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ORIGINAL RESEARCH

Mandibular morphology in four species of insectivorous bats: the impact of sexual dimorphism and geographical differentiation

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Keywords

Chiroptera; geometric morphometrics; morphology; ecogeography; sexual dimorphism; mandibles.

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Introduction

Temporal and spatial variation are increasingly recognised as major factors in explaining morphological changes in the mammalian skull at both micro and macroevolutionary scale. Abundant evidence of ecogeographical patterns have now been recognised in species and genera belonging to multiple clades including primates (Cáceres et al., 2014; Cardini et al., 2007; Meloro et al., 2014), carnivorans (Bubadué et al., 2016; Meiri et al., 2005; Meloro et al., 2017), ungulates (Terada et al., 2012) and marsupials (Magnus et al., 2017) to name a few groups. Interestingly, not much has been explored in Chiroptera, the second largest mammalian group, currently including 1,400 species covering broad dietary and ecological adaptations (Fenton & Simmons, 2015; Jiang et al., 2019).

The rationale to test the impact of geographical variation on skull morphology comes from the well-established Bergmann's rule, which suggests that mammals at higher latitudes evolve to be larger, optimising their surface/volume ratio (McNab, 1971). Bats, inherently volant, generally exhibit broader geographical range size than many terrestrial mammals. It is hypothesised that

Abstract

Chiroptera is the only mammalian order that has adapted to active flight, offering a unique platform to study ecomorphological adaptations. While bats exhibit a diverse diet, the focus of this study is on insectivorous bats, specifically four species: *Myotis daubentonii*, *Nyctalus noctula*, *Plecotus austriacus* and *Rhinolophus ferrumequinum*. It is important to note that despite sharing an insectivorous diet, these species occupy different ecological niches, perform distinct feeding strategies and explore varied habitats to capture prey. Using 2-D geometric morphometrics, we analysed a sample of mandibles to identify differences in size and shape among these species. We also investigated ecogeographical variation within their overlapping distribution across continental Europe. Significant differences in both mandibular size and shape were found among the four species. Sexual dimorphism influenced only the mandibular shape of *R. ferrumequinum*. A latitudinal gradient in mandibular size was found solely in *N. noctula*, while longitude significantly explained shape variation in *M. daubentonii*. These findings suggest that even within the ecological guild of insectivorous bats, there exists a diverse range of morphological adaptations that allow these species to occupy distinct ecological niches.

their morphology would reflect adaptations to latitudinal changes (Herreid, 1964). However, a departure from Bergmann's rule is seen in Safi et al. (2013), where body mass of bats was found to be inversely correlated with latitude. This suggests a complex interplay between geographical factors and inherent phylogenetic tendencies in bats. Given these intricacies in bats' adaptation, it becomes imperative to explore morphological traits which could be responsive to these geographical and ecological changes.

Skull size has been suggested as a key chiropteran trait that responds consistently to latitudinal changes (Kryštufek, 1993). There is increasing evidence that shape changes occur in relation to ecological feeding adaptations (Bogdanowicz et al., 1999; Clavel & Morlon, 2020; Freeman, 1981; Giacomini et al., 2022; Nogueira et al., 2009). In this regard, the mandible is perhaps more susceptible to allow detection of ecogeographical variation considering its pivotal role in mammalian chewing (Herring, 1993). Within Chiroptera, mandibular shape has been consistently identified as a trait that strongly covaries with bite force at macroevolutionary scale (Manhães et al., 2017) and general feeding adaptations (Arbour et al., 2019). López-Aguirre et al. (2015) identified geographical trends in the mandible of

the tropical genus *Carollia*. These patterns detected in subtropical species perhaps can also be explored in temperate taxa.

Here we explore a subsample of mandibles belonging to three species of the Vespertilionidae family: Daubenton's bat (*Myotis daubentonii*), the grey long-eared bat (*Plecotus austriacus*) and the noctule (*Nyctalus noctula*) and one of Rhinolophidae, the greater horseshoe bat (*Rhinolophus ferrumequinum*) in order to identify possible geographical trends in their morphologies.

These species were selected because they are all considered insectivorous and their geographical ranges broadly overlap covering Western Europe to East Asia, reaching as far south as Morocco and as far north as Sweden. Because the ecological adaptations of these taxa differ to some degree (see section below), we expect to identify several differences in mandibular morphology for both size and shape traits (Freeman, 1981). We are also testing for sexual dimorphism within each selected species. Myers (1978) noted that in several vespertilionid bats females are larger than males due to the increasing energy demand on pregnant females (Williams & Findley, 1979); same applies for Rhinolophidae (Dietz et al., 2006). Since body mass significantly influences mammalian skeletal morphology (Damuth, 1990), we expect sexual dimorphism at least in bat mandibular size with females bigger than males. If sexual difference also occurs in mandible shape, it could support intra-specific diet partitioning or differences in allometric (=the influence of size on shape traits) variation between males and females (Fairbairn, 1997).

Study species

Daubenton's bat *Myotis daubentonii* (Kuhl, 1817) is the smallest of the study species (5–10 g) with a geographical range encompassing the majority of the palearctic region, with the most northern edge located in Finland (Dietz et al., 2009; Lučan & Radil, 2010; Vesterinen et al., 2016). The species is common throughout most of its European range (Mitchell-Jones et al., 1999) with a flexible diet that mainly consists of aquatic insects (Vaughan et al., 1997). However, during the midnight sun period in Finland, they feed in woodland due to the higher predation risk over open water (Jones & Rydell, 1994; Nyholm, 1965). They mainly feed on Chironomidae, a family of nematoceran flies, but also consume Diptera, Trichoptera, Lepidoptera and other insects (Flavin et al., 2001; Swift & Racey, 1983). They have been noted for their ecological flexibility (Nissen et al., 2013). Males consume a broader variety of prey species than females (Vesterinen et al., 2013, 2016). Jones and Kokurewicz (1994) reported female Daubenton's bats from Poland to be significantly larger than males in both body mass and wing morphology. Kokurewicz (2004) confirmed the body mass female:male difference before and after hibernation period while Encarnaç o et al. (2005) also found differences between sexes in roost-site selection.

