

APPLICATION OF LANDMARK MORPHOMETRICS TO SKULLS REPRESENTING THE ORDERS OF LIVING MAMMALS

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ABSTRACT Statistical analyses of geometric morphometric data have been generally restricted to the Euclidean space tangent to curved shape space. This approach is based on the knowledge that such an approximation does not affect statistical and biological conclusions, when differences among specimens' shapes are not too large. We examined the wide variation of shapes within the vertebrate class Mammalia to determine the tangent space approximation by comparing Procrustes distances in Kendall shape space to tangent space distances among 53 mammal skulls and articulated jaws belonging to almost all of the living orders. Previous studies have been restricted to relatively low taxonomic levels, implying a narrower range of shapes.

Thirty-five three-dimensional (3D) landmarks on the sagittal plane and right side of each specimen were digitized using a MicroScribe 3DX. Procrustes and tangent space distances between all specimens were compared using the program TPSSMALL (Rohlf, 1998b). The correlations between these distances were always greater than 0.99. Lower jaw and brain subsets of the landmarks gave similar results, while the face subset had more scatter, but nearly the same correlation. The 3D shapes, as summarized by the landmarks, were clustered and the dendrogram was compared to a currently hypothesized phylogeny. We also point out that data from landmark morphometrics are as appropriate as morphological and molecular data for cladistic analysis.

INTRODUCTION

A firm foundation for landmark morphometrics has now been built (Bookstein, 1998; Marcus *et al.* 1996; Dryden and Mardia, 1998) and we are more confident than ever on how to proceed in the analysis of biological shape data (Bookstein, 1996). Procrustes distances in curved shape space give us statistics for describing and comparing shapes of organisms. Statistical inference in curved shape space is difficult, but an approximation—tangent space, tangent at the

mean specimen or consensus—has provided an adjustment of data so that classical multivariate statistical analyses of shapes based on the aligned landmark coordinates are appropriate (Rohlf and Marcus, 1993; Rohlf, this volume; Dryden and Mardia, 1998).

While most biological objects are three dimensional, it is frequently satisfactory to look at 2D projections or 2D sections. It is relatively easy to collect two-dimensional data using imaging, or photographs and digitizing tablets. For larger specimens, three-

dimensional digitizers have now become more affordable (Dean, 1996; Marcus et al. 1997; Corner et al., 1992), but it is still expensive and difficult to collect 3D data for small organisms (Dean, 1996 and Fadda and Corti, this volume).

Informal investigation of the distortions in shape introduced due to using tangent space and speculation about all possible shape differences suggests that tangent space is a good approximation for shape space when the shape dispersion in a given study is small enough (Dryden and Mardia, 1998; MacLeod, 1999). The ensuing multivariate analyses of shapes and inferences based on the statistics proceed in the same way as for traditional morphometrics (Marcus, 1990), with some adjustments for the alignment process (Rohlf, 1998a).

We examined the relationship between tangent space and curved shape space using a set of representative skulls of almost all of the orders of living mammals. We believed this data would provide a good test for the tangent space approximation, as the digitized shapes encompass the upper limit of shape variation typical of most analyses. Up to now landmark morphometrics studies have looked at variation within species, genera or at most families of vertebrates and a few other groups. Seldom have higher taxonomic levels been investigated (Marcus *et al.*, 1996), with the exception of the comparison of Loricarioid catfish by Schaefer and Lauder (1996). Greater differences in shape might occur in growth studies, but so far such studies have been restricted to growth of fish, and from neonates to adults for mammals (Zelditch et al., 1995; Zelditch et al., 1993, Monteiro et al., 1999; Hingst-Zaher et al., this volume). A major problem in studying shapes that are extremely different—either among taxa or among growth stages—is that as the shapes become more diverse, the number of equivalent landmarks decreases. Therefore there may be relatively few comparable and equivalent land-

marks available in a study encompassing a broad diversity of shape (see Results and Discussion for the “homologous” status of landmarks).

Our goals in this study are twofold: 1) to test how closely curved shape space distances are approximated by tangent space distances for missing orders a data set includes highly diverse **skull** shapes; and 2) to evaluate the phylogenetic signal in the landmark data set.

MATERIAL AND METHODS

We sampled a total of 23 ordinal mammalian groups as recognized by McKenna and Bell (1997), or Wilson and Reeder (1993). The four missing orders Paucituberculata (rat opossums), Notoryctemorphia (marsupial moles), Chrysochloridea (golden moles), and Scandentia (tree shrews) that we were not able to record, were rather small for the device we used, very rare, or we could not easily find all of the landmarks. Our data included landmarks for 53 specimens (Table 1). A representative sample of skull shapes were included from some orders exhibiting a greater diversity in skull shape.

We used a MicroScribe 3DX to digitize the landmarks with a repeatability of about 0.25 mm (Marcus et al., 1997). While the manufacturer claims a maximum size range of 1 meter, practical limitations are for objects up to 600 mm (in maximum diameter). These boundary conditions limited our ability to record coordinates on very large and very small skulls. We selected adult specimens for all orders except Proboscidea, for which a young elephant (*Elephus maximus*) was measured as the representative. The smallest specimen digitized was the marsupial *Dromiciops gliroides*, (Monito del Monte) with a maximum distance between landmarks of 31 mm., while the largest was a perissodactyl, *Tapirus terrestris* (South American tapir) with a maximum of 498 mm.

We designated 35 landmarks to cover as much as possible of the sagittal plane and

Table 1. Specimens digitized. Note the number appended to the common name is the sequence in the data base, and the numbers used in Figures 8 and 9. Common names follow Nowak (1991). A = adult; Y = young.

