PRELIMINARY RESULTS OF MORPHOLOGICAL VARIABILITY OF THE GENUS *PSEUDOPHOXINUS* [TELEOSTEI, CYPRINIDAE] IN TUNISIAN FRESHWATERS

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ملخص

النتائج الاولية حول التغيرات الشكلية لدى الاسماك من جنس *Pseudophoxinus* في المياه العذبة التونسية : تمت دراسة التنوع الايكوبيولوجي للأسماك من جنس *Pseudophoxinus* في المياه العذبة التونسية بواسطة طريقتين إحصائيتين. الأولى ذات بعد واحد ترتكز على اختباري Fisher و Duncan و مكنتنا من تبيين العناصر التي تضفي على نوع *punicus* خاصية الانفراد بعدد مرتفع للأسنان الخيشومية و للحراشف فوق الخط الأفقي مقارنة بالمجموعات الأخرى التي تبرز كوحدة بيولوجيا متشابهة. الثانية متعددة الأبعاد وترتكز على تحليل العلاقات بين هذه المجموعات بناء على عناصر بيولوجية و ايكولوجية و مكنت من توحيد المجموعات ذات خاصيات ايكولوجية مشتركة ضمن نوع واحد *callensis*. وهو ما يجعلنا نستخلص أن جنس *Pseudophoxinus* يتكون من نوعين اثنين: *punicus* و *callensis* باعتبار و ان معيار التمييز هو ايكولوجي في انتظار القيام بدارسة جزيئية لتأكيد التركيبة النوعية لهذا الجنس.

كلمات مفاتيح: *Pseudophoxinus*، تنوع، ايكوبيولوجي، مياه عذبة، تونس

RESUME

Résultats préliminaires sur la variabilité morphométrique chez le genre *Pseudophoxinus* (Teleostei, Cyprinidae) dans les eaux douces tunisiennes : L'analyse de la variabilité bioécologique des populations de poissons du genre *Pseudophoxinus* a été réalisée par deux méthodes statistiques. La première univariée basée sur les tests de Fisher et de Duncan et a permis de distinguer l'espèce *Pseudophoxinus punicus* qui s'individualise par des valeurs très élevées de nombre de branchiospines au premier arc branchial gauche et d'écailles à la ligne latérale par rapport aux autres formant un groupe biologiquement homogène. La deuxième méthode, multivariée, a permis d'une part d'isoler *P. punicus* des autres populations et d'autre part de séparer les populations ayant des caractères écologiques spécifiques, mettant ainsi en évidence l'effet milieu sur la répartition des populations appartenant au genre *Pseudophoxinus*. Ce qui nous mène à conclure que le genre *Pseudophoxinus* serait formé de deux espèces *punicus* et *callensis*, en attendant de procéder à une étude moléculaire pour confirmer sa composition spécifique.

Mots clés : *Pseudophoxinus*, variabilité, éco-biologie, eaux douces, Tunisie

ABSTRACT

This paper aimed to study the morphological variability of several *Pseudophoxinus* populations in Tunisian freshwaters, by 2 statistical methods. The first one unvaried allowed distinguishing the species *Pseudophoxinus punicus* which is individualized higher numbers of gillrakers and scales on the lateral line compared to the other individuals who constitute a more or less homogeneous group corresponding to the complex *Pseudophoxinus callensis / Pseudophoxinus chaignoni.* The second method which combined the morphological and ecological factors allowed to distinguish off *P. punicus* from the others and also to gather the populations which have similar ecological factors, highlighting their effect on the *Pseudophoxinus* populations distribution. Thus, this study allowed us to gather that genus *Pseudophoxinus* should be formed by two species: *punicus* and *callensis*, waiting for a molecular study to confirm that.

***Key words*:** *Pseudophoxinus,* variability, morph-anatomy, ecology, freshwater, Tunisia.

INTRODUCTION

The genus *Pseudophoxinus* (Bleeker, 1860) belongs to the family of "Cyprinidae" and makes up the kind of endemic fish, second to *Barbus* in Tunisian freshwaters. Having a Siberian origin (Darlington, 1957; Banarescu, 1972), the *Pseudophoxinus* fish were considered among the migrants who reached the "Ibery" towards the end of Oligocene and North Africa towards Miocene (Almaça, 1979). In Tunisia, the systematic of this genus offers a lot of shadow zones. In fact, the specific statute of *P. punicus* (Fig. 1) was confirmed in earlier studies.

