Systematic value of seedling morphology of some members of the tribe Spermacoceae of Rubiaceae

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Abstract
The taxonomic implication of seedling morphology has been emphasized from an investigation of six members of the Tribe Spermacoceae (Rubiaceae) collected mostly from the East Himalayan region. The seedlings are basically epigeal, phanerocotylar and distinguishable on the basis of characters of paracotyledons, eophylls, hypocotyl, internodes, stipules, primary veins (number and pattern), etc. which may be used in identification of taxa. These seedling morphological data of the investigated taxa can be correlated with other botanical disciplines in deducing taxonomic affinity.

Key words: Rubiaceae, Seedling morphology, Mitracarpus, Richardia, Spermacoce.

INTRODUCTION
In its currently accepted circumscription, the tribe Spermacoceae is essentially an herbaceous lineage of the family Rubiaceae, representing about 61 genera and 1235 species (Lens et al. 2009). Hooker (1882) reported 7 species under 3 genera of Spermacoceae in the Flora of British India. The majority of the species are herbaceous. Tetramerous flowers in combination with fimbriate stipules, solitary ovules attached to the middle of the septum in cell, valvate corolla lobes, dry fruits with valvular capsules and pluricolpate pollen grains characterize the tribe (Verdcourt 1958; Bremekamp 1966; Dwyer 1980; Chaw & Peng 1987). The delimitations of Spermacoceae have varied from a long recognized morphologically well-defined tribe (Spermacoceae sensu stricto; Hooker 1873; Bremekamp 1952, 1966; Verdcourt 1958; Robbrecht 1988) to a wider interpretation stemming from molecular analyses and including the traditionally recognized tribes Hedyotideae, Knoxieae, Manettieae, and Triainolepideae (Bremer 1996; Bremer & Manen 2000). Four genera of Spermacoceae, Dioidia Linnaeus, Richardia Linnaeus, Spermacoe Linnaeus (including Borreria G.F.W. Meyer), and Mitracarpus Zuccarini are widespread in tropical Asia (Chaw & Sivarajan 1989). Chaw & Peng (1987) and Sivarajan et al. (1987) elucidated general morphology and taxonomy of Spermacoceae. Seed coat micromorphology of the Asiatic Spermacoceae has been studied by Chaw & Sivarajan (1989). The phylogenetic utility of chloroplast (atpB-rbcL, petD, rps16, trnL-F) and nuclear (ETS, ITS) DNA regions was investigated for the tribe Spermacoceae by Karehed et al. (2008). Phylogeny of the Tribe based on plastid DNA data is studied by Groeninckx et al. (2009). In none of the above accounts, the seedling morphology of Spermacoceae was considered. Seedling morphology is useful for identification and delimitation of taxa (Ladiges et al. 1981, 1984; Sampathkumar...
It has been also shown that juvenile morphological characters can be useful as that of other characters like morphological, anatomical, cytological, palynological, reproductive, etc. for the identification of a plant. As such, the present attempt is the first Indian contribution to the knowledge of seedling morphology, which represents a glimpse of taxonomic implications to be considered within the investigated members of the Tribe Spermacoceae distributed usually in the East Himalayan region.

MATERIALS AND METHODS

In the present investigation, seedlings of six species belonging to three genera representing the tribe Spermacoceae of the family Rubiaceae occurring in natural forested habitats in Lataguri, Chalsa, Chapramari areas of Jalpaiguri district and Siliguri, Lebong area of Darjeeling district were collected and studied. Seeds of all these taxa were also collected from the identified adult plants and grown in the Experimental Botanic Garden of the Department of Botany, University of Calcutta. The different developmental stages of each seedling taxon were considered. The seedlings both raised and collected from natural habitats were compared. Similar data on seedling morphology were noted in both the cases. Ten to fifteen specimens of different growth forms were studied taking from different habitats. All the specimens were documented in the form of herbarium sheets, which have been deposited in the Calcutta University Herbarium (CUH). The morphology of seedlings was described following the terminology as proposed by Burger (1972), Hickey (1973) and Vogel (1980). For method of description of seedlings, Paria et al. (1990), Kamilya & Paria (1993, 1994) were followed.

