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Good Vibrations: The Evolution of Whisking in Small Mammals

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ABSTRACT

While most mammals have whiskers, some tactile specialists—mainly small, nocturnal, and arboreal species—can actively move their whiskers in a symmetrical, cyclic movement called whisking. Whisking enables mammals to rapidly, tactually scan their environment to efficiently guide locomotion and foraging in complex habitats. The muscle architecture that enables whisking is preserved from marsupials to primates, prompting researchers to suggest that a common ancestor might have had moveable whiskers. Studying the evolution of whisker touch sensing is difficult, and we suggest that measuring an aspect of skull morphology that correlates with whisking would enable comparisons between extinct and extant mammals. We find that whisking mammals have larger infraorbital foramen (IOF) areas, which indicates larger infraorbital nerves and an increase in sensory acuity. While this relationship is quite variable and IOF area cannot be used to solely predict the presence of whisking, whisking mammals all have large IOF areas. Generally, this pattern holds true regardless of an animal's substrate preferences or activity patterns. Data from fossil mammals and ancestral character state reconstruction and tracing techniques for extant mammals suggest that whisking is not the ancestral state for therian mammals. Instead, whisking appears to have evolved independently as many as seven times across the clades Marsupialia, Afrosoricida, Eulipotyphla, and Rodentia, with Xenarthra the only placental superordinal clade lacking whisking species. However, the term whisking only captures symmetrical and rhythmic movements of the whiskers, rather than all possible whisker movements, and early mammals may still have had moveable whiskers. Anat Rec, 303:89–99, 2020. © 2018 American Association for Anatomy

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INTRODUCTION

Most mammals have facial whiskers or vibrissae (from the Latin "*vibrio*" to vibrate) (Vincent, 1912; Ahl, 1986), which are thick, tactile hairs that sit within a highly innervated follicle. Facial whiskers are arranged in a grid-like pattern made up of rows and columns. Small, social, nocturnal, arboreal mammals are thought to have more whiskers that are longer and more regularly arranged (Fig. 1B, Figure 2C, see symbol δ) than those of diurnal or terrestrial mammals (Fig. 1C, Figure 2C, see symbols ε and ζ) (Pocock, 1914; Lyne, 1959; Ahl, 1986; Muchlinski et al., 2013). Many small mammals are termed *whisker specialists* and move their whiskers backward and forward in a symmetric and cyclic movement called *whisking* (Vincent, 1912; Wineski, 1985; Prescott et al., 2011). Whisking is thought to guide behaviors, such as locomotion and foraging, in animals that survive in dark, complex habitats (Grant and Arkley, 2016; Grant et al., 2018). It is a major innovation in tactile specialists, enabling rapid sampling of their environments during spatial exploration (Knutsen, 2015), which boosts the quality and quantity of sensory information. Indeed, whisking can occur at speeds of 25 Hz and is one of the fastest movements that mammals can make (Grant and Arkley, 2016).

Specialist sets of intrinsic and extrinsic muscles drive whisker movements (Dörfl, 1982; Haidarliu et al., 2010; Grant et al., 2013a). Some interspecific variations exist within the muscle architecture with arboreal, nocturnal animals having much more pronounced and regularly arranged intrinsic muscles than diurnal and terrestrial mammals (Fig. 2C) (Muchlinski et al., 2013; Grant et al., 2017). Diurnal mammals, such as some primates, horses and deer lack organized whiskers, have very thin whiskers and a reduced whisker follicle without intrinsic muscles (Fig. 2C) (Muchlinski et al., 2013). The intrinsic muscle architecture has been preserved from marsupials (Grant et al., 2013a) to rodents (Haidarliu et al., 2010) to nocturnal primates (Muchlinski et al., 2013), even though their last common ancestor has been dated to be at least from the Late Jurassic with the fossil eutherian *Juramaia* (Luo et al., 2011), or even the Early Jurassic according to phylogenomic scale molecular clock analyses (dos Reis et al., 2014). This has prompted some researchers to suggest that the first nocturnal, arboreal mammals might have had moveable whiskers (Mitchinson et al., 2011; Grant et al., 2013a).

It is challenging to explore the evolution of whiskers and whisking. Whiskers are very rarely preserved in fossils, and their associated musculature preservation is even less likely, so a bony structure correlated with whisker processing and movements would make a good surrogate measure. One candidate structure is the infraorbital foramen (IOF) (Fig. 1A,D). The IOF is a small hole in the skull through which the infraorbital nerve passes (Muchlinski, 2008). Sensory information from the whiskers is transmitted *via* the infraorbital nerve to the brain (Muchlinski, 2010a). The size of the IOF is extremely well-correlated to the size of the infraorbital nerve, and is a good measure of maxillary somatosensory acuity (Muchlinski, 2008). Indeed, IOF area has been associated with whisker sensing in 25 mammalian orders, and is correlated to whisker counts; however, IOF area alone is not enough to reliably predict whisker counts (Muchlinski, 2010b). Nocturnal, arboreal mammals have more

