples of dependent trait usage that should be avoided include the simultaneous use of the characteristic 'chewing the cud' (i.e. being a functional ruminant) and a coding for the quantity of methane production (O'Leary et al. 2013), because ruminating mammals usually produce more methane than nonruminant mammals (Franz et al. 2010; Franz et al. 2011); or the simultaneous use of both the characteristic of 'chewing the cud' (i.e. being a functional ruminant) and the presence of a reticulum (O'Leary et al. 2013), because – even ignoring the question whether the corresponding stomach compartments are really homologues in Ruminantia and Tylopoda (cf. Langer 1988) – rumination requires a sorting mechanisms that is located in the stomach compartments of these groups referred to by that name (Lechner-Doll & von Engelhardt 1989; Clauss et al. 2009a). Note that these two criticisms of the O'Leary et al. (2013) trait collection refer to traits that I feel sufficiently informed about to judge.

In practice, there appears to be little control for trait independence, and I am not aware of standardised protocols used routinely to that effect before the inclusion of a trait in a character matrix for phylogeny reconstruction. Additional evident examples of violations of trait independence are situations where absolute measurements (such as gestation length, methane production, length of an osseous or soft tissue structure) are used to derive traits and are used in combination, and/or in combination with body mass or body length. It is logical that larger animals have larger structures, and any coding for such measurements would have to include, prior to the classification, a correction for body size. Less evident examples of such violations of trait independence may be characteristics that are conceived as qualitative, but that are nevertheless related to body size. For example, the lobated outer appearance of kidneys as well as the internal structure of kidneys appears to be related to size (Sperber 1944). Similarly, the degree of furrowing on the cerebral cortex is clearly a function of body size (Hackethal 1981). Using such information indiscriminately, without testing for associations with body size proxies, will lead to the inclusion of non-independent traits.

4.1.3 Trait representativity

4.1.3.1 Traits that occur in single species:

Traits that only occur in single or few species that represent a higher taxonomic unit are apparently sometimes added to data matrices, as for example the deciduous horn sheet of Antilocapridae (Hassanin & Douzery 2003). This raises the question whether one would also include such traits in matrices if they were not, incidentally, representative for a monotypic group, but typical for a single member of a speciose taxon – such as the unique shape of the ruminal papillae or the tongue in *Rangifer tarandus* (3.2.6.3 and 7). Deciding to use the former information but not the latter in constructing a character matrix represents a decision that may be reasonable and practical, but that does not originate from the data per se but from some preconceived notion of phylogenetic relationships. Ideally, phylogenetic approaches should have a clearly stated strategy in the selection of character traits before those traits are searched for. If the only criterion is to use every information possible, information such as on the reindeer papillae and tongue would have to be included.

4.1.3.2 Traits that are assigned at higher-than-species level:

When certain traits are not entered in matrices on a species level, but considered a taxon-specific trait, such as the absence of a gall bladder and an ileocaecal gland in the Cervidae and their presence in the Bovidae, or the absence of 'abdominal musk glands' in any ruminant except the Moschidae (Janis & Scott 1987; Hassanin & Douzery 2003), the question arises about the defined rules that have to be met to allow such classifications. The question is whether we can accept such an opinion-based classification that is not based on a systematic investigation (but on 'general knowledge') as a trait? How much circular reasoning is involved in that process? Can we assign a state ('gall bladder present') to a taxon (Bovidae) even if only one single exception has been reported? How many exceptions have to be reported before we have to revise that classification (actually, at least six exceptions are recorded – all investigated Cephalophinae species; not counting the other maybe spurious exceptions in 3.2.6.20)? How do we reconcile the description of the musk gland resembling preputial glands in other ruminants (3.2.4.1) with the putative uniqueness of Moschidae in this respect? In my personal view, these examples suggest that the allocation of traits must be made on a species level, and not on a level of higher taxa.

4.1.4 Sample size

Another important issue in collating morphological information is the sample size that one will accept as an indication of a reliable, species-specific trait characterisation. In theory, this would have to be assessed for any molecular, microscopic or macroscopic morphophysiological trait. Intuitively, one may assume that certain features that are of a 'more general' bauplan relevance, such as the number of subdivisions of a stomach compartement, to be less subject to intra-specific variation than 'less general' bauplan features, such as the exact location of branching points in blood vessel systems or the number of omasal leaves (Frewein et al. 2004), the exact placement and number of coils of the colonic spiral (Westerling 1975), the exact shape of the furrows of the cerebral cortex (Oboussier 1966) that all show relevant intra-specific variation. Even traits one might consider less flexible, such as the presence or absence of a gall bladder (see the *Giraffa camelopardalis* example in 3.2.6.20), or the presence of certain skin glands (Quay 1971), may be subject to substantial intra-specific variation. In the case of quantitative measures, where data from several individuals can be combined by averaging, one would have to ensure that any cut-off threshold that leads to a categorical classification does not only apply for the species average, but also for all individual measurements of that species.

