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Research article

Palynological remarks on the taxonomic status of *Ludwigia octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven: LM and FESEM studies

Arti Garg[1], Swati Tripathi[2]*, Anjum Farooqui[2], A. N. Shukla[1] and Arya Pandey[2]

[1]Botanical Survey of India (BSI), Central Regional Centre, Allahabad, Uttar Pradesh, India
[2]Birbal Sahni Institute of Palaeosciences (BSIP), 53 University Road, Lucknow, Uttar Pradesh, India

*Corresponding Author: swati.tripathi@bsip.res.in [Accepted: 15 December 2021]

Abstract: The long-existing taxonomic controversy of *Ludwigia octovalvis* subsps. *octovalvis* and *Ludwigia octovalvis* subsps. *sessiliflora* is resolved on the basis of pollen morphometric characters supported by multivariate Principal Component Analysis (PCA). Pollen is 3-colporate having three distinct slit-like colpi in both the subspecies, however, the sexine pattern was the chief identifiable feature to distinguish the two subspecies of *L. octovalvis*. In *L. octovalvis* subsp. *octovalvis*, reti-rugulate sexine pattern was observed with sparsely arranged relatively larger rugulae near the pore, whereas, in *L. octovalvis* subsp. *sessilifora*, a typical rugulate character was noticed with compactly arranged similar size rugulae at the pore side. The relatively longer slit-like colpi (pollen aperture) with a typical rugulate pattern forms the *L. octovalvis* subsp. *sessiliflora*, an advanced subspecies as compared to *L. octovalvis* subsp. *octovalvis*. Variations in primary, secondary and tertiary pollen characters of diagnostic value amply justified an independent species status to the subspecies *sessiliflora*, which is thus raised to the status of *Ludwigia sessiliflora* (Micheli) Arti Garg comb. et stat. nov.

Keywords: comb. et stat. nov. - *Ludwigia octovalvis* subsp. *octovalvis* - PCA - Pollen morphometry.

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INTRODUCTION

The cosmopolitan genus *Ludwigia* L. is globally represented by 82 species (Mabberley 2017), mostly distributed in Tropical America, of which, eight species and one infraspecific taxon occur in India (Barua 2010). While resolving the taxonomic status of the genus *Ludwigia* L., Raven (1961) affirmed the combination of the genus *Jussiaea* L. with *Ludwigia* L., on basis of consistent characters stated by Brenan (1953). In doing so, the first reported *Oenothera octovalvis* Jacq., which was transferred to the genus *Jussiaea* L. as *Jussiaea octovalvis* (Jacq.) Sw. (Swartz 1791) became *Ludwigia octovalvis* (Jacq.) P.H. Raven (Raven 1961), thus made three infraspecific combinations of *Ludwigia octovalvis* (Jacq.) P.H. Raven *viz.*, *L. octovalvis* subsp. *brevisepala* (Brenan) P.H. Raven, *L. octovalvis* subsp. *macropoda* (Presl.) P.H. Raven and *L. octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven. Of these, the species proper, *L. octovalvis* (Jacq.) P.H. Raven subsp. *octovalvis* and the subspecies *L. octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven occur in India along with seven other species *viz*. *L. adscendens* (L.) H. Hara, *L. decurrens* Walt., *L. linifolia* (Vahl) R.S. Rao, *L. perennis* (L.) P.H. Raven, *L. peruviana* (L.) H. Hara., *L. prostrata* Roxb., and *L. hyssopifolia* (G.Don) Exell distributed in central, north-east and south India, Manipur, Andaman Islands, Western Ghats, and Goa (Barua 2010).

Raven (1963) in his work on new combinations of *Ludwigia* L. also stated, 'it is desirable to make the following new combinations available at present time, due to the importance of the species involved, especially the one that has long been known as *Jussiaea suffruticosa* L.' indicating the taxonomic significance of *J. suffruticosa* L. (*non*-Walt.) which thereafter became *Ludwigia octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven.

