

Pollen morphology of members of southern African *Boerhavia* and *Commicarpus* (Nyctaginaceae)

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ABSTRACT

In southern Africa, *Boerhavia* L. and *Commicarpus* Standl. are the most species-rich genera of the Nyctaginaceae. Hitherto, the pollen morphology of only three southern African species of each of these genera has been described. A palynological study of the remaining species is therefore necessary to complete the information for the taxa. The pollen morphology of sixteen species was studied with light, scanning electron and transmission electron microscopy. Pollen grains of *Boerhavia* and *Commicarpus* are uniform in shape and sculpturing. Grains are spheroidal and pantoporate, and the tectum tubuliferous and spinulose. Pollen grains of the *Boerhavia* are 52–91 µm in diameter and those of *Commicarpus* 52–129 µm. Spinules in both genera are 1–5 µm long. Pore plates have one or two spinules. In both genera the exine is 3.5–11.5 µm thick, the tectum 0.9–3.9 µm thick and the collumellae 0.6–2.8 µm long. Foot layers are 0.9–5.1 µm thick and the endexine is barely visible. Size variation of the pollen grain, pore diameter and exine thickness overlap and cannot be used to distinguish between the two genera or the individual species.

INTRODUCTION

Nyctaginaceae Jussieu, commonly known as the four-o'clocks, are a relatively small family of about 30 genera and 300–400 species (Levin *et al.* 2001; Douglas & Manos 2007). It is distributed throughout the tropical and subtropical regions of the New World (Bittrich & Kühn 1993; Jordaan 2000), but mainly in the Americas (Stannard 1988). In southern Africa, south of the Zambezi River (Botswana, Lesotho, southern Mozambique, Namibia, South Africa, Swaziland and Zimbabwe), five genera and 20 species occur of which sixteen species are found in Namibia (Germishuizen & Meyer 2003; Struwig 2012). Namibia is therefore the centre of diversity for the family in southern Africa. *Boerhavia* L. and *Commicarpus* Standl. are the largest genera of the family in southern Africa, with seven and nine species respectively (Germishuizen & Meyer 2003).

Pollen grains of Nyctaginaceae are 17–200 µm in diameter, spheroidal, prolate or oblate and 3(4)-colpate, pantocolpate or pantoporate (Bittrich & Kühn 1993). Pores are covered by a pore plate, which can either be roughened or spinulose (Bittrich & Kühn 1993). The exine is very thick and varies in sculpturing (spinulose, tubuliferous or coarsely reticulate) (Nowicke 1970; Bogle 1974).

Heimerl (1934) used pollen morphology to divide the Nyctagineae into four subtribes. Subtribe Nyctagininae (to which *Boerhavia* and *Commicarpus* belong) is characterised by large, spheroidal, pantoporate pollen grains with thick walls and a tubuliferous and spinulose exine (Nowicke 1970; Nowicke & Luikart 1971). Nowicke (1970) found that the shape and size of the grains, and the thickness of the exine, form a continuum within gen-

era and species of subtribe Nyctagininae, so much so that these characters are of limited taxonomic significance. Nowicke & Luikart (1971) came to the same conclusion for the other subtribes. Recently, a phylogenetic study of the family (Douglas & Manos 2007) found that pollen morphology is homoplasious among genera and can therefore not be used to support the tribal and subtribal divisions of Heimerl (1934).

Pollen grains of 13 of the 40 species of *Boerhavia* and three of the 35 species of *Commicarpus* were described by Nowicke (1970) as spheroidal, pantoporate and the sexine as tubuliferous and spinulose. The spinules are 1.0–2.5 µm long. Grains of *Boerhavia* are 70–138 µm in diameter, with 18–40 pores. Pores are 2.4–7.0 µm in diameter and the pore plates have a roughened base with 1 or 2 spinules. Sexine is 2.5–6.0 µm thick and the nexine is 4–7 µm. Grains of *Commicarpus* are 84–112 µm in diameter with 27–39 pores. Pores are 3.0–5.5 µm in diameter and the pore plates have 1 or 2 spinules. Sexine is 2.5–5.5 µm thick and the nexine is 2.5–5.5 µm.

Pollen of southern African members of *Boerhavia* and *Commicarpus* has not been described extensively, except for the naturalised *B. diffusa* var. *diffusa* and *B. erecta*, and the native *B. repens* subsp. *repens*, *C. fruticosus*, *C. helenae* var. *helenae* and *C. pentandrus* (Nowicke 1970; Perveen & Qaiser 2001). A palynological study was therefore conducted to provide a detailed description of the pollen morphology of other native members. As previous studies (Nowicke 1970; Perveen & Qaiser 2001) have suggested that pollen morphology of these two genera is uniform and of limited taxonomic value at the generic or species level, the poorly known southern Africa species provided an opportunity to report further evidence regarding the taxonomic significance of palynological characters.

MATERIALS AND METHODS

Pollen from herbarium specimens and fresh plant material collected *in situ* in Namibia and South Africa

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was investigated with light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Pollen grains were acetolysed according to the method of Erdtman (1969). For LM, pollen was mounted in glycerine jelly and sealed with entellan (Product 7961, E. Merck, Darmstadt) according to the method of Fripp (1983). For SEM, a drop of 96% ethanol/pollen mixture was pipetted on specimen stubs, dried and sputter-coated with gold/palladium. Specimens were examined with a FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM) or a JEOL JSM 840 SEM. A minimum of eight pollen grains from three individuals per species were used to measure the diameter of the grain, pores and the length of the spinules.