Classification = Order, Family. except when indicated

Classification by Wilson and Reeder	Classification by McKenna and Bell	Species	No. and Common name	AMNH.	Locality	sex	age
Monotremata, Tachyglossidae	Tachyglossa, Tachyglossidae	<i>Zaglossus bruijni</i>	8 Long nosed echidna	190862	New Guinea	?	A
Monotremata, Ornithorhynchidae	Platypoda, Ornithorhynchidae	<i>Ornithorhynchus anatinus</i>	9 Duck billed platypus	200255	no data	?	A
Didelphimorphia, Didelphidae	Didelphimorphia, Didelphidae	<i>Didelphis albiventris</i>	5 Opossum	39006	Rolovia	m	A
Microbiotheria, Microbiotheriidae	(Superorder) Microbiotheria, Microbiotheriidae	<i>Dromiciops gliroides</i>	7 Monito del Monte	92147	Chile	f	A
Dasyuromorphia, Thylacinidae	(Grandorder) Dasyuromorphia, Thylacinidae	<i>Thylacinus cynocephalus</i>	2 Tasmanian wolf	35822	zoo	?	A
Dasyuromorphia, Dasyuridae	(Grandorder) Dasyuromorphia, Dasyuridae	<i>Sarcophilus laniarius</i>	3 Tasmanian devil	65673	Tasmania	f	A
Peramelemorphia, Peramelidae	Peramelia, Peramelidae	<i>Isodon macrourus</i>	6 Short nosed bandicoot	104486	New Guinea	m	A
Diprotodontia, Phascolarctidae	Diprotodontia, Phascolarctidae	<i>Phascolarctos cinereus</i>	4 Koala	173704	Australia	?	A
Diprotodontia, Vomhatidae	Diprotodontia, Vomhatidae	<i>Vombatus ursinus</i>	1 Common wombat	200234	Australia	?	A
Xenarthra, Bradypodidae	Pilosa, Bradypodidae	<i>Bradypus variegatus</i>	39 Three-toed tree sloth	32700	Colombia	m	A
Xenarthra, Megalonychidae	Pilosa, Megalonychidae	<i>Choloepus didactylus</i>	38 Two-toed tree sloth	60648	Ecuador	?	A
Xenarthra, Dasypodidae	Cingulata, Dasypodidae	<i>Dasypus novemcinctus</i>	40 Long nosed armadillo	93116	Brazil	m	A
Xenarthra, Myrmecophagidae	Pilosa, Myrmecophagidae	<i>Myrmecophaga tridactyla</i>	42 Giant anteater	133489	Brazil	m	A
Xenarthra, Myrmecophagidae	Pilosa, Myrmecophagidae	<i>Tamandua tetradactyla</i>	41 Lesser anteater	176664	Mexico	?	A
Insectivora, Solcnodontidae	Soricomorpha, Solenodontidae	<i>Solenodon paradoxus</i>	49 Solenodon	77752	Dominican R.	m	A
Insectivora, Tenrecidae	Soricomorpha, Tenrecidae	<i>Tenrec ecaudatus</i>	50 Tenrec	212913	no data	?	A
Insectivora, Erinaceidae	Erinaceomorpha, Erinaceidae	<i>Erinaceus amurensis</i>	51 Hedgehog	57219	China	m	A
Dermoptera, Cynocephalidae	Primates, Galeopithecidae	<i>Cynocephalus volans</i>	52 Colugo, Flying lemur	187861	Philippines	f	A
Chiroptera, Pteropodidae	Chiroptera, Pteropodidae	<i>Pteropus vampyrus</i>	36 Flying fox	102064	Java	f	A
Chiroptera, Phyllostomidae	Chiroptera, Phyllostomidae	<i>Vampyrum spectrum</i>	37 Spectral bat	267446	French Guianam		A
Primates, Indridae	Primates, Indridae	<i>Propithecus verreauxi</i>	27 Sifaka	100540	Madagascar	m	A
Primates, Cchidae	Primates, Atelidae	<i>Aotus azarae</i>	28 Night monkey	211457	Bolivia	m	A
Primates, Cercopithecidae	Primates, Cercopithecidae	<i>Papio hamadryas</i>	26 Baboon	89365	Africa	?	A
Primates, Hominiidae	Primates, Hominiad	<i>Gorilla gorilla</i>	25 Gorila	167334	Africa	m	A
Carnivora, Felidae	Carnivora, Felidae	<i>Acynonix jubatus</i>	19 Cheetah	35998	Kenya	?	A
Carnivora, Herpestidae	Carnivora, Herpestidae	<i>Suricata suricatta</i>	16 Suricate	81756	S. Africa	m	A
Carnivora, Hyaenidae	Carnivora, Hyaenidae	<i>Proteles cristatus</i>	17 Aardwolf	173512	no data	?	A
Carnivora, Mustelidae	Carivora, Mustelidae	<i>Enhydra lutris</i>	20 Sea otter	146618	Alaska	?	A
Carnicora, Odoheniidae	Carnivora, Phocidae	<i>Odobenus rosmarus</i>	23 Walrus	14069	no data	?	Y

Classification = Order, Family, except when indicated

Classification by Wilson and Reeder	Classification by McKenna and Bell	Species	No. and Common name	AMNH.	Locality	sex	age
Carnivora, Phocidae	Carnivora, Phocidae	<i>Phoca vitulina</i>	22 Harbor seal	232406	Mass.	♂	A
Carnivora, Otariidae	Carnivora, Otariidae	<i>Callorhinus ursinus</i>	24 Northern fur seal	77796	zoo	?	A
Carnivora, Procyonidae	Carnivora, Procyonidae	<i>Procyon cancrivorus</i>	21 Raccoon	96119	Brazil	?	A
Carnivora, Ursidae	Carnivora, Ursidae	<i>Ailuropoda melanoleuca</i>	14 Giant panda	110454	China	?	A
Carnivora, Ursidae	Carnivora, Procyonidae	<i>Ailurus fulgens</i>	18 Lesser panda	110455	W. China	?	A
Carnivora, Ursidae	Carnivora, Ursidae	<i>Ursus americanus</i>	15 American black bear	41329	Alaska	?	A
Cetacea, Delphinidae	Cete, Delphinidae	<i>Delphinus delphis</i>	46 Common dolphin	77931	aquarium	?	A
Sirenia, Dugongidae	Uranotheria, Dugongidae	<i>Dugong dugon</i>	34 Dugong	88392	Adaman Isl.	?	A
Sirenia, Trichechidae	Uranotheria, Trichechidae	<i>Trichechus manatus</i>	35 Manatee	70363	aquarium	?	Y
Proboscidea, Elephantidae	Uranotheria, Elephantidae	<i>Elephas maximus</i>	48 Asiatic elephant	70266	no data	f	Y
Perissodactyla, Equidae	Perissodactyla, Equidae	<i>Equus zebra</i>	33 Mountain zebra	82326	Zululand	?	A
Perissodactyla, Tapiridae	Perissodactyla, Tapiridae	<i>Tapirus terrestris</i>	32 South American tapir	70322	zoo	?	A
Hyracoidea, Procaviidae	Uranotheria, Procaviidae	<i>Heterohyrax brucei</i>	47 Gray hyrax	165758	Namibia	f	A
Tubulidentata, Orycteropodidae	Tubulidentata, Orycteropodidae	<i>Orycteropus afer</i>	45 Aardvark	150398	LOO	?	A
Artiodactyla, Suidae	Artiodactyla, Suidae	<i>Babrousa babyrussa</i>	31 Babirusa	152851	Celebes	m	A
Artiodactyla, Hippopotamidae	Artiodactyla, Hippopotamidae	<i>Hexaprotodon liberiensis</i>	29 Pygmy Hippopotamus	146849	zoo	f	A
Artiodactyla, Camelidae	Artiodactyla, Camelidae	<i>Lama glama</i>	30 Llama	248739	Bolivia	m	A
Pholidota, Manidae	Cinolesta, Manidae	<i>Manis gigantea</i>	44 Pangolin	53848	Africa	?	A
Rodentia, Castoridae	Rodentia, Castoridae	<i>Castor canadensis</i>	13 Beaver	77848	no data	♂	A
Rodentia, Muridae	Rodentia, Muridae	<i>Ondatra zibethicus</i>	12 Muskrat	270055	Pennsylvania	m	A
Rodentia, Hystricidae	Rodentia, Hystricidae	<i>Hystrix africae</i>	10 African porcupine	70360	S. Africa	f	A
Rodentia, Hydrochaeridae	Rodentia, Hydrochaeridae	<i>Hydrochaeris hydrochaeris</i>	11 Capybara	75888	Brazil	u	A
Lagomorpha, Leporidae	Lagomorpha, Leporidae	<i>Lepus arcticus</i>	43 Arctic hare	42140	no data	u	A
Macroscelidea, Macroscelididae	(Mirorder) Macroscelidea, Macroscelididae	<i>Rhynchocyon cirnei</i>	53 Checkered Elephant shrew	49447	Zaire	m	A

Table 2. Details of Landmarks (see Figure 1).

Description of landmarks for the Orders of Mammals (Letters after numbers refer to Jaw, Face, and Braincase subsets)

Mandible (right mandibles digitized in all cases):

- 1J. anterior end of the symphysis between the dentary bones, ventral surface.
- 2J. posterior end of the symphysis between the dentary bones, ventral surface.
- 3J. top of the coronoid process.
- 4J. lower point of the arch between coronoid and condyloid processes
- 5J. posteroventral tip of the angular process
- 65. most anterior border of mental foramen (when multiple, most anterior foramen)

Skull, on sagittal plane.

- 7B. rearmost point of interparietal-(inion)
- 8B. frontal-parietal suture.
- 9F. frontal-nasal suture.
- 10F. anterior end of suture between nasal bones.
- 11F. anterior point of suture between the premaxillaries, at the anteriormost portion of the roof of the palate.
- 12F. premaxilla-maxillae suture, on the palate.
- 13F. maxilla-palatine suture.
- 14F. rear most point of palatines.
- 15B. sphenoccipital suture.
- 16B. anterior edge of foramen magnum
- 17B. posterior edge of foramen magnum

Skull, right side.

