Morphological Study and Analysis Methods

Biodiversity Informatics Research Methods Workshop

National Museums of Kenya

23-27 September 2013

Taxonomic Revision Methods

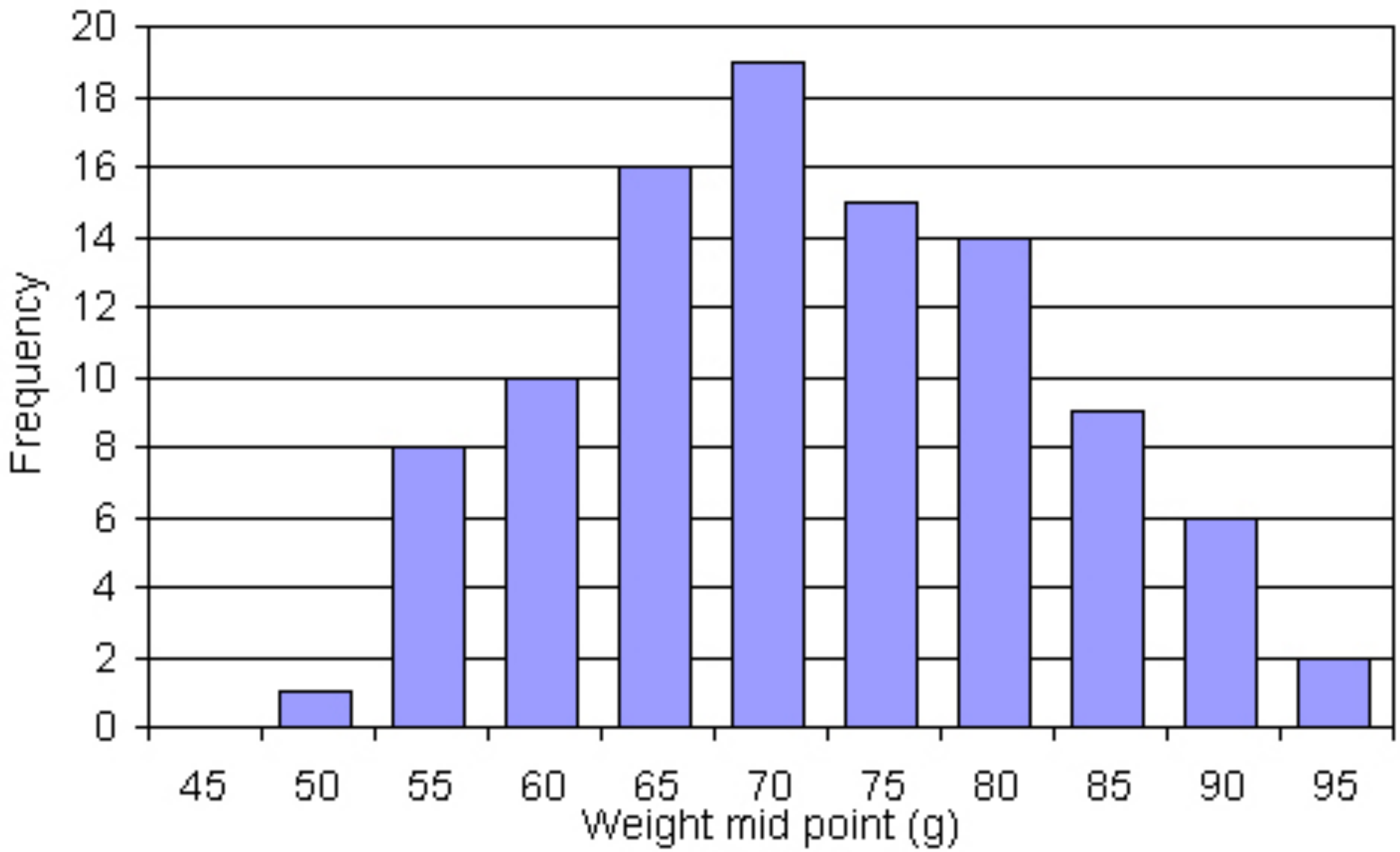
Taxonomic Revision

- Mentioned earlier that traditional studies of morphological variation of species attempt to infer evolutionary change at the genetic level based on patterns of variation in external or internal) morphological characters.
- Taxonomists gather data on multiple morphological characters in an attempt to identify small subsets of characters diagnostic of species.
- Deal with characters determined by multiple genes (polygenic traits).

Polygenic Traits

- External morphological traits (such as weights, shapes, scale counts on lizards or fish) are polygenic, i.e., they are determined by multiple genes.
- Expression of the genes controlling polygenic traits usually results in a range of phenotypes which tend to follow a normal distribution, with traits of most individuals conforming to the mode of the distribution and smaller numbers of individuals exhibiting extremes (tails of the distribution).

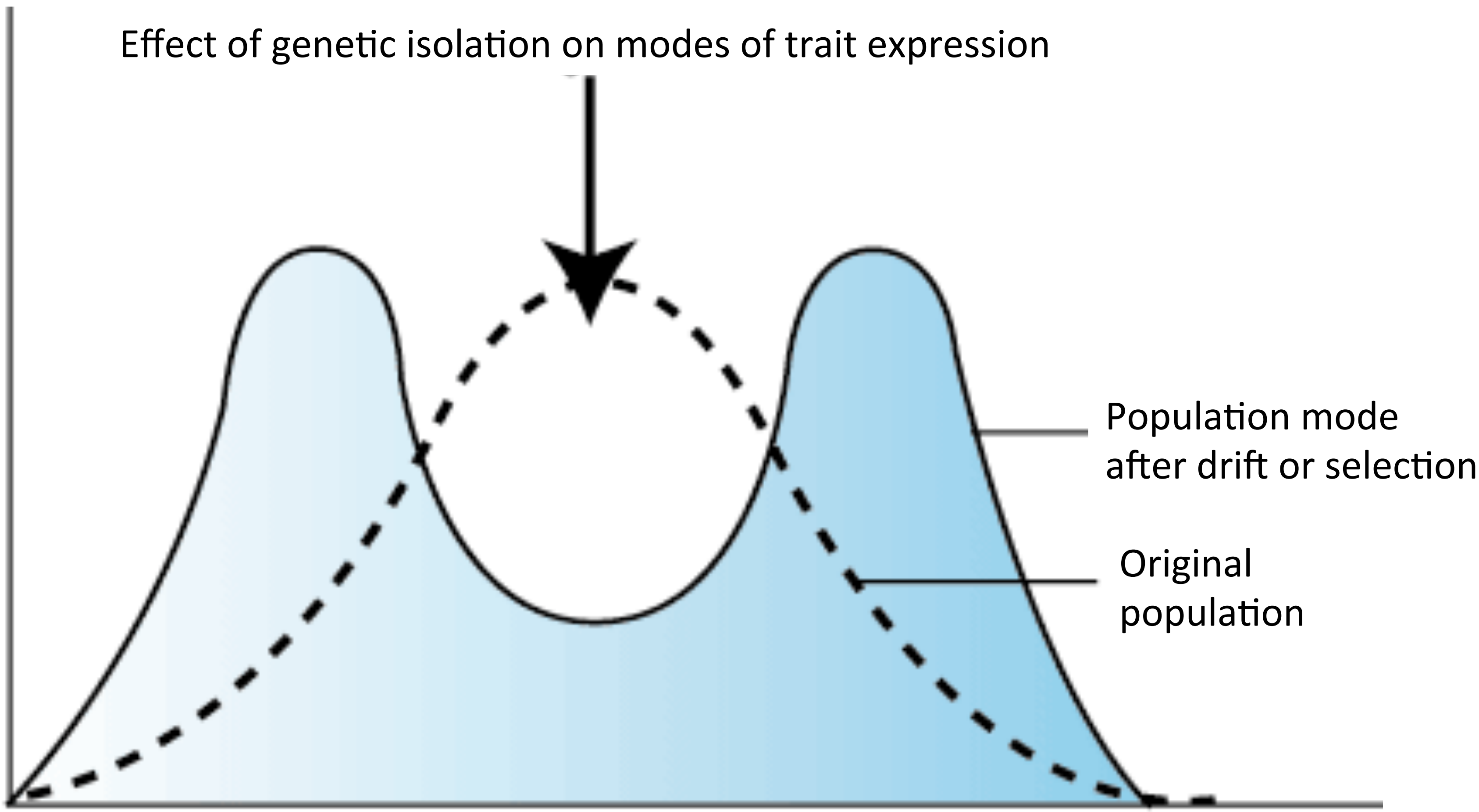
Expression of Polygenic Traits



Effect of Genetic Isolation

- Genetic isolation causes genes controlling polygenic traits to diverge in their expression patterns due to random genetic drift or local adaptation.
- Over time, isolation causes the modes of phenotype expression patterns of populations to shift, ultimately resulting in **stepped clines**.
- Conversely, gene flow among populations homongnizes phenotype expression patterns resulting in clinal variation.