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To resolve the taxonomic status of the two subspecies of *Ludwigia octovalvis* it was found that *L. octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven differed from *L. octovalvis* (Jacq.) P.H. Raven subsp. *octovalvis* only in vegetative characters, the former having long, erect trichomes on stem and leaves and narrowly ovate to linear leaves in contrast to the latter species proper which had adpressed trichomes on stem and leaves with usually subovate leaves. However, these morphological characters were not cogent enough to resolve the existing taxa complexity, therefore a palyno-taxonomic character analysis of the two subspecies was carried out as the pollen phenotype of any taxonomic entity is genetically determined and most reliable diagnostic trait for species discrimination (Datta *et al*. 2006).

The detailed pollen morphological analysis of *Bougainvillea*, *Ceiba* and *Bombax* species from India using LM and FESEM has been performed that significantly contribute to the taxonomic characterization of these species (Tripathi *et al*. 2019, 2017). Farooqui *et al*. (2019) carried out an extensive pollen morphometrical analysis of seven living Indian species of *Ludwigia* and their comparison with two fossil counterparts. Thus, in our present research work, detailed pollen morphometric analysis with the aid of a light microscope (LM) and field emission scanning electron microscope (FESEM) was conducted in *L. octovalvis* subsps. *octovalvis* and *L. octovalvis* subsps. *sessiliflora* in order to resolve the long ongoing taxonomic controversy. The statistical analysis (PCA) was applied to the numerical data obtained from pollen morphometry of the two subspecies of *L. octovalvis* for observing the overall variations.

MATERIAL AND METHODS

Pollen analysis

Polliniferous material for the present study was obtained from correctly identified and authenticated herbarium specimens of the two subspecies *Ludwigia octovalvis* (Jacq.) P.H. Raven subsp. *octovalvis* (No.: P.C. Pant 25230) and *L. octovalvis* subsp. *sessiliflora* (Micheli) Raven (No.: G. Panigrahi 12651) housed in Botanical Survey of India (BSI), Allahabad (BSA). Mature flower buds were carefully extracted from the duplicate specimens, soaked in 70% alcohol for 48 hrs, and anthers were dissected out, gently crushed in centrifuge tubes with a small amount of water, followed by sieving through 150 mesh sieve net and the filtrate was discarded. The residue containing pollen material was acetolysed following Erdtman's method (Erdtman 1960). One part of the acetolysed pollen were mounted in phenolated glycerine jelly to make permanent slides for Light microscope (LM) studies and deposited in the pollen herbarium of BSIP (BSIP slide no. 16464–16468), while the other part was mounted on brass stubs, gold-coated, observed and photographed under high vacuum field emission electron microscope (FESEM) and JEOL-JSM Scanning Electron Microscope (Figs. 1 & 2). For detailed 3-dimensional and Light microscopic analysis of pollen, temporary slides were also made in glycerol which was discarded after completing the observations. Characteristic morphological features, phenology, conservation, evolutionary status and nativity of the studied subspecies of *L. octovalvis* are provided in table 1.

Table 1. Characteristic morphological features, phenology, conservation, evolutionary status and nativity of studied plants.

Characters	***Ludwigia octavalvis* (Jacq.) P.H. Raven**	***Ludwigia octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven**
Height and Trunk	Undershrubs, erect, up to 2.5 m high, well branched	Undershrubs, erect, up to 2.3 m high, well branched
Flower morphology	Flowers 1.5–2.1 cm across; pedicels *ca.* 4 mm long. Sepals *ca.* 7.0 × 3.2 mm. Petals *ca.* 9.5 × 7.3 mm. Filaments 2.0–2.5 mm long; anthers *ca*. 1 mm long.	Flowers 2.0–2.3 cm across; pedicels 4–5 mm long. Sepals *ca*. 7.8 × 4.3 mm. Petals *ca*. 10.8 × 9.3 mm. Filaments 2.3–2.8 mm long; anthers *ca*. 1 mm long.
Flowering Period	August to February	June to February
Conservation status	Common	Uncommon
Evolutionary aspect	Primitive	Advanced
Nativity	Central America, Australia, South-East Asia	South America (Brazil, Peru), North America (West indies,) Mexico

Pollen photographs under LM were taken in different orientations using Olympus DP 25 digital camera attached to Olympus BX 61 microscope. The pollen morphometric analysis included observation and measurements of (i) primary characters of apertures - the colpi number, nature, size (length and breadth), location, endopore size, aspis length and breadth, (ii) secondary characters, the exine ornamentation, basic pattern, nature and size of individual rugulae on the general surface and at apertural aspis and (iii) the tertiary

characters of pollen shape and size (Table 2). All average pollen dimensions were estimated after studying 30 pollen grains and pollen size was determined as Polar (P) length and/or Equatorial diameter (E). For determining pollen shape, the formula P/E x 100 was used. The terminology for pollen description was based on (Punt *et al*. 2007) and (Erdtman 1952).

