

# Sexual dimorphism in *Leptodactylus latinasus* (Anura, Leptodactylidae): nasal capsule anatomy, morphometric characters and performance associated with burrowing behavior

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## Abstract

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In anurans, the ability to burrow has been associated with the characteristics of external morphology, with modifications in the musculoskeletal system and even in some brain regions. Male *Leptodactylus fuscus* construct subterranean chambers with the snout, where the eggs are deposited and early larval stages develop. *Leptodactylus latinasus*, a member of the *L. fuscus* group, is a good model to test the relationship among digging behavior of males, sexual divergence in the morphology of the nasal region, cranial-dimensions, and performance as digger. The goal of this work is to provide a detailed morphological description of the olfactory region, which allows detecting sexually dimorphic characters, to evaluate sexual divergence with head dimension data, and to test whether differences exist in the digging performance of each sex. Our data do not clearly indicate sexual dimorphism in head size, nasal region morphology or digging performance that can be linked with the burrowing behavior. Thus, the only unequivocal sexually dimorphic character associated with the construction of the nuptial chamber by males would be the rigid, chisel-like snout, present exclusively in males.

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## Introduction

It is generally accepted that an organism's design is the result of a trade-off between adaptation to the local environment and phylogenetic constraints (Wainwright 1996; Irschick *et al.* 1997). For several vertebrate taxa with burrowing habits, the relationship between body design and digger performance has been emphasized (Fernandez *et al.* 2000; Measey and Herrel 2006; Van Daele *et al.* 2008). In anurans, the ability to burrow has been associated with the characteristics of external morphology, such as a spade-like metatarsal tubercle (Emerson 1976; Trueb and Duellman 1978; Kley and Kearney 2007), and the development of a rigid chisel-like snout (Heyer 1978). Burrowing behavior has also been

associated with the modifications in the musculoskeletal system, mainly in hindlimbs and pectoral-cranial morphology (Emerson 1976), and even in some regions of the brain (reduced optic tecta, large main olfactory bulbs and small accessory olfactory bulbs, and also a large torus semi-circularis) (Taylor *et al.* 1995).

Many studies have reported differences between the sexes in morphological, physiological, and endocrinological variables that are likely to affect diverse animal abilities. The most popular hypothesis for the origin of sexual dimorphism is sexual selection (Van Damme *et al.* 2008). Although the possible origin of sexual dimorphism in body size and shape has been extensively studied, the source of inter-sexual divergence in animal performance has been infrequently investigated

(Lailvaux *et al.* 2003). This is surprising, because the study of the causes and consequences of sexual dimorphism in performance may give insight into the mechanism of natural and sexual selection (Van Damme *et al.* 2008).

Heyer (1978) hypothesized a relationship between burrowing habits and sexual dimorphism for the species of the *Leptodactylus fuscus* group. The males of the species of this group construct subterranean chambers where the eggs are deposited and early larval stages develop. These nuptial chambers generally are made with the snout (Philibosian *et al.* 1974; Prado *et al.* 2002; Reading and Jofré 2003; Kokubum and Giaretta 2005). Consequently, in the species where this behavior is present, it has been stated that the male's head is longer than the female's (Heyer 1978). Also, the use of the snout in digging would imply a strengthening of bones of the nasal region (Heyer 1969). In fact, in this group, osteological features that reflect the degree of strength of this skull area showed a tendency toward increased ossification when analyzed within a phylogenetic framework (Ponssa 2008).

For several reasons, *Leptodactylus latinasus*, a member of the *L. fuscus* group, is a good model to test whether the digging behavior of males is reflected in sexual divergence in the morphology of the nasal region, head dimensions, and performance as a digger. First, it has been noticed that in this species, the nuptial chamber is constructed by the male (Gallardo 1958, 1964). Second, the males of this species have a configuration of the snout rigid and chisel-like (Heyer 1978), which frames the question whether this external design would have a correlation with internal morphology. A mineralized septum nasi, tectum nasi, and solum nasi have been described for this species and others of the *L. fuscus* group (Ponssa 2008); nevertheless, a more detailed description of the olfactory apparatus has not been published. Thus, the first goal of this work was to provide a detailed description of the skeletal morphology of the olfactory region, which allows detecting sexually dimorphic characters. The second goal was to recognize sexual divergence in head dimensions, externally as well as for skull measurements. Finally, we test whether differences exist in the digging performance of each sex.

## Materials and Methods

### Specimens

*Leptodactylus latinasus* (Fig. 1) specimens were used for the study, 13 specimens (seven males and six females) for the description of the morphology of the olfactory region and skull measurement analysis and 42 specimens (24 males and 18 females) for the head morphometric analysis. The force measurements were based on specimens (males,  $N = 11$ ; females,  $N = 10$ ) captured in Yerba Buena, and in El Ceibal and Finca Nougés, Lules, Tucumán, Argentina.

All studied specimens belong to the collection of the Instituto de Herpetología, Fundación Miguel Lillo (FML), and to



Fig. 1—Male of *Leptodactylus latinasus* from the El Ceibal, San Pablo, Lules, Tucumán, Argentina.

the private collection of María Laura Ponssa (L) and Sebastián Barrionuevo (SB) (Appendix I).