The grey long-eared bat *Plecotus austriacus* (Fischer, 1829), weighing 8–10 g, is characterised by its expanded ear and broad wings (Nowak, 1997; Stebbings, 1970). Common throughout Southern and Central Europe, it is absent from most of the UK, North Africa and East Eurasia

(Stebbing, 1970; Wilson & Reeder, 1993). This species captures insects in flight using a pouch developed from its tail membrane (Leen, 1969). As in the other species, *P. austriacus* is known to roost in urban areas (Altringham, 1996). It mainly feeds on Lepidoptera and occasionally woodland Diptera, with a relatively small dietary niche (soft food items) (Jennings, 2008). Spitzenberger et al. (2001) report for most of the species within the *Plecotus* genus complex, male skulls to be generally smaller than females although no significant sexual dimorphism was noted for *P. austriacus*. Razgour (2021) data revision equally support males to be smaller than females in forearm length but differences in body mass are quite small and change depending on the population.

The noctule bat *Nyctalus noctula* (Schreber, 1774) is one of the largest Holarctic insectivores (25–30 g), widely distributed throughout most of Europe, aside from the most northern and southern regions (Braun & Dieterlen, 2003; Dietz & von Helverson, 2004). It generally occurs near woodland areas (Rachwald, 1992) and its European distribution generally resembles that of deciduous and mixed forests (Petit et al., 1999). However, its distribution is currently expanding, potentially due to the higher frequency of taller buildings (Godlevska, 2015) which can provide substitute roosts if similar enough to the natural cavities (Bihari, 2004). In terms of diet, the species feeds on a range of taxa: Trichoptera, Ephemeroptera, Coleoptera and Hemiptera and also Lepidoptera, Neuroptera and Diptera (Rydell & Petersons, 1998). The species is adapted to aerial hawking (prey is pursued and caught in flight) rather than gleaning (prey is taken from leaves or the ground) (Norberg & Rayner, 1987). This restricts the size of prey items that they can consume (Black, 1974). Sexual size dimorphism in numerous body dimensions has been reported for this species (Lindecke et al., 2020; O'Mara et al., 2016).

The greater horseshoe bat *Rhinolophus ferrumequinum* (Schreber, 1774) is relatively large (16.6–29.3 g) and widespread throughout the majority of the Palaearctic (Csorba et al., 2003; Jiang et al., 2019) but is experiencing significant declines, especially in Northern Europe, due to habitat fragmentation (Tournayre et al., 2019). The species has a varied diet, including Lepidoptera and Diptera and other insects such as Hymenoptera and Neuroptera. However, it mainly feeds on Coleoptera (hard food item) (Song & Yoo, 2007). It generally forages in covered areas such as deciduous forests, with seasonal variation in habitat selection due to the influence of season on food availability (Wang et al., 2010). Female biased dimorphism in body mass and forearm length has been noted in this species by Wu et al. (2014), although Ikeda et al. (2020) report no sexual dimorphism in mandible size and shape.

Despite having overlapping diets, the four species have considerably different feeding strategies and the proportions of each food type in their diet greatly vary. *Nyctalus noctula* and *M. daubentonii* seem to have more generalist diets compared to the specialised feeding of *R. ferrumequinum* and particularly *P. austriacus* leading us to expect great differentiation in mandible shape and size to reflect their differing diets.

Considering their ecological adaptations and previous investigations, we expect to identify a latitudinal trend at least in *Myotis* and *Rhinolophus* (Kryštufek, 1993; Moratelli &

Oliveira, 2011). Using geometric morphometrics (Adams et al., 2004, 2013), we will test for both size and shape differences in the mandible of these bat species together with sexual dimorphism and geographical trends. This approach conveniently allows the separation of size from shape variation into components that can be explored quantitatively and qualitatively through visualisation of deformation grids. This method has been successfully employed to test similar hypotheses in different bat species using both the cranium and the mandible (López-Aguirre et al., 2015; Ospina-Garcés & León-Paniagua, 2021). The latter is here explored bi-dimensionally considering that its variation is equally approximated in 3D (Cardini, 2013).

Materials and methods

Size and shape data were collected from 245 mandibles belonging to four different bat species from different areas of their ranges within Europe, Turkey and North Africa (Fig. 1).

We managed to cover 36% of the total range for *M. daubentonii*, 65% for *P. austriacus*, 27% for *N. noctula* and 28% for *R. ferrumequinum*. This limitation was mainly due to specimen availability within the visited collection. However, we still covered a similar latitudinal range in all the taxa from 30 to ca. 56 degrees north (39–51 in *M. daubentonii*, 39–56 in *N. noctula*,

39–51 in *P. austriacus* and 31–50 in *R. ferrumequinum*). For each specimen, the geographical coordinates of its field collection point were recorded. There was fair sampling of males and females ($n = 129$ and 125 respectively) and a comparable number of individuals per species (Table 1). For full details of each specimen, see Table S1. The mandibles were photographed in lateral view using a 24 mega-pixel digital SLR Nikon D5300 camera attached to a Nikkor 60 mm macro lens and mounted on a Manfrotto tripod at a minimum 1 m distance from the above. A Petri dish with micron scale bar was adapted to allow each hemi-mandible to lay horizontally flat. The Manfrotto tripod allowed the camera optical plane to be positioned parallel to the specimen using a spirit level. Digital photographs were land-marked with the software tpsDig2 (Rohlf, 2015).

Thirteen homologous landmarks were digitised (see Fig. 2). These incorporated the majority of the important biological points as utilised in other studies which explore mandible variation in bats (Bookstein et al., 1985; López-Aguirre et al., 2015; Monteiro & Nogueira, 2011). Landmarks 1–2 described the relative position of the canine, while 3–4 the position of m1. Landmark 5 identifies the limit of the dental row and the beginning of ramus mandibulae, while 6 and 7 were added to quantify corpus depth. Landmarks 8–9–10 identify the angular process, 11–12 the condyle and 13 was positioned at the highest edge of the ramus mandibulae.

