

## Morphological and molecular characterization of *Endophyllum* species on perennial asteraceous plants in South Africa

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*Endophyllum osteospermi* is an autoecious, endocyclic rust fungus, which has only been recorded on *Chrysanthemoides monilifera* ssp. *monilifera* Asteraceae, Calendulae, a perennial woody shrub. Both organisms are indigenous to South Africa. Because *E. osteospermi* is being considered for release in Australia as a biocontrol agent against *C. monilifera* ssp. *monilifera*, it is necessary to determine its host range and natural distribution in South Africa. To address this, natural stands of *Chrysanthemoides* species, as well as other South African asteraceous plants, were monitored for *E. osteospermi* between 1992 and 2003. A morphological and molecular comparison of specimens referable to *Endophyllum* as undertaken. Based on these results, *E. osteospermi* as recorded on *C. monilifera* ssp. *monilifera*, *pisifera*, *rotundata*, *canescens*, and *subcanescens*, *C. incana*, and an undescribed taxon. *E. osteospermi* as also recorded on *Osteospermum ciliatum*, *O. polygaloides*, and *O. potbergense*. Furthermore, a closely related but previously undescribed species, *E. dimorphothecae* sp. nov. is described on *Dimorphotheca cuneata*. *Aecidium elytopappi* is transferred to *Endophyllum* as *E. elytopappi* comb. nov., being recorded on *Elytropappus rhinocerotis* and *Stoebe plumosa*. This study shows that in South Africa *E. osteospermi* is restricted to a small group of related plant species in the Calenduleae. This rust is therefore considered suitable as a candidate agent for the biocontrol of *C. monilifera* ssp. *monilifera*, and pending the results of host specificity testing, would most likely be safe to introduce into Australia.

### INTRODUCTION

The autoecious, microcyclic endocyclic rust fungus *Endophyllum osteospermi* has up to the present been recorded only on the perennial woody shrub *Chrysanthemoides monilifera* ssp. *monilifera* Asteraceae, Calendulae, both the plant and the fungus species being indigenous to South Africa Doidge 1950, Morris 1982, Crous, Phillips *et al.* 2000. Witchbrooms develop on plants that are systemically invaded with mycelium, and from which pycnia and aecidioid telia develop. Upon germination the aecidioid teliospores produce a metabasidium with 2–4 vesicle-like modified basidiospores Morris 1982, Wood 1998. Infection is associated with a reduction in growth and reproduction in host plants going in the wild Wood 2002, which in severe cases can lead to plant death Morris 1982.

*C. monilifera* ssp. *monilifera* has become naturalised in southeastern Australia here it is an invasive weed, and is threatening native vegetation and wildlife

Parsons Cuthbertson 1992, Stahle 1997. The invasive success of this plant is due to its capacity for vigorous growth, copious production of seed, and rapid regeneration after release in the absence of natural enemies Parsons Cuthbertson 1992. A biocontrol programme targeting these plants has been initiated, as part of which *E. osteospermi* is considered to be a potential biological control agent Scott Adair 1995, Adair Edards 1996.

Records of the distribution of *E. osteospermi* are limited to specimens deposited at the South African National Collection of Fungi PREMARC Plant Protection Research Institute, Pretoria Doidge 1950. A better understanding of the natural distribution and host range of *E. osteospermi* in South Africa is therefore necessary to determine the risk posed if this rust fungus is to be introduced into Australia as a biocontrol agent.

To address this question, stands of *Chrysanthemoides* species were surveyed between 1992 and 2003 in the Western Cape, Eastern Cape, and KwaZulu-Natal Provinces of South Africa. Other indigenous asteraceous

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plants that occurred within the natural distribution range of *C. monilifera* in these provinces were also screened during the same period.

Currently, subspecies of *Chrysanthemoides monilifera* are recognised Norlindh 1943 spp. *monilifera*, *pisifera*, *rotundata*, *canescens*, *subcanescens*, and *septentrionalis*. However, *C. monilifera* ssp. *pisifera* as presently circumscribed encompasses several distinct taxa (Griffioen 1995), and is in need of revision. Populations of one of the recognised subspecies were observed for the presence of itchesbrooms, especially ssp. *monilifera* and several morphs of ssp. *pisifera*. No observations of ssp. *septentrionalis* were made as this subspecies occurs outside of the area of study. Several morphs of *C. incana* were also observed.

Rust fungi causing itchesbrooms or galls and with aeciospores which on germination proved to be aecidioid teliospores, and hence were referable to the genus *Endophyllum*, were found on a number of perennial asteraceous plants in addition to *Chrysanthemoides* species. These rust specimens proved to be morphologically similar to *E. osteospermi*. Plant hosts on which they were collected included *Dimorphotheca cuneata* Calendulae, *Elytropappus rhinocerotis* Gnaphalieae, *Osteospermum ciliatum*, *O. polygaloides*, *O. potbergense* Calendulae, and *Stoebe plumosa* Gnaphalieae. The rust fungus on *E. rhinocerotis* is currently non as *Aecidium elytrapappi*, and in addition to several collections on this host in South Africa Doidge 1950, it has also been recorded from *Stoebe ilimandsharica* in Tanzania Henderson 1972. None of the other plant species listed has previously been recorded as hosts of rust fungi. One other species of *Endophyllum* as found, namely *Endophyllum macoaniamum* on *Conyza scabrida* Astereae Wood 2004. This species is morphologically distinct, is not systemic causing galls or itchesbrooms, produces typical basidiospores as opposed to the vesicellie modified basidiospores common to the species treated here and is not further treated here. To resolve the specific identity of the various collections, specimens were compared based on morphological and molecular data. The latter was based on sequences of the internal transcribed spacer ITS1–5.8S–ITS2 region of the ribosomal DNA, which was obtained from selected specimens.

## MATERIALS AND METHODS

### Morphology

Fresh aecidioid teliospores were dusted on water agar 1.5 agar, iolab, Midrand, South Africa in 6 cm diam Petri dishes, after which the plates were sealed and incubated at 15 °C for 24 h. Germinated spores were examined with a eiss Aiosop light microscope and photographed using a eiss MC63 camera.

Fresh or dried aecidioid telia were mounted in Tissue Freezing Medium™ Leica Instruments, Nubloch, Germany, and 10 mm thick transverse

sections cut with a Leica CM1100 Cryostat microtome. Sections were mounted in 70% lactic acid aqueous solution, and 25 aecidioid teliospores and peridial cells per specimen were measured at 1000× magnification. In addition, 50 aecidioid teliospores from each of the selected, dried specimens collected throughout the distribution of *E. osteospermi* were also measured. The length to width ratio of each aecidioid teliospore and the 95% confidence limits of the means were calculated. Measurements are stated as the average 95% confidence limits with the minimum and maximum given in parentheses. In addition, type specimens of *E. osteospermi* and *A. elytrapappi* were also examined. Another species, *A. metalasiae*, is morphologically similar Doidge 1927. Unfortunately, despite searching for this species it was not recollected. The type specimen of *A. metalasiae* was, however, examined.

Whole and cross sections of aecidioid telia and pycnia were prepared according to the method of Adendorff & Rijkenberg (2000) and observed with a Hitachi S-570 scanning electron microscope SEM at 10 kV.

### Molecular analysis

Fresh rust samples were collected in the field, placed in plastic bags and processed in the laboratory within 3 d after collection. Where abundant aecidioid telia were produced on individual large itchesbrooms, these were removed from the plant material and used for DNA extraction. Where the above was not possible, parts of developing itchesbrooms or galls bearing aecidioid telia were used. The collections used are listed in Table 1, and identified by a sign in the listings of specimens examined.

Specimens were placed in 1.5 ml Eppendorf tubes in 100 µl extraction buffer, and manually ground using a sterile conical tissue grinder elArt Products, Peunnoc, N. After grinding, an additional 400 µl extraction buffer was added. The extraction buffer was prepared with equal volumes of 100 mM Tris pH 9.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, and 2% CTAB cetyltrimethylammonium bromide. The suspension was passed through three freeze-thaw cycles of 10 min in ice then 3 min in a water bath at 100 °C, then incubated in a water bath at 65 °C for 60 min. The isolation protocol of Lee Taylor 1990 was used to extract genomic DNA from these aecidioid telia or infected plant samples. The primer pairs ITS1f and ITS4b Gardes runs 1993, and ITS5 White et al. 1990 and ITS4b were used to amplify part of the nuclear rRNA operon using the PCR conditions recommended by the authors White et al. 1990, Gardes runs 1993. Seven to and four PCR products were obtained for each locality, each from individual itchesbrooms. The PCR products were separated by electrophoresis at 95 °C for 1 h in a 1.5% agarose gel in 0.5× TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light

Table 1. Specimens of Endophyllum species subjected to DNA analysis.

