A NEW FRAMEWORK FOR MORPHOLOGICAL AND MORPHOMETRIC STUDY OF FISH SPECIES BASED ON GROUPWISE REGISTRATION OF OTOLITH IMAGES

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ABSTRACT

Morphology of bones, teeth, and some particular structures are widely used for categorizing species and studying their evolution. In this paper, we used groupwise registration to provide a representative image from the set of the image samples that represents its typical morphology. We also provided perturbation map which indicates the deviation of each point through the ensemble. These images support qualitative discussions about the morphology of structures in different species. The perturbation map can be further exploited for determining appropriate landmarks for morphometric analysis. Knowing the deformation between the prototype and each image sample from the species, the framework allows for automatic detection of corresponding points. Once the user puts a landmark on the prototype image, the corresponding points on all the image samples will be determined. This maximizes the accuracy in measuring the morphological indices by eliminating the human error due to uncertainty in locating landmarks.

Index Terms— Groupwise registration, Morphology, Morphometric, Evolution, Otolith.

1. INTRODUCTION

1.1. Evolution study and categorization

Form of particular structures and their morphology are of fundamental importance in categorization of species (or populations) and studying their stock, distribution and evolution [1]. Morphometrics refers to the quantitative analysis of form based on different attributes including ratio of linear dimensions, Eigen shape analysis etc. A major objective of morphometrics is to test hypotheses about the factors that affect shape. It is used to quantify a trait of evolutionary significance by detecting changes in the shape.

Otolith is a single massive calcareous concretion in each of the three semicircular canals of the inner ear (Fig. 1). They are important organs in sound reception in most fish species. However, it applies in biosciences to study evolutionary relationships, age determination, life history, stock assessment and systematic. In case of otolith, the morphometric parameters vary substantially among species. Being species-specific, the shape and pattern of the teleost otoliths are ideal for studying populations of species [2].

Begg et al. used morphometrics with shape analysis to identify fish species [3]. Some features like elliptical Fourier analysis with techniques of pattern recognition and clustering are applied to otolith by Stransky et al. [4], and Tracy et al. [5]. However, the conventional approach in the literature of bioscience is qualitative morphological analysis by including enough samples of the underlying structure and measuring some morphometric indices and then analyzing them statistically [2]. The overall morphology and relationships of different parts of otoliths are compared by Waessle et al. [6] using landmark method. One significant issue of this approach is the intra-species variability of otolith. Studies on otolith morphology have shown that the otoliths can represent different sizes and shapes between and within fish species. Therefore, it is required to understand the intra- and inter-species variation for species discrimination and evolutionary studies [7][8]. The other significant issue is repeating measurements manually for each sample that makes the results susceptible to human error.

In this paper, we propose a framework based on groupwise registration. In comparison with pairwise registration, groupwise registration [9] allows for registering a group of images into a common reference domain. Based on this we provide a representative image from the available otolith samples. The representative images can be referred when comparing different species as they are representing all of the collected samples of each species. We also provide the perturbation map which determines the variation of pixel's position to help morphological analysis. The perturbation map indicates the intra-species variation and together with the representative image provides required information for qualitative morphology. It also tells if one pixel is fixed enough to be a landmark or not due to its intra-species variation. Furthermore, following groupwise registration the transformations from the representative image to each image sample are known. Therefore if the user measures some indices on the representative images, in our framework the measurement on all of the image samples perform automatically by finding the corresponding points. This technique removes the need for repeating the measurements for each image sample and eliminates associating human error. The proposed framework is applied to fish otolith and the results are reported in this paper.

2. GROUPWISE REGISTRATION FOR MORPHOLOGICAL STUDY

Assume a set of image samples form a population, in this section we propose a framework for morphological analysis of structures based on groupwise registration.

2.1. Groupwise registration

Given a set of images $\{I_1, I_2, \cdots, I_N\}$ with $I_i: \Omega_i \to R$ and $\Omega \subset R^2$, the objective is to find a representative image which is close to all images in the set but it is not necessarily one of them. This is the significant difference between groupwise and pairwise registration in which the moving image is tried to be registered to a particular reference image.

