EDINBURGH JOURNAL OF BOTANY 66 (3): 371–390 (2009)

doi:10.1017/S0960428609990035

THE *HEDYOTIS–OLDENLANDIA–KOHAUTIA* COMPLEX (*RUBIACEAE*) IN NEPAL: A STUDY OF FRUIT, SEED AND POLLEN CHARACTERS AND THEIR TAXONOMIC SIGNIFICANCE

S. Neupane[1], S. Dessein[2] & T. J. Motley[3]

Pollen and seed characters of 12 Nepalese representatives of the *Hedyotis–Oldenlandia–Kohautia* complex (*Spermacoceae* s.l.; *Rubiaceae*) were investigated morphologically using scanning electron and light microscopy. The members of the complex were found to show remarkable variation in fruit, seed and pollen features. Pollen grains were all colporate with the aperture number varying from 3–4 to occasionally 5. The ectoaperture was a colpus, and the endoaperture was an endocingulum, a lalongate endocolpus or an endocolpus combined with an annulus around the mesoporus. Sexine ornamentation was variable, being perforate, reticulate or microreticulate. Three species were found to have a double reticulum. Supratectal elements were generally absent, but sometimes muri were beset with granules. Seeds were numerous per capsule, small and non-crateriform. Three types of seed were distinguished based on shape: (1) lenticular with a narrow wing-like margin, (2) trigonous, and (3) globose/subglobose. Trigonous seeds exhibited marked variation in colour, size and shape. On the basis of the pollen and seed characters, used in combination with the type of fruit dehiscence, five natural groups are identified for Nepalese taxa. The generic status of *Hedyotis*, *Oldenlandia* and *Kohautia* is maintained but some species are transferred from *Hedyotis* to *Oldenlandia*. Pollen and seed morphology, together with the type of fruit dehiscence, proved to be helpful in delimiting supra- and infrageneric groups within the *Hedyotis–Oldenlandia–Kohautia* complex.

Keywords. *Hedyotis*, *Kohautia*, morphology, *Oldenlandia*, palynology, plant systematics, *Rubiaceae*, seed, *Spermacoceae*, taxonomy, typification.

Introduction

Hedyotis L., *Oldenlandia* L. and *Kohautia* Cham. & Schltdl. are three closely related genera of the *Rubiaceae* traditionally referred to the tribe *Hedyotideae* Cham. & Schltdl. ex DC. (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988). However, molecular data have shown that the tribe *Spermacoceae* Cham. & Schltdl. ex DC.

[1] Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Current address: Department of Biological Sciences, Old Dominion University, 45th Street, 110 Mills Godwin Building, Norfolk, VA 23529-0266, USA. E-mail: sneup003@odu.edu
[2] National Botanic Garden of Belgium, Domein van Bouchout, 1860 Meise, Belgium.
[3] Department of Biological Sciences, Old Dominion University, 45th Street, 110 Mills Godwin Building, Norfolk, VA 23529-0266, USA.

sensu stricto makes the *Hedyotideae* paraphyletic. *Spermacoceae* has been expanded to include most of the former *Hedyotideae* genera, including *Hedyotis*, *Oldenlandia* and *Kohautia* (Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009). The three genera have a tropical and subtropical distribution with most species in the Old World. Globally the complex comprises just over 400 species of which 112 are in *Hedyotis*, 262 in *Oldenlandia* and 36 in *Kohautia* (Govaerts *et al.*, 2006). Superficially, the three genera are very similar, sharing a herbaceous habit, relatively small flowers, and dry, usually capsular fruits with few to many small seeds. In Nepal, the complex is represented by 13 species (Neupane, 2006).

The circumscriptions and classifications of *Hedyotis*, *Oldenlandia*, *Kohautia* and numerous related genera such as *Neanotis* W.H.Lewis and *Houstonia* L. have been the focus of much taxonomic debate and confusion. Some taxonomists ('lumpers'; e.g. Lamarck, 1792; Torrey & Gray, 1841; Dutta & Deb, 2004) have favoured wide circumscriptions of the genera by proposing an all-inclusive *Hedyotis* or by recognising two to three large genera *Hedyotis*, *Oldenlandia* and *Houstonia*. Others ('splitters'; e.g. Willdenow, 1798; de Candolle, 1830; Bremekamp, 1952; Terrell, 2001a, 2001b) have favoured recognising many, often small, genera, sister to *Oldenlandia*, *Houstonia* and *Hedyotis* (see taxonomic history section below).

There are no global studies of the *Hedyotis–Oldenlandia–Kohautia* complex. Only partial revisions are available, so far all based on non-Asiatic species. The present paper aims to shed light on the morphological variation observed among the Nepalese representatives of the complex in order to contribute to a clearer delimitation of the genera involved.

Brief taxonomic history of Hedyotis–Oldenlandia–Kohautia *and related genera*

Since the description of *Hedyotis* and *Oldenlandia* by Linnaeus in *Species Plantarum* in 1753, there has been confusion, discussion and disagreement over the delimitation of the two genera (Halford, 1992). Linnaeus (1753) described *Hedyotis* as a genus of three Asian species (*H. auricularia* L., *H. fruticosa* L. and *H. herbacea* L.) and *Oldenlandia* with four species (*O. biflora* L., *O. corymbosa* L., *O. umbellata* L. and *O. uniflora* L.); he also described a third exclusively American genus, *Houstonia* L., containing two species (*Houstonia caerulea* L. and *H. purpurea* L.), related to *Hedyotis* and *Oldenlandia*. This last genus has also been the subject of debate (for an overview see Terrell, 1996; Church, 2003), but will not be discussed in detail here. Lamarck (1792) considered *Oldenlandia* and *Hedyotis* congeneric and used the name *Hedyotis* for the resulting unit. This opinion was followed by Wight & Arnott (1834), Torrey & Gray (1841) and others. Other authors, however, maintained *Oldenlandia* as a distinct genus (Willdenow, 1798; Roxburgh, 1820; de Candolle, 1830). Gray (1859) stated that, based on fruit dehiscence and seed structure, 'all three Linnaean genera (*Oldenlandia*, *Houstonia* and *Hedyotis*) equally merit restoration'.

The taxonomic discussion over the generic delimitation of *Oldenlandia* and *Hedyotis* was further complicated by a controversy concerning the type of *Hedyotis*.

The problems began when Linnaeus in *Genera Plantarum* (1754) described *Hedyotis* as a genus with dehiscent fruits whereas *Hedyotis auricularia* had been described earlier by him in *Species Plantarum* (1753) as having indehiscent fruits. *Hedyoti*s was first lectotypified by Chamisso & Schlechtendal (1829) with *H. auricularia*. However, Bremekamp (1952) proposed *Hedyotis fruticosa* as its lectotype because the indehiscent capsules of *H. auricularia* did not comply with Linnaeus's (1754) generic description of *Hedyotis*. The issue was recently resolved by selecting *Hedyotis fruticosa* as a conserved type (Jarvis, 1992; see also McNeill *et al.*, 2006, App. III).

The third genus under discussion, *Kohautia*, with the type species *K. senegalensis* Cham. & Schltdl., was proposed by Chamisso & Schlechtendal (1829) in their newly erected suprageneric *Hedyotideae*. *Kohautia* is easily separated from *Hedyotis* and *Oldenlandia* by having a style which always overtops the anthers. Although generally accepted as a well-defined group, *Kohautia* has sometimes been included in *Hedyotis* (Wight & Arnott, 1834) or *Oldenlandia* (Hooker, 1873; Schumann, 1891).

Bremekamp (1952), in his revision of the African *Oldenlandia* species, accepted both *Hedyotis* and *Kohautia* as distinct genera. He also recognised many small satellite genera for African taxa traditionally referred to as *Oldenlandia*. Lewis (1962), in contrast, in a chromosomal study of North American *Hedyotis* s.l., treated *Oldenlandia* and *Houstonia* as subgenera of *Hedyotis*, namely as *Hedyotis* subgen. *Oldenlandia* (L.) Torrey & A.Gray and *Hedyotis* subgen. *Edrisia* (Raf.) Lewis, respectively. However, in an extensive cytopalynological study of African *Hedyotideae*, Lewis (1965b) accepted many of the genera Bremekamp separated from *Oldenlandia*. Similarly, Verdcourt (1976) maintained most of Bremekamp's segregate genera and supported the separate recognition of *Hedyotis*, *Oldenlandia* and *Kohautia*.