Statistical analysis

To get species differentiation information from pollen morphological data statistically, Principal Component Analysis (PCA) was applied to the quantified pollen morphometrical analyses of the two subspecies of *Ludwigia* L., using Canoco version 5 software (Smilauer & Leps 2014) and the significance of the data was evaluated at p-value ≤0.05. The obtained figure was modified further in Corel-draw-12 software for minor editing of data labels (Fig. 3).

RESULTS

Pollen morphological characters of the two taxa *Ludwigia octovalvis* subsp. *octovalvis* and *L. octovalvis* subsp. *sessiliflora* are described below and shown in table 2. The key characters of taxa delineation are as follows:

Key to taxa

1. Stem and leaf trichomes adpressed; leaves usually subovate. Pollen 70–72 µm diam., surface reti-rugulate, aspis 6 × 13 µm; colpi 20–22 µm long, endopore 2.5–3.0 µm diam.; exine >3 µm thick; AMB triangulate-spheroidal ……..…..... *L. octovalvis* subsp. *octovalvis*

1. Stem and leaf trichomes erect; leaves narrowly ovate–linear. Pollen 75–77 µm diam., surface rugulate, aspis 8 × 18 µm; colpi 26–30 µm long; endopore 4–6 µm diam.; exine <3 µm thick; AMB sub–spheroidal ..... *L. octovalvis* subsp. *sessiliflora*

Table 2. Pollen morphometric analyses of *Ludwigia octovalvis* (Jacq.) P.H. Raven subsp. *octovalvis* and *L. octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven.

Characters	***Ludwigia octavalvis* (Jacq.) P.H. Raven**	***Ludwigia octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven**
Average colpi length	21 µm	29 µm
Average endopore diameter	2.7 µm	5.5 µm
Average aspis size	6 µm × 13 µm	8 µm × 18 µm
Exine ornamentation	Reti-rugulate	rugulate
Apertural rugulae	Larger than surface	Similar to surface
Nature of surface	Heterogenous	Homogenous
Average exine thickness	3.1 µm	2.8 µm
Average grain size	71 µm	76 µm
Shape	Triangulate-spheroidal	Sub-spheroidal

Pollen description

L. octovalvis (Jacq.) P.H. Raven subsp. *octovalvis* (Fig. 1; Table 2)

Grains 3-colporate, aspidote. Aspis crater-like, *ca*. 6 × 13 µm, sharply raised with steep edges, rim deeply invaginated (Fig. 1C, E), atrium 2–3 µm deep. Colpi distinct, 20–22 µm long, slit-like, margins smooth, co-aligned in tetrad (Fig. 1B); pore sunken, 2.5–3.0 µm diam. Exine 3.0–3.2 µm thick; surface reti-rugulate with faintly visible grooves and ridges, heterogeneous (Fig. 1D); rugulae channelled, hemicylindrical, sharply curved, merging to form a reticulum, relatively large and branched at colpi margins. Grains 70–72 µm diam., triangulate-spheroidal (Fig. 1A) Viscin threads were visible.

Ludwigia sessiliflora (Micheli) Arti Garg, comb. et stat. nov. (Fig. 2; Table 2)

Jussiaea octonervia f. *sessiliflora* Micheli, Fl. Bras. (Martius) 13(2): 171. 1875. *Jussiaea octonervia* var. *sessiliflora* (Micheli) Micheli, Fl. Bras. (Martius) 13(2): 180, t. 35. 1875. *Jussiaea suffruticosa* var. *sessiliflora* (Micheli) Hassl., Bull. Soc. Bot. Genève ser. 2, 5: 271. 1913. *Ludwigia pubescens* var. *sessiliflora* (Micheli) H. Hara, J. Jap. Bot. 28(10): 293. 1953. *Ludwigia octovalvis* subsp. *sessiliflora* (Micheli) P. H. Raven, Kew Bull. 15(3): 476. 1962. *Ludwigia octovalvis* var. *sessiliflora* (Micheli) Shinners, Sida 1(6): 385. 1964.

