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Exormotheca bulbigena sp. nov. (Hepaticae, Marchantiales) and its relation to *E. holstii* in southern Africa

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Keywords: *Exormotheca bulbigena, E. holstii*, Hepaticae, Marchantiales, southern Africa

ABSTRACT

A new species *Exormotheca* **bulbigena** is described from southern Africa and its relation to *E. holstii* Steph. is discussed. Morphologically these species are very similar and can be distinguished only when fertile. The chromosome numbers, however, n = 32 for *E.* **bulbigena** and n = 18 for *E. holstii*, distinguish sterile living plants.

INTRODUCTION

Stephani (1899), in his *Species hepaticarum*, described a new species, *Exormotheca holstii*, from Muse (Tanzania). Subsequently, Marquand (1930), apparently unaware of Stephani's publication, described *E. megastomata* from a site near Middelburg, Mpumalanga (Transvaal), South Africa, as a new species. Finally Arnell (1953) published a new species, *E. youngii*, from Pilgrim's Rest, Mpumalanga, (Transvaal) South Africa, which he himself later placed in synonymy under *E. holstii* in 1963. After a morphological examination of all the respective herbarium samples Perold (1994) sank *E. megastomata* under *E. holstii*.

Chromosome numbers of *Exormotheca* samples from the *loci classici* of Marquand and of Arnell and from other sites in eastern Africa as well, always yielded a chromosome number of n = 18. However, *Exormotheca* plants collected by Volk at Gaikos and Otjua in Namibia have a chromosome number of n = 32. Nevertheless, all other morphological characters of the sterile plants were identical to those from the eastern sites. *A priori*, different chromosome counts cannot be considered as distinctive on species level, because there are many different species in the Marchantiales with the same chromosome number. On the other hand, some species with as many as six different caryotypes are also reported (Bornefeld 1989; Fritsch 1982).

By cultivation in a greenhouse, Volk succeeded in growing fertile plants from material collected in the eastern localities and in Namibia. The present study is based on the examination of living and fertile plants, and only those specimens are considered for which the chromosome number and/or the spores are known. The study of the sexual organs and their products and the asexual reproduction of *Exormotheca* has shown that the sinking of *E. megastomata* under *E. holstii* (Perold 1994) is justified. However, an additional new species, *E. bulbigena*, has to be established.

MATERIALS AND METHODS

Dry herbarium samples of *Exormotheca* from southern Africa were cultivated in a greenhouse on a mixture of garden mould and sand over a base of peat.

For chromosome counts, thallus tips were fixed, extracted and stained with orceine as described earlier (Bornefeld 1984). In the present study only samples with known chromosome numbers are considered, although the number of *Exormotheca* localities is far greater (Perold 1994).

The localities are listed according to Edwards & Leistner (1971).

For SEM studies the samples were fixed in a mixture of 70% ethanol/glacial acetic acid/40% formalin = 90/5/5 v/v/v at room temperature for 24 hrs, and then dehydrated in an acetone series. After critical point drying with CO_2 and sputtering with gold, SEM micrographs were taken with a DSM 962 model by Zeiss.

Exormotheca bulbigena *Bornefeld, O.H.Volk & R.Wolf*, sp. nov.

Thallus monoicus. Frons hyalina, usque ad 20 mm longa, simplex vel furcata, linearis, antica plana, crassa. *Costa* maxima, strato antico aequialta, postice valde rotundata, lateribus convexo-adscendentibus. *Squamae* posticae magnae, cellulis longissimis (50–600 µm) formatae, uno latere ad basin grosse lacinulatae, oblique oblongae acuminatae obtusae. *Stratum anticum* costae aequialtum, in fundo fila aggregata gerens. *Stomata* densissima, altissima, ad $^2/_3$ coalita, tertio supero libera, cylindrica, obtusa, vertice poro oblongo (± 50 × 140 µm) perforata. Frons ad apicem stolonum bulbillos pulviniformes ± 2–4 mm longos et latos 2–3 m altos gerens. Antheridia minuta in duabus lineis (30 × 40 µm) caudaliter et sagittaliter carpocephali, in strato antico, stomatibus contecta, disposita. *Ostiola* ± 160 µm lata, e sulco prominentes. *Carpocephala* in fundo alveoli nudis, strato antico recedente et ante di-

