



Short Note

Mandibular and cranial modularity in the greater horseshoe bat *Rhinolophus ferrumequinum* (Chiroptera: Rhinolophidae)

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Abstract

We report the first evaluation of mandibular and cranial modularity in the greater horseshoe bat (*Rhinolophus ferrumequinum*). Although some authors found no modular pattern of these morphological structures in mammals, we discovered that traits integration in *R. ferrumequinum* is not uniform throughout the mandible and cranium, but structured into two distinct modules. Allometry does not affect mandibular and cranial modularity in *R. ferrumequinum* probably as a result of the low fraction of shape variation explained by size. Observed at the static level, mandible and cranium modularity in this species could be either functional or developmental or both, but we cannot rule out a genetic nature for underlying mandibular and cranial trait interactions. Evolutionary modularity among related taxa is influenced by genetic and functional modularity. Therefore, future studies on cranial integration and modularity at multiple levels of variation may shed more light on these important features of morphological variability in bats, including *R. ferrumequinum*.

Modularity is a property of biological systems to be built of units that are integrated internally and relatively independent from other such units (Cheverud, 1996; Wagner, 1996; Klingenberg, 2005). Processes contributing to morphological integration and modularity can be of developmental, functional, genetic and environmental nature. Although empirical studies have mainly focused on the level of variation among individuals from the same species and ontogenetic stage (static level), modularity can also be studied across multiple growth stages within particular species (ontogenetic level), as well as across multiple taxa at any given ontogenetic stage (evolutionary level) (Klingenberg, 2014). Morphological integration and modularity have important implications for the evolutionary potential of morphological traits. Therefore, investigations on the patterns of integration and modularity at defined levels and comparisons of these patterns across multiple levels can contribute significantly to knowledge about the processes involved in morphological integration and modularity as well as those responsible for morphological diversification at these levels.

Within mammals, modularity has most frequently been evaluated on the skull of rodents, carnivores, shrews and primates (Klingenberg, 2013). In bats there are only a few such studies, mostly conducted at the evolutionary level. One of them tested hypotheses of ecological versus developmental factors underlying patterns of within-species and evolutionary integration in the mandible of phyllostomid bats (Monteiro and Nogueira, 2010). Others examined different modularity hypotheses regarding the cranium of rhinolophids (Santana and Lofgren, 2013) and the palate in phyllostomids and pteropodids (Sorensen et al., 2014).

We investigated mandibular and cranial modularity in the static context, i.e. in adults of the greater horseshoe bat, *Rhinolophus ferrumequinum* (Schreber, 1774), one among the thirty bat species distributed in the territory of Serbia (Paunović et al., 2011; Budinski,

pers. comm.). According to previous studies of modularity in mammals, at least two functional and developmental units should be recognized within the mandible and cranium; the ascending ramus and alveolar region within the mandible; the basicranium and face within the cranium. However, some authors reported on the absence of a modular pattern of mandibular and cranial trait integration in mammals (for review see Klingenberg, 2013). Bearing in mind the discrepancies found in these previous studies, we tested whether the mandible and the cranium of *R. ferrumequinum* are integrated as a whole, or characterized by a two-module organization.

Using geometric morphometric approaches, we examined 208 mandibles and 181 crania from the collection of the Natural History Museum (Belgrade, Serbia). Budinski et al. (2015) had analyzed this sample of crania previously and the extra mandibles used herein originated from the same three geographic regions of the central Balkans considered there. We obtained digital images (4000 × 3000 pixels resolution) of mandibles in the labial view and crania in the ventral view using Canon PowerShot SX20 IS. Two-dimensional landmarks (15 on the right mandible and 18 on the right side of the cranium) were digitized with TpsDig software (Rohlf, 2010) (Fig. 1). We aligned the landmark coordinates using Generalized Procrustes Analysis (GPA) (Rohlf and Slice, 1990; Dryden and Mardia, 1998) to extract size (centroid size – CS) and shape (Procrustes coordinates) information from landmark data. However, by affecting all traits of the structure jointly, the influence of size on shape (allometry) can counteract modularity (Klingenberg, 2009). A previous study of the cranial variation of *R. ferrumequinum* reported a marked effect of allometry, together with significant sexual shape dimorphism and cranial shape differences among bats from different geographic regions (Budinski et al., 2015). To eliminate the influence of allometry we performed a multivariate regression of shape on size. Subsequently, residuals from this regression were used to generate pooled within-sex and geographic region cov-

