THE IDENTITY AND OCCURRENCE OF *PHYLLANTHUS HOOKERI* MUELL.-ARG. AND *P. NOZERANII* ROSSIGNOL & HAICOUR (EUPHORBIACEAE) IN INDIA

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Abstract

There are 16 species of *Phyllanthus* subgenus *Phyllanthus* reported from India. The present paper adds two more species that are invasive weeds in the paddy fields and on forest floors, namely *Phyllanthus hookeri* Muell.-Arg. and *P. nozeranii* Rossignol & Haicour of sect. Urinaria subsect. Urinaria of *Phyllanthus*. The former is somewhat woody and perennial whereas the latter is slender and monsoonal. The presence of these two taxa in India was brought to light in 1987 by Rossignol *et al.* based on the herbarium specimens collected earlier to 1863 and deposited at Paris from northeastern and southeastern India. Whilst *Phyllanthus hookeri* is overlooked or underrated by the taxonomists, *P. nozeranii* is misidentified and considered conspecific with *P. urinaria* L.

Introduction

The biovulate Linnean *Phyllanthus* L. (Euphorbiaceae) is not only a well-known medicinal plant genus with its diverse biomolecules but also has given the name to the recently resurrected segregate family Phyllanthaceae Martinov, by molecular taxonomists. The first comprehensive taxonomic treatment of this genus for India was provided by Hooker (1887) in his *Flora of British India*. Earlier, Roxburgh (1832) described 25 species of *Phyllanthus*. Chiefly confined to humid tropics of the world, the genus comprises 833 species (Govaerts *et al.*, 2000). In India, it is represented by 53 species (Gangopadhyay *et al.*, 2007).

Phyllanthus L. (*s.l.*) is often divided into a number of subgenera, namely, *Cicca, Emblica, Eriococcus, Isocladus, Kirganelia, Phyllanthus, Xylophylla,* etc. The subgenus *Phyllanthus* is characterized by herbs or low woody shrubs bearing colporate pollen grains, tricarpellary capsular fruits with six, striate and/or foveolate seeds. It is represented by 16 species in India (Gangopadhyay *et al.*, 2007).

The present paper reports *Phyllanthus hookeri* and *P. nozeranii* of subgenus *Phyllanthus* as additions to the Indian flora. These, in fact, were reported earlier but either overlooked by authors or often misidentified, or treated conspecific with *Phyllanthus urinaria*; the fourth of the six species described by Linnaeus in his *Species Plantarum* (1753). Later, Roxburgh (1832: 660) considered *P. urinaria* as the second species under Section II (*Leaves pinnate*; obviously, Roxburgh mistook the phyllanthoid branchelets as pinnate leaves). *Phyllanthus urinaria* L. was kept in section Urinaria by

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Webster while Central Asia is conceived as the centre of origin of this section which is of interest due to hepatoprotective and other medicinal uses (Lee *et al.*, 2006; Komuraiah *et al.*, 2009). Chaudhary and Khan (2003) studied the SEM of seeds of nine herbaceous species of *Phyllanthus* from India and described these under three morphotypes, with *Phyllanthus urinaria* placed in *P. urinaria* type.

It is the experience of the taxonomists in general and of experts of the genus in India in particular that there exist specimens of *Phyllanthus* evincing affinity with *P. urinaria* but do not match wholly with the technical description of it in the regional floras. Rossignol et al. (1987) were the first to draw the attention to the discontinuities within the section Urinaria in the characteristics like pilosity, length of the internodes and of plagiotrophic (phyllanthoid) shoots, the number of leaves on it and the number of colpii of the pollen, the presence or absence of foveoles on the lateral sides of seeds, ploidy level, etc. On the basis of morphology, cytology, genetics and biometry, a new classification was presented by Rossignol et al. (1987) in which the allied species of P. urinaria or "urinaria complex", are placed in the subsection Urinaria Haicour & Rossignol, recognizing two subgroups within it on spermoderm ornamentation. Each of these lines is represented by two species which differ from either in somatic chromosome number: 50 (P. nozeranii) and 100 (P. embergeri) in the "spiraled" line, 50 (P. urinaria) and 100 (P. hookeri) in the "radiated" line. The members of the subsection are characterized by 4-5 colporate, prolate pollen with bi-reticulate ornamentation (Chen and Wu, 1997; Chen et al., 2009).

