# FISH SCALE IDENTIFICATION: FROM INDIVIDUAL TO SPECIES-SPECIFIC SHAPE VARIABILITY

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**Background.** Species identification from recovered fish scales, e.g., from the sediment or stomach contents, has proven to be problematic for clupeid species. To ensure reliable species discrimination within Clupeidae, we attempted to reveal inter- and intra-specific, as well as, the intra-individual variability of scale shape in two sympatric clupeid species the European pilchard, *Sardina pilchardus* (Walbaum, 1792) and the round sardinella, *Sardinella aurita* Valenciennes, 1847. Our aim was to test, whether the landmark-based geometric morphometric method is reliable for species identification from mixed samples.

**Material and methods.** The specimens of European pilchard and round sardinella were collected between May and October 2014 from the Gulf of Ambracia, Greece. The scale samples were taken from ten body areas (marked by letters A–J) of 487 specimens in total. A multiple-step analysis was conducted on randomly selected blind samples. The differences between body areas were assessed with canonical variate analysis (CVA) and discriminant function analysis (DFA) to compute generalized Mahalanobis distances ($D$) and discriminant functions ($T^2$), respectively.

**Results.** The CVA based on the total scale samples showed that in sardinella, all body areas differed significantly from each other ($D = 2.54 \pm 0.79$, $P < 0.001$) except for one (area I vs. J: $D = 22.36$, $P = 0.085$). In pilchard, two body area pairs represented non-significant group pairs (area D vs. G: $D = 1.03$, $P = 0.12$; and area F vs. G: $D = 1.21$, $P = 0.06$), whereas all other areas were significantly different ($D = 2.43 \pm 0.88$, $P = 0.003$). At the last step of the blind sampling procedure, all scales were correctly classified by species with an average discrimination rate as high as 96.3%.

**Conclusion.** In contrast to scale identification based merely on morphology, the geometric morphometric method is capable of detecting differences in scale shape from mixed samples that are unattainable with the former approach. Therefore, a more reliable and effective species identification can be accomplished from recovered scales, especially when discriminating species within the same family or genus.

**Keywords:** Clupeidae, fish scale identification, geometric morphometrics, pilchard, sardinella

## INTRODUCTION

Scales are characteristic features for fishes and they are present in all major fish groups. Due to their morphological variability across taxa, fish scales are of high value for systematics and phylogenetic studies. Scale identification dates back over 160 years. Early research by Williamson (1851), Cockerell (1913), Chu (1935), and Lagler (1947) had demonstrated the value of scales in fish taxonomy. Later on, the development of keys and atlases based on scale characteristics allowed for more accurate identification to species level (Batts 1964, Mosher 1969, Casteel 1972, 1973, Patterson et al. 2002).

Traditionally, species identification is based on morphological characteristics. Scale characteristics such as the overall shape and structural features have proven to be useful not only for species but even for population identification, e.g., in walleye, *Sander vitreus* (Mitchill, 1818) (see Jarvis et al. 1978); lake whitefish, *Coregonus clupeaformis* (Mitchill, 1818) (see Casselman et al. 1981); Atlantic salmon, *Salmo salar* Linnaeus, 1758 (see de Pontual and Prouzet 1987); and striped bass, *Morone saxatilis* (Walbaum, 1792) (see Richards and Esteves 1997). Scale morphology allows discriminating even among fish

populations at large spatial scales, e.g., within the great lakes system (Casselman et al. 1981) or among rivers from different drainage basins (Richards and Esteves 1997). Advances in image processing and analytical approaches nowadays allow using scale shape as a discriminator among local populations. Morphometric analysis, based on landmark data (geometric morphometric method) has been proven to be a reliable tool to differentiate congeneric species (Ibáñez et al. 2007, 2012), allowing for the identification of populations on a local and regional scale (Staszny et al. 2012, Bräger et al. 2016b) as well as among sympatric phenotypes (Garduño-Paz et al. 2010). The geometric morphometric analysis is only sensitive enough to reveal inter- and intra-specific memberships, however, when sample comparability is ensured by deriving them from the same sampling area on the fish body, usually the left flank, and consistency among samples is guaranteed (Ibáñez et al. 2007).