For TEM, unacetolized grains were fixed in 4% aqueous paraformaldehyde and rinsed in three changes of 0.05 M cacodylate buffer, pre-stained in 2% uranyl acetate (pH 2), dehydrated in a graded ethanol series and embedded in resin (L.R. White™ Wirsam/London Resin Company). Sections were made with a Reichert-Jung Ultracut E microtome and contrasted with 2% uranyl acetate (pH 2) and lead citrate (Anala R). Sections were examined with a Philips CM10 Transmission Electron Microscope.

Pollen terminology follows Punt *et al.* (2007).

RESULTS

Pollen grains of the southern African *Boerhavia* and *Commicarpus* species are spheroidal, pantoporate and the tectum is tubuliferous and spinulose. Spinules are (1.12–)2.70(–5.43) μm long and the pores are covered with a pore plate with one to two spinules (Figures 1, 2, 3). Exine is (3.35–)6.55(–11.45) μm thick. The tectum is (0.88–)2.09(–3.93) μm thick and tubuliferous, the collumellae are short [(0.64–)1.33(–2.82) μm], the foot layer is (0.86–)2.67(–5.12) μm thick and the endexine is barely perceptible (Figure 4).

Pollen grains of the *Boerhavia* species are (51.59–)64.59(–91.48) μm diam.; the pores are (2.77–)4.46(–7.67) μm diam. and the exine (3.35–)6.99(–11.45) μm thick. The tectum is (0.88–)1.89(–3.78) μm thick and tubuliferous, the collumellae are short [(0.64–)1.32(–2.80) μm], and the foot layer is (1.42–)2.98(–5.12) μm thick. Pollen grains of *B. deserticola* are the largest and those of *B. repens* subsp. *repens* the smallest (Table 1). Exine of the naturalised *B. diffusa* var. *diffusa* is the thickest (Table 1). *Boerhavia hereroensis* is the indigenous species with the thickest exine, while *B. repens* subsp. *repens* has the thinnest (Table 1).

Pollen grains of the *Commicarpus* species are (51.59–)79.80(–129.28) μm diam.; the pores are (2.59–)5.62(–10.64) μm diam. and the exine (4.05–)6.16(–9.10)

