

CLADISTICS AND PHENETICS ON SOME TAXA OF POLYGONACEAE JUSS.

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Abstract

Polygonaceae is a large dicot family of approximately 48 genera and 1200 species worldwide. The main objective of the current investigation is to compare between the pathways of evolution and the degree of similarity among the present polygonaceous taxa. To achieve the intended target, two character data sets were tested; morphological and molecular traits. Winclada/Nona programs were used for cladistic analysis while NTSYS-PC software for phenetic analysis. The resulted cladogram supports the monophyly of Polygonaceae with its five lineages, while the obtained phenogram comprises five groups. It was concluded that there was a close concordance between the results of phylogenetic and phenetic studies on Polygonaceae.

Key words: Phylogenetic analysis, Numerical taxonomy, Polygonaceae.

Introduction

Polygonaceae is a large dicot family of approximately 48 genera and 1200 species with a worldwide distribution (Sanchez & Kron, 2008). It is represented in flora of Egypt by 22 wild species under six genera *Viz.* *Atraphaxis* L., *Calligonum* L., *Emex* Neck., *Oxygonum* Burch., *Polygonum* L. and *Rumex* L. (Montasir & Hassib, 1956). Täckholm (1974) has recognized 28 species and added one more genus *Bilderdykia* Dumort. to the genera cited by Montasir & Hassib (1956). Boulos (1999) has recorded eight genera (*Atraphaxis*, *Calligonum*, *Emex*, *Fallopia* Adans, *Oxygonum*, *Persicaria* (L.) Mill., *Polygonum* and *Rumex*) with 25 species, four subspecies and one variety. Horticultural genera in Egypt are represented by the following genera *Antigonon* Endl., *Coccoloba* P.Browne, *Muehlenbeckia* Meisn. and *Ruprechtia* C.A.Mey. Polygonaceae have been recognized as a distinct family (Jussieu, 1789) due to the presence of ochrea, five or six tepals of the perianth, and the achene fruit (Janelle & Adriana, 2011).

At infra-familial level, the subfamily Eriogonoideae was first proposed by Meissner (1856) that divided Polygonaceae into four subfamilies *Viz.* Eriogoneae, Polygonoeae, Brunnichieae and Symmerieae. Dammer (1893) divided it into three subfamilies *Viz.* Rumicoideae, Polygonoideae and Coccoloboideae.

Moreover Polygonaceae is divided into Eriogonoideae and Polygonoideae according to Haraldson (1978) and Anon., (2009) while another third subfamily Coccoloboideae was added by (Gross, 1913 & Brandbyge, 1993) and the fourth subfamily was also included Calligonoideae (Roberty & Vautier, 1964).

At tribal level, Polygonaceae was divided into several tribes according to many current systems of classification. The minimum number of its tribes was three *Viz.* Eriogoneae, Pterogocarpae and Apterocarpae *sensu* Meissner (1856). Whereas the maximum number of them was proposed by Roberty & Vautier (1964) which included nine tribe *viz.*, Eriogoneae, Rumiceae, Polygoneae, Rheae, Coccolobeae, Triplareae, Calligoneae, Brunnichieae and Antigoneae.

Morphology of Polygonaceae has been studied intensively by (Galle, 1977; Hamed & Tantawy, 1991;

Tantawy *et al.*, 2005). Many approaches have been centered to identify systematically informative leaf and seed characteristics that allow species to be recognized on the basis of leaves and seeds (Hickey & Taylor, 1991; Tantawy, 2000; Tantawy & Rabie, 2000). Inamdar (1971) and Hamed & Tantawy (1990) described epidermal characters and stomata in 15 species of Polygonaceae and observed diversity of trichomes and epidermal cells, in addition, Ghazalah *et al.*, (2010) studied the leaf epidermal characters of eleven polygonaceous species and concluded that these traits have taxonomic importance. The stomata were mostly anisocytic, anomocytic and paracytic type. Molecular data have been used to address the phylogenetic relationships within families such as Polygonaceae (Sanchez & Kron, 2009). Phenetic is a discipline that depends on the phenotypic similarity or variations between the taxa and infers heavily from the methods of numerical taxonomy (Sneath & Sokal, 1973). Lamb-Frye & Kron (2003) established phylogenetic relationship within Polygonaceae, particularly in *Polygonum*, *Emex*, *Persicaria* and *Polygonella* Michx. They were of the opinion that presently accepted two subfamilies (Polygonoideae and Eriogonoideae) are not monophyletic.

The specific objectives of the present investigation are to construct and compare the phenetic and phylogenetic relationships in addition to explore the contribution of morphological and molecular traits in systematics of Polygonaceae and to assess the monophyly within the studied family.

Materials and Methods

Sampling: Vegetative shoots of 20 taxa (19 of Polygonaceae belonging to nine genera and *Plumbago auriculata* Lam. as an outgroup) were used due to the strong support of sister relationship between Polygonaceae and Plumbaginaceae (Cuénoud *et al.*, 2002). Nine taxa were gathered from some Egyptian botanical gardens while the rest were collected from different natural habitats within Egypt (Table 1). The accurate authentication of wild taxa was done with the help of Täckholm (1974) and Boulos (1999). Whereas the horticultural taxa with the help of Bailey & Bailey (1976). The taxa were further matched with the authentically

identified specimens housed in the Herbaria at Ain Shams University, Faculty of Science (CAIA), Cairo University, Faculty of Science (CAI), Flora and Phytotaxonomy Research Department (CAIM) and Orman Botanical Garden (Giza). Voucher specimens of the studied taxa were deposited in CAIA.

Morphological investigation: Macromorphological characters of whole plants were examined. Lamina vein architecture investigation was carried out with chemical treatments; HNO₃ was used for about 6-24 h, washed and then stained with (1% safranin), for documentation a binocular stereo zoom light microscope Bel Photonics and

illuminated glass box designed by the authors (Abdel-Hameed, 2014) were used. All photographs were taken using digital camera Cannon Power Shot G12 (Fig. 1, a-e). Stomatography investigation was carried on according to Stace (1965). The photomicrographs were obtained using a Reichert Microstar IV microscope (Fig. 2, a-f). For SEM small pieces (7mm²) of the leaf materials were glued on SEM stubs, gold was used for coating in SPI-Module sputter coater, examined in Jeol JSM 5200 at magnifications ranged from 750 X- 1500X (Fig. 3, a-c). Terminology for description of leaf architecture and epidermal characteristics were dependent on Metcalfe & Chalk (1950), Anon., (1999) and Prabhakar (2004).