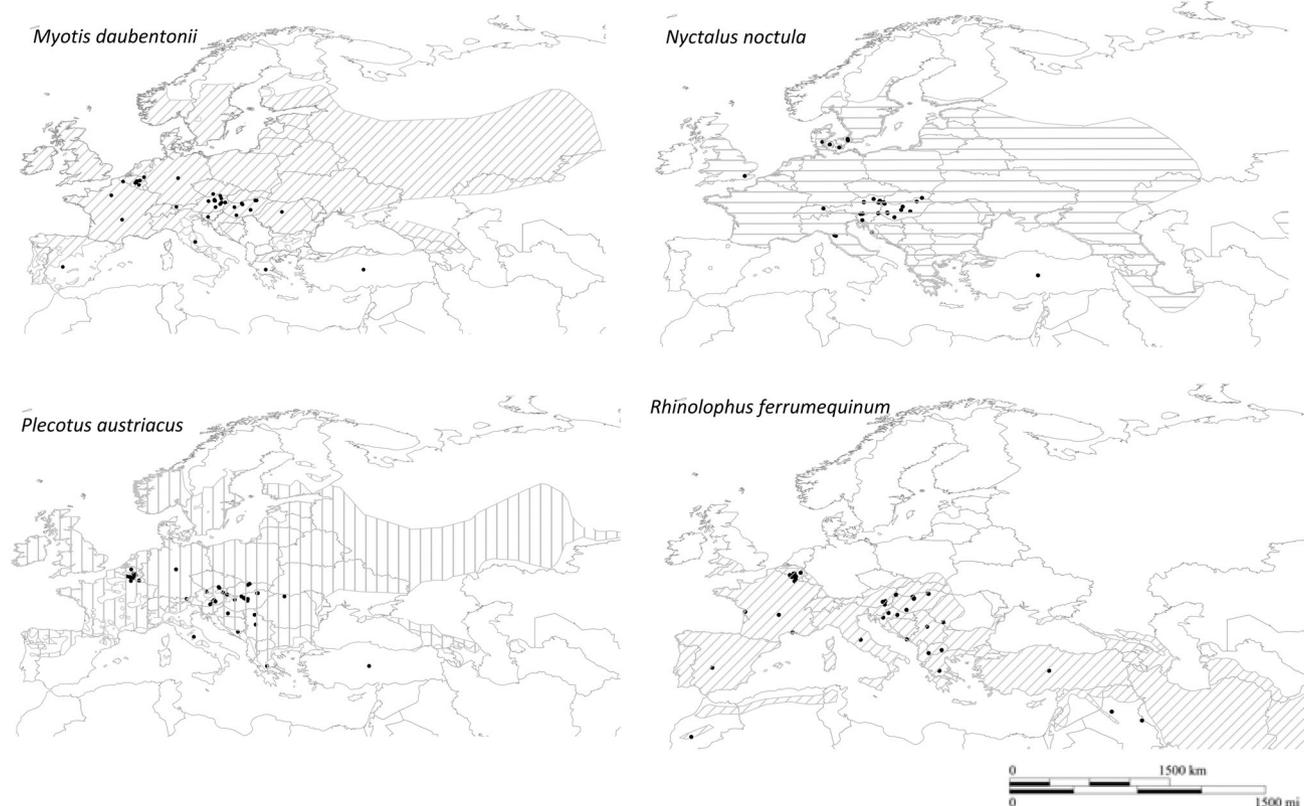


Figure 1 Geographical distribution of mandible specimens analysed for four bat species across Eurasia and North Africa. For each species, the geographical range is shown in light grey as from IUCN official maps (<https://www.iucnredlist.org/resources/spatial-data-download>).

Table 1 Sample size and sex composition for each of the four bat species studied

Species	# Specimens	# Females	# Males	# Undetermined
<i>Myotis daubentonii</i>	57	25	29	3
<i>Plecotus austriacus</i>	55	29	25	1
<i>Nyctalus noctula</i>	61	27	33	1
<i>Rhinolophus ferrumequinum</i>	72	33	35	4
Total	245	114	122	9

The geomorph R package (version 3.2.2) (Adams et al., 2021), a statistical package commonly used for geometric morphometric studies (Hart et al., 2020), was employed for all the statistical analyses of the 2D landmark coordinates.

A generalised procrustes analysis (GPA) was carried out using the `gpa()` function. This procedure allows the removal of non-shape variation, through superimposing, scaling, rotation and translation of the original landmark coordinates (Rohlf & Slice, 1990) into a new set of shape coordinates: the Procrustes coordinates, which are used as a proxy for shape (Zelditch et al., 2004). The size of the landmark configuration of each specimen was recorded as the centroid size, which is the square root of the sum of squared distances between each landmark and the configuration centroid (Bookstein, 1989; Adams et al., 2004).

Shape variation was first explored using principal component analysis. This allowed summarising variation between specimens into orthogonal vectors that can be biologically interpreted in conjunction with thin-plate spline to allow visualisation of shape changes relative to the mean configuration (Zelditch et al., 2004). We first tested hypotheses about significant differences in size and shape between species. These are expected considering the different body masses exhibited by the species analysed.

Procrustes ANOVA and ANOVA (using the function `procD.lm()`; Adams & Collyer, 2015) were employed on the whole sample ($n = 245$) to test for taxonomic differences in mandibular shape and size.

The impact of sex on mandibular variation was equally explored restricting the analyses only to the sexed individuals. Again, the function `procD.lm` was employed to test the impact of sex in conjunction with taxonomy. The interaction term (taxonomy \times sex) was equally tested to identify possible

differences in level of dimorphism between the species. Eventually, sex was tested separately for each species.

Allometric variation (Klingenberg, 2016) in the whole sample and for each species was explored in order to identify the impact of size on shape variation. The model shape-size \times species was tested to identify differences in allometric trajectories. If these did not occur, residuals of shape coordinates were eventually analysed to explore shape differences between species irrespective of allometry. Finally, geographical variation was explored following Cardini et al. (2007). Size and shape data were averaged for each geographical location aiming to avoid pseudo-replication. Latitude and longitude were tested as factors (singularly and in conjunction) on both size and shape, separately. Based on the work of Kryštufek (1993) and Bogdanowicz (1990), we expected latitude to have an effect on the mandible shape and/or size of *R. ferrumequinum* and *M. daubentonii*.

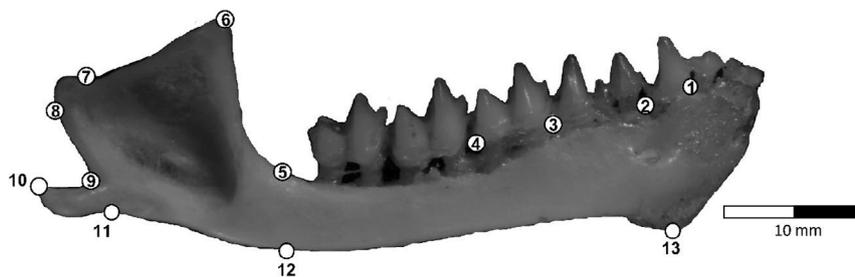
Additionally, for all test permutations were used to account for small sample size, and P -values were Bonferroni corrected in all the post-hoc tests. Type III sum-of-squares were implemented in all cases for the `procD.lm` models to ensure testable factors were equally weighted (see Gálvez-López et al., 2022).

