Geometric morphometrics

Outline analyses

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Section 1

Introduction

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- We use the x- and y-coordinates (semi-landmarks) of the outline of the structure
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- **Disadvantage:** Dismisses all information other then silhouette shape

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- Ideal for structures with little internal characteristics

Carcharadontosaurus saharicus

http://www.fossilmall.com

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We extract the outline of a structure

- Ideal for structures with little internal characteristics
- Identify the object of interest in the image
- Extraxt x- and y coordinates along outline
- The first outline point is mostly a **well defined homologue structure**

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That was easy . . . now it gets complicated

Problem 1: Unequal number of outline points

Specimens vary in size and image resolution

We have a number of x- and y-coordinates, but their number differs between specimens → **we have now way to compare them**

Hanafi Idris et al. (2008) J. Biol. Sci. 8: 882–8

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Even if we force the same number of points for all specimens, their **individual positions are not comparable** because the specimens are not identical

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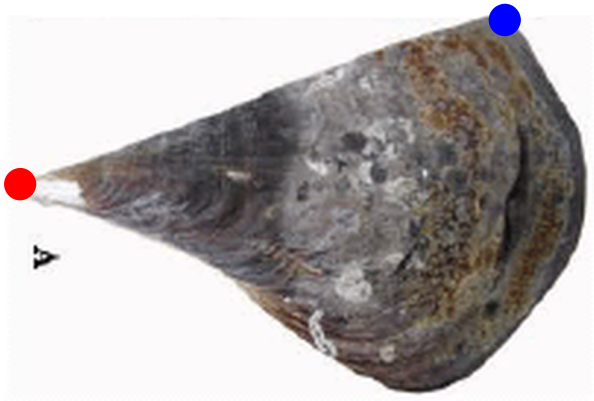

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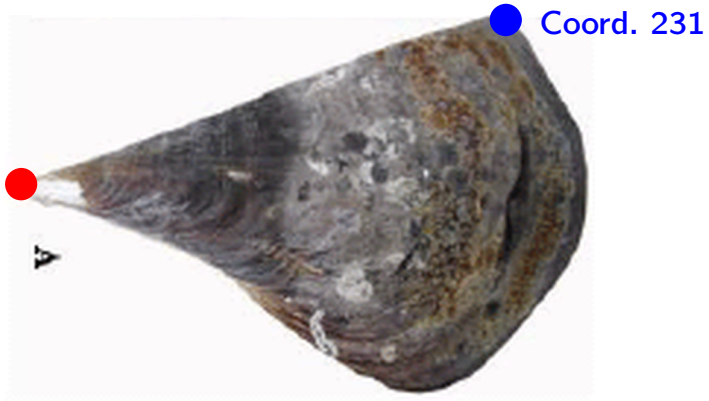

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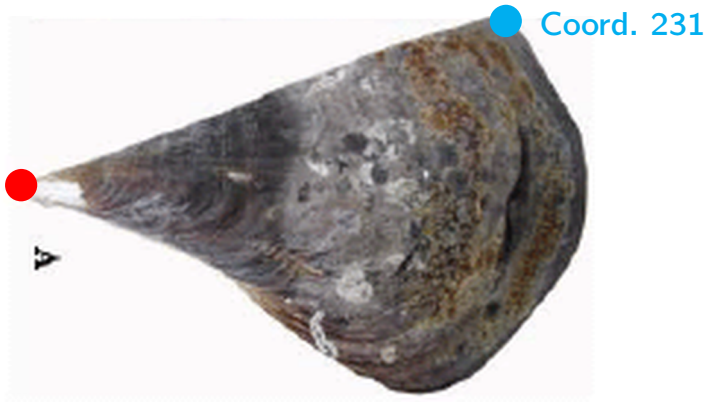

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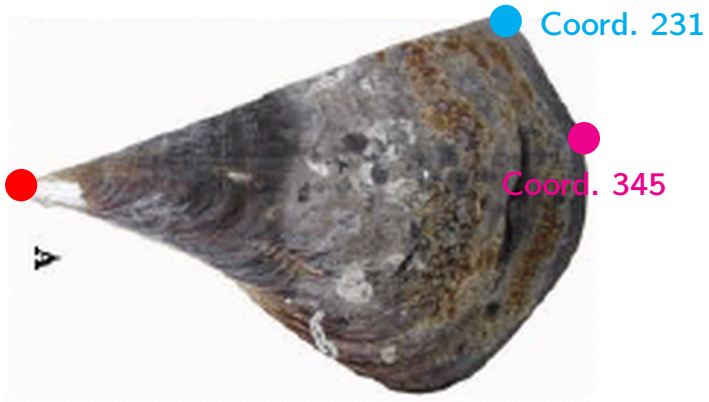

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How do we solve this predicament?

- We have some problems . . .
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- . . . and a potential solution
  1. We re-describe the outlines in a way that makes them comparable $\Rightarrow$ **the same number-position should describe the same morphological characteristic in all specimens**

Section 2

Fourier transformation et al.

The brilliant idea of a French gentleman

- Complex waveforms are difficult to describe

Jean-Baptiste Joseph Fourier

https://en.wikipedia.org

The brilliant idea of a French gentleman

- Complex waveforms are difficult to describe
- What if we describe them as a sum of simpler waveforms?

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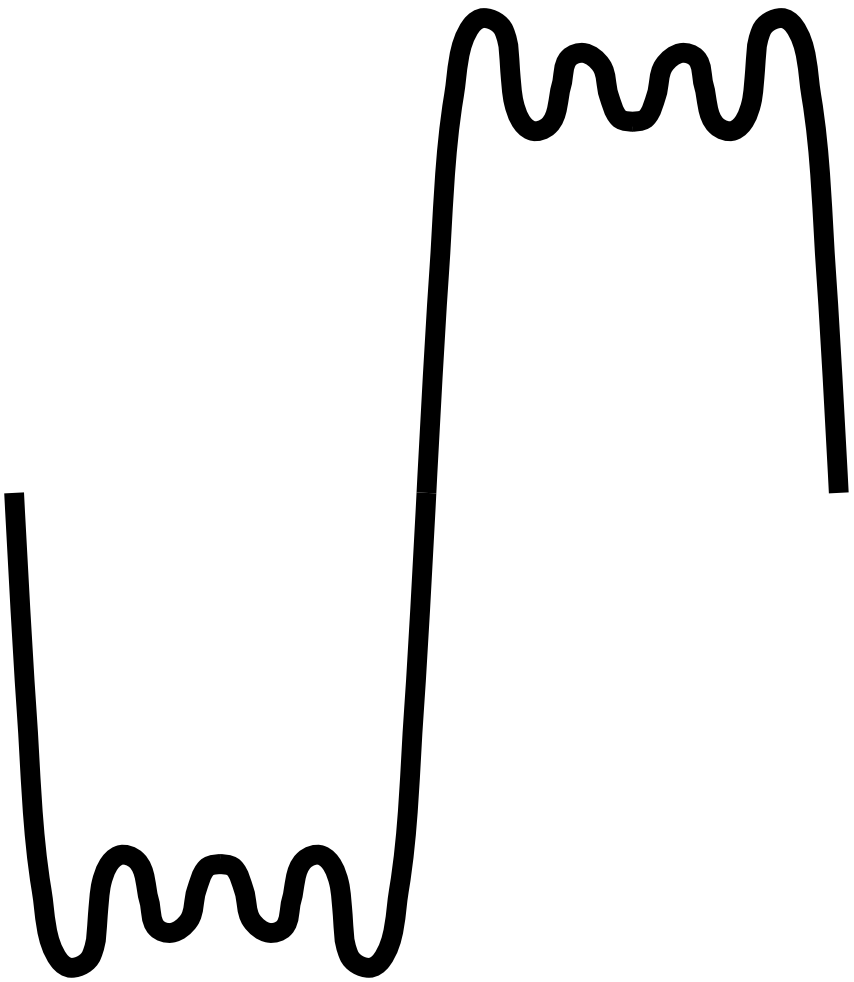
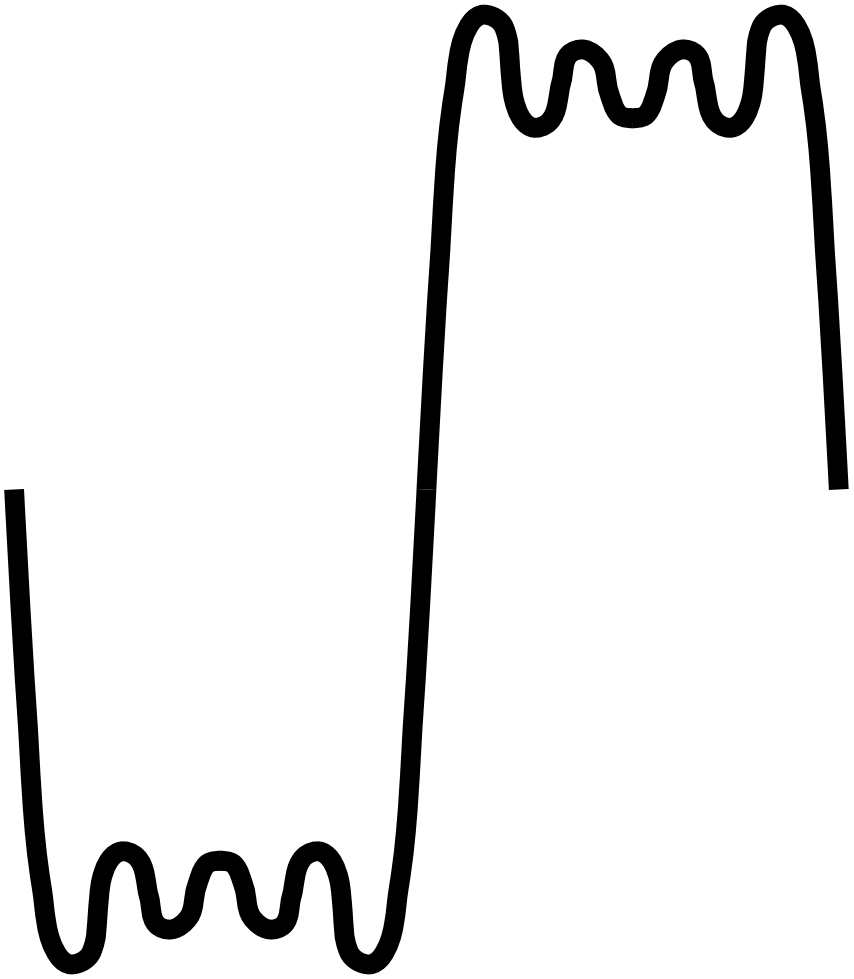
The idea of Fourier analyses

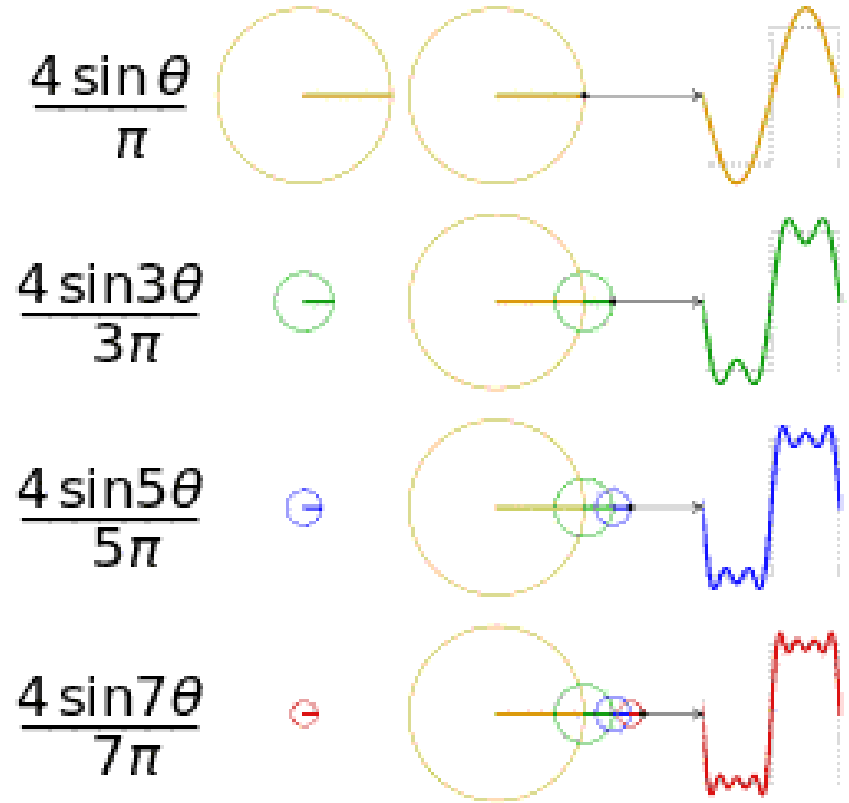
You have a complex form . . .



The idea of Fourier analyses

You have a complex form . . . and describe it as a sum sin- and cos-functions





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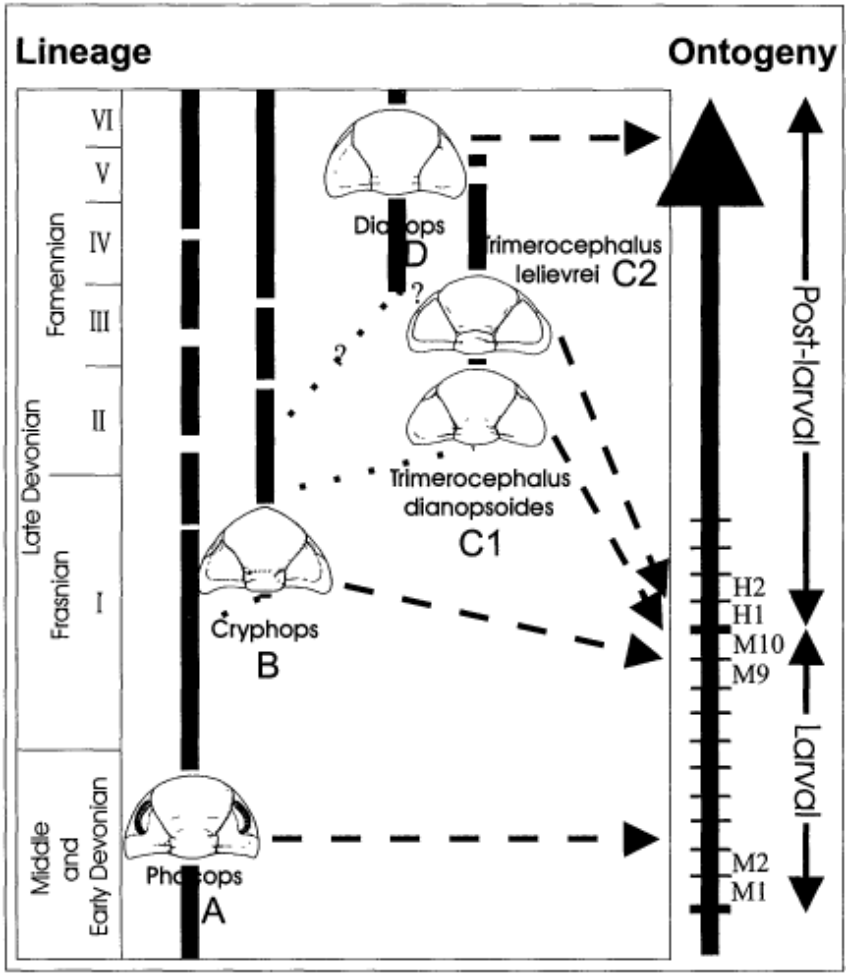
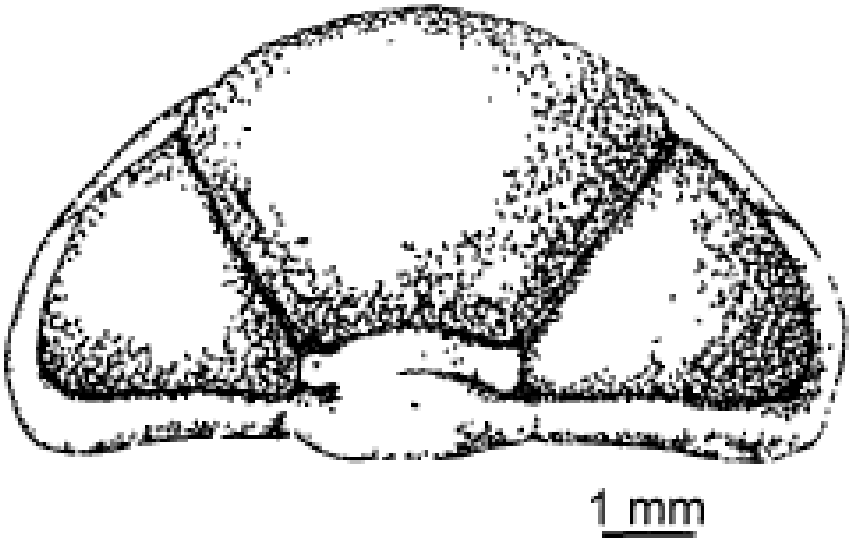
An example: Trilobite cephalons