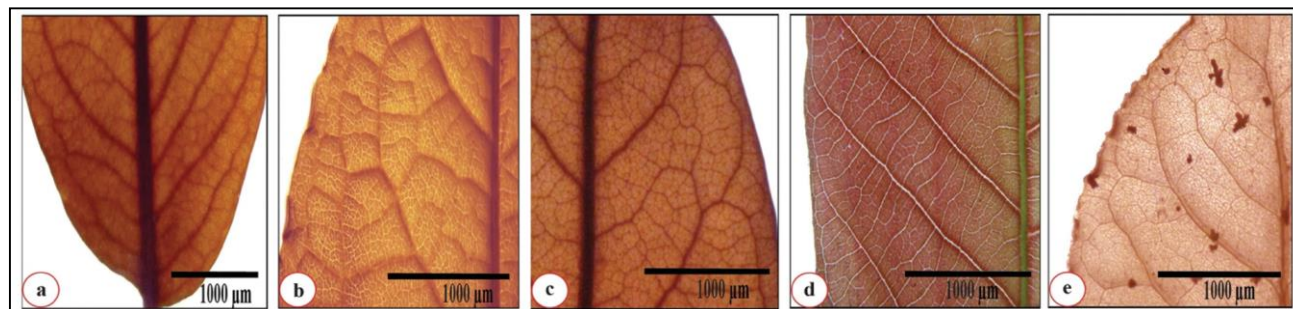


Fig. 1. Major categories of lamina vein architecture and stomatography (LM and SEM); a. Pinnate 1° V. (*Ruprechtia salicifolia*). b. Festooned brochidodromous 2° V. (*Antigonon leptopus*). c. Brochidodromous 2° V. (*Ruprechtia apetala*). d. Mixed opposite/alternate percurrent 3° V. (*Triplaris cumingiana*). e. Alternate percurrent 3° V. (*Rumex vesicarius*).

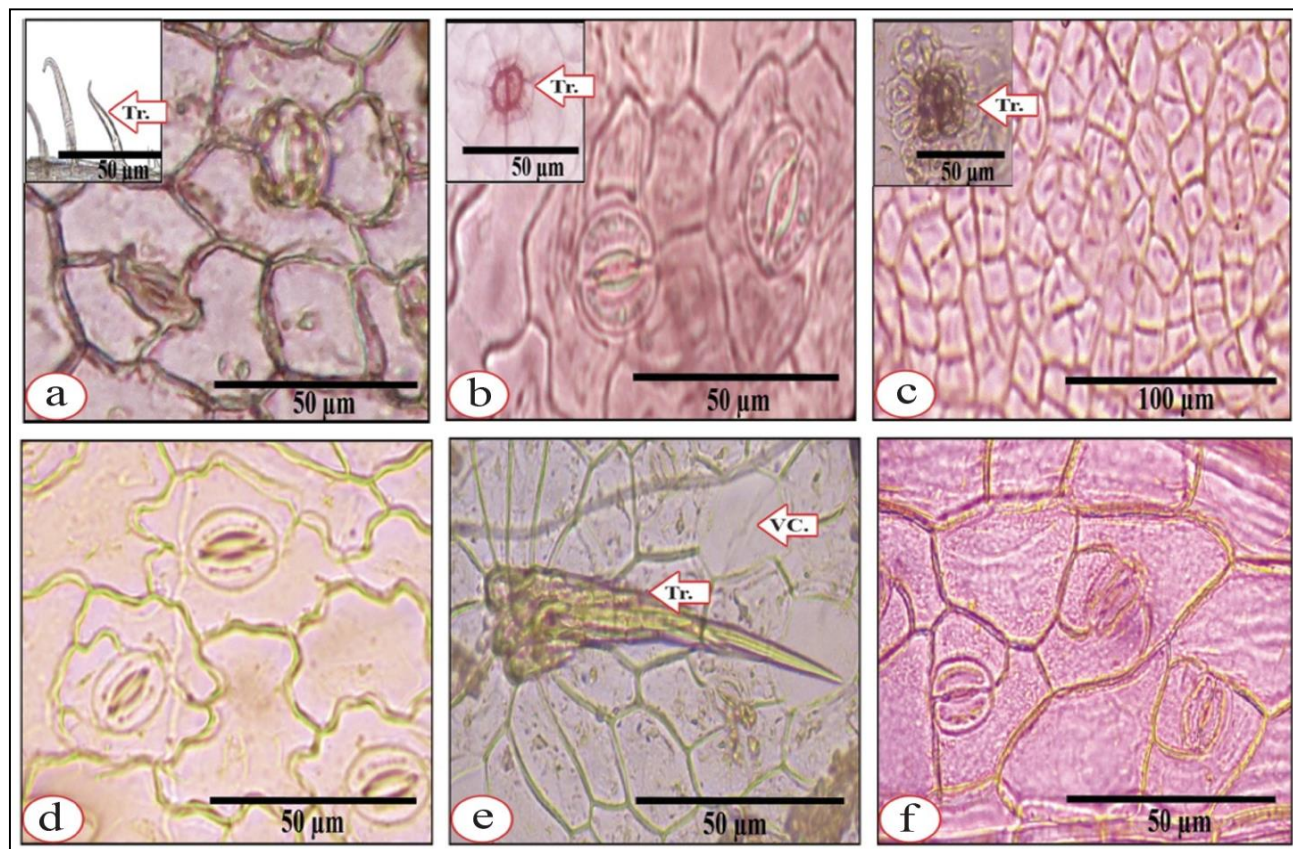


Fig. 2. Major categories of stomatography as revealed with LM; a. Anisocytic stomata, curved anticlinal wall, unbranched uniseriate (uni-, bi- & multicellular) eglandular trichome (*Antigonon leptopus*). b. Anomocytic & anisocytic stomata, sinous anticlinal wall, peltate two celled sessile glandular trichome (*Muehlenbeckia complexa*). c. Straight anticlinal wall, peltate four celled sessile glandular trichome (*Ruprechtia apetala*). d. Paracytic & anisocytic stomata (*Persicaria decipiens*). e. Bunch of unicellular with swollen base trichome, vesicular cell (VC.) (*Persicaria lapathifolia*). f. pericytic stomata (*Polygonum equisetiforme*).

Table 1. Collection data of the studied taxa.

