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# Morphological variability of the cranium of *Lontra longicaudis* (Carnivora: Mustelidae): a morphometric and geographic analysis

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## Abstract

**Background:** Studies of morphometric variation make it possible to delimit species and geographic intraspecific variation, mainly in species with wide distribution ranges. In the Neotropical otter, *Lontra longicaudis*, variation in the shape of the rhinarium of three potential subspecies has been described but it is not known whether there is a pattern to the morphometric variation in the skull throughout the distribution of this species. We analyzed morphological variation in the cranium (ventral view) and the mandible (lateral view) of the Neotropical otter, comparing male and female specimens and evaluating the differences between specified geographic units utilizing methods from geometric morphometrics. Specimens from the entire distribution of the species were analyzed. Between sexes, variability in the shape was determined by calculating the Procrustes distances and using Goodall's *F* test. Geographic variation was analyzed using a discriminant analysis, an analysis of variance (ANOVA) on a matrix of partial warp scores, and a cluster analysis with Mahalanobis distances, allowing for similarities in shape to be identified between different geographic units. Variation in the size of the two structures was calculated based on the values for centroid size using a one-way ANOVA with a Bonferroni correction and a 95 % confidence interval.

**Results:** There was sexual dimorphism in shape for both views, with males the largest. In general, there was geographic variation in the shape and size of both the cranium and the mandible in the Neotropical otter, exhibiting a pattern that resembled Bergmann's rule. Variation in shape between geographic units could result from the presence of geographic barriers, the spatial configuration of hydrological regions, and/or the large distances between populations throughout this species' distribution.

**Conclusions:** The Neotropical otter exhibits dimorphism in the size, but not in the shape of the skull. There is geographic variation between geographic units, and our results suggest that *L. longicaudis* could be a group of species. An integrative study using molecular and morphological data could elucidate its taxonomy.

**Keywords:** Geographic variation; Geometric morphometrics; *Lontra longicaudis*; Sexual dimorphism

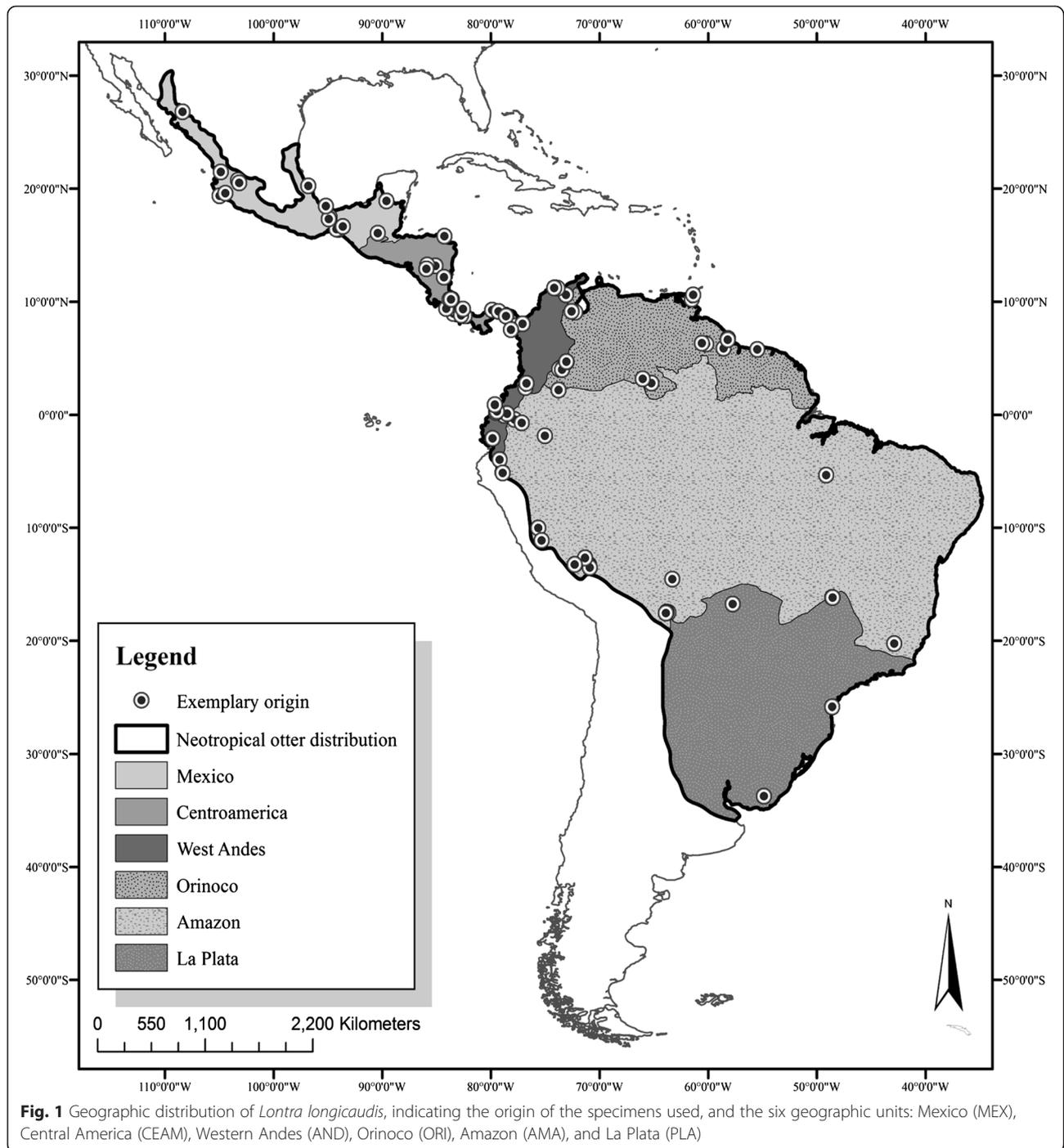
## Background

The Neotropical otter (*Lontra longicaudis*) is an aquatic species with a wide distribution that is limited to the terrestrial-aquatic ecotone that corresponds to lagoon and riparian systems (Gallo-Reynoso et al. 2008). This species is Neotropical in origin and has a continuous distribution from Argentina in South America to the Trans-Mexican Volcanic Belt (Fig. 1). The latter is a mountain range that divides the country in two, follows

the outline of both the Atlantic and Pacific slopes, and ultimately reaches its northern extreme in the state of Sonora on the Pacific slope with its other extreme in Tamaulipas on the Gulf of Mexico slope (Gallo-Reynoso 1997). Sexual dimorphism has been reported for *L. longicaudis*, with males larger than females (Kruuk 2006). Along its distribution, this otter occupies habitats such as coastal lagoons, rivers, and lakes that are located in distinct geographic and climatic regions, from arid zones to wetlands and from tropical to temperate forests (Larivière 1999). The composition of its diet is also variable between regions