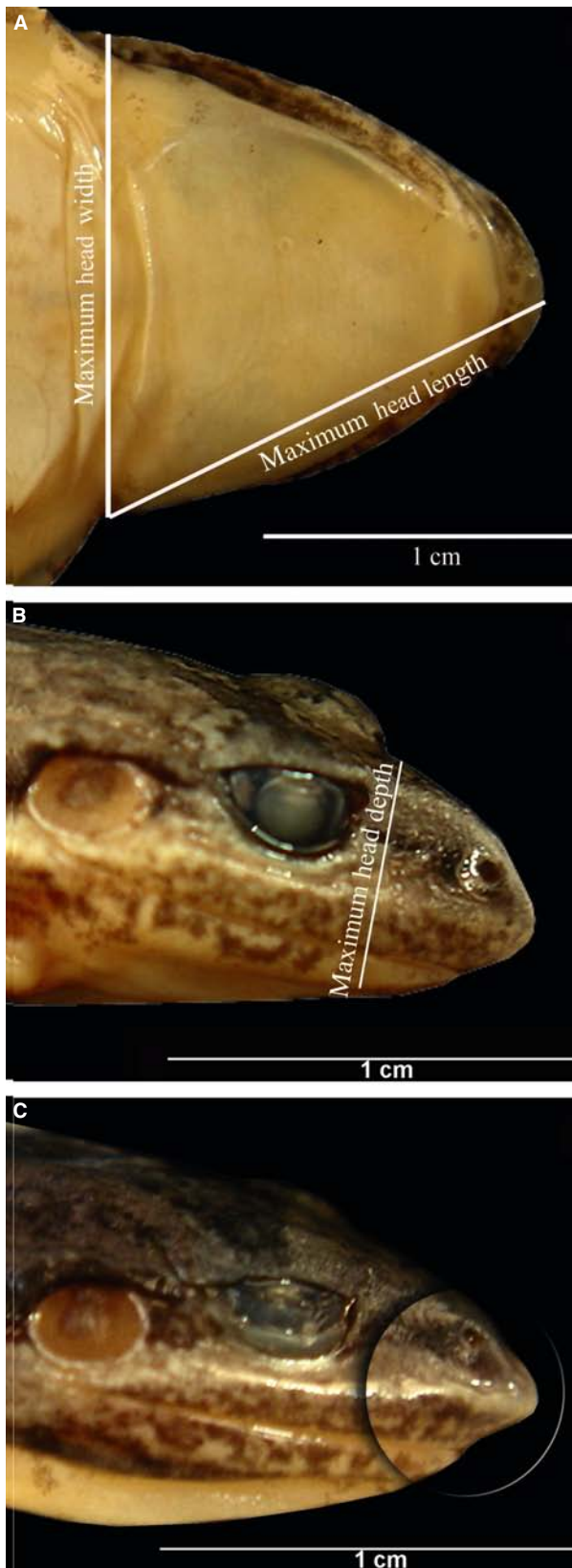
### Morphology

The specimens were eviscerated, cleared, and double-stained for cartilage and bone following methods adapted from Wassersug (1976). All descriptions and illustrations were made with the use of a Carl Zeiss (Discovery.V8) stereoscope with an attached camera lucida and 5-megapixel digital camera. Terminology of the olfactory region follows that of Pugener and Maglia (2007) and Maglia *et al.* (2007).

### Morphometrics

External body and head dimensions were measured with a digital caliper (Mitutoyo CD-30C and CD-15B;  $\pm 0.01$  mm), including the snout-vent length (SVL) and length, width, and depth of the head (Fig. 2). We evaluate differences in morphometric variables of the head between sexes by performing an analysis of covariance (ANCOVA), with SVL entered as covariate to reduce total size influence. All measures were log<sub>10</sub>-transformed to fulfill assumptions of normality, and each variable was analyzed separately.

The bone angles were calculated on photographs with Image Tool software, version 3.0 (University of Texas Health Science Center, San Antonio, Texas, USA). We calculated the angle formed by the pars alaris of the premaxillary bone with the horizontal axis of the skull in lateral view and the angle formed by the union of the premaxillary and the maxillary bones in ventral view (Fig. 3A,B) to detect morphometric sexual differences in the snout region of the skull. Analysis of variance (ANOVA) tests were used to analyze the morphometric variables.



**Fig. 2**—Specimens of *Leptodactylus latinasus* showing the external measurements analyzed. —**A**. Ventral view of the head showing the measurements of the width and length. —**B**. Lateral view of the head showing the measurement of the depth of the head. —**C**. Lateral view of the head of a male specimen showing the rigid chisel-like snout.

#### Digging force

Excavation force of the head of the specimens was recorded using a DINDIG Series Digital Push Pull Force Gauge (range G100 N), SCHWYZ. Animals were handheld and introduced in a plastic tunnel of a proportional diameter appropriate for the volume of the animal. Then, the specimens were motivated to push a short metal plate that was bolted to the force platform. Measurements were repeated five times for each animal. The maximal value obtained during such a recording session was considered to be the maximal excavation force for that individual. All the animals were weighed the same day *in vivo* to the nearest 0.01 g with an electronic balance (OHAUS-Adventure). We evaluate differences in digging performance of the head between sexes by performing an analysis of covariance (ANCOVA), with weight entered as covariate to reduce total size influence. All measures were log<sub>10</sub>-transformed to fulfill assumptions of normality, and each variable was analyzed separately.

The analyses of external morphology, skull angles, and digging performance were carried out with the SPSS software package, version 9.0 (1998) (SPSS Inc., Chicago, Illinois, USA).