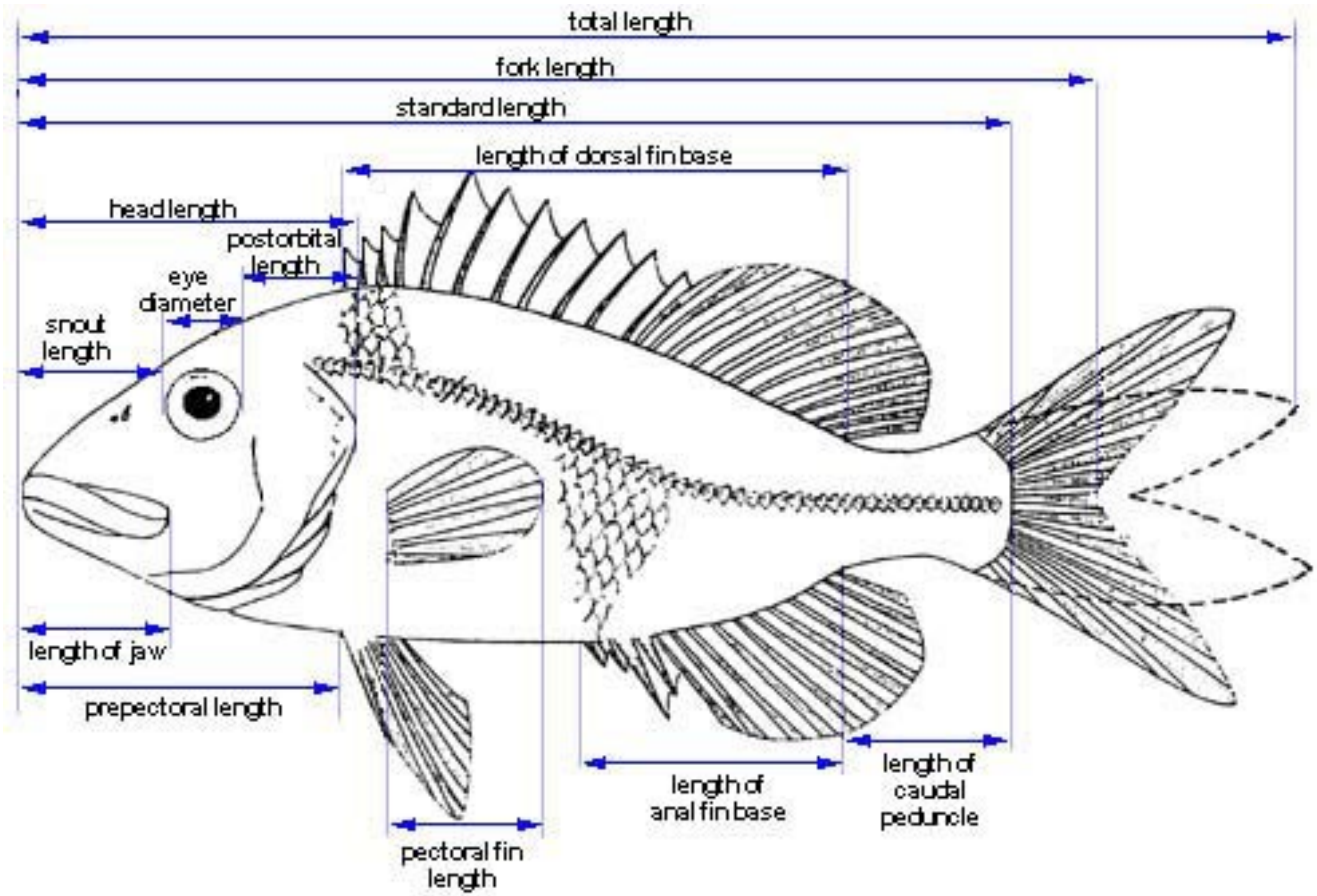
Effect of Genetic Isolation



Counts and Measurements

- In taxonomic revisions, we quantify variation in polygenic traits by making counts and measurements on samples taken from populations in different geographic areas.
- Meristic (countable) characters (scale counts, teeth, fin rays) have discrete values (e.g., whole numbers of teeth, scales, fin rays, etc.).
- Morphometric (mensural) characters (body proportions) exhibit continuous variation (including fractions of whole numbers).

Standard Fish Counts and Measurements



http://www.flmnh.ufl.edu/fish/Education/Diagrams/FishDimensions.html

Expression of Scale Counts in Kenyan *Labeobarbus* species

Lateral Line Scales	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**$\bar{x}$**
Athi River						13	10	2	1												26.65
Tana River	1	1	0	5	6	6	4	3			1										25.63
Ewaso Nyiro		2	5	6	2	6	1	3													24.80
Kerio River								2	4	14	17	10	5	3							31.18
Yala River										16	21	10	8		1	1					31.33
Miriu River										5	10	7	1	2							31.38
Mara River								1	1	3	9	16	20	6	1	1		1		1	32.52

Expression of Scale Counts in Kenyan *Barbus* species

					Lateral Line Scales					
Species/Population	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**x̄**
Barbus cf. *kerstenii*										
Tana (Meru)	1	8	13	2						21.67
Tana (Sagana)				5	4	7	3	1		24.55
Tana (Ragati)				2	4	7	4			24.76
Athi (Nairobi Park)			1	3	12	26	19	2	2	25.12
Athi (Yuu)				3	18	7	2			24.27
Lake Naivasha				1	2	3		1		24.71
Ewaso N'giro				6	5					23.45
Lake Victoria (Nyando)			1	6	4	2				23.54
Lake Victoria (Mara)			2	14	8	3	1			23.70
Barbus neumayeri										
Ewaso Nyiro					1	8	6	7	2	25.92

Mesural Data

Body Measurements

- Standard body measurements are made with calipers (dial or digital).
- Measurements taken with most digital calipers can be input directly into spreadsheets to reduce transcription errors.
- Because organisms differ is size, measurements must be standardized based on some standard body size measurement and expressed as proportions of that measurement (e.g., proportion of standard length).
- Because of size dependence, body measurements are strongly correlated; want body measurements that are size independent.

Body Proportions

- Resulting proportions are ratios that may not conform to normal distribution assumptions.
- Can regress measurement on standard body size measurement and retain only the residual as data (~removing the effect of body size).
- A standard body measurement, such as standard length, does not provide a complete estimate of body size.
- Kenya *Barbus* body measurements

Analyzing Body Proportions

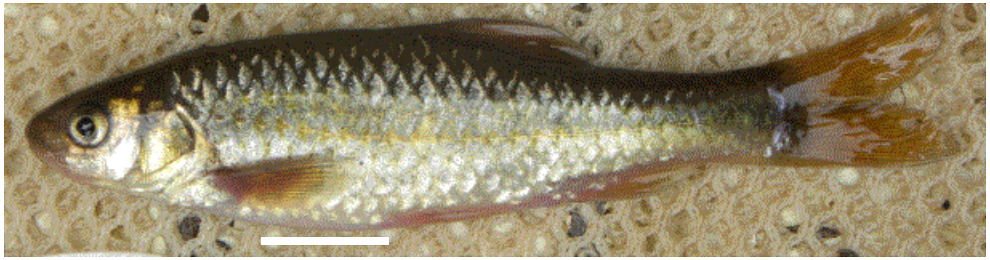
- Body proportions can be analyzed using standard univariate (ANOVA) or multivariate (MANOA) statistics, best to subject the data to multivariate analyses such as Principal Components Analysis (PCA) to examine general trends and then perform more detailed analyses later.
- As with meristic data, the aim is to identify diagnostic characters.