Rust species	Host species	Locality	Genan accession no. <sup>a</sup>	PREM no.
Endophyllum osteospermi	Chrysanthemoides monilifera ssp. monilifera	Kirstenbosch National Botanic Garden, Cape Town	AY652755	57893
	C. monilifera ssp. monilifera	HouHoë Pass, Ot River	AY652752	57895
	C. monilifera ssp. pisifera	Gouritz River, Albertinia	AY652754	57888
	C. monilifera ssp. pisifera	eteen ellvidere and rentononSea, Knysna	AY652753	57887
	Chrysanthemoides undescribed	Cape Point	AY652750	57894
	Chrysanthemoides incana	Rietvlei, Milnerton	AY652756	57898
E. dimorphothecae	Osteospermum polygaloides	Sartberg Pass, Prince Albert	AY652757	57902
	Dimorpotheca cuneata	erlateloof Pass, Sutherland	AY652758	57918
E. elytopappi	Dimorpotheca cuneata	oplaas farm, Sutherland	AY652759	57919
	Elytropappus rhinocerotis	lesfontein farm, Sutherland	AY652761	57917
	Elytropappus rhinocerotis	order of Kaapzicht and eenacht ine estates, Kuils River	AY652760	57915

<sup>a</sup> Alignment in TreeASE SN1916.

using a GeneGenius Gel Documentation and Analysis System Syngene, Cambridge, UK following ethidium bromide staining.

The PCR products were purified using a Nucleo Spin<sup>1</sup> Extract 2in1 kit MachereyNagel, Düren, Germany. The purified products were sequenced in both directions using the PCR primers and the cycle sequencing reaction as carried out as recommended by the manufacturer with an ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Ready Reaction Kit PE Biosystems, Foster City, CA containing AmpliTaq DNA Polymerase. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer Perkin Elmer, Norwalk, CN.

Sequences were assembled and added to sequences obtained from Genbank (<http://ncbi.nlm.nih.gov>) (Sutherier et al. 1996, Roy et al. 1998, Fogler et al. 1998, Pfunder, Schurch Roy 2001, Hernandez, Palm Castlebury 2002, Weber, Webster Engel 2003, Chung, Tsuiboshi Kaishima, unpubl., Szabo, unpubl. using Sequence Alignment Editor v2.0a11 Rambaut 2002, and manual adjustments for improvement were made here necessary. Pucciniastrum goepeltianum L76509 as included as the outgroup. The phylogenetic analyses of sequence data were done using PAUP version 4.0b10 (Swofford 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis as performed for the data set using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-sapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated. Neighbour-joining analysis as performed using uncorrected p-distance and Jukes-Cantor substitution models. Alignment gaps were treated as

missing data, all characters were unordered and of equal weight and any ties encountered were broken randomly. The robustness of the resulting trees was evaluated by 1000 bootstrap replications Hillis et al. 1993. The trees were printed with TreeView version 1.6.6 Page 1996.

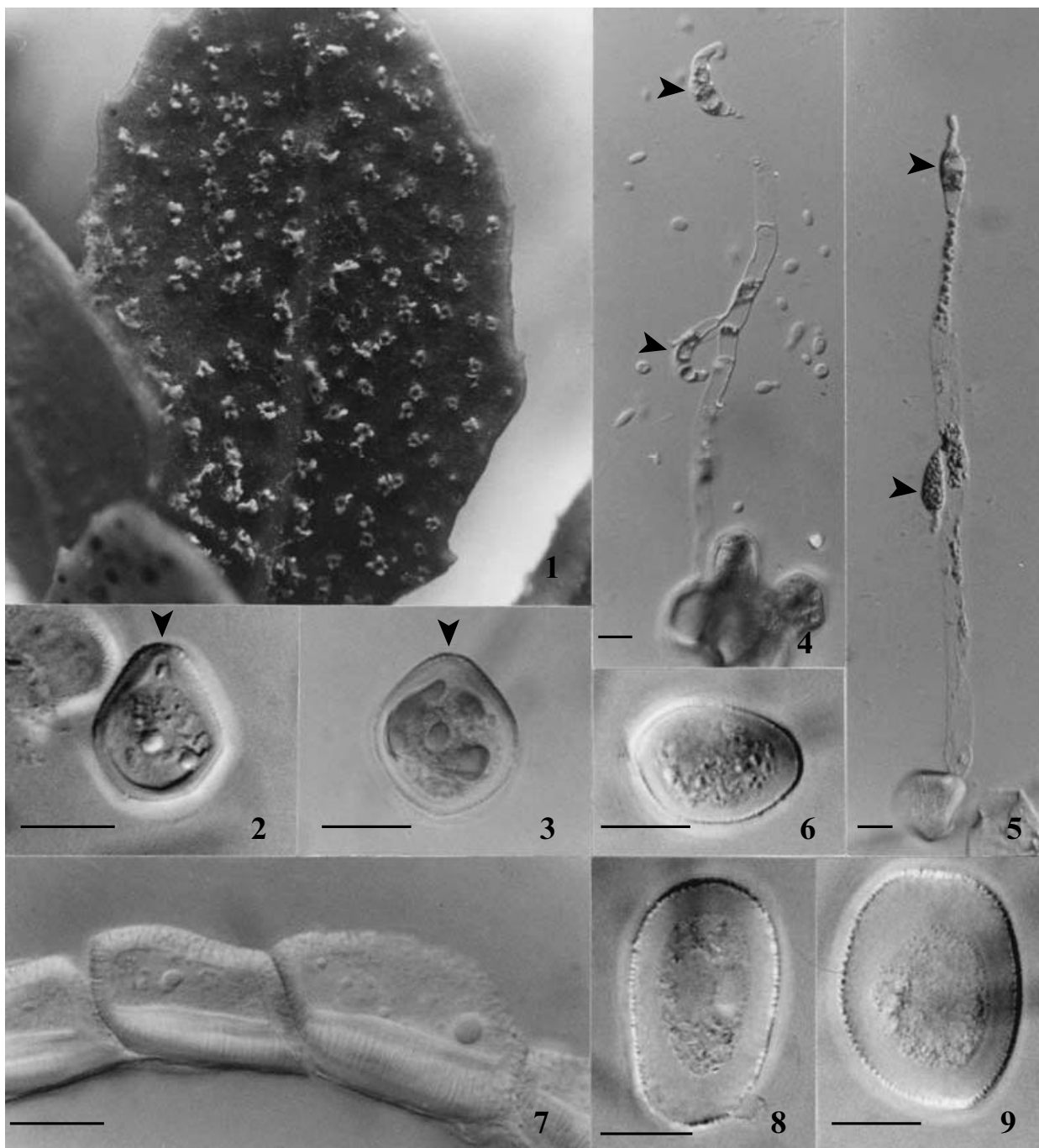
## TAXONOMY

*Endophyllum osteospermi* Doidge A. R. Wood, S. Afr. Bot. Soc. Trans. 64:146 1998. Figs 1–7

*Asionym* *Acidium osteospermi* Doidge, *Phytologia* 21:171 1927.

Infections systemic, causing itchesbrooms, host stems galled at base of itchesbrooms. Pycnia amphigenous but mostly hypophyllous, scattered unevenly, not in lesions, preceding the aecidioid telia 150–210 µm diam. Aecidioid telia ass-shaped with ostiolar trichomes. Aecidioid telia amphigenous and on stems but mostly hypophyllous, scattered unevenly, not in lesions aecidioid, orange, cylindrical, up to 0.5 mm long, 200–375 µm diam. Peridial margin hite, reeded, deeply incised. Peridial cells firmly joined together, irregular oblong to subrhomboid, 19–26–27.5–39 µm r 12–19–20–29 µm outer all striate, 7–10–11–15 µm thicker all coarsely verrucose, 3–4–6 µm thick. Aecidioid teliospores irregular angular globose, ovate to ellipsoid, 18–21.5–22–28 µm r 14–17–18–23 µm, length/width ratio 1.1–1.75 spore wall all hyaline, evenly verruculose, 1–2–2.5 µm thick apically slightly thickened with a distinct germ pore, 2–4 µm thick upon germination producing 2–4 vesiculate modified basidiospores.

