Gastrointestinal and dental morphology of herbivorous mammals: where does the Laotian rock rat fit?

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We studied dental complexity in Laonastes aenigmamus to place this unique rodent species into a comparative context. The complexity of cheek teeth of Laonastes are within the range of that for omnivorous mammals feeding predominantly on plants or that for herbivorous mammals. In a comparative sample of predominantly herbivorous hystricomorph rodents, only the degu (Octodon degus) and the chinchilla (Chinchilla chinchilla) have less complex cheek teeth than Laonastes. We also used ethanol fixed stomachs and a silicone cast of stomach of five Laonastes to study its macroscopic and microscopic gut anatomy. The stomach of Laonastes has four elongated chambers that are partially sacculated. The stomach of Laonastes differs microscopically from the typical rodent stomach: most of the epithelium is glandular, and non-glandular stratified squamous epithelium can be found only in the sulcus that is microscopically homogenous. Microscopic screenings showed that the digesta was similar in all stomach compartments. The mean renal medullary thickness (RMT) in this species is low indicating that its ability to concentrate electrolytes in the urine is low. We discuss the dentition and the stomach morphology of Laonastes in relation to observations on its natural diet.

Introduction

Laonastes aenigmamus is the only living species of the Diatomyidae family, otherwise represented by four genera and nine species, all extinct and distributed across Asia and Arabia from the Early Oligocene to Late Miocene (34–11 Ma) (Dawson et al. 2006, Lopez-Antonanzas 2011). The geographical distribution of Laonastes encompasses only the mountains of Khammouan karst, in the Central Lao People’s Democratic Republic (Keovichit et al. 2011).
Laonastes postcranial skeleton has been noted to be relatively unremarkable (Jenkins et al. 2005, Dawson et al. 2006, Huchon et al. 2007) but the jaws, masticatory musculature, and dentition are highly distinctive (Jenkins et al. 2005, Dawson et al. 2006, Hautier et al. 2011, Herrel et al. 2012). The mandible of Laonastes presents an intermediate association of features that could be considered neither sciurognathous nor as hystricognathous (Hautier et al. 2011). As compared with fetal crania and musculature, in adult Laonastes the rostral part of the skull elongates and the zygomaticomandibularis muscle develops disproportionately (Herrel et al. 2012).

Dry leaves, especially of Euphorbiaceae form the main part of the diet of Laonastes, with additional consumption of structures built by termites and some insects (Jenkins et al. 2005, Keovichit et al. 2011, J.-P. Hugot pers. obs.). In an analysis of tooth wear indicating the probable diet of the last few meals (Jenkins et al. 2005), some differences were reported between individuals. One individual had wear on the teeth suggesting that leaves formed the last meals, while there was evidence of grass and seeds in the diet of a second individual.

Recent studies on the gastrointestinal tract of the Laotian rock rat Laonastes aenigmamus (Keovichit et al. 2011, Scopin et al. 2011) have revealed that these animals appear to digest plant fibre with macroscopically compartmentalized stomachs (Figs. 1 and 2) characterized by a distinct sulcus (Fig. 3). These findings, together with the relatively small body size of Laonastes (maximum body weight about 500 grams), beg the question of how the gastrointestinal morphology of Laonastes compares to that of other mammals.

Mammal species show remarkable variation in their stomach morphology in terms of compartmentalization and the type of mucosal surface (Barry 1977, Carleton 1973, Langer 2002, Wang et al. 2003, Stevens & Hume 2004, Kotze et al. 2006). The monogastric compound stomach of the rat (Rattus norwegicus) for example, is divided by the margo plicatus into the proven-tricular (non-glandular) and the glandular parts (Baker et al. 1979). With an elaborated gastric wall topography, and placement of different types of glandular cells (Abdel-Magied & Taha 2003), even further functional compartmentalization of the mammalian stomach can be achieved.

