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Seed coat micromorphology of Indian species of *Nymphoides* (Menyanthaceae)

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Abstract. Seeds of eight Indian species of *Nymphoides* were studied using scanning electron microscope with respect to their surface characters in relation to taxonomy. Seeds are either flat and compressed, or discoid in shape. The compressed seeds of *N. peltata* are ciliate along the margins while those of other species are tuberculate or aculeate all over the surface. Epidermal cells are polygonal or irregularly shaped with raised or depressed, straight or radially sinuate junctures. These characters can usefully be utilized in the identification of species, especially while working with herbarium materials.

Key words: India; *Limnanthemum*; Menyanthaceae; *Nymphoides*; Seed coat micromorphology; Taxonomy.

Introduction

Nymphoides Seguier is an interesting aquatic genus of about 20 species (Cook *et al.*, 1974), widely distributed in the tropical and temperate regions of both the Old World and the New World. However, it is a difficult group to be studied with dried herbarium materials, because most species are indistinguishable vegetatively and the flowers dry so poorly that they are useless for identification purposes. As a result the materials are often confused and misidentified in most of the herbaria. Our primary objective, hence, has been to discover characters which are usually well preserved in herbarium materials that can be usefully utilized for identification of species. It is in this background that our attention fell on the seed surface characteristics of the Indian representatives of this genus.

Seed surface ornamentation and epidermal patterns have been classically and widely used for taxonomic purposes in a variety of plant groups. In Menyanthaceae, these characters have been studied by Marais and Verdoorn (1963), Aston (1969) and Raynal (1974) using light microscope (LM), who have concluded that these are highly variable and taxonomically unreliable. Marais and Verdoorn (1963) have shown a complete gradient from smooth to bristly seeds with intermediates having only a few, feeble warts on the hull of seeds leaving the general seed surface mostly smooth, in the South African species of the genus. Aston's (1969) studies on the Australian species of *Villarsia* Vent. have demonstrated that the epidermal cells of their seeds are also variable. However, in the African and Madagascar species of *Nymphoides*, Raynal (1974) recognized two different groups of species characterized by polygonal and sinuate epidermal cells and a third group where they are variable and hence unreliable for taxonomic purposes.

But, these studies have been mainly based on LM studies where finer distinction among various types of ornamentations are not feasible. So, we scanned the seed materials with a view to discriminate among dif-

ferent types of seed surface ornamentations rather than their distribution and abundance, as an aid for identification of various species, and the results have been rather encouraging, as can be seen from the text and photographs.

Clarke (1883), in J. D. Hooker's Flora of British India, described six species of the genus (under the generic name *Limnanthemum*) in the erstwhile Bristish India. Of these, *L. forbesianum* Griseb. now stands reduced to *Nymphoides aurantiacum* (Cramer, 1981). *Nymphoides peltata* (Gmel.) O. Kuntze [= *L. nymphaeoides* Link] is restricted to western Himalayas and Kashmir. *Nymphoides hydrophylla* (Lour.) O. Kuntze [= *L. cristatum* Griseb.] and *N. indica* (Linn.) O. Kuntze [= *L. indicum* (Linn.) Griseb.] are distributed throughout India, while *N. aurantiacum* (Dalz.) O. Kuntze [= *L. aurantiacum* Dalz.] and *N. parvifolium* (Griseb.) O. Kuntze [= *L. parvifolium* Griseb.] are restricted to Deccan peninsula and Malabar. Subsequently, Vasudevan (1968) discovered and described a new species *N. macrospermum* from the flooded low lands of Malabar coast. Joseph (in press) and Joseph and Sivarajan (in press) have recently discovered two more new species from the same area which are being published with the names *Nymphoides sivarajanii* and *N. krishnakesara* respectively, bringing the total number of species in India to eight.

Materials and Methods

Seeds prepared for examination under scanning electron microscope (SEM) were collected from herbarium sheets using a Nikon SMZ-2T dissecting LM. After cleaning with a fine Chinese brush dipped in 95% ethanol, the seeds were kept in a Toshiba DC-85 dessiccator (relative humidity 45-50%) for at least 24 hours, then coated with gold, and examined with a Zeiss 950 SEM at an accelerating voltage of 15 KV.