Specimens examined South Africa KwaZulu-Natal Province Cathedral Peak, Mons Col Nature Reserve, 29°44' S 29°12' E, on *Chrysanthemoides monilifera* ssp. *canescens*, 26 Jan. 1997, A. R. Wood 61 PREM 56794



Figs 1–7. *Endophyllum osteospermi*. Fig. 1. Aecidioid telia on the abaxial surface of a leaf from an itchesbroom on *Chrysanthemoides monilifera*. Figs 2–3. Aecidioid teliospore showing a distinctly thickened apex and germ pore. Figs 4–5. Germinated aecidioid teliospore with vesiculate modified basidiospores. Fig. 6. Aecidioid teliospore showing a distinctly thickened apex. Fig. 7. Peridial cells of an aecidioid telium. Figs 8–9. Aecidioid teliospore of *Aecidium metalasiae*. Scale bars = 10  $\mu$ m.

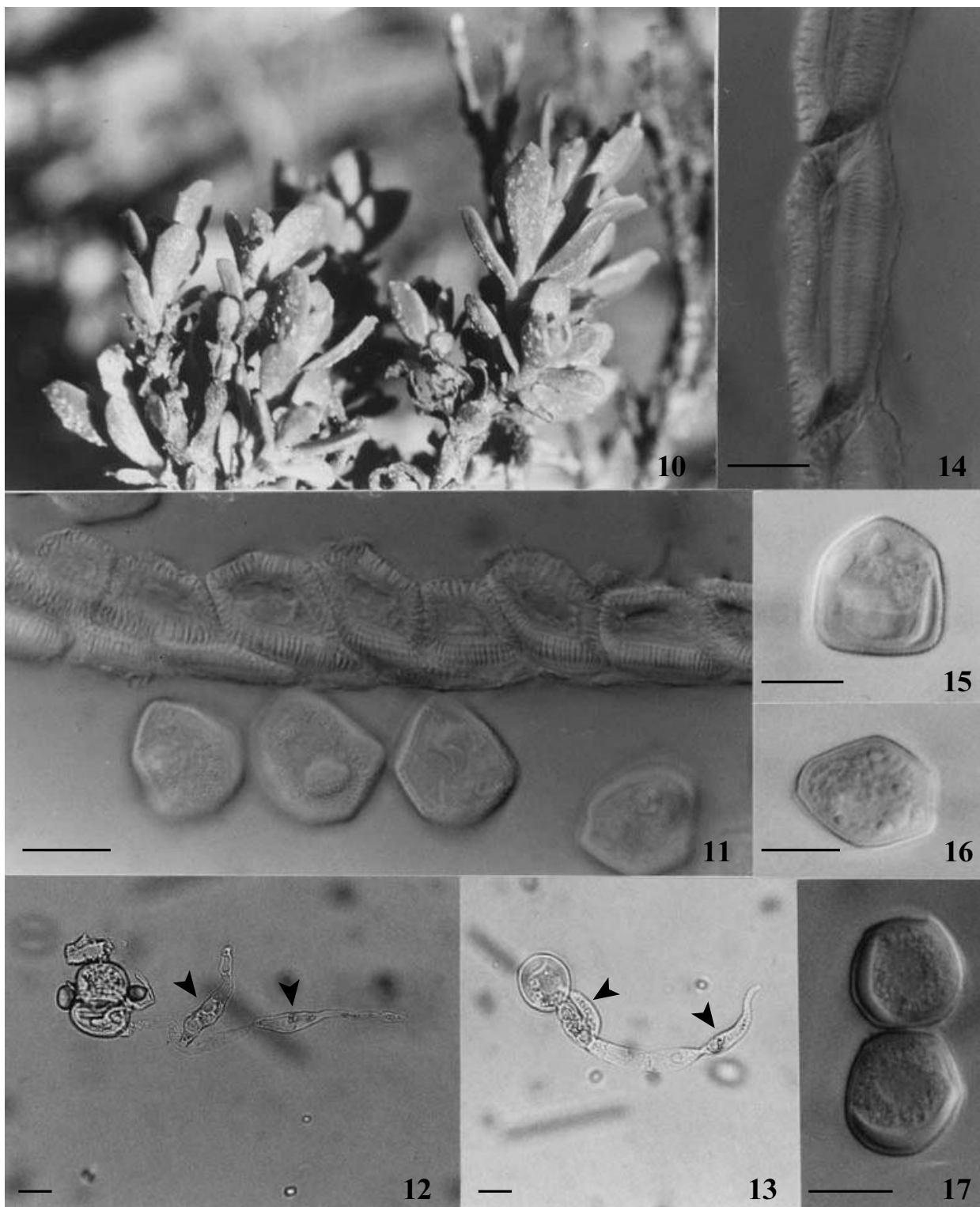
Eastern Cape Province Grahamstown, 33° 18' S 26° 31' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 6 Oct. 1998, A. R. Wood 82 (PREM 56815); Jefferys Bay, Kabeljou River Nature Reserve, 33° 59' S 24° 56' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 7 Oct. 1998, A. R. Wood 83 (PREM 56816) 6 m W of Haney/Humansdorp intersection on N2, 33° 59' S 24° 42' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 6 Oct. 1998, A. R. Wood 84 (PREM 56817) N of Addo, 25° 35' S 33° 19' E, on *Chrysanthemoides*

*monilifera* ssp. *pisifera*, 15 Feb. 2000, A. R. Wood 92 (PREM 56825) 3 m W of Alexandria along R27, 33° 38' S 26° 23' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 20 Aug. 2002, A. R. Wood 384 (PREM 57889) Kiddy's each, 33° 09' S 27° 41' E, on *Chrysanthemoides monilifera* ssp. *rotundata*, 20 Aug. 2002, A. R. Wood 385 (PREM 57886) Kaysers each, 33° 12' S 27° 36' E, on *Chrysanthemoides monilifera* ssp. *rotundata*, 20 Aug. 2002, A. R. Wood 386 (PREM 57890) Western Cape Province Cape Town, lower slopes of Lions

Head, on *Chrysanthemoides monilifera* ssp. *monilifera*, 13 Nov. 1912, W. Saton PREM 6587, Holotyperedasdorp District, Mierraal, on *Chrysanthemoides monilifera* ssp. *monilifera*, 16 Nov. 1927, C. A. Smith PREM 228392 m S of Simons Ton, 34 13 S 18 23 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 7 ul. 1997, A. R. Wood 65 PREM 56798 sin loc. on *Chrysanthemoides monilifera* ssp. *monilifera*, Oct. 1997, A. R. Wood 66 PREM 56799Paarl, Afriaans Language Monument, 33 46 S 18 57 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 27 un. 1997, A. R. Wood 68 PREM 56081near old tunnel along N1, DuToits Pass, 33 44 S 19 09 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 18 ul. 1997, A. R. Wood 69 PREM 56802redasdorp, Heuningberg Nature Reserve, 34 35 S 20 02 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 6 Aug. 1997, A. R. Wood 73 PREM 56806 Caledon, Caledon otanical Garden, 34 13 S 19 25 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 28. Sep. 1997, A. R. Wood 70 PREM 56803E of redasdorp, De Hoop Nature Reserve, Potberg Education Centre, 34 23 S 30 32 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 29 Sep. 1997, A. R. Wood 75 PREM 56808W of ot River, HouHoePass, 34 13 S 19 11 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 29 Sep. 1997, A. R. Wood 76 PREM 56809 sin loc. on *Chrysanthemoides monilifera* ssp. *monilifera*, 5 May 2003, A. R. Wood 516 PREM 57895E of redasdorp, De Hoop Nature Reserve, 1 m S of homestead, 34 28 E 20 24 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 10 Dec. 1997, A. R. Wood 74 PREM 56807 Hermanus, Fernloof Nature Reserve, 34 23 S 19 15 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 16 Feb. 1998, A. R. Wood 71 PREM 568041.5 m N of Stellenbosch, Helshoogte, 33 55 S 18 54 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 7 Sep. 1998, A. R. Wood 72 PREM 56805Cape Ton, Rhodes Memorial, 33 58 S 18 30 E, 16 on *Chrysanthemoides monilifera* ssp. *monilifera*, Aug. 1999, A. R. Wood 67 PREM 56800Somerset West, Helderberg Nature Reserve, 34 03 S 18 52 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 10 Dec. 2000, A. R. Wood 298 PREM 57336 sin loc. on *Chrysanthemoides monilifera* ssp. *monilifera*, 16 Mar. 2003, A. R. Wood 509 PREM 57892N of Ceres, Gydo Pass, 33 14 S 19 20 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 9 Feb. 2003, A. R. Wood 504 PREM 57891 Cape Ton, Kirstenbosch National otanic Garden, 34 00 S 18 24 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 2 May 2003, A. R. Wood 514 PREM 57893E of Paarl, ains Kloof Pass, Steenbo Par, 33 32 S 19 08 E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 16 May 2003, A. R. Wood 519 PREM 57896 12 m from Cango Mountain Resort on road to De Rust, 33 24 S 22 22 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 19 Mar. 1998, A. R. Wood 86 PREM 56819Sartberg Pass, 33 21 S 22 06 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 20 Mar. 1998, A. R. Wood 85 PREM 568182 m E of Gouritz River along N2, 34 12 S 21 46 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, A. R. Wood 77 PREM 568101 m W of Albertinia, 34 12 S 21 34 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, A. R. Wood 78 PREM 56811W of Heidelberg, Nieershe Suurbraaintersection on N1, 34 05 S 20 40 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, A. R. Wood 79 PREM 56812E of Sedgeeld, road to Karatara, 34 01 S 22 51 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, A. R. Wood 80 PREM 56813N of