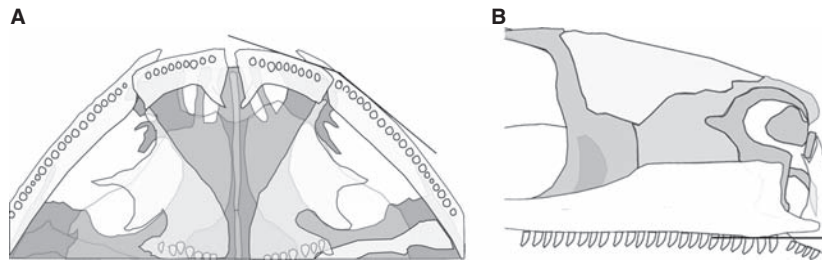
#### Results

##### Nasal region morphology

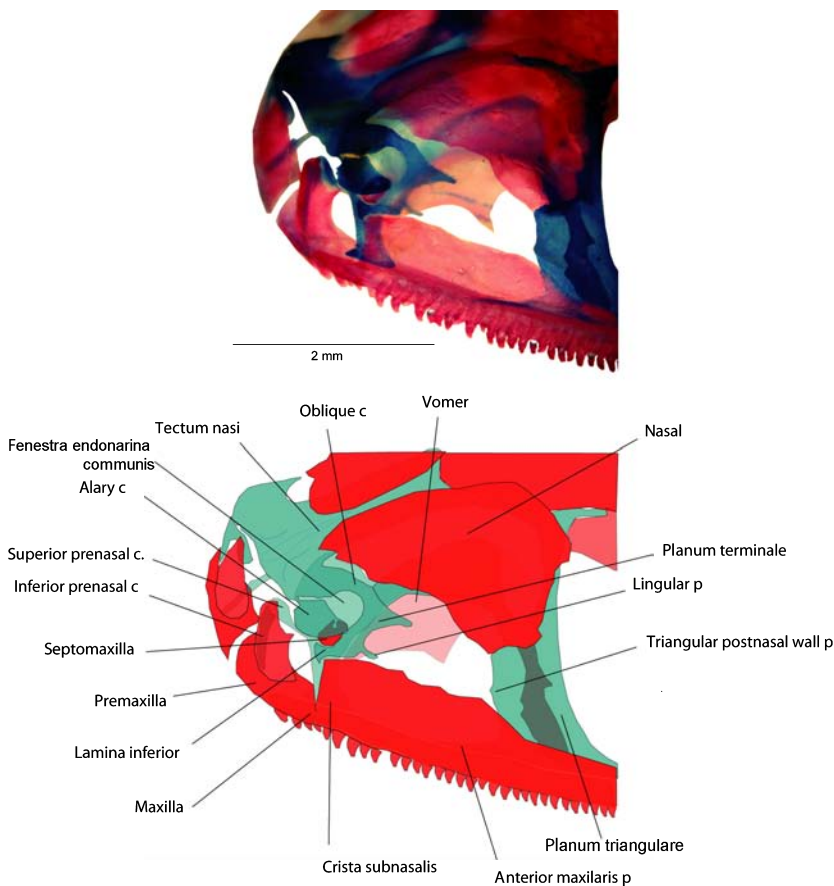
The paired nasal (olfactory) capsules are located in the foremost section of the cranium, the olfactory region, which composes the anterior fourth of the skull. The olfactory capsules house the olfactory organs and lie anterior to the sphenethmoid within an area enclosed by the premaxillae and maxillae. These bones, along with the nasals and vomers, give support to the nasal cartilages. The skeleton of the nasal capsules is mostly cartilaginous, and the septomaxillae are the only bony elements internal to the nasal capsules.

Each nasal capsule has two openings, the apertura nasalis externa and the apertura nasalis interna. The apertura nasalis externa lies on the anterodorsal surface of the cranium and opens through the fenestra endonarina communis into the main cavity of the nasal capsule (Fig. 4). Ventrally, the apertura nasalis interna opens through the fenestra endochoanalis into the buccal cavity, where it is covered by the vomer (Fig. 5).

*Septum nasi*. The septum nasi is a narrow, vertical plate that separates the nasal capsules throughout their entire length (Fig. 5). Anteriorly, the septum nasi protrudes through the



**Fig. 3**—Anterior region of the skull of *Leptodactylus latinasus* showing the osteological angles analyzed. —**A.** Angle formed by the union of the premaxilla and the maxilla in ventral view. —**B.** Angle formed by the pars alaris of the premaxilla with the horizontal axis of the skull in lateral view.



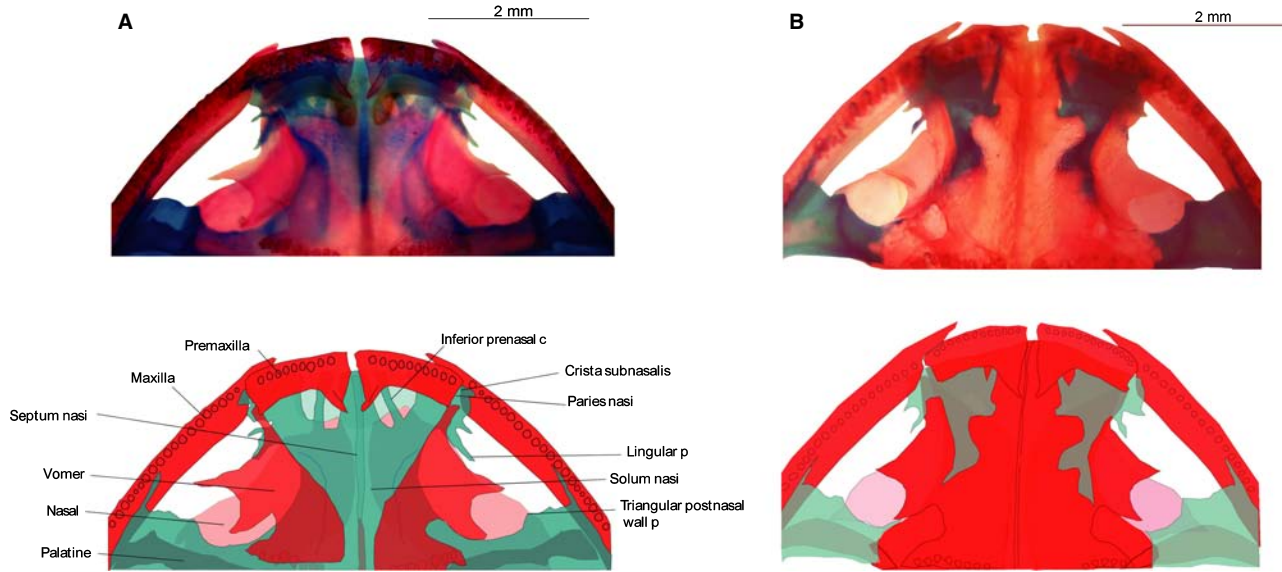
**Fig. 4**—Lateral view of left nasal capsule of *Leptodactylus latinasus* (L691). Blue denotes nasal cartilage; red denotes bones. c, cartilage; p, process.

anterior nasal wall and forms a prenasal process, which can be projected or absent (Table 1). The septum nasi extends anteriorly, between or behind the alary processes of the premaxillae. The anterior border of the prenasal process is cartilaginous in some specimens (83% of females; 14% of males) (Table 1). Dorsally, the septum nasi is continuous with the tectum nasi.