Let Ω_R be the common reference image domain and assume of transformations $\{T_i(x): \Omega_R \to \Omega_i\}$. In other words the transform $T_i(x)$ maps each point $x \in \Omega_R$ in the common reference domain to image point $x' \in \Omega_i$ in the domain of image I_i. Following a proper transformation $I_i(x') = I_i\big(T_i(x)\big)$. In this paper we used the approach of Zollei et al. [10] for affine transforms and the method of Balci et al [9] for B-splines free form deformations. Both of these methods are founded on the stack entropy cost function and we describe it here briefly for the case of free form deformation. The justification for the stack entropy cost function is that the entropy distribution of intensities at corresponding points is low after proper alignment of images. Let $H\left(I\big(T(x)\big)\right)$ when $x \in \Omega_R$ be the univariate entropy of the stack of pixel intensities $\big\{I_1\big(T_1(x)\big), \cdots, I_N\big(T_N(x)\big)\big\}$ at pixel x. Based on Parzen window density estimation with Gaussian kernel G_σ, the cost function for estimation of free form transforms in [9] is defined as:

$$E = -\sum_x \frac{1}{N}\sum_{i=1}^{N} \log \frac{1}{N}\sum_{j=1}^{N} G_\sigma\big(d_{ij}(x)\big) \qquad (1)$$

where $d_{ij}(x)$ is the distance between intensity values of a pair of images at x in the reference domain.

$$d_{ij}(x) = I_i\big(T_i(x)\big) - I_j\left(T_j(x)\right) \qquad (2)$$

The special definition of eq. (1) does not use a reference image and all the images are simultaneously driven to the common tendency of the population as it achieves its minimum when the intensity differences are small.

The free form B-spline deformation model with regular grid of control points $\Phi_{i,j}$ can be written as the 2D tensor product of 1-D cubic splines

$$T(x) = x + \sum_{l=0}^{3}\sum_{m=0}^{3} B_l(u)B_m(v)\Phi_{i+l,j+m} \qquad (3)$$

where $i = \lfloor x/x_n \rfloor - 1$, $j = \lfloor y/y_n \rfloor - 1$, $u = x/x_n - \lfloor x/x_n \rfloor$, $v = y/y_n - \lfloor y/y_n \rfloor$ and B_l is the l^{th} cubic B-spline function. Refer to [10] for the gradient descent minimization of the cost function and the required derivatives.

For implementation in this paper, we first performed a groupwise affine registration, followed by free form groupwise registration.

2.2. Representative and Perturbation images

Assume the set of images $\{I_1, I_2, \cdots, I_N\}$ be aligned after groupwise affine registration. Groupwise free form registration of these images yields the set of deformations $\{T_1(x), T_2(x), \cdots, T_N(x)\}$ from the reference domain to each image. The representative image R can be defined as:

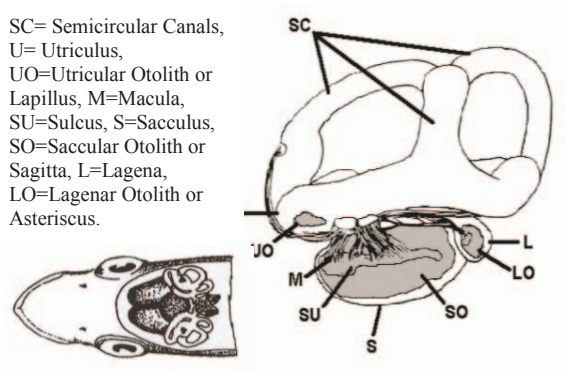


Fig. 1. Inner Ear of Fishes and position of otolith, Lateral View

$$R(x) = \frac{1}{N}\sum_{i=1}^{N} I_i\big(T_i(x)\big) \qquad (4)$$

where $x \in \Omega_R$. The representative image $R(x)$ in eq. (4) is indeed the average of the registered images and it can be used for qualitative discussion about the morphology. Given such a representative image, one does not need to show all the samples in database for visual comparison that is imprecise and difficult.

Corresponding to the representation image, a perturbation image is defined as

$$P(x) = \frac{1}{N}\sum_{i=1}^{N} \|T_i(x) - x\|^2 \qquad (5)$$

The perturbation image $P(x)$ is the map of the variation in the position of each pixel. It determines if one pixel is fixed in the images or its position is varying. The perturbation map helps better understanding the morphology of the underlying structure as it shows if one corner or curvature is similar in all images. The perturbation map also suggests proper landmarks for measurements of morphometric analysis.

2.3. Finding corresponding points

The transformation function $T_i(x)$ maps each point of the reference image domain $x \in \Omega_R$ to the domain of image I_i. Therefore, if x_0 be a landmark on the reference image $R(x)$, then $\{T_1(x_0), T_2(x_0), \cdots, T_N(x_0)\}$ is the set of corresponding points in the images of dataset. The conventional approach for morphometric analysis is measuring some indices such as lengths and angles and manually repeating the measurements in all images. In our framework we propose to use the representative image and the estimated transformation to find corresponding points automatically and consistently for measurements. This eliminates the human error in locating the corresponding positions of landmarks in different images. This also removes the labor of repeating the measurements manually. As a result of the quantitative morphometric analysis can perform accurately and the results are more reliable.