Knysna, road to Noetzie, 34 03 S 23 07 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, A. R. Wood 81 PREM 56814S of Calitzdorp, Rooiberg Pass, 33 40 S 21 38 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 3 Feb. 2000, A. R. Wood 91 PREM 56824W of Heidelberg, 2 m NE of Duienhos River along road to GysmanshoePass, 33 58 S 21 00 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 21 Dec. 2000, A. R. Wood 299 PREM 57335 SW of Knysna, beteen ellvidere and rentononSea, 34 05 S 23 00 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 3 un. 2002, A. R. Wood 376 PREM 57887 2 m W of Gouritz R. ridge along N2, 34 12 S 21 44 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 un. 2002, A. R. Wood 380 PREM 57888Tsitsiamma National Par, 34 01 S 23 53 E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 28 May 2003, A. R. Wood 524 PREM 57897S of Calitzdorp along road to Hot Springs at unction ith road to Rooiberg Pass, 33 21 42 E, on *Chrysanthemoides monilifera* ssp. *subcanescens*, 3 Feb. 2000, A. R. Wood 90 PREM 56823N of Milnerton, beteen the ocean and Rietvlei, 33 50 S 18 29 E, on *Chrysanthemoides incana*, 9 Sep. 1998, A. R. Wood 64 PREM 56797 sin loc. on *Chrysanthemoides incana*, 13 ul. 2003, A. R. Wood 525 PREM 57898N of Melbostrand, Koeberg Nature Reserve, 33 40 S 18 26 E, on *Chrysanthemoides incana*, 12 un. 1997, A. R. Wood 62 PREM 56795 sin loc. 9 Sep. 1998, A. R. Wood 63 PREM 56796Simons Ton, Red Hill Pass, 34 11 S 18 25 E, on *Chrysanthemoides undescrbed taon*, 14 ul. 1998, A. R. Wood 87 PREM 56820beteen ermaaliheid and Stillbaai, 34 20 S 21 02 E, on *Chrysanthemoides undescrbed taon*, 9 Oct. 1998, A. R. Wood 88 PREM 56821Stillbaai, 34 23 E 21 25 E, on *Chrysanthemoides undescrbed taon*, 9 Oct. 1998, A. R. Wood 89 PREM 56822 Cape Peninsula, 4.7 m NW of Cape Point Nature Reserve, 34 13 S 18 24 E, on *Chrysanthemoides undescrbed taon*, 2 May 2003, A. R. Wood 515 PREM 578945 m W of Arniston, 34 38 S 20 11 E, on *Chrysanthemoides undescrbed taon*, 19 Oct. 2003, A. R. Wood 530 PREM 57899Stellenbosch, onershoeNature Reserve, 33 59 S 18 59 E, on *Osteospermum ciliatum*, 18 Sep. 1994, A. R. Wood 93 PREM 579032 m S of Simons Ton, 34 13 S 18 28 E, on *Osteospermum ciliatum*, Aug. 1996, A. R. Wood 59 PREM 56856 sin loc. on *Osteospermum ciliatum*, 3 Oct. 1996, A. R. Wood 56 PREM 56852 sin loc. on *Osteospermum ciliatum*, 7 ul. 1997, A. R. Wood 60 PREM 56857E of redasdorp, De Hoop Nature Reserve, near Potberg Education Centre, 34 23 S 20 32 E, on *Osteospermum potbergense*, ul. 1995, A. R. Wood 57 PREM 56853 sin loc. on *Osteospermum potbergense*, 24 un. 1998, A. R. Wood 58 PREM 56855 sin loc. on *Osteospermum potbergense*, un. 2002, A. R. Wood 374 PREM 57904N of McGregor, oesmans Pass, on *Osteospermum polygaloides*, 14 Aug. 1976, L. Hugo 786 NG Sartberg Pass, 33 19 S 22 02 E, on *Osteospermum polygaloides*, 21 Mar. 1998, A. R. Wood 49 PREM 57900 sin loc. on *Osteospermum polygaloides*, 18 un. 2002, A. R. Wood 383 PREM 57902Caledon, Caledon Wild Floer reserve, 34 13 E 19 25 E, on *Osteospermum polygaloides*, 1 Aug. 1999, A. R. Wood 151 PREM 57901.

Notes. *Chrysanthemoides incana*, *C. monilifera* ssp. *canescens*, *C. monilifera* ssp. *pisifera*, *C. monilifera* ssp. *rotundata*, *C. monilifera* ssp. *subcanescens*, *Osteospermum ciliatum*, *O. polygaloides* and *O. potbergense* are all nehost records for this rust fungus.



Figs 10–17. *Endophyllum dimorphothecae*. Fig. 10. Aecidioid telia on a itchesbroom on *Dimorphotheca cuneata*. Figs 11–12. Peridial cells of an aecidioid telium. Figs 13–14. Germinated aecidioid teliospore with vesicellie modied basidiospores. Figs 15–17. Aecidioid teliospores. Figs 10–12 10 mm, 13–14 25 mm, 15–17 10 mm.

*Endophyllum dimorphothecae* A. R. Wood Crous, sp. nov. Figs 10–17

Etym. Named after the host plant.

*Pycnia amphigena*, plerumque hypophylla, dispersa, prius auctore telia aecidioida apparentia, 135–200 mm diam. Telia

*aecidioida amphigena*, plerumque hypophylla, dispersa, aurantiaca, cylindrica, ad 0.4 mm alta, 135–200 mm diam. Cellulae peridiales forma variabili, oblongae vel sub rhomboideae, 17–23–26–35 r 12–17–19–23 mm paries externus striatus, 5–7–8–9 mm crassus, paries internus verrucosissimus, 2–3–4–5 mm crassus. Teliosporae aecidioidae irregulariter angulares, globosae vel ellipsoideae,

16–20–21–28 r 12–16–17–22 mm, ratio longitudo latitudo 1.1–1.85 paries hyalinus, verruculosus, 1–2 mm crassus, apendistinctus vel modice inspissatus ad 1.5–2 mm germinantes 2–4 basidiosporas vesiculiformes proferunt.

Typus South Africa Western Cape Province between Laingsburg and Ladismith, Anysberg Nature Reserve, 33 27 S 20 42 E, on *Dimorphotheca cuneata*, 14 Sep. 2003, A. R. Wood 527 PREM 57921 – holotypus KM122450, PUR N4781 – isotypi.

Infections systemic, causing itchesbrooms. Pycnia amphigenous but mostly hypophyllous, scattered unevenly, not in lesions, preceding the aecidioid telia 135–200 mm diam as shaped with ostiolar trichomes. Aecidioid telia amphigenous but mostly hypophyllous, scattered unevenly, not in lesions aecidioid, orange, cylindrical, up to 0.4 mm high, 135–200 mm diam, peridial margin hite, reeed, incised. Peridial cells rmly oined together, irregular oblong to subrhomboid, 17–23–26–35 r 12–17–19–23 mm outer all striate, 5–7–8–9 mm thic inner all coarsely verrucose, 2–3–4–5 mm thic. Aecidioid teliospores irregular angular globose to ellipsoid, 16–20–21–28 r 12–16–17–22 mm, length:width ratio 1.1–1.85 spore all hyaline, verruculose, 1–2 mm thic, apex indistinct or apeslightly thickened, 1.5–2 mm thic upon germination producing 2–4 vesiclelike modified basidiospores.