Results

Shape and size variation

Principal component analysis identified a quite distinctive pattern in shape variation among the four species. PC1 (54.58% of variance) effectively separated species, with *R. ferrumequinum* occupying the most positive extreme scores and *N. noctula* the most negative. This axis described shape changes relative to corpus depth (thick on the most anterior edge of the canine in *N. noctula*, thin in *R. ferrumequinum*) and angular process (that is much more developed and posteriorly projected in *R. ferrumequinum*). Along PC2 (19.33% of variance) shape changes occurred especially in the most posterior region of the corpus, that was much thinner in *M. daubentonii* and *P. austriacus* when compared to *N. noctula* and *R. ferrumequinum*. This axis shape variation also related to the relative expansion of the condyle and ramus region (Fig. 3).

Taxonomy explained a significant component (76%) of shape variation ($n = 245$, $SS = 0.72621$, $MS = 0.24207$, $R^2 = 0.76023$, $F = 245.71$, $p < 0.001$), and when only the sample of sexed individuals ($n = 236$) was analysed, a significant interaction between sex and species could be identified (Table 2).

**Figure 2** Landmark configuration. Location of the 13 landmarks on the right hemimandible of a *Myotis daubentonii* mandible.

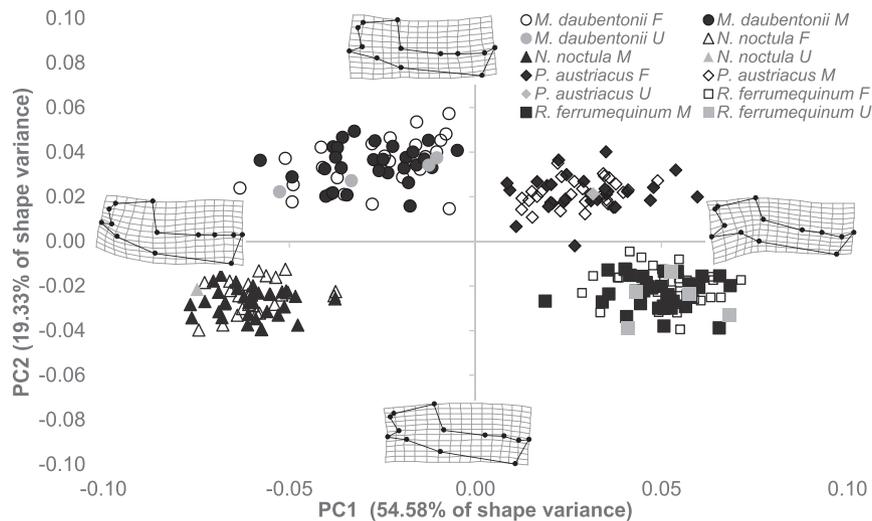


Figure 3 Scatterplot for the first two principal components obtained from the Procrustes coordinates. Species samples are separated into distinct clusters. Deformation grids along each PC axis show relative shape changes from the mean based on thin plate spline.

Table 2 Procrustes ANOVA to test the impact of taxonomy and sex (and their interaction) on mandible shape and size for the 236 sexed bat specimens

		d.f.	SS	MS	R^2	F	Z	p
Shape	Species	3	0.330	0.110	0.359	111.9797	8.607	0.001
	Sex	1	0.001	0.001	0.001	0.6464	-0.8288	0.794
	Species × Sex	3	0.004	0.001	0.005	1.5810	2.1301	0.021
	Residuals	228	0.214	0.001	0.248			
	Total	235	0.918					
Size	Species	3	3.8673	1.28911	0.444	2034.206	23.489	0.001
	Sex	1	0.006	0.006	0.001	9.226	2.388	0.004
	Species × Sex	3	0.007	0.002	0.001	3.937	2.224	0.011
	Residuals	228	0.144	0.200	0.017			
	Total	235	8.715					

Significance is highlighted in bold.

Sex had a significant influence on mandible shape only for *R. ferrumequinum*, explaining 5.9% of shape variation (Table 3).

Size also significantly differed between species, with *R. ferrumequinum* and *N. noctula* being larger than *P. austriacus* and the smallest being *M. daubentonii* ($P < 0.0001$). (Table 2; Fig. 4). *Post hoc* comparisons confirmed that the mean sizes for each species pair were significantly different ($P < 0.001$ for all species). Sex was equally a significant factor in mandibular size of *M. daubentonii* and *P. austriacus*, but not for *N. noctula* and *R. ferrumequinum* (Table 3). When significant, females were larger than males.

Allometry and size-free shape variation

Size explained a significant proportion of shape in the entire sample ($n = 254$, $SS = 0.187$, $R^2 = 0.196$, $F = 59.194$, $P < 0.0001$). However, this effect disappeared when the factor 'species' was added into the model (Table 4). Similarly, when exploring allometric influence for each species separately, there

was no effect of size on shape (0.078 (*R. ferr.*) $< P < 0.704$ (*N. noct.*)). This was confirmed by the lack of significant interaction between size and species in the Procrustes ANOVA model (Table 4).

The shape residuals obtained from the model size–shape applied to the total sample still showed a significant difference between species ($SS = 0.30848$, $MS = 102.825$, $R^2 = 0.66258$, $F(3, 144) = 94.254$, $P < 0.001$).

Ecogeographical variation

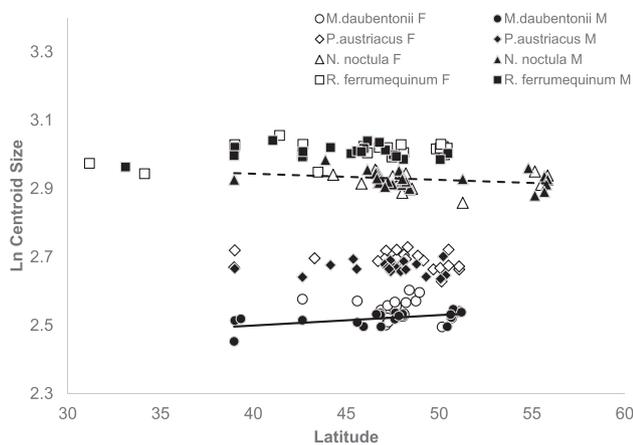
One hundred forty-eight geographical locations were available for all 245 bat specimens.

