

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 was 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* was undertaken. Based on these results, *E. osteospermi* was recorded on *C. monilifera* ssp. *monilifera*, *pisifera*, *rotundata*, *canescens*, and *subcanescens*, *C. incana*, and an undescribed taxon. *E. osteospermi* was 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 rhinoceros* 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 & Baxter 2000). Witches' brooms 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 growing in the field (Wood 2002), which in severe cases can lead to plant death (Morris 1982).

C. monilifera ssp. *monilifera* has become naturalised in south-eastern Australia where 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 fire in the absence of natural enemies (Parsons & Cuthbertson 1992). A biocontrol programme targeting these plant has been initiated, as part of which *E. osteospermi* is considered to be a potential biological control agent (Scott & Adair 1995, Adair & Edwards 1996).

Records of the distribution of *E. osteospermi* are limited to two specimens deposited at the South African National Collection of Fungi (PREM; ARC-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 risks 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, six subspecies of *Chrysanthemoides monilifera* are recognised (Norlin 1943): ssp. *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 five of the recognised subspecies were observed for the presence of witches' brooms, 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 witches' brooms 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 known as *Aecidium elytropappi*, and in addition to several collections on this host in South Africa (Doidge 1950), it has also been recorded from *Stoebe kilimandsharica* 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* was found, namely *Endophyllum macowanianum* on *Conyza scabrata* (*Astereae*) (Wood 2004). This species is morphologically distinct, is not systemic causing galls or witches' brooms, produces typical basidiospores (as opposed to the vesicle-like 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, Biolab, 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 Zeiss Axioskop light microscope and photographed using a Zeiss MC63 camera.

Fresh or dried aecidioid telia were mounted in Jung Tissue Freezing Medium™ (Leica Instruments, Nußloch, Germany), and 10 µm 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. elytropappi* 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 witches' brooms, these were removed from the plant material and used for DNA extraction. Where the above was not possible, parts of developing witches' brooms 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 (Bel-Art Products, Pequannock, NJ). 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 °, then incubated in a water bath at 65 ° 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 & Bruns 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 & Bruns 1993). Between two and four PCR products were obtained for each locality, each from individual witches' brooms. The PCR products were separated by electrophoresis at 95 V for 1 h in a 1.5% (w/v) 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	GenBank accession no. ^a	PREM no.
<i>Endophyllum osteospermi</i>	<i>Chrysanthemoides monilifera</i> ssp. <i>monilifera</i>	Kirstenbosch National Botanic Garden, Cape Town	AY652755	57893
	<i>C. monilifera</i> ssp. <i>monilifera</i>	Houw Hoek Pass, Bot River	AY652752	57895
	<i>C. monilifera</i> ssp. <i>pisifera</i>	Gouritz River, Albertinia	AY652754	57888
	<i>C. monilifera</i> ssp. <i>pisifera</i>	Between Bellvidere and Brenton-on-Sea, Knysna	AY652753	57887
	<i>Chrysanthemoides</i> undescribed taxon	Cape Point	AY652750 & AY652751	57894
	<i>Chrysanthemoides incana</i>	Rietvlei, Milnerton	AY652756	57898
	<i>Osteospermum polygaloides</i>	Swartberg Pass, Prince Albert	AY652757	57902
<i>E. dimorphothecae</i>	<i>Dimorphotheca cuneata</i>	Verlatekloof Pass, Sutherland	AY652758	57918
	<i>Dimorphotheca cuneata</i>	Boplaas farm, Sutherland	AY652759	57919
<i>E. elytropappi</i>	<i>Elytropappus rhinocerotis</i>	Blesfontein farm, Sutherland	AY652761	57917
	<i>Elytropappus rhinocerotis</i>	Border of Kaapzicht and Zewenwacht wine estates, Kuils River	AY652760	57915