Additional specimens examined South Africa Northern Cape Province S of Sutherland, summit of Erlateloo Pass, 32 31 S 20 38 E, on *Dimorphotheca cuneata*, 16 June 2002, A. R. Wood 381 PREM 57918 SW of Sutherland, Roggeveld escarpment, farm lesfontein, 32 27 S 20 26 E, on *Dimorphotheca cuneata*, 16 June 2002, A. R. Wood 382 PREM 57920 sin. loc. on *Dimorphotheca cuneata*, 23 Feb. 2003, A. R. Wood 507 PREM 57920, PUR N4778 SW of Sutherland, Roggeveld escarpment, farm oplaas, 32 31 S 20 24 E, on *Dimorphotheca cuneata*, 23 Feb. 2003, A. R. Wood 505 PREM 57919, KM122451.

*Endophyllum elytropappi* Henn. A. R. Wood Crous, comb. nov. Figs 18–23

asionym *Aecidium elytropappi* Henn., *Hedigia* 37: 294 1898.

Infections systemic, causing fusiform stem galls up to 5 cm long or itchesbrooms depending on host. Pycnia not seen. Aecidioid telia on stems, closely crowded, not in lesions aecidioid, orange, cylindrical, up to 2 mm long, 225–275 mm diam peridium extending well beyond epidermis, peridial margin hite, not reeed, not or shallowly incised. Peridial cells irregular oblong to subrhomboid, 21–28.5–30–39 r 15–21–22–30 mm outer all striate, 10–13–14–17 mm thic inner all coarsely verrucose, 4–5–7 mm thic. Aecidioid teliospores irregular angular globose, ovate to ellipsoid, 21–25.5–26–32 r 16–20.5–21–25 mm, length:width ratio 1.1–1.81 spore all hyaline, verruculose, 1.5–2–3 mm thic apeslightly thickened with a distinct germ pore,

2–3–5 mm thick upon germination producing 3–4 vesiclelike modified basidiospores.

Specimens examined South Africa Eastern Cape Province W of Patensie, Aviaansloof Wilderness Area, ergplaas plateau, 33 37 S 24 28 E, on *Elytropappus rhinocerotis*, an. 2000, A. R. Wood 168 PREM 57913 Northern Cape Province SW of Sutherland, farm lesfontein, 32 27 S 20 26 E, on *Elytropappus rhinocerotis*, 23 Feb. 2003, A. R. Wood 506 PREM 57917, KM122448, PUR N4777 Western Cape Province of River, on *Elytropappus rhinocerotis*, Nov. 1896, Schlechter s.n. KM110309 – isotype hills above Darling, 33 22 S 18 22 E, on *Elytropappus rhinocerotis*, 1 an. 1999, A. R. Wood 130 PREM 57912, KM122447, PUR N4775N of Wellington, Elandsberg Nature Reserve, 33 27 S 19 04 E, on *Elytropappus rhinocerotis*, 2 Feb. 2003, A. R. Wood 501 PREM 57914 near Kuils River, border of farms Kaapzicht and eenacht in the estates, 33 56 S 18 44 E, on *Elytropappus rhinocerotis*, 5 Feb. 2003, A. R. Wood 502 PREM 57915 between Laingsburg and Ladismith, Anysberg Nature Reserve, 33 28 S 20 35 E, on *Elytropappus rhinocerotis*, 12 Sep. 2003, A. R. Wood 528 PREM 57916 NE of Paarl, near old tunnel along Du Toits Pass, 33 44 S 19 09 E, on *Stoebe plumosa*, 18 ul. 1997, A. R. Wood 371 PREM 57908 Stellenbosch, an Marais Nature Reserve, 33 55 S 18 53 E, on *Stoebe plumosa*, 4 Dec. 2000, A. R. Wood 257 PREM 57905 sin. loc. on *Stoebe plumosa*, 23 May 2002, A. R. Wood 372 PREM 57909N of George, Montague Pass, near Toll House, 33 54 S 22 25 E, on *Stoebe plumosa*, 25 ul. 2001, A. R. Wood 336 PREM 57906 George, 2 m E of Outeniqua Nature Reserve offices, towards Tierkop, 33 55 S 22 26 E, on *Stoebe plumosa*, 4 un. 2002, A. R. Wood 377 PREM 57910 Stellenbosch, onershoe Plantation, 33 58 S 18 56 E, on *Stoebe plumosa*, 5 Feb. 2003, A. R. Wood 503 PREM 57911.

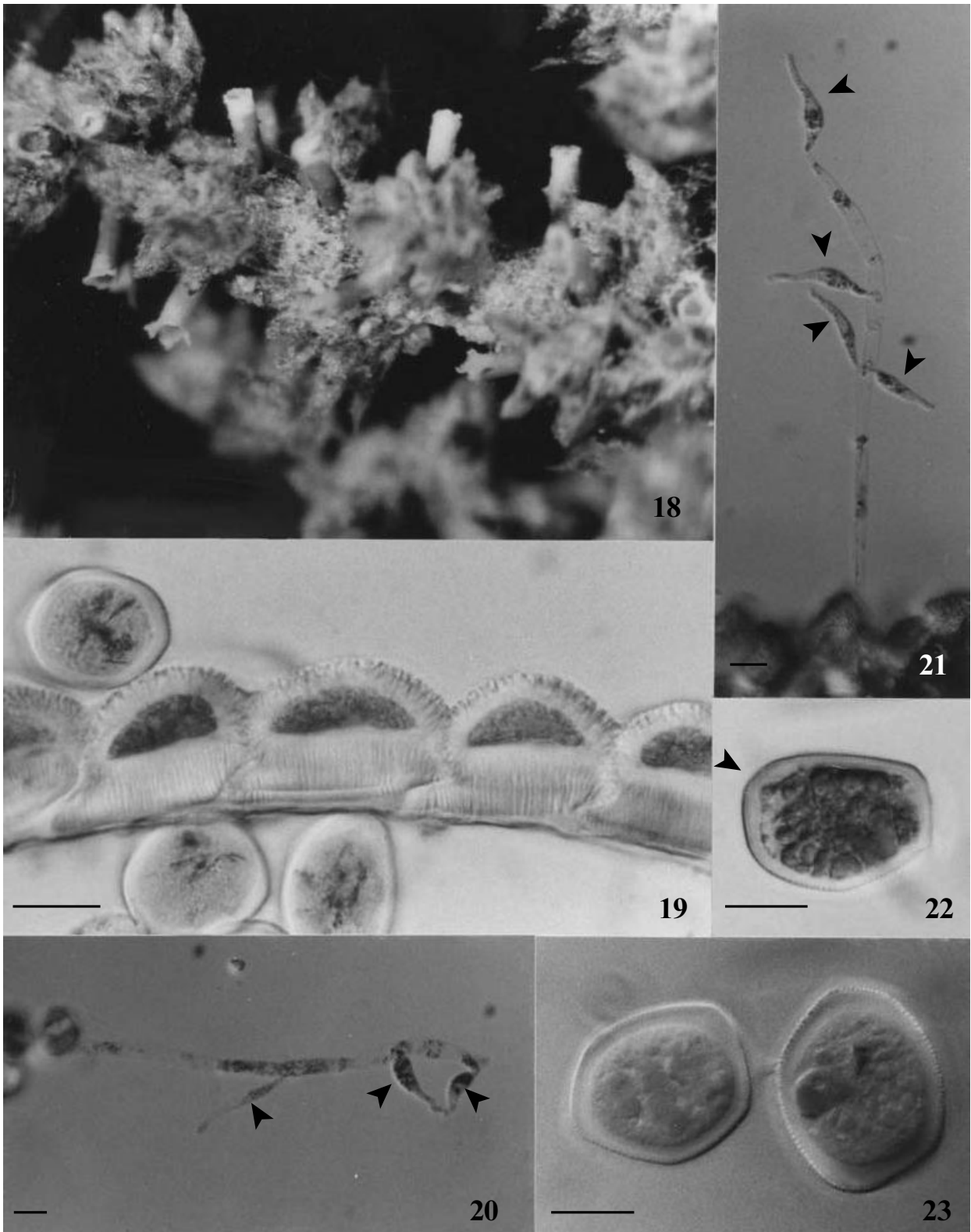
Note. *Stoebe plumosa* is a neohost record for this fungus.

*Aecidium metalasiae* Syd. P. Syd., *Annls Mycol.* 10: 35 1912. Figs 8–9

Infections systemic, causing fusiform galls on host stem, up to 2 cm long. Pycnia not seen. Aecia closely crowded on galls, deeply immersed aecidioid, orange, cylindrical, up to 2 mm long, approx. 200 mm diam peridial margin hite, not reeed, not or shallowly incised. Peridial cells rmly oined together, irregular oblong to subrhomboid, 23–25–27–30 r 13–18–21–28 mm outer all striate, 5–6–7–10 mm thic inner all coarsely verrucose, 2–3–4–5 mm thic. Aeciospores irregular ovate to ellipsoid to angular globose, orange, 21–24–26–32 r 18–20–22–24 mm, length:width ratio 1.04–1.52 spore all hyaline, verruculose, 3–4–5 mm thic, germination not observed.