However, the two other species *P. callensis* (Fig. 2) and *P. chaignoni* are the subjects of contradictory hypotheses because of their mutual similarities on the morph anatomic plan. Some authors such as (Vaillant, 1904; Boulenger, 1911; Pellegrin, 1920; Dieuzeide and Champagne, 1950; Collares-Pereira, 1983) relied on morphometric characters including the colour of body and fins and the form of scales, so that they could prove the co-existence of those two species. Despite, other authors such as (Bertin and Esteve, 1948; Almaça, 1979; Kraïem, 1983; Boumaïza and Quignard, 1996) found out that the meristic parameters show wide overlaps, and the morphometric characters are too insufficient to distinguish the two forms as different species. Thus, they

have classified *P. chaignoni* as sub-species or ecotype of *P. callensis*. Our study aims to analyze the morphometric

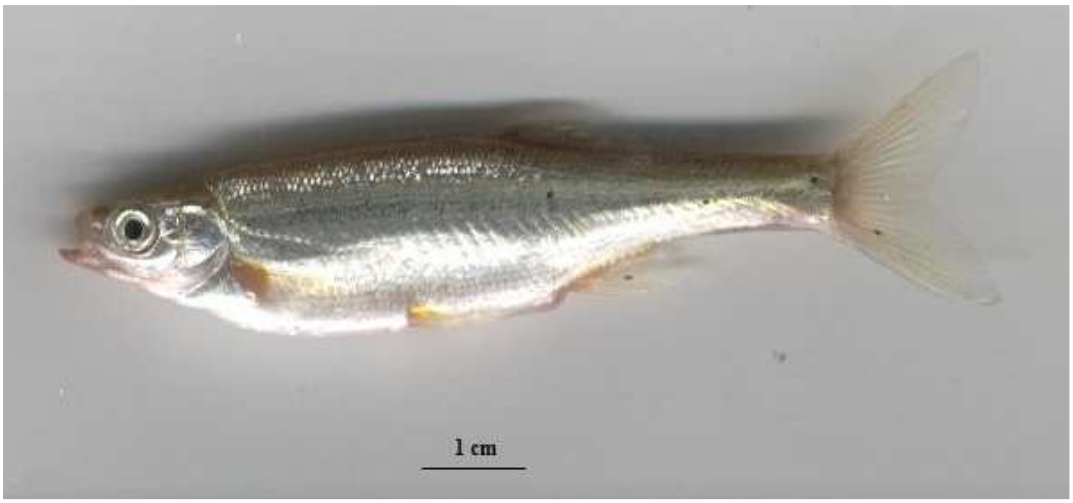

Fig. 1: Photo of the specie *Pseudophoxinus punicus* (Pellegrin, 1920) in Tunisia

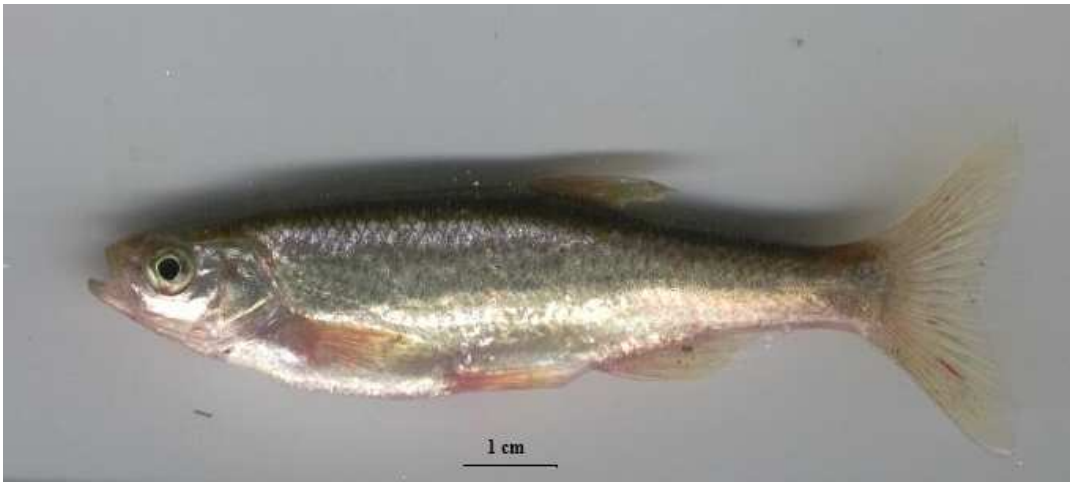

Fig. 2: Photo of the specie *Pseudophoxinus callensis* (Guichenot, 1850) in Tunisia