Sample sizes are often not indicated in anatomical studies, or are notoriously small. The example of the discussion about the presence or absence of a gall bladder in *Giraffa camelopardalis* (3.2.6.20) could serve as exhortation that deriving traits from small sample sizes might create relevant bias, especially if results are used to extrapolate a trait beyond the species level.

4.1.5 Quantitative traits

A general problem in using measures of functional anatomy is that they commonly describe features that scale with body mass; a difference in function is then often linked to differences as described by the quantitative measure relative to body mass between species. Because of differences in the scaling relationship of body mass and the measure in question – due to geometry alone, a length measure should scale to mass$^{0.33}$, an area measure to mass$^{0.67}$, and a volume or mass measure to mass$^{1.0}$ – one cannot use the simple 'proportion of body mass' as a default data transformation. Rather, the residuals of a regression analysis of the (log-transformed) data should be used. In a second step, this information has to be transformed into categories for the use in the matrix for phylogeny reconstruction. This process appears to be particularly prone to circular reasoning, if thresholds are decided based on differences between perceived clades. As a less subjective measure, an a priori scale could be applied on standardized residuals that define categories in terms of the magnitude of their deviation from the mean. By normalising the residuals, and grouping them into the maximum number of character states that the software allows, quantitative traits can possibly be coded by retaining much of their quantitative information.

4.2 Data availability

When assessing the availability of data, soft tissue traits 3.2.1.1 (frontal sinus), 3.2.4.1 (pelage coloration), 3.2.4.3 (horn and antler information), 3.2.5 (brain masses), 3.2.6.1 (salivary gland mass), 3.2.6.4-10 (masseter mass, forestomach composition, rumen shape, rumen papillation, rumen pillar thickness, reticular crest height, omasum anatomy), 3.2.6.12 (rumen physiology) 3.2.6.20 (gall bladder) represent traits that are either already coded or available as original data that can be coded, e.g. using a process of residual calculation and classification as outlined in 4.1.4. For other sources, criteria by which the scattered available data can be evaluated would have to be defined first. This evidently creates a bias towards the digestive tract. As long as no new data are available, an approach that attempts to classify all available traits appears justified but represents a major endeavour. The value of adding such information to molecular data should be evaluated.

5. Conclusions

In conclusion, coding of morphological characters for phylogenetic studies should not be made based on a quick literature screening by researchers alien to the anatomical/ physiological topic in question, especially not if no pre-coded data exists (as for most organ systems described here). The kind, and the reliability of the information used, should be based on a priori defined quality measures, and these should include definitions on whether traits can be read from drawings or photographs. Ideally, coding should include a blinded procedure, where the person applying the coding rules is unaware of the species, to avoid circular reasoning. Independence assessment should follow a defined protocol and should always consider the influence of body size. Ascribing traits on higher than species level may often lead to erroneous ascriptions, and must be justified in terms of a defined methodology. For most cases where soft tissue data is wanted for phylogenetic evaluations, these characteristics will have to be investigated and described anew in a comparative approach.

The generation of such data would be an extremely valuable project; unfortunately, in order to generate high-quality data, such a project would require logistical and financial efforts that make it a difficult prospect, including field weighing and dissection facilities and a substantial travel budget (and salary) for a group of anatomists, and provisions for extensive sample transport and storage. Even comprehensive collections, as e.g. for primate digestive tracts in general (Chivers & Hladik 1980), or for ruminant-specific measures (such as in Hofmann 1973; Hofmann 1988; Hofmann et al. 2008), represent the work of decades, are nevertheless confined to a very limited number of anatomical measures (in the case of the named examples, only measures related to the digestive system), and include reports on species with sample sizes of one. Datasets that represent measurements on various organ systems from the same individual animals (and hence possibly reducing data variation due to body mass, body or reproductive condition, sex, locality, time of measurement, subspecies status, or anatomical definitions and measurement technique) do not exist for ruminants or other groups of large mammals. Nevertheless, the diversity of ruminants, their global representation in basically all biomes, and the occurrence of larger numbers of species at certain geographical locations with hunting operations in place, would make such a project both worthwhile and feasible.

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