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and seasons, though different studies have shown its diet is largely made up of fish and crustaceans and to a lesser extent amphibians, mammals, birds, and turtles (Gallo-Reynoso et al. 2008; Carvalho-Junior et al. 2010; Chemes et al. 2010; Platt and Rainwater 2011). Owing to these factors and the variation in its habitat in terms of vegetation, climate, and food availability throughout its extensive distribution, potential changes in the cranial morphology of *L.*

*longicaudis* may be interpreted as local adaptations to environmental variables such as different types of prey.

The wide geographic distribution of *L. longicaudis* and the variation in its aquatic habitats give rise to two hypotheses of morphological variation. The first hypothesis is that there should be a differentiation in the shape and size of the cranium and mandible between the sexes, and the second is that these variables differ between

populations of the different geographic regions throughout its distribution.

In order to test these hypotheses, we used techniques based on geometric morphometrics (Kendall 1984; Bookstein 1991; Lawing and Polly 2010) which describe and interpret changes in the shape of different anatomical structures of organisms, based on comparisons of homologous structures (Adams et al. 2004). This analysis of anatomical shape allows for a better understanding of morphological evolution and adaptive effects (Zelditch et al. 2004) since variation in shape is influenced by environmental factors (Rohlf and Marcus 1993).

Morphometric analysis of the cranium of several species has indicated that patterns of morphological variation in species with large distribution areas might be adaptations to the range of environmental conditions occurring throughout their distribution (Zelditch et al. 2004; Cardini et al. 2007). This pattern has been observed in mammals such as the puma (*Puma concolor*), the tiger (*Panthera tigris*), and the brown bear (*Ursus arctos*), for which variation in the size and configuration of the cranium are correlated with the different geographic regions throughout which their populations are distributed (Gay and Best 1996; Baryshnikov and Puzachenko 2011; Mazák 2011). These patterns of morphological variation are associated with habitat variables such as temperature, precipitation, and latitude (Platz et al. 2011). Patterns of change in the mandible associated with contrasting habitats have been observed in several species of rodents (Duarte et al. 2000). This also occurs for species like the titi monkey, genus *Leontopithecus* (Freitas et al. 1999), and for bats of the genus *Monophyllus* (Mancina et al. 2010). And for the green monkey, *Cercopithecus aethiops*, there is a close relationship between rainfall pattern and the length of its face (clinal variation) (Cardini et al. 2007).

Patterns of morphological change associated with geographic regions are even more notable in species with a high level of habitat specialization, such as aquatic species (Langerhans et al. 2003; Jonsson and Jonsson 2001). For the cichlid *Biotodoma wavrini* and the characid *Bryconops caudomaculatus*, distinct morphological adaptations have been found for their two contrasting habitats, river channels and lagoons, where morphological variation increases with increasing geographic distance between populations and with variation in other environmental factors (Langerhans et al. 2003).

Morphological variation in the Eurasian otter (*Lutra lutra*) has only been assessed using linear morphometric analysis. For example, Ruiz-Olmo et al. (1998) reported that the age of *L. lutra* may be determined on the basis of its cranial morphology. And variation in cranium size has also been found over the range of this species, with more variability in animals in the tropics (Baryshnikov and

Puzachenko 2012). This technique demonstrated that there is a significant difference in cranial morphology between healthy populations of otters and populations considered at risk due to decreased population size and habitat quality (Pertoldi et al. 2000). In addition, these studies have made it possible to explore any effects of contaminants on the morphology and ontogenesis of the cranium of *L. lutra* (Pertoldi et al. 1998) and other species such as *Ursus maritimus* (Pertoldi et al. 2012).

Several taxonomic studies of *L. longicaudis* have postulated that it is potentially composed of a number of species based primarily on the differentiation of its rhinarium (Pohle 1920; Cabrera 1957; Harris 1968). The most recent studies have suggested that populations with distinct rhinarium morphology may be conspecific and that these populations may be subdivided geographically into three subspecies: (I) *L. longicaudis annectens*, found from Mexico to Colombia and Ecuador; (II) *L. longicaudis enudris*, distributed in French Guiana, Suriname, Peru, and Trinidad and Tobago; and (III) *L. longicaudis longicaudis*, distributed throughout most of South America from Brazil to Uruguay and Argentina (Van Zyll 1972; Larivière 1999). However, this classification has also been questioned recently by Trinca et al. (2012) as a result of new insights into the phylogenetic structure of the South American populations based on mitochondrial sequence analysis. These authors mentioned that an intra-specific subdivision continues to be controversial due to genetic differentiation within the different geographic regions, and indicated that an integrative study would be necessary to examine morphological variation in the species, including an analysis of cranial morphometrics.

The present study has the objectives of evaluating changes in the morphology of the cranium and mandible of *L. longicaudis* using geometric morphometric techniques and examining the variation in the configuration (shape and size) of the cranium and the mandible between sexes and among the different geographic regions of its distribution. Morphological variation associated with distinct geographic regions throughout which the otter is distributed would support previously reported genetic differences.

## Methods

### Sampling

The crania of 151 specimens of *L. longicaudis* from localities throughout its distribution were examined (Fig. 1). The specimens were housed in the National Collection of Mammals of the National Autonomous University of Mexico, the Mammal Collection of the Autonomous University of Campeche, the Vertebrate Collection of the Biological Station of the National Autonomous University of Mexico in Los Tuxtlas, the Mammal Collection of the University of Kansas, the American Museum of Natural

History, the Mammal Collection of the Field Museum, the National Museum of Natural History of the Smithsonian Institute, the Carnegie Museum, and the Michigan University Museum (see list of specimens in Additional file 1). Since the Neotropical otter is a threatened species and protected in the majority of the countries where it is found, the analysis was limited to cranium samples from the aforementioned collections.