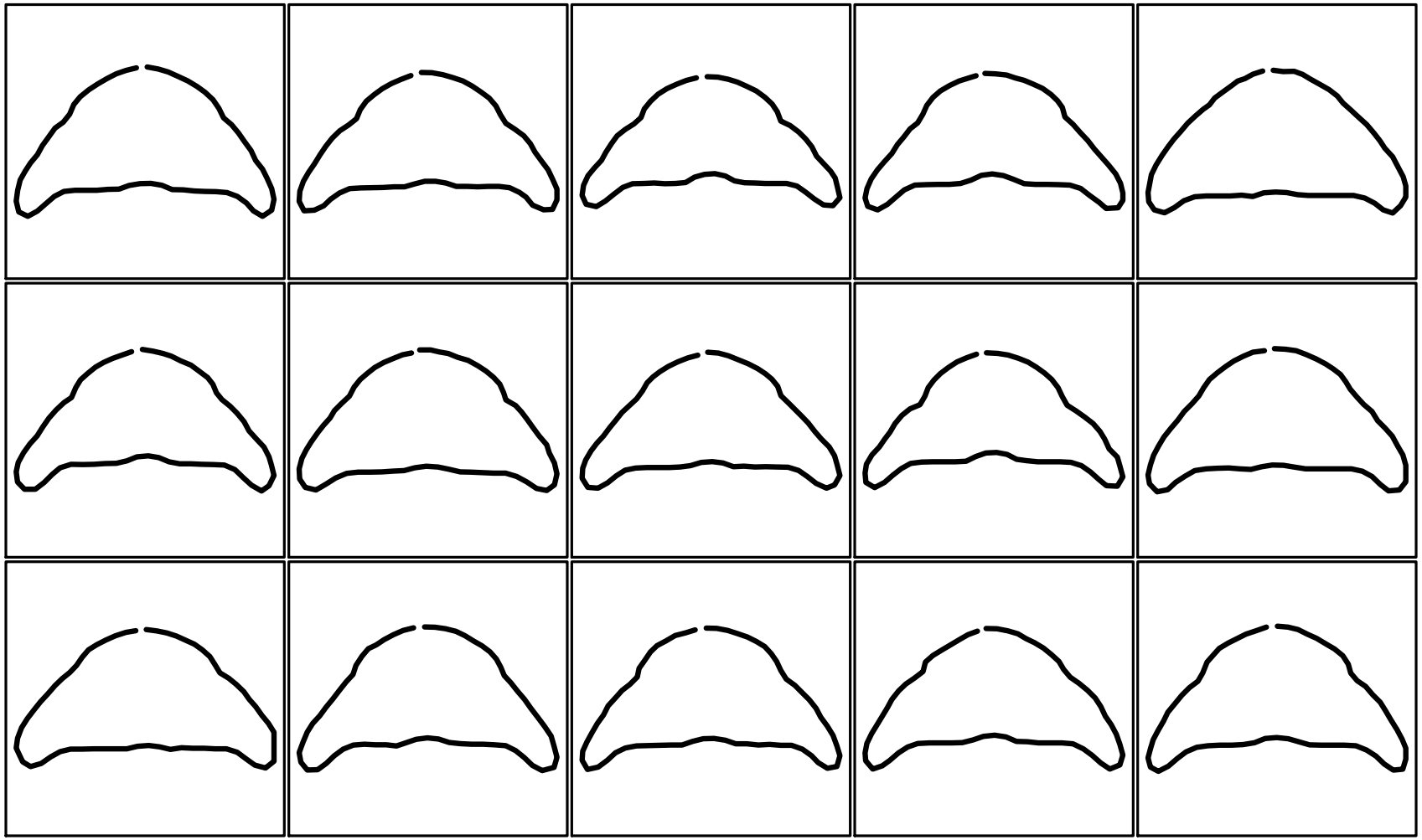
Crônier et al. (1998) *Paleobiology* 24 (3): 359–70





An example: Trilobite cephalons

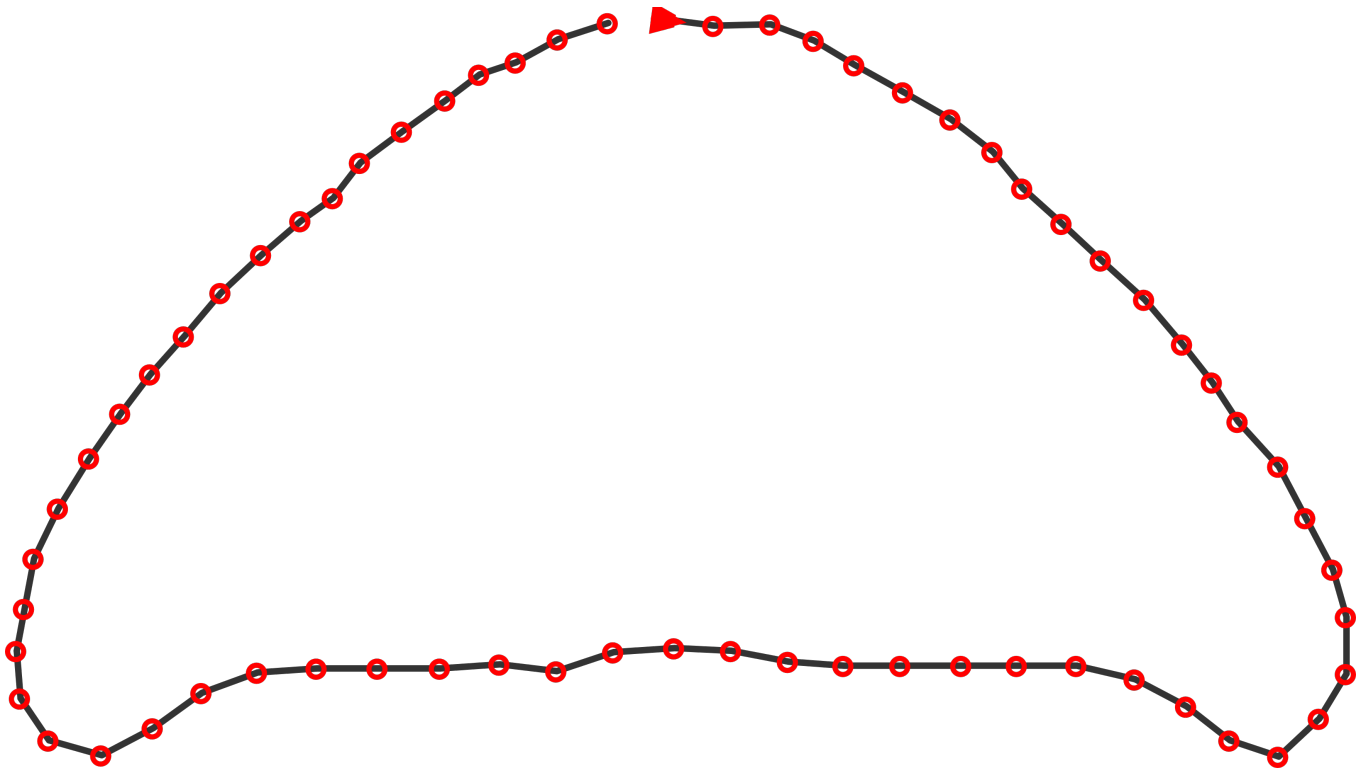
Outlines of the cephalon of 50 specimens of *Trimerocephalus lelievrei*



The original Fourier shape analysis

Step 1

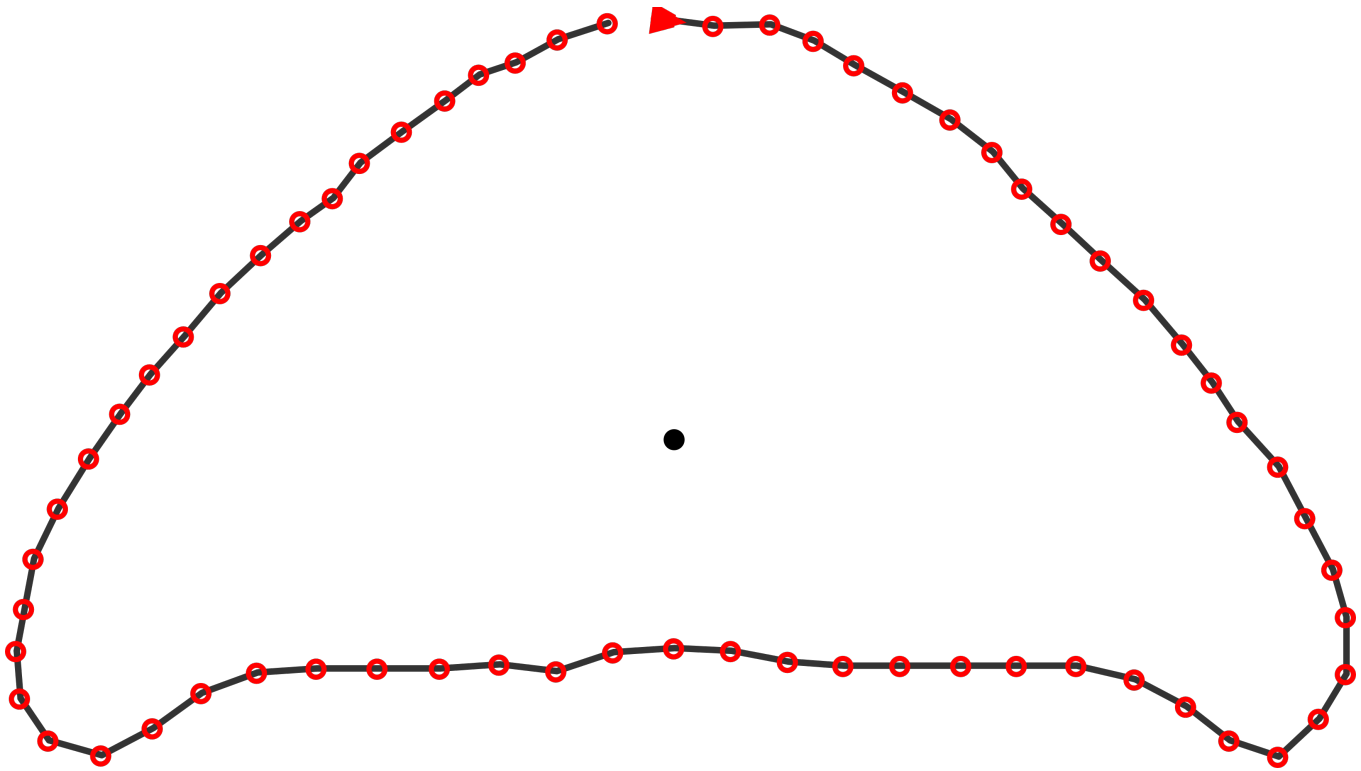
Resample outline for fixed number of equidistant points



The original Fourier shape analysis

Step 2

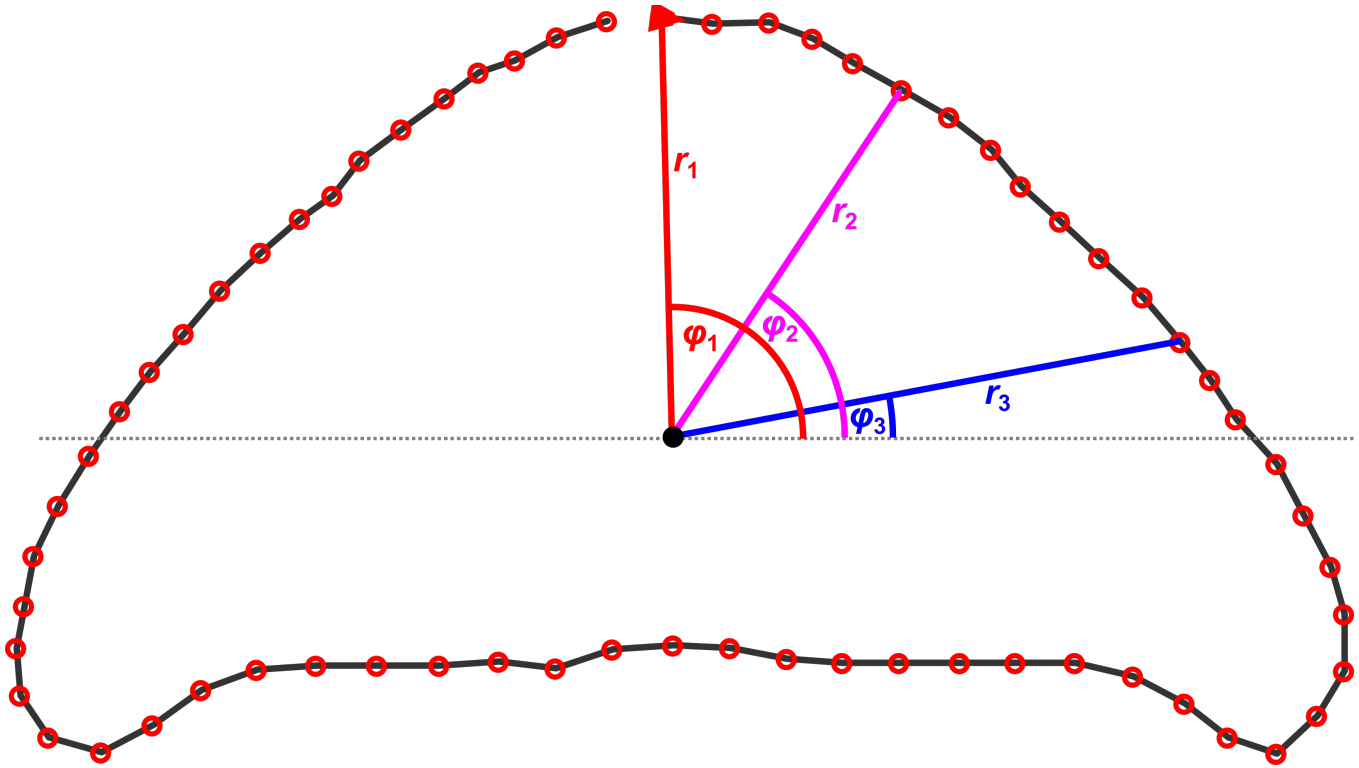
Find the centre of the shape



The original Fourier shape analysis

Step 3

Draw vectors from centre to each point



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1. Each radius you draw gives you an angle ϕ from the horizontal and a length r between the centre point and the outline point

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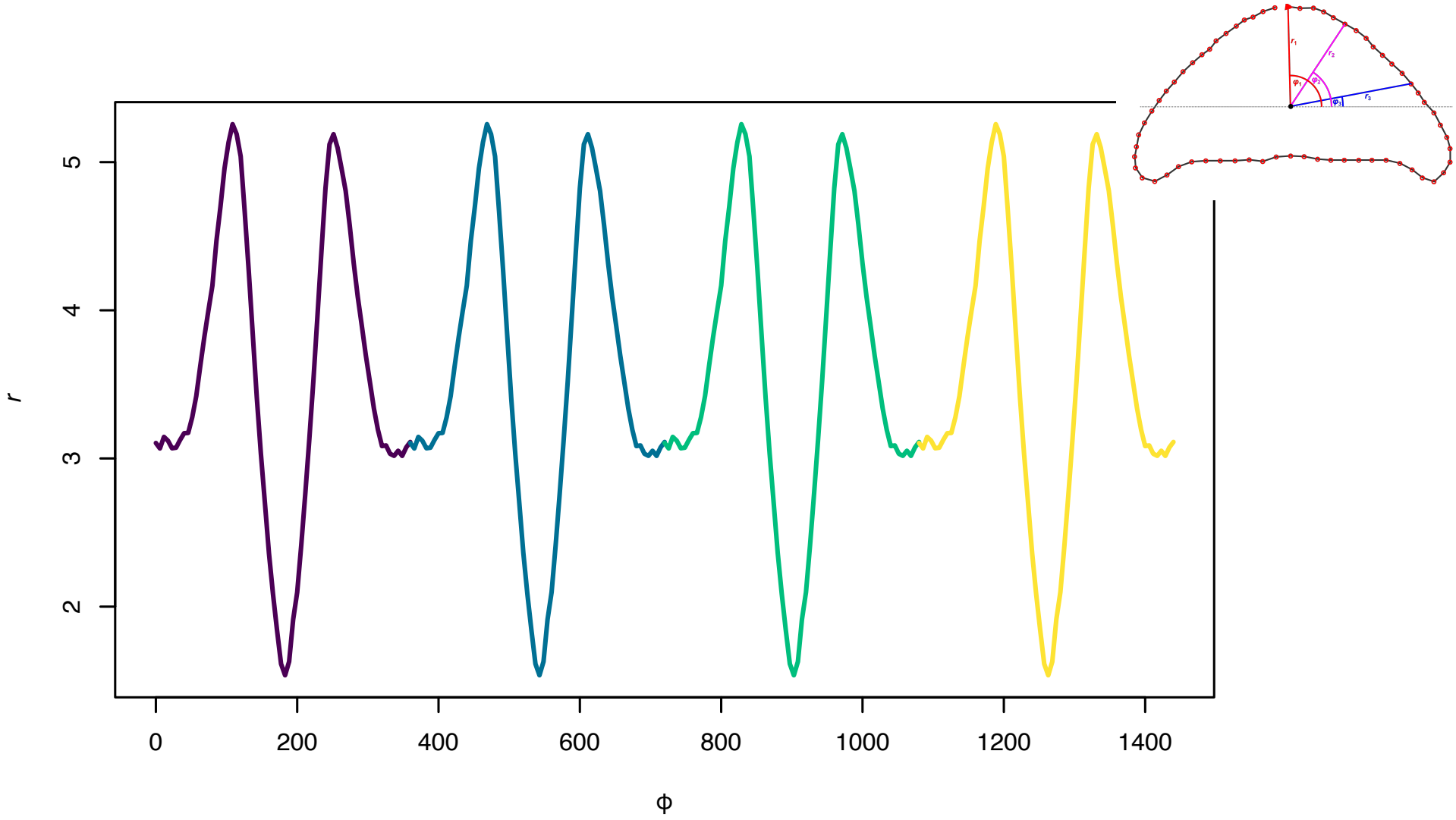
2. The radius will depend on the angle, because it is the distance between centre and outline of the shape at that angle $\Rightarrow$ r is a function of ϕ: $r(\phi)$
3. When you reach the last point in the outline and go a step further, you end up where you began $\Rightarrow$ the function $r(\phi)$ is periodic and can be described by Fourier functions

Fourier re-description of the shape



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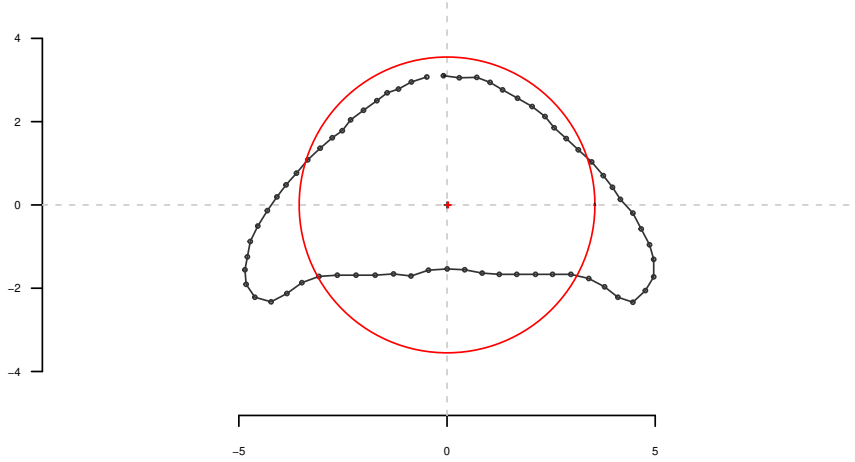

