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JUSTIFICATION OF THE TAXONOMIC ASSIGNMENT OF MUNGBEAN PLANT TO THE GENUS *VIGNA*, SAVII. PHYTOCHEMICAL FEATURES

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ABSTRACT

This investigation was carried out to justify the assignment of mungbean plant from the genus *Phaseolus* to the genus *Vigna*. Electrophoretic studies on seed storage proteins (albumin, globulin and total soluble proteins) using SDS-PAGE proved that mungbean plant is more related to cowpea plant with similarity of 21% compared with 9% between plants of mungbean and kidney bean, suggesting the assignment of mungbean plant to the genus *Vigna*.

Further phytochemical studies were carried out to compare between plants of mungbean and cowpea. Percentages of the main chemical components in seeds were as follows:

Total carbohydrates 67.93, 65.65; crude protein 23.90, 24.62; lipids 1.20, 1.30; fibres 4.07, 5.07; ash 2.90, 3.36 and moisture 6.06, 6.16% for mungbean and cowpea, respectively. Moreover, seeds of mungbean and cowpea positively shared the presence of 16 amino acids. These results strengthen the assignment of mungbean plant to the genus *Vigna*.

Electrophoretic analysis of seed storage proteins, and main chemical components of seeds suggest that mungbean plant is more closely related to the genus *Vigna*. Hence the name *Vigna radiata* (L.) Wilczek might be accepted as the name of choice for mungbean plant. Morphological and anatomical features through the consecutive ages of 2 growing seasons of mungbean and cowpea plants are considered in the following two parts of this study as extra evidences in this concern.

Keywords: *Vigna*, *Phaseolus*, mungbean, cowpea, kidney bean, electrophoresis, seed chemical components.

INTRODUCTION

The mungbean is a leguminous species, or pulse crop, grown principally for its protein-rich edible seeds. Duke (1981) reported that dried whole seeds of mungbean contain (per 100 g): 341 calories, 10.6% moisture, 22.9 g protein, 1.2 g fat, 61.8 g total carbohydrate, 4.4 g fibre, 3.5 g ash, 105 mg Ca, 330 mg P, 7.1 mg Fe, 6 mg Na, 1132 mg K, 55 mg *B*-carotene equivalent, 0.53 mg thiamine, 0.26 mg riboflavin, 2.5 mg niacin and 4 mg ascorbic acid. However, raw mature seeds of cowpea typically contain (per 100 g): *ca*. 11.4 % moisture, 338 calories, 22.5 g protein, 1.4 g fat, 61.0 g total carbohydrate, 5.4 g fibre, 3.7 g ash, 104 mg Ca, 416 mg P, 0.08 mg thiamine, 0.09 mg riboflavin, 4.0 mg niacin and 2.0 mg ascorbic acid. In results at IITA, based on several thousand distinct cultivars, protein averaged 23-25%, ranged from 18 to 29%, with potential for perhaps 35%. The proteins consist of 90% water-insoluble globulin and 10% water-soluble albumins.

For food, the seeds of mungbean are prepared by cooking, fermenting, milling, or sprouting. Seeds are utilized in making soups, curries, bread, sweets, noodles, salads and many other culinary products. Among the pulses, the mungbean is favoured for children and the elderly due to its easy

digestibility and low production of flatulence protein in the seeds averages around 24% (Poehlman, 1991).

The mungbean is native to the India-Burma area of Southeast Asia. From Asia, it spread into the Middle East, the Pacific Islands, East Africa, Australia and the Americas, but Asia continues to be the region for major production. Fruitful efforts have been made by egyptian investigators to benefit from mungbean as a pulse crop to be cultivated under local conditions. These efforts resulted in producing the local mungbean cv. Kawmy 1 being registered and certified in 1997 by the Egyptian Ministry of Agriculture.

The nomenclature of mungbean has been confusing. For many years the species was classified in the genus *Phaseolus* along with the common bean. However, Piper and Morse (1914) and Bose (1932) stated that Roxburgh in 1832 transposed the original Linnean names and designated the green seeded mungbean, *Phaseolus mungo* Willd., the yellow seeded mungbean, *P. aureus* Roxb. In 1903, Prain called mungbean *P. radiatus* L.. Later, Bose (1939) referred to mungbean as *P. radiatus* L. syn. *P. aureus* Roxb. During the next 30 to 40 years the names *P. radiatus* L. *P. aureus* Roxb. were both used for mungbean in the literature.

