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Seed morphology and surface microstructure of the genus *Plectranthus* L. (Lamiaceae) in Arabian Peninsula highlighting on their systematic implications

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Abstract

Seed coat morphology of 13 species of *Plectranthus* L. in Arabian Peninsula was comparatively examined using scanning and light microscopy methods. The diagnostic values for systematic were studies. Macro- and micro-morphological characteristics, including seed shape, colour, size, seed surface, epidermal cell shape, anticlinal boundaries, and periclinal cell wall are presented. Descriptions of seed size, shape, colour, surface, and seed coat types are summarised for the genus. Taxonomic phylogenetic implications of the seed coat micromorphology are also discussed in comparison with the available gross morphological and molecular data. Results of the seed character analyses offered useful data for evaluating the taxonomy of *plectranthus* both on subgeneric and sectional levels. Monophyly of both subgenus *Burnatastrum* (Briq) Codd, and *Germanea* (Benth.) Briq. is also supported here. The remarkable result of this study was to identify *P. arabicus* as a separate group. The results suggested that it should be treated as a separate subgenus because of having reddish-brown colour seeds and flat-to-convex periclinal cell wall which is distinguishable from the rest of the species. Likewise, the endemic species of *P. asirensis*, *P. ovatus*, and *P. hijazensis* group and *P. hyemalis* and *P. hadiensis* group should be homogeneous groups. A key for the identification of the investigated taxa based on seed characters is also provided here.

Keywords: Arabian Peninsula, morphology, *Plectranthus*, seed, systematic implications.
Abbreviations: SEM_Scanning Electron Microscope, UPGMA_Unweighted Pair Group Method with Arithmetic Mean, trnL_intron; trnL-trnF_inter gene spacer, *rps*_ribosomal protein S, RAPD_Random amplified polymorphic DNA.

Introduction

Lamiaceae is a large family that extended, widely spread and adapted to nearly all habitats and altitudes. The genus *Plectranthus* is one of the largest genera of Lamiaceae, belonging to the subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae. It comprising about 300 species distributed in both tropical and warm regions of the Old World (Codd, 1985; Retief, 2000). A phylogenetic study of the tribe Ocimeae, based on plastid genes to which *Plectranthus* belongs, found that *Plectranthus* is paraphyletic (Paton et al., 2004). Bentham (1832, 1848) monographed the species of *Plectranthus*, and divided *Plectranthus* into seven sections: *Germanea* (Lam.) Benth., *Coleoides* Benth., *Heterocylix* Benth., *Melissoides* Benth., *Isodon* Schrad. Ex Benth. *Pyramidium* Benth. and *Amethvstoides* Benth. However, In Bentham and Hooker, (1876), Bentham latter revised this arrangement, recognizing two primary groups: Sect. *Germanea* and sect. *Isodon.* In sect. *Germanea*, with *Germanea* and *Coleoides* as subsections (in which the great majority of conventional *Plectranthus* spp. are placed), the calyx is 2-lipped with the upper lip consisting of a single broad tooth and the lower lip of four narrower acute or acuminate teeth: the cymes are usually sessile with the pedicels arising from the axis of the inflorescence. Sect. *Isodon*, with *Isodon*, *Pyramidium*, *Amethvstoides* and *Melissoides* as subsections, was distinguished by the calyx being equally 5-toothed, in some groups more or less 2-lipped with the upper lip composed of 3 teeth and the lower lip of 2 teeth: the cymes are pedunculate and branched. A similar classification was accepted by Briquit (1897); however, *Germanea* and *Isodon* were treated as subgenera. In each subgenus the largest sections, *Coleoides* and *Isodon*, respectively, were subdivided into a number of series. Codd (1975) revised the genus in South Africa and divided *Plectranthus* into five subgenera: *Nodiflorus* Codd, *Burnatastrum* (Briq) Codd, *Coleus* (Lour.) Codd, *Calceolanthus* Codd and *Plectranthus* based on the inflorescence characters. Lukhoba et al. (2006) reviewed the genus *Plectranthus* and its ethno-botanical uses extensively, providing a comprehensive understanding of the global ethno botany of *Plectranthus*. Their review touches on the horticultural uses of the genus. The Arabian Peninsula, the great part of which is hot desert, encompasses very few Labiate. However, there are mountains where Forsskohl and tribe Ocimoideae were found, providing several new Labiatae belonging to that tribe. In the present study, 13 species cover and describe all native *Plectranthus* plants of the Arabian Peninsula (Saudi Arabia, Yemen, and Oman). In the Flora of Yemen, Wood (1997) revised *Plectranthus*, and reported 12 species. However, in the flora of Saudi Arabia, Collenette (1999) reported *Plectranthus* by seven species: *P. arabicus*, *P. cylindraceus*, *P. tenuiflorus*, *P. comosus*, *P. barbatus*, *P. pseudomarrubioides* and *P. asirensis*, but Chaudhary (2001)

accepted only 6 species viz. *P. arabicus, P. cylindraceus, P. tenuiflorus, P. lanuginosus, P. barbatus,* and *P. asirensis*. Correspondingly, in Flora of Oman, Ghazanfar (2003) reported only *P. barbatus, P. cylindraceus* and *P. hadiensis*. Some species of *Plecteranthus* are difficult to identify because of a lack of clear cut morphological criteria to discriminate not only among species within the genus but also among the closely related genera. This has resulted in numerous taxonomic problems in the naming of species with the result that species have often been placed in several closely related genera like *Coleus* (Paton et al., 2004).

Seed morphology provides a number of characteristics that are potentially useful for species identification, phylogenetic implication, and character-state evolution (Barthlott, 1981, 1984; Moazzeni et al., 2007; Abdel Khalik, 2010, 2013; Abdel Khalik and Hassan, 2012; Hassan and Abdel Khalik, 2014). However, there is no information dealing with seed morphology of *Plectranthus*. The aim of the present study is to estimate the importance of seed micromorphological characters for the infrageneric classification of *Plectranthus*, by means of cluster analyses and to determine whether data on seed micromorphology can contribute additional knowledge about seed shape and seed coat in the studied taxa.

