Ontogeny of the cranial system in Laonastes aenigmamus

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Abstract

Rodents, together with bats, are among the ecologically most diverse and most speciose groups of mammals. Moreover, rodents show elaborate specializations of the feeding apparatus in response to the predominantly fore-aft movements of the lower jaw. The Laotian rock rat Laonastes aenigmamus was recently discovered and originally thought to belong to a new family. The difficulties in classifying L. aenigmamus based on morphological characters stem from the fact that it presents a mixture of sciurognathous and hystricognathous characteristics, including the morphology of the jaw adductors. The origin of the unusual muscular organization in this species remains, however, unclear. Here, we investigate the development of the masticatory system in Laonastes to better understand the origin of its derived morphology relative to other rodents. Our analyses show that skull and mandible development is characterized by an overall elongation of the snout region. Muscle mass increases with positive allometry during development and growth, and so does the force-generating capacity of the jaw adductor muscles (i.e. physiological cross-sectional area). Whereas fetal crania and musculature are more similar to those of typical rodents, adults diverge in the elongation of the rostral part of the skull and the disproportionate development of the zygomaticomandibularis. Our data suggest a functional signal in the development of the unusual cranial morphology, possibly associated with the folivorous trophic ecology of the species.

Key words: feeding; jaw muscle; mammal; ontogeny; scaling; skull.

Introduction

Rodents, together with bats, are among the ecologically most diverse and most speciose groups of mammals (Wilson & Reeder, 2005). Moreover, rodents show one of the most extreme specializations of the feeding apparatus, with a single pair of highly specialized upper and lower incisors used for gnawing, and a small number of cheek teeth used for chewing. These specializations are associated with a specialized musculature (Schumacher, 1961) facilitating the antero-posterior jaw movements typical of rodents (propaliny; see Bech, 1953). The exceptional diversity of rodents is, moreover, associated with a diverse array of specializations of the jaw musculature (Wejs, 1973; Woods & Hermanson, 1985; Offermans & De Vree, 1989; Druzinsky, 2010a,b; Cox & Jeffery, 2011; Hautier et al. 2011a). Variations in the masseter complex, and the associated modifications of the skull, have traditionally been used as diagnostic characters to classify rodents (Hautier et al. 2011a).

The Laotian rock rat Laonastes aenigmamus was recently discovered in the Lao People’s Democratic Republic (Jenkins et al. 2005). Although this species was originally thought to belong to a new family, a reexamination of the specimen (Dawson et al. 2006) showed that it belongs to the
otherwise extinct family Diatomyidae. Moreover, recent molecular analyses confirmed the placement of *L. aenigmamus* as a sister taxon to the Ctenodactylidae (Huchon et al. 2007). The difficulties in classifying *L. aenigmamus* using morphological characters stem from the fact that it presents a mixture of sciurognathous and hystricognathous characteristics (Jenkins et al. 2005; Dawson et al. 2006; Huchon et al. 2007). Indeed, a recent analysis of the anatomy of adult Laonastes demonstrated that the pars reflexa of the masseter complex in Laonastes has evolved independently in this species (Hautier & Saksiri, 2009). This suggests that the masseter complexity contains a functional rather than a phylogenetic signal, and that variation in the jaw adductors may be tuned to functional demands. However, the origin of the unusual muscular organization in this species remains unclear given that all of its closest relatives are extinct. Here, we propose to investigate the development of the masticatory system in Laonastes to better understand the origin of its derived morphology and potential functional specializations relative to other rodents.