Terrell (1975), in a comparative analysis of type species of *Hedyotis*, *Oldenlandia* and *Houstonia*, found important differences in fundamental characteristics of the fruits and seeds which according to him 'strongly suggests the existence of three genera rather than one'. Halford (1992), in a review of Australian *Oldenlandia*, identified the genera *Hedyotis*, *Oldenlandia*, *Kohautia* and *Synaptantha* Hook.f. He distinguished *Hedyotis* from *Oldenlandia* on the basis of fruit dehiscence type. Terrell & Robinson (2003) commented that 'the *Hedyotis* species in Mexico and southwestern United States do not resemble either the Asian or the Hawaiian species, nor do the latter two resemble each other'. In the same paper, they reduced *Exallage* Bremek., another satellite genus defined by Bremekamp (1952) based on its indehiscent fruits, to a subgenus of *Oldenlandia*.

Molecular studies by Bremer (1996) and Bremer & Manen (2000) found *Hedyotis* and *Oldenlandia* to be paraphyletic whereas studies by Andersson & Rova (1999) and Andersson *et al.* (2002) found them to be polyphyletic. Recently, in a more sample-rich study focusing on the *Spermacoceae*, Groeninckx *et al.* (2009), based on chloroplast data, and Kårehed *et al.* (2008), based on combined nuclear and chloroplast data, found *Hedyotis* and *Oldenlandia* polyphyletic and *Kohautia* biphyletic.

This taxonomic survey indicates that systematists have not yet reached a consensus on the delimitation of *Hedyotis*, *Oldenlandia* and *Kohautia*. However, recent studies usually favour the recognition of the three genera, often in addition to many other (small) genera (Bremekamp, 1952; Terrell *et al.*, 1986; Terrell, 1991, 1996, 2001a, 2001b, 2001c; Terrell & Robinson, 2003; Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009).

Data from pollen, seed and fruit morphology, together with chromosome numbers, have been found to be most accurate in classifying the species formerly referred to the tribe *Hedyotideae* (Gray, 1859; Lewis, 1965a, 1965b; Terrell, 1975, 1996, 2001a, 2001b, 2001c; Terrell *et al.*, 1986; Terrell & Wunderlin, 2002; Terrell & Robinson, 2003). This inspired us to conduct the present morphological study on the Nepalese representatives of *Hedyotis*, *Oldenlandia* and *Kohautia* to provide a better understanding of the species in this difficult lineage.

Materials and Methods

This study was based mainly on material from personal collections, herbarium material from Tribhuvan University Central Herbarium (TUCH) and some Nepalese specimens deposited at the Natural History Museum, London (BM). The pollen of 17 specimens belonging to 11 species and the seeds of 16 specimens belonging to 11 species were investigated (Table 1). Pollen and seed material was studied using both light microscopy (LM, including the use of a stereomicroscope) and scanning electron microscopy (SEM). The LM and stereomicroscope study was conducted at TUCH. Scanning electron micrographs of nine species of *Hedyotis*, *Oldenlandia* and *Kohautia* were produced at the Laboratory of Plant Systematics, Belgium. The acetolysis method (Erdtman, 1966, 1969) was used for the study of pollen under LM, with the acetolysed pollen mounted in glycerin jelly sealed with paraffin. For the SEM study, pollen was acetolysed following Reitsma's 'wetting agent method' (Reitsma, 1969). Acetolysed pollen was rinsed in 70% or 96% ethanol, pipetted onto a stub, and left to dry. The stubs were coated with gold using an SPI-MODULE™ sputter coater. Observations and digital images were made under a Jeol JSM 5800 LV microscope. Seeds were directly mounted on stubs, coated with gold and observed under SEM. Illustrations of seeds were made using a stereomicroscope. Pollen terminology is based on Punt *et al.* (2007) and partly on Dessein *et al.* (2005) for the description of the tectum. For seed morphology, the terminology is partly based on Stearn (1966: 506–507) and Dessein (2003).

Results

Pollen morphology

The results of the pollen morphology study are shown in Table 2.

TABLE 1. Specimens of *Hedyotis*, *Oldenlandia* and *Kohautia* examined for the pollen and seed study, with figure references

Taxon	Collector(s)	Locality	Institution	Figure references
Hedyotis scandens	*S. Neupane* 25	Suryabinayak, Bhaktpur	TUCH	Pollen: Fig. 1A, B, Fig. 2A, Fig. 3A
Hedyotis scandens	*J. Shrestha* 22	Nagarjun, Kathmandu, 1960 m	TUCH	Seed: Fig. 4A
Oldenlandia auricularia	*S. Neupane* 16	Gorkha, Manakamana, way to Kageshowr Mahadev, 1300 m	TUCH	Pollen: Fig. 1C, D, Fig. 3B
Oldenlandia auricularia	*S. Pahari* 56	Kaski, Kande, 1645 m	TUCH	Seed: Fig. 4C, Fig. 5A, B
Oldenlandia biflora	*J. Williams* 54	Sunsari, Borampur, near Dharan, 228 m	BM	Pollen: Fig. 1G, H, Fig. 2B
Oldenlandia biflora	*S. Neupane* 20	Kailali, Ghodaghodi Tal, 145 m	TUCH	Seed: Fig. 4F, Fig. 5C, Fig. 6B
Oldenlandia brachypoda	*S. Neupane* 1	Kathmandu, Gokarna, 1300 m	TUCH	Pollen: Fig. 1K, L
Oldenlandia brachypoda	*S. Neupane* 1	Kathmandu, Gokarna, 1300 m	TUCH	Seed: Fig. 4I, Fig. 6E
Oldenlandia diffusa	*S. Neupane* 15	Bhaktapur, Balkot, VDC, Ward no. 5	TUCH	Pollen: Fig. 1I, J
Oldenlandia diffusa	*S. Neupane* 15	Bhaktapur, Balkot, VDC, Ward no. 5	TUCH	Seed: Fig. 4J, Fig. 5I, Fig. 6F
Oldenlandia erecta	*S. Neupane* 3	Kathmandu, Gokarna, 1300 m	TUCH	Pollen: Fig. 1M, N, Fig. 2C, Fig. 3F
Oldenlandia erecta	*S. Neupane* 3	Kathmandu, Gokarna, 1300 m	TUCH	Seed: Fig. 4H, Fig. 5J, K, Fig. 6G
Oldenlandia lineata	*S. Neupane* 9	Chitwan, Bishhazari Tal	TUCH	Pollen: Fig. 1E, F, Fig. 3C
Oldenlandia lineata	*S. Neupane* 8	Chitwan, Bishhazari Tal	TUCH	Seed: Fig. 4B
Oldenlandia ovatifolia	*S. Neupane* 23	Chitwan, way to Bishhazari Tal	TUCH	Pollen: Fig. 3D
Oldenlandia ovatifolia	*M. Mikage et al.* 9552771	Parsa, Parsa Wildlife Reserve, Mahadev Khola, 190 m	BM	Seed: Fig. 4G, Fig. 5D
Oldenlandia pinifolia	*Norkett* 8008	Tumlingtar, beneath cliff, on shore of Sabbaya River, 1800 ft	BM	Seed: Fig. 4E, Fig. 5G, H, Fig. 6B
Oldenlandia verticillata	*K. Weschi* 71702	Chitwan, Ramnagar, 300 m	BM	Pollen: Fig. 3E
Oldenlandia verticillata	*S. Neupane* 5	Chitwan, way to Bishhazari Tal	TUCH	Seed: Fig. 4D, Fig. 5E, F
Kohautia coccinea	*Polunin, Sykes & Williams* 3219	Between Warlu and Ilu, Bheri River, 6500 ft	BM	Pollen: Fig. 1Q, R, Fig. 2E
Kohautia coccinea	*Polunin, Sykes & Williams* 4965	Lithu, E of Jumla, 8000 ft	BM	Seed: Fig. 4K
Kohautia gracilis	*Polunin, Sykes & Williams* 3953	Jumla, Karnali valley, between Badal Khet and Tila, 5000 ft	BM	Pollen: Fig. 1O, P, Fig. 2D