Two to twelve seeds of each sample were mounted and examined. The seed photographed was in all cases judged to be typical for the sample. Sizes of seeds were measured under a dissecting LM. For each species 6 to 155 seeds were measured depending on the availability of materials. Descriptive terms mainly followed those of Murley (1951). All voucher specimens studied are listed after the description of each species.

Observations

Nymphoides peltata (Gmel.) O. Kuntze [= *Limnanthemum nymphaeoides* Link] Figs. 1-6.

Seeds 20-25 per capsule, brown, flat and compressed, ovoid, rounded at distal end, obtuse or truncate at proximal end, 4.0-5.2 mm long, 2.7-3.3 mm wide, ciliate at margin, the cilia pusticulate and tuberculate toward distal ends, the tubercles depressed at tip; testa cells slightly convex, psilate or gemmate or with minute favulariate thickenings near cell junctures, the junctures depressed and strongly sinuate.

Specimen examined: *Muenscher & Justice 859, Rao 828* (CAL).

N. indica (Linn.) O. Kuntze [= *L. indicum* (Linn.) Griseb.)] Figs. 7-11.

Seeds 18-25 per capsule, light brown to brown, discoid, 1.5-1.7 mm long, ca. 1.5 mm wide, concave at funiculus scar; surface with scattered clusters of tuberculate protuberances, the tubercles 15-50 μm long, often depressed and rugulose; testa cells rather flat, variously shaped, with dendroid thickenings and thin, raised, gemmate junctures.

Specimen examined: *Joseph 38961* (CALI).

L. wightianum Griseb. Figs. 12-14.

Seeds closely similar to those of *N. indica* in appearance, but have more sparsely distributed, solitary tubercles. The tubercles rounded at the distal ends, 60-70 μm long. The testa cells are variously shaped, rather flat with gemmate protuberances at the junctures. The surface is foveolate.

Specimen examined: *Wight 2579* (LE).