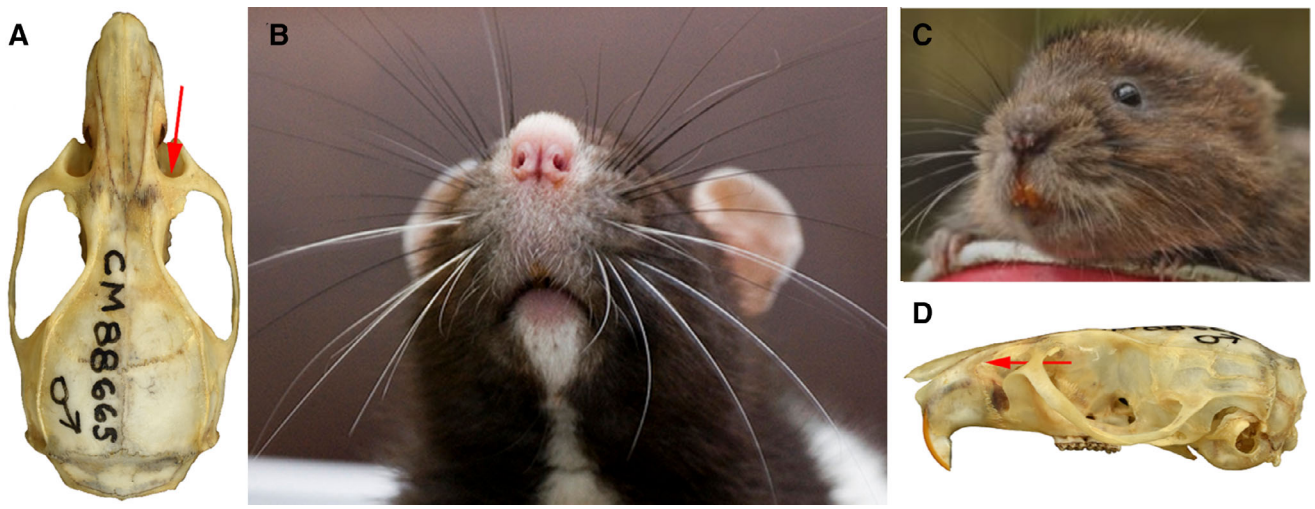

Fig. 1. Rodent whiskers and skull example. a and d show a rodent skull from *Rattus rattus* with the IOF indicated by a red arrow in the dorsal (**A**) and lateral (**D**) view (Carnegie Museum of Natural History specimen CM 88665). **B**) shows whiskers of whisking, nocturnal, arboreal *Rattus rattus* (Picture courtesy of B. Mitchinson) and **C**) whisking, nonaboreal, diurnal, and semiaquatic *Arvicola amphibius* (Picture courtesy of the Wildwood Trust).

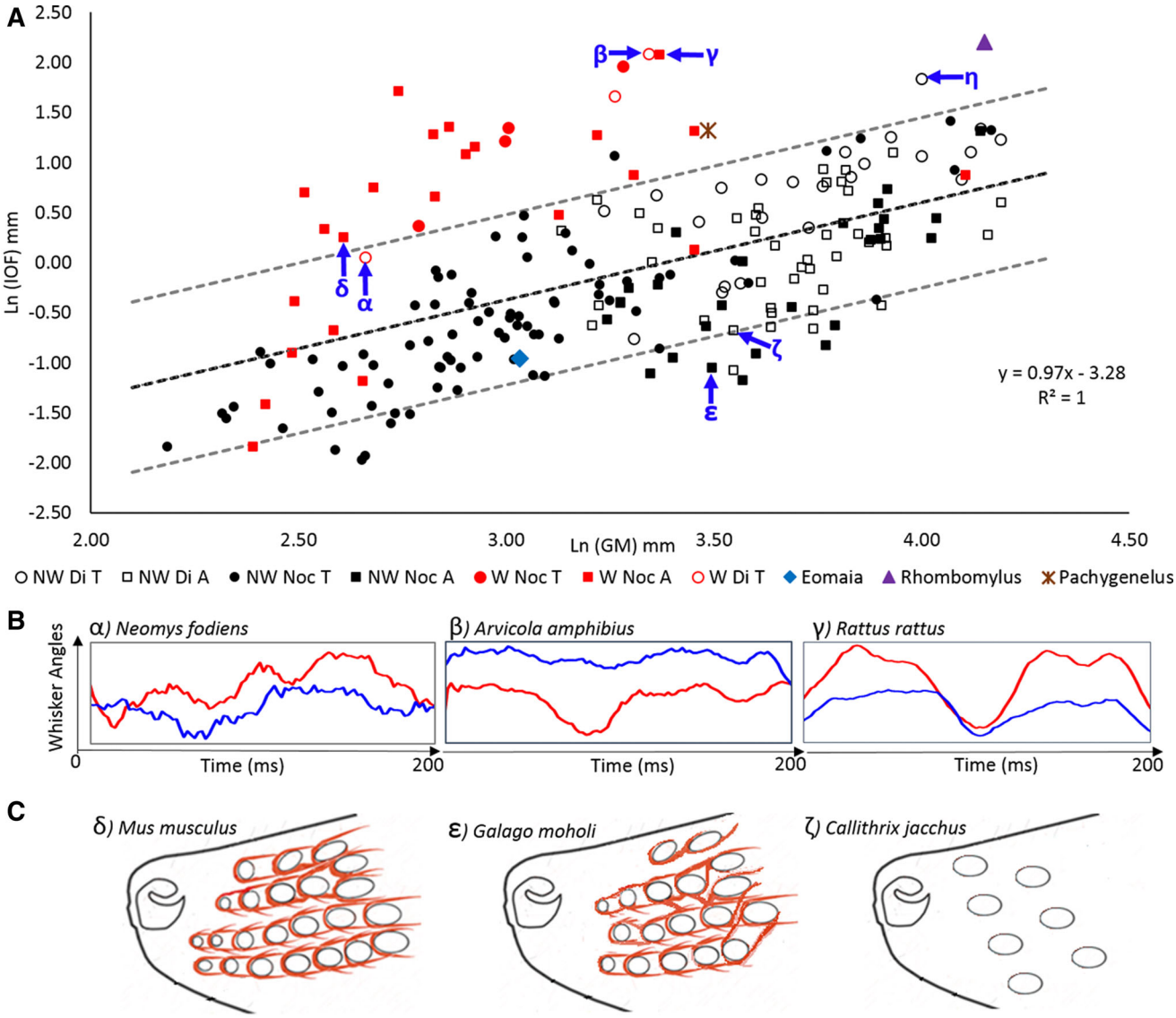

Fig. 2. Data summary figure. **A**) ln IOF (Infraorbital foramen area) and ln GM (Geometric Mean) scattergraph for whisking (in red) and nonwhisking (in black) extant mammals. Circles indicate terrestrial mammals and squares indicate arboreal mammals. Filled markers indicate nocturnal mammals and empty markers indicate diurnal and cathemeral mammals. The black line represents the findings from a GLS regression and the dotted black lines the 95% confidence intervals of the regression line (GLS: ln IOF area = −3.28 + 0.97 lnGM, $r^2 = 0.29$, $P < 0.0001$). *Eomaia scansoria* indicated with a blue diamond marker, *Rhombomylus turpanensis* with a purple triangle and *Pachygenelus monus* with a green asterisk. **B**) Whisker traces from three extant mammals (indicated as α, β, γ in A) over 200 ms for the left (blue) and right (red) whiskers. Nocturnal, arboreal *Rattus rattus* (γ) has large amplitude, regular whisking, compared to both diurnal, terrestrial *Neomys fodiens* (α) and *Arvicola amphibius* (β), who have more irregular, and smaller amplitude whisking movements. **C**) Example whisker muscle diagrams for three extant mammals (indicated as δ, ε, ζ in A), showing general examples for a nocturnal arboreal mammal (based on Haidarliu et al., 2010) (δ) a diurnal, terrestrial rodent, (ε) a noctural, arboreal primate (based on Grant et al. 2017; Muchlinski et al. 2010a), and (ζ) a diurnal, arboreal primate (η) is a nonwhisking lagomorph, *Lepus americanus*.

whiskers, and therefore, are likely to have larger IOF areas (Muchlinski et al., 2013; Grant et al., 2017); they are also thought to be more likely to whisk as they use their whiskers to guide navigation around their complex habitats at night (Grant and Arkley, 2016; Arkley et al., 2017; Grant et al., 2017; Grant et al., 2018). In addition, that some animals are actively using their touch sensing system by whisking (Grant et al., 2009; Grant et al., 2014) implies that their whiskers are more of a primary sense, and therefore may also have higher sensory acuity.