TABLE 2. Pollen morphology of *Hedyotis*, *Oldenlandia* and *Kohautia*

Taxon	P (μm)	E (μm)	Equatorial shape	NC	Sexine	Supratectal elements	Ectoaperture	CL (μm)	Endoaperture	Annulus
Hedyotis scandens	30–(30.5)–32	24–(26.9)–30	Prolate-spheroidal to subprolate	4	Double reticulum	Present on inside of muri	Long colpus	16	Endocingulum	Absent
Oldenlandia auricularia	28–(30.7)–34	28–(30.1)–33	Prolate-spheroidal	3 + 4	Double reticulum	Present on inside of muri	Long colpus	15	Endocingulum	Absent
Oldenlandia biflora	20–(21.9)–23	17–(19.8)–21	Prolate-spheroidal to subprolate	3 + 4	Perforate to microreticulate	Absent	Long colpus	10	Endocolpus	Absent
Oldenlandia brachypoda	26–(28.6)–32	28–(31.0)–35	Oblate-spheroidal	3(4)	Reticulate	Granules	Long colpus	10	Endocolpus	Absent
Oldenlandia diffusa	27–(28.3)–30	26–(29.6)–34	Oblate-spheroidal	3(4)	Reticulate	Granules	Long colpus	9	Endocolpus	Absent
Oldenlandia erecta	29–(32.3)–34	26–(27.9)–30	Prolate-spheroidal to subprolate	3 + 4	(Micro) reticulate	Absent	Long colpus	13	Endocolpus	Absent
Oldenlandia lineata	26.6	23.3	Prolate-spheroidal to subprolate	3 + 4	Double reticulum	Present on inside of muri	Long colpus	16	Endocingulum	Absent
Oldenlandia ovatifolia	33.3	30	Prolate-spheroidal	3 + 4	Not studied	Not studied	Not studied	Not studied	Endocolpus	Absent
Oldenlandia verticillata	28	31	Oblate-spheroidal	3 + 4	Not studied	Not studied	Not studied	Not studied	Endocolpus	Absent
Kohautia coccinea	20–(21.8)–23	17–(18.5)–22	Prolate-spheroidal to subprolate	(3)4	Reticulate	Absent	Long colpus	10	Endocolpus	Present
Kohautia gracilis	25	22–(23.7)–28	Prolate-spheroidal to subprolate	4(5)	Reticulate	Absent	Long colpus	11	Endocolpus	Present

P, polar diameter; E, equatorial diameter (values in parentheses represent average lengths). NC, no. of apertures (values in parentheses were not found in all specimens). CL, colpus length.

Polarity, symmetry and size

Pollen grains of *Hedyotis*, *Oldenlandia* and *Kohautia* in Nepalese specimens were all isopolar and radially symmetrical. The average equatorial diameter ranged from 18.5 μm (in *Kohautia coccinea* Royle) to 31 μm (*Oldenlandia brachypoda* DC.; Table 2). Size variation, often exceeding 20%, was also observed and documented within a specimen or between specimens of a single population.

Shape

The pollen shape in the equatorial view is described by calculating the ratio of the polar axis (P) and the equatorial diameter (E). The P/E ratio in the complex varies between 0.88 and 1.25. They were oblate-spheroidal (P/E between 0.88 and 1; Fig. 1I, K), or prolate-spheroidal to subprolate (P/E between 1–1.14 and 1.14–1.33; Fig. 1A, C, E, G, M, O, Q). In the polar view the pollen grains are circular in outline, sometimes slightly lobed due to the sunken colpi (Fig. 2A–E).

Apertures

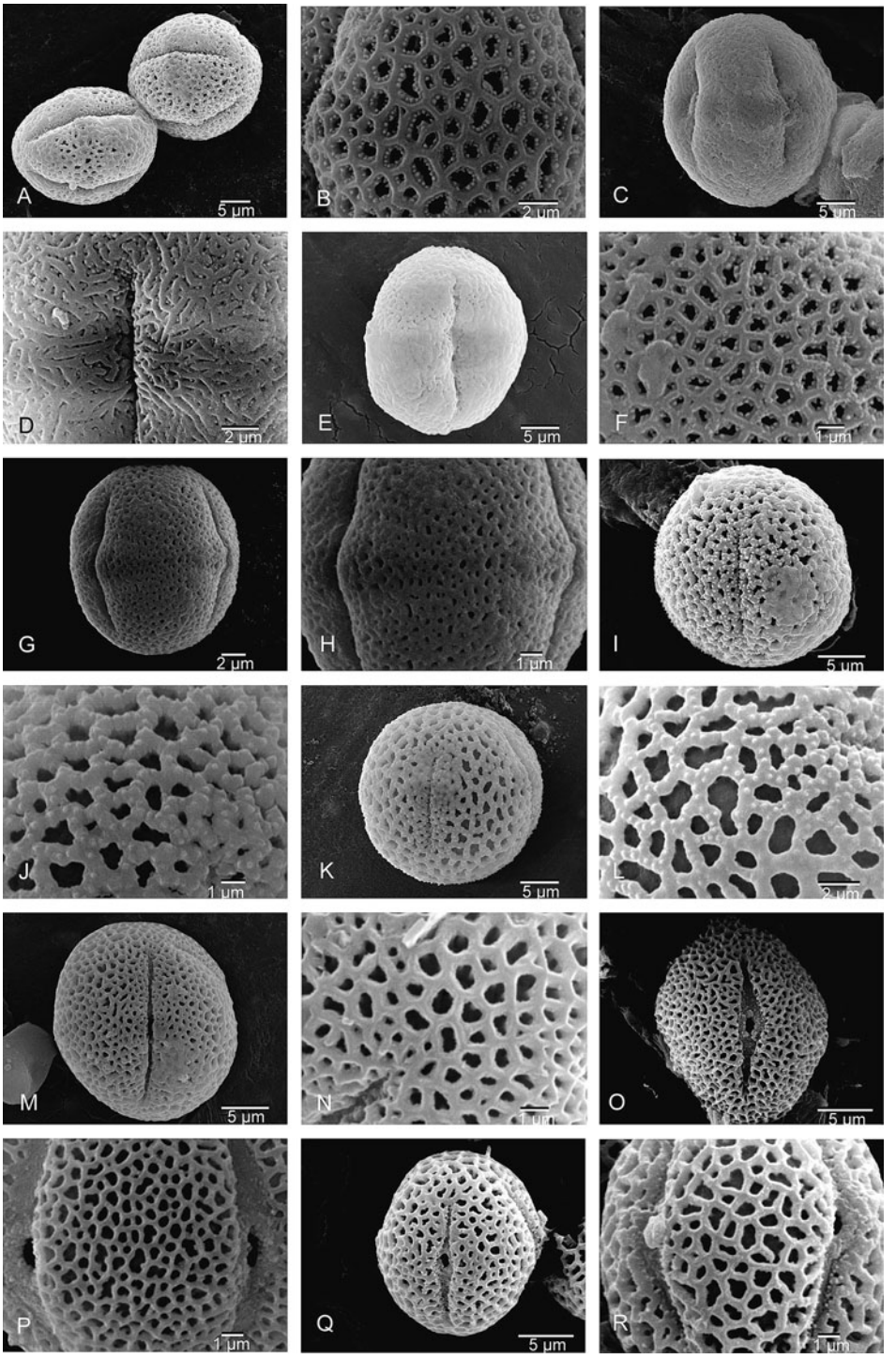
Position and number. In all species apertures were positioned along the equator (zonoaperturate). The number of colpi was 3 or 4 in *Oldenlandia auricularia* (L.) K.Schum., *O. biflora*, *O. erecta* (Manilal & Sivar.) R.R.Mill, *O. lineata* (Roxb.) Kuntze, *O. ovatifolia* (Cav.) DC., *O. verticillata* L.; 3 to occasionally 4 in *O. brachypoda* DC. and *O. diffusa* (Willd.) Roxb.; 4 to occasionally 3 in *Kohautia coccinea*; 4 in *Hedyotis scandens* Roxb.; and 4 to occasionally 5 in *Kohautia gracilis* (Wall.) DC.

Type of aperture. In Nepal, pollen apertures in all members of the complex were found to be compound, comprising an ectocolpus, a mesoporus and an endoaperture. The mesoporus of the *Kohautia* species showed an annulus (Fig. 1O, Q), a feature not observed in Nepalese members of *Hedyotis* and *Oldenlandia*. Endoapertures may be an endocingulum as in *Oldenlandia auricularia*, *O. lineata* and *Hedyotis scandens*, or a lalongate endocolpus as in *Oldenlandia biflora*, *O. brachypoda*, *O. diffusa*, *O. erecta*, *O. ovatifolia* and *O. verticillata* (categorised as Os type A by Lewis, 1965a, 1965b). In the *Kohautia* species there was an endocolpus combined with a marked thickening around the inside of the mesoaperture (Os type B in Lewis, 1965a, 1965b). The thickening around the mesoaperture on the inside coincided with the thickening around the mesoaperture on the outside and hence formed one single structure. We prefer to name this structure an annulus.