Grains 3-colporate, aspidote. Aspis crater-like, 7–8 × 17–18 µm, gently raised with sloping edges, rim shallowly invaginated (Fig. 2 C, E), atrium 4–5 µm deep. Colpi distinct, 28–30 × 3–4 µm, broad in the centre,

tapering towards ends, margins ill-defined; pore sunken, 5–6 µm diam. (Fig. 2E). Exine 2.7–2.9 µm thick; surface rugulate, homogenous; rugulae elongated, 1–6 µm long, tightly interwoven forming prominent grooves and mounds of ridges (Fig. 2D), channelled, almost straight or slightly curving and unbranched at pore margins with almost similar size rugulae. Grains 75–77 µm diam., sub–spheroidal (Fig. 2A, B).

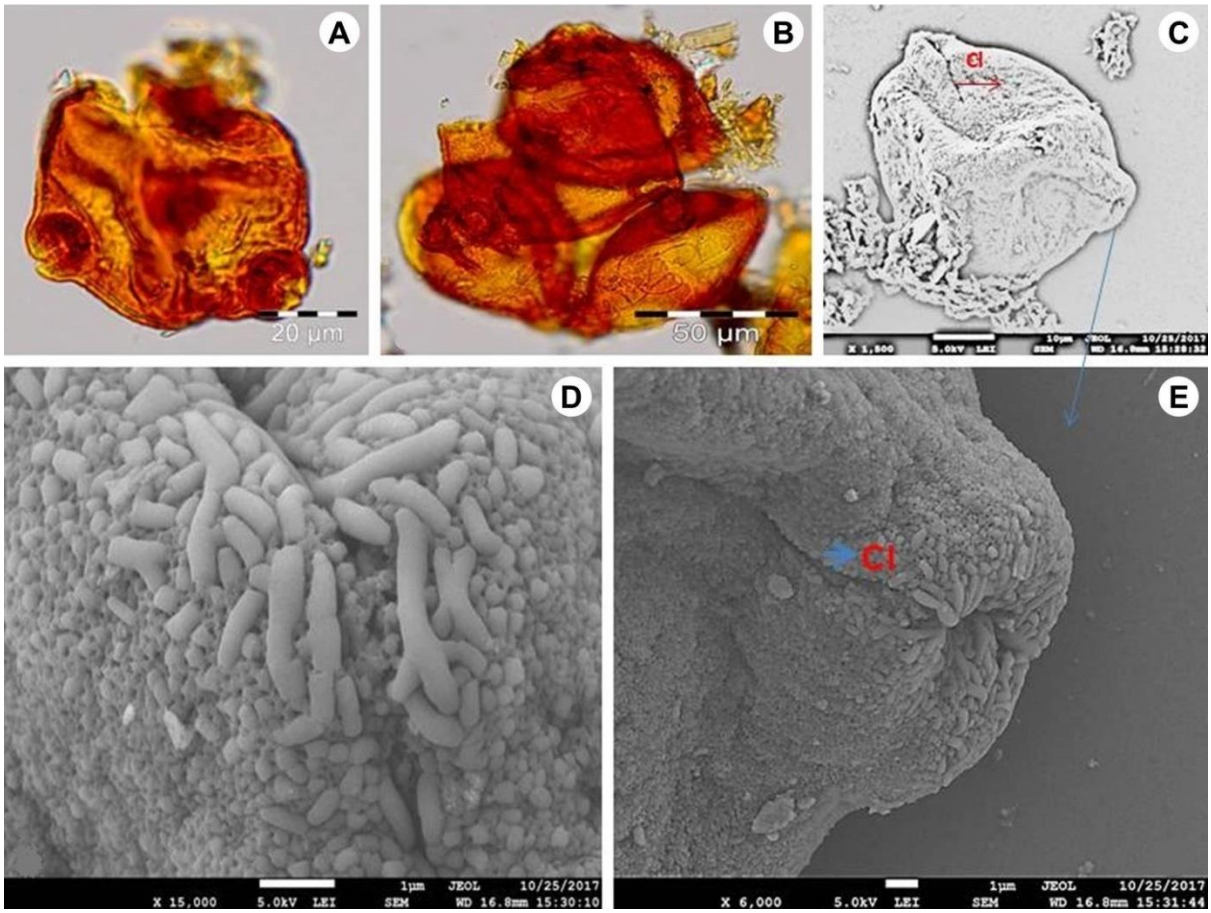

Figure 1. LM and FESEM images of *Ludwigia octovalvis* (Jacq.) P. H. Raven var. *octovalvis*: **A**, LM- Tricolporate sub-spheroidal pollen in polar view; **B**, LM- Pollen attached through aspidote pore in tetrad; **C**, FESEM- Tricolporate pollen showing slit like colpi (Cl) in polar view; **D**, FESEM- Long and branched rugulae of the apertural region on aspis; **E**, FESEM- Slit like colpi extending beyond aspis (Cl).

PCA results

The PCA of pollen morphometry data of the two taxa of *Ludwigia* L., *L. octovalvis* subsp. *octovalvis* and *L. octovalvis* subsp. *sessiliflora* revealed considerable variation in pollen morphological values which produced different clustering of the two taxa (Fig. 3). Their PCA scores and loading plot indicated 81% variance in principal components of the two subspecies. The principal character of pollen that was responsible for such variance was the reti-rugulate sexine pattern in *L. octovalvis* subsp. *octovalvis* and typically rugulate sexine in *L. octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven along with other determinant character variations in colpi nature, endopore size and aspis dimensions, supporting our view of according independent species status to *L. octovalvis* subsp. *sessiliflora*.