The distinction between the genera *Phaseolus* L. and *Vigna* Savi was partially based on the degree that the beak of the keel was incurved and on the form of a bearded style. Upon re-examination, new evidence suggested that certain asiatic species placed in *Phaseolus* by the above criterion more closely resembled species of *Vigna* than the american species *Phaseolus*. This led Wilczek (1954) to transfer mungbean and other closely related Asian species formerly classified in *Phaseolus* L. to *Vigna* Savi.

Reclassification of the species from *Phaseolus* to *Vigna* has been supported by some later studies (Taylor, 1966; Sahai and Rana, 1977 and Renganayaki *et al*., 1987).

This study is an attempt to justify the assignment of mungbean from the genus *Phaseolus* to the genus *Vigna.* The present paper, which is the first of 3 parts study, deals with electrophoretic analysis of seed storage protein, in addition to determination of percentages of main chemical constituents in seeds of mungbean, cowpea and kidney bean to define which of cowpea and kidney bean is more related to mungbean. In the following two parts of this study comparative morphological and anatomical features of mungbean and the genus being more closely related will be carried out. Such study might conceivably add precise evidence to the taxonomic identity of mungbean plant.

MATERIALS AND METHODS

Source of seeds: Seeds of mungbean (*Vigna radiata* (L.) Wilzek cv. Kawmy 1) were procured from Field Crop Research Institute, National Research Centre, Dokki, Giza, Egypt. Whereas, seeds of cowpea (*Vigna sinensis* Savi cv. Dokki 331) and kidney bean (*Phaseolus vulgaris* L. cv. Giza 6) were

secured from Department of Vegetable Research, Horticultural Research Institute, Agricultural Research Centre, Giza, Egypt.

Electrophoretic identification of seed protein: Identification of protein in seeds of mungbean, cowpea and kidney bean was carried out in Agricultural Analysis Unit, Faculty of Agriculture, Cairo University, Giza, Egypt. Seed samples of the investigated plants were subjected to protein identification by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS – PAGE) according to the method of Laemmli (1970). Data of the comparative chemical studies of protein in the seeds of investigated plant taxa were subjected to regenerate similarity coefficients according to Jaccard (1908). The similarity coefficients were used to construct dendrograms through the clustering method of UPGMA (Unweighed Pair-Group Method with Arithmetical Averages) using NTSYS-PC (Rohlf, 1993).

Chemical analysis of main constituents in seeds: The following determinations were carried out on dry weight basis:

Determination of total carbohydrates: Total carbohydrates were determined spectrophotometrically (as glucose) after acid hydrolysis using phenol sulphuric acid reagent (Dubois *et al*., 1960).

Determination of crude protein: Total nitrogen content was determined using the modified micro-Kjeldahl method described by Pregl (1945). Nitrogen content of seeds was multiplied by 6.25 to calculate the crude protein (Anon., 1975).

Determination of total lipids: Total lipids were determined as petroleum ether soluble fraction (Anon., 1975).

Total amino acids detection: Detection of amino acids was performed (Anon., 1987). The apparatus used was Spectra – Physics Analytic, Inc. A 0099-600 with spectra focus optical scanning detector and spectra system UV 2000 detector and ultrasphere C18 Beckman column. The analysis was carried out using a gradient of Pico - Tag solvent A & B at 40 ºC and flow rate 1 ml/min. Detection of the separated Pico-Tag amino acids was carried out at 254 nm wave length. Before injection of the sample, the illustrate was calibrated by two injections of the standards.

Crude fibres, ash and moisture content were determined as well (Anon., 1975).

RESULTS AND DISCUSSION

Electrophoretic identification of seed storage protein:

Seeds of kidney bean, cowpea and mungbean are photographed in Figure (1). An electrophoretic separation of protein in these seeds using SDS-PAGE was performed (Figure 2), The protein in seeds of each of the three investigated taxa was extracted using different solvents, namely; H_2O (albumin), NaCl 1% (globulin) and tris-boric buffer (total soluble proteins).