Three pollen types were distinguished on the basis of the endoaperture:

Type 1: endocingulum in *Hedyotis scandens*, *Oldenlandia auricularia* and *O. lineata* (Fig. 3A–C);

Type 2: lalongate endocolpus in *Oldenlandia biflora*, *O. brachypoda*, *O. diffusa*, *O. erecta*, *O. ovatifolia* and *O. verticillata* (Fig. 3D–F);


A
5 µm
B
2 µm
C
5 µm
D
2 µm
E
5 µm
F
1 µm
G
2 µm
H
1 µm
I
5 µm
J
1 µm
K
5 µm
L
2 µm
M
5 µm
N
1 µm
O
5 µm
P
1 µm
Q
5 µm
R
1 µm

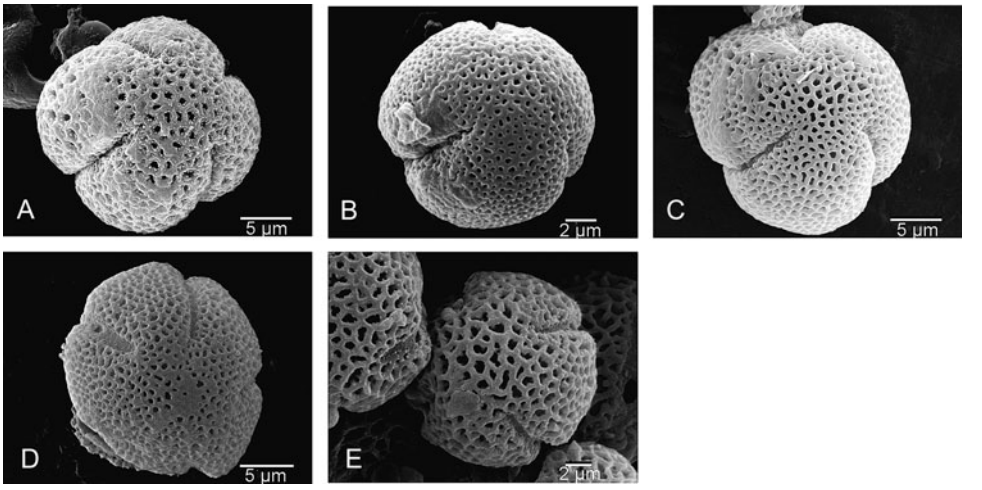


FIG. 2. Polar view of pollen grains in SEM. A: *Hedyotis scandens*. B: *Oldenlandia biflora*; C: *O. erecta*. D: *Kohautia gracilis*; E: *K. coccinea*.

Type 3: endocolpus combined with an annulus around the mesoporus in *Kohautia coccinea* and *K. gracilis* (Fig. 1O, Q).

Pollen wall stratification

The exine layer was composed of a nexine and a sexine layer with a row of columellae and a tectum with or without supratectal elements. The nexine was often thickened along the ectocolpi.

Tectum. The tectum was reticulate (lumina $>$ 1 µm and larger than muri) in *Oldenlandia brachypoda*, *O. diffusa*, *Kohautia coccinea* and *K. gracilis* (Fig. 1J, L, P, R). *Hedyotis scandens*, *Oldenlandia auricularia* and *O. lineata* were characterised by having a double reticulum, i.e. with an infra- and a suprareticulum (Fig. 1B, D, F). The reticulation often tended to be of the striate-reticulate type in *Oldenlandia auricularia* (Fig. 1D). *Oldenlandia biflora* (Fig. 1H) showed a transition between perforate (perforations $<$ 1 µm and smaller than the muri) and microreticulate

FIG. 1. Equatorial view of pollen grains in SEM. A, B: *Hedyotis scandens*. C, D: *Oldenlandia auricularia*; E, F: *O. lineata*; G, H: *O. biflora*; I, J: *O. diffusa*; K, L: *O. brachypoda*; M, N: *O. erecta*. O, P: *Kohautia gracilis*; Q, R: *K. coccinea*. B & F: Note the presence of supratectal elements on inside of muri. D: Detail of mesocolpium with striate-reticulate sexine. H: Perforate to microreticulate tectum. J & L: Note the presence of granules on the surface of muri. N: Detail of apocolpium indicating the absence of supratectal elements. O & Q: External colpus; also note the annulus around the mesoporus. P & R: Detail of mesocolpium with reticulate sexine.

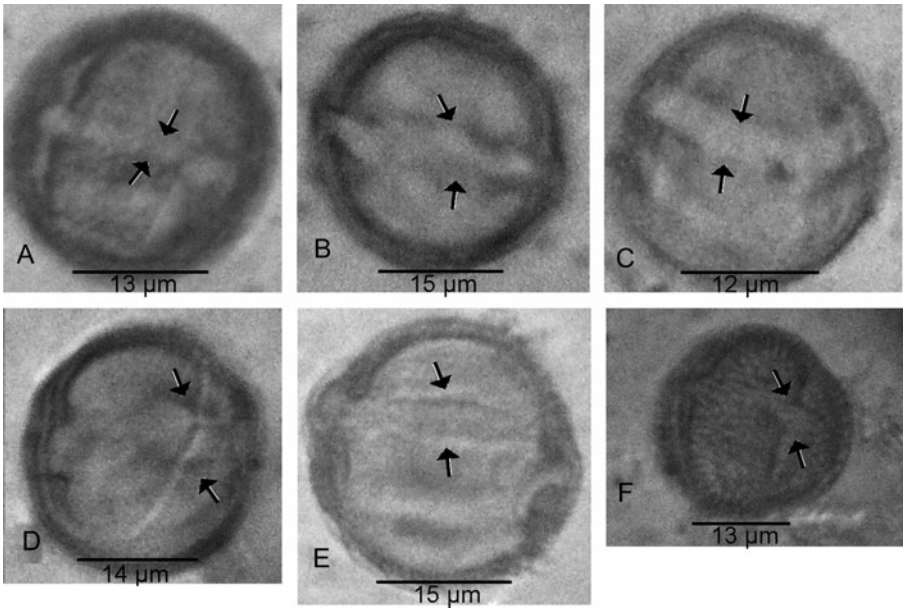


FIG. 3. Equatorial view of pollen grains showing endoaperture types in LM. A: *Hedyotis scandens*. B: *Oldenlandia auricularia*; C: *O. lineata*; D: *O. ovatifolia*; E: *O. verticillata*; F: *O. erecta*. A–C: Note the brighter zone between arrows indicating position of endocingulum. D–F: Area within arrows indicates the presence of lalongate endocolpus.

(perforations $<$ 1 µm and larger than the muri), and *O. erecta* exhibited a transition between microreticulate and reticulate (Fig. 1N).

Supratectal elements. Supratectal elements were absent in *Oldenlandia erecta*, *O. biflora* and *Kohautia* species whereas in *Hedyotis scandens*, *Oldenlandia auricularia* and *O. lineata* supratectal elements were present on the inside of the muri (Fig. 1B, D, F). Pollen of *Oldenlandia diffusa* and *O. brachypoda* were beset with granules (Fig. 1J, L).

Fruit dehiscence and seed morphology

The results of the fruit dehiscence and seed morphology studies are shown in Table 3.

Fruit dehiscence

The only species with septicidally dehiscent capsules (the defining characteristic of the genus *Hedyotis*) was found to be *Hedyotis scandens*, which had a beak protruding beyond the calyx lobes. This is loculicidally dehiscent from the top but then divides septicidally into two valves. The capsules were indehiscent in *Oldenlandia auricularia* and *O. lineata*, whereas in *Oldenlandia biflora*, *O. brachypoda*, *O. corymbosa*, *O. diffusa*, *O. erecta*, *O. ovatifolia*, *O. pinifolia* (Wall. ex G.Don)

TABLE 3. Fruit dehiscence and seed shape and surface in *Hedyotis*, *Oldenlandia* and *Kohautia*

Taxon	Fruit dehiscence	Seed shape	Seed surface
Hedyotis scandens	Septicidal	Lenticular with wing-like margin	Reticulate
Oldenlandia auricularia	Indehiscent	Trigonous	Reticulate
Oldenlandia biflora	Loculicidal	Globose/subglobose	Reticulate-foveate
Oldenlandia brachypoda	Loculicidal	Trigonous	Reticulate
Oldenlandia diffusa	Loculicidal	Trigonous	Reticulate
Oldenlandia erecta	Loculicidal	Trigonous	Reticulate
Oldenlandia lineata	Indehiscent	Trigonous	Reticulate
Oldenlandia ovatifolia	Loculicidal	Globose/subglobose	Reticulate-foveate
Oldenlandia pinifolia	Loculicidal	Trigonous	Reticulate-areolate
Oldenlandia verticillata	Loculicidal	Trigonous	Reticulate-areolate
Kohautia coccinea	Loculicidal	Trigonous	Reticulate

Kuntze, *O. verticillata*, *Kohautia coccinea* and *K. gracilis* capsules were loculicidally dehiscent from the top.