DISCUSSION

Importance of pollen morphological characters

The importance of pollen morphological characters in taxonomy and evolutionary patterns of plant kingdom was first formulated by Wodehouse (1935) and later by Nair (1964), Chaloner (1967), Muller (1970), and Walker & Doyle (1975). With the growth of palynological science, pollen–spore morphology became inevitable in studies of comparative morphology, taxonomy and evolution of plants and in solving various taxonomic complexities (Persson *et al*. 1996, Giveyrel *et al*. 2000, Carlo & Paula 2004). In doing so, the pollen apertural characters were considered to be of primary significance, exine sculpture secondary and size and shape of the grains as tertiary, in the order of importance. Being genetically determined and phenotypically expressed, the pollen morphology is a constant and unique indicator of their parent species. Pollen grains are therefore the most reliable, micromorphological, single-celled entities with immense diagnostic traits which are preserved in fossil

pollen (Moore & Webb 1978).

The family Onagraceae possesses morphologically unique 3-zono-colporate, aspidote pollen grains which are mostly dispersed as tetrads attached tightly at aspis, and bound together by long viscin threads, which are often present even in individual monad grains after separation from their tetrads. In tetrads, the colpi of different grains are co-aligned, a character that can be ascertained only in tetrad conditions and can be seen under LM and FESEM. In monad form, although this character is not discernable, but the typical aspidote condition and distinct shape of pollen remain unaltered which makes the Onagraceae pollen easily identifiable from other plant groups. The colpi are located on variously oriented aspis' of different elevations and ornamentation in different species. The endopores are generally lo-longate and sunken in the aspis cavity (atrium).