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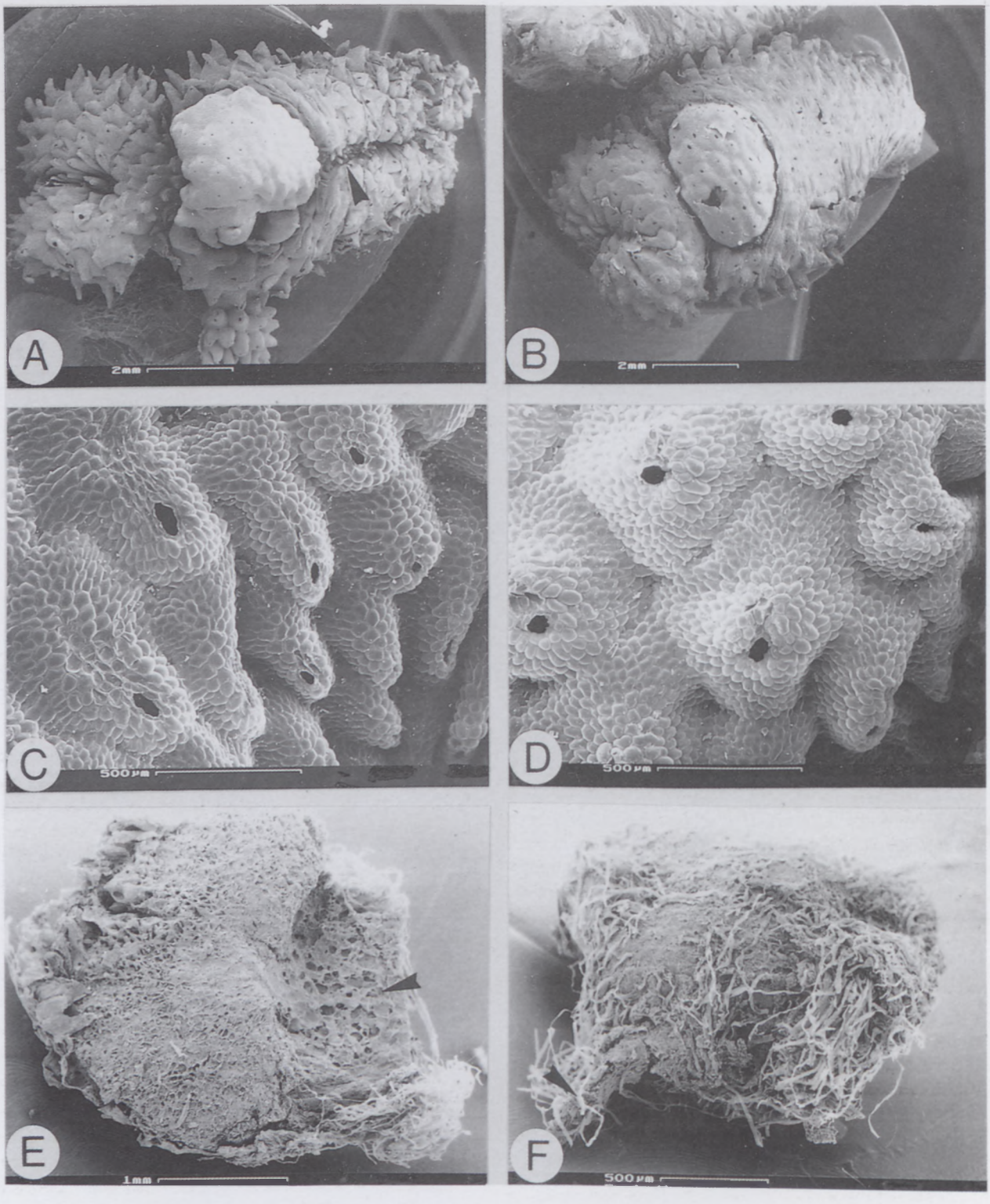

FIGURE 1.—*Exormotheca bulbigena*: A, thallus with carpocephalum; arrow shows antheridial necks. C, stomata with pores. E, F, bulb: E, dorsal view, where surface is broken (arrow), spongy interior tissue is visible; F, ventral view, arrow shows remnant of stolon which formed bulb. *E. holstii*: B, female thallus. D, stomata with pores near thallus tip. Scale bars: A, B, 2 mm; C, D, 500 µm; E, 1 mm; F, 500 µm.