Seed morphology

Seeds in the Nepalese species were all found to be non-crateriform, i.e. they lack a ventral cavity or depression as found in North American *Houstonia*. Seeds were small in size (0.2–0.8 mm in length) and numerous, sometimes more than 100 per capsule (e.g. *Oldenlandia diffusa*).

Shape. Based on their shape three basic types of seed could be identified within the complex:

Type 1: dorsiventrally flattened, lenticular seeds with an irregularly narrow wing-like margin. This is the seed type observed in *Hedyotis fruticosa* and among Nepalese species is found only in *H. scandens* (Fig. 4A);

Type 2: subglobose or bluntly angular seeds turning globose in *Oldenlandia biflora* and *O. ovatifolia* (Fig. 4F, G, Fig. 5C, D);

Type 3: trigonous or obconical seeds found in *Oldenlandia auricularia*, *O. brachypoda*, *O. diffusa*, *O. erecta*, *O. lineata*, *O. pinifolia*, *O. verticillata* and *Kohautia coccinea* (Fig. 4B–E, H–K, Fig. 5A, B, E–K). Seeds are also trigonous in *Kohautia gracilis* (Dutta & Deb, 2004).

The trigonous seeds also exhibited a range of morphological variation in size, colour and shape. The lateral sides of the seeds were more concave in *Oldenlandia auricularia*, *O. lineata*, *O. pinifolia*, *O. verticillata* and *Kohautia* species (Fig. 5A, F, H) compared with the *Oldenlandia corymbosa–diffusa* complex (including *Oldenlandia brachypoda*, *O. corymbosa*, *O. diffusa* and *O. erecta*; Fig. 5I–K). The latter group was also characterised by very small seeds (< 0.5 mm) compared with the other species in this study.

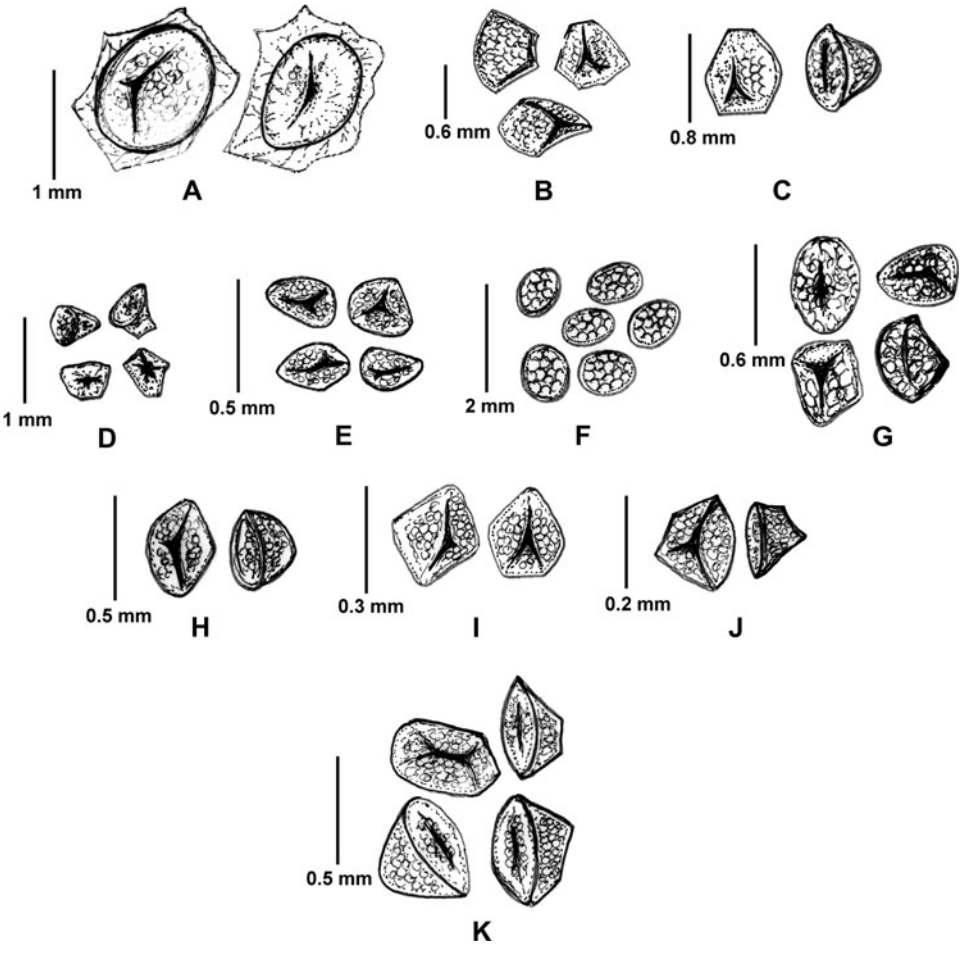


FIG. 4. Variation in seeds (as seen under stereomicroscope ×20) among the members of *Hedyotis*, *Oldenlandia* and *Kohautia*. A: *Hedyotis scandens*. B: *Oldenlandia lineata*; C: *O. auricularia*; D: *O. verticillata*; E: *O. pinifolia*; F: *O. biflora*; G: *O. ovatifolia*; H: *O. erecta*; I: *O. brachypoda*; J: *O. diffusa*. K: *Kohautia coccinea*.

Seed surface. The seed surface was of three types: reticulate (reticulate pattern with distinctly elevated ribs that were readily visible to the naked eye; Fig. 6A, C–G), reticulate-foveate (reticulate pattern with broader walls and appearing pitted) and reticulate-areolate (reticulate pattern of an obscure type that is generally only visible under 40× magnification). The reticulate pattern was found in *Oldenlandia auricularia*, *O. brachypoda*, *O. diffusa*, *O. erecta*, *O. lineata*, *Kohautia coccinea* and *K. gracilis*. *Oldenlandia biflora* and *O. ovatifolia* were characterised by a reticulate-foveate seed coat surface with hexagonal pits (Fig. 6B). *Oldenlandia pinifolia* and *O. verticillata* had the obscure type of reticulation termed reticulate-areolate (Fig. 6C, D). Seed coat cells were punctated in all members of the *Hedyotis–Oldenlandia–Kohautia* complex.