- 18F. middle of anterior edge of incisive foramen.
 - 19F. middle of posterior edge of incisive foramen.
 - 20F. premaxillary-maxillary suture on the palatal-facial border
 - 21F. posterior tip of premaxilla, where it meets the nasal.
 - 22E. middle of posterior-external edge of infraorbital canal.
 - 23F. most anterior point on ventral surface of the jugal
 - 24F. most anterior point on the dorsal surface of the jugal
 - 25B. triple point of squamosal, parietal and frontal suture, or middle of the line formed between parietal and frontal when the superior squamosal is large.
 - 265. Anterior-posterior and mid-lateral center of the glenoid fossa
 - 27B. middle of superior edge of auditory canal.
 - 28J. inferior tip of the zygomatic process of the pterigoid
 - 29B. inferior tip of the paraoccipital process
 - 30B. most exterior lateral point of the the occipital condyle
 - 31B. most lateral point on the edge of the foramen magnum
 - 32B. Hypoglossal canal for the 12th nerve
 - 33F. most anterior point on the orbital-facial border.
 - 34F. middle point at the anterior edge of the lacrimal foramen.
 - 35B. optic foramen.
-

right side of the skull (including the attached lower jaw) from dorsal, ventral, and lateral perspectives. After digitizing lower jaw landmarks, the jaw was disarticulated from the skull to allow sampling points on the palate and glenoid fossa (Figure 1). Six landmarks were taken on the jaw, 13 landmarks on the braincase, and 16 on the face (Table 2, and Fig 1). Thirteen landmarks were on the saggital plane (or nearly so) and the remaining 22 were on the right side of the skull. Classifying by type, according to O'Higgins and Jones (1998): 16 of the 35 are type I, biologically equivalent, localized at intersections of bones; six are type II, whose equivalence is supported by geometric evidence, and 12 are of mixed type I/II (but see section on homology assessment and the use of geometric morphometric data in phylogenetic analysis). On adults, fusion of bones made some suture-based landmarks difficult to locate. Juveniles of identical species with unfused elements served as a guide for locating landmarks on the adult skull.

The diversity of mammal skulls presented some difficulties regarding choice of landmarks, and demanded some arbitrary choices, since we have no way to deal with "missing" landmarks. For example many orders are edentulous so this ruled out any landmarks based on teeth. In some orders the orbits are not clearly demarcated, so that only a point on the anterior orbital margin could be recorded. The jugal is missing in some orders, while in others it is part of the zygomatic arch. We digitized the dorsal and ventral anterior points of the jugal when present, and dorsal and ventral points on the maxillary portion of the zygomatic arch when the jugal was missing. We tried to record landmarks at or on the same structure, even if this structure is not topologically equivalent in some specimens. For example landmark 14, the "posterior point of the palatines", is not always the most posterior point on the hard palate. The pangolins (Pholidata) and anteaters (Myrmecophagidae) have the most

posterior palatal point between the pterygoids. The lachrymal is reduced or missing in some marine mammals and in the Monotremata. In the first case, we used the middle of the lachrymal bone, and in the latter, the suture intersection of the maxillary, jugal and frontal. In adult *Ornithorhynchus* (platypus), the premaxillae do not meet anteriorly on the midline, as they do in young specimens, so we digitized the landmark by projecting a midline point.

In order to visualize the data we took advantage of the software packages (the TPS series of Rohlf) that produce thin-plate splines. However these programs only accept 2D data. To construct useful 2D representations of the data, the 3D points were projected onto horizontal and saggital planes. This was

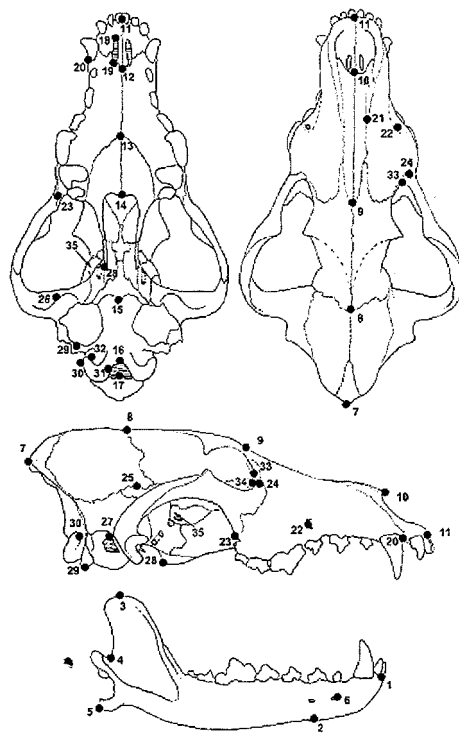
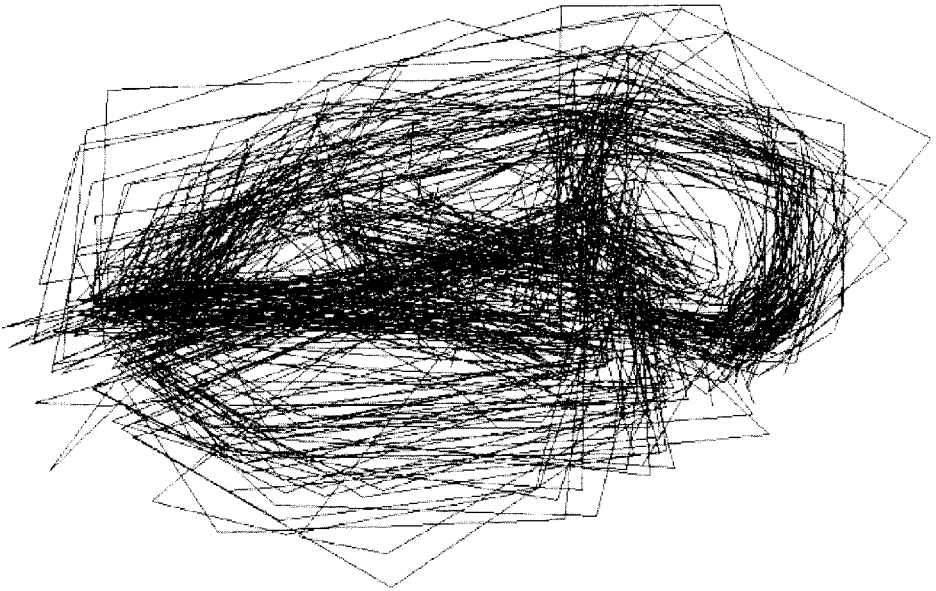


Figure 1. Landmark locations on a *Canis* skull. See Table 2 for anatomical locations of landmarks.

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b

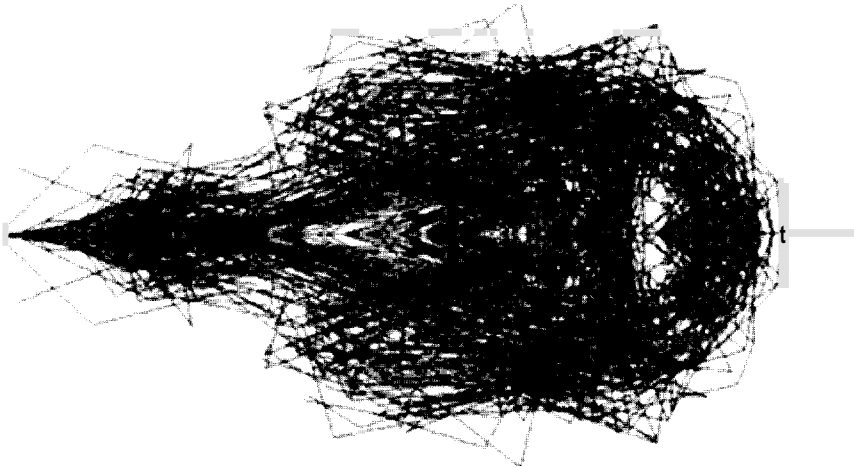


Figure 2. Superimposition of all specimens with some landmarks connected to visualize shapes a) 2D projection in the sagittal plane; b) 2D projection in the horizontal plane

done by a) aligning planes defined by triplets of points in the desired projection plane (using the option SC in GRF-ND, Slice, 1993)

and b) dropping the third coordinate from the resulting data. For the horizontal plane, we first duplicated the 3D landmarks from the