2.4. Framework for morphological study and morphometric analysis

Fig. 2 shows the block diagram of the proposed framework for morphological and morphometric study of species. The initial groupwise affine registration aligns the input images and resolves different imaging conditions. It compensates for the size, rotation and translational shift of images. Then the groupwise deformable registration is applied which provides the set of non-rigid dense transformations required for computing the representative and the perturbation images from eq. (4) and eq. (5).
Finally, the representative image and the set of transformations are used for finding the corresponding points based on the landmarks on the representative image.

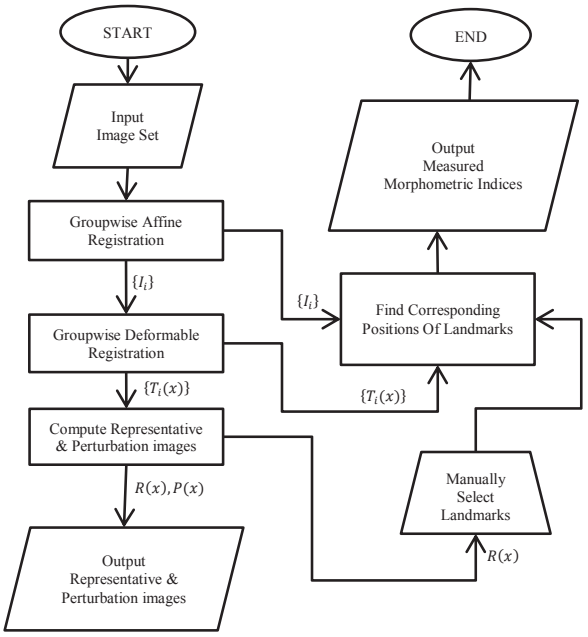


Fig 2. Proposed framework for morphological and morphometric study of species based on groupwise registration

3. EXPERIMENTAL RESULTS

In collaboration with the Department of Earth- and Environmental Sciences, Palaeontology & Geobiology of the Ludwig-Maximilians-University, the proposed framework is applied to morphology of otoliths from left ear of freshwater fishes from four populations. The materials are fish otoliths from four countries i.e. Iran (from Mehran River), Oman (from Creek), Saudi Arabia (from Alkhari spring) and Bahrain. A number of 11-15 samples were collected from each population.
Scanning Electronic Microscopy (SEM) was used to study the otolith morphology and images with the size of 640×480 were taken. SEM images were captured with a LEO 1430 VP at the Zoological State Collection Munich (ZSM). Otoliths were positioned with their lateral face down on plasticine,. The images were taken with the DFC 295 camera and Leica Image Software IMAGIC 1000. The morphometry of otoliths was performed based on the technique introduced in [2] including eight linear distances and three angles (Fig. 3, Table 1). Linear measurements were standardized and, together with the values of the angles, used for statistical analyses.

The proposed framework was implemented on a 64-bit core i7 machine with 4 GB of RAM by using Matlab coding and Mevislab visual programming. For groupwise registration we used the application developed by Yigitsoy et al. which was later extended for spatio-temporal groupwise registration [11]. The whole process of consecutive groupwise affine and then deformable registration took 17 seconds on average. The representative image and the perturbation map were computed as illustrated in section 2 and together with the transformations, were exported to another application with graphical user interface for measurement of morphometric indices as shown in Fig. 3.
Fig. 4 shows average and std. images from one population in comparison with the corresponding representative and std images from the proposed process. The average image in Fig. 4-a, is simply the average of images before registration. The std. images are the standard deviations before registration (Fig. 4-b) and after registration (Fig. 4-d). Fig.5 shows the representative and perturbation images resulted from each population beside some samples of the images in each column.

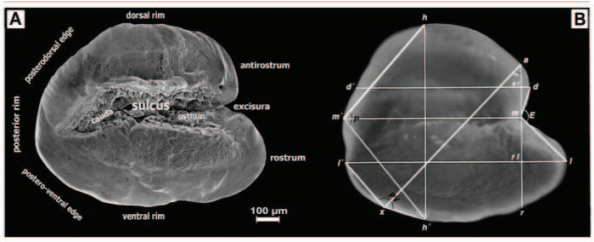
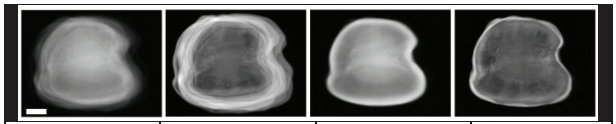


Fig. 3. Terminology of otolith of a fish specimen and linear measurements of morphometric indices [2].