(A) (B) (C)

Fig. (1): Mature seeds of (A) Kidney bean cv. Giza 6, (B) Cowpea cv. Dokki 331 and (C) Mungbean cv. Kawmy 1.

A B C

1 2 3 4 5 6 7 8 9 M

Fig. (2): SDS-PAGE 12% separation of seed protein subunits of (A): Kidney bean cv. Giza 6, (B): Cowpea cv. Dokki 331 (C): Mungbean cv. Kawmy 1; where: 1,4 and 7: H_2O extract (albumin), 2,5 and 8: NaCl 1% extract (globulin) and 3, 6 and 9: Tris-boric buffer extract (total soluble proteins). (M): Low molecular weight protein marker (Pharmacia).

Data of SDS-PAGE of albumin in seeds of the three investigated plant species are given in Table (1) and Figure (2). It is evident that of 15 bands, kidney bean and cowpea showed 4 bands, all of them were monomorphic in case of kidney bean. As to cowpea, 3 bands were monomorphic. However, mungbean recorded 8 bands; 7 of them were monomorphic. Kidney bean did not share either cowpea or mungbean in any of the recorded bands.

Table (1): Densitometer analysis of albumin SDS-PAGE in seeds of the three investigated taxa.

Band No.	Relative front (Rf)	M.W. (KD)	Kidney bean	Cowpea	Mungbean
1	0.067	107.963	+	-	-
2	0.079	105.220	-	-	+
3	0.091	102.608	-	+	-
4	0.122	96.159	-	-	+
5	0.183	84.552	-	-	+
6	0.207	80.311	-	-	+
7	0.271	70.164	-	-	+
8	0.283	68.506	-	+	-
9	0.304	62.827	-	+	-
10	0.482	33.282	+	-	-
11	0.524	30.248	-	-	+
12	0.591	24.368	+	-	-
13	0.620	22.029	-	+	+
14	0.637	20.811	+	-	-
15	0.799	15.013	-	-	+

In contrast, cowpea and mungbean shared 1 band at M.W. 22.029 KD, and Rf 0.620. This implies that cowpea and mungbean are related to each other more than to kidney bean.

Results of SDS-PAGE of globulin in seeds of the three investigated taxa are given in Table (2) and Figure (2). It is obvious that 19 bands were recorded. Kidney bean recorded 4 bands of this total number of bands. However, both of cowpea and mungbean recorded 10 bands. Cowpea and mungbean shared 4 out of these 10 bands at M.W. 120.049, 42.119, 21956 and 19.209 KD and Rf 0.017, 0.376, 0.621 and 0.677, respectively. Kidney bean and mungbean shared 1 band at M.W. 106.878 KD and Rf 0.072. It is worthy to mention that kidney bean proved monomorphic for 3 bands. However, cowpea was monomorphic for 6 bands and mungbean was monomorphic for 5 bands.

Data of SDS-PAGE of total soluble proteins in seeds of the three investigated taxa are shown in Table (3) and Figure (2). It is evident that of 24 bands, kidney bean, cowpea and mungbean recorded 7, 12 and 14 bands, respectively. Cowpea and mungbean shared 5 bands at M.W. 113.941, 78.160, 30.236, 22.108 and 18.103 KD and Rf 0.042, 0.220, 0.525, 0.619 and 0.607, respectively. The band at M.W. 78.160 KD and Rf 0.220 was common in kidney bean as well. Kidney bean shared cowpea 1 band at M.W. 25.326 KD, and Rf 0.581. However, kidney bean and mungbean shared 2 bands at M.W. 106.488 and 12.418 KD and Rf 0.074 and 0.893, respectively. It is worthy to mention that there were 3, 6 and 7 monomorphic bands in case of kidney bean, cowpea and mungbean, respectively.

These results prove the existence of wide range of differences among kidney bean and both of cowpea and mungbean. In the mean time, the latter two species were more similar.

Table (2): Densitometer analysis of globulin SDS-PAGE in seeds of the three investigated taxa.