*Tectum nasi.* The tectum nasi is an unpaired, horizontal plate that forms the roof of the nasal capsules (Fig. 6). The tectum nasi gets gradually wider anteriorly and covers the olfactory capsule posteromedially. The intramembranous nasal bone

overlies the anterolateral margins of the tectum nasi. This element is completely ossified or cartilaginous with mineralization (Table 1) (Fig. 6), in some specimens it even becomes coossified with the overlying, intramembranously derived nasal. The lateral margin of the tectum nasi is notched by a wide fenestra nasolateralis; the anterodorsal corner of the fenestra has a deep, rostrally directed indentation. The fenestra nasolateralis lacks a ventral cartilaginous boundary.

*Solum nasi.* The solum nasi extends posteriorly from the level of the premaxillae to the anterior ossification of the sphenethmoid. This is an unpaired, horizontal plate that forms the



**Fig. 5**—Ventral view of nasal capsule of *Leptodactylus latinasus*. —**A.** Female specimen (L691). —**B.** Male specimen (FML2410-2). Blue denotes nasal cartilage; red denotes bones. c, cartilage; p, process.

**Table 1** Characters sexually dimorphic in nasal region of *Leptodactylus latinasus*

	L700	L599	L687	L690	L691	L665	L643	L425	SB249	L674	L675	L420	FML2410-2
	(F)	(F)	(F)	(F)	(F)	(F)	(M)	(M)	(M)	(M)	(M)	(M)	(M)
<b>Prenasal process</b>													
1 Degree of prolongation in relation to the alary process: (0) absent (1) at the same level	1	0	0	0	1	1	0	1	1	1	1	0	1
2 Shape: (0) straight (1) slightly convex (2) triangular	0	1	0	0	0	1	1	2	1	1	2	1	1
3 Margin (0) cartilaginous (1) ossified	1	0	0	0	0	0	1	1	1	1	1	0	1
<b>Tectum nasi</b>													
4 Coossification: (0) Absent, differentiated from the nasal (1) Coossified with nasal	1	0	0	0	0	1	1	1	1	1	1	0	1
5 Degree of ossification: (0) Uniformly ossified (1) Diffuse spots of calcium on a cartilaginous matrix	0	1	1	1	1	0	0	0	0	0	0	1	0
<b>Solum nasi</b>													
6 Degree of ossification (0) Uniformly ossified (1) Diffuse spots of calcium on a cartilaginous matrix	0	1	1	1	1	0	0	0	0	0	0	1	0
7 Coossification (0) Differentiated from vomer (1) Coossified with vomer	1	0	0	0	0	1	1	1	1	1	1	0	1

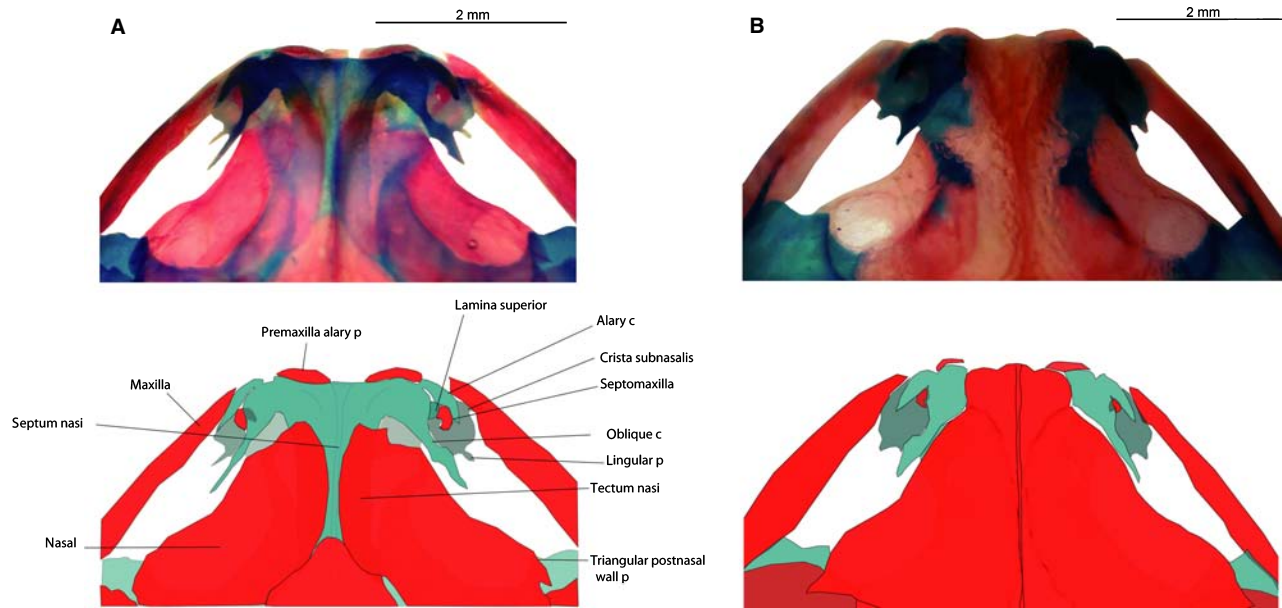
Shaded cells (light grey): character states without association with burrowing behavior in females; shaded cells (dark grey): character states with association with burrowing behavior in males.  
F, female; M, male.

floor of the nasal capsules (Fig. 5). In most individuals (about 62%), the solum nasi is completely ossified or even coossified with the vomers; but in the remaining specimens, an abundant mineralization is conspicuous in a cartilaginous matrix (Table 1) (Fig. 5). Medially, the solum nasi is continuous with the septum nasi. Most of the solum nasi is underlain by the large vomer.

**Anterior nasal wall.** Each anterior nasal wall is continuous with the septum nasi medially, the tectum nasi dorsomedially, the

solum nasi ventrally, and the crista subnasalis posterolaterally. Most of the anterior nasal wall is cartilaginous, although in some male individuals, ossification from the septum nasi may invade the medial portion of the wall.

