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A Systematic Revision of the Genus *Plectranthus* L. (Lamiaceae) in Saudi Arabia Based on Morphological, Palynological, and Micromorphological Characters of Trichomes

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Abstract

This study aims to investigate the morphology and ultrastructure characters of pollen grains and trichomes in order to evaluate their systematic value of these characters in specific and intraspecific separation of the Saudi Arabian *Plectranthus* species. A critical systematic revision of 7 species of *Plectranthus* (Lamiaceae) in Saudi Arabia was conducted by means of numerical analyses based on thirty-one morphological characters, including vegetative parts, seeds, pollen grains, and trichomes. Macro- and micro-morphological characters, including seed and pollen shape, size, coat sculpture, trichome structure, were studied. It reveals the presence of seven species, including two endemic species. The pollen grains were zonocolpate, hexacolpate, prolate to subprolate. Three types of exine ornamentation were recognized. Also, two trichome types could be distinguished and classified into glandular and non-glandular. The glandular trichomes could be distinguished as peltate, capitate and digitiform. The eglandular trichomes were single, uniseriate, multicellular. Pollen and trichome characters were found to be valuable, while seed characters presented only minor taxonomic value. On the basis of UPGMA clustering analysis four branches and clusters were distinguished. The results offer useful data for evaluating the taxonomy of *Plectranthus* both at subgeneric and sectional levels. Our results indicated some degree of similarity among the species of subgenus *Burnatastrum*. *Plectranthus arabicus* is considered as a separate group and may be treated as separate subgenus. Furthermore, the endemic species *Plectranthus asirensis* and

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***Plectranthus hijazensis* constitute a monophyletic group and there are close relationships between this group and *Plectranthus tenuiflorus*. A key for the identification of the investigated taxa based on studied characters is provided.**

Keywords

Morphological, Palynology, *S*eed, Taxonomic Revision, Trichomes

1. Introduction

Lamiaceae is a large family that is widely spread and in nearly all habitats and altitudes its species can be found. The genus *Plectranthus* L'Hér. is one of the largest genera of Lamiaceae, belonging to the subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae. It comprises about 300 species distributed in both tropical and warm regions of the Old World [1] [2]. A phylogenetic study of the tribe Ocimeae, based on plastid genes to which *Plectranthus* belongs, showed that *Plectranthus* was paraphyletic [3]. [4] [5] 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 [6], 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 [7], 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. [8] revised the genus in South Africa and based on the inflorescence characters he divided *Plectranthus* into five subgenera: *Nodiflorus* Codd, *Burnatastrum* (Briq) Codd, *Coleus* (Lour.) Codd, *Calceolanthus* Codd and *Plectranthus* based on the inflorescence characters. [9] reviewed the genus *Plectranthus* and its ethnobotanical used broadly, providing a complete understanding of the global ethno-botany of *Plectranthus*. Their review touches on the horticultural uses of the genus. In Saudi Arabia, *Plectranthus* species are used of economic and traditional medicine and have potential for development towards their use in the primary health care system. In Saudi Arabia, *Plectranthus barbatus* Andr. is the most important species of the genus, and is used as a remedy for stomach, intestine, liver disorders, heart problems, respiratory diseases and it is also resistant to insect attack [10]. Also, *P. tenuiflorus* (Vatke) Agnew is cultivated as ornamental. The leaf extract of *P. tenuiflorus* is also used to treat ear infections, and the leaves of *P. asirensis* Wood J. R. I. and *P. cylindraceus* Benth. are used as antiseptic and deodorant dressing for wounds [11] [12]. Some species of *Plectranthus* are problematic to identify because of a lack of clear cut morphological criteria to differentiate not only among species within the genus but also among the closely related genera. This has resulted in many taxonomic difficulties in the naming of species with the result that species have often been placed in some closely related genera such as *Coleus* Lour. [3]. In the Flora of Saudi Arabia, [13] reported *Plectranthus* by seven species: *P. arabicus* Bruce E. A., *P. cylindraceus*, *P. tenuiflorus*, *P. comosus* Sims, *P. barbatus*, *P. pseudomarrubioides* Willemse R.H. and *P. asirensis*, but [14] accepted only 6 species viz. *P. arabicus*, *P. cylindraceus*, *P. tenuiflorus*, *P. lanuginosus* (Benth.) Agnew, *P. barbatus*, and *P. asirensis*.

Pollen morphology provide a number of characteristics that are potentially useful for species identification, phylogenetic implication, and character-state evolution [15]-[22]. However, there are no works dealing with seed and pollen morphology of *Plectranthus*. Moreover, trichomes are broadly dispersed over the aerial reproductive and vegetative parts of the members of Lamiaceae and are generally distinguished as glandular and non-glandular trichomes. [17] found that trichome micromorphology was useful for systematics and reconstructing the phylogeny of Lamiaceae. In some genera of Lamiaceae, the trichome morphology is helpful in

infrageneric classification [23] [24]. Unfortunately, there is not much information on trichome morphology in *Plectranthus*. Only a few studies have reported on the structure and meaning of the trichomes occurring in *Plectranthus* and include the species *Plectranthus madagascariensis* Benth. [25], *Plectranthus ornatus* Codd [26], *Plectranthus neochilus* schltr. [27], *P. laxiflorus* Benth. [28] and *P. tenuiflorus* [29]. Therefore, this study aims to investigate the morphology and ultrastructure characters of pollen grains and trichomes in order to evaluate their systematic value of these characters in specific and intraspecific separation of the Saudi Arabian *Plectranthus* species.

2. Materials and Methods

2.1. Plant Material

Plants were collected during the flowering stages from different places in Saudi Arabia (Appendix 1). The identification was simplified according to [13] [14], and the voucher specimens are deposited at the Herbaria of King Saud University, Kew and Edinburgh (KSU, K and E).