^a Alignment in TreeBASE 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[®] Extract 2-in-1 kit (Macherey-Nagel, Düren, Germany). The purified products were sequenced in both directions using the PCR primers and the cycle sequencing reaction was carried out as recommended by the manufacturer with an ABI PRISM Big Dye 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://www.ncbi.nlm.nih.gov>; Berthier *et al.* 1996, Roy *et al.* 1998, Vogler & Bruns 1998, Pfunder, Schürch & Roy 2001, Hernández, Palm & Castlebury 2002, Weber, Webster & Engel 2003, Chung, Tsukiboshi & Kakishima, unpubl., Szabo, unpubl.) using Sequence Alignment Editor v2.0a11 (Rambaut 2002), and manual adjustments for improvement were made where necessary. *Pucciniastrum goepeltianum* (L76509) was 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 was performed for the data set using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping 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 was performed using uncorrected ("p") 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 & Bull 1993). The trees were printed with TreeView Version 1.6.6 (Page 1996).

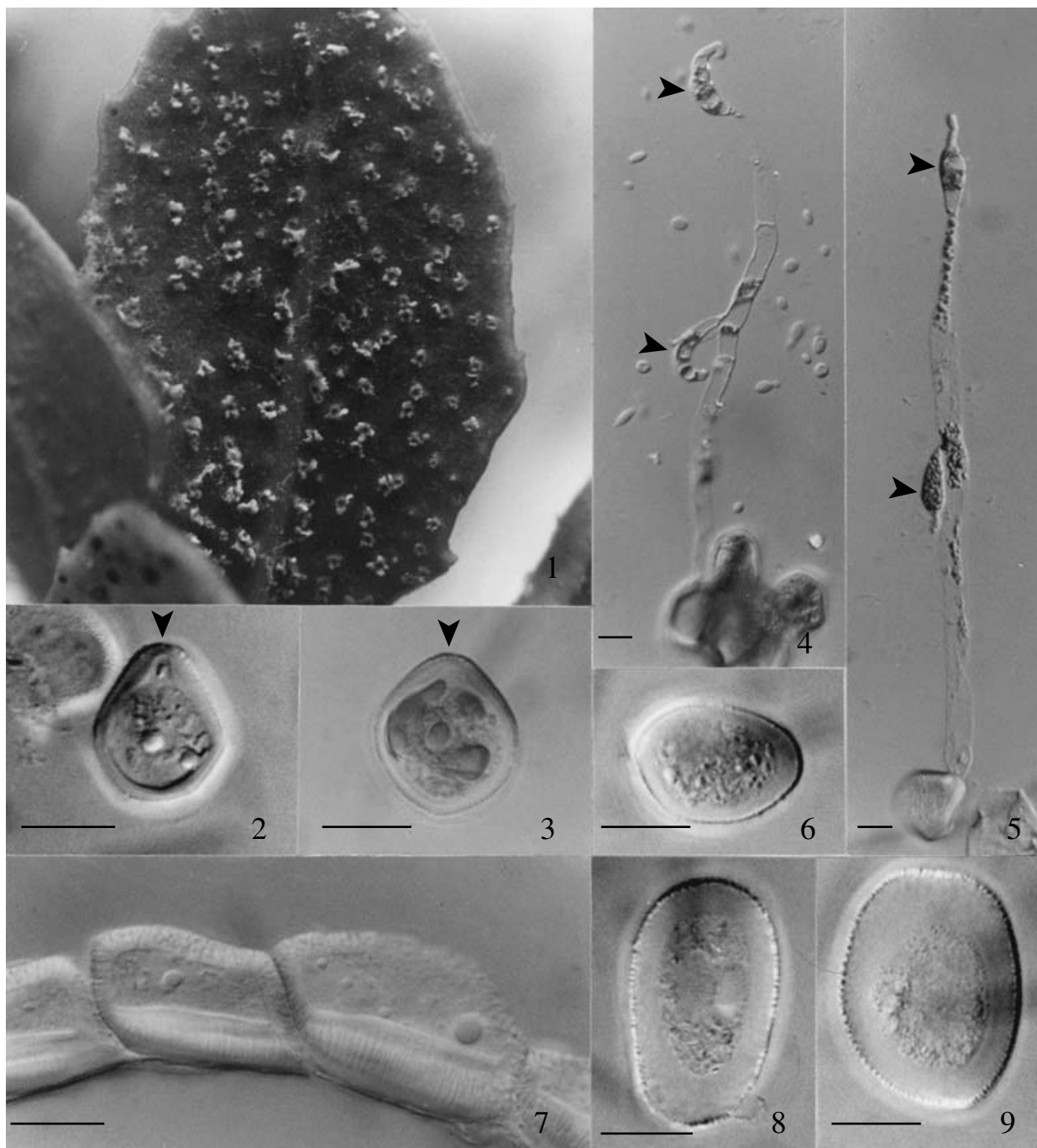
TAXONOMY

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

Basionym: *Aecidium osteospermi* Doidge, *Bothalia* **2**: 171 (1927).

Infections systemic, causing witches' brooms, host stems galled at base of witches' brooms. *Pycnia* amphigenous but mostly hypophyllous, scattered unevenly, not in lesions, preceding the aecidioid telia; 150–210 µm diam; flask-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 white, reflexed, deeply incised. *Peridial cells* firmly joined together, irregular oblong to sub-rhomboid, (19–)26–27.5(–39) × (12–)19–20(–29) µm; outer wall striate, (7–)10–11(–15) µm thick; inner wall coarsely verrucose, (3–)4(–6) µm thick. *Aecidioid teliospores* irregular angular-globose, ovate to ellipsoid, (18–)21.5–22(–28) × (14–)17–18(–23) µm, length/width ratio 1:1–1.75; spore wall hyaline, evenly verruculose, 1–2(–2.5) µm thick; apex slightly thickened with a distinct germ pore, 2–4 µm thick; upon germination producing 2(–4) vesicle-like modified basidiospores.

Specimens examined: **South Africa**: KwaZulu-Natal Province: Cathedral Peak, Monks Cowl 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.** Acceidoid telia on the abaxial surface of a leaf from a witches' broom on *Chrysanthemoides monilifera*. **Figs 2–3.** Acceidoid teliospore showing a distinctly thickened apex and germ pore. **Figs 4–5.** Germinated acceidoid teliospore with two vesicle-like modified basidiospores. **Fig. 6.** Acceidoid teliospore showing a distinctly thickened apex. **Fig. 7.** Peridial cells of an acceidoid telium. **Figs 8–9.** Acceidoid teliospore of *Accidium metalasiae*. Bar = 10 μ m.

