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IDENTIFICATION OF TWO SPECIES OF SUBFAMILY (BAGRINEA) NAMELY: CHRYSICHTHYS RUEPPELLI AND CHRYSICHTHYS AURATUS USING ISOELECTRIC FOCUSING TECHNIQUE

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ABSTRACT

Identification of ***Chrysichthys rueppelli*** and **C. *auratus*** (subfamily BAGRINEA) was carried out morphologically and by using isoelectric focusing technique of soluble protein of the eye lens, flesh and skin.

Results indicated that ***C. rueppelli*** and ***C. auratus*** are two completely separate species with major differences in both morphological and isoelectric point focusing values (PI). It was also found that, in each organ (eye lens, flesh and skin), there are characteristic protein fractions showing clear taxonomic relationship between the two examined species.

INTRODUCTION

Chrysichthys rueppelli and ***Chrysichthys auratus***, are commercially important fish species in the fresh water fisheries of Egypt.They look very close to each other from the morphological point of view.However, biochemical analysis can give an accurate identification for both species under investigation.

Isoelectric focusing technique is an important aspect in studying the pattern of tissue protein. It can reveal a high degree of species specificity when separated by slab Polyacrylamide disc electrophoresis (Herzberg and Pasteur, 1974), or by thin layer isoelectric focusing (IEF) with Polyacrylamide gels (Carpene ***et al.***, 1983; Kamel, 1987; El-Gharabawy and Zaki, 1990;

El-Gharabawy, 1991. Isoelectric focusing technique was introduced as a tool for identification of fish species. So, the complex genetic constitutions and the specific protein profiles could be used for identification of several fish species (Lundstrom, 1980, 1981).

Lundstrom (1980), (1981) and (1983) used thin layer Polyacrylamide and Agarose gel isoelectric focusing method for identification of fish species. He described the nature of the monk fish protein pattern variation and provided a means for the reliable identification of monk fish. Monk fish showed three slightly different patterns. Each of the variant patterns was reproducible enough to allow identification of monk fish fillets by comparison of known and unknown patterns.

Basaglia (1989), studied the soluble lens protein from fifteen Sparid species. El-Gharabawy and Zaki (1990) used phase-system isoelectric focusing (IEF) method as fast method for identification the various character of protein pattern of the fish organ tissue (gonad, liver and muscle) of ***Mugil capito*** and ***Mugil cephalus.*** Moreover El-Gharabawy (1991) used IEF technique to identify five ***sole*** species in the Egyptian Mediterranean water from fish muscle and skin. In (1991), Basaglia and Marchetti studied the soluble white muscle proteins from fifteen Sparid species.

Assem (1992) employed isoelectric focusing to determine the variation which occurs in gonads, plasma and pituitary gland during maturation stages for both sexes of ***Oblada melanura.*** She found that the plasma, gonads and pituitary proteins for male and female have great differences. Also, there are differences in electrophoretic pattern according to the maturation stages of both female and male with characteristic band for every stage of maturity.

El-Gharabawy (1995) studied the soluble white muscle proteins of twelve Sparid species by electrophoresis and isoelectric focusing in the Egyptian Mediterranean water.

The aim of this work is to identify the two species by morphological differences and by the analysis of soluble buffered protein in the eye lens, flesh and skin of both species (***Chrysichthys rueppelli*** & ***Chrysichthys auratus***) by using phast gel isoelectric focusing from (3-9) to identify the two species under investigation.

MATERIAL AND METHODS

Sample preparation:

Samples of eye lens, flesh and skin were taken from some specimen of ***Chrysichthys rueppelli*** and ***Chrysichthys auratus***. 0.22 gm of each sample was homogenated individually with 2 ml of Tris-HCl buffer of PH 8.6. The homogenate solution was centrifugated at 6,000 rpm for 10 min. The clear supernatant was pipetted into vials and either immediately examined or kept frozen at -20 °C until required. Phast system apparatus (pharmacia LKB, S-75182, 1987) was utilized for separation and staining the protein bands. The frozen extracted samples were thawed at room temperature then applied onto the gel plates (50x43 mm in diameter) and (0.35 mm thick) at pH from (3-9), using sample application 1μ.