Results

Seed characters

The seed morphological characters of the studied taxa of the genus *Plectranthus* as shown by light microscopy and SEM are revised in (Table 1 and Figs 1-5).

Seed shape

The seed shape varies from ovoid in *P. amboinicus* (Fig. 1 A1), *P. asirensis* (Fig. 1 A3), *P. barbatus* (Fig. 2 A4), *P. hijazensis* (Fig. 3 A7), *P. hyemalis* (Fig. 3 A8) and *P. lanuginosus* (Fig. 4 A11), sub-spherical in the *P. arabicus* (Fig. 1 A2), *P. cylindraceus* (Fig. 2 A5), *P. pseudom arrubioides* (Fig. 4 A12) and *P. tenuiflorus* (Fig. 4 A13), obovoid in *P. ignarius (*Fig. 3 A9), to elliptical in *P. hadiensis* (Fig. 2 A6) and *P. ovatus* (Fig. 4 A11).

Seed color

The colors of the seeds are of less diagnostic and systematic value among species. The colors vary from yellowish-brown in *P. cylindraceus* and *P. pseudomarrubioides*, grayish brown in *P. ignarius* and *P. lanuginosus*, brown in *P. amboinicus*, black in *P. barbatus*, to reddish-brown in the rest of taxa.

Seed surface (ridges)

Seed ridges showed great variation among the studied taxa. They vary from seeds with narrow ridges in *P. hadiensis* (Fig. 2 A6) and *P. hyemalis* (Fig. 3 A8) to no ridges (wingless) in the rest of the species.

Seed size

Seed measurements vary significantly among the examined taxa. The largest seeds were recorded in *P. asirensis, P. barbatus, P. hijazensis, P. ignarius* and *P. lanuginosus* with ca 1.1-1.6 X 0.7-1.4 mm and the smallest ones in *P. amboinicus, P. arabicus, P. cylindraceus* and *P. pseudomarrubioides* measuring ca 0.6-0.8 × 0.5-0.6 mm, but in *P. hadiensis, P. hyemalis* and *P. tenuiflorus* has slightly larger seeds, measuring 0.8-1 × 0.7-0.9 mm.

Outer epidermal cell shape

The cellular shapes can be of considerable diagnostic systematic value. The epidermal cells are polygonal, varying from isodiametric to poly gonals in *P. hadiensis* (Fig. 2 B6) and *P. hijazensis* (Fig. 3 B7); isodiametric and 5-6 gonals in *P. amboinicus* (Fig. 1 B1), *P. arabicus* (Fig. 1 B2), *P. asirensis* (Fig. 1 B3), *P. barbatus* (Fig. 2 B4) and *P. cylindraceus* (Fig. 2 B5), *P. ignarius* (Fig. 3 B9) and *P. ovatus* (Fig. 4 B11); 5, 6 gonals in *P. pseudomarrubioide* (Fig. 4 B12) and *P. tenuiflorus* (Fig. 4 B13), to irregular to poly gonals in *P. hyemalis* (Fig. 3 B8) and *P. lanuginosus* (Fig. 4 B10).

Anticlinal cell wall boundaries

These are mostly well developed. There are 3 types of cell wall boundaries: the first type is undulate in *P. hyemalis* (Fig. 2 B8); the second type is straight to slightly sinuous in *P. barbatus* (Fig. 2 B4), *P. hadiensis* (Fig. 2 B6), *P. hijazensis* (Fig. 3 B7) and *P. lanuginosus* (Fig. 4 B10); the third type is straight in the rest of the taxa (Figs. 1-4 B1, B2, B9, B11, B12, B13). Based on the relief of cell wall boundaries, there are 3 types of boundaries: slightly raised in *P. lanuginosus* (Fig. 4 B10); raised as in *P. hyemalis* (Fig. 3 B8); and channelled as in the rest of the taxa. The sculpture of the anticlinal walls of the studied taxa is of less significant. It varies from fine folds in *P. barbatus* (Fig. 2 B4), *P. hadiensis* (Fig. 2 B6), *P. hijazensis* (Fig. 3 B7), *P. hyemalis* (Fig. 3 B8), *P. lanuginosus* (Fig. 4 B10) and *P. ovatus* (Fig. 4 B11) to smooth in the rest of the taxa.

Periclinal cell wall

The curvature of the outer wall can serve as a good diagnostic character. There are 4 different shapes for the outer periclinal cell wall: (i) flat in *P. asirensis* (Fig. 1 B3), *P. barbatus* (Fig. 2 B4), *P. ignarius* (Fig. 3 B9) and *P. tenuiflorus* (Fig. 5 B13); (ii) flat to slightly concave in *P. cylindraceus* (Fig. 2 B5) and *P. pseudomarrubioides* (Fig. 4 B12); (iii) convex in *P. hadiensis* (Fig. 2 B6) and (V) flat to convex in *P. amboinicus* (Fig. 1 B1), *P. arabicus* (Fig. 1 B2), *P. hijazensis* (Fig. 3 B7), *P. lanuginosus* (Fig. 4 B10) and *P. ovatus* (Fig. 4 B11). The outer surface of the cell wall shows less variation among the studied species. It is fine folds in *P. asirensis* (Fig. 21 B3), *P. barbatus* (Fig. 2 B4), *P. hadiensis* (Fig. 2 B6), *P. hijazensis* (Fig. 3 B7), *P. hyemalis* (Fig. 3 B8), *P. lanuginosus* (Fig. 4 B10) and *P. ovatus* (Fig. 4 B11), and smooth in the rest of the taxa.

Cluster analysis

The results of the cluster analyses are presented in (Figure 6, Table 2). In the UPGMA dendrogram, 5 major clusters and branches (A–E) with approximately 0.60 similarity are distinguished:1) branch A includes *P. amboinicus* and *P. ignarius*; 2) cluster B contains *P. asirensis*, *P. barbatus*, *P. ovatus* and *P. hijazensis*; 3) group C comprises *P. hyemalis*, *P. hadiensis*, *P. lanuginosus* and *P. tenuiflorus*; 4) group D includes *P. cylindraceus* and *P. pseudomarrubioides*; and 5) branch includes only *P. arabicus*. The subgenera and sections show intra variability among themselves. In general,

Table 1. Seed characters of the investigated taxa of *Plectranthus* in Arabian Peninsula .