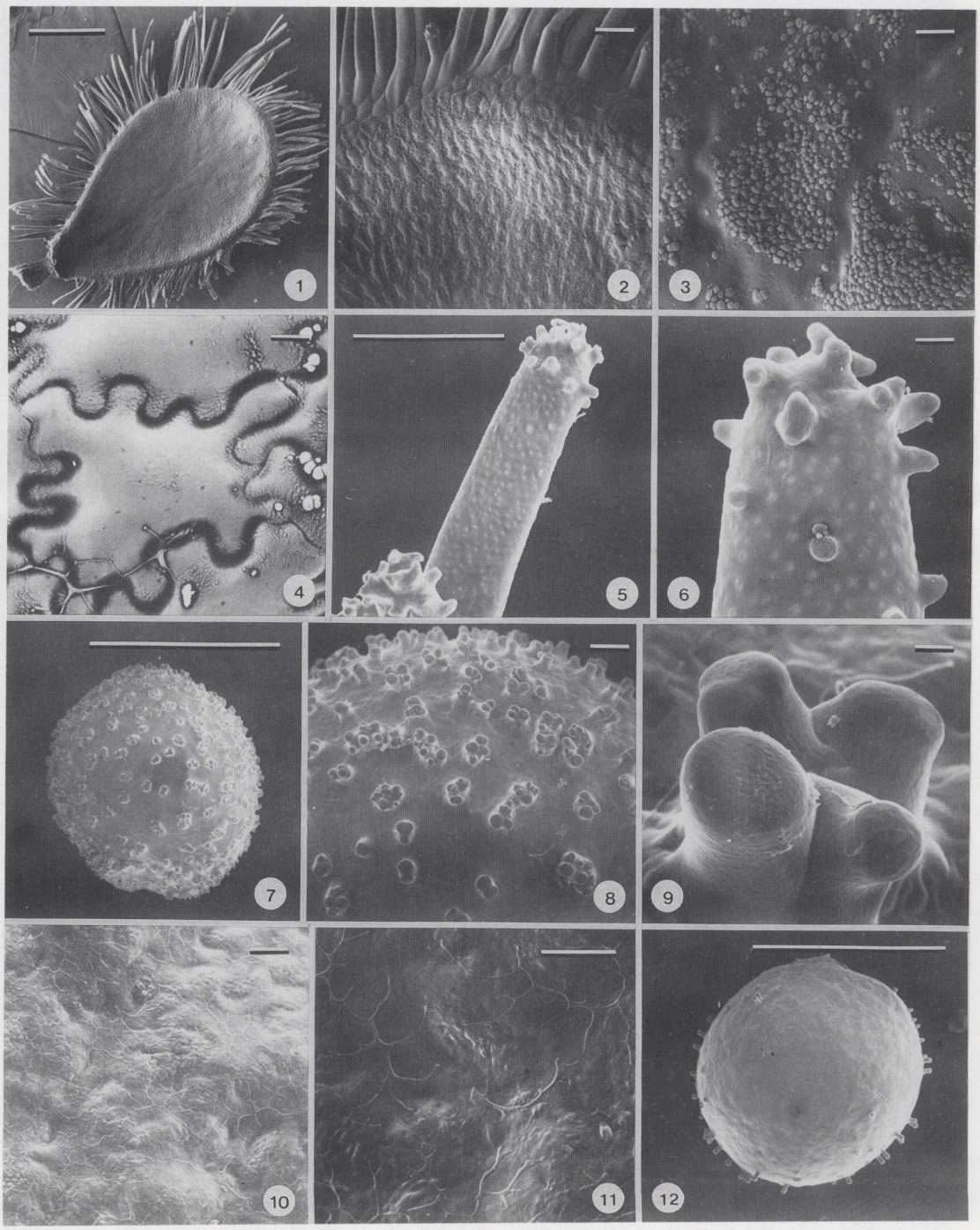
N. parvifolium (Griseb.) O. Kuntze Figs. 15-17.

Seeds 8-15 per capsule, light brown, discoid; 0.8-1 mm diam., usually with persistent funiculus; surface tuberculate, tubercles 10-21 μm long, solitary or coherent in various numbers, apex mostly depressed; testa cells foveolate, the junctures raised and faintly sinulate.