and ecological variability of *Pseudophoxinus* populations, of Tunisian freshwaters to check if this genus is represented by three species (*P. punicus*, *P. callensis* and *P. chaignoni*) or by just two species (*P. Punicus* and *P. Callensis*) showing a phenotypic plasticity. The complexity of the Pseudophoxinus genus has been mentioned by numerous authors and its systematic status is being always reviewed (Kottelat and Barbieri, 2004; Freyhof and Özulug, 2006; Bogutskaya et al., 2007; Kottelat and Freyhof, 2007; Küçük, 2007).

MATERIAL AND METHODS

The samples of "*Pseudophoxinus*" were collected from 8 sites (Fig. 3) which belong to three different hydrographical basins (Tab. I): the Ichkeul basin (oued Joumine); the Medjerda basin (oued Ghezala and Kasseb on the North banks, and Aïn Bou Saadya source on oued Siliana flowing from the south bank) and the east basin

(oued Meliane, oued el kebir and the Lebna reservoir). These basins reflect a good quality of water with a satisfactory oxygenation, a clear transparency and absence of pollution. Nevertheless, he also presents varied morph-dynamic faces which characterize different biotopes (Dkhil-Abbes, 2004) and they show different physical-chemical characters.

Two kinds of nets have been used for fishing:

* One net to collect alevin with a mesh of 10 mm, easy to use in shallow streams,
* One multi-mesh net (with meshes of 10, 15, 26, 30, 40, 60, 80 mm, of which only the first one is able to fish the *Pseudophoxinus*), used in deep oued and dams.

In total, 387 fish were collected in 8 different sites as presented in table I. In laboratory, the fish were examined, measured and weighed. Two kinds of

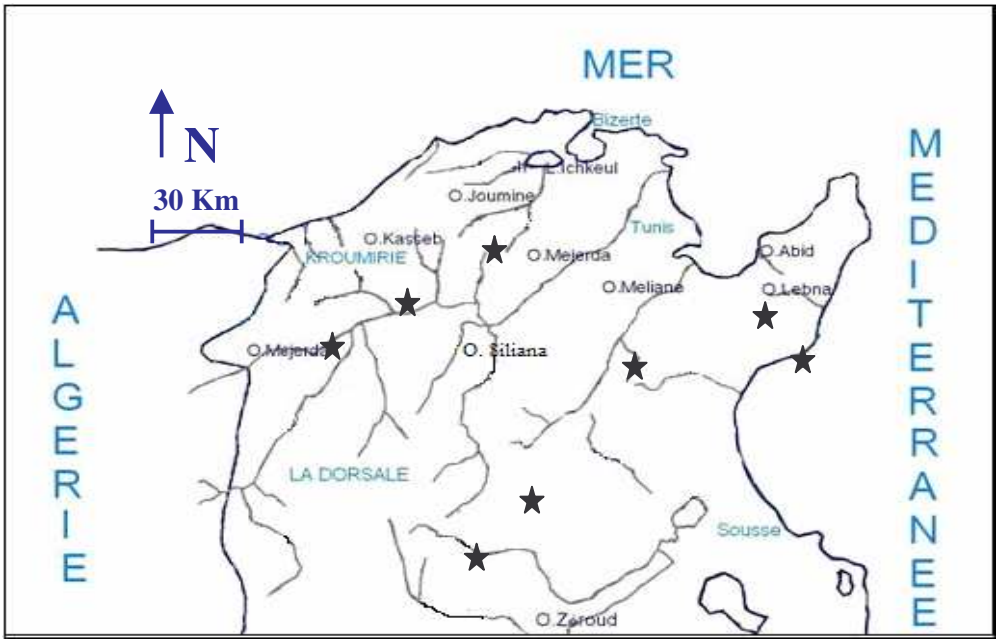

Fig.3: Tunisian hydrographical network map and location of the studied sites (★)