| No. | Studied Taxa | Date | Locality |
|-----|--|-----------|----------|
| 1. | <i>Antigonon leptopus</i> Hook. & Arn. --Bot. Beechey Voy. 308, t. 69. 1838 [Dec 1838] (IK) = <i>Antigonon amabie</i> K.Koch = <i>Antigonon platypus</i> Hook. & Arn. = <i>Antigonon cinerascens</i> M.Martens & Galeotti = <i>Antigonon cordatum</i> M.Martens & Galeotti = <i>Corculum leptopus</i> Stuntz | 15/9/2013 | A |
| 2. | <i>Coccoloba peltata</i> Schott --Syst. Veg. (ed. 16) [Sprengel] 4(2, Cur. Post.): 405. 1827 [Jan-Jun 1827] (IK) = <i>Coccoloba martii</i> Meisn.= <i>Coccoloba nymphaeifolia</i> Linden = <i>Coccoloba peltigera</i> Meisn. = <i>Uvifera peltata</i> Kuntze Revis. | 24/2/2013 | B |
| 3. | <i>Coccoloba uvifera</i> L. -- Syst. Nat., ed. 10. 2: 1007. 1759 [7 Jun 1759] (IK) = <i>Coccolobis uvifera</i> (L.) Crantz = <i>Polygonum uviferum</i> L. | " | " |
| 4. | <i>Emex spinosa</i> (L.) Campd. -- Monogr. Rumex 58, t. 1, f. 1. 1819 (IK) = <i>Rumex spinosus</i> L. = <i>Rumex glaber</i> Forssk. = <i>Centopodium spinosum</i> (L.) Burch. = <i>Vibo spinosa</i> (L.) Medik. | 2/4/2014 | D |
| 5. | <i>Muehlenbeckia complexa</i> Meisn. -- Pl. Vasc. Gen. [Meisner] 2: 227. 1841 (IK) = <i>Polygonum complexum</i> A. Cunn. = <i>Calacinum complexum</i> (A.Cunn.) J.F.Macbr. = <i>Sarcogonum complexum</i> (A.Cunn.) Kunze | 24/2/2013 | B |
| 6. | <i>Muehlenbeckia platyclada</i> (F. J. Müll.) Meisn. -- Bot. Zeitung (Berlin) 23: 313. 1865 (as "platyclados") (IK)= <i>Polygonum platycladum</i> F. Muell. = <i>Homalocladium platycladum</i> (F. Muell.) L. H. Bailey = <i>Calacinum platycladum</i> (F.Muell.) J.F.Macbr. = <i>Sarcogonum platycladum</i> (F.Muell.) | 15/9/2013 | A |
| 7. | <i>Persicaria decipiens</i> (R. Br.) K. L. Wilson -- Telopea 3(2): 178 (1988): (IK) = <i>Persicaria salicifoliola</i> (Brouss. ex Willd.) Assenov = <i>Polygonum decipiens</i> R. Br. = <i>Polygonum salicifolium</i> Brouss. Ex Willd. = <i>Polygonum serrulatum</i> Lag. | 20/6/2014 | G |
| 8. | <i>Persicaria lanigera</i> (R. Br.) Soják -- Preslia 46(2): 153. 1974 (IK) = <i>Polygonum lanigerum</i> R. Br. | 16/2/2014 | E |
| 9. | <i>Persicaria lapathifolia</i> (L.) Gray -- Nat. Arr. Brit. Pl. ii. 270. (IK) = <i>Polygonum lapathifolium</i> L. = <i>Polygonum tomentosum</i> Schrank | 13/6/2014 | F |
| 10. | <i>Persicaria senegalensis</i> (Meisn.) Soják -- Preslia 46(2): 155. 1974 (IK) = <i>Polygonum senegalense</i> Meisn. | 10/5/2015 | N |
| 11. | <i>Polygonum equisetiforme</i> Mayer ex Ten. -- Syll. Pl. Fl. Neapol. 195. 1831 (IK) | 2/4/2014 | D |
| 12. | <i>Polygonum patulum</i> M. Bieb. -- Fl. Taur.-Caucas. 1: 304. 1808 (IK) = <i>Polygonum bellardii</i> All. = <i>Polygonum senegalense</i> Meisn. | 21/3/2014 | H |
| 13. | <i>Rumex dentatus</i> L. subsp. <i>dentatus</i> -- Mant. Pl. Altera 226. 1771 [Oct 1771] (IK) | 16/5/2014 | I |
| 14. | <i>Rumex pictus</i> Forssk. -- Fl. Aegypt.-Arab. 76. 1775 [1 Oct 1775] (IK) = <i>Rumex lacerus</i> Balb = <i>Acetosa bipinnata</i> Chaz. = <i>Acetosa picta</i> (Forssk.) Á.Löve & Kapoor = <i>Analiton bipinnatus</i> Raf. | 21/3/2014 | J |
| 15. | <i>Rumex vesicarius</i> L. -- Sp. Pl. 1: 336. 1753 [1 May 1753] (IK) = <i>Acetosa vesicaria</i> (L.) Á. Löve | 22/4/2013 | K |
| 16. | <i>Ruprechtia apetala</i> Wedd. -- Ann. Sci. Nat., Bot. sér. 3, 13: 268. 1850(IK) = <i>Ruprechtia boliviensis</i> Herzog = <i>Ruprechtia corylifolia</i> Griseb. = <i>Ruprechtia excelsa</i> Griseb. = <i>Ruprechtia fagifolia</i> Meisn. = <i>Ruprechtia mollis</i> Wedd. = <i>Magonia apetala</i> (Wedd.) Kuntze = <i>Magonia corylifolia</i> (Griseb.) Kuntze = <i>Magonia excelsa</i> (Griseb.) Kuntze = <i>Magonia fagifolia</i> (Meisn.) Kuntze = <i>Magonia mollis</i> (Wedd.) Kuntze | 12/5/2013 | L |
| 17. | <i>Ruprechtia laxiflora</i> Meisn. -- Fl. Bras. (Martius) 5(1): 56. 1855 [1 Jan 1855] (IK) = <i>Ruprechtia polystachya</i> Griseb = <i>Magonia laxiflora</i> (Meisn.) Kuntze = <i>Magonia polystachya</i> (Griseb.) Kuntze = <i>Magonia viraru</i> (Griseb.) Kuntze = <i>Ruprechtia viraru</i> Griseb. = <i>Triplaris polystachya</i> (Griseb.) Kuntze | 14/5/2013 | B |
| 18. | <i>Ruprechtia salicifolia</i> C. A. Mey. -- Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 6(2, Bot.): 150. 1840 [Nov 1840]; this is vol. 4 of the Sci. Nat. series; reprinted as Bem. Polygon: 16. (IK) = <i>Magonia salicifolia</i> (Cham. & Schltdl.) Kuntze = <i>Triplaris salicifolia</i> Cham. & Schltdl. | " | " |
| 19. | <i>Triplaris cumingiana</i> Fisch. & C. A. Mey. ex C.A.Mey. -- Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 6(2, Bot.): 148. 1840 [Nov 1840]; this is vol. 4 of the Sci. Nat. series; reprinted as Bem. Polygon: 14. (IK) = <i>Triplaris armottiana</i> Meisn. = <i>Triplaris auriculata</i> Meisn. = <i>Triplaris guayaquilensis</i> Wedd. = <i>Triplaris lindeniana</i> Wedd. | 4/3/2015 | M |
| 20. | <i>Plumbago auriculata</i> lam. -- Encycl. [J. Lamarck & al.] 2(1): 270. 1786 [16 Oct 1786] (IK) = <i>Plumbago capensis</i> Thunb. | 15/9/2013 | A |

A: Botanical Garden, Botany Department, Faculty of Science, Ain Shams University, Alabbassia, Cairo. B: Orman Botanical Garden, Giza. D: Mersa Matrouh, El- Gharam Sea Shore. E: Cairo Alexandria Cultivated Road. F: Kafr El-Sheikh Canal Banks. G: El-Kanater Irrigation Canals. H: Rashid, under the International Costal Road. I: El-Mariottia Irrigation Canals, Al-Haram, Giza. J: Rashid, Al-Bossaili, Alexandria. K: Saint Katherine, South Sinai. L: El-Zohria Botanical Garden, Giza. M: Mazhar botanic garden. N: El Waraq Island