Fourier re-description of the shape

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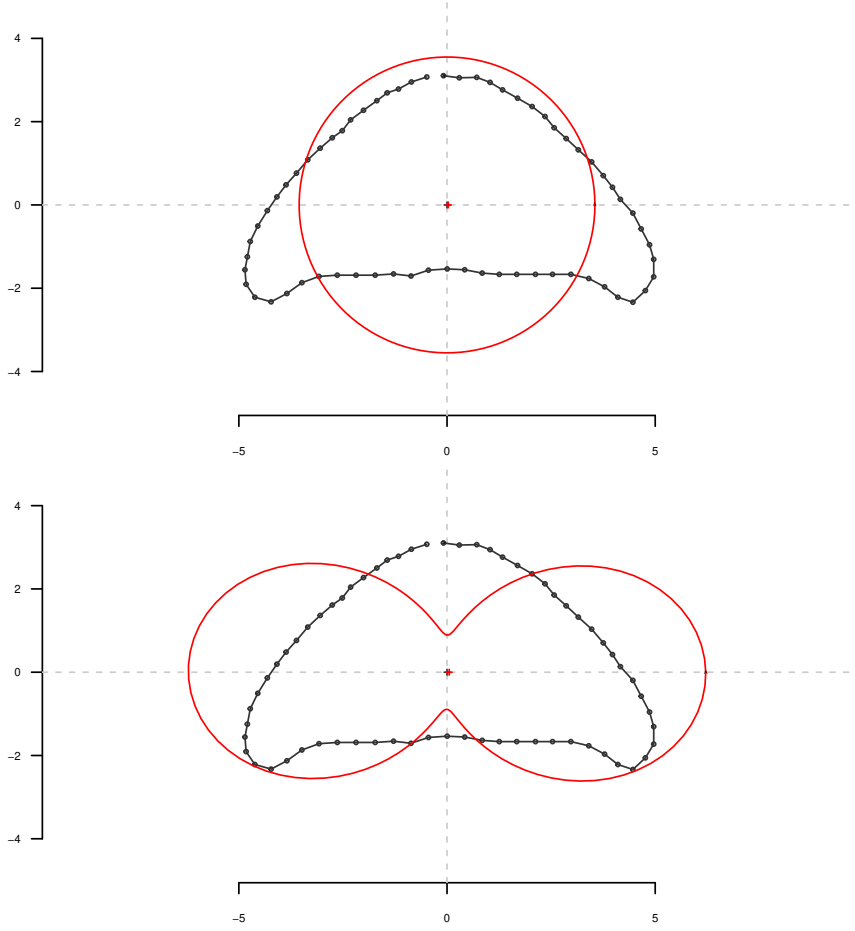
  1. One harmonic can only describe a circle
  2. Two harmonics can describe an ovoid shape



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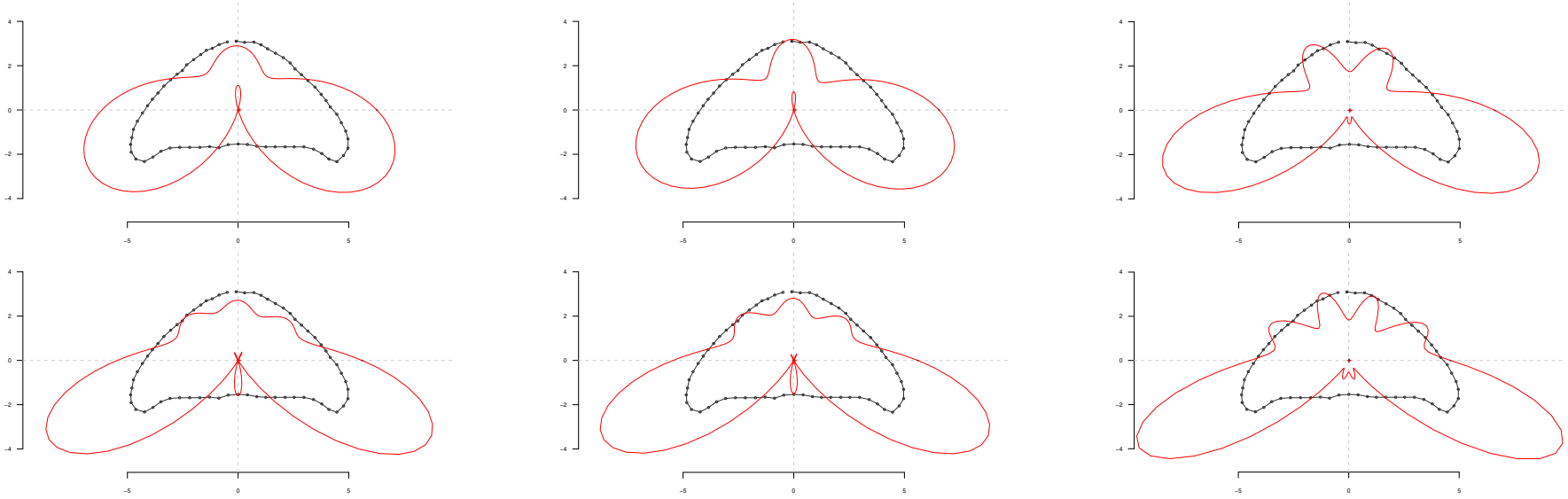

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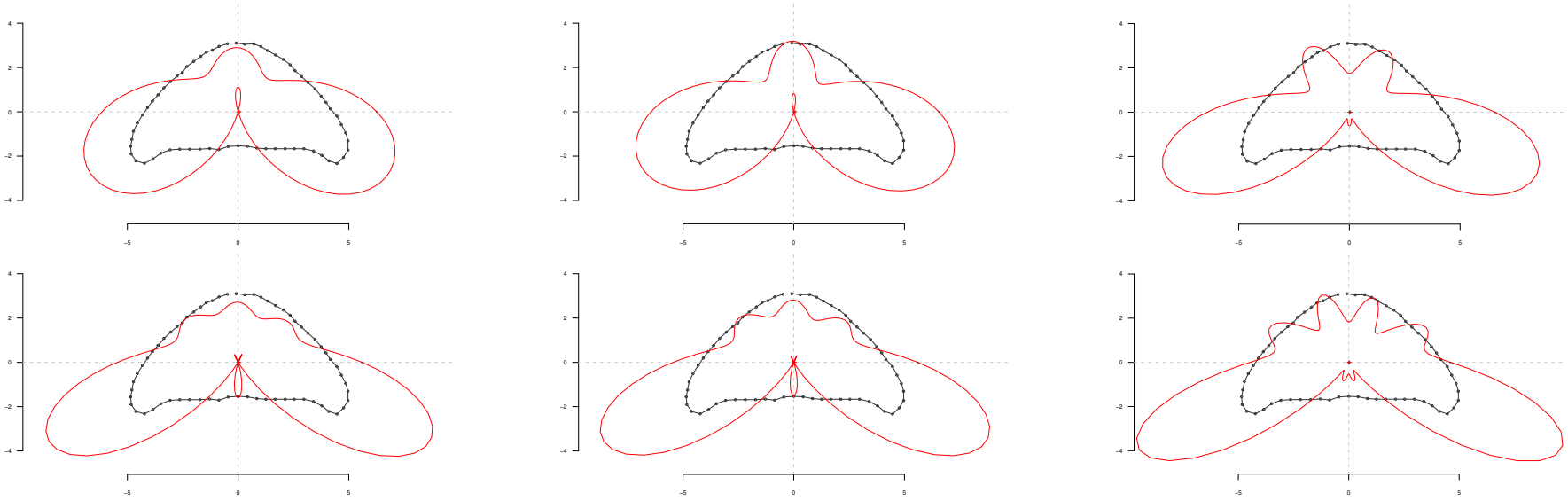
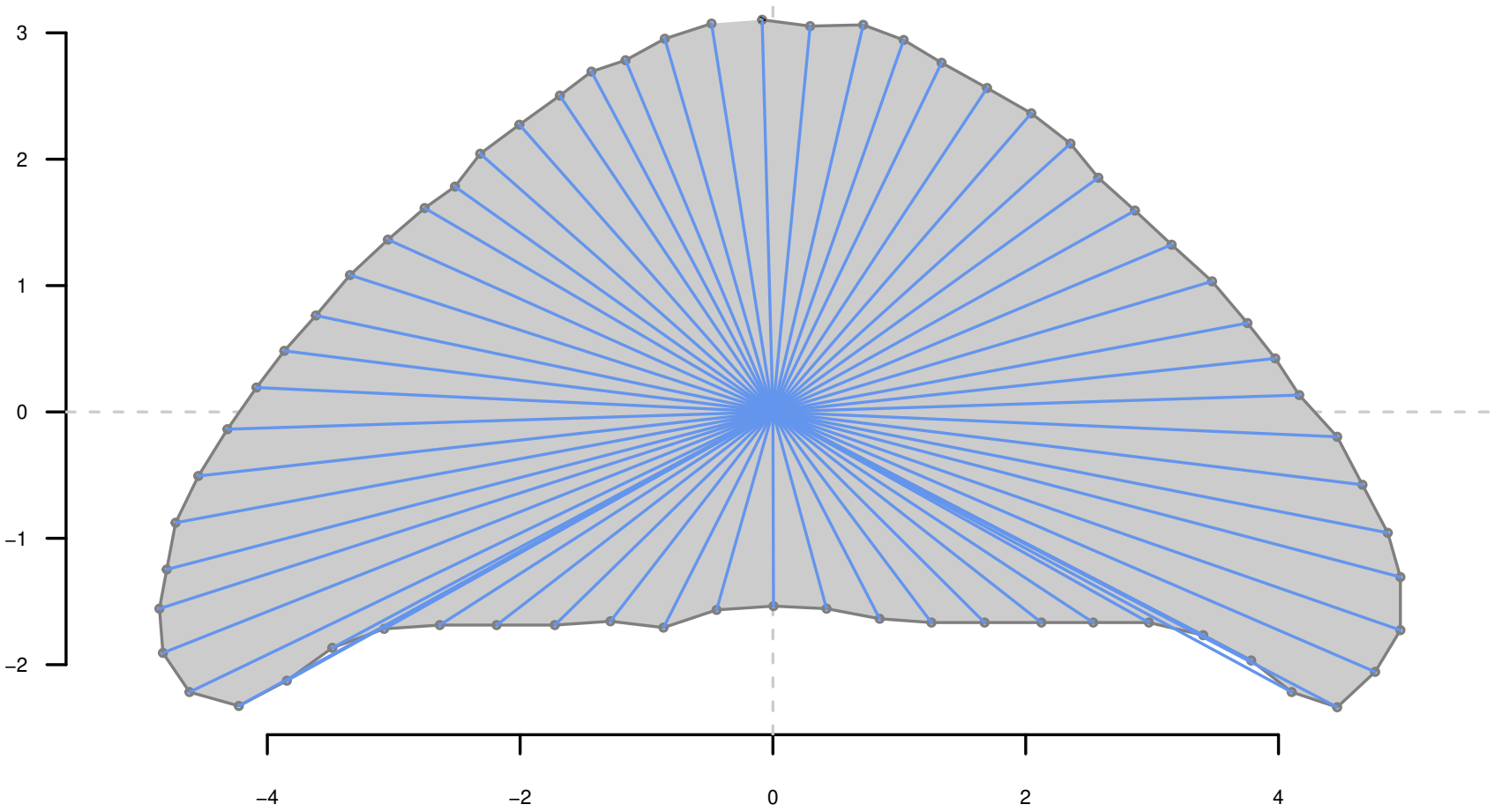


Problem 1: This simple approach is not very good at describing even only fairly complex shapes

The other problem with radii Fourier analysis



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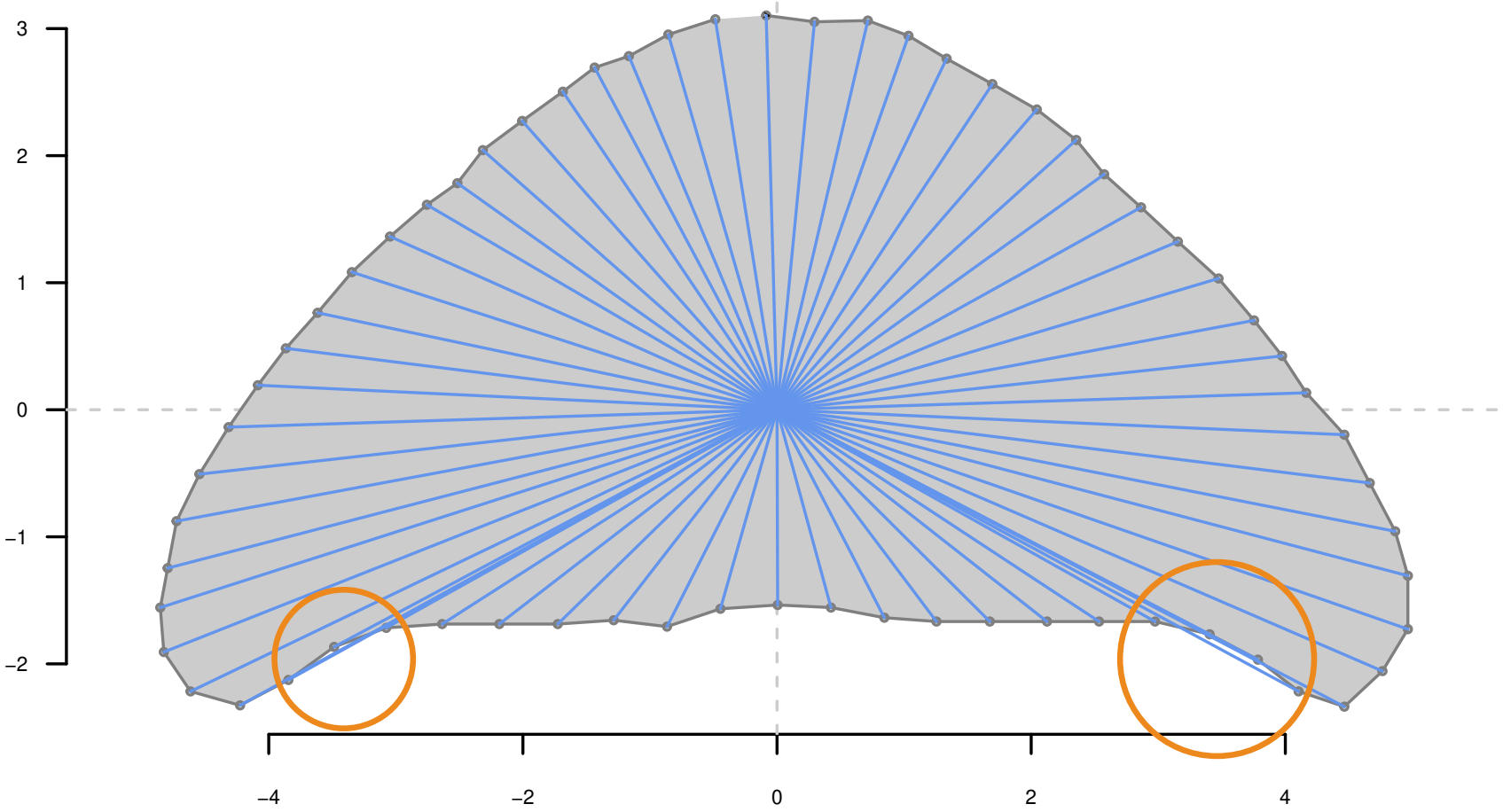

Elliptic Fourier analysis

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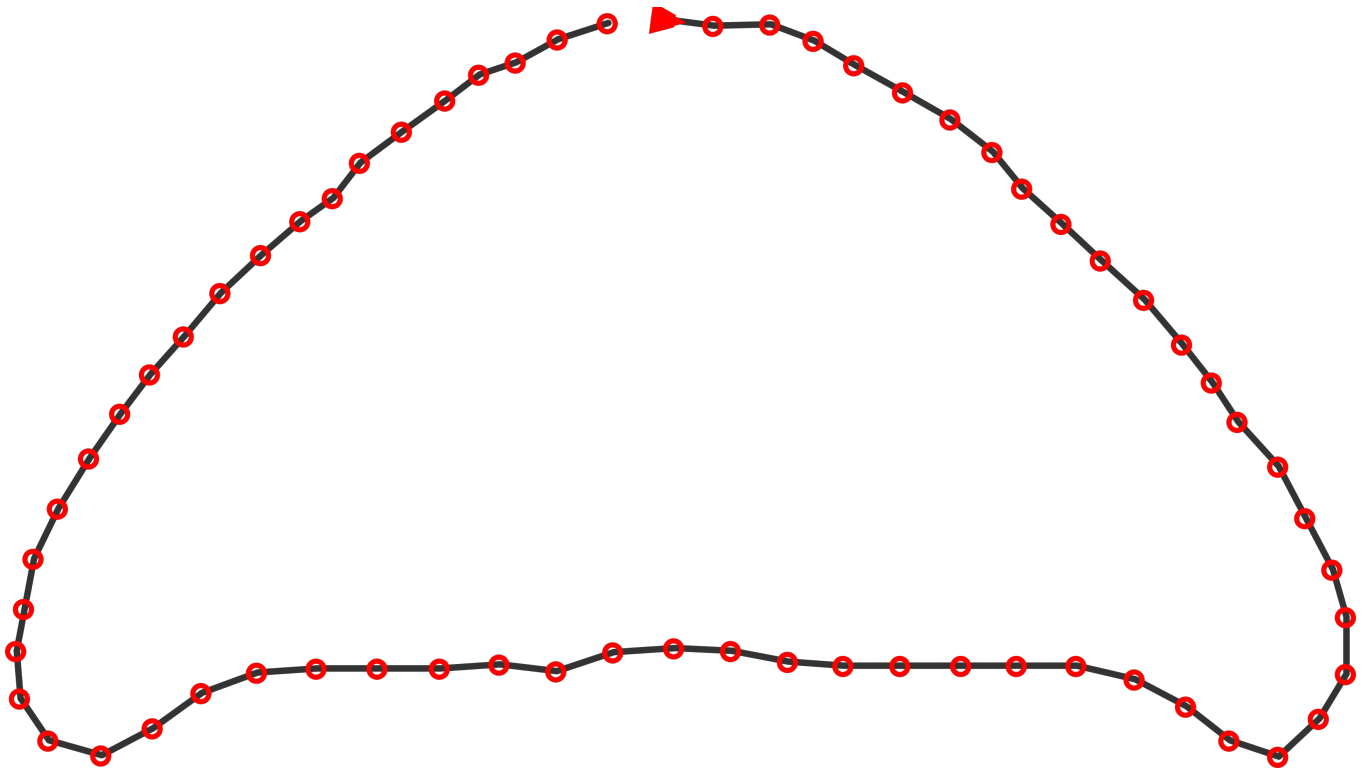
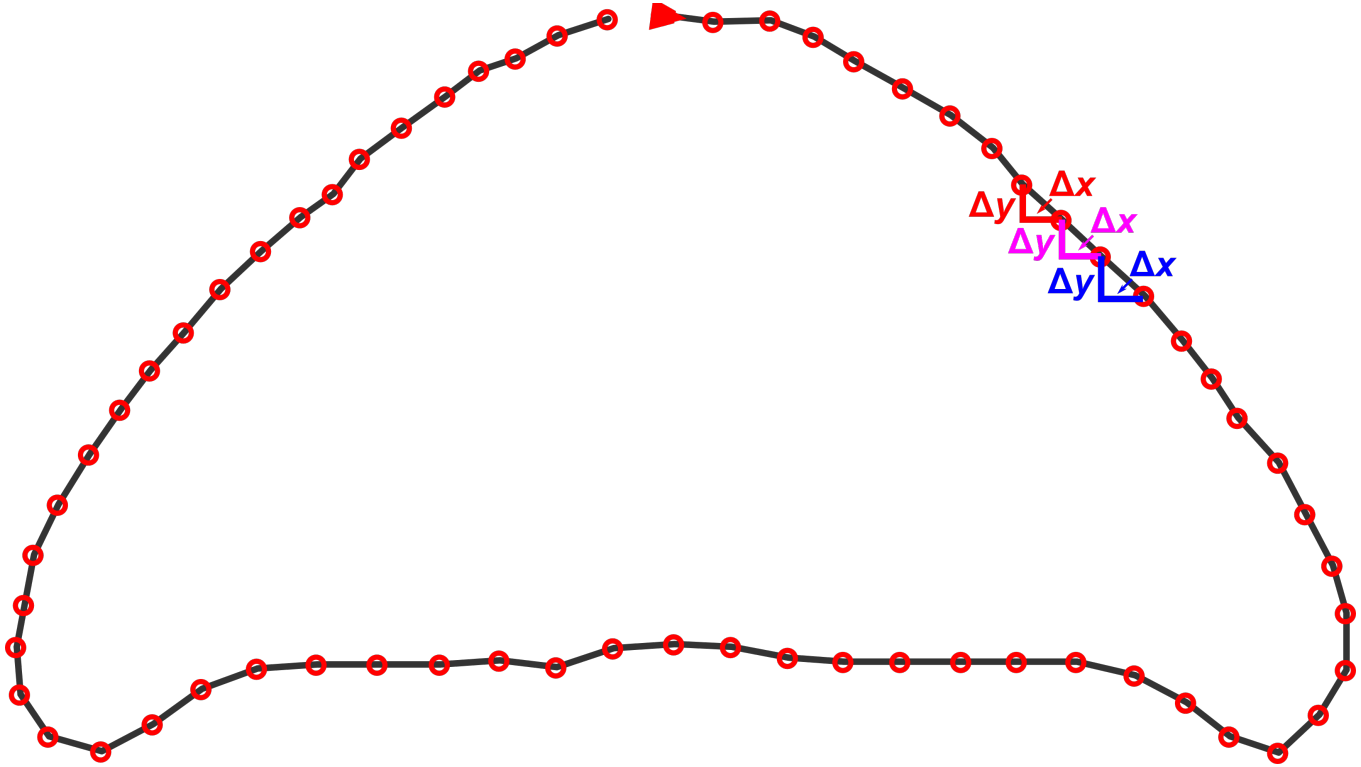