DIAGNOSES OF SEEDLINGS

*Mitracarpus hirtus* (Linnaeus) DC.
(Plate-I a; Plate-II a)

*Seedlings* epigeal, phanerocotylar. *Taproot* terete, long, 2.5 – 5 cm, herbaceous, yellowish-white, glabrous; lateral roots many, branched, slender, greyish-white. *Hypocotyl* slightly bent at base, short, 0.4 – 0.7 cm, pinkish-green, hairy, terete. *Paracotyledons* two, opposite, herbaceous, persistent up to 8th to 10th leaves stages, stipulate, petiolate, pale green, glabrous; petiole 0.05 – 0.15 cm long, almost flat, glabrous; blade orbicular to suborbicular, 0.2 – 0.3 cm x 0.18 – 0.28 cm, entire, base and apex rounded, primary veins three, actinodromous. *Internodes* straight or curved, pinkish-green, hairy on angles, 4-angular, length of first, second and third internodes 0.4 – 1.4 cm, 1 – 1.6 cm and 1.5 – 1.8 cm respectively. *First two leaves* opposite-decussate, simple, stipulate, herbaceous, pale green, hairy; petiole 0.1 – 0.25 cm long; blade narrow-ovate, 0.35 – 0.6 cm x 0.2 – 0.35 cm, entire, acute, base cuneate, primary vein one, eucamptodromous. *Subsequent leaves* gradually enlarging, wide-ovate with obtuse base and acuminate apex; other characters almost similar to that of first two leaves.

*Specimens examined:* Lataguri forest, Jalpaiguri (W.B.), Mukhopadhyay 317, dated 06.09.1996.

*Richardia brasiliensis* Gomes
(Plate-I d; Plate-II b)
PLATE - I: Different stages of seedlings (a) *Mitracarpus hirtus* (Linnaeus) DC.; (b) *Spermacoce ocymoides* Burman f.; (c) *Richardia scabra* Linnaeus; (d) *Richardia brasiliensis* Gomes; (e) *Spermacoce latifolia* Aublet; (f) *Spermacoce exilis* (L.O.Williams) C.D. Adams ex W.C.Burger & C.M.Taylor

Seedlings epigeal, phanerocotylar. Taproots terete, long, 2.5 – 4.5 cm, herbaceous, brownish-white, glabrous; lateral roots many, highly branched, slender, soft, greyish-white. Hypocotyl slightly curved, short, 0.25 – 0.4 cm, brownish-white, glabrous, terete, herbaceous. Paracotyledons two, opposite, succulent, persistent up to 4th to 8th leaves stages, stipulate,
petiolate, pale green, glabrous; petiole glabrous, 0.15 – 0.25 cm long, flat; blade orbicular to suborbicular, 0.3 – 0.4 cm x 0.3 – 0.4 cm, entire, base and apex rounded, primary vein three, actinodromous. *Internodes* straight or slightly bent, pale green, densely hairy, 4-angular, length of first, second and third internodes 0.1 – 0.3 cm, 0.7 – 1.2 cm and 0.5 – 0.8 cm respectively. *First two leaves* opposite-decussate, simple, stipulate, herbaceous, pale-green, densely hairy; petiole 0.2 – 0.3 cm long; blade ovate to narrow-ovate, 0.6 – 1.4 cm x 0.3 – 0.8 cm, entire, acute, base obtuse, primary vein one, eucamptodromous. *Subsequent leaves* gradually enlarging; other characters almost similar to that of first two leaves.

**Specimens examined:** Chalsa forest, Jalpaiguri (W.B.), *Mukhopadhyay 378*, dated 23.08.1995.

**Richardia scabra** Linnaeus
(Plate-I c; Plate-II c)

*Seedlings* epigeal, phanerocotylar. *Taproot* terete, long, 5 – 8 cm, herbaceous, brownish-white, glabrous; lateral roots many, highly branched, slender, soft, whitish. *Hypocotyl* slightly curved, short, 0.5 – 1 cm, greenish-white (upper part) or pinkish-green (lower part), glabrous, terete, herbaceous. *Paracotyledons* two, opposite, succulent, persistent up to 6th to 8th leaves stages (flowering stage), stipulate, petiolate, yellowish-green, glabrous; petiole glabrous, 0.4 – 0.7 cm long, flat; blade narrow ovate to ovate lanceolate, 0.45 – 0.9 cm x 0.25 – 0.45 cm, entire, subrounded, base obtuse, primary veins three, actinodromous. *Internodes* slightly zigzag, pale green, hairy, 4-angular, slightly twisted, length of first, second and third internodes 0.3 – 1.2 cm, 1.4 – 1.8 cm and 2 – 2.5 cm respectively. *First two leaves*, opposite-decussate, simple, stipulate, succulent, pale green, hairy; petiole 0.1 – 0.25 cm long; blade ovate to narrow-ovate, 0.8 – 1.2 cm x 0.4 – 0.6 cm, entire, acute, base obtuse, primary vein one, brochidodromous. *Subsequent leaves* gradually enlarging; elliptic or oblong; other characters almost similar to that of first two leaves.