2.2. Pollen Grain and Seed Characters

Pollen samples of each studied species were composed from herbarium specimens. All investigations were carried out on acetolysed pollen grains according to [30]. For light microscopy (LM), the pollen grains were observed by an Olympus type BH-2. Photomicrographs were taken with an Olympus photomicroscope. The measurements are based on 20 readings from each slide. The polar axis (P), equatorial diameter (E) and P/E ratio were calculated. For scanning electron microscopy (SEM), acetolysed pollen grains were dehydrated in ethanol sequences and mounted on a metallic stub in few drops of ethanol. The specimens were coated with gold in Apolaron E1100 ion sputtering Device, then viewed at 25 - 30 KV in a JOEL JSM 5300 scanning electron microscope of the Central Laboratory Faculty of Science, Sohag University, Egypt. The terminology and main morphological ideas are based on [31]-[33].

Only mature seeds were taken for investigation. The dried seeds were examined by light microscope (Olympus type BH-2), and 10 - 15 seeds for each taxon were chosen to cover the range of variation. The terminology used here follows authors such as [15] [16] [34].

2.3. Trichome Morphology

For trichomes morphology, dried specimens were used. Small portions of leaves or stems were taken and mounted on a stub using double adhesive tape. The specimens were processed as described above. The terminology used follows [25] [26] with some modification.

2.4. Morphological Characters Observations

Table 3 shows the characters and character states scored for plant, pollen, trichome and seed morphology, averaged for each OTU. In total 31 characters were taken into consideration, comprising 5 quantitative and 26 qualitative characters. Fifteen of the qualitative characters were scored as binary and the remaining were scored as multi-state characters.

2.5. Data Analysis

One type of analyses was performed with NTSYS-pc 2.02k software (Applied Biostatistics Inc., Setauket, New York, USA). I performed a cluster analysis using average taxonomic distance and UPGMA clustering (procedures SIMINT, SAHN, and TREE). To decrease the effects of different scales of measurement for different characters, the values for each character were standardized with procedure STAND, according to the formula: yI, STD = (yi − AVGyi)/STDyi), Where the default value in NTSYS-pc (STAND) for yi = the value to be standardized, AVGyi = the average of all values for the character, and STDyi = the standard deviation. The cophenetic relationship coefficient between the distance matrix and the tree matrix was calculated to examine the goodness of fit of the cluster analysis to the distance matrix (procedures COPH and MXCOMP) [35].

3. Results

3.1. Seed Characters

The seed morphological characters of the studied taxa of the genus *Plectranthus* as shown by light microscopy are presented in (**Table 1**).

3.1.1. Seed Shape

The seed shape varies from ovoid in *P. asirensis*, *P. barbatus* and *P. hijazensis* Abdel Khalik, to sub-spherical in the *P. arabicus*, *P. cylindraceus*, *P. pseudomarrubioides* and *P. tenuiflorus*.

3.1.2. Seed Size

Seed measurements vary significantly among the examined taxa. The largest seeds were recorded in *P. asirensis*, *P. barbatus* and *P. hijazensis* with ca 1.1 - 1.5 × 0.7 - 1.2 mm and the smallest ones in *P. arabicus*, *P. cylindraceus* and *P. pseudomarrubioides* measuring 0.6 - 0.8 × 0.5 - 0.6 mm, but in *P. tenuiflorus* has slightly larger seeds, measuring 0.8 - 1 × 0.7 - 0.9 mm.

Table 1. Seed, pollen and trichome characters of the investigated taxa of *Plectranthus* in Saudi Arabia.

Characters	*P. arabicus*	*P. asirensis*	*P. barbatus*	*P. cylindraceus*	*P. hijazensis*	*P. pseudomarrubioides*	*P. tenuiflorus*
Seed morphology							
1. Seed shape (mm)	Sub spherical	Ovoid	Ovoid	Sub spherical	Ovoid	Sub spherical	Sub spherical
2. Seed size	0.7 - 0.8 × 0.6 - 0.7	1.3 - 1.5 × 1.1 - 1.2	1.2 - 1.5 × 0.8 - 1	0.7 - 0.8 × 0.6 - 0.7	1.1 - 1.5 × 0.7-1	0.6 - 0.7 × 0.5 - 0.6	0.8 - 1 × 0.7 - 0.9
3. Seed color	Reddish-brown	Reddish- brown	Black	Yellow- brown	Reddish- brown	Yellow-brown	Reddish-brown
Pollen morphology							
4. Polar axis (*P* µm)	(20 - 26) 23	(22 - 30) 26	(26 - 36) 31	(20 - 26) 23	(21 - 25) 23	(16 - 24) 20	(37 - 44) 41
5. Equatorial diameter (E µm)	(15 - 21) 18	(20 - 25) 22	(20 - 28) 24	(15 - 21) 18	(13 - 16) 15	(14 - 18) 16	(26 - 32) 29
6. P/E	1.28	1.18	1.29	1.28	1.5	1.25	1.41
7. Pollen shape	Subprolate	Subprolate	Subprolate	Subprolate	Prolate	Subprolate	Prolate
8. pollen type	Hexa-Zonocolpate	Hexa-Zonocolpate	Hexa-Zonocolpate	Hexa-Zonocolpate	Hexa-Zonocolpate	Hexa-Zonocolpate	Hexa-Zonocolpate
9. Exine sculpture	Bi-reticulate	Bi-reticulate	Primary lumina is reticulate and secondary is granulate-perforate	Bi-reticulate	Primary lumina is reticulate and secondary is microreticulate	Primary lumina is reticulate and secondary is microreticulate	Primary lumina is reticulate and secondary is microreticulate
Trichomes morphology							
10. Unbranched multicellularhairs	+	+	+	+	+	+	+
11. Bi-cellular hairs	+	-	-	+	-	-	-
12. Branched multicellular hairs	+	-	-	-	-	-	-
13. Multicellular glandular hairs	+	-	-	-	-	-	-
14. Long-stalked capitate hairs	-	-	-	-	+	-	-
15. Peltate hairs	-	-	-	+		+	-

3.1.3. Seed Color

The colors of the seeds are of low diagnostic and systematic value among species. The colors vary from reddish-brown in *P. arabicus*, *P. asirensis P. hijazensis* and *P. tenuiflorus*, yellowish-brown in *P. cylindraceus* and *P. pseudomarrubioides*, to black in *P. barbatus*.