Although developmental data may provide profound insights into the origin and homology of derived anatomical patterns, relatively few studies have investigated the ontogeny of the cranial system in mammals in general, and rodents in particular. Indeed, despite a recent revival of

### Table 1
Body and cranial dimensions of the individuals dissected in this study.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Sex</th>
<th>Body length (mm)</th>
<th>Skull length (mm)</th>
<th>Skull width (mm)</th>
<th>Skull depth (mm)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY161</td>
<td>F</td>
<td>255</td>
<td>66.91</td>
<td>26.85</td>
<td>24.76</td>
<td>D</td>
</tr>
<tr>
<td>KY180</td>
<td>F</td>
<td>175</td>
<td>56.80</td>
<td>24.23</td>
<td>20.59</td>
<td>D, MO</td>
</tr>
<tr>
<td>KY132</td>
<td>M</td>
<td>231</td>
<td>62.35</td>
<td>25.71</td>
<td>23.73</td>
<td>D</td>
</tr>
<tr>
<td>KY211</td>
<td>M</td>
<td>248</td>
<td>66.73</td>
<td>27.6</td>
<td>24.34</td>
<td>D, MO</td>
</tr>
<tr>
<td>KY213</td>
<td>F</td>
<td>237</td>
<td>65.28</td>
<td>26.35</td>
<td>23.77</td>
<td>D, MO</td>
</tr>
<tr>
<td>KY167</td>
<td>M</td>
<td>245</td>
<td>62.32</td>
<td>25.15</td>
<td>16.92</td>
<td>D</td>
</tr>
<tr>
<td>KY186</td>
<td>M</td>
<td>257</td>
<td>67.52</td>
<td>27.12</td>
<td>18.05</td>
<td>MO</td>
</tr>
<tr>
<td>KY110</td>
<td>M</td>
<td>277</td>
<td>56.39</td>
<td>24.65</td>
<td>16.04</td>
<td>MO</td>
</tr>
<tr>
<td>KY038</td>
<td>F</td>
<td>255</td>
<td>56.57</td>
<td>24.33</td>
<td>16.29</td>
<td>MO</td>
</tr>
<tr>
<td>KYJ</td>
<td>J</td>
<td>234</td>
<td>58.17</td>
<td>23.90</td>
<td>16.59</td>
<td>MO</td>
</tr>
<tr>
<td>KY087</td>
<td>Fetus</td>
<td>67.55</td>
<td>28.98</td>
<td>16.89</td>
<td>14.53</td>
<td>D, MO</td>
</tr>
<tr>
<td>KY080</td>
<td>Fetus*</td>
<td>67.55</td>
<td>28.98</td>
<td>16.89</td>
<td>14.53</td>
<td>MO</td>
</tr>
</tbody>
</table>

Table entries include measurements taken on the preserved specimens.

D, dissection; F, female; J, unsexed juvenile; M, male; MO, morphometrics.

*Scanned inside uterus, no dimensions available.

![Fig. 1](image1.png) Figure illustrating the landmarks used to quantify ontogenetic variation in the skull. Illustrated are a lateral (bottom), ventral (top left) and dorsal view of the skull. See Table 2 for landmark definitions.

![Fig. 2](image2.png) Figure illustrating the landmarks used to quantify ontogenetic variation in the mandible. Illustrated are a dorsal (top), lingual (middle) and lateral view of the mandible. See Table 2 for landmark definitions.
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Table 2 Definitions of the landmarks used for geometric morphometrics.