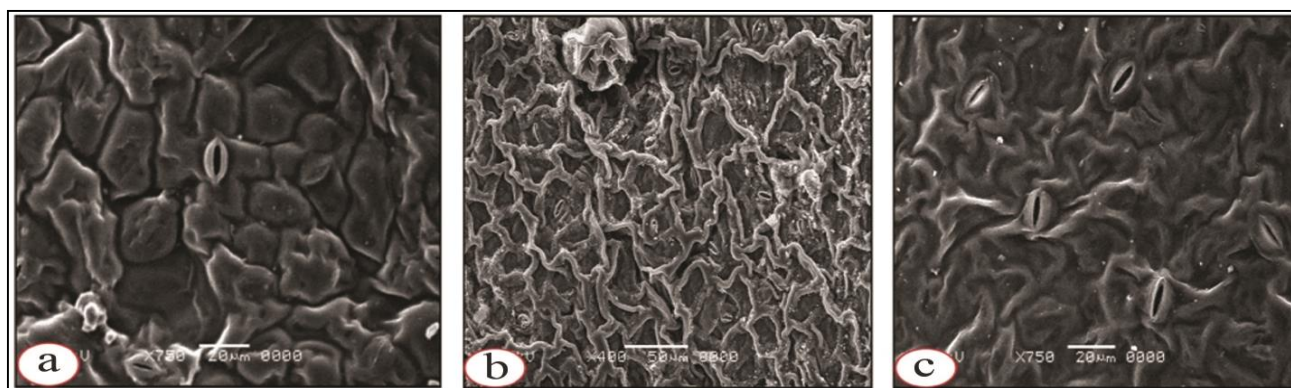


Fig. 3. Major categories of stomatograpy as revealed with SEM; a. Colliculate sculpture, narrow depressed anticlinal wall, raised granulate periclinal wall (*Antigonon leptopus*). b. Reticulate sculpture, wide raised anticlinal wall, depressed wrinkled periclinal wall (*Persicaria lapathifolia*). c. Ruminant sculpture, narrow depressed anticlinal wall, raised smooth periclinal wall (*Muehlenbeckia platyclada*).

Table 2. Types of bands and percentage of polymorphism of primers applied to the studied taxa of Polygonaceae.

| Primer | ISSR 1 | ISSR 2 | ISSR 4 | ISSR 5 | ISSR 6 | ISSR 7 | ISSR 8 | ISSR 9 | ISSR 15 | ISSR 18 |
|------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| Monomorphic bands | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 0 |
| Polymorphic bands (without unique) | 13 | 9 | 9 | 13 | 12 | 14 | 10 | 3 | 6 | 13 |
| Unique bands | 0 | 0 | 2 | 0 | 1 | 3 | 1 | 1 | 2 | 1 |
| Polymorphic bands (with unique) | 13 | 9 | 11 | 13 | 13 | 17 | 11 | 5 | 8 | 14 |
| Total number of bands | 14 | 10 | 11 | 13 | 13 | 17 | 12 | 7 | 11 | 14 |
| % Of polymorphism | 93 % | 90 % | 100 % | 100 % | 100 % | 100 % | 92 % | 71 % | 73 % | 100 % |

Molecular investigation: Total DNA extraction was performed as indicated by Qigene multisource Genomic DNA Mini-Prep Kit (USA, cat. No. Ap-MN-MS-GDNA-50). An initial screening of 20 ISSR primers (successfully utilized in other plant species) was performed in order to test their readability and amplification profiles for polymorphism. Ten ISSR primers were selected (Table 2). Polymerase chain reactions (PCR) were performed according to Whitty *et al.*, (1994). The ISSR products based were detected on agarose gel (1.2% in 1× TBE buffer) and then ethidium bromide (0.3 ug/ml) was used for staining. Sizes of amplicon were estimated by 1 Kb DNA standard (Bioron, Germany). Reproducible bands visualized on the gels (Fig. 4) were scored as a binary code (1/0) for their presence or absence, based on the UVP gel documentation system (Gel Works ID advanced software, UVP).

Phylogenetic analysis: Character/taxon matrix was prepared in WinClada Software (Nixon, 1999), and analyzed using NONA 1.6 (Goloboff, 1993). The cladistic analyses was conducted using heuristic searches.

Phenetic analysis: The estimation of character states variation was done by Unweighted Pair-Group Method using Arithmetic Averages with SAHN function due to Sneath & Sokal (1973) and characters states were analyzed as binary states. The grouping process was depended on the similarity values. All computations were performed by the aid of NTSYS-PC version 2.02 (Rohlf, 2000).

Results and Discussion

The morphological and molecular characters of all the taxa under investigation in addition to their states were extracted and tracked on the resulted dendrograms to detect its importance in delimiting taxa. The type of bands and percentage of polymorphism of each primer applied to the studied taxa of Polygonaceae were shown in Table 2.

The obtained cladogram (Fig. 5) showed that within the investigated taxa of Polygonaceae there was a support for five lineages *Viz.* *Muehlenbeckia* lineage (*M. platyclada*, *Emex spinosa* and *Rumex pictus*), *Ruprechtia* lineage (*R. salicifolia*, *Triplaris cumingiana*, *R. apetala* and *R. laxiflora*), *Coccoloba* lineage (*C. peltata*, *C. uvifera*, *Antigonon leptopus* and *Muehlenbeckia complexa*), *Rumex* lineage (*R. vesicarius*, *Polygonum patulum* and *Rumex dentatus sub. dentatus*) and *Polygonum* lineage (*P. equisetiforme*, *Persicaria senegalensis*, *P. lapathifolia*, *P. decipiens* and *P. lanigera*).

The resulted phenogram (Fig. 6) showed that out of all studied taxa, the exemplars of *Persicaria* were segregated into a distinct group at a taxonomic distance of 0.53, all the rest of the studied taxa were diffused into four groups, the first one was distinguished as a separate phenetic line at 0.59 taxonomic distance including *Muehlenbeckia platyclada*, *Antigonon leptopus*, *M. complexa*, *Coccoloba peltata* and *C. uvifera*. Whereas *Triplaris cumingiana*, *Ruprechtia apetala*, *R. salicifolia* and *R. laxiflora* were clustered together in a second group at a taxonomic distance 0.69. The third group that was separated at a taxonomic distance 0.6 included *Emex spinosa* and *Rumex dentatus subsp. dentatus*, while the fourth one was divided into two separate phenetic lines at a taxonomic distance 0.63, the first one was of *Polygonum equisetiforme* and *P. patulum* at 0.69 while the second consisted of *Rumex pictus* and *R. vesicarius* at 0.7.

In phylogenetic context, the clade comprising all the studied taxa received 100% bootstrap value, expressing strong support for the monophyly of the family. The cladogram was divided into five lineages based on morphological and molecular synapomorphies. The same result was obtained by phenetic analysis where all studied taxa are distributed into five groups which was in agreement with Cuénoud *et al.* (2002) and Lamb-Frye & Kron (2003).