Tab. I: presentation of *Pseudophoxinus's* samples studied

Samples	Designations	Effectifs	Basin bancs
O.Joumine	J	48	Nord (Ichkeul)
O.Kasseb	K	34	Nord Ouest (Mejerda)
O.Ghézala	G	58	Nord Ouest (Mejerda)
Ain Bou Saadya	S	50	Nord Ouest (Mejerda)
O.Kébir	E	50	Nord Est (Mhamdia)
O.Méliane	M	50	Nord Est (Méliane)
O.Abid	A	50	Nord Est (Cap Bon)
B.Lebna	L	47	Nord Est (Cap Bon)

parameters were studied: morphometric measures (Fig. 4) and meristic counts (Tab. II). Like many other authors, mainly Kraïem (1994), Boumaïza (1994), Gharred and Ktari (2001), we have used the report of different measurements of different part of the body by the standard length. Otherwise, these parameters were treated by two types of statistical analysis with the SAS software (SAS, 1990) to determine, in the first time, the characters which discriminate better the populations and then to identify the correlations between them concerning each character separately. We first used the unvaried analysis based on the Fisher and the Duncan tests. These analyses led us to determine the possible causes of the variability within the complex (*P. callensis*/*P. chaignoni*) which could be due to its phonotypical plasticity (only one species existed, *P. callensis*) or to its individualization into many species (*P. callensis* and *P. chaignoni*). In a second time, we used the correspondence factorial analysis (AFC) which allowed us to determine the relationship between the characters of the studied populations and the ecological factors of their localities.

RESULTS

A/ Result of unvaried analysis:

The analysis of variance by the Fisher test was used to identify the characters which discriminate the studied populations and the Duncan test was used to make a hierarchical classification for each character separately.

Tab. II: meristics and morph metrics parameters counted on the Pseudophoxinus

Parameters studied	Codes utilisés
❖ **Morphometric parameters :**	
Total length ;	LT
Standard length ;	LS
Head length ;	Lt
Eyes diameter ;	DO
Predorsale distance ;	PD
preventable distance;	PV
pectoral length ;	LP
height of body ;	H
Distance between the anal and ventral.	DVA
❖ **Numeric parameters :**	
Number of scales on the lateral line;	Ell
Number of scales between dorsal and lateral line ;	ED ll
Number of scales between ventral and lateral line ;	EV ll
Number of osseous and soft ray of dorsal ;	ROM D
Number of osseous and soft ray of ventral;	ROM V
Number of osseous and soft ray of pectoral;	ROM P
Number of osseous and soft ray of anal;	ROM A
Number of gillrakers on the 1st left gill bone ;	Branch.

