Workshop

**Methods
in Ostracodology**

CONTRIBUTION TO
GEOMETRIC MORPHOMETRICS

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Workshop
"Methods in Ostracodology"

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CONTRIBUTION TO GEOMETRIC MORPHOMETRICS

Dedicated to Roger L. Kaesler

Organisation

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Austrian Academy of Sciences, Commission for the Stratigraphical & Palaeontological
Research of Austria
Landesmuseum Joanneum, Department of Geology & Palaeontology

Preface

The aim of the present workshop "Methods in Ostracodology" is to intensify communication between specialists, both palaeontologists and neontologists interested in taxonomy, evolution and (palaeo)ecology of ostracods. This goal can be achieved if we are aware or if we adopt some of the new research tools useful for the description of ostracods and their use for environmental reconstruction. Therefore, our intention is to make more available techniques used for three types of purposes:

1. sampling sediments to gain a high resolution spatial and temporal record,
2. applying geometric morphometrics for ostracod description and taxonomy,
3. using stable isotope composition of ostracod valves for further use in (palaeo)environmental reconstructions.

Morphometrics, the quantitative description, analysis and interpretation of form, is especially suited for ostracods, particularly when combined with conventional multivariate-statistical techniques and with methods used in computer graphical analysis. As F. J. Rohlf noted (Ann. Rev. Ecol. Syst., 1990: 299) "*morphometrics is a fundamental area of research*" and "*techniques of description and comparison of shapes of structures are needed in any systematic study ... based on the morphology of organisms*".

The present volume presents a series of contributions in this scientific field: Dr. A. Baltanás (Madrid) provides a general overview on morphometrics including traditional methods but focussing on geometric morphometrics and its various aspects. The major part of this volume deals with an alternative geometric morphometric method newly developed in Austria, mainly by specialists from the University of Salzburg (Prof. J. Linhart and his students). This method is documented herein with its mathematical background, a detailed programme description and some practical examples as well, and is introduced - besides the workshop attendees - to a broad scientific community.

In addition to this focus on morphometrics, the workshop will also provide information on and demonstration of specific field sampling and analytical techniques for high resolution studies. Such field techniques will be demonstrated in the well studied clay pit of Mataschen (Styria).

Ecological interpretations and environmental reconstructions are based on morphological characters but are increasingly supported by and combined with analyses of the stable isotope composition of the ostracod valves, preferably of $\delta^{18}O$ and $\delta^{13}C$. On the application of these geochemical techniques in ostracodology special emphasis will be placed, covering different aspects from the mere analytical procedure to the influence of diagenesis on the isotopic values (Dr. J. Boomer, Birmingham, Dr. C. Latal, Graz).

These methodological aspects will be supplemented by contributions of most workshop participants on various ostracod and environment topics.

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Martin Gross, Dan L. Danielopol, Werner E. Piller

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GEOMETRIC MORPHOMETRICS –

A contribution to the study of shape variability in Ostracods

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We have told each other so often and with such force and such eloquence of the uses to which the study of ostracodes has been applied that we have overlooked one startling fact: almost no one uses ostracodes for anything.
R.L. Kaesler (1983)

Why shape?

Biodiversity is an issue of main concern not only for scientists but for the whole society as well. Taxonomic richness is but one of the many ways we use to express biological diversity. Morphological disparity – the amount of shape variability within a clade – is another. And given that both features do not necessarily correlate (Cherry et al. 1979, Foote 1993a), their comparison can provide further evidence about ecological and evolutionary processes involved in the production and maintenance of biodiversity.

Morphological disparity can be explored at a wide range of taxonomic levels. Indeed, there has been a growing interest in methods addressing morphological disparity in recent years (Foote 1997, McGhee 1999, Ciampaglio et al. 2001, Wills 2001, Zelditch et al. 2004). Some 'classic' studies concern the morphospace occupied by spiral (Raup 1967) or planar branch systems (McKinney 1981) aiming to understand macroevolutionary patterns at high-rank taxonomic levels. Studies in ecomorphology, however, commonly focus at lower taxonomic levels (mainly, closely related species) seeking for correlations between morphology and ecological requirements (Norberg 1994) or for the effects of competitive selective pressures assumed to occur between (Dayan et al. 1990). At the species level, between-populations disparity and its correlation with environmental conditions is used to evaluate adaptation to local conditions (Loik and Noble 1993); and at further detail, morphological variability within a population can be related to the niche concept and dynamics (Pulliam 1986) or to sexual selection (Møller 1994).

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Finally, there is an increasing interest in exploring the potential for shape change of a given genotype – the phenotypic plasticity of morphological features –, as well as its adaptive value (Schlichting and Pigliucci 1998).

The amount of morphospace occupied by a set of clades has been used as indicator of ecological diversity (Warheit et al. 1999), evolutionary radiation (McGhee 1999), morphological convergence in distant communities (Ricklefs and Miles 1994), or selective extinctions (Roy and Foote 1997). Less frequent is its use as tracer of environmental conditions or dispersal routes of groups below the species level (populations, clones, ...).

Why Ostracods?

Most of the issues outlined above can be extensively addressed using ostracods. Indeed, this group of organisms can be labelled as ideal for a morphometric approach because of its high taxonomic richness and the diversity of habitats occupied. In addition, ostracods have an extensive fossil record, a feature that allows the examination of shape-environment relationships back into evolutionary time scale.

Morphometric study of ostracods mainly focuses on the analysis of carapace shape. Ostracod carapace has a marked functional meaning; it is the interface between the organism and its environment (Benson 1981). Hence, ostracod carapaces can be considered as engineering solutions, a compromise between design and materials, developed to match specific environmental conditions (Benson 1981). Consequently, it is assumed that ostracod carapace is subject to selection pressures (i.e. has adaptive value).

At the specific level ostracod carapaces include such a number of features (tubercles, ribs, nodes, spines, …) and are conservative enough to be used for taxonomical identification in both neontological and, specially, paleontological studies. Carapaces, however, are not invariant morphological features at the specific level; indeed, valve shape variability has been extensively documented both within- and between-populations.

Methods for the study of shape change and variability

Form and shape

‘Form’ is an attribute of organisms that is made of two components: size and shape (Benson 1975, Bookstein 1989, Foote 1995, Baltanás et al. 2000). To discern between ‘form’

and 'shape' is not a trivial matter given that we frequently deal with information regarding 'shape' which, in fact, is related to 'size' (allometry). This is particularly the case when studying ontogenetic processes or in comparisons between individuals grown under different environmental conditions (Rohlf and Bookstein 1987).

Concerning methods and techniques available for the study of shape change and variability, several approaches exist but we will here concentrate in two: Traditional Morphometrics and Geometric Morphometrics.

Traditional Morphometrics

This approach, also named Multivariate Morphometry (Reyment 1985, Foote 1995) and Multivariate Biometry (Bookstein 1993), is an application of multivariate statistics to morphometric issues. Although widely used, these techniques have a main flaw: they do not recognize de geometric origin of the data under scrutiny. Variables used in this approach — distances, angles and ratios—are out of context both geometrically and biologically (Bookstein 1993). In other words, the set of variables used in these procedures preclude the reconstruction of the original shape out of their values. Such loss of information makes these methods of limited value.

Statistical techniques aimed to study relationships between morphological features (length, height, weight, …) developed well before the term 'biometry' was coined (Galton 1869, 1889). Examples can be found in the works of Montbeillard, Quetelet and Galton; as well as in later contributions by Edgeworth, Pearson, Fisher and Wright.

The many multivariate techniques existing, which have been applied to numerous sets of meristic data derived from a plethora of organisms, emphasize the structure of the covariance matrix over other aspects of the measurements and lack any connection to the geometrical arrangement of such measurements, their biological meaning or the functional processes related to the organism development (Bookstein 1993). Such situation can be noticed in the first publications that use the term 'morphometry' in its current use (Blackith 1965, Blackith et al. 1971).

In addition, traditional morphometrics has some severe limitations (Lestrel 1997): (a) it is highly subjective; (b) it does not preserve information on, *i.e.* it is not possible to recover the original shape out of morphometric variables used (distances, angles and ratios); and (c) all variables used are but a small amount of all information about shape contained in a biological object.

Aware of such circumstances, several scientists (Jolicoeur 1963, Burnaby 1966, Mosimann 1970) tried to put additional emphasis on the biological foundations of morphometric data. Their attempt, however, was not successful enough. The actual turnover occurs at the beginning of the '80s with the rise of the so-called Geometric Morphometrics (Rohlf 1990a, Rohlf and Marcus 1993, Bookstein 1991, 1993).

Geometric Morphometrics

Geometric morphometrics inspire, partially at least, in the work of D'Arcy W. Thompson (1942) who approached the study of biological shape change as distortions occurring in a cartesian coordinate system which have been previously selected on the basis of its biological homology. Shape is a definite entity, a configuration of points that keep geometric relationships among them and cannot be split into isolated items (like length or height). Confronted with a biological shape, the morphometrician will attempt to describe it in terms of transformation from an original reference shape. Although the approach proposed by Thompson was very appealing and promising it was not accompanied by any analytical procedure. It was the arrival of the computer age, several decades later, that makes it possible to develop application for morphometric analysis based on Thompson's ideas feasible (Bookstein 1993).

Within geometric morphometrics, comparisons between organic forms are addressed by collecting information concerning the location of discrete points, called *landmarks*. A set of homologous points, *landmarks*, provides information of the biological form given they are distributed homogeneously on the organism and bear some biological meaning (Goodall 1983, Bookstein 1984, 1986, Chapman 1990, Rohlf and Slice 1990, Schweitzer and Lohmann 1990, Reilly 1990, Bookstein 1991, 1993, Reyment and Abe 1995, Foote 1995, Stone 1998). The analysis of configurations of *landmarks* allows the study of shape change without decomposing it into artificial variables. There are several landmark-based methods (fig. 1) and an updated review can be found in Zelditch et al. 2004. Concerning ostracods, there are several studies that apply landmark methods (Kaesler and Foster 1987, Reyment et al. 1988, Abe et al. 1988, Reyment and Bookstein 1993, Reyment 1995, 1997, Elewa 2004).

For a large number of ostracod species, however, it is not possible to identify *landmarks,* or, at least, a number of landmarks large enough to make that approach feasible. Under such circumstances there is an option: *Outline Analysis* (Rohlf 1990b). Outline analysis operates on the following basis: (1) when landmarks are not available one should record the

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positions of a rather high number of points along the contour of the studied object; (2) a mathematical function must the be fitted to such observationsin order to (3) explore differences between shapes through the analysis of the mathematical descriptors fitted to them. This approach includes a variety of specific methods (fig. 1), among others '*Eigenshape*' analysis (Lohmann 1983, Schweitzer et al. 1986, Lohmann and Schweitzer 1990), standard Fourier descriptors (Kaesler and Waters 1972), and Elliptic Fourier Analysis (Kuhl and Giardina 1982, Kaesler and Maddocks 1984, Rohlf and Archie 1984, Foote 1989, Rohlf 1995, Lestrel 1997, McLellan and Endler 1998, Baltanás and Geiger 1998).
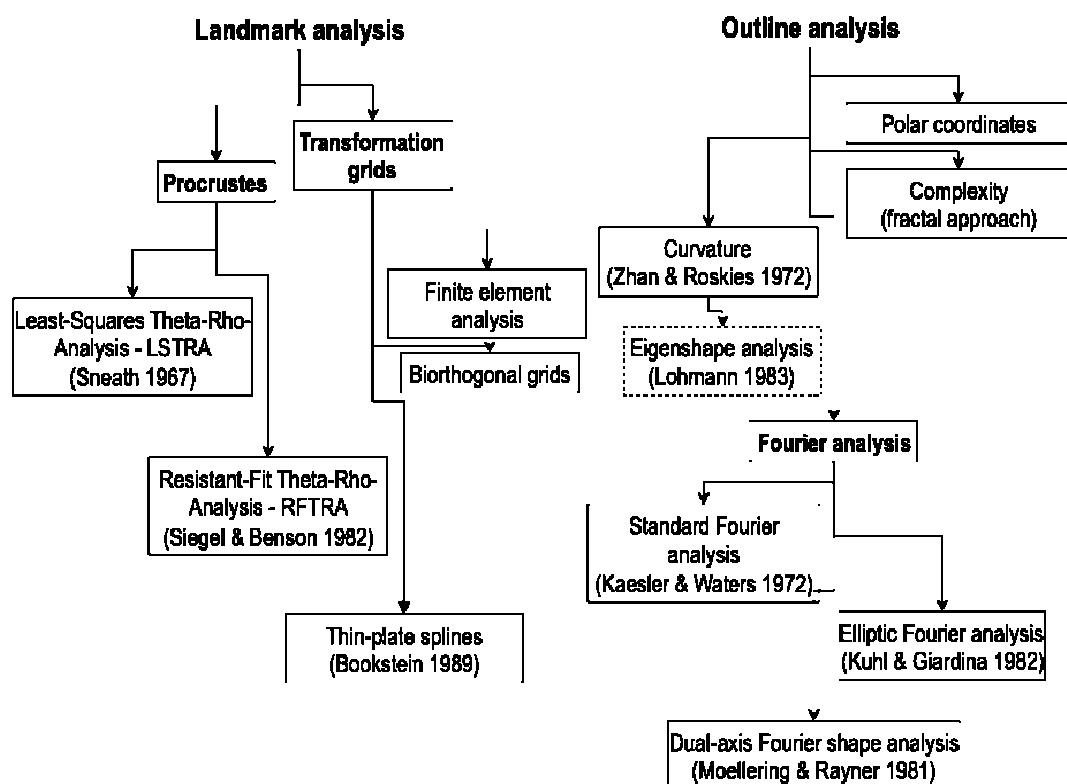
Figure 1: Sketch of relationships between some methods in the realm of Geometric Morphometrics.

Course Outline

Sessions in the course will offer a close view to some of the methods mentioned above together with exercises dealing with related aspects like 'Data Acquisition Procedures' and 'Multivariate Analysis of Shape Descriptors'.

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Introduction to the Computer Programme MORPHOMATICA

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MORPHOMATICA is a user-friendly computer programme designed for the morphometric analysis of the shape of ostracods with a more or less smooth outline. The software package (Linhart et al. 2006) with the same name is available at http://palstrat.uni-graz.at.

For the mathematical description of outline shapes MORPHOMATICA uses an original solution, here called "the Linhart algorithm" (cf. details in the next chapter), based on a B-spline method, a popular technique in computer aided geometric design (cf. Hill 1990) which has been applied to morphometric analysis of human skulls by Guéziec (1996).

The MORPHOMATICA project, which started in 2001 at the Limnological Institute, Austrian Academy of Sciences, in Mondsee, and at the Department of Mathematics, University of Salzburg, is a spin-off of a larger project on the morphometrics of non-marine ostracods initiated ten years ago by one of us (A.B.) and from which various publications issued (cf. inter alia Baltanás and Geiger 1998, Baltanás et al. 2002, 2003, Danielopol et al. 2002, Sánchez-González et al. 2004). One should see the computer programme described here as a complement to other computer programmes using alternative approaches, like Eigenshape analysis and/or Fourier analysis (see a review of morphometric methods used by ostracodologists in Danielopol et al. 2002).

MORPHOMATICA and the Linhart's B-spline algorithm have interesting features as compared with other programmes: (1) it uses a reduced number of parameters for the mathematical reconstruction of form; (2) one can identify the segments of the outline described by the B-spline functions; (3) the computation is not excessively long for the solution proposed; (4) it allows to estimate the precision with which the B-spline curve fits the original digitised outline; and (5) it allows to produce virtual "arte–factual" outlines useful

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for various topics dealing with theoretical and/or applied morphology. MORPHOMATICA, as other programmes do (cf. those of the Eigenshape analysis method discussed by Rohlf 1996), suffers also from limitations: it performs badly when outlines are much angulated or very heterogeneous. In such cases it seems that Elliptic Fourier analysis -included in programmes like EFA - Eliptic Fourier Analysis (cf. Rohlf 1990) or MAO – Morphometrica Analysis of Outlines, developed by one of us (A.B.), performs better.

The presentation of MORPHOMATICA is here offered in several contributions: the mathematical part presented by W. Neubauer and J. Linhart, the programme description *sensu strictu* written by W. Brauneis, W. Neubauer, A. Stracke, the practical description of creating "tps.dig files" by A. Strake. Finally the presentation of a series of worked examples for morphometric analysis of outlines, prepared by A. Stracke, W. Neubauer, L. Picot and D. Danielopol, are intended to demonstrate the utility of MORPHOMATICA for descriptive work within two research directions: comparative morphology and taxonomy (1st example), morphological variability potentially related to ecological cues (2nd example).

One should note that the information presented with MORPHOMATICA is related to the utilisation of other computer programmes too. MORPHOMATICA uses the digitised information of the valve outlines captured with the programme "Tps.dig" (Rohlf 2001). We used for this programme the version 1.43, which was downloaded from the web site http://life.bio.sunysb.edu/morph/soft-dataacq.html. Additionally the data obtained from the superimposition of outlines allows the computation of the amount of morphological differences (represented by vector dimensions and Euclidean distances). This latter data is further analysed using multivariate statistical methods. In the examples we present it is shown how using non-metric multi dimensional scaling and/or hierarchical cluster analysis one can visualise the data within the framework of morphological spaces. We use since several years the computer package "Primer", with its versions 5 and 6 (Clarke and Gorley 2001, Clarke, K.R. and Gorley, R.N. 2001. Primer v5: User manual/tutorial. Primer-E Ltd., Plymouth 2006) specially designed for multivariate statistical analysis. Note that there are other packages which can be as useful as the one mentioned here. Our preference for "Primer" is due to the user-friendly structure of the programmes, to the excellent manual produced by Clarke and Warwick (2001).

Finally, we recommend to those interested in additional information on geometric morphometrics the various books issued during Morphometric Symposia, like those of Marcus et al. (1996). An excellent introductory text, which has to be consulted, is "Geometric morphometrics for biologists, a primer" (Zelditch et al. 2004).

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For the practical use of MORPHOMATICA programme one should consult inter alia also Iepure et al. (2007, 2008), Minati et al. (2008), Danielopol et al. (2008), Gross et al. (2008).

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Approximating and Distinguishing Ostracoda by the Use of B-Splines

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1. Preface

Biological outlines may be investigated by various mathematical models. There is a well-developed theory of shape (Zelditch 2004) for the case of sufficiently many good landmarks. For the morphometrics of outlines with few or no landmarks sometimes simple polygonal curves (Bézier curves) have been used (see for instance Loy 2000), but this may lead to polynomials of rather high degree, which tend to oscillate in an undesirable way. This is a well-known problem in many technical applications, especially in computer aided geometric design (CAGD), where it is usually overcome by the use of splines (Hoschek and Lasser 1993, Farin 1990).

Splines consist of several polynomial curves of low degree that smoothly fit together. Two of the most widely used splines are Bézier splines and B-splines. These are not different curves, but only different representations of the same curves; they may be transformed into one another (Hoschek and Lasser 1993). In both cases the shape of the curve is determined by a list of geometrically meaningful control points, but for B-splines fewer control points are needed.

The name B-spline was coined by Isaac Jacob Schoenberg and it is used as an abbreviation for basic spline (de Boor 1978, Farin 2001). The authoritative person for the development of the theory of B-spline curves and surfaces was Carl de Boor due to his researches at General Motors.

In the 50s and 60s of the last century the automobile industry in particular had to face the difficulty that freeform curves and surfaces couldn't be exactly reproduced owing to a lack of a proper mathematical description. Carl de Boor solved this problem by depicting the shape of component parts as parametric curves, defined piecewise
by polynomials.

As a result, the curve's shape is determined by its so-called control points or de-Boor points, which are the vertices of the control polygon. It turned out that this way of characterizing curves and shapes was advantageous for other fields and generalized versions and enhancements of B-splines were developed shortly after. Representing curves and shapes using B-splines and their further development is still employed in Computer-Aided-Design systems. Nowadays the areas of application are manifold and diversified and reach far beyond technical mould design and construction (Bartels 1987, Farin 2001, Hoschek and Lasser 1993).

For our task, the approximation of outline data, B-spline curves offer several advantages. B-spline curves are invariant under affine transformations, the pixel data can be approximated by a numerically stable and accurate algorithm and, we obtain an enormous data reduction. The primary outline data, consisting of approximately 1000 to 1400 pixels, can be excellently depicted by a B-spline curve determined by just 16 control points. A further important fact to mention in advance is a property called "local control". Thereby the shifting of a single control point of the B-spline curve does not cause the change of the entire curve progression but just a deviation in the surrounding of the concerning control point. This characteristic has proved very useful for the examination of morphological structures just having an effect on single parts of the outline.

This article gives an overview of the theoretical background of approximating B-spline curves, following the elaborations in Bartels (1987), de Boor (1978), Hoschek and Lasser (1993), Piegl (1995). A detailed description of the algorithm for the approximation of ostracods' outlines is presented in Bayer et al. (2002) and Neubauer (2007). The shape of a carapace offers the possibility of a fast and stable computation. Moreover, the area deviation provides us with demonstrative results for the application in palaeontology since a perspicuous graphical representation of the stated area is feasible and the output is in units of square micrometers.

The last section deals with a method of distinguishing two populations of ostracods by using the data of a distance matrix, which frequently finds application in biological research.

