

Geometric morphometrics in herpetology: modern tools for enhancing the study of morphological variation in amphibians and reptiles

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The use of geometric morphometrics for studying phenotypic variation in amphibians and reptiles has visibly increased in the last decade. These modern tools provide a robust statistical framework to study organismal shape while preserving the geometric properties of the studied structures and thus improve our capacity for investigating patterns of morphological variation, and understanding their ecological and historical causes. Their application in herpetology has shed new light to the remarkable diversity observed among extant and extinct amphibians and reptiles. Here I first briefly consider the historical emergence of geometric morphometric methods, trying to provide a practical guide for herpetologists interested in implementing these tools to their investigation. I then review the wide array of published studies using geometric morphometrics to investigate morphological patterns in amphibians and reptiles. Across different investigation fields, an emergent pattern is the existence of general similarities, but also profound differences, among members of higher taxonomic groups. Size-shape allometry is a common pattern in many groups, but remarkable variation of allometric trajectories exists among closely related taxa. Sexual dimorphism has been extensively studied in reptiles, but less so in amphibians, while the contrary is true for phenotypic plasticity. The use of geometric morphometrics has allowed the detection of potentially adaptive shape patterns and the investigation of their causes. Finally, these methods have been invaluable in the study of fossils and have provided a better understanding of the paleobiology of extinct taxa.

Key words: adaptation; allometry; geometric morphometrics; paleontology; phenotypic plasticity; sexual dimorphism.

Morfometría geométrica en herpetología: nuevas herramientas para promover el estudio de la variación morfológica en anfibios y reptiles. El uso de la morfometría geométrica para estudiar la variación fenotípica en anfibios y reptiles ha aumentado notablemente durante la última década. Esta nueva herramienta proporciona un marco estadístico sólido para estudiar la forma de los organismos preservando las propiedades geométricas de las estructuras analizadas, mejorando así la comprensión de los factores ecológicos e históricos que explican los patrones de variación morfológica. Su aplicación en herpetología proporciona una nueva forma de explorar la diversidad morfológica de anfibios y reptiles tanto actuales como extintos. En esta revisión comienzo examinando la secuencia histórica que llevó a la aparición de la morfometría geométrica, tratando de ofrecer también una guía práctica para aquellos herpetólogos interesados en incorporar esta herramienta en su investigación. Después reviso un amplio muestrario de trabajos en los que la morfometría geométrica se usa para estudiar patrones morfológicos en anfibios y reptiles. Una pauta que emerge repetidamente es la existencia de similitudes generales, pero también de profundas diferencias, entre los miembros de los grupos taxonómicos de mayor rango. La existencia de una relación alométrica entre tamaño y forma es común en muchos grupos, pero también se observa una variabilidad considerable en las trayectorias alométricas entre taxones hermanados. El dimorfismo sexual se ha estudiado extensivamente en reptiles, pero no tanto en anfibios, mientras que con la plasticidad fenotípica ocurre lo contrario. El uso de la morfometría geométrica permite la detección de variaciones adaptativas en los patrones morfológicos y la investigación de sus causas. Finalmente, estos métodos tienen un valor incalculable para el estudio de organismos fósiles y proporcionan una mejor comprensión de su paleobiología.

Key words: adaptación; alometría; dimorfismo sexual; morfometría geométrica; paleontología; plasticidad fenotípica.

Morphology is undoubtedly one of the main components of an organism's phenotype. As such, morphological traits have always been at the centre of attention of biological research, comprising important pieces of evidence for a wide variety of investigation fields. From traditional taxonomy and the modern school of systematics, through physiology, development, ecology, biogeography, and all the way to modern evolutionary biology, practically all biological fields include questions and hypotheses related to how morphological variation emerges and how it is distributed across temporal and spatial scales. Geometric morphometrics (GM) revolutionised the way we measure, study and perceive morphological diversity (ROHLF & MARCUS, 1993; ADAMS *et al.*, 2004). By establishing a solid mathematical basis for the study of organismal shape, while preserving the geometric properties of the structures of interest, GM methods provide a powerful tool for depicting and studying morphological variation in a more realistic and integrated manner than previous morphometric tools (BOOKSTEIN, 1996; ROHLF, 2000a). Amphibians and reptiles, being excellent model organisms, have been the subject of extensive morphological research, often serving as paradigmatic cases in the study of phenotypic variation. Accordingly, over the past two decades, herpetologists have taken advantage of the modern toolkit of GM to further enhance our understanding of the astonishing morphological variety that exists across amphibian and reptile taxa. Since the early development of GM tools, most extant – and several extinct – herpetofaunal groups have been investigated using these methods.

This review aims at putting together an up-to-date account of how GM methods have been implemented in herpetology until today, also providing a comprehensive basis for herpetologists interested in exploring these methods in their research. Although I have tried to include all studies to which I had access and provide examples from all herpetofaunal groups, this is not meant to be an exhaustive account from a taxonomical perspective, but rather to give insight on how GM methods have advanced our understanding of the patterns and processes underlying the phenotypic diversification of our study organisms.

GEOMETRIC MORPHOMETRICS: WHY AND HOW?

The birth of GM or how GM is different

Morphometrics, the quantitative study of biological shape variation and its covariation with other variables (BOOKSTEIN, 1991; DRYDEN & MARDIA, 1998), may be seen as the successful outcome of the long-standing interest in organismal form. Ever since Aristotle biologists have been intrigued by the huge diversity of forms we encounter in nature and the causes and processes that create it. However, such interest was only put on a quantitative basis with the statistical advances made during the 19th and 20th centuries, which led to the emergence of biometry as a formal discipline (SOKAL & ROHLF, 1995; SLICE, 2005; MITTEROECKER & GUNZ, 2009). The development of statistical tools for analysing multivariate data opened a new door to the description and study of complex phenotypes. This was achieved through the quantification of a number of linear distances,

counts, ratios or angles, describing the properties of a morphological trait of interest (ADAMS *et al.*, 2004; SLICE, 2005). The application of multivariate statistics on the above biometric variables then allowed for the testing of specific biological hypotheses about an organism's multivariate phenotype (i.e. "traditional morphometrics"; MARCUS, 1990). However, morphometrics still suffered some shortcomings which troubled morphometricians and urged for solutions. These problems deserve our attention, since their resolution was the basic motivation for the development of the field of GM and they provide direct insight into how this new methodology differs from traditional morphometrics.