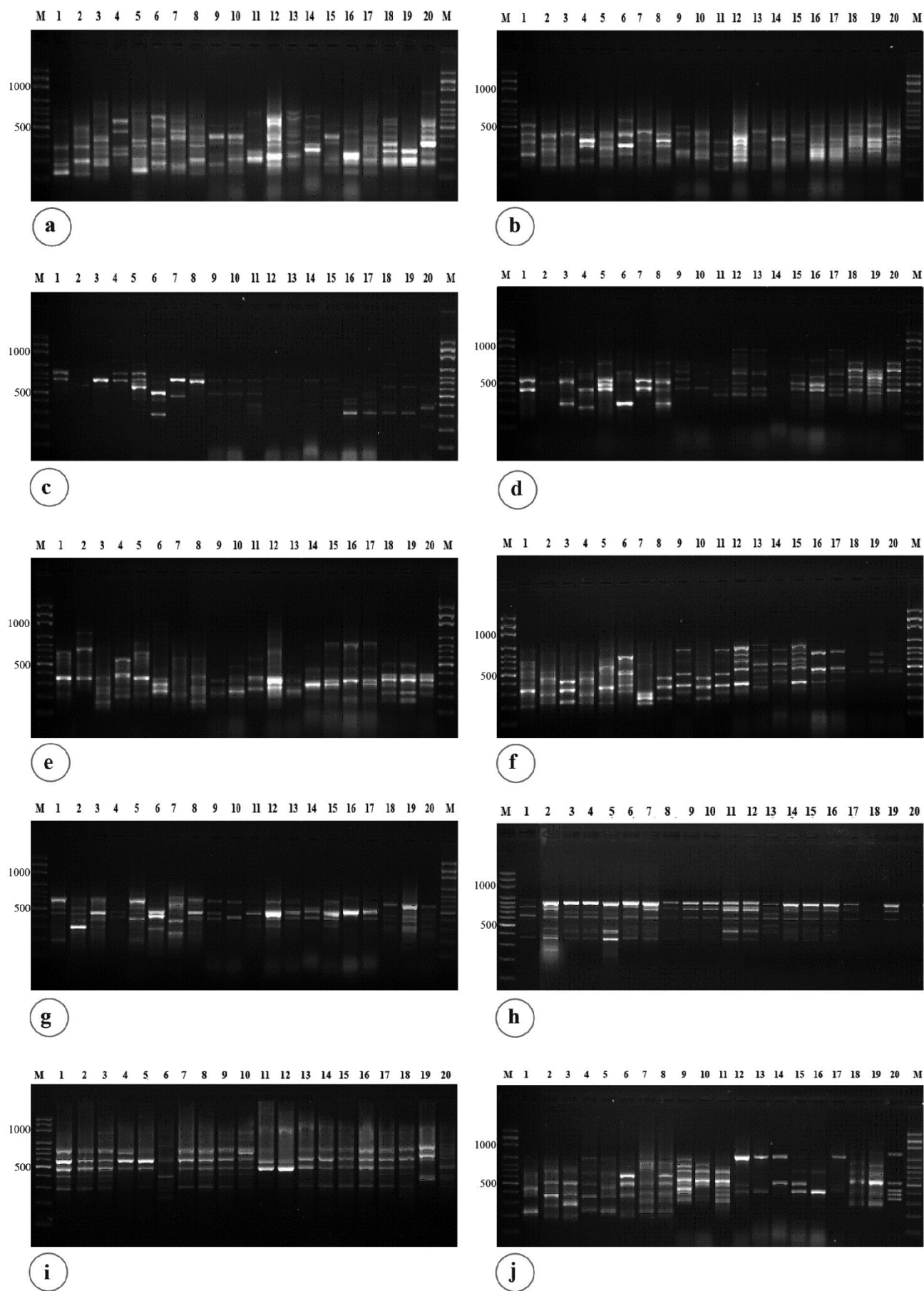


Fig. 4. ISSR profile of the studied taxa of Polygonaceae by the primer generated by a. primer ISSR 1. b. primer ISSR 2. c. primer ISSR 4. d. primer ISSR 5. e. primer ISSR 6. f. primer ISSR 7. g. primer ISSR 8. h. primer ISSR 9. i. primer ISSR 15. j. primer ISSR 18.

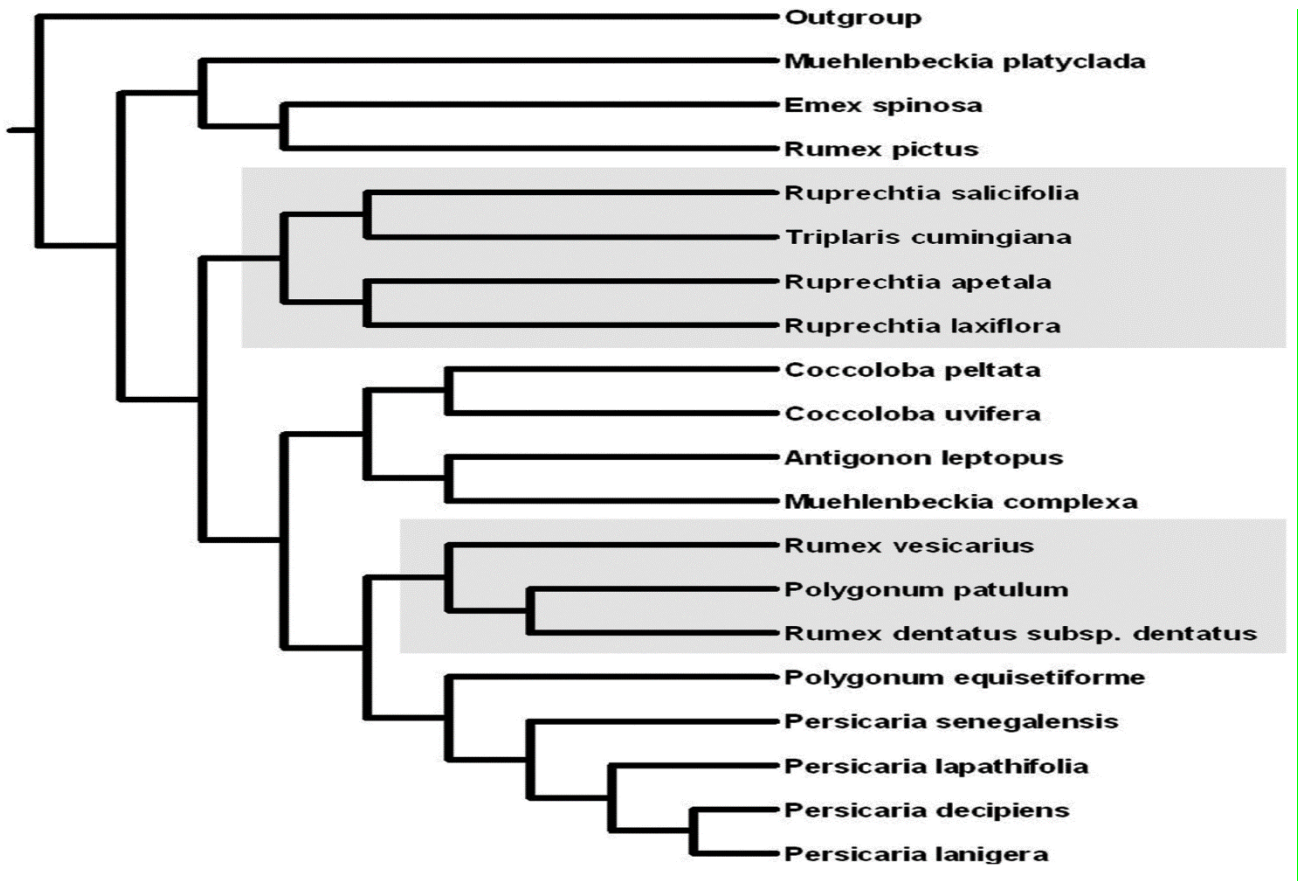


Fig. 5. Cladogram based on morphological and molecular traits of Polygonaceae comprising five lineages.

