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Research Article.

Biosystematics study of Clupeidae from the West coast of Algeria

Houria Handjar [(1)] and Nawel Khelil [(1)].

[(1)] Laboratory Management and Valorization of Agricultural and Aquatic Ecosystems. Natural and Life Sciences Department. University Center of Tipaza Morsli Abdellah, Oued Merzoug 42200, Tipaza. Algeria.

Corresponding author: handjarhouria@gmail.com

Abstract

One of the taxonomically complex families that has not been the subject of significant research in Algeria is that of the Clupeidae, which are extremely varied in terms of both morphology and anatomy: Many uncertainties persist in their classification, in particular *S. aurita* and *S. pilchadus*. The main objective is to contribute to the systematics of the family and to the precise identification of the species of this genus targeted by fishing. An examination of faunistic notes and treatises shows a heterogeneity in the inventories carried out on clupeidae populations in the Mediterranean and leads to an extremely confused synonymy. Morphological, morphometric and geomorphometric analyses were carried out. Data were collected during regular outings from July 2014 to May 2016 in various fishing ports and sales outlets in the western region of Algeria. A sample of 520 specimens was obtained from commercial catches. A Principal Component Analysis (PCA) was performed on the biometric data. The cephalic skeleton (neurocranium and splanchnocranium) was taken into consideration. The various methods used enabled inter and intraspecific comparisons to be made. The PCA clearly separates the genera and species of scorpionfish, and highlights the differences between representatives of *S. aurita* and *S. pilchardus*. This discontinuity is confirmed by morphology and geomorphology.

Keywords

Clupeidae;
Morphology;
Morphogeometry;
Oosteology.

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1 Introduction

The Clupeidae are small to medium-sized fish, with bodies that are generally fusiform and subcylindrical, but sometimes very compressed laterally; a row of scouts is present on the ventral profile (Fischer *et al.,* 1987).

The Clupeidae family, best known for its marine forms (sardines, herring and anchovies), includes several genera and species that have adapted to fresh and brackish waters, and others that enter lagoons.

Clupeidae are characterized by an apophyseal connection between the swim bladder and the inner ear, which greatly enhances auditory perception. Most Clupeidae have one or more ventral or pelvic escutcheons, allowing rapid identification of the family (Leveque *et al*., 1989).

Gourene (1988) carried out a systematic review of the freshwater Clupeidae of West and Central Africa. Six genera were selected from the area under consideration: four from freshwater and two from brackish water (Teugels *et al*., 1988; Leveque *et al*., 1989).

Many species of Clupeidae, particularly those from tropical and subtropical waters where diversity is highest, are difficult to identify, as their identification requires microscopic observation (for the number of branchiospines, shape of the second supra-maxillary, etc.) and the taxonomy of some genera is not sufficiently well understood (e.g. the Clupeidae genera Sardinella and Herklotsichthys, Anchoviella) (Fischer *et al.,* 1987).

This family has attracted our particular attention for several reasons: its economic importance, the difficulty of recognizing the species belonging to this family due to the strong resemblance, and the fact that each species has been described by most professionals under different names. This leads us to consider the possibility of recognizing the frontier of a typological concept of the species, according to which it is possible to define a standard type of the species from an individual chosen as a type.

Examination of faunistic notes and treatises (Fisher *et al*., 1987; Bauchot, 1980) show that there are three genera comprising 5 species of Clupeidar in the Algerian basin: *Alosa alosa, Alosa fallax, Sardina pilchardus, Sardinella aurita*, and *Sardinella maderensis*.

We will try to answer the above-mentioned questions in the following sections: (**1**) a morphometric study using a multivariate analysis method (Principal Component Analysis or PCA); (**2**) a morphogeometric study's benchmarks used for all individuals in the MorphoJ program®.

2 Materials and methods

2.1 Sampling site

Five hundred and twenty (520) individuals belonging to 4 species of Clupeidae were collected from sales outlets in the western region (landings came from Oran, Ain témochante, Tlemcen and Mostaganem).

2.2 Characteristics studied

All race differentiation criteria within a species define the notion of meristic characters. Many authors have noted variations in vertebral mean, fin radii or branchiospines within independent populations (Brahimi, 2009). These latter characters were the focus of this study. Some twenty individuals were processed for *Sardinella aurita* and *S. pilchardus*. A single individual was analyzed for *A. fallax* and eleven for *S. maderensis*.

2.2.1 Branchiospines

The number of gills on the dorsal and ventral branches was determined from the first right gillarch.