Elliptic Fourier analysis

Step 2

Gather step size (Δx, Δy) from point to point



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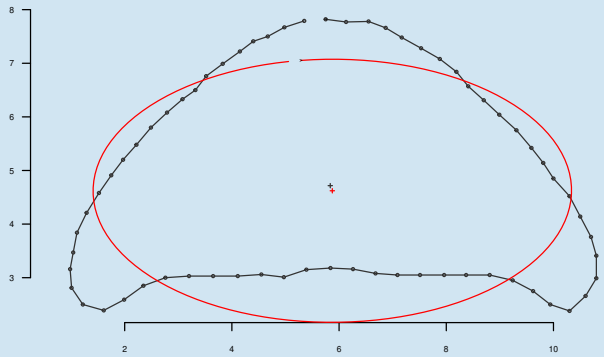
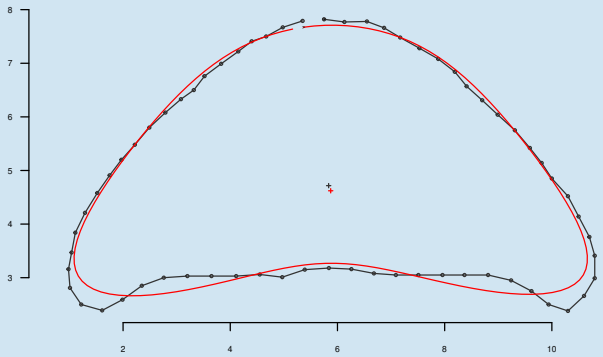
- You still have many data points, indeed four times as many as original outline points, but these **data points are now comparable with each other**

Much better performance via radii variation method

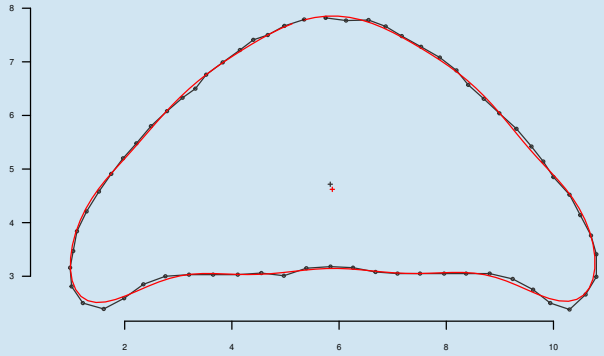
1 Harmonic



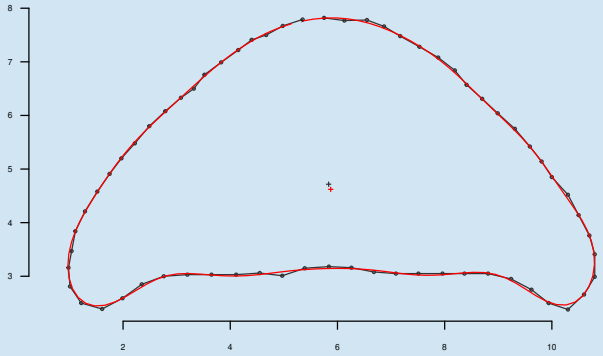
4 Harmonics



8 Harmonics



12 Harmonics



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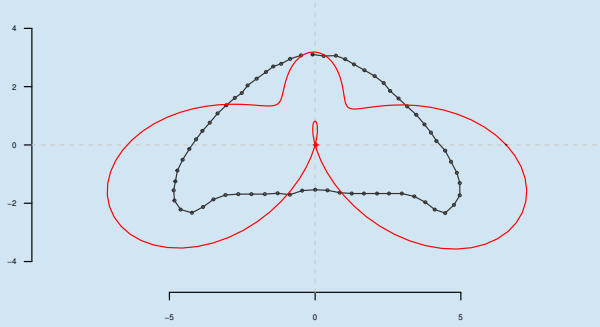

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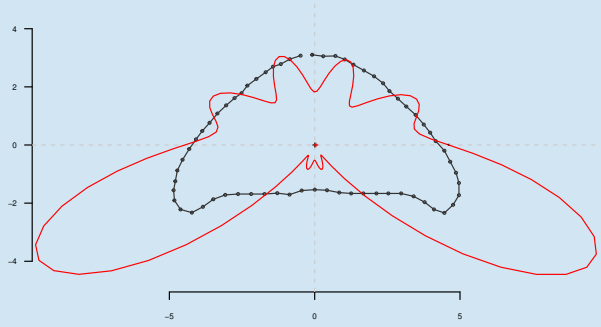

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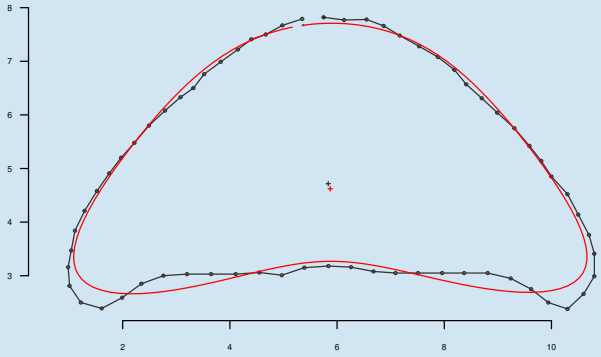

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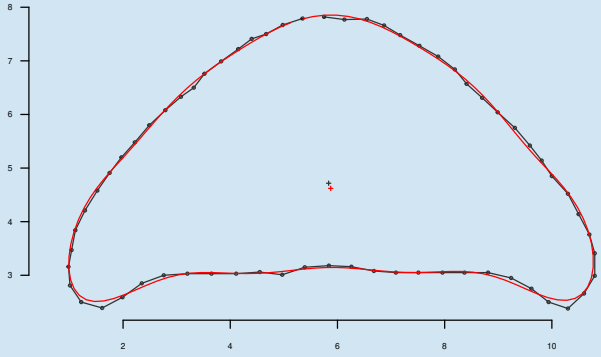
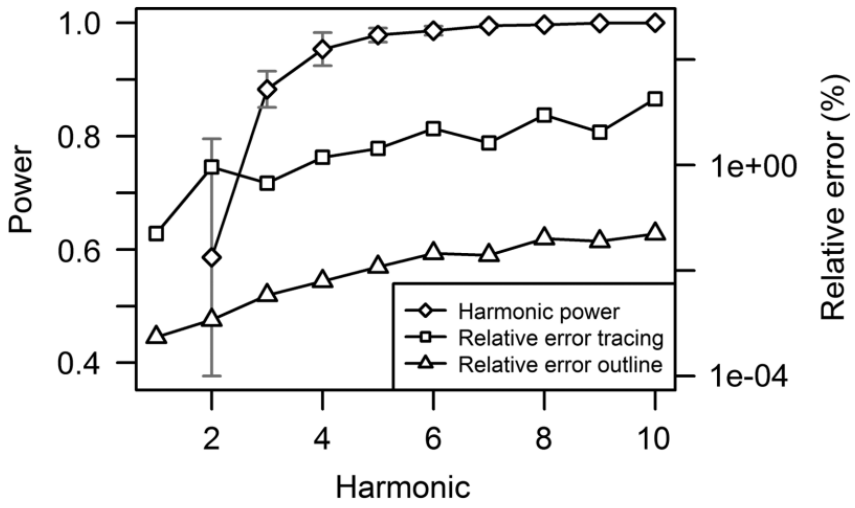


Enough is enough

Finding the optimal number of harmonics

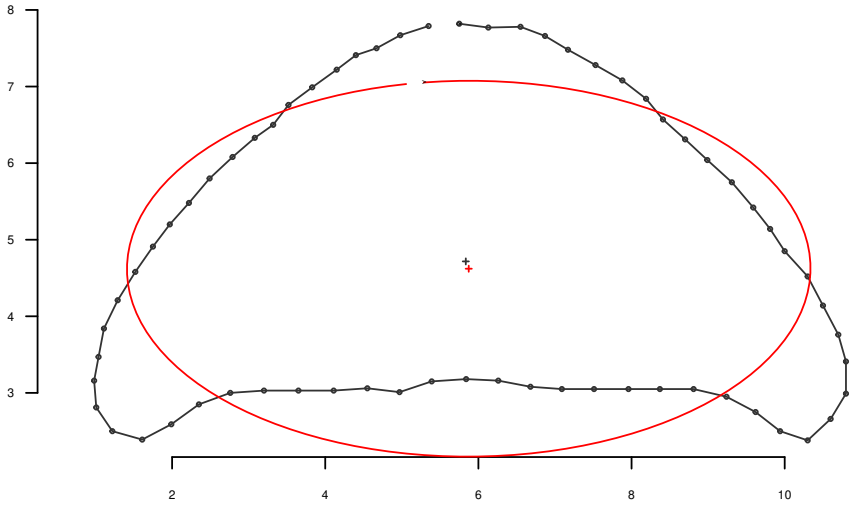
- Cumulative descriptive power of harmonics can be calculated for each specimen
- Optimal number of harmonics can be chosen at level where average or minimum power of harmonic is above a defined threshold across all specimens
- Threshold often chosen as 99 %, but can be different



Hoffmann et al. (2017) Paleobiology 43 (2): 304–20

Normalized EFA

- Usually, outlines are rotated during analysis to eliminate arbitrary differences due to object orientation in the image
  1. Based on a well-defined baseline (requires two reliable landmarks)



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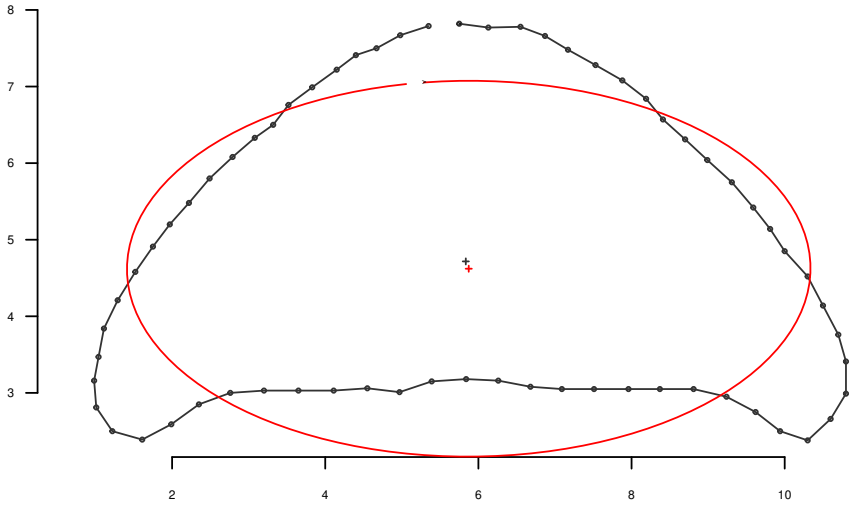

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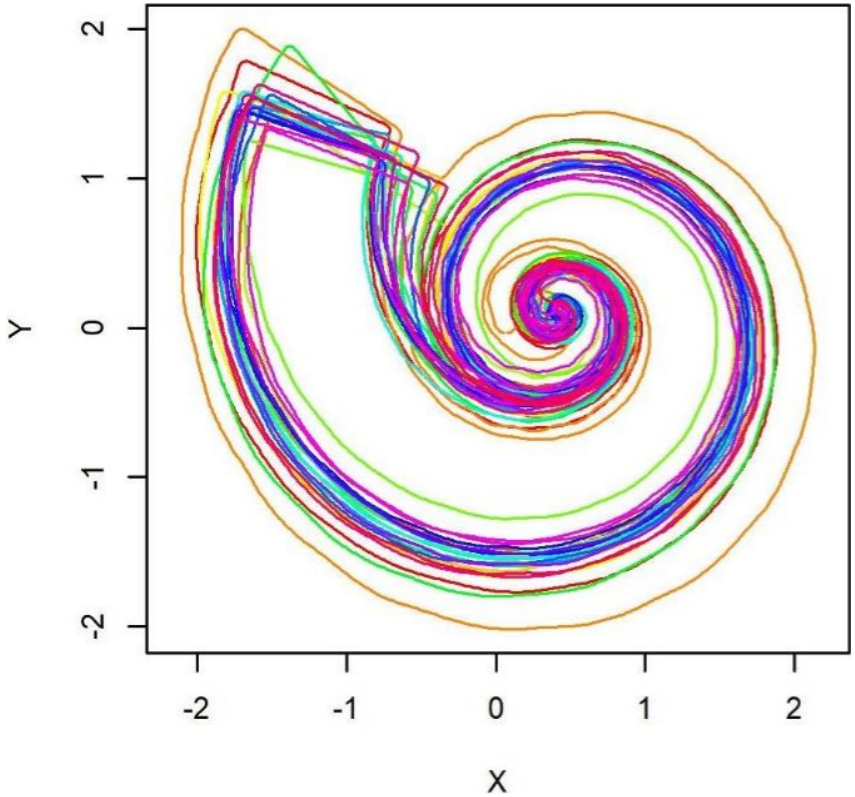
- Normalization ensures that only relevant shape information is used for analyses



Hoffmann et al. (2021) J. Molluscan Stud. 87: eyab001

Other outline procedures

- There exist other methods of outline analyses as well, for instance
  1. Zahn–Roskies Fourier analysis
  2. Fast Fourier transform analysis
  3. Eigenshape analysis
- All methods have advantages and disadvantages, but elliptic Fourier analysis is mostly a good choice

R

Example of outline data preparation

For a look at outline data preparation in R, we move on to exercise № 2

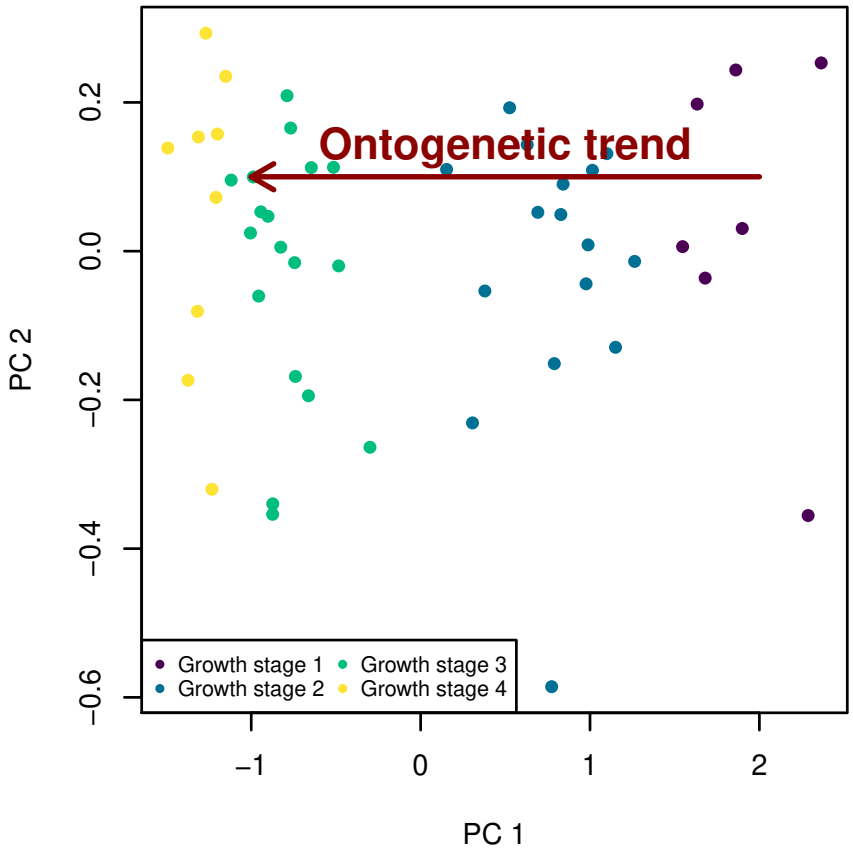
Open the exercise sheet for instructions and code examples

Section 3

Outline data analysis

We now have a multivariate dataset

- We can analyse the data in the same way as we analyse other multivariate data
  1. PCA to visualize differences
  2. Cluster analyses to define groups
  3. LDA/CVA to distinguish *a priori* defined groups
  4. MANOVA to test for between-group differences



