Studying the morphological diversity of *Bunium paucifolium* and some *Elwendia* species (Apiaceae)

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Studying the morphological diversity of *Bunium paucifolium* and some *Elwenda* species (Apiaceae)

Najmeh Adelifar¹ and Farkhondeh Rezanejad¹*

**Abstract:** The genus *Bunium* L. (Apiaceae) has about 50 species in the world, distributed in Asia, Europe and North Africa. Recently, *Bunium* has been divided into two separate genera *Elwenda* and *Bunium*. Kerman province is the largest province in Iran and has a wide distribution of these genera especially *E. persica*, as the most important medicinal and spice species. There are a few published studies on the morphology of these genera, especially on flower and fruit morphology. 20 morphological traits were studied to analyze the differences between species. The most important traits in the first principal component analysis (PCA1) were the number of bracts and umbellets per umbel, stylopodial length, corolla shape, empty fruits and the number of flowers and fruits per umbellet. Corolla shape was raised and vertical in *B. paucifolium* while horizontal and wide in three other species (*E. cylindrica, E. persica* and *E. wolffii*). There are 3 to 4 male flowers in the center of umbellet in *E. cylindrica, E. wolffii* and *B. paucifolium* while in *E. Persica*, no male flowers were observed. This study revealed that morphological traits particularly corolla shape are consistent with the molecular phylogenetic results reported by other researchers.

**Subjects:** Plant Biology; Plant Morphology; Evaluation/ Program Evaluation

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**PUBLIC INTEREST STATEMENT**

The genus *Bunium* L. (Apiaceae) has about 50 species in the world and has a wide distribution in Iran especially in Kerman province. Recently, *Bunium* has been divided into two separate genera *Elwenda* and *Bunium*. Due to the absence of a comprehensive study on quantitative and qualitative traits, in this survey, morphological traits of flower, pollen and fruit in two genera *Elwenda* (*E. cylindrica, E. persica* and *E. wolffii*) and *Bunium* (*B. paucifolium*) were investigated. The most important traits in the principal component analysis (PCA) were the number of bracts and umbellets per umbel, stylopodial length, corolla shape, empty fruits and the number of flowers and fruits per umbellet. Our study revealed that morphological traits, particularly corolla shape, are consistent with the molecular phylogenetic results reported by other researchers and confirms the studies in the separation of *Bunium* to two genera *Bunium* and *Elwenda*.
Keywords: Apiaceae; Bunium; corolla shape; flower; Fruit; Elwendia