Eight samples were applied to each gel plate including two standard proteins. Protein bands were silver stained according to Heukeshoven and Dernick. Hoefer scientific instruments (GS 300) with GS 365 W, Electrophoresis data system, version 3.0% was used in the determination.

Relative quantitative and qualitative protein bands were analyzed on the basis of number, position, density and width of each band. Values of isoelectric points (PI's) were calculated according to the pharmacia PI calibration kit. No. (H-B-045-02).

RESULTS

Morphological identification:

The two species ***C. rueppelli*** and ***C. auratus*** belong to family Siluridae, which includes fishes with naked body or with bony plates which are considered as secondary squamation. One to four pairs of barbells, sometimes very long. Pectoral fins inserted very low down, folding like the ventral, often armed, like the dorsal, with a strong bony spine. An adipose fin is often present (Boulangea 1907). Also these two species belong to subfamily BAGRINAE which is characterized by short dorsal fin, presence of adipose fin or transformed into second rayed fin, short anal fin and finally gill membranes free from the isthmus.

These two species belong to genus ***Chrysichthys***, which is presented by feebly compressed body and the dorsal fin consisting of a spine and 5 or 6 soft rays and followed by an adipose fin. Ventral fin with 6 rays. Four pairs of barbells (nasals and maxillary), and two memdibulars. Eyes superolateral with free border. (Fig. 1).

The *C. **rueppelli*** differs from ***C. auratus*** by having smaller eye, longer adipose dorsal fin, shorter and rounded lobe of the caudal fin. The first soft ray of the dorsal fin is not produced, this character is not in any way connected with age. While ***C. rueppelli*** has the same color of ***C. auratus*** which tend more or less to dull puffish gray, silvery white beneath, pectoral, ventral and anal fins sometimes tinged with pale yellowish orange, the caudal fin sometimes tinged with pink or red color (Fig. 1).

Identification of fish species using conventional morphological technique is sometime difficult, since many morphological characteristics are overlapping among the two species. Isoelectric focusing has the power of differentiating protein fractions with highly specific identification for each species. Four protein bands were separated from the eye lens of the ***Chrysichthys rueppelli*** at PI's 3.25, 7.60, 8.90, and 9.40, with relative quantitative values varied between 58.8 maximally and 3.9 minimum. This species was identified by two specific bands separated at isoelectric point (PI's) values of 3.25 and 7.60 with relative quantitative values 33.1 and 3.9 respectively. Table (1) and Fig. (2). While in the second species ***Chrysichthys auratus***, seven protein bands were separated from the same tissue (Eye lens) and were identified by two specific bands separated at PI's values of 3.35 and 5.80 with relative quantitative values 23.40 and 4.7 respectively.

Nine proteins fractions were separated from the flesh of ***Chrysichthys rueppelli***. This species was identified by two specific protein fractions separated at PI's 5.45 & 7.30 with relative quantitative value 7.4 & 15.6 respectively. For ***Chrysichthys auratus*** eight protein fractions were separated from flesh protein extract table (1) Fig. (2). The quantitative value varied between 40.3 & 0.5.

Table (1): The isoelectric point (PI's) values and the percentage of quantitative values for scanning protein fraction in three types of tissue (eye lens, flesh and *skin*) *Chrysichthys rueppeli* and *Chrysichthys auratus*.

	Eye lens		Flesh		Skin	
PI	*C. rueppeli*	*C. auratus*	*C. rueppeli*	*C. auratus*	*C. rueppeli*	*C. auratus*
3.20	-	-	-	-	-	*12.6
3.25	* 33.1	-	-	-	-	-
3.30	-	-	-	-	-	*20.5
3.35	-	*23.4	-	-	-	-
3.40	-	-	-	* 18.3	-	-
3.45	-	-	-	-	-	* 8.9
3.50	-	-	-	* 10.7	-	-
3.85	-	3.3	# 19.2	# 0.7	-	-
4.00	-	11.7	# 5.9	# 25.00	25.6	-
4.45	-	-	7.8	-	14.3	-
4.50	-	-	6.7	-	4.9	-
5.45	-	-	* 7.4	-	-	-
5.80	-	* 4.7	-	-	-	-
6.50	-	-	-	-	* 7.3	-
7.30	-	-	* 15.6	-	-	-
7.60	* 3.9	-	-	-	-	-
8.01	-	-	-	-	-	* 4.7
8.05	-	3.6	3.5	-	-	-
8.65	-	-	-	-	-	* 13.7
8.80	-	-	-	* 0.5	-	-
8.90	# 4.3	# 4.7	-	0.8	-	-
9.10	-	-	-	* 3.7	-	-
9.20	-	-	8.5	-	1.5	-
9.40	58.8	-	# 25.3	# 40.3	-	39.4
9.50	-	48.5	-	-	46.3	-