The birth of GM is tightly linked to four considerations related to biological form: size, homology, shape description and visualisation. With size and its effect on other morphological traits being of central importance to the evolution of all living organisms (GOULD, 1966), it was soon evident that in order to study biological form, a full mathematical definition of size was in order, due both to practical and to theoretical reasons (BOOKSTEIN, 1989a; SLICE, 2005). Numerous solutions were proposed (see ROHLF & BOOKSTEIN, 1987 for review and comparison), but no solid argument could definitely support the use of a single method. A similar problem existed as to the homology of the studied traits, in the sense of the operational, reproducible definition of the quantities to be measured and compared (BOOKSTEIN, 1982; SLICE, 2005). Yet an additional concern regarded the selection of variables used for shape description; since the geometric relationships between linear measurements were not included in the dataset, one could neither predict

nor guarantee the statistical differentiation of shapes known to be different (BOOKSTEIN, 1982, 1996; SLICE, 2005). Finally, since the geometry of the studied objects was not captured, morphometric data could not be effectively used to directly visualise shape variation related to other biological variables of interest (ADAMS *et al.*, 2004). All the above adversities led to the development of GM methods.

What is GM and how to use it

The "revolution in morphometrics" (ROHLF & MARCUS, 1993) started by advances in the application of outline and landmark tools for the geometrical study of organismal shape (BOOKSTEIN, 1986; ROHLF, 1986) and the simultaneous development of statistical theory for shape analysis (KENDALL, 1984, 1985). The combination of landmark techniques for capturing organismal form with a newly introduced, statistically robust shape theory led to the growth of a set of morphometric methods that preserved the geometric properties of the studied objects, namely GM (SLICE, 2005; MITTEROECKER & GUNZ, 2009). The raw data used for GM consist of outline data describing the bounding edge of the structure of interest or, more frequently, of Cartesian coordinates of landmark locations (SLICE, 2005; MITTEROECKER & GUNZ, 2009). Outline data were the first to be used, but they were later largely abandoned, especially after the introduction of semi-landmark methods that incorporate boundary curve information directly into landmark-based analyses (BOOKSTEIN, 1997; MITTEROECKER & GUNZ, 2009). Since two- and three-dimensional landmark-based GM methods are more frequently used today, I will concen-

trate on these methods for a practical review. However, the interested reader can explore the published bibliography on outline methods (see among others ROHLF & ARCHIE, 1984; FERSON *et al.*, 1985; ROHLF, 1986, 1990; ADAMS *et al.*, 2004).

Landmark-based GM analysis begins with the definition of landmarks (Table 1, Fig. 1a). Simplistic as this may seem, it is a central part of biological inference: “Landmarks are the points at which one’s explanations of biological processes are grounded” (BOOKSTEIN, 1991). It is through the definition of landmarks, based on biological intuition and previous observation of the organisms of interest, that the biologist will manage to fully capture the shape of interest. Once landmarks have been defined, these are digitised in a number

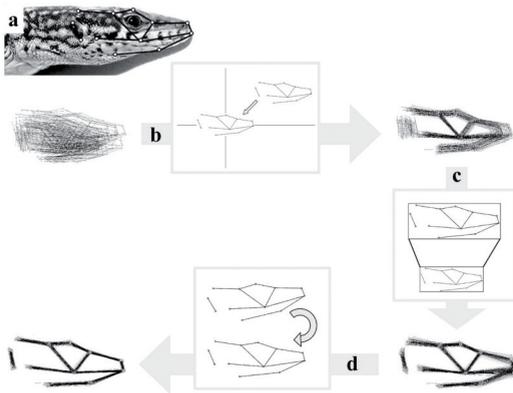


Figure 1: Removing non-shape variation from landmark coordinates through Generalized Procrustes Analysis (GPA: ROHLF & SLICE, 1990). Once landmarks have been defined (a) and digitised in a number of specimens, their coordinates are first translated (b) by placing their centroid to the origin of a Cartesian system. Then they are scaled (c) to unit centroid size. Finally, they are rotated (d) using a least-squares criterion. This way landmark coordinates are superimposed to a common coordinate system and non-shape variation is removed.

of specimens, resulting in a collection of Cartesian coordinates. These coordinates still include non-shape information (size, orientation and position) and need to be mathematically processed in order to obtain shape variables. The dominant procedure today uses shape variables lying in Kendall’s shape space (or most frequently an approximation in a space tangent to the mean shape of the studied sample), Procrustes distance being the associated metric (Table 1). While several methods were proposed in the past for obtaining shape variables from landmark coordinates, methods using Kendall’s space have been proven to be the most powerful and statistically robust (ROHLF, 1999, 2000a,b, 2003). In order to obtain shape variables, landmark configurations are first superimposed using a least-squares procedure, namely Generalized Procrustes Analysis (GPA, Table 1, ROHLF & SLICE, 1990). GPA removes variation due to digitizing location, scale and orientation through the optimal translation (Fig. 1b), scaling (Fig. 1c) and rotation (Fig. 1d) of landmark coordinates. The specimen points aligned through this procedure can then be projected into a linear shape space tangent to Kendall’s shape space (Table 1; ROHLF, 1999; SLICE, 2001, 2005; MITTEROECKER & GUNZ, 2009), where the Euclidean distances between observations closely approximate Procrustes distances in Kendall’s space. One should be aware that, in addition to GPA, several other superimposition techniques exist, with potential benefits in particular datasets (SIEGEL & BENSON, 1982; BOOKSTEIN, 1991; ZELDITCH *et al.*, 2004; SLICE, 2005).

Once non-shape variation has been removed, the superimposed landmark coordinates of specimens not differing in shape will per-

Table 1: Glossary of terms frequently used in geometric morphometrics*.