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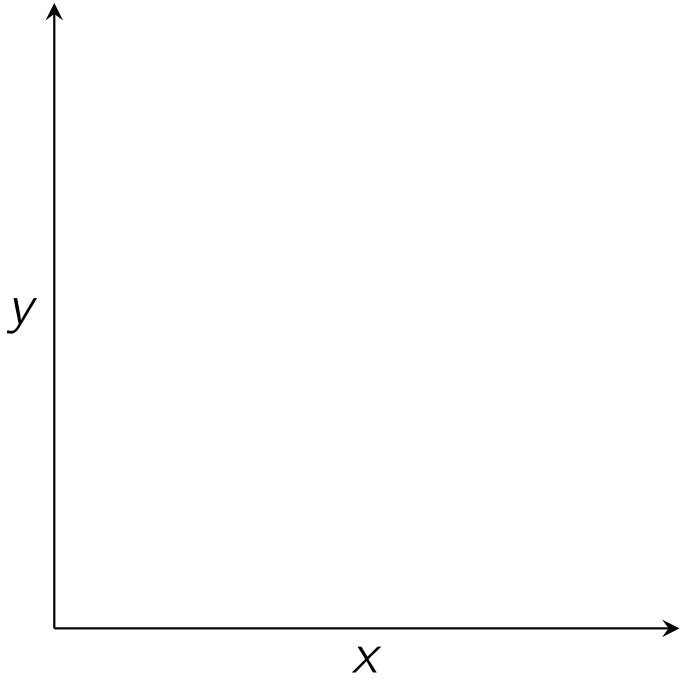
- The PCA allows to see the two or three most important components of the morphological description to see differences and/or clusters of points
- The PCA shows the **morphospace** of the analysed organisms
- PCA is **only data visualization** for data mining, **not statistics**

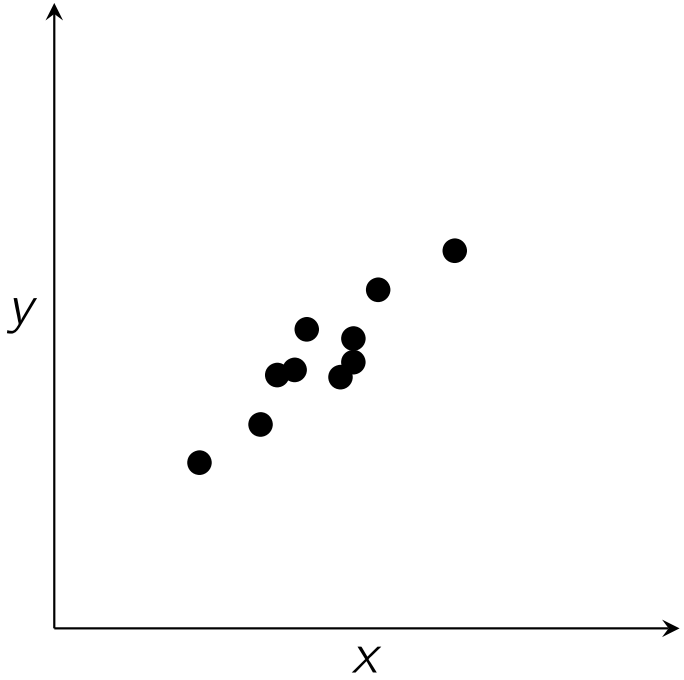
Principal component analysis

Example in 2 D



Principal component analysis

Example in 2 D



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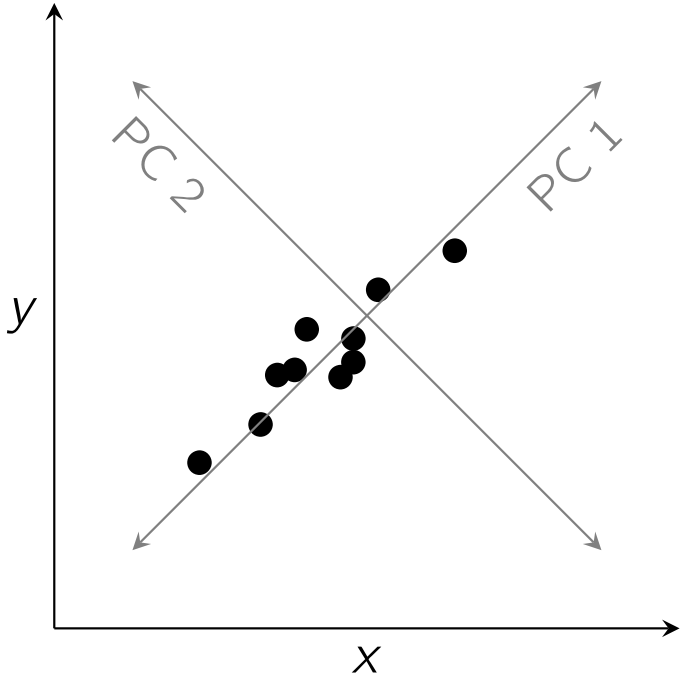

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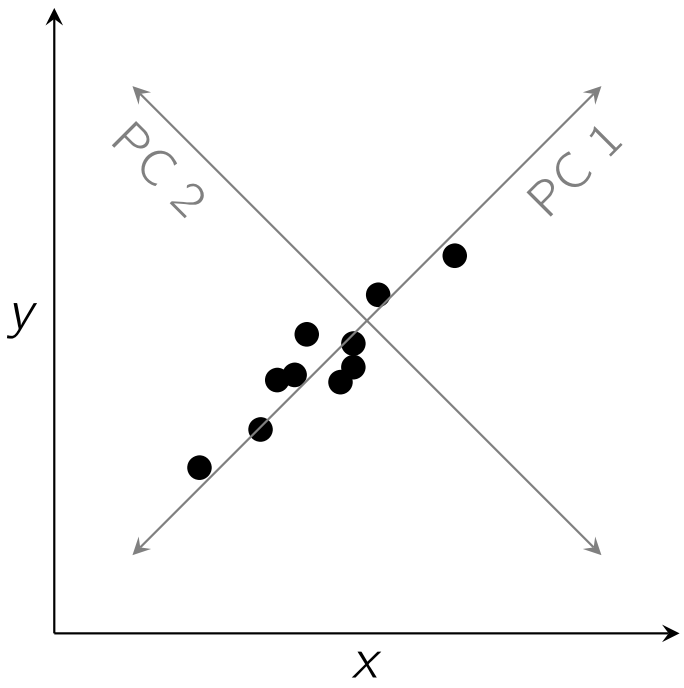
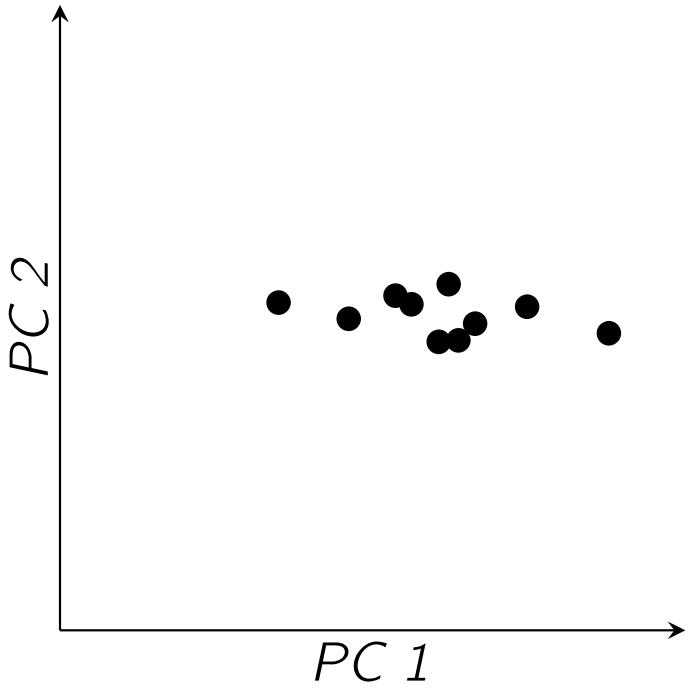
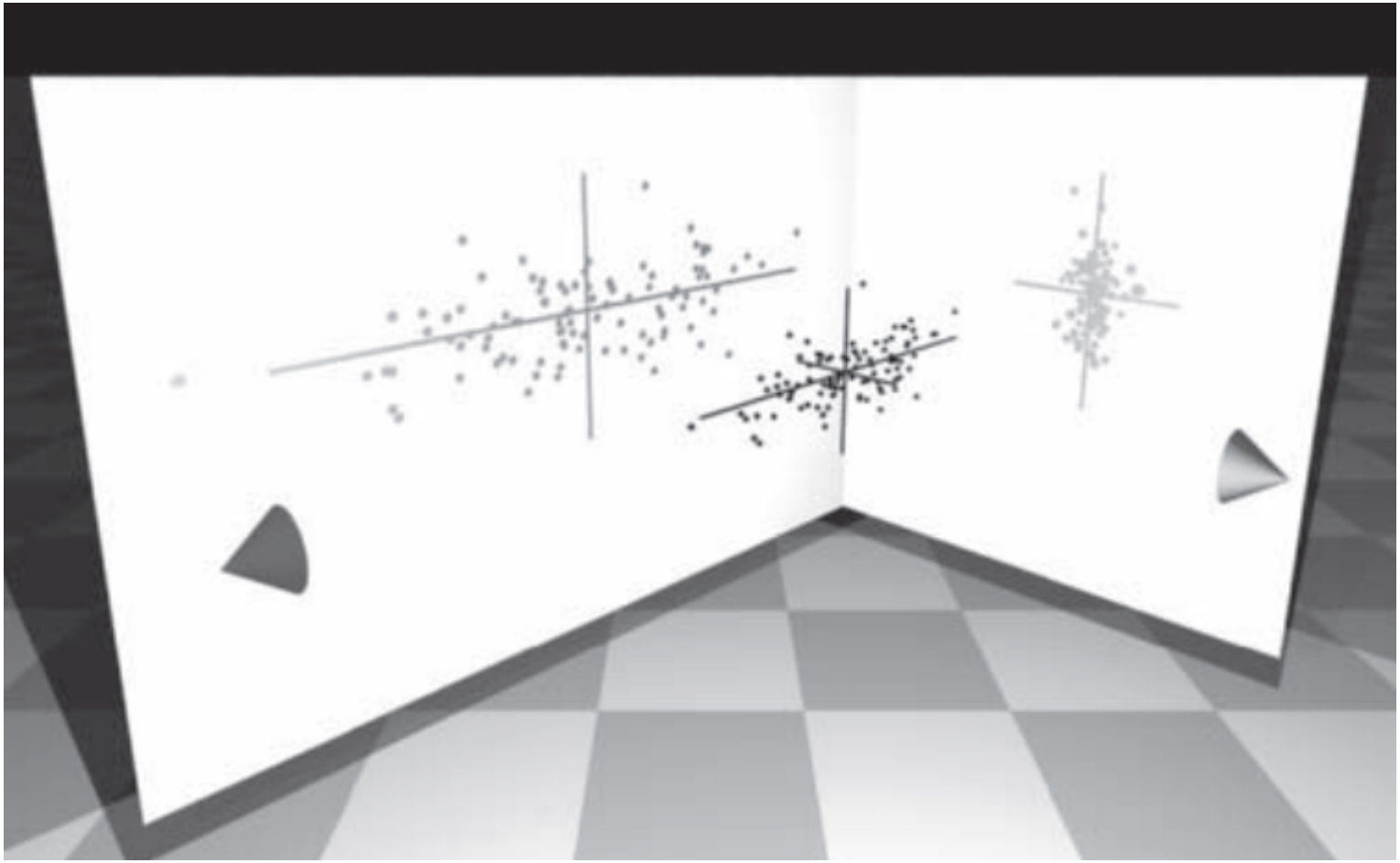




Principal component analysis

Example in 3 D



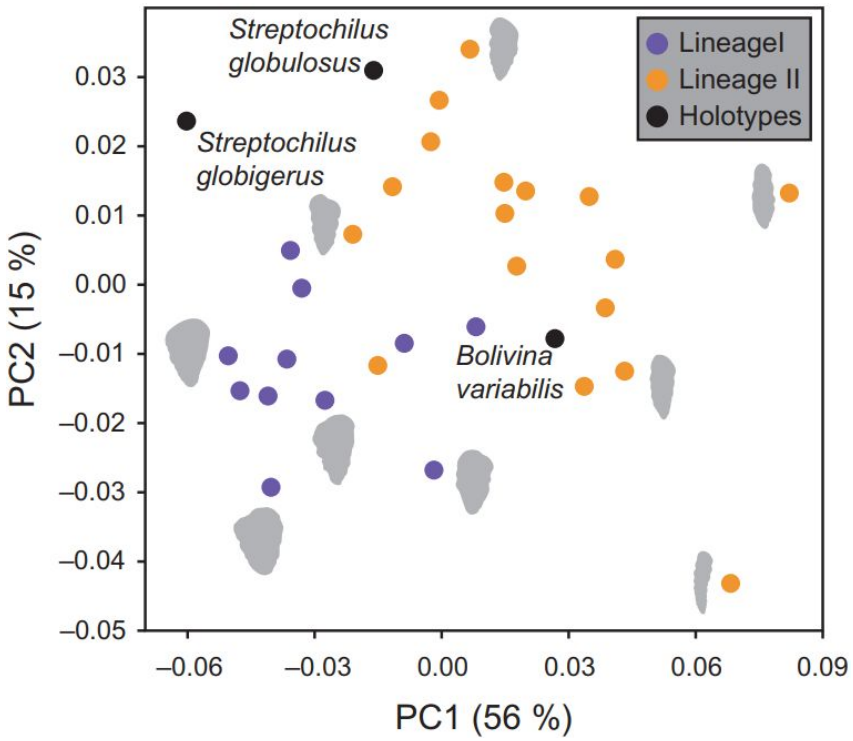
Hammer and Harper (2006) Palaeontological Data Analysis. (Blackwell Publishing: Malden, Oxford, Carlton)

Main output of a PCA

- **Scores**: The coordinates for each specimen in the entire PCA space (i.e. one value per PC)—corresponds to position of this specimen within the morphospace. **Scores can be used as variables for follow-up analyses**
- **Loadings**: The contribution of each parameter to each PC axis. In outline analysis, this is rarely interesting, but it can be used to check the dominance of Harmonic 1
- **Standard deviation of each PC axis**: This is helpful to estimate the degree of dispersion of data points along this axis

Interpretation of PCA

- PCA can be performed on the data without any prior knowledge of grouping
- If *a priori* groups are known, they can be indicated in the PCA plot but do not affect the ordination solution
- PCA can be investigated for visible group-clusters or trends, which may be further interpreted



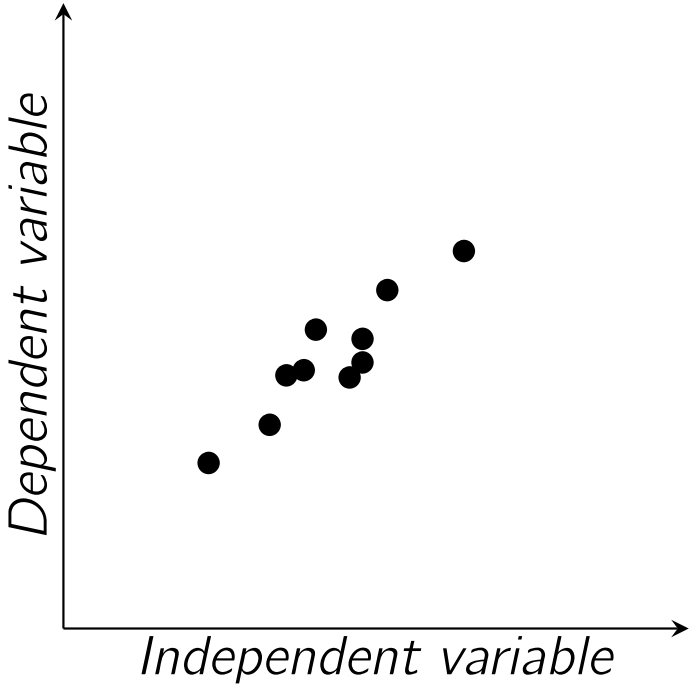
Kucera et al. (2017) J. Plankton. Res. 39 (3): 436–49

PC scores as analytical variable

- The scores of a morphometric PCA can also be used as variable for further analyses
- Normally, the scores of specimens along PC 1 (the axis that explains the greatest amount of variation) are used
- If you have good reason to believe that PC 1 is dominated by some underlying factor (e.g. environmental parameters), you can also use higher PCs for this kind of analysis
- Follow-up analyses can involve practically every type of standard analysis, but regression analyses are probably most common

Regression analyses

- Regression checks for causal relationship between independent (x) and dependent (y) variable



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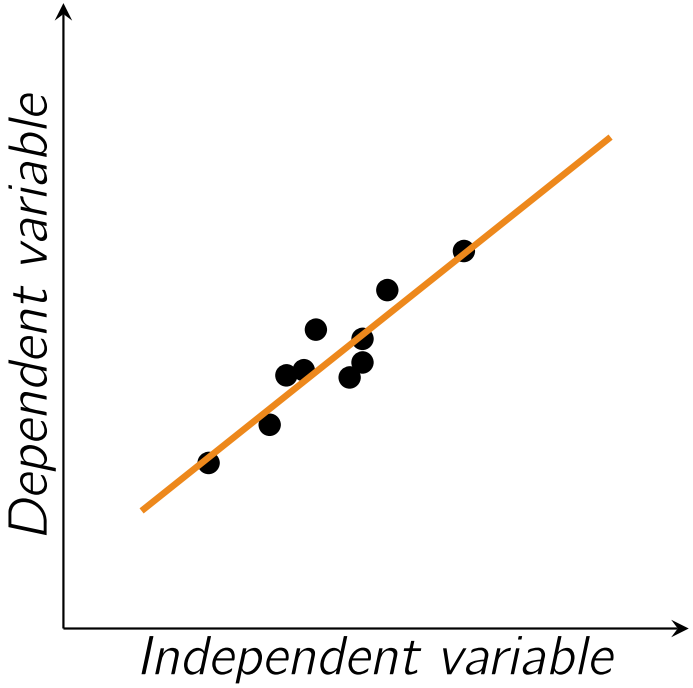