When fish scales are recovered from environmental or biological sources, e.g., sediment records or stomach contents, and the samples include scales of two or more species from mixed body areas ("mixed sample"), as is usually the case, the identification, until recently, was exclusively possible using the characteristic features of scale morphology. The usefulness of fish scales in the sedimentary records has been proven by several studies, e.g., in the reconstruction of paleoclimate and paleoceanographic conditions (Patterson et al. 2005); in the determination of environmental changes (Valdés et al. 2008); or in the assessment of paleoproductivity (Soutar and Isaacs 1969, Drago et al. 2009). Scales have provided similarly valuable information in studies of diet and feeding ecology by helping to identify prey items in fish (e.g., Mauchline and Gordon 1984), in birds (e.g., Ewins et al. 1994, Correia et al. 2016), and in mammals (e.g., Cottrell et al. 1996, Ford and Ellis 2006). Screening a large number of fish scales for species identification merely based on scale morphology, however, is rather time consuming. Furthermore, species determination among closely related species remains problematic (Bräger et al. 2016a).

Clupeiform species such as anchovies, herrings, shads, sardines, and their relatives possess derived cycloid scales (Bräger and Moritz 2016) that are easily shed and thus can be found in high densities in sediments or piscivorous species (Patterson et al. 2002, Correia et al. 2016, respectively). To distinguish clupeoid scales from other fish taxa is fairly easy, but the high degree of similarity in their scale morphology and the plasticity within single individuals impede species identification within families (Patterson et al. 2002, Bräger and Moritz 2016). In a previous study, Bräger et al. (2016b) showed that the scales of two sympatric clupeid species, i.e., European pilchard, *Sardina pilchardus* (Walbaum, 1792) and the round sardinella, *Sardinella aurita* Valenciennes, 1847, can be reliably discriminated using the landmark-based geometric morphometric method on scales of known origin. To ensure reliable species identification within Clupeidae, here we attempted to reveal inter- and intra-specific, as well as, the intra-individual variability of scale shape in European pilchard and round sardinella. Our aim was to test, whether the method can also be used reliably for the species identification from mixed samples using blind sampling procedure.

## MATERIAL AND METHODS

**Scale sampling and preparation.** Scale samples from European pilchard and round sardinella were collected between May and October 2014. Specimens were purchased from commercial fishermen operating in the Gulf of Ambracia, a shallow and semi-enclosed gulf with an area of about 400 km$^2$ in north-western Greece (Fig. 1). In total, 219 adult individuals of pilchard (mean SL ± SD: 81.7 ± 4.68 mm) and 268 adult individuals of sardinella (mean SL ± SD: 155.4 ± 8.51 mm) were measured and sampled. For scale sampling, we followed the ten body areas suggested by Bräger and Moritz (2016) that cover the entire body surface.
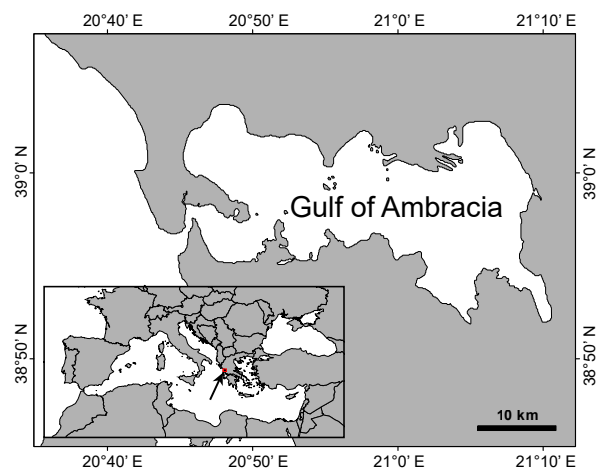


**Fig. 1.** The study area in north-western Greece

The exact location of these ten body areas is indicated in Fig. 2 (body areas A–J). For each specimen, only a single scale was collected from one body area on the left side of the body to avoid auto-correlation problems among scales. The number of scale samples (sampled individuals) varied for the ten sampling areas between 16 and 28 for pilchard, and from 24 to 30 for sardinella (Table 1). The scale material was stored in 70% ethanol upon collection then processed in the laboratory following the procedure described in Bräger et al. (2016b).