* Specific band

common band

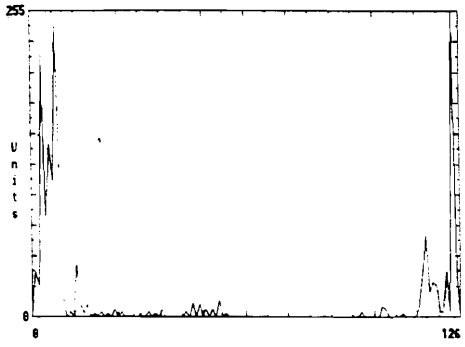
Fig. (1): Photomicrograph of two species in Chrysichthys (Lateral view) showing:

(A) Chrysichthys rueppelli with:

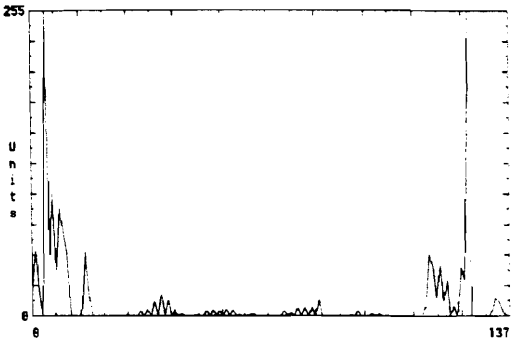
1) small eye
2) Long adipose dorsal fin
3) Short and rounded caudal fin
4) First soft ray of the dorsal fin is not produced

(B) Chrysichthys auratus; with

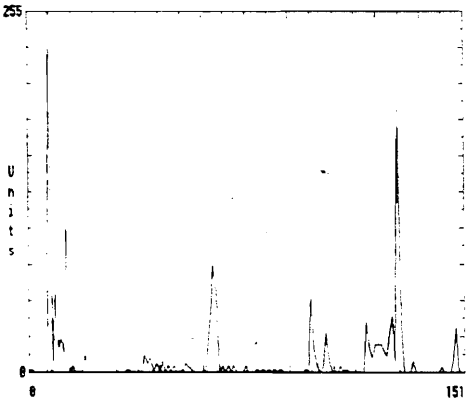
1) oval eye
2) adipose dorsal fin
3) caudal fin