chotomiam frondis. Complura carpocephala sequentialiter et aequidistantes (± 3–4 mm) in una frons inserta esse possunt. *Receptacula* sessilia globosa, vertice haud porosa stratoque chlorophyllifero tecta; subtus obconico-angustata, utroque latere involucrata; *involucra* 2, opposita, capituli vertice convexo-prominente separata, oblique adscendentia, conchaeformia, antice subcarinata, subtus apiceque aperta, labiis late hiantibus usque ad basin decurrentibus; *capsula* longius pedicellata, irregulariter quadrivalvata, valvae rufo-brunneae, maxime incrassatae. *Sporae* ± 140 µm, papulae distales irregulariter vermiculiformes. *Elateres* degeneratae (20 µm latae et 50–150 µm longae) cum anulis et spiralibus brevibus. *Chromosomatum numerus*: n = 32.

TYPE.—Namibia, 2216 (Otjimbingwe) Farm Otjua, granitic outcrop, (–AA), *Volk 85/766* c. fr. (M, holo.; M, PRE).

DISCUSSION

In her study of *Exormotheca holstii* samples from southern Africa, Perold (1994) necessarily used dry material from various localities collected in different years. In our studies, samples of *E. holstii* from Namibia and Mpumalanga (eastern Transvaal) and of *E. bulbigena*, grown under identical conditions, developed carpocephala (Figure 1A, B). Other characters such as overall size and shape, shape in cross-section, cell shape of the assimilatory tissue, width and height of the stomata [the papillae on top of (or) side of which stomata are located], shape of the ventral scales, and colour were all the same. Because these characters are meticulously described by Perold (1994) they are not repeated here, where the differences between the two species are emphasized. The only morphological feature which differs in the sterile thalli are the pores of the stomata; they are more elongate in *E. bulbigena* than in *E. holstii* (Figure 1C, D; Table 1). Whether or not these relations are affected by ecological factors in the field is unknown and therefore these differences should not be overestimated.

The formation of small bulbs is known for *E. tuberifera* (Kashyap 1914) and for *Corbierella* (= *Exormotheca*) *algeriensis* Douin & Trabut (1919). Therefore we investigated our cultures for such organs and indeed found these in *E. bulbigena* (*inde nomen*). They are cushion-shaped, 2–4 mm long and broad, and 2–3 mm thick. The bulbs consist of a chlorophylliferous, spongy tissue with some oil cells, somewhat more compact ventrally. When mature these little bulbs are no longer attached to the thallus and occur isolated in the ground. When dry they shrivel up and are easily overlooked in the field. Figure 1E & F shows the dorsal and ventral view of bulbs which were rehydrated for one day after seven months of desiccation. On the lower side the remnant of the stolon which formed the bulb is visible (arrow). *Exormotheca holstii* also forms short stolons with a slightly enlarged terminal bud, but these are not drought tolerant and upon remoistening become covered by mould.

As there appears to be little variation in the caryotype, the main character for discrimination of sterile plants remains the chromosome number: n = 32 for *Exormotheca bulbigena* and n = 18 for *E. holstii* (Figure 2A, C). Chromosome analysis (Bornefeld 1984) reveals that *E. bulbigena* is eutetraploid to a basic number of eight chro- mosomes and *E. holstii* is eudiploid to a basic number of nine chromosomes (Figure 2B; D). The difference in the basic numbers is not surprising when taking into account that for *Exormotheca fimbriata* (Brazil) both caryotypes are reported: n = 8 (Jovet-Ast 1976) and n = 9 (Bornefeld unpubl.).

TABLE 1.—Size relations of stomatal pores of *Exormotheca bulbigena* and *E. holstii* grown simultaneously under identical conditions (n = 16)

	E. bulbigena	*E. holstii*
long axis	138.1 ± 37.9 µm	127.8 ± 24.6 µm
short axis	52.9 ± 20.1 µm	88.1 ± 13.6 µm
long axis : short axis	2.61	1.45