**Postnasal wall.** The paired postnasal walls are cartilaginous, anteriorly concave structures that form the posterior limit of the nasal capsules. They are dorsomedially continuous with the sphenethmoid, dorsally with the tectum nasi, ventrally with the solum nasi, and laterally with the planum triangulare (Fig. 4).



**Fig. 6**—Dorsal view of nasal capsule of *Leptodactylus latinasus*. —**A**. Female specimen (L691). —**B**. Male specimen (FML2410-2). Blue denotes nasal cartilage; red denotes bones. c, cartilage; p, process.

The anterior margin of the planum antorbitale is concave and constitutes the posterior wall of the nasal cavity, whereas the ventrolateral posterior border completes the anterior margin of the orbit. The planum triangulare – the ventrolateral portion of the planum antorbitale – invests the anteromedial portion of the pars facialis of the maxilla and bears both an anterior and a posterior maxillary process (Figs 4 and 7). The anterior maxillary process is very thin, projects anteriorly to the level of the middle ramus of vomer, and invests the inferior margin of the medial face of the pars facialis of the maxilla. The posterior maxillary process extends posteriorly, investing the maxilla laterally and the anterior ramus of the pterygoid medially. The posterior end of this process is synchondrotically fused to the pterygoid process of the palatoquadrate, which extends posteriorly toward the suspensorium. Proximally, the planum triangulare is pierced by the lateral orbitonasal foramen.

*Alary cartilage and prenasal cartilages.* The alary cartilages are quadrangular in shape, with the ventral margin extending posteriorly (Fig. 4); each one is dorsal and partially overlaps the septomaxilla and anterolateral to the oblique cartilage. This cartilage also serves as the anterior margin of the fenestra endonarina (Fig. 4). The anterior, convex face of the alary cartilage is not fused to the superior prenasal cartilage. The dorsomedial margin of the alary cartilage is in contact with the dorsolateral edge of the oblique cartilage. The superior prenasal cartilages are ovoid structures; each superior prenasal cartilage extends anteriorly to abut the dorsomedial margin of the posterior face of the alary process of the premaxilla. The paired inferior prenasal cartilages are

ventrally arcuate, forming an angle of about  $90^\circ$ . Each inferior prenasal cartilage is fused to the ventral portion of the solum nasi at the level of the anterior margin of the paries nasi. The limit between both is evident because of the mineralization of the solum nasi (Fig. 5). This fusion is restricted to the terminus of the inferior prenasal cartilage. Distally, the inferior prenasal cartilage is club-shaped. It extends anteroventrally to the level of the crista subnasalis and abuts 90% of the posterior aspect of the alary process of the premaxilla. The inferior prenasal cartilages are significantly longer and thinner than the superior prenasal cartilages.

*Crista intermedia, lamina superior, and lamina inferior.* The cristae intermedia are located ventral to the alary cartilage. The dorsomedial margin of the crista intermedia is fused to the transitional zone between the septum nasi and the tectum nasi, whereas the ventrolateral margin bears the lamina superior and the lamina inferior. The anterior ramus of the septomaxilla is attached to the posterior margin of the lamina superior. The lamina inferior lies ventral to the septomaxilla (Fig. 4); its posterior margin extends more posteriorly than the posterior margin of the lamina superior and is parallel to the septum nasi. Anteriorly, the dorsal margin of the lateral wall of the lamina inferior is fused to the lateral edge of the lamina superior. Posteriorly, there is no connection with the lamina superior, and the dorsal margin of the lamina inferior is free.

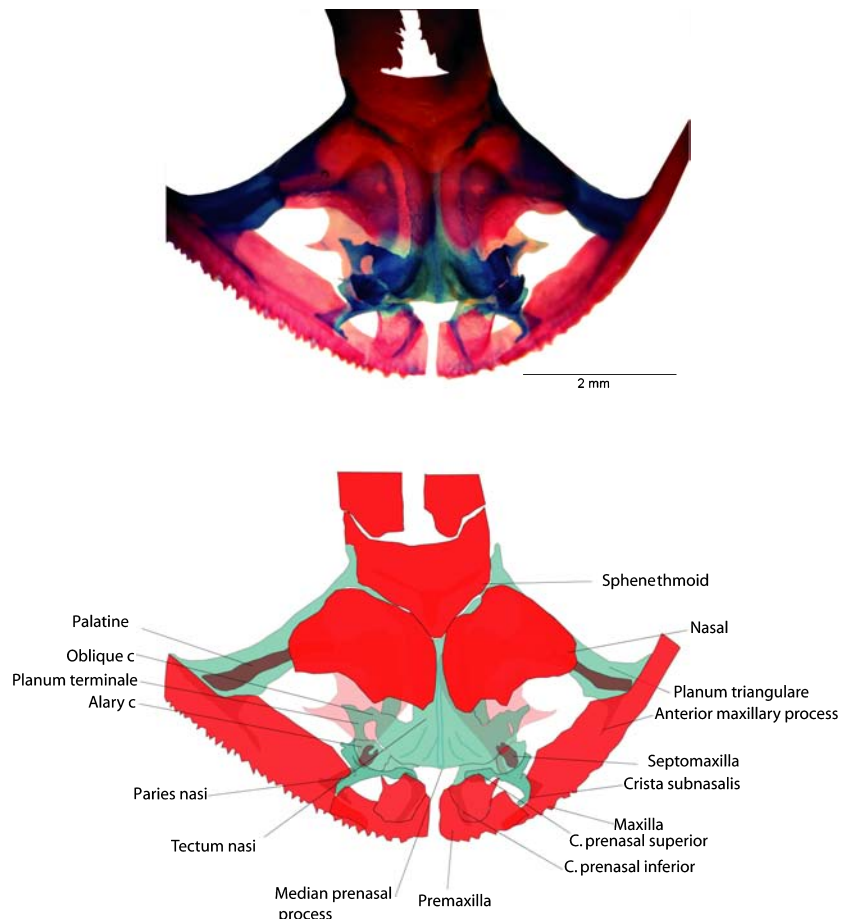
*Oblique cartilage and planum terminale.* A semi-circular cartilaginous sheet lies lateral to each side of the septum nasi.