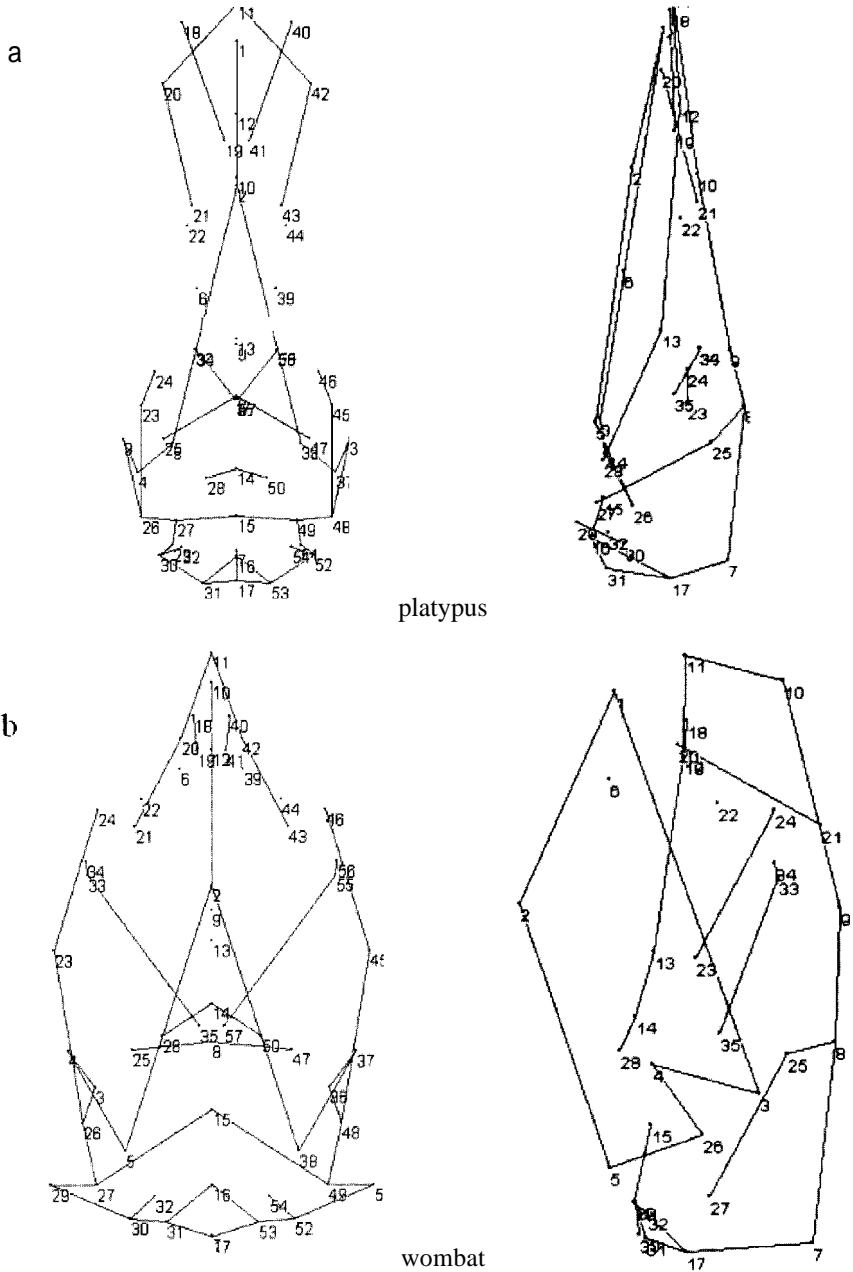
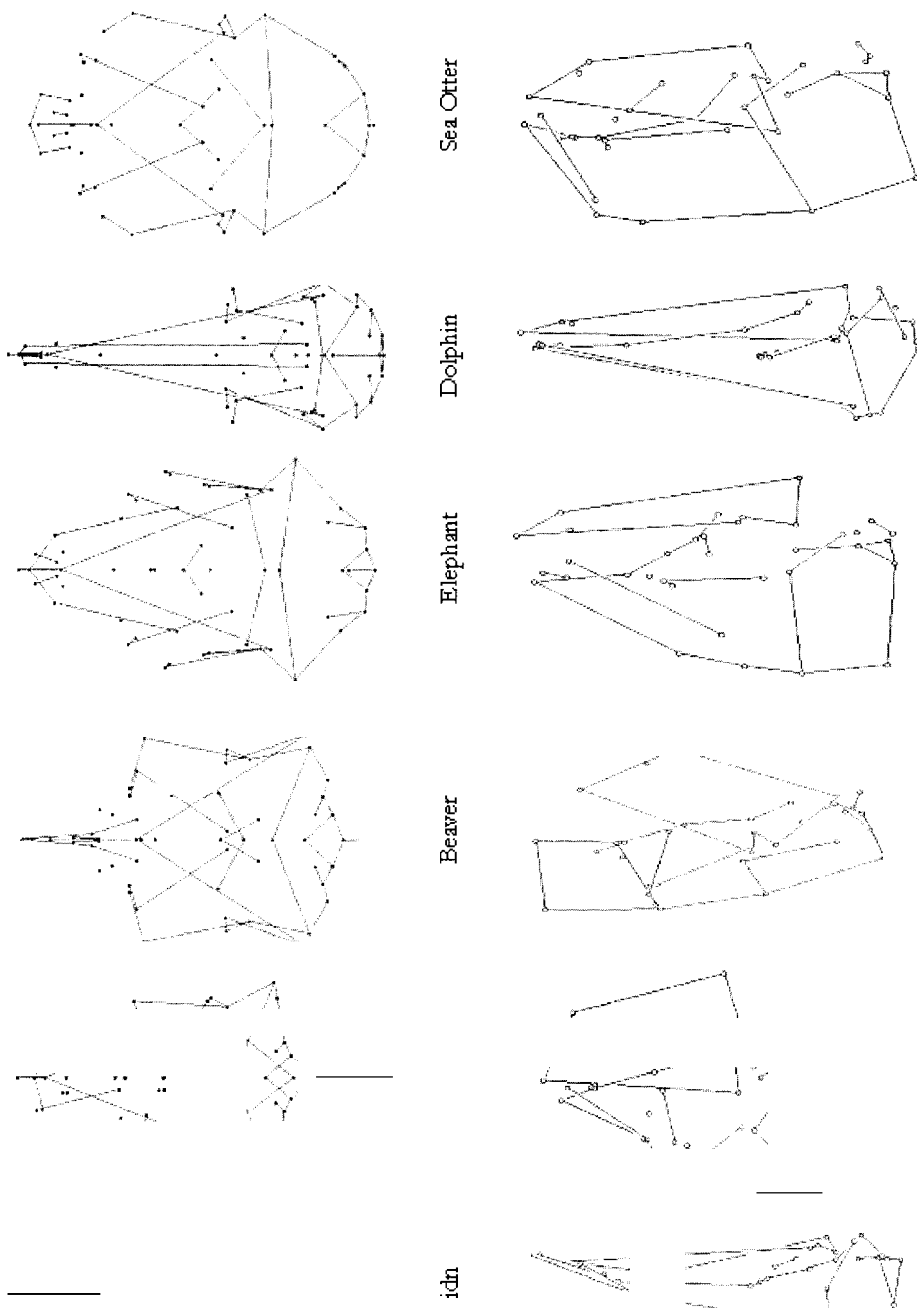


Figure 3. Numbered landmarks used in the analysis. See Table 2 for a complete description of the landmarks. Landmarks 1-6 are on the jaw; 1, 2, 7 through 17 on the saggital plane; 18 to 35 on the right side of the skull. Landmarks 40-57 are reflections of 18 to 35 to the left side.



among the 53 specimens digitized. Both horizontal and sagittal 2D projections are given.