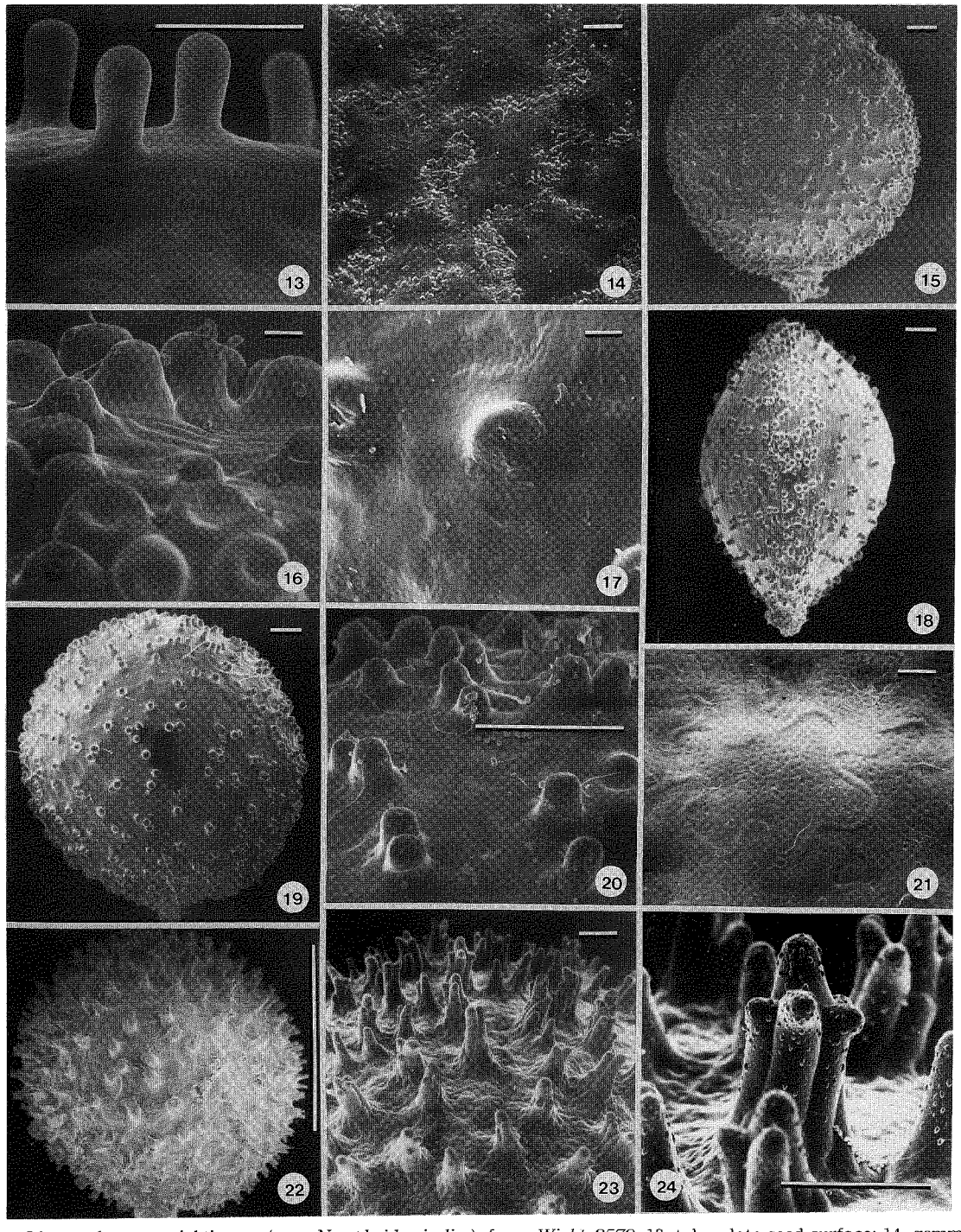
Specimen examined: *Sivarajan 431* (CALI).

N. sivarajanii Joseph (in press) Figs. 18-21.

Seeds 15-20 in each capsule, cream to dark brown, discoid, 0.8-1 mm diam., usually with persistent funiculus; surface tuberculate, the tubercles single



s. 1–6. *Nymphoides peltata*: 1–3 from *Muenscher & Justice 859*, 4–6 from *Rao 828*. 1, entire seed; 2, enlarged view of seed surface; 3–4, testa cells; 5–6, cilia with pusticulate and tuberculate distal ends. Figs. 7–11. *N. indica*: from *Joseph 38961*. 7, entire seed; 8, tuberculate seed surface; 9, a single cluster of tubercles; 10–11, testa cells. Fig. 12. *Limnanthemum wightianum* (now *N. indica*): from *Wight 2579*; entire seed. Scale bars = 10 μm in 3, 4, 6, 9, 10, 11; 100 μm in 2, 5, 8; and 1000 μm in 1, 7, 12.



Figs. 13-14. *Limnanthemum wightianum* (now *Nymphoides indica*): from *Wight 2579*. 13, tuberulate seed surface; 14, gemmate junctures of testa cells. Figs. 15-17. *N. parvifolium*: from *Sivarajan 431*. 15, entire seed; 16, tubercules; 17, foveolate seed surface. Figs. 18-21. *N. sivarajanii*: from *Joseph 30243*. 18-19, entire seed; 20, tubercles on the seed surface; 21, testa cells with sinuate junctures and foveolate surface. Figs. 22-24. *N. hydrophylla*: from *Joseph 38631*. 22, entire seed; 23, seed surface with aculeate prickles; 24, prickles with gemmate protuberances towards distal end. Scale bars = 10 μm in 14, 16, 17, 21; 100 μm in 13, 15, 18, 19, 20, 23, 24; 1000 μm in 22.