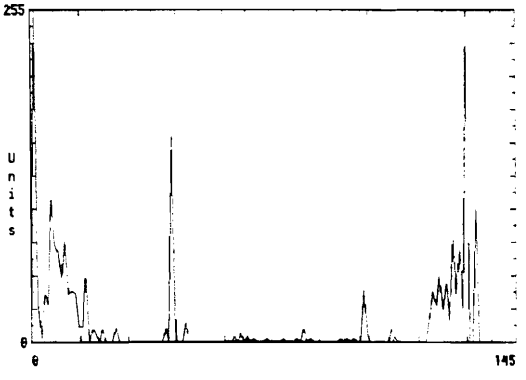
Eye lens *C. rueppelli*



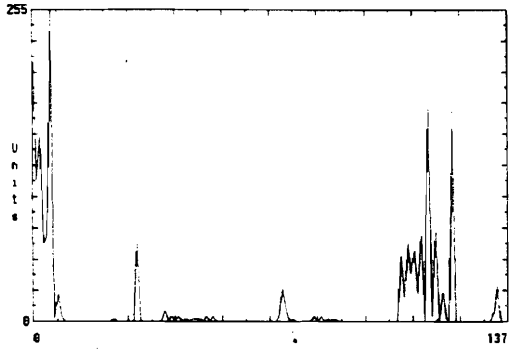
Eye lens *C. auratus*



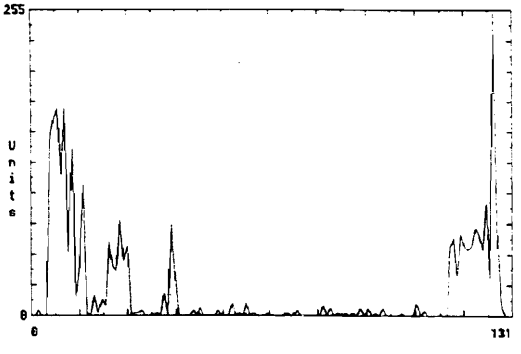
Flesh *C. rueppelli*



Flesh *C. auratus*



Skin *C. rueppelli*



Skin *C. auratus*

Fig. (2): IEF of phast Gel (IEF 3-9) of protein pattern in three type of tissue in (eye lens, flesh and skin ***Chrysichthys rueppelli*** and ***Chrysichthys auratus***.

The flesh protein of ***Chrysichthys auratus*** was identified by four specific protein patterns separated at PI's 3.40, 3.50, 8.80 and 9.10 with relative quantitative value 18.3, 10.7, 0.5 and 3.7 respectively. Six protein fractions were separated from skin of ***C. rueppelli.*** The relative quantitative value varied from 46.3 to 25.6 as maximum and minimum value, table (1) Fig. (2).

The skin protein of ***Chrysichthys rueppelli*** was identified by one specific protein fraction separated at PI 6.50 with relative quantitative value 7.3, while the protein fraction of skin in ***Chrysichthys auratus*** was identified by five specific protein fractions separated at PI's 3.20, 3.30, 3.45, 8.01 and 8.65 with relative quantitative value of 12.6, 20.5, 8.9, 4.7 and 13.7 respectively.

DISCUSSION

Chrysichthys rueppelli and ***Chrysichthys auratus*** are generally considered as two species of subfamily Bagrinea and are found in the coastal, lakes, Nile river, fresh water canals and streams.

Biochemical characterization of soluble protein has proved to have advantage in identifying interspecific differences and has made it possible to identify species specific patterns (Jeng ***et al.*** 1973 and Carpene ***et al.***, 1983). In the present study the utilization of isoelectric focusing technique at pH ranged from (3-9) of soluble protein in eye lens, flesh and skin has easily allowed the accurate identification of ***C. rueppelli*** and ***C. auratus*** as shown in table (1).

The comparison between the two species were facilitated by protein migration to specific pH points in a gradient that corresponds to their isoelectric points (PI's). The differences in the electrophoretic patterns presented in the present work were the character of the investigated species and can be used in comparative study of protein.

There was only one common band in the eye lens tissue in the two species and three common bands in the flesh tissue, that may act as a characteristic feature of subfamily Bagrinea. Each tissue exhibited a distinct protein pattern and could be clearly differentiated between two species by a number of specific bands. In skin tissue there was only specific bands, which confirms that electrophoretic patterns of fish skin extracts are species specific.

The skin protein in *C. rueppelli* was characterized by only one specific band, while *C. auratus* was characterized by five specific bands.*C. rueppelli* and *C. auratus* were also identified and differentiated by two specific bands in the eye lens.

The soluble protein in the flesh of the *C. rueppelli* was characterized by also two specific bands, while in *C. auratus* four specific bands were present. Fig. (2), Table (1).

The differences in the isoelectric focusing patterns in these two species appear to reflect the morphological and behavioral differentiation taking place among these closely related taxa. Starmach, (1977) explained that the arrangement and number of bands depend on the structure of the investigated protein, differentiated according to the genetic code.

Identification of other different species of fish using isoelectric focusing were studied by many authors (Lundstrom, 1983; Ukishima, 1984; NG *et al*, 1986; El-Gharabawy and Zaki 1990a,b; El-Gharabawy, 1991), they concluded that each species of fish had characteristic species specific bands.

The results obtained from the present study have also emphasized the usefulness of electrophoretic technique for phylogenetic purposes. This trend is consistent with the concepts presented for multispecies (Carpene *et al*, 1983); Sunfish species (Whitmore 1986) *Mugil* species (El-Gharabawy *et al* 1991) *Sparidae* species, (Basaglia 1991).

The precise identification of fish species is of prime importance to clarify the taxonomic position of such species in Egypt, as pre-step for rearing, artificial spawning and hybridization required for fish farming.