2. B-Spline Representation

2.1. B-Spline Curves

Definition 2.1. A pth-degree B-spline curve is defined by

$$\mathbf{C}(t) = \sum_{i=0}^{n} N_{i,p}(t)\,\mathbf{P}_i \qquad a \leq t \leq b.$$

The $\mathbf{P}_i$ are the control points, and the $N_{i,p}(t)$ are the pth-degree B-spline basis functions defined by

$$
\begin{aligned}
N_{i,0}(t) &= \begin{cases} 1 & \textit{if } u_i \leq t < u_{i+1} \\ 0 & \textit{otherwise} \end{cases} \\
N_{i,p}(t) &= \frac{t - u_i}{u_{i+p} - u_i} N_{i,p-1}(t) + \frac{u_{i+p+1} - t}{u_{i+p+1} - u_{i+1}} N_{i+1,p-1}(t),
\end{aligned}
$$

where $U = (u_0,...,u_m)$ is a nondecreasing sequence of real numbers, i.e., $u_i \leq u_{i+1}$, $i = 0,...,m\text{-}1$, called knot vector. The items u_i are called knots and the polygon formed by the $\mathbf{P}_i$ is the control polygon.

Particularly we use B-spline curves of degree $p = 2$, also called quadratic B-spline curves, for the approximation of ostracods' outlines. Their basis functions are, already calculated, given by

$$
N_{i,2}(t) = \begin{cases}
\frac{(t-u_i)^2}{(u_{i+2}-u_i)(u_{i+1}-u_i)} & \text{for } u_i \leq t < u_{i+1} \\[2mm]
\frac{(u-u_i)(u_{i+2}-t)}{(u_{i+2}-u_i)(u_{i+2}-u_{i+1})} + \frac{(u-u_{i+1})(u_{i+3}-t)}{(u_{i+3}-u_{i+1})(u_{i+2}-u_{i+1})} & \text{for } u_{i+1} \leq t < u_{i+2} \\[2mm]
\frac{(u_{i+3}-t)^2}{(u_{i+3}-u_{i+1})(u_{i+3}-u_{i+2})} & \text{for } u_{i+2} \leq t < u_{i+3}
\end{cases}
$$

and $N_{i,2}(t) = 0$ for t outside the interval $[u_i, u_{i+3})$. In these formulas, division by zero may occur. When this is the case, the result of the division is set equal to zero.

A common choice for the knot vector of pth-degree basis functions is setting the first $p+1$ knots 0, the last $p+1$ knots 1 and the interior knots equally spaced;

$$
\begin{aligned}
u_0 &= u_1 = ... = u_p = 0 \\
u_j &= \frac{j - p}{m - 2p} \quad \text{for} \quad j = p+1,...,m-p-1 \\
u_{m-p} &= ... = u_m = 1.
\end{aligned}
$$

This so-called uniform knot vector has, referring to our application with $p = 2$, the form

$$U = \{0, 0, 0, \frac{1}{m-4}, ..., \frac{m-5}{m-4}, 1, 1, 1\}.$$

As an example, for $n = 6$ the basis functions of degree 2 generated by an uniform knot vector are depicted below (fig. 1).
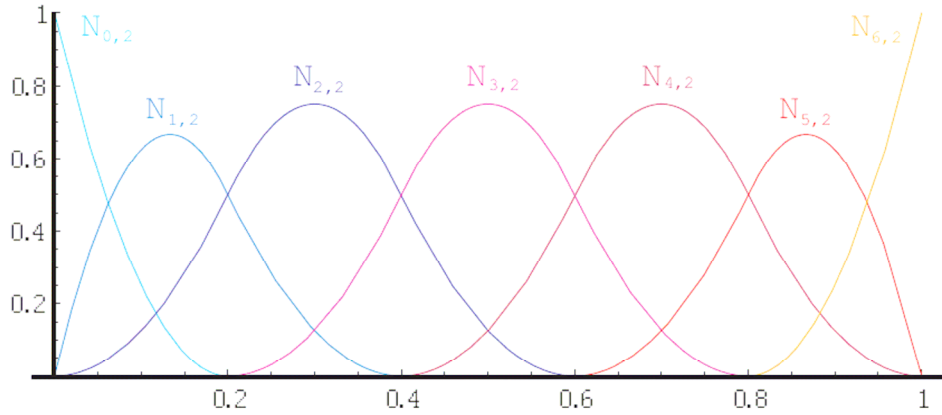
Figure 1: The nonzero second-degree basis functions generated by a knot vector $U = (0, 0, 0, 1/5, 2/5, 3/5, 4/5, 1, 1, 1)$.

To give an introductory idea of B-spline techniques we contemplate the following example. Let U be a uniform knot vector as defined in the equation above, $p = 2$ and the set of control points

$$\{\mathbf{P}_i\} = \{(-1, 0), (-0.9, 1), (-0.2, 1.3), (-0.5, -1), (0.7, -1), (0.1, 0.5), (1, 1)\}.$$

Figure 2 shows the resulting B-spline curve.
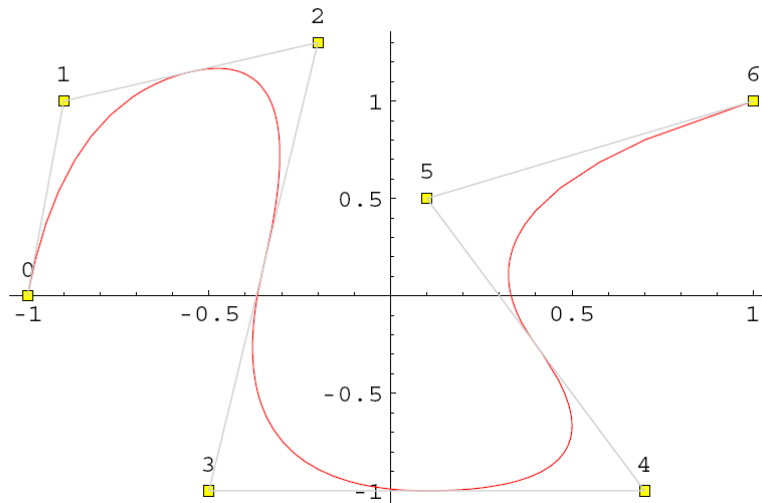
Figure 2: B-spline curve using the conditions of the example above.

Below a number of properties of B-spline curves following mostly from the definition 2.1 are given.

Property 2.2. Endpoint interpolation: $\mathbf{C}(0) = \mathbf{P}_0$ and $\mathbf{C}(1) = \mathbf{P}_n$.

Property 2.3. Affine invariance: An affine transformation, including translation, rotation and scaling, is applied to the curve by applying it to the control points.

Property 2.4. Local support: Moving $\mathbf{P}_i$ changes $\mathbf{C}(t)$ only in the interval $[u_i, u_{i+p+1})$ (fig. 3). This follows from the fact that $N_{i,p}(t) = 0$ for $t \notin [u_i, u_{i+p+1})$, since $N_{i,p}(t)$ is a just linear combination of $N_{i,0}(t), ..., N_{i+p,0}(t)$ (see definition 2.1) and those zero-degree basis functions are 0 outside $[u_{i+m}, u_{i+m+1})$, $m = 0, ..., p$. The triangular scheme illustrates this fact for the basis function $N_{1,2}(t)$.

$$
\begin{array}{ccccc}
 & & N_{1,2} & & \\
 & \swarrow & & \searrow & \\
 & N_{1,1} & & N_{2,1} & \\
 \swarrow & & \searrow \; \swarrow & & \searrow \\
N_{1,0} & & N_{2,0} & & N_{3,0}
\end{array}
$$
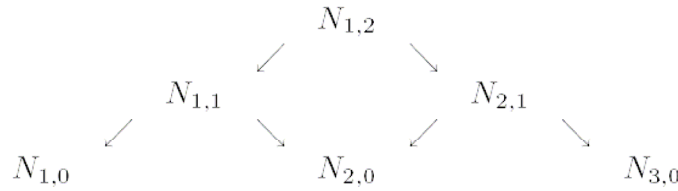
$N_{1,2}(t)$ is a combination of $N_{1,0}(t)$, $N_{2,0}(t)$, and $N_{3,0}(t)$. Thus, $N_{1,2}(t)$ is nonzero only for $t \in [u_1, u_4)$.

Conversely, in any given knot span $[u_j, u_{j+1})$ at most $p+1$ of the $N_{i,p}(t)$ are nonzero, namely the functions $N_{j-p,p}, ..., N_{j,p}$.
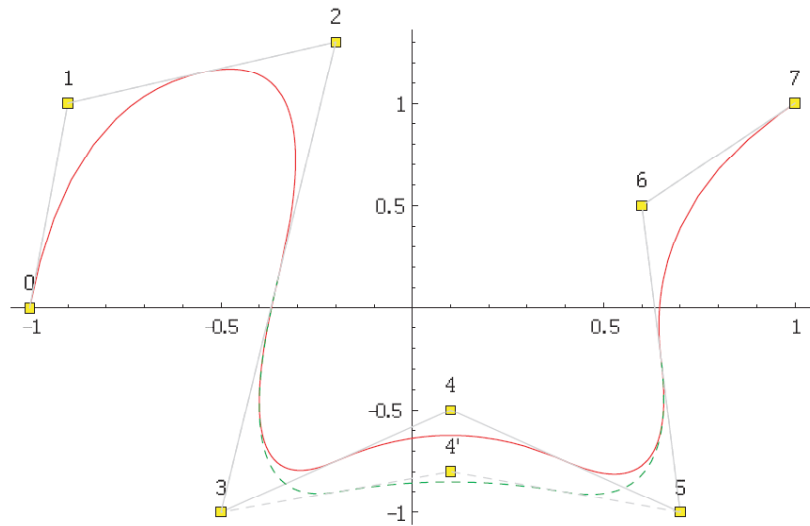
Figure 3: A curve with degree $p = 2$ on $U = (0,0,0,1/6,2/6,3/6,4/6,5/6,1,1,1)$; moving $\mathbf{P}_4$ to $\mathbf{P}_{4'}$ changes the curve in the interval $[2/6, 5/6)$.

Moving along the curve from $t = 0$ to $t = 1$, the functions $N_{i,p}(t)$ act like switches. As the parameter t moves past a knot, one $N_{i,p}(t)$ and the corresponding $\mathbf{P}_i$ switches off and $\mathbf{P}_{i+p+1}$ switches on.

Property 2.5. The B-spline curve tangents each segment of the control polygon.

2.2. Approximation to Outline Pixel Data with B-Spline Curves

In this section we study the construction of B-spline curves that should fit a rather arbitrary set of geometric data, such as pixels of an ostracod's outline, following the elaborations in Hoschek and Lasser (1993), Piegl (1995), Bayer et al. (2002), Deuflhard and Hohmann (2002). The aim is to construct curves that do not necessarily satisfy the given data in an exact way, but only approximately. In some applications - such as ours - a large number of points is generated, which can contain measurement errors or computational noise. In this case, it is important for the curve to capture the "shape" of the data, but not to "wiggle" its way through each single point.

Given is an array of sequenced pixels $\{\mathbf{Q}_k\}$, $k = 0,...,l$, which we want to approximate with a second-degree B-spline curve. If we assign a parameter value t_k to each pixel $\mathbf{Q}_k$, and select the knot vector $U = (u_0,...,u_m)$ to be uniform, we can set up a system of linear equations

$$\mathbf{Q}_k = \mathbf{C}(t_k) = \sum_{i=0}^{n} N_{i,p}(t_k)\,\mathbf{P}_i$$

with $l+1$ equations, where the control points $\mathbf{P}_i$ are the $n+1$ unknowns. The choice of the parameter values t_k enormously affects the shape of the curve. An adequate method provides the chordal parameterisation. It assigns the parameter values t_k proportionally to the total length of the outline. Let d be the total chord length

$$d = \sum_{k=1}^{l} \|\mathbf{Q}_k - \mathbf{Q}_{k-1}\|.$$

The parameter values are given by

$$
\begin{aligned}
t_0 &= 0 \\
t_k &= t_{k-1} + \frac{\|\mathbf{Q}_k - \mathbf{Q}_{k-1}\|}{d} \qquad k = 1,...,l-1 \\
t_l &= 1.
\end{aligned}
$$

Typically, it is necessary to find a B-spline curve approximating a large number of points. In general, the number of pixels l to approximate is much higher than the number of desired control points n. Hence, the system of equations is overdetermined and we will not get an exact solution.

The best way to overcome this difficulty is to solve the system in the sense of minimizing the sum of the squared differences between the given set of pixels $\{\mathbf{Q}_k\}$ and the appropriate values of the B-spline curve $\mathbf{C}(t_k)$,

$$\sum_{k=0}^{l} \|\mathbf{C}(t_k) - \mathbf{Q}_k\|^2 = \sum_{k=0}^{l} \| \sum_{i=0}^{n} N_{i,p}(t_k) \mathbf{P}_i - \mathbf{Q}_k\|^2 \longrightarrow \min.$$

There are manifold possibilities to get a solution for this least square problem. A numerical stable computation of the minimizing problem results from the pseudo-inverse matrix gathered from the singular value decomposition of the system matrix (see Deuflhard and Hohmann 2002, Hogben 2007, Strang 1998). This method is implemented in MORPHOMATICA to approximate outlines.

3. Implementation of the Formal Methods to Ostracod Outlines

3.1. Data Structure

A photograph of an ostracod's valve is taken under a microscope. Afterwards, the so-called Tps-dig (Rohlf 2001) saves the outline in a data set. This program creates a file with the pairs of coordinates of the outlining pixels of the picture. Also other information, such as potential landmarks and the file name of the picture, is saved in this file. The illustration below schematically shows the structure of such a file.

```
LM=0
OUTLINES=1

POINTS=1387
630 78
631 79
631 80
632 81
633 82
...
627 74
627 75
628 76
629 77

IMAGE=J001.tif
ID=J001
```

The file describes an outline with no specified landmarks (LM = 0) and one contour (OUTLINES = 1) with 1387 pixels (POINTS = 1387).

3.2. Centre of Gravity and Axes of Inertia

The positions of the valves on the pictures vary widely and the tps-files do not tag any basing points. Nevertheless, a meaningful comparison of two valves should be independent of alignment and position in the picture. Therefore, to compare the shape of two outlines, they first have to be superimposed. Ideally, this should be done in such a way that the "difference" between them is as small as possible. But this would be a very difficult task, so we choose to position the two outlines in such a way that the centroids and the main axes of inertia coincide.

The structure of the tps-data suggests computing the centroid based on the pixels using the arithmetic mean of the coordinate vectors of the points $\mathbf{Q}_k$, that is

$$\mathbf{S} = \frac{1}{l} \sum_{k=0}^{l} \mathbf{Q}_k.$$

But this is the centroid of these points and not of the whole outline. Consequently, this only makes sense if the points are distributed very uniformly. If parts of the outline are a little bit rugged or jagged, there will be relatively many points $\mathbf{Q}_k$ concentrated in these parts and $\mathbf{S}$ tends to move towards them. So the centroids of two very similar outlines may be rather different, if only one has some rugged parts. Figure 4 illustrates this difficulty.
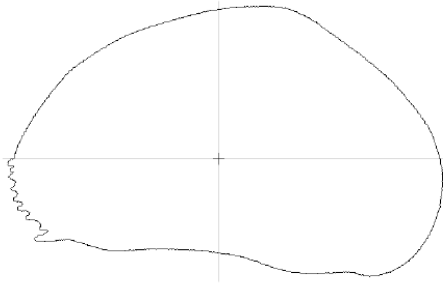
Figure 4a: Centroid and main axes computed with the outline points.
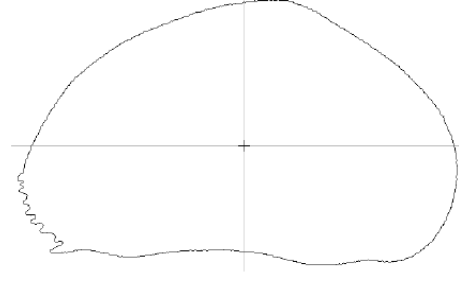
Figure 4b: The origin now is the centre of gravity of the domain and the axes correspond with the main axes of inertia.

This is why the above centroid should be replaced by the centre of gravity of the domain A surrounded by the given outline. This centre is defined by

$$\mathbf{S} = (s_x, s_y) = \frac{1}{a} \int_A (x, y) \, dx \, dy,$$

where s_x and s_y denote the coordinates of $\mathbf{S}$, and a is the area of the domain A. It turns out that $\mathbf{S}$ can be computed in the following rather simple way, if x_k and y_k denote the coordinates of $\mathbf{Q}_k$, and the points are given in counterclockwise order,

$$s_x = -\frac{1}{6a} \sum_{i=0}^{n} (y_{i+1} - y_i) \left(x_{i+1}^2 + x_i x_{i+1} + x_i^2 \right).$$

s_y is computed in a similar way with x and y interchanged and the whole expression multiplied by -1. Of course, (x_{l+1}, y_{l+1}) is understood to be equal to (x_1, y_1).

Analogical to the centroid, the axes of inertia should also be calculated not only for the points, but for the whole outline or, more precisely, for the domain surrounded by the outline. The moment of inertia with regard to a certain axis (passing through the origin) is defined to be the integral of the squared distance from this axis, taken over the considered domain. It may be computed in a similar, but somewhat more complicated way as the centre of gravity above.

If the moment of inertia is not equal for all directions of axes, there is a unique direction, which yields the minimum moment, and this is taken to be the new x-axis. This direction is given by an eigenvector of a certain 2 by 2 matrix and thus it is not difficult to compute.

The new *y*-axis is, of course, perpendicular to the new *x*-axis and corresponds to the maximum moment of inertia.

Finally, we have to define a congruence transformation *f*, which moves the points of the contour as mentioned above. Shifting **S** to the point **Y** = (0,0) and rotating the vectors **E**$_1$ = (e_{11}, e_{12}), specifying the axis with minimum moment of inertia, and **E**$_2$ = (e_{21}, e_{22}), specifying the axis with maximum moment, to (1,0) resp. (0,1) can be done by a transformation

$$f(\mathbf{X}) = \begin{pmatrix} e_{11} & e_{12} \\ e_{21} & e_{22} \end{pmatrix} (\mathbf{X} - \mathbf{S}).$$

Applying this transformation to each point moves the contour into the desired position.

3.3. Approximation to Contour Data

To prepare the point data for a good and meaningful approximation, it is necessary to divide the contour into two halves. If the B-spline approximation is applied in a straightforward way to an outline, it may happen that two very similar outlines lead to rather different control points. This is the case, for instance, with the two artificial elliptical outlines of figure 5. One should note that this phenomenon occurs due to the fact that moving the control points simultaneously in a suitable way around the curve has only little influence on the shape of the curve.
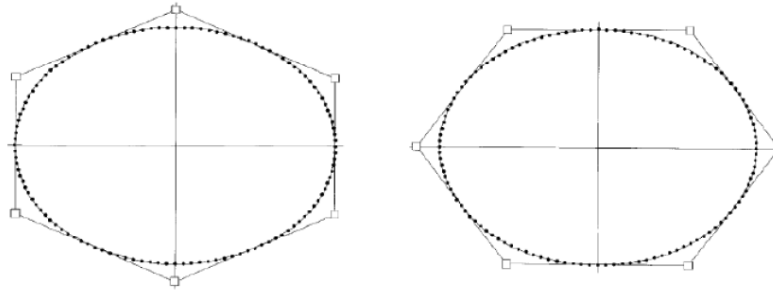
Figure 5: Two rather similar elliptic outlines with different control points.

To avoid this problem, we cut the outline in two pieces and approximate each half separately under the condition that the resulting curves fit together.

After the standardisation this can easily be done by using the *x*-axis as dividing line. Concretely, all points **P**$_i$ = (x_i, y_i) with $x_i > 0$ will be assigned to the dorsal region and all points with $x_i < 0$ to the ventral region. Therefore we must assume that the contour crosses the *x*-axis

at most at two points, what is usually the case for ostracods' outlines (fig. 6). Hence there are only two pairs of consecutive points in the contour where $x_i \geq 0$ and $x_{i+1} < 0$.

We pass a line through these points, determine the intersection points with the *x*-axis and add them to the contour. These intersections will be the starting and end points of the approximating B-spline curves.
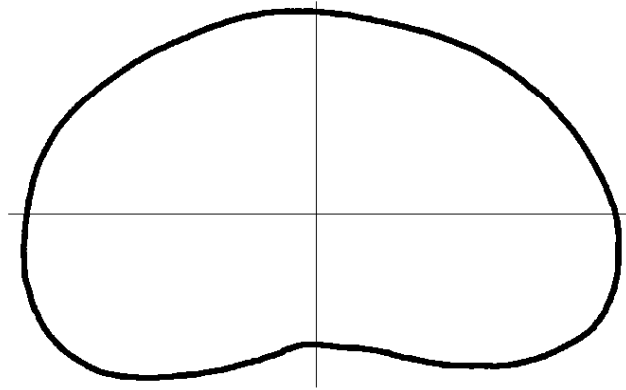
Figure 6: Ostracod contour after standardisation.