**Specimens examined:** Siliguri, Darjeeling (W.B.), *Mukhopadhyay 103*, dated 25.09.1994.

**Spermacoce latifolia** Aublet
(Plate-I e; Plate-II e)

*Seedlings* epigeal, phanerocotylar. *Taproot* terete, elongating, 4 – 8 cm, semi-woody, greyish-brown, glabrous; lateral roots many, branched, fibrous, pale brown. *Hypocotyl* straight or slightly bent, long, 2 – 3.2 cm, pale-green, glabrous, terete, lower part traversed by branched adventitious roots. *Paracotyledons* two, opposite, herbaceous, persistent up to 8th to 10th leaves stages, stipulate, petiolate, pale green, glabrous; petiole 0.25 – 0.4 cm long, adaxially channelled, glabrous; blade wide ovate, 0.8 – 1.3 cm x 0.7 – 1 cm, entire, retuse, base subrounded to subcordate, primary vein one, brochidodromous. *Internodes* straight or slightly curved, pale green, 4-angular, angles prominently winged and hairy, length of first, second and third internodes 1.2 – 4.3 cm, 2 – 5 cm and 3 – 4.5 cm respectively. *First two leaves* opposite-decussate, simple, stipulate, hairy; petiole 0.3 – 0.5 cm long; blade ovate-elliptic, 2 – 3 cm x 0.8 – 1.4 cm, entire or variously lobed, acute, base cuneate, primary vein one, eucamptodromous. *Subsequent leaves* gradually enlarging; other characters almost similar to that of first two leaves.

**Specimens examined:** Lataguri forest, Jalpaiguri (W.B.), *Mukhopadhyay 362*, dated 03.09.1996; Chalsa forest, Jalpaiguri (W.B.), *Mukhopadhyay 368*, dated 21.08.1995.

**Spermacoce exilis** (L.O. Williams) C.D. Adams ex W.C. Burger & C.M. Taylor
(Plate-I f; Plate-II d)
Seedlings epigeal, phanerocotylar. Taproot terete, elongating, 4.5 – 7 cm, herbaceous, greyish-brown, glabrous, lateral roots many, branched, slender and fibrous, pale-brown. Hypocotyl straight or slightly curved, short, 0.6 – 1 cm, pale-green, glabrous, terete, lowest part sometimes traversed by adventitious roots. Paracotyledons two, opposite, subcoriaceous, persistent up to 8th to 10th leaves stages (flowering stages), stipulate, petiolate, pale green, glabrous; petiole very short, 0.1 – 0.15 cm, glabrous; blade wide-ovate to rhomboid (0.4 – 0.55 cm x 0.3 – 0.4 cm), entire, truncate, base subrounded, primary vein one, eucamptodromous. Internodes straight or slightly bent, pale green, 4-angular, hairy along angles, length of first, second and third internodes 1.5 – 2.3 cm, 1.8 – 2.5 cm and 1.5 – 2 cm respectively. First two leaves opposite-decussate, simple, stipulate, glabrous; petiole 0.1 – 0.2 cm long; blade ovate-elliptic, 0.8 – 1.3 cm x 0.35 – 0.6 cm, entire, acute, base cuneate, primary vein one, eucamptodromous. Subsequent leaves gradually enlarging; other characters almost similar to that of first two leaves.


Spermacoce ocymoides Burman f.
(Plate-I b; Plate-II f)
ARTIFICIAL KEYS
(valid for taxa mentioned)

Key to Genera:
1a. Paracotyledons with 3 primary veins, actinodromous .................................. 2
1b. Paracotyledons with 1 primary vein, other than actinodromous ............

Key to Species of Richardia:
1a. Veins of first two leaves eucamptodromous; subsequent leaves ovate to narrow-ovate .................................................. Richardia brasiliensis
1b. Veins of first two leaves brochidodromous; subsequent leaves elliptic or oblong ....