**Data analysis.** The landmark-based geometric morphometric analysis was used to determine, whether the differences in clupeid scale shape were sufficient to enable a reliable discrimination among body areas from mixed samples in pilchard and sardinella. Seven landmarks were recorded on each scale (Bräger et al. 2016b) using "tpsUtil" v. 1.60 (Rohlf 2015a) and "tpsDig2" v. 2.17 (Rohlf 2015b) utility programs to identify the key features as suggested by Staszny et al. (2012). Shape data of the scales were analysed with the software program "MorphoJ" v. 1.06d (Klingenberg 2011). Group identities (by species and by

body areas) were assigned to raw landmark coordinates. The centroid size (CS) was used as the size metric of the scales since it constitutes the only mathematically shape-free size variable (Zelditch et al. 2004). To rotate, to scale and to align the raw coordinates into new shape variables, a Generalized Procrustes Analysis (GPA) was performed. Furthermore, a multivariate linear regression of shape (Procrustes coordinates) on size (logCS) was undertaken for each group to remove possible allometric effects. The significance of the relation, i.e., the presence of an allometric effect, was evaluated by using a permutation test against the null hypothesis of independence (10 000 iterations). As data were free of allometric effects associated with growth, residuals of this regression provided the basis for further analyses (Mitteroecker et al. 2013). Finally, the differences between groups were assessed with a non-parametric canonical variate analysis (CVA) and a parametric discriminant function analysis (DFA) to compute generalized Mahalanobis distances ($D$) and discriminant functions ($T^2$), respectively. The reliability of the results was examined with permutation tests with 10 000 iterations.

The detailed procedure of the multi-step scale classification is as follows:

**Table 1**

Number of scale samples (= specimens) and body size variation between European pilchard, *Sardina pilchardus* and round sardinella, *Sardinella aurita*

| Body area | Pilchard | | Sardinella | |
|---|---|---|---|---|
| | N | SL [mm] | N | SL [mm] |
| A | 21 | 82.1 ± 5.45 | 30 | 153.0 ± 5.86 |
| B | 27 | 80.6 ± 4.05 | 30 | 154.0 ± 3.99 |
| C | 17 | 83.2 ± 2.35 | 30 | 156.2 ± 5.76 |
| D | 21 | 82.1 ± 5.50 | 25 | 155.2 ± 6.70 |
| E | 16 | 83.0 ± 5.56 | 24 | 151.8 ± 4.34 |
| F | 20 | 81.7 ± 5.18 | 26 | 156.9 ± 4.15 |
| G | 27 | 80.7 ± 4.34 | 26 | 155.2 ± 5.34 |
| H | 28 | 81.1 ± 4.68 | 25 | 156.1 ± 5.69 |
| I | 16 | 82.9 ± 5.51 | 26 | 154.0 ± 2.87 |
| J | 26 | 80.9 ± 3.83 | 26 | 156.9 ± 4.03 |
| Total | 219 | 81.7 ± 4.68 | 268 | 155.4 ± 8.51 |