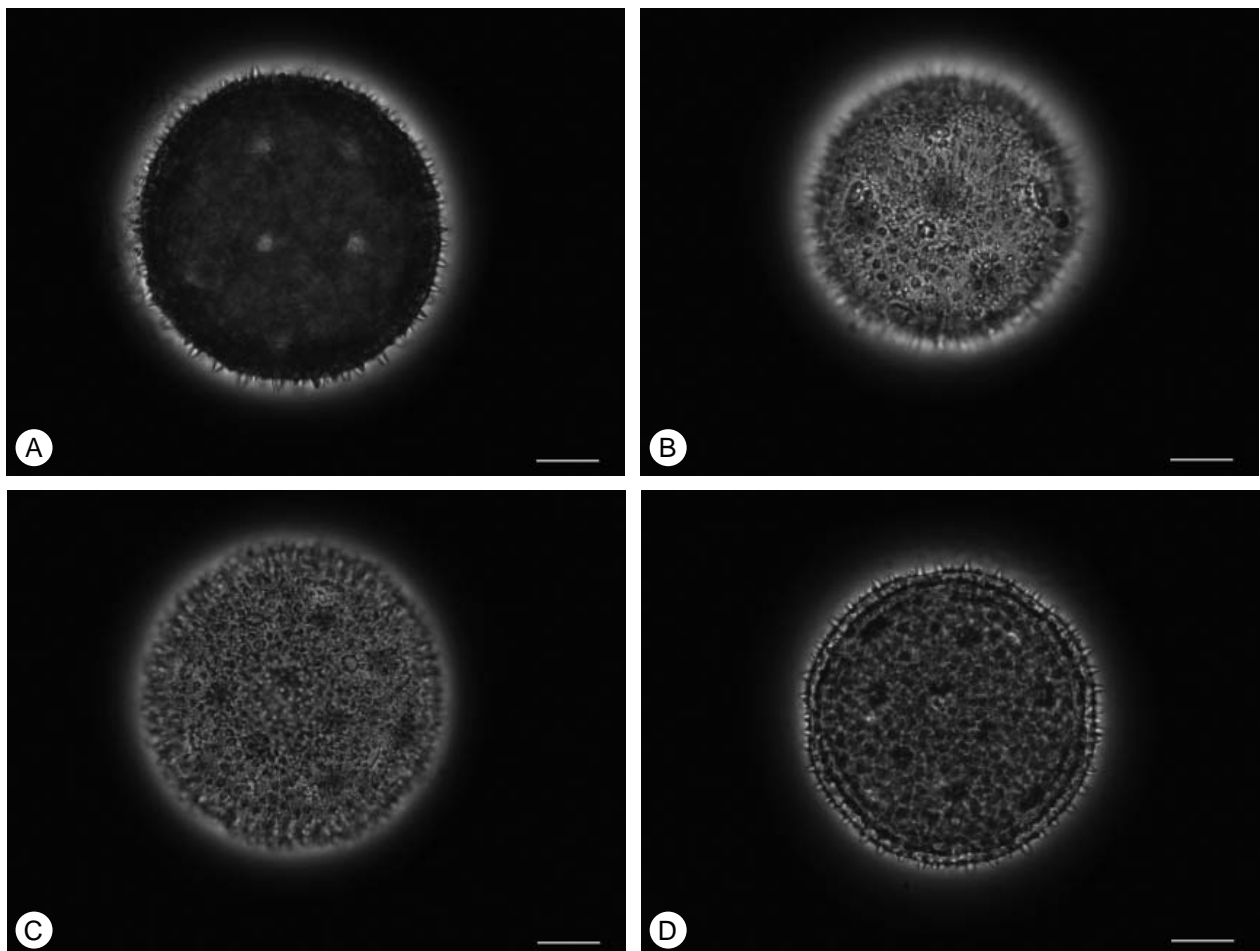


FIGURE 1.—Light micrographs of pollen grains of A, *Boerhavia deserticola* (Struwig 42); B, *B. hereroensis* (Struwig 34); C, *Commicarpus fallacissimus* (Struwig 43); D, *C. squarrosus* (Struwig 41). Scale bars 20 μm .

μm thick. The tectum is (1.19–)2.28(–3.93) μm thick and tubuliferous, the collumellae are short [(0.65–)1.34(–2.82) μm] and the foot layer is (0.86–)2.37(–3.82) μm thick. Pollen grains of *C. decipiens* are the largest and those of *C. helenae* var. *helenae* the smallest (Table 1). Exine of *C. pilosus* is the thickest and that of *C. helenae* var. *helenae* the thinnest (Table 1).

DISCUSSION

Pollen grain size range in the southern African species of *Boerhavia* is smaller than but overlapping the range reported by Nowicke (1970) for *Boerhavia* from the Americas (Table 1; Table 2). Nowicke (1970) studied *B. erecta*, which also occurs naturalised in southern