2.2.2 Vertebrae

To count the number of vertebrae, we boiled the individuals for a few minutes, making it easy to separate the vertebral column from the flesh using a scalpel. We counted all vertebrae from the occipital condyle to the urostyle.

2.3 Morphometric characteristics

The main characters that have been considered are shown in figure 1, 306 observations and 14 quantitative variables have been analyzed morphometrically: (1) STL: standard length, from tip of muzzle to base of caudal fin; (2) FL: fork length, from tip of muzzle to fork; (3) TL: total length, from tip of rostrum to tip of longest lobe of extended caudal fin; (3) Pd: pre-dorsal distance, from tip of rostrum to anterior end of dorsal fin; (5) rd: dorsal ray length; (6) Ld: length of dorsal fin, from base of first spiny ray to last soft ray; (7) Pplv: pre-pelvic length from tip of rostrum to anterior end of pelvic fin; (8) plv: length of pelvic fin, from base to tip; (9) Pa: pre-anal length from tip of rostrum to anterior end of anal fin; (10) La: length of anal fin, from the base of the first spiny ray to the last; (11) Lt: length of head, from tip of snout to tip of horizontal opercular spine; (12) LO: eye length; (13) LC: body width, greatest distance between flanks; and (14) HC: body height.

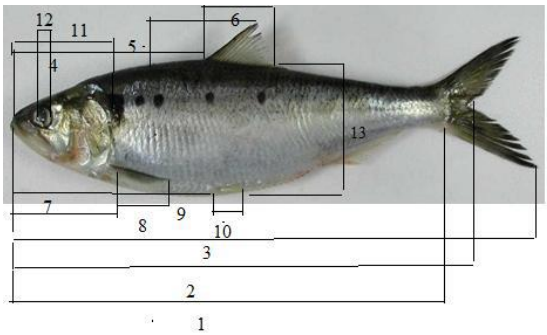

Figure 1. Morphometric characteristics measured on representatives of the Clupeidae

We chose to standardize our variables, using the Bacha *et al.* (2014) method described by Lleonart *et al.* (2000) to eliminate the effect of individual size and relative growth from a matrix of multi-variate morpho-metric data. The standardization relationship is described by the following relation:

$$MS = MO\ (\frac{Tl}{TL})^b$$

where, **MO**: the original morphometric measurement, **Tl**: average total length of all individuals, **TL**: total length, and **b**: slope.

2.4 Morphogeometry

A morphogeometric study was carried out using MorphoJ ®, an integrated software package for geometric morphometric operations.

2.5 Statistical analysis

A Principal Component Analysis (PCA) was performed on these standardized morphometric data. The analysis was carried out using Statistica 5, a software package capable of performing analyses on matrices. These methods are particularly powerful for exploring the structure of data, taking into account their multidimensional nature. They have been described by several authors, including Daget (1976), Legendre and Legendre (1979, 2006), Dagnelie (1973, 1975), Gilbert (1978), Laforge (1981), Philipeau (1986), Lagarde (1983), Bouroche and Saporta (1992), Georgin (2007), and others cited by Ladoul (2011): Schwartz (1983), Duby and Robin (2006).

PCA is a technique for reducing a complex system of correlations to a smaller number of dimensions. The principal components method is also known as the principal axis's method.

3 Results and Discussion

The table 1 shows the numbers of different species and their size ranges.

3.1 Characteristic features

3.1.1 Branchiospines

The number of branchiospines is shown in Table 2; the number of branchiospines on the gill arch varies from 47 to 96 in *S. pilchardus*, with an average of 78.1 branchiospines; while for *S. aurita*, it fluctuates around an average of 104. from 54 to 186 cm.

The average branchiospines established from the individuals sampled characterize the populations of clupeidae, living in the Algerian basin (Table 1). The range of variability of branchiospines in *S. aurita* is very large and probably distinguishes the two sardinella populations.

Table 1. Number of branchiospines and vertebrae for Clupeidae

Species	*S. pilchardus*		*S .aurita*		*A. falax*		*S. maderensis*	
			Branchiospines					
Size range	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
	47	96	54	186	52	90	82	96
Mean	54	186	104.13		71		88.5	
s.d.	9.12		36.18		12.67		3.56	
FAO	44	106	> 80		30	80	>70	
			Vertebrae					
Size range	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
	26	56	24	56	49	55	30	36
Mean	35.00		48.69		52.00		28.95	
s.d.	6.76		2.98		2.00		1.87	

3.1.2 Vertebrae

The number of vertebrae for each species are shown in Table 2. The number of vertebrae varies from 26 to 56 in *S. aurita*, with an average of 35, while in *S. pilchardus* it fluctuates between 24 and 56, with an average of 48.