Each specimen was assigned to one of the three age categories using the criteria of Van Zyll (1972): juvenile (presence of deciduous teeth), subadult (a full set of fully erupted teeth, presence of nasal sutures, and occipital-parietal sutures), and adult (absence of maxillary-nasal and occipital-parietal sutures). For the morphometric analysis, only adult samples were used to avoid the errors associated with the modifications that the cranium undergoes when the animal is growing (Caumul and Polly 2005).

The samples were grouped a priori into six geographic units (GUs): Mexico, Central America, Western Andes, Orinoco, Amazon, and La Plata (Fig. 1) based on the main hydrological delimitations throughout this otter's distribution and on patterns of morphological and genetic structure observed among the different populations of other aquatic species in South America (Banguera-Hinestroza et al. 2002; Hubert and Renno 2006; Arzamendia and Giraud 2009), including the Neotropical otter (Trinca et al. 2012).

#### **Digitization of images, recording landmarks, and semi-landmarks**

The analysis of variation in cranial morphology was based on digital images of the cranium in ventral view ( $n = 64$ ) and the mandible in lateral view ( $n = 87$ ). All photographs were taken by the same person to avoid any variation associated with possible different points of view of observers. Specimen placement was standardized, and the crania were photographed on the right side of the face, from a distance of 40 cm from the camera lens and oriented at an angle of 90°. All images were captured using a Sony Alpha 200 reflex camera with a resolution of 3872 × 2592 pixels. A ruler was included in each photograph as a reference scale.

The use of only the right side of the cranium and the mandible to locate the landmarks assumes bilateral symmetry in these structures. The configuration of the cranium and the mandible was recorded using landmarks (LMs), which are natural points located on homologous sites and that can be easily located on any specimen (Bookstein 1997), and semi-landmarks (SLMs), which are located along the homologous outlines of the structures and are based on templates drawn between the LM points (Bookstein 1991; Gunz and Mitteroecker 2013). Outlines were drawn using

MakeFan6 software (Sheets 2003) to facilitate the location of the SLMs and were based on two LMs (numbers 1 and 8 for the mandible and 1 and 16 for the cranium, Fig. 2). LMs and SLMs were digitized for each specimen using the TpsDig 2.15 software (Rohlf 2001), and configuration matrices obtained for each view. For these matrices, a generalized Procrustes superposition adjustment (Rohlf and Slice 1990) was applied using the software CoorGen6f (Sheets 2005a), where variation associated with the effects of positioning, orientation, and scale is minimized by calculating the sum of the squared differences between corresponding landmarks (Bookstein 1991; Zelditch et al. 2004). The placement of the outlines for the positioning of the SLMs results in a shape distortion during the Procrustes adjustment (Bookstein 1996; Gunz and Mitteroecker 2013), so a second adjustment was made to eliminate variation by sliding the SLMs based on the alignment distance criteria of the Semiland software (Sheets 2002).

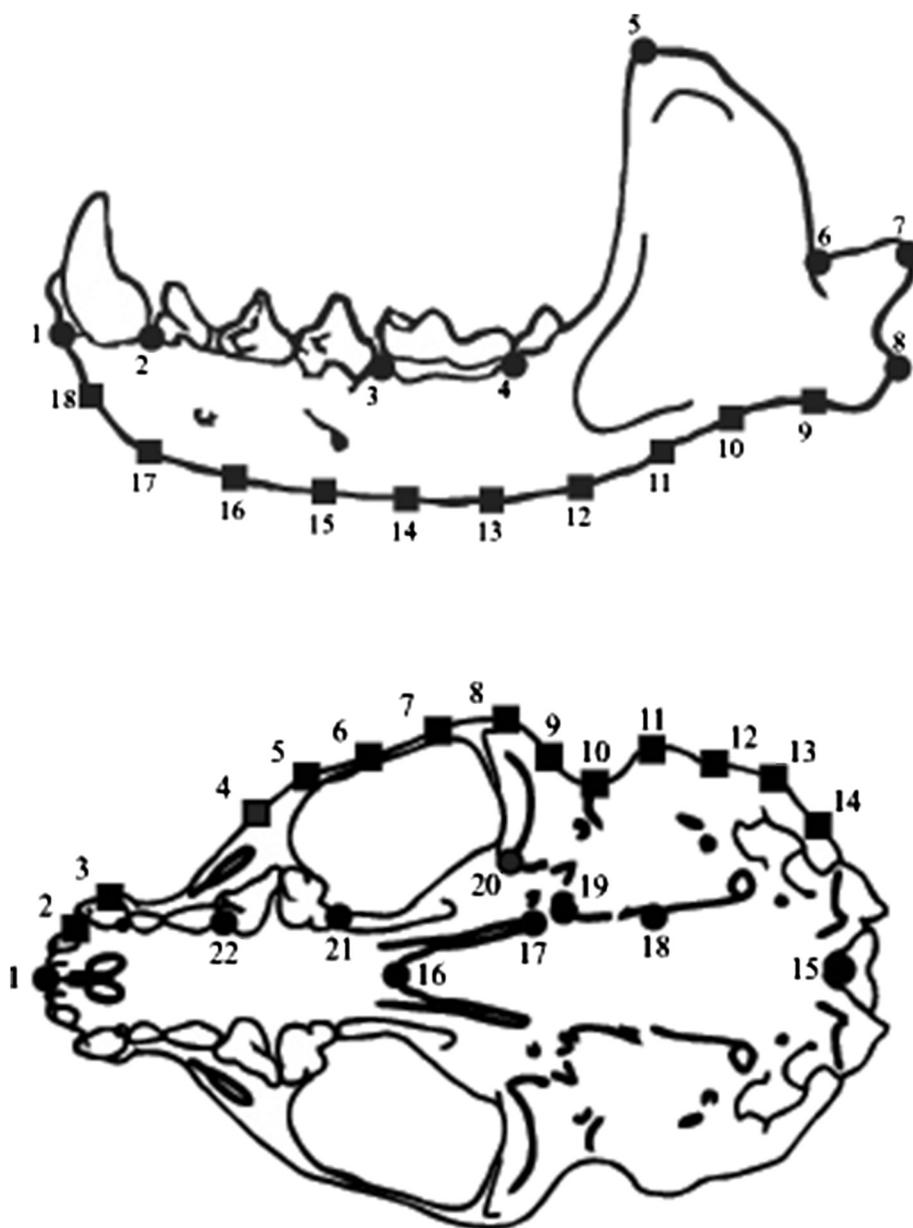
For each configuration of LMs and SLMs (2 LMs and 21 SLMs on the ventral cranium view; 2 LMs and 16 SLMs on the lateral mandible view), an eigen analysis was done following the methodology of Bookstein (1991) and Rohlf (1996). This analysis allows a tangent space to be converted to a Euclidian space, making it possible to use conventional multivariate statistical analyses (Rohlf 1996). The average general shape was used as a reference, and each sample was described in terms of its deviation from the average form for all specimens. The resulting matrices describe change in shape (partial warp scores, PWS) and size as measured from the centroid size (CS). These variables were used in subsequent analyses to describe the change in shape and size of the skull and mandible.