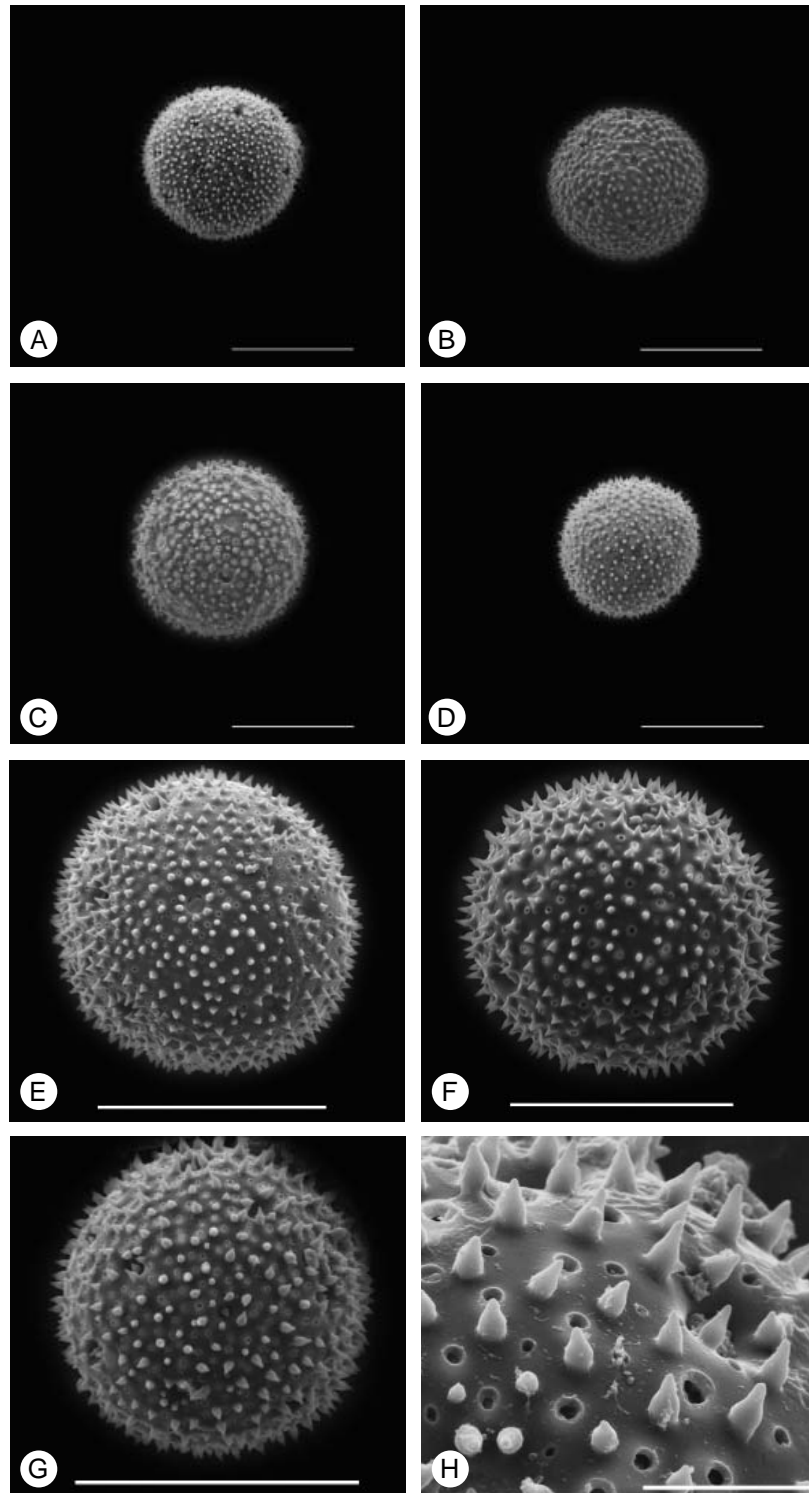


FIGURE 2.—Scanning electron micrographs of pollen grains of southern African *Boerhavia* species. A, *B. coccinea* var. *coccinea* (Struwig 55); B, *B. cordobensis* (Straub 499); C, *B. deserticola* (Struwig 42); D, *B. diffusa* var. *diffusa* (Struwig 88); E, *B. erecta* (Struwig 133); F, *B. hereroensis* (Struwig 34); G, *B. repens* subsp. *repens* (Acocks 21788); H, surface of a pollen grain of *B. deserticola* at higher magnification (Struwig 42). Scale bars A–G, 50 μm ; H, 10 μm .

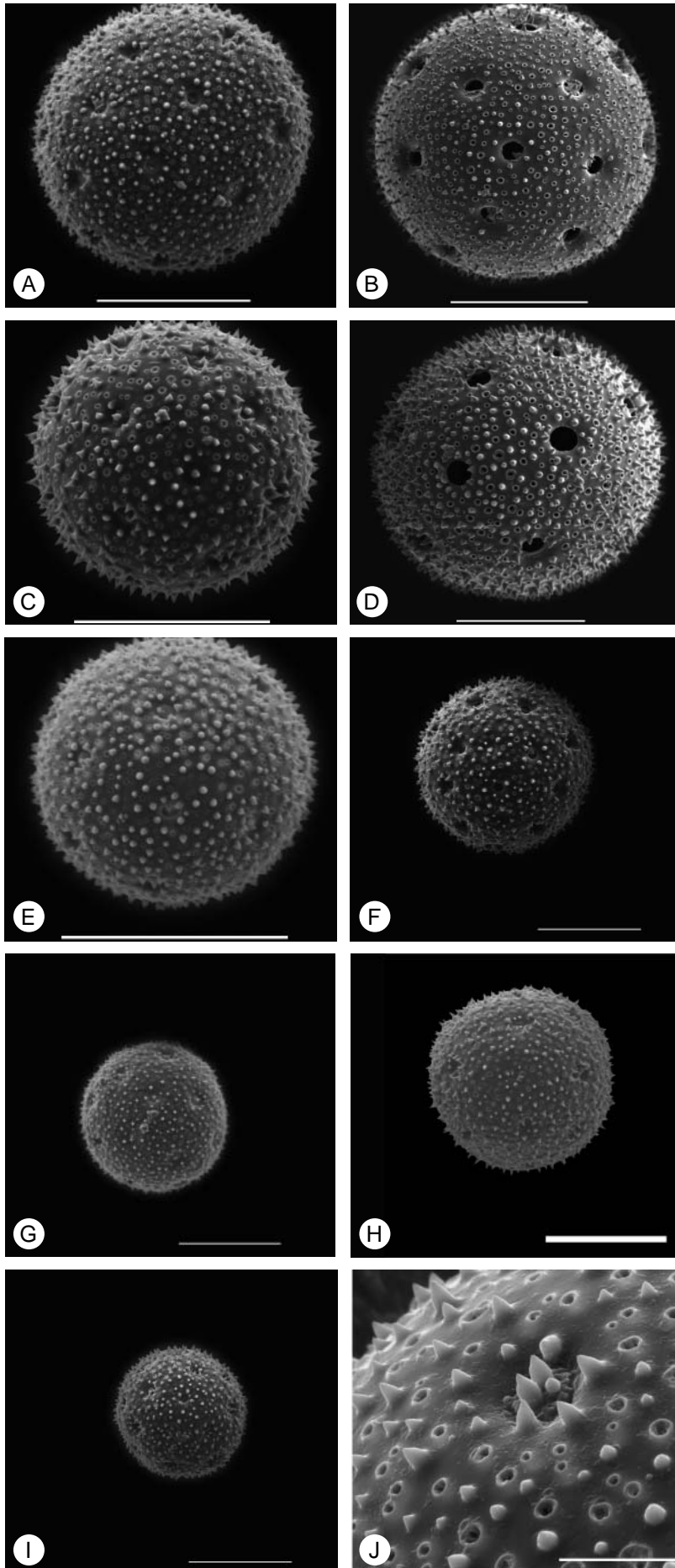


FIGURE 3.—Scanning electron micrographs of pollen grains of southern African *Commicarpus* species. A, *C. chinensis* subsp. *natalensis* (Struwig 63); B, *C. decipiens* (Struwig 181); C, *C. fallacissimus* (Struwig 46); D, *C. squarrosus* var. *fruticosus* (Struwig 160); E, *C. helenae* var. *helenae* (Struwig 44); F, *C. pentandrus* (Struwig 52); G, *C. pilosus* (Straub 609); H, *C. plumbagineus* var. *plumbagineus* (Struwig 106); I, *C. squarrosus* var. *squarrosus* (Struwig 41); J, surface of a pollen grain of *C. decipiens* at higher magnification (Struwig 181). Scale bars A, C, F–I, 50 μ m; B, 65 μ m; D, 35 μ m; J, 10 μ m; E, 20 μ m.