Body areas (A–J) are visualised in Fig. 2 (after Bräger and Moritz 2016); $N$ = number of scales, SL = standard length (mean ± standard deviation).
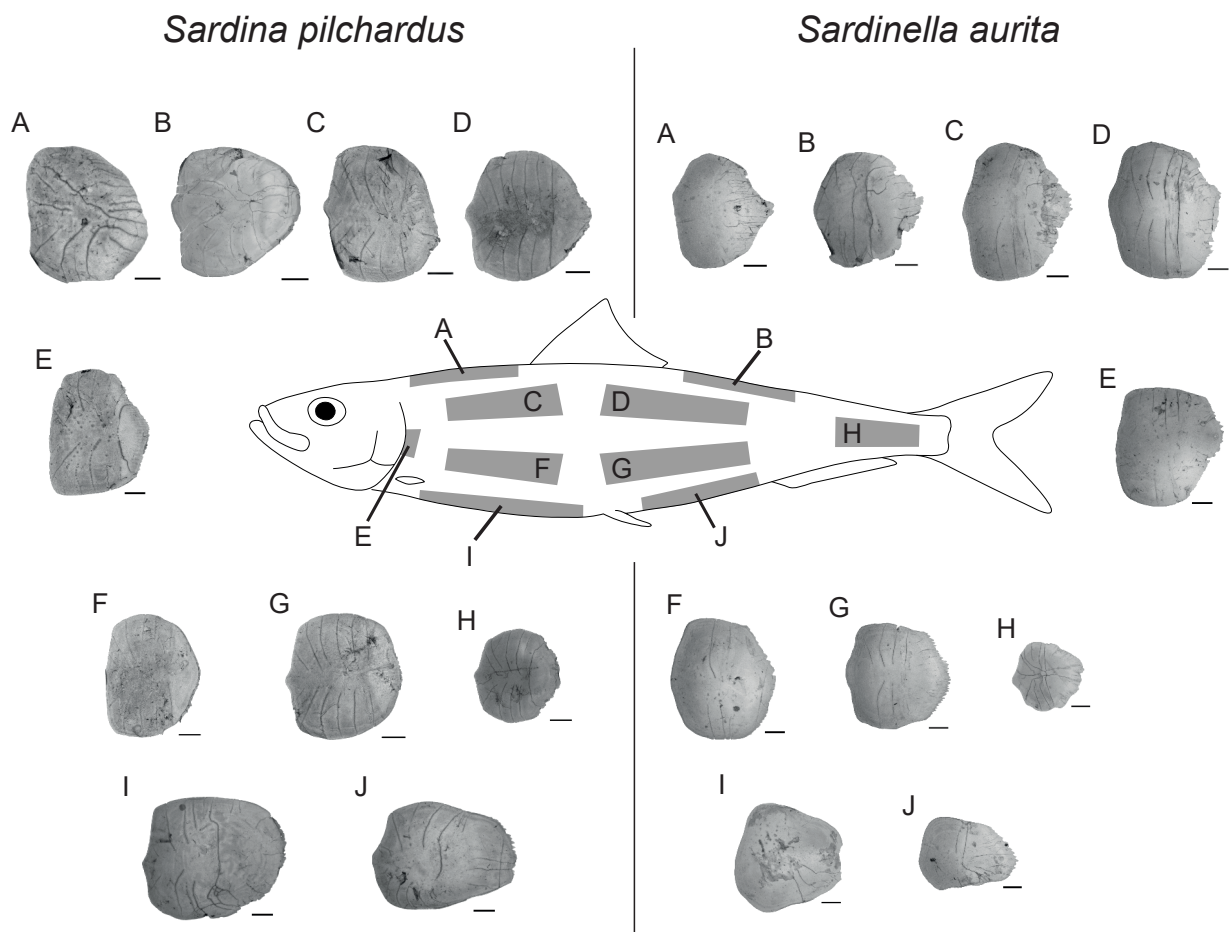


**Fig. 2.** Scale shape variation among body areas in European pilchard, *Sardina pilchardus*, and round sardinella, *Sardinella aurita*; scales are oriented with the anterior (rostral) part to the left of the image; scale bar = 1 mm; the schematic drawing of a clupeid fish with the sampling areas is adopted from Bräger and Moritz (2016)

- Examining the discrimination of scale shapes among body areas within species.
- Randomly selecting 5 scales within each body area in both species and using them as blind samples.
- Testing whether each blind sample can be identified on species level:
  - Blind sample compared to the scale shapes of the full reference database (i.e., all body areas) of the two species (classification Type I).
  - Blind sample compared to the scale shapes of the body areas (independently of the species).
  - Comparison of scale shapes from body areas that showed non-significant differences at the species level (classification Type II).

Although the Type II classification was not necessary to be run in every case, for testing purposes it was performed for the entire dataset. With this iterative, "step-by-step" comparison, the species-level identification was also possible in those cases, when Type I classification failed (e.g., if significant differences between the blind samples and their original species were present).