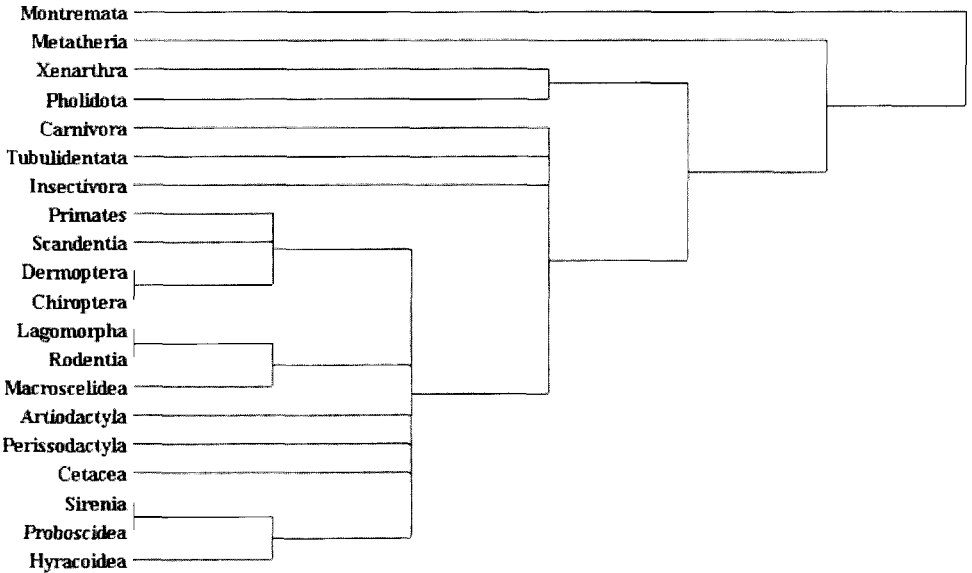


Figure 5. Cladogram for the orders of mammals based on Novacek (1990).

right side across the sagittal plane to the left side (increasing the total number of landmarks to 57). To define this plane the landmarks used were tops of the left and right superior auditory canals (landmarks 27 and 49), and the anterior pre-maxillary suture on the sagittal plane (11). For the sagittal plane the landmarks used were the tip of the premaxillary (landmark 11),inion (7) and the posterior edge of the foramen magnum (17).

For the 2D data all 53 specimens were superimposed and projected in the sagittal and horizontal plane to produce Figure 2 (a and b respectively). Figure 3 indicates the location of the landmarks listed in Table 2 for the platypus and wombat. Figure 4 shows several different specimens in 2D horizontal and sagittal projections.

The 3D coordinates were aligned using Generalized Least Squares (GLS) in the program TPSSMALL (Rohlf, 1998b) and projected into tangent space using the program Options: Size = 1; Projection = orthogonal (options available in all of the current ver-

sions of the TPS software for Windows - see Discussion for earlier and alternative options). We give results for some other options for comparison (Tables 3 and 4). The 3D aligned data were saved for all further analysis using the options CS=1 and orthogonal projections.

Using TPSSMALL (Rohlf, 1998b) we computed Procrustes distances (Slice et al., 1996) for the 3D landmarks in Kendall shape space (Slice et al, 1996; Rohlf, this volume) and compared them to Euclidean distance in tangent space. TPSSMALL was also used to compute these distances for the subsets of landmarks forming the jaw, face and braincase.. The visualization program, Morpheus (Slice, 1998) has been used to generate most of the graphics. All other computations have been done using NTSYSpC for windows (Rohlf 1999), Rohlf's TPS series of programs for the PC, the SAS statistical package, and Excel.

We used the phylogeny of Novacek (1989, 1990, 1992, 1993) based on a number of

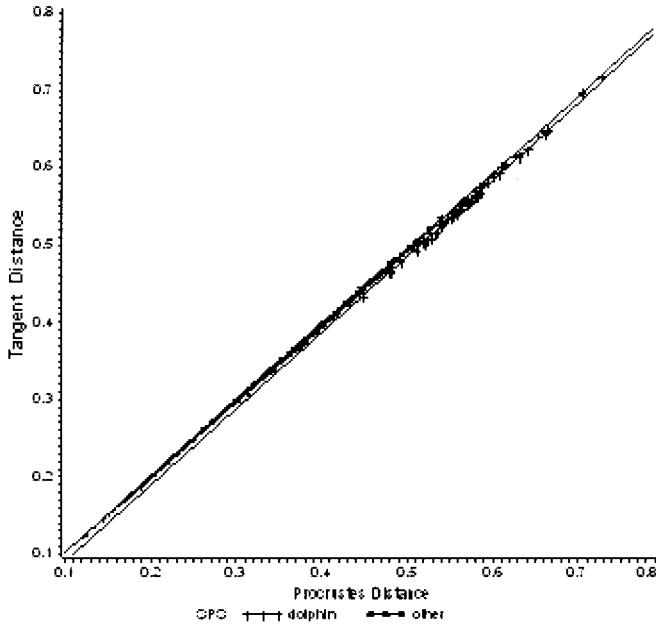


Figure 6. Scatter plot for all 3D data of Procrustes Distance against Euclidean tangent space distance (Tangent Distance) with best fitting lines through the origin for $CS = 1$; and Orthogonal Projection, tangent at consensus. Symbols: + dolphin comparisons; ? all other comparisons.

cranial and soft part anatomical characters to compare to our results based on partial and relative warps ($\alpha = 0$). His phylogeny does not go below the ordinal level, except for a few contentious groups. Both Novacek's phylogenies and McKenna and Bell's (1997) classification agree that the mammals form a monophyletic group, with a nested hierarchy shown in Figure 5 (based on Novacek, 1990). We examined the partial warps for the horizontal and sagittal projections as well as the relative warps in two dimensions but only report on one partial warp and two relative warps, that depict shape differences. Some partial warps seem to be informative for defining characters that might be used in a phylogenetic framework, in the sense of Fink and Zelditch (1995) and Zelditch *et al.* (1995).

An attempt was also made to see how the 3D Procrustes distances related to the currently accepted phylogenies of ordinal relations among

mammals. We coded Novacek's cladogram and the cladograms "implied" in McKenna's classification in NEXUS format (Rohlf, 1999) to create trees. We used phenetic clustering algorithms, including UPGMA, single linkage, and complete linkage on the 3D Procrustes distance data for all of the landmarks – to find the level of consensus with the cladogram.

RESULTS AND DISCUSSION

When we compared the 3D Procrustes distances to the tangent space distances (i.e. Euclidean distances in tangent space) for mammalian skull shape, the relation was very close to linear for all of the data. However, comparative distances to one species, the common dolphin fell on a line slightly below that for all other comparisons (Figure 6).

Table 3 gives the range and mean Procrustes and Euclidean distances for both the orthogonal and stereographic projections for the 3D data for CS (centroid size) = 1 and also for

Table 3. Comparison of Procrustes distances to approximations for the complete data set. Tangent at the consensus.

Between Specimens	*Procrustes distance ρ	CS=1		CS=cos(ρ)	
		+Orthog	Stereo	Orthog	Stereo
Tangent Distances					
Min	0.1202	0.1201	0.1210	0.1193	0.1202
Mean	0.3394	0.3364	0.3515	0.3223	0.3337
Max	0.7306	0.7167	0.7994	0.6433	0.7152
Slope		0.9896	1.0398	0.9429	0.9926
Corr		0.9999	0.9998	0.9995	0.9999
To Reference					
Min	0.1087	0.1085	0.1092	0.1074	0.10823
Mean	0.2358	0.2327	0.2422	0.2236	0.2348
Max	0.5066	0.4852	0.5550	0.4250	0.5003
Slope		0.9841	1.0339	0.9373	0.9951

* Note the maximum possible Procrustes distance between two object is $(\pi/2 \cong 1.57)$ radians.