Specimen examined South Africa Western Cape Province Cape Ton, Lions Head, on *Metalasia* sp., Dec. 1908, I Pole Evans s.n. PREM 686 – holotype.

Notes. The host as originally identified as *M. muricata*. Since the original report the concept of this plant species has changed, and this epithet is no applied to a

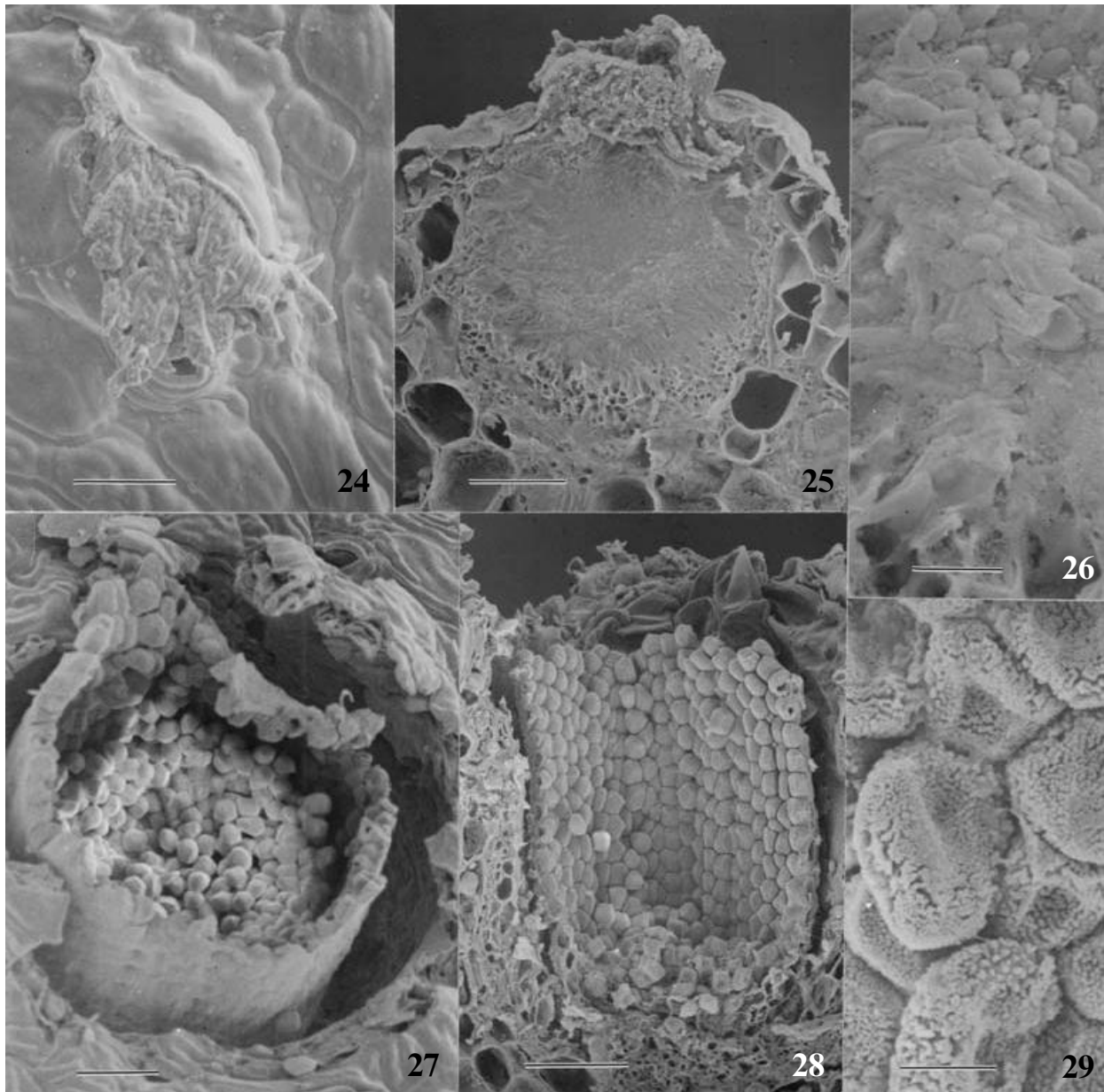


Figs 18–23. *Endophyllum elytrpappi*. Fig. 18. Aecidioid telia on a itchesbroom on *Stoebe plumosa*. Fig. 19. Peridial cells of an aecidioid telium. Figs 20–21. Germinated aecidioid teliospore with four vesicle-like modified basidiospores. Fig. 22. Aecidioid teliospore showing a distinctly thickened apical germ pore. Fig. 23. Two aecidioid teliospores. Scale bars = 10 μm.

plant that is restricted to coastal sands. Furthermore, it does not occur on mountain slopes Karis 1989, which is the habitat at the type locality of *A. metalasiae*. It is possible, therefore, that the host is *M. densa*, a common

and widespread species that is non from the type locality of *A. metalasiae* Karis 1989. However, as the type specimen has no others its identity remains uncertain.





Figs 24–29. *Endophyllum osteospermi* on *Chrysanthemoides monilifera* ssp. *monilifera*. Fig. 24. Surface view of the epidermis of a leaf showing the emerging eous hyphae, pycniospores and honeycomb of a pycnium. Fig. 25. Transverse section through a pycnium. Fig. 26. Enlarged view of transverse section through a pycnium showing pycniospores and pycniosporophores embedded in a mucous matrix. Fig. 27. Surface view of the epidermis of a leaf showing an aecidioid telium. Fig. 28. Transverse section through an aecidioid telium, showing peridium and some aecidioid teliospores. Fig. 29. Aecidioid teliospores. Bars: 1 30  $\mu$ m, 25 40  $\mu$ m, 26 7  $\mu$ m, 27 50  $\mu$ m, 28 68  $\mu$ m, 29 10  $\mu$ m.

**Key to *Endophyllum* spp. with vesiculate modified basidiospores occurring on tannin Asteraceae**

- 1 Peridermium extends just beyond host epidermis usually no more than 0.5 mm, and rarely up to 1 mm, aecidioid teliospore all 1–2.5 mm thick. . . . . 2
- Peridermium extends well beyond host epidermis to 3 mm, aecidioid teliospore all 1.5–5 mm thick. . . . . 3
- 2 1 Aecidioid teliospore all 1–2–2.5 mm thick, apically thickened 2–4 mm thick outer all of peridial cells 7–15 mm thick . . . . . *osteospermi*
- Aecidioid teliospore all 1–2 mm thick, apically indistinct or slightly thickened 1.5–2 mm thick outer all of peridial cells 5–9 mm thick . . . . . *dimorphothecae*
- 3 1 Aecidioid teliospore all 1.5–2–3 mm thick, apically thickened 2–3–5 mm thick outer all of peridial cells 10–17 mm thick . . . . . *elytropappi*
- Aecidioid teliospore all uniformly 3–4–5 mm thick, no distinct apical thickening outer all of peridial cells 5–10 mm thick . . . . . *metalasiae*

Table 2. Measurements of *Endophyllum osteospermi* acidioid teliospores from various localities.

Locality	Host <sup>a</sup>	PREM no.	lr min.–ma.	<sup>b</sup>	lr 95 conf. lim.	<sup>c</sup>
Lions Head, Cape Ton holotype	C.m.m.	6587	19–28r	15–21	22–24r	17–19
Gydo Pass, Ceres	C.m.m.	57891	18–26r	15–22	22–23r	18–19
HouHoePass, ot River	C.m.m.	57895	19–24r	16–21	21–22r	18–19
Kirstenbosch, Cape Ton	C.m.m.	57893	18–25r	16–20	21–22r	17–18
Simons Ton	C.m.m.	56799	23–39r	18–34	30–32r	25–27
Paarl	C.m.m.	56801	20–27r	15–24	22–23r	19–20
De Hoop Nature Reserve	C.m.m.	56808	22–30r	18–23	24–25r	20–21
Grahamston	C.m.p.	56815	19–29r	14–20	21–22r	17–18
Heidelberg	C.m.p.	56812	17–27r	16–22	21–22r	18–19
Jeffrey's Bay	C.m.p.	56816	19–27r	14–22	21–22r	17–18
Heidelberg	C.m.p.	57333	18–31r	14–20	21–22r	17–18
Sedgeeld	C.m.p.	56813	18–29r	16–21	22–23r	17–18
Cathedral Pea	C.m.c.	56794	22–29r	16–25	24–25r	19–20
Calitzdorp	C.m.s.	56823	20–27r	16–22	23–24r	19–20
Stilbaai	C.sp.	56822	18–24r	14–22	21–22r	18–19
Koeborg Nature Reserve	C.i.	56795	20–28r	16–24	24–25r	20–20
Stellenbosch	O.c.	57903	19–25r	14–19	21–23r	16–18
Sartberg Pass	O.p.	57900	18–26r	15–23	20–22r	17–18

<sup>a</sup> C.m.m., *Chrysanthemoides monilifera* ssp. *monilifera* C.m.p., ssp. *pisifera* C.m.s., ssp. *subcanescens* C.m.c., ssp. *canescens* C.sp., *Chrysanthemoides* sp. C.i., *C. incana* O.c., *Osteospermum ciliatum* O.p., and *Osteospermum polygaloides*.