Different gastrointestinal morphologies are linked to the fermentation of fibrous food components by microbes (Stevens & Hume 1998). Since fermentation takes time, the specialized gut compartments assist the retention of fibrous materials for efficient digestion. There are two broad morphophysiological types of mammalian herbivores that are defined according to whether fermentation takes place in the forestomach cranial to the acid-stomach (as in ruminant and non-ruminant foregut fermenters), or in the hind-gut caudal to the acid-stomach (as in colon and cecum fermenters, Stevens & Hume 1998). Each of these systems have their own benefits and
drawbacks with regard to digestive efficiency, but for both types of herbivore, body size is thought to be a key factor in their ecology and evolution through its impacts of energy/nutrients requirements and gut capacity (Demment & Van Soest 1985, Müller et al. 2013).

Many small mammalian herbivores minimize faecal protein losses (in the form of bacterial matter) by selectively collecting bacteria in their hindgut by special retention mechanisms (Hume & Sakaguchi 1991), excreting these in a controlled fashion (e.g., by distinctively formed, special faeces) and re-ingesting them (Franz et al. 2011). Clauss et al. (2007a) suggested that coprophagy may be assumed per default in any herbivorous rodent unless proven otherwise.

Although we described the general characteristics of Laonastes morphology above, our purpose was to further investigate Laonastes stomach characteristics as well as quantify functional aspects of dental form for comparison to other rodent species. We also aimed to compare the known morphology of this species to our behavioral observations on captive Laonastes individuals.

Since an important aspect of the gastric function is the physical separation of constituents of digesta (Langer 1984, Munn et al. 2012), we screened the stomach chambers of Laonastes to see whether there is any significant retention of coarse particles in the cranial stomach chambers. We also examined the stomach microanatomy of Laonastes, and used behavioral observations in an effort to find direct evidence that Laonastes is coprophagous.

Finally, since the diet of Laonastes consists mainly of dry items, we studied the ability of this species to concentrate electrolytes in its urine (Schmidt-Nielsen & O’Dell 1961), and compared this with the corresponding ability in other mammalian species in order to obtain an estimation on how well Laonastes is adapted to dry environments.

**Material and methods**

*Laonastes aenigmamus* has been protected in Laos since 2008, and it has been on the International Union for Conservation of Nature (IUCN) Red list since 2009. The Lao authorities have taken the responsibility to inform local human populations of this new legislation (*Laonastes* was trapped locally for food). During the transitional period the capture of this species was tolerated to allow its scientific study. With an exceptional letter of authorization (no. 1183, 9 June 2008) obtained from the Lao Government (Ministry of Planning and Investment), we were able to acquire five adult specimens (found dead in local hunters’ traps in the Khammouane Province) which provided us with some additional information on the teeth and gastrointestinal morphology of *Laonastes*. We also had access to some additional skulls from earlier studies for teeth analyses.

On necropsy, we placed the entire gastrointestinal tracts in ethanol for later studies done.
in laboratory in Finland. Additionally, skulls ($n = 9$) were cleaned and also shipped to Finland for analyses of the teeth. In order to quantify the dental form, we measured three-dimensional dental complexity of *Laonastes aenigmamus* cheek tooth rows using geographic information systems (GIS) analysis (for details see Evans et al. 2007: fig. 4). This method, termed Orientation Patch Count (OPC), and which measures the number and orientation of patches on tooth crown surfaces, does not require tooth feature homologies to be established, and approximates the number of tools on the tooth that can break down food material (Evans et al. 2007). It has been previously shown to provide estimations of diet in rodents, carnivores, primates, and bats (Evans et al. 2007, Santana et al. 2011, Godfrey et al. 2012), and used to infer the diet of extinct mammalian taxa such as multituberculates, and plesiadapid and lemuriform primates (Boyer et al. 2010, Godfrey et al. 2012, Wilson et al. 2012). Because OPC has previously been determined for murine rodents, we additionally scanned and analyzed a selection of hystricomorph rodents. Three-dimensional laser scans were obtained and processed as previously described (Evans et al. 2007, Wilson et al. 2012).