A significant impact of latitude on shape and size occurred for the entire sample, accounting for 0.1 and 0.3% of the variance respectively (Table 5). When looking at the species separately, latitude had no influence on mandibular shape variation for any of the four species except in the subsample of *P. austriacus* males ($n = 20$). This result was mainly driven by the interaction

Table 3 Procrustes ANOVA to test the impact of sex on mandible shape and size for each of the four species, omitting the specimens with undetermined sex: 53 mandibles for *Myotis daubentonii*, 54 for *Plecotus austriacus*, 67 for *Rhinolophus ferrumequinum* and 60 for *Nyctalus noctula*

			d.f.	SS	MS	R^2	F	Z	<i>p</i>
<i>M. daubentonii</i>	Size	Sex	1	0.005847	0.005847	0.1392	8.4089	2.3237	0.0045
		Residuals	52	0.036155	0.000695	0.8608			
		Total	53	0.042002					
	Shape	Sex	1	0.000608	0.000608	0.00868	0.4556	-1.4457	0.9235
		Residuals	52	0.069355	0.001334	0.99132			
		Total	53	0.069963					
<i>P. austriacus</i>	Size	Sex	1	0.006215	0.006215	0.18624	11.901	2.6882	7.00E-04
		Residuals	52	0.027158	0.000522	0.81376			
		Total	53	0.033373					
	Shape	Sex	1	0.001122	0.001122	0.02436	1.2983	0.82348	0.203
		Residuals	52	0.044936	0.000864	0.97564			
		Total	53	0.046058					
<i>N. noctula</i>	Size	Sex	1	0.000452	0.000452	0.01118	0.6557	0.24444	0.4216
		Residuals	58	0.039966	0.000689	0.98882			
		Total	59	0.040417					
	Shape	Sex	1	0.001169	0.001169	0.02609	1.5536	1.2557	0.1051
		Residuals	58	0.043632	0.000752	0.97391			
		Total	59	0.044801					
<i>R. ferrumequinum</i>	Size	Sex	1	0.000312	0.000312	0.00751	0.4991	0.072221	0.482
		Residuals	66	0.041209	0.000624	0.99249			
		Total	67	0.041521					
	Shape	Sex	1	0.003518	0.003518	0.05871	4.1168	3.6563	3.00E-04
		Residuals	66	0.056407	0.000855	0.94129			
		Total	67	0.059925					

Significant highlighted in bold.

**Figure 4** Scatterplot of latitude versus natural log transformed centroid size for male and female bat mandibular specimens averaged by geographical location. Trendlines were superimposed on the data that showed a significant correlation between latitude and centroid size (positive in male *Myotis daubentonii* and negative in male *Nyctalus noctula*).

of latitude with longitude in the regression model. The model shape~latitude was non-significant (SS = 0.0005, MS = 0.0005, $R^2 = 0.035$, $F(1,18) = 0.651$, $Z = -0.765$, $P = 0.784$) for *P. austriacus* males. Non-parametric correlation identified

positive latitudinal effect on size variation in the averaged sample of *M. daubentonii* while an opposite negative trend occurred in the *N. noctula* (mainly driven by females, Fig. 4, Table 6).

Longitude significantly impacted shape variation for the entire sample but only to a very limited extent (0.5% of the variation). Individually the mandible shape of the averaged subsample of *M. daubentonii* and of the male subsample of *P. austriacus* were significantly influenced by longitude, accounting for 5.2 and 12.1% of the variation respectively. In the *M. daubentonii* subsample, longitudinal shape variation was associated with deformation in corpus, coronoid, angular process and canine regions. At small longitudes, mandibles (mainly sample from Spanish locations) exhibit relatively thick corpus behind the molar region and short coronoid, angular process and canine region (Fig. 5a). Similar shape deformation occurred in *P. austriacus* males that at smaller longitudes (locations from Belgium) showed a relatively wider condyle and shorter angular process when compared with specimens from Greece. Only in the *P. austriacus* female subsample did longitude have a positive association with size (Table 6).

Discussion

The four analysed species of insectivorous bats showed a consistent difference in mandibular morphology in both size and shape. This pattern was not an artefact of allometric variation and supports the ecological niche partitioning of these species in relation

Table 4 Test for an allometric influence on the mandible shape for the entire sample ($n = 245$) and only sexed individuals ($n = 236$)

		d.f.	SS	MS	R^2	F	Z	p
Shape	Size	1	0.001	0.001	0.001	1.126	0.476	0.318
	Species	3	0.005	0.001	0.004	1.237	1.004	0.159
	Size × Species	3	0.003	0.001	0.003	1.159	0.73	0.232
	Residuals	237	0.225	0.001	0.235			
	Total	244	0.955					
Size	Size	1	0.001	0.001	0.001	1.118	0.451	0.327
	Species	3	0.52	0.173	0.566	183.139	8.369	<0.001
	Sex	1	0.002	0.002	0.002	1.965	1.921	0.003
	Residuals	230	0.218	0.001	0.237			
	Total	235	0.918					

Significance in bold ($p < 0.05$).**Table 5** Test for a geographical influence on the mandible shape and size for the entire sample with data averaged by location

		d.f.	SS	MS	R^2	F	Z	P
Shape	Species	3	0.006	0.002	0.010	2.419	3.618	<0.001
	Latitude	1	0.001	0.001	0.003	1.902	1.681	0.046
	Latitude × Species	3	0.003	0.001	0.005	1.157	0.690	0.244
	Residuals	140	0.108	0.001	0.201			
	Total	147	0.539					
Size	Species	3	0.044	0.015	0.008	27.178	6.251	<0.001
	Latitude	1	0.003	0.003	0.001	6.087	2.001	0.016
	Latitude × Species	3	0.007	0.002	0.001	4.212	2.397	0.007
	Residuals	140	0.076	0.001	0.014			
	Total	147	5.280					
Shape	Species	3	0.089	0.030	0.165	39.015928	8.190	<0.001
	Longitude	1	0.003	0.003	0.005	3.427	3.312	0.001
	Longitude × Species	3	0.003	0.001	0.006	1.334	1.263	0.105
	Residuals	140	0.106	0.001	0.197			
	Total	147	0.539					
Size	Species	3	1.428	0.476	0.271	818.671	21.723	0.001
	Longitude	1	0.001	0.001	0.001	1.7021	0.887	0.200
	Longitude × Species	3	0.003	0.001	0.001	1.414	0.701	0.242
	Residuals	140	0.081	0.001	0.015			
	Total	147	5.279					

Significance in bold ($p < 0.05$).

to different feeding strategies. The mandibular morphospace (Fig. 3) shows expected similarities depicted along PC1 scores that separate *M. daubentonii* and *N. noctula* (negative scores) from *R. ferrumequinum* and *P. austriacus* (predominantly positive scores).