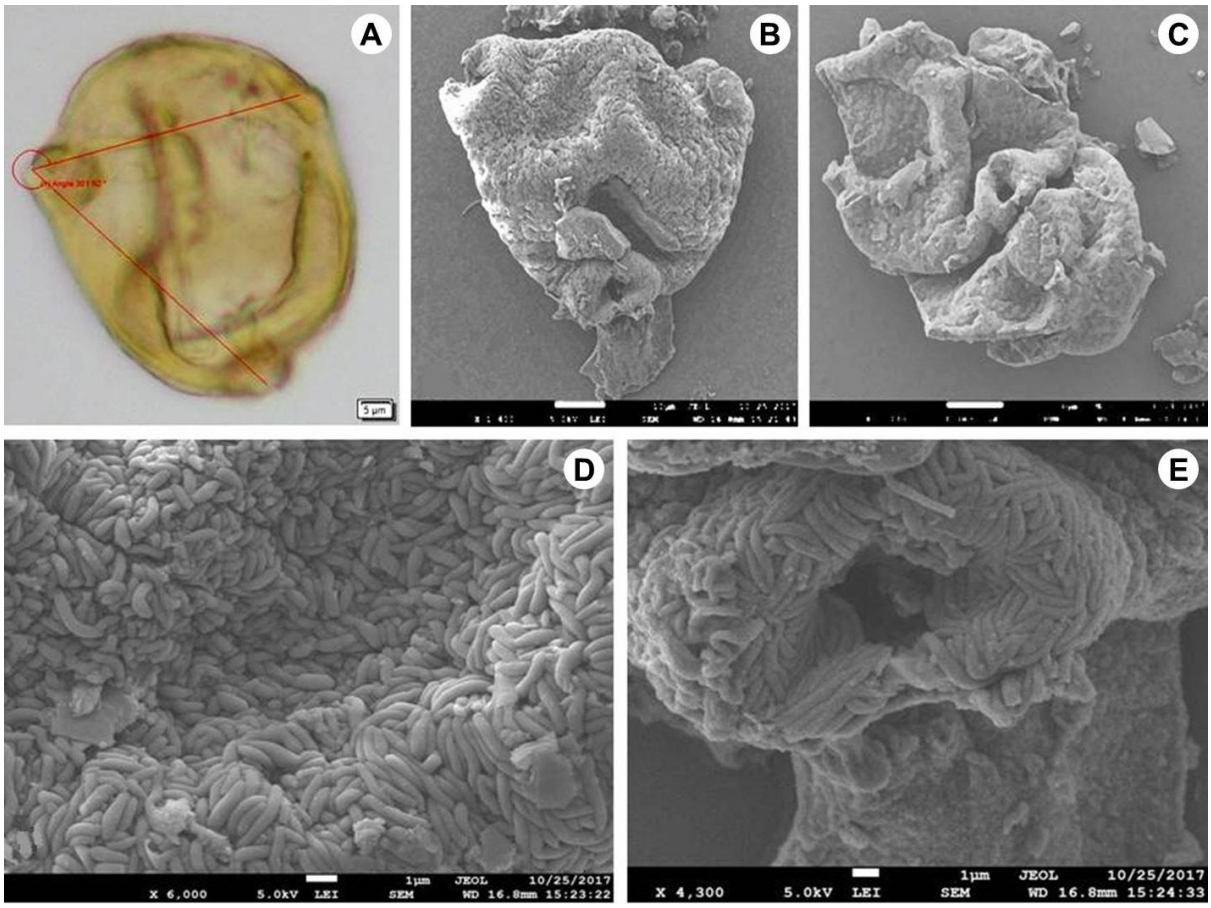

Figure 2. LM and FESEM images of *L. octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven raised to *L. sessiliflora* (Micheli) Arti: **A**, LM- Image of tricolporate sub-spheroidal pollen in polar view; **B**, FESEM- Aspidote pollen grain showing slit-like Colpi extending towards polar region; **C**, FESEM- Two pollen grains attached through aspidote pore in polar view; **D**, FESEM- Surface showing short and long (0–02–4 µm long), sometimes branched rugulae; **E**, FESEM- Aspis showing invaginated ectoaperture.

Pollen morphological characters to upgrade the taxonomic status of L. octovalvis subsp. *sessiliflora*

The pollen grains of *Ludwigia octovalvis* subsp. *sessiliflora* differed considerably from *L. octovalvis* subsp. *octovalvis* in almost all diagnostic characters at primary (apertural), secondary (surface ornamentation) and tertiary (size and shape) levels (Table 2; Figs. 1 & 2). While the colpi in subspecies proper were located on a steeply raised aspis, was shorter 20 to 22 µm and slit-like with small (2.5–3.0 µm) endopore, the corresponding colpi in subsp. *sessiliflora* was located on a gently sloping aspis, was much longer 28–30 µm with broad ora, tapering ends and a larger (5–6 µm) endopore. Similarly, the surface ornamentation in the species proper was reti-rugulate, comprised of a heterogeneous assemblage of hemicylindrical rugulae which were relatively large and branched at colpi margins (Fig. 1D), while the surface ornamentation in subspecies *sessiliflora* was typically rugulate, comprised of a homogenous assemblage of tightly interwoven, elongated rugulae which were unbranched at colpi margins. Besides, occasionally slightly short rugulae were also visible at the surface of *L. octovalvis* subsp. *sessiliflora* (Fig. 2D). Further, the grains of *L. octovalvis* subsp. *octovalvis* were smaller (70–72 µm diam.) with triangulate-spheroidal AMB and thick (3.0–3.2 µm) exine while those of *L. octovalvis* subsp. *sessiliflora* were much larger (75–77 µm diam.) with sub-spheroidal AMB and thin (2.7–2.9 µm) exine. The overall differences in the primary and secondary pollen morphological characters of the two subspecies bearing

with the 81% variance in their principal components sharply validate an independent species status of *L. octovalvis* subsp. *sessiliflora*.