The outlining points of the respective region get numbered consecutively clockwise and are approximated by a B-spline curve of degree $p = 2$ with a uniform knot vector and parameter value gathered by a chordal parameterisation. The procedure is explained in sections 2.1 and 2.2. Figure 7 plots the contour data of an ostracod with its approximating B-spline curve.
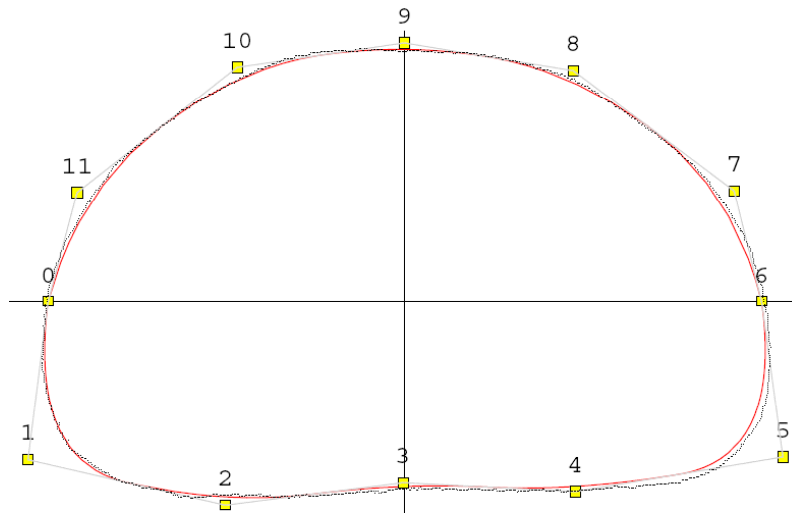
Figure 7: Ostracod contour approximated by a second-degree B-spline curve and its control polygon.

However, this approximation scheme does not bring an optimum result. A better solution can be achieved if the vector $\mathbf{C}(t_i) - \mathbf{Q}_i$, t_i indicating the corresponding chord length parameter of $\mathbf{Q}_i$, is perpendicular to the curve. Therefore we alter the parameter t_i iteratively by adding a value λ_i until $\mathbf{C}(t_i) - \mathbf{Q}_i$ is roughly perpendicular to the tangent $\mathbf{C}'(t_i)$.

The comparison in figure 8 shows the desired result. After 6 iterations the approximating B-spline curve approaches the contour data in an observable better way.
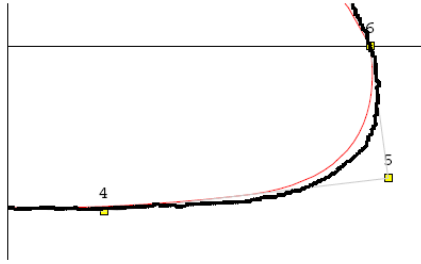
Figure 8a: Before adjusting the parameter.
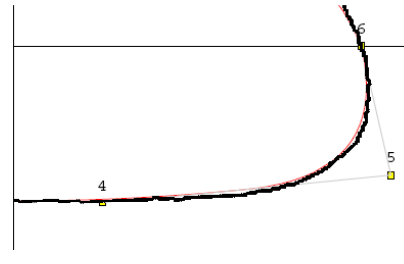
Figure 8b: After 6 iterative steps a better approach is guaranteed.

To compare biological aspects of ostracoda it is sometimes useful to adjust the sizes of the standardized outlines. This is essential for comparing, e.g., ostracods of different age.

Transforming the control points of the B-spline curve in such a way that the end points $\mathbf{P}_0 = (x_0,0)$, $\mathbf{P}_n = (x_n,0)$, lying on the *x*-axis, get the coordinates $\mathbf{P}_0 = (-1,0)$, $\mathbf{P}_n = (1,0)$ is one possibility. This method has one slight shortcoming. The centre of gravity shifts out of the origin, what can cause difficulties if the contour is rather anomalous or pear-shaped.

We obtain a better solution if we use a transformation

$$s\left(\mathbf{X}\right) = \sqrt{a}\mathbf{X},$$

where *a* is the ratio of the areas a_1, a_2 enclosed by the B-spline curves,

$$a = \frac{a_1}{a_2}.$$

This method keeps the centre of gravity in the origin and guarantees a better comparability.

4. Distinguishing Outlines

4.1. Distance of the Corresponding Control Points

At first sight, the following concept of a distance between two B-spline curves seems to be quite natural.

Definition 4.1. Let $\mathbf{C}$, $\mathbf{D}$ be two B-spline curves with control point sequences $\mathbf{P}_0,...,\mathbf{P}_n$ resp. $\mathbf{Q}_0,...,\mathbf{Q}_n$. We define the distance between $\mathbf{C}$ and $\mathbf{D}$ as the square root of the sum of all squared Euclidean distances between the corresponding control points divided by the number of control points

$$d\left(\mathbf{C},\mathbf{D}\right) := \frac{1}{n}\sqrt{\sum_{i=0}^{n}\|\mathbf{P}_i - \mathbf{Q}_i\|^2}.$$

Defining the difference between two B-spline curves in this way has a number of coherent reasons. First of all, two superimposed B-spline curves are identical if their corresponding control points coincide. Property 2.3 (Affine invariance) implies that a translation or rotation is applied to the curve by applying it to the control points. Both indicate a reasonable measure. A further evidence provides the attribute that the control polygon represents a kind of approximation to the B-spline curve. So, to some extent, the control points describe the shape of the curve (cf. Baltanás et al. 2003).

A further advantage of distinguishing B-spline curves by using the distance of the corresponding control points arises from property 2.4 (Local support). A single control point takes effect just on a part of the B-spline curve. By measuring the Euclidean distance of two corresponding control points we should be able to determine whether the respective regions differ significantly or not.

Moreover, our measure d is of special interest for users from the field of biology or palaeontology, since it is a tangible, intelligible and easy to visualize tool. The computing time is extremely short and the approximation of the outline pixel data generates a unique sequence of control points. Their distances may have an explanatory power of the difference in the curves shape.

Nevertheless, the computation of the difference of two superimposed B-spline curves with the measure defined above has some shortcomings, which are discussed in this section. At first, this distance function does not induce a metric. This is easy to comprehend by imaging a B-spline curve whose control points are located on a straight line.

The resulting B-spline curve corresponds with this line. After moving one or several control points along the line, our measurement d gets positive, but the B-spline curve is still the same line.

Additionally, the measure d only considers the magnitude of the difference vector between two corresponding points, but not the direction. To comprehend the effect of shifting a control point, we examine the following testing arrangement. Let $\mathbf{P}_0,...,\mathbf{P}_4$ be control points put on a semicircle of radius 1 in uniformly distributed angles, that is

$$\mathbf{P}_0 = (-1,0) \quad \mathbf{P}_1 = \left(-\tfrac{1}{\sqrt{2}}, \tfrac{1}{\sqrt{2}}\right) \quad \mathbf{P}_2 = (0,1) \quad \mathbf{P}_3 = \left(\tfrac{1}{\sqrt{2}}, \tfrac{1}{\sqrt{2}}\right) \quad \mathbf{P}_4 = (1,0).$$

By selecting various vectors $\mathbf{V}_j$ with the same length $\|\mathbf{V}_j\| = a$ for all j and adding them to control point $\mathbf{P}_2$, we obtain a B-spline curve $\mathbf{C}_j(t)$ for each vector. Owing to the measure defined above,

$$\begin{aligned} d\left(\mathbf{C}, \mathbf{C}^j\right) &= \frac{1}{n}\sqrt{\sum_{i=0}^{n}\left\|\mathbf{P}_i - \mathbf{P}_i^j\right\|^2} \\ &= \frac{1}{5}\sqrt{\left\|\mathbf{P}_2 - \mathbf{P}_2^j\right\|^2} \\ &= \frac{a}{5} \end{aligned}$$

for any vector of length a, although, intuitively speaking, some of the curves deviate much more from $\mathbf{C}(t)$ than others (fig. 9).
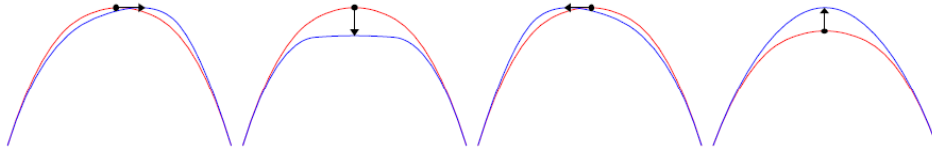
Figure 9: The primal B-spline curve (red) and various B-spline curves (blue) generated by adding different vectors with the same length to a control point.

This difficulty is of particular importance if we adjust several control points. Each point of the curve is determined by at least 3 control points (for degree ≥ 2), see property 2.4. Thereby, neighbouring control points can be positioned in such a way that an almost identical curve emerges. Figure 5 shows this effect. The left B-spline curve results from the right curve by rotating the control points by 30°. It is obvious, that both curves are nearly similar, but our difference measure yields a high value of dissimilarity.

4.2. Area Deviation

The B-spline curves we use to approximate ostracods' outlines feature some "nice" characteristics. We want to use these characteristics for computing a demonstrative and tangible measure to distinguish contours. Our approximating B-spline curves have no loops, self-intersections or other anomalies. We obtain solely so-called simply-closed curves given by

Definition 4.2. A curve $\mathbf{C}(t)$, $t \in [a,b]$, in the plane is called simply-closed if it has no self-intersections,

$$\mathbf{C}(t_1) \neq \mathbf{C}(t_2) \ for \ t_1 \neq t_2, \ t_1, t_2 \in [a,b],$$

and the endpoints coincide,

$$\mathbf{C}(a) = \mathbf{C}(b).$$

Accordingly, a simply-closed curve $\mathbf{C}$ constitutes a bounded part of the plane A surrounded by the curve. A can also be seen as the interior of $\mathbf{C}$. To distinguish two simply-closed curves $\mathbf{C}$ and $\mathbf{D}$ with their interiors A and B, we consider the area of the part of the plane, which is contained in exactly one of the domains A and B and may be viewed as the area "between" the outlines. This corresponds to the area of the symmetric difference $\mathbf{A} \Delta \mathbf{B}$, which is called the area deviation of A and B, illustrated in figure 10.
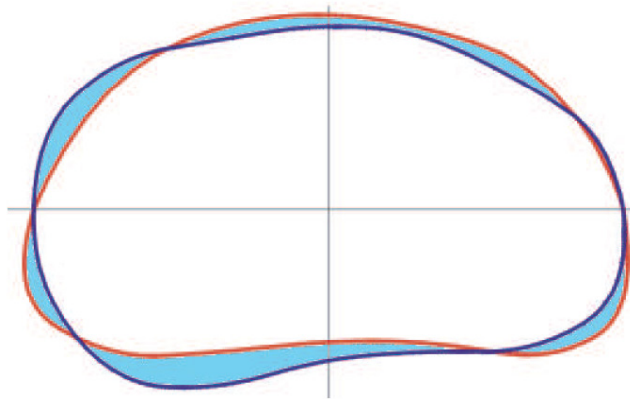
Figure 10: The light blue area is the area deviation of two superimposed simple-closed B-spline curves.

The area deviation offers several advantages for the use in ostracodology. This common and very natural measure is demonstrative and tangible; the resulting differences are in square micrometers, what doubtless contributes to a better conceivability. Furthermore, the measure is rather inured to possible data errors and measuring faults.

As mentioned above, ostracods' outlines feature a good characteristic for a fast computation without difficulties. In general, the approximating polygons are not convex, but the angles between the *x*-axis and a straight line from the origin to the vertices are in ascending order. This fact makes it possible to reduce the number of computations for possible points of intersection substantially.

Every specimen is approximated by two open B-spline curves describing two regions, a dorsal and a ventral one, fixed by the main axes of inertia with the minimum moment. To calculate an approximative value for the enclosed area "between" the B-spline curves of two specimen-halves, in the present implementation 51 points on each curve, corresponding to equally spaced parameter values, are computed. We denote them with $\mathbf{C}_i$ for the first curve and $\mathbf{D}_j$ for the second ($i,j = 0,...,51$). This yields a polygon with 50 line segments substituting each B-spline curve, with $S_i = \mathbf{C}_i\mathbf{C}_{i+1}$ denoting the segments of the first polygon and $T_j = \mathbf{D}_j\mathbf{D}_{j+1}$ denoting the segments of the second.

To evaluate the area deviation, the points of intersection of the two superimposed polygons are of importance. In principle, it would be necessary to determine the points of intersection of each segment of the first polygon with each segment of the second polygon, what requires $50 \times 50 = 2500$ comparisons for each specimen-half. This goes beyond the scope of a tolerable computing time.

To accelerate the process, the cotangent $\cot(\varphi) = x/y$ is assigned to every vertex $\mathbf{C}_i$ resp. $\mathbf{D}_j$. For this purpose, let φ_i be the angle between the *x*-axis and the vector $\mathbf{OC}_i$, pointing from the origin to the vertex $\mathbf{C}_i$, and ψ_j be the angle between the *x*-axis and the vector $\mathbf{OD}_j$. If the cotangent values are in ascending order, this means

$$\cot(\varphi_i) < \cot(\varphi_{i+1})$$

and

$$\cot(\psi_j) < \cot(\psi_{j+1}),$$

we can confidently assume that points of intersection on a segment S_i can only be possible for segments T_j where

$$\cot(\psi_{j+1}) > \cot(\varphi_i)$$

and

$$\cot(\psi_j) < \cot(\varphi_{i+1}),$$

see figure 11.
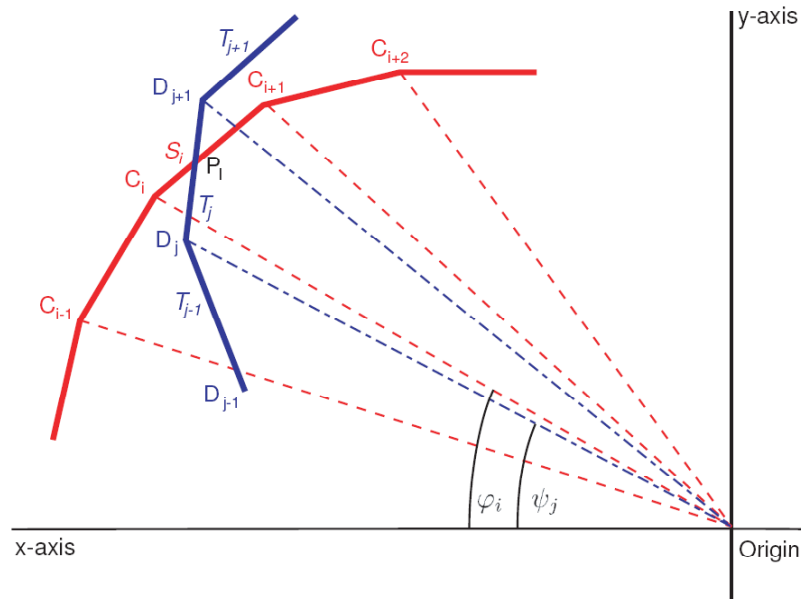
Figure 11: For possible points of intersection of the segment S_i only the segments T_j and T_{j+1} of the other polygon are worth considering.

Usually, a segment of a polygon has to be compared with 2 or 3 segments of the other polygon. This makes it possible to reduce the number of comparisons to about 150 as opposed to 2500 mentioned above.

Due to the structure of the algorithm certain outlines of specimens cannot be treated. As described above the cotangent-values of the successive vertices of the spline-approximating polygon must be in ascending order. In other words, the B-spline considered as a clockwise directed curve must not change its direction (viewed from the origin). As a rule, such shapes occur only in faulty datasets. To get a general idea of such uncalculable shapes, a couple of exemplary specimens with their approximating B-splines are indicated below.
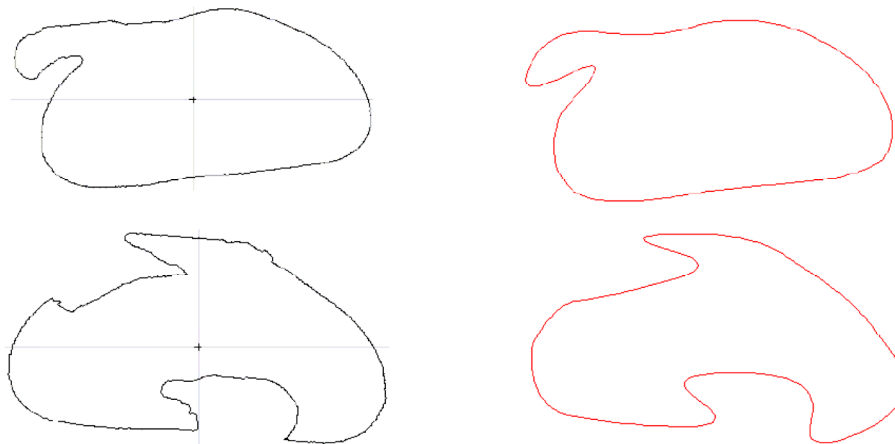
Figure 12: Examples of uncalculable outlines (black) and their approximating B-spline curves (red).

The distinction of ostracods using the area deviation is implemented in the current version of the program MORPHOMATICA (Linhart et al. 2006) and a brief documentation of the algorithm was introduced for the first time by us in Minati et al. (2008).

The program additionally offers the possibility to distinguish the valves by examining only the dorsal resp. ventral region.

5. Classification of Populations

In many fields of ostracodology a comparison of whole groups or populations of ostracods is wanted and necessary. In the following a measure for the dissimilarity of two populations is explained, which is proposed by one of us (J. L.). It is supposed that for any two individuals a kind of "distance" is available. So let $P = p_1,...,p_n$ and $Q = q_1,...,q_m$ be two populations and d a distance defined on $P \cup Q$. The *dissimilarity index* of P and Q is then defined by

$$\text{diss}(P,Q) := \frac{\frac{1}{nm} s(P,Q)}{\frac{1}{n^2} s(P) + \frac{1}{m^2} s(Q)},$$

where

$$s(P,Q) := \sum_{i=1}^{n} \sum_{k=1}^{m} d(p_i, q_k)$$

and

$$s(P) := \sum_{i=1}^{n-1} \sum_{k=i+1}^{n} d(p_i, p_k), \qquad s(Q) := \sum_{i=1}^{m-1} \sum_{k=i+1}^{m} d(q_i, q_k).$$

Some essential properties of this dissimilarity index are:

1.   diss(P,Q) only depends on the distances $d(x,y)$ with $x,y \in P \cup Q$.

2.   diss(P,Q) is invariant to scaling transformations.

3.   diss(P,Q) $= 1$ if $P = Q$.

4.   If the underlying distance d is a so-called hypermetric (see Kelly 1970), diss(P,Q) is always ≥ 1. The area deviation (cf. chapter 4.2) is a typical example for a hypermetric (see also Kelly 1970).

The dissimilarity index of P and Q may be viewed as the ratio of the average distance between individuals of different populations to the average distance between individuals of the same population. Perhaps this becomes more clear if the denominator of the expression defining diss(P,Q) is written in the following form:

$$\frac{\frac{1}{n^2/2}s(P) + \frac{1}{m^2/2}s(Q)}{2}.$$

At first sight, it seems that here $n^2/2$ should be replaced by $n(n\text{-}1)/2$, since this is the number of distances within the population P (and analogously for Q). But then the value of diss(P,Q) would be smaller than 1 for $P = Q$, which does not make sense. Intuitively, one might think that to a certain extent also the distances between identical individuals (which are of course equal to zero) should be taken into account.

For several purposes it will be more convenient to consider the natural logarithm of the dissimilarity index,

$$\ln \text{diss}(P,Q),$$

which yields a value in the range $[0,\infty)$ instead of $[1,\infty)$.

The current version (1.6) of "Morphomatica" creates a "resemblance matrix" with entries representing the pairwise distances given by the area deviation. Table 1 shows a resemblance matrix comparing the valves of respectively 8 specimens of the species Pseudocandona danubialis from Ada-Kaleh (fig. 13) and Pseudocandona eremita from Aştileu (fig. 14), both localities in Romania. The data and pictures originate from a comparative study of Iepure et al. 2007, investigating the morphology of valves belonging to populations from Romania.

Figure 13: Lateral view of female valves belonging to the species Pseudocandona danubialis from Ada-Kaleh.

	AK1	AK2	AK3	AK4	AK5	AK6	AK7	AK8	Ast1	Ast2	Ast3	Ast4	Ast5	Ast6	Ast7	Ast8
AK1	–															
AK2	50.71	–														
AK3	25.11	38.77	–													
AK4	25.03	38.26	0.71	–												
AK5	9.47	51.62	25.54	25.42	–											
AK6	30.89	23.25	25.6	25.24	34.16	–										
AK7	24.45	48.31	16.59	16.65	23.44	35.46	–									
AK8	42.94	29.36	43.08	42.67	47.45	18.52	50.5	–								
Ast1	64.12	46.03	67.33	66.89	66.35	43.68	72.62	29.96	–							
Ast2	60.38	40.56	62.21	61.78	63.82	38.48	68.47	28.28	12.81	–						
Ast3	47.58	49.18	59.68	59.39	48.81	39.46	58.66	33.03	22.38	21.1	–					
Ast4	55.3	35.71	56.31	55.86	57.99	32.2	63.33	28.5	15.9	10.25	21.82	–				
Ast5	51.62	36.57	54.24	53.86	53.57	31.47	58.85	29.97	16.89	11.91	18.69	6.35	–			
Ast6	59.79	49.27	66.54	66.22	62.89	43.41	69.25	32.05	12.84	11.92	17.27	15.47	15.8	–		
Ast7	58.18	41.82	61.3	60.94	60.58	38.13	65.92	34.11	14.41	10.13	23.31	7.6	8.33	13.51	–	
Ast8	73.64	44.79	73.77	73.39	75.73	49.97	81.57	37.58	17.31	16.65	31.63	19.31	23.35	19.25	18.37	–

Table 1: Area deviation between valves of the Ada-Kaleh and Aştileu populations.
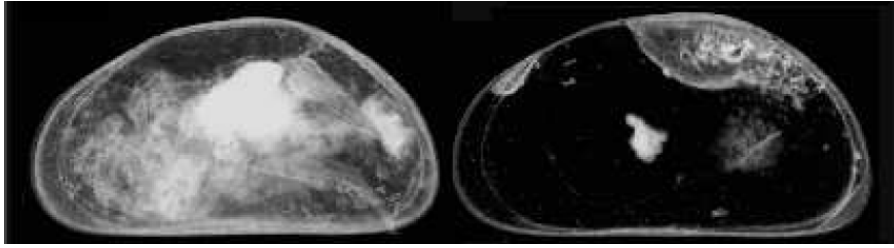
Figure 14: Lateral view of female valves belonging to the species Pseudocandona eremita from Aştileu.