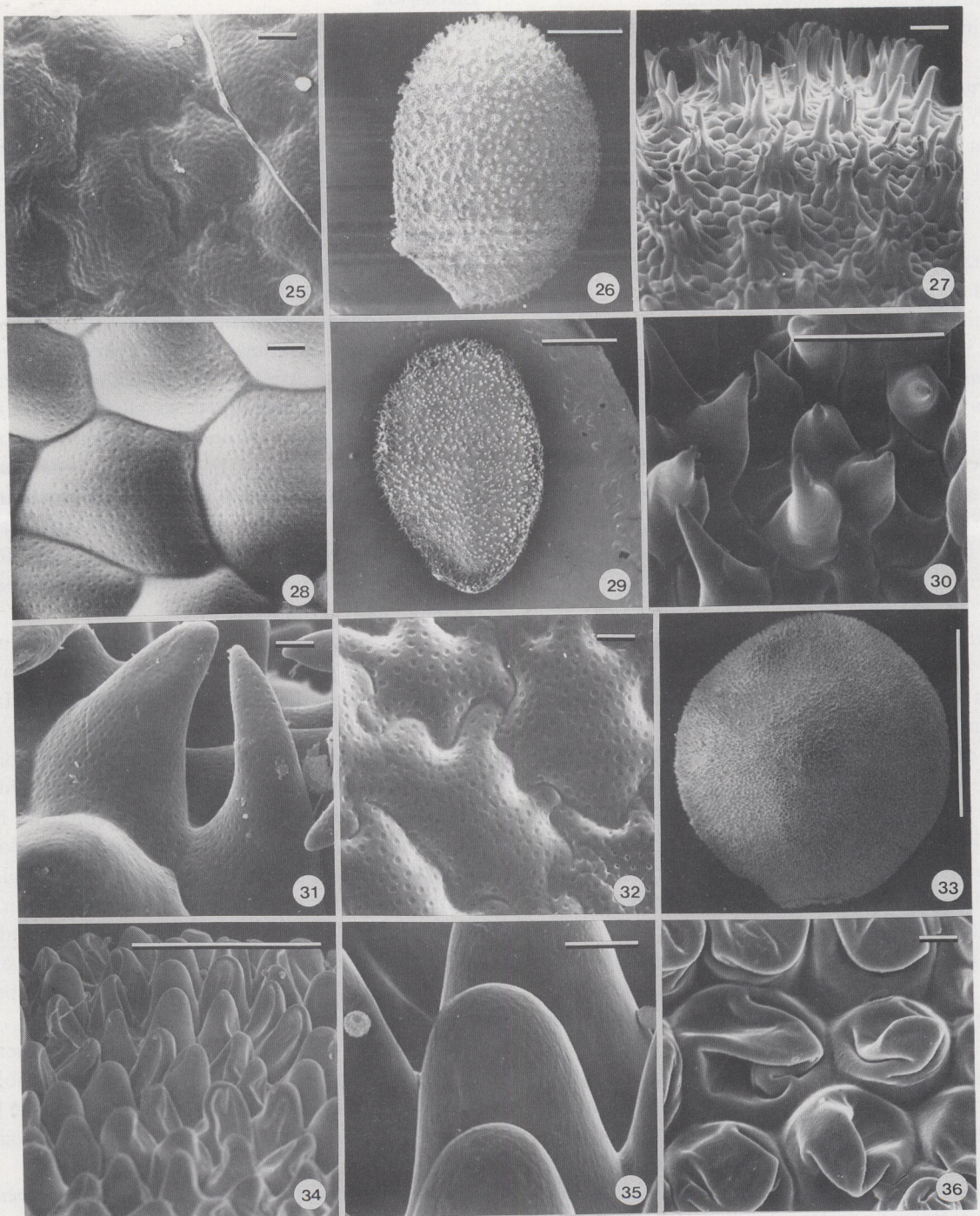


Fig. 25. *Nymphoides hydrophylla*: from *Joseph 38631;* testa cells. Figs. 26-28. *N. macrospermum*: from *Joseph 38636*. 26, entire seed; 27, aculeate seed surface; 28, testa cells. Figs. 29-32. *N. krishnakesara*: from *Joseph 43001*. 29, entire seed; 30, seed surface with bulbous-based aculeate prickles; 31, foveate aculea; 32, foveate testa cells with sinuate junctures. Figs. 33-36. *N. aurantiacum*: from *Revathy 1039*. 33, entire seed; 34, aculeate seed surface; 35, aculea on the peripheral seed surface; 36, aculea on the central seed surface. Scale bars = 10 μm in 25, 28, 31, 32, 35, 36; 100 μm in 27, 30, 34; 1000 μm in 26, 29, 33.

or clustered, 13-24 μm long; testa cells foveolate, rather flat with raised, sinuate junctures.

Specimen examined: *Joseph 30243* (CALI).

N. hydrophylla (Lour.) O. Kuntze [= *L. cristatum* Griseb.; *N. cristatum* (Roxb.) O. Kuntze] Figs. 22-25.

Seeds 4-6 in each capsule, brown, discoid with persistent funiculi, ca. 2 mm in diam.; surface aculeate, the prickles 80-160 μm long, in a bundle of 3-7 with gemmate protuberances toward the distal part; testa cells convex and rugose, the junctures depressed and variously undulate.

Specimen examined: *Joseph 38631* (CALI).

N. macrospermum Vasudevan Figs. 26-28.

Seeds 2-6 per capsule, cream to yellow-brown, ovoid or ellipsoid, 3.8-4.4 mm long, 3.2-3.6 mm wide; surface aculeate, aculea 70-120 μm long with a blunt tip, foveolate, usually fused in bundles of 2-5 with gemmate protuberances; testa cells polygonal, convex, and foveolate with straight, depressed junctures.

Specimen examined: *Joseph 38636* (CALI).

N. krishnakesara Joseph & Sivarajan (in press) Figs. 29-32.