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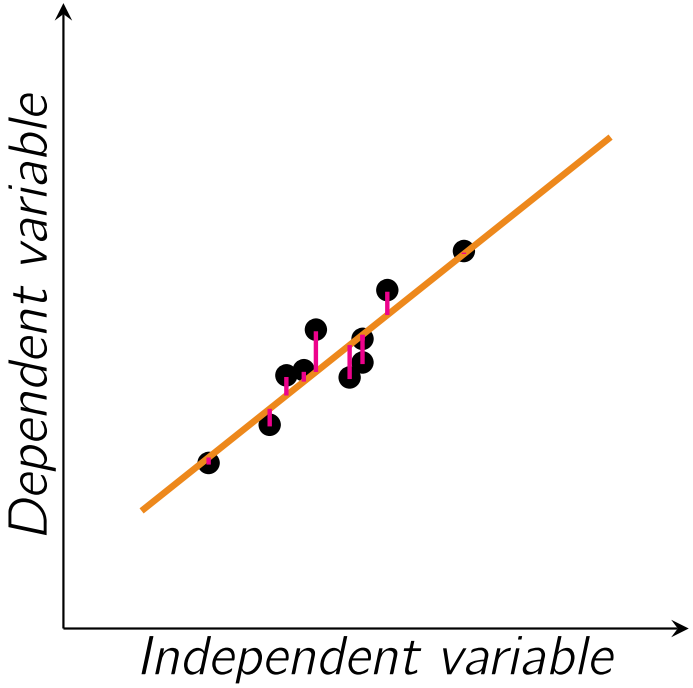
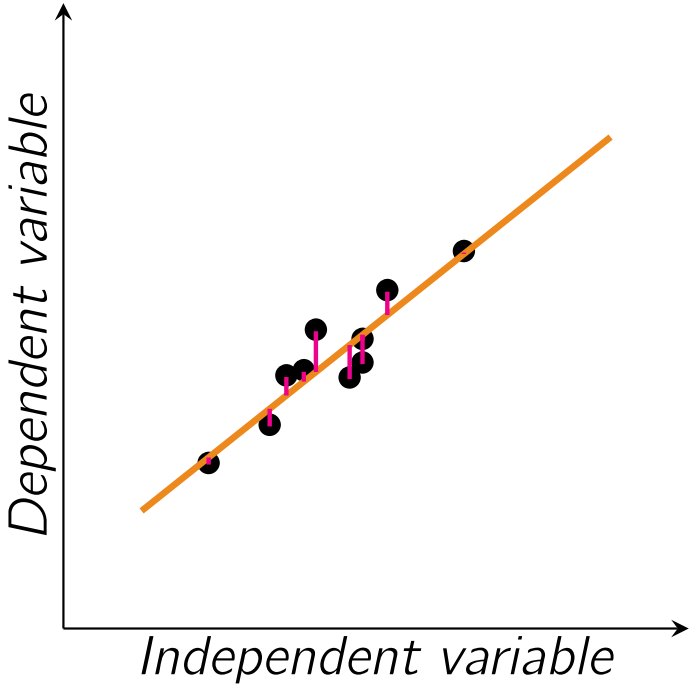
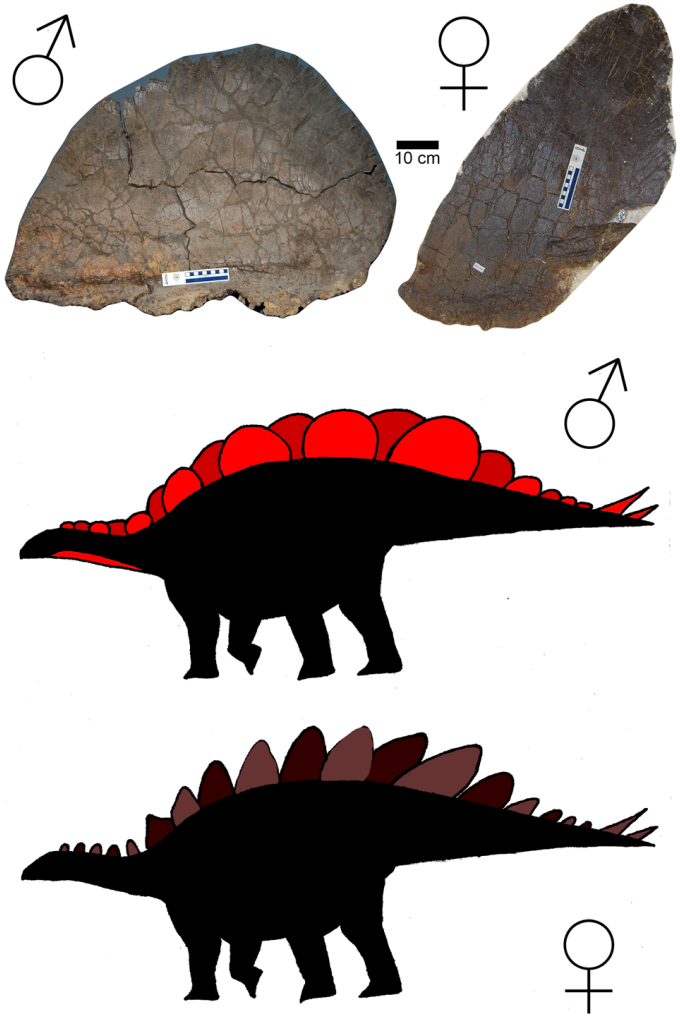


Regression analyses

- Regression checks for causal relationship between independent (x) and dependent (y) variable
- A predictive equation is fitted to the data with overall distance to all data points minimal
  - p-value: Probabilistic support of the null-hypothesis H_0
  - R^2-value (coefficient of determination): Fraction of variation explained
  - Correlation coefficient: Measure of effect size



Excursion: The probabilistic *p*-value



http://viralportal.net

1. You start working on *Stegosaurus* and observe, that there are males and females to be found

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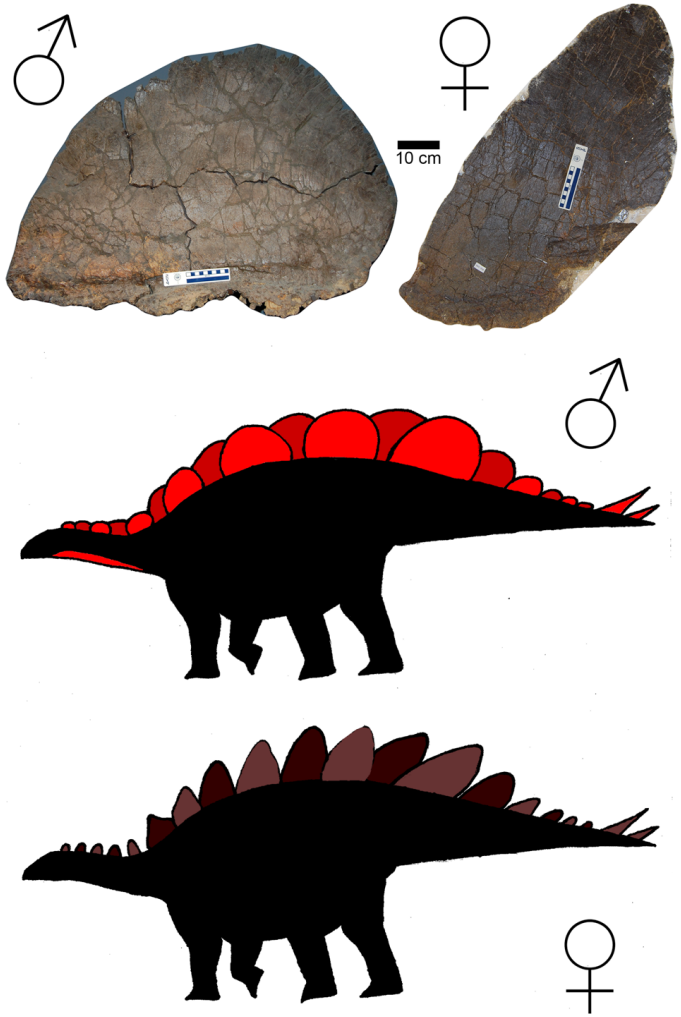

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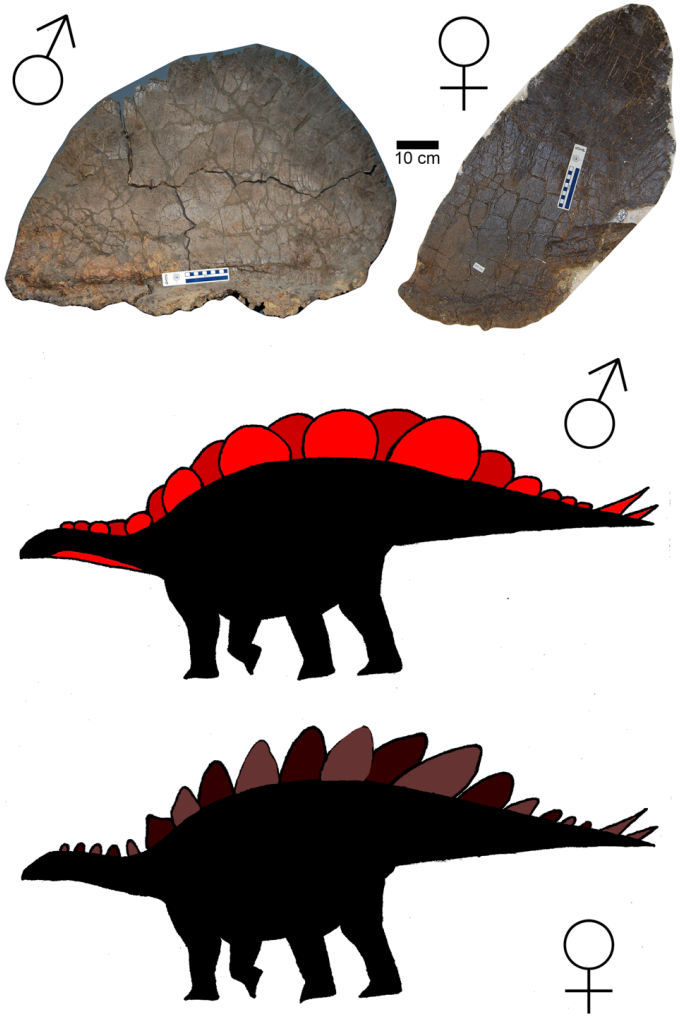

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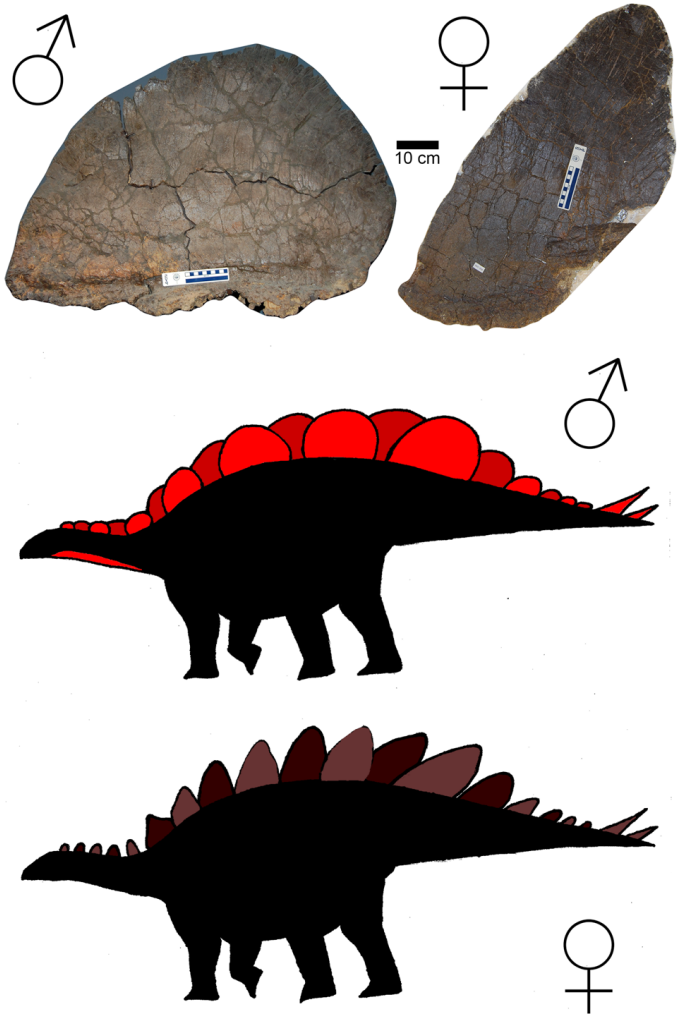

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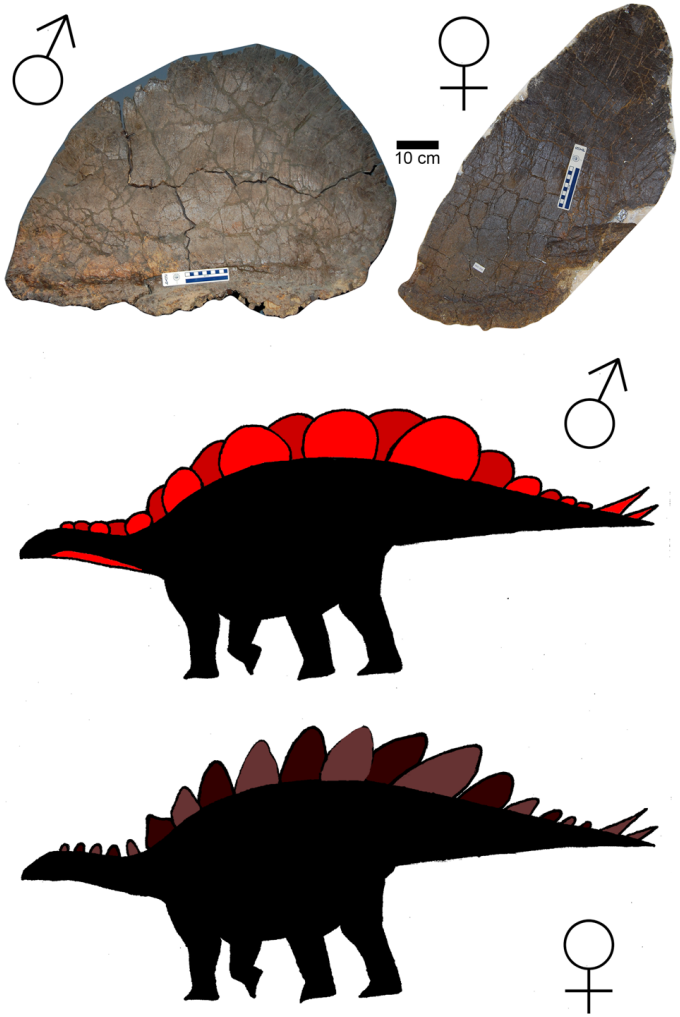

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5. You try to **disprove** H_0

Excursion: The probabilistic *p*-value

The process of hypothesis evaluation

- You go in the field and calculate a statistic: For instance, you look at 10 *Stegosaurus* and count how many are male and female

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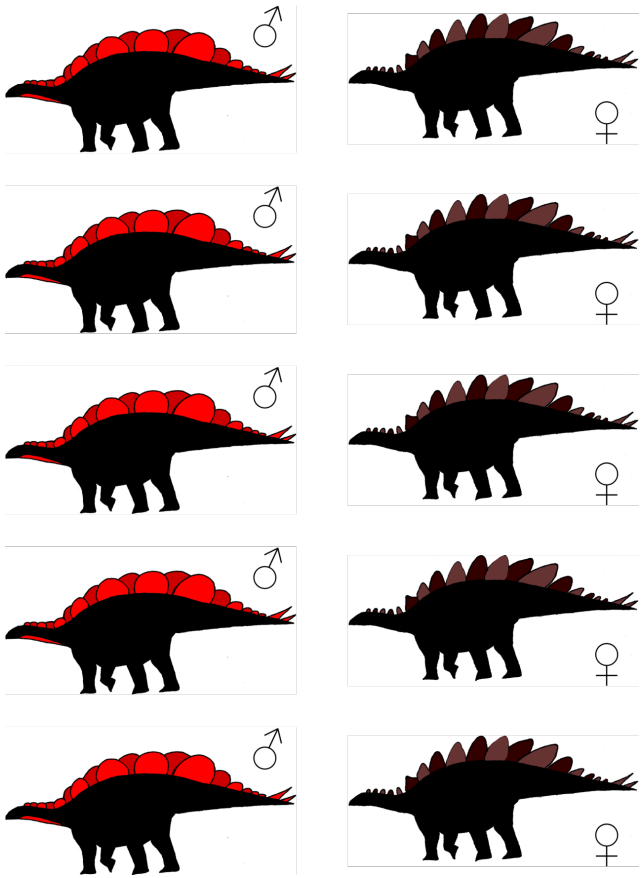
- In natural sciences, this threshold is normally $\alpha = 0.05$

Excursion: The probabilistic *p*-value

The sex-distribution in *Stegosaurus*

Assumption of H_0



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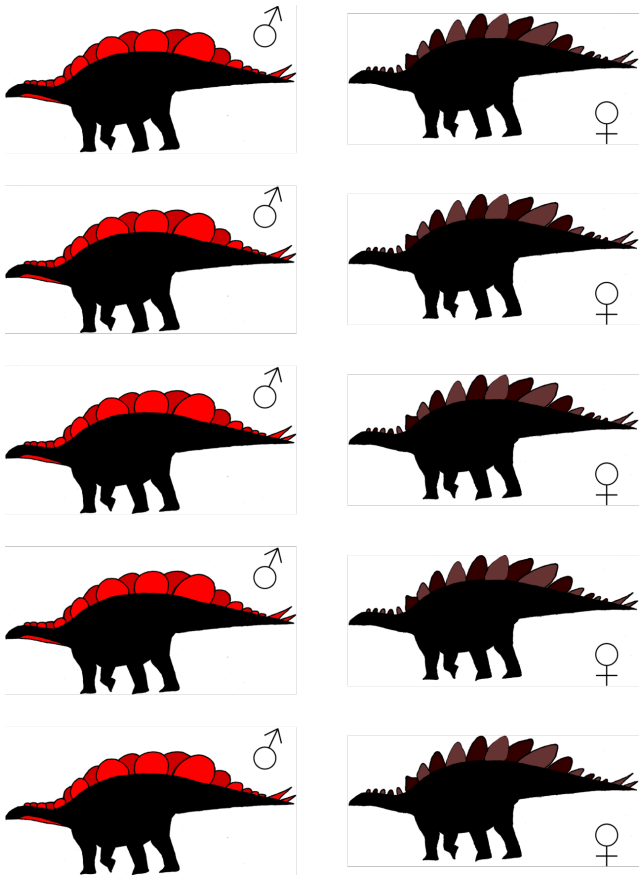
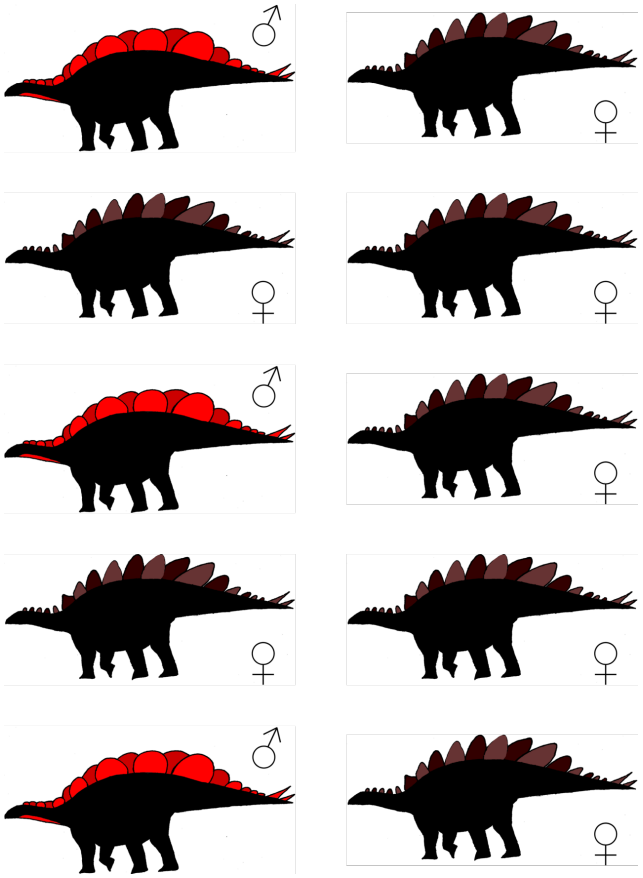


Observation



Excursion: The probabilistic p-value

How do we interpret that?