We microscopically screened ethanol fixed stomach chambers of *Laonastes* in an effort to see if there was physical separation of particles of different sizes in different stomach compartments. Standard histological sections ($5 \mu m$) were prepared from each of the stomach chambers of *Laonastes*, and stained with Hematoxylin-eosin. Sections were studied by microscope at 200x magnification. Here our aim was to determine how the glandular and non-glandular sections of the stomach were located.

In an effort to estimate how well *Laonastes* concentrates electrolytes in its urine, we measured the renal medullary thickness (RMT, Sperber 1944) of two *Laonastes* specimens as described in Laakkonen (2002). Behavioral data were gathered by observing captive *Laonastes* (Keovichit et al. 2011). Anatomical terminology is in accordance with the International Committee on Veterinary Cross Anatomical Nomenclature (2012).

**Results**

**Teeth**

The Orientation Patch Count for *Laonastes* cheek tooth (Fig. 4 and Table 1) rows corresponds to the OPC range of taxa among murine that are omnivorous but feed predominantly on plant-dominated diet or are strictly herbivorous (Evans et al. 2007). The OPC values peak in adult *Laonastes* individuals as compared with those in juvenile and senescent specimens (Table 1), and, at least for lower toothrows, adult mean OPC (lower toothrow = 206, upper toothrow = 155) falls within the herbivory category of Evans et al. (2007). In contrast to nearly all murine and hystricomorph rodents analyzed to date, the lower toothrow of *Laonastes* appears to have higher OPC values than the upper toothrow.

**Stomach anatomy**

Our histological examination of the stomach anatomy of *Laonastes* showed that each of the stomach chambers of *Laonastes* (not shown) is microscopically similar, consisting of glandular (simple columnar) epithelium. A gastric sulcus (*sulcus ventriculi*) runs along the stomach wall at the lesser curvature (*curvature ventriculi minor*)
from the oesophagus towards the pylorus, ending shortly before the latter (Scopin et al. 2011: fig. 3). The gastric sulcus (sulcus ventriculi) is lined by macroscopically and microscopically visible low folds of tissue (bordering fold in the terminology of Carleton 1973) separating the sulcus from the glandular part of the stomach. Microscopically the sulcus was similar throughout its length in adult Laonastes consisting of stratified squamous epithelium. At the caudal part of the stomach Laonastes has a well-developed pyloric sphincter muscle (not shown).

In microscopic screenings of food particles from various ethanol fixed stomach chambers of Laonastes, we saw no significant differences in digesta particle size patterns between different stomach regions. We saw no sign of Laonastes eating their own feces but we frequently saw them licking their perianal areas (J.-P. Hugers pers. obs.).

The RMTs of Laonastes were 3.18 and 4.24 (mean 3.71).

Discussion

Placing the dentition into a broad comparative context, the bilophodonty of Laonastes cheek teeth is not in itself indicative of extreme specialization for plant fibre consumption. The teeth, however, are mesodont and cusps are somewhat lamellar (Fig. 4), features that suggest increased functional durability and functional efficiency, respectively (Janis & Fortelius 1988).

The number of patches or dental complexity (OPC value) recorded for a cheek tooth row