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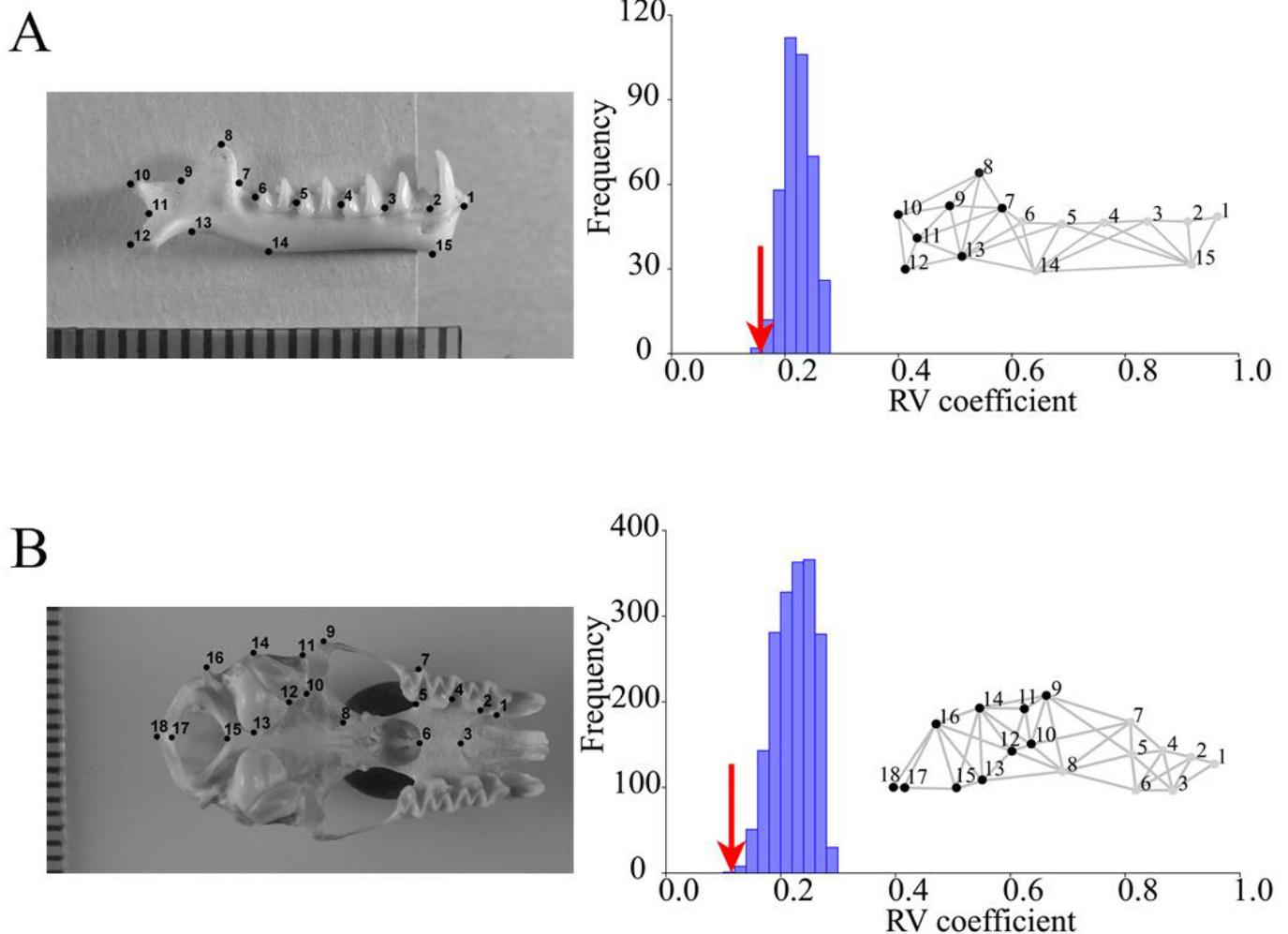


Figure 1 – Landmarks recorded on the analyzed morphological structures and evaluation of the a priori mandibular and cranial hypotheses of modularity in the greater horseshoe bat, *Rhinolophus ferrumequinum*. Partitions of (A) the mandible configuration into the ascending ramus (black dots) and alveolar region (grey dots) and (B) the cranium configuration into basicranial (black dots) and facial (grey dots) regions (including the adjacency graphs). The values of RV coefficients observed for the partition into hypothesized modules (mandible: RV=0.1569; cranium: RV=0.1142) and proportions (P) of partitions with RV lower than or equal to the a priori hypothesis (mandible: P=0.0052; cranium: P=0.0005) are indicated by arrows.

ariance matrix to remove the effects of these external factors (Klingenberg, 2009).