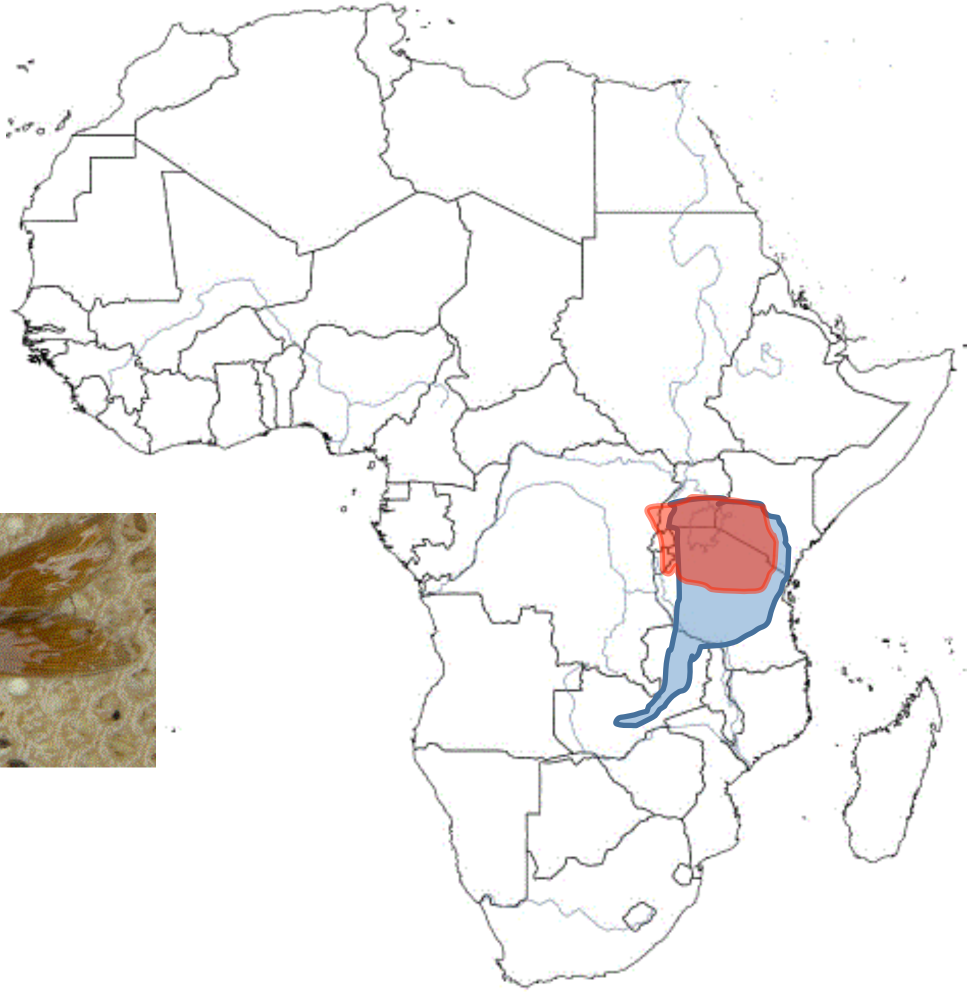
PCA Demonstration using PAST

Barbus kerstenii Peters 1868

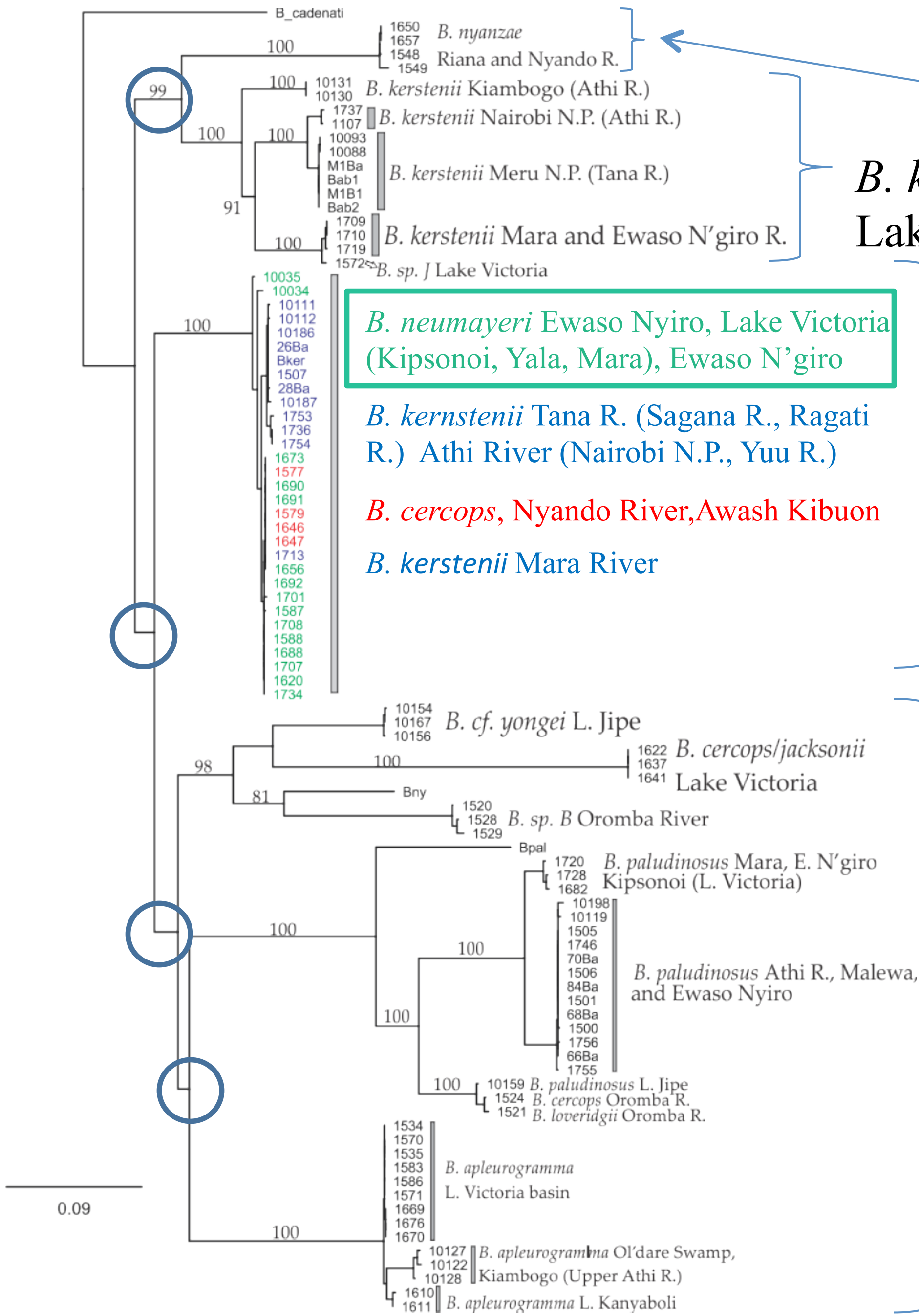
- Type Locality – “route between the coast opposite Zanzibar and Mount Kilimanjaro, Tanzania” (Pangani River system).
- Small, slender bodied, low scale counts, often darkly pigmented with distinct red-orange spot on opercle.
- Central and East Africa, including Lake Victoria basin, Lake Naivasha, Tana, Ewaso Nyiro, and Athi river basins of Kenya, Lake Tanganyika, Lake Kivu, Zambian Congo system, Okavango, upper Zambezi