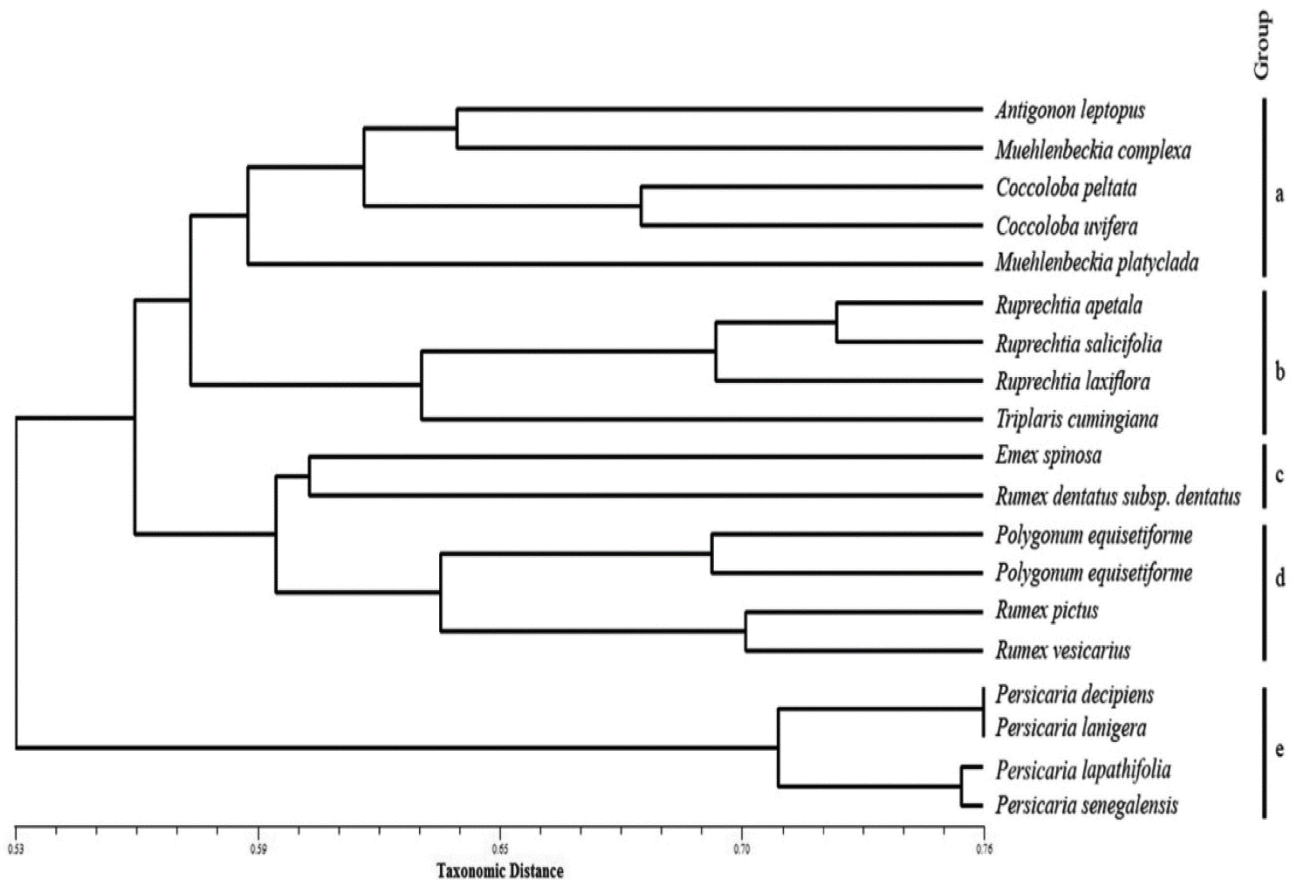


Fig. 6. Phenogram based on morphological and molecular traits of Polygonaceae comprising five groups.

Phylogenetically, *Muehlenbeckia platyclada* occupied the basal position to *Emex spinosa* and *Rumex pictus* that have sister-group relationship. These taxa formed a monophyletic group owing to ochreate stipules, three carpels, one style, curved adaxial anticlinal walls, narrow abaxial anticlinal walls width, smooth abaxial periclinal walls surface, brochidodromous 2° vein category, in addition to 18 common bands of variable molecular weights produced by eight primers (ISSR 1, 4, 5, 6, 7, 8, 9 and 18) as synapomorphic characters. This is in agreement with placing of *Muehlenbeckia* and *Rumex* in tribe Apterocarpaceae under subfamily Polygonoideae (Meissner, 1865) and *Muehlenbeckia*, *Emex* and *Rumex* together under subfamily Polygonoideae (Haraldson, 1978 and Anon., 2009). On the other hand Bentham & Hooker (1883), Dammer (1893), Perdrigeat (1900), Gross (1913), Roberty & Vautier (1964) and Brandbyge (1993) segregated them in different tribes and subfamilies. Gross (1913) put *Emex* and *Rumex* in tribe Rumiceae which was strongly supported as monophyletic group by Sanchez (2011) and *Muehlenbeckia* in another tribe Coccolobeae. Brandbyge (1993) put them in two distinct subfamilies; Polygonoideae (*Emex* and *Rumex*) and Coccoloboideae (*Muehlenbeckia*) and this was supported by our result obtained from the phenetic analysis where *M. platyclada* segregated in a distinct group away from *E. spinosa* and *R. pictus*.

Ruprechtia apetala, *R. laxiflora* and *R. salicifolia* were nested in one lineage with *Triplaris cumingiana* supporting the monophyly of that lineage. This is in accord with Sanchez and Kron (2008) through the synapomorphic characters; perennial tree, stem erect, glabrous, terete, leaves simple, alternate, petiolate, cuneate lamina base, flowers with six perianth, three carpels, three style, achene fruit, lamina epidermis type hypostomatic, polygonal ab-, adaxial cell shape, brochidodromous secondary vein category, irregular secondary vein spacing, secondary vein angle increasing toward base, mixed opposite/alternate percurrent 3° vein category, exmedially ramified 3° vein course, in addition to ten common bands of variable molecular weights produced by six primers (ISSR 2, 4, 6, 7, 8 and 15). The same results were obtained upon phenetic analysis where the three studied taxa of *Ruprechtia* and *T. cumingiana* were nested together in the same phenetic group. This confirms that *Triplaris* and *Ruprechtia*, were previously placed in the same tribe Triplarieae or subtribe Triplarieae (Meissner, 1856; Bentham & Hooker, 1883; Dammer, 1893; Gross, 1913; Roberty & Vautier, 1964; Haraldson, 1978; Brandbyge, 1993) and in the same subfamily Eriogonoideae (Anon., 2009). According to Burke *et al.*, (2010), *Triplaris* and *Ruprechtia* form a highly supported clade (95-99%) bootstrap; indicating that *Triplaris* was monophyletic while *Ruprechtia* was not and that was also confirmed by the data in the present study. *R. salicifolia* & *Triplaris cumingiana* showed a sister-group relationships as the same as *R. apetala* & *R. laxiflora*.