We tested the a priori hypotheses of modularity according to the procedure introduced by Klingenberg (2009). The mandible configuration was divided into subsets of 7 (ascending ramus) and 8 (alveolar region) landmarks (Fig. 1A), while cranium configuration was split into subsets of 10 (basicranium) and 8 (face) landmarks (Fig. 1B). We compared the strength of the association between the hypothesized modules, as estimated by the RV coefficient (Escoufier, 1973), to RVs obtained for all alternative partitions with the same number of landmarks as in the hypothesized modules. We used adjacency graphs (Fig. 1) to provide spatial contiguity of alternative partitions. The values of RV coefficient range from zero to one. The hypothesis of modularity is confirmed if the RV coefficient between the hypothesized modules is the lowest or is within the lower tail of the distribution of RVs observed for alternative partitions. To check whether allometry counteracts mandibular and cranial modularity in *R. ferrumequinum*, we retested the a priori hypotheses of modularity using pooled within-sex and geographic region covariance matrix of Procrustes coordinates (allometry-included shape variables). All analyses were carried out using MorphoJ software (Klingenberg, 2011).

The hypotheses of two-module organization were confirmed for both the mandible and the cranium (Fig. 1). For the mandible, two out of 386 alternative partitions had RVs lower than or equal to the RV coefficient calculated for the partition into the ascending ramus and alveolar region (Fig. 1A). For the cranium, only for one out of 1850 alternative partitions was the RV coefficient lower than or equal to that observed

for the partition into basicranial and facial regions (Fig. 1B). Modularity tests on allometry-included shape data gave similar results (mandible: RV = 0.1606, P = 0.0052; cranium: RV = 0.1236, P = 0.0005) indicating that allometry does not affect mandibular and cranial modularity. This is probably due to the low fraction of shape variation explained by size (Budinski et al., 2015). Within mammals, the hypotheses of two-module organization of the mandible and cranium tested herein have most frequently been examined in rodents, particularly in mice. While some of these studies failed to recognize the ascending ramus and alveolar region as separate modules, others documented the presence of several modules within the mandible or even the absence of mandibular modularity. However, the majority of them found evidence to support the ascending ramus-alveolar region hypothesis (see Jojić et al., 2012 and references therein). Exploring mandibular and cranial modularity in three phyllostomid bats (*Carollia perspicillata*, *C. brevicauda* and *C. castanea*), López Aguirre et al. (2015) observed that in *C. perspicillata* and *C. brevicauda* none of 10000 random partitions had RV coefficients lower than that observed for the partition into the ascending ramus and alveolar region. This undoubtedly confirmed the hypothesis of a two-module organization of the mandible in these bat species. As with mandibular modularity, numerous studies dealing with cranial modularity in rodents revealed contrasting results, with some supporting and others opposing the basicranium-face hypothesis (Klingenberg, 2013). To the best of our knowledge, there are only two studies that have investigated cranial modularity in bats (Santana and Lofgren, 2013; López Aguirre et al., 2015). Santana and Lofgren (2013) found strong evidence supporting the hypothesis that two sep-

arate modules, braincase and rostrum, can be recognized within the cranium from the ventral view in 22 rhinolophid species, as none of the 10000 random partitions had an RV coefficient lower than that observed for partition into the hypothesized modules. Contrary to this and our finding, the neurocranium-splanchnocranium hypothesis was rejected in each of the analyzed *Carollia* species (López Aguirre et al., 2015).

While Santana and Lofgren (2013) confirmed the hypothesis of mammalian cranial modularity for rhinolophid bats, they did not include *R. ferrumequinum*. Moreover, these authors studied cranial modularity at the evolutionary level. Patterns of morphological variation or covariation observed at the evolutionary level could be similar to or different from those observed at the static level. Although some investigators reported on the absence of a modular pattern of these morphological structures in mammals, we found that integration of traits in *R. ferrumequinum* is not uniform throughout the mandible and cranium, but structured into two distinct modules. Morphological traits are integrated with each other because they develop, function, inherit and evolve together (Olson and Miller, 1958; Cheverud, 1996; Klingenberg, 2008). By appropriate study designs, the processes responsible for morphological integration and modularity can be inferred from morphometric data (Klingenberg, 2013). From the design of our study (within species integration – static level), we can conclude that the observed mandibular and cranial modularity in *R. ferrumequinum*, i.e. the nature of underlying mandibular and cranial trait interactions, could be functional or developmental or of both kinds. Since developmental interactions can mediate the expression of genetic variation in phenotypic traits and genetic variation can influence developmental modularity (Klingenberg, 2005, 2008), a relationship exists between developmental and genetic modularity. Consequently, the genetic nature of underlying mandibular and cranial trait interactions in *R. ferrumequinum* cannot be ruled out. Both genetic and functional modularity influence evolutionary modularity among related taxa. Therefore, future studies of cranial integration and modularity at multiple levels of variation and their comparison across levels (Klingenberg, 2013, 2014) may shed more light on these important features of morphological variability in bats, including *R. ferrumequinum*. ☞

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