Term	Description
Allometry	Shape change associated to size change.
Centroid size	The size measure used in GM. It is calculated as the square root of the sum of squared distances of each landmark from the centroid of the landmark configuration.
Consensus configuration	A landmark configuration intended to represent the central tendency (for example mean shape) of an observed sample. Often a consensus configuration is computed to optimize some measure of fit to the full sample: in particular, the Procrustes mean shape is computed to minimize the sum of squared Procrustes distances from the consensus landmarks to those of the sample.
Deformation grid	The visual representation of a shape transformation as modelled using the thin-plate spline, based on D'Arcy Thompson's idea of using grids to represent a shape difference.
Generalized Procrustes Analysis (GPA)	A generalized superimposition method that works by minimising the partial Procrustes distance over all sampled shapes by a least-squares fitting procedure.
Kendall's shape space	The geometric setting for analyses of Procrustes distances among arbitrary sets of landmarks. Each point in this shape space represents the shape of a configuration of points in some Euclidean space, irrespective of size, position, and orientation.
Landmark	A specific point on a biological form or image of a form located according to some rule. BOOKSTEIN (1991) recognised three categories of landmarks, depending on the criteria used for their definition: <i>Type I landmarks</i> correspond to points clearly defined by some local property, such as the juxtaposition of different tissues; <i>Type II landmarks</i> are defined purely based on geometric, not biological, properties, such as the maximum curvature of a structure; finally, <i>Type III landmarks</i> are the less robustly defined ones, since they are located in relation to other points in the structure. Type III landmarks can be incorporated in GM analyses, but are not in fact considered true landmarks and caution should be taken when using them.
Partial warps	An auxiliary structure for the interpretation of shape changes and shape variation in sets of landmarks. They are eigenvectors of the bending energy matrix that describes the net local information in a deformation along each coordinate axis.
Principal warps	Eigenfunctions of the bending-energy matrix interpreted as actual warped surfaces (thin-plate splines) over the picture of the original landmark configuration.
Procrustes distance	Approximately, the square root of the sum of squared differences between the positions of the landmarks in two optimally (by least-squares) superimposed configurations at centroid size. This is the metric for Kendall's shape space, and thus the distance measure used in GM.
Procrustes residuals	The set of vectors connecting the landmarks of a specimen to corresponding landmarks in the consensus configuration after a Procrustes fit. The sum of squared lengths of these vectors is approximately the squared Procrustes distance between the specimen and the consensus in Kendall's shape space. The partial warp scores are an orthogonal rotation of the full set of these residuals.
Relative warps	Principal components of partial warp scores. In a relative warps analysis, the parameter α can be used to weight shape variation by the geometric scale of shape differences.
Shape	The geometric properties of a configuration of points that are invariant to changes in translation, rotation, and scale.
Thin-plate spline	An interpolation function used to model the difference in shape between two objects by minimising the bending energy of the deformation. It provides a unique solution to the construction of D'Arcy Thompson-type deformation grids for data in the form of two landmark configurations.

* Compiled and augmented from SLICE *et al.* (1996) and ZELDITCH *et al.* (2004).

fectly coincide. In turn, specimens of different shape will present at least some differences in landmark positions. The larger the shape difference between specimens, the larger the difference in the positions of homologous landmarks after superimposition. Such shape difference is quantified through the Procrustes distance metric, which allows for statistical comparisons and hypothesis testing (SLICE, 2005; MITTEROECKER & GUNZ, 2009). In this sense, then, Procrustes residuals (i.e. landmark coordinates after superimposition) can be used as shape variables to investigate shape variation. However, Procrustes residuals suffer the statistical adversities of not being a full-rank set of variables (due to superimposition) and of being non-Euclidean in nature, which frequently complicates their statistical treatment since they cannot be subjected to analysis using linear models. While this adversity can be overcome through projection into a tangent, Euclidean space, the usual approach is to perform a series of mathematical operations to model shape variation. This is done using the thin-plate spline. The thin-plate spline is an interpolation technique borrowed for use in morphometrics from the fields of computational surface theory and computer graphics (BOOKSTEIN, 1989b, 1991). Imagine that the shape of interest, represented by a configuration of landmarks, lies on an infinitely thin, flat, metal plate of infinite size. The change into another shape can be obtained through a set of vertical displacements of the metal plate in a direction perpendicular to its surface, one Cartesian coordinate at a time. By minimising the energy necessary to bend the metal plate between two shapes (bending energy) we obtain a criterion for parsimoniously describing and modelling shape chan-

ge. The descriptors resulting from the application of the thin-plate spline are partial warps (Table 1), which are in fact the eigenvectors of the bending-energy matrix and are orthogonal components describing shape variation according to spatial scale. The partial warp scores (together with the uniform components of shape variation) of each individual can then be used as shape variables for multivariate statistical analyses (SLICE, 2005).

In addition to providing Euclidean shape variables for statistical hypothesis testing, the thin-plate spline is an essential tool for visualising shape variation in an integrated and intuitive manner (SLICE, 2005). Since the thin-plate spline is in fact an interpolation function, it can be used to map the deformation in shape between two objects (BOOKSTEIN, 1991). This is done through a mathematically formal realization of D'Arcy Thompson's idea of transformation grids (THOMPSON, 1917), a solution long sought by morphometricians (BOOKSTEIN, 1996). These maps of shape change, referred to as deformation grids (Table 1), use a visual representation of a wire mesh to depict the bending necessary to transform one shape into another, a procedure known as warping (Fig. 2). This is one of the most important advances provided by GM methods: since the geometry of the studied objects is preserved throughout the analysis, shape differences between objects can be directly described in terms of differences in the deformation grids representing these objects (ADAMS *et al.*, 2004; SLICE, 2005; MITTEROECKER & GUNZ, 2009). Several software packages are available for conducting all the above GM analyses, performing statistical comparisons and visualising the results (see Appendix 1).

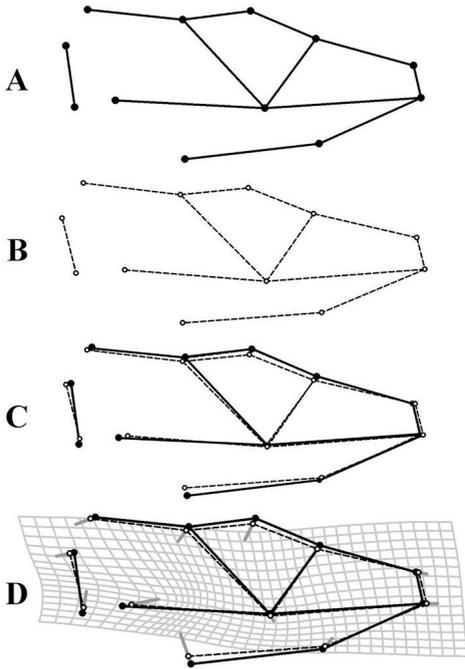


Figure 2: How the thin-plate spline can be used to visualize shape differences found from landmark-based GM methods. In this example, shapes A and B are being compared. Once landmark configurations have been aligned through superimposition (C), shape differences between them can be visualised as the deformation caused on a wire mesh which is bended from one shape to the other (D). Linear vectors on the landmarks represent the direction of change for each of them from shape A into shape B (exaggerated five-fold to enhance visualisation).

**GM IN HERPETOLOGY:
A TAXONOMICAL ACCOUNT**

Herpetologists have been increasingly using GM techniques to study morphological variation in amphibians and reptiles over the last decade. Focusing on the studies considered here, a visible increase in the use of GM methods for studying extant or extinct amphibians and reptiles is observed after the

year 2000, from less than five publications per year between 2000 and 2003 to an average of about 13 publications per year after 2007 (Fig. 3). Among amphibians, both anurans and urodeles have been investigated, but GM methods have not yet been applied – to the best of my knowledge – for studying caecilians. Among reptiles, saurians are the most studied group, with a total of 25 publications, followed by chelonians (19 studies). Other groups are visibly less explored, with only three studies in snakes and three in crocodiles, while, as far as I am aware, amphisbaenians are still to be studied. GM tools are also extensively used for studying extinct amphibian and reptile taxa, with an important contribution to the total number of studies (Fig. 3) and remarkable results (see below).