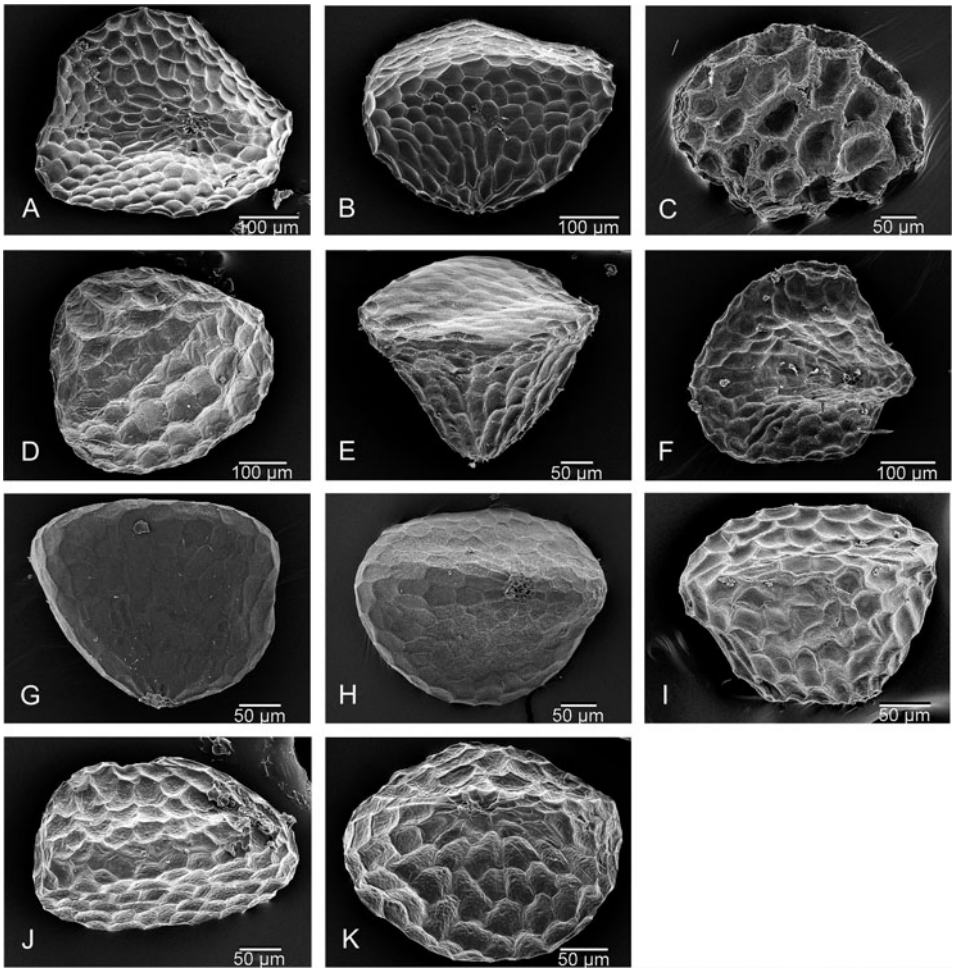


FIG. 5. Seed morphology in SEM. A, B: *Oldenlandia auricularia*; C: *O. biflora*; D: *O. ovatifolia*; E, F: *O. verticillata*; G, H: *O. pinifolia*; I: *O. diffusa*; J, K: *O. erecta*. C & D: Note subglobose shape in *O. biflora* and *O. ovatifolia*.

Punctation was comparatively dense and more distinct in *Oldenlandia brachypoda* and *O. diffusa* (Fig. 6E, F) than in *O. erecta* (Fig. 6G).

DISCUSSION

Hedyotis: sensu stricto *or* sensu lato*?*

The selection of *Hedyotis fruticosa* L. as a conserved type for *Hedyotis* necessitates the redefinition of the generic limits of *Hedyotis* and consequently several other (possibly new) genera around the world. *Hedyotis* species can be segregated from

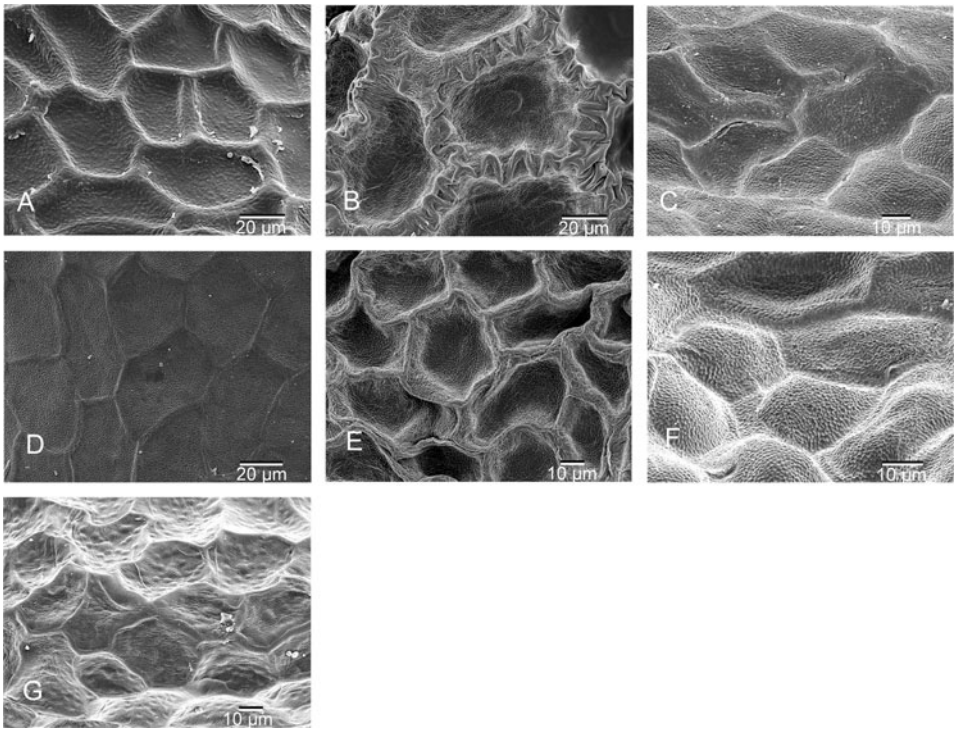


FIG. 6. Detail of seed coat surface in SEM. A: *Oldenlandia auricularia*; B: *O. biflora*; C: *O. verticillata*; D: *O. pinifolia*; E: *O. brachypoda*; F: *O. diffusa*; G: *O. erecta*. A, E–G: Seed coat surface reticulate. B: Seed coat surface reticulate-foveate. C & D: Seed coat surface reticulate-areolate. E & F: Note the well-marked punctations in seed coat cells of *O. brachypoda* and *O. diffusa*.

Oldenlandia by the septicidally dehiscent capsule, which splits into two distinct valves, and by the dorsiventrally flattened seeds with thin or winged margins. In Nepal this new delimitation of *Hedyotis* requires the transfer of many taxa from *Hedyotis* L. to *Oldenlandia* L., all of which already have combinations in *Oldenlandia*. These are *Oldenlandia auricularia* (L.) K.Schum. (syn.: *Hedyotis auricularia* L.), *O. biflora* L. (syn.: *H. biflora* (L.) Lam.), *O. brachypoda* DC. (syn.: *H. brachypoda* (DC.) Sivar. & Biju), *O. corymbosa* L. (syn.: *H. corymbosa* (L.) Lam.), *O. diffusa* (Willd.) Roxb. (syn.: *H. diffusa* Willd.), *O. erecta* (Manilal & Sivar.) R.R.Mill (syn.: *H. erecta* Manilal & Sivar.), *O. lineata* (Roxb.) Kuntze (syn.: *H. lineata* Roxb.), *O. ovatifolia* (Cav.) DC. (syn.: *H. ovatifolia* Cav.), *O. pinifolia* (Wall. ex G.Don) K.Schum. (syn.: *H. pinifolia* Wall. ex G.Don) and *O. verticillata* L. (syn.: *H. verticillata* (L.) Lam.). The key characters for *Hedyotis* mentioned earlier (septicidally dehiscent capsules and dorsiventrally flattened seeds) fit well for the Asian and Pacific species of *Hedyotis* but cannot be applied with certainty to the taxa in the rest of the world, especially with respect to the '*Hedyotis*' species of the

Hawaiian Islands and of North and South America which Terrell & Robinson (2003) suggested might require generic separation.

In Nepal, the *Hedyotis–Oldenlandia–Kohautia* complex can be characterised by a herbaceous or subshrub habit; bilocular ovary with numerous ovules in each locule; pollen grains 3–4(–5)-colporate; and seeds small (0.2–0.8 mm) and numerous per fruit. The seeds are generally angular and trigonous, dorsiventrally flattened, although are lenticular with an irregular wing-like margin or subglobose in some species.

The variation in fruit type and dehiscence together with seed and pollen morphology are important taxonomic characters in the *Hedyotis–Oldenlandia–Kohautia* complex. On the basis of these characters five homogeneous groups have been identified within the complex from Nepal. These groups largely coincide with sections identified by Wight & Arnott (1834). The five groups are as follows:

Group 1 (*Hedyotis fruticosa* group): This group is represented by a single species, *Hedyotis scandens*, in Nepal. It has diplophragmous capsules (which are characterised by partly loculicidal dehiscence from the top and then septicidal dehiscence along the septum into two distinct valves) and *H. fruticosa*-type seeds (dorsiventrally flattened, lenticular with irregularly narrow wing-like margin; Fig. 4A, Table 3). Terrell & Robinson (2003), in their comparative morphological survey of Asian and Pacific species of *Hedyotis*, found that most of the Sri Lankan and Micronesian taxa have diplophragmous capsules and *fruticosa*-type seeds. For these species, they recognised a new group as *Hedyotis* subgen. *Hedyotis*. These characters are absent in Hawaiian and North and South American taxa but could be important for defining *Hedyotis* in its narrower sense. Flowers in *Hedyotis scandens* are heterostylous, a regular feature in the *Rubiaceae* (Anderson, 1973). The pollen is colporate with an endocingulum as endoaperture (Fig. 3A, Table 2). The tectum is of the double reticulate type and supratectal elements are present on the inside of the muri (Fig. 1B). The representatives of the *Hedyotis fruticosa* group are distributed throughout Sri Lanka, India, China, Southeast Asia, Indonesia and Micronesia.