<table>
<thead>
<tr>
<th>Cranium</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Most anterior point where the premaxillar suture meets the anterior part of alveolar margin of the incisives, midline</td>
<td></td>
</tr>
<tr>
<td>2 Most antero-dorsal point of the premaxillar suture, left side</td>
<td></td>
</tr>
<tr>
<td>3 Most antero-dorsal point of the premaxillar suture, right side</td>
<td></td>
</tr>
<tr>
<td>4 Most anterior point of the infra-orbital canal, right side</td>
<td></td>
</tr>
<tr>
<td>5 Most anterior point of the infra-orbital canal, left side</td>
<td></td>
</tr>
<tr>
<td>6 Most anterior point of the pterygoid apophysis that meets the alisphenoid, left side</td>
<td></td>
</tr>
<tr>
<td>7 Most anterior point of the palatine, midline</td>
<td></td>
</tr>
<tr>
<td>8 Most anterior point of the pterygoid apophysis that meets the alisphenoid, right side</td>
<td></td>
</tr>
<tr>
<td>9 Most ventral point jugal/maxilla suture, right side</td>
<td></td>
</tr>
<tr>
<td>10 Most ventral point jugal/maxilla suture, left side</td>
<td></td>
</tr>
<tr>
<td>11 Most posterior point of insertion of the maxilla part of the zygomatic arch to the tooth row, right side</td>
<td></td>
</tr>
<tr>
<td>12 Most posterior point of insertion of the maxilla part of the zygomatic arch to the tooth row, left side</td>
<td></td>
</tr>
<tr>
<td>13 Most posterior point of insertion of the squamosal part of the zygomatic arch to the cephalic capsule, left side</td>
<td></td>
</tr>
<tr>
<td>14 Most anterior point of insertion of the squamosal part of the zygomatic arch to the cephalic capsule, right side</td>
<td></td>
</tr>
<tr>
<td>15 Most posterior point of insertion of the squamosal part of the zygomatic arch to the cephalic capsule, left side</td>
<td></td>
</tr>
<tr>
<td>16 Most posterior point of insertion of the squamosal part of the zygomatic arch to the cephalic capsule, right side</td>
<td></td>
</tr>
<tr>
<td>17 Most posterior point of the zygomatic arch (jugal), right side</td>
<td></td>
</tr>
<tr>
<td>18 Most posterior point of the zygomatic arch (jugal), left side</td>
<td></td>
</tr>
<tr>
<td>19 Point of frontal/lacrimal suture, left side</td>
<td></td>
</tr>
<tr>
<td>20 Point of frontal/lacrimal suture, right side</td>
<td></td>
</tr>
<tr>
<td>21 Tip of the paroccipital apophysis, left side</td>
<td></td>
</tr>
<tr>
<td>22 Tip of the paroccipital apophysis, right side</td>
<td></td>
</tr>
<tr>
<td>23 Most antero-ventral point of the foramen magnum, midline</td>
<td></td>
</tr>
<tr>
<td>24 Most postero-ventral point of the occipital crest, midline</td>
<td></td>
</tr>
<tr>
<td>25 Most posterior point of the interparietal, midline</td>
<td></td>
</tr>
<tr>
<td>26 Most lateral point of the exoccipital/parietal/squamosal suture, left side</td>
<td></td>
</tr>
<tr>
<td>27 Most lateral point of the exoccipital/parietal/squamosal suture, right side</td>
<td></td>
</tr>
<tr>
<td>28 Most antero-dorsal point of insertion of the zygomatic arch to the snout, left side</td>
<td></td>
</tr>
<tr>
<td>29 Most antero-dorsal point of insertion of the zygomatic arch to the snout, right side</td>
<td></td>
</tr>
<tr>
<td>30 Most posterior point of maxilla/nasal/frontal, left side</td>
<td></td>
</tr>
<tr>
<td>31 Most posterior point of maxilla/nasal/frontal, right side</td>
<td></td>
</tr>
<tr>
<td>32 Most antero-lateral point of the basioccipital that meets the basisphenoid and the tympanic bulla, left side</td>
<td></td>
</tr>
<tr>
<td>33 Most antero-lateral point of the basioccipital that meets the basisphenoid and the tympanic bulla, right side</td>
<td></td>
</tr>
<tr>
<td>34 Most antero-central point of the basioccipital/basisphenoid suture, midline</td>
<td></td>
</tr>
<tr>
<td>35 Most lateral point of foramen magnum, left side</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 (Continued)