N	Taxon	Seed shape	Seed color	Seed surface (ridge)	Seed size (long x wide) mm	Outer of epidermal cell shape	Anticlinal cell wall boundaries	Periclinal cell wall
1	*Plectranthus amboinicus* (Lour.) Spreng	Ovoid	Brown	Flat, smooth, wingless	0.6-0.7 x 0.4-0.5	Isodiametric, 5, 6 gonals	Straight, channeled, smooth	Flat to convex, smooth
2	*Plectranthus arabicus* E. A. Bruce	Sub spherical	Reddish-brown	Flat, smooth, wingless	0.7-0.8 x 0.6-0.7	Isodiametric, 5, 6 gonals	Straight, channeled, smooth	Flat to convex, smooth
3	*Plectranthus asirensis* J. R. I. Wood	Ovoid	Reddish-brown	Flat, smooth to fine folds, wingless	1.3 - 1.5 x 1.1 - 1.2	Isodiametric, 5, 6 gonals	Straight, channeled, smooth	Flat, fine folds
4	*Plectranthus barbatus* Andrews	Ovoid	Black	Flat, folded, wingless	1.2-1.5 x 0.8-1	Isodiametric, 5, 6 gonals	Straight to slightly sinuous, channeled, fine folded	Flat, folded
5	*Plectranthus cylindraceus* Hochst. ex Benth	Sub spherical	Yellow-brown	Flat, smooth, wingless	0.7-0.8 x 0.6-0.7	Isodiametric, 5, 6 gonals	Straight, channeled, smooth	Flat to concave, smooth
6	*Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger	Elliptic	Reddish-brown	Flat, smooth to fine folds, with narrow ridge (wing)	0.9-1x 0.3-0.4	Isodiametric, polygonals	Straight to slightly sinuous, channeled, fine folded	Convex, smooth to fine folds
7	*Plectranthus hijazensis* K. Abdel Khalik	Ovoid	Reddish-brown	Flat, smooth to fine folds, wingless	1.1-1.5 X 0.7-1	Isodiametric, polygonals	Straight to slightly sinuous , channeled, fine folded	Flat to convex , fine folds
8	*Plectranthus hyemalis* J.R.I.Wood	Ovoid	Reddish-brown	Flat, smooth to fine folds, with narrow ridge (wing)	0.8-1 x 0.6-0.9	Irregular, polygonal cells	Undulate, raised, folded	Flat to concave, fine folds
9	*Plectranthus ignarius* (Schweinf.) Agnew	Obovoid	Grayish brown	Flat, smooth, wingless	1.4-1.6 x 1.1-1.4	Isodiametric, 5, 6 gonals	Straight, channeled, smooth	Flat, smooth
10	*Plectranthus lanuginosus* (Hochst.ex Benth.) Agnew	Ovoid	Grayish brown	Flat, smooth to fine folds, wingless	1.1–1.5 x 1-1.2	Irregular, polygonal cells	Straight to slightly sinuous, slightly raised; fine folds	Flat to slightly convex; folded
11	*Plectranthus ovatus* Benth.	Elliptic	Reddish-brown	Flat, folded, wingless	1.3-1.4 x 0.8-1	Isodiametric, 5, 6 gonals	Straight, channeled, fine folds	Flat to slightly convex; folded
12	*Plectranthus pseudomarrubioides* R. H. Willemse	Sub spherical	Yellow-brown	Flat, smooth, wingless	0.6-0.7 x 0.5-0.6	5, 6 gonals	Straight, channeled, smooth	Flat to concave, smooth
13	*Plectranthus tenuiflorus* (Vatke) Agnew	Sub spherical	Reddish-brown	Flat, smooth, wingless	0.8-1 x 0.7-0.9	5, 6 gonals	Straight, channeled, smooth	Flat, smooth

the UPGMA indicates that our study follows the currently applied subgenera sectional classification of *Plectranthus* by Codd (1975) and Paton et al. (2004).

Key to the identification of Plectranthus L. species from Saudi Arabia, based on seed characters

1a. Seeds ovoid to sub-spherical…… ..….....4
1b. Seeds obovoid to elliptical……..…….....2
2a. Seed obovoid; 1.4–1.6 × 1.1–1.4 mm; grayish brown……. *P. ignarius*
2b. Seed elliptical; 0.9-1.3 × 0.3-1mm; reddish brown.....……... 3
3a. Seed with narrow ridge (wing); 0.9-1 x 0.3-0.4 mm; periclinal cell wall convex……....…….…… ……*P. hadiensis*
3b. Seed without ridge (wingless); 1.3-1.4 x 0.8-1 mm; periclinal cell wall flat to slightly convex…..... *P. ovatus*
4a. Seed ovoid; seed with narrow ridge or wingless.. … …...8
4b. Seed subspherical; seed wingless.....……...…5
a. Outer of epidermal cell shape isodiametric to 5-6 gonals…...…….6
5b. Outer of epidermal cell shape 5-6 gonals……7