**STUDYING PHENOTYPIC VARIATION:
FIELDS OF APPLICATION**

The integrated study of shape through GM motivated an explosion of morphological investigation in amphibians and reptiles and expanded our knowledge of patterns of variation and their causes. In the following section, I provide a question-based review of published studies, aiming at describing general shape patterns observed across herpetofaunal taxa and discussing their potential causes as seen by herpetologists.

Allometric patterns: shape change due to size

GM tools have been a cornerstone contribution to the study of development and ontogenetic shape change. By enhancing shape quantification and shape change visualisation, GM methods have provided the possibility not only

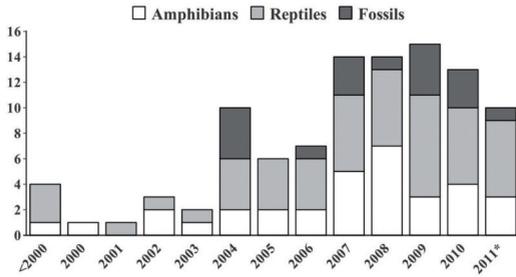


Figure 3: Yearly evolution of the number of publications using GM methods to analyse morphological variation in extant and extinct amphibians and reptiles. Data retrieved from Google Scholar and Isi Web of Science. *Records concerning 2011 include studies published or in press until July 31.

of accurately describing complex shapes, but also of understanding exactly how shape changes throughout an organism's development (LAWING & POLLY, 2010). Most studies investigating allometric relationships, both in an ontogenetic and static context of allometry (i.e. GOULD, 1966), indicate that large part of the shape variation observed is attributable to size-shape allometry (see MONTEIRO & ABE, 1997; BIRCH, 1999; BONNAN, 2007; SMITH & COLLYER, 2008; PIRAS *et al.*, 2010; CHIARI & CLAUDE, 2011; IVANOVIĆ *et al.*, 2011 for illustrative examples from different groups). Caution is advised to avoid conceptual misunderstandings: while GM methods remove size variation through GPA or similar superimposition procedures, the allometric dependence of shape on size still remains in the data and can be investigated (MONTEIRO, 1999). Statistically, since shape variables produced through GM techniques are size-free, any significant association with size (either represented by centroid size or any other size variable) indicates deviation from isometry (ZELDITCH *et al.*, 2004).

Extensive research about ontogenetic shape change at different developmental stages has

been carried out mainly in amphibians, revealing both similarities and profound differences among groups. Pre-metamorphic shape ontogeny of the chondrocranium in anurans seems to generally follow a common pattern, at least in species of the genera *Bufo*, *Pelodytes*, *Rana* and *Telmatobius* (LARSON 2002, 2004, 2005; CANDIOTI, 2008; GARRIGA & LLORENTE, in press). In these anuran species, general patterns of skull development include the reduction of the sensory capsules and a hypermetric or isometric growth of trophic structures (LARSON, 2002, 2004), in line with predictions made for all tetrapods (EMERSON & BRAMBLE, 1993). Interestingly, these studies also attest that the development of the chondrocranium does not seem to be tightly linked to that of the hind limb, thus rendering Gosner stages – a developmental staging system frequently used in anurans (GOSNER, 1960) – a relatively poor indicator of chondrocranial differentiation (LARSON, 2002). Similarly, species of newts studied show generally congruent patterns, the skull base and rostral portion being the areas more profoundly modified throughout ontogeny (IVANOVIĆ *et al.*, 2007, 2008). However, while general trends appear relatively uniform, allometric trajectories frequently vary among closely related species (LARSON, 2005; IVANOVIĆ *et al.*, 2007, 2008), providing a potentially important mechanism of morphological differentiation, both in extant and extinct taxa (WITZMANN *et al.*, 2009). The same is true for body shape variation before and through metamorphosis in newts (VAN BUSKIRK, 2009; IVANOVIĆ *et al.*, 2011), although further studies should investigate the generality of the results obtained for *Triturus*, since remarkable variation of allometric patterns has been observed in some cases (VAN

BUSKIRK, 2009). Finally, caution should be taken when extrapolating between groups or even populations of the same species, since allometric trajectories of amphibian shape have been found to present radical modifications in both plastic and adaptive responses to environmental variation (see below).

Allometric shape variation has been also extensively investigated in reptiles, again revealing concordance of general trends, but also significant variation between closely related species. In lizards, the general pattern of skull allometry, both in an ontogenetic (MONTEIRO & ABE, 1997; KALIONTZOPOULOU *et al.*, 2008; RAIA *et al.*, 2010) and static (BRUNER & COSTANTINI, 2007; COSTANTINI *et al.*, 2010; LJUBISAVLJEVIĆ *et al.*, 2011; ZUFFI *et al.*, 2011) context, includes a relative shortening of the anterior area and an enlargement of the posterior region. Interestingly, some studies have reported a lack of an allometric relationship between head shape and size in some lizard species (VIDAL *et al.*, 2005, 2006); however, these results should be considered with caution, since in the above studies, allometry was investigated by bivariate regression of the first principal component of shape variation on centroid size, thus potentially providing an incomplete view of allometric patterns. In fact, multivariate regression of all shape variables (i.e. partial warps) on centroid size or other size measures are better suited for investigating size-shape relationships in a GM context (KLINGENBERG, 1996; MONTEIRO, 1999), since size may significantly contribute to the observed shape patterns even without being the main source of variation (captured by principal components analysis). As for amphibians, allometric trajectories of the skull have been found to vary extensively among species

(LJUBISAVLJEVIĆ *et al.*, 2010), but intraspecific variation seems to be less common.