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$$p(3) = \frac{10!}{3! \times (10-3)!} \times 0.5^3 \times 0.5^{10-3}$$

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We observe that $p > 0.05$ and therefore accept H_0

Excursion: The probabilistic p-value

Beware of the limitations

- The p-value gives you a probability, no clear answer: If you choose $\alpha = 0.05$ you will falsely reject the null-hypothesis in 5 % of all cases (Type I error)

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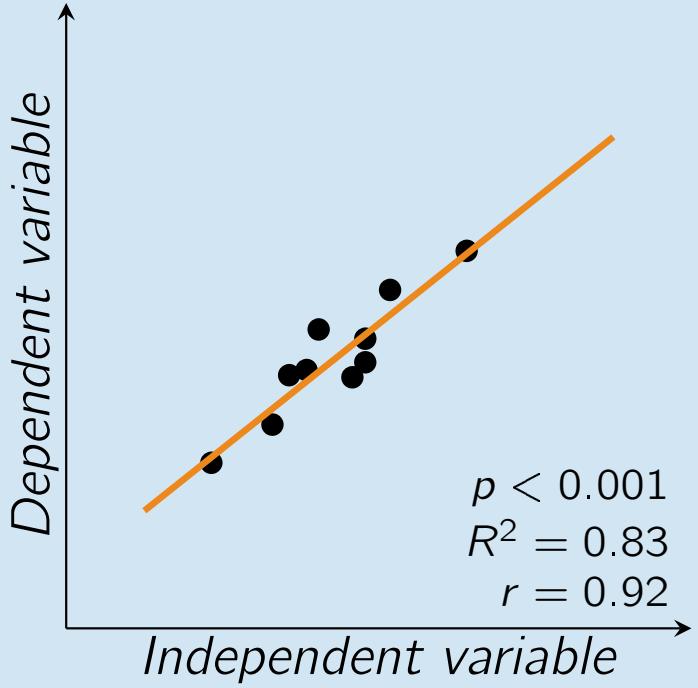
- The equations will become much more complex for other data distributions and more complex tests: Do not calculate by hand, that is what software is for
- Besides frequentist statistics there exist maximum likelihood statistics and Bayesian statistics: They have advantages and disadvantages of their own

Regression analyses

How to interpret regression results

Low spread, significant



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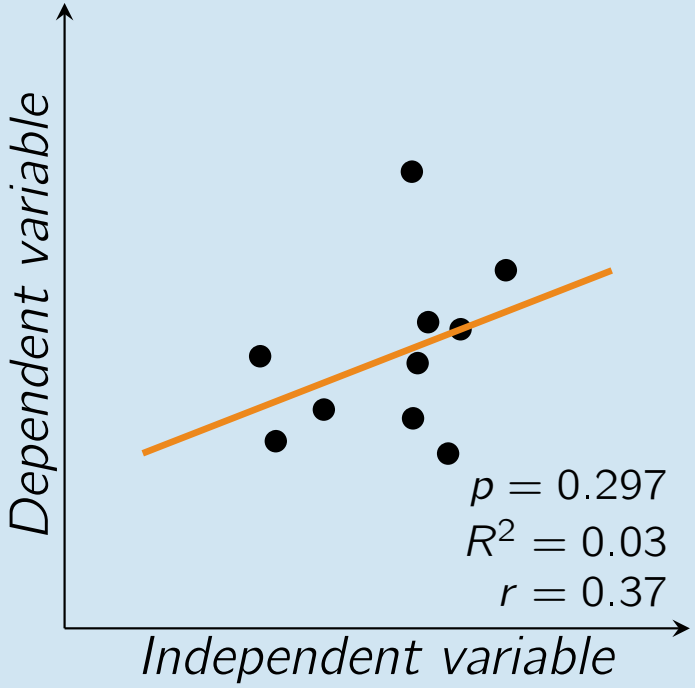
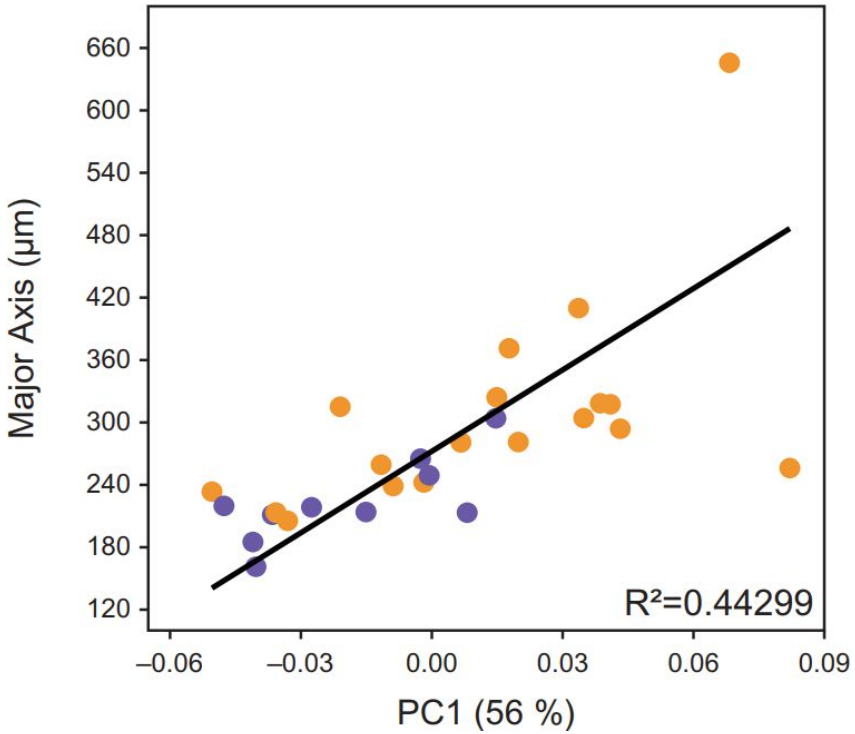


Regression analyses in morphometrics

Checking for ontogenetic trends

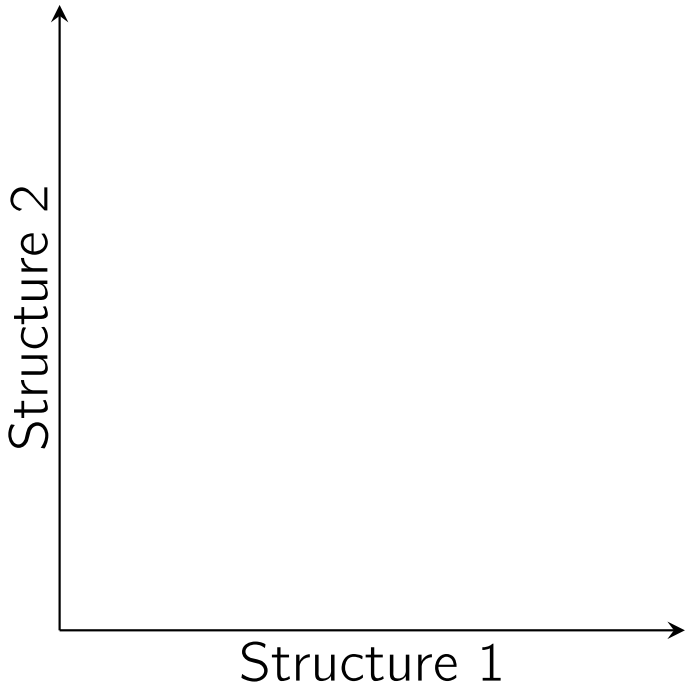
- Investigate the relationship of morphology with other parameters
  1. With environmental factors: **Ecophenotypes**
  2. With size parameter: **Ontogenetic trends**
  3. With geological sample age: **Anagenetic trends**
  4. With any other continuous (and technically ordinal) variable



Kucera et al. (2017) J. Plankton. Res. 39 (3): 436–49

Linear discriminant analysis

Example in 2 D



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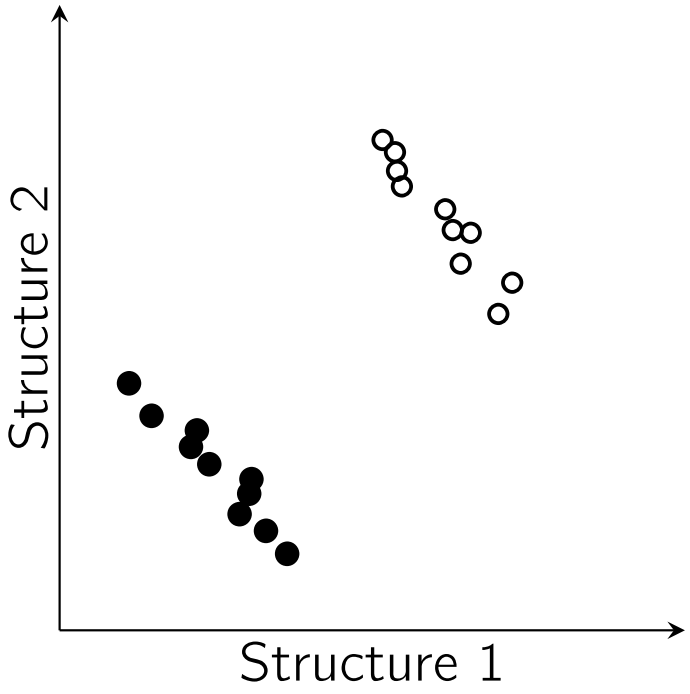

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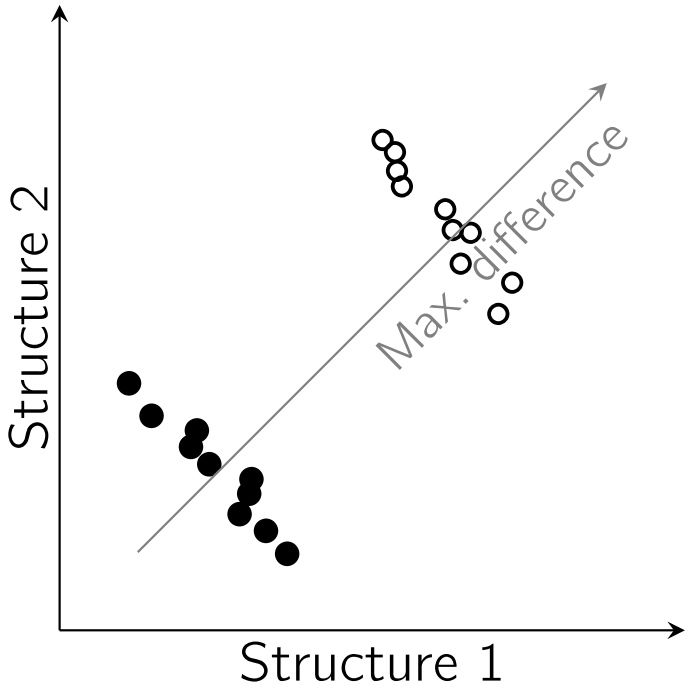

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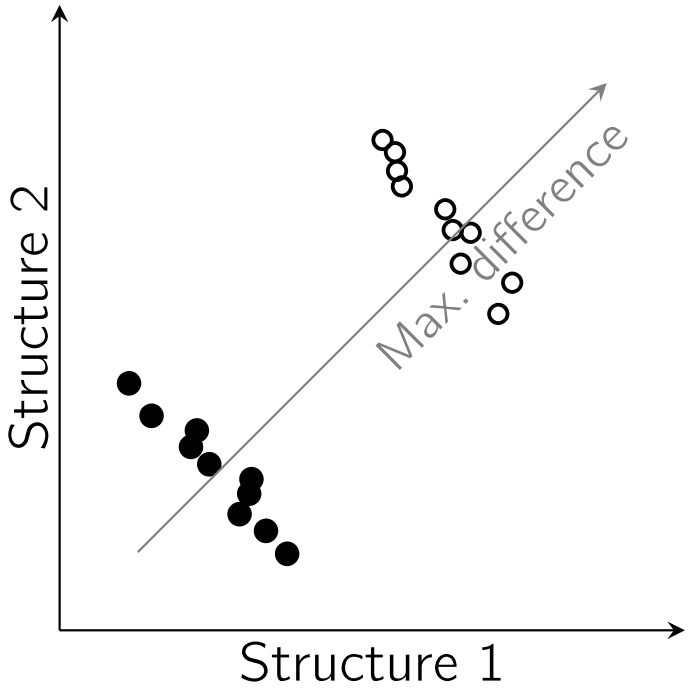

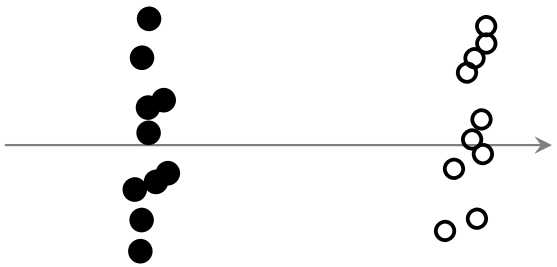

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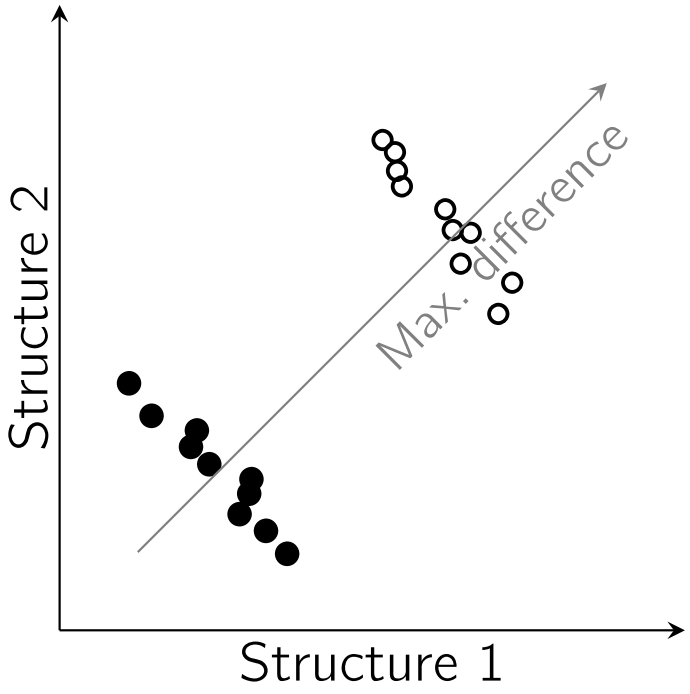

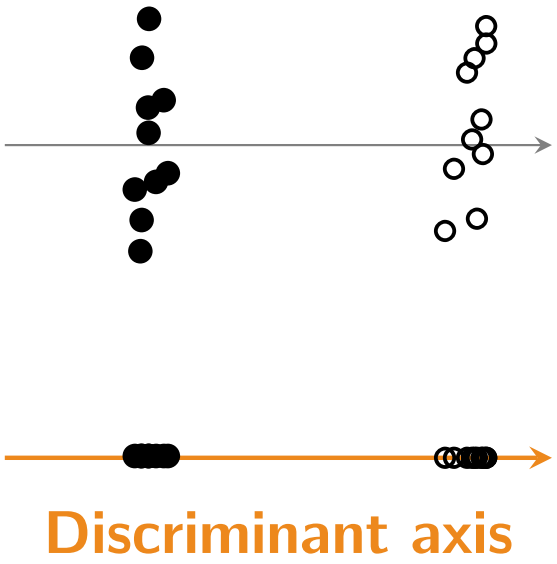

Linear discriminant analysis

Example: Brachiopods of the genus *Glottidia*

http://paleopolis.rediris.es

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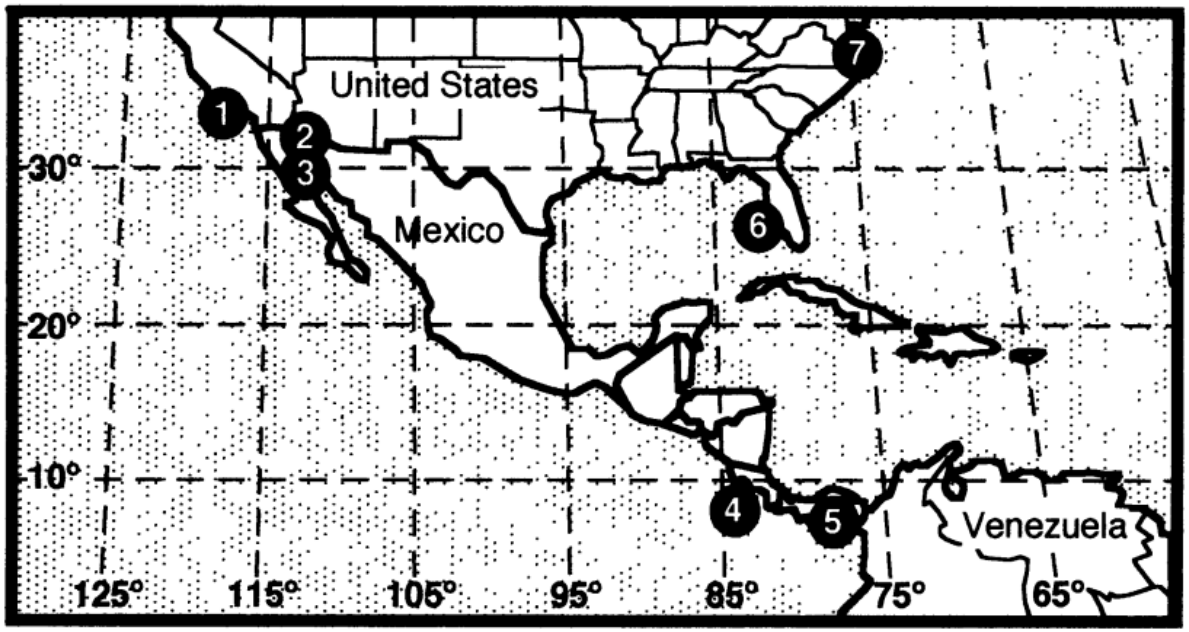