Certain of the herbarium specimens of *Phyllanthus urinaria* complex available at Madras Herbarium, Coimbatore (MH) and Kakatiya University Herbarium, Warangal (KUH) including the live plants in the botanical garden, Kakatiya University campus belong to the less-known but described species, viz. *Phyllanthus hookeri* Muell.-Arg. and *P. nozeranii* Rossignol & Haicour. Curiously, there was no mention of *P. nozeranii* in the recent account of *Phyllanthus* from India by Gangopadhyay *et al.* (2007). However, *Phyllanthus hookeri* Muell.-Arg., *P. urinaria* var. *hookeri* (Muell.-Arg.) Hook. f. and *P. urinaria* var. *oblongifolia* Muell.-Arg. were treated conspecific with *P. urinaria* L.

Ramla (1995) described and segregated the seeds of *P. urinaria* complex in Kerala State as *P. urinaria* – 'spiraled' (S) and 'radiated' (R) on seed coat ornamentation, as has been done by Rossignol *et al.* (1984, 1987). But, she did not go further to distinguish them at the species level though well aware of the work of Rossignol *et al.* (1987). Later, Chaudhary and Rao (2002) treated *P. urinaria*, without commenting on the apparent morphological variation. Chaudhary and Khan (2003:118) stated that the seed of *P. urinaria* has 12-15 transverse ridges and 1-3 circular pits on the sides (a routine description copied from floras, e.g. Philcox, 1997) while their photographs show only *single large pit*.

The following is the key (modified after Rossignol *et al.*, 1987) to segregate the species of *Phyllanthus urinaria* complex:

| 1. See | eds without foveoles | 2 |
|---|---|--------------------------------------|
| 1. See | eds with foveole/s | 3 |
| 2. 0 | Capsules mamillate-rugose with fleshy or thin scales; pollen grains with 5 colpi | hookeri |
| 2. 0 | Capsules smooth skinned, sub-globose; pollen grain with 4 colpi | urinaria ssp. nudicarpus |
| 3. Seeds with large, single foveole | | |
| 3. Seeds with 2-4, small foveoles; pollen grains with 4 colpi | | <i>urinaria</i> ssp. <i>urinaria</i> |
| 4. | Staminate flowers with tepals hispid abaxiallly; plagiotropic shoots highly hispidulous; pollen grains with 4 colpi | nozeranii |
| 4. | Staminate flowers with tepals glabrous abaxiallly; plagiotropic shoots scarcel | у |
| | hispidulous; pollen grains with 5 colpi | embergeri |

A decade after the work of Rossignol *et al.* (1987) on this group, Chen and Wu (1997) clearly reiterated that *Phyllanthus embergeri* Rossignol & Haicour, *P. hookeri* Muell.-Arg. and *P. urinaria* ssp. *nudicarpus* Rossignol & Haicour are distinct taxa in Taiwan on pollen characters. The present account deals with *P. hookeri* and *P. nozeranii*, which can be easily identified in the field as well as in the herbarium, and even on other evidence as demonstrated below:

1. Phyllanthus hookeri Muell.-Arg. in Linnaea 32: 19 (1863) *et* in DC., Prodr. 15(2): 366 (1866); Rossignol *et al.*, Amer. J. Bot. 74: 1862 (1988).

Type: Eastern India, Khasia Mountains (3000-4000 ft), J.D. Hooker *et* T. Thomson sub phyllantho no. 71 in hb. DC. (*Holotype* P; *Isotype* VIL).

Phyllanthus leprocarpus Wight, Icon. Pl. Ind. Or. 5: 25, t. 1895, f.4 (1852).

Phyllanthus urinaria var. hookeri (Muell.-Arg.) Hook. f., Fl. Brit. India 5:294(1887).

Diasperus hookeri (Muell.-Arg.) Kuntze, Revis. Gen. Pl. 2: 599 (1891). (Fig. 1a, b)

Perennial erect herbs, up to 60 cm high. Main stem woody, hard, smooth; cataphylls arranged spirally; internodes relatively short, angular. Leaves narrowly oblong, distichous, hispidulous along the margins; leaf base slightly oblique, $1.1-1.4 \times 0.3-0.4$ cm; petiole 1 mm long; stipules acuminate. Phyllanthoid branchlets plagiotropic up to 15 cm long, with 47-50 leaves. Plants monoecious; 22-25 proximal nodes with axillary, solitary, pistillate flowers while the distal nodes 30-37 with solitary or at the most 2 staminate flowers. Staminate flowers: pedicels 0.5 mm long, tepals 6, white with red strip along the midvein, elliptic-oblong, entire, obtuse; filaments form a column (less than 0.5 mm); anthers free, divergent, sub-globose, yellow, dithecous, extrorse and dehiscing by longitudinal slits; disk glands discrete, alternating the tepals, granular; pollen grains 5- colporate. Pistillate flowers: pedicels 0.5 mm long, tepals 6, white, linear-oblong, apex rounded; glabrous below; upper rim of disk finely crenulate. Fruits capsular, depressed globose, exocarp mamillate-rugulose, with fleshy scales. Seeds 6, brown, trigonous, 1.3-