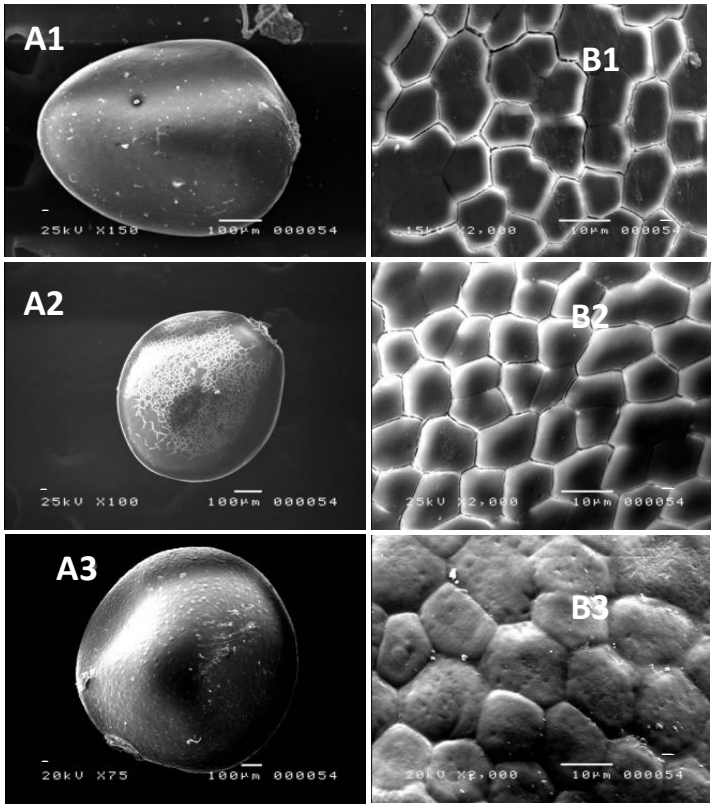

Fig 1. SEM micrographs of seeds in the *Plectranthus* taxa examined (A1, B1) *p. amboinicus*, (A2, B2) *P. arabicus*, (A3, B3) *P. asirensis*, (A) entire seed, (B) enlargement of seed coat.

6a. Seed reddish-brown; periclinal cell wall flat to convex.....*P. arabicus*
6b. Seed yellow-brown; periclinal cell wall flat to concave.....*P. cylindraceus*
7a. Seed yellow-brown; 0.6-0.7 x 0.5-0.6 mm; periclinal cell wall flat to concave.....…..*P. pseudomarrubioides*
7b. Seed reddish-brown; 0.8-1 x 0.7-0.9 mm; periclinal cell wall flat ……...…*P. tenuiflorus*
8a. Seed size 0.6-1 x 0.4-0.9 mm……...…9
8b. Seed size 1.1-1.6 x 0.7-1.4 mm…….……..109a. Seed reddish-brown; with narrow ridge; outer of epidermal cell shape irregular to polygonal cells; anticlinal cell wall boundaries undulate, raised……..*P. hyemalis*
9b. Seed brown; without narrow ridge; outer of epidermal cell shape isodiametric to 5-6 gonals; anticlinal cell wall boundaries straight, channeled…… *P. amboinicus*
10a. Seed reddish-brown……...…11
10b. Seed black or grayish-brown……...12
11a. Anticlinal cell wall boundaries straight, smooth; periclinal cell wall flat……*P. asirensis*
11b. Anticlinal cell wall boundaries straight to slightly sinuous, fine folds; periclinal cell wall flat to convex…….....*P. hijazensis*
12a. Seed black; outer of epidermal cell shape isodiametric to 5-6 gonals; anticlinal cell wall boundaries channeled; periclinal cell wall flat……....*P. barbatus*
12b. Seed grayish-brown; outer of epidermal cell shape irregular to polygonal cells; anticlinal cell wall boundaries slightly raised; periclinal cell wall flat to convex…….……
……..*P. lanuginosus*

Discussion

Some authors have tried to provide an accepted system to split the genus *Plectranthus* into subgenera, sections and series (Bentham, 1832, 1848; Bentham and Hooker, 1876; Briquet, 1897; Codd 1975; Paton et al., 2004, see Table 2) These studies were based on 1 or 2 traits from these morphological characters such as calyx forms, flower, and inflorescence structure. In the present study a number of seed characters were used based on the details of seed coat structure. In overall, the results show that different forms of seed morphology are helpful in distinguishing various species (Table 3); they do not confirm the subgenera and sectional classification of the genus *Plectranthus* suggested by Bentham's classifications (Bentham, 1832, 1848; Bentham and Hooker, 1876) and Briquit (1897) and somewhat confirm the subgenera and sectional classification of *Plectranthus* by Codd (1975) and Paton et al. (2004).

Subgenus Coleus (Lour.) Codd (group A)

Within the group A, *P. amboinicus* and *P. ignarius* have been recognized with 0.98 morphological similarities. These species can be obviously defined on the basis of various features: seed without ridge (wingless), isodiametric to 5-6 gonals epidermal cell shape, straight, channeled, smooth anticlinal cell wall boundaries.

They correspond to previously recognized position within subgenus *Germanea* section *Germanea* (Bentham and

Table 2. Taxa arranged in alphabetical order according to Wood (1997), Collenette (1999) and Chaudhary (2001). The table compares traditional Bentham (1832-36), Bentham and Hooker (1876), Briquit (1897) and more recent Codd (1975) classifications.

N	Taxon	Bentham (1832-1836)	Benth. & Hook. (1876)	Briquit (1897)	Codd (1975)	Present study
1	*Plectranthus amboinicus* (Lour.) Spreng	Section: Aromaria (*Coleus*)	Section: Germanea	Subgenus: Germanea	Subgen. Coleus	Group A
2	*Plectranthus arabicus* E. A. Bruce	Section: Isodon	Section: Isodon	Subgenus: Isodon	-	Group D
3	*Plectranthus asirensis* J. R. I. Wood	Section: Isodon	Section: Isodon	Subgenus: Isodon	-	Group B
4	*Plectranthus barbatus* Andrews	Section: Calceolus (*Coleus*)	Section: Germanea	Subgenus: Germanea	Subgen. Calceolanthus	Group B
5	*Plectranthus cylindraceus* Hochst. ex Benth	Section: Germanea	Section: Germanea	Subgenus: Germanea	Subgen. Burnatastrum	Group E
6	*Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger	Section: Coleoides	Section: Germanea	Subgenus: Germanea	Subgen. Plectranthus	Group D
7	*Plectranthus hijazensis* K. Abdel Khalik	-	-	-	-	Group B
8	*Plectranthus hyemalis* J.R.I.Wood	-	-	-	-	Group C
9	*Plectranthus ignarius* (Schweinf.) Agnew	-	-	-	Subgen.Coleus	Group A
10	*Plectranthus lanuginosus* (Hochst.ex Benth.) Agnew	Section: Germanea	Section: Germanea	Subgenus: Germanea	Subgen. Calceolanthus	Group C
11	*Plectranthus ovatus* Benth	Section: Germanea	Section: Germanea	Subgenus: Germanea	-	Group B
12	*Plectranthus Pseudomarrubioides* R.H. Willemse	Section: Germanea	Section: Germanea	Subgenus: Germanea	Subgen. Burnatastrum	Group C
13	*Plectranthus tenuiflorus* (Vatke) Agnew	Section: Aromaria (*Coleus*)	Section: Germanea	Subgenus: Germanea	Subgen. Coleus	Group C