Extant mammals typically have a single IOF per side, although anomalies occur (*Procyon lotor*, Carnegie Museum of Natural History specimen CM 34215 has two per side). In contrast, many non-therian cynodonts typically have more than one (Kemp, 1982; Krause et al., 2014). A recent study analyzed the evolution of the IOF in nonmammaliaform cynodonts using CT-data (Benoit et al., 2016). They reported that a change in the pattern of the infraorbital nerve occurred in Early Jurassic trity-lodontids and tritheledontids, the late surviving

nonmammalian cynodont groups that are more closely related to mammals (Matsuoka et al., 2016; Rodrigues et al., 2018). Although multiple foramina were present, only one was likely to transmit the infraorbital nerve, as the other openings had an independent origin in the orbit. The taxa in question, the tritylodontid *Tritylodon longaevus* and the tritheledontid *Pachygenelus monus*, were said to have an IOF similar to mammals that also transmitted the infraorbital nerve (see Fig. 1F in Benoit et al., 2016). The large IOF areas of these species (7.03 and 3.75 mm^2, respectively), are equivalent to rodent IOF values, indicating that they may well have had facial tactile hairs (Benoit et al., 2016). This would represent the earliest occurrence of whiskers, but it is not known whether these whiskers may have been moveable or not. Whether the findings of Benoit et al. (2016) are applicable to Mesozoic mammals with multiple foramina in the maxilla is yet to be tested.

This article aims to investigate whether whisking mammals have larger relative IOF areas, indicating a higher sensory acuity. We focus here on small mammals, as they are much more likely to whisk. We go on to explore the occurrence of whisking with being nocturnal and arboreal. We then use ancestral character state reconstruction and tracing techniques to discuss the evolution of whisking and compare IOF areas of fossils to those of extant mammals. If whisking is an advantage for gathering sensory information, this will be reflected in a larger infraorbital nerve and larger IOF area; therefore, we predict that IOF area will be larger in small mammals that whisk.

MATERIALS AND METHODS

Samples

Two hundred and nine species were included in this study: 174 extant nonwhisking mammals, 31 extant whisking mammals, and 4 fossils: *Eomaia scansoria* (Ji et al., 2002), *Rhombomylus turpanensis* (Meng et al., 2003), *Pachygenelus monus* (Benoit et al., 2016), and *Tritylodon longaevus* (Benoit et al., 2016). Extant mammal samples were cleaned skulls that were obtained from museums around the United States and the UK. Hystricomorphs and myomorphs were not included in the IOF size study as they are characterized by having an enlarged IOF because a muscle (M. masseter medialis, pars anterior) runs through the foramen (Wood, 1972; Hautier et al., 2015); however, hystricomorphous members of Ctenohystrica were included in the phylogeny in Figure 4 to assist in resolving the ancestral state for all rodents. The Late Jurassic fossil eutherian *Juramaia* type specimen cast and photos (Luo et al., 2011) did not have a fully exposed IOF so could not be used in this study. As whisking mammals tend to be small (Muchlinski et al., 2013), only small species were included in the study, defined as having a geometric mean (GM) of cranial shape <67 mm (see section on Measurements for more information).

Whisking, Activity, and Substrate Preferences

Each species was allocated a score for whisking or nonwhisking. A score was considered *certain* if it was obtained from the literature (Woolsey et al., 1975; Haidaliu and Ahissar, 1997; Mitchinson et al., 2011; Grant et al., 2017; Arkley et al., 2017; Grant et al., 2018), collected high-speed video footage or direct author observations. A score was considered *less certain* if the authors had directly observed animals that were much related, or evolutionary and morphologically similar. For example, no chiropterans or primates have ever been found to whisk and many species would have been given a score of *less certain* as the authors had not directly observed all of the species that we used. Any species that were we were *not certain* of consisted of animals belonging to a group that the authors had not observed directly and were therefore removed from the sample and any further analysis. Our values of certainty can be seen in Supporting Information 1. Some high-speed video footage of whisking mammals was collected and tracked using the BIOTACT Whisker Tracking Toolbox (Perkon et al., 2011) for the whisker traces in Figure 2B (as per methods in Arkley et al., 2017).

Species were also scored as to whether they are diurnal, cathemeral, or nocturnal (termed *activity pattern*), and arboreal or terrestrial (termed *substrate preference*). These were obtained from author observations, personal communications from animal keepers and the literature.

Measurements

The majority of IOFs of extant mammals were measured first by creating a mold of the IOF outlet using a flexible and injectable molding material (Coltène President Plus, Regular Body Molding Material). These molds were sectioned and photographed with a scale under a stereomicroscope. From these images, IOF area (in mm^2) was calculated using Scion Image® software (for details see Muchlinski, 2010b). Some of the samples were not able to be used for molds, especially if they were very small and delicate (i.e., *Pipistrellus pipistrellus, Sorex minutus*). Therefore, the skull was placed under a size-calibrated, light microscope (Lumar V12 Microscope and AxioCam MRc) and the IOF area was measured manually, by drawing around the hole using Axio-Vision Software (version 4.8.2), which automatically calculated the area. This was done three times and a mean was taken, to reduce any human error component. To evaluate whether these two measurement techniques would yield adequately similar results, the IOFs of 15 specimens were measured using the two methods described above and compared. A Spearman's rank correlation shows that that the two measurement methods correlated significantly ($n = 15$, $rho = 0.99$, $P < 0.0001$). Therefore, results from both techniques could be combined.