 1.5×1.5 mm; ornamentation radiating from the rounded hilum with 5-7 ridges on lateral faces and 13-14 distinct transverse ridges on dorsal side; non-foveolate.

Flowering and fruiting: Throughout the year.

Illustration: Phyllanthus urinaria auct. non L.: Matthew, Further Ill. Fl. Tamilnadu Carnatic f. 580. 1998; Pullaiah and Babu, Fl. Andhra Pradesh 4: 1808, f. 458 (1998).

Geographical distribution: Asia: India to Philippines.

Specimens examined: Andhra Pradesh: Chittoor district, Satyavedu to Madras (150 m): 7.10.1974, M. Chandrabose 45259; Cuddapah district, on the side of Gunjam river (190 m): 9.11.1962, J. L. Ellis 14981. East Godavari district: Kotha Isukapalli, V.S. Raju 1615 (KUH); Warangal district: KU campus, Hanamkonda: V.S. Raju & S. Suthari 1851 (KUH). Assam: Locality?: Masters, MH 70925. Chattisgarh, Bastar district, Kondagaon, in paddy fields (767 m): 19.11.1958, K. Subramanyam 7185; North Kanger valley, Kutamsar (530 m): 26.8.1959, K. Subramanyam 8630. Karnataka, South Canara, Sullia: 25.10.1900, C.A. Barber 2070. Kerala: Cannanore district, Tolpetty (800 m): 9.7.1978, V.S. Ramachandran 57516; Idukki district, Churuly on hill slopes in Cardamom plantation: 22.8.1981, V.S. Raju 71145; Edayar-Pooyamkutty (125 m), 15.12.1988, P. Bhargavan 89934. Madhya Pradesh: Rewa distrct, Rewa town, Khatonlia forest (400 m): 14.9.1959, K.M. Sebastine 8766. Nagaland: Herb. Hort. Bot. Calcuttensis, Flora of Naga Hills: 22.10.1886, Dr. D. Prain, MH 70921. Sikkim: Flora of Bengal (Sikkim): July, 1882. J.S. Gamble 10460; Flora of the Sikkim Himalaya (locality? 187 m). G. King 187. Southeast India: Coromandel coast: Coll.? 108, date? (P). Tamil Nadu: Tirunevelly district, Courtallam: 14.9.1915, Coll.? 12158, MH 46773; 21.10.1919, K.C. Jacob 16224; Mundanthurai to Karyar: 17.9.1915, Coll.? 12219; Papanasam: 12.7.1907, Coll.? 8371. West Bengal: Hook.f. & Thoms., MH 70923.

Note: Within the subsect. Urinaria, *P. hookeri* shows striking resemblance to *P. urinaria* var. *urinaria* and *P. urinaria* ssp. *nudicarpus* in habit. But, the tepals of staminate flowers are larger, obovate-crenulate and the seeds foveolate (cf. Silva and Sales, 2007) in the former and capsules are smooth and pollen 4-colporate in the latter (cf. Chen and Wu, 1997). However, Govaerts *et al.* (2000) considered it as a variety under *P. urinaria* though *Phyllanthus hookeri* was accepted as a distinct species by Chen and Wu (1997) on pollen morphology. *Phyllanthus hookeri* is distinct from *P. nozeranii* on ovule structure, seed and seedling morphology (cf. Ramla, 1995) besides the other differences already stated.

 Phyllanthus nozeranii Rossignol & Haicour, Amer. J. Bot. 74: 1858 (1987) (publ. 1988).
(Fig. 1c, d)

Vernacular (Telugu): Erra usirikee.