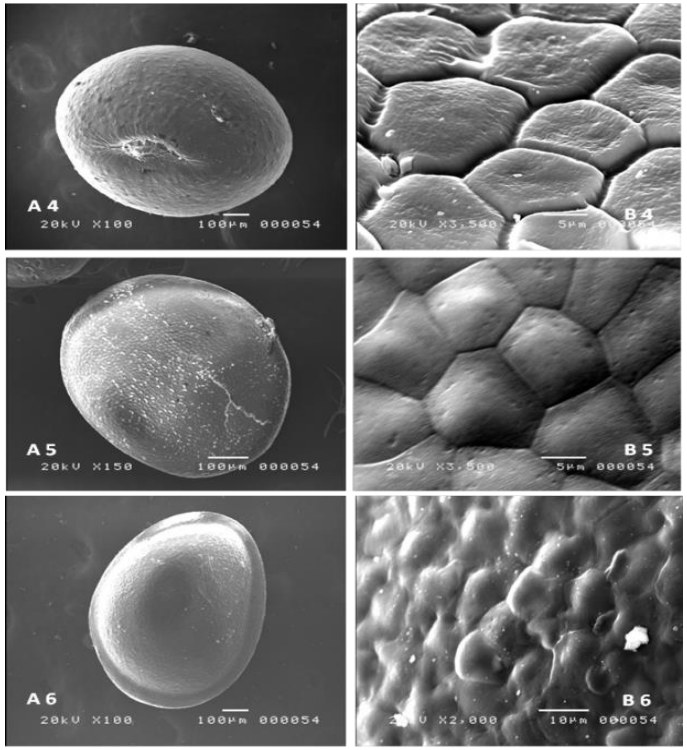

Fig 2. SEM micrographs of seeds in the *Plectranthus* taxa examined (A4, B4) *P. barbatus*, (A5, B5) *P. cylindraceus*, (A6, B6) *P. hadiensis*, (A) entire seed, (B) enlargement of seed coat.

Table 3. Characters and character states used in the morphometric analysis of the *Plectranthus*.

N	Character	Character state	Code
1	Seed shape	Ovoid	1
		Sub spherical	2
		Obovoid	3
		Elliptical	4
2	Seed color	Reddish- brown	1
		Yellow-brown	2
		Grayish brown	3
		Brown	4
		Black	5
3	Seed surface (ridge)	Flat, smooth , wingless	1
		Flat, smooth to fine folds, wingless	2
		Flat, folded, wingless	3
		Flat, smooth to fine folds, with narrow ridge (wing)	4
4	Seed size in mm (Length x width)	0.6-0.8 x 0.5-0.7	1
		0.8-1.1 x 0.7-0.9	2
		1.2-1.6 x 0.8-1.4	3
5	Epidermal cell patterns	Isodiametric to 5-6 gonals	1
		Isodiametric, polygonals	2
		5-6 gonals	3
		Irregular, polygonal cells	4
6	Anticlinal walls	Straight	1
		Straight to slightly sinuous	2
		Undulate	3
7	Relief of cell wall boundaries	Channeled	1
		Slightly raised	2
		Raised	3
8	Sculpture of anticlinal boundaries	Smooth	1
		Fine folds	2
		Folded	3
9	Curvature of outer periclinal cell wall	Flat	1
		Flat to concave	2
		Flat to convex	3
		Convex	4
10	Sculpture of periclinal cell wall	Smooth	1
		Smooth to fine folded	2
		Folded	3

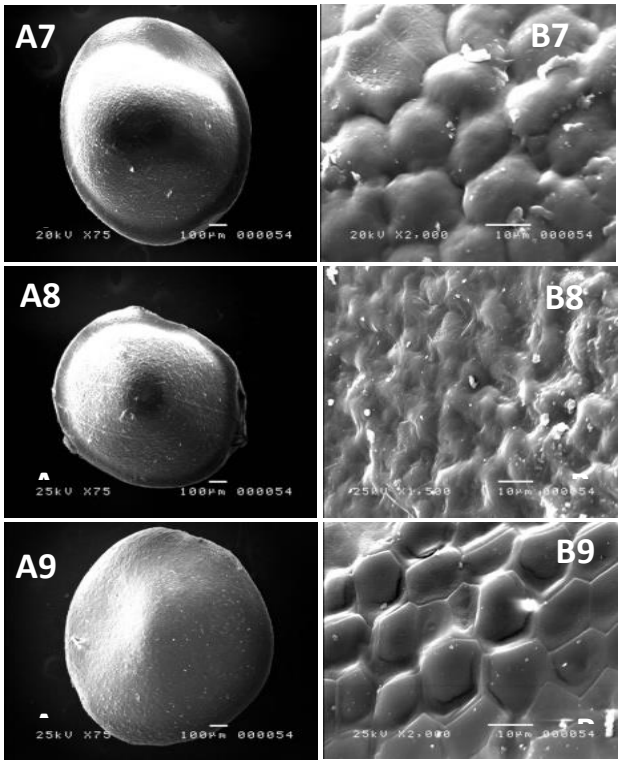

Fig 3. SEM micrographs of seeds in the *Plectranthus* taxa examined (A7, B7) *P. hijazensis*, (A8, B8) *P. hyemalis*, (A9, B9) *P. ignarius*, (A) entire seed, (B) enlargement of seed coat.