Group 2 (*Oldenlandia auricularia* and *O. lineata* group): This group includes members of the former genus *Exallage*, a genus segregated from *Hedyotis* by Bremekamp (1952). The members are characterised by the seeds possessing 'oldenlandioid' characters (trigonous seeds with concave lateral sides; Fig. 4B, C, Fig. 5A, B) and indehiscent capsules (see Table 3). Terrell & Robinson (2003) proposed the inclusion of *Exallage* under *Oldenlandia* as *Oldenlandia* subgen. *Exallage* (Bremek.) Terrell & H.Rob. This group was also included under *Hedyotis* sect. *Euhedyotis* by Wight & Arnott (1834) and Hooker (1880). The pollen is colporate with an endocingulum as endoaperture (Fig. 3B, C, Table 2). The tectum is of a double reticulate type and supratectal elements are present on the inside of the muri (Fig. 1D, F). Members of this group are strong aluminium accumulators, which is thought to be a relatively primitive feature (Jansen *et al.*, 2000).

Group 3 (*Oldenlandia corymbosa–diffusa* complex and *O. pinifolia*, *O. verticillata* group): This group is characterised by loculicidal dehiscent capsules and oldenlandioid seeds. The pollen is colporate and endoapertures are lalongate endocolpi (Fig. 3E, F, Table 2). Based on gross morphology this group can be further divided into two subgroups:

Subgroup **3a** (*Oldenlandia corymbosa–diffusa* complex): This subgroup comprises *Oldenlandia brachypoda*, *O. corymbosa*, *O. diffusa* and *O. erecta* which are all annual herbs and are characterised by loculicidal capsules with very small-sized oldenlandioid seeds (< 0.5 mm) compared with the other species (Table 3). The concavity of the lateral sides of the seeds is less pronounced in the trigonous seeds of this group (Fig. 4H–J, Fig. 5I–K). Some members of this group (e.g. *Oldenlandia diffusa*, *O. brachypoda*; Fig. 6E, F) have distinct punctations on the surface of the seed coat, whereas punctations are less obvious in *O. erecta*.

Subgroup **3b** (*Oldenlandia pinifolia* and *O. verticillata*): These species are characterised by sessile and capitate cymes, crustaceous and loculicidally dehiscent capsules, erect calyx teeth, and seeds of the oldenlandioid (trigonous) type (Table 3) with strongly concave lateral sides (Fig. 4D, E, Fig. 5F, H). The seed coat is reticulate-areolate (Fig. 5E, G). Wight & Arnott (1834) suggested placing these taxa under *Hedyotis* sect. *Scleromitrion* Wight & Arn. Govaerts *et al.* (2006) included *Oldenlandia verticillata* and *O. pinifolia* in *Hedyotis*.

Group 4 (*Oldenlandia biflora* and *O. ovatifolia* group): The members of this group have 4-angular capsules and globose or subglobose seeds with the seed coat surface of the reticulate-foveate type forming hexagonal pits (Fig. 5C, D, Table 3). This distinguishes the group from the rest of *Hedyotis* and *Oldenlandia*. The pollen is colporate and endoapertures are lalongate endocolpi (Fig. 3D, Table 2). Santapau & Wagh (1964) accepted the group as a distinct genus, *Gonotheca* Blume ex A.DC. However, *Gonotheca* Blume ex A.DC. is a later homonym of *Gonotheca* Raf. Therefore Babu (1971) renamed the genus *Thecagonum* Babu. Ridsdale (1998) treated them as *Oldenlandia* but Mill (1999) placed these species under *Hedyotis*. Groeninckx *et al.* (2009) found *Oldenlandia biflora* to be sister to the *Kadua* clade (Polynesian taxa often considered under *Hedyotis* s.l.). The study did not include *Oldenlandia ovatifolia* so further sampling is needed to see whether *O. biflora* and *O. ovatifolia* should be transferred to *Kadua* Cham. & Schltdl.

Group 5 (*Kohautia* group): This group includes *Kohautia gracilis* and *K. coccinea* which are distinct from *Hedyotis* and *Oldenlandia* by their thyrse-like inflorescence and rather large funnel-shaped corolla tubes with stamens and style included. Capsule and seed characters resemble true *Oldenlandia* (i.e. loculicidal capsules and trigonous seeds; Fig. 4K, Table 3). Endoapertures are endocolpi with a distinct annulus around the mesoaperture (visible both outside and inside). Groeninckx *et al.* (2009) and Kårehed *et al.* (2008) found *Kohautia* biphyletic, forming two well-supported clades corresponding to the two subgenera of *Kohautia*, namely *Kohautia*

and *Pachystigma*. Palynologically these two subgenera are distinct because subgenus *Kohautia* has an annulus whereas *Pachystigma* has not (Lewis, 1965a, 1965b).

Concluding Remarks

These results clearly indicate that the Nepalese members of the *Hedyotis–Oldenlandia–Kohautia* complex represent diverse lineages of species. However, some members of the complex (*Oldenlandia auricularia*, *O. biflora*, *O. lineata* and *O. ovatifolia*) exhibit morphological characters intermediate between *Hedyotis* and *Oldenlandia.* We have provided a useful method for identifying the presently known species for inventories and ecological and floristic studies in Nepal. Further study is required, preferably using molecular phylogenetic techniques, to clearly establish the natural units within this wide-ranging complex. With our current knowledge, it is proposed here to accept the generic status of *Hedyotis*, *Oldenlandia* and *Kohautia*. This is somewhat in agreement with the treatment of Mill (1999) in the *Flora of Bhutan* but with the removal of some species of *Hedyotis* to *Oldenlandia* as explained above.

Acknowledgements

We thank the administration of the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal for providing access to laboratory facilities. We also thank Roy Vickery, Curator of the Natural History Museum, London (BM), for providing herbarium material and Dr Peter Hoen and Dr Wim Punt, Laboratory of Palaeobotany and Palynology, The Netherlands, for help with description of the apertures in *Kohautia*. In addition, the first author thanks his former adviser, Dr Krishna Kumar Shrestha, Tribhuvan University, Kathmandu, for comments and support during the course of this study.

References

Anderson, W. R. (1973). A morphological hypothesis for the origin of heterostyly in the Rubiaceae. *Taxon* 22: 537–542.

Andersson, L. & Rova, J. H. E. (1999). The *rps*16 intron and the phylogeny of the Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 214: 161–186.

Andersson, L., Rova, J. H. E. & Alzate Guarin, F. (2002). Relationships, circumscription, and biogeography of *Arcytophyllum* (Rubiaceae) based on evidence from cpDNA. *Brittonia* 54: 40–49.

Babu, C. R. (1971). *Thecagonum* Babu: a new generic name in *Rubiaceae*. *Bull. Bot. Surv. India* 11(1–2): 213–214.

Bremekamp, C. E. B. (1952). The African species of *Oldenlandia* L. sensu Hiern and K. Schumann. *Verh. Kon. Akad. Wetensch. Afd. Natuurk., Sect. 2* 48: 1–297.

Bremekamp, C. E. B. (1966). Remarks on the position, the delimitation and the subdivision of the Rubiaceae. *Acta Bot. Neerl.* 15: 1–33.

BREMER, B. (1996). Phylogenetic studies within Rubiaceae and relationships to other families based on molecular data. *Opera Bot. Belg.* 7: 33–50.

BREMER, B. & MANEN, J. F. (2000). Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 225: 43–72.

CANDOLLE, A. P. DE (1830). Rubiaceae, Hedyoteae. *Prodromus Systematis Naturalis Regni Vegetabilis* 4: 419–439. Paris: Treuttel and Wurtz.

CHAMISSO, A. & SCHLECHTENDAL, D. VON (1829). Hedyotideae. In: De plantis in expeditione speculatoria Romanzoffiana observatis. *Linnaea* 4: 1–596.

CHURCH, S. A. (2003). Molecular phylogenetics of *Houstonia* (Rubiaceae): descending aneuploidy and breeding system evolution in the radiation of the lineage across North America. *Molec. Phylogenet. Evol.* 27: 223–238.

DESSEIN, S. (2003). *Systematic studies in the Spermacoceae (Rubiaceae)*. PhD thesis, Katholieke University, Leuven, Belgium.

DESSEIN, S., OCHOTERENA, H., DE BLOCK, P., LENS, F., ROBBRECHT, E., SCHOLS, P. *et al*. (2005). Palynological characters and their phylogenetic signal in Rubiaceae. *Bot. Rev.* 71: 354–414.

DUTTA, R. & DEB, D. B. (2004). *Taxonomic Revision of* Hedyotis *L. (Rubiaceae) in Indian Subcontinent*. Kolkata: Botanical Survey of India.