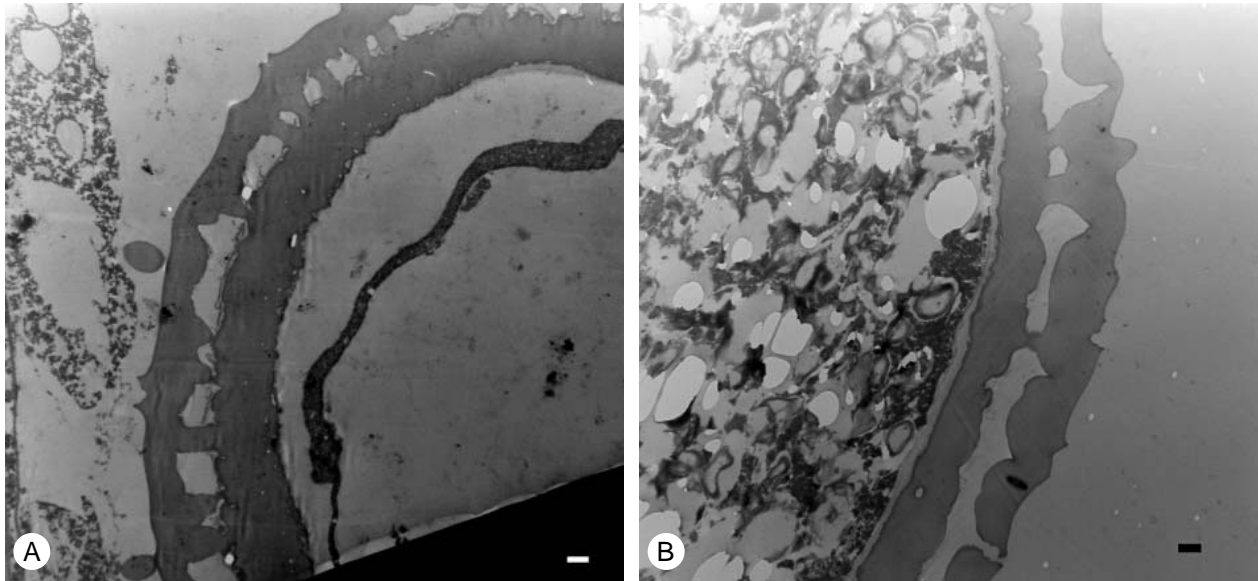


FIGURE 4.—Transmission electron micrographs showing the exine of portions of pollen grains of A, *Boerhavia deserticola* (Struwig 43); B, *Commicarpus decipiens* (Struwig 176). (C, columellae; E, endexine; F, foot layer; T, tectum). Scale bar 1 μ m.

Africa, and recorded the diameter of the grains to be nearly twice as large as that measured for the present study (Table 1; Table 2). The shape and sculpturing are, however, the same. Nowicke (1970) also studied three *Commicarpus* species, namely *C. brandegei* from Mexico, and *C. fruticosus* and *C. pentandrus* from southern Africa. The pollen shape and sculpturing of the latter two (Nowicke 1970) correspond with the findings of the present study, although the size range measurements of the grain diameters are less than that measured by Nowicke (1970) and the pore diameter is larger than that measured by Nowicke (1970) (Table 1; Table 2).

Perveen & Qaiser (2001) studied the pollen morphology of the Nyctaginaceae in Pakistan and included species also found in southern Africa, namely *B. diffusa* var. *diffusa*, *B. repens* subsp. *repens* and *C. helenae* var. *helenae*. The present study confirms the pollen shape and sculpturing of these three species given by Perveen & Qaiser (2001), but in some instances the diameter of the pollen grains, the pore diameter and the exine thickness differ. Slight differences in measurements between this study and that of Perveen & Qaiser (2001) can be ascribed to natural variation within the species and between geographical areas.

Pollen grain diameter of *B. repens* subsp. *repens* (Table 1) is substantially less than that reported by Perveen & Qaiser (2001) (Table 2). Pore diameters of *B. diffusa* var. *diffusa* and of *B. repens* subsp. *repens* (Table 1) show a slight overlap with the measurements of Perveen & Qaiser (2001) (Table 2). Exine of *B. diffusa* var. *diffusa* (Table 1) is significantly thicker than reported by Perveen & Qaiser (2001) (Table 2).-

The columellae of the studied species are short, the foot layer is thick and the endexine is barely perceptible (Table 1). This is consistent with palynological studies on members of the Nyctaginaceae by Skvarla & Nowicke (1976) and Nowicke & Skvarla (1979), involving *Salpianthus arenarius* Humb. & Bonpl., *Pisonia aculeata* L. and *B. erecta*. Skvarla & Nowicke (1976)

specifically noted that the endexine of *Boerhavia* is barely perceptible.