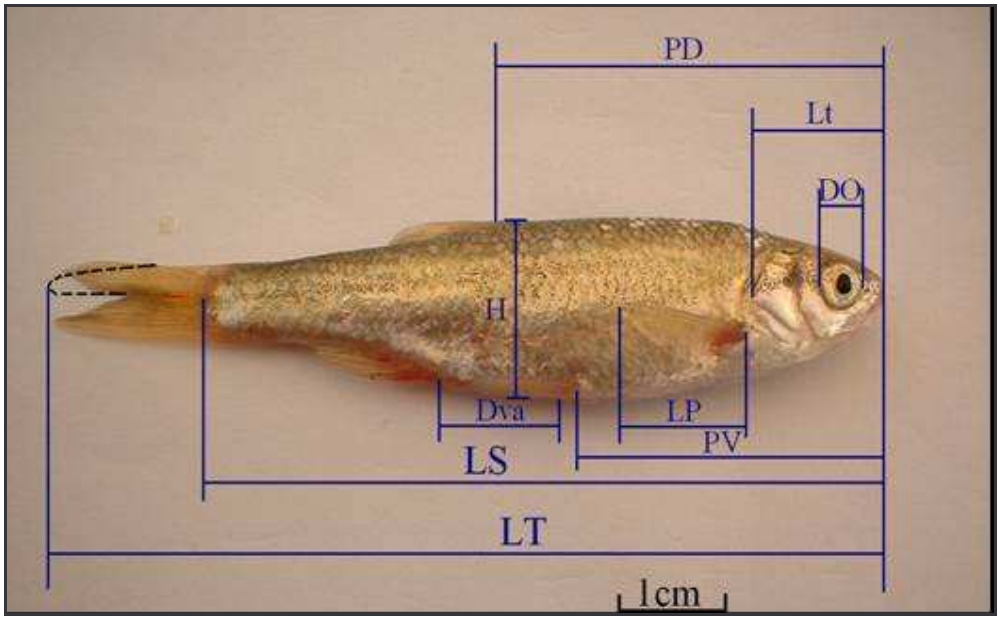

Fig. 4: Different morph metrics characters measured on Pseudophoxinus
(See legend in the text)

1- Variance analysis by Fisher's test:
The results obtained by this test (Tab. III) indicated that the meristic parameters showed an inter-population variability with highly significant differences (P<0,001).The number of gillrakers on the first left gill bone seemed the best criterion of discrimination between the fish populations according to the very high value of calculated F (Fc= 3766.7>>F =15.02). The number of scales on the lateral line was also a good criterion (Fc=463. 38 > F =15.02). Moreover, the ratios (Lt/LS and DO/Lt) showed inter-population variability with high significant differences especially between the fish populations collected in oueds or in dams.

2- Comparison of means by Duncan's test:
The statistical model of multi comparison using the

Tab. III: results of the variance analysis of all the samples

Ns: non significant in P>0.5 - * : significant in P<0.5 - **: very significant in P<0.01 - ***: Highly significant in P< 0.001 - CM: mean of square - DDL: degree of freedom - F: F: Fisher test - RMA: soft rays on anal - Branch. : Number of gillrakers on the 1st left gill bone

Parameters measured	CM	DDL	F	F signification
Lt/LS	0.05	8	75.29	***
DO/Lt	0.09	8	41.34	***
LP/LS	0.028	8	1.49	ns
DVA/LS	0.035	8	1.5	ns
PD/LS	0.058	8	0.59	ns
PV/LS	0.052	8	0.82	ns
ELL	2596.34	8	463.38	***
RMA	19.42	8	40.68	***
Branch.	23493.41	8	3766.7	***

duncan test showed correlations between the populations for each parameter considered independently.The obtained results (Tab. IV) indicated that the characters "number of gillrakers on the first left gill bone" and "the number of scales on the lateral line" allowed to individualize a population in the oued Gheazala called G2.This population was characterized by a relatively high number of gillrakers (**84**) and number of scales (**69**) and corresponds to the species *P. punicus,* already well-studied by former works (Vaillant, 1904; Boulenger, 1911; Pellegrin, 1920; Dieuzeide and Champagne, 1950; Almaça, 1977, 1979; Collares-Pereira, 1983; Boumaïza, 1994; Kraïem, 1983, 1991, 1994). It occupied a small area limited to the streams of the Kroumirie region in North-West of Tunisia where water is cool with a good chemical quality and relatively fast flow.

The other portion of the population from oued Ghezala constituted a single group with a number of gillrakers varying between 13 and 17 and a number of scales varying between 44 and 49. Thus, would correspond to a complex *P. callensis*/*P. chaignoni* (Kraïem, 1983, 1991; Boumaïza and Quignard, 1996). Otherwise, the character "number of scales on the lateral line", allowed distinguishing the eastern populations of *Pseudophoxinus* from the other individuals collected from the western hydrographical network of north Tunisia. However, this difference is not statistically significant.

B/ Results of multivariate analysis

The multivariate analysis was used to determine the correlations between populations for all considered variables. That's why, we have simultaneously considered ecological and biological parameters in order to recognize conformities between these parameters, and to explain –if ever it exists- the differences between the populations in each location.

a- Definition of factorial axis and variability by these axis:

Based on table V, we noticed that the first axis was defined by the biological parameters and it absorbed 64, 87% of total variability. The second axis was correlated to the following ecological factors: the nature of the environment, the current depth, riparian vegetation and lightness, and accumulated 14, 25% of the total variability. The 3rd axis was defined by the nature of substratum and the current water-course, and it absorbed 10, 97% of total variability. Therefore, we limited the analysis to the plan formed by the first two factorial axes since they represented more than 79% of the total variability.

b- Projection of populations on the factorial axis:

The projection of populations and the variables studied on the 1st axis (Fig. 5) showed the separation of two groups:

* The 1st group combined the G2 population of oued Ghezala a "the number of gillrakers". This showed that this population was distant from others by a biological criterion. That's why the axis F1 constituted the biological component of this analysis.