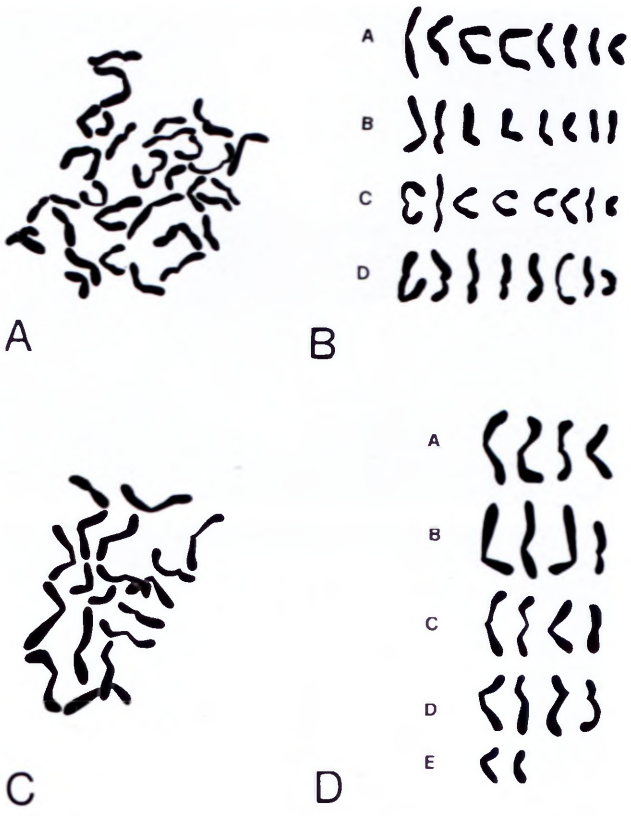

FIGURE 2.—*Exormotheca bulbigena*: A, original arrangement of chromosome set; B, chromosomes sorted by types. *E. holstii*: C, original arrangement of chromosome set; D, chromosomes sorted by types.

The type of *Exormotheca holstii* is dioecious; the types of Marquand (1930) and of Arnell (1953) are sterile. In our cultures out of 12 samples from South Africa only one contained both sexes; four were female, three were male, and four remained sterile. When comparing fertile plants of *E. bulbigena* and *E. holstii* the most obvious difference is the deep sulcus caudal to the carpocephalum of the monoecious *E. bulbigena* (Figure 1A) which is lacking in the female *E. holstii* plant (Figure 1B). Closer inspection of the sulcus shows the antheridial necks which are composed of eight rows of cells (Figure 3A). The whole structure has a diameter of ± 160 µm. The antheridial necks of *E. holstii* (Figure 3B, D) have a diameter of only 100 µm. A series of sagittal sections of the carpocephalum of *E. bulbigena* (Figure 4A, B) reveals the presence of two rows of additional 'microantheridia' 40 × 30 µm; the respective values for the main antheridia in the depth of the sulcus, which are arranged in two parallel rows, are 350 and 150 µm. These microantheridia are a unique feature not described as yet for liverworts. The reinforcing bands in the sporangium wall are very variable within one sporangium and thus are of no value for distinguishing between the two species. The spores of both species are about the same size (120–150 µm), dark brown to black, anisopolar, without a wing. The ornamentation of the distal face of *E. bulbigena* can be described as 'vermiculate' (sensu Perold 1989) (Figure 5A). If the ridges become very short (e.g. Perold 1994, fig. 6A) it can form a papillate pattern. The corresponding structure of *E. holstii*, very broad, indented papillae, could be described as 'polygonal'. Figure 5C shows a spore of the sample *Crosby 1115* from Zoetvlei and its similarity to that of the type of *E. holstii* (Figure 5E) proves the cor-