Computing the above dissimilarity index for the Ada-Kaleh and Aştileu populations (resemblance matrix table 1) yields

$$
\begin{aligned}
n &= 8 & m &= 8 \\
s(P) &= 869.2 & s(Q) &= 454.56 \\
s(P,Q) &= 3412.94
\end{aligned}
$$

and

$$
\begin{aligned}
\operatorname{diss}(P,Q) &= 2.58, \\
\ln \operatorname{diss}(P,Q) &= 0.947.
\end{aligned}
$$

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MORPHOMATICA – Programme Description

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The programme MORPHOMATICA was initially designed and implemented by one of us (W. B.), with the mathematical framework provided by Prof. Linhart (Department of Mathematics, University of Salzburg). The functionality was strongly dependent of the requirements and suggestions of two other colleagues, Dr. Dan L. Danielopol and Dr. Angel Baltanás (UAM, Madrid) always with the biological requirements and the desired output in mind.

MORPHOMATICA, version 1.5, written by W. B. and version 1.6, expanded by W. N., uses the B-splines algorithm adapted to ostracod outlines by Johann Linhart. The mathematical background of the B-splines for an approximate description of ostracod outlines is presented in Bayer et al. (2002), Baltanás et al. (2003) and Neubauer (2007).

The program is a software application running under Microsoft Windows 98, NT 4.0, 2000, XP, which enables the user to apply the above described method to digitised ostracod outlines. The design of the program is based on experiences made during a bachelor project at the University of Salzburg (Bayer et al., 2002). The software package with the same name (cf. Linhart et al. 2006) is available at http://palstrat.uni-graz.at.

Working with MORPHOMATICA

The following topics will be introduced:

- Create a new MORPHOMATICA document
- Insert a specimen from a file and how to work with it
- Calculate the approximation for the outline of a specimen
- Combine several specimen to a cluster, calculate the cluster mean and corresponding delta vectors

- Export specimen and cluster data for import into other applications

- Print specimen outlines and computational data

- Save a MORPHOMATICA document

The User Interface of MORPHOMATICA

The user interface consists of a main application window, which is partitioned into two adjoining areas, and several dialog objects, which are displayed when additional input is required or parameters are to be manipulated. In the left part of the main window, the file names of the imported ostracod outlines and the associated approximations and clusters are displayed in the style of a directory tree. In this tree view, the user can select the file he intends to work on.
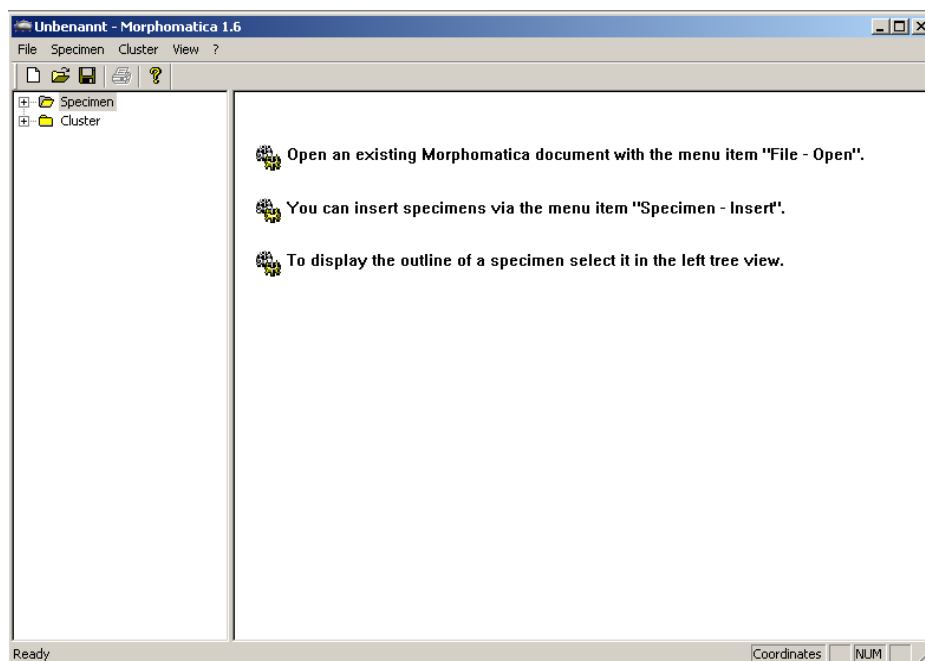
The contents of the right part of the window depend on the selected entry in the tree view. If an imported ostracod outline in the specimen directory of the tree view is selected, the right pane - the specimen view - displays its outline and if an approximation was calculated for the specimen, the according B-spline is drawn.
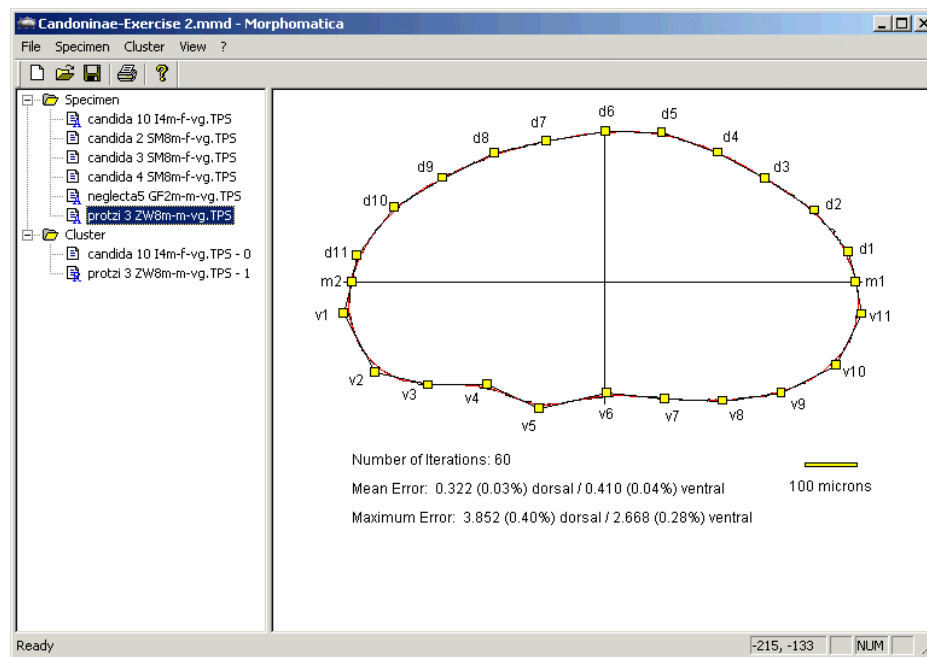
If alternatively an entry in the cluster directory of the tree view is selected, then the approximations of all specimens contained in the cluster, the associated delta vectors, and the optional mean specimen outline are displayed in the cluster view in the right pane.

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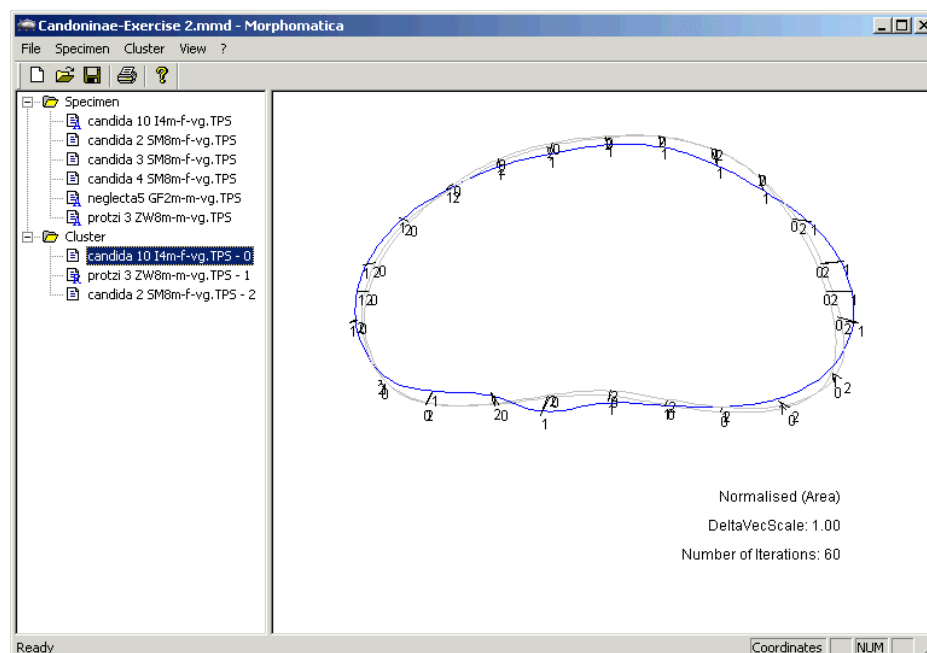
Create a new MORPHOMATICA document

A new document is created every time MORPHOMATICA is executed. This means, that the document does not contain any specimen or corresponding approximation, and that all program parameters are initialised with certain predefined reasonable values. Alternatively, the user can create a new document with the menu item *File - New* at any time. If there is already an existing document being worked with, the user will be asked if he intends to save it. Without saving the contents of the previous document are discarded.

After the creation of a new document, the main program window looks as following:
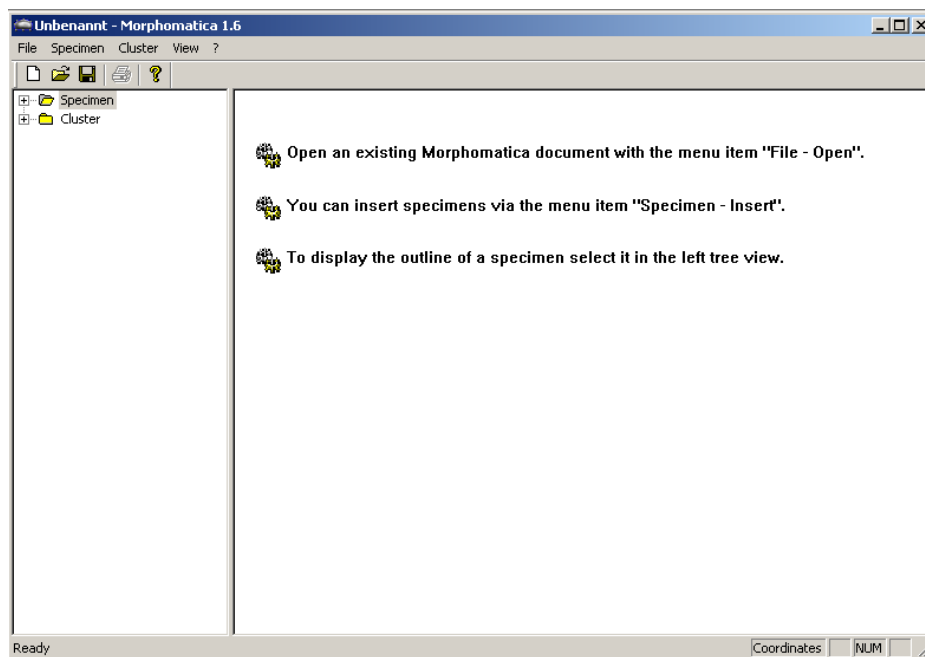
As mentioned before, no specimens are contained in a new document. Now, you have to import specimens from an appropriate file format into the document.

Working with a specimen

Insert a specimen into the document

To insert one or more specimens from a file into the document, use the menu item *Specimen - Insert*. MORPHOMATICA supports the file formats

- *.tps, a file format used by DigiTPS.
- *.msd, files created with the program Morphosys - in case that the file extension is different at your system, enter the appropriate extension in the "Filename" field.

In the next step, an open dialog will be displayed in which you select the file(s) with the ostracod(s) you intend to import. The open dialog allows you to select one ore more files at the same time:
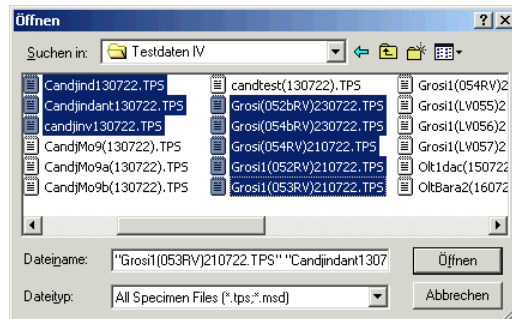
After confirming the dialog with "open", the specimens contained in the selected files are inserted into the document. Those specimens will be added to the specimen directory in the tree view with their related file names. If more than one specimen is stored in the imported file, the entries in the tree view are extended with an enumeration.

Display the outline of a specimen

To display the outline of an imported specimen you just have to select its name in the tree view on the left. The currently selected specimen is marked in the tree view with a blue background. The outline is displayed in the specimen view with additional information like the corresponding scaling of the outline or the coordinate axes.
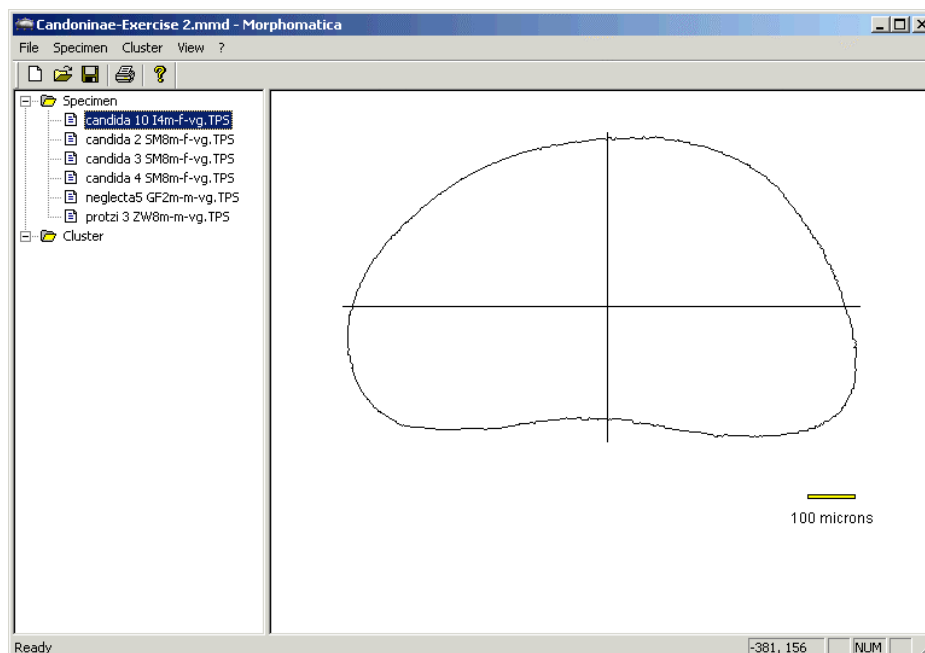
Remark: If a scale is stored for a specimen in the imported file, the scale labelled "100 micro meters" is shown, otherwise a scale labelled "100 pixel" is displayed.

Remark: Basically, a rotation and translation is automatically applied to every imported specimen such that the centroid of the specimen is located in the origin of the coordinate system and that the main axes of inertia are parallel to the coordinate axes.

Display file information for imported specimen

The file properties of a specimen can be shown with the menu entry *Specimen - Properties* and are located within the first property page *File* (see below, left).

The path of the file where the specimen was imported from is displayed at the top of the dialog, immediately followed by the file type. Additional information of the outline is displayed as well.
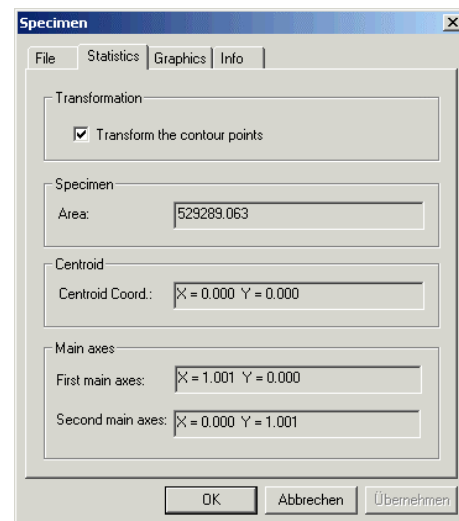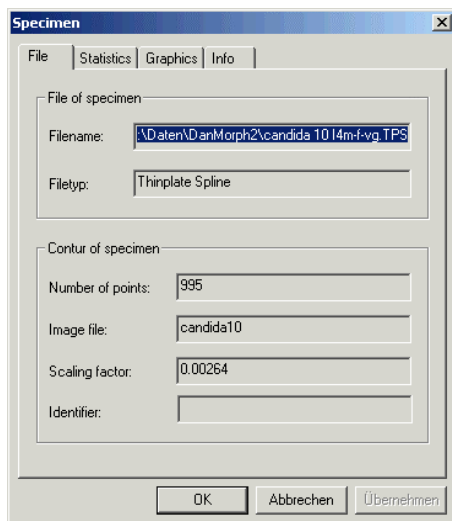
Display statistical data for specimen

Mathematical parameters, which are calculated from the outline defined in the specimen data file, are displayed within the property page *Statistics* (see above, right). As already mentioned, an imported specimen is transformed to meet certain conditions. The mathematical parameters for the transformation are listed below. If you do not want the outline to be transformed you can deselect the check box at the top of the dialog. In that case, the outline is displayed using the coordinates as defined in the data file.

Settings for the graphical representation

There are several options for the representation of the outline of a specimen. To set the options for a specific specimen, select its name in the tree view and choose the menu item *Specimen - Properties*. After opening the property page called *Graphics* the following options are shown:
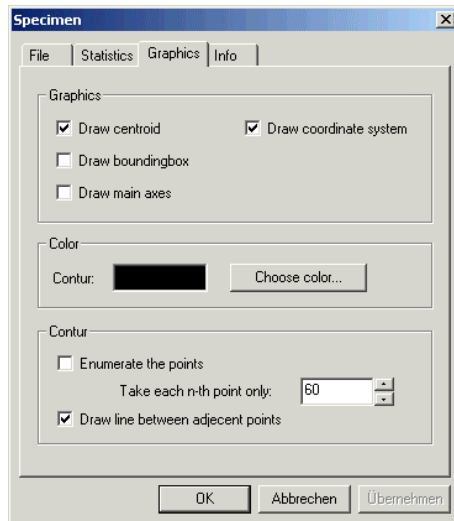
It is possible to change the following options:

- *Draw centroid:* If the check box is activated, the centroid of the specimen is marked.
- *Draw bounding box:* The bounding box enclosing all points of the outline of the specimen, as well as the coordinates of the corner points of this rectangle are displayed.
- *Draw main axes:* Selects, whether the main axes of inertia are drawn or not. This option is useful if the outline is not transformed to normal position.
- *Draw coordinate system:* Option to draw the axes of the coordinate system.
- *Colour:* To choose the colour for the specimen.
- Enumerate the points (of contour): If the option is activated, then the points of the outline will be enumerated (only the number of each n-th point will be printed).
- *Draw line between adjacent points:* Given that the distance between the points of the outline is to large to form a continuous outline, selecting this option will connect the points with straight line segments.

Additional morphological data

Not only the imported outline of a specimen can be saved in a MORPHOMATICA document, it's also possible to specify additional morphological data.

For this, choose the menu item *Specimen - Properties* and in that dialog the page *Info*.
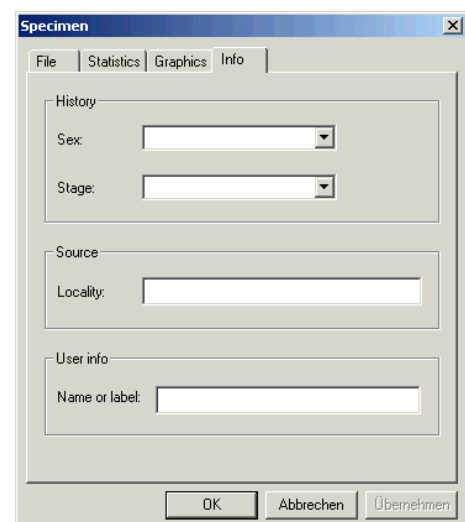
Remove a specimen from a MORPHOMATICA document

A specimen can be removed from the document at any time by selecting its name in the tree view and selecting the menu item *Specimen - Delete*. Alternatively, pressing the „Del" - Key deletes the selected specimen.

Approximate a specimen

The outline of each specimen can be approximated with the B-spline method described in the Mathematical background section. The calculated approximation is drawn together with the outline in the specimen view.