The IOF was also examined in the Early Cretaceous fossil eutherian *Eomaia scansoria* type specimen, from casts and photographs (Ji et al., 2002). It was visible in both the main part and counterpart of the specimen, above the upper third premolar. The counterpart had a maximum IOF diameter of 0.67 mm. The main part had a maximum IOF diameter of around 0.6 mm, but there was slight damage and distortion to this side; therefore, only the counterpart measurement was used. The IOF area for this specimen was calculated using the diameter and assuming a circular IOF. As most IOF shapes are oval in nature (Fig. 1A,D), this would give the *maximal IOF area* of 0.38 mm^2. The IOF was also examined in the early Eocene fossil *Rhombomylus turpanensis*, a relative of lagomorphs (Asher et al., 2005; O'Leary et al., 2013),

using an IOF area from the literature of 9.12 mm^2 (Muchlinski and Kirk, 2017, specimen V5278), calculated from light microscopy measurements. The IOF areas of the Early Jurassic fossil nonmammalian therapsids *Pachygenelus monus* and *Tritylodon longaevus* were also taken from the literature at 3.75 mm^2 and 7.03mm^2, respectively (Benoit et al., 2016, specimens BPI/1/5691 and BPI/1/5289).

IOF area is positively correlated with body mass (Muchlinski, 2010a, 2010b). Accordingly, IOF area measurements must be size-adjusted to compare IOF area across a wide range of body sizes. The GM of cranial shape was chosen for IOF area size standardization. The GM was derived by measuring maximum cranial length (mm) (the linear distance between prosthion and opisthocranion) and bizygomatic width (mm) (the linear distance between the most lateral points of the zygomatic arches) in all the skull and fossil specimens. Bizygomatic width and cranial length were multiplied by one another, and the square root of that value was taken to give the GM (in mm). The GM of cranial shape was found to significantly correlate with body mass in mammals (Muchlinski, 2010a, 2010b), it also scales with slight negative allometry, but the confidence intervals do include isometry (Muchlinski, 2010b).

The GM of *Eomaia scanosia* was calculated from the skull length measurement from the type specimen; however, as the specimen is flattened the width could not be measured. A measure of width was approximated from observing the shape of the skull in Figure 1 of Ji et al. (2002), and comparing to that of extant insectivorans and rodents, to give an approximated ratio of length: width of around 1.96:1, yielding a GM of 20.73 mm. The values for the *Rhombomylus turpanensis* specimen V5278 also only contained a length measurement (Meng et al., 2003), as the width was not measured from the zygomatic arches. Therefore, a ratio of length:width was approximated from the specimen from Figure 27F in Meng et al. (2003) as 1.75:1, yielding a GM of 63.5 mm. The GM of *Pachygenelus monus* was extracted from the CT data provided in the literature (Benoit et al., 2016), and given at 32.69 mm. The GM for *Tritylodon longaevus* was measured from photographs of specimen BPI/1/5289; skull length of 153.38 mm and width of 101.76 mm give a GM of 124.94 mm.

Analytical Methods

Both conventional and phylogenetic statistical methods were used in this study. All data analyses were performed using species mean data from 204 extant mammal species. Conventional statistics were run using JMP® version 13 and phylogenetically adjusted statistics were run using the R packages *ape* (Paradis et al., 2004), *caper* (Arnold et al., 2010; Orme, 2013; Team, 2014), *geiger* (Harmon et al., 2007), *nlme* (Pinheiro et al., 2009), and *picante* (Kembel et al., 2010). The phylogenetic branching sequence used in these analyses follow Bininda-Emonds et al. (2007) (Supporting Information 2). All graphs were created in JMP®.

To compare relative IOF sizes between whisking and nonwhisking mammals, residual IOF area was calculated for each species using either a GLS or a PGLS regression of ln IOF area versus ln(geometric mean). The regression line was fitted to species mean data for all extant mammals. Residual IOF areas of the various taxa were compared using both conventional Student's t-tests and phylogenetic Student's t-tests (adjusted for multiple pairwise comparisons; phylotools: see Revell, 2012) (Table 1). Because extant rodents have significantly larger residual IOF areas than all the other mammalian groups in our comparative sample (Muchlinski and Kirk, 2017), a separate t-test was run to compare whisking and nonwhisking rodents.

To examine the ancestral state of whisking in therian mammals, we implemented Mesquite's *Trace Character History* function to graphically represent character state evolution on the tree. Mesquite ancestral state modeling uses the "Mk1 (est.)"; specifically, the one-parameter Markov k-state model; a generalization of the Jukes-Cantor model. We used a pruned phylogeny and stored characters in a likelihood reconstruction. See Supporting Information 2 for more details on phylogeny and likelihood estimates.

RESULTS

Whisking Mammals Have Larger IOFs, Regardless of Activity Pattern or Substrate Preference

Whisking mammals have larger IOF areas than nonwhisking mammals (Figs. 2A and 3A). Although there is overlap between the clusters of points representing nonwhisking (black symbols) and whisking species (red symbols) (Fig. 2A), these two behavioral groups appear to be distinct when considering their relative IOFs values. The significant differences in relative IOF area between whisking and nonwhisking mammals is confirmed by t-test (conventional statistics: $t = 7.22$, $P < 0.001$; phylogenetic test statistics: $t = 8.51$, $P < 0.001$) and is also illustrated in Figure 3A. The findings from a GLS regression indicate that ln GM can be used to account for 29% of the variance in ln IOF area (GLS: ln IOF area = −3.28 + 0.97 lnGM, $r^2 = 0.29$, $P < 0.001$). The lambda value from a PGLS regression (PGLS: ln IOF area = −4.53 + 1.39 lnGM, $r^2 = 0.44$, $\lambda = 0.91$) indicates that phylogeny needs to be considered when evaluating the findings. Results with a PGLS regression line can be seen in Supporting Information 3.

In general, whisking animals have significantly larger relative mean IOFs than nonwhisking animals, regardless of activity pattern or substrate preference (see Fig. 3B, Table 1). The only species that was not statistically significantly different in mean IOF area from both nonwhisking and whisking animals was the whisking, terrestrial, cathemeral, semiaquatic shrew (Eulipotyphla), *Neomys fodiens (*Fig. 3B; Table 1). The nonwhisking, terrestrial, cathemeral, semiaquatic rodent, *Ondatra zibethicus*, had a relative IOF area that was significantly larger than the means of all other nonwhisking mammals, but did not differ significantly from the mean values of whisking mammals (Fig. 3B).