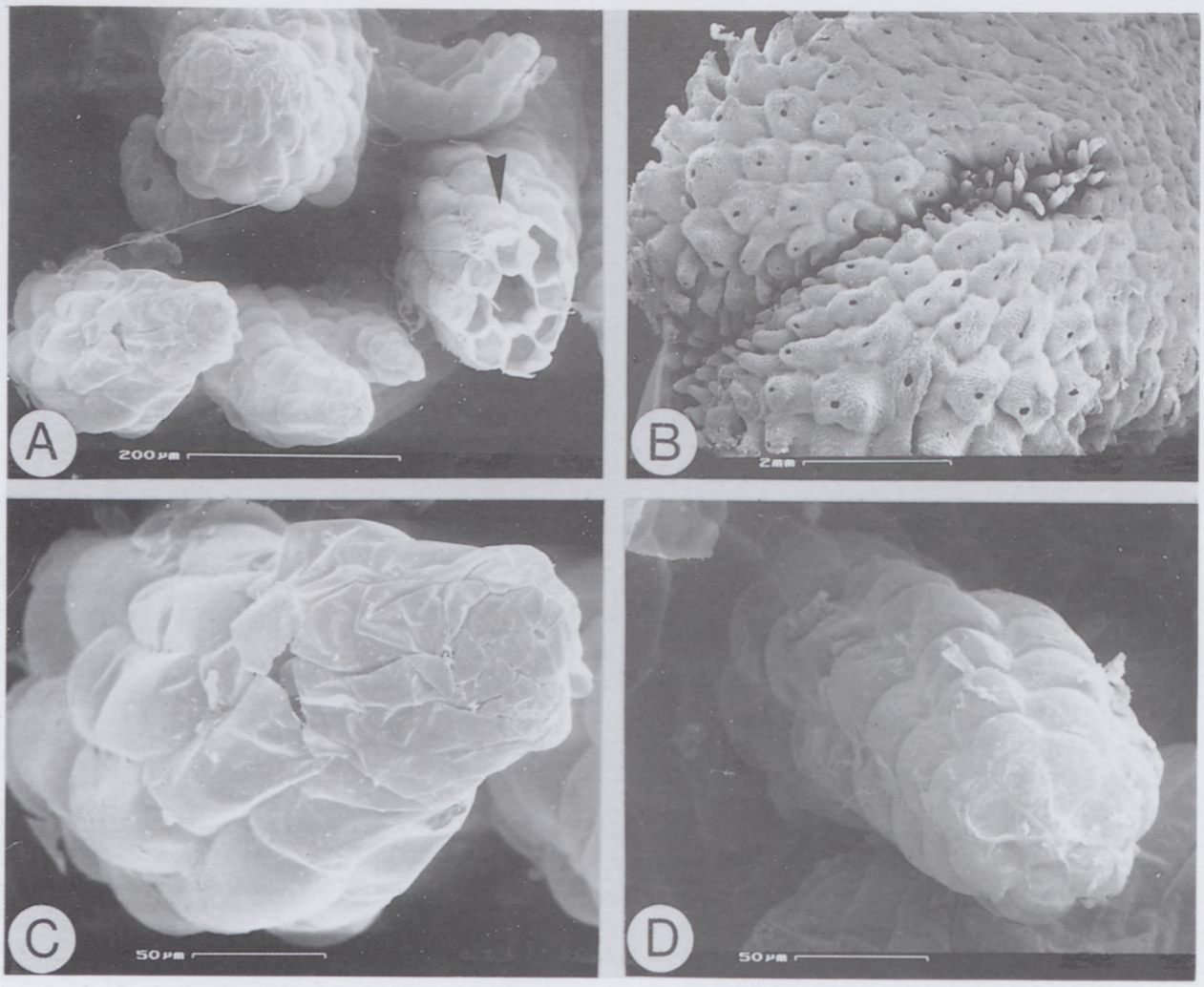

FIGURE 3.—Antheridial necks. A, C, *E. bulbigena*: A, from sulcus, arrow shows broken neck with 8 rows of cells forming the channel. B, D, *E. holstii*: B, male thallus with antheridial necks. Scale bars: A, 200 µm; B, 2 mm; C, D, 50 µm.

rectness of the identification by Perold (1994). In the description of *E. holstii*, with respect to the papillae of the spores, Stephani (1899) mentions 'papulis saepe rostratis'. In Figure 5E no rostrum-like structures can be detected on the papillae and it remains unclear to which structures Stephani refers; elaters stuck to the papillae probably led to the remark mentioned. The ornamentation of the proximal face of the spores is rather fine and the triradiate mark is inconspicuous (Figure 5B, D, F). The differences of the distal face become visible in another way by dark-field microscopy where the spores are seen in a bright orange colour. With this method structures are visible on the ridges of the ornaments which in *E. bulbigena* are punctiform or very short grooves, for *E. holstii* they may be