#### **Statistical analysis**

A two-way analysis of variance (ANOVA) for centroid size and a two-way multivariate analysis of variance (MANOVA) for shape were performed to test a priori the differences between sexes and among GUs. Specimens from La Plata were excluded from both analyses of the ventral view because only females were available for this GU. These analyses were run using Statistica 8.0 software (StatSoft 2007).

#### **Sexual dimorphism in shape and size**

Because sexual dimorphism has been reported for several species of otter (Kruuk 2006), we analyzed shape and size differences between sexes, based on 70 mandible images (36 male and 34 female) and 56 cranium images (29 male and 27 female). To this end, a principal component analysis (PCA) and two-way MANOVA were run to test for sexual dimorphism in the shape of the mandible and the cranium. Subsequently, a



**Fig. 2** Landmarks used to capture the configuration of the mandible (LM = 8 and SLM = 10) and the ventral view of the cranium (LM = 9 and SLM = 14) of *Lontra longicaudis*. Circles are LM and squares SLM

permutation test was performed using Goodall's *F* test, designed to compare Procrustes coordinates (Goodall 1991, Zelditch et al. 2004), as implemented in the software TwoGroup6 (IMP series; Sheets and Zelditch 2001). The significance of the differences was tested with 2500 bootstrap permutations. The variation in size between the sexes was analyzed using an ANOVA with Bonferroni correction and a 95 % confidence interval and run in Statistica 8.0 (StatSoft 2007). These analyses were run to determine whether the data for

males and females could be pooled for subsequent tests.

**Geographic variation in shape and size**

Due to the low number of specimens used for the analysis of geographic variation and the fact that no significant sexual dimorphism was found for either structures (see "Results"), the data for both sexes were combined to analyze for geographic variation.

Canonical variables were extracted in a multidimensional space in order to explain the majority of the variation between groups for which the partial warp scores matrix was used. To assess shape variation between GUs in both views, a canonical variate analysis (CVA) was run in CVAGen6 software (Sheets 2005b). We obtained thin-plate spline plots (deformation grids describing shape changes) by regressing the PWS onto the first and second canonical variables. Subsequently, a discriminant function analysis (DFA) using Mahalanobis distances was calculated to assess the validity of the a priori classification of the specimens into six GUs.

Applying a MANOVA, a significant difference in shape was detected between the distinct GUs. To describe morphological similarity between GUs, a cluster analysis with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used, using the Mahalanobis distance between GUs. The DFA and UPGMA were run in Statistica 8.0 (StatSoft 2007).

Variation in cranium and mandible size between the six GUs of *L. longicaudis* was analyzed using a one-way ANOVA, with log-natural-transformed values of centroid size. Post hoc comparisons among GUs were run using a Bonferroni correction and a 95 % confidence interval in Statistica 8.0 (StatSoft 2007).

## Results

### Sexual dimorphism in shape and size

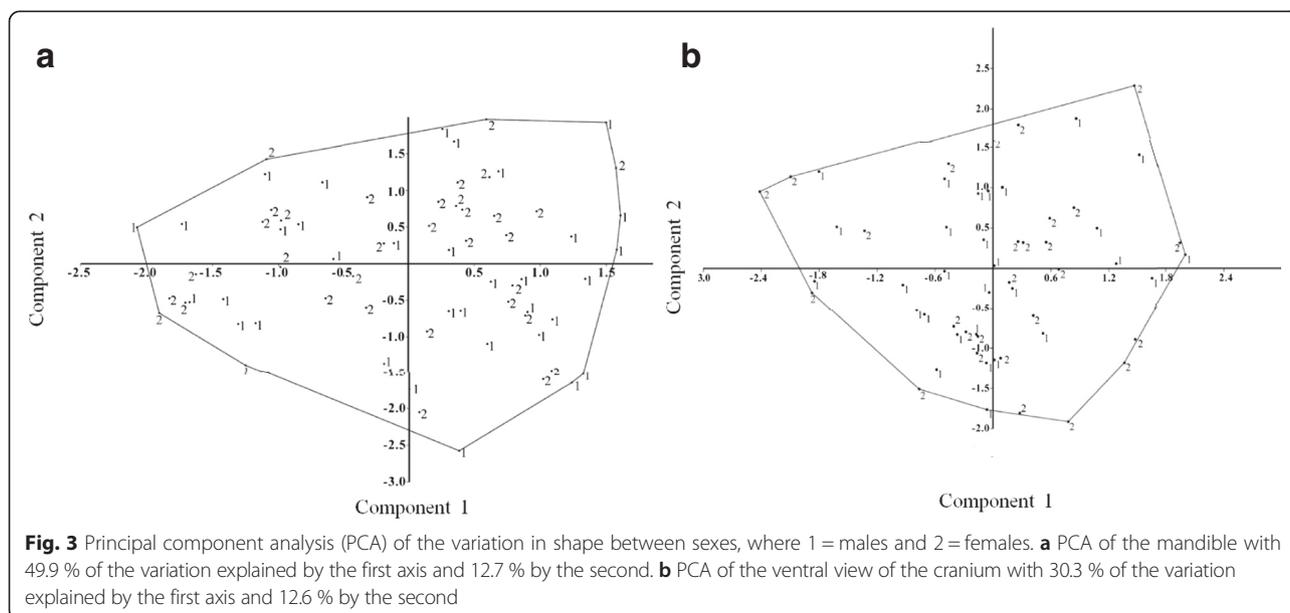
The PCA of the skull and the mandible indicate a small proportion of the variance was explained by the first two principal components, 63.6 and 42.9 %, respectively. Plots of these two components for each structure showed continuous variation between male and females, indicating the absence of sexual dimorphism (Fig. 3). This