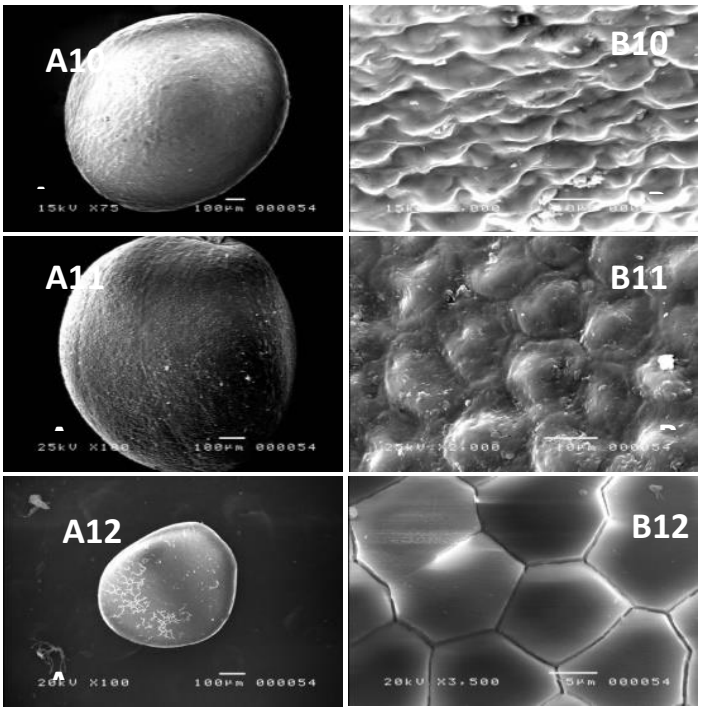

Fig 4. SEM micrographs of seeds in the *Plectranthus* taxa examined (A10, B10) *P. lanuginosus*, (A11, B11) *P. ovatus*, (A12, B12) *P. pseudomarrubioides*, (A) entire seed, (B) enlargement of seed coat.

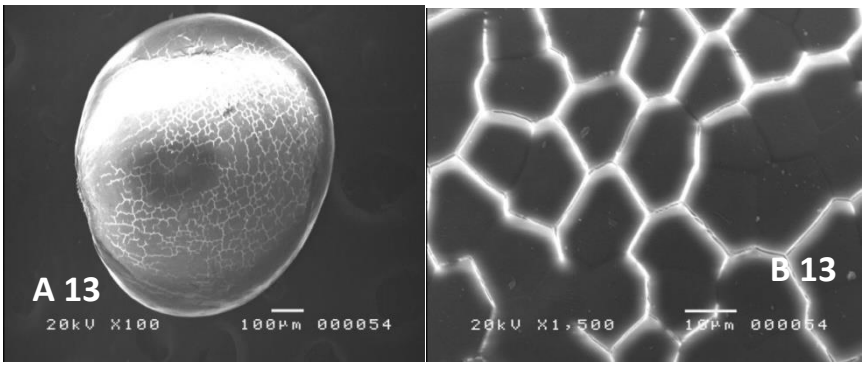

Fig 5. SEM micrographs of seeds in the *Plectranthus* taxa examined (A13, B13) *P. tenuiflorus*, (A) entire seed, (B) enlargement of seed coat.

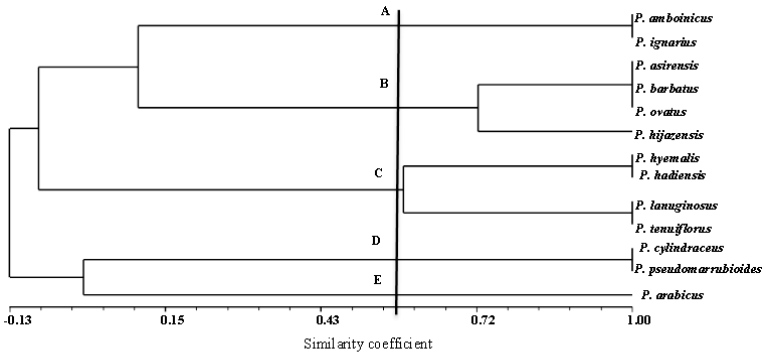

Fig 6. Dendrogram of distance between 13 species of *Plectranthus* as obtained by the unweight pair group method with arithmetic means (UPGMA) based on average taxonomic distance calculated on seed morphological characters.

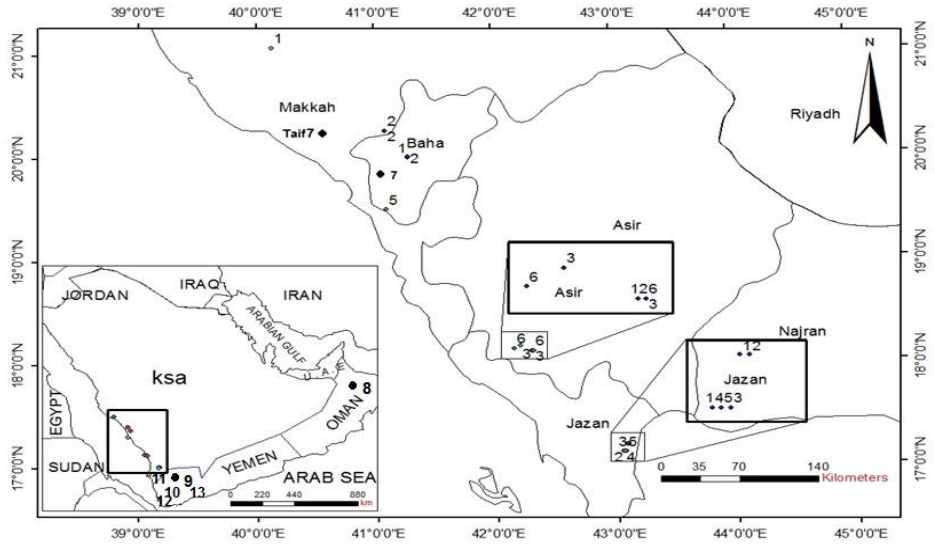

Fig 7. Location map of *Plectranthus* in Arabian Peninsula.1. *P. barbatus*, 2. *P. asirensis*, 3. *P. tenuiflorus*, 4. *P. arabicus*, 5. P. pseudomarrubioides, 6. *P. cylindraceus*, 7. *P. hijazensis*, 8. *P. hadiensis*, 9. *P. amboinicus*, 10. *P. ovatus*, 11. *P. ignarius*, 12. *P. lanuginosus*, 13. *P. hyemalis*.