<sup>b</sup> Minimum and maximum range of length and width of 50 spores from each locality.

<sup>c</sup> 95 confidence limits of the average length and width of 50 spores from each locality.

## RESULTS

### Morphology

In all specimens studied, fresh acidioid teliospores germinated to produce metabasidia with two to four vesicle-like modified basidiospores. These basidiospores are not dispersed (Figs 4–5, 13–14, 20–21). *Endophyllum osteospermi* and *E. dimorphothecae* predominantly produced two basidiospores, whereas *E. elytropappi* predominantly produced four basidiospores.

The pycnia of *E. osteospermi* and *E. dimorphothecae* are morphologically typical of type 4 pycnia (Hiratsua Cummins 1963), being defined as subepidermal, with determinate growth and a strongly convoluted hymenium (Figs 24–26). Pycnia were not observed on *E. elytropappi*, but may well be produced. The acidioid telia (Figs 27–28) and acidioid teliospores (Fig. 29) of all four species observed are morphologically typical of the *Aecidium* type of aecia (Sato Sato 1985) and aeciospores. The surface ornamentation of the acidioid teliospores corresponds to the verrucose type of Sato Sato 1982, and type 1 of Savile 1973. This latter type is defined as having uniformly small, or a mixture of small and large arts verruculae, and with no pore plugs or refractile granules. The verruculae in these four species are all uniformly small (Fig. 29). There is some variation in acidioid teliospore dimensions from different specimens collected throughout the non range of *E. osteospermi* (Table 2). This variation is, however, not associated with geographic location. Acidioid teliospores from drier localities are slightly smaller than those from wetter localities. These types of pycnia, acidioid telia and acidioid teliospore morphologies are typical of the Pucciniaceae (Cummins Hiratsua 2003).

Although the four species of *Endophyllum* treated here are morphologically very similar, they can be distinguished according to the accompanying ey.

### Molecular analysis

Except at one locality (Cape Peninsula), all sequences obtained from each locality were identical to the others from that locality. Both parsimony and neighbour joining analysis supported the distinction of three clades, one for each of the species *Endophyllum dimorphothecae*, *E. elytropappi* and *E. osteospermi* (Figs 30–31). The sequence obtained from the rust fungus on the host plant *Osteospermum polygaloides* fitted within the *E. osteospermi* clade, confirming the morphological conclusion. Attempts were made to sequence DNA extractions from herbarium specimens of *Endophyllum* on this and other *Osteospermum* species. Unfortunately, none of these attempts yielded sequences of sufficient quality to include in the analysis.

## DISCUSSION

Prior to this work, published distribution records of *Endophyllum osteospermi* consisted only of two specimens on *Chrysanthemoides monilifera* ssp. *monilifera* (Doide 1950; Morris 1982) recorded populations on *C. monilifera* ssp. *monilifera* from the Cape Peninsula (Constantia), and in the Stellenbosch, Franschhoek and Illiersdorp districts. Unfortunately no voucher specimens from these localities were preserved. Furthermore, these records are all in the inter rainfall areas in the eastern part of the Western Cape Province, South Africa. The above specimens listed

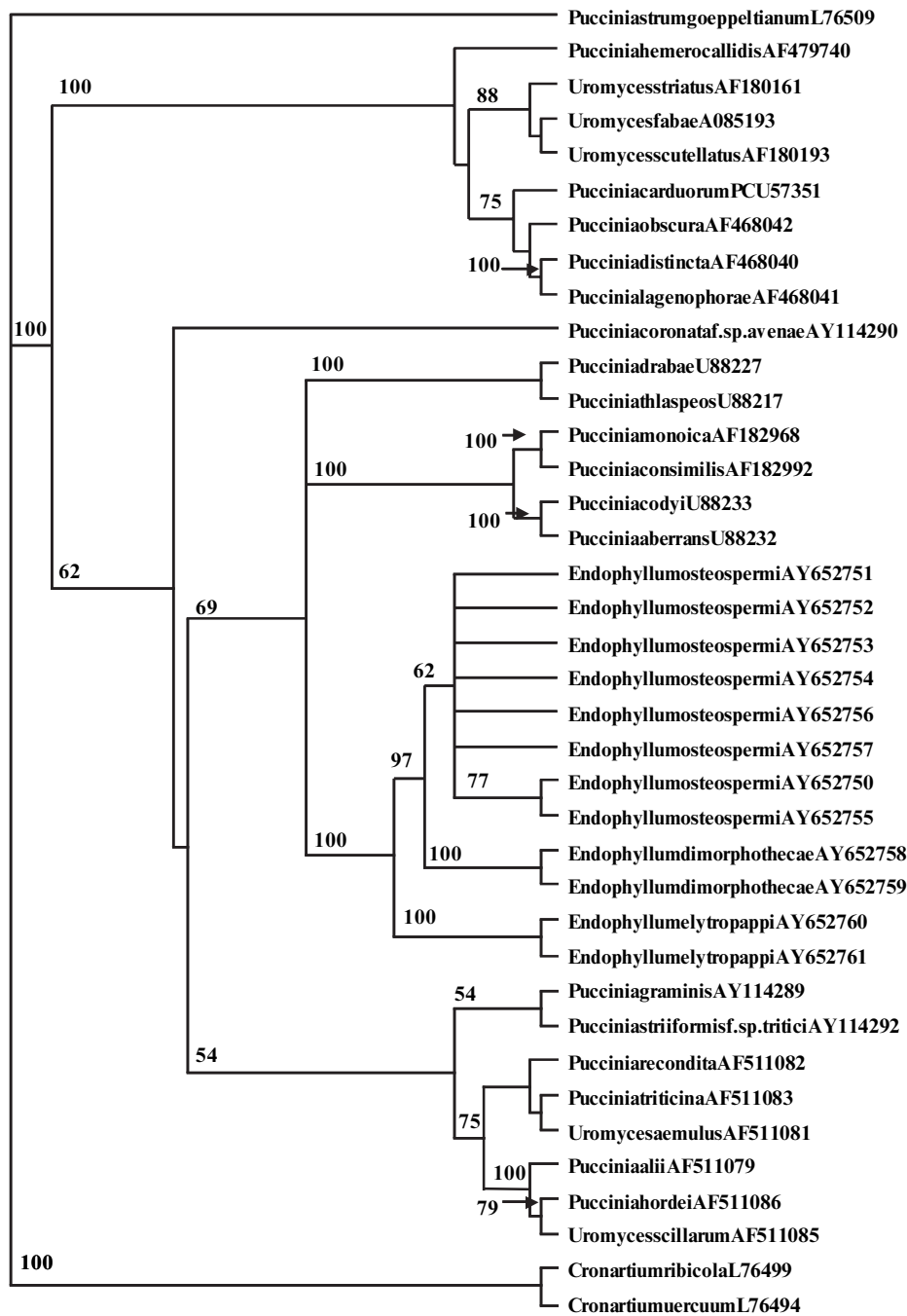


Fig. 30. Strict consensus of 167 most parsimonious trees of the ITS1–5.8S–ITS2 rDNA region of *Endophyllum dimorphothecae*, *E. elytropappi*, and *E. osteospermi*. Bootstrap support values 1000 replicates are shown at the nodes.

therefore represent a range extension of approximately 1300 m eastwards. They also indicate that *E. osteospermi* is not restricted to an inter-rainfall region. All forms of *Chrysanthemoides* are probably susceptible to this rust fungus, but forms that occur in semi-arid regions, or arid humid regions, possibly avoid infection due to a climate unfavourable to infection by *E. osteospermi* Wood, Crous & Lennox 2004.