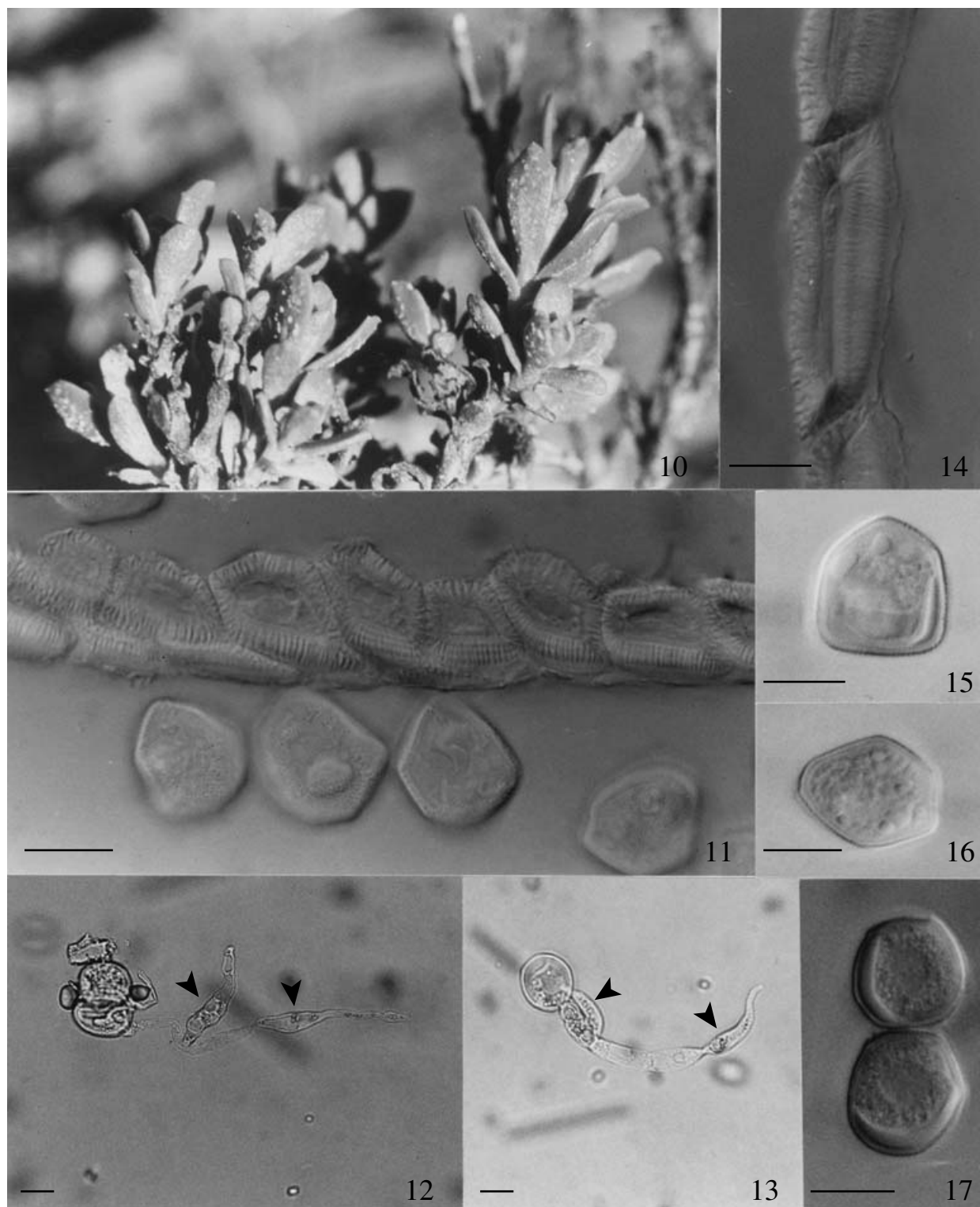
Eastern Cape Province: Grahamstown, 33° 18' S 26° 31' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 6 Oct. 1998, A. R. Wood 82 (PREM 56815); Jefferys Bay, Kabeljou River Nature Reserve, 33° 59' S 24° 56' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 7 Oct. 1998, A. R. Wood 83 (PREM 56816); 8 km W of Hankey/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, Zuurberg Pass, 25° 35' S 33° 19' E, on *Chrysanthemoides*

monilifera ssp. *pisifera*, 15 Feb. 2000, A. R. Wood 92 (PREM 56825); 3 km 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); Kidds Beach, 33° 09' S 27° 41' E, on *Chrysanthemoides monilifera* ssp. *rotundata*, 20 Aug. 2002, A. R. Wood 385 (PREM 57886); Kaysers Beach, 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 Lion's