Coccoloba peltata, *C. uvifera*, *Antigonon leptopus* and *Muehlenbeckia complexa* have a close relationship and formed a monophyletic group owing to perennial habit, leaves simple, petiolate, flowers with five perianth, eight stamens, three carpels, three styles, achene fruit, lamina epidermis type hypostomatic, narrow abaxial anticlinal

wall, irregular 2° vein spacing, mixed opposite/alternate percurrent 3° vein category, in addition to 13 common bands of variable molecular weights produced by six primers (ISSR 1, 4, 7, 8, 9 and 15). Phenetically *M. platyclada* is added to the preceding taxa in a single phenetic line. This is in agreement with traditional placement of *Muehlenbeckia* in tribe Coccolobeae along with *Antigonon* and *Coccoloba* (Bentham & Hooker, 1883; Dammer, 1893; Gross, 1913; Haraldson, 1978; Brandbyge, 1993). On the other hand Meissner (1856), Perdrigeat (1900) and Roberty & Vautier (1964) placed them into more than one tribe. Anon., (2009) treated the two genera *Antigonon* and *Coccoloba* in subfamily Eriogonoideae and placed *Muehlenbeckia* in subfamily Polygonoideae indicating that *Muehlenbeckia* is not monophyletic group. This disagrees with Schuster (2011) where *M. platyclada* is nested in another lineage in the present study.

Phylogenetically, *Rumex* lineage includes *R. vesicarius*, *R. dentatus* and *Polygonum patulum*. Phenetically, these taxa in addition to *Emex spinosa*, *Polygonum equisetiforme* and *Rumex pictus* are clustered together in a distinct cluster. This is in accordance with Roberty & Vautier (1964), Haraldson (1978), Brandbyge (1993) and Anon., (2009), where *Rumex* and *Polygonum* are included in the same subfamily Polygonoideae while the most current taxonomic systems distribute them in different subfamilies, tribes and subtribes. To cite a few, Bentham & Hooker (1883) treated *Rumex* in tribe Rumiceae and *Polygonum* in another tribe Eupolygoneae while Gross (1913) put them in two distinct subfamilies; Eriogonoideae and Polygonoideae respectively. *R. pictus* is segregated in *Muehlenbeckia* lineage indicated that this genus is not monophyletic group. This disagrees with Sanchez (2011).

Emex spinosa and *Rumex dentatus* subsp. *dentatus* were clustered together in a distinct phenetic group. This agrees with placing of *Emex* and *Rumex* as a separate genera in the same tribe Rumiceae (Bentham & Hooker, 1883; Dammer, 1893; Perdrigeat, 1900; Gross, 1913; Haraldson, 1978; Brandbyge, 1993) and in the same subfamily Polygonoideae (Anon., 2009). On the other hand Roberty & Vautier (1964) segregated them in two distinct tribes Polygoneae and Rumiceae respectively. *Polygonum equisetiforme* is clustered with *Polygonum patulum* in the same phenetic subgroup and this is in agreement with Ronse De Craene & Akeroyd (1988).

Persicaria decipiens, *Persicaria lanigera*, *Persicaria lapathifolia* and *Persicaria senegalensis* were grouped in a single phenetic group. Phylogenetically, these taxa along with *Polygonum equisetiforme*, were nested in one lineage through the synapomorphic characters; herb habit, stem terete, leaves simple, ochreate, lanceolate, cuneate lamina base, amphistomatic lamina epidermis type, curved adaxial anticlinal wall, brochidodromous 2° vein category, in addition to eight common bands of variable molecular weights produced by eight primers (ISSR 1, 2, 4, 5, 8, 9, 15 and 18). This was in accord with Gross (1913) that supported the inclusion of *Persicaria* and *Polygonum* in the tribe Polygoneae. Haraldson (1978) and Brandbyge (1993) segregated them in two distinct tribes; Persicarieae and Polygoneae respectively.

Polygonum equisetiforme occupied the basal position of the lineage and the other species *Polygonum patulum* was segregated in *Rumex* lineage, indicating that this genus was not monophyletic group. Biomolecular studies revealed that *P. sensu lato* was polyphyletic and should be divided into several genera (Cuénoud *et al.*, 2002; Lamb-Frye & Kron, 2003; Kim & Donoghue, 2008; Sanchez & Kron, 2008).

Gross (1913) was the first one who recognized *Persicaria* as an independent genus. However, it was considered as section of the *Polygonum* by Roberty & Vautier (1964). The molecular studies carried out by Kim & Donoghue (2008) segregated *Persicaria* as a distinct genus as mentioned in the morphological and anatomical studies carried out by Haraldson (1978) and Brandbyge (1993). To some extent, this is in an accordance with the present study.

Conclusion

While comparing the generated trees using phylogenetic and phenetic analyses, it is revealed that the comparability between them was about 63% on the same studied taxa. The tree derived from the phylogenetic analysis supported the clustering of *Antigonon leptopus*, *Coccoloba peltata*, *C. uvifera* and *Muehlenbeckia complexa* in a single lineage (*Coccoloba*) while in phenetic context *M. platyclada* was added to all the preceding taxa forming a single group. *Ruprechtia apetala*, *R. laxiflora* and *R. salicifolia* are grouped together and clustered with *Triplaris cumingiana* in the same lineage and in a single group using both cladistic and phenetic methods. *Emex spinosa*, the studied species of *Polygonum* and *Rumex* were scattered into different lineages phylogenetically, whereas in phenetic method they were grouped together phenetically at the same cluster. Phylogenetically, *Polygonum equisetiforme* was grouped with *Persicaria senegalensis*, *P. decipiens*, *P. lanigera* and *P. lapathifolia*. While phenetically it was excluded from this group.