More specifically, the PC1 negative values correspond to a dorsally displaced angular process (i.e. short moment arm of the superficial masseter, associated with wider gapes; Herring & Herring, 1974) and a deep anterior corpus (associated with stronger bites with the anterior teeth; Gálvez-López & Cox, 2022). Combined, these traits suggest strong anterior bites at wide gapes, which are congruent with the aerial hawking of *M. daubentonii* and the *N. noctula*. On the other hand, positive PC1 values correspond to a ventrally displaced angular process (i.e. long moment arm for the superficial masseter) and an overall robust corpus, which suggests strong bites at the

cheek (Gálvez-López & Cox, 2022) and would be useful for both processing large prey (as in the moth specialist *P. austriacus*) and dealing with hard prey (i.e., *R. ferrumequinum*). Expanded corpus in relation to tough food consumption has been observed also in other mammalian groups such as carnivores (Meloro et al., 2008; Raia, 2004) or hypsodont ungulates (Raia et al., 2010), suggesting that insectivorous bats are no exception to this chewing adaptation.

If we also consider that relative bite force is another factor influenced by bat cranio-mandibular morphology (Nogueira et al., 2009), our results are equally informative with *P. austriacus* showing the most gracile mandible and the lowest bite force of the four (1.19 Newton after Giacomini et al., 2022) analysed species. *Myotis daubentonii* also has a low bite force (1.68) while *N. noctula* and *R. ferrumequinum* have the largest bite forces (8.78 and 7.55 respectively). This variation is depicted by PC2

Table 6 Spearman's rho parameters testing correlation between latitude and longitude versus natural log transformed centroid size for each species after averaging data by location and separating sexes

Species	Sex	# Locations	Latitude	Longitude
<i>M. daubentonii</i>	Males	21	0.61319	-0.10783
	Females	19	-0.05263	-0.09474
	Avg	34	0.30726	-0.08602
<i>P. austriacus</i>	Males	20	-0.15038	0.32632
	Females	23	-0.31818	0.42688
	Avg	36	-0.15032	0.28443
<i>N. noctula</i>	Males	28	-0.43514	-0.03394
	Females	21	-0.23117	-0.15974
	Avg	39	-0.46964	-0.10182
<i>R. ferrumequinum</i>	Males	20	-0.13985	-0.05414
	Females	21	0.16623	0.11039
	Avg	37	0.1496	0.073495

Significant correlation parameters ($P < 0.05$) are highlighted in bold.

that partition species based on the relative expansion of the ramus region (for providing increasing surface in temporalis and masseter muscle attachment) and mandibular corpus thickness (Fig. 3).

Allometry

When exploring shape variation, it is crucial to account for allometry. For instance, Budinski et al. (2015) noted that although climate variables correlated with cranial shape and size in *R. ferrumequinum*, this relationship did not persist after accounting for allometry. Similarly, Kokurewicz (2004) suggested that allometry could complicate observed sexual dimorphism in *M. daubentonii*, and several studies have shown that a significant amount of variation within or between species can be attributed to differences in allometric trajectories (Abdala et al., 2001; Gould, 1975; Reig, 1992; Vinyard & Ravosa, 1998).

However, our results showed that size impacted shape variation once taxonomy is included in the model, only in the overall combined sample. When species were considered separately, the effect of size was never significant. Previous authors identified a significant allometric component in cranial shape of the greater horseshoe bat (Budinski et al., 2015), however no attempt has yet been made to explore this issue in the mandible.

The only significant size component detected in our total sample was due to body mass differences between species, with *Myotis* being the smallest and *Rhinolophus* the largest. We suggest this supports that shape differences in the mandibular morphology of the analysed insectivorous bats are not a by-product of size differences, but that they reflect functional differences in food acquisition and bite force production. This hypothesis is corroborated by the analysis of 'size-free' residual shape variables which confirmed strong differences between species.

Sexual dimorphism

The only species that we could identify to be sexual dimorphic in mandible shape was *R. ferrumequinum*, for which sex explained ca. 5.8% of variation. Previous work covering a broader geographical range (including Asia) for this species found shape dimorphism in the cranium but not in the mandible (Ikeda et al., 2020). This was explained in relation to the fact that subtle ecological factors might influence shape differences between sexes in the genus *Rhinolophus* including interspecific competition (Dragu et al., 2019). Our result is not due to allometric patterns or body size differences between males and females (Wu et al., 2014), so we argue that mandible shape differences might potentially reflect distinct ecological specialisations particularly for the European and Turkish populations analysed. Jones et al. (2009) found that *R. ferrumequinum* exhibits a less diverse diet especially during the summer period corresponding to female late pregnancy. Heavier females are more restricted into their flight ability thus explaining their more specialised dietary niche. This should apply to all the

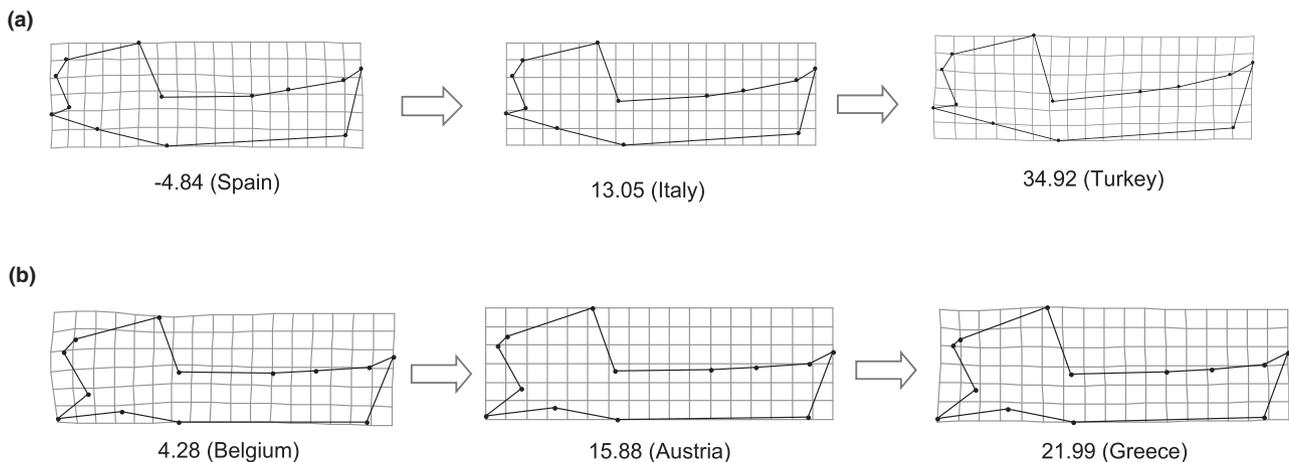


Figure 5 Deformation grids showing the mandible shape change in *Myotis daubentonii* (averaged individuals) and *Plecotus austriacus* (male averages) associated with longitude, from the most negative to the most positive locations.