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen ID</th>
<th>OPC of lower teeth</th>
<th>OPC of upper teeth</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carnivores</strong> (n = 2)</td>
<td>See Evans et al. (2007)</td>
<td>179–192</td>
<td>150–170</td>
<td>Plant dominated omnivory</td>
</tr>
<tr>
<td><strong>Carnivores</strong> (n = 2)</td>
<td>See Evans et al. (2007)</td>
<td>195–257</td>
<td>270–342</td>
<td>Herbivory</td>
</tr>
<tr>
<td><strong>Rodents</strong> (n = 33)</td>
<td>See Evans et al. (2007)</td>
<td>133–287</td>
<td>162–309</td>
<td>Plant dominated omnivory</td>
</tr>
<tr>
<td>Octodon degus</td>
<td>UN2285</td>
<td>136 (161)</td>
<td>143 (142)</td>
<td>Grass and shrub foliage, seeds</td>
</tr>
<tr>
<td>Chinchilla chinchilla</td>
<td>UN2288</td>
<td>136 (152)</td>
<td>157 (200)</td>
<td>Mostly grasses</td>
</tr>
<tr>
<td>Lagostomus maximus</td>
<td>UN2291</td>
<td>201 (218)</td>
<td>304 (318)</td>
<td>Mostly grasses</td>
</tr>
<tr>
<td>Myocastor coypus</td>
<td>UN2292</td>
<td>226 (241)</td>
<td>349 (371)</td>
<td>Grasses, sedges, roots</td>
</tr>
<tr>
<td>Dasypodictis leporine</td>
<td>UN2287</td>
<td>228 (265)</td>
<td>265 (300)</td>
<td>Fruits, nuts, seeds, insects</td>
</tr>
<tr>
<td>Cavia porcellus</td>
<td>UN2286</td>
<td>230 (271)</td>
<td>205 (222)</td>
<td>Mostly grasses</td>
</tr>
<tr>
<td>Erethizon dorsatum</td>
<td>UN2290</td>
<td>272 (288)</td>
<td>330 (251)</td>
<td>Generalist herbivore</td>
</tr>
<tr>
<td>Cuniculus paca</td>
<td>3/1960</td>
<td>294 (291)</td>
<td>330 (381)</td>
<td>Fruits, nuts, seeds, leaves, tubers</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>91 (juvenile, m3 partially erupted)</td>
<td>162 (164)</td>
<td>146 (148)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>75 (juvenile)</td>
<td>186 (205)</td>
<td>155 (178)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>114 (juvenile)</td>
<td>154 (166)</td>
<td>142 (141)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>113 (adult)</td>
<td>187 (202)</td>
<td>147 (202)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>78 (adult)</td>
<td>231 (254)</td>
<td>140 (167)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>86 (adult)</td>
<td>200 (206)</td>
<td>177 (215)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>108 (old)</td>
<td>166 (177)</td>
<td>158 (225)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>120 (old)</td>
<td>179 (192)</td>
<td>155 (177)</td>
<td>Dry leaves, some insects</td>
</tr>
<tr>
<td>Laonastes aenigmamus</td>
<td>73 (old)</td>
<td>173 (159)</td>
<td>152 (206)</td>
<td>Dry leaves, some insects</td>
</tr>
</tbody>
</table>
increases with increasingly fibrous diets, from a low count in hypercarnivores, increasing through carnivory, animal-dominated omnivory and plant-dominated omnivory, and with the highest values recorded for fibre specialists such as the bamboo-eating giant panda, *Ailuropoda melanoleuca*. The value for *Laonastes* cheek tooth rows corresponds to the OPC range of predominantly herbivorous taxa among those mammalian species examined previously (Fig. 4), agreeing with the field observations (J.-P. Hugot pers. obs.). Furthermore, OPC values of *Laonastes* are lower than those of the most specialized hindgut fermenting herbivores analyzed by Evans *et al.* (2007), and could implicate the role of its compartmentalized stomach in the digestion of high fibre content plant material. Comparison with hystricomorphs, however, shows that *Laonastes* OPC values are well within the range of these herbivorous grass eaters (Table 1). It is perhaps noteworthy that *Octodon* and *Chinchilla*, which are specialized in eating dry and high-fibre-content plants, have even lower OPC values than *Laonastes*. *Octodon* and *Chinchilla* are also coprophagous but currently it is not known whether dental form is affected by coprophagy.

*Laonastes* has a voluminous stomach characterized by sacculcation (Keovichit *et al.*, 2011, Scopin *et al.* 2011; Figs. 1 and 2) partly resulting from the constriction caused by the strong peritoneal folds originating in the lesser curvature. This kind of stomach morphology, called by Langer (1985) plurilocular (multichambered), provides the capacity to hold a portion of the digesta for long periods of time for thorough microbial digestion. In this study, we found no significant differences in digesta particle size patterns between different stomach regions. Jenkins *et al.* (2005) reported that the stomach contents consisted mainly of very fine particulate matter (plant remains). In other mammal species with multichambered stomachs, a differential passage of solute and particles has been documented in hippopotamus (Clauss *et al.* 2004), macropods (Munn *et al.* 2012) and functional ruminants (ruminants and camelids, reviewed in Müller *et al.* 2011), but apart from ruminants and camelids, no differential passage of different-sized particles (Schwarm *et al.* 2009, Munn *et al.* 2012) or systematic differences in particles sizes in the different forestomach compartments has been identified (Schwarm *et al.* 2013).