Head, on *Chrysanthemoides monilifera* ssp. *monilifera*, 13 Nov. 1912, *W. J. Saxton* (PREM 6587, Holotype); Bredasdorp District, Mierkraal, on *Chrysanthemoides monilifera* ssp. *monilifera*, 16 Nov. 1927, *C. A. Smith* (PREM 22839); 2 km S of Simon's Town, 34° 13' S 18° 23' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 7 Jul. 1997, *A. R. Wood* 65 (PREM 56798); *sin loc.* on *Chrysanthemoides monilifera* ssp. *monilifera*, Oct. 1997, *A. R. Wood* 66 (PREM 56799); Paarl, Afrikaans Language Monument, 33° 46' S 18° 57' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 27 Jun. 1997, *A. R. Wood* 68 (PREM 56081); near old tunnel along N1, DuToit's Pass, 33° 44' S 19° 09' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 18 Jul. 1997, *A. R. Wood* 69 (PREM 56802); Bredasdorp, 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 Botanical Garden, 34° 13' S 19° 25' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 28. Sep. 1997, *A. R. Wood* 70 (PREM 56803); E of Bredasdorp, 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 56808); W of Bot River, Houw Hoek Pass, 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 57895); E of Bredasdorp, De Hoop Nature Reserve, 1 km S of homestead, 34° 28' E 20° 24' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 10 Dec. 1997, *A. R. Wood* 74 (PREM 56807); Hermanus, Fernkloof Nature Reserve, 34° 23' S 19° 15' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 16 Feb. 1998, *A. R. Wood* 71 (PREM 56804); 1.5 km N of Stellenbosch, Helshoogte, 33° 55' S 18° 54' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 7 Sep. 1998, *A. R. Wood* 72 (PREM 56805); Cape Town, Rhodes Memorial, 33° 58' S 18° 30' E, 16 on *Chrysanthemoides monilifera* ssp. *monilifera*, Aug. 1999, *A. R. Wood* 67 (PREM 56800); Somerset 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 57892); N 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 Town, Kirstenbosch National Botanic Garden, 34° 00' S 18° 24' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 2 May 2003, *A. R. Wood* 514 (PREM 57893); E of Paarl, Bain's Kloof Pass, Steenbok Park, 33° 32' S 19° 08' E, on *Chrysanthemoides monilifera* ssp. *monilifera*, 16 May 2003, *A. R. Wood* 519 (PREM 57896); 12 km 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 56819); Swartberg Pass, 33° 21' S 22° 06' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 20 Mar. 1998, *A. R. Wood* 85 (PREM 56818); 2 km 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 56810); 1 km W of Albertinia, 34° 12' S 21° 34' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, *A. R. Wood* 78 (PREM 56811); W of Heidelberg, Niekershek / Suurbraak intersection on N1, 34° 05' S 20° 40' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, *A. R. Wood* 79 (PREM 56812); E of Sedgfield, road to Karatara, 34° 01' S 22° 51' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, *A. R. Wood* 80 (PREM 56813); N of