Band No.	Relative front (Rf)	M.W. (KD)	Kidney bean	Cowpea	Mungbean
1	0.017	120.049	-	+	+
2	0.037	115.081	-	-	+
3	0.046	112.935	+	-	-
4	0.055	110.854	-	+	-
5	0.072	106.878	+	-	+
6	0.088	103.326	-	+	-
7	0.117	97.258	-	-	+
8	0.178	85.451	-	-	+
9	0.197	82.083	-	+	-
10	0.282	68.573	-	-	+
11	0.299	64.802	+	-	-
12	0.312	59.828	-	+	-
13	0.359	45.215	-	-	+
14	0.376	42.119	-	+	+
15	0.527	30.054	-	+	-
16	0.567	26.609	+	-	-
17	0.579	25.508	-	+	-
18	0.621	21.956	-	+	+
19	0.677	19.209	-	+	+

Table (3): Densitometer analysis of total soluble proteins SDS-PAGE in seeds of the three investigated taxa.

Band No.	Relative front (Rf)	M.W. (KD)	Kidney bean	Cowpea	Mungbean
1	0.012	121.205	-	+	-
2	0.019	119.594	-	-	+
3	0.042	113.941	-	+	+
4	0.058	110.107	+	-	-
5	0.074	106.488	+	-	+
6	0.086	103.818	-	+	-
7	0.118	97.036	-	-	+
8	0.153	90.080	-	+	-
9	0.198	81.881	-	-	+
10	0.220	78.160	+	+	+
11	0.309	61.004	-	+	-
12	0.322	56.398	-	-	+
13	0.340	50.502	+	-	-
14	0.362	44.321	-	-	+
15	0.384	41.430	-	-	+
16	0.477	33.651	-	-	+
17	0.525	30.236	-	+	+
18	0.581	25.326	+	+	-
19	0.619	22.108	-	+	+
20	0.663	19.769	+	-	-
21	0.607	18.103	-	+	+
22	0.847	13.616	-	+	-
23	0.983	12.418	+	-	+
24	0.976	10.503	-	+	-

Similarity indices of seed storage protein of the three investigated plant species based on SDS-PAGE are represented in Table (4). This is also illustrated dendrogrammatically in Figure (3).

The albumin dendrogram indicates that the three tested plant species were classified into two main clusters. The first cluster contained kidney bean alone. The second cluster included both cowpea and mungbean. These results ensure the densitometer analysis of albumin and strengthen the likelihood that cowpea and mungbean are related to each other (9%).

The globulin dendrogram showed the same trend. Two clusters were detected. The first cluster included kidney bean only. While the second cluster grouped both cowpea and mungbean together. This indicates that both cowpea and mungbean are more related to each other (25%), compared with the similarity between kidney bean and mungbean (8%).

Furthermore, dendrogram of the total soluble proteins supported the previously recorded relationship. Mungbean was more related to cowpea with similarity of 19%. However, the similarity between mungbean and kidney bean was lesser (11%).

Data represent summation of the previous electrophoretic studies of seed storage proteins (albumin, globulin and total soluble proteins) of the three investigated plant species (Table, 4 and Figure, 3) prove that mungbean and cowpea are more closely related to each other with similarity of 21%. However, similarity between mungbean and kidney bean was 9% and that between cowpea and kidney bean was 6%.

Further phytochemical studies dealing with the main chemical constituents in seeds of mungbean and cowpea are given in the following to support the previous findings.

Table (4): Similarity among the three studied taxa based on SDS-PAGE of seed storage protein

Characters	Kidney bean	Cowpea	Mungbean
1- Albumin:			
Kidney bean	1.00		
Cowpea	0.00	1.00	
Mungbean	0.00	0.09	1.00
2- Globulin:			
Kidney bean	1.00		
Cowpea	0.00	1.00	
Mungbean	0.08	0.25	1.00
3- Total soluble proteins:			
Kidney bean	1.00		
Cowpea	0.11	1.00	
Mungbean	0.11	0.19	1.00
The sum of 1, 2 and 3:			
Kidney bean	1.00		
Cowpea	0.06	1.00	
Mungbean	0.09	0.21	1.00

Chemical constituents of seeds.

Data on quantitative determination of carbohydrates, proteins, lipids, fibres, ash and moisture in mungbean and cowpea seeds are given in Table (5). It is evident that total carbohydrates recorded the highest percentage among constituents of seeds followed by crude protein. In contrast, lipids were the lowest in this respect. Worthy to note that percentages of

constituents in seeds of both mungbean and cowpea were almost similar. The mentioned percentages of chemical constituents of seeds are in harmony with those given by Duke (1981).