Calculation of the approximation

To calculate the approximation from a selected specimen select the menu item *Specimen - Approximation - Calculate*.
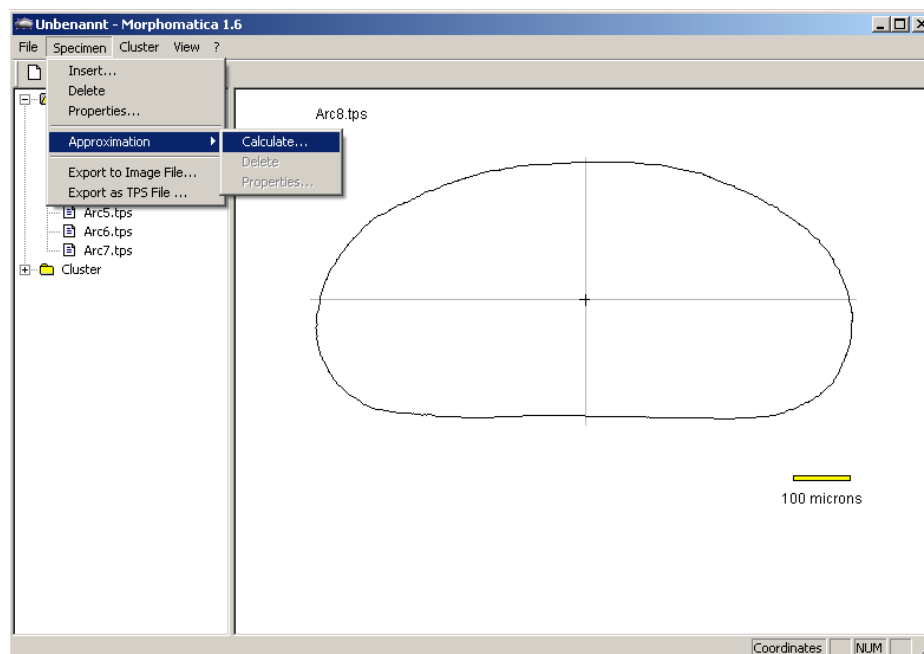
Parameters for the approximation

In the up-coming dialog, several parameters for the approximation can be varied.

It is possible to specify the number of control points to be calculated and the number of iterations for the parameter correction to be applied.

Next, the approximating B-splines are drawn over the outline of the specimen. Additionally, the control points of the upper and lower half of the B-splines are displayed and enumerated, as well as the corresponding control polygon. The calculated values for the approximation (such as the number of iterations, mean and maximum approximation error of the upper and lower half) are shown in the lower left corner of the specimen view.
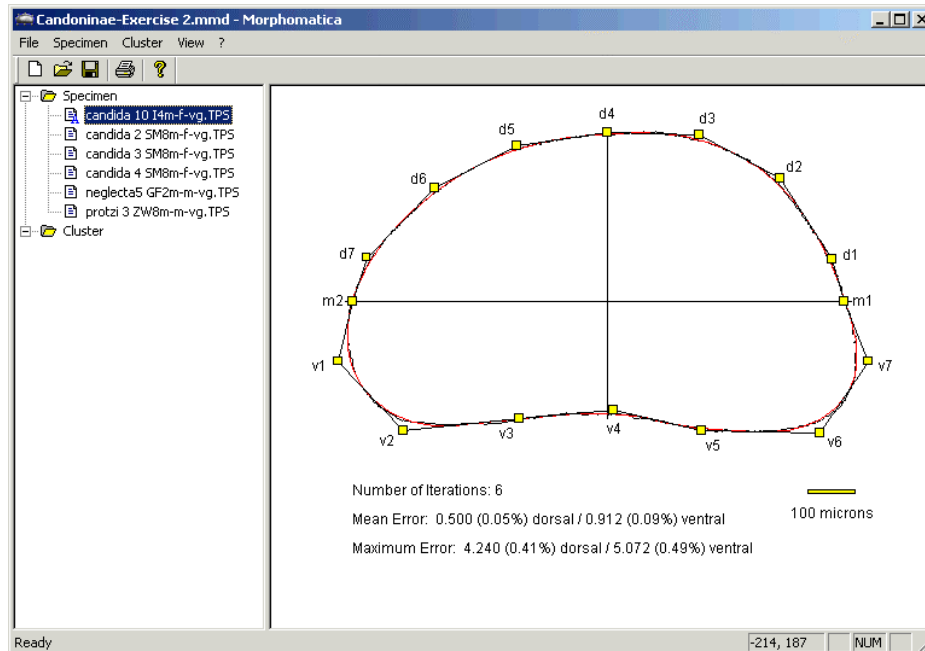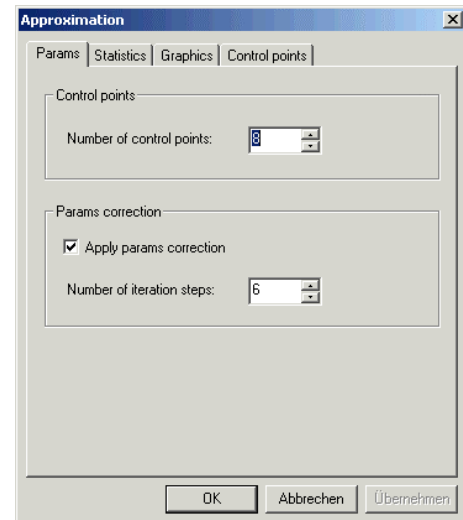
Remark: A specimen for which an approximation is fitted, is marked with a blue "A" next to its file name in the tree view.

Statistical data of the approximation

The calculated approximation values are listed in the property page *Statistics* too.

There it is also possible to select, whether the error values are to be displayed in the specimen view or not.
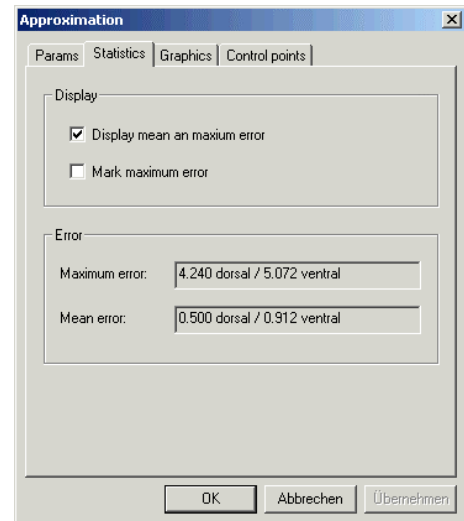
Settings for the graphical representation of the B-splines
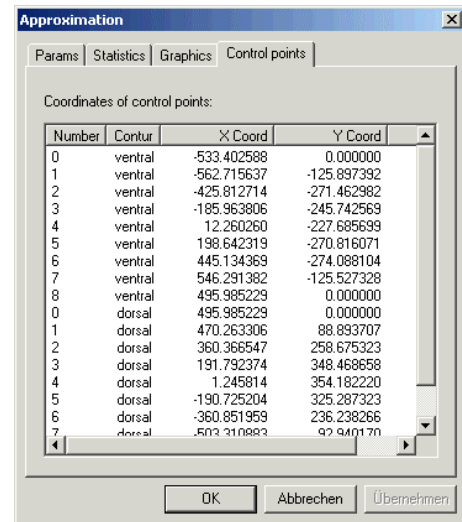
As with the representation of the outline of a specimen, there are several options for the representation of the approximating B-splines, which are accessible under the menu *Specimen - Approximation - Properties*. After selecting this menu item the following options are display in the property page *Graphics*. It is possible to vary the following options:

- *Draw spline:* If this check box is activated, the control points are drawn over the outline of the specimen. It is also possible to change the colour of the B-spline there.
- *Draw polygon:* By selecting this entry, the control polygon is drawn.
- *Mark points:* The control points are marked with yellow-filled rectangles. If the cursor is over any of those rectangles, the coordinates of the corresponding control point are displayed.
- *Index the points:* Each control point is enumerated.
- *Display coordinate values:* The coordinates of each control point are written next to it.
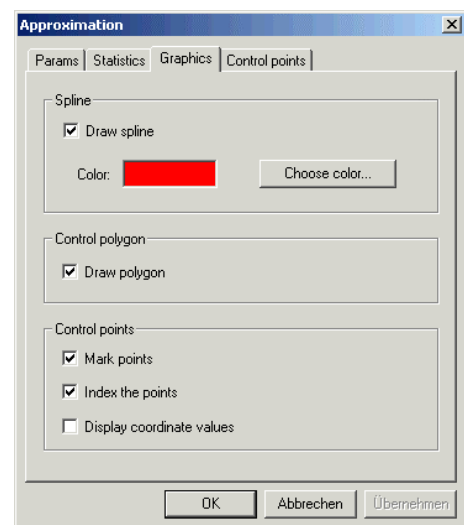
Display the calculated control points coordinates

The calculated control points can also be requested via the menu item *Specimen - Approximation - Calculate*.

Number	Contur	X Coord	Y Coord
0	ventral	-533.402588	0.000000
1	ventral	-562.715637	-125.897392
2	ventral	-425.812714	-271.462982
3	ventral	-185.963806	-245.742569
4	ventral	12.260260	-227.685699
5	ventral	198.642319	-270.816071
6	ventral	445.134369	-274.088104
7	ventral	546.291382	-125.527328
8	ventral	495.985229	0.000000
0	dorsal	495.985229	0.000000
1	dorsal	470.263306	88.893707
2	dorsal	360.366547	258.675323
3	dorsal	191.792374	348.468658
4	dorsal	1.245814	354.182220
5	dorsal	-190.725204	325.287323
6	dorsal	-360.851959	236.238266
7	dorsal	-503.310883	92.940170

Changing the approximation parameters

The parameters of an existing approximation can be changed with the menu item *Specimen - Approximation - Properties* at any time. In the general case a parameter change forces a recalculation of the approximation.

Delete the approximation of a specimen

An approximation of a specimen can be deleted with the menu item *Specimen - Approximation - Delete*.

Change a control point interactively

The user can change the calculated control points interactively. To do this click on the yellow rectangle representing the control point you want the coordinates to change. The fields of the upcoming dialog are initialised with the actual position. You can now change the position by entering the new coordinates directly or by pressing the up and down cursor buttons next to the fields.
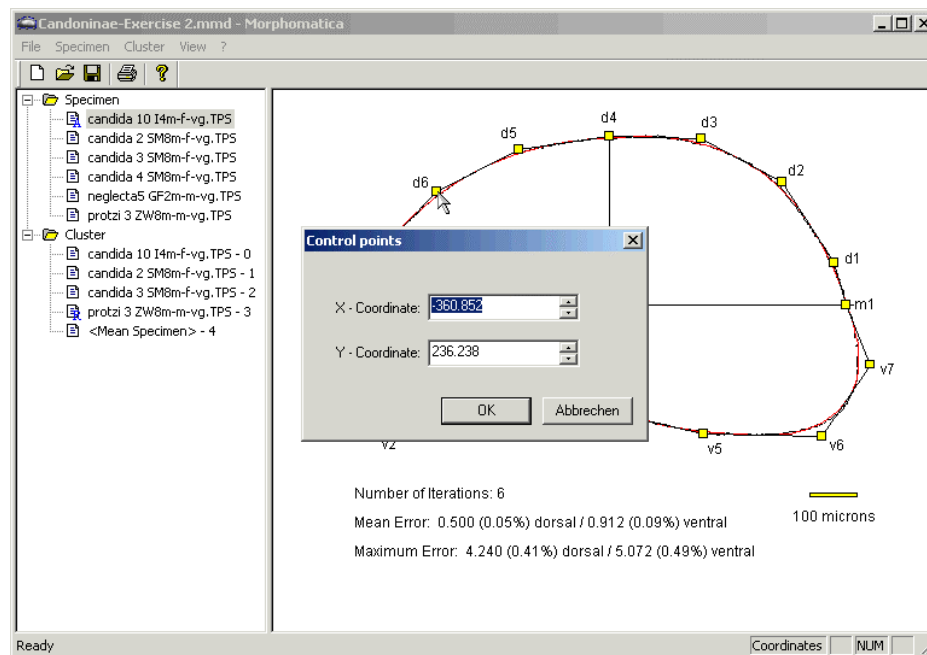
After closing the dialog with Ok, the B-spline is recalculated and the new approximation is drawn.

Remark: Place the mouse cursor over the yellow rectangle representing a control point if you want to know its coordinate values.
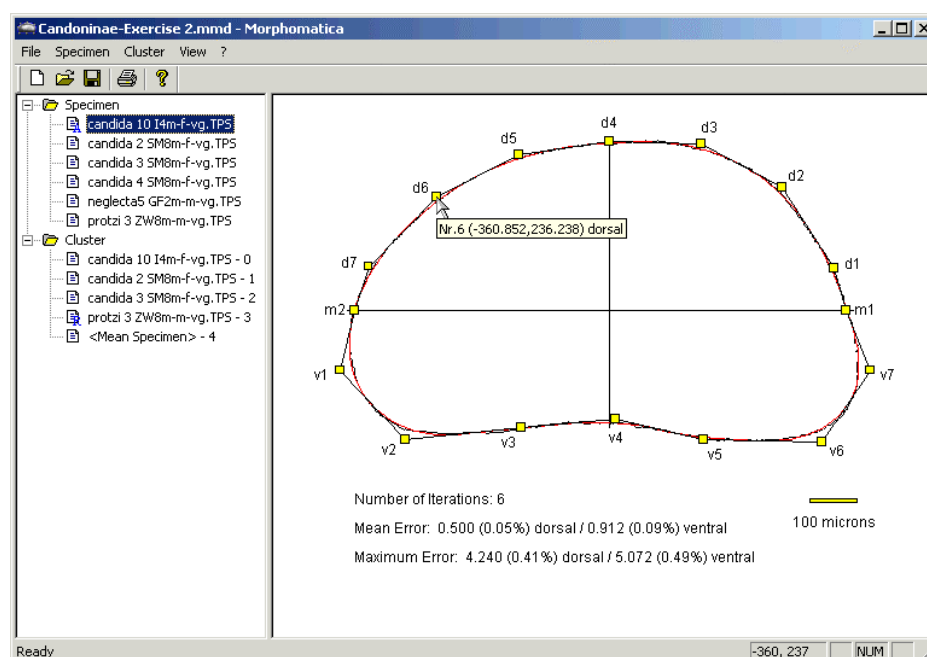
Cluster view of specimens

In the cluster view the approximating B-splines of all specimens in the cluster are simultaneously drawn over each other. This allows an easier comparison between several specimens in interest.

Add a specimen to the cluster

To insert a specimen from the specimen list into the cluster, mark its name in specimen directory of the tree view. Next insert the specimen with the menu item *Cluster - Insert*.

The filename of the specimen is appended in the cluster directory of the tree view.
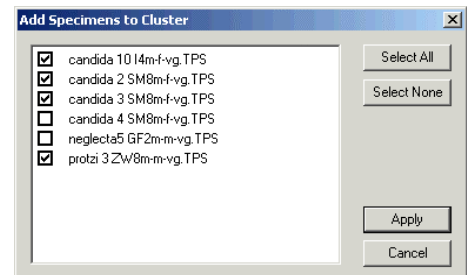
Alternatively, a specimen can be inserted to the cluster with a typical drag and drop operation within the tree view from the specimen directory into the cluster directory. If more specimens are to be inserted into the cluster, a convenient way is via the menu item *Cluster - Select*.

At the same time you add a specimen to the cluster the corresponding cluster approximation is calculated. Containing several specimens, the cluster view looks like the following:
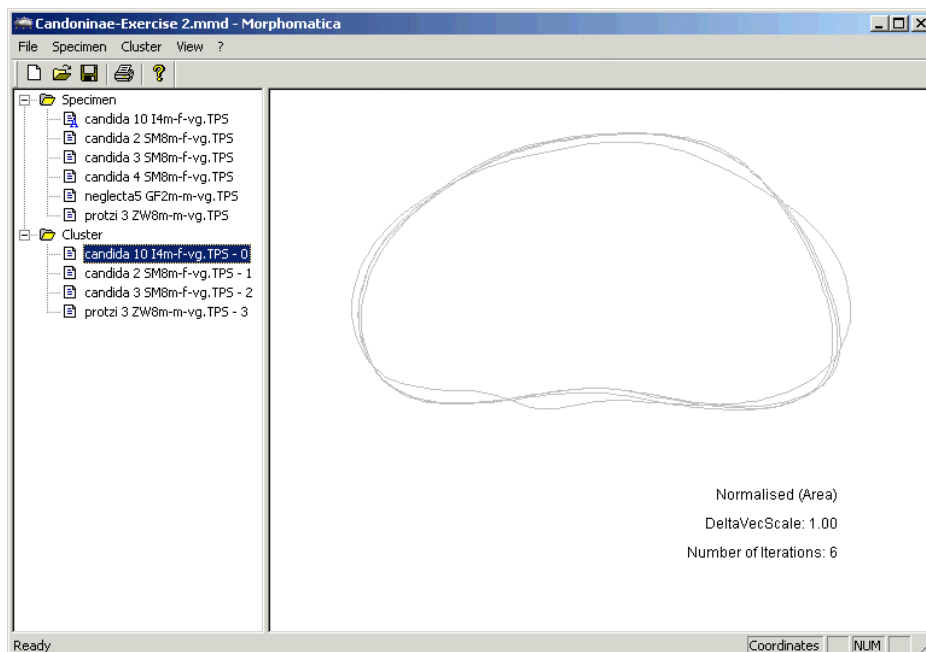
Calculation of the cluster mean

As soon as one or more specimens are added to the cluster, the mean specimen of these can be calculated with the menu item *Cluster - Calculate mean specimen*. The outline of this mean specimen is drawn in the cluster view too, using a different colour. An entry named "<Mean Specimen>" is added in the cluster directory of the tree view representing this mean specimen.
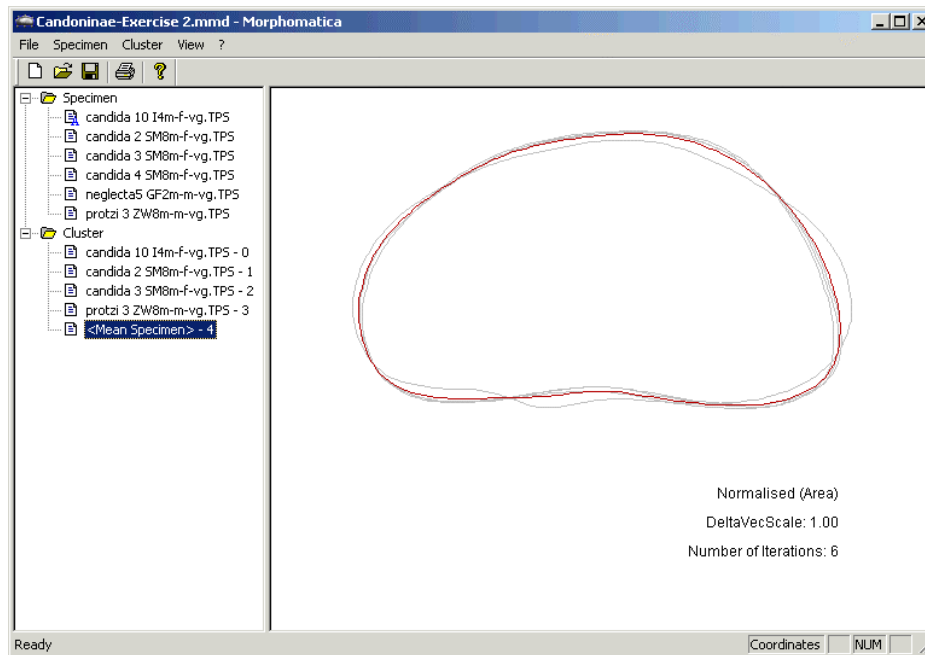
Remark: The mean specimen is not recalculated if new specimens are added to the cluster or specimens are removed from it. This behaviour is deliberate, such that multiple ways of comparison with the mean specimen are possible.

Selecting a specimen as reference

After two ore more specimens are present in the cluster, one of these can be defined as the cluster reference. To define a specimen in the cluster as reference, select its name in the cluster directory of the tree view and use the menu item *Cluster - Mark as reference*.

Automatically with the selection of a specimen as cluster reference, the delta vectors between this cluster reference specimen and all other specimens in the cluster are calculated and displayed. The name in the cluster directory of the reference specimen is further marked with a blue "R" next to it, to identify it as reference.