<table>
<thead>
<tr>
<th>Mandible</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Most antero-dorsal point of the mandibular symphysis that meets the posterior part of the alveolar margin of the incisive</td>
<td></td>
</tr>
<tr>
<td>2 Most antero-ventral point of the mandibular symphysis that meets the anterior part of the alveolar margin of the incisive</td>
<td></td>
</tr>
<tr>
<td>3 Tip of the angular process</td>
<td></td>
</tr>
<tr>
<td>4 Point that is at the maximum of concavity between the condyloid and the angular process</td>
<td></td>
</tr>
<tr>
<td>5 Most posterior point of the edge of the articular surface condyle</td>
<td></td>
</tr>
<tr>
<td>6 Most anterior point of the edge of the articular surface condyle</td>
<td></td>
</tr>
<tr>
<td>7 Most posterior point of the alveolar margin of the premolar-molar row</td>
<td></td>
</tr>
<tr>
<td>8 Most anterior point of the alveolar margin of the premolar-molar row</td>
<td></td>
</tr>
<tr>
<td>9 Most postero-ventral point of symphysis of the left dentary</td>
<td></td>
</tr>
<tr>
<td>10 Point of maximum of convexity of the antero-ventral part of the dentary</td>
<td></td>
</tr>
<tr>
<td>11 Most ventral point of insertion of the molar row to the medial body of the mandible</td>
<td></td>
</tr>
<tr>
<td>12 Point of insertion of the medial dental ridge to the condyloid process</td>
<td></td>
</tr>
<tr>
<td>13 Point of maximum of concavity of the ventral margin of the mandible</td>
<td></td>
</tr>
<tr>
<td>14 Most anterior point of the groove of the medial part of the angular process</td>
<td></td>
</tr>
</tbody>
</table>

Numbers correspond to the landmark numbers as illustrated in Fig. 1.