**RESULTS**

In both clupeid species, the general pattern of scale shape changed according to the area of origin on the fish body. The most typical shapes among body areas varied from circular, cordate, to oval, even to slightly quadrilateral. Scales derived from the mid-lateral region of the fish body (areas C, D, E, F, and G) were extended in the dorsoventral axis, whereas scales from the dorsal or ventral edges of the body (areas A, B or I, J, respectively) were rather compressed or elongated in the anteroposterior axis. This elongation was found to be the most prominent in the ventral areas (I and J). Caudal peduncle scales (area H) were characterized by being relatively shorter in the anteroposterior axis resulting in a circular appearance of the scales (Fig. 2).

The regression of scale shape (using Procrustes coordinates) on scale centroid size indicated notable allometry (dependence of shape on size) among body areas in both species (in pilchard: 2.54%, $P < 0.001$; in sardinella: 1.01%, $P = 0.016$). The mean scale shape among body areas of the two species showed different levels of overlap in the CVA with definite separation of the two species (Fig. 3). The areas of the mid-lateral region, i.e., C–B, D–B, G–B, D–G, and F–G in case of pilchard, as well as areas F and G in case of sardinella proved to be the most similar in their average scale shape (e.g., lowest Mahalanobis distances), whereas certain pairs of areas B–I, C–I, and H–I in pilchard and E–I and D–I in sardinella differed most from each other. The CVA based on total scale samples (see Table 2) showed that in pilchard two body area-pairs represented a non-significant group pairs (area D vs. area G: $D = 1.03$, $P = 0.12$; and area G vs. area F: $D = 1.21$, $P = 0.06$), whereas all other areas were significantly different from each other ($D = 2.43 \pm 0.88$, $P < 0.001$). In sardinella, two body areas represented a non-significant pair (area I vs. J: $D = 22.36$, $P = 0.085$), whereas all other areas were significantly different from each other ($D = 2.54 \pm 0.79$, $P < 0.001$). Between the two
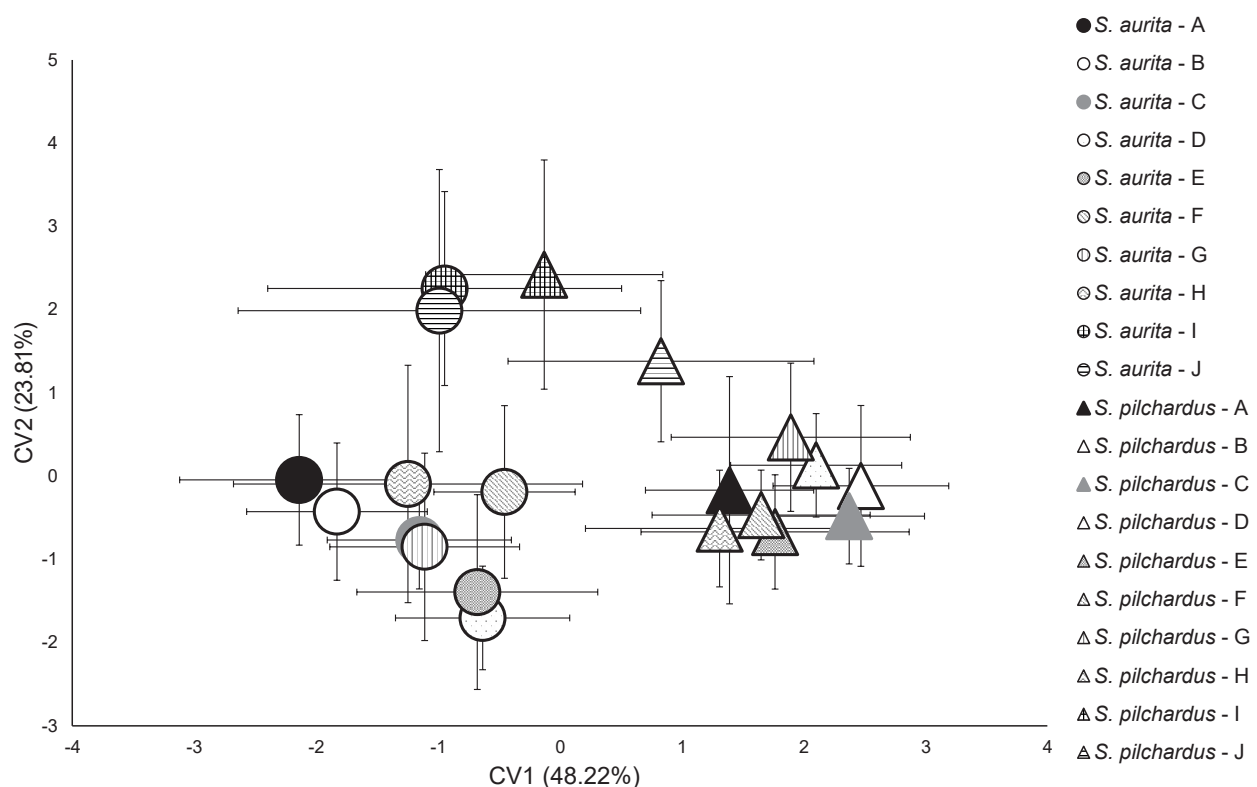