Knysna, road to Noetzie, 34° 03' S 23° 07' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Oct. 1998, *A. R. Wood* 81 (PREM 56814); S of Calitzdorp, Rooiberg Pass, 33° 40' S 21° 38' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 3 Feb. 2000, *A. R. Wood* 91 (PREM 56824); W of Heidelberg, 2 km NE of Duiwenhoks River along road to Gysmanshoek Pass, 33° 58' S 21° 00' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 21 Dec. 2000, *A. R. Wood* 299 (PREM 57335); †SW of Knysna, between Bellvidere and Brenton-on-Sea, 34° 05' S 23° 00' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 3 Jun. 2002, *A. R. Wood* 376 (PREM 57887); †2 km W of Gouritz R. Bridge along N2, 34° 12' S 21° 44' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 5 Jun. 2002, *A. R. Wood* 380 (PREM 57888); Tsitsikamma National Park, 34° 01' S 23° 53' E, on *Chrysanthemoides monilifera* ssp. *pisifera*, 28 May 2003, *A. R. Wood* 524 (PREM 57897); S of Calitzdorp along road to Hot Springs at junction with road to Rooiberg Pass, 33° 38' S 21° 42' E, on *Chrysanthemoides monilifera* ssp. *subcanescens*, 3 Feb. 2000, *A. R. Wood* 90 (PREM 56823); N of Milnerton, between 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 Jul. 2003, *A. R. Wood* 525 (PREM 57898); N of Melkbostrand, Koeberg Nature Reserve, 33° 40' S 18° 26' E, on *Chrysanthemoides incana*, 12 Jun. 1997, *A. R. Wood* 62 (PREM 56795); *sin loc.* 9 Sep. 1998, *A. R. Wood* 63 (PREM 56796); Simon's Town, Red Hill Pass, 34° 11' S 18° 25' E, on *Chrysanthemoides* undescribed taxon, 14 Jul. 1998, *A. R. Wood* 87 (PREM 56820); between Vermaaklikheid and Stillbaai, 34° 20' S 21° 02' E, on *Chrysanthemoides* undescribed taxon, 9 Oct. 1998, *A. R. Wood* 88 (PREM 56821); Stillbaai, 34° 23' E 21° 25' E, on *Chrysanthemoides* undescribed taxon, 9 Oct. 1998, *A. R. Wood* 89 (PREM 56822); †Cape Peninsula, 4.7 km NW of Cape Point Nature Reserve, 34° 13' S 18° 24' E, on *Chrysanthemoides* undescribed taxon, 2 May 2003, *A. R. Wood* 515 (PREM 57894); 5 km W of Arniston, 34° 38' S 20° 11' E, on *Chrysanthemoides* undescribed taxon, 19 Oct. 2003, *A. R. Wood* 530 (PREM 57899); Stellenbosch, Jonkershoek Nature Reserve, 33° 59' S 18° 59' E, on *Osteospermum ciliatum*, 18 Sep. 1994, *A. R. Wood* 93 (PREM 57903); 2 km S of Simon's Town, 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 Jul. 1997, *A. R. Wood* 60 (PREM 56857); E of Bredasdorp, De Hoop Nature Reserve, near Potberg Education Centre, 34° 23' S 20° 32' E, on *Osteospermum potbergense*, Jul. 1995, *A. R. Wood* 57 (PREM 56853); *sin loc.* on *Osteospermum potbergense*, 24 Jun. 1998, *A. R. Wood* 58 (PREM 56855); *sin loc.* on *Osteospermum potbergense*, Jun. 2002, *A. R. Wood* 374 (PREM 57904); N of McGregor, Boesmans Pass, on *Osteospermum polygaloides*, 14 Aug. 1976, *L. Hugo* 786 (NBG); Swartberg 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 Jun. 2002, *A. R. Wood* 383 (PREM 57902); Caledon, Caledon Wild Flower 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 new host records for this rust fungus.