+ Slope for dolphin compared to all other skulls is 0.9731 for the CS = 1, Orthog column

CS=cos(ρ). Note the small differences between the values, and the very high correlation in every case when comparing specimens. The range of values were 0.120 (Bear to Aardwolf) to 0.731 (Dolphin to muskrat). Separate analyses of distances with orders showed that the Xenarthra (5 species) were most variable, with Procrustes distance between specimens varying from 0.169 to 0.539. Marsupials (7 species) and carnivores (11 species) were the most homogeneous with Procrustes distances varying from 0.144 to 0.278 for the former and 0.120 to 0.273 for the latter. Rodents (4 species) had Procrustes distances between 0.228 and 0.351; Primates (5 species) 0.168 to 0.322; Uranotheres (4 species) 0.245 to 0.443; Perissodactyls (2 species) 0.298 and Artiodactyls (3 species) 0.235 to 0.278; and Insectivores (in the broad sense, 3 species) had very similar Procrustes distances—0.209 to 0.213. Therefore most of the larger Procrustes distances were inter-ordinal comparisons, and the single species with the most distances

above 0.50 was the dolphin. The minimum distance from the dolphin to any other species was 0.449 (to the llama), the elephant was next with 0.295 (to the manatee) and then all others had minimum distances equal or below 0.263. Generally the long snouted mammals - including the monotremes, aardvark, lesser and giant anteater, pangolin, and armadillo - had among the larger distances to other mammal specimens. Comparing interordinal distance the Uranotheres - manatee, elephant, and dugong were quite distant from shapes for other orders.

The relation of tangent space and Procrustes distances for braincase and jaws produced closer fits than the overall data set, however the face subset shows a higher scatter (Figure 7) than the overall data set. The stereographic projection for the face gives the greatest differences of tangent space distances from Procrustes distances of any of the data sets analyzed using the consensus as the reference..

Table 4. Comparison of Procrustes distances to approximations for the face data set. Tangent at the consensus.

Between Specimens	Procrustes distance ρ	CS=1		CS=cos(ρ)	
		+Orthog	Stereo	Orthog	Stereo
Projected Tangent Space Distances					
Min	0.1509	0.1513	0.1534	0.1494	0.1513
Mean	0.5012	0.4918	0.5441	0.4468	0.4934
Max	1.1309	1.0730	1.3425	0.8584	1.0495
Slope		0.9783	1.0956	0.8784	0.9812
Corr		0.9999	0.9988	0.9975	0.9999
To Reference					
Min	0.1625	0.1618	0.1639	0.1576	0.1601
Mean	0.3522	0.3422	0.3751	0.3131	0.3484
Max	0.6974	0.6422	0.8378	0.4931	0.6765
Slope		0.9650	1.0808	0.8649	0.9869

Procrustes distances for the face data become quite large, but the tangent space values still predict Procrustes distance with little error (Table 4).

For the entire 3D data set, one can also see how the choice of a reference specimen other than the consensus affects the tangent space approximations. Reference specimens near the consensus give results comparable to that for the consensus specimen. However, when an extreme specimen is chosen as the reference the scatter in the plot of tangent space distance to Procrustes distance increases considerably. Figure 8 shows the relation for CS=1 and orthogonal projection when the dolphin - the most extreme specimen from the over all consensus - is chosen as the reference. Table 5 gives the comparisons of the distances.

Alignment Considerations.

Rohlf (this volume and in press) has shown that the way specimen landmarks are aligned and scaled makes a difference in shape comparisons and statistical analyses. Earlier suggestions and conventions used in the DOS

versions of TPS, and also in GRF-ND (Slice, 1993) software scaled centroid size to $\cos(\rho)$ where ρ is the Procrustes distance to the consensus. This procedure places the specimens in a curved shape space, and does not place them in tangent space. The process of translation, scaling and rotation to best fit residuals "uses up" seven dimensions for 3D coordinates (4 for 2D coordinates), in the aligned coordinates. In tangent space this corresponds to a reduction in rank of the data as a singular value decomposition of the aligned

Table 5. Comparison of Procrustes distances to Tangent space approximation. Tangent at the dolphin shape.

Between Specimens	Procrustes distance ρ	Tangent Distances Orthogonal, CS = 1
Min	0.1202	0.1224
Mean	0.3394	0.3383
Max	0.7306	0.6714
Slope		0.9947
Corr		0.9997

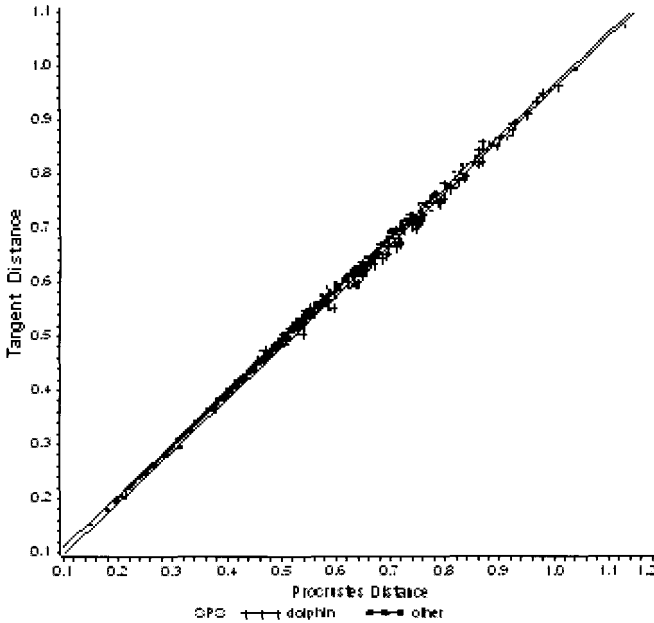


Figure 7. Scatter plot of 3D face data of Procrustes distance (y) against tangent space distance (x) with best fitting line through the origin for $CS = 1$; and Orthogonal Projection, tangent at consensus. Symbols: + dolphin comparisons; ? all other comparisons

coordinates will show. However, some portion of the inherent curvature of the space is left in the coordinates aligned using $\cos(\rho)$ which increases the rank of the aligned data in curved space (Rohlf, this volume).

We chose to project the aligned 3D coordinates orthogonally into tangent space, even though it provides distances smaller than Procrustes distances. However specimens with very large distances from the consensus might be barely distinguishable even though they might be quite dissimilar. On the other hand, stereographic projection exaggerates the distance between objects and the consensus, and also between pairs of shapes—more than may be acceptable for large differences in shape. In fact for stereographic projection, the tangent space distance has a potential of becoming infinite (Rohlf, 1998a), while curved shape space distance is never more than $(\pi/2 \cong 1.57)$.

Orthogonal projection had earlier been called the "shrunken $1/\cos(\rho)$ " option in Rohlf's TPS software for Windows up to early 1998 (for example this terminology was used in Marcus, 1998).

Is There Phylogenetic Signal in the Present Landmarks Data Set?