The majority of whisking mammals in our sample belong to the order Rodentia, and rodents are characterized by having relatively larger IOFs than most other mammals (Muchlinski and Kirk, 2017). To better understand the observed pattern between whisking behavioral differences and IOF area, we ran a t-test between whisking and nonwhisking rodents and can confirm that

TABLE 1. Student's *t*-test comparing relative infraorbital foramen (IOF) area between behavioral groups

	NW. N. T.	NW. N. A.	NW. D. T.	NW. D. A.	NW. C. T.	NW. C. A.	W. N. T.	W. N. A.	W. D. T.	W. C. T.
NW. N. T.		t = 1.40; SE = 0.13 *P* = 0.17	t = −4.75; SE = 0.13 *P* < 0.0001	t = −0.29; SE = 0.11 *P* = 0.76	t = −3.73; SE = 0.55 *P* = 0.0003	t = 1.13; SE = 0.55 *P* = 0.20	t = 5.66; SE = 0.32 *P* = 0.002	t = 3.23; SE = 0.15 *P* = 0.002	t = 5.64; SE = 0.40 *P* < 0.0001	t = 4.23; SE = 0.67 *P* < 0.0001
NW. N. A.	t = 3.77; SE = 0.12 *P* = 0.0002		t = −5.05; SE = 0.67 *P* < 0.0001	t = −1.50; SE = 0.14 *P* = 0.13	t = −3.99; SE = 0.55 *P* = 0.42	t = 0.79; SE = 0.56 *P* = 0.42	t = 5.99; SE = 0.33 *P* < 0.0001	t = 8.41; SE = 0.17 *P* < 0.0001	t = 5.95; SE = 0.39 *P* < 0.0001	t = 1.82; SE = 0.55 *P* = 0.07
NW. D. T.	t = −2.47; SE = 0.12 *P* = 0.99	t = −5.05; SE = 0.15 *P* < 0.0001		t = 4.13; SE = 0.14 *P* < 0.0001	t = −2.54; SE = 0.56 *P* = 0.99	t = −4.41; SE = 0.53 *P* < 0.0001	t = 3.57; SE = 0.34 *P* = 0.0004	t = 8.18; SE = 0.14 *P* < 0.0001	t = 3.92; SE = 0.40 *P* < 0.0001	t = 0.66; SE = 0.56 *P* = 0.51
NW. D. A.	t = 2.24; SE = 0.10 *P* = 0.0002	t = −1.71; SE = 0.10 *P* = 0.09	t = 3.91; SE = 0.14 *P* = 0.001		t = −3.65; SE = 0.53 *P* = 0.0003	t = −0.51; SE = 1.56 *P* = 0.24	t = 5.48; SE = 0.53 *P* < 0.0001	t = 3.57; SE = 0.71 *P* = 0.002	t = 5.51; SE = 0.39 *P* < 0.0001	t = 1.76; SE = 0.76 *P* = 0.17
NW. C. T.	t = −3.99; SE = 0.53 *P* = 0.01	t = −4.81; SE = 0.54 *P* < 0.0001	t = −3.35; SE = 0.54 *P* = 0.001	t = −4.41; SE = 0.50 *P* < 0.001		t = 3.46; SE = 0.76 *P* = 0.0007	t = −3.63; SE = 0.63 *P* = 0.72	t = 7.57; SE = 0.17 *P* < 0.0001	t = 0.24; SE = 0.67 *P* = 0.81	t = −1.36; SE = 0.39 *P* < 0.0001
NW. C. A.	t = 1.88; SE = 0.53 *P* = 0.09	t = 1.00; SE = 0.54 *P* = 0.32	t = 2.44; SE = 0.54 *P* = 0.016	t = 1.44; SE = 0.53 *P* = 0.15	t = 4.19; SE = 0.75 *P* < 0.0001	t = 4.62; SE = 0.61 *P* = 0.0001	t = −3.87; SE = 0.63 *P* = 0.61	t = −1.55; SE = 0.55 *P* = 0.94	t = 4.23; SE = 0.67 *P* = 0.01	t = 2.09; SE = 0.77 *P* = 0.04
W. N. T.	t = 5.84; SE = 0.31 *P* = 0.0001	t = 7.06; SE = 0.30 *P* < 0.0001	t = 4.61; SE = 0.32 *P* = 0.016	t = 6.48; SE = 0.32 *P* < 0.0001	t = −0.51; SE = 0.61 *P* = 0.51	t = 1.12; SE = 0.61 *P* < 0.0001		t = 1.88; SE = 0.34 *P* = 0.06	t = −0.78; SE = 0.50 *P* = 0.44	t = 1.30; SE = 0.63 *P* = 0.19
W. N. A.	t = 9.04; SE = 0.14 *P* < 0.0001	t = 10.53; SE = 0.16 *P* < 0.0001	t = 5.52; SE = 0.15 *P* = 0.001	t = 9.95; SE = 0.15 *P* < 0.0001	t = −1.65; SE = 0.54 *P* = 0.10	t = 4.12; SE = 0.64 *P* < 0.0001	t = −1.78; SE = 0.33 *P* = 0.07		t = −2.53; SE = 0.40 *P* = 0.99	t = 0.33; SE = 0.56 *P* = 0.75
W. D. T.	t = 5.51; SE = 0.38 *P* < 0.0001	t = 6.57; SE = 0.39 *P* < 0.0001	t = 4.54; SE = 0.39 *P* < 0.0001	t = 6.06; SE = 0.38 *P* < 0.0001	t = −0.06; SE = 0.65 *P* = 0.95	t = 3.46; SE = 0.76 *P* = 0.0007	t = 1.56; SE = 0.48 *P* = 0.57	t = −2.18; SE = 0.39 *P* = 0.98		t = 1.81; SE = 0.67 *P* = 0.07
W. C. T.	t = 2.16; SE = 0.53 *P* = 0.03	t = 2.99; SE = 0.54 *P* = 0.003	t = 1.54; SE = 0.54 *P* = 0.003	t = 2.58; SE = 0.53 *P* = 0.01	t = −1.31; SE = 0.75 *P* = 0.19	t = 2.58; SE = 0.75 *P* = 0.005	t = −1.09; SE = 0.61 *P* = 0.27	t = 0.15; SE = 0.54 *P* = 0.88	t = 1.45; SE = 0.65 *P* = 0.15	

The top triangle shows results obtained using conventional statistics. The bottom triangle represents the results of the phylogenetic *t*-test. To compare relative IOF sizes between taxa, residual IOF area was calculated for each species using a GLS or PGLS regression of ln IOF Area on ln Geometric Mean. NW = nonwhisking, W = whisking, D = diurnal, C = cathemeral, N = nocturnal, A = arboreal, T = terrestrial. Significant results ($P < 0.05$) are indicated by the gray shaded cells.

whisking rodents also have larger IOFs than non-whisking rodents (conventional statistics: t = 9.25, $P < 0.0001$; phylogenetic test statistics: t = 11.77, $P < 0.0001$) (Fig. 3C).