* the second group combined all the other populations which did not express major differences on the biological scale explaining their belonging to a different species in the event *P. chaignon*i suggested by Vaillant, 1904; Boulenger, 1911; Pellegrin, 1920; Dieuzeide and Champagne, 1950; Collares-Pereira, 1983. Therefore, this led us to talk about the *P. callensis*/ *P. chaignoni* complex.

The projection on the axis F2 allowed separating the 3 other groups:

Tab. IV: Results of the Duncan test related to all the *Pseudophoxinus*'s samples collected

Lt/LS		Abid	Kébir	Méliane	G1	Joumine	Kasseb	Lebna	S	G2
	X	0.27	0.23	0.22	0.22	0.21	0.2	0.18	0.18	0.16
	N	50	50	50	17	48	34	47	50	41
DO/Lt		Abid	Méliane	Joumine	G1	Kébir	Kasseb	Lebna	G2	S
	X	0.55	0.5	0.49	0.488	0.486	0.455	0.437	0.427	0.41
	N	50	50	48	17	50	34	47	41	50
Ell		G2	S	Kasseb	G1	Lebna	Joumine	Kébir	Abid	Méliane
	X	68.97	48.94	46.79	46.59	46.42	44.81	44.44	44.4	44.18
	N	41	50	34	17	47	48	50	50	50
RM NA		G2	G1	Joumine	Méliane	Lebna	Kasseb	Abid	S	Kébir
	X	12.32	10.94	10.93	10.82	10.44	10.44	10.3	10.28	10.04
	N	41	17	48	50	47	34	50	50	50
Branch.		G2	Joumine	Abid	G1	Kasseb	Méliane	Kébir	Lebna	S
	X	84.34	16.95	15.94	15.11	15.08	14.36	14.12	13.85	13.46
	N	41	48	50	17	34	50	50	47	5

- The 1st one was formed by the population streaming from Lebna reservoir and had the ecological characteristics "the nature of locality, the depth and lightening". This population was the only one collected from a dam which was characterized by a maximal illumination on its surface and an important depth.
- The 2nd group was formed by the population sampled from Aïn Bou Saadya source and explained the effect of the factor "riparian vegetation" on its remoteness.
- The 3rd group occupied a central position and linked the remaining populations (Abid, El Kébir, Meliane, Joumine, Kasseb, and Ghezala G1) which represented similar biological and ecological characteristics.

Thus, the axis F2 constituted the ecological component of this analysis.

Tab. V: definition of the factorials axis and absorption of the global variability by this

Axis	Egeinvalue	Proportions (%)	Cumulée (%)	Variables	Eigenvalue
1	0.29	64.87 %	64.87 %	Lt/LS	0.002
				DO/Lt	0.004
				LP/LLS	0.0025
				DVA/LS	0.0024
				PD/LS	0.005
				PV/LS	0.005
				Ell	0.113
				RM A	0.056
				Branch.	0.718
2	0.14	14.25 %	79.12 %	Mil	0.165
				Prof	0.322
				Vég	0.336
				Ecl	0.115
3	0.12	10.97 %	90.09 %	Sub	0.463
				Cou	0.166