developmental studies investigating the early development and patterning of the cranial musculature in mammals (Smith, 1994; Goswami, 2007; Sánchez-Villagra et al. 2008; Wilson & Sánchez-Villagra, 2009; Hautier et al. 2011b), almost nothing is known about the late development and postnatal growth of the cranial system (but see, e.g. Strong, 1926; De Beer, 1937; Hughes et al. 1978; Maier et al. 2003; Flores et al. 2010), and even less about the development of the associated musculature. From a functional perspective, however, such studies can provide profound insights into the selective patterns operating during early ontogeny, which ultimately determine the adult form of an organism (e.g. Herrel et al. 2005; Wyckmans et al. 2007; Herrel & Holanova, 2008; Genbrugge et al. 2011) and may help...
Table 3 Results of regressions of muscle data vs. cranial length.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Slope ± SE</th>
<th>Intercept</th>
<th>( r^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. temporalis**</td>
<td>4.22 ± 0.29</td>
<td>−9.08</td>
<td>0.98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. temporalis pars orbitalis</td>
<td>3.92 ± 0.47</td>
<td>−8.82</td>
<td>0.95</td>
<td>0.001</td>
</tr>
<tr>
<td>M. temporalis pars posterior</td>
<td>3.62 ± 0.30</td>
<td>−8.27</td>
<td>0.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. masseter superciliaris pars anterior</td>
<td>4.42 ± 0.51</td>
<td>−9.16</td>
<td>0.95</td>
<td>0.001</td>
</tr>
<tr>
<td>M. masseter superciliaris pars reflexa**</td>
<td>4.91 ± 0.17</td>
<td>−9.38</td>
<td>0.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. masseter superciliaris pars reflexa**</td>
<td>4.36 ± 0.74</td>
<td>−8.90</td>
<td>0.90</td>
<td>0.004</td>
</tr>
<tr>
<td>M. temporalis pars anterior</td>
<td>4.93 ± 0.33</td>
<td>−9.72</td>
<td>0.98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. masseter posterior**</td>
<td>3.90 ± 0.34</td>
<td>−8.67</td>
<td>0.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. masseter pars inferoarbitals**</td>
<td>4.49 ± 0.40</td>
<td>−8.77</td>
<td>0.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. masseter pars zygomatica**</td>
<td>5.75 ± 0.26</td>
<td>−11.09</td>
<td>0.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. pterygoideus pars internus**</td>
<td>4.76 ± 0.19</td>
<td>−9.34</td>
<td>0.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. pterygoideus pars externus**</td>
<td>4.22 ± 0.23</td>
<td>−8.68</td>
<td>0.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. digastricus**</td>
<td>3.85 ± 0.12</td>
<td>−7.99</td>
<td>0.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fiber length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. temporalis*</td>
<td>1.34 ± 0.10</td>
<td>−1.52</td>
<td>0.98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M. temporalis pars orbitalis</td>
<td>1.21 ± 0.23</td>
<td>−1.45</td>
<td>0.88</td>
<td>0.006</td>
</tr>
<tr>
<td>M. temporalis pars posterior</td>
<td>0.98 ± 0.54</td>
<td>−0.94</td>
<td>0.45</td>
<td>NS</td>
</tr>
<tr>
<td>M. masseter superciliaris pars anterior</td>
<td>1.42 ± 0.25</td>
<td>−1.64</td>
<td>0.89</td>
<td>0.005</td>
</tr>
<tr>
<td>M. masseter superciliaris pars reflexa**</td>
<td>1.15 ± 0.21</td>
<td>−1.16</td>
<td>0.89</td>
<td>0.005</td>
</tr>
<tr>
<td>M. masseter superciliaris pars reflexa**</td>
<td>0.99 ± 0.15</td>
<td>−0.92</td>
<td>0.92</td>
<td>0.002</td>
</tr>
<tr>
<td>M. masseter superciliaris pars anterior</td>
<td>1.22 ± 0.19</td>
<td>−1.39</td>
<td>0.91</td>
<td>0.003</td>
</tr>
<tr>
<td>M. masseter posterior</td>
<td>1.48 ± 0.37</td>
<td>−1.93</td>
<td>0.80</td>
<td>0.02</td>
</tr>
<tr>
<td>M. masseter pars inferoarbitals**</td>
<td>1.05 ± 0.36</td>
<td>−1.02</td>
<td>0.68</td>
<td>0.04</td>
</tr>
<tr>
<td>M. masseter pars zygomatica</td>
<td>0.98 ± 0.17</td>
<td>−0.91</td>
<td>0.90</td>
<td>0.004</td>
</tr>
<tr>
<td>M. pterygoideus pars internus**</td>
<td>0.86 ± 0.23</td>
<td>−0.83</td>
<td>0.78</td>
<td>0.02</td>
</tr>
<tr>
<td>M. pterygoideus pars externus**</td>
<td>0.55 ± 0.20</td>
<td>−0.27</td>
<td>0.65</td>
<td>0.05</td>
</tr>
<tr>
<td>M. digastricus**</td>
<td>1.03 ± 0.13</td>
<td>−1.04</td>
<td>0.94</td>
<td>0.001</td>
</tr>
<tr>
<td>Physiological cross-sectional area (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. temporalis*</td>
<td>2.88 ± 0.31</td>
<td>−6.53</td>
<td>0.96</td>
<td>0.001</td>
</tr>
<tr>
<td>M. temporalis pars orbitalis</td>
<td>2.71 ± 0.48</td>
<td>−6.35</td>
<td>0.89</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\( P \) represents \( P \)-value for the significance of a test evaluating whether the regression is different from 0.

*Indicates slopes significantly different from the slopes predicted by geometric scaling (i.e. 3 for mass, 1 for fiber length and 2 for physiological cross-sectional area) at \( a = 0.05 \).

**Indicates significance at \( a = 0.01 \).

understand the systematic position of taxa characterized by highly derived anatomical features (Maier et al. 2003). Here, we study ontogenetic changes in the shape of the cranium and mandible in the Laotian rock rat. Moreover, we explore whether the observed ontogenetic changes in skull shape are associated with ontogenetic changes in the jaw adductor musculature. Finally, functionally relevant aspects of the jaw adductor anatomy are compared with other rodents and interpreted in the context of what is known about the ecology of Laonastes.