1. Introduction

Bunium genus is an important medicinal and spice plant belonging to Apiaceae family, Apioideae subfamily and Apiaeae tribe (Mozaffarian, 2007). The Apiaceae family is classified into three subfamilies that the largest of them is the Apioideae (Plunkett & Downie, 1999). Apiaceae family includes about 450 genera and 3700 species in all around the world that are rich in secondary metabolites including many valuable genera in terms of economic and medical importance (Duran et al., 2010; Olle & Bender, 2010). The plants belonging to the Apiaceae family have its own distinctive features such as umbel inflorescences, actionomorphic and five merous corolla, inferior ovary with two locules and the schisocarp fruits that are separated from each other by carpophor. In fact, these characters in the Apiaceae family are in the late evolutionary stages of the series of dialypetalous (Ghahreman, 1993; Mozaffarian, 2007; Plunkett & Downie, 1999). Morphological traits are important as a source of taxonomic features. Different species are identified based on characteristics such as the number umbel per plants, shape and number of bracts and bracteoles, sexuality and shape of flowers, shape and number of fruits, the length of the mericarp, the number and shape of the furrows of the mericarp, secretory ducts, commissural width, carpophore and stylopodium (Chahota et al., 2017; Ghahremaninejad et al., 2014). Although some researches have presented stenopolynotypic type of pollen in this family (Erdtman, 1952; Klimko et al., 2012) but other studies have stated significant importance of pollen in family taxonomy. Cerceau Larrival and Roland Heydacker (1976), Van Zeist and Bottema (1977) have grouped this family into several types based on the pollen features. Studies of the pollen structure and size, state and size of pores, mesocolpium and apocolpium structure are considered as important parameters in determining of phylogenetic relationships of different species of this family (De Leonardis et al., 2009). The Bunium L. has about 50 species in the world, distributed in Asia, Europe and North Africa (Degtjareva et al., 2009). Recently, Bunium genus has been divided into two separate genera Elwendia and Bunium genus; so some species were transferred from Bunium to Elwendia (Degtjareva et al., 2013; Pimenov, 2017). One of the most important species is Kerman black zira or Elwendia persica (Boiss.). Kerman province is the largest province in Iran, and has the highest distribution of E. persica (synonym B. persica). Although E. persica is a well-known species that is used in medicinal and nutrition industries, there are a few data on morphological traits of flower, fruit and pollen in these two genera in Iran. Initially, the studies of Degtjareva et al. (2009) on some Bunium species distributed in ancient Mediterranean based on ITS and psbA-trnH sequences and morphological characters (the number of cotyledons, the width of mericarp commissure) showed that Bunium species are divided into two main Clade including Bunium-I and Bunium-II. According their results, B. persica, B. wolffii and B. cylindrica are placed in first calde (Bunium-I) whereas B. paucifolium is placed in Bunium-II clade (Degtjareva et al., 2009). Recently, these researchers transferred Bunium I clade to the genus Elwendia Boiss. and Bunium II Clade to the genus Bunium (Degtjareva et al., 2013; Pimenov, 2017). Morphological characteristics, along with molecular characteristics can be useful and effective in taxonomy and understanding the evolutionary relationships. There are no published studies on inflorescence, flower and fruit traits these genera except for a few basic level studies. The reproductive features tend to be much less variable at least in the basic structure. Therefore, reproductive characters are regarded as more useful for plant classification and understanding the evolutionary relationships than vegetative characters. Therefore, due to the absence of a comprehensive study on quantitative and qualitative traits of reproductive feature, in this survey, morphological traits of flower, pollen and fruit in two genera Elwendia (E. cylindrica, E. persica and E. wolffii) and Bunium (B. paucifolium) were studied.

2. Materials and methods

2.1. Plant material

Several field collections showed that two genera Elwendia and Bunium have been distributed in different parts of Kerman province. Species identification was performed using conventional keys
and reports from previous researchers. Table 1 shows different species and their location in this study.

2.2. Morphological evaluation

Nineteen morphological traits cited were recorded during the growing season at different populations of studied genera. These characteristics were studied using naked eye, stereo microscope)OLYMPUS TL2 (and light microscopy (OLYMPUS BH-2) connected to camera. Mature pollen grains at dehiscence stage were dusted onto a drop of water on a microscope slide. The size of pollen was compared by measuring the length of polar (P) and equatorial (E) axes of 10 pollen grains on each slide randomly. The measurements were made with an ocular micrometer (Rezanejad, 2007; Rezanejad & Majd, 2012). The shape category is determined by dividing the equatorial width into the polar length of the pollen grain (P/E) and according to the classification of Punt et al. (2007). Of the Nineteen traits, thirteen were quantitatively measured, while six were qualitative. The quantitatively measured traits were Umbels per plant (UPP), Bracts per umbel (BPU), Umbellets per umbel (ULPU), Bracteolates per umbellet (BPRU), Flower per umbellet (FL), Fruits per umbellet (FR), Filled fruits per umbellet (FFR), Empty fruits per umbellet (EFR), Fruit length (FRL), Pedicel length (PEL), Polar axis (P), Equatorial axis (E), Stylopodia length (SL). The qualitative traits were Bush color (BCO), Corolla shape (CSH), Stylopodium shape (SSH), Fruit shape (FSH), Mericarp rib shape (MRS), Male flower (MF).

2.3. Statistical analysis

Data for each quantitative morphological trait were randomly recorded on ten replications. The mean values of these replications were used for data analysis. For qualitative variables, mode values were considered and coded for statistical analysis. Different measures such as variances, correlation and principal component analysis (PCA) were determined using the software SPSS Ver.24 by PCA method as well as NT SYS Ver.2.11a software by the unweighted pair-group (UPGM) method (Moghaddam & Pirbalouti, 2017).