Table (5): Percentage of constituents in seeds of mungbean and cowpea

Species	Total carbohydrates	Crude protein	Lipids	Fibres	Ash	Moisture
Mungbean	67.93	23.90	1.20	4.07	2.90	6.06
Cowpea	65.65	24.62	1.30	5.07	3.36	6.15

Data concerning analysis of amino acids in seeds of mungbean and cowpea are given in Table (6). These results prove that mungbean seeds contain 16 amino acids of 23.90%. Worthy to note that tyrosine (10.06%) and glutamic (4.67%) are limiting amino acids in seeds of mungbean. They recorded the highest percentage among amino acids detected in mungbean seeds.

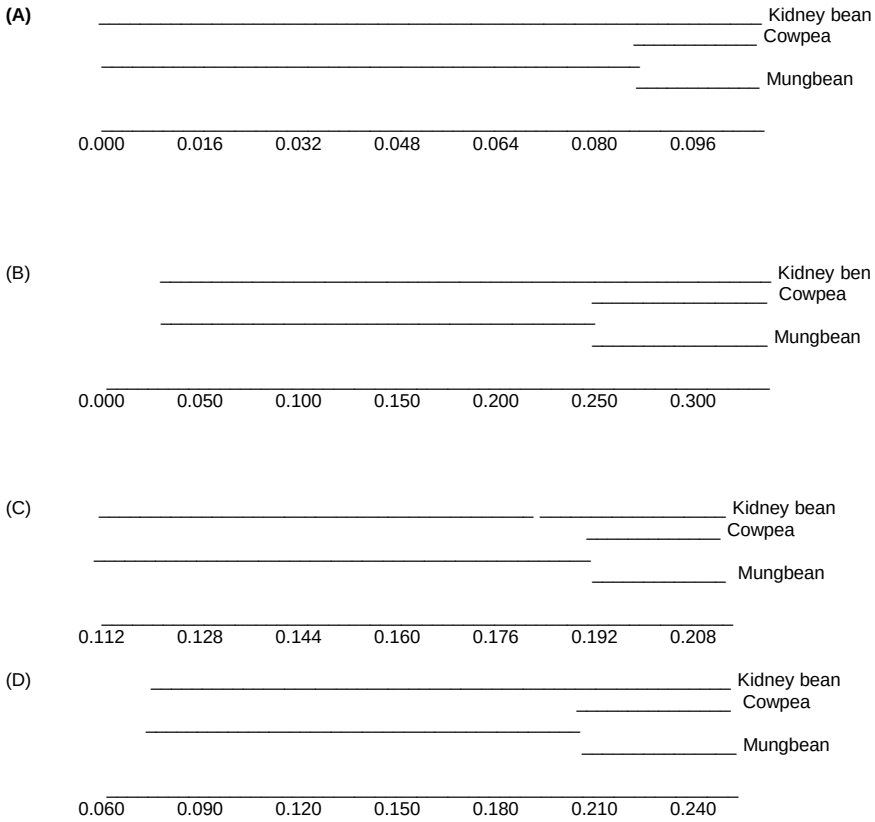

Fig. (3): Dendrograms of Jaccard similarity obtained by the UPGMA method showing relationship among the three studied plant species based on SDS-

PAGE of: (A): albumin, (B): globulin, (C): total soluble proteins and (D): the sum of A, B and C.

Methionine (0.0063%) and cystine (0.0509%) showed the least percentage of amino acids in mungbean seeds, being found in minute fraction. However, the remainder amino acids in seeds ranged between 0.3 – 2.6%.

It is evident that among 17 recorded amino acids, cowpea seeds are rich in the amino acids phenylalanine (5.71%), proline (3.61%), tyrosine (2.93%), glutamic (2.93%) and isoluecine (2.49%). In contrast, some other amino acids were found as traces. These are alanine (0.0022%), therionine (0.0047%), arginine (0.0088%) and histedine (0.0224%). However, the remainder of amino acids in cowpea seeds existed at a moderate percentage ranging from 0.19 to 1.99. Worthy to mention that, phenylalanine is a limiting amino acid in seeds of cowpea.