The average number of vertebrae established from the individuals sampled characterizes the population of clupeidae, living in the Algerian basin (table. 2); the extent of variability of vertebrae in *S. aurita* and *S. pilchardus*, is very important and probably distinguishes the two populations of sardinella and sardine.

3.2 Morphometric analysis

Principal component analysis was performed on 306 observations and 14 quantitative variables. Analysis of the correlation matrix shows that some variables are highly correlated, generating redundant information. From this point of view, only 6 variables were considered. The first two axes generate 67% of the information. For our analysis, we will consider axes I, II and III (81% of total variance) forming factorial planes I-II, I-III.

The projection of individuals (Table 2) on the I-II plane (LC, Ol and HC) highlights Clupeidae species with morphometric similarities (Figure. 2, 3): these are *Sardina pilchardus*, *Sardinella aurita*. While *Alosa fallax* and *S. madernsis* are well individualized.

Table 2. Contribution of initial variables to the formation of axes 1, 2 and 3

	Axe 1	Axe 2	Axe 3
LT	0.479	0.314	0.198
Rd	0.199	0.171	0.122
Lt	0.083	0.060	0.986
LC	0.746	-0.270	0.060
HC	0.091	0.936	0.070
Ol	0.861	0.316	0.106

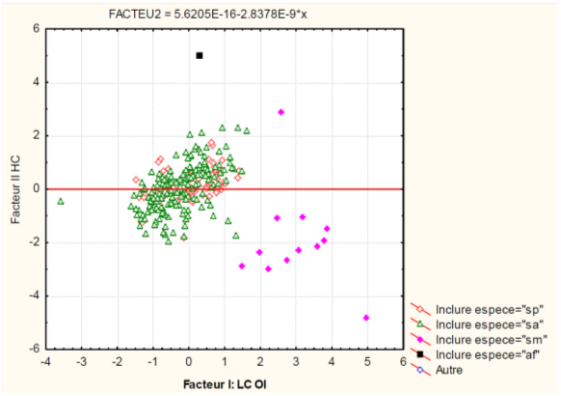

Figure 2. Projection of individuals on the factorial plane I-II

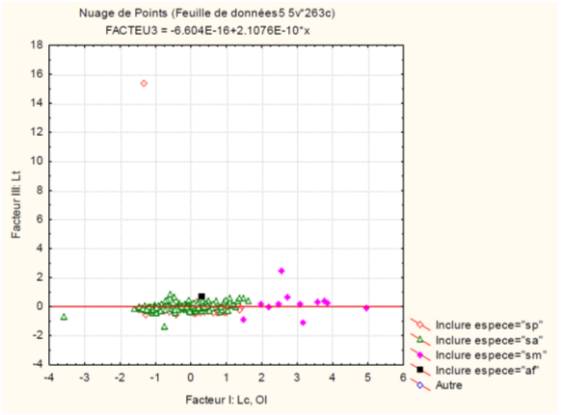

Figure 3. Projection of individuals on the factorial plane I-III

3.2.1 Morphogeometric analysis by gender

According to the projection on the CVA1 axis, which explains 94% of the variance, we distinguished two groups (Figure 4): A first group represented by the species *Alosa fallax*, and a second group comprising two species *Sardinella aurita* and *Sardina pilchardus*.

The Mahalanobis distance (Table 3) is a distance measure based on the correlation between variables by which different models can be identified and analyzed.

The closest species are *S. aurita* and *S. pilchardus* with the smallest distance (39.02) and the farthest are A. *fallax* with a distance of (927.83).

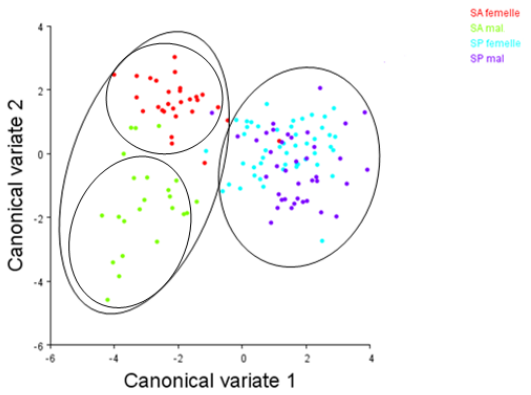

Figure 4. Morpho-geometry results by sex

According to the projection on the CVA1 axis, which explains 89.48% of the variance, we have distinguished two distant groups; *A. fallax* clearly separates itself from the other species, although there is an overlap between the two other clupeidae species, with discrimination between the two sexes (Figure. 4).