Broadly overlapping distribution





Cyt b Tree



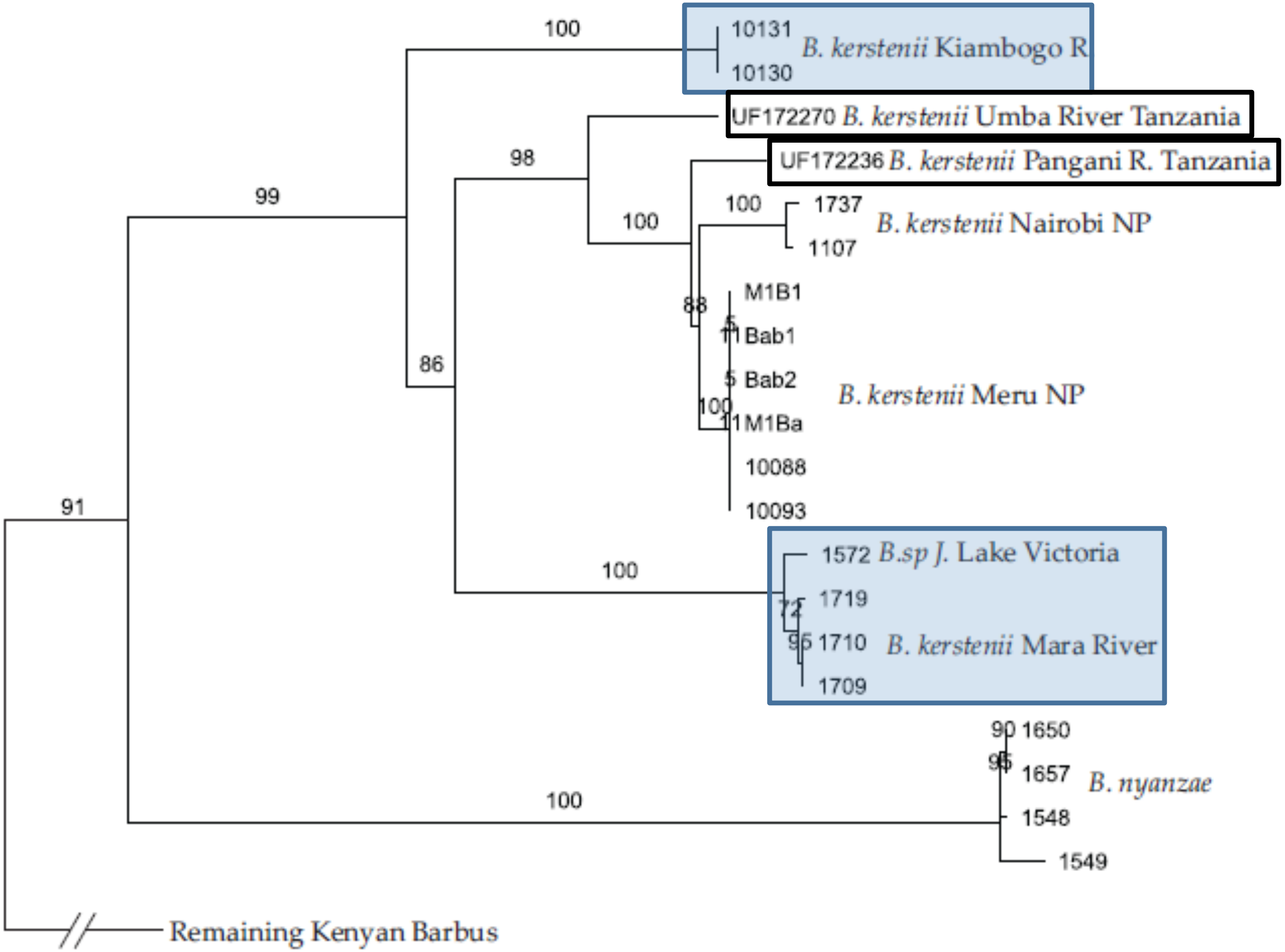
B. nyanzae Lake Victoria

B. kerstenii Athi, Tana, Ewaso N'giro, Lake Victoria

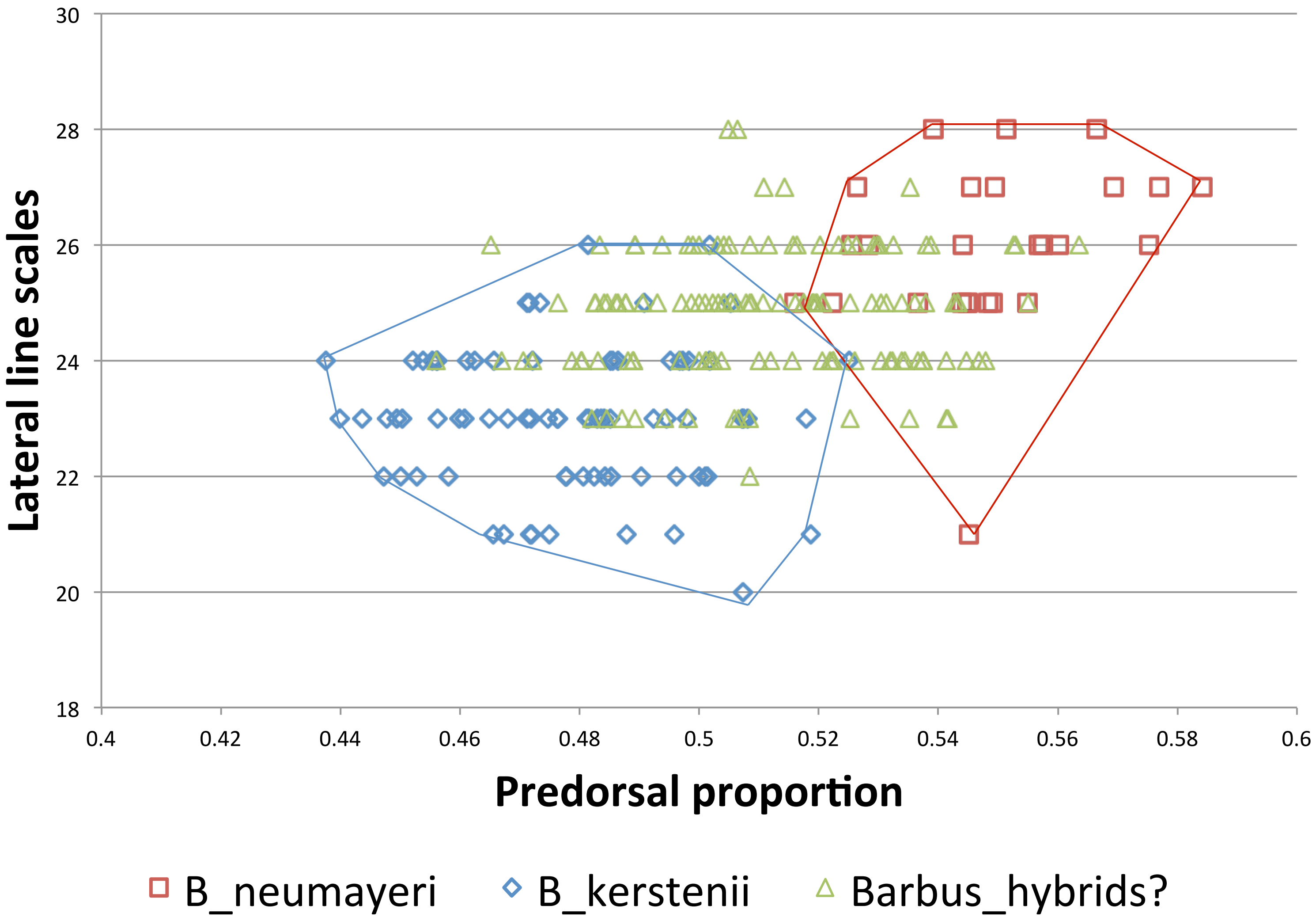
mostly *B. neumayeri*, but fish with other morphotypes are introgressed with *B. neumayeri* mtDNA

Other Kenyan *Barbus*

B. kerstenii clade



Species & Hybrid Identity

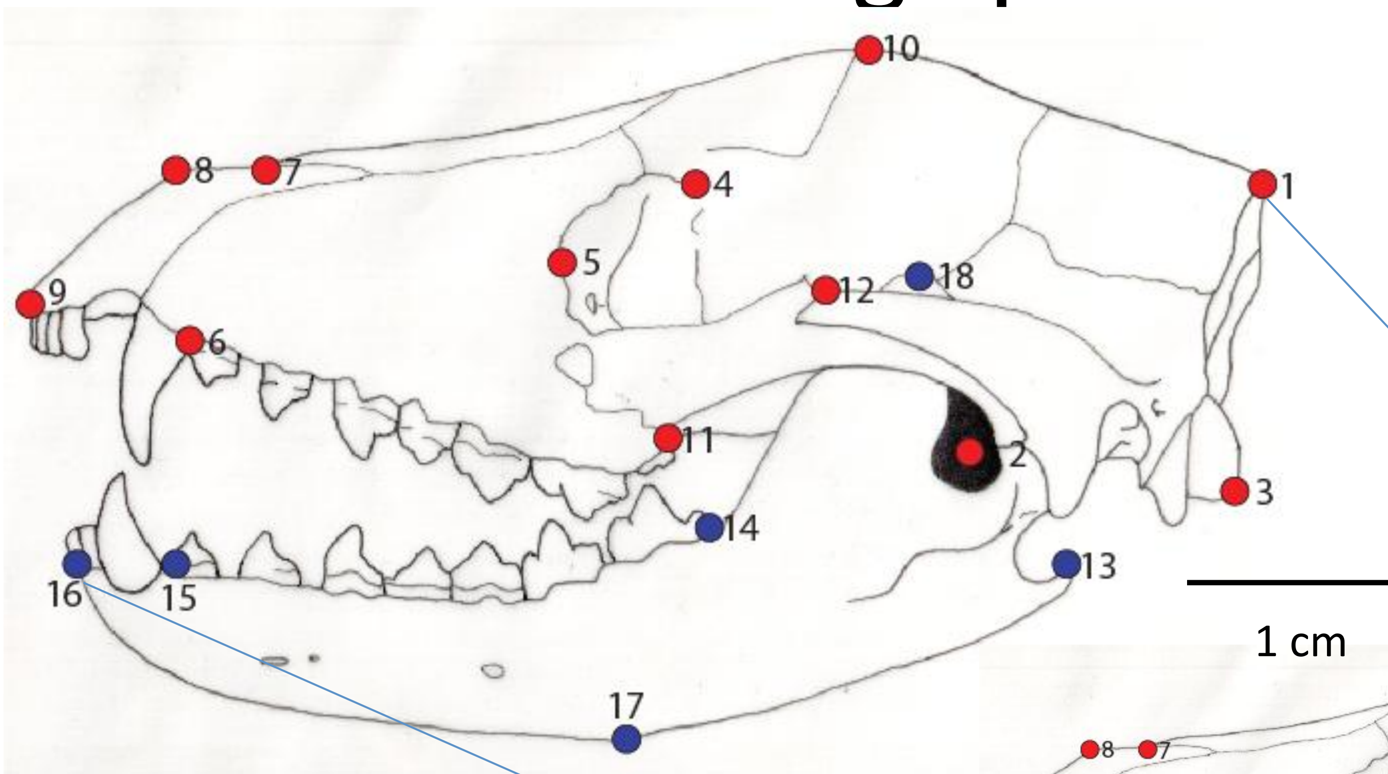


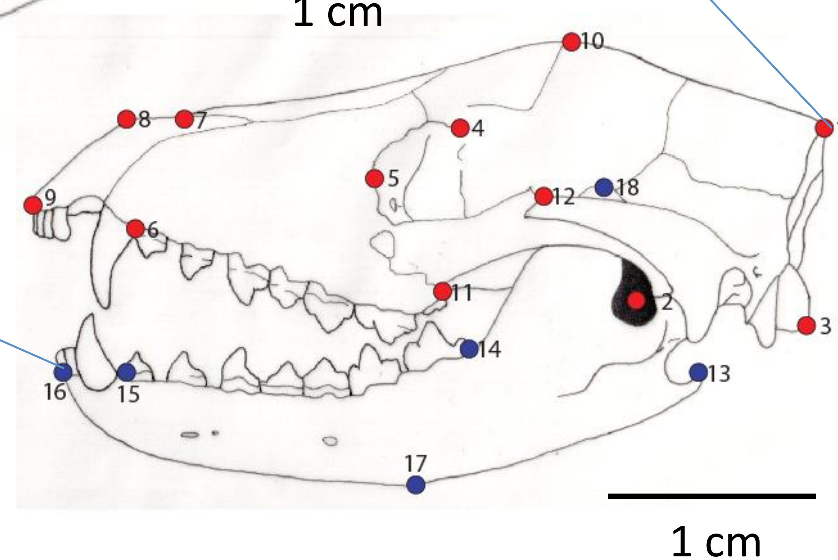
Digital Morphometrics

Digitizing specimens

- Computer tools have long been used to aid researchers in studying attributes of specimens measured on 2-D images of specimens.
- Most methods involve analysis of aspects of specimen shape (body proportions or morphometry) by placing landmarks on scaled, 2-D images.
- Scale data permits statistically appropriate adjustment of images to common size and removal of size-related variation.