Key to Species of Spermacoce:
1a. Veins of first two leaves brochidodromous; hypocotyl 4-angular without adventitious roots .......................................................... Spermacoce ocymoides
1b. Veins of first two leaves eucamptodromous; hypocotyl terete with adventitious roots ........................................ 2
2a. Veins of paracotyledon brochidodromous, paracotyledons retuse; hypocotyl long (2 – 3.2 cm) .................................................. Spermacoce latifolia
2b. Veins of paracotyledon eucamptodromous, paracotyledons truncate; hypocotyl short (0.6 – 1 cm) .................................................. Spermacoce exilis

DISCUSSION
In the present investigation on the seedling morphology of six species, representing three genera of Spermacoceae have been studied. The taxa display wide range of seedling morphological features in respect of shape, primary vein number, and venation pattern of paracotyledons; first two leaves and subsequent leaves as shown in Table 1. Using these characters in combination with some others, the six species can be easily recognised. Three genera have been placed in two different categories. Category I includes Mitracarpus hirtus...
Table 1. Seedling morphological data of the investigated species of the Tribe Spermacoceae

<table>
<thead>
<tr>
<th>Species</th>
<th>Mitracarpus hirtus</th>
<th>Richenda brasiensis</th>
<th>Richenda subrata</th>
<th>Spermacoce latifolia</th>
<th>Spermacoce exilis</th>
<th>Spermacoce osyomoides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seedling type</strong></td>
<td>epigal, phanerocotyl</td>
<td>epigal, phanerocotyl</td>
<td>epigal, phanerocotyl</td>
<td>epigal, phanerocotyl</td>
<td>epigal, phanerocotyl</td>
<td>epigal, phanerocotyl</td>
</tr>
<tr>
<td><strong>Hypocotyl</strong></td>
<td>short (0.4–0.7 cm), hairy</td>
<td>short (0.2–0.4 cm), glabrous</td>
<td>short (0.2–1 cm), glabrous</td>
<td>long (1–3.2 cm), glabrous</td>
<td>short (0.6–1 cm), glabrous</td>
<td>short (0.3–0.8 cm), glabrous</td>
</tr>
<tr>
<td><strong>Phyllotaxy</strong></td>
<td>opposite</td>
<td>opposite</td>
<td>opposite</td>
<td>opposite</td>
<td>opposite</td>
<td>opposite</td>
</tr>
<tr>
<td><strong>Blade shape</strong></td>
<td>orbicular to suborbicular</td>
<td>orbicular to suborbicular</td>
<td>narrow ovate to ovate</td>
<td>wide-ovate</td>
<td>wide-ovate to rhomboid</td>
<td>ovate</td>
</tr>
<tr>
<td><strong>Margin</strong></td>
<td>entire</td>
<td>entire</td>
<td>entire</td>
<td>entire</td>
<td>entire</td>
<td>entire</td>
</tr>
<tr>
<td><strong>Apex</strong></td>
<td>rounded</td>
<td>rounded</td>
<td>subrounded</td>
<td>subrounded</td>
<td>subacute</td>
<td>subacute</td>
</tr>
<tr>
<td><strong>Base</strong></td>
<td>rounded</td>
<td>rounded</td>
<td>orbiculate</td>
<td>subrounded-ovate</td>
<td>subrounded</td>
<td>subrounded</td>
</tr>
<tr>
<td><strong>Primary vein no.</strong></td>
<td>three</td>
<td>three</td>
<td>three</td>
<td>one</td>
<td>one</td>
<td>one</td>
</tr>
<tr>
<td><strong>Vesicular</strong></td>
<td>actinodromous</td>
<td>actinodromous</td>
<td>actinodromous</td>
<td>brachidodromous</td>
<td>ecaepactodromous</td>
<td>Brachidodromous</td>
</tr>
<tr>
<td><strong>Surface</strong></td>
<td>glabrous</td>
<td>glabrous</td>
<td>glabrous</td>
<td>glabrous</td>
<td>glabrous</td>
<td>glabrous</td>
</tr>
<tr>
<td><strong>Stipule</strong></td>
<td>stipulate</td>
<td>stipulate</td>
<td>stipulate</td>
<td>stipulate</td>
<td>stipulate</td>
<td>stipulate</td>
</tr>
<tr>
<td><strong>Internodes</strong></td>
<td>hairy on angles, 4-angular</td>
<td>hairy, 4-angular</td>
<td>hairy, 4-angular</td>
<td>hairy, 4-angular</td>
<td>hairy, 4-angular</td>
<td>hairy, 4-angular</td>
</tr>
<tr>