**Fig. 3.** Canonical variate analysis results comparing scale shapes among body areas in European pilchard, *Sardina pilchardus*, and round sardinella, *Sardinella aurita*; symbols show the group means of body areas (see Fig. 2); crosshairs show standard deviations

**Table 2**

Statistical differences of scale shapes among the ten body areas (A–J) in European pilchard, *Sardina pilchardus* (Sp) and round sardinella, *Sardinella aurita* (Sa) based on canonical variate analysis

|  | Sa/A | Sa/B | Sa/C | Sa/D | Sa/E | Sa/F | Sa/G | Sa/H | Sa/I | Sa/J | Sp/A | Sp/B | Sp/C | Sp/D | Sp/E | Sp/F | Sp/G | Sp/H | Sp/I | Sp/J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sa/A |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/B | 1.416 |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/C | 1.740 | 1.378 |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/D | 3.086 | 2.196 | 2.001 |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/E | 2.525 | 2.400 | 2.214 | 2.749 |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/F | 1.984 | 1.747 | 1.357 | 2.061 | 2.094 |  | 0.007 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/G | 2.131 | 1.687 | 1.593 | 1.567 | 2.077 | 1.228 |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/H | 2.827 | 1.921 | 2.574 | 2.224 | 3.534 | 2.289 | 2.289 |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/I | 3.008 | 3.095 | 3.182 | 4.170 | 4.262 | 2.717 | 3.355 | 3.302 |  | **0.335** | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sa/J | 2.715 | 2.886 | 3.160 | 4.025 | 3.885 | 2.519 | 3.093 | 3.370 | **1.225** |  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sp/A | 4.030 | 3.672 | 3.214 | 3.201 | 3.116 | 2.381 | 3.129 | 3.724 | 3.941 | 3.657 |  | <0.001 | <0.001 | <0.001 | <0.001 | 0.007 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sp/B | 4.713 | 4.392 | 3.919 | 3.819 | 3.928 | 3.094 | 3.969 | 4.273 | 4.407 | 4.196 | 1.982 |  | 0.014 | 0.002 | <0.001 | <0.001 | 0.038 | <0.001 | <0.001 | <0.001 |
| Sp/C | 4.649 | 4.345 | 3.731 | 3.785 | 3.619 | 3.137 | 3.980 | 4.431 | 4.464 | 4.390 | 2.160 | 1.154 |  | <0.001 | <0.001 | 0.001 | 0.005 | <0.001 | <0.001 | <0.001 |
| Sp/D | 4.491 | 4.095 | 3.621 | 3.471 | 4.009 | 2.874 | 3.682 | 3.825 | 3.836 | 3.823 | 2.373 | 1.159 | 1.447 |  | <0.001 | <0.001 | **0.121** | <0.001 | <0.001 | <0.001 |
| Sp/E | 4.255 | 4.153 | 3.498 | 3.795 | 2.981 | 2.745 | 3.405 | 4.415 | 4.562 | 4.427 | 2.684 | 2.311 | 2.298 | 2.583 |  | 0.005 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sp/F | 3.938 | 3.663 | 2.912 | 3.015 | 3.035 | 2.198 | 2.998 | 3.735 | 3.901 | 3.838 | 1.599 | 1.508 | 1.450 | 1.510 | 1.647 |  | **0.060** | <0.001 | <0.001 | <0.001 |
| Sp/G | 4.172 | 3.893 | 3.417 | 3.608 | 3.583 | 2.512 | 3.447 | 3.868 | 3.491 | 3.376 | 1.888 | 1.134 | 1.502 | **1.029** | 2.035 | **1.211** |  | 0.001 | <0.001 | <0.001 |
| Sp/H | 4.034 | 3.478 | 3.005 | 2.447 | 3.402 | 2.347 | 2.766 | 3.068 | 3.848 | 3.919 | 2.617 | 2.211 | 2.213 | 1.485 | 2.458 | 1.588 | 1.880 |  | <0.001 | <0.001 |
| Sp/I | 3.512 | 3.714 | 3.844 | 4.703 | 4.274 | 2.961 | 3.832 | 3.533 | 2.400 | 2.459 | 3.568 | 4.008 | 4.348 | 3.842 | 3.958 | 3.669 | 3.247 | 4.004 |  | 0.004 |
| Sp/J | 4.045 | 3.553 | 3.534 | 3.868 | 3.815 | 2.654 | 3.413 | 3.126 | 2.869 | 3.081 | 2.690 | 3.108 | 3.316 | 2.806 | 3.131 | 2.693 | 2.278 | 2.772 | 2.192 |  |