Remark: If the cluster reference specimen is changed, the delta vectors are automatically recalculated and actualised in the cluster view.
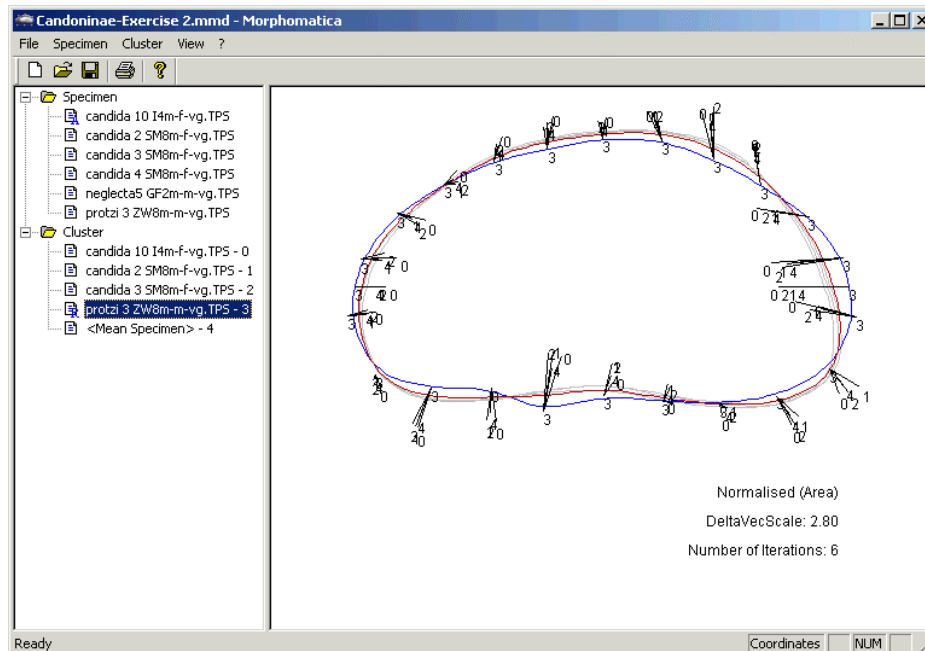
Settings for the cluster approximation parameters

If you need to change the parameters of the cluster, select the menu item *Cluster - Properties*. You can set the parameters for the approximation (of all specimens in the cluster) in the upcoming dialog (like before in the approximation for a single specimen).
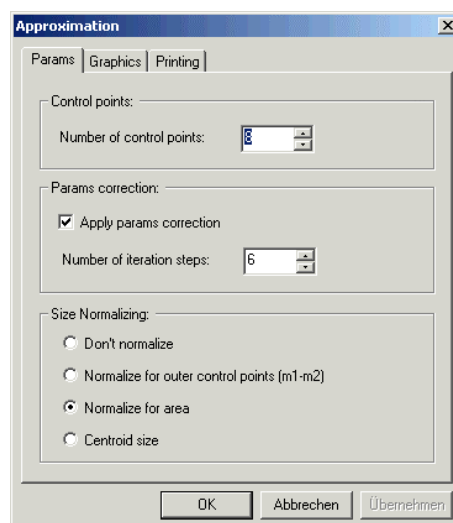
The options are as follows:

- Number of control points for upper and lower B-spline

- Number of steps in the parameter correction (the mathematical method used to improve the calculation of the approximation)

- Apply various normalisation operations. This allows for a size independent comparison of specimens.

  o *Normalize for outer control points:* The specimen approximations (i.e. the two B-splines) are transformed, such that the cluster points m1 on the left side and m2 on the right side of all specimens fit together.

  o *Normalize for area:* The specimen approximations are transformed, such that all have equal area (1000).

  o *Normalize for centroid size:* The specimen approximations are transformed, such that all have equal values for the centroid size of the outlines (the square root of the sum of squared Euclidean distances from each contour point to the centroid divided by the number of contour points k).

Settings for the cluster representation

It is possible to change the graphical representation with the menu item *Cluster - Properties*. The following options are shown in the property page *Graphics* of the property sheet.
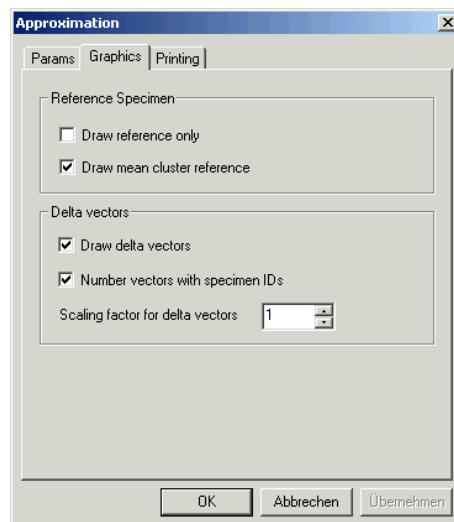
The following options can be set:

- *Draw reference only:* If this option is selected, only the reference specimen (but together with all the delta vectors) is drawn.

- *Draw mean cluster reference:* If the check box is activated, the mean specimen is drawn.

- *Draw delta vectors:* Whether the delta vectors should be displayed or not.

- *Number vectors with specimen Ids:* The endings of the delta vectors can be marked with the number of the specimen in the cluster view corresponding to the number in the cluster directory of the tree view.

- *Scaling factor for delta vectors:* Use this to scale the delta vectors appropriately. If two specimens are hardly different, this option can be used for a better comparison.

Settings for the print parameters

It is possible to change the printing settings in the property page *Printing* from the menu item *Cluster - Properties*.
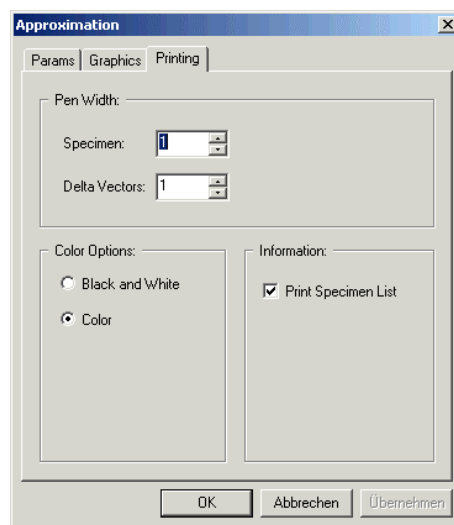
The following settings can be applied:

- *Pen Width Specimen:* Specifies the pen width for the specimen in the printout.

- *Pen Width Delta Vectors:* Specifies the pen width for the delta vectors in the printout.

- *Colour Options:* Whether colour (e.g. for a overhead beamer presentation) or only black/white (e.g. for a paper) should be used.

- *Print Specimen List:* Should a separate list with the names of the specimens in the cluster be printed?

Listening the calculated cluster data

The calculated cluster data can be displayed with the menu item *Cluster - Display coordinates*. This replaces the cluster view in the right pane of the main application window with a spread sheet view, which lists all the relevant data of the cluster (such as the names of specimens, all calculated control points and the delta vectors to the reference specimen).

The meanings of the column headers are as follows:

- *Nr:* Number of specimen according to its number in tree view.
- *Specimen:* Shorted file name of the specimen (including its position inside the file if necessary).
- *Area total:* Is the area deviation between the given specimen and the reference specimen.
- *Area dorsal:* Area total applied to the dorsal region only.
- *Area ventral:* Area total applied to the ventral region only.
- *Sum Delta:* Is the sum of all Euclidian distances between control points for the given specimen and those of the reference specimen.
- *Mean Delta:* Is the sum of the delta lengths divided by the number of control points.
- *Max Delta:* Is the maximum value in the set of Euclidian distances (delta length) between control points for the given specimen and those of the reference specimen.

- *Sum Delta-Quad:* Is the sum of all squared Delta Lengths (i.e. the Euclidian distances between the control points of a specimen and the corresponding homologous of the reference outline).

- *Mean Delta-Quad:* Is the square root of the Sum Delta-Quad divided by the number of control points.

- *Remaining columns:* Coordinates of the calculated control points (d...dorsal, v...ventral).

The specimen selected in the tree view, is marked in the cluster data view with a grey background.

The rows of the table can be sorted depending on the values in a column by clicking on this column header.

It is possible to copy either the whole sheet of displayed data or selected columns. For this action for instance select *Cluster - Display coordinates*, click on a column (Fig. below, see b), press the right key-mouse, move arrow to *Copy column* (Fig. below, see c). Open in Excel a new file and click *Insert*.
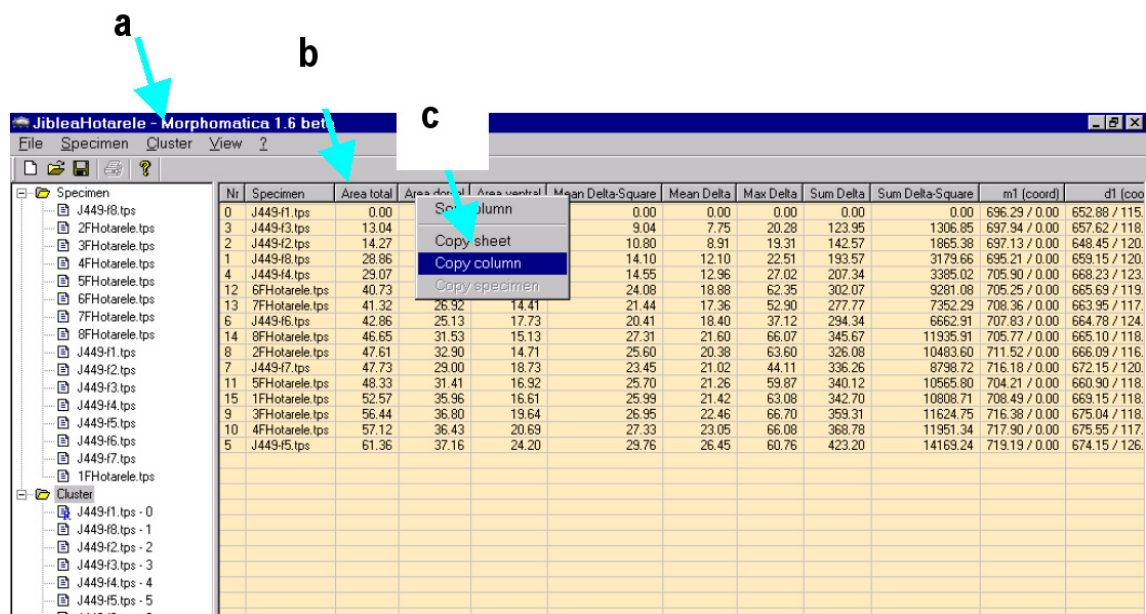
Evaluating of pairwise differences

The menu *Cluster – Display differences* shows a comparison of each two approximating B-splines of all specimens. The method of calculation can be chosen before. The result relies on the selected size normalization. The following methods are available:

- *Area total*: Evaluates the area between two approximating B-splines.

- *Area dorsal*: Area total applied to the dorsal region only.

- *Area ventral*: Area total applied to the ventral region only.

- *Mean delta square*: Is the square root of the sum of all Euclidian distances between the control points of two B-splines divided by the number of control points.

The possible methods of calculating and evaluating differences appear after choosing the menu item.
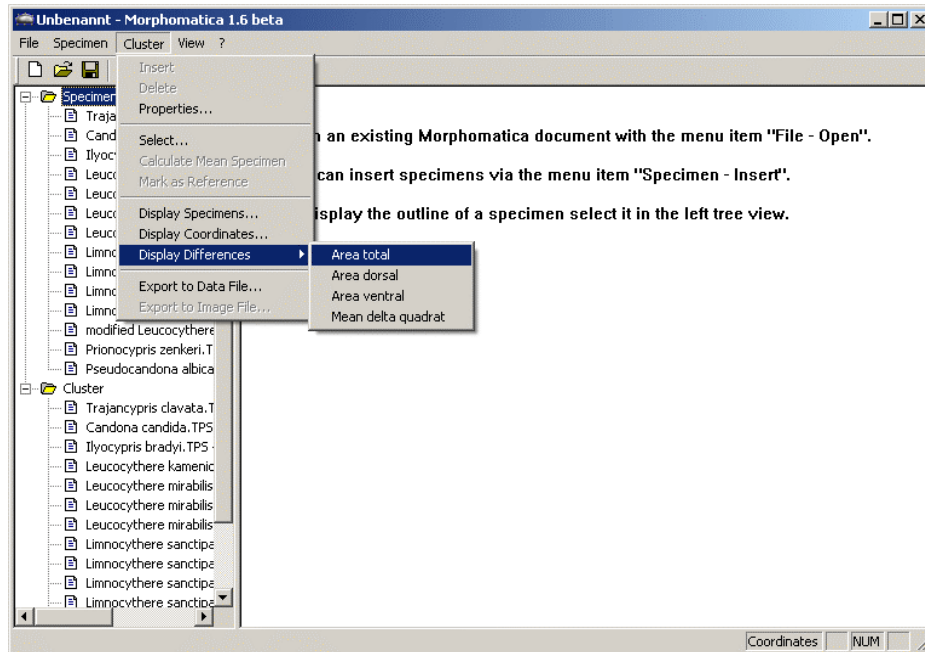
After selecting the method a matrix with the calculated values is printed out.

By clicking the right mouse button into the matrix a menu, including options for processing appears.
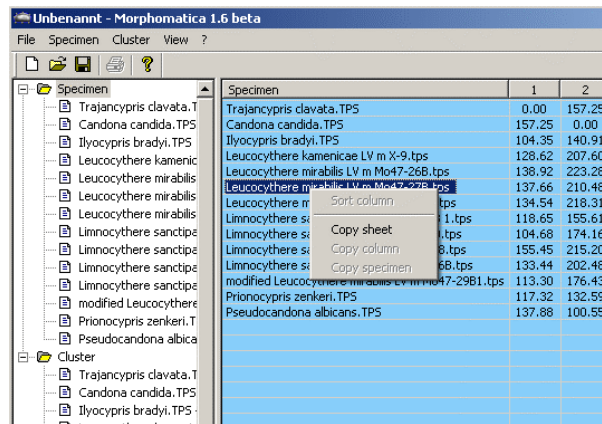
Choosing the menu item *Copy sheet* will copy the matrix into the clipboard where the data are available for further work in other applications (e.g. MS Excel, Primer).

Example of an exported sheet opened with MS Excel:

Remark: Due to the structure of the algorithm for computing the area deviation of shapes where the approximating B-spline changes direction cannot be calculated. In this case an error message comes up and you get the entry **"n.c."** for "not calculable" into the concerning cell. As a rule, such shapes occur only in faulty datasets.

Export specimen and cluster data

The calculated data of the cluster can be exported as a text file by using the menu item *Cluster - Export data*. The contents to export depend on the selected entry in the tree view.
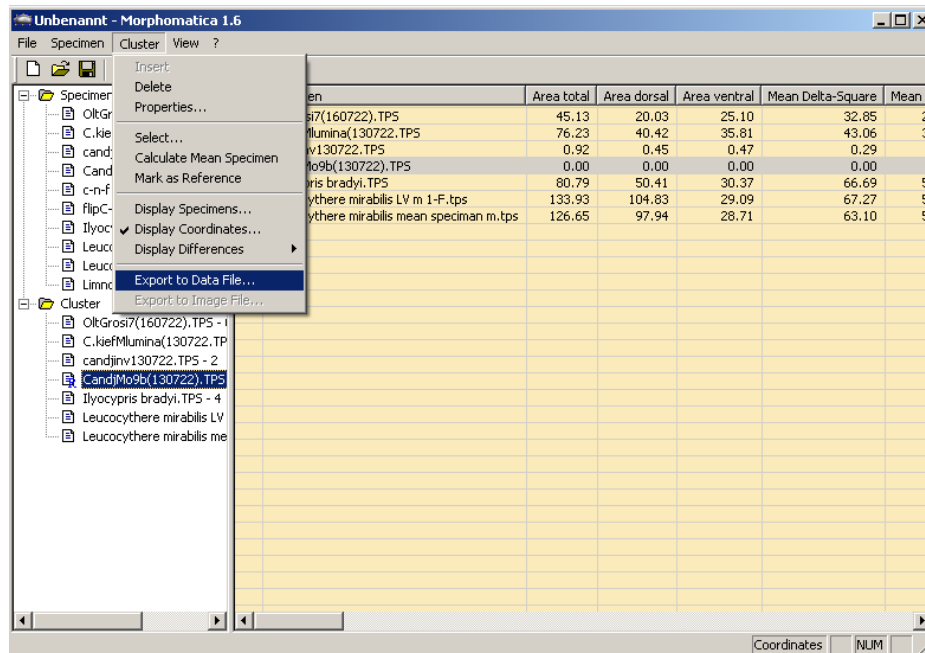
In the upcoming dialog, you have to specify a file name for the export file.
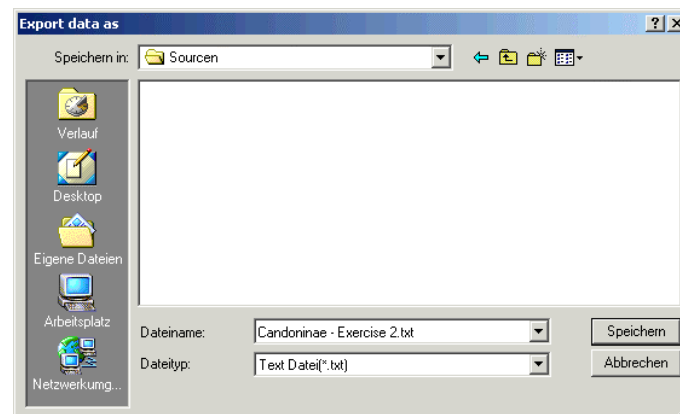
After saving, this text file can be opened and further worked with (e.g. with MS Excel or other statistical programs).

Example of an exported text file opened with MS Excel:

Print a MORPHOMATICA document

The contents of a MORPHOMATICA document can be printed with the menu item *File - Print*. The contents to be printed depend on the selected entry in the tree view.
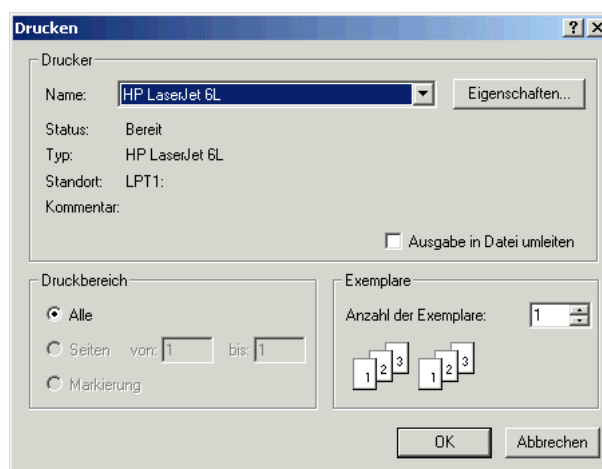
You can check the output before printing with the menu item *File - Print Preview*.
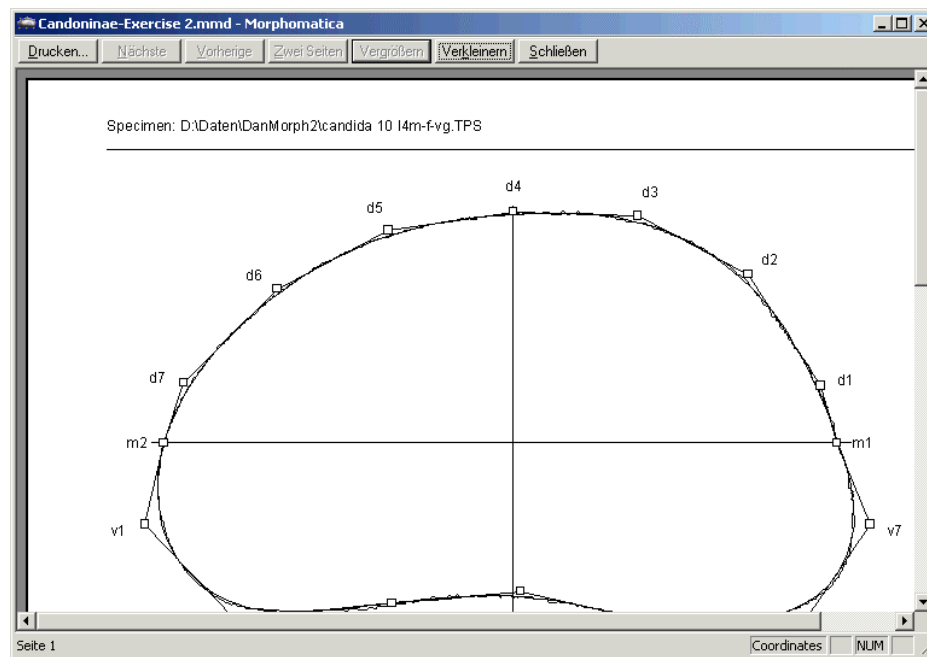
The printer settings (portrait, landscape, A4 or A3 format, etc.) can be done with the menu item *File - Print Setup*.

Save a MORPHOMATICA document

A MORPHOMATICA document can be save with the menu item *File - Save*.

You have to specify the desired path and file name (the file is saved in a XML file format).
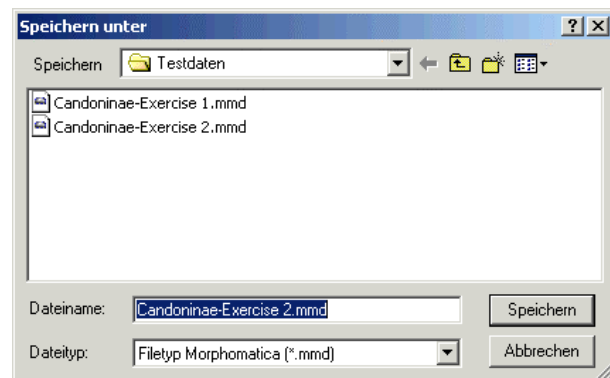
If MORPHOMATICA will be closed and the current document is changed but not saved, the user is informed and the document can be saved immediately.
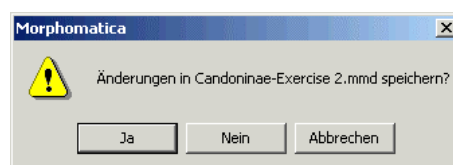
The complete contents of the MORPHOMATICA document are saved in a XML file. The files from which the specimens where imported are therefore no longer necessary. So, it's e.g. possible to send the XML file (with the extension *.mmd) alone per email to another user of MORPHOMATICA.

Remark: It is also possible to extract the specimens again in the TPS file format (Menu: Specimen -Export as TPS file).

Acknowledgements

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From the photography to the digitalized outline suitable for MORPHOMATICA

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Below is presented a technique that we used successfully at the Limnological Institute in Mondsee during the last years. Various amendments to this procedure can be applied.

For digitising the valve we first insert the microphotograph in the computer programme "Adobe Photoshop" as jpg file.

It is necessary to correct the outline of the valves with Photoshop in order to digitalize it with tps.dig. If the outline has a sharp edge on a clean background very few modifications are necessary, but many outlines need to be 'cleaned'.