Seeds 5-10 per capsule, cream or yellow-brown or gray, flat and compressed, obovoid, rounded or obtuse at distal end, subacute at proximal end, 3.2-4.0 mm long, 2.2-2.5 mm wide, ca. 0.4 mm thick with an obliquely truncate funicular scar; surface aculeate, prickles bulbous based, sharply pointed at apex, 80-100 μm long, solitary or in bundles of 2-5, foveate; testa cells variously shaped, convex, foveate, junctures sinuate, depressed.

Specimen examined: *Joseph 43001* (CALI).

N. aurantiacum (Dalz.) O. Kuntze [= *L. aurantiacum* Dalz.] Figs. 33-36.

Seeds 12-15 in each capsule, light brown, discoid, ca. 2 mm in diam., with a distinct funicular scar; surface densely aculeate, almost every testa cell developing into an aculeum, aculea ca. 40 μm long, obtuse at apex, those at the central region of seed surface not expanded; testa cells faintly favulariate with obscure junctures.

Specimen examined: *Revathy 1039* (CALI).

Discussion

Seed ornamentation patterns have been taxonomically used since the last century, because they are evident to superficial observation. The test has been whether the seeds are smooth, warty, echinate, spinulate etc. and whether these ornamentations are abundant or not. It has already been shown that the ornamentations in this group of plants are epidermal, the cells developing into elongate, digitiform structures perpendicular to the seed surface, often in coherent clusters from adjacent epidermal cells. However, such a lot of variations in this character were encountered in the African and Madagascar species of this genus that Raynal (1974) concluded that it is taxonomically unusable. Aston (1969) observed two main types of epidermal cells in Australian *Villarsia*, one group with polygonal cells with straight walls and the other with irregularly shaped cells with radially sinuate walls. However, in yet another group, Raynal (1974) reported that this is variable and hence become taxonomically of doubtful merit.