*P* = values are given in the upper triangle; *D* = values are given in the lower triangle.

*D* = Mahalanobis distance, *P* = significance level; bold print denotes non-significant differences; body areas (A–J) are visualised in Fig. 2 (after Bräger and Moritz 2016).

species, scales of all body areas differed significantly and at much higher statistical distances ($D = 3.61 \pm 0.59$, $P < 0.001$). The validity of the results was confirmed by the large proportion of correct classifications with an average discrimination rate of 96.3% based on cross-validation. The detailed results of the blind sample classification based on randomly selected scales within each of the body areas are summarized in Tables 3 and 4. In most cases (in pilchard: body area B, C, D, E, F, G, H, J; in sardinella: body area A, B, C, D, E, F, I, J), the Type I classification was sufficient to classify a blind sample correctly at species-level (e.g., with a non-significant difference from own species). In those cases, when the blind sample differed significantly from the entire sample of the species (i.e., in pilchard: area A and area I; in sardinella: area G and area H), the Type II classification could classify the blind sample well to the species (i.e., non-significant differences from the species).

## DISCUSSION

Scales of many fish taxa show variable shapes and possess a high degree of morphological plasticity within species and specimens, often making clear identification at species level difficult from recovered scale samples. This applies, particularly to clupeiform fishes. Several small pelagic fish species belonging to this order support some of the largest fisheries on Earth (Anonymous 2016) and play a crucial role in marine ecosystems, because their large biomass poses a critical link in marine food webs

(Ganias 2014). Reliable and rapid species identification of this group from environmental samples would provide valuable information, for example, for fisheries (e.g., by discriminating species and stocks), foraging ecology (e.g., by identifying them as prey items) and paleoclimatic and paleoceanographic investigations (e.g., by determining species composition in sediment samples).

In the presently reported study, scales from different body areas of the European pilchard and the round sardinella were successfully classified and discriminated in mixed samples. Although there are a few body areas that show similarities in scale shape within the same species, the last step of our multi-step analysis correctly classified among the body areas of each of the two species. Only scales from body area J (posterior ventral area) in pilchard could not be discriminated reliably from sardinella in the final step (Type II) but were already classified correctly in an earlier step (Type I). The DFA showed a significant difference between the two species for area J when the analysis was run again for sardinella. To increase the reliability of the analysis, elongated scales derived from either the dorsal or ventral edges of the fish body (areas A, B and I, J, respectively) might be disregarded for identification purposes. Our findings agree with those of Ibáñez and O'Higgins (2011) who also found scales of the mid-section of fish to be most useful for this identification method.