The stomach chambers of *Laonastes* all appeared microscopically similar, consisting of glandular (simple columnar) epithelium. Since the *Laonastes* used in this study were found dead in traps, the stomach epithelium had started to dissolve and was not in optimum condition for histological examination. Stomach specimens collected and fixed immediately after the death of an individual would be needed to study the microanatomy of *Laonastes* in more detail.

Non-ruminating mammals with large stomachs show variability in the microanatomy of their stomachs. In macropods, the foregut contains regions of squamous epithelium and cardiac glandular mucosa (Smith 2009), and in the pigmy hippopotamus the walls of the forestomach are covered with villi and a non-glandular mucous membrane (Macdonald & Hartman 1983).

Of other rodent species, several African rodent species have markedly sacculated stomachs and a conspicuous folded edge of the mucous membrane separating the highly modified corpus (numerous papillae or several diverticula) from a glandular antrum (Perrin & Maddock 1983). Of these species, *Thallomys paedulcus* is a specialist folivore feeding on fibrous leaves. It has an elaborate oesophageal groove system and fornical diverticula (Perrin & Maddock 1983). None of these African rodent species has a stomach sulcus similar to that of *Laonastes*. The microanatomy of *Laonastes* stomach appears dissimilar from that of any other rodent or mammal species.

Microscopically the sulcus of *Laonastes* was similar throughout its length in adult *Laonastes* and consisted of stratified squamous epithelium. As in other mammals that have a stomach sulcus, also in *Laonastes* this groove like structure may be an adaptation to easily bypass milk in juveniles straight to the caudal part of the stomach. A study done in ruminants indicates that it is unlikely that even soluble food components are bypassed in such a structure (Lechner *et al.* 2009). *Laonastes* have been observed to occasionally chew while resting (J.-P. Hugot pers. obs.). Whether this is indicative of a regurgitation/remastication behaviour remains to be investigated.
Due to the competition of internal organs for the available space within the abdominal cavity, the large size of the stomach usually limits the size of the cecum and colon (for example in hippopotamus Stevens and Hume 2004), which decreases the ability for colonic water absorption. Despite the large stomach, Laonastes has a distinct although undifferentiated cecum (Scopin et al. 2011) that may be needed to compensate for the possible lack of fermentation in the non-glandular stomach.

The low RMT (mean = 3.71) indicates that Laonastes is not particularly well adapted to dry environments (Al-kahtani et al. 2004), and has to rely on habitat choice and behavioral adaptations to survive the dry season. Captive Laonastes seldom use water made available for them but can consume moist food items (fruits) without apparent diarrhea or change in the consistency of fecal droppings (J.-P. Hugot pers. obs.). However, this species has been observed to rapidly ingest dry leaves after a period in captivity with only softer food items made available (J.-P. Hugot pers. obs.).

At present, there is no direct evidence that Laonastes is coprophagous but this species has frequently been observed to lick their perianal areas (J.-P. Hugot pers. obs.). Whether this behavior is part of the process of ingesting feces, as documented in other coprophagous species (e.g. Kenagy & Hoyt 1980), is presently not known.

Large body size has often been suggested to make the use of food items high in crude fibre content (such as plant cell walls) easier due to the lower relative energy requirements of large mammals as compared to those of mammals with small body size. Recent studies suggest however, that in herbivores the difference could be less marked than predicted simply on the basis of the body size-metabolic rate relationship (Müller et al. 2013). Instead, interactions between food intake and food residence time may be more important for understanding the evolution and ecology of herbivores (Clauss et al. 2007b).

The combination of relatively complex teeth, unique gut morphology and relatively small body size of Laonastes provide intriguing possibilities for further studies on the relation of body size, diet choice and gastrointestinal morphophysiology in herbivorous mammals.

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