3.2. Pollen Grain Characters

The main features of the investigated pollen grains are summarized in (**Table 1**). Selected LM and SEM micrographs of pollen grains studied are presented in (**Figures 1-3**).

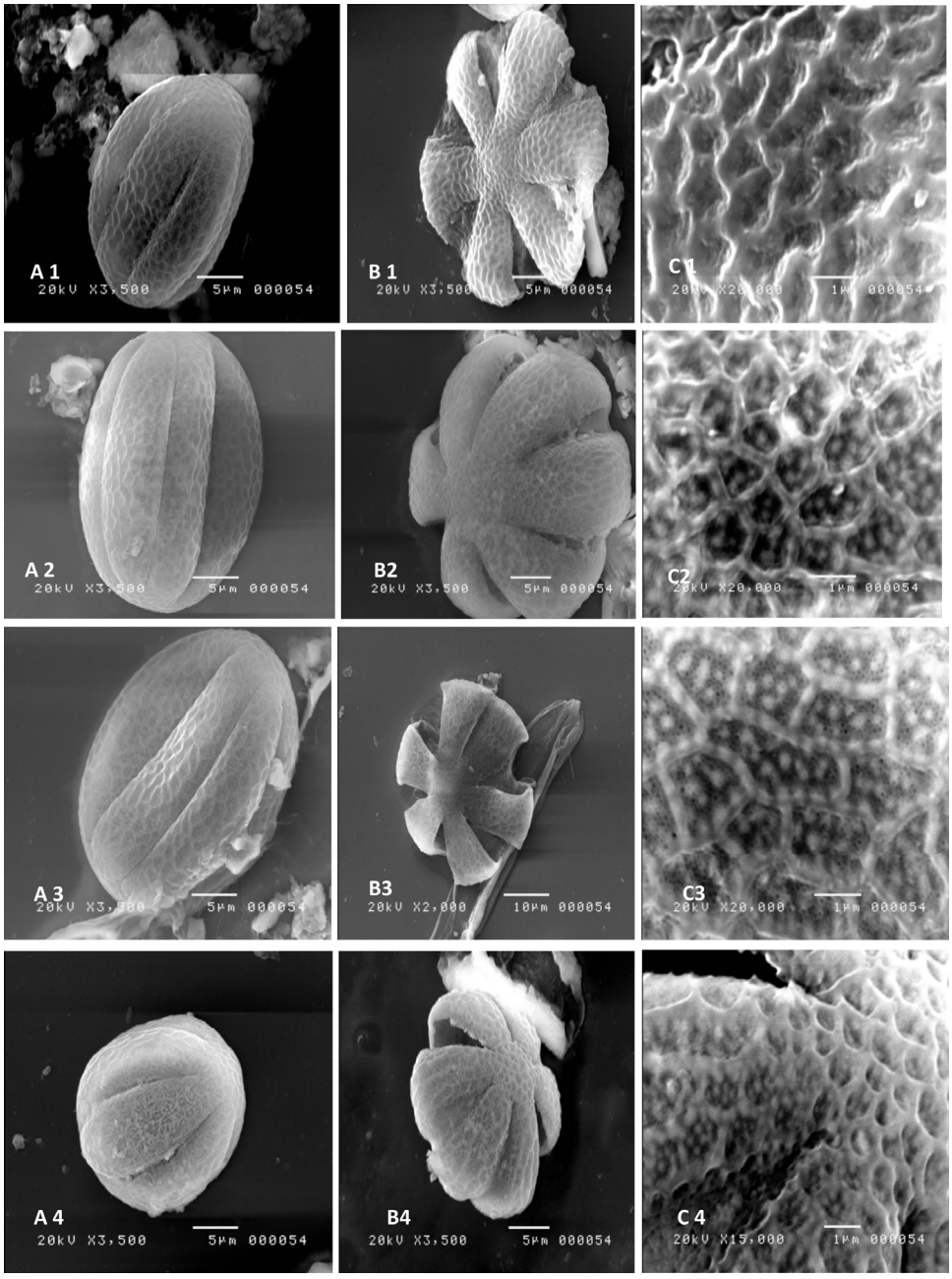

Figure 1. SEM photographs of pollen grains. A: equatorial view; B: polar view; C: enlargement part of pollen grain exine. (A1, B1, C1) *P. arabicus*, (A2, B2, C2) *P. asirensis*, (A3, B3, C3) *P. barbatus*, (A4, B4, C4) *P. cylindraceus*.

3.2.1. Pollen Size

The size of pollen grains varies considerably among the examined taxa (**Figure 1**, **Figure 2**), the largest pollen grains in *P. tenuiflorus* have a polar axis of 41 µm and an equatorial diameter of 29 µm (**Figure 2(A7)**, **Figure 3(B7)**) and the smallest grains have an average $P \times E$ value ranging from 20 × 16 µm in *P. pseudomarrubioides* (**Figure 2(A6)**, **Figure 2(B6)**), while the other species have slightly larger grains of 23 - 31 × 18 - 24 µm (see **Table 1**).

3.2.2. Pollen Shape

The ratio between the mean polar axis (P) and the mean equatorial diameter (E) can be used to assign the pollen grains to shape classes.

In the examined species, there is no wide variation in pollen shape. Pollen grains are commonly sub-prolate or prolate. It is prolate in *P. tenuiflorus* (**Figure 2(A7)**, **Figure 2(B7)**) and subprolate in the rest of the taxa (**Figures 1-3**).

3.2.3. Pollen Apertures

Apertures are simple. All species investigated are zonocolpate. The colpi are narrow to slit-like after acetolysis, they are usually widest at the equatorial view and gradually narrow towards the poles. The number of colpi is hexacolpate.