Fink and Zelditch (1995) and Zelditch et al. (1995) use 2D partial warps to find phylogenetic features in fish. Rohlf (1998a) and Zelditch et al. (1998) debate their use in such analyses, the first saying there is no biological meaning to partial warps, as they are a function of the principal warps and therefore of the reference configuration of landmarks, and the second defending their use as cladistic characters, on the basis of hypotheses of primary homology. In our data set, some partial warps show differences that might help define shared character states. For ex-

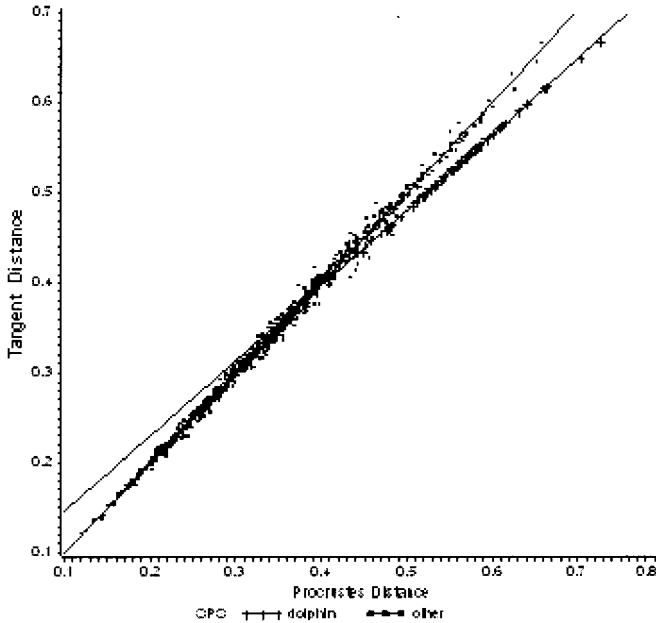


Figure 8. Scatter plot for all 3D data of Procrustes distance (y) against tangent space distance (x) with best fitting line through the origin for $CS = 1$; and Orthogonal Projection, tangent at dolphin. Symbols: + dolphin comparisons; ? all other comparisons

ample in the horizontal 2D projection. in partial warp $\times 10$ (Figure 9) the Baboon and Gorilla at one extreme have the foramen magnum (landmarks 16 and 17) relatively far forward compared to inion (7), contrasted with inion anterior to the foramen magnum in a number of aquatic mammals, as well as the Pangolin. Apparently (see Figure 9) only landmarks 7 (inion) and 17 (the rear edge of the foramen magnum) are involved in this partial warp. The partial warps depend to a large degree on nearness or distance apart of landmarks on the consensus (Rohlf, 1998a) which is a function of their average position—landmarks 7 and 18 are nearly coincident there. It would seem that a more informative character might involve the position of inion (7) relative to the four landmarks associated with the foramen magnum, but no such partial warp is evident. Among the relative warps, the first few show

a number of shape differences distributed over the skull - and the latter ones are too subtle to show features (Figure 10). Since the relative warps (with the uniform component retained) are the same as the principal components of the aligned coordinates (Rohlf, 1998a), this result is not surprising. The possible phylogenetic signal in our landmarks data is very low and difficult to ascertain. An initial constraint is that there are only as many partial warps+uniform components as the number of adjusted landmarks in our two dimensional projections. In addition, as pointed out in the introduction, whenever diversity in any given study group increases, it becomes more difficult to find the equivalent landmarks across that diversity, thus reducing drastically the number of possible landmarks. However, even if a large number of landmarks could be used, this number will probably not satisfy the minimal

number of informative characters needed to account for a data matrix with a large number of taxa with diverse shapes. This point represents a severe limitation of landmark based data and perhaps any type of analysis, and seems to undermine any attempt of using exclusively any one type of data such as geometric morphometrics data in a cladistic analysis. This reinforces the view that landmark based data are better used along with other characters (discrete morphological and molecular data) (e.g., Burke et al., 1996). One other possible explanation is that the shape variation examined in this study is not informative for phylogenetic inference at that level of universality (i.e., among the orders of mammals). Such correspondance between characters and their taxonomic level of expression is well known by systematists (e.g., Hennig, 1966; Wiley, 1981) and geometric morphometric data are not different in that sense

Finally, our results may reflect in part our sparse, and sometimes unequal, sampling of mammalian diversity. For some orders, we have only one representative, and the maximum number of specimens sampled for an order is 11.

Comparison of Aligned Data to Phylogenies using phenetic clustering.

The algorithm that gave the best consensus was complete linkage, though the unweighted pair group method using averaging (UP-

GMA, Rohlf, 1999) gave very similar results. There was no consensual resolution of the cladogram above the intra-ordinal level, except for the Glires - rodents and rabbits. Neighbor joining did not produce a better result, and the face, braincase, and jaw subsets all show less consensus than the complete data set. The UPGMA dendrogram for Procrustes distances is given in Figure 11.

Homology assessment and the use of geometric morphometric data in phylogenetic analysis.

Much has been said about landmarks and homology (Smith, 1990; Bookstein, 1994; Zelditch et al., 1995). However, the imprecise use of the adjective “homologous” when applied to landmarks from two or more different taxa, widely seen in the literature, is misleading. Landmarks are not the attributes of the organisms to be compared but rather the tools which help capture these attributes. In this regard, partial warps (as in Zelditch et al., 1995, for example) and their deformations, i.e. shape variables (characters and character states, respectively) – not landmarks - are the attributes in question from which propositions of primary homology are generated. Therefore, the adjective “equivalent” (see O’Higgins and Jones, 1998) is the designation we use for the chosen landmarks, as they correspond to the same topographical feature in the compared structures

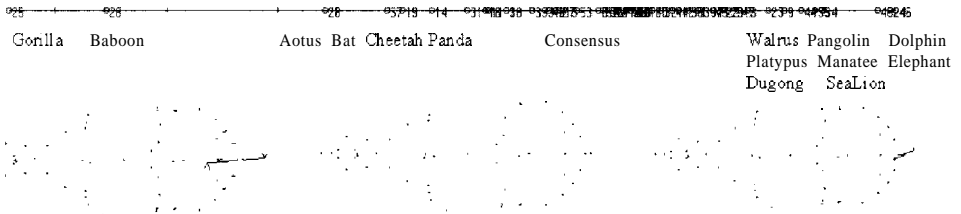


Figure 9. Partial warp x10 for horizontal projections of landmark data. a) Partial warp scores - 25-baboon, 26-gorilla. 46-dolphin etc., b) Landmarks involved are 16 - anterior edge of foramen magnum. and 11 inion c) depicted as thin plate spline

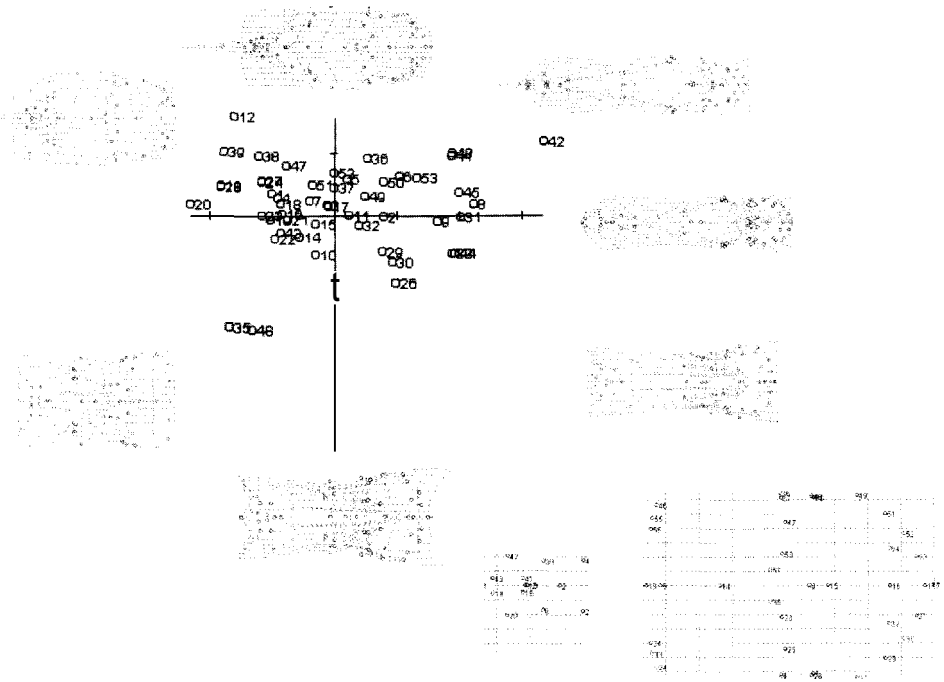


Figure 10. Relative warp 1 vs. 2 for horizontal projection 2D data. Scores for specimens and representative splines; consensus in lower right corner.

or organisms, but are not part of the hypothesis of primary homology (see below). The word “homology” should be applied only when referring to the features derived from the decomposition of shape differences.