Hooker, 1876; Briquit, 1897). However, Codd (1975) reviewed the genus and he put these species within subgenus *Coleus*. Furthermore, Paton et al. (2004) presented a phylogenetic analysis of basils and allies using the DNA sequence of the chloroplast *trnL, trnL-trnF* inter gene spacer and *rps* 16. They indicated that *Plectranthus* is paraphyletic, and within *Plectranthus*, they show that *P. amboinicus* clade is well supported (96% support). Moreover, Bandeira et al. (2010) analyzed the interspecific diversity among four species of the *Plectranthus* (*P. grandis*, *P. barbatus*, *P. neochilus* and *P. amboinicus*) and the intraspecific diversity of *P. barbatus* in southern Brazil using RAPD technique. They observed a higher genetic similarity between *P. amboinicus* and *P. neochilus* (80%). Moreover, Lukhoba et al. (2006) treated *P. amboinicus* and *P. ignaius* other related within one sub-clade (1b) but in two different groups (1 & 8). Generally, these data are supported and congruent with those of Bentham and Hooker (1876), Briquit (1897), Codd (1975), Paton et al. (2004), and Lukhoba et al. (2006).

Subgenus Calceolanthus Codd (Cluster B)

Within group B, one major clusters and branches with 0.75 similarities were identified. The first cluster includes *P. asirensis*, *P. barbatus* and *P. ovatus*. However, the second clade includes the endemic *P. hijazensis* to Saudi Arabia. Inside the cluster of *P. asirensis*, *P. barbatus* and *P. ovatus* have been recognized with 0.98 morphological similarities. These species can be clearly defined on the basis of various structures: seed without ridge (wingless), seed size 0.6-0.8 x 0.5-0.7 mm, isodiametric to 5-6 gonals epidermal cell shape, and channeled anticlinal cell wall boundaries. Another branch of the species represented by *P. hijazensis* shares the same seed without ridge (wingless), seed size 0.6-0.8 x 0.5-0.7 mm, and channeled anticlinal cell wall boundaries, but differs in isodiametric to polygonal epidermal cell shape, and straight to slightly sinuous anticlinal cell wall.

Bentham and Hooker (1876) treated *P. asirensis* as a separate section *Isodon*. However, Briquit (1897) treated this species as subgenus *Isodon*. Furthermore, *P. barbatus* and *P. ovatus* correspond to previously recognized position within subgenus *Germanea* section *Germanea* (Bentham and Hooker, 1876; Briquit, 1897). However, Codd (1975) reviewed the genus and put these species within subgenus *Calceolanthus*. Furthermore, Paton et al. (2004) presented a phylogenetic analysis of basils and allies using the DNA sequence of the chloroplast *trnL, trnL-trnF* inter gene spacer and *rps* 16. They indicated that *Plectranthus* is paraphyletic, and within *Plectranthus*, they show that *P. barbatus* clade is well supported (98% support). Likewise, Lukhoba et al. (2006) provided an informal classification that divided the species into two main clades. Clade 1, the *Coleus* clade of Paton et al. (2004), broadly corresponds to the formally recognized genus *Coleus*, which is divided into two subclades, clades 1a and b. Clade 2 is recognized as the *Plectranthus* clade. They treated *P. asirensis*, *P. barbatus* and other related within clade 1 but in two separated sub-clades (1a and 1b). Additionally, Bandeira et al. (2010) analyzed the interspecific diversity among four species of the *Plectranthus* (*P. grandis*, *P. barbatus*, *P. neochilus* and *P. amboinicus*) and the intraspecific diversity of *P. barbatus* in southern Brazil, using RAPD technique. They observed a higher genetic similarity between *P. grandis* and *P. barbatus* (77%), and they showed *P. barbatus* genotypes from different locations in Brazil showed a genetic similarity 96-100%. Current observations confirmed the possibility that *P. asirensis*, *P. barbatus* and *P. ovatus* should be monophyletic group and there are close relationships between these species and *P. hijazensis*. These results are in agreement with the results of Bentham and Hooker (1876), Briquit (1897), Codd (1975), and Lukhoba et al. (2006).

Subgenus Germanea (Benth.) Briq. (Cluster C)

Within group C, there is a close relationship with 0.60 similarity. Two major clusters with 0.98 similarities were identified. The first cluster includes *P. hyemalis* and *P. hadiensis*. These species can be clearly defined on the basis of various features: reddish- brown seeds; narrow ridge (wing); seed size 0.8-1 × 0.3-0.9 mm; and smooth to fine folded outer of periclinal cell wall. The second cluster includes *P. lanuginosus* and *P. tenuiflorus*. Specializations in

seed morphology include seed without ridge (wingless); and flat to slightly convex periclinal cell wall.

Within group C, *P. tenuiflorus* corresponds to the previously recognized position within subgenus *Germanea* (Briquit, 1897), and subgenus *Coleus* (Codd, 1975) belonging to *Plecranthus*. Moreover, Bentham and Hooker (1876), and Briquit (1897) treated *P. hadiensis* and *P. lanuginosus* as subgenus *Germanea*, section *Germanea*. However, Codd (1975) reviewed the genus and put these species within subgenus *Plectranthus* and *Calceolanthus*, respectively.

Lukhoba et al. (2006) treated *P. tenuiflorus* (*P. aegyptiacus*), *P. lanuginosus* within one sub-clade (1b) but in two groups (2, 8). Furthermore, they treated *P. hadiensis* into another sub-clade (1a) but in the same group (8). Our results are congruent with other author reports of phylogenetic and morphological studies such as Bentham and Hooker (1876), Briquit (1897), and Lukhoba et al. (2006) regarding relationships among these species in an enlarged concept of subgenus *Germanea*, but in disagreement with Codd (1975).