3.2.4. Exine Sculpture

The exine ornamentation has a complex structure. Generally, the sexine is thicker than nexine. The tectum of the pollen grains in almost all species examined is double-reticulate. The lumina of the main reticulum are in filled by a substratum that is either perforate or reticulate. The main reticulum is referred to as the primary reticulum

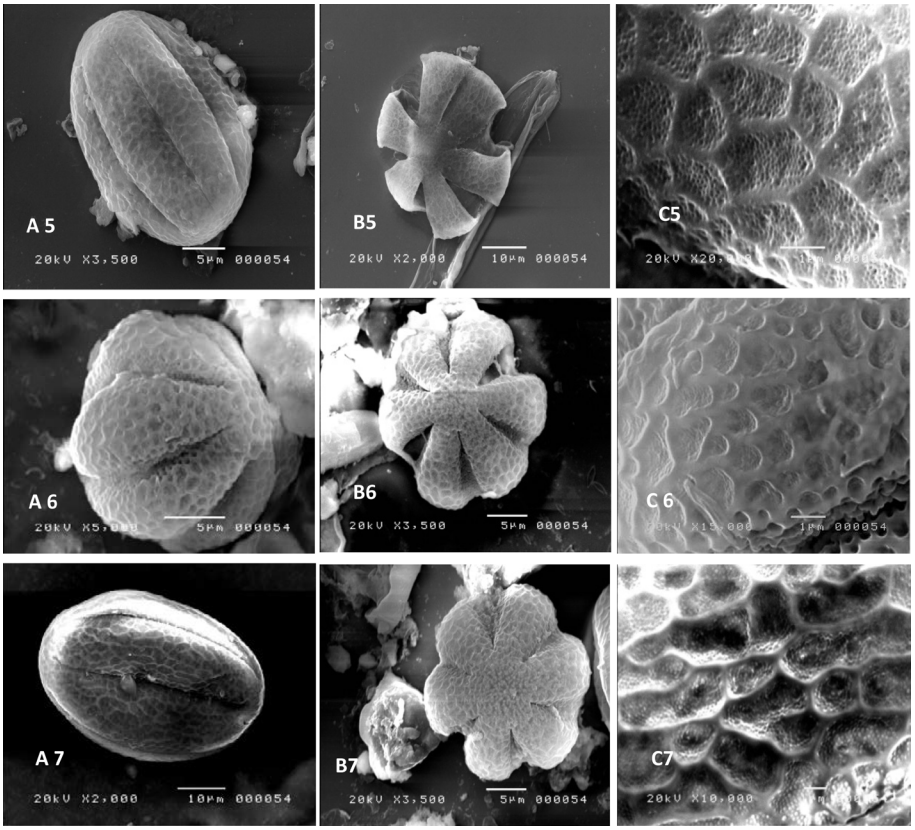

Figure 2. SEM photographs of pollen grains. A: equatorial view; B: polar view; C: enlargement part of pollen grain exine. (A5, B5, C5) *P. hijazensis*, (A6, B6, C6) *P. pseudomarrubioides*, (A7, B7, C7) *P. tenuiflorus*.

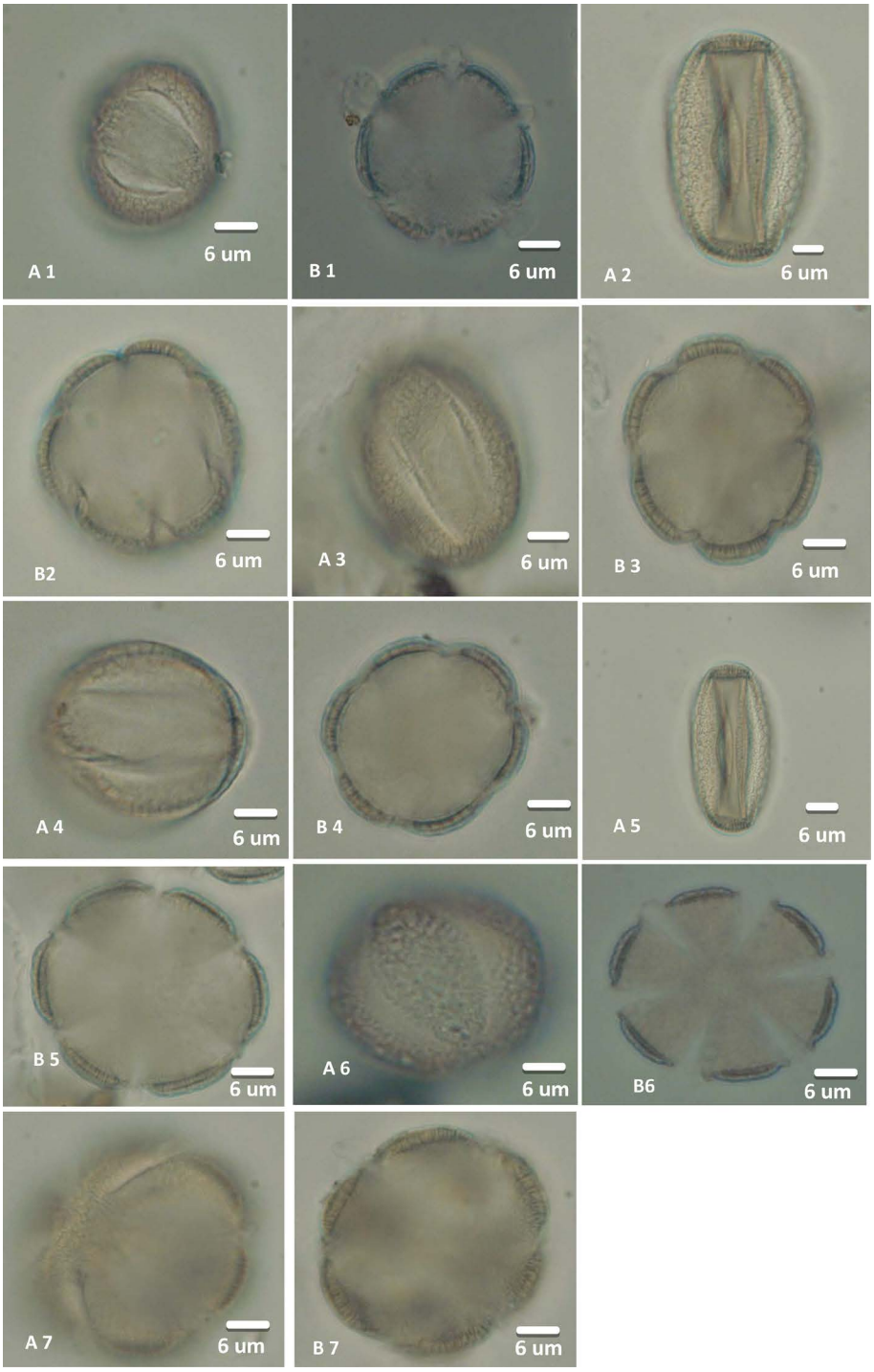

Figure 3. Light microscope photographs of pollen grains. A: equatorial view; B: polar view. (A1, B1) *P. arabicus*, (A2, B2) *P. asirensis*, (A3, B3) *P. barbatus*, (A4, B4) *P. cylindraceus*, (A5, B5) *P. hijazensis*, (A6, B6) *P. pseudomarrubioides*, (A7, B7) *P. tenuiflorus*.