Regarding other reptile groups, size variation also seems to be a main determinant of shape variation in turtles, including ontogenetic, static and evolutionary allometric effects (CLAUDE *et al.*, 2003, 2004; DEPECKER *et al.*, 2006; ANGIELCZYK, 2007; MYERS *et al.*, 2007; NISHIZAWA *et al.*, 2010; ANGIELCZYK *et al.*, 2011; CHIARI & CLAUDE, 2011). Different characters show varying degrees of variation in allometric trajectories. For example, evolutionary allometry of skull shape seems to be constrained, and similar to intraspecific allometry (CLAUDE *et al.*, 2004), while extensive variation is observed between ecologically distinct groups in the shoulder girdle (DEPECKER *et al.*, 2006). The trait most frequently studied in turtles, the shell, also shows varying degrees of allometric modifications. For instance, CHIARI & CLAUDE (2011) described substantial modification of growth trajectories between two closely related lineages of Galápagos tortoises, while the same seems to be the case for the miniaturised species of emydine turtles (ANGIELCZYK, 2007). Other reptile groups have been less investigated; however, extensive variation seems to exist in skull ontogenetic patterns of crocodiles (MONTEIRO *et al.*, 1997; PIRAS *et al.*, 2010), while size variation seems to be a moderate source of skull shape variation as compared to other factors in the rhynchocephalians (JONES, 2008).

Sexual dimorphism

Sexual dimorphism (SD) is a ubiquitous feature of many animal taxa and the application of GM has importantly enhanced our understanding of the proximate and evolution-

nary causes of shape SD. Surprisingly, studies using GM methods to address SD in amphibians are very limited. Some studies investigated the effect of sex on shape and reported significant SD (IVANOVIĆ *et al.*, 2007, 2008, 2009), but in these cases sex was treated as a side variable, rather than being the main focus of interest. This markedly contrasts with the extensive investigation of shape SD using GM in reptiles, and particularly in lizards. As is true for allometric patterns (see above), the analysis of lizard SD using GM has focused mainly on head and skull shape and has revealed a general resemblance of global patterns, but also significant variation across groups, although the number of studies is visibly skewed towards the lacertids. Indeed, head/skull shape is sexually dimorphic in all lacertid lizards that have been examined (i.e. *Algyroides*: LJUBISAVLJEVIĆ *et al.*, 2011; *Dalmatolacerta* and *Dinarolacerta*: LJUBISAVLJEVIĆ *et al.*, 2010; *Lacerta*: BRUNER *et al.*, 2005; COSTANTINI *et al.* 2007; *Podarcis*: KALIONTZOPOULOU *et al.*, 2007, 2008; LJUBISAVLJEVIĆ *et al.*, 2010; RAIA *et al.*, 2010). In all of the aforementioned genera, sexual shape variation is mainly located at the posterior region of the head, males always presenting a more enlarged parietal (dorsally) and tympanic (laterally) areas as compared to females (Fig. 4). The same trend is also observed in other phylogenetically disparate lizard groups, such as iguanids of the genus *Liolaemus* (VIDAL *et al.*, 2005) and *Tarentola* geckos (ZUFFI *et al.*, 2011). Additionally, in all the above examples, investigation of the proximate causes of shape SD indicated that males and females follow common allometric slopes when considering the relationship between head shape and size (as represented by centroid size). Interestingly,

however, when size variation is taken into account, some studies indicate size-independent differentiation of head shape between the sexes (i.e. difference of allometric regression intercepts, KALIONTZOPOULOU *et al.*, 2008), while others indicate size SD as the only source of shape SD (BRUNER *et al.*, 2005; LJUBISAVLJEVIĆ *et al.*, 2010). This, together with the variation of allometric slopes observed among closely related species (Ljubisavljević *et al.*, 2010) and the complete lack of head shape dimorphism observed in some instances (MONTEIRO & Abe, 1997), indicates that important variation may exist across species, urging for further investigation.

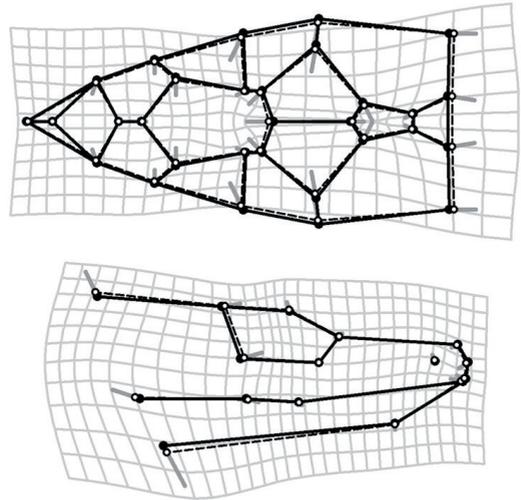


Figure 4: Typical pattern of head shape sexual dimorphism in lacertid lizards, characterised by a relative enlargement of the posterior region and reduction of the anterior area in males. In this case, the transformation of female (filled symbols, continuous line) to male (open symbols, dashed line) shape in *Podarcis bocagei* is represented through deformation grids for the dorsal (top) and lateral (bottom) view of the head (modified from KALIONTZOPOULOU *et al.*, 2008). Shape differences are exaggerated five-fold to enhance visualisation.

A promising line of research may be the elucidation of the functional significance of head shape SD as captured by GM methods, in order to provide further evidence to the long-standing hypothesis of sexual selection acting on bite force as the main determinant of the observed morphological patterns (HERREL *et al.*, 2007; HUYGHE *et al.*, 2009; KALIONTZOPOULOU *et al.*, in press).

The investigation of SD using GM is relatively limited in turtles and snakes, but some general conclusions may be drawn. In turtles, some studies have reported significant SD in shell shape, which is the only trait that has been examined. However, there is a marked discordance among authors regarding the comparison between traditional and geometric morphometrics for quantifying shape in turtles; while some authors use GM as a powerful method for capturing patterns invisible to traditional approaches, such as SD in hatchlings (VALENZUELA *et al.*, 2004; LUBIANA & FERREIRA JÚNIOR, 2009), others report a weaker SD in geometric shape as compared to that observed using linear measurements (CHIARI & CLAUDE, 2011). Such a disagreement may be due to differences in the degree and direction of shape SD in different taxa, a possibility supported by the limited data available (VALENZUELA *et al.*, 2004; CEBALLOS & VALENZUELA, 2011). A different pattern is observed in snakes, although the reduced number of studies once again hinders the extraction of definite conclusions. The available studies indicate that head/skull shape SD in snakes is non-existent or of relatively low importance, at least as compared to other sources of variation, such as geographic locality (MANIER, 2004; SMITH & COLLYER, 2008) or phylogenetic signal (GENTILI *et al.*, 2009). Clearly, patterns of

shape SD and its variation in turtles and snakes needs to be further investigated in the future.

Shape evolution: adaptation and phenotypic plasticity

The search for causal factors that may explain the extensive morphological variation we observe in many animal groups has always been a fascinating field of investigation. GM methods have enhanced our capacity of describing shape variation and associating it with both ecological factors and performance measures, thus providing evidence of the adaptive potential of certain shape traits. Extensive herpetological research has focused on the search for such variation, shedding new light on the way amphibians and reptiles respond morphologically to environmental disparity.