In addition to *Chrysanthemoides* species, *E. osteospermi* is also found on several species of *Osteospermum*, which are closely related to *Chrysanthemoides*. It appears that *O. potbergense* is not a normal host of *E. osteospermi*, as itchesbrooms are only found on

plants occurring amongst bushes of *C. monilifera* on which *E. osteospermi* is abundant Wood & Nordenstam 2004. The aecidoid telia are not observed to mature and open on *O. potbergense*, neither are itchesbrooms found on plants further away from infected *C. monilifera*. A similar situation occurred in *O. ciliatum* at one locality 2 m S of Simons Ton, but at another locality no infected *C. monilifera* plants were found near infected *O. ciliatum* plants on the shoe Nature Reserve. At both these sites the aecidoid telia matured and opened normally. Where infected *O. polygaloides* plants were found, no infected *C. monilifera* plants were found nearby at least

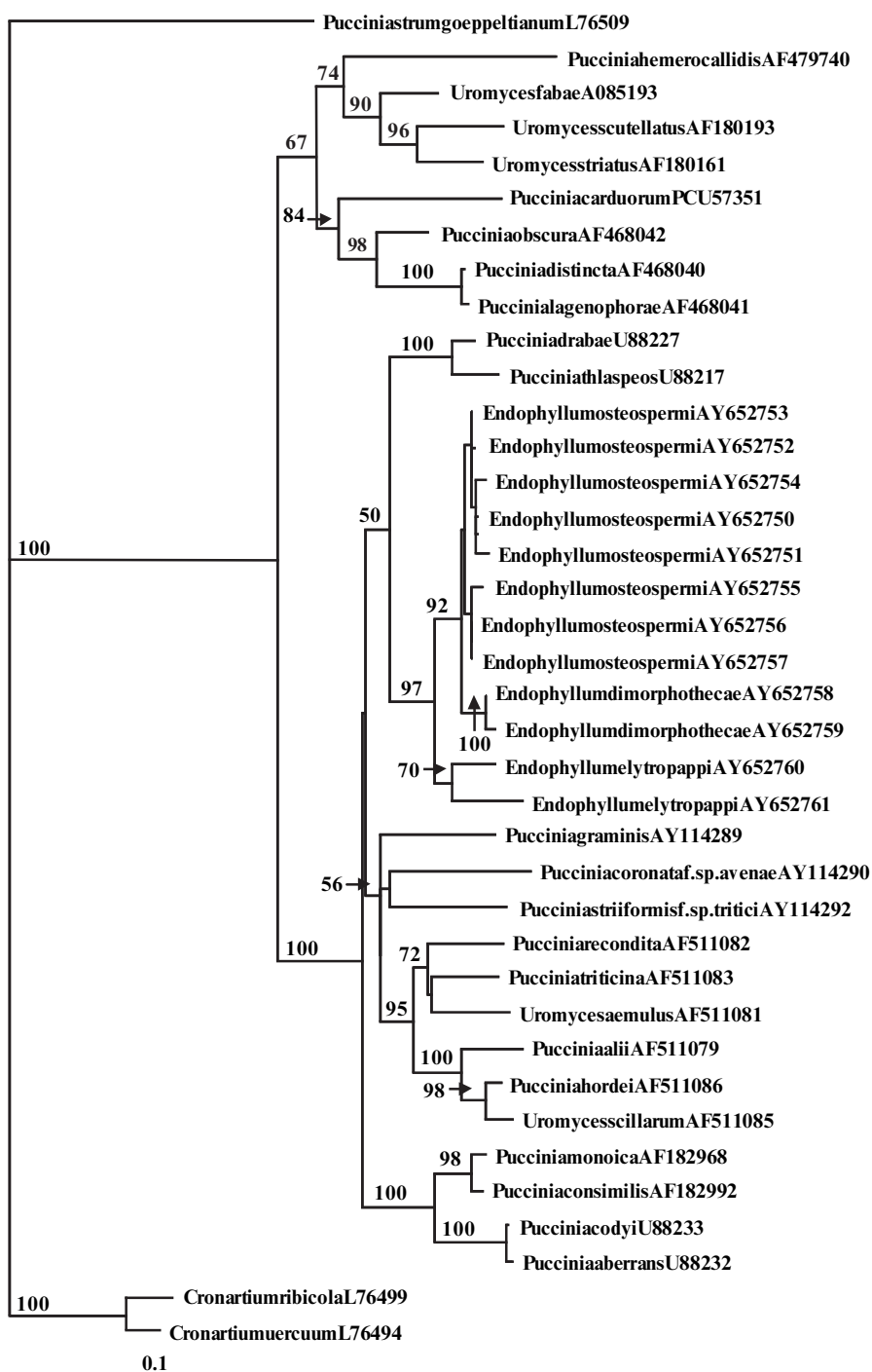


Fig. 31. Neighbouring tree of the ITS1–5.8S–ITS2 rDNA region of *Endophyllum dimorphothecae*, *E. elytropappi*, and *E. oteospermi*. Bootstrap support values 1000 replicates are shown at the nodes.

within 1 m. Therefore, *O. ciliatum* and *O. polygaloides* can be considered as true hosts of this rust fungus. Sequence data obtained from *O. polygaloides* confirmed that the species infecting this plant is indeed *E. oteospermi*. No other species of *Osteospermum* species were found to be infected with *E. oteospermi*.

Recent field observations on the morphology of cypselas of various species of *Osteospermum* indicate that the single character of the fleshy pericarp surrounding the cypselas used to distinguish *Chrysanthemoides*

from *Osteospermum* (Norlindh 1943) is insufficient to distinguish between these two genera, and that the status of *Chrysanthemoides* must be reexamined (Wood & Nordenstam 2004). The above-mentioned hosts of *E. oteospermi* may possibly be congeneric, depending on the revised status of *Chrysanthemoides*.

No other host plants of *E. oteospermi* are known, despite many observations made over the years on related plants. The rust fungus on *D. cuneata*, also in the *Calendulae*, proved to be distinct, and as therefore described as a new species, *E. dimorphothecae*. This

host plant is ecologically distinct from *C. monilifera*, occurring in areas much drier than those here *C. monilifera* Gros Norlindh 1943. The molecular data supported the distinction between *E. osteospermi* and *E. dimorphothecae*, also suggesting that these two species possibly shared a common ancestor in the recent past. All other asteraceous plants found in South Africa that are hosts of *Endophyllum* species, are host to *E. elytrappi* *Elytropappus* and *Stoebe* spp. or *E. macoanum* *Conyza scabrida* Wood 2005. The only other species of rust fungus found in South Africa that caused itchesbrooms on asteraceous hosts as an undescribed species of *Uromyces* on species of *Euryops* *Senecioneae* A.R.W., unpubl. Unfortunately, no specimens of *A. metalasiae* were collected, despite a number of surveys made to try and recollect it. The type locality is in the same area Lions Head, Cape Town from which the type of *E. osteospermi* also originated. The latter species, however, as found to still be present in that area, as is the host plant *Metalasia densa*. *Aecidium metalasiae* is morphologically very similar to *E. elytrappi*, but is readily distinguished by its thicker, aecidioid teliospores, and is therefore maintained as a separate species. However, it may be only an aberrant specimen of *E. elytrappi*. If this species does turn out to be a distinct species, it may also possess an endocyclic life cycle.

A morphological development pattern and nuclear cycle similar to that of the aecidioid teliospores of *E. osteospermi* has been reported for a number of other rust fungi, including *Prospodium transformans* Shuttleworth 1953, *Puccinia vitata* Gardner 1988, and *Racospermyces oasyn* *Atelocauda oasyn* Gardner 1981, Chen, Gardner Webb 1996, Waler 2001. *Aecidium metalasiae* modified basidiospores appear to be rare amongst rust fungi. This, together with the morphology and molecular data presented here indicates that these species form a phylogenetically closely related complex.

In South Africa *E. osteospermi* is naturally restricted to a small group of closely related plant hosts in the tribe *Calenduleae*. The *Calenduleae* only occur in Africa and the Mediterranean Norlindh 1943, and therefore no closely related plants occur naturally in Australia. This rust species is therefore considered to be a candidate agent for the biological control of *C. monilifera* ssp. *monilifera*, due to both its restricted natural host range and its negative impact on growth and reproduction Wood 2002 of *C. monilifera* ssp. *monilifera*. Pending host specificity testing of a range of asteraceous plants, this rust fungus would most likely be safe to introduce into Australia.

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