Plants herbaceous, 20-35 cm high, ephemeral, monsoonal. Young stem bearing a rosette of 5-7 assimilatory leaves; cataphylls arranged spirally above it; internodes

slightly angular, finely pilose, 8-15 mm long. Phyllanthoid branchlets plagiotropic, dorsiventrally flattended, 6.2-8.0 cm long with 15-16 distichous leaves. Leaves obovate, purplish along the margins and veins (young leaves more purplish abaxially); base oblique, $1.3-1.8 \times 0.6-0.7$ cm. Staminate flowers: 0.5-2.0 mm in diameter (in full bloom),



Fig. 1a,b: *Phyllanthus hookeri* (a) Whole plant and seed (b) Seedling c, d: *Phyllanthus nozeranii* (c) Whole plant and seed (d) Seedling.

pedicels below 0.5 mm long, tepals 6, white; stamens with filaments fused, anthers free, divergent, sub-globose, yellow, dehiscing by longitudinal slits; pollen grains 4-colporate. Pistillate flowers: axillary, solitary, pedicels below 0.5 mm long; tepals 6, apex rounded. Fruits capsular, depressed-globose, 2 mm in diameter, very scaly. Seeds 6, brown, 1.2-1.5 x 1-1.2 mm; with 13-15 transversal ridges on their convex face; lateral faces with a central, conspicuous, crescent-shaped foveole.

Geographical distribution: Southeast Asia, Peninsular India.

Specimens examined: Andhra Pradesh: East Godavari district, Locality? (125 m): 19.9.1980, G.V. Subbarao 67560; Khammam district: Vazeedu, V.S. Raju 1616 (KUH); Kurnool district, Locality? (375 m): 29.8.1965, J.L. Ellis 25575; Lukke (590 m): 9.8.1980, J.L. Ellis 42224; Visakhapatnam district: Forest near S. Kota (175 m): 4.9.1960, N.P. Balakrishnan 11018; Warangal district: Mangapet, V.S. Raju 1624 (KUH); KU campus: V.S. Raju & S. Suthari 1852 (KUH). Karnataka: South Canara, Sullia, 25.1.1900, C.A. Barber 2070; Sampagi: 10.11.1900, C.A. Barber 2192. Kerala: Trivandrum district, Near Nilamil (125 m): 12.8.1978, M. Mohanan 54798; Trichur district, Thunakadavu submergible area (667 m): 24.7.1964, K.M. Sebastine 20929. Tamil Nadu: Tirunevelly district, Courtallam: 13.9.1915, Coll.? 12036, MH46781. Peninsular India: Coromandel coast (without locality): Macé s.n. (P).

The specimens cited above for the two species, other than those indicated as KUH and P, are all available at MH.

Discussion

Among the eight secondary metabolites screened for *Phyllanthus hookeri*, *P. nozeranii* and *P. urinaria*, alkaloids are present while iridoids absent in all. Steroids are exclusive to *P. urinaria*, lignins, methylene-dioxy compounds and triterpenoids to *P. hookeri* and ellagic acid to *P. nozeranii*. However, tannins are shared by *P. hookeri* and *P. nozeranii*. The distribution of 21 known and 24 unknown amino acids, 10 known and 4 unknown phenolic acids and 8 secondary metabolites scored for 17 species of *Phyllanthus* revealed 25.0 paired affinity between *P. nozeranii* and *P. urinaria*, 23.0 between *P. hookeri* and *P. nozeranii*, and 15.3 between *P. hookeri* and *P. urinaria*. The isolation values are 50 for *P. urinaria*, 30.7 for *P. hookeri* and 22.2 for *P. nozeranii* (Komuraiah, 2009).

Leaf proteins in *P. hookeri* and *P. nozeranii* were studied using SDS-PAGE. Based on the mobility of the protein bands, the species of *Phyllanthus* were categorized into **A** (Slow: $R_f 0.4-1.5$), **B** (Intermediate: $R_f 1.6-2.1$), **C** (Fast: $R_f 2.8-3.9$) and **D** (Very fast: R_f 4.0-5.1). *P. hookeri* showed exclusive bands in **A** and **B** while *P. nozeranii* developed a band is **C** (Fast) while both shared bands in **D** (Very Fast). When the paired affinity and isolations were calculated for 11 species of *Phyllanthus*, *P. hookeri* and *P. nozeranii* showed zero paired affinity whereas *P. hookeri* evinced highest isolation value of 54.5.

Conversely, *Phyllanthus hookeri*, *P. nozeranii and P. urinaria* are distinct not only in external morphology as demonstrated but also in cytology (Rossignol *et al.*, 1984, 1987) and phytochemistry (Komuraiah, 2009), even on their antimicrobial properties (Komuraiah *et al.*, 2009).

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