analysed bat species even if sexual dimorphism in dietary habits has not been extensively analysed, yet. Work on *M. daubentonii* reported males to consume a wider variety of prey species than females (Vesterinen et al., 2013, 2016). It has been proposed that this pattern of diet sexual dimorphism is due to distinct foraging behaviour with males being capable of flying higher and longer, hence increasing their chance to feed on more diverse prey, although we found no mandible shape differences in this taxon.

We identified mandibular size dimorphism for both *M. daubentonii* and *P. austriacus* confirming observations based on body mass data with females being larger than males (Gaisler & Zúkal, 2004; Razgour, 2021). Sexual size dimorphism did not occur in the mandible sample of *N. noctula* and greater horseshoe bat. Budinski et al. (2015) suggested that distinct morphological structures under different sex-specific selective pressures may explain the absence of differences in cranium size between male and female of horseshoe bats, despite differences in body and wing size. This explanation may also apply to the *N. noctula* whose sexual dimorphism is generally small and varies from trait to trait. Lindecke et al., (2023) reported for *N. noctula* males from Austria longer crania and mandibles when compared to females, suggesting that regional variation might occur in this species characterised by a broad geographical range and high rate of female-biased migration (Lehnert et al., 2018).

Budinski et al. (2015) suggested that distinct morphological structures under different sex-specific selective pressures may explain the absence of differences in cranium size between male and female of horseshoe bats, despite differences in body and wing size. This explanation may also apply to the noctule whose sexual dimorphism is generally small and varies from trait to trait. Lindecke et al., (2023) reported that for noctules from Austria, males have longer crania and mandibles when compared with females suggesting that regional variation might occur in this species characterised by a broad geographical range and high rate of female-biased migration (Lehnert et al., 2018).

Our data only partially support the theory proposed by Williams and Findley (1979) for vespertilionid bats whose female tend to have higher energetic demands associated with maternity, hence bigger body size traits. More data are clearly needed to identify a unifying theory of sexual size and shape dimorphism in insectivorous bats, whose subtle sexual differences might change not only between species but also between populations.

Geographical variation

Although our total sample showed a significant impact of both latitude and longitude on mandibular size and shape, this pattern proved to be valid only for some species. The *N. noctula* mandibles were smaller at higher latitudes, contradicting the pattern expected from Bergmann's rule (Fig. 4). Interestingly, this result is equally supported by previous research on *R. ferrumequinum*, which also showed a negative association between other body dimensions and latitude (Jiang et al., 2019; Kryštufek, 1993). Bats have different ways to cope with thermoregulation which include wing vascularisation

and accumulation of body fat. This might prevent Bergmann's rule being detected in the skeletal morphology as recently demonstrated for other neotropical species (Castillo-Figueroa, 2022). Studies on *M. daubentonii* have shown increased size, based on cranial and dental measurements, from south to north (Bogdanowicz, 1990) and this pattern equally occurs in our mandibular sample although it does not emerge if sexes are analysed separately (Table 7).

Longitude also had a significant impact on mandible shape of *M. daubentonii* perhaps reflecting broader sampling variation from West to East rather than North to South. This trend could also be interpreted in relation to differences in dietary niche and feeding behaviour of the studied species. *M. daubentonii* is known to have a generalist diet and feeds on a wide variety of prey types (Beck, 1995; Flavin et al., 2001; Vesterinen et al., 2013), which may require a more adaptable mandible.

The significant negative impact of latitude on mandible size of *N. noctula* (particularly females) could reflect the differences in prey group proportions found across different regions. In Slovakia and the Czech Republic, for instance, *N. noctula* feeds mainly on Lepidoptera, followed by Diptera, Coleoptera and Araneida, while in Switzerland, the species feeds mainly on Trichoptera and Diptera, but switches to Coleoptera in Spring and Autumn when Trichoptera are rarer (Gloor et al., 1995; Kaňuch et al., 2005). *Rhinolophus ferrumequinum* had no significant effect of latitude or longitude on mandible size or shape, which could be the consequence of their highly specialised diet on Coleoptera (Beck, 1995).

For *P. austriacus*, we were only able to find a significant impact of longitude on the subsample of males. Why this pattern emerges in just the males remains to be clarified. This species has a consistent diet across various locations in Europe, with the largest proportion always consisting of Lepidoptera and the remainder primarily composed of Diptera with smaller proportions of other groups (Borg & Sammut, 2002; Feldman et al., 2000; Mayer & von Helverson, 2001; Razgour, 2012; Razgour et al., 2011; Riccucci & Lanza, 2018). The high proportion of moths varies seasonally and across regions with data from Central Europe supporting relatively less consumption of Lepidoptera when compared to populations from Switzerland and Turkey. Our sample of males included locations as far east as Greece where higher consumptions of Lepidoptera might be potentially expected. A relatively smaller condyle and a ventrally expanded angular process associated with relatively narrower gape (Herring & Herring, 1974) might potentially limit diet variation in east populations of *P. austriacus*.