**Materials and methods**

**Specimen collection**

The collection and use of *Laonastes aenigmamus* has been regulated in Lao PDR since November 2008. Since January 2009 this species is listed as ‘Endangered’ on the IUCN Red List. For our study we obtained a research and collecting permit (letter of authorization N° 1183, 9 June 2008) from the Lao Government. The Khammuan Province Agriculture and Forestry Office (PAFO) validated our field collection schedule and an officer escorted us during collection trips. All animals used in this study were collected in Khammouane province and are deposited in the collections of the Muséum National d’Histoire Naturelle.
Specimens and scanning

Six individuals of formaldehyde-preserved specimens of *L. aenigmamus* including adults, juveniles and one fetus were used for dissection (skull length: 29–67 mm; Table 1). For comparative purposes two adult male *Rattus rattus* (skull length: 47.81 ± 0.16 mm) and two adult male *Cavia porcellus* (skull length: 56.55 ± 0.10 mm) were also dissected.

In addition, 10 specimens of *L. aenigmamus* of different ontogenetic stages (two fetuses at different stages of development but of unknown age, three juveniles and five adults) were included in our morphometric analysis. The two fetuses and three of the adults were scanned at the UGCT scanning facility at Ghent University (http://www.ugct.ugent.be) using a micro-focus transmission-type X-ray tube. Depending on sample size, tube voltage was chosen between 80 and 100 kV, and an open type dual-head tube (Feinfocus FXE160.50 and FXE160.51) was used providing sufficiently small spot size. Specimens were mounted on a controllable rotating table (MICOS, UPR160F-AIR). For each specimen a series of 1000 projections of 1496 pixels was recorded, covering 360. Reconstruction of the tomographic projection data was done using the in-house developed Octopus-package (Vlassenbroeck et al. 2007). Volume and surface rendering was performed using AVIZO 7.0 (VSG). The remainder of the specimens (cleaned skulls of three juveniles and two adults) was scanned using a surface scanner (stereoSCAN3D; Breuckman GmbH, Meersburg, Germany) at the morphometrics platform of the Muséum National d’Histoire Naturelle.

Morphology

Specimens were measured using digital calipers (skull length, skull width and skull height; ± 0.01 mm Mitutoyo CD-15B), weighed and µCT scanned. Cranial muscles were removed under a binocular microscope (M5 Wild; Wild Heerbrugg, Gais, Switzerland). Muscles were removed on one side and transferred to labeled vials containing a 70% aqueous ethanol solution. Muscles were blotted dry and weighed to the nearest 0.01 mg using a microbalance (Mettler Toledo MTS; Mettler-Toledo, Columbus, OH, USA). Next, muscles were transferred to a 30% aqueous nitric acid solution for 24–28 h, after which the solution was replaced by a 50% aqueous glycerin solution. Individual fibers were teased apart using blunt-tipped glass needles, and 10–15 fibers were selected randomly and drawn using a binocular microscope with attached camera lucida (MT5 Wild). Drawings were scanned and fiber lengths determined using the ImageJ V1.31 software. Physiological cross-sectional areas were calculated by multiplying muscle mass by its density (1060 kg m$^{-3}$; Méndez & Keys, 1960) and dividing the thus calculated muscle volume by the average fiber length (Herrel et al. 2008). No correction for pennation angle was included as complex muscles were separated into their constituent parts defined by a single fiber orientation.