Landmarking Specimens

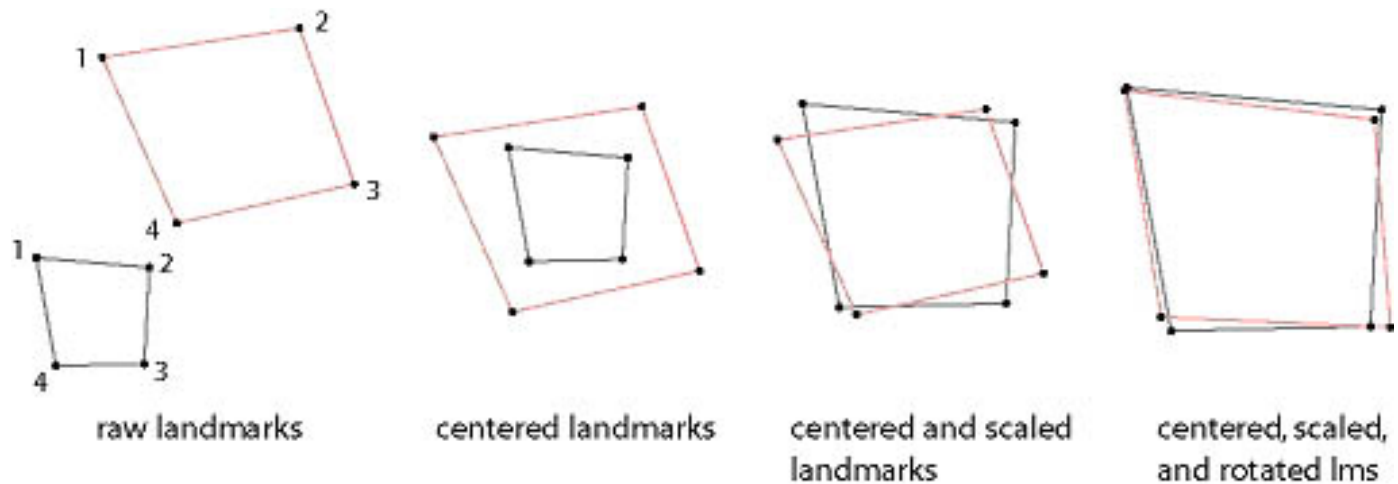




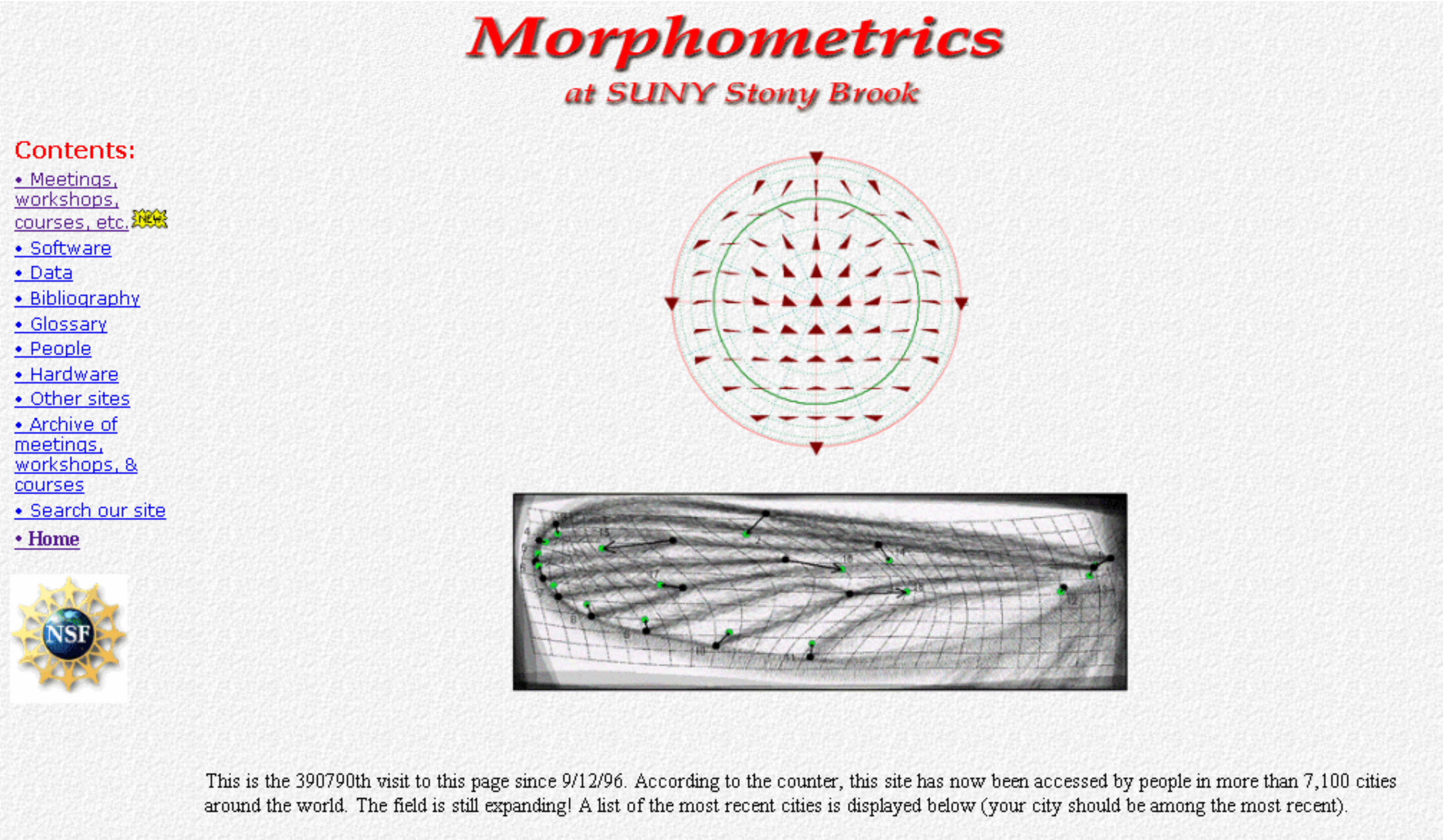
- Landmarks placed at homologous points.
- Saved as x, y coordinates.

Procrustes Superposition Analysis

- A form of statistical analysis of a set of shapes
- Involves optimally translating, rotating and uniformly scaling the objects so that like landmarks align.