This cartilage, the oblique cartilage, extends from the middle-lateral to the dorsomedial aspect of the nasal capsule (Figs 4 and 7). This cartilage has both anterior and posterior irregular margins. The anterior margin of the oblique cartilage forms the posterodorsal limit of the fenestra endonarina. The anterior end of the oblique cartilage is expanded and globe-shaped. The anterodorsal part of the margin provides support to a section of the rim of the external narial aperture. The posterolateral margin of the oblique cartilage forms the anterior margin of the fenestra nasolateralis. The ventrolateral portion of the oblique cartilage, the planum terminale, is a vertical plate of cartilage that forms the lateral wall of the nasal capsule and is fused to the lamina inferior. The dorso-medial edge of the oblique cartilage is fused with the antermost portion of the lateral margin of the tectum nasi; both structures complement one another by forming a dorsal cover to the anterior nasal capsule. The area of fusion between the oblique cartilage and the tectum nasi is relatively wide. The inferior edge of the planum terminale has a posterior, rod-shaped, lingular process. A narrow band of cartilage extends anteriorly to the lingular process to fuse with the lamina inferior and the lateral margin of the dorsal wall of the crista

subnasalis. Anteriormost, close to the anterior point of this fusion, the cartilages expand. The posteroventral corner of the planum terminale bears a short, posteriorly directed triangular process.

*Crista subnasalis.* The crista subnasalis is an elongated structure with a posteriorly opened V-shaped channel (Figs 4 and 7). The distal end of the crista subnasalis is bifurcate: a short, ventrally directed process articulates with the superior margin of the posterior end of the premaxilla, and a slightly longer, posteriorly directed process traverses along the pars palatina of the maxilla to abut the medial wall of the pars facialis of the maxilla (Fig. 4).

*Parietes nasi.* The parietes nasi are paired, elongate, ventrolaterally oriented cartilaginous structures that extend to the premaxilla–maxilla articulation (Fig. 7). Each paries nasi merges with the solum nasi medially, the anterior nasal wall anteriorly, and the lamina inferior dorsally. Ventral to the paries nasi, the crista subnasalis abuts the posterior end of the premaxilla anteroventrally and the medial wall of the pars facialis of the maxilla posteroventrally.



**Fig. 7**—Dorsofrontal view of nasal capsule of *Leptodactylus latinasus* (L691). Blue denotes nasal cartilage; red denotes bones. c, cartilage.

**Septomaxilla.** One pair of intramembranous bones, the septomaxillae, are located within the nasal capsules, embedded in the nasal cartilages (Figs 4, 6 and 7). Each septomaxilla is located posteroventral to the corresponding alary cartilage, and dorsal to the lamina inferior. In lateral view, the septomaxilla has a U-shaped corpus; the apex projects anteroventrally. The superior ramus is more slender than the inferior ramus.

**Premaxillae.** The premaxillae are paired, tooth-bearing bones that form the anterior margin of the maxillary arcade. The premaxillae are narrowly separated from one another medially, whereas laterally they are slightly overlapped by the maxillae. In anterior aspect, each premaxilla resembles an inverted T. The leg of the T is a thin, bony shaft, the alary process that projects dorsally and slightly posteriorly from the head of the T. The head of the T is formed by the well-ossified pars dentalis. Posteriorly, the premaxilla bears a narrow lingual ledge, the pars palatina. Two of the premaxillary parts, the pars palatina and the alary process, are in direct contact with the nasal cartilages. The pars palatina arises near the posteroventral margin of the pars dentalis and is narrower at its mid-region than at the posterolateral end of the premaxilla. The posterolateral portion of the pars palatina is braced by the anteroventral end of the crista subnasalis. Medially, the pars palatina bears a palatine process that forms a right triangle; this process is bifid, with the inner ramus shorter than the external ramus (Fig. 5). The alary processes are subrectangular, oriented dorsally, and parallel to each other (Fig. 7). The base of the alary process is narrower than the superior margin. The posterior face of the alary process is slightly concave and is invested dorsomedially by the superior prenasal cartilage and ventromedially by the inferior prenasal cartilage. The pars dentalis is rectangular and bears about 9–11 fang-like, recurved, pedicellate teeth throughout its entire ventral margin.

**Maxillae.** The maxillae are paired, tooth-bearing bones located on the lateral sides of the maxillary arcade, posterior to the premaxillae. These elements provide bony protection for the ventrolateral aspect of the nasal capsules. The anterior tip of the maxilla bears a lateral projection that overlies the premaxilla. Like the premaxillae, each maxilla also has three distinct sections: (1) a dentate element, the pars dentalis, (2) a palatal ledge, the pars palatina, and (3) a dorsolateral facial flange, the pars facialis. Of the three sections, the pars facialis, which protects the lateral aspect of the nasal capsule, is most closely associated with the nasal cartilages. The pars facialis is well developed, lacks pre- and postorbital processes, and extends for approximately less than half the length of the maxilla, from the anterior margin of the bone to the level of the planum triangulare (Fig. 7). The anterior margin of the pars facialis invests the entire posterior margin of the corresponding paries nasi.

**Nasals.** The nasals are paired, triangular to elongated bones that roof the medial and posterolateral region of each olfactory organ covering the tectum nasi. The nasals are separated narrowly from one another; thus, in cleared-and-stained specimens, the septum nasi is obvious between them (Fig. 6). A small space separates the nasals from the oblique cartilage of the nasal capsule. The nasals overlap the sphenethmoid and postnasal walls, but do not come in contact with the premaxillae or the maxillae. A short, blunt maxillary process extends laterally.

**Vomer.** The vomers are paired, palatal bones that lie ventral to the olfactory region and help to floor the nasal capsules. These bones underlie the sphenethmoid and are medially separated from one another (Fig. 5). Each vomer is composed of four distinct portions, which are the anterior, dentigerous, prechoanal, and postchoanal processes. The anterior blunt process of the vomer extends anterolaterally, reaching the premaxilla-maxilla articulation, continuing beyond the level of the lingual process of the planum terminale.