Figs 10–17. *Endophyllum dimorphothecae*. **Fig. 10.** Accidioid telia on a witches' broom on *Dimorphotheca cuneata*.

Figs 11–12. Peridial cells of an accidioid telium. **Figs 13–14.** Germinated accidioid teliospore with two vesicle-like modified basidiospores. **Figs 15–17.** Accidioid teliospore(s). Bars: 10–12 = 10 μm , 13–14 = 25 μm , 15–17 = 10 μm .

Endophyllum dimorphothecae A. R. Wood & Crous,
sp. nov. (Figs 10–17)

Etym.: Named after the host plant.

Pycnia amphigena, plerumque hypophylla, dispersa, prius
aquam telia accidioidea apparentia, 135–200 μm diam. Telia

accidioidea amphigena, plerumque hypophylla, dispersa,
aurantiaca, cylindrica, ad 0.4 mm alta, 135–200 μm diam.
Cellulae peridiales forma variabili, oblongae vel sub-
rhomboideae, (17–)23–26(–35) \times (12–)17–19(–23) μm ; paries
externus striatus, (5–)7–8(–9) μm crassus, paries internus
verrucosissimus, (2–)3–4(–5) μm crassus. Teliosporae acci-
dioideae irregulariter angulares, globosae vel ellipsoideae,

(16–)20–21(–28) × (12–)16–17(–22) µm, ratio longitudo/latitudo 1: 1–1.85; paries hyalinus, verruculosus, 1(–2) µm crassus, apex indistinctus vel modice inspissatus ad 1.5–2 µm; 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; K(M) 122450, PUR N4781 – isotypi).

Infections systemic, causing witches' brooms. *Pycnia* amphigenous but mostly hypophyllous, scattered unevenly, not in lesions, preceding the aecidioid telia; 135–200 µm diam; flask-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 µm diam, peridial margin white, reflexed, incised. *Peridial cells* firmly joined together, irregular oblong to sub-rhomboid, (17–)23–26(–35) × (12–)17–19(–23) µm; outer wall striate, (5–)7–8(–9) µm thick; inner wall coarsely verrucose, (2–)3–4(–5) µm thick. *Aecidioid teliospores* irregular angular-globose to ellipsoid, (16–)20–21(–28) × (12–)16–17(–22) µm, length/width ratio 1:1–1.85; spore wall hyaline, verrucose, 1(–2) µm thick, apex indistinct or apex slightly thickened, 1.5–2 µm thick; upon germination producing 2(–4) vesicle-like modified basidiospores.