GM-based studies of the evolution of turtle shell shape provide an exemplar system to the study of adaptation. The shell represents a fundamental component of the turtle phenotype and it is recognised as one of the most remarkable novelties among tetrapods (BURKE, 1989). Moreover, the shape of the shell has been shown to present an important heritable component, thus holding strong evolutionary potential (MYERS *et al.*, 2006). At the same time, turtles have diversified to occupy a wide range of ecological niches, while preserving a basic body plan, thus constituting exceptional model organisms for studying the consequences of ecological diversification on morphological traits. Several components of habitat use have been shown to directly influence turtle body shape. The differentiation between aquatic and terrestrial life is undoubtedly one of the main causes of turtle phenotypic diversification, being reflected in the shape of the carapace (CLAUDE

et al., 2003; RIVERA & CLAUDE, 2008; STAYTON, 2011), the plastron (CLAUDE *et al.*, 2003; ANGIELCZYK *et al.*, 2011), the skull (CLAUDE *et al.*, 2004) and the shoulder articulation (DEPECKER *et al.*, 2006). Diet is an additional niche dimension involved in skull differentiation in turtles (CLAUDE *et al.*, 2004), while anti-predatory strategies, specifically as represented by plastral kinesis (PRITCHARD, 2008), seem to be a main factor of shell differentiation, at least among emydines (ANGIELCZYK *et al.*, 2011). By contrast, phylogenetic inertia seems to play a subsidiary role in turtle shape differentiation, at least in comparison with ecological factors (CLAUDE *et al.*, 2003, 2004; ANGIELCZYK *et al.*, 2011). Although less frequent, intraspecific studies of shape variation also support the importance of habitat effects for turtle morphology. Not only does the shell of aquatic turtles differ from that of terrestrial ones, it is also highly susceptible to the characteristics of water flow. Indeed, RIVERA (2008) showed that freshwater turtles of the genus *Pseudemys* inhabiting fast-flowing water regimes present a significantly more streamlined shell, while those inhabiting slow-flowing regimes are more domed (Fig. 5). Further supporting an adaptive explanation for the observed

patterns, the shape typical of fast-flowing regimes importantly reduced drag during swimming. Interestingly, a trade-off was also shown to exist between this hydrodynamic efficiency and mechanical strength. Flattened, hydrodynamic shells were more fragile (RIVERA & STAYTON, 2011), a pattern observed also between aquatic and terrestrial emydids (STAYTON, 2011). The functional relevance of shell shape has also been confirmed in terms of swimming speed, where slider turtles with a relatively wider and shorter plastron attained higher speeds than elongated ones (MYERS *et al.*, 2007).

A wide range of studies have also used GM to decipher the effects of environmental variation on the morphology of amphibians. As is common for this group (WELLS, 2007), multiple studies support the existence of extensive phenotypic plasticity in both head and body shape. Temperature (JORGENSEN & SHEIL, 2008), predation risk (JOHNSON *et al.*, 2008; VAN BUSKIRK, 2009; HOSSIE *et al.*, 2010), competition (GARRIGA & LLORENTE, in press), as well as numerous other habitat components (VAN BUSKIRK, 2009) have been shown to produce plastic responses on larval body shape and ontogenetic shape allometry. Furthermore, body shape has been shown to

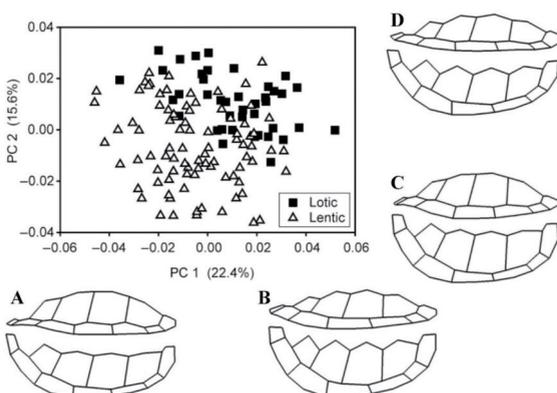


Figure 5: Three-dimensional shell shape variation observed between male *Pseudemys* turtles inhabiting fast-flowing and slow-flowing water environments, as visualised through a PCA analysis of the three-dimensional coordinates of the carapace. Individuals of both sexes from lotic habitats are more stream-lined, a shape modification that has also been shown to reduce drag, providing empirical evidence for an adaptive causation of the observed differences (modified with permission from RIVERA, 2008). Deformation grids represent the extreme values observed across the first (A, B) and second (C, D) principal component axes.

be tightly related to swimming performance in anuran larvae (DAYTON *et al.*, 2005; ARENDT, 2010), although the plastic shape modifications observed due to predation do not seem to enhance speed, but may rather be related to tail lure anti-predator display (JOHNSON *et al.*, 2008).

While plasticity studies are dominated by anurans, the investigation of evolutionary shape change as a response to environmental factors is clearly skewed towards salamanders, and particularly those of the genus *Plethodon*, which have served as an important model system for the evolutionary application of GM tools. Character displacement due to competition has been shown to be a major force of head shape diversification in this genus, which has been associated to both biomechanical (ADAMS & ROHLF, 2000) and behavioural (ADAMS, 2004) modifications. Undoubtedly, head shape is a trait with strong evolutionary potential in this salamander group, an observation corroborated by the existence of an important heritable component (ADAMS, 2011). However, the detailed studies conducted also reveal that competitive interactions, while repeatable across space when the same pair of species is involved (ADAMS, 2010), are also characterised by important variability (ADAMS *et al.*, 2007; ARIF *et al.*, 2007; MYERS & ADAMS, 2008), indicating that extrapolation from one pair of species to another can be problematic. While species interactions seem to be of central importance in shaping morphological variation in *Plethodon*, other factors are also involved. For example, MAERZ *et al.* (2006) reported significant intraspecific variation associated to trophic polymorphism. Diet also seems to be significantly associated to shape

variation of the hyobranchial skeleton of anuran larvae (CANDIOTI, 2006). Other patterns of shape variation in salamanders concern potential adaptation to structural environment, where shape variation among closely related taxa also involves the modification of shape allometries. In a very interesting contrast of evolutionary patterns, JAEKEL & WAKE (2007) and ADAMS & NISTRÌ (2010) respectively described how divergence or convergence of ontogenetic allometries can be modified to produce foot shapes that match the structural environments of *Bolitoglossa* and *Hydromantes* salamanders. These two examples nicely illustrate how GM can be used to describe potentially adaptive morphological variation (foot webbing) and associate it to the functional advantages gained by certain shape modifications (capacity for climbing), while also remarking on the importance of ontogenetic trajectories for understanding the evolution of shape – and other – traits (KLINGENBERG, 2010).