Each variable function of landmarks (e.g., partial warp or other function of landmarks), as any hypothesis of primary homology, can be subjected to the test of congruence in order to be legitimized as a secondary homolog [see de Pinna (1991) for definitions of primary and secondary homologies, see also Rieppel (1988)] However, the initial choice of landmarks should be sufficiently precise in order to capture relevant homologous shapes, a procedure approached by choosing corresponding sets of landmarks placed in topographically equivalent positions. The choice of landmarks can thus be equated to the procedure of choosing the

features that will compose the frame of reference which will guide the establishment of primary homology, thus satisfying the only prerequisite for searching and defining characters (=primary homology assessment). In other words, both landmarks and morphological features are expected to be comparable among organisms and thus should be topographically equivalent.

Recently, Brower and Schawaroch (1996) proposed the terms “topographic identity” and “character state identity” to characterize two distinct steps previously conflated under de Pinna’s (1991) primary homology definition. The distinction of the two steps permits a more precise understanding of the procedure dealing with homology discovery for both morphological and molecular data. Under this view of homology assessment, geometric morphometric data are not different

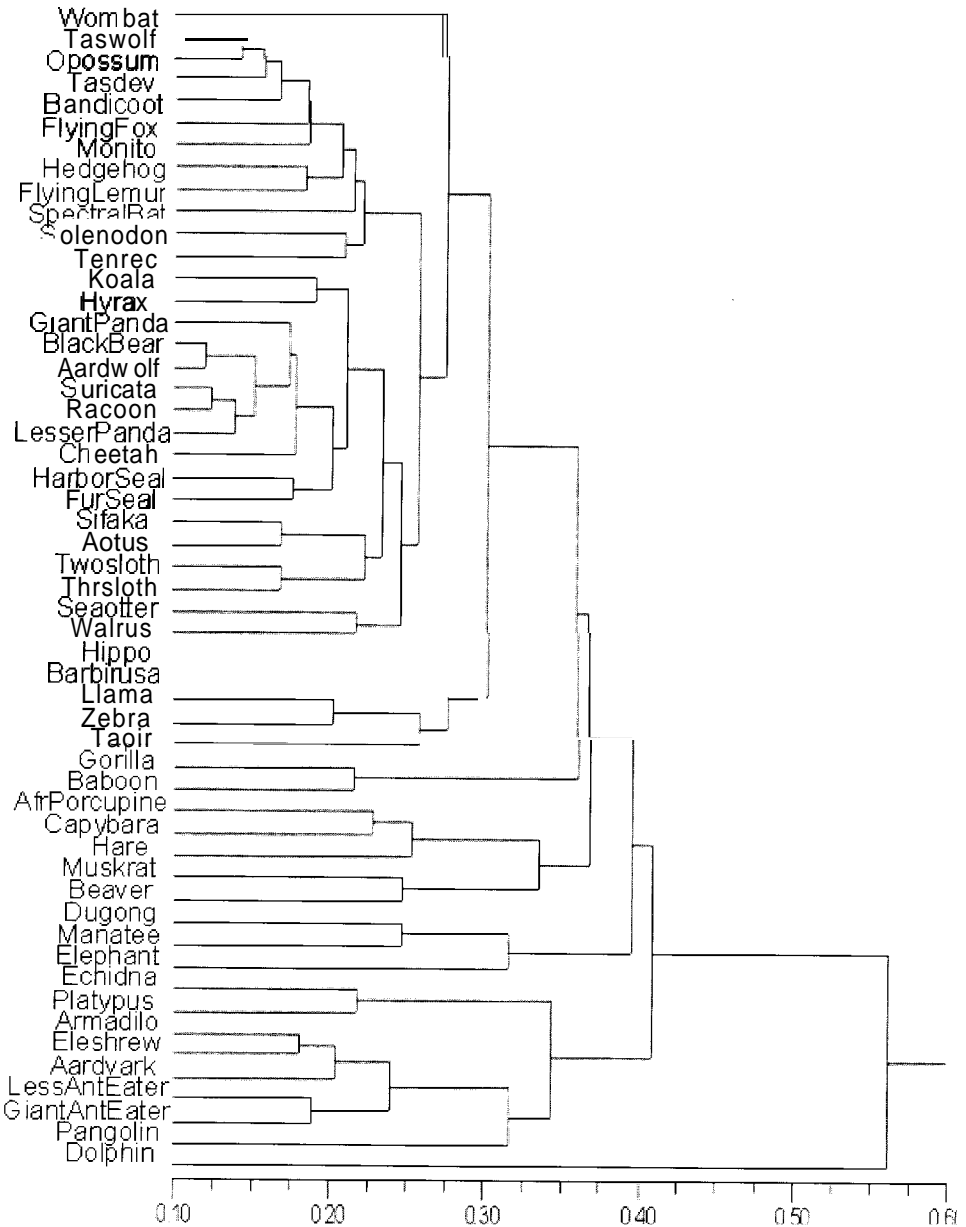


Figure 11. Dendrogram using UPGMA for Procrustes distances among all specimens.

from morphological or molecular data. The identification step via topographical identity corresponds to the initial choice of topographically identical landmarks which capture the essential shape of the organism or

structure. After landmarks are identified, the shape variables (such as partial warps scores: e.g., Zelditch et al., 1995: fig. 5) are hypothesized to be identical (=character state identity) and coded as character states which are

assigned to the terminal taxa under study. When addressing cladistic concerns landmark based methods of geometric morphometrics have to be viewed as any other tool or method to assess morphological similarity and the different expressions of features in organisms. As argued by de Pinna (1991: 377). "similarity is the phenomenon that systematics addresses, and as any true phenomenon its origin lies outside the method that attempts to understand it". We view this assertion as a basic tenet of systematic principles which invalidates Bookstein's (1994: 224) claim that the way modern biologists determine morphological similarity (=primary homologies) "is not compatible with the algebra or geometry of the curving manifolds that house our morphometric measurements". Discovery of morphological similarity does not require any operational definition other than "similarity as detected by topographical equivalence". Geometric morphometric tools can depict shape similarity with accuracy, being thus useful in phylogenetic analysis, although we agree with Rohlf (1998a) (see also Adams and Rosenberg, 1998) that one of the basic problems faced with geometric morphometrics in cladistics are the limitations and artificiality of the methods (i.e., partial warps) used to find discontinuous geometric morphometric characters. The "creases", or local deformation grids recently elaborated by Bookstein (1999), may be a promising new way of visualizing characters of potential phylogenetic value without the theoretical and methodological limitations of partial warps. However, this approach is beyond the scope of the present study. In conclusion, geometric morphometric characters meet the prerequisites of any other class of characters (see also Zelditch et al., 1995). Proposition and testing of homology for these characters under an explicit cladistic framework (i.e., legitimation of homology hypotheses through congruence) is possible and desirable.

SUMMARY AND CONCLUSIONS.

The great diversity of shape among mammalian skulls is generally larger than is commonly treated in most morphometric comparisons in zoological shape. This data provides an empirical comparison of tangent space distances to Procrustes distances and shows that statistical comparisons in tangent space will not be greatly affected by this approximation in most cases. In this example relatively large values of Procrustes distance are well approximated using a tangent point at the consensus shape. The importance of using a reference shape near the consensus in tangent space comparisons is supported, as shapes far from the consensus introduce distortion. For example, comparison between the dolphin, or some of the long faced ant-eating mammals and other mammals will be more distorted than other comparisons

The overall comparison of shapes by Procrustes distance shows almost no consensus with current ordinal level phylogenies as constructed from traditional morphology. The partitioning of the variability into partial and relative warp scores may suggest some characters that can contribute to phylogenetic analysis, but partial warp scores or the characters they suggest partition the landmarks associations in terms of patterns of nearness in the reference, rather than any structural constraints in the skull itself. Of course our results reflect our choice of landmarks and specimens, but with this amount of diversity it is difficult to find as many informative landmarks as one would like.

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