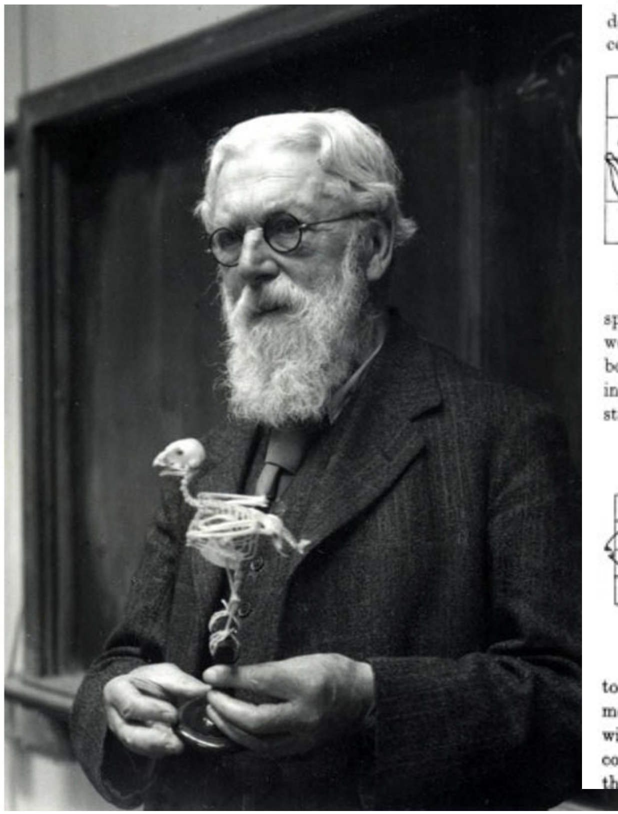


Geometric Morphometrics



http://life.bio.sunysb.edu/morph/

D'Arcy Thompson's Theory of Transformation



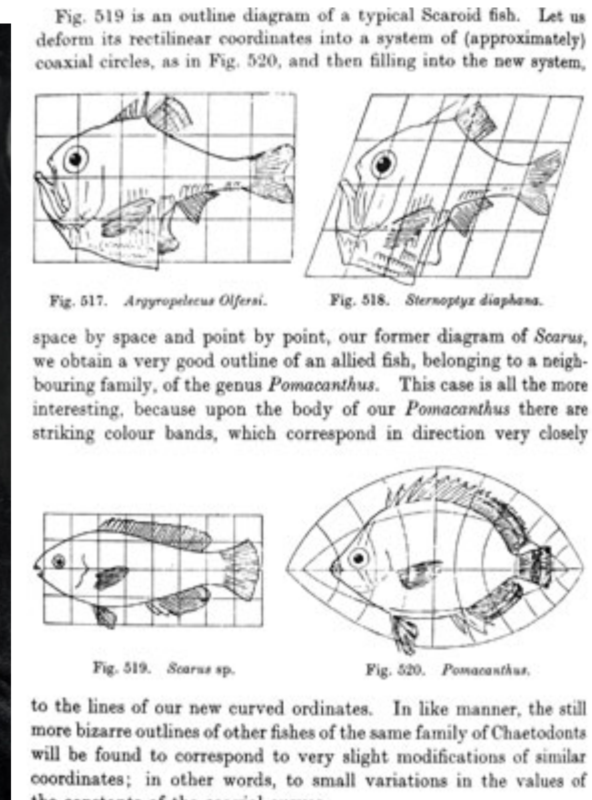


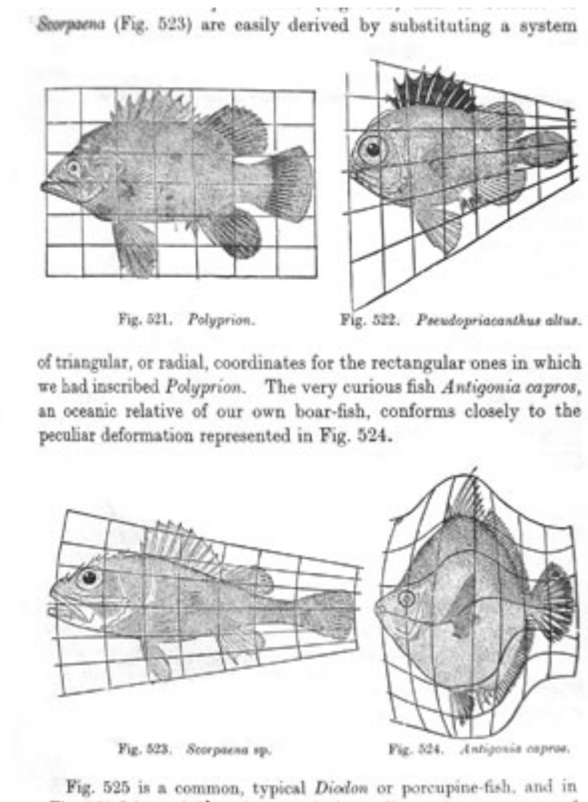
Fig. 519 is an outline diagram of a typical Scaroid fish. Let us deform its rectilinear coordinates into a system of (approximately) coaxial circles, as in Fig. 520, and then filling into the new system,

Fig. 517. *Argyropelecus Olfersi.* Fig. 518. *Sternoptyx diaphana.*

space by space and point by point, our former diagram of *Scarus*, we obtain a very good outline of an allied fish, belonging to a neighbouring family, of the genus *Pomacanthus*. This case is all the more interesting, because upon the body of our *Pomacanthus* there are striking colour bands, which correspond in direction very closely

Fig. 519. *Scarus* sp. Fig. 520. *Pomacanthus.*

to the lines of our new curved ordinates. In like manner, the still more bizarre outlines of other fishes of the same family of Chaetodonts will be found to correspond to very slight modifications of similar coordinates; in other words, to small variations in the values of the constants of the coaxial curves.



Scorpaena (Fig. 523) are easily derived by substituting a system

Fig. 521. *Polyprion.* Fig. 522. *Pseudopriacanthus altus.*

of triangular, or radial, coordinates for the rectangular ones in which we had inscribed *Polyprion*. The very curious fish *Antigonia capros*, an oceanic relative of our own boar-fish, conforms closely to the peculiar deformation represented in Fig. 524.

Fig. 523. *Scorpaena* sp. Fig. 524. *Antigonia capros.*

Fig. 525 is a common, typical *Diodon* or porcupine-fish, and in

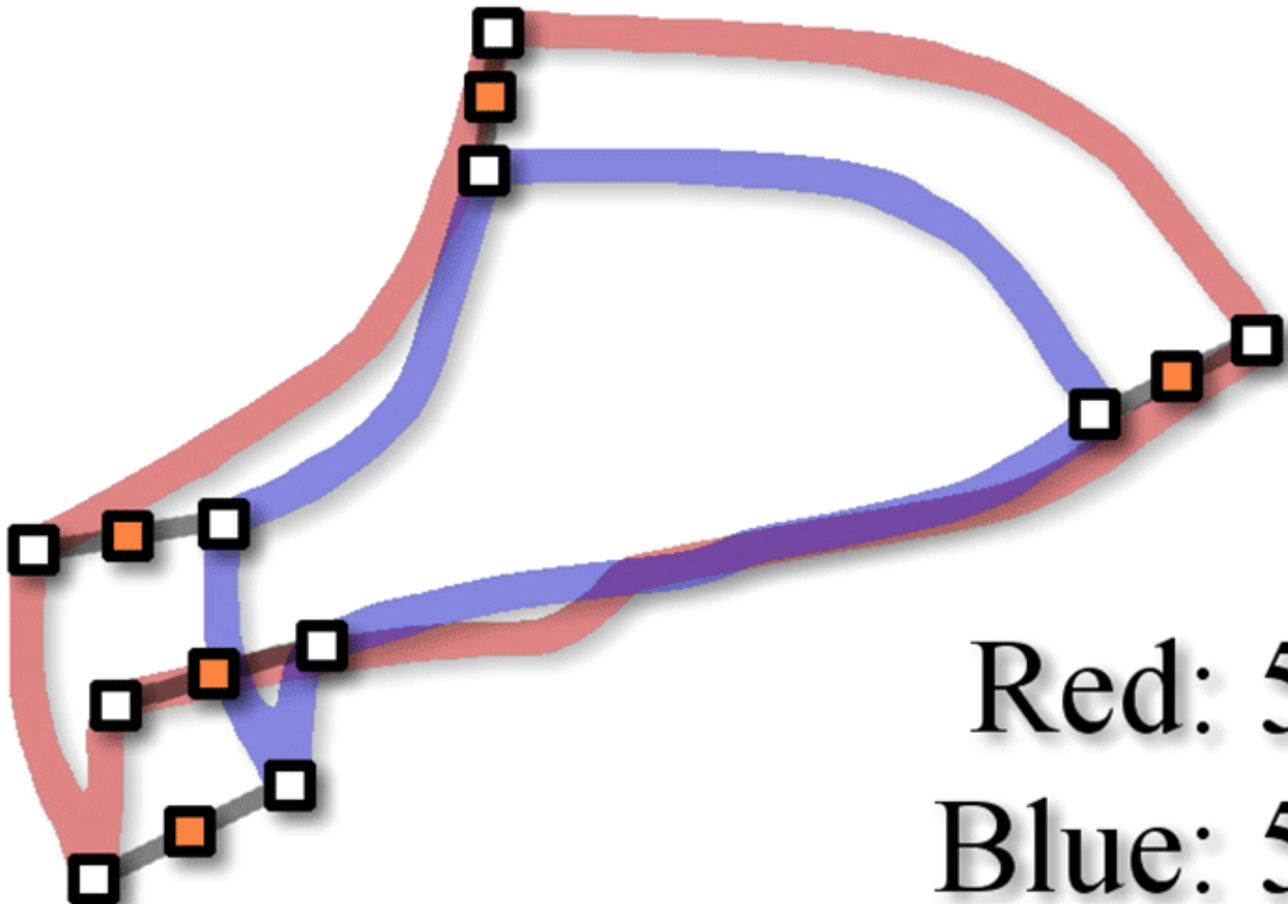
Thin Plate Splines

- Physical analogy involving the bending (deformation) of a thin sheet of metal.
- Geometric morphometrics applies this idea to the problem of shape (landmark coordinate) transformation.
- One interprets the lifting of the plate as a displacement of the *x* or *y* coordinates within the plane.

Partial Warp

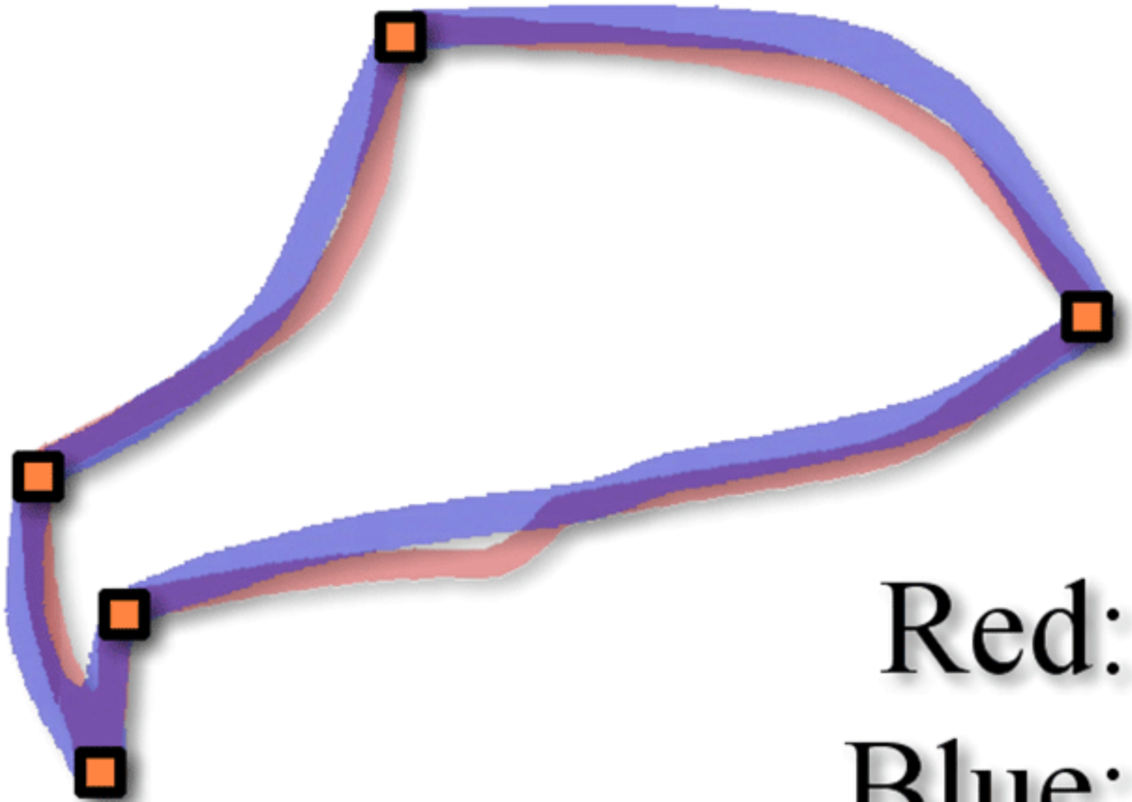
- Thin-plate spline interpolation of the non-uniform component of shape deformation.
- Auxiliary structures for the interpretation of shape changes and shape variation in sets of landmarks.
- Eigenvectors of the bending energy matrix that describes the net local information in a deformation along each coordinate axis