First the picture is opened and the valve righted, so that the ventral contour is vertical. *Menubar – Image – Rotate Canvas – Arbitrary.*
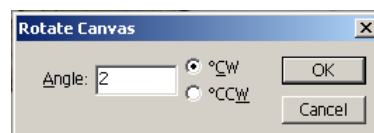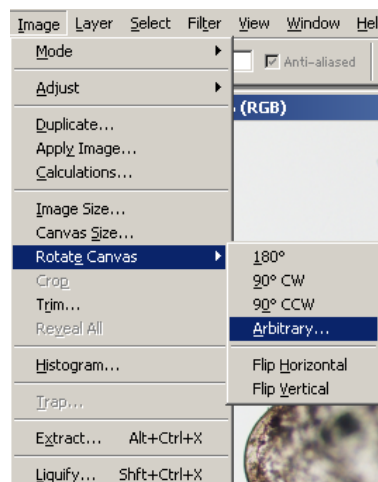
Next the scale, if not there, is inserted. If it is necessary to use a scale from another picture, open it and use the Rectangular Marquee tool of the toolbar on the left side of the screen to cut out 0.1 mm of the measure (below, left).
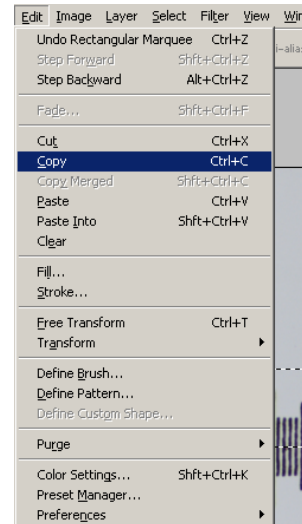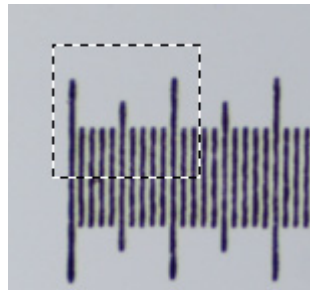
Edit – Copy – click the picture where you want to insert – *Edit – Paste* (above, right). With the Move tool the measure is positioned.

Next all colour information is discarded. *Image – Mode – Grayscale –* a window asking you to first flatten opens – *Flatten*. (Prior to flattening, the picture and the measure are independent layers, after flattening the layers are permanently joined).
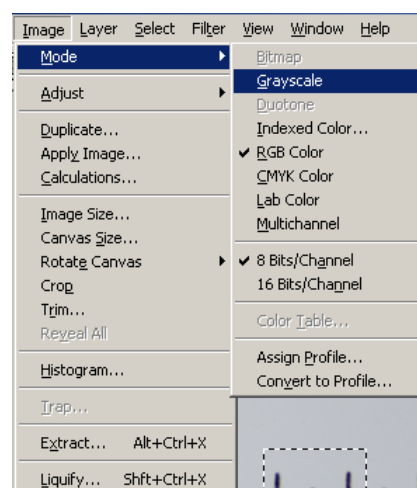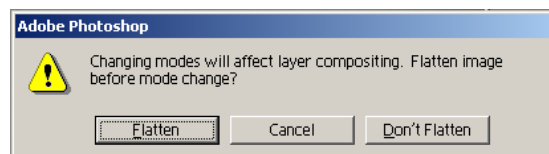
All valves need to be oriented in the same way to compare the outlines; right valves need to be flipped horizontally to be comparable to left valves.

Image – Rotate Canvas – Flip Horizontal.
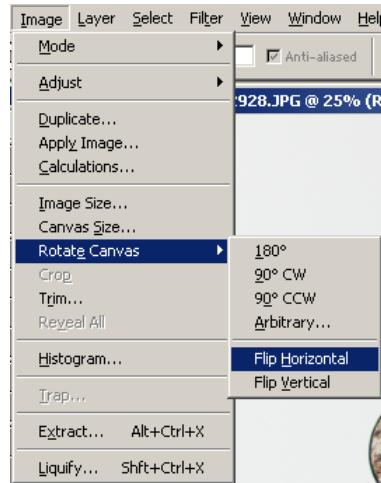
The outline is checked; any pieces of dirt that are connected to the valve are erased with the Eraser tool (dirt somewhere in the background is ignored).
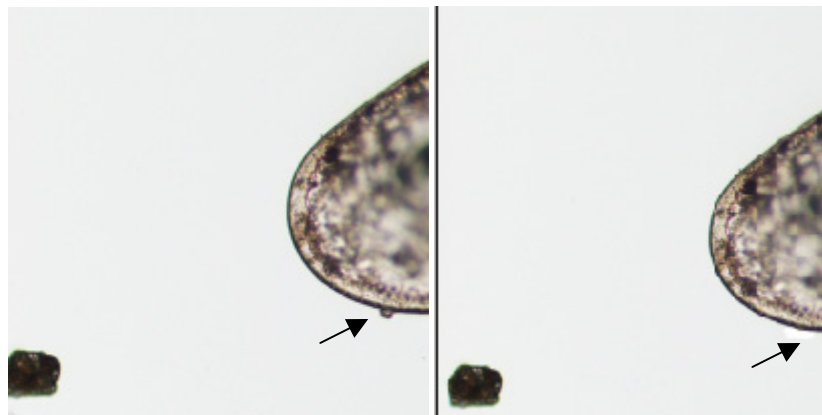
If the outline is very translucent there is too little contrast and the edge needs to be outlined with the Paintbrush Tool , select the thickness of the stroke in the toolbar below the menubar.
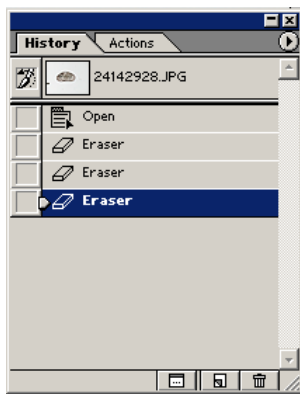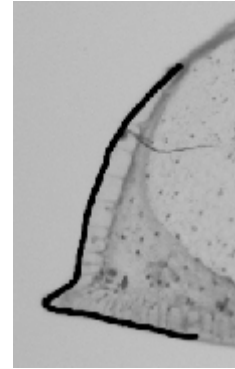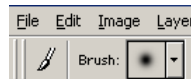
In the menubar under Window there is a heading Show History, if selected a window is shown where the modification steps are listed, it is possible to undo the last 20 changes.

The original is shown in the header bar of the history window and can always gone back to.
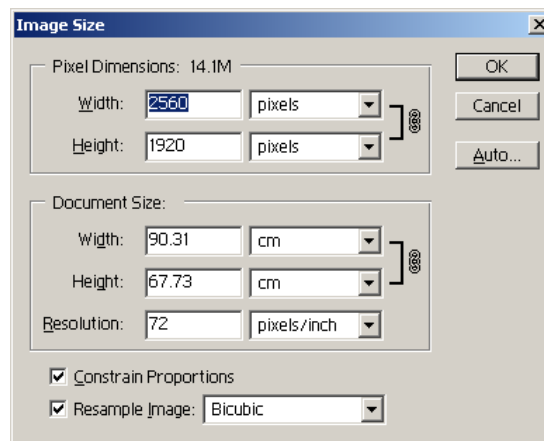
The Image Size (under *Image* in the menubar) is very important for the number of digitised points the outline is made up out of. Think about an appropriate number (> 1000 pts) before digitalizing, since MORPHOMATICA is sensible to variations of points used to calculate the outline. The number of points depends on the size of the picture and the resolution. Size and resolution should be adapted prior to producing the bitmap. No example for resolution and size can be given, because these parameters vary greatly, depending on the way the picture was produced.

The outline is digitalized from a bitmap of the valve, to get the bitmap go to *Image – Mode – Bitmap*.

Choose 50% Threshold for the bitmap. Check, if the valve has a continuous outline, wherever there is a small gap it will disturb the digitalizing of the outline. Make changes not in the bitmap, but go back in the history to the last point prior to bitmap to make modifications.
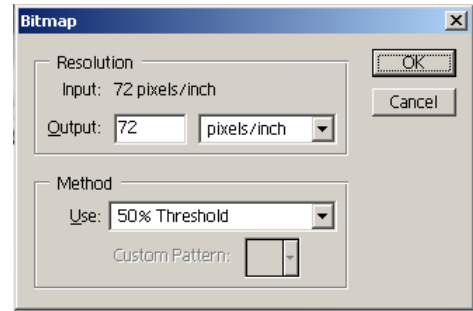
Finally the modified image is stored, go to *File – Save as* and store it either under a new name or a different format, do not simply *Save* since this way the original picture will be lost. This step can be performed first, depending on your personal preferences.

The computer program "Tps.Dig"

This program is a simple means to digitalize objects. If you have to download it from the internet choose:

Rohlf, F J. 2001. tpsDIG, Program version 1.43. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.: http://life.bio.sunysb.edu/morph/soft-dataacq.html (1/17/04).

Open your picture under *File – Input Source – File*; select the format you used for storing the modified picture.
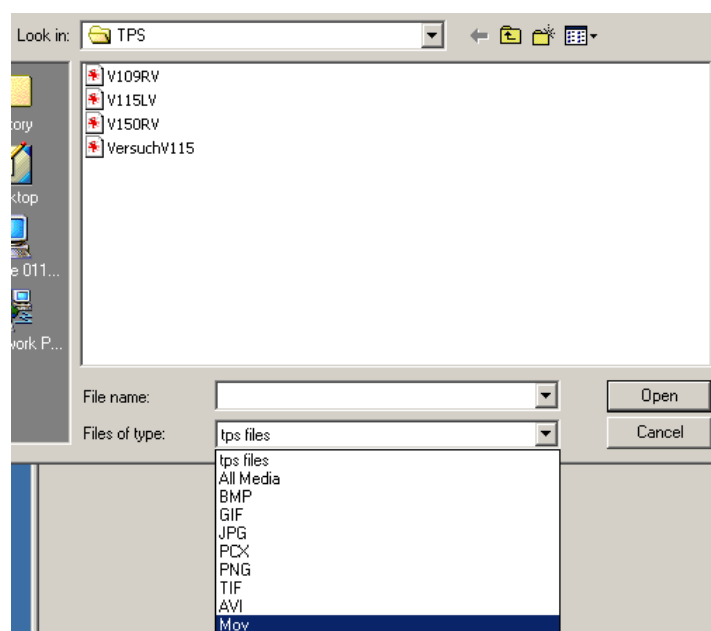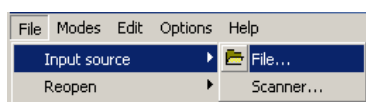
Go to *Options – Set Scale,* change the size to 0.1 (not comma, but point) and click the beginning and end of the measure on the picture.
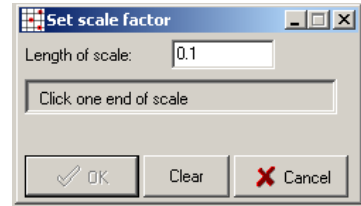
The line must be parallel to that of the scale on the picture, be careful to work accurately since small mistakes here will result in artificial differences in size in MORPHOMATICA. Click the outline button 🔲 to activate the tool for digitalizing the contour. Place the tip of the arrow right of the valve, make sure that there are no pieces of dirt between the tip of the tool and the valve and click it. A red line marks the outline of the object, as recognized by the program.

If the line is partially on the inside of the valve, the contour was `leaky´ and has to be corrected in Photoshop (changes must be saved in Photoshop and the valve reopened in tps.dig to adopt the changes). Right click the mouse and choose *Save as XY coords.*, the number of points for the outline appears in a window and has to be accepted, an additional yellow outline appears.
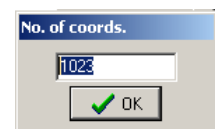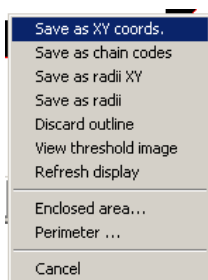
If the number of digitised points is suitable (should be around 1000 - 1500 points), store the information under *File – Save data as*, if not go back to Photoshop to change the Image Size and Resolution to fit your ideas.

Comparison of *Fabaeformiscandona caudata* (Kaufmann) and *Fabaeformiscandona lozeki* (Absolon) from the sublittoral of Lake Mondsee

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[3] Avenue des Vendeens 71, F-50400 Grandville (E-Mail: laurent.picot@lavache.com).
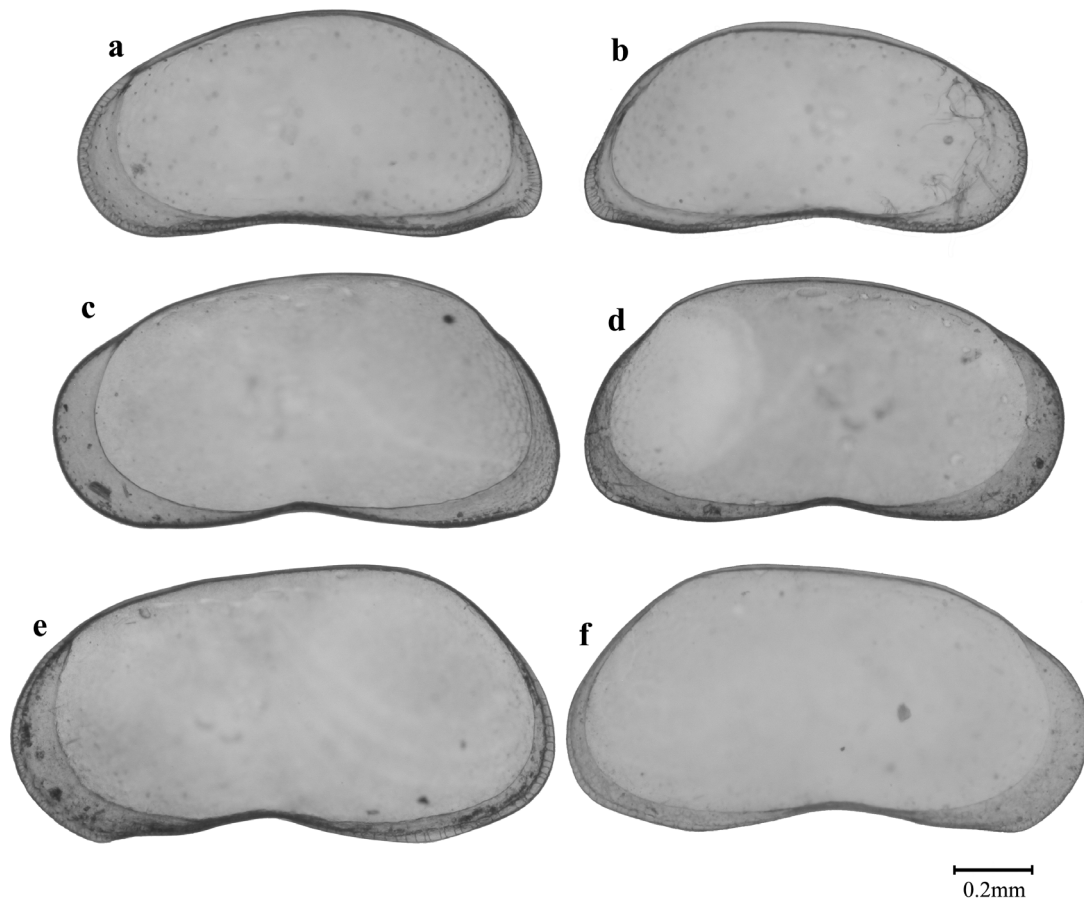
Fabaeformiscandona caudata: left valve (a), right valve (b); *Fabaeformiscandona* lozeki: female left valve (c), right valve (d), male left valve (e), right valve (f).

Above, you see microphotographs representing typical specimen of the two species.

The pictures are transformed to a bitmap in a program such as Adobe Photoshop, to enable digitalizing with tps.dig. Collect the specimen you want to compare in one folder, this is not obligatory, but will help keep things organised.

Open MORPHOMATICA, click on *Specimen* in the menubar and choose *Insert*. A dialog field opens where the samples that you want to compare are selected.
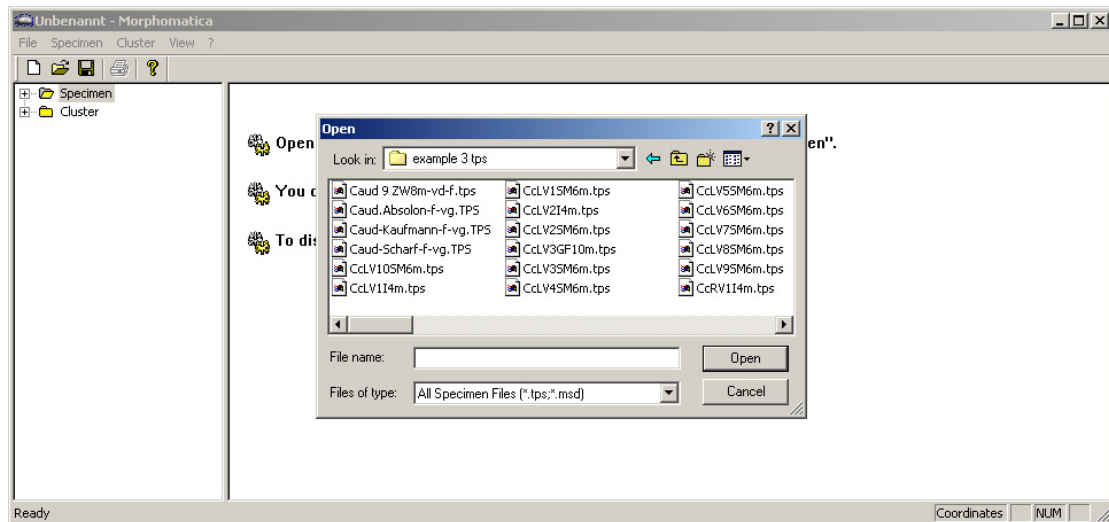
To see the fit of the calculated outline select *Approximation* under *Specimen* in the menubar.
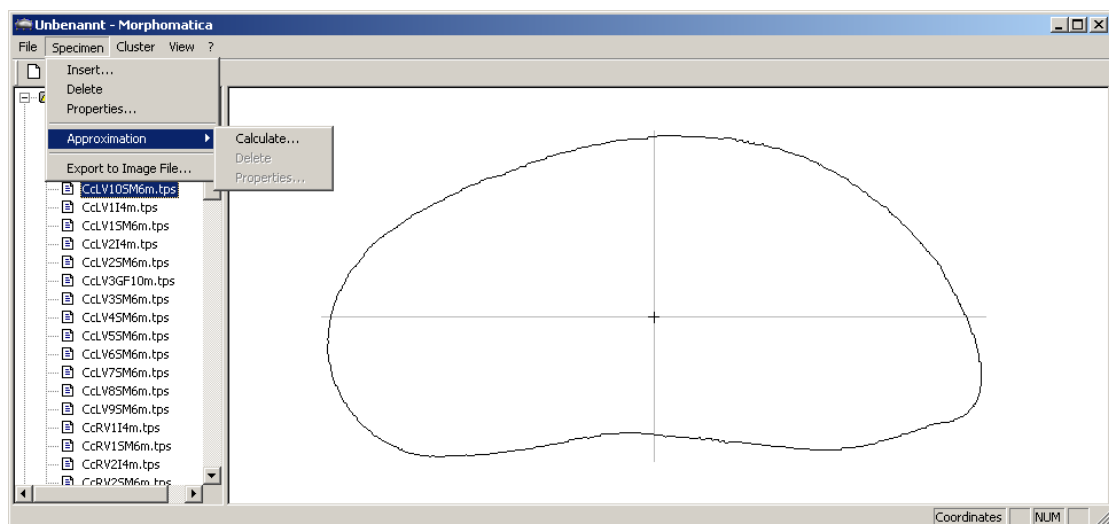
It might be useful to change the number of control points to get a better resemblance between the calculated and the real shape.
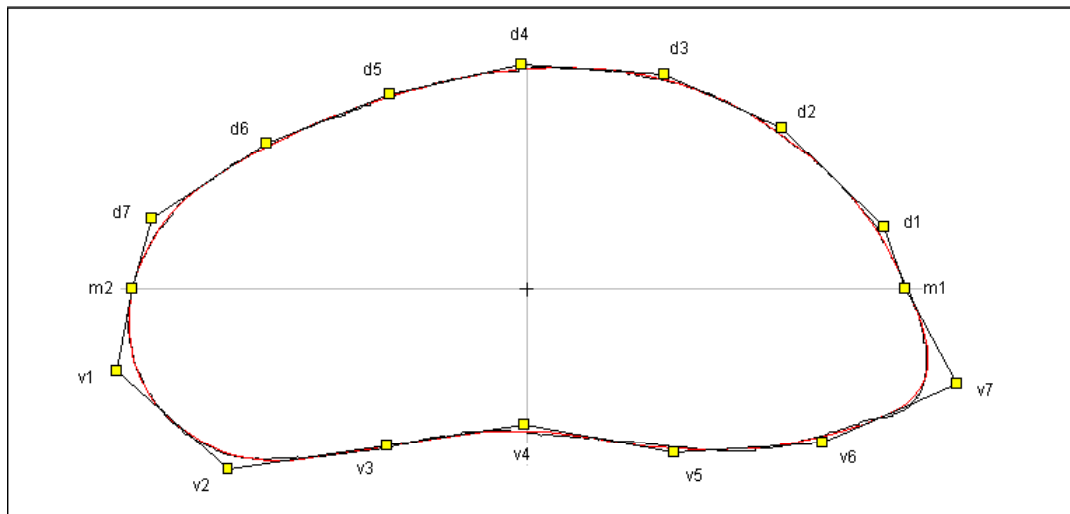
Mark the Cluster folder, select the specimen you want to compare and click *Apply*.
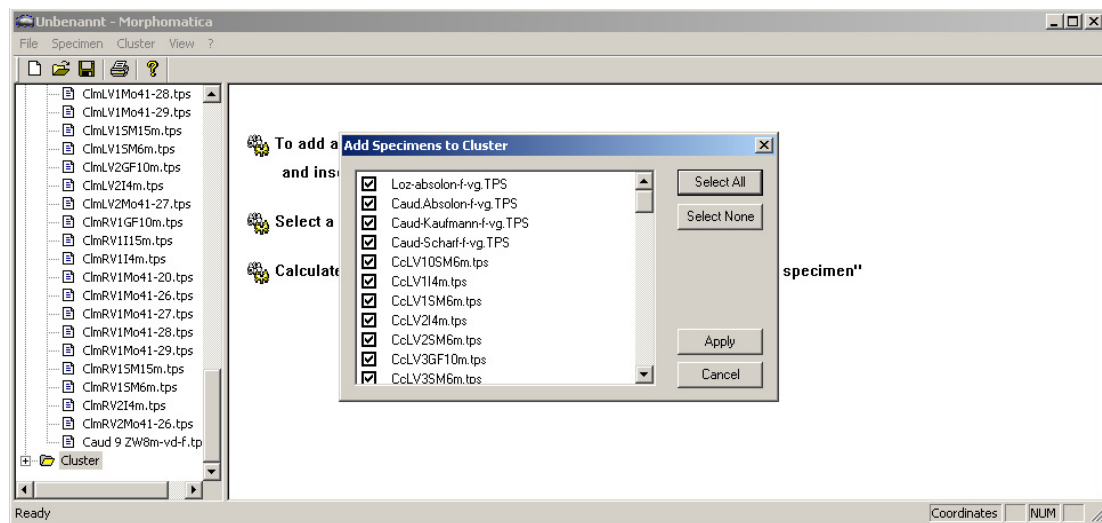
Set the control points to the value that you determined earlier (usually 8 control points on each half of the valve give a good result).