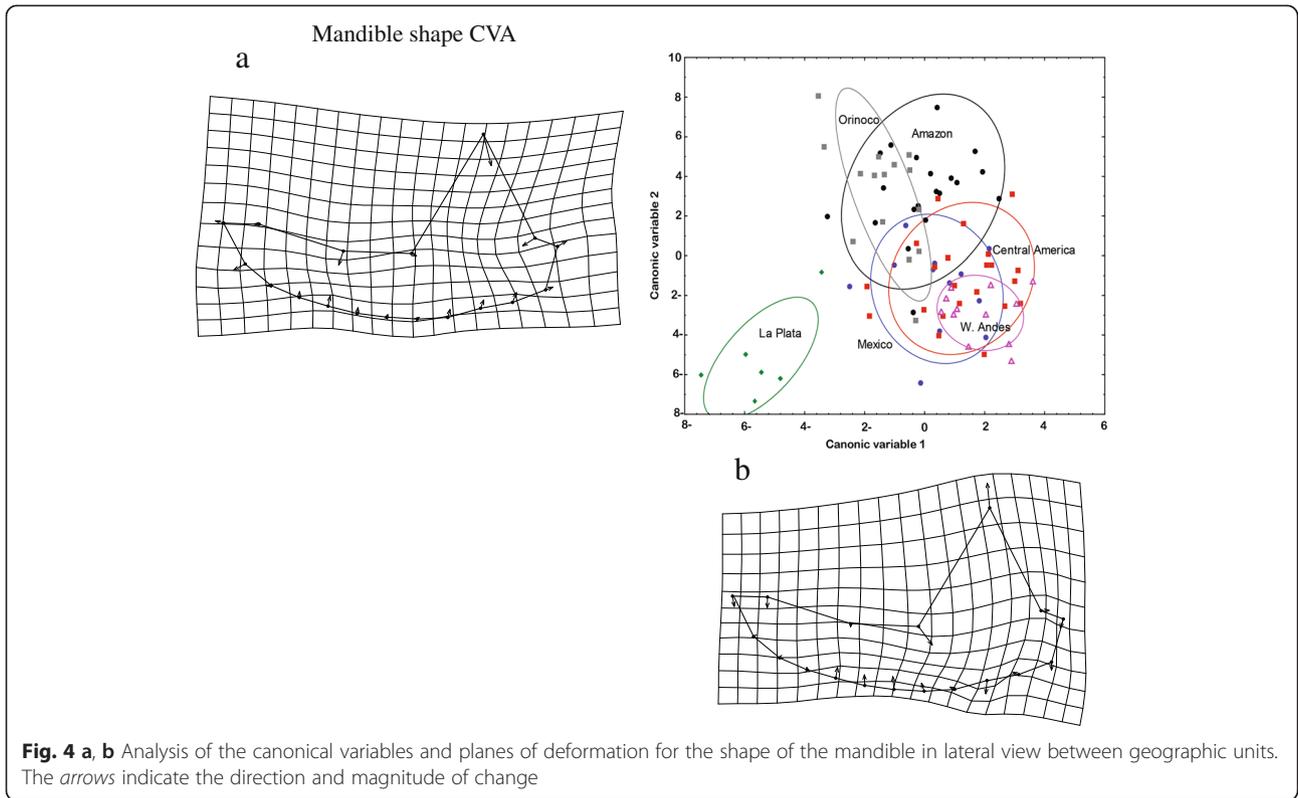
was confirmed by Goodall's  $F$  tests which showed that there were no significant differences in the shape of either structure between sexes (mandible: Goodall's  $F_{(32)} = 0.75$ ,  $p = 0.84224$ ; skull: Goodall's  $F_{(42)} = 1.62$ ,  $p = 0.1271$ ). Hence, we pooled female and male samples in subsequent analyses. The results of the ANOVA for centroid size indicated that males are significantly different from females in both skull size ( $F_{(1,54)} = 6.2030$ ,  $p = 0.0158$ ) and mandible size ( $F_{(1,68)} = 9.8040$ ,  $p = 0.0025$ ), with males larger (median CS mandible = 103.76, median CS skull = 169.76) than females (median CS mandible = 95.76, median CS skull = 161.53) for both views.

### Geographic variation in the shape of the mandible and the cranium

The MANOVA detected significant differences in skull (Wilks'  $\lambda = 0.014$ ,  $F = 1.362$ ,  $p < 0.05$ ) and mandible (Wilks'  $\lambda = 0.013$ ,  $F = 1.606$ ,  $p < 0.001$ ) shapes between GUs. The CVA plots depict the patterns of change between the GUs defined in this study for both structures (Fig. 4). For both views, the samples from La Plata differed the most from the samples from the other GUs. The shape of the cranium had the most marked differences between GUs. For the change in the deformation axes of the mandible between GUs, the VC1 values indicate a change in the height of the coronoid process, which includes the emergence of the first and second molars, and in the width of the set of teeth. For the second canonical correlation (VC2), a change in shape was found between landmarks 1 and 8, which corresponds to the length of the mandible.