Table 1. Otolith variables and angles and explanations, see Fig. 3

Otolith variables	Measurement	Description
Rel. length of dorsal rim	d-d´	(d-d´)/(l-l´)
Rel. length of medial part	m-m´	(m-m´)/(l-l´)
Rel. height of rostrum	m-r	(m-r)/(h-h´)
Rel. height of antirostrum	m-a	(m-a)/(h-h´)
Rel. length of antirostrum	al-d	(al-d)/(l-l´)
Rel. length of rostrum	rl-l	(rl-l)/(l-l´)
L/H-index	l-l´ and h-h´	(l-l´)/(h-h´)
Posterior angle	Angle P	h-m´-h´
Excisura angle	Angle E	d–m–l
Postero-ventral angle	Angle PV	l´–x–h´



(a)	(b)	(c)	(d)

Fig 4. Typical experimental result. (a) is average and (b) is std. image before registration and (c) and (d) are the resulted representative and perturbation images, respectively.

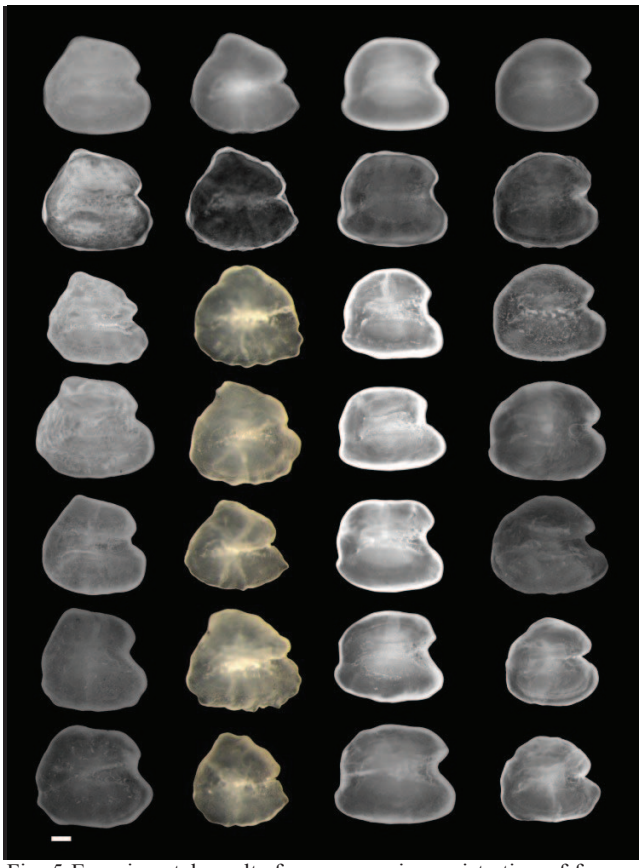


Fig. 5 Experimental results from groupwise registration of four population in four columns. The representative image is shown in the first row, the perturbation map in second row and in the lower rows samples are shown.

4. DISCUSSION

The std. image in Fig. 4-b shows there was a considerable intra-species variation in the shape of the otolith samples which affects its morphology. Due to the large intra-species variation, qualitative descriptions about the shape of the otolith is not reliable before registration as Fig. 4-a shows. The std. image in Fig. 4-d implies proper registration and consequently representative image in Fig.4-c is sharp. The representative image is not necessarily one of the images in the set of image samples but it is close to all of them. This can be inferred from comparing the resulted representative image and the samples from each of populations. Hence, the representative image is suitable for morphology.

The several here studied morphometric characters considered to be important in differentiation of populations. Comparison of the previous morphometric with the proposed groupwise registration suggested the possible errors in previous morphometric analysis.

Comparison of the morphometric analyses with the AVE and STD images depict some other important obvious characters, which are essential for the morphological and evolutionary studies because it is not possible to measure those characters by the mophometric analyses. Moreover, and because of the intra population variation in the otolith shape, occurrence of errors would be usually inevitable in linear measurements. Therefore, the proposed framework improves the morphometric method and makes it more precisely to study morphological differentiation of species.

For example, the relative height of antirostrum is significant to separate the populations when we did morphometric analysis, while this part shows high variation (high SD) in the studied otoliths of each population when groupwise registration was applied (Fig. 5).

Meanwhile, the presence of tip in dorsal rim of the otoliths in some populations (see Fig. 5) affects the other related characters like relative length/height and posterior angle and makes errors. The groupwise registration can be used to minimize the errors before doing morphometric analyses. In conclusion, groupwise registration as a new approach is profitable for comparative morphological study of different structures in animal species, especially the structures that are being used as key characters in species differentiation.

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