To see the coordinates of the vectors and the differences between the control points, mark one valve as reference and select *Display Coordinates*.

To export the pairwise area deviation of the whole outline select *Display Differences – Area total*.

The resulting sheet is a classical matrix that can easily be exported to Excel. Right click the sheet and choose *Copy Sheet*, open a new Excel table and paste the sheet into the field A3. Into field A1 write a title, copy the names of the specimen and paste it with *Paste Special – Transform* into field B2. Save the Excel file.

Start Primer, select *Open* and choose your Excel table; in the pop up dialog field click *Similarities*, on the second surface, *Dissimilarities*, check that the right number of lines is imported, if not most likely a labelling mistake occurred. If everything is correct, click OK. The matrix is displayed.


Under the header *Edit* choose *Factors*, a list of the specimen is displayed, click *Factors* and select *Add*.

You are now asked to give a name for the factor you are about to make, the name for the factor is important if you give the samples several factors (e.g. species and sex/species, sex plus individual identifier/species, sex plus origin, etc.).

The same factors are used for the statistical methods, such as Anosim or Cluster. It is possible to produce the factor lists in Excel and copy/paste them into Primer (paste only works with the menu or the keys and not the right mouse button); this is helpful since it might speed up the process of labelling. In the Factors menu you can also define a plot key plus you can move the given factors up and down, which makes the legend easier to interpret.

To produce a MDS plot of your matrix click on *Analyse* and select *MDS*.
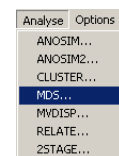
The program asks for the number of restarts and starts calculating. The more restarts you have the more reliable the results are, but the longer the calculation takes, ten restarts are usually sufficient. The MDS will be displayed in a new window, go to *Graph* and select *Properties*. Choose the factor and whether you want labels and/or factors displayed. The graph can be rotated in order to give the best display of the data.
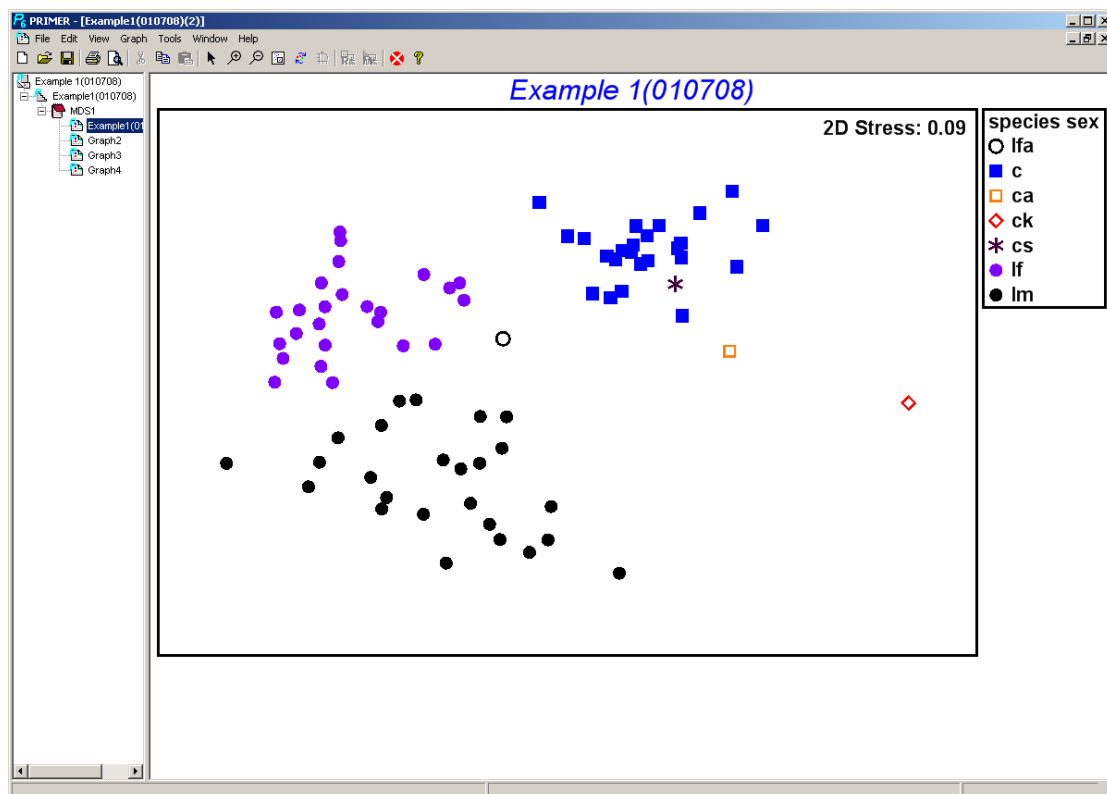
ca	*F .caudata* det. A. Absolon (Absolon 1973)
ck	*F. caudata* det. A. Kaufmann (Kaufmann 1900)
cs	*F. caudata,* det. B. Scharf (Scharf and Keyser 1993)
c	*F. caudata*, lake Mondsee (det. D. Danielopol)
lf	*F. lozeki* female, lake Mondsee (det. D. Danielopol)
lm	*F. lozeki* male, lake Mondsee (det. D. Danielopol)
lfa	*F. lozeki* female, det A. Absolon (Absolon 1973)

Further calculations such as Cluster or ANOSIM can be performed using "Primer" as well.

References

Absolon, A. 1973. Ostracoden aus einigen Profilen spät- und postglazialer Karbonatablagerungen in Mitteleuropa. Mitt. Bayer. Staatssamml. Paläont. hist. Geol., 13: 47-94.

Kaufmann, A. 1900. Cyprididen und Darwinuliden aus der Schweiz. Rev. Suisse Zool., 8: 209-423.

Scharf, B. W. and Keyser, D. 1993. Living and subfossil Ostracoda (Crustaccea) from lac du Bouchet (France, Auvergne). Doc. CERLAT Mem., 2: 387-391.

Comparative study of *Candona neglecta* valves from the shallow and deep sites of Lake Mondsee

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60 valves of Candona neglecta female from different depths of lake Mondsee are compared to see if there is a difference between specimens from deep (65 m) and shallow (3 m-6 m) sites.
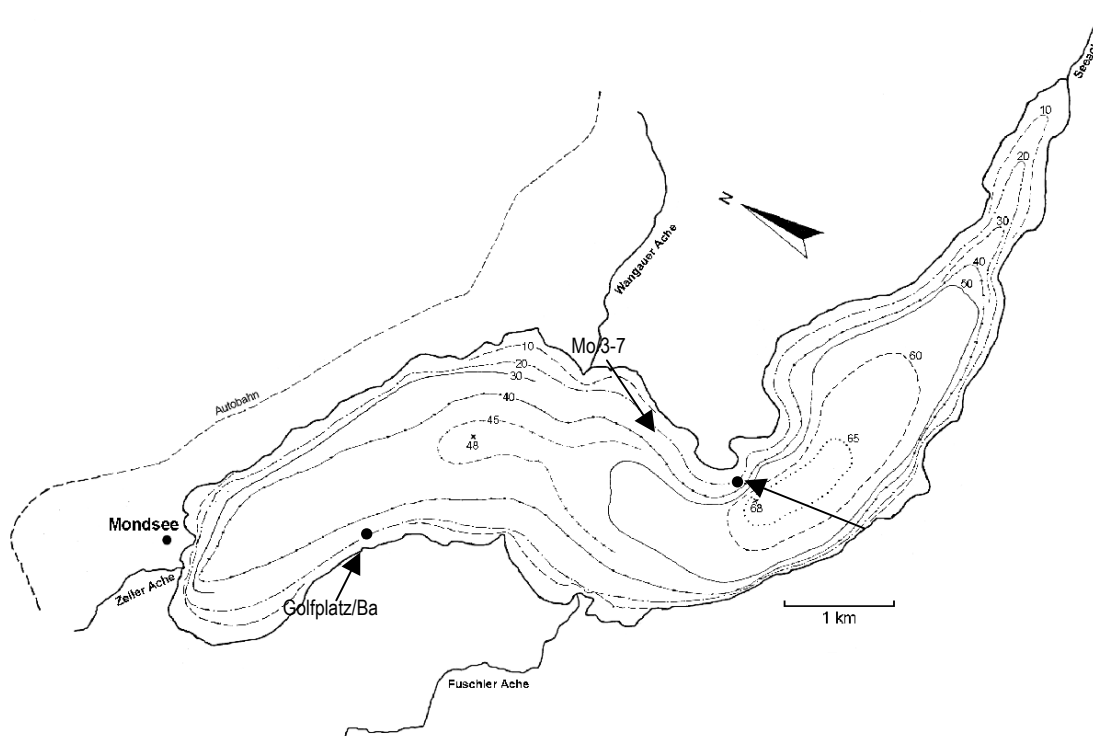
Lake Mondsee.

Open MORPHOMATICA to insert the digitalized specimen into the specimen folder. Activate the Cluster folder, go to *Cluster* in the menubar and choose *Select*, click the *Select All* button and *Apply*. Check under *Cluster – Properties* if *Normalize for Area* is selected. The valves are projected on top of each other. The difference between the valves is small, to see if there is a difference between the valves from the shallow parts and the deep parts, select valves from one depth only.

Valves from the deep/shallow sites, Normalized for Area.

More information concerning the differences between the different origins can be obtained, if the valves are compared without normalization. Activate *Cluster*, in *Properties* change to *Don't normalize* and select the valves from the different sites again. The valves from the deep sediment show a greater diversity in size than those from the shallow parts of the lake.

Valves from the deep/shallow sediment, No Normalization.

The data obtained with MORPHOMATICA can be used for further analysis in other programs. In both cases (Normalize for Area, Don't Normalize) all specimen are used for the Cluster analysis.

Select *Display Differences – Area Total* and copy the sheet with a click on the right mouse button. Paste the matrix into field A3 of an Excel worksheet, copy the list of specimen and insert it with *Paste special – Transpose* into field B2, into field A1 fill a title. Save the Excel book. Repeat the copy and paste with the other normalization and insert it into the next sheet of the workbook.

We use for further analysis the program Primer. Start Primer and select Excel files to be opened, in the dialog box choose sheet 1 and pick *Similarities – Ok* and *Dissimilarities – Ok*.

Make sure that the number of rows corresponds to the number of specimen that was used (if it does not, check in the Excel worksheet if something went wrong with copying the list of specimen, any blank field in column A and row 2 other than A2 will be the signal that it is the end of the matrix).

Go to *Edit* and select *Factors*, in the dialog box under *Factors* add a new factor and name it. Fill in the column with abbreviations, under *Factors* pick *Plot key* to give the abbreviations corresponding symbols that you like and sort the abbreviations with the up/down arrows to have them displayed on the side of the plot in the right order. When you are done confirm the abbreviations and plot key with OK. Select *Analyse* in the menubar and *MDS...*, ten restarts are usually sufficient.

The resulting plot needs to be modified, go to *Graph* and *Properties*, select *Display Symbol and Factor*, in the neighbouring field *Label and None*. If you made several columns with factors it is possible to select a factor, confirm with Ok. The plot can be rotated to display the results best (plot on the following page). The first plots are based on a matrix with normalization for area.

dl	Candona neglecta, deep sites, left valve
dr	Candona neglecta, deep sites, right valve
sl	Candona neglecta, shallow sites, left valve
sr	Candona neglecta, shallow sites, right valve

Abbreviations used in the plots below.
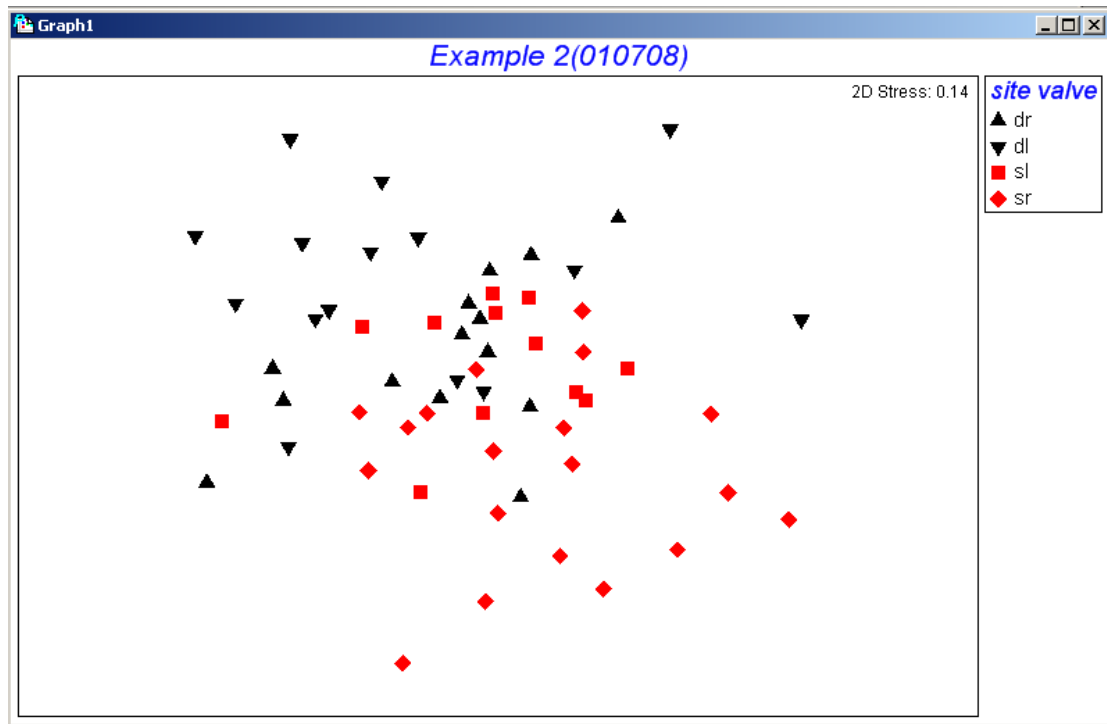
MDS plot using *Normalized for Area*.

In this case, a three dimensional display helps to better understand the differences between the populations (see figure next page).

Go *Graph* and *Properties* again and select 3D instead of 2D in the dimensions field. The resulting graph shows the samples positioned in a three dimensional space. Most symbols have a thin line either going up or down that helps to position the symbols. There is an imaginary plane and the samples are either in, above or beneath that plane (imagine balloons filled with helium = above, or water = below) – the lines connect the symbols with this plane. The graph can be viewed from all around to find the best view of the results.

Most of the valves from the deep sediment of lake Mondsee are below the plane, right and left valves are grouped, whereas the valves form the shallow parts of the lake are mostly within or above the plane.
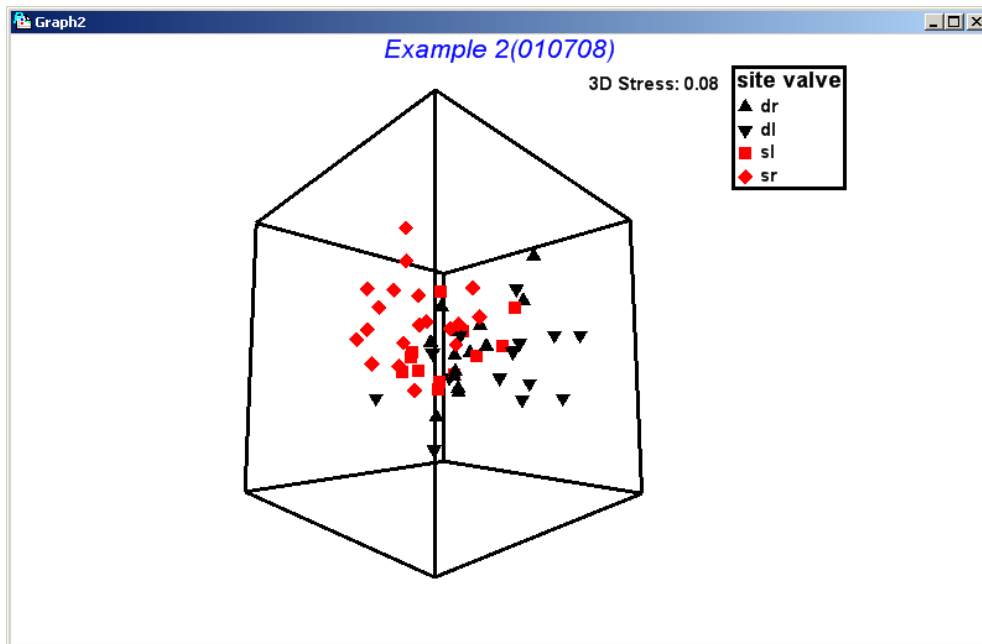
MDS-Plot in 3D.

To open the matrix containing the data from *Don't Normalize* it is necessary to save the first matrix as a Primer file or close it to enable the program to open the same Excel workbook again. In the first dialog box select sheet two as the source otherwise continue as before. Copy the factor list from the first matrix and use it for the new matrix.

The same effect as was shown in MORPHOMATICA above is visible in the plot, the variability is higher in the group of valves from the deep site.
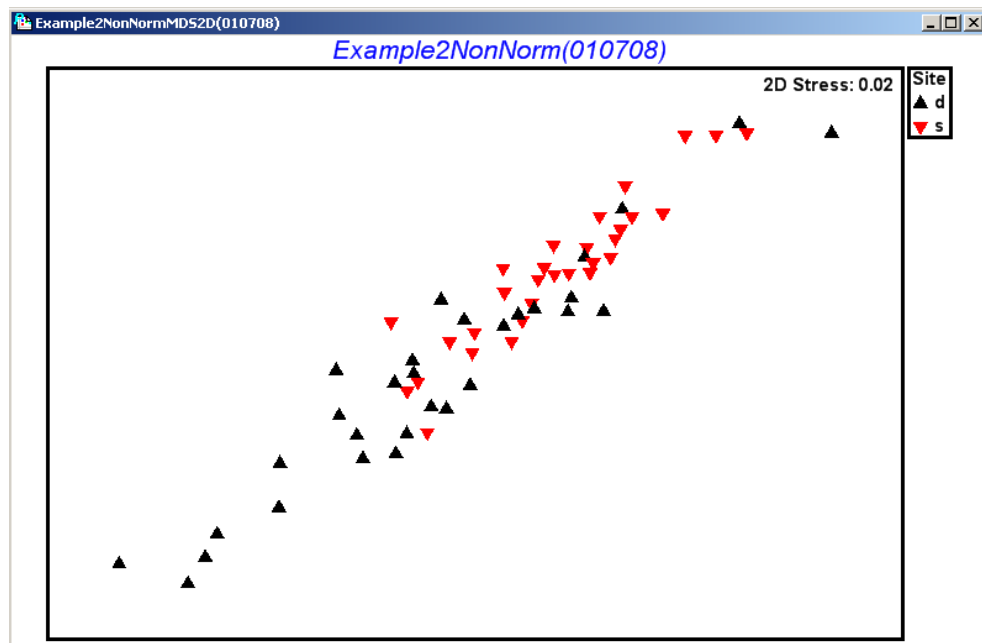
MDS-plot with *No Normalization.*

Primer offers a randomisation test (Anosim) to evaluate the statistical difference between the populations. The example below was performed on the data produced with the setting *Normalize for Area*.

Global Test
Sample statistic (Global R): 0.25
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
dr, dl	0.137	1.7	77558760	999	16
dr, sl	0.145	1.4	9657700	999	13
dr, sr	0.206	0.4	818809200	999	3
dl, sl	0.22	0.6	17383860	999	5
dl, sr	0.51	0.1	Very large	999	0
sl, sr	0.217	0.4	141120525	999	3

Result section of the Anosim analysis performed with Primer.

There is a rather high difference between left valves from the deep sediment compared to the right valves from the shallow sediment as compares to the other pair-combinations.

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