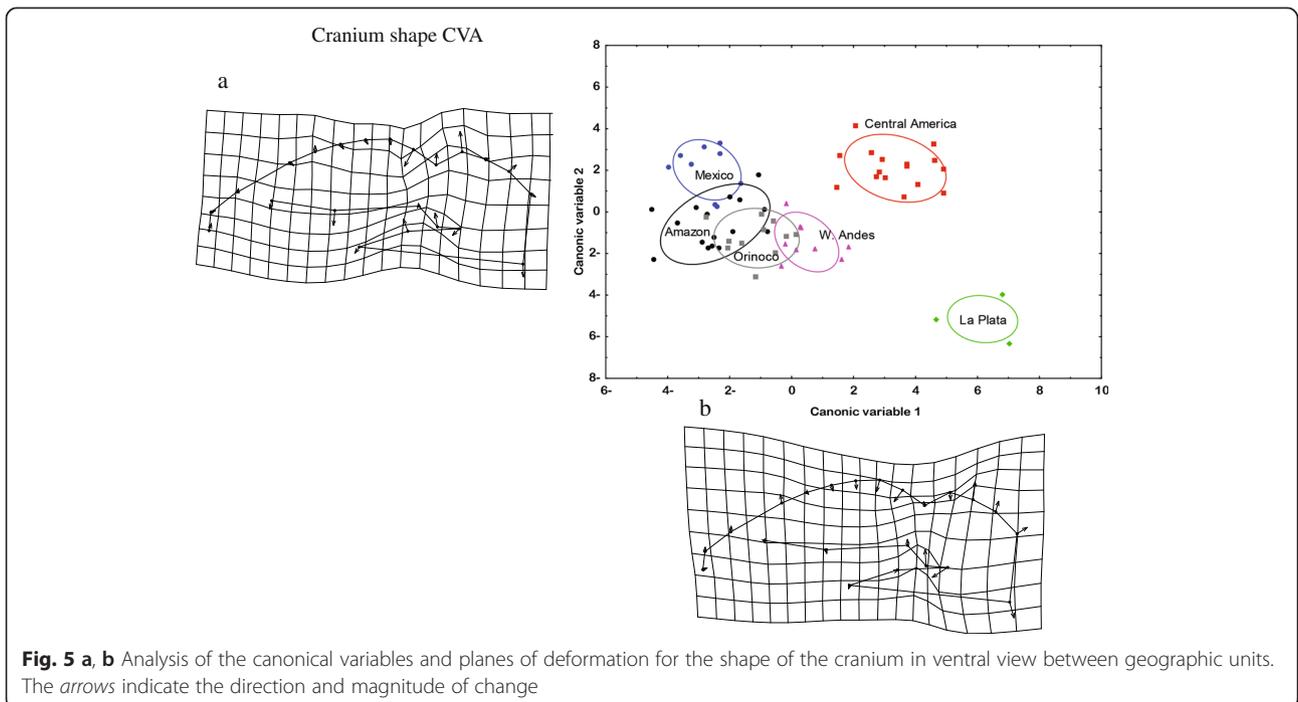
The deformation grid of the cranium between the GUs along VC1 (Fig. 5) shows a change in the shape of the posterior neurocranium (LMs 12–14) and the foramen





magnum (LM 15), in addition to a decrease in the width of the zygomatic arch. For VC2, there is an increase in the length of the mastoid bone (LM 11), along with an increase in the distance between the pterygoid muscles (LM 17) and a decrease in the distance between the

rows of molars and premolars (LMs 21 and 22). The DFA of the mandible demonstrated that the a priori grouping of the specimens in six GUs is appropriate, with the majority of mandible samples correctly assigned (89.6 %) (Fig. 6). The analysis of the mandible for



specimens from the six GUs using the Mahalanobis distances resulted in three groups. The first group is formed by the Amazon region, the Western Andes, Central America, and Mexico, Mexico being the region with the most differences within this grouping. The second group has the specimens from the Orinoco, and the third group is formed by the specimens from La Plata, the region with the lowest level of similarity with respect to the other GUs (Fig. 6).

The deformation grid of the cranium between the GUs along VC1 (Fig. 5) shows a change in the shape of the posterior neurocranium (LMs 12–14) and the foramen magnum (LM 15), in addition to a decrease in the width of the zygomatic arch. For VC2, there is an increase in the length of the mastoid bone (LM 11), along with an increase in the distance between the pterygoid muscles (LM 17) and a decrease in the distance between the rows of molars and premolars (LMs 21 and 22).

The DFA of the cranium samples indicated that 98.43 % were correctly assigned to the GUs by the discriminant analysis (Fig. 6). The analysis of the grouping of the six GUs using the Mahalanobis distances shows that the variation in shape of the mandible of *L. longicaudis* allowed the specimens to be classified into three groups. The first group includes specimens from the Amazon, Orinoco, and Mexico, Mexico being the unit with the most differences in shape within this larger grouping. The second most different group is that of the Western Andes and Central America. The third group has the samples from La Plata, the region with the lowest similarity with respect to the other GUs (Fig. 6).

#### Geographic variation in size

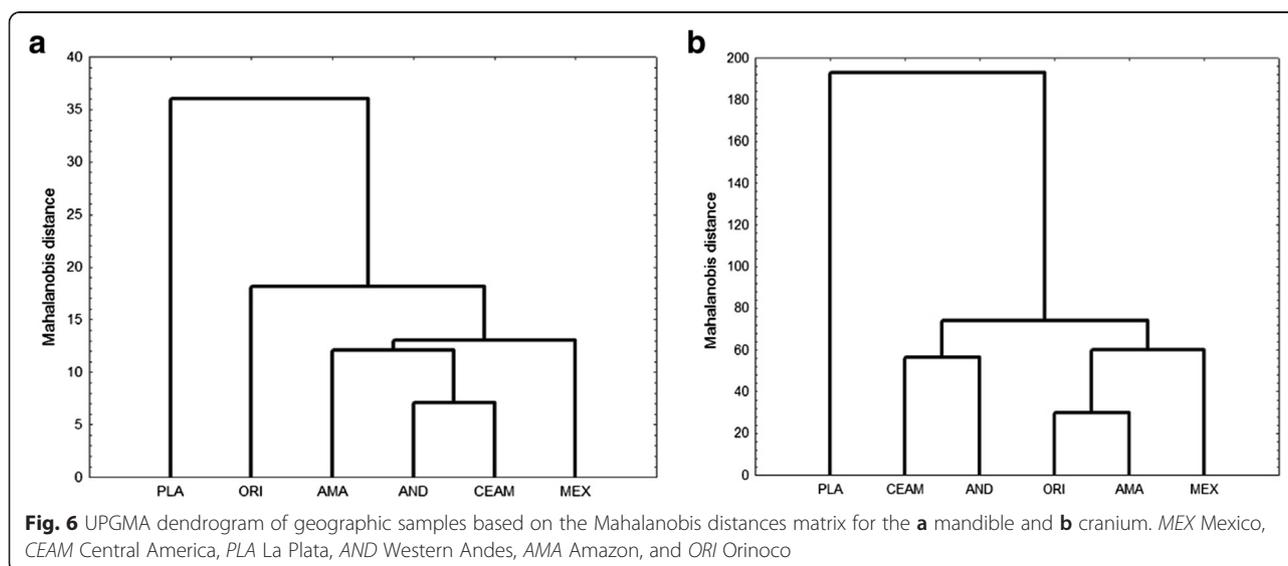
Differences were found ( $F_{(5,81)} = 3.9603$ ,  $p = 0.0029$ ) in the size of the mandible between the specimens from distinct

GUs. The multiple comparison test with a Bonferroni correction classified the GUs into three groups. The first group included Western Andes, the Amazon, and Central America (average centroid size 107.27); the second Orinoco and La Plata (average centroid size 112.28), and the third Mexico (average centroid size 122.59). There were marginally significant differences in size of the cranium ( $F_{(5,58)} = 2.3123$ ,  $p = 0.0553$ ) between the GUs.