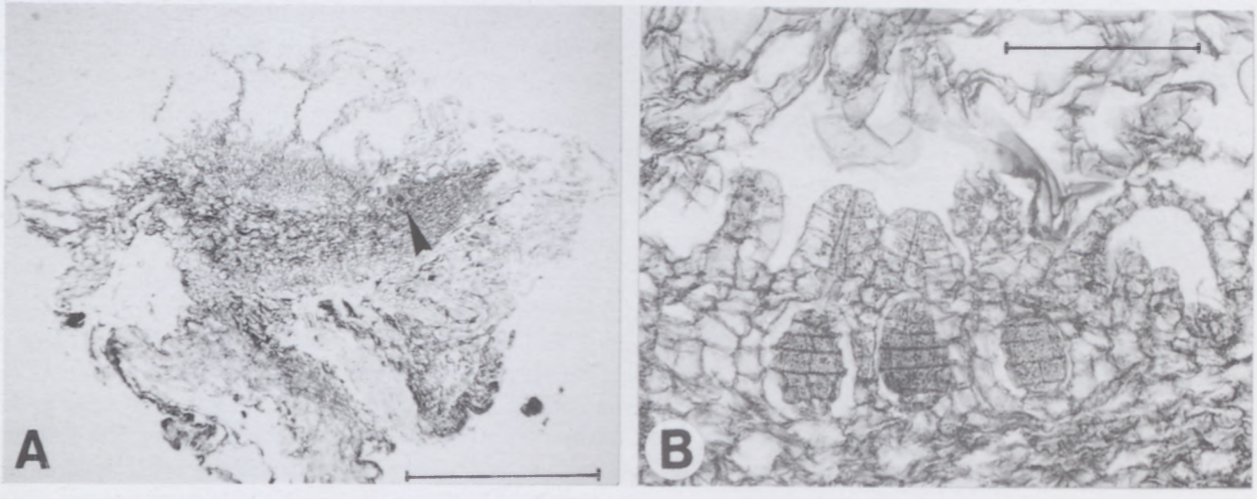

FIGURE 4.—*E. bulbigena*: A, sagittal section through carpocephalum; arrow shows site of microantheridia. B, microantheridia. Scale bars: A, 1 mm; B, 100 µm.