With such a great degree of variability in seed-coat patterns in this group of plants, we have no pretentions that our sampling is adequate. May be more intensive studies on a population basis might yield even more valuable information on the taxonomic usefulness of these characters. The evidences provided here, therefore, should be taken as preliminary rather than conclusive, especially for species delimitation and identification, which should be based on multiple correlation rather than on seed coat alone. Our preliminary studies, however, seem to provide valuable supporting characters for species recognition among Indian materials and reveal interesting correlations, as could be seen from the text and photographs.

Grisebach (1839) is probably the only author who has attempted a subgeneric classification of *Nymphoides*. This classification has largely been based on inflorescence and seed characters. He recognized two sections in this genus as follows:

Sect. *Waldschmidtia*: "*Semina pauca, complanata, alata, ciliolata. Inflorescentia axillaris*"

Sect. *Nymphoides*: "*Semina subglobosa, exalata, laevial, muricata. Inflorescentia petiolaris*"

and included only one species (*N. peltata*) in the former and all others in the latter. Our observations on the

seed surface characters almost corroborate his point of subgeneric classification and probably even call for its generic separation, because as in other morphological characters, in seed characters also, there is no other close relative for *N. peltata* in the genus *Nymphoides*. Ornduff (1970 b) has also shown that this species is hexaploid.

Nymphoides indica [= *Limnanthemum indicum*] is a complex group distributed in both the New World and the Old World, but have been referred to under different binomials mainly depending upon the flower color. Grisebach (1839) has recognized several segregate species in this group, like *L. kleinianum*, *L. wightianum*, both from India and *L. humboldtianum* from the New World. Clarke (1883) has already reduced *L. kleinianum* and *L. wightianum* into the synonymy of *L. indicum* and Ornduff (1970 a, b) has concluded that *L. humboldtianum* (now *N. humboldtianum*) also is conspecific with the latter. Despite the fact that the New World taxa are consistently tetraploid and the Old World ones diploid, there are no consistent morphological differences to separate *N. humboldtianum* from *N. indica* (Ornduff, 1970 a).

For the present study we have taken seeds from Malabar materials of *Nymphoides indica*. During a recent visit to Komarov Botanical Institute, Leningrad, one of us (VVS) could examine materials of *L. kleinianum* (Assam: *Masters s.n.*, LE; Peninsular India: *Wight 1846,* LE; Tenasserim & Andamans: *Helfer 5842,* LE) and *L. wightianum* (Kerala: *Wight 2579,* LE) all from India and got convinced that they are conspecific. The seed materials from *L. kleinianum* were unripe and not suitable for scanning. However, we got some ripe seeds from *L. wightianum* for our studies, which do not seem to be substantially different from those of *N. indica*. Nevertheless, it differs in having more sparsely distributed, solitary tubercles on the seed surface and in having foveolate testa surface with indistinct junctures. The variability points to the need of adequate sampling and more intensive studies on the seed coat patterns of *N. indica* complex.

Nymphoides parvifolium with its dimorphic leaves and homostylous flowers, is morphologically quite distinct from *N. indica*. But seed coat studies betray close relationship between the two. Recently we collected a material locally (*Joseph 30243,* CALI), which resembled *N. parvifolium* in some respects, but differed in its more profusely branched habit, distinctly veined floating leaves, profusely fimbriate corolla lobes and larger fruits with more seeds. After detailed studies, this is being published as a new species with the name *N. sivarajanii* Joseph (in Press). Seed coat surface patterns also suggest that the closest relative of this species is to be found in *N. parvifolium*.

Nymphoides hydrophylla [= *N. cristatum*] is a gynodioecious species with hermaphroditic and female flowers (Vasudevan Nair, 1975). Its relationship with other Asiatic species is not clear, but Ornduff (1970 b) has precluded any relationship with *N. indica*. Seed-coat patterns tend to corroborate this viewpoint. The seed surface is beset with clustered, aculeate protuberances, much longer than those of *N. indica*, *N. parvifolium* and *N. sivarajanii*, and distinctively shaped with gemmate protuberances at least towards the distal ends. The convex and rugose testa cells with their depressed junctures also preclude any relationship of *N. hydrophylla* with the aforesaid species.