3. Results and discussion

Morphological and anatomical fruit characteristics, as well as molecular and phylogenetic studies are important and useful for plant identification and classification (Liu et al., 2016). Sampling studies showed that in some regions, all studied species were seen together (Table 1). The inflorescences in all species are compound umbels with a long tail, the rays

| No. | Location         | Species                  | Latitude (N) | Longitude (E) | Altitude (m) |
|-----|------------------|--------------------------|--------------|---------------|--------------|
| 1   | Bidkhun (Bardsir)| *E. cylindrica* E. wolffii| 29° 34’ 15”  | 56° 28’ 60”  | 3523.7928    |
| 2   | Chenarueeyeh (Zarand) | *E. persica*            | 30° 47’ 40”  | 56° 32’ 80”  | 2442.3624    |
| 3   | Darb- Behesht (Jiroft) | *E. cylindrica* E. wolffii | 29° 17’ 22”  | 57° 23’ 13”  | 2609.088     |
| 4   | DehBala (Kerman)  | *E. persica*             | 30° 16’ 64”  | 57° 18’ 58”  | 2589.276     |
| 5   | Dumar (Jebalbarez) | *B. paucifolium*         | 28° 50’ 10.45” | 57° 55’ 54.60” | 2022.0432 |
| 6   | Fashko (Dehbaki)  | *B. paucifolium* E. persica E. wolffii | 29° 11’ 89”  | 57° 48’ 30”  | 3349.4472    |
| 7   | Hanza (Rabor)     | *E. cylindrica*          | 29° 17’ 11”  | 57° 11’ 16”  | 2599.0296    |
of the umbels are unequal and extensive (Figures 1 and 2). Underground parts are irregularly spherical, the small to large depending to age; leaves are basal or alternately arranged along the stem, and leaf blades are often pinnate (Figure 1). The color of bush in three species of *E. cylindrica*, *E. persica* and *B. paucifolium* is green, while various populations of *E. wolffii* are purple specially during flower and fruit development (Figure 1). Each compound umbel in maturity stage has been composed of 7–10 unequal umbellets depends on the species (Figure 2, Table 2). They have 2–6 involucral bracts which are linear, sharp, herbarceous and caduceus; however, most of umbels are without bracts in *B. persica* (Figure 2). Each umbellet has 10–20 flowers depending on the species type. The involucels have 5–7

![Figure 1. Morphological view of (A) B. Paucifolium, B) E. cylindrica, (C) E. persica and (d) E. wolffii.](image-url)
bracteoles that are smaller than bracts. Bracteoles length in each involucl is different (heteromorphy) (Figure 3, Table 2). There are 3 to 4 male flowers in the center of umbellet in *E. cylindrica*, *E. wolffii*, *E. persica* and *B. paucifolium*. However, some populations of *E. persica* like the DehBala population had no male flowers (Figure 3). Patterns of flower development in andromonoeious species of Apioideae have been discussed by Ajani et al. (2016). They reported functionally male flowers show the same development pattern as hermaphroditic flowers except for the gynoecium, which gets inhibited at a late stage of development (Ajani et al., 2016). The accumulation and density of flowers in the inflorescences is an important biological trait that is dependent to the species type. The accumulation of flowers in large umbel inflorescences attracts insects from long distance; and this matter is helpful for pollination (Linder, 1998).

No discernible and grown calyx was observed in the flowers studied populations (Figures 3 and 4). Ajani et al. (2016) reported that from the functional point of view, flowers in Apioideae do not necessarily need large sepals. The outer flowers are usually protected by involucral and/or involuclellar bracts and the inner ones are densely packed and protected by neighboring meristems (Ajani et al., 2016). The corolla shape showed obvious differences between species so that it was raised and vertical in *B. paucifolium*, while horizontal and wide in three other species (Figures 3 and 4, Table 3). The corolla in all studied populations had five valvate petals with bent vertices into the flower which has similarly been reported in *Pycnocyciea* (Sheikhabahaei et al., 2014). The mid part of each petal is purple with white edge;
the density of purple color in the dorsal surface of the petal is more than its ventral part. The androecium has five stamens with dorsifixed anthers; gyroecium is syncarpous with stylodium at apex of ovary. During fruit development, bracts and bracteoles fall (Figure 5). The stylodium is conical in *E. cylindrica*, path conical in *E. persica* and *E. wolffii* and flat in *B. paucifolium* (Figure 6).