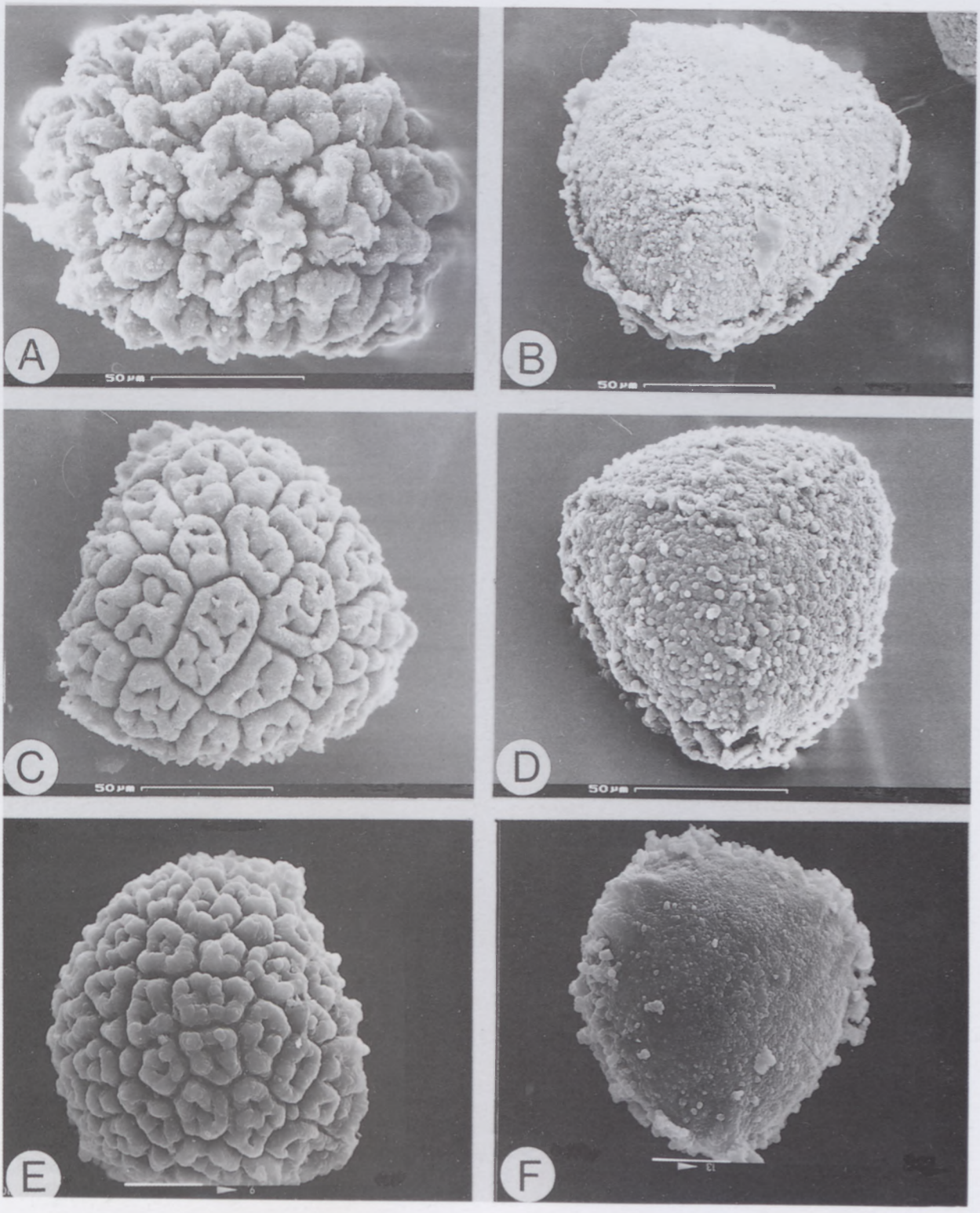

FIGURE 5.—Spores. A, B, *E. bulbigena*: A, distal view; B, proximal view. C–F, *E. holstii*, *Crosby 1115*, Zoetvlei: C, distal view, D, proximal view; E, F, type specimen, E, distal view, F, proximal view. Scale bars: A–D, 50 µm; E, F, 50 µm.

described as long, finely branched furrows (Figure 6A, B). The elaters of both species are degenerate, about 20 µm in diameter and between 50 and 150 µm long, with rings or incomplete spirals. Summarising all the differences between *E. bulbigena* to *E. holstii* (Table 2) we consider it obligatory to consider the former as a distinct species, even though sterile plants are very similar.

Consideration of the climate diagrams (Walter & Lieth 1967) for typical sites of the two species (Figure 7A, B) suggests that *E. bulbigena* is better adapted to a hotter and drier climate than *E. holstii*. The site of the latter at Rietfontein in Namibia is near a fountain and thus not contrary to this suggestion.

The immediate influence of external ecological factors on physiological activity of these plants is difficult to estimate. Both species form droplets of condensed water inside the stomata which consist of living cells. By cooling down the air within the stomata by evaporation and

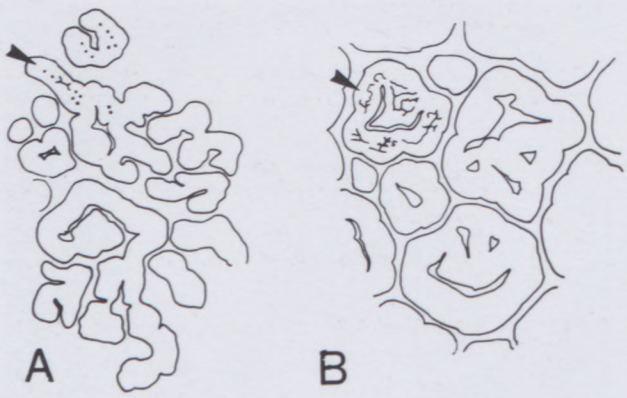

FIGURE 6.—Ornamentation of the distal spore faces as seen by dark-field microscopy. A, *E. bulbigena*; B, *E. holstii*. Arrows: fine structure on ridges of ornaments.

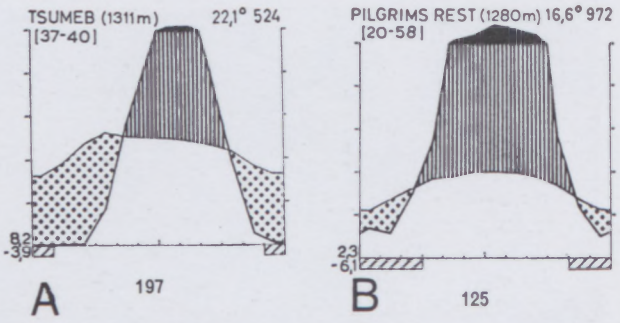

FIGURE 7.—Climatic diagrams of typical sites. A, in Tsumeb, Namibia for *E. bulbigena*; B, in Pilgrim's Rest, Mpumalanga (Transvaal) for *E. holstii*.