<td><strong>First two leaves</strong></td>
<td>petiolate</td>
<td>petiolate</td>
<td>petiolate</td>
<td>petiolate</td>
<td>petiolate</td>
<td>petiolate</td>
</tr>
<tr>
<td><strong>Blade shape</strong></td>
<td>narrow-ovate</td>
<td>ovate to narrow-ovate</td>
<td>ovate to narrow-ovate</td>
<td>ovate-elliptic</td>
<td>ovate-elliptic</td>
<td>ovate</td>
</tr>
<tr>
<td><strong>Margin</strong></td>
<td>entire</td>
<td>entire</td>
<td>entire</td>
<td>enter or lobed</td>
<td>entire</td>
<td>entire</td>
</tr>
<tr>
<td><strong>Base</strong></td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
</tr>
<tr>
<td><strong>Primary vein no.</strong></td>
<td>one</td>
<td>one</td>
<td>one</td>
<td>one</td>
<td>one</td>
<td>one</td>
</tr>
<tr>
<td><strong>Vessels</strong></td>
<td>ecaepactodromous</td>
<td>ecaepactodromous</td>
<td>xochidodromous</td>
<td>ecaepactodromous</td>
<td>ecaepactodromous</td>
<td>Brachidodromous</td>
</tr>
<tr>
<td><strong>Surface</strong></td>
<td>hairy</td>
<td>densely hairy</td>
<td>hairy</td>
<td>hairy</td>
<td>glabrous</td>
<td>hairy</td>
</tr>
<tr>
<td><strong>Subsequent</strong></td>
<td>ovate to narrow-ovate</td>
<td>ovate to narrow-ovate</td>
<td>elliptic or oblong</td>
<td>ovate-elliptic</td>
<td>ovate-elliptic</td>
<td>ovate</td>
</tr>
<tr>
<td><strong>Apex</strong></td>
<td>acuminate</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
</tr>
<tr>
<td><strong>Base</strong></td>
<td>obtuse</td>
<td>obtuse</td>
<td>obtuse</td>
<td>obtuse</td>
<td>obtuse</td>
<td>obtuse</td>
</tr>
<tr>
<td><strong>Vesicular</strong></td>
<td>ecaepactodromous</td>
<td>ecaepactodromous</td>
<td>xochidodromous</td>
<td>ecaepactodromous</td>
<td>ecaepactodromous</td>
<td>Brachidodromous</td>
</tr>
</tbody>
</table>
and Richardia spp. where the paracotyledons have three primary veins with actinodromous venation pattern. Category II having primary vein one with brochidodromous and eucamptodromous venation pattern consists of three species of Spermacoce only. Mitracarpus hirtus and Richardia spp. can be distinguished based on shape and apex of subsequent leaves and surface of hypocotyl. The investigated species of Richardia spp. can be differentiated with the help of some characters, viz., shape of subsequent leaves and paracotyledons, venation pattern of first two leaves. In the category II, Spermacoce latifolia and S. exilis appears distinct from S. ocyoides in having terete hypocotyl with adventitious roots and eucamptodromous venation pattern for first two leaves. Further, S. latifolia and S. exilis separable from each other based on venation pattern of paracotyledons and relative length of hypocotyl.

The seedling morphological characters are significant like other botanical disciplines. It is revealed that Spermacoce spp., Mitracarpus sp. and Richardia spp. sharply differ from each other by morphological characters of fruits and seeds (Sharma & Paine 1989). Interestingly, these taxa can be differentiated with the help of detail seedling characters. Under the genus Richardia, two species R. brasiliensis and R. scabra can be differentiated based on mericarp as well as seedling characters. Palynology of the genus Spermacoce sp. has been studied and commented by Dessein et al. (2002, 2005). This study revealed the remarkable variation of pollen characters. Likewise, there is diversity of seedling morphological characters being represented in different degrees by the investigated taxa.

Thus, within this limited scope of study, it is evident that the morphological features of seedlings are of value and important as that of adult vegetative and reproductive characters and as such may be used for taxonomic delimitation of taxa within the Tribe.

**LITERATURE CITED**