Several examples also illustrate the ecomorphological relevance of head/skull shape in lizards. While visibly less integrated than the examples on *Plethodon* above, the available studies provide evidence to the importance of different ecological factors for shape evolution in various lizard taxa. In an insightful theoretical consideration of convergence in multiple niche dimensions, HARMON *et al.* (2005) used GM on head shape in *Anolis* lizards, together with other character sets, to test the hypothesis that if multidimensional convergence really occurs in response to different aspects of the environment, different character systems will show different patterns of convergence among species. In fact, character systems differed and variation in head shape was suggested to be

due to differences in diet, aggressive or anti-predatory behaviour among habitat types (HARMON *et al.*, 2005). The hypothesis that diet may profoundly influence head shape in lizards is also supported by the observation that parallel and convergent evolution occurs between groups specialised in a certain type of diet (STAYTON, 2005, 2006), while the same observation stands for crocodiles (PIERCE *et al.*, 2008). Nevertheless, the strength of such an influence seems to vary across taxonomic levels, since similar studies among gekkotans (DAZA *et al.*, 2009) and the rhynchocephalians (JONES, 2008) indicate that, although head shape is associated to diet, phylogenetic affinity visibly dominates over feeding behaviour as a factor of skull shape differentiation. Considering other factors, both habitat type (KALIONTZOPOULOU *et al.*, 2010) and insularity (BĂNCILĂ *et al.*, 2010; RAIA *et al.*, 2010) have been shown to influence head shape in lacertid lizards, but these observations should be further tested in other lizard groups.

Systematics, taxonomy and phylogenetic signal

Due to their increased effectiveness for capturing shape variation, GM methods have been used for species discrimination and for describing morphological variation between closely related, and in many cases cryptic, taxa. For instance, LEACHÉ *et al.* (2009) used GM on cranial horn shape of the coast horned lizard species complex, combined with a large number of other biologically meaningful traits, to characterise the process of lineage formation in this group. In a similar approach, CHIARI & CLAUDE (2011) used GM to study carapace size and shape variation in Galápagos tortoises and confirmed the morphological differentia-

tion between two genetically distinct lineages, which had been described to differ morphologically using linear methods. GM tools have also been used to analyse intraspecific geographic variation (MANIER, 2004; VIDAL *et al.*, 2005; CLEMENTE-CARVALHO *et al.*, 2008; SMITH & COLLYER, 2008), investigate the strength of phylogenetic signal in shape data (GENTILLI *et al.*, 2009) and examine the degree of concordance between phylogenetic relatedness and morphological similarity (IVANOVIĆ *et al.*, 2008, 2009). From a purely taxonomical perspective, JAMNICZKY & RUSSELL (2004) used GM to investigate the “batagurine process”, a potential diagnostic character of the turtle family Bataguridae, while VIEIRA *et al.* (2008) investigated the morphological differentiation between colour morphs of a toad population to examine the taxonomical implications of the observed polymorphism.

While the above studies pose biologically meaningful questions and most use GM in a statistically robust framework to test specific hypotheses, the use of shape characters in phylogeny and systematics has been questioned extensively (ADAMS & ROSENBERG, 1998; ROHLF, 1998; KLINGENBERG & GIDASZEWSKI, 2010) and caution is advised when moving in this area of investigation. This predicament lies on both practical and theoretical grounds and is mainly associated to the use of shape variables (either partial warps or their principal components) as cladistic characters (ADAMS *et al.*, 2011). The main difficulty presented is that of transforming continuously varied, multivariate data as shape into discrete character states for parsimony inference (ROHLF, 1998; MONTEIRO, 2000). Additional problems regard the effect of a reference form, which is of central importance to the operations necessary to obtain shape

variables, and which has been repeatedly shown to deeply influence the results obtained, thus rendering GM shape variables of doubtful usefulness for phylogenetic inference (BOOKSTEIN, 1994; ADAMS & ROSENBERG, 1998). This of course does not mean that organismal shape as described by GM methods cannot be analysed in a phylogenetic context, or used to investigate the morphological affinity of closely related species and compare it to their known phylogenetic relationships. Moreover, GM methods often provide a useful tool for obtaining further evidence to support or reject phylogenetic hypotheses (as implemented for example in PIRAS *et al.*, 2010) or to complement the evidence provided by molecular studies (CLEMENTE-CARVALHO *et al.*, 2011) but caution should be taken for correct implementation.

Paleontology

GM-based methods have been of great utility in paleontology, providing an innovative view of morphological patterns in extinct amphibian and reptile taxa (ELEWA, 2004). Practically all the aforementioned fields have partners in paleontological research, which implement GM as a powerful tool to obtain shape data from samples that frequently suffer in terms of structural quality (BASZIO & WEBER, 2002; ANGIELCZYK & SHEETS, 2007). New methods have been developed for the identification of fossils and used to specify the structural position of paleontological findings in snakes (POLLY & HEAD, 2004), as well as being implemented as an indirect tool of estimating the size of extinct taxa (HEAD *et al.*, 2009). Furthermore, GM methods have aided the investigation of sexual dimorphism in archosaurs, providing evidence for potentially

dimorphic structures (BARDEN & MAIDMENT, 2011) and allowing corroboration of the results through comparison with their extant relatives (i.e. *Alligator*; PRIETO-MARQUEZ *et al.*, 2007; BONNAN *et al.*, 2008). Numerous studies have taken advantage of the tool-kit of GM to characterise morphological disparity patterns and investigate their temporal variation and to examine macroevolutionary trends (CANUDO & CUENCA-BESCÓS, 2004; STAYTON & RUTA, 2006; PIERCE *et al.*, 2009a; YOUNG & LARVAN, 2010; YOUNG *et al.* 2010). As for extant groups, shape variation has also been investigated in the light of its functional implications, thus providing a deeper understanding of the paleobiology and paleoecology of archosaur taxa (BONNAN, 2004, 2007; PIERCE *et al.*, 2009b; BONNAN *et al.*, 2010). Finally, a very interesting contribution in terms of the originality of the studied shapes is that of RODRIGUES & FARIA DOS SANTOS (2004), who used GM to investigate the variation of sauropod tracks.