Evolution of Whisking

Ancestral character state reconstruction and tracing techniques on our data suggest that the first therian mammal was non-whisking, with a likelihood of 99.33%; the likelihood of whisking was only 0.67%. Our mapping of whisking state onto a phylogeny reveals that whisking evolved multiple times rather than being the ancestral state for therian mammals (Fig. 4). With our limited sample, whisking appears to have evolved independently at least twice in marsupials, once in Afrosoricida (in tenrecs), once in Eulipotyphla (in shrews), and three times in Rodentia, within each of the three major rodent clades ("squirrel related," "mouse related," Ctenohystrica—Blanga-Kanfi et al., 2009) (Fig. 4). These seven independent evolutions of whisking within therian mammals cover marsupials plus each of the superordinal placental mammalian clades other than Xenarthra, showing the wide range of therian clades that whisking has evolved in.

Eomaia scansoria, an early eutherian mammal (gray circle, Fig. 4), has a relatively small IOF at 0.38 mm at its maximum (blue square in Fig. 2A), which is also well within the nonwhisking IOF values (Fig. 2A). While a small number of whisking mammals have similar IOF values and also fall below the regression line, such as *Suncus minutus*, *Geogale aurita* and *Sorex araneus*, these taxa have much smaller skull size than *Eomaia scansoria* (Fig. 2A), and no whisking animals of comparable size fall directly around the values of *Eomaia* in Figure 2A. *Rhombomylus turpanensis*, which is thought to be close to the ancestry of lagomorphs and rodents (gray circle, Fig. 4), has a large IOF area of 9.12 mm^2, which sits well above the one-standard deviation line from the line of best fit through the non-whisking mammals (purple triangle in Fig. 2A). Only whisking mammals have IOF areas this large, but the nonwhisking lagomorph, *Lepus americanus* (Fig. 2A, see symbol η), is also relatively close to this value (6.33 mm^2) and lies closest to *Rhombomylus* in Figure 2A.

The nonmammalian therapsid *Pachygenelus monus* also has a fairly large IOF area (3.75 mm^2), which sits above the regression upper confidence interval (Fig. 2A), where only 2 individuals are non-whisking (the lagomorphs *Lepus americanus* and *Oryctolagus cuniculus*). Indeed, its IOF area is exactly the same as the whisking, nocturnal, arboreal tenrec *Setifer setosus* (the point next to *Pachygenelus monus* in Fig. 2A). The much larger *Tritylodon* is beyond the extant data in size, but extrapolating the regression line shows it would have been above the regression line, although below the 95% confidence interval.

DISCUSSION

Our results show that extant whisking mammals have larger relative IOF areas than non-whisking mammals (Table 1, Fig. 2A, Figure 3A–C). This is a robust result that can be observed irrespective of activity pattern and substrate preference (Fig. 3B), and even when only the

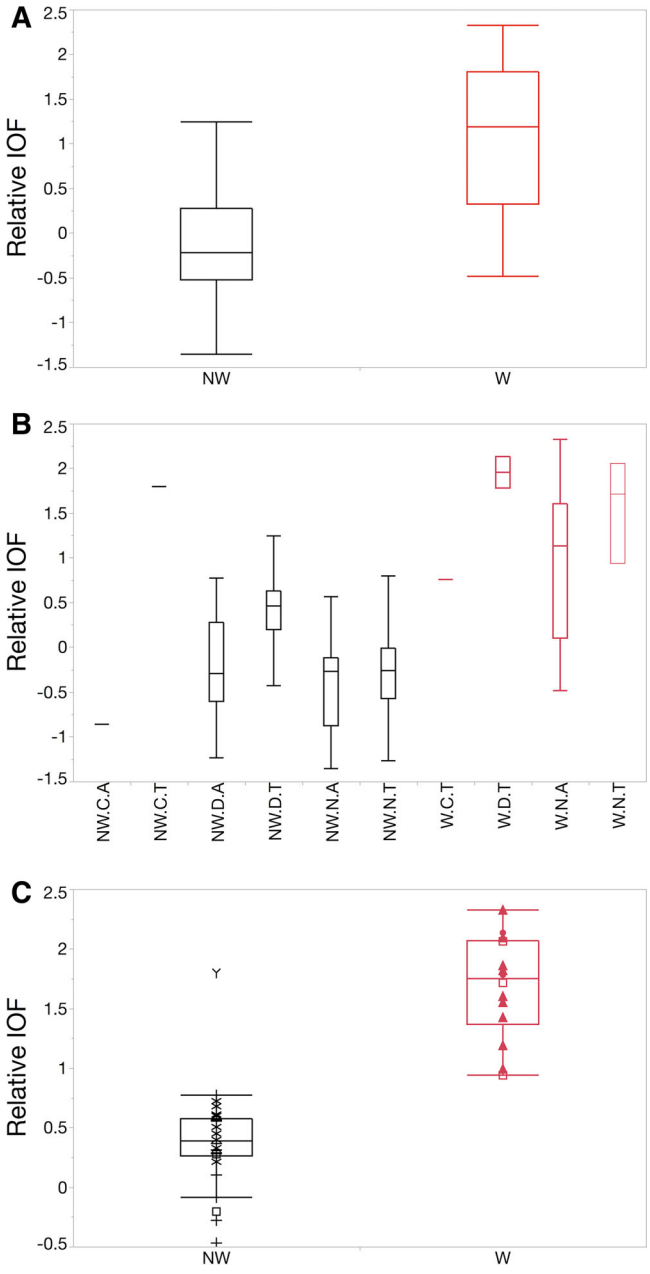

Fig. 3. Boxplots revealing that whisking mammals have larger IOF areas. **A**) Box plot of IOF area for all whisking (in red) and nonwhisking (in black) mammals. **B**) Breakdown of grouping (whisking, activity, and substrate) on IOF Area; **C**) including only the rodents in the analysis. Whisking animals robustly have significantly larger IOF areas. NW = nonwhisking, W = whisking, D = diurnal, C = cathemeral, N = nocturnal, A = arboreal, T = terrestrial. * = NW.D.T, + = NW.D.A, ■ = NW.N.T, □ = NW.N.A, Y = *Ondatra zibethicus*, ● = W.D.T, ○ = W.N.T, ◁ = W.N.A. Relative IOF area was normalized to GM values.