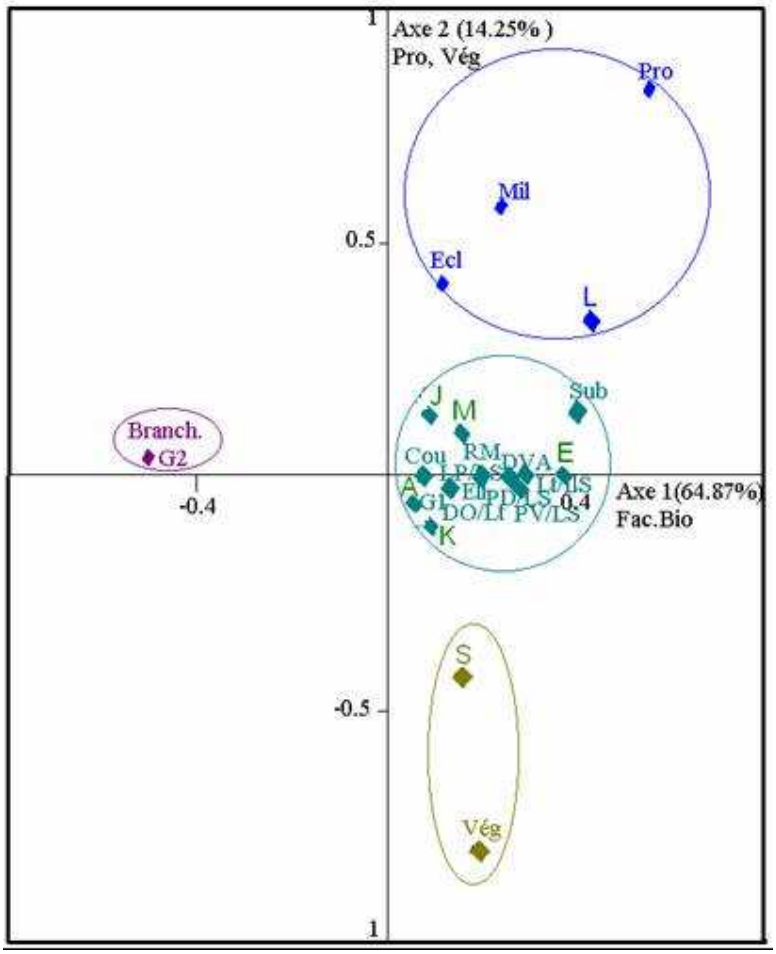

Fig. 5: AFC graphical presentation of ecological and biological parameters

DISCUSSION

Our research's objective was to clarify the specific composition of *Pseudophoxinus* genus in Tunisian freshwater by two types of analysis: the unvaried analysis and the multivariate one.

- The unvaried analysis, based on the Fisher and Duncan tests, allowed determining the characters which discriminate the populations; they consisted in the meristic ones: "the number of gillrakers on the first left gill bone, the number of soft rays on the anal fin and the number of scales on the lateral line". Moreover, it led to a separation of *P. punicus* species and the complex *P. chaignoni/P. callensis*.

- The multivariate analysis, based on the AFC, showed that the ecobiological variability of *Pseudophoxinus* populations could be analysed according to two criteria:

* The first criterion is biological. It allowed confirming the specific statute of *P. punicus*, all the other populations which constituted a more or less homogeneous group with many biological similarities. Therefore, we suggested to admit the complex *P. callensis/P. chaignoni* excluding the individualization of *P. chaignoni* as a 3rd species as supported by numerous previous studies (Vaillant, 1904; Boulenger, 1911; Pellegrin, 1920; Dieuzeide and Champagne, 1950; Collares-Pereira, 1983). The present work claimed that the morphologic variability observed within the group is due to a phenotypical plasticity related to ecological factors.

* The 2nd criterion is ecological. It showed that within the *P. callensis/P. chaignoni* complex, despite the biological affinities, it is possible to discriminate the populations taking into account the ecological characters inducing the formation of ecotypes. It was particularly the case of population collected from the spring Aïn Bou Saadya and the dam of Lebna which were far from the rest of other populations (Fig. 6) as they are derived from 2 areas having different nature (Spring and reservoir) and belonging to 2 different basin banks, which prevented any inter-circulation of genes flow. This corroborated the advanced idea of several authors (Bertin and Estève, 1948; Almaça, 1979; Kraïem, 1983; Boumaïza and Quignard, 1996) who pointed out that the body colour is a variable character within populations and that the meristic characters show extended overlapping. Thus, they do not give enough distinction to admit the existence of the two species *P. callensis* and *P. chaignoni.*

However, it's necessary to signal that despite of excluding the hypotheses claiming the individualization of a third species, it's difficult to confirm the existence of only two species of *Pseudophoxinus* (*P. callensis* and *P. punicus*). A genetic analysis based on molecular markers and cytochrome sequencing seems to be indispensable to clarify this ambiguity.

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