In conclusion the results reported in the present study for both species *C. rueppelli* and *C. auratus* have shown that each species has a characteristic, species-specific electrophoretic pattern of the protein fractions in the eye lens, flesh and skin.

REFERENCES

Basaglia, F., 1989a. Comparative investigation of the soluble proteins of eye-lens of some species of genera sparus and pagellus (Sparidae, Toleostei). Comparative biochemistry and Physiology; 93(b): 873-876.

Basaglia, F, 1989b. Study of the soluble lens protein from fifteen Sparidae species. Comparative biochemistry and physiology; 94 (13): 625-626.

Basaglia, F., and Marchetti, M.G., 1991. Study of soluble white muscle tissue proteins from fifteen Sparidae species. Journal of Fish Biology, 38: 763-772.

Boulengea, G.A., 1907. The Zoology of Egypt: The fishes of the Nile. Publ. by Hugh Reet, Ltd., London.

Carpene, E. Hakim, G and Cortes, P., 1983. Isoelectric focusing of lateral muscle myogen and hemoglobin of two species of mugilidae. Comp. Bioch. and Physiol 74 (6): 487-491.

El-Gharabawy, M.M and M.I. Zaki, 1990. Comparative study of protein pattern in gonads and pituitary gland of ***Mugil Capito*** in relation to maturation stage using Phast system isoelectric focusing. Assiut Journal of Agricul. Sci. 31 (3): 283-299.

El-Gharabawy, M.M and M.I, Zaki, 1991. Phast system isoelectric focusing as a method for identification of two species of family mugilidae. Assiut. J. Agric. Sci. Vol. 21 (3): 301-311.

El-Gharabawy, M.M., 1991. Identification of five sole species from the Egyptian Mediterranean water by isoelectric focusing. Assuit. J. of Agricultural Sciences, 22 (1): 49-64.

El-Gharabawy, M.M., 1995. Comparative study of soluble white muscle proteins of twelve Sparid species by electrophoresis and isoelectric focusing in the Egyptian Mediterranean water. Bull. Fac. Sci., Assuit Univ. 24 (1-E): 227-248.

Henkeshoven, J and R. Dernick, 1985. Simplified method for silver staining of proteins in Polyacrylamide gels and the mechanism of silver staining. Electrophoresis, 6: 103-112.

Herzberg, A. and Pasteur, R., 1974. The identification of gray mullet species by the disc electrophoresis. Aquaculture, 5: 99-106.

Jeng, S.S.; Lee, Y.S.; Yee, Y.S.; Tamg, T.M. and Wang, T.H., 1973. Studies on polymorphism of scomber australasicus are revealed by electrophoresis of red muscle protein. Bull. of Japanese Society for Scientific Fisheries, 39: 295-298.

Kamel, S.A., 1987. Identification of some fish species from the Egyptian Mediterranean water by isoelectric focusing. Proc. Zool. Soc. A.R. Egypt. 14: 169-177.

Lundstrom, R.C., 1980. Fish species identification by thin layer Polyacrylamide gel isoelectric focusing collaborative study. J. Assoc. off. Anal. Chem 63(1): 69-73.

Lundstrom, R.C., 1981. Fish species identification by isoelectric focusing. Sarcoplasmic protein polymorphism in monkfish (***Liphius americanus***) J. Assoc. off. Anal. Chem. 64 (1): 32-37.

Lundstrom, R.C., 1983. Identification of the pacific Rockfish (Sebatue) species by isoelectric focusing. J. Assoc. Off. Anal. Chem. 66 (4): 974-980.

Ng, C.S.; Low, L.K.; Lam, C.P. and Yamada, J., 1986. Thin layer Polyacrylamide isoelectric focusing as a method for species identification of tropical fishes. Singapore. J. Pri. Ind. (141): 36-45.

Starmach, J., 1977. Elektrofortely rozdzial no zelu poliakryloamidowym bialek surowicy krwi siedmiu rodzin karpi-electrophoretic separatation of blood serum proteins on polycrylamide gel in seven corp families. Acta Hydrobiolo. 19, (2): 163-167.

Ukishima, Y. Narita, H. Hasui, T. and Nara, M., 1984. Studies on the application of thin layer isoelectric focusing to food analysis. I. Identification of fish species of sliced escolor and castor oil fish. Eisei Kagaku; 30 (4): 198-193.