Alignment and Consensus



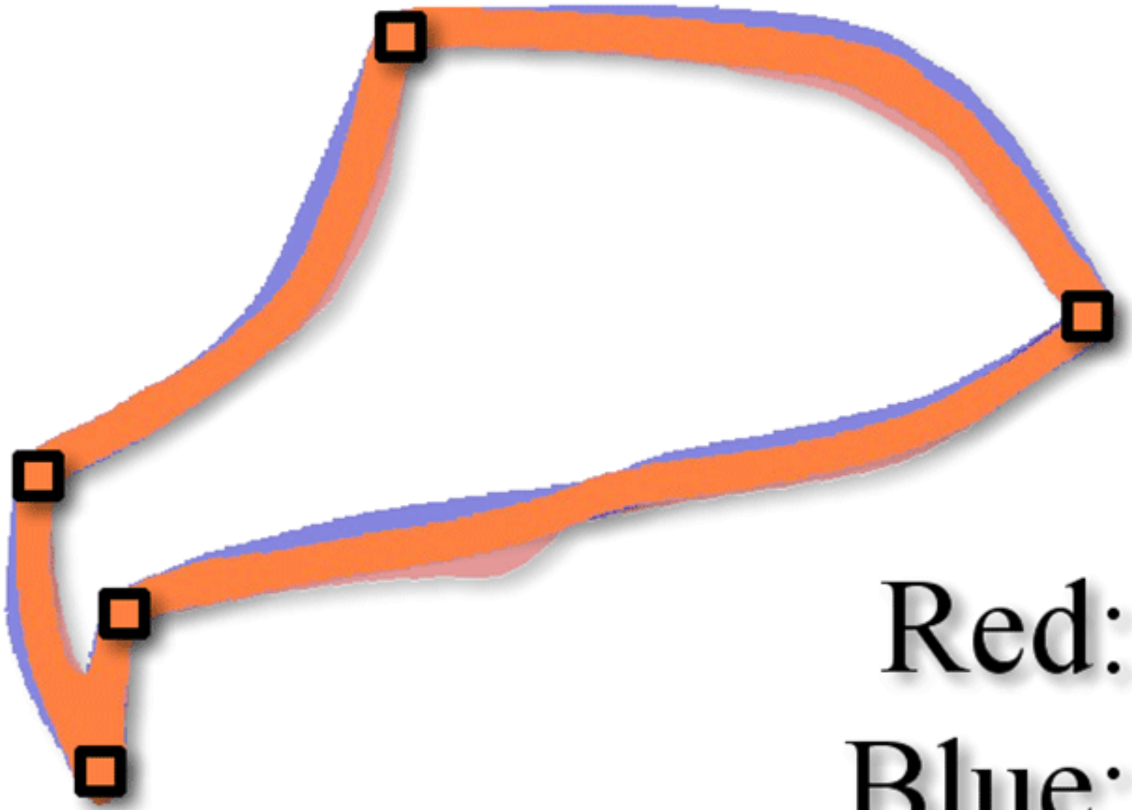
Red: 50%
Blue: 50%

Warping



Red: 50%
Blue: 50%

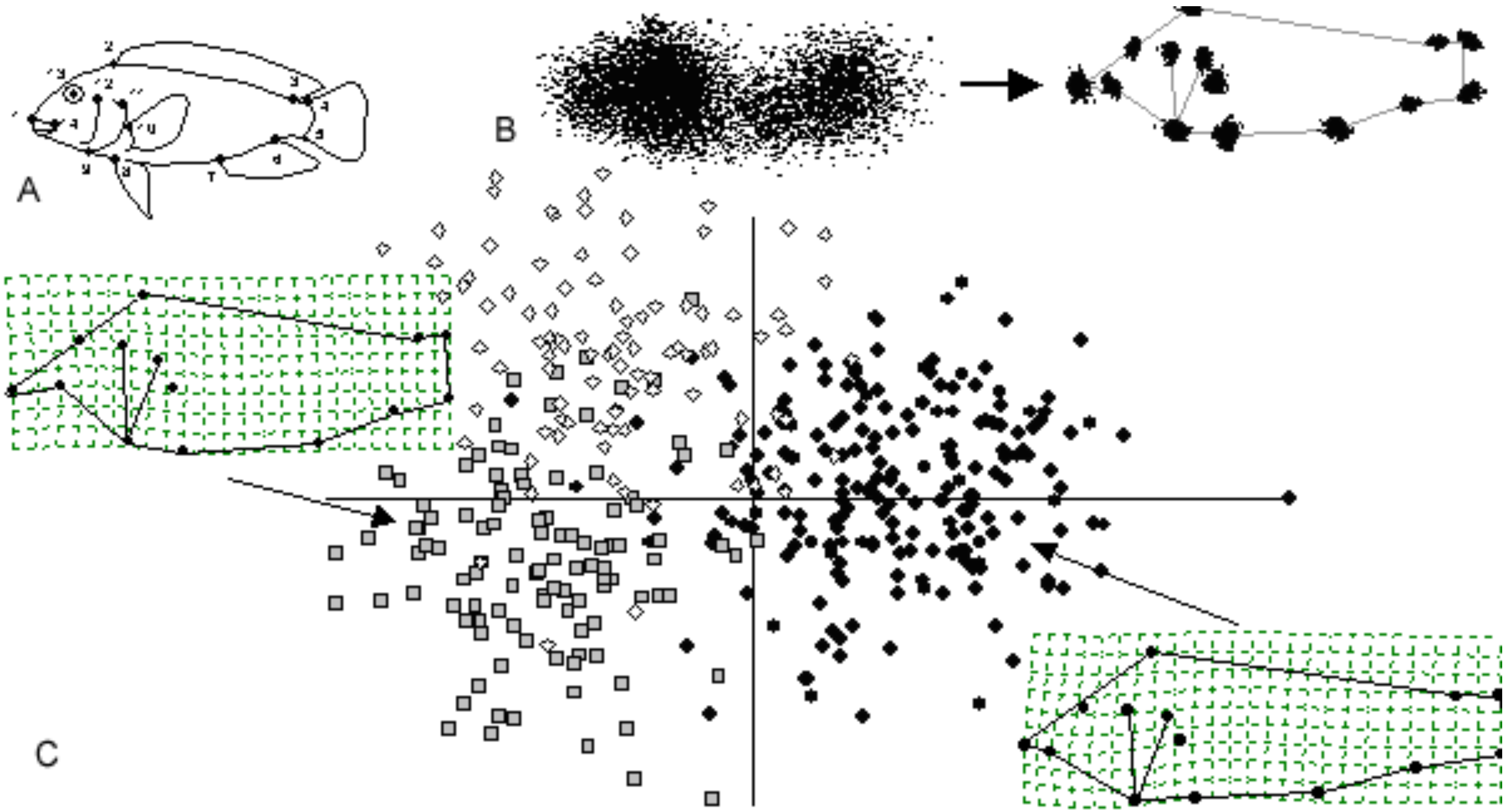
Merging and Extremal



Red: 50%

Blue: 50%

Geometric Morphometric Analysis



http://life.bio.sunysb.edu/morph/review/review.html

Carpiodes shape variation

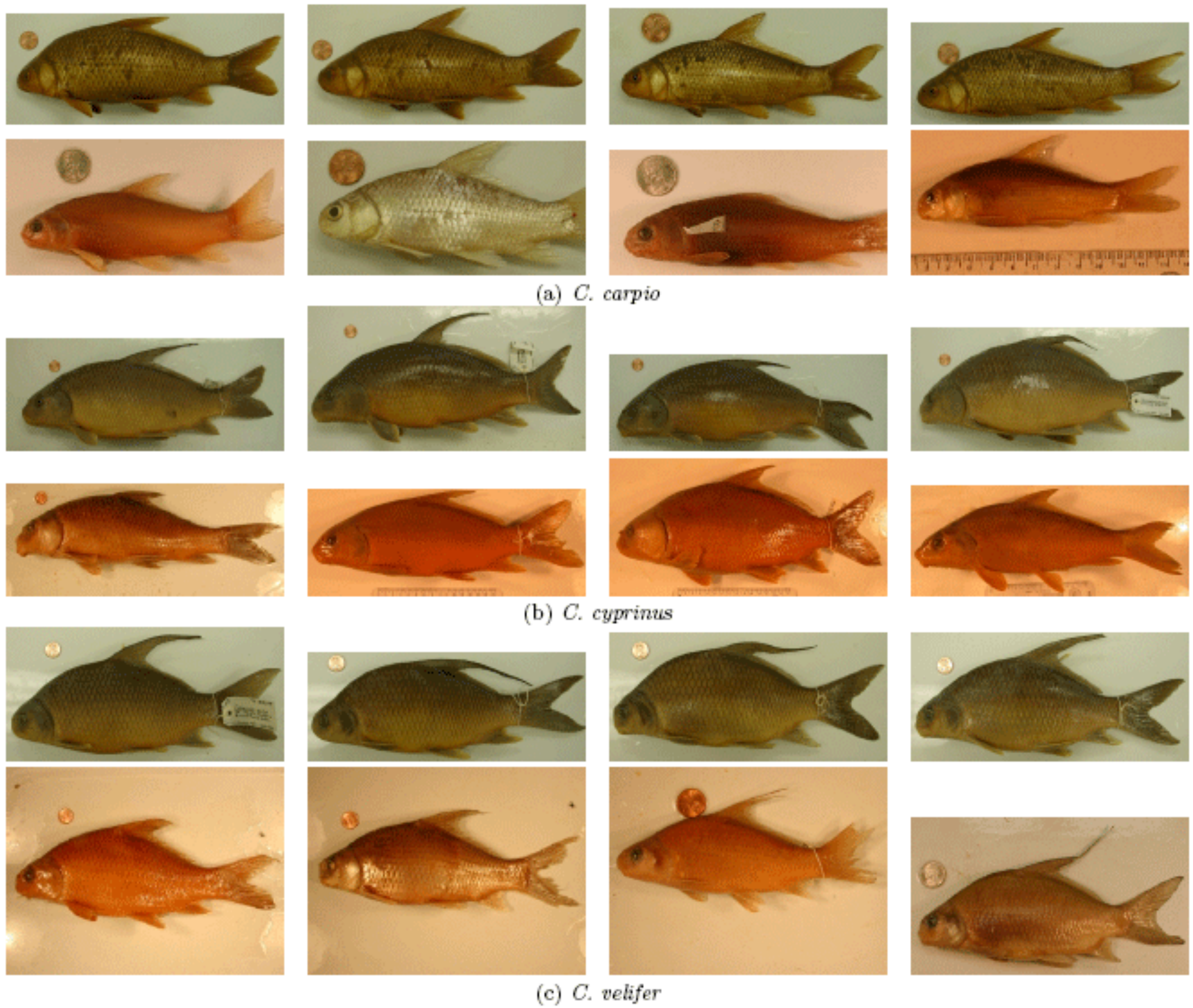
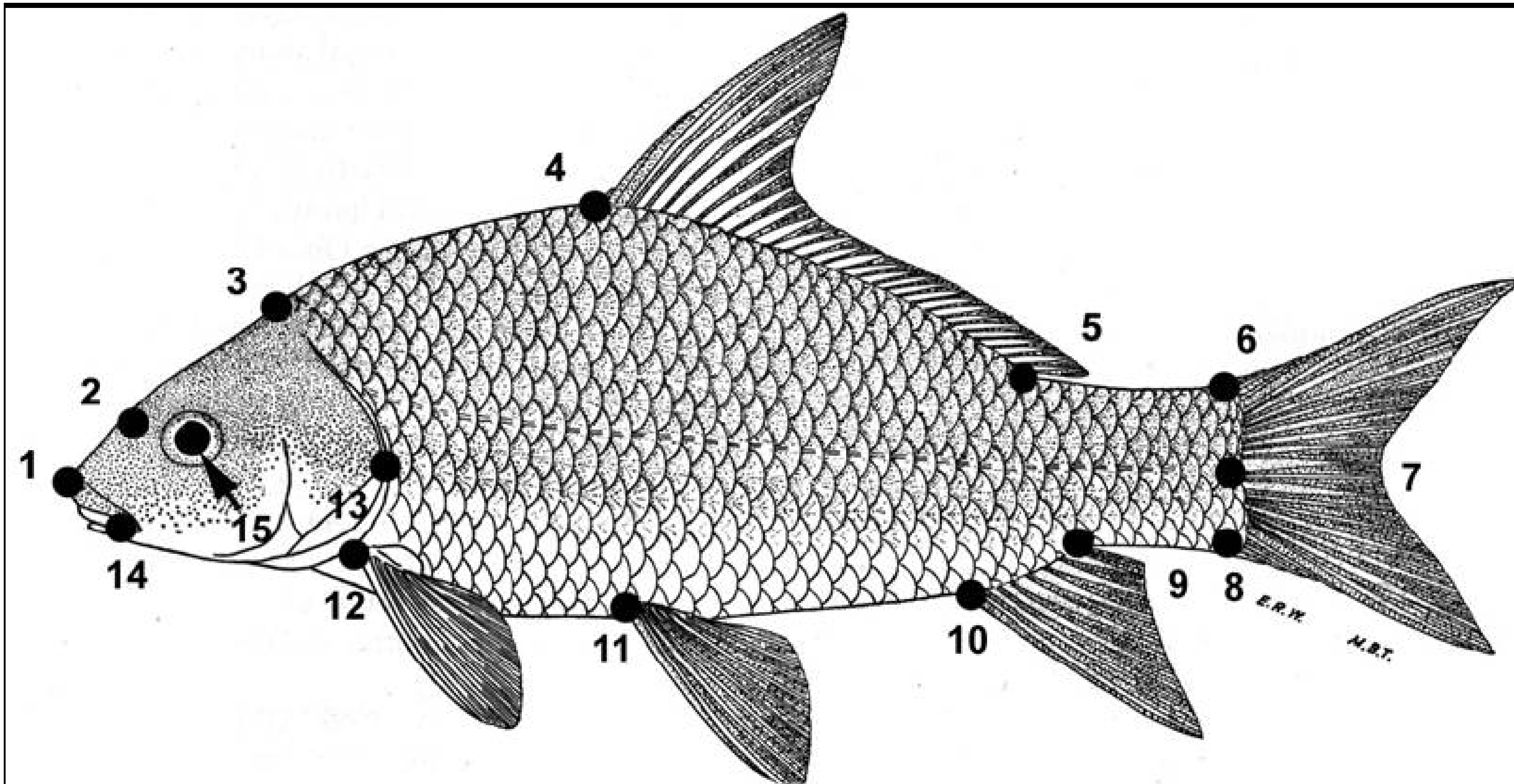
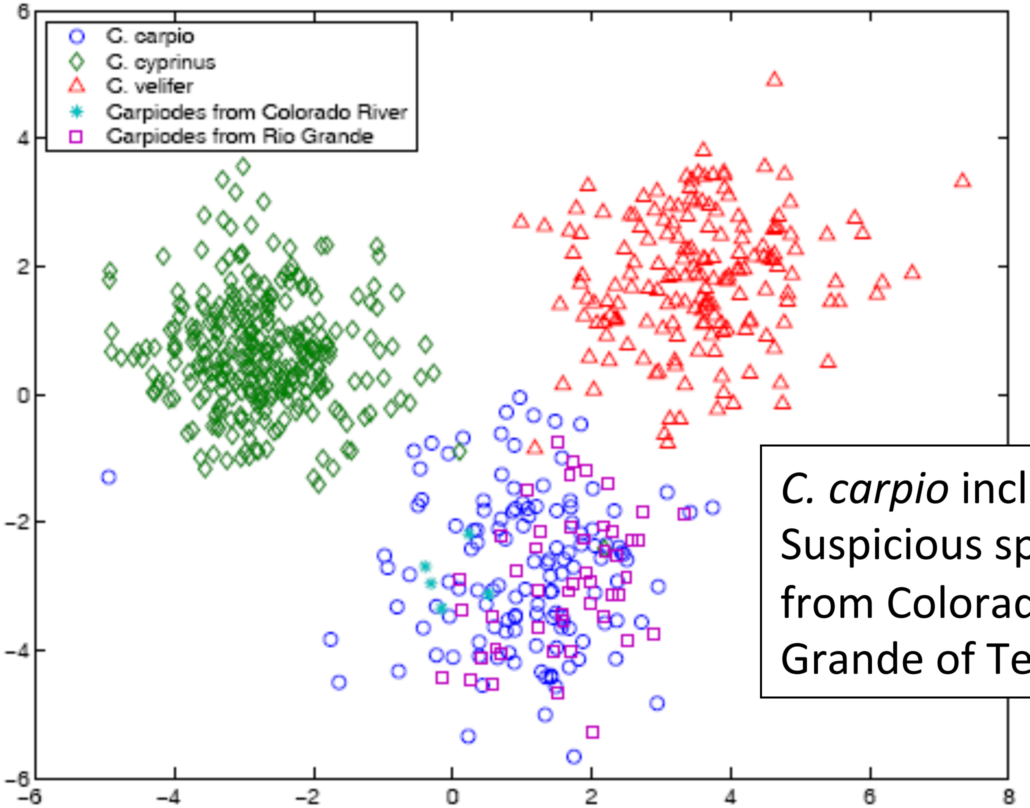


Figure 2: **Images of specimens from three species of the genus *Carpiodes*: *C. Carpio*, *C. cyprinus*, and *C. velifer*.**

Landmarking specimens



Multivariate discrimination of *Carpiodes* species



C. carpio includes 53 Suspicious specimens from Colorado River and Rio Grande of Texas

Figure 4: Plot of 650 *Carpiodes* specimens representing three distinct morphotypes on the first two canonical variate axes based on derived shape variables from geometric morphometric analysis of landmark data.

Landmarking Demonstration using tpsDig