The prechoanal and postchoanal processes extend laterally and posteriorly from the anterior process. The prechoanal process forms the anterior and the medial portion of the anterior margin of the apertura nasalis interna. The main body of the prechoanal process is straight, its proximal end is slightly wider than the distal portion and is oriented posterolaterally. The postchoanal process is short and triangular and extends posteriorly from the prechoanal process to form the medial margin of the apertura nasalis interna. The transverse dentigerous process lies medial to the apertura nasalis interna and usually bears approximately 11–14 true teeth. Medially, the vomers are widely separated from one another.

The degree of polymorphism in the characters possibly associated with the burrowing habits does not allow us to detect an unequivocal pattern of sexual dimorphism. Nevertheless, an obvious tendency is manifest (Table 1). The shape of the prenasal process shows a more specialized morphology in males than in females (character 1: about 71% of males with a prenasal process anteriorly prolonged, in opposition to only 50% in females; character 2: 100% of males with a prenasal process with a defined shape, against about 33% in females). In addition, the degree of ossification is greater in males (Figs 5 and 6) than in females. Characters 3, 4, 5, 6, 7 show uniform ossification and coossification, which imply hyperossification in approximately 87% of males and 33% of females.

#### Head dimensions

Morphometric variables recorded are in Table 2. The analysis of covariance (ANCOVA), considering sex as factor and SVL as a co-variable, indicate that the three head measures (length, width, and depth) do not differ significantly between sexes (Table 3). Regarding angles of the bones of the snout region of the skull, although box plots show that some differences



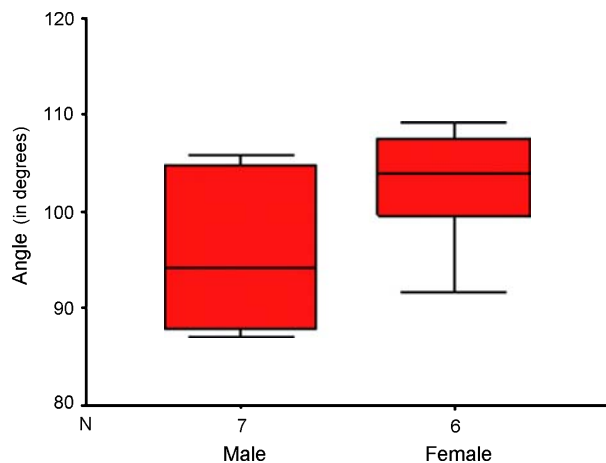
**Table 2** Means  $\pm$  standard deviations of the seven variables measured in the specimens of *Leptodactylus latinasus* of each sex for the quantitative analysis

	Female (mean $\pm$ SD)	Male (mean $\pm$ SD)
SVL (mm)	32.2 $\pm$ 2.2	30.3 $\pm$ 0.9
HL (mm)	12.5 $\pm$ 0.8	12 $\pm$ 0.6
HW (mm)	11.7 $\pm$ 1.3	11.1 $\pm$ 0.6
HD (mm)	6.1 $\pm$ 0.7	6.2 $\pm$ 0.6
PA	102.8 $\pm$ 6.5	95.7 $\pm$ 8.3
PMX-MX	153 $\pm$ 3.3	150 $\pm$ 2.3
PF (N)	3.1 $\pm$ 0.5	2.3 $\pm$ 0.3

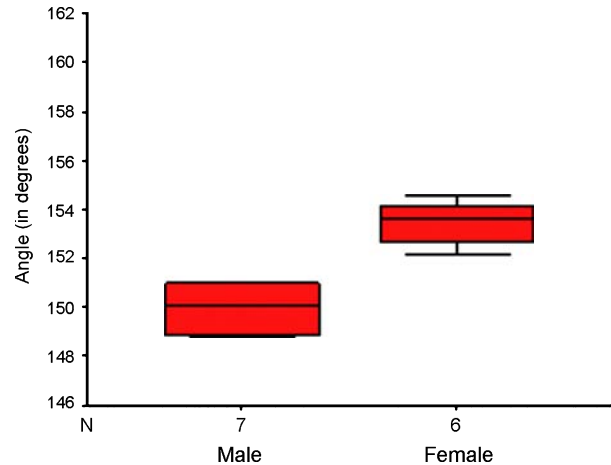
SVL, snout-vent length, HL, head length; HW, head width, HD, head depth; PA, angle formed by the pars alaris of the premaxilla with the horizontal axis of the skull in lateral view; PMX-MX, the angle formed by the union of the premaxilla and the maxilla in ventral view; PF, push force.

**Table 3** Results of the ANCOVA analysis of the external head dimensions

	$F_{1,34}$	Significance
Head length	0.596	0.446
Head width	2.231	0.145
Head depth	0.972	0.331

**Fig. 8**—Boxplot representing the angle that the pars alaris of the premaxilla forms with the line from alongside the pars dentalis of premaxilla and maxilla, measured in lateral view. In general, the angle is larger in females than in males, in which the snout is slightly sharper, although these values are not significantly different.

exist between sexes, especially the angle formed by the premaxillae and maxillae in dorsal view (Figs 8 and 9), those differences are not significant, as was demonstrated in the ANOVA analysis of those angles (Table 4).

**Fig. 9**—Boxplot representing the angle formed in the point of union between the premaxilla and the maxilla, in ventral view. In females the angle is greater than in males, although these values are not significantly different.**Table 4** Results of the ANOVA analysis of the skull dimensions

	$F_{1,12}$	Significance
Alary process angle	2.994	0.112
Premax-max angle	3.206	0.101

#### Digging force

The analysis of covariance (ANCOVA), considering sex as factor and weight as a co-variable, indicate no differences in push force between the two groups ( $F_{1,17} = 0.44$ ,  $P = 0.514$ ).