Subgenus Burnatastrum (Briq) Codd (group D)

In this group (D), *P. cylindraceus* and *P. pseudomarrubioides* have been recognized with around 0.98 morphological similarities. These species can be obviously defined on the basis of various features: sub-spherical seed, yellow-brown, without ridge, seed size 0.6-0.8 × 0.5-0.7 mm, straight, channeled, smooth anticlinal cell wall boundaries, flat to concave, smooth periclinal cell wall. *Plectranthus cylindraceus* and *P. pseudomarrubioides* matches to the previously recognized position within subgenus *Germanea* section *Germanea* (Bentham and Hooker, 1876; Briquit, 1897). Furthermore, Codd (1975) treated these species as subgenus *Burnatastrum*. Paton et al. (2004) presented a phylogenetic analysis of basils and allies using the DNA sequence of the chloroplast *trnL, trnL-trnF, rps* 16 and augmented by morphological data. They illustrated that *P. cylindraceus* and *P. buchananii* separated from the rest of the species of *Plectranthus* with 98% support. Lukhoba et al. (2006) extensively studied the phylogeny of 62 species of *Plectranthus* and its ethnobotanical uses. They provided an informal classification that divided the species into two main clades. Clade 1, the *Coleus* clade of Paton et al. (2004), broadly corresponding to the formally recognized genus *Coleus*, is divided into two subclades, clades 1a and b. Clade 2 is recognized as the *Plectranthus* clade. Within subclade 1b, they proved that *P. cylindraceus* and *P. pseudomarrubioides* are well separated (group 7) from the rest of taxa. Moreover, Grayer et al. (2010) surveyed 34 species of *Plectranthus* for exudate flavonoids to understand whether the distribution of these compounds would support a recent classification of the genus based on molecular and morphological characters. They identified two major groups, the *Coleus* and *Plectranthus* clades. They found that flavanones were restricted to only five species of the *Plectranthus*, and flavonols were only found in two species of the *Coleus* clade, *P. cylindraceus* (*P. montanus*) and *P. pseudomarrubioides*. Our results are congruent with those of Bentham and Hooker (1876), Briquit (1897), Codd (1975), Paton et al. (2004), Lukhoba et al. (2006), and Grayer et al. (2010).

In clade E (P. arabicus)

Within clade E, Bentham and Hooker (1876) treated *P. arabicus* and *P. asirensis* as a separate section *Isodon*. However, Briquit (1897) treated these species as subgenus *Isodon*. Our results do not support the placement of *P. arabicus* with *P. asirensis* in the subgenus (section) *Isodon*. This is due to the placement of *P. arabicus* within a separate branch with high genetic similarities and distinguishing from the rest of the species by having: seed reddish-brown, flat to convex periclinal cell wall, annual life form; plant length (15 cm); watery-succulent; sessile leaves; Branched and glandular multicellular hairs. In general, these results disagree with those of Bentham and Hooker (1876), and Briquit (1897) regarding placement of *P. arabicus* and *P. asirensis* in an enlarged concept of subgenus *Isodon* section *Isodon* and suggesting that *p. arabicus* should be treated as separate subgenus.

Materials and Methods

Plant material

Plants were collected during the flowering stages from different places in Saudi Arabia and other taken from the herbarium as loan (Fig 7). The identification was simplified according to (Wood, 1997; Collenette, 1999; Chaudhary, 2001). After comparison with voucher, specimens were deposited at the Herbaria of King Saud University, Kew and Edinburgh herbarium (KSU, K and E). http://www.kew.org/kew-science/collections/herbarium and http://www.rbge.org.uk/science/herbarium.

Seed material

In total, the seed micro-sculpture of 13 species of *Plectranthus* were investigated and reanalyzed. Only clearly visible, measurable characters were recognized. Some of the investigated seeds were collected from mature capsules of living plants in Saudi Arabia, and others were taken from either herbarium specimens or from abroad as a loan. A list of voucher specimen localities is presented in the supplementary data. Only mature seeds were used for investigation. The dried seeds were first examined by dissecting scope (Olympus type BH-2), and 10–15 seeds for each taxa were chosen to cover the range of variation. For scanning electron microscopy (SEM), seeds were mounted on stubs with double adhesive tape. The stubs were sputter-coated with gold for 5 min in an E1100 (Polaron Equipment). After coating, the specimens were examined with a JEOL JSM 5300 scanning electron microscope using accelerating voltages at 20–25 kV. All photomicrographs were taken at the central laboratory of the Faculty of Science, Sohag University, Egypt. The terminology used here follows that of authors such as Barthlott (1981, 1984), Abdel Khalik and Maesen (2002), and Abdel Khalik (2013) for description of seed shape, cell shape, and seed coat ornamentation.

Character selection coding

The principles for character selection were the independency of the characters and their stability within the taxa analysed (Stuessy, 1990; Davitashvili and Karrer, 2010). Seeds provide several qualitative and few quantitative characters. The focus was on qualitative characters of seed micromorphology that were easy to detect. One quantitative character (character 4) was measured for bigger samples, and means were grouped in magnitudes that could be treated statistically as qualitative characters.

Analysis of seed data

A total of 10 characters were measured in each species. The UPGMA analysis was performed with NTSYS-pc 2.02k software (Applied Biostatistics Inc., Setauket, NY, USA). The cluster analysis was conducted by average taxonomic distance and UPGMA clustering (procedures SIMINT, SAHN, and TREE). The characters and character states scored and obtained from seed morphological trats (Table 3).

Conclusions

The structure of seed coat offers a set of useful characters for the taxonomy of the *Plectranthus*. The present study showed that seeds of *Plectranthus* display high diversity in shape, color, size, surface, epidermal cell characters, anticlinal cell wall boundaries, and periclinal cell wall. Some species even have specialized structures. Seed coat morphology also provided some evidence for infra generic classification and partly corresponds with the phylogenetic, ethnobotanical morphology and phytochemistry. Our results indicated some degree of similarity among the species of subgenus *Burnatastrum* (Briq) Codd, *Germanea* (Benth.) Briq.**,** and support the monophyly of these subgenera as previously suggested by some authors. A remarkable result from this study was to identify *P. arabicus* as a separate group, suggesting that it should be treated as a separate subgenus. Likewise, the endemic species of *P. asirensis*, *P. ovatus*, and *P. hijazensis* group and *P. hyemalis* and *P. hadiensis* group should be homogeneous groups. Finally, seed coat analysis confirmed that developmental variation in seed characters is taxonomically useful, not only because it gives a better understanding of sculpture development, but also because it allows us to formulate the taxonomy of *Plectranthus* on the subgenera and sectional levels, and it is useful for constructing an identification key.

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