Amphibians and reptiles as models for the development of new methods

The multivariate nature of GM data frequently challenges statistical methods and has stimulated morphometricians to extend existing methods for studying complex shapes. Apart from addressing biological questions, several authors have used amphibians and reptiles as model organisms to develop new methodological approaches that utilise the toolbox of GM and further enhance our capacity of investigating morphological variation. For example, MONTEIRO (1999) used GM data on sexual and ontogenetic variation of the skull of tegu lizards to provide a comprehensive review of how multivariate regression tech-

niques can be implemented to understand the processes and causes underlying shape changes. Along the same lines, MAGWENE (2001) used turtle shells as an example to illustrate how growth fields as characterised by a set of growth vectors could be studied and compared in order to understand the developmental processes involved in ontogenetic shape change. In a conceptually similar approach, COLLYER & ADAMS (2007) examined character displacement in *Plethodon* salamanders (ADAMS, 2004) to exemplify the use of shape change vectors for the study of two-state multivariate phenotypic change, an approach later generalized to multi-state phenotypic trajectories (ADAMS & COLLYER, 2009). Also trying to characterise the direction of phenotypic change, STAYTON (2006) proposed a new method for quantifying data disparity patterns and used it to test for convergence in skull shape among herbivorous lizards. In the field of quantitative genetics of shape, MYERS *et al.* (2006) generalized the univariate approximation of shape heritability based on Procrustes distance (MONTEIRO *et al.*, 2002) for application with unequal sample sizes among families, thus complementing other existing multivariate methods (i.e. KLINGENBERG, 2003; KLINGENBERG & MONTEIRO, 2005), and used it to study patterns of plastron shape heritability in slider turtles. A very promising methodology for the field of biomechanics was put forward by PIERCE *et al.* (2008) and STAYTON (2009), who joined shape theory and finite element models to study the mechanical properties of the crocodilian skull and three-dimensional turtle shell shape correspondingly. Finally, although not directly linked to the study of shape variation, it is worth mentioning the use of GM tools as a means of standardising specimen position for

studying colour patterns in salamanders (ASHLOCK *et al.*, 2003; COSTA *et al.*, 2009).

CONCLUDING REMARKS

The aforementioned studies illustrate the wide range of questions for which GM may be implemented, facilitating the integrated study of shape variation and its causes using amphibians and reptiles as model organisms. Many of these studies have compared traditional morphometric methods to GM and have reached the conclusion that GM techniques are frequently more powerful than linear measurement data for detecting and describing organismal shape variation (VALENZUELA *et al.*, 2004; BONNAN *et al.*, 2008; KALIONTZOPOULOU *et al.*, 2008; ARENDT, 2010). This is both an advantage and a potential pitfall. On one hand, GM methods are expected to be statistically more powerful in detecting subtle shape variation, since the number of variables analysed is usually much higher than that examined in traditional morphometrics. On the other, caution should be taken in the implementation of such a sensitive tool, to keep with strong biological inference and avoid missing focus of biological hypotheses. As modern technological resources become increasingly available for use in biological sciences, a vast amount of new techniques for obtaining shape data can be explored, including for example computerised tomography (CT) scans and three-dimensional surface scanning. Herpetologists have until now taken full advantage of the technical and statistical tools available for the analysis of shape variation, providing new insights to the evolution of shape and frequently put-

ting forward new methods for data analysis. Nevertheless, the exploration of GM methods for understanding shape variation in amphibians and reptiles is still an open field, with promising perspectives for future contributions. I hope the above review has provided an informed view of the questions that have been explored and the answers obtained, and point to directions for further inquiry.

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APPENDIX 1: SOFTWARE RESOURCES

Several software packages have been developed for the application of GM methods, including data acquisition (digitising landmarks), obtaining shape variables and performing exploratory analyses and hypothesis-testing. A detailed account of all available software, as well as numerous other useful resources, can be found in the SUNY Morphometrics webpage developed and maintained by E.J. Rohlf (<http://life.bio.sunysb.edu/morph>). All programs are freely available for download. Here I briefly provide an account of the main software packages more frequently used in recent publications. Apart from the software mentioned below, all GM procedures can also be carried out in R language (R DEVELOPMENT CORE TEAM, 2010), with an extremely high flexibility for data visualisation and analysis. The book "Morphometrics with R" (CLAUDE, 2008) is the essential guide for this purpose.

The tps series (ROHLF, 2011): Developed since the early days of GM, this is a series of software packages that aid the user in treating different aspects related to the acquisition and analysis of GM data. Rather than an integrated package, this is a series of programs, each thought for carrying out specific operations or answering relevant biological questions. In this sense, the tps programs are organised conceptually, depending on the operations or statistical analyses of interest. Different programs provide utility operations (tpsUtil) and landmark acquisition (tpsDig), superimposition methods (tpsSuper), modelling shape variation through the thin-plate spline and related methods (tpsRelW; tpsSplin) and a wide array of more specific

exploratory and statistical procedures such as the visualization of thin-plate splines on trees (tpsTree), regression of shape onto independent variables and regression-related hypothesis testing (tpsRegr) and two-block partial least squares analysis (tpsPLS). Although some users might find the separation in different programs troublesome, this is a very question-driven software series, specifically designed for answering shape-related questions and accompanied by extremely thorough help pages that provide the user with both a conceptual and mathematical understanding of the operations involved. Both 2D and 3D analyses are supported. Designed for use in Windows, but will run without problems in Linux using Wine.

MorphoJ (KLINGENBERG, 2011): A user-friendly integrated software package that provides a platform for the most important types of analyses usually carried out with GM data. These include 2D and 3D Procrustes superimposition with and without object symmetry, utility operations, generation of covariance matrices, matrix correlation, principal components analysis, two-block partial least squares, canonical variate and linear discriminant analyses, regression analysis, mapping shapes onto a phylogeny and calculation of phylogenetic independent contrasts and analyses related to the quantitative genetics of shape. Written in Java, it is a practically platform-independent program that will run on all Windows, Macintosh OS X and Linux.

IMP (SHEETS, 2000): This is a set of six basic and several other auxiliary programs for analysing GM data. It will perform all the usual GM operations, including the generation of shape coordinates, principal components analysis, canonical variate analysis,

shape regression and pair-wise multivariate shape comparisons. Both 2D and 3D analyses are supported. It is based on MATLAB, but will work without this software being installed on the computer. It is a Windows-based program, but will apparently run well on MAC and Linux through emulators.

Morphologika (O' HIGGINS & JONES, 2006): A set of integrated tools for examining size and shape variation among objects described by configurations of both 2D and 3D landmark coordinates. It enables generalised Procrustes fitting of configurations, tangent

space projection, PCA of shape or size and shape, multivariate regression of shape on an independent variable, visualization of size and shape variations by warping of the mean or computation of transformation grids. As for the tps-series, shape variables can be exported and more mainstream multivariate analyses are to be carried out in external statistical software. Although still frequently used, *morphologika* is no longer maintained and has rather been replaced by the EVAN toolbox (<http://www.evan-society.org>), which is more oriented to 3D shape analysis.