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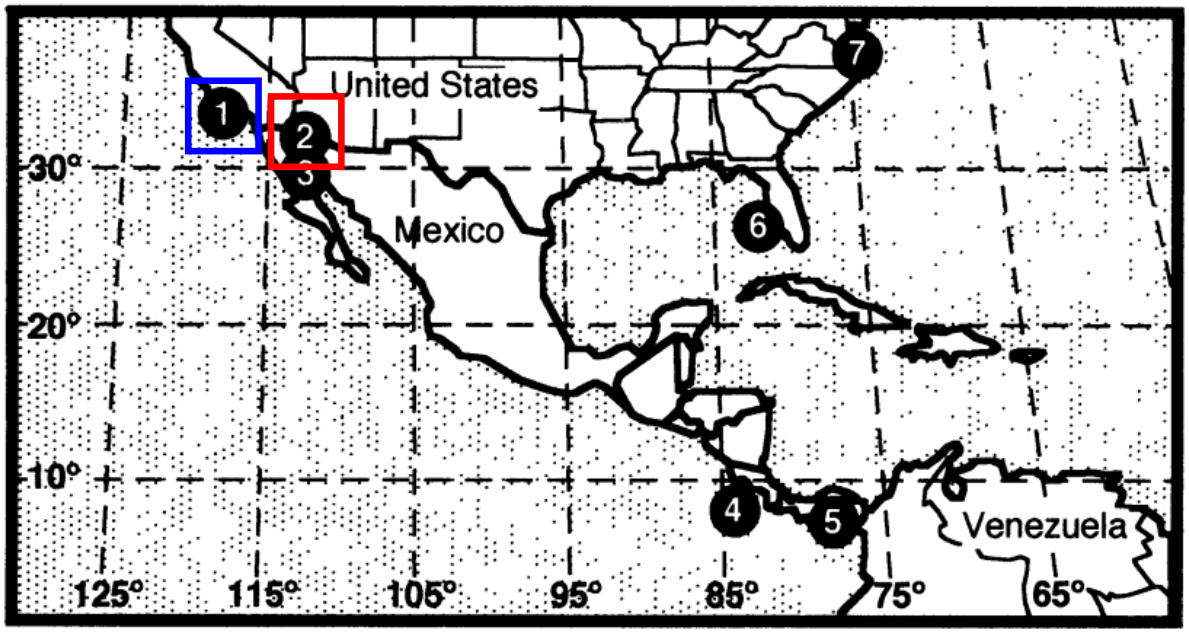

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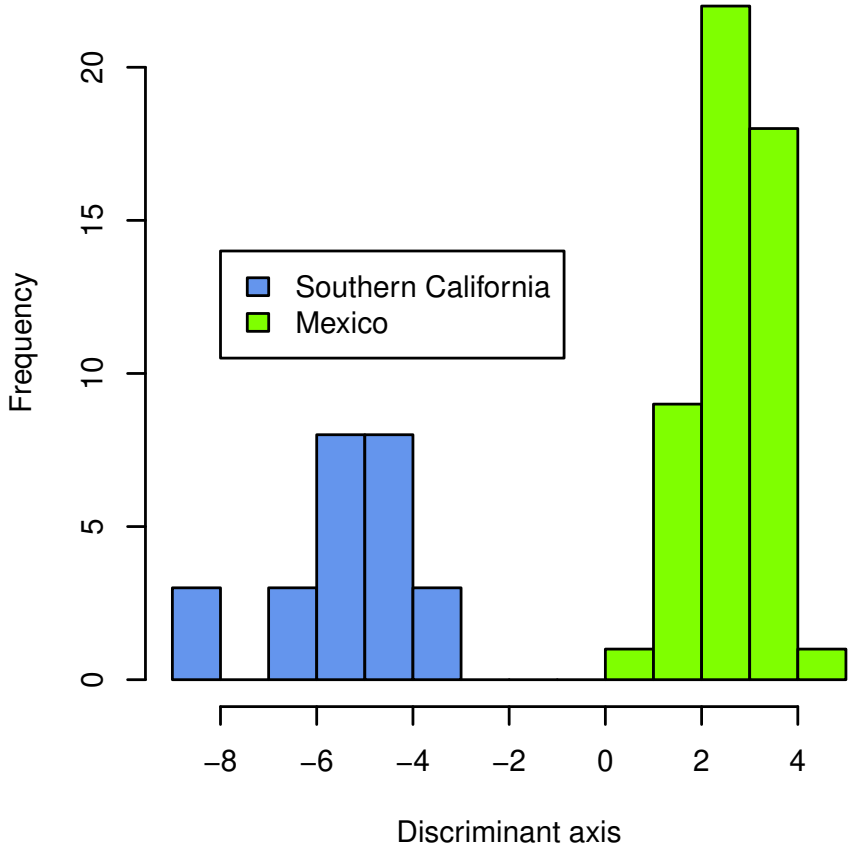
LDA: Compare morphology between two groups



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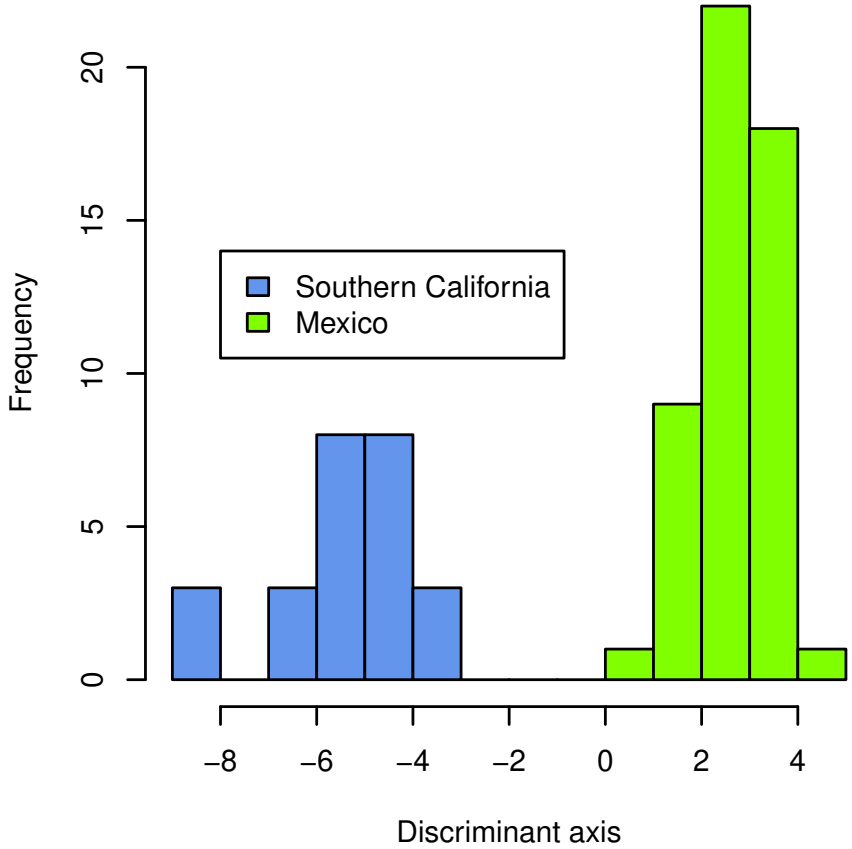

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  2. The *a posteriori* allocations of specimens to groups to validate the quality of the LDA solution

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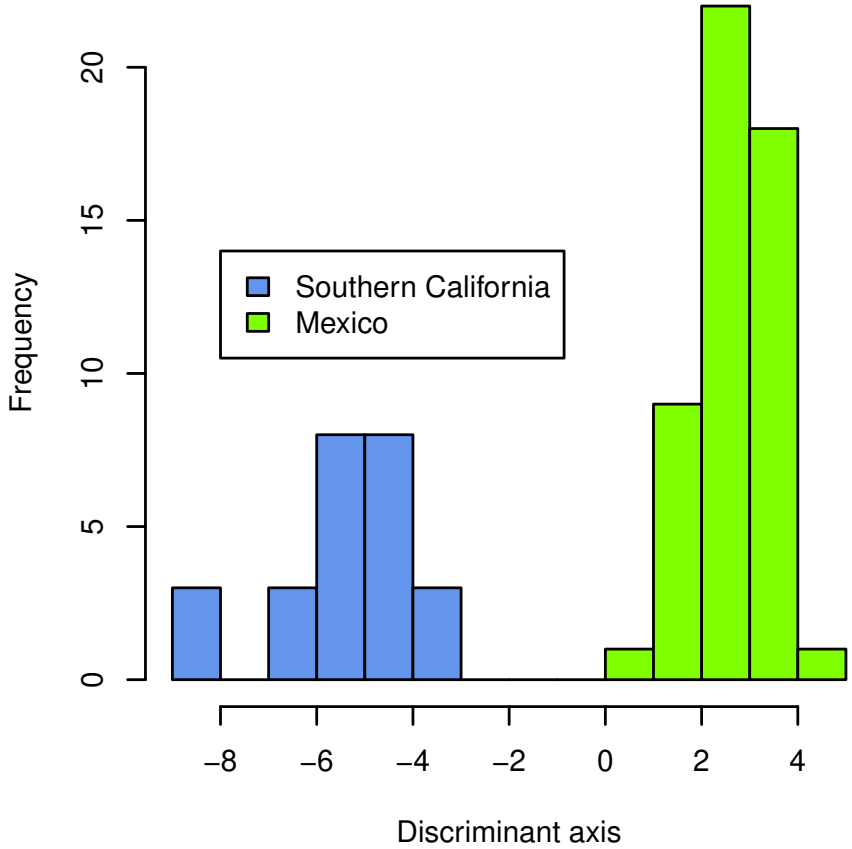

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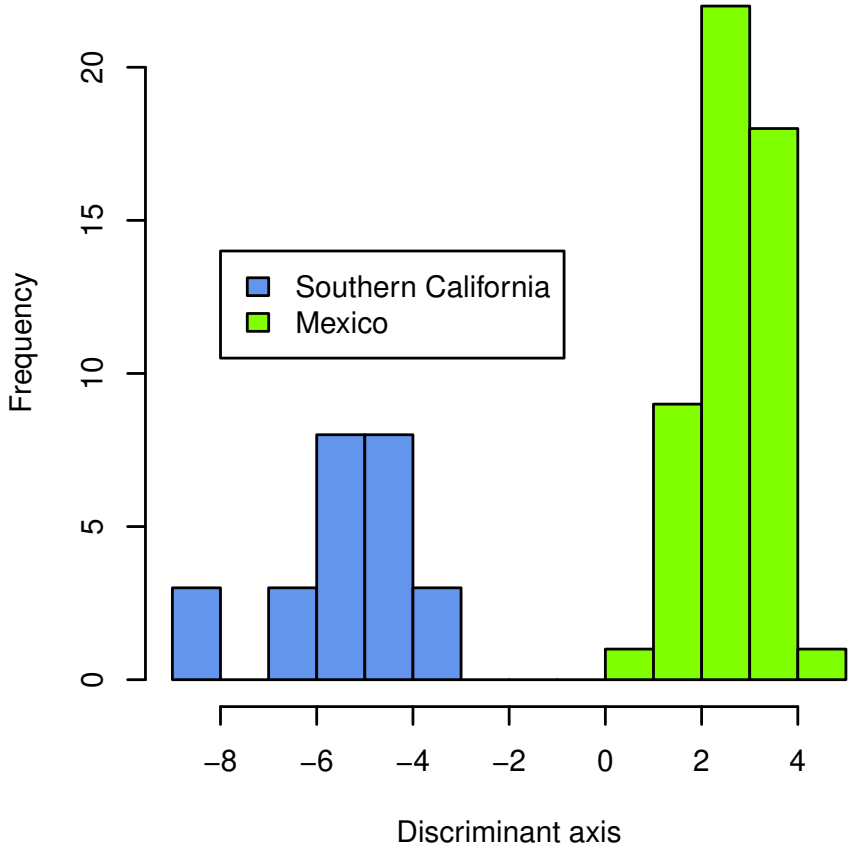

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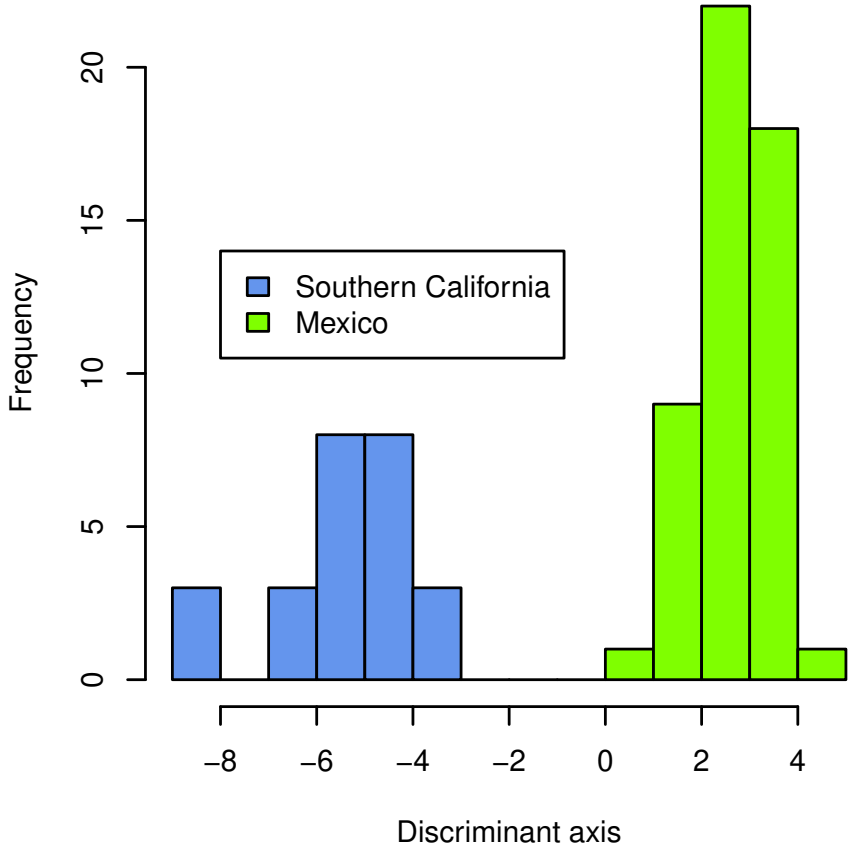

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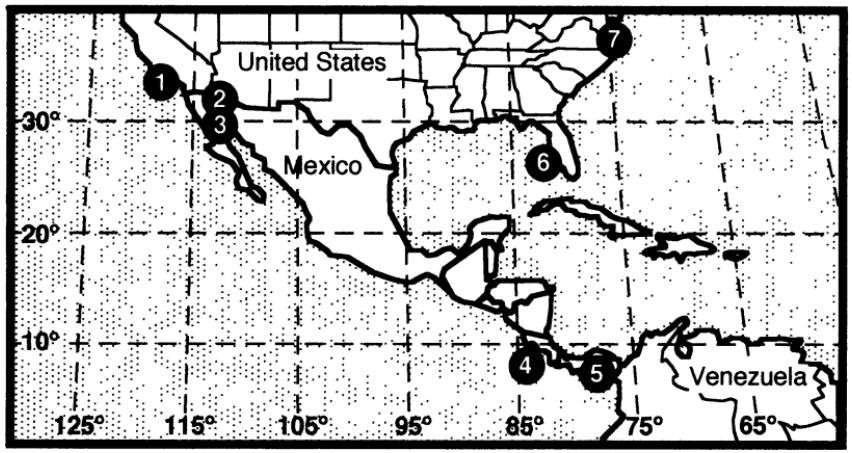
The brachipods from southern California and Mexico differ significantly in their morphology

Canonical variates analysis

The extension of LDA for more than two groups



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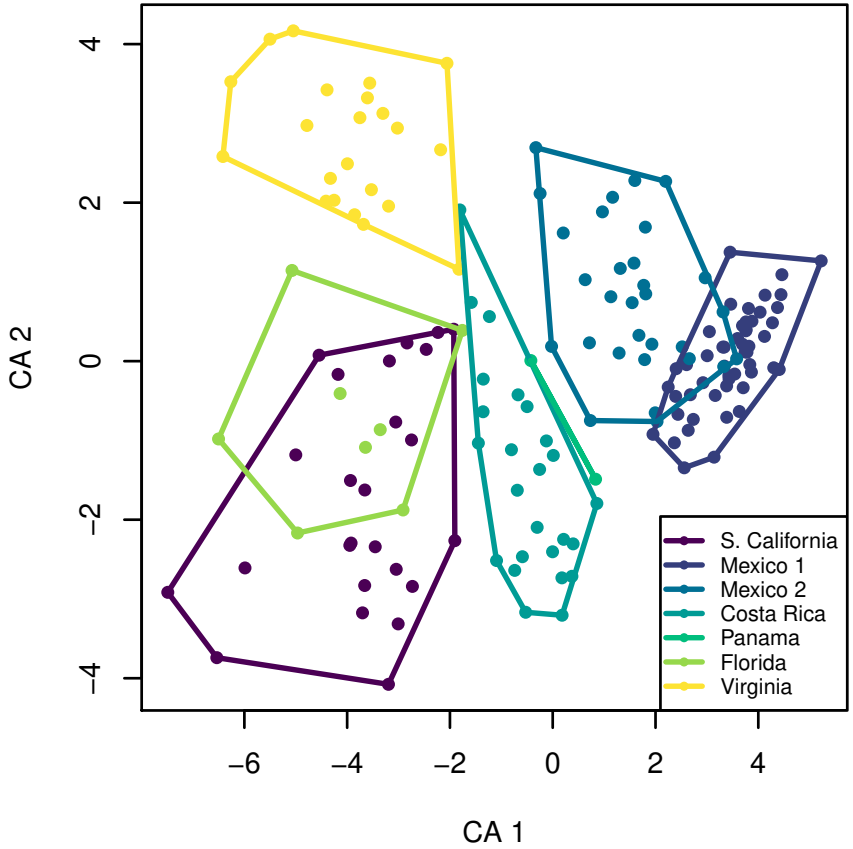

Of sharpshooters and misconceptions

- LDA/CVA and MANOVA can only be used when the groups were defined **a priori** and **independently** of the morphological measures

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- LDA/CVA and MANOVA can only be used when the groups were defined **a priori** and **independently** of the morphological measures
- DO: Test for instance if groups from different localities/times or species defined based on genetics differ morphologically

Of sharpshooters and misconceptions

- LDA/CVA and MANOVA can only be used when the groups were defined **a priori** and **independently** of the morphological measures
- DO: Test for instance if groups from different localities/times or species defined based on genetics differ morphologically
- **DO NOT:** Test if groups differ morphologically that you defined **based on the same morphological data** (e.g. via PCA)

R

Example of outline data analysis

For a look at outline data analysis in R, we move on to exercise № 3

Open the exercise sheet for instructions and code examples