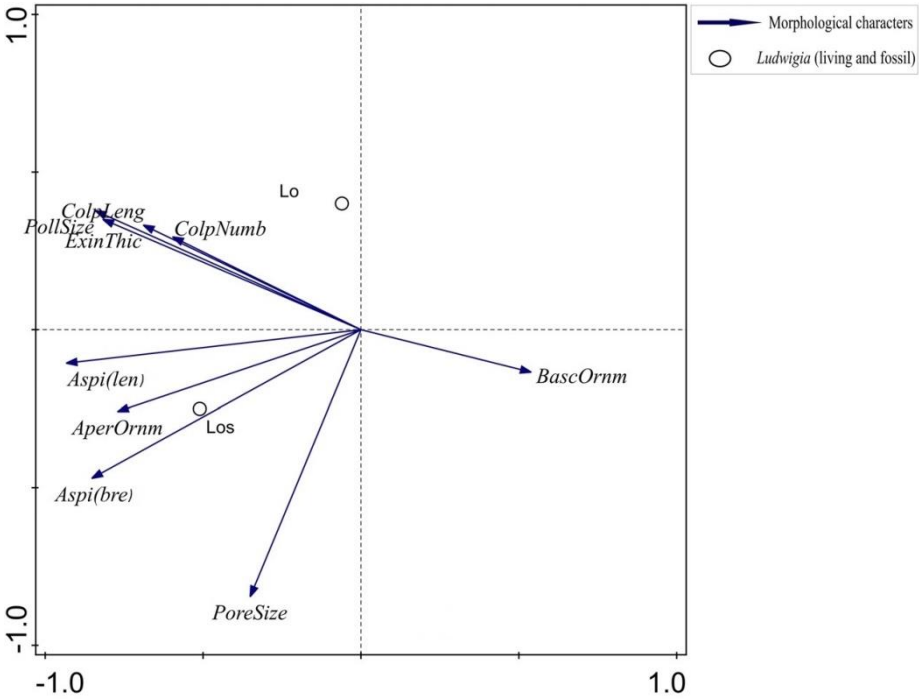

Figure 3. PCA data of *Ludwigia octovalvis* (Jacq.) P.H. Raven subsp. *octovalvis* and *L. octovalvis* subsp. *sessiliflora* (Micheli) P.H. Raven (abbreviations used: Lo - *Ludwigia octovalvis* subsp. *octovalvis*; Los - *L. octovalvis* subsp. *sessiliflora*; BascOrnm – Basic ornamentation; PollSize – pollen size; ColpLeng – Colpi length; ColpNumb – Number of colpi; ExinThic – exine thickness; Aspi len – aspis length; AperOrnm – ornamentation at apertural region; Aspi bre – aspis breadth; PoreSize – endopore diameter. Arrow (→) – pollen morphological character; Circle (O) – Name of the Taxon.

CONCLUSIONS

The detailed pollen morphometrical analysis of the two subspecies of *Ludwigia octovalvis* (*L. octovalvis* subsp. *octovalvis* and *L. octovalvis* subsp. *sessiliflora*) was carried out to solve the ongoing taxonomic complexity subjected to their similar plant morphological characters except for the differences in leaf and stem trichomes. The PCA results of 81% variance in morphological characters were in concurrence with the observed pollen morphometrical analysis of both the subspecies and amply authenticated the variation between principal components of these two subspecies. Hence the consistent variations in diagnostic morphometrical characters (especially of sexine pattern) of pollen grains evidenced in the two subspecies of *Ludwigia octovalvis*, supported by the significance statistical variance, strongly substantiate an independent species status of *L. octovalvis* subsp. *sessiliflora* under the genus *Ludwigia* L. as *Ludwigia sessiliflora* (Micheli) Arti Garg comb. et stat. nov.

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