Morphometric analysis

Landmarks were always taken by the same person (A-CF) on the surface of 3D scans using the software package Idav Landmark.
(Wiley et al. 2005). Forty-two anatomical landmarks for the crania and 14 landmarks for the left mandible accurately describing the complex shape of the skull and mandible were chosen (Figs 1 and 2; Table 2 for definitions of landmarks). Note that we did not use the coronoid process as a landmark in our analysis of lower jaw shape due to the absence of a marked coronoid process in Laonastes. Two repetitions were done for each individual in order to assess measurement error, which was found to be low. Shape variation of the skull and mandible was assessed using geometric morphometric approaches allowing an analysis of size and shape components independently (Zelditch, 2004). Generalized Procrustes superimposition (Rohlf & Slice, 1990) was performed on the point coordinates using the package Rmorph (Baylac, 2012) in the program R (R Development Core Team, 2010). The Evan toolbox (http://www.evan.at) was used to perform a principal component analysis (PCA) and a visualization of shape variation along each axis using thin-plate splines in three dimensions. These visualizations were obtained by warping the consensus surface to the extremes of the PCA axes by minimizing the bending energy (Gunz et al. 2005).

Statistical analyses
All data were Log10-transformed before analysis. To explore scaling relationships and growth of the masticatory muscles in Laonastes, we regressed the mass, fiber length and physiological cross-sectional area of each muscle against cranial length (Table 3). To test whether slopes differed from predictions of geometric growth (slope of 1 for length vs. length, 2 for area vs. length, and 3 for mass vs. length) we used a two-tailed t-test (Table 3).

Results
Skull development and growth
Overall skull development in Laonastes is typical of rodents (Strong, 1926) and characterized by an initial fusion of the cranial vault bones along the midline (Fig. 3a,b). Subsequently, the parietals fuse with the frontals. Fusion of the occipitals with the parietals takes place later in development, which is atypical for hystricognath rodents (Wilson & Sanchez-Villagra, 2009). Postnatal growth of the skull involves the increase of the cranial ridges that serve as insertion areas for the jaw adductor muscles and a continued growth of the facial region (Fig. 3c,d).

Morphometrics
Our analyses of cranial and mandibular shape indicate significant shape changes during growth (Figs 3–5). A PCA performed on the shape data extracted two axes that together describe over 75% of the variation in the data set for the cranium and over 64% of the overall variation in the mandible (Fig. 4). For both the cranium and the mandible, the first axis distinguishes between fetal specimens vs. juveniles and adults. The second axis discriminates between the fetuses and juveniles vs. adults. This suggests that most of the variation is driven by shape changes during early development (Fig. 3a–c). Figure 3 illustrates the shape changes in the cranium, and shows how during early development shape changes in the skull are characterized by a narrowing of the brain case and an elongation of the snout and the orbital region. The second axis indicates shape changes from juveniles to adults (Fig. 4), and is driven by a further narrowing of the brain case, a widening of the zygomatic arches, an elongation of the orbital region and a further elongation of the snout (Fig. 5a). Interestingly, shape changes in the snout region are exacerbated in the insertion area of the infraorbital part of the m. masseter.

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(Fig. 5a). With respect to the mandible, the first axis describing the shape change from fetal to adult specimens is characterized by a forward displacement of the jaw articulation relative to the angular process, a decrease in the height of the body of the mandible, an elongation of the diastema and a narrowing of the tooth row (Fig. 5b). The second axis, characterizing shape changes taking place from juveniles to adults, is characterized mostly by an increase of the curvature and a decrease in depth of the diastema. Overall, the lower jaw in adults is more slender, has a longer diastema, yet a larger insertion area for the internal pterygoid and the pars reflexa of the masseter.

Muscle anatomy and scaling

Here we follow the terminology and subdivisions of the adductor musculature as described by Hautier & Saksiri (2009). The overall anatomy of the jaw adductor musculature in \textit{Laonastes} was similar to that described previously (Hautier & Saksiri, 2009). The scaling of the jaw muscles follows allometric patterns of growth, with all muscles except the m. temporalis pars orbitalis and the m. temporalis pars posterior showing positive allometry (Table 3; Fig. 6). Fiber lengths, on the other hand, showed mostly isometric growth, with the exception of the fibers of the main part of the m. temporalis where fibers get relatively longer as skulls get bigger (Table 3). As a consequence, the physiological cross-sectional areas of most muscles increased with positive allometry, indicating that the jaw adductors in adults have a greater force-generating capacity relative to body size than in fetal and juvenile animals (Table 3; Fig. 6).