and the substratum as the secondary reticulum [36]. Concerning the sculpturing of the exine three types can be distinguished:

Type (A): Bi-reticulate (double-reticulate). The primary lumina and the secondary one (substratum) are reticulate in *P. arabicus*, *P. asirensis* and *P. cylindraceus* (**Figure 1(C1)**, **Figure 1(C2)**, **Figure 1(C4)**).

Type (B): Granulate-perforate. The primary lumina are reticulate and the secondary one is granulate-perforate in *P*. *barbatus* (**Figure 1(C3)**).

Type (C): Micro-reticulate. The primary lumina is reticulate and the secondary one is micro-reticulate in *P*. *hijazensis*, *P*. *pseudomarrubioides* and *P*. *tenuiflorus* (**Figure 2(C5)-(C7)**).

3.3. Basic Types of Trichomes

Observations with Light and Scanning Electron Microscope (LM and SEM) revealed that non-glandular and glandular trichomes on the plant surfaces (**Figure 4**).

3.3.1. Non-Glandular Trichomes

The eglandular trichomes are single, uniseriate, multicellular, pointed, erect or leaning towards the leaf apex. It

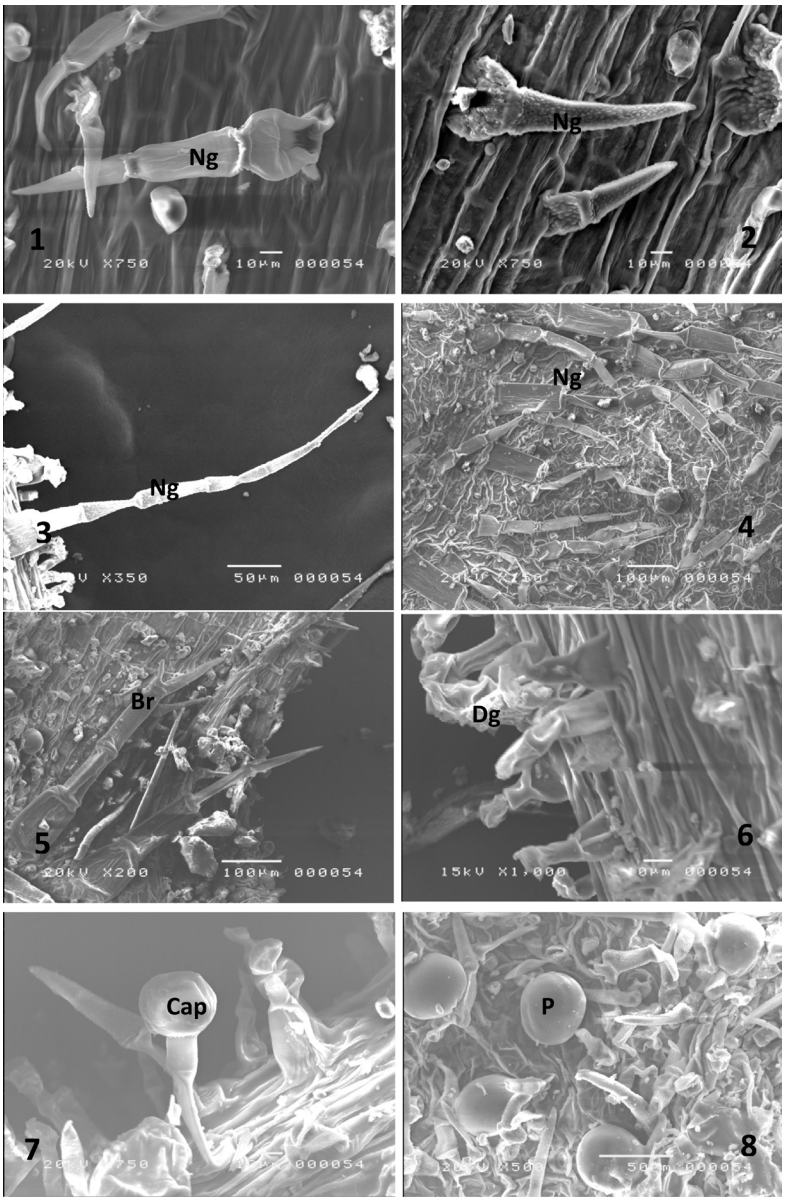

Figure 4. SEM micrographs showing different types of hairs in the genus *Plectranthus*. (1)-(4) Non-glandular, unbranched multicellularhairs (Ng), (5) Branched multicellular hairs (Br), (6) Digitiform hairs (Dg), (7) Long-stalked Capitate hairs (Cap), (8) Peltate hairs (P).

consists of two to eight cells. Each non-glandular trichome appears to be supported by a basal cellular pedestal cells. SEM observation revealed the presence of two types of non-glandular trichomes-branched with two fids in *P. arabicus* (**Figure 4(5)**) and unbranched trichomes which is consisted of 2 - 8 cells in all the species of *Plectranthus* (**Figure 4(1-4)**).

3.3.2. Glandular Trichomes

The glandular trichomes are of three main types:

1) Peltate trichomes

This type consists of a basal epidermal cell, a very short stalk cell and a large round head in *P. cylindraceus* and *P. pseudomarrubioides* (**Figure 4(8)**).