Fig. 4. Ancestral trait and phylogeny. Species or clade circles are from observation of whisking or non-whisking in extant taxa; ancestral node circles provide an estimate for the presence of "whisking" (white = nonwhisking; black = whisking). With percentage likelihoods from (see Supporting Information 2 for likelihood estimates). Two fossil specimens (gray tip circles) were included in the phylogeny, but not in the ancestral character state analysis. Based on the behavior of living mammals, the ancestral state for therian mammals is reconstructed as nonwhisking, and whisking evolved seven times independently.

rodent species are analyzed (Fig. 3C). Our results also suggest that whisking was not an ancestral trait in therian mammals, but has evolved independently on at least seven occasions, and widely across the therian mammal clade.

The larger relative IOF areas in whisking species indicate that these mammals have higher maxillary somatosensory acuity. It might be that using sensors actively, by whisking, is associated with higher sensitivity in these structures. Rice et al. (1986) measured the degree of innervation in whisker follicles in whisking (rats, mice, hamsters) and nonwhisking (rabbits, guinea pig, cat) mammals, and did not find a difference in the number of axons in the deep vibrissal nerve. However, there was a decrease in innervation in the deep area of the follicle in nonwhisking mammals, specifically in the cat and guinea pig. It could be that these small increases in innervation, as well as the increased number of whiskers in whisking mammals (Grant et al., 2017), gives rise to a larger infraorbital nerve, and hence IOF area, in whisking mammals.

However, just because the relative IOF area is large, this does not necessarily indicate that an animal whisks. There is a large spread of the data, and an overlap of whisking and nonwhisking distributions (Fig. 2A). Therefore, relative IOF area cannot be used alone to predict the presence of whisking. For example, Figure 2A shows that the nonwhisking, diurnal, terrestrial *Lepus americanus* (Fig. 2A, see symbol η), has a large relative IOF value (6.33 mm^2), similar to many other whisking mammals, such as the whisking, nocturnal, arboreal *Rattus rattus* (8.08 mm^2) and whisking, diurnal, terrestrial *Arvicola amphibius* (8.15 mm^2).

Moreover, the whisking movements themselves are very variable between species, and the relative IOF area is not able to infer any information about whisking amplitude or symmetry. *Arvicola amphibius* (Fig. 1C, Figure 2B, see symbol β) and *Rattus rattus* (Fig. 1B, Figure 2B, see symbol γ) have very similar, large

relative IOF areas; however, nocturnal, arboreal *Rattus rattus* whisks symmetrically with large amplitude movements, and diurnal, terrestrial *Arvicola amphibius* whisks at much lower amplitudes (Grant et al., 2018). Figure 2B shows whisker traces of the whisking, cathemeral, terrestrial *Neomys fodiens* (Fig. 2A, see symbol α), which has a much smaller IOF than *Arvicola amphibius* (Fig. 2A, see symbol β), but whisks with similarly low amplitude movements. Indeed, the relative IOF area of *Neomys fodiens* is very similar to the nocturnal, arboreal *Mus musculus*, which is known to whisk, with highly motile whisker movements (Mitchinson et al., 2011) that are similar to the whisking movements of *Rattus rattus*, despite the smaller relative IOF area of *Mus musculus.* Perhaps, rather than thinking of whisker movements as simply whisking or nonwhisking, it might be better to think of them as a continuum with varying degrees of amplitude, symmetry and rhythmicity. Whisking really only captures the symmetrical and rhythmic movements of the whiskers; perhaps including other measures of amplitude or angular position would account for some of the variation within the data. It is clear that whisking behavior varies among whisking mammals. Moreover, whiskers are likely to be important and functional in nonwhisking animals (Grant et al., 2017).

Semiaquatic and aquatic mammals are all nonwhisking, however, they have long, densely arranged whiskers, with the highest innervation (Dehnhardt et al., 1999; Dehnhardt, 2001), which suggests their whiskers are an important sense. Certainly, we see relatively large IOF areas in the semi-aquatic *Arvicola amphibius* (Fig. 1C, Figure 2B), *Neomys fodiens* (Fig. 3B) and *Ondatra zibethicus* (Fig. 3B). Just like in nocturnal, arboreal mammals, these species use their whiskers to guide foraging and navigation in dark, complex environments; but they do not tend to cyclically whisk underwater, due to the high energetics associated with moving in water. However, these mammals still may move their whiskers somewhat to position them for sensing (Grant et al., 2013b; Milne and Grant, 2014).

While we suggest an active sense is likely to be associated with higher sensory acuity, the relative IOF area cannot be used to make any suggestions about the exact intrinsic muscle architecture. This is not surprising as muscle activation occurs through the facial nerve (Klein and Rhoades, 2004), and not through the infraorbital nerve. Whisking mammals do tend to have large, regular intrinsic muscles (Muchlinski et al., 2013; Grant et al., 2017) and larger IOFs (Figs. 2A, C, see symbol δ). Indeed, whisking, nocturnal and arboreal *Mus musculus* has regular, large intrinsic muscles (Dörfl, 1982) (Fig. 2C, see symbol δ), and a much higher IOF area (1.29 mm^2) than the nonwhisking, nocturnal, arboreal *Galago moholi* (Fig. 2A, see symbols c and ε) and nonwhisking, diurnal, arboreal *Callithrix jacchus* (Fig. 2 a, see symbols c and ζ) (0.53 mm^2 and 0.51 mm^2, respectively). However, Muchlinski et al., (2013) showed that that *Galago moholi* (v), has small, disorganized intrinsic muscles, and *Callithrix jacchus* (vi) does not have any intrinsic muscles (Muchlinski et al., 2013) (Fig. 2C); despite them both having very similar IOF areas. The layout of the intrinsic mystacial muscles are not known for the majority of mammals, so we were not able to analyze the links between relative IOF size and musculature here. Comparative muscle anatomy studies might be a good place from which to explore links between movement and sensory acuity.

Whisking is mainly associated with being nocturnal and arboreal (Mitchinson et al., 2011; Arkley et al., 2017; Grant et al., 2017). The majority of our whisking species were, indeed, nocturnal (87%, compared to 61% in nonwhisking species) and arboreal (74%, compared to 40% in nonwhisking species) (Fig. 2A). However, we show here that whisking animals have significantly larger IOFs than nonwhisking animals regardless of activity pattern or substrate preference (Fig. 3B). Animals with many, long whiskers are also associated with being small and social (Muchlinski, 2010b); therefore, perhaps whisking is prevalent in these species too. An investigation in to the association of whisking with body size could be tested in future analyses.