Pollen morphology (shape and sculpturing) of *Boerhavia* and *Commicarpus* is not diagnostic, and although pollen grains of the *Boerhavia* species are smaller than those of the *Commicarpus* species (Table 1), the ranges overlap substantially, making it impossible to distinguish between the genera with the aid of numerical measurements alone. Pore diameter of *Commicarpus* species has a higher range than that of *Boerhavia* species and the exine of *Commicarpus* species has a lower range in thickness than that of *Boerhavia* species (Table 1). However, the overlap in size ranges of the pollen grains, pore diameter and thickness of the exine does not provide sufficient discontinuity to distinguish between the two genera, nor the individual species.

CONCLUSION

The pollen morphology of ten southern African species of *Boerhavia* and *Commicarpus* was described for the first time and proved to be essentially uniform in shape and sculpturing. Pollen grains are spheroidal and pantoporate and the tectum is tubuliferous and spinulose. Pollen grains of *Boerhavia* are generally smaller than those of *Commicarpus* species. Pore diameter in *Commicarpus* is generally larger than that in *Boerhavia* and the exine in *Commicarpus* is thinner than in *Boerhavia*. However, the use of grain dimensions to distinguish among taxa is unreliable due to the extensive overlap in size ranges.

Although pollen morphology distinguishes broadly between the genera, the shape and size of the pollen grains show too much variation within species to be useful for distinguishing between species. Therefore, this southern African study confirms earlier reports that palynological characters are of limited taxonomic value in distinguishing between *Boerhavia* and *Commicarpus* and their species.

Table 1.—Measurements of pollen grains of *Boerhavia* and *Commicarpus* species

Taxon	Diameter of grains (µm)	Diameter of pores (µm)	Length of spinules (µm)	Thickness of exine (µm)	Thickness of the tectum (µm)	Length of the col-lumella (µm)	Thickness of the footlayer (µm)
<i>Boerhavia coccinea</i> var. <i>coccinea</i>	(53.08–) 66.48 (-91.48)	(3.48–) 4.44 (-5.97)	(1.72–) 2.61 (-3.75)	(6.30–) 6.70 (-7.74)	(1.59–) 2.37 (-3.33)	(0.64–) 1.06 (-1.53)	(2.33–) 3.66 (-5.27)
<i>Boerhavia cordobensis</i>	(56.34–) 66.59 (-80.65)	(3.38–) 4.08 (-4.71)	(1.77–) 2.71 (-3.61)	(6.50–) 7.02 (-7.46)	(1.39–) 2.50 (-3.78)	(0.98–) 1.23 (-1.69)	(2.53–) 3.30 (-4.25)
<i>Boerhavia deserticola</i>	(65.42–) 74.55 (-82.54)	(4.67–) 5.34 (-6.12)	(1.83–) 3.58 (-5.43)	(4.48–) 6.58 (-8.10)	(1.01–) 1.79 (-2.82)	(0.67–) 1.52 (-2.38)	(1.42–) 2.12 (-3.10)
<i>Boerhavia diffusa</i> var. <i>diffusa</i>	(51.99–) 63.26 (-76.19)	(3.13–) 4.31 (-4.73)	(1.82–) 3.04 (-3.84)	(7.75–) 8.78 (-9.69)	(1.17–) 1.54 (-1.81)	(0.86–) 1.23 (-1.49)	(3.02–) 3.90 (-5.12)
<i>Boerhavia erecta</i>	(57.09–) 64.15 (-69.15)	(3.08–) 4.23 (-4.59)	(1.83–) 2.16 (-2.86)	(4.76–) 6.81 (-10.22)	(1.15–) 1.59 (-2.25)	(1.06–) 1.38 (-1.72)	(1.15–) 1.59 (-2.25)
<i>Boerhavia hereroensis</i>	(49.06–) 60.77 (-74.62)	(3.30–) 4.79 (-7.67)	(1.47–) 2.70 (-4.01)	(5.64–) 8.57 (-11.45)	(1.66–) 1.98 (-2.45)	(0.98–) 1.67 (-2.80)	(2.13–) 3.00 (-4.45)
<i>Boerhavia repens</i> subsp. <i>repens</i>	(45.07–) 57.89 (-68.25)	(2.77–) 4.03 (-5.41)	(2.03–) 3.05 (-3.98)	(3.35–) 4.02 (-4.96)	(0.88–) 1.19 (-1.93)	(0.64–) 1.03 (-1.55)	(1.67–) 2.08 (-2.43)
<i>Commicarpus chinensis</i> subsp. <i>natalensis</i>	(70.26–) 97.04 (-121.04)	(3.40–) 5.46 (-7.37)	(1.78–) 2.76 (-3.43)	(4.13–) 5.30 (-6.71)	(1.19–) 2.00 (-2.82)	(0.75–) 1.56 (-2.16)	(1.39–) 2.00 (-2.60)
<i>Commicarpus decipiens</i>	(97.40–) 116.64 (-129.28)	(4.81–) 5.03 (-5.37)	(1.45–) 2.43 (-3.01)	(5.79–) 7.35 (-9.10)	(2.35–) 2.84 (-3.35)	(1.39–) 1.7 (-2.82)	(1.45–) 2.78 (-3.82)
<i>Commicarpus fallacissimus</i>	(63.88–) 76.62 (-88.46)	(4.35–) 5.25 (-5.92)	(2.18–) 3.18 (-3.78)	(4.76–) 4.81 (-4.93)	(1.21–) 1.49 (-1.86)	(0.81–) 1.21 (-1.42)	(1.4–) 1.59 (-1.87)
<i>Commicarpus helena</i> var. <i>helena</i>	(53.76–) 60.60 (-71.29)	(2.59–) 4.35 (-5.00)	(1.12–) 1.72 (-1.87)	(3.57–) 4.26 (-5.11)	(1.05–) 1.73 (-2.67)	(1.01–) 0.95 (-1.07)	(0.86–) 1.51 (-1.86)
<i>Commicarpus pentandrus</i>	(63.74–) 84.23 (-100.12)	(3.47–) 5.76 (-8.45)	(2.39–) 3.36 (-4.84)	(4.65–) 6.40 (-8.28)	(1.45–) 2.55 (-3.20)	(0.65–) 1.25 (-1.66)	(1.60–) 2.39 (-2.84)
<i>Commicarpus pilosus</i>	(67.55–) 71.97 (-77.66)	(4.55–) 5.04 (-5.49)	(1.88–) 2.55 (-3.26)	(6.69–) 7.45 (-9.06)	(1.48–) 2.00 (-3.11)	(0.78–) 1.20 (-1.92)	(2.20–) 2.66 (-3.53)
<i>Commicarpus plumbagineus</i>	(71.99–) 85.26 (-105.27)	(3.70–) 5.82 (-8.30)	(1.19–) 1.95 (-2.77)	(4.93–) 6.02 (-6.99)	(2.36) 2.4 (2.52)	(1.59–) 1.76 (-2.03)	(2.05–) 2.53 (-3.09)
<i>Commicarpus squarrosus</i> var. <i>fruticosus</i>	(64.64–) 76.23 (-87.89)	(4.36–) 7.11 (-10.64)	(1.95–) 2.49 (-4.02)	5.00	(2.03–) 2.79 (-3.93)	(0.97–) 1.26 (-1.76)	(2.73–) 3.08 (-3.97)
<i>Commicarpus squarrosus</i> var. <i>squarrosus</i>	(45.55–) 65.49 (-82.54)	(3.30–) 4.77 (-6.12)	(1.47–) 2.89 (-5.43)	(3.88–) 5.23 (-6.16)	(1.19) 1.77 (2.43)	(0.78–) 1.13 (-1.39)	(1.09–) 1.76 (-2.30)