TABLE 2.—List of characters differing in *Exormotheca bulbigena* and *E. holstii*

	E. bulbigena	*E. holstii*
sterile plants		
chromosome number	n = 32	n = 18
shape of pores	oblong	shortly elliptical
small bulbs	+	–
fertile plants		
sex distribution	monoecious	dioecious
fertile (female) thallus	with sulcus	without sulcus
spore ornamentation distal face	vermiculate	polygonal
width of antheridial necks	160 µm	100 µm
microantheridia	+	–

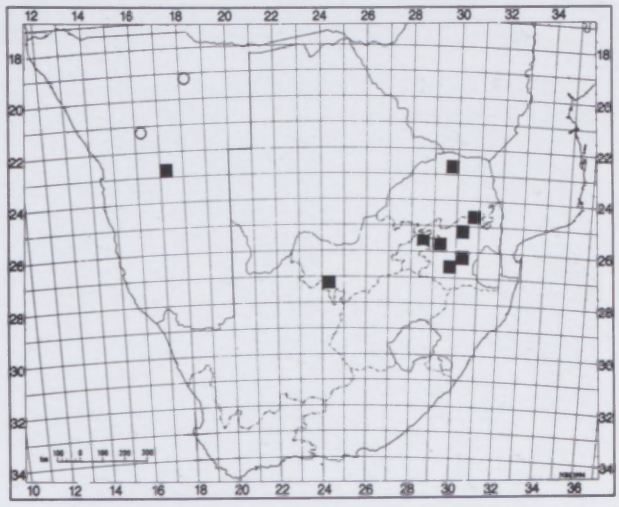

FIGURE 8.—Distribution map of *E. bulbigena*, ○; and *E. holstii*, ■, in southern Africa. Only samples with known spores and/or chromosome numbers were considered.

by their sheltered humidity the plants seem to establish a greenhouse-like microclimate of their own, certainly favourable for photosynthesis. Figure 8 shows the distribution of the two species in southern Africa. So far *E. bulbigena* has only been found in Namibia and may thus be considered endemic to this region.

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SPECIMENS EXAMINED

Exormotheca bulbigena, n = 32

NAMIBIA.—1918 (Grootfontein): Gaikos, on quartzite sand, (–AD), *Volk 81/124* (M, PRE). 2116 (Otjimbingwe): Otjua, granitic outcrop, (–AA), *Volk 84/696, 85/766, 88/030* (M, PRE).

Exormotheca holstii, n = 18

NAMIBIA.—2217 (Windhoek): Rietfontein, Granitzersatz, durch Sickerwasser zeitweise feucht bis nass, (–CA), *Volk 01160* (B=L, PRE).

NORTHERN PROVINCE.—2229 (Waterpoort): Lokovhela 793 Farm, Soutpansberg, (–DD), *Glen 2650* (PRE).

MPUMALANGA.—2430 (Pilgrim's Rest): on R532 to Bourke's Luck Potholes ± 1 km before coming to SADF Dog Training Centre, on dry sandy slope above stream, between grass, (–DB), *S.M. Perold* 2702 (PRE). 2529 (Witbank): Felsiger Hang über Elefantenfluss, (–CD), *Volk 88/026*. 2530 (Lydenburg): S of Lydenburg, Spitskop, drier ledge above waterfall, (–AB), *Perold & Koekemoer 2872* (PRE). 2629 (Bethal): Ermelo, (–DB), *s.n.* 2630 (Carolina): Knock Dhu Farm, 13 km SE of Lake Chrissie on Lothair road, common on rich black loamy soil, in grassland, (–AD), *Germishuizen 2839* (PRE).

GAUTENG.—2528 (Pretoria): Donkerhoek, 22.5 km E of Pretoria along Pretoria–Witbank Freeway, just beyond road cutting, seepage area, (–CD), *Germishuizen 5624*; *S.M. Perold 2795, 2796*.

NORTH-WEST.—2724 (Taung): Farm Zoetvlei, ± 50 km W of pan, on higher ground, (–AA), *Crosby 1115* (PRE).

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