Whisking in Early Mammals

Despite the variation in our data, fossil measurements of IOF area, in tandem with the ancestral character state phylogenetic data, might be used to suggest the whisking status of fossil mammals at key nodes on the phylogenetic tree of early mammals. As well as previously unrecognized ecological and dietary niches in early mammals (Luo, 2007; Wilson et al., 2012; Gill et al., 2014), a wide range of locomotor modes and substrate preference has been discovered and inferred in stem and crown group mammals from the Middle Jurassic onwards (Luo, 2007; Chen and Wilson, 2015), with arboreal or scansorial adaptations well represented (Goswami et al., 2011; Bi et al., 2014; Meng et al., 2015). While our data shows there are associations with whisking, and being nocturnal and arboreal, these are in no way dependent on one another. The fossil *Eomaia scansoria* is an arboreal basal eutherian mammal (Ji et al., 2002), but both our ancestral character state reconstruction and tracing (Fig. 4) and IOF area comparisons with extant mammals (Fig. 2A) suggest that *Eomaia* would not have whisked. However, as intrinsic muscles are preserved in both marsupial and placental mammals (Grant et al., 2013a), our data does not rule out that *Eomaia* might have had moveable whiskers. Certainly, whisker movements are more diverse than just whisking (Grant et al., 2017; Grant et al., 2018).

The relative IOF area of the nonmammalian therapsid *Pachygenelus monus* is large, and sits above the regression line upper confidence intervals in Figure 2A, where only two nonwhisking lagomorph species can be found (*Lepus americanus* and *Oryctolagus cuniculus*). All other mammals in this area of the graph are whisking. The early Jurassic nonmammalian therapsid *Tritylodon longaevus* has a very large IOF area of 7.03 mm^2 (Benoit et al., 2016). While this species was slightly too large to include in our selection of small mammals (GM = 124.9 mm), the IOF area of *Tritylodon longaevus* would also have been above an extrapolated regression line, although below the upper confidence intervals, in Figure 2A. These two data points would seem to confirm the presence of whiskers in nonmammaliaform mammaliamorphs 200 million years ago in the Lower Jurassic, and investigating further the evolution of IOF size across the phylogeny from mammaliamorphs to crown group mammals would be of interest for determining the early evolution of whiskers and whisking.

The literature has suggested that whisker movements might be an ancestral trait in therian mammals (Mitchinson et al., 2011; Grant et al., 2013a). We show here that whisking was not recovered as the ancestral state of therian mammals; rather, we see whisking evolving later and independently in a number of therian groups, including marsupials, tenrecs, shrews, and rodents. The rodents contain the largest group of whisking mammals in our data, and the ancestral character state reconstruction analysis suggests that whisking evolved independently at least three times, in each of the three major rodent clades ("squirrel related," "mouse-related," and Ctenohystrica) of Blanga-Kanfi et al., (2009) (Fig. 4). There is still uncertainty regarding which of these three clades represents the first branching, earliest diverging rodent clade, that is the sister group of all other rodents (Blanga-Kanfi et al., 2009; Fabre et al., 2012, 2015). The only one of the three that we reconstruct as likely having been ancestrally whisking is the 'mouse-related' clade (node likelihood = 81%; see Supporting Information 2 Ancestral States likelihood data, and Figure showing node numbers). However, the mouse-related clade is the least likely group to be at the base of the rodent tree (Fabre et al., 2015), with the most likely being the squirrel-related clade (Blanga-Kanfi et al., 2009; Fabre et al., 2012, 2015), which is also what we base our phylogeny on (Bininda-Emonds et al., 2007). We therefore reconstruct the common ancestral character state of all rodents as nonwhisking.

Rhombomylus turpanensis is a basal member of Glires, the clade containing rodents and lagomorphs. The phylogenetic position of *Rhombomylus* was initially described as closer to rodents than lagomorphs, but more recent analyses suggest that it was more closely related to lagomorphs (Fig. 4) (Meng et al., 2003; Asher et al., 2005; O'Leary et al., 2013). *Rhombomylus* has a large IOF area of 9.12 mm^2 (upper second molar area = 11.1 mm^2) (Muchlinski and Kirk, 2017), and is further above the upper confidence limit of the regression line than all but two nonwhisking extant mammals and similar to one nonwhisking lagomorph (Fig. 2B, see symbol η). A study by Muchlinski and Kirk (2017) presented data from another small basal member of Glires, *Tribosphenomys minutus* (O'Leary et al., 2013), with an IOF area of 0.25 mm^2 (upper second molar area = 1.13 mm^2), which is similar to some of our small whisking mammals, such as *Suncus minutus* (IOF = 0.16 mm^2, GM = 10.91 mm) and *Sorex araneus* (IOF = 0.31 mm^2, GM = 14.20 mm). Certainly, it would be of interest to examine the associations between the IOF area and whisker movements in Glires in more depth. Lagomorphs and many hystricomorph rodents, such as *Cavia porcellus*, do not seem to rhythmically whisk their whiskers, but are capable of some asymmetric whisker movements (Grant et al., 2017). Some hystricomorph rodents are capable of whisking, including *Chinchilla lanigera* (Woolsey et al., 1975). Indeed, Rodentia contains both whisking and nonwhisking species, with perhaps the most whisking species of any order, and whisking evolved at least three times independently within the clade. As well as whisking, rodents also make a variety of other whisker movements at varying amplitudes and symmetries (see whisker traces of *Cavia porcellus* in Grant et al., 2017 and Grant et al., 2018; *Muscardinus avellanarius* in Arkley et al., 2017 and Grant et al., 2018). Incorporating other aspects of whisker movement might tease apart some of the overlap that can be seen in the whisking and nonwhisking data in Figure 2A. We posit that whisker movements should be thought of as more of a continuum and not just a binary, whisking or nonwhisking trait.

CONCLUSIONS

This is the first study to associate whisking behavior with a morphological skull measurement. We find that whisking mammals have significantly larger relative IOF areas, which indicates larger infraorbital nerves and an increase in sensory acuity in whisking mammals. This relationship is quite variable, however, so IOF area cannot be used alone to predict whisker movements or aspects of mystacial musculature in small mammals. Whisking mammals tend to be nocturnal and arboreal, but are not constrained to these substrate preferences or activity patterns; regardless of substrate preferences or activity patterns, whisking mammals have on average larger relative IOF areas. Whisking is not the ancestral state of therian mammals, but evolved independently multiple times, in Marsupiala, Afrosoricida, Eulipotyphla, and Rodentia. To better understand the relationship between whisker movements and associated increases in sensory acuity, other aspects of movement should be considered, as the term whisking only captures symmetrical and rhythmic movements.

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