## Discussion

### Sexual dimorphism in shape and size

This is the first study to use geometric morphometric techniques to describe the sexual and geographic variations in the cranium and mandible of *L. longicaudis* throughout its distribution. The results indicate that there is no sexual dimorphism in the shape of the mandible or the skull of the Neotropical otter, though both structures do differ in size. This difference corresponds to a pattern of sexual dimorphism common in the Mustelidae family where males are generally larger than females (Moors 1980). The Eurasian otter *L. lutra* (Lynch and O'Sullivan 1993; Lynch et al. 1996; Baryshnikov and Puzachenko 2012) and the river otter *Lontra canadensis* (Pertoldi et al. 2000; Kruuk 2006) also have this pattern. Given that the difference in the size of the cranium and the mandible of the otters is correlated with body size (Pertoldi et al. 1998), the difference in size between sexes of *L. longicaudis* could be associated with its characteristically solitary habit and territorial behavior. The males of *L. lutra* must defend their territory, and a larger body ensures a higher position in the hierarchy and potentially better territory (Pertoldi et al. 1997; Kruuk 2006). For *L. canadensis*, social behavior has been reported and there are even cases of overlapping territories between males (Blundell et al. 2002; Melquist et al. 2003), though the territories of the males are larger



**Fig. 6** UPGMA dendrogram of geographic samples based on the Mahalanobis distances matrix for the **a** mandible and **b** cranium. MEX Mexico, CEAM Central America, PLA La Plata, AND Western Andes, AMA Amazon, and ORI Orinoco

in size than those of the females possibly due to the larger energetic requirement of having a larger body (Sandell 1996; Thom et al. 2004). On the other hand, females do not engage in territorial disputes and are smaller, with lower energy requirements for daily maintenance. It is thought that the excess of energy is allocated to raising the litters (Moors 1980; Pertoldi et al. 1997; Gorman et al. 2006).

#### Geographic variation in the size and shape of the skull and mandible

The size of the mandible differs between GUs: animals from Mexico and La Plata have larger mandibles relative to those from the other GUs. These two GUs are the most northern (Mexico) and southern (La Plata) distributions of *L. longicaudis*. This variation is congruent with the size patterns reported for other species, like the American puma, *P. concolor*, which in addition to having a large distribution, like the Neotropical otter, also has a larger cranium size in temperate regions and with increasing latitudinal distance from the Equator (Iriarte et al. 1990; Gay and Best 1996). These patterns of change correspond to Bergmann's rule, which dictates that populations located furthest from the Equator have a relatively larger body. It is assumed that this pattern is the result of an adaptation that allows the animal to maintain its body temperature and confers greater endurance during aestivation (Blackburn and Hawkins 2004).

Cranium size does not follow the pattern observed for the mandible: the specimens from Mexico had a larger centroid size, while for the specimens from La Plata, it was smaller. In this study, only four samples were used from the La Plata region, so it is necessary to increase the sample size to determine whether this pattern would still be observed.

Our results indicated that significant variation in the shape of the cranium and the mandible of the Neotropical otter is associated with geography. In other species of Mustelidae such as weasels, European minks, and European badgers (Lee and Mill 2004), it is assumed that this type of variation responds to the variation in environmental conditions throughout the distribution of the species (Kruuk 2006). The morphology of a species responds to select factors in the environment, in addition to limitations in the response of the phenotype to such factors. This has led to the proposal that the ecological and geographic distribution of populations is a reflection of the ecological range of a phenotype (Ricklefs and Miles 1994). In contrast, it has been reported that geographic differences do not always lead to a homologous effect on the shape and size of an anatomical structure, in this case the cranium, due to the high levels of plasticity and adaptation in size that have been observed in other mammals (Cardini et al. 2007). Our data indicates

that for *L. longicaudis*, there are changes in shape as well as size between the GUs studied, suggesting the existence of different selective pressures to which the shape and size of the cranium and the mandible respond and different means of adaptation.

In general, variation in shape corresponded to an increase in distance between the GUs. For example, the shortest Mahalanobis distances were between the Orinoco and the Amazon for the cranium and between Central America and the Western Andes for the mandible. In both cases, the GUs are adjacent hydrological regions and the proximity of the regions could allow for regional migration. The similarity between neighboring hydrological regions is exemplified by the genetic distance between the giant otter populations of *Pteronura brasiliensis*, located in the Amazon Basin, the Orinoco, and Guyana, suggesting that these populations originated from an ancestral population that inhabited the central region of the Amazon. Several populations of giant otter subsequently diverged and were partially isolated, although similarity may have been maintained between them by gene flow (Pickles et al. 2011). Likewise, for those GUs that are geographically most distant, such as Mexico and La Plata, greater Mahalanobis distances were also found for both structures. To elaborate, it was expected that these two regions would have the greatest differences in shape for the two views: not only due to geographic distance but also because these GUs are located at the limits of the Neotropical otter's distribution. Organisms that live at the limits of their distribution generally exhibit the greatest morphological change, as they are generally subject to suboptimal conditions that may challenge their limits of tolerance (Gay and Best 1996).

Morphological variation is not always associated with geographic distance, as in some cases it may be explained by the presence of different evolutionary units that are delimited by geographic barriers that successively give origin to vicariance events (Patton and Smith 1994; De Queiroz and Good 1997; Smith et al. 1997). This pattern is observed in the GUs of the Western Andes, for which there was less variation in shape compared with Central America than with the adjacent regions of the Orinoco and the Amazon. The Andes mountain range is a potential geographic barrier that may give rise to vicariance events.