We have two dioecious species of *Nymphoides* in *India*: *N. macrospermum* and *N. krishnakesara*. The latter is closely similar to the former, but differs in its vertical rhizome, dimorphic leaves, distinctly blue anthers, female flowers with two whorls of radiating stigmatic hairs and in several other respects. Ornduff (1970 b) opines that *N. macrospermum* is closely related to the distylous *N. indica*. But the seed surface patterns are very different in these two. The aculeate testa and the convex testa cells with their depressed junctures relate it better with *N. hydrophylla* from which it differs in the smooth aculea (those of *N. hydrophylla* have gemmate protuberances on them) and polygonal epidermal cells with straight walls. Despite close similarity with *N. macrospermum*, the seeds of *N. krishnakesara* differs from those of the former in having bulbous-based aculeate protuberances on the seed surface and irregularly shaped testa cells with sinuate walls. *N. aurantiacum* stands apart from all species in having almost every testa cell developed into an aculeate protuberances, virtually rendering the testa cells invisible.

In short one can recognize here among the Indian materials, three different groups of species based on seed-coat patterns.

1. Seeds flat, compressed, ciliate along margins, cilia tuberculate on the distal parts, epidermal cells a little raised with depressed, sinuate junctures. . . . . *N. peltata*

2. Seeds discoid, non-ciliate, surface tuberculate, epidermal cells rather flat with raised junctures. . . . . *N. indica*, *N. parvifolium*, *N. sivarajanii*
3. Seeds discoid or compressed and flat, non-ciliate, surface aculeate, epidermal cells convex with depressed junctures. . *N. hydrophylla*, *N. macrospermum*, *N. krishnakesara*, *N. aurantiacum.*

The eight Indian species of *Nymphoides* can be recognized in herbarium materials, using ripe seeds as follows:

1. Seeds compressed, ciliate at margins . *N. peltata*
1. Seeds compressed or discoid, non-ciliate . . . 2
2. Seeds discoid, tuberculate on the surface . . . 3
2. Seeds discoid or compressed, aculeate on the surface 5
3. Testa cell walls obscure, cells foveolate . . . . . *N. parvifolium*
3. Testa cell walls distinct, cells foveolate or not. 4
4. Testa cell walls with dendroid thickenings, walls not radially sinuate. . . . . *N. indica*
4. Testa cell walls without dendroid thickings, walls radially sinuate. . . . . *N. sivarajanii*
5. Aculea tuberculate towards distal ends . . . . . *N. hydrophylla*
5. Aculea not tuberculate 6
6. Every testa cell bearing an aculeum . . . . . *N. aurantiacum*
6. Not every testa cell bearing an aculeum 7
7. Testa cell walls straight . . . *N. macrospermum*
7. Testa cell walls sinuate . . . . *N. krishnakesara*

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印度荇菜屬(荇菜科)植物之種皮微細形態

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荇菜屬(*Nymphoides* Seguier)是泛佈於熱帶和亞熱帶地區的水生植物，約有 20 種。因本屬大部份種類的莖葉皆相當類似；且花一經乾燥與新鮮植物差異頗大，很難再作爲分類的依據。故標本館中所收藏的該屬標本常被錯誤的歸類，也因此造成本屬在植物標本鑑定上的不易。爲此，本文嘗試以掃描電子顯微鏡研究印度的 8 種荇菜屬植物之種皮形態，期探討種子表面的特徵與種間的分類關係。這 8 種植物的種子爲扁平狀或鐵餅狀。荇菜(*N. peltata* (Gmel.) O. Kuntze)的扁平種子周圍具纖毛最爲特殊；其它種類則整個種子表面有瘤狀或刺狀的突起物。本屬種子的表皮細胞皆爲多邊形或不規則形；具有突起的或下陷的，直的或輻射波曲狀的細胞接縫。上述的這些種皮性狀在鑑別種類上相當管用，尤其當觀察乾燥的植物標本時更形重要。