Scales that represent the typical round shape properties for a clupeid species are generally located on the flanks

**Table 3**

Results of the blind sample classification in European pilchard, *Sardina pilchardus*

| Classification | Body area | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| Type I ($D/P$) | 2.04/0.041 | 1.33/0.48 | 3.43/0.37 | 1.39/0.42 | 0.99/0.85 | 0.76/0.97 | 1.12/0.74 | 1.81/0.11 | 2.94/<0.001 | 1.78/0.13 |
| Non-significant differences | A, G | B, G, H | B, C, E, G | D, E, G | E, G | A, B, C, E, F, G, H, J | A, B, E, G | G, H | I | H, J |
| Type II ($D/P$) | 1.93/0.15 | 1.06/0.77 | 1.41/0.5 | 1.08/0.83 | 1.14/0.82 | 0.81/0.96 | 0.94/0.95 | 1.44/**0.29** | 1.08/0.91 | 1.56/**0.21** |
| Type II ($T^2/P$) | 15.14/0.29 | 9.54/0.58 | 7.54/0.74 | 7.12/0.79 | 4.55/0.95 | 3.08/0.98 | 3.44/0.98 | 10.84/0.53 | 6.25/0.95 | 12.24/**0.43** |

Body areas (A–J) are visualised in Fig. 2 (after Bräger and Moritz 2016); classification of groups: Type I = CVA-based on the total scale samples, Type II = CVA- and DFA-based on the non-significant sampling areas, CVA = canonical variate analysis, DFA = discriminant function analysis; $D$ = Mahalanobis distance, $T^2$ = discriminant functions, $P$ = significance level; bold print denotes non-significant differences in body areas between pilchard and sardinella.

**Table 4**

Results of the blind sample classification in round sardinella, *Sardinella aurita*;

| Classification | Body area | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| Type I ($D/P$) | 1.86/0.12 | 1.5/0.35 | 1.21/0.63 | 2.03/0.07 | 1.76/0.17 | 0.62/0.99 | 2.48/0.01 | 2.48/0.01 | 2.08/0.06 | 1.96/0.1 |
| Non-significant differences | A, J | A, B, H, J | A, B, C, E, G, H | D, H | E | A, B, C, E, F, G, H, J | H | H | I, J | H, I, J |
| Type II ($D/P$) | 1.4/0.41 | 1.26/0.6 | 1.12/0.74 | 1.17/0.83 | 1.00/0.94 | 0.53/0.99 | 1.52/0.71 | 1.52/0.71 | 1.28/0.64 | 1.27/**0.69** |
| Type II ($T^2/P$) | 9.65/0.59 | 9.07/0.59 | 5.83/0.85 | 5.37/0.92 | 6.6/0.91 | 1.34/0.99 | 7.26/0.87 | 7.26/0.87 | 6.99/0.82 | 7.35/0.76 |

Body areas (A–J) are visualised in Fig. 2 (after Bräger and Moritz 2016); classification of groups: Type I = CVA-based on the total scale samples, Type II = CVA- and DFA-based on the non-significant sampling areas, CVA = canonical variate analysis, DFA = discriminant function analysis; $D$ = Mahalanobis distance, $T^2$ = discriminant functions, $P$ = significance level; bold print denotes non-significant differences in body areas between pilchard and sardinella.

and the lateral mid-section of the fish body (i.e., areas C, D, F, and G). According to the study of Shackleton (1988), scales from these body areas (i.e., "typical scales") were observed in high numbers in the sediments, as well as in samples collected during predatory behaviour of bottlenose dolphins (Bräger et al. 2016a). Clupeiformes tend to lose their scales easily (Patterson et al. 2002), thus the occurrence of typical clupeiform scales in sediments or biological samples is high (Drago et al. 2009, Bräger et al. 2016a). Our investigation showed that the rate of correct classification of scales from the above-mentioned body areas (i.e., lateral mid-section of the fish body) was the highest; therefore the reliability of identifying them among recovered scales has a high likelihood. The large proportion of correct classifications, as well as the high number of examined scale samples, further increase the efficiency of species identification from mixed samples in environmental or biological sources. The geometric morphometric method eliminates any effect of allometric growth when using the centroid size of the scales as the size metric, followed by a linear regression and permutation tests. Therefore, knowing the exact length of the specimen that the scales originate from, which is an essential component of the standardizing process in the traditional morphometrics, is no longer needed for the analysis. Our results provide a reliable and efficient way for scale identification in paleontological and dietary studies that can help to overcome the limitations of morphological analyses used in former studies (e.g., Drago et al. 2009, Bräger et al. 2016a).

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