ERDTMAN, G. (1966). *Pollen Morphology and Plant Taxonomy*. New York and London: Hafner Publishing Company.

ERDTMAN, G. (1969). *Handbook of Palynology: An Introduction to the Study of Pollen Grains and Spores*. Copenhagen: Munksgaard.

GOVAERTS, R., RUHSAM, M., ANDERSSON, L., ROBBRECHT, E., BRIDSON, D., DAVIS, A. *et al*. (2006). *World Checklist of Rubiaceae*. The Board of Trustees of the Royal Botanic Gardens, Kew. http://www.kew.org/wcsp/rubiaceae/, accessed 9 December 2008.

GRAY, A. (1859). *Hedyoteae. Proc. Amer. Acad. Arts* 4: 312–318.

GROENINCKX, I., DESSEIN, S., OCHOTERENA, H., PERSSON, C., MOTLEY, T. J., KÅREHED, J. *et al*. (2009). Phylogeny of the herbaceous tribe Spermacoceae (Rubiaceae) based on plastid DNA data. *Ann. Missouri Bot. Gard.* 96(1): 109–132.

HALFORD, D. A. (1992). Review of the genus *Oldenlandia* L. (Rubiaceae) and related genera in Australia. *Austrobaileya* 3: 683–722.

HOOKER, J. D. (1873). Ordo LXXXIV. Rubiaceae. In: BENTHAM, G. & HOOKER, J. D. (eds) *Genera plantarum ad exemplaria imprimis in herbariis kewensibus servata defirmata*, pp. 7–151. London.

HOOKER, J. D. (1880). Rubiaceae, *Hedyotis* and *Oldenlandia. Flora of British India* 3: 49–70. London: L. Reeve and Co.

JANSEN, S., DESSEIN, S., PIESSCHAERT, F., ROBBRECHT, E. & SMETS, E. (2000). Aluminium accumulation in leaves of Rubiaceae: systematic and phylogenetic implications. *Ann. Bot.* 85: 91–101.

JARVIS, C. E. (1992). Seventy-two proposals for the conservation of types of selected Linnaean generic names, the report of Subcommittee 3C on the lectotypification of Linnaean generic names. *Taxon* 41: 552–583.

KÅREHED, J., GROENINCKX, I., DESSEIN, S., MOTLEY, T. J. & BREMER, B. (2008). The phylogenetic utility of chloroplast and nuclear DNA markers and the phylogeny of the Rubiaceae tribe Spermacoceae. *Molec. Phylogenet. Evol.* 49: 843–866.

LAMARCK, J. B. A. P. M. DE (1792). *Hedyotis. Tabl. Encycl.* 1: 269–272.

LEWIS, W. H. (1962). Phylogenetic study of *Hedyotis* (Rubiaceae) in North America. *Amer. J. Bot*. 49: 855–865.

LEWIS, W. H. (1965a). Pollen morphology and evolution in *Hedyotis* subgenus *Edrisia* (Rubiaceae). *Amer. J. Bot*. 52(3): 257–264.

LEWIS, W. H. (1965b). Cytopalynological study of African Hedyotideae (Rubiaceae). *Ann. Missouri Bot. Gard.* 52: 182–211.
LINNAEUS, C. (1753). *Hedyotis* and *Oldenlandia. Species Plantarum* 1: 101–110. Stockholm.
LINNAEUS, C. (1754). *Hedyotis. Genera Plantarum* ed. 5: 44. Stockholm.
MCNEILL, J., BARRIE, F. R., BURDET, H. M., DEMOULIN, V., HAWKSWORTH, D. L., MARHOLD, K. *et al*. (eds) (2006). *International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress, Vienna, Austria, July 2005*. Ruggell: A. R. G. Gantner Verlag KG [Regnum Veg. vol. 146].
MILL, R. R. (1999). *Hedyotis*, *Oldenlandia*, *Kohautia*. In: GRIERSON, A. J. C. & LONG, D. G. (eds) *Flora of Bhutan* 2: 757–772. Royal Botanic Garden Edinburgh and Royal Government of Bhutan.
NEUPANE, S. (2006). *Taxonomy of* Hedyotis –Oldenlandia–Kohautia *complex (Rubiaceae) in Nepal*. Master's dissertation, Central Department of Botany, Tribhuvan University, Nepal.
PUNT, W., HOEN, P. P., BLACKMORE, S., NILSSON, S. & LE THOMAS, A. (2007). *Glossary of Pollen and Spore Terminology*. LPP Foundation, Laboratory of Palaeobotany and Palynology, University of Utrecht, Utrecht, The Netherlands. http://www3.bio.uu.nl/palaeo/glossary/glos-int.htm, accessed 6 April 2009.
REITSMA, T. (1969). Size modifications of recent pollen grains under different treatments. *Rev. Paleobot. Palynol*. 9: 175–202.
RIDSDALE, C. E. (1998). Rubiaceae, Tribe Hedyotideae. In: DASSANAYAKE, M. D. (ed.) *A Revised Handbook to the Flora of Ceylon* 12: 236–284. Rotterdam: A. A. Balkema.
ROBBRECHT, E. (1988). Tropical woody Rubiaceae: Characteristic features and progressions: Contributions to a new subfamilial classification. *Opera Bot. Belg.* 1: 1–272.
ROXBURGH, W. (1820). *Hedyotis* and *Oldenlandia. Flora Indica* 1: 368–446. Serampore: Mission Press.
SANTAPAU, H. & WAGH, S. K. (1964). Nomenclatural changes in Indian plants. *Bull. Bot. Surv. India* 5: 105–109.
SCHUMANN, K. (1891). Rubiaceae. In: ENGLER, A. & PRANTL, K. (eds) *Die natürlichen Pflanzenfamilien*, pp. 1–156. Leipzig: Engelmann.
STEARN, W. T. (1966). *Botanical Latin*. London: Nelson.
TERRELL, E. E. (1975). Relationships of *Hedyotis fruticosa* L. to *Houstonia* L. and *Oldenlandia* L. *Phytologia* 31: 418–424.
TERRELL, E. E. (1991). Overview and annotated list of North American species of *Hedyotis*, *Houstonia*, *Oldenlandia* (Rubiaceae), and related genera. *Phytologia* 71(3): 211–243.
TERRELL, E. E. (1996). Revision of *Houstonia* (Rubiaceae–Hedyotideae). *Syst. Bot. Monogr.* 48: 1–118.
TERRELL, E. E. (2001a). Taxonomy of *Stenaria* (Rubiaceae: Hedyotideae), a new genus including *Hedyotis nigricans*. *Sida* 19: 591–614.
TERRELL, E. E. (2001b). *Stenotis* (Rubiaceae), a new segregate genus from Baja California, Mexico. *Sida* 19: 899–911.
TERRELL, E. E. (2001c). Taxonomic review of *Houstonia acerosa* and *H. palmeri*, with notes on *Hedyotis* and *Oldenlandia* (Rubiaceae). *Sida* 19: 913–922.
TERRELL, E. E., LEWIS, W. H., ROBINSON, H. & NOWICKE, J. W. (1986). Phylogenetic implications of diverse seed types, chromosome numbers and pollen morphology in *Houstonia* (Rubiaceae). *Amer. J. Bot.* 73(1): 103–115.
TERRELL, E. E. & WUNDERLIN, R. P. (2002). Seed and fruit characters in selected Spermacoceae and comparison with Hedyotideae (Rubiaceae). *Sida* 20: 549–557.
TERRELL, E. E. & ROBINSON, H. (2003). Survey of Asian and Pacific species of *Hedyotis* and *Exallage* (Rubiaceae) with nomenclature notes on *Hedyotis* types. *Taxon* 52: 775–782.

TORREY, J. & GRAY, A. (1841). Hedyotideae. *A Flora of North America* 2: 37–43. New York: Wiley and Putman.
VERDCOURT, B. (1958). Remarks on the classification of the Rubiaceae. *Bull. Jard. Bot. État Bruxelles* 28: 209–290.
VERDCOURT, B. (1976). Rubiaceae (Part 1), *Oldenlandia*. In: POLHILL, R. M. (ed.) *Flora of Tropical East Africa*, pp. 268–315. London: Crown Agents.
WIGHT, R. & ARNOTT, G. A. W. (1834). *Hedyotis. Prodromus Florae Peninsulae Indiae Orientalis* 1: 405–418. London: Parbury, Allen, & Co.
WILLDENOW, C. L. (1798). *Hedyotis. Species Plantarum* 1: 566. Berlin: G. C. Nauk.

Received 21 April 2008; accepted for publication 14 May 2009