According to Table. 3, the closest species are male *S. pilchardus* and female *S. pilchardus* with the smallest distance (16.43) and the farthest are female *A. fallax* and female *S. pilchardus* with a distance of (775.66).

Table 3. Mahalanobis distance between species by sex

	1. SA	2. SP ind	3. SP femelle	4. SP mal	5.SM
1. SA	33.48				
2. SP ind	37.54	47.05			
3. SP femelle	42.55	49.84	16.43		
4. SM	769.01	767.51	775.66	767.96	

When projected on the CVA1 axis, which explains 89.48% of the variance, we distinguished two groups (Figure. 4). Group one *S. aurita* and a second group comprising *S. pilchardus.*

3.2.2 Morphogeometric analysis by region

According to the projection on the CVA1 axis, which explains 46.94% of the variance, we have distinguished three groups (Figure. 4). A first group identifies the species *A. fallax* center, a second group characterizes *S. aurita* center and west and S. pilchardus center and a third group represents S. pilchardus west.

In our case study, our results demonstrated the existence of at least two different morphotypes in S. pilchardus. Morphological observation and analysis of meristic and morphometric characters revealed a fairly marked discontinuity in *S. pilchardus* and *S. aurita.* Morphometric analysis confirmed the morphological observation.

According to the results we have obtained, we conclude that there is a separation between the two sexes of the *Sardinella aurita* species, but we note that there are a few individuals that do not follow the norm, proving the morphometric discontinuity.

The morphometric study confirms the morphological observations. The results of the biometric analysis enable us to identify Clupeidae species on the basis of a few characteristic mophometric parameters: head length and body height. The morphometric study reveals a morphometric similarity between the species S. pilchardus and S. aurita; and a fairly pronounced morphometric discontinuity, especially in S. aurita. The explanation for the intraspecific variability could be explained by the region according to the biometric analysis; the morphometric difference according to sex seems less obvious and has been reinforced by morphogeometry. Morphometric analysis reveals a separation between two different regions (central, western) of the same species (Sardina pilchardus), but we note that there are a few individuals that do not correspond to the standard prototype of the species that asserts morphometric discontinuity, so it goes

without saying that a separation between individuals of the two species *Sardina pilchardus* and *Sardinella aurita.*

The results obtained from the morpho-geometric analysis show a clear difference between two groups for each species. One group encompassing the populations of the western region, and a second encompassing the population of the central region for *S. pilchardus* and *S. aurita*, for its part, showed a distance between the two sexes. This spatial inconsistency could be explained by environmental conditions, notably pollution and feeding conditions.

Our results need to be backed up by genetic studies to settle the issue.

4 Conclusion

The Clupeidae are small fish with oblong bodies that are more or less compressed. Little research has been done on them in the Algerian basin. The morphological similarity of this family has always been a problem. Our work was inspired by this problem, which led us to tackle the problem of intra- and inter-specific comparison of the analysis of morphological, meristic and morphometric characteristics. It was important to determine an exhaustive list of Clupeid species caught by all types of fishing gear and from all over the region: *Alosa fallax, Sardina pilchardus, Sardinella aurita and S. maderensis.*

The problem with this family stems from the confusion that arises between the species: the sardine can be confused with young shad, because of its striated operculum. It can be distinguished from them by the last two rays of its anal fin, which are longer than the others, and by the posterior end of its mouth located in front of the vertical line passing through the centre of the eye. As well as the notch in the snout of *A. fallax*.

We attempted to describe the intraspecific morphological variability observed in the *S. pilchardus* species, which shows two forms: the presence of one or two lines of dark black spots along the lateral line.

The meristics study revealed fairly pronounced intra- and inter-specific variability in the number of vertebrae, branchiospines and fin rays.

Morphometric examination using principal component analysis clearly separates the Clupeidae species, but reveals great similarity between *S. aurita* and *S. pilchardus*, and clear discrimination from the other species. Similarly, the PCA affirms the morphological observation, clearly highlighting the intra-specific variability of the Clupeidae.

It was difficult to separate these two species thanks to some of the mophometric parameters that characterise them: body height, width, eye diameter, etc. However, it should be emphasised that the length of the head is characteristic of each species.

In addition, the results obtained by morphogeometric analysis show a difference between the two species, and highlight a discrimination between males and females of *Sardinella aurita* as well as a clear distinction between western and central sardines.

- **Conflict interest**

No conflict interest.

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