The structure and size of fruit showed differences in various species. They were narrow and cylindrical with two narrowed ends in *E. cylindrica*, ovate to broad in *E. persica*, cylindrical in *B. wolffii* and elliptic to ovate in *B. paucifolium* (Figure 6). There are five similar ribs in each fruit which are undulate in *E. cylindrica* and *E. persica*, wavy in *B. wolffii* and ovate in *B. paucifolium* (Figure 6). The fruit length is 5.14, 2.83, 3.89 and 2.53 mm in *E. cylindrica*, *E. persica*, *E. wolffii* and *B. paucifolium*, respectively (Figures 5 and 6, Table 2). Therefore, *E. cylindrica* and *B. paucifolium* show the highest and lowest size in length respectively. Some morphological, genetic and phytochemical variations have been reported for some populations of *B. persica* in India (Chahota et al., 2017; Dar et al., 2011). However, no comprehensive study was found on the features of inflorescence, flower, pollen and fruit in studied species.

The studies of pollen morphology on Apiaceae family have pointed to the effective role of pollen grains in determining the relationship of phylogenetic (De Leonardis et al., 2009). The data on pollen characteristics showed that polar axis is 21.75 µm, 22 µm, 16.75 µm and 22.50 µm while equatorial axis is 7 µm, 6.50 µm, 6.25 µm and 6.75 µm in *E. cylindrica*, *E. persica*, *E. wolffii* and *B. paucifolium* respectively. Therefore, pollen grains have small size. The ratio of the length of the polar axis to the equatorial diameter (P/E) is 3.1, 3.38, 2.68 and 3.33 µm in *E. cylindrica*, *E. persica*, *B. wolffii* and *B. paucifolium* respectively (Figure 7, Table 2). According to the classification of Punt et al. (2007), these pollen grains are described Perprolate (Pererect) in which P/E is more than 2 µm (Table 2). There are various reports on pollen characteristics of this family. Cerceau Larival and Roland Heydacker (1976), has

**Table 2. Quantitative morphological traits studied in *Bunium paucifolium*, *E. cylindrica*, *E. persica* and *E. wolffii* (mean ± s. e. mm)**

| Traits abbreviation | *B. paucifolium* | *E. cylindrica* | *E. persica* | *E. wolffii* |
|---------------------|-----------------|----------------|-------------|-------------|
| UPP                 | 10 ± 5 a        | 9 ± 4 a        | 7 ± 2 a     | 9 ± 2 a     |
| BPU                 | 6 ± 1 a         | 4 ± 2 b        | 1 ± 0.5 d   | 2 ± 1 c     |
| ULPU                | 7 ± 1 b         | 8 ± 2 b        | 9 ± 2 a     | 8 ± 2 ab    |
| BPRU                | 7 ± 1 a         | 5 ± 1 b        | 7 ± 1 a     | 5 ± 1 b     |
| FL                  | 14 ± 2 c        | 14 ± 2 d       | 18 ± 1 a    | 14 ± 1 b    |
| FR                  | 14 ± 2 b        | 13 ± 2 a       | 18 ± 2 b    | 13 ± 1 b    |
| FFR                 | 10 ± 2 b        | 5 ± 3 b        | 10 ± 4 a    | 5 ± 2 b     |
| EFR                 | 4 ± 2 b         | 9 ± 4 a        | 10 ± 4 a    | 8 ± 3 a     |
| FRL                 | 3 ± 0 c         | 5 ± 1 a        | 3 ± 0.5 c   | 4 ± 0 b     |
| PEL                 | 6 ± 1 a         | 3 ± 1 c        | 4 ± 1 bc    | 5 ± 2 b     |
| P                   | 23 ± 2 a        | 22 ± 2 a       | 22 ± 2 a    | 17 ± 3 b    |
| E                   | 7 ± 1 a         | 7 ± 2 a        | 7 ± 1 a     | 6 ± 1 a     |
| SL                  | 3 ± 1 a         | 1 ± 0 c        | 1 ± 0.5 c   | 2 ± 0 b     |

Umbels per plant (UPP), Bracts per umbel (BPU), Umbellets per umbel (ULPU), Bracteoles per umbellet (BPRU), Flower per umbellet (FL), Fruits per umbellet (FR), Filled fruits per umbellet (FFR), Empty fruits per umbellet (EFR), Fruit length (FRL), Pedicel length (PEL), Polar axis (P), Equatorial axis (E), Stylopodia length (SL). Values in the same column with different subscript letters represent significant differences between species at each trait at p < 0.05 by Duncan’s test (n = 10).
classified pollen of this family into four types based on the ratio of the polar and equatorial axis (P/E). In addition, Van Zeist and Bottema (1977) classified this family into 9 types based on the pollen grains. Azizian et al. (2003) reported that pollen grains in *Diplotaenia* (Apiaceae) are prolate in shape. Thus, it seems that pollen shape has significant value in the family and genera classification. However, there are also the reports showing that pollen grains are similar in this family (Erdtman, 1952).