Additional specimens examined: South Africa: Northern Cape Province: †S of Sutherland, summit of Verlatekloof 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 Blesfontein, 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 Boplaas, 32° 31' S 20° 24' E, on *Dimorphotheca cuneata*, 23 Feb. 2003, A. R. Wood 505 (PREM 57919, K(M) 122451).

Endophyllum elytrappi (Henn.) A. R. Wood & Crous, **comb. nov.** (Figs 18–23)

Basionym: *Aecidium elytrappi* Henn., *Hedwigia* **37**: 294 (1898).

Infections systemic, causing fusiform stem galls up to 5 cm long or witches' brooms 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 µm diam; peridium extending well beyond epidermis, peridial margin white, not reflexed, not or shallowly incised. *Peridial cells* irregular oblong to sub-rhomboid, (21–)28.5–30(–39) × (15–)21–22(–30) µm; outer wall striate, (10–)13–14(–17) µm thick; inner wall coarsely verrucose, (4–)5(–7) µm thick. *Aecidioid teliospores* irregular angular-globose, ovate to ellipsoid, (21–)25.5–26(–32) × (16–)20.5–21(–25) µm, length/width ratio 1: 1–1.81; spore wall hyaline, verrucose, 1.5–2(–3) µm thick; apex slightly thickened with a distinct germ pore,

(2–)3–5 µm thick; upon germination producing (3–)4 vesicle-like modified basidiospores.

Specimens examined: South Africa: Eastern Cape Province: W of Patensie, Baviaanskloof Wilderness Area, Bergplaa plateau, 33° 37' S 24° 28' E, on *Elytropappus rhinocerotis*, Jan. 2000, A. R. Wood 168 (PREM 57913); *Northern Cape Province:* †SW of Sutherland, farm Blesfontein, 32° 27' S 20° 26' E, on *Elytropappus rhinocerotis*, 23 Feb. 2003, A. R. Wood 506 (PREM 57917, K(M) 122448, PUR N4777); *Western Cape Province:* Bot River, on *Elytropappus rhinocerotis*, Nov. 1896, *Schlechter s.n.* (K(M) 110309 – isotype); hills above Darling, 33° 22' S 18° 22' E, on *Elytropappus rhinocerotis*, 1 Jan. 1999, A. R. Wood 130 (PREM 57912, K(M) 122447, PUR N4775); N 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 Zewenwacht wine 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 Toit's Pass, 33° 44' S 19° 09' E, on *Stoebe plumosa*, 18 Jul. 1997, A. R. Wood 371 (PREM 57908); Stellenbosch, Jan 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 57909); N of George, Montague Pass, near Toll House, 33° 54' S 22° 25' E, on *Stoebe plumosa*, 25 Jul. 2001, A. R. Wood 336 (PREM 57906); George, 2 km E of Outeniqua Nature Reserve offices, towards Tierkop, 33° 55' S 22° 26' E, on *Stoebe plumosa*, 4 Jun. 2002, A. R. Wood 377 (PREM 57910); Stellenbosch, Jonkershoek Plantation, 33° 58' S 18° 56' E, on *Stoebe plumosa*, 5 Feb. 2003, A. R. Wood 503 (PREM 57911).