Discussion

Our data show distinct shape changes in the cranium and mandible of \textit{Laonastes} during development and growth. From a more general rodent-like shape, the rostral part of the cranium elongates, while the mandible shape changes to become more slender and with a more posteriorly positioned angular process. These shape changes appear to coincide with ontogenetic changes in muscle development, with jaw adductor muscles (notably the masseter complex) becoming heavier and disproportionately stronger in adults. These results are in contrast to data on the scaling of functionally important elements of the locomotor system, with juveniles often showing a more advanced development and function of the musculoskeletal system relative to adults (Carrier, 1983, 1995, 1996; Herrel & Gibb, 2006). Our data suggest that cranial development and growth in \textit{Laonastes} is more similar to carnivores, which show a late maturation of cranial form and performance (e.g. Tanner et al. 2010; La Croix et al. 2011). However, in contrast to carnivores such as...
Hyenas (Tanner et al. 2010), Laonastes shows an elongation of the rostrum with size, and an increase in the insertion area for the masseter rather than the temporalis. Although the specific increase in the insertion area of the masseter during growth is undoubtedly related to the massteric dominance among the jaw adductors of rodents (Schumacher, 1961), the elongated rostrum may be related to the folivorous feeding habits of Laonastes (Scopin et al. 2011).

Indeed, a comparison of the allocation of the jaw adductor mass to the different functional groups shows that as adults Laonastes are divergent from rodents such as rats or guinea pigs (Fig. 7). Whereas the masseter sensu stricto is the biggest muscle in all rodents, in adult Laonastes the zygomaticomandibularis comprises 30% of the total jaw adductor mass in contrast to the 10–15% observed in rats and guinea pigs (Fig. 7). However, qualitative descriptions of the jaw adductors in members of the New World genus Proechimys suggest that these may have similar muscle allocation patterns (Woods, 1972; Hautier & Saksiri, 2009). Moreover, Ctenodactylus vali (Hautier, 2010; Fig. 7) also shows a disproportionate development of the zygomaticomandibularis muscle relative to rats or guinea pigs. Interestingly, the fetal Laonastes specimen dissected in this study shows a muscle allocation that is more similar to that observed in other rodents, with a relatively larger temporalis and smaller zygomaticomandibularis muscle. Yet, the coronoid process never develops as suggested by our fetal specimens.

These observations suggest that the unusual anatomy of Laonastes is likely related to aspects of its life-history and the use of the feeding system as adults. Previous examinations of the digestive tract anatomy of Laonastes have suggested that the digestive system may be specialized for folivory, a trait unusual for rodents. Indeed, even compared with the closely related gundis (Ctenodactylus), Laonastes has an exceptionally long rostrum and extensive development of the infraorbital part of the masseter muscle (Hautier & Saksiri, 2009; Hautier, 2010). From a functional perspective, the elongation of the snout and anterior insertion of the infraorbital part of the masseter likely provides a greater horizontal force component upon contraction, which will result in a more extensive anteriod displacement of the lower jaw during jaw closure. Moreover, the strong development of the masseter complex relative to the temporalis and the rather horizontal position of the temporalis indicate an optimal functioning of the
jaw system at very low gape angles, which would accord well with the suggested folivorous feeding habit (Wright et al. 2008). The relatively strong development of the anterior portion of the masseter also assures a uniform force generation across the entire tooth row, which may be beneficial in reducing fibrous plant material such as leaves (Onoda et al. 2011). Adaptive hypotheses regarding the specialization of the masticatory system in the function of a folivorous diet could be tested by careful biomechanical modeling of representative rodents with different feeding ecologies.

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