2) Capitate trichomes

This type with long stalked possesses one basal cell, along two to three celled stalk variable length and a unicellular bulb-shaped head in *P. hijazensis* (**Figure 4(7)**).

3) Digitiform trichomes

These consist of two to three cells, in line of similar diameter and approximately equal length. There is no clear distinction between head and stalk cells. Therefore, these glandular trichomes are made up of one basal cell, one or two stalk cells and one apical secretory (head-like) cell in *P. tenuiflorus* (**Figure 4(6)**).

3.4. Cluster Analysis

The results of the cluster analyses are presented in (**Figure 5**, **Table 2**). In the UPGMA dendrogram, 4 major branches and clusters (A-D) with approximately 97% similarity are distinguished: 1) branch A includes only *Plectranthus arabicus*; 2) branch B contains only *P. barbatus*; 3) group C comprises *P. cylindraceus* and *P. pseudomarrubioides*; and 4) group D includes group of *P. asirensis*, *P. hijazensis* and branch of *P. tenuiflorus*. The subgenera and sections show intra variability among themselves. In general, UPGMA indicates that our study follows the currently applied subgenera sectional classification of *Plectranthus* by [3] [8].

Key to *Plectranthus* L. species from Saudi Arabia, based on morphological characters

1a. Plant annual, up to 16 cm long; leaves sessile.....*P. arabicus*

1b. Plant perennial or sub shrub, up to 150 cm long; leaves petiolated……..……2

2a. Flower 4 - 9 mm long; seed size (0.6 - 0.8 × 0.5 - 0.7) mm…….……....3

2b. Flower larger than above 10 - 16 mm long; seed size (0.8 - 1.5 × 0.7 - 1.2) mm…….4

3a. Flower ≤ 4 mm long; pollen exine sculpture is bi-reticulate……..…*P. cylindraceus*

3b. Flower 4 - 10 mm long; pollen exine sculpture with primary lumina is reticulate and secondary one is microreticulate.....*P. pseudomarrubioides*

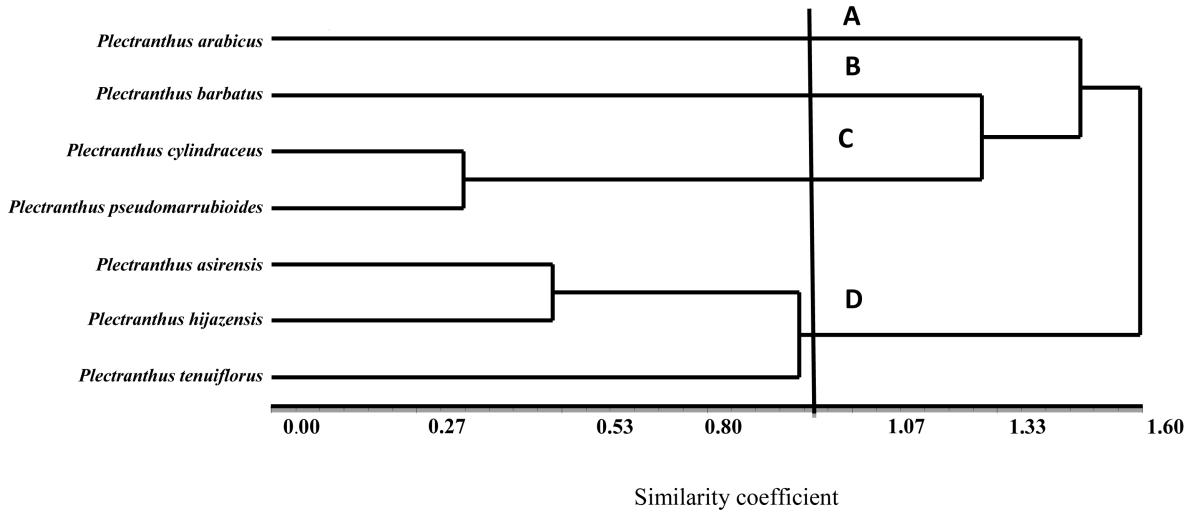

Figure 5. Dendrogram illustrating the relationships of the investigated species of *Plectranthus* based on morphological characters.

Table 2. Taxa arranged in alphabetical order according to [13] [14]. The table compares traditional Bentham [4]-[7] and more recent [8] classifications.

No	Taxon	Bentham (1832-1836)	Benth. & Hook. (1876)	Briquit (1897)	Codd (1975)	Present study
1	*Plectranthus arabicus* E. A. Bruce	Section: Isodon	Section: Isodon	Subgenus: Isodon	-	Group A
2	*Plectranthus asirensis* J. R. I. Wood	-	-	-	-	Group D
3	*Plectranthus barbatus* Andrews	Section: Calceolus (*Coleus*)	Section: Germanea	Subgenus: Germanea	Subgen. Calceolanthus	Group B
4	*Plectranthus cylindraceus* Hochst. ex Benth	Section: Germanea	Section: Germanea	Subgenus: Germanea	Subgen. Burnatastrum	Group C
5	*Plectranthus hijazensis* Abdel Khalik, K	-	-	-	-	Group D
6	*Plectranthus Pseudo marrubioides* R.H. Willemse	Section: Germanea	Section: Germanea	Subgenus: Germanea	Subgen. Burnatastrum	Group C
7	*Plectranthus tenuiflorus* (Vatke) Agnew	Section: Aromaria (*Coleus*)	Section: Germanea	Subgenus: Germanea	Subgen. Coleus	Group D

4a. Plant succulent; flower with long-pedicels up to 11 mm……..*P. tenuiflorus*

4b. Plant not succulent; flower with short-pedicels up to 7 mm ……5

5a. Leaves lanceolate; fruiting pedicels appressed to inflorescence axis; seed black; pollen exine sculpture with primary lumina reticulate and secondary one granulate-perforate……*P. barbatus*

5b. Leaves ovate to obovate; fruiting pedicels spreading-erect; seed reddish-brown; pollen exine sculpture with primary lumina reticulate and secondary one either reticulate or microreticulate…….....…6

6a. Inflorescence axis branched, covered with long capitate hairs; pollen grain prolate……..*P. hijazensis*

6b. Inflorescence axis un-branched, covered with long white non-glandular hairs; pollen grain subprolate……..*P. asirensis*

4. Discussion

Many authors have tried to provide an accepted system to split the genus *Plectranthus* into subgenera, sections and series [3]-[8], see **Table 2**). These studies were based on 1 or 2 characters from these morphological characters such as calyx forms, flower, and inflorescence structure. In the present study a large number of morphological, palynological, seed and trichomes characters were used. In general, the results show that different patterns of the studied characters are helpful in distinguishing various species (**Table 3**); they do not confirm the subgenera and sectional classification of the genus *Plectranthus* proposed by Bentham's classifications [4]-[7] but confirm somewhat the subgenera and sectional classification of *Plectranthus* by [3] [8].