References

- Abdel-Hameed, U.K. 2014. Delimitation of *Azadirachta indica* A. Juss. From *Melia azedarach* L. (Meliaceae Juss.) based on leaf morphology. *Phyton*, (83): 363-367.
- Anonymous. 1999. Leaf Architecture Working Group [LAWG]. *Manual of leaf Architecture*. Morphological Description and Categorization of Dicotyledonous and net veined Monocotyledonous Angiosperms. Smithsonian Institution, Washington, D.C., USA. Pp: 26-44.
- Anonymous. 2009. Angiosperm Phylogeny Group [APG III]. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Bot. J. Linn. Soc.* 161: 105-121.
- Bailey, L.H. and E.Z. Bailey. 1976. *A Concise Dictionary of Plants Cultivated in the U. S. and Canada*. Hortus Third. Macmillan Company, New York. Pp: 744: 1127.
- Bentham, G. and J.D. Hooker. 1883. *Genera Plantarum*. Reeve and Williams and Nargate, London, England. Vol. 3, part 1, Pp. 88-105.
- Boulos, L. 1999. *Flora of Egypt*. Vol. 1 (Azollaceae-Oxalidaceae). Al Hadara publishing, Cairo, Egypt. Pp: 21-34.
- Brandbyge, J. 1993. "Polygonaceae". In: Klaus Kubitzki (editor); Jens G. Rohwer, and Volker Bittrich (volume editors). *The Families and Genera of Vascular Plants*. Springer-Verlag: Berlin, Heidelberg, Germany. 2: 531-544.
- Burke, J.M., A. Sanchez, K.A. Kron and M. Luckow. 2010. Placing the woody tropical genera of Polygonaceae: A hypothesis of character evolution and phylogeny. *Amer. J. Bot.* 97: 1377-1390.
- Cuénoud, P., V. Savolainen, L.W. Chatrou, M. Powell, R.J. Grayer and M.W. Chase. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *Amer. J. Bot.*, 89: 132-144.
- Dammer, U. 1893. Polygonaceae. In A. Engler & K. Prantle, *Die Natürliche Pflanzenfamilien*. 3: 1-36. Leipzig.
- Galle, P. 1977. Untersuchungen zur Blütenentwicklung der Polygonaceen. *Botanische Jahrbücher für Systematik* 98: 449-489.
- Ghazalah, Y., A.K. Mir, N. Shahin and Q.H. Mohamed. 2010. Micromorphological investigation of foliar anatomy of *Fagopyrum* Mill., and *Rumex* L. of Polygonaceae. *Pak. J. Bot.*, 42(1): 47-75.
- Goloboff, P. 1993. Nona Software and documentation by the author, Tucoeman, Argentina. Windows OS. Pp: 83-91.
- Gross, H. 1913. *Beiträge zur Kenntnis der Polygonaceen*. *Botanische Jahrbücher für Systematik Pflanzengeschichte und Pflanzengeographie* 49: 234-339. Leipzig.
- Hamed, K.A. and M.E. Tantawy. 1990. The stem anatomy of some Polygonaceae and its diagnostic significance in identification. *J. Fac. Educ.*, 15: 249-268.
- Hamed, K.A. and M.E. Tantawy. 1991. The microcharacters of the petiole and blade of certain Polygonaceae as an identificatory tool. *ASU Bull.*, 28B: 390-406.
- Haraldson, K. 1978. Anatomy and taxonomy in Polygonaceae subfamily Polygonoideae Meissn. Emend. Jaretsky. *Symb. Bot.* Vol. 22. pp93.
- Hickey, L.J. and D.W. Taylor. 1991. The leaf architecture of *Ticodendron* and the application of foliar character in discerning its relationships. *Ann. Mo. Bot. Gard.*, 78: 105-130.
- Inamdar, J.A. 1971. Epidermal structure and development of stomata in some Polygonaceae. *Proc. Ind. Acad. Sci.*, 72: 91-98.
- Janelle, M.B. and S. Adriana. 2011. Revised subfamily classification for Polygonaceae, with a tribal classification for Eriogonoideae. *Brittonia*, 63 (4): 510-520.
- Jussieu, A.L.D. 1789. *Genera plantarum secundum ordines naturales disposita*. Paris: Herrissant & Barrois. Pp: 82-83.
- Kim, S.T. and M.J. Donoghue. 2008. Molecular phylogeny of *Persicaria* (Persicarieae, Polygonaceae). *Syst. Bot.*, 33: 77-86.
- Lamb-Frye, A.S. and K.A. Kron. 2003. Phylogeny and character evolution in Polygonaceae. *Syst. Bot.*, 28: 326-332.
- Meissner, C.F. 1856. Polygonaceae. In: A. de Candolle, *Prodromus systematis naturalist regni vegetabilis*, 14: 1-4, 28-186.
- Metcalfe, C.R. and L. Chalk. 1950. *Anatomy of the Dicotyledons*. Clarendon Press, Oxford. vol. 2. Pp: 1092-1110.
- Montasir, A.H. and M. Hassib. 1956. *Illustrated Manual Flora of Egypt*. Bull. Fac. Sci. ASU. Part I, pp. 440-443.
- Nixon, K.C. 1999. The parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics*, 15, Pp: 407-414.
- Perdrigeat, M.C.A. 1900. Anatomie comparee des Polygonees et sus rapport avec la morphologie at la classification. *Act. Soc. Linn. Bord.*, 55: 1-91.
- Prabhakar, M. 2004. Structure, Delimitation, Nomenclature and Classification of Stomata. *Acta Bot. Sin.*, 46(2): 242-252.
- Roberty, G. and S. Vautier. 1964. Les genres de Polygonacees. *Boiseiera*, 10: 1-128.

- Rohlf, P.J. 2000. NTSYS-PC Numerical Taxonomy and Multivariate Analysis Systems Exeter Publishing, New York. Pp: 6-31.
- Ronse De Craene, L.P. and J.R. Akeroyd. 1988. Generic limits in Polygonum and related genera (Polygonaceae) on the basis of floral characters. *Bot. J. Linn. Soc.*, 98: 321-371.
- Sanchez, A. 2011. Evolutionary relationships in polygonaceae with emphasis on *Triplaris*. North Carolina. Pp: 110-127.
- Sanchez, A. and K.A. Kron. 2009. Phylogenetic relationships of *Afrobrunnichia* Hutch. & Dalziel (Polygonaceae) based on three chloroplast genes and ITS. *Taxon*, 58:781-792.
- Sanchez, I. and K.A. Kron. 2008. Phylogenetics of Polygonaceae with an account on the evolution of Eriogonoideae. *Syst. Bot.*, 33(1): 87-96.
- Schuster, T.M. 2011. Evolutionary relationships in polygoneae (polygonaceae) with an emphasis on the amphi-pacific *Muehlenbeckia* Meisn. North Carolina. Pp: 101-158.
- Sneath, P.H. and R.R. Sokal. 1973. Numerical Taxonomy. Freeman, San Fransisco, P. 573.
- Stace, C.A. 1965. Cuticular studies as an aid to plant taxonomy. *Bull. Br. Mus. (Nat. Hist.), Bot.*, 4: 3-78.
- Täckholm, V. 1974. *Student's Flora of Egypt*, ed. 2, Cairo University. Pp: 58-68.
- Tantawy, M.E. 2000. Taxonomic significance of seed characters in some species of *Rumex* L. (Polygonaceae) with special references to the seed coat sculpture. *Egypt. J. Biotechnol.*, 8: 38-50.
- Tantawy, M.E. and S.H. Rabei. 2000. Scanning electron microscope studies on seed coat of some species of *Polygonum* L. s. lat. (Polygonaceae). *Bull. Fac. Sci., Assiut Univ. University* 29 (2D): 365-376.
- Tantawy, M.E., K.A. Hamed and U.I. El-Magliy. 2005. Floral morphology of some taxa of Polygonaceae in Egypt. *Egypt. J. Biotechnol.*, 21: 308-330.
- Whitty, P.W., W. Powell and J.I. Sprent. 1994. Molecular separation of genera in Cassiinae (Leguminosae), and analysis of variation in the noulating species of *Chamaecrista*. *Mol. Ecol.*, 3: 507-515.

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