We were unable to find any examples of past literature that directly link geographically influenced mammalian mandible morphology with a more generalist diet. However, there have been studies on non-mammalian species presenting varied results about the relationships between diet specialisation and morphology across different geographical locations. In fishes, research indicates that generalist species exhibited less variation in mouth morphology in diverse aquatic habitats compared to specialists (Smith & Skúlason, 1996). Similarly, in insects, a study on beetles revealed that geographical variation in shape

Table 7 Test for geographical influence on the mandible shape for each species with data averaged by location and after separating sexes

		d.f.	SS	MS	R ²	F	Z	Pr(>F)
<i>Myotis daubentonii</i> Avg	Latitude	1	0.001394	0.001394	0.04033	1.4866	0.98788	0.164
	Longitude	1	0.001804	0.001804	0.05219	1.9241	1.63893	0.049
	Latitude:Longitude	1	0.002213	0.002213	0.06403	2.3604	2.11282	0.015
	Residuals	30	0.028121	0.000937	0.81378			
	Total	33	0.034556					
<i>M. daubentonii</i> Female	Latitude	1	0.000939	0.000939	0.04888	0.954	0.10142	0.467
	Longitude	1	0.000971	0.000971	0.05055	0.9867	0.19908	0.419
	Latitude:Longitude	1	0.000944	0.000944	0.04915	0.9593	0.15338	0.435
	Residuals	15	0.014762	0.000984	0.76849			
	Total	18	0.019209					
<i>M. daubentonii</i> Male	Latitude	1	0.001135	0.001135	0.04112	0.8358	-0.047443	0.521
	Longitude	1	0.001048	0.001048	0.03797	0.7719	-0.22368	0.575
	Latitude:Longitude	1	0.00125	0.00125	0.04531	0.921	0.06736	0.459
	Residuals	17	0.023076	0.001357	0.83629			
	Total	20	0.027593					
<i>Plecotusaustriacus</i> Avg	Latitude	1	0.000682	0.000681	0.02332	0.8266	-0.18346	0.561
	Longitude	1	0.000831	0.000831	0.02845	1.0084	0.22461	0.402
	Latitude:Longitude	1	0.00096	0.00096	0.03285	1.1643	0.49829	0.321
	Residuals	32	0.026382	0.000824	0.90271			
	Total	35	0.029225					
<i>P. austriacus</i> Female	Latitude	1	0.000513	0.000512	0.02617	0.5516	-0.97392	0.828
	Longitude	1	0.0006	0.0006	0.03064	0.6458	-0.61603	0.738
	Latitude:Longitude	1	0.000668	0.000668	0.0341	0.7187	-0.42985	0.67
	Residuals	19	0.017651	0.000929	0.90153			
	Total	22	0.019579					
<i>P. austriacus</i> Male	Latitude	1	0.001736	0.001736	0.123	2.6559	2.935	0.003
	Longitude	1	0.001718	0.001718	0.12174	2.6286	2.8398	0.004
	Latitude:Longitude	1	0.00183	0.00183	0.12972	2.8008	3.0058	0.001
	Residuals	16	0.010455	0.000653	0.74102			
	Total	19	0.014109					
<i>Nyctalus noctula</i> Avg	Latitude	1	0.000885	0.000885	0.03715	1.4108	0.94958	0.179
	Longitude	1	0.000419	0.000419	0.01758	0.6678	-0.43604	0.643
	Latitude:Longitude	1	0.000391	0.000391	0.01644	0.6243	-0.6097	0.717
	Residuals	35	0.021946	0.000627	0.92158			
	Total	38	0.023813					
<i>N. noctula</i> Female	Latitude	1	0.000934	0.000934	0.06942	1.4254	0.90515	0.196
	Longitude	1	0.000891	0.000891	0.06622	1.3598	0.80613	0.228
	Latitude:Longitude	1	0.000902	0.000902	0.06701	1.3761	0.83082	0.222
	Residuals	17	0.011144	0.000656	0.82789			
	Total	20	0.013461					
<i>N. noctula</i> Male	Latitude	1	0.001019	0.001019	0.05662	1.6012	1.34293	0.092
	Longitude	1	0.000448	0.000448	0.02488	0.7037	-0.52655	0.694
	Latitude:Longitude	1	0.000394	0.000394	0.02191	0.6196	-0.83591	0.806
	Residuals	24	0.015273	0.000636	0.84863			
	Total	27	0.017997					
<i>Rhinolophus ferrumequinum</i> Avg	Latitude	1	0.000514	0.000514	0.05287	1.0836	0.30141	0.382
	Longitude	1	0.000467	0.000467	0.04805	0.9848	0.03903	0.49
	Latitude:Longitude	1	0.000555	0.000555	0.05707	1.1697	0.48745	0.306
	Residuals	16	0.007589	0.000474	0.78071			
	Total	19	0.00972					
<i>R. ferrumequinum</i> Female	Latitude	1	0.000997	0.000997	0.06281	1.2705	0.68138	0.248
	Longitude	1	0.000908	0.000908	0.05717	1.1564	0.55077	0.29
	Latitude:Longitude	1	0.000974	0.000974	0.06132	1.2404	0.66809	0.247
	Residuals	17	0.013345	0.000785	0.84037			
	Total	20	0.01588					

(continues)

Table 7 (Continued)

		d.f.	SS	MS	R^2	F	Z	$Pr(>F)$
<i>R. ferrumequinum</i> Male	Latitude	1	0.001019	0.001019	0.05662	1.6012	1.34293	0.092
	Longitude	1	0.000448	0.000448	0.02488	0.7037	-0.52655	0.694
	Latitude:Longitude	1	0.000394	0.000394	0.02191	0.6196	-0.83591	0.806
	Residuals	24	0.015273	0.000636	0.84863			
	Total	27	0.017997					

Significance in bold ($p < 0.05$).

of mandibles was more pronounced in generalist species than in specialists. (Emlen et al., 2005). These findings suggest that the relationship may vary across different animal groups.

In conclusion, our data show mandible shape differences in the studied insectivorous bat species potentially reflecting different hunting techniques and bite force production. Mandible size does not have a strong effect on shape variation, and conformity to the expected Bergmann's rule was found only in male *M. daubentonii*, while female *N. noctula* showed the opposite patterns of latitudinal size variation. A small degree of sexual dimorphism was found in the mandible shape of the greater horseshoe bat, while females of *M. daubentonii* and *P. austriacus* showed bigger mandibles than males. This subtle dimorphic pattern warrants caution in the general interpretation of geographical trends in mandibular morphologies of vespertilionid and rhinolophid bats. We found longitude to impact shape of male *P. austriacus* as well as the averaged shape of *M. daubentonii*. A complex interplay of factors might influence intraspecific morphological variation of insectivorous bat species and more detailed ecological information is needed to assess ecological variation between sexes and across populations.

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Author contributions

CM and GG developed the working hypotheses and data collection. GG collected the data. DC analysed and interpreted the data. DC and CM wrote and finalised the paper.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Details for specimens of study.