In branch A (*P. arabicus*)

Within branch A, [6] treated *P. arabicus* and *P. asirensis* as a separate section *Isodon*. [7] 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 annual life form; plant length (15 cm); watery- succulent; sessile leaves; Branched and glandular multicellular hairs.

[37] investigated anatomical characters of the stem and leaf in *Plectranthus* and distinguished *P. arabicus* from the rest of the species by having terete stem, narrow cortex with only parenchyma, 6 bundles, and covered with bi-multicellular and branched non glandular hairs. Generally, these results disagree with those of [6] [7] regarding placement of *P. arabicus* and *P. asirensis* in an enlarged concept of subgenus *Isodon* section *Isodon* and agree with [37] to suggest that *P. arabicus* should be treated as separate subgenus.

Subgenus Calceolanthus Codd (*P. barbatus*) (branch B)

Within branch B, *P. barbatus* corresponds to previously recognized position within subgenus *Germanea* section *Germanea* [6] [7]. However, [8] reviewed the genus and he put this species within subgenus *Calceolanthus*. Furthermore, [3] 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

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

N	Character	Character state	Code
1	Life cycle	Annual Perennial or sub shrub	1 2
2	Plant nature	Watery-succulent Not succulent	1 2
3	Plant length (mean length in cm)		
4	Plant growth	Decumbent to ascending Erect	1 2
5	Plant surface	Glabrous to sparsely hairs Densely hairs	1 2
6	Stem nature	Herbaceous Woody at the base Woody	1 2 3
7	Stem outline shape	Terete Quadrangular Terete to quadrangular	1 2 3
	Trichome characters		
8	Branched multicellular hairs	Present Absent	1 2
9	Digitiformhairs	Present Absent	1 2
10	Long-stalked capitate hairs	Present Absent	1 2
11	Peltate hairs	Present Absent	1 2
	Leaf characters		
12	Leaf nature	Succulent Not succulent	1 2
13	Leaf petiole measurements (mean length in mm)		
14	Leaf shape	Ovate Oblong to lanceolate Ovate to elliptical	1 2 3
15	Leaf apex	Acute Obtuse	1 2
16	Leaf margin	Serrate Dentate Crenate Lobed	1 2 3 4
	Inflorescence characters		
17	Inflorescence branching	Un branched Branched at the base Branched at the axis	1 2 3
18	Inflorescence position	Terminal Terminal and axillary	1 2
19	Inflorescence axis surface	Densely glandular Glabrous to non-glandular hairs	1 2
20	Bract presence	Permanent Deciduous	1 2

Continued

		Flower characters	
21		Flower pedicle (mean length in mm)	
22		Sepal length (mean length in mm)	
23	Sepal apex	Acuminate Acute Obtuse	1 2 3
24	Sepal surface	Glabrous to sparsely hairs Hairy with glandular	1 2
25		Petal length (mean length in mm)	
26	Petal colour	White Purple blue Purplish-blue	1 2 3 4
		Pllen grain characters	
27	Pollen shape	Subprolate P/E = 1.14 - 1.33 µ ProlateP/E = 1.33 - 2 µ	1 2
28	Exine sculpture	Bi-reticulate The primary lumina reticulate and secondary is micro-reticulate The primary lumina reticulate and secondary is granulate-perforate	1 2 3
		Seed characters	
29	Seed shape	Ovoid Sub spherical	1 2
30	Seed color	Yellow-brown Reddish-brown Black	1 2 3
31	Seed size in mm (Length x width)	0.6 - 0.8 × 0.5 - 0.7 0.8 - 1 × 0.7 - 0.9 1.2 - 1.5 × 0.8 - 1.2	1 2 3

Plectranthus, they show that *P. barbatus* clade is well supported (98% support). Moreover, [38] analyzed the interspecific diversity among four species of *Plectranthus*: *P. grandis* (Cramer L. H.) Willemse R. H., *P. barbatus*, *P. neochilus* Schltr., and *P. amboinicus* (Lour.) Spreng., and the intraspecific diversity of *P. barbatus* in southern Brazil, by means of the 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%.

The results do not support the situation of *P. barbatus* with other taxa in the subgenus (section) *Germanea*. This is due to the placement of *P. barbatus* within a distinct branch with high genetic similarities and split from the rest of the subgenera on the basis of the species with quadrangular stem, not succulent leaf, terminal inflorescence, black seed, exine sculpture of pollen grain with primary lumina is reticulate and secondary is granulate-perforate. These results disagree with those of [6] [7], but agree with [3] [8] [38].

Subgenus Burnatastrum (Briq) Codd (group C)

In this group (C), *P. cylindraceus* and *P. pseudomarrubioides* have been recognized with around 0.39 morphological similarities. These species can be clearly defined on the basis of various features: Annual, watery-succulent, glabrous to sparsely hairs, herbaceous, quadrangular stem, covered with peltate hairs, inflorescence branched at the axis, obtuse sepal apex, sub-spherical seed, small seed size (0.6 - 0.8 × 0.5 - 0.7).

Plectranthus cylindraceus and *P. pseudomarrubioides* match to the previously recognized position within subgenus *Germanea* section *Germanea* [6] [7]. Furthermore, [8] treated these species as subgenus *Calceolan-*

thus. [3] presented a phylogenetic investigation 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* Baker was separated from the rest of the species of *Plectranthus* with 98% support. [9] studied the phylogeny of 62 species of *Plectranthus* and its ethno-botanical uses extensively. They provided an informal classification that separated the species into two main clades. Clade 1, the *Coleus* clade of [3], 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*. *pseudo- marrubioides* are well separated (group 7) from the rest of taxa.