#### Discussion

The data gathered in this study do not clearly indicate a sexual dimorphism in head size, nasal morphology, and digging performance that can be linked with burrowing behavior. The only unequivocal sexually dimorphic character associated with the construction of the nuptial chamber by males is the rigid chisel-like snout, present exclusively in males. In spite of the evident sharp edge of the snout in the males observed (Fig. 2C), the head length was not sexually dimorphic.

Curiously, the osteological measurements and the other derived characters that were previously linked with the burrowing habit, typical of the *L. fuscus* group of the genus *Leptodactylus*, i.e. degree of ossification of the tectum, solum, and septum nasi, prenasal process configuration, etc. (Ponssa 2008), show a suggestive tendency toward a dimorphic pattern. This tendency is just superficially manifest; the statistical analyses do not support a conclusive statement about the occurrence of dimorphism in the considered measurements,

and the qualitative features analyzed show degree of polymorphism that do not allow the formulation of a definitive conclusion. The survival of the offspring will depend greatly on the efficiency with which the nuptial chamber is built, because the eggs and early larval stages develop inside these cavities. So, why does head size, morphology of snout, and digging performance lack sexual dimorphism? These features would be predicted to be related to the construction of an optimal incubation chamber, and consequently, the features should be under greater sexual selection pressure. A parsimonious explanation of our results is that the digging behavior of *L. latinasus* does not require particular head dimensions or osseous morphology of the nasal capsules in the males. Thus, there has not been sexual selection for morphology or performance in the males of *L. latinasus*. In other words, although selection has favored a digging behavior in males to construct incubation chambers, it has not operated in relation to the morphological features studied herein. The rigid, chisel-like snout of the males appears to be adequate for the construction of the nuptial chamber. The data presented here allow us to propose the hypothetical events leading to the snout morphology in *L. latinasus*. As the females can push as strongly as the males, an initial selection for increased digging force in both sexes enabled the exploitation of the terrestrial habitats for reproduction (Heyer 1969), which seems likely. This selection would act on the performance of males and females and also on head morphology, deduced from the characters associated with the burrowing habits described for both sexes (e. g. position of prenasal process in relation to alary process of premaxilla, degree of ossification of tectum and solum nasi) (Ponssa 2008; this work). In a next step, a selective pressure arose on males to develop a sharp edge of the snout, allowing them to construct a more optimal incubation chamber. This hypothesis remains to be tested. We know that the nasal capsule of *L. latinasus* and other species of the *fuscus* group have a morphology implying a strengthening of bones of the nasal region, which is absent in species of the other groups of the genus that do not exhibit digging behavior (Ponssa 2006, 2008; this work); but are there differences in the push performance between the species of the groups with more terrestrial versus aquatic reproductive modes? The other question to be tested is whether the spatulate snout guarantees a nuptial chamber more optimal for the protection and successful development of the offspring.

*Leptodactylus latinasus* belongs to the *fuscus* group, the most speciose group of the genus. This group has been characterized by a reproductive mode linked to terrestrial habitat, because the deposition of the nest is on land in an incubation chamber; the males call from this burrow, where the mating and the first stages of development take place (Heyer 1969). Nevertheless, the observations of the incubation chamber construction are quite rare and lacking for most of the species of the group. Reports about which sex excavates the burrow exist for *Leptodactylus bufonius* (Ceï 1949; Reading and Jofré 2003), *Leptodactylus mystacinus* (Oliveira Filho and

Giaretta 2008), *L. fuscus* (Ceï 1949; Martins 1988; Giaretta and Kokubum 2004), *Leptodactylus mystaceus* (Caldwell and Lopez 1989), and *L. latinasus* (Gallardo 1958, 1964). If we had found sexual dimorphism in the nasal capsules or head dimensions of *L. latinasus*, then the relationship of these characters with the construction of the incubation chamber might have been used to predict that sex is involved in this behavior for species in the group for which there are not behavioral observations. Nevertheless, our results indicate that we cannot relate digging behavior with sexual dimorphism, other than the spatulate snout. In a few species of the genus *Leptodactylus*, males and females participate in chamber construction equally, with the contribution of each at different stages of construction (Ceï 1949; Silva *et al.* 2005). Even in *L. fuscus*, one of the species of the genus whose reproductive aspects have been studied in greatest detail (Oliveira Filho *et al.* 2005; Arzabe and Prado 2006), the participation of the female in the process of nuptial cavity building was recently described, specifically in the closing of the chamber: 'Initially she pressed her nose against the substrate, pushing small grains of moist sand toward the burrow entrance. With movements of her legs, she loosened the sand, which she then pushed with her nose; while with her front limbs and with her chin, she compacted the loose sand as she stretched and contracted her body' (Lucas *et al.* 2008, p. 9). These observations encourage one to make more detailed observations on the reproductive behavior of *L. latinasus*.

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## Appendix I

Material examined for morphological data collection. \*Specimens that were double-stained and cleared. <sup>f</sup>Specimens used in the excavation performance.

Males: FML12046, 12047, 12048, 12049, 12050, 4832-3, 4832-4, 4832-5, 4832-6, 4832-7, 12240, 12241, 12242, 12243, 12244, 12245, 12246, 12247, 2410-2\*, SB 249\*, L425\*, 249\*, 674\*, 675\*, 420\*, 643\*, 8<sup>f</sup>, 9<sup>f</sup>, 11<sup>f</sup>, 13<sup>f</sup>, 14<sup>f</sup>, 760<sup>f</sup>, 761<sup>f</sup>, 763<sup>f</sup>, 764<sup>f</sup>, 772<sup>f</sup>, 776<sup>f</sup>.

Females: FML21702, 21703, 21690, 21704, 21686, 22594, 22595, 22596, 4832-8, 4832-9, 4832-10, 16009, 16010, 9166, 9167, 10636; L700\*, 599\*, 687\*, 690\*, 691\*, 665\*, 768<sup>f</sup>, 10<sup>f</sup>, 767<sup>f</sup>, 15<sup>f</sup>, 18<sup>f</sup>, 765<sup>f</sup>, 770<sup>f</sup>, 777<sup>f</sup>, 778<sup>f</sup>, 30<sup>f</sup>.