Table 2.—Measurements made of *Boerhavia* and *Commicarpus* pollen grains by ^aNowicke (1970) and ^bPerveen & Qaiser (2001)

Taxon	Diameter of grains (µm)	Diameter of pores (µm)	Length of spinules (µm)	Thickness of the nexine (µm)	Thickness of the sexine (µm)	Thickness of the exine (µm)
^a <i>Boerhavia</i>	70–138	2.4–7	1–5	4–7	2.5–6	-
^b <i>B. diffusa</i> var. <i>diffusa</i>	50–62.5	2.5–7.5	4.25–5.25	-	-	2.22–3.33
^a <i>B. erecta</i>	121–138	6–7	4–5	5–5.5	2.5–3	-
^b <i>B. repens</i> subsp. <i>repens</i>	61.03–100.5	3.23–3.94	3.59–7.18)	-	-	3.23–3.59
^a <i>Commicarpus</i>	84–112	3–5.5	1–2.5	2.5–5.5	2.5–5.5	-
^a <i>Commicarpus fruticosus</i>	84–92	3–4	2–2.5	2.5–3	2.5	-
^b <i>C. helenae</i> var. <i>helenae</i>	50–60	2.5–5.1	3.75–5.01	-	-	1.11–2.22
^a <i>Commicarpus pentandrus</i>	98–110	4.5–5.5	2–2.5	4.5–5	4	-