The correlation coefficients quantitative traits of different populations *Elwendungia* and *Bunium* were presented in Table 4. The high positive and significant correlation value was obtained between filled fruits and bracteolates per umbellet. The stylodia length was positively and significantly associated with bracts per umbel and empty fruits per umbellet. The principal component analysis (PCA) of species indicated five principal components. The high positive correlation coefficients were obtained for 23.76% of total variance and the important traits included in the first principal component (PC1) were bracts per umbel, stylodia length, empty fruits, flowers per umbellet, fruits per umbellet and umbellets per umbel. The second principal component (PC2) had high contributing 22.08% variance and traits included in this group were filled fruits, bracteolates per umbellet, fruit length, fruits per umbellet and flowers per umbellet. The third principal component (PC3) was accounted for 10.24% of the total variation, with high factor loading for polar axis and pedicel length. Similarly, The fourth principal component (PC4) and fifth principal component (PC5) are showing 8.97% and 7.34% variance with major traits.
of umbel per plant and equatorial axis (Table 5). Chahota et al. (2017) reported in the fourteen populations of B. persica in Hymalaya, inflorescence, seed and branch traits were most significant in detecting variation. Also, the studies of Dor et al. (2011) showed that wide range of variation was found between six ecotypes of this species growing in Kashmir (India) for various morphological traits. Phenotypic coefficient of variation for all the traits was higher than the genotypic coefficient of variation, indicating that all these characters interacted with the
| Traits abbreviation | Traits characteristics | B. paucifolium | E. cylindrica | E. persica | E. wolffii |
|---------------------|------------------------|---------------|--------------|------------|-----------|
| BCO                 | Green                  | Green         | Green        | Purple     |
| CSH                 | Raised and vertical    | Horizontal and wide | Horizontal and wide | Horizontal and wide |
| SSH                 | Flat                   | Conical       | Path conical | Path conical |
| FSH                 | Elliptic to ovate      | Narrow and cylindrical | Oval to broad | Cylindrical |
| MRSH                | Flat                   | Undulate      | Undulate     | Wavy       |
| MF                  | Present                | Present       | Absent/Present | Present    |

Bush color (BCO), Corolla shape (CSH), Stylpodium shape (SSH), Fruit shape (FSH), Mericarp rib shape (MRSH), Male flower (MF).

Figure 5. The structure of umblets in (A) B. paucifolium, (B) E. cylindrica, (C) E. persica and (D) E. wolffii in maturation stage of fruit. Purple color of E. wolffii is obvious.
Figure 6. The view of mature fruit in (A) *E. cylindrica* (narrow and cylindrical; ribs: undulate), (B) *B. paucifolium* (elliptic to ovate; ribs: flat), (C) *E. persica* (ovate to broad; ribs: undulate) and (D) *E. wolffii* (cylindrical; ribs: wavy).

Figure 7. The structure of dumbbell-shaped pollen grains in (A) *E. cylindrica*, (B) *B. paucifolium*, (C) *E. persica* and (D) *E. wolffii*.
Table 4. Correlation coefficients among quantitative traits in Bunium paucifolium, E. cylindrica, E. persica and E. wolffii