Table (6): Percentage of amino acids in seeds of mungbean and cowpea

No.	Amino acids	Actual %	
		Mungbean	Cowpea
1	Aspartic	2.6012	0.7420
2	Glutamic	4.6742	2.9272
3	Serine	0.5938	0.1875
4	Glycine	0.2560	0.6640
5	Histedine	0.8063	0.0224
6	Arginine	0.3072	0.0088
7	Therionine	-	0.0047
8	Alanine	0.3121	0.0022
9	Proline	2.1772	3.6076
10	Tyrosine	10.0635	2.9330
11	Valine	0.4618	1.4771
12	Methionine	0.0063	1.0683
13	Cystine	0.0509	0.2267
14	Isoluecine	0.4121	2.4881
15	Luecine	0.3395	1.9946
16	Phenylalanine	0.5342	5.7090
17	Lysine	0.3037	0.5568
	Crude protein	23.90	24.62

CONCLUSION

An electophoretic analysis was carried out for the three studied plant species using SDS-PAGE of albumin, globulin and total soluble proteins of seeds to find out their relationship to each other. Data obtained were subjected to regenerate similarity coefficients and construction of dendrograms showing the suggested relationships among the investigated plant species. The resulting data proved that cowpea and mungbean are more

closely related to each other justifying their high degree of similarity (21%). On the other hand, it was evident that kidney bean did not share cowpea (6%) and mungbean (9%) such relationship. In addition, the percentage of the main chemical constituents in seeds of mungbean and cowpea were almost similar. Moreover, seeds of mungbean and cowpea positively shared the presence of 16 amino acids.

Hence, the concept of belonging mungbean to the same genus of cowpea is reasonable, and the suggested scientific name of mungbean: *Vigna radiata* (L.) Wilczek might be accepted. Such concept is subjected to further investigations in the following 2 parts of this study dealing with morphological and anatomical features of mungbean and cowpea plants throughout the consecutive ages of 2 growing seasons in an attempt to gain additional evidences in this respect.

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تقنين الوضع التصنيفي لنبات فول المانج وتبعيته الى جنس اللوبيا ,*Vigna* Savi

أولا: الخصائص الكيميائية النباتية

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أجريت هذه الدراسة لتقنين نقل نبات فول المانج من جنس الفاصوليا ***Phaseolus*** الي جنس اللوبيا ***Vigna***، ولقد برهنت دراسات التفريد الكهربي للبروتينات المخزنة بالبذرة (الالبيومينات ، الجلوبيولينات والبروتينات الكلية الذائبة) باستخدام SDS-PAGE أن نبات فول المانج أكثر قرابة الى اللوبيا وبتماثل قدره ٢١% مقارنة بتماثل ٩% بين نباتات فول المانج والفاصوليا ، مما يرجح تبعية نبات فول المانج الى جنس اللوبيا.

تم اجراء المزيد من الدراسات الكيميائية النباتية للمقارنة بين نباتات فول المانج واللوبيا ووجد أن النسبة المئوية للمكونات الكيميائية الرئيسية فى البذور كالتالى:

الكربوهيدرات الكلية ٦٧,٩٣ و٦٥,٦٥ - البروتين الخام ٢٣,٩٠ و ٢٤,٦٢ – الدهون ١,٢٠ و ١,٣٠ – الالياف ٤,٠٧ و ٥,٠٧ – الرماد ٢,٩٠ و ٣,٣٦ – الرطوبة ٦,٠٦ و ٦,١٦ % فى كل من فول المانج واللوبيا على التوالي. بالأضافة الي ذلك فقد تماثل وجود عدد ١٦ حمضاً أمينياً فى كل من فول المانج واللوبيا، وتدعم هذه النتائج تبعية نبات فول المانج الى جنس اللوبيا.

تقترح دراسات التفريد الكهربي للبروتينات المخزنة بالبذور بالاضافة الى المكونات الكيميائية الرئيسية بالبذور أن نبات فول المانج أكثر قرابة الى جنس اللوبيا ولذلك فأن الاسم العلمى:

***Vigna radiata* (L.) Wilczek** المقترح لنبات فول المانج يمكن قبوله من الناحية التصنيفية. وسوف يتم اجراء دراسة للخصائص المورفولوجيه التشريحية خلال الاعمار المتتالية لموسمي نمو فى الجزئين التاليين من هذه الدراسة لتوفير المزيد من الدلائل فى هذا الشأن.