**Commicarpus fruticosus* syn. of *C. squarrosus* var. *fruticosus*

Specimens examined

NAMIBIA.—**1813** (Opuwo): Joubert Pass to Opuwo, against slope in stony/rocky soil, (–DD), 9 Feb. 2009, *Struwig 46* (PUC, WIND). **1913** (Sesfontein): Khowarib Rest Camp, behind the tents underneath acacia and mopane trees, (–BD), 9 Feb. 2009, *Struwig 44* (PUC, WIND); on road to Sesfontein, next to road in dry stream bank, (–DB), 8 Mar. 2009, *Struwig 43* (PUC, WIND). **1917** (Tsumeb): Tsumeb, at junction of D3022 and D2863, Otavi mountains, (–BD), 10 Feb. 2009, *Struwig 48* (PUC, WIND). **2014** (Khorixas): Twyfelfontein Lodge, hills behind lodge, SW of main building (–CB), 7 Feb. 2009, *Struwig 40, 41* (PUC, WIND); Twyfelfontein, village near Twyfelfontein Lodge, in dry streambed, (–CB), 8 Feb. 2009, *Struwig 42* (PUC, WIND). **2016** (Otjiwarongo): Otjiwarongo, Gobabis road, corner of C30 and C22, (–DB), 11 Feb. 2009, *Struwig 52* (PUC, WIND). **2017** (Waterberg): Otjiwarongo, Klein Waterberg, at foot of mountain in shade of trees, (–CA), 11 Apr. 2010, *Struwig 181* (PUC, WIND). **2114** (Uis): Uis, Brandberg, near entrance to Tsisab gorge, (–AA), 6 Feb. 2009, *Struwig 38* (PUC, WIND). **2115** (Trekopje): Omaruru, Loskop farm, (–BD), 11 Apr. 2010, *Struwig 176* (PUC, WIND); Usakos, Klein Spitzkuppe, against mountain slope amongst rocks, (–CC), 5 Feb. 2009, *Struwig 35, 36* (PUC, WIND); Karibib, Klippenberg Country Club, rocky hill behind restaurant, (–DD), 4 Feb. 2009, *Struwig 34* (PUC, WIND). **2116** (Okahandja): Okahandja, D2110, first dry stream bank after entrance to farm Okatjiho, (–DD), 12 Feb. 2009, *Struwig 54, 55* (PUC, WIND). **2217** (Windhoek): Windhoek National Botanical Garden, Lily Walk, (–CA), 3 Feb. 2009, *Struwig 33* (PUC, WIND); D1463, at T-junction sign just before entrance to Aris Farm, (–CC), 13 Feb. 2009, *Struwig 57* (PUC, WIND). **2416** (Maltahöhe): C 14, Naukluft Mtns, mountain slope behind the river, (–AA), 13 Feb. 2009, 7 Apr. 2010, 8 Apr. 2010, *Struwig 160, 163* (PUC, PRE, WIND); C19, Tsaris Mtns, (–AB), 8 Apr. 2010, *Struwig 164* (PUC, WIND); Maltahöhe, D850, next to road, (–DB), 9 Apr. 2010, *Struwig 168* (PUC, WIND).

LIMPOPO.—**2229** (Waterpoort): Mapungubwe National Park, Rhodesdrift, garden of Section Ranger, (–AA), 18 Nov. 2009, *Struwig 117* (PRE, PUC); Pont Drift, Breslau 2MS, dam wall area, (–AC), 28 Jul. 1998, *Straub 499* (PRE); Pont Drift, Breslau 2MS, NE base of Pyramid koppie, (–AC), 14 May 1997, *Straub 609* (PRE); On Waterpoort–Alldays road, (–CD), 17 Nov. 2009, *Struwig 111* (PRE, PUC); 9 km on Waterpoort–Alldays road, (–DC), 17 Nov. 2009, *Struwig 110* (PRE, PUC); Louis Trichardt (Makhado), just after tunnel coming from Louis Trichardt on farm Klein Afrika, on riverbank, (–DD), 17 Nov. 2009, *Struwig 106* (PUC, PRE). **2230** (Messina): R525, 4 km after Tspise, (–CA), 19 Nov. 2009, *Struwig 122* (PRE, PUC);

NORTH-WEST.—**2626** (Klerksdorp): Klerksdorp, Doringkruin, pavement on the corner of Moepel Avenue and Oleander Avenue, (–DC), 16 Jan. 2010, *Struwig 132* (PRE, PUC). **2627** (Potchefstroom): Potchefstroom, N12 Johannesburg road at Alpha Fruit and Veggie, Plot 283, next to road, (–CA), 4 Dec. 2008, *Struwig 23* (PRE, PUC); Potchefstroom, Bailliepark, 108 Steyn Street, (–CA), 2 Feb. 2010, *Struwig 133* (PRE, PUC).

MPUMALANGA.—**2431** (Acornhoek): Kruger National Park, H4-1 from Skukuza to Nkuhlu, along Sabie River, (–DC), 21 Apr. 2009, *Siebert 3970* (PUC); Kruger National Park, in Skukuza Research Camp, behind tents and bungalows underneath trees, (–DC), 16 Mar. 2010, *Struwig 143* (KNP, PUC).

KWAZULU-NATAL.—**2831** (Nkandla): Richards Bay, Mtuzini Nature Reserve, next to road opposite entrance to Inkwazi Campsite, (–DD), 12 Mar. 2009, *Struwig 88* (NH, PUC). **2832** (Mtubatuba): Richards Bay, Naval Hill, (–CC), 12 Mar. 2009, *Struwig 62* (PUC, NH); Richards Bay, Richards Bay Camping Site, Block E & F, in vegetation forming borders around campsites, (–CC), 12 Mar. 2009, *Struwig 63* (PUC, NH). **2931** (Stanger): Umhlanga Rocks, trail past the bridge going over the lagoon, against dune slope facing the beach, (–CA), 10 Mar. 2009, *Struwig 61* (NH, PUC).

NORTHERN CAPE.—**2824** (Kimberley): Hay division, 11 Mar. 1937, *Acocks 1978* (KMG, PRE). **2921** (Kenhardt): Kenhardt division, 25.8 miles SW by W of Kenhardt, (–AC), 4 Feb. 1961, *Acocks 21788* (PRE).

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