|       | UPP | BPU | ULPU | BPRU | FL  | FR  | FFR | EFR | FRL | PEL | P  | E  | SL |
|-------|-----|-----|------|------|-----|-----|-----|-----|-----|-----|----|----|----|
| UPP   | 1   |     |      |      |     |     |     |     |     |     |    |    |    |
| BPU   | 0.135 | 1    |      |      |     |     |     |     |     |     |    |    |    |
| ULPU  | -0.162 | -0.338* | 1    |      |     |     |     |     |     |     |    |    |    |
| BPRU  | -0.196 | -0.075 | -0.002 | 1    |     |     |     |     |     |     |    |    |    |
| FL    | -0.186 | -0.535** | 0.525** | 0.450** | 1  |     |     |     |     |     |    |    |    |
| FR    | 0.015 | -0.465** | 0.179 | 0.486** | 0.453** | 1  |     |     |     |     |    |    |    |
| FFR   | -0.084 | 0.100 | 0.137 | 0.692** | 0.378* | 0.415** | 1  |     |     |     |    |    |    |
| EFR   | 0.139 | -0.531** | 0.349* | -0.155 | 0.371* | 0.349* | -0.272 | 1  |     |     |    |    |    |
| FRL   | -0.018 | -0.194 | -0.051 | -0.372* | -0.331* | -0.292 | -0.583** | 0.225 | 1  |     |    |    |    |
| PEL   | 0.180 | 0.261 | -0.233 | 0.221 | 0.086 | 0.068 | 0.221 | -0.304 | -0.450** | 1  |     |    |    |
| P     | -0.157 | 0.274 | -0.166 | 0.419** | -0.162 | 0.230 | 0.493** | -0.313* | -0.102 | 0.127 | 1  |     |    |
| E     | -0.027 | 0.161 | -0.007 | -0.075 | -0.144 | -0.076 | -0.014 | -0.086 | 0.036 | -0.002 | 0.097 | 1  |     |
| SL    | 0.163 | 0.684** | -0.395* | -0.145 | -0.295 | -0.465** | 0.121 | -0.629** | -0.432** | 0.488** | -0.035 | 0.067 | 1  |

**Significant with P < 0.01; *significant with P < 0.05.
Table 5. Eigen vector values of the first five principal components of quantitatively measured traits

| Morphological traits | Component 1 | Component 2 | Component 3 | Component 4 | Component 5 |
|----------------------|-------------|-------------|-------------|-------------|-------------|
| UPP                  | .324        | -.002       | -.248       | .644        | -.200       |
| BPU                  | .867        | .024        | .058        | -.101       | .235        |
| ULPU                 | -.595       | .110        | -.041       | -.260       | .316        |
| BPRU                 | -.105       | .817        | .338        | .010        | -.097       |
| FL                   | -.680       | .536        | -.269       | -.073       | .004        |
| FR                   | -.606       | .560        | .059        | .191        | .071        |
| FFR                  | .060        | .851        | .196        | -.109       | .169        |
| EFR                  | -.763       | -.201       | -.101       | .182        | -.105       |
| FRL                  | -.072       | -.722       | .441        | .244        | .156        |
| PEL                  | .389        | .475        | -.488       | .123        | -.159       |
| P                    | .229        | .469        | .703        | .121        | .123        |
| E                    | .178        | -.044       | .045        | .469        | .580        |
| SL                   | .799        | .452        | -.211       | -.075       | -.062       |
| Eigenvector          | 3.565       | 3.313       | 1.5366      | 1.346       | 1.101       |
| Variance %           | 23.763      | 22.083      | 10.240      | 8.974       | 7.342       |
| Cumulative %         | 23.763      | 45.847      | 56.087      | 65.061      | 72.403      |
environment (Dar et al., 2011). The studies on morphological, genetic traits and phytochemical composition of Bunium persica populations showed that inflorescence, seed and branch traits were most significant in detecting variation (Chahota et al., 2017).

The variability in both qualitative and quantitative traits was found in B. paucifolium and Elwendia species. The distribution plot of species based on quantitative traits showed that E. cylindrica and E. wolffii overlap somewhat and E. persica is close to them (without overlap); however, B. paucifolium is different from the three other species (Figure 8). Therefore, according this plot, B. paucifolium is placed in a group separate from the others. The obtained results of the study on the qualitative characteristics of the examined samples in the NTSYS software confirm the results obtained of quantitative traits and show that B. paucifolium is separated from other species (Figure 9).

In conclusion, the results of this study showed that corolla shape obviously shows the difference between Bunium and Elwendia species and confirms molecular studies performed by Degtjareva et al. (2013) and Pimenov (2017) in the separation of Bunium to two genera Bunium and Elwendia. However, B. paucifolium in some traits such as plant color, presence/absence of male flowers and bracts, is similar to Elwendia species.
Figure 9. Diagram of species distribution based on qualitative data by UPGM method.

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