The hypothesis of vicariance events caused by the Andes supports the genetic distances reported by Trinca et al. (2012), who found large genetic distances between the phylogenetic groups of South America and those of the Western Andes, where genetic distances are greater between populations of *L. longicaudis* than with its sister species, *Lontra provocax*. This separation also corresponds to a subspecies division: *L. longicaudis annectens* is distributed throughout the Western Andes and *L. longicaudis enudris*

is distributed throughout the Eastern Andes (Larivière 1999). The effect of population separation by the Andes mountain range has also been reported for other vertebrate species including some South American fish (Hubert et al. 2007) and bats belonging to the genus *Artibeus* (Larsen et al. 2007), where the elevation of the Andes prevents gene flow and has resulted in different species on each side of the mountain range. The results of the present study offer morphological information that concurs with the molecular data from Trinca et al. (2012) on the separation of populations of the Eastern and Western Andes. This indicates that sufficient time has passed for both the morphological and the genetic divergence of the populations of *L. longicaudis* on both sides of the mountain range.

Another important pattern that reinforces the possibility of divergence events for the Neotropical otter was the notable differentiation in the shape of the mandible and cranium of the animals from La Plata with respect to those from the other five GUs. This reinforces the possibility of divergence events for the Neotropical otter. This dissimilarity for La Plata corresponds to the geographic group labeled Eastern South America and reported by Trinca et al. (2012), which was formed by a clade that does not share a single haplotype with the other clades that were reported on by the authors. This pattern of differentiation corresponds to the genetic variation between the populations of Pantanal, which belongs to the hydrological region of La Plata, and the other populations of the giant otter *P. brasiliensis* (Pickles et al. 2011).

In addition, this pattern of differentiation in shape may be influenced by the two biogeographically distinct regions in the La Plata Basin—tropical in the north and subtropical-temperate in the south—where climatic zoning has promoted changes in distribution and diversification patterns in both aquatic and terrestrial (Arzamendia and Giraudo 2009) species, and has possibly had the same effect on the Neotropical otter. Added to this effect is the presence of prairies that may be functioning as a barrier between the Amazon and La Plata during the dry season when bodies of water become scarce, preventing the Neotropical otter from migrating from one hydrological region to another.

Finally, it is important to address the pattern found for the Mahalanobis distance values and for the UPGMA, and the variation in the shape of the mandible and the cranium for specimens found in Mexico. These specimens showed no association with any of the other GUs, thereby indicating that the otters of this region could be under selective forces that are allowing for this differentiation. A similar pattern occurred in the morphology of the cranium of *Panthera onca* (Larson 1997), confirmed by molecular analysis (Eizirik et al. 2001), for which there is also a pattern of geographic division for

populations in Mexico and Central America in comparison to the populations of South America. This pattern has also been found for the ocelot, *Leopardus pardalis*, and the margay, *Leopardus wiedii*, using molecular data (Eizirik et al. 1998), species which both have distributions similar to that of the Neotropical otter.

This supports the idea that the otters in Mexico and Central America have been separated from the more southern populations and are subject to a distinct set of selective pressures, resulting in important morphological variations that are reflected in the shape and size of the cranium and the mandible. On the other hand, since the Pacific and Gulf of Mexico slopes where *L. longicaudis* is distributed are separated by the Central Mexican Plateau, it is possible that the shape and size of the cranium also vary between the two coastlines. We recommend that a morphometric study be undertaken to compare populations from the two regions separated by the Trans-Mexican Volcanic Belt that bifurcates and creates the Pacific and the Atlantic ridge. This would allow for a better description of any vicariance event that has happened between these hydrological regions of Mexico.

The hypothesis that *L. longicaudis* is a species complex is supported by the geographic distances, the presence of geographic barriers, and the morphological adaptations to distinct environmental conditions that exist throughout its distribution, as well as by the congruence between the morphological differences we report here and the genetic information reported in Trinca et al. (2012). This hypothesis should be further tested using an integrative approach that includes phylogeographic information and geometric morphometrics with an appropriate number of sequences and representative sampling for the entire distribution of the Neotropical otter, especially in Mexico and the La Plata region. This would elucidate the current status of *L. longicaudis* as a possible group of species, as proposed by other authors based on the form of the rhinarium (Pohle 1920; Cabrera 1957; Harris 1968). The results of such a study would have important consequences for the knowledge and conservation of this species given that *L. longicaudis* is on the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) and is currently categorized as data deficient, due to uncertainty about the rate decline of its populations and debate over its taxonomic status (IUCN 2015).

## Conclusions

The present results add new information about morphometric skull variation in *L. longicaudis*. There is significant variation in the size of the cranium between male and female Neotropical otters, with the males' crania the biggest. In contrast, the variation in shape is not significant between sexes.

The cranial and mandible shape analysis indicated the morphometric distance was greatest between the La Plata and the other GUs, whereas the smallest distance was found between the Western Andes and Central America for the cranium and Amazonia and Orinoco for the mandible.

For size variation, both views indicated that specimens from Mexico are the biggest relative to the other GUs, in concurrence with Bergmann's rule. The presence of geographic barriers and the large distances between the GUs could have given rise to the morphological variation observed in the Neotropical otter. The results of this study and previous research on genetic variability suggest that *L. longicaudis* could actually be a group of species and therefore requires an integrative study to clarify its taxonomy.

## Additional file

**Additional file 1: Additional material: origin of specimens.** Examined specimens of *Lontra longicaudis* for the mandible. National Mammal Collection of the Biological Institute of the Autonomous National University of Mexico (NMC-UNAM), Vertebrate Collection of the Autonomous University of Campeche (VC-AUC), Mammal Collection of the Biological Research Station of Los Tuxtlas, Autonomous National University of Mexico (MCT-UNAM), American Natural History Museum (ANHM), Carnegie Museum of Natural History (CM), Field Museum of Chicago (FIELD), National Museum of Natural History-Smithsonian (SM), Museum of Michigan State University (MSU), Mammal Collection of Kansas University (KU), and private collections (PC).

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

PCHR conceived this study and acquired the data. PCHR and JAG coordinated this study and performed data analysis. PCHR, JAG, and CV interpreted the data and drafted and revised the manuscript. All authors read and approved the final manuscript.

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