Moreover, [10] surveyed 34 species of *Plectranthus* for exudate flavonoids to see whether the scattering 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* and *P*. *pseudomarrubioides*. Generally, these data are support and congruent with those of [3] [6]-[10].

Subgenus Coleus (Lour.) Codd (group D)

Within group D, one major cluster and branch with 0.97 similarities were identified. The first cluster includes the endemic *P*. *asirensis* and *P*. *hijazensis*. However, the second branch includes *P*. *tenuiflorus* treated as section *Aromaria* Benth. that belongs to the genus *Coleus*, as subgenus *Germanea*, and subgenus *Coleus* that belong to *Plecranthus*. [9] treated *P*. *tenuiflorus*, *P*. *asirensis* and other connected within one group (group 8) but in two separated sub-clades (1a and 1b).

Inside the cluster of the endemic *P*. *asirensis* and *P*. *hijazensis* have been recognized with 0.45 morphological similarities. These species can be clearly defined on the basis of various structures: sub-shrubs, not succulent, erect, woody stem at the base, terete to quadrangular stem outline, petiolate leaves, serrate leaf margins, bracts deciduous, ovoid seed, seed size (1.2 - 1.5 × 0.8 - 1.2). Moreover, [37] showed that the anatomical characters of the stem and leaf might be useful in distinguishing *P*. *asirensis* and *P*. *hijazensis* from the rest of the species by their shared terete to undulate stem, wide cortex with angular collenchyma and parenchyma, numerous bundles.

An another branch of the species represented by *P*. *tenuiflorus* shares the same densely hairs surface, woody stem, terete to quadrangular stem outline, petiolate leaves, terminal inflorescence, bract deciduous, hairy with glandular sepal surface, reddish-brown seed.

Current observations confirmed the possibility that *P*. *asirensis* and *P*. *hijazensis* should be a monophyletic group and there are close relationships between these species and *P*. *tenuiflorus* subgenus *Germanea*, and subgenus *Coleus* and these results are in agreement with the results of [8] [9] [37].

5. Conclusion

A taxonomic revision of seven *Plectranthus* species (Lamiaceae) in Saudi Arabia was conducted by means of numerical analysis based on thirty-one morphological characters, including vegetative parts, seeds, pollen grains, and trichomes. Macro- and micro-morphological characters, including seed and pollen shape, size, coat sculpture, trichome structure, were studied. Two endemic species exist. The data presented here considerably underline the importance of seed, pollen and trichome characters within the genus *Plectranthus* both on subgeneric and sectional levels. Some species of *Plectranthus* have similar trichome shapes, pollen grains and seed characters, but are quite different in gross morphology and can easily be recognized as distinct species. The molecular systematic study on *Plectranthus* [3] [9] including some species distributed in Saudi Arabia, allows an interpretation of the usefulness of pollen, trichome and seed characters in *Plectranthus*, these can be considered as phylogenetic informative characters. Our results indicate some degree of similarity among the species of subgenus *Burnatastrum* (Briq) Codd and support the monophyly of the subgenus as suggested by [8] [9]. A remarkable result from this study was identifying *P*. *arabicus* as a separate group and suggesting that it should be treated as a separate subgenus. Furthermore, the endemic *P*. *asirensis* and *P*. *hijazensis* appear to be a homogeneous group and there are close relationships between this group and *P*. *tenuiflorus*. A key for the identification of the investigated taxa based on studied characters is provided.

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Appendix 1

List of *Plectranthus* taxa and specimens studied [13] [14].

1. *Plectranthus arabicus* E. A. Bruce: Saudi Arabia. Jazan: Gabal Fayfa, Abdel Khalik s. n. (UQU); Gabal Fayfa, 100 km NE of Trajam, on steep SW approach road, S. Collenette 3570 (E, K).

2. *Plectranthus asirensis* J. R. I. Wood: Saudi Arabia. Abha: Wadi Al-ouse, A.K. Nashar IH 98 (E); Jazan: Jabal Fayfa, Taku Miyazaki 10203 JF H20 (E); Jazan: Gabal Fayfa, Abdel Khalik and Al-Ozekii s.n. (UQU); Abha: Raydah village, city, Taku Miyazaki 10205RV 2445 (E).

3. *Plectranthus barbatus* Andrews: Saudi Arabia. Jazan: Gabal Fayfa, Abdel Khalik s.n. (UQU); Jazan: Jabal Fayfa, *S. Chaudary 385* (E); Taif: near Al Hadda, S. Collenette 2387 (K); Abha: Raydah village, Taku Miyazaki 990906R4 (E).

4. *Plectranthus cylindraceus* Hochst. ex Benth: Saudi Arabia. Abha: Wadi Al-ouse, Abdel Khalik and Howldars s. n. (UQU); Wadi Al-ouse, Jabal Sawdah, near police station, S. Collenette 6364 (E, K); Al-Baha: Jabal Shada, 25 Km north of Mikhas, *S.* Collenette 9306 (E, K).

5. *Plectranthus pseudomarrubioides* R. H. Willemse: Saudi Arabia. Jazan: Gabal Fayfa, Abdel Khalik and Al-Ozekii s.n. (UQU).

6. *Plectranthus tenuiflorus* (Vatke) Agnew: Saudi Arabia. Jazan: in area of Gabal Fayfa, Abdel Khalik s.n. (UQU); Abha: Wadi Al-ouse, Jabal Sawdah, near police station, Collenette 3119 (K, E).

7. *Plectranthus hijazensis* K. Abdel Khalik: Saudi Arabia. Al Baha: in area of Saad Medhas, Abdel Khalik and Howldar s.n. (UQU); Agabat Al-Abna, SE Buljurashi, Fayed 13922 (K); Near Taif, 15 Km SW of Al-Hadda, S. Collenette 1554 (K); Near Jabal Ibrahim of Taif, Al Baha road , S. Collenette 3552(K, E).