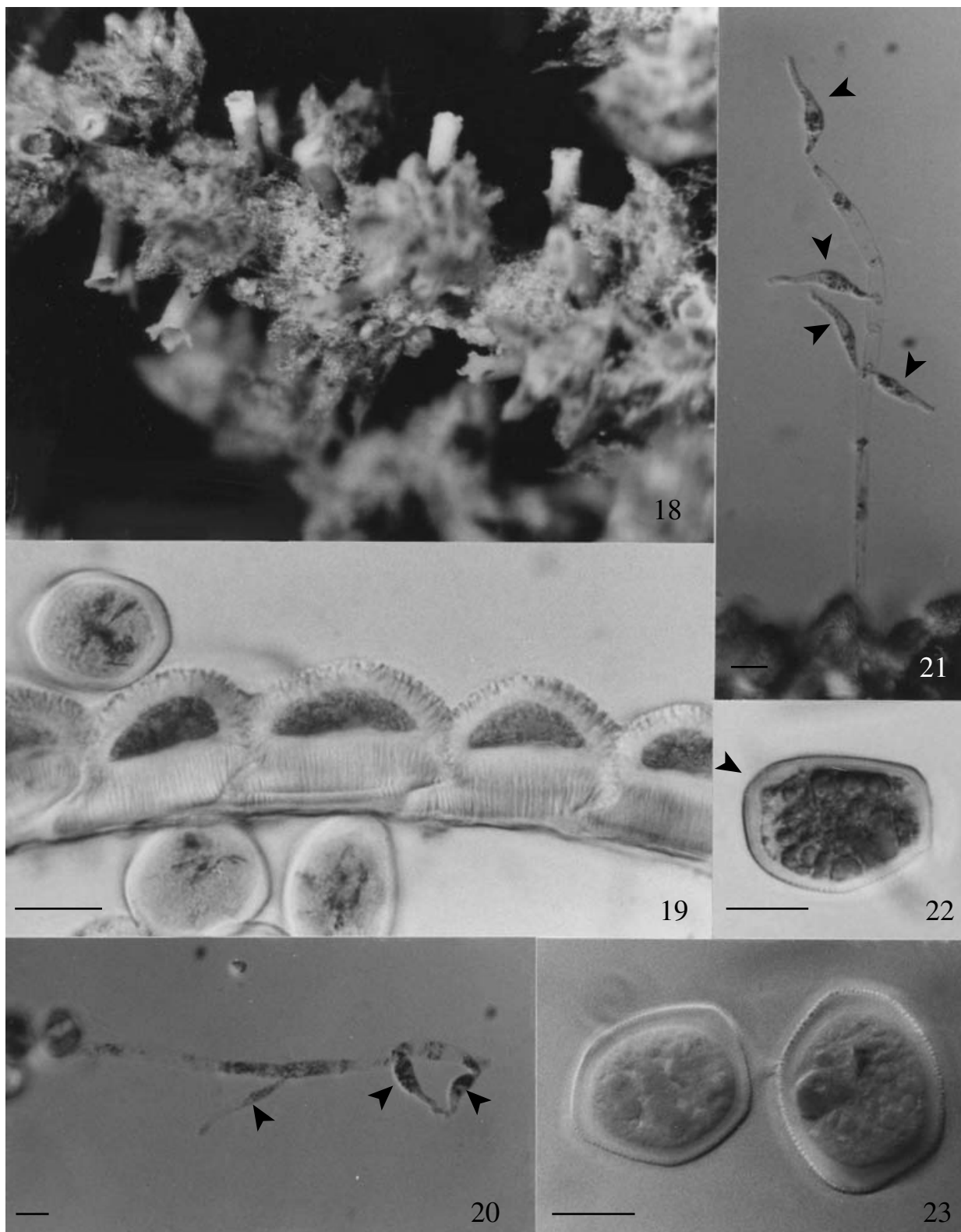
Note. *Stoebe plumosa* is a new host record for this fungus.

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 µm diam; peridial margin white, not reflexed, not or shallowly incised. *Peridial cells* firmly joined together, irregular oblong to sub-rhomboid, (23–)25–27(–30) × (13–)18–21(–28) µm; outer wall striate, (5–)6–7(–10) µm thick; inner wall coarsely verrucose, (2–)3–4(–5) µm thick. *Aeciospores* irregular ovate to ellipsoid to angular-globose, orange, (21–)24–26(–32) × (18–)20–22(–24) µm, length/width ratio 1:1.04–1.52; spore wall hyaline, verrucose, (3–)4(–5) µm thick, germination not observed.

Specimen examined: South Africa: Western Cape Province: Cape Town, Lions Head, on *Metalasia* sp., Dec. 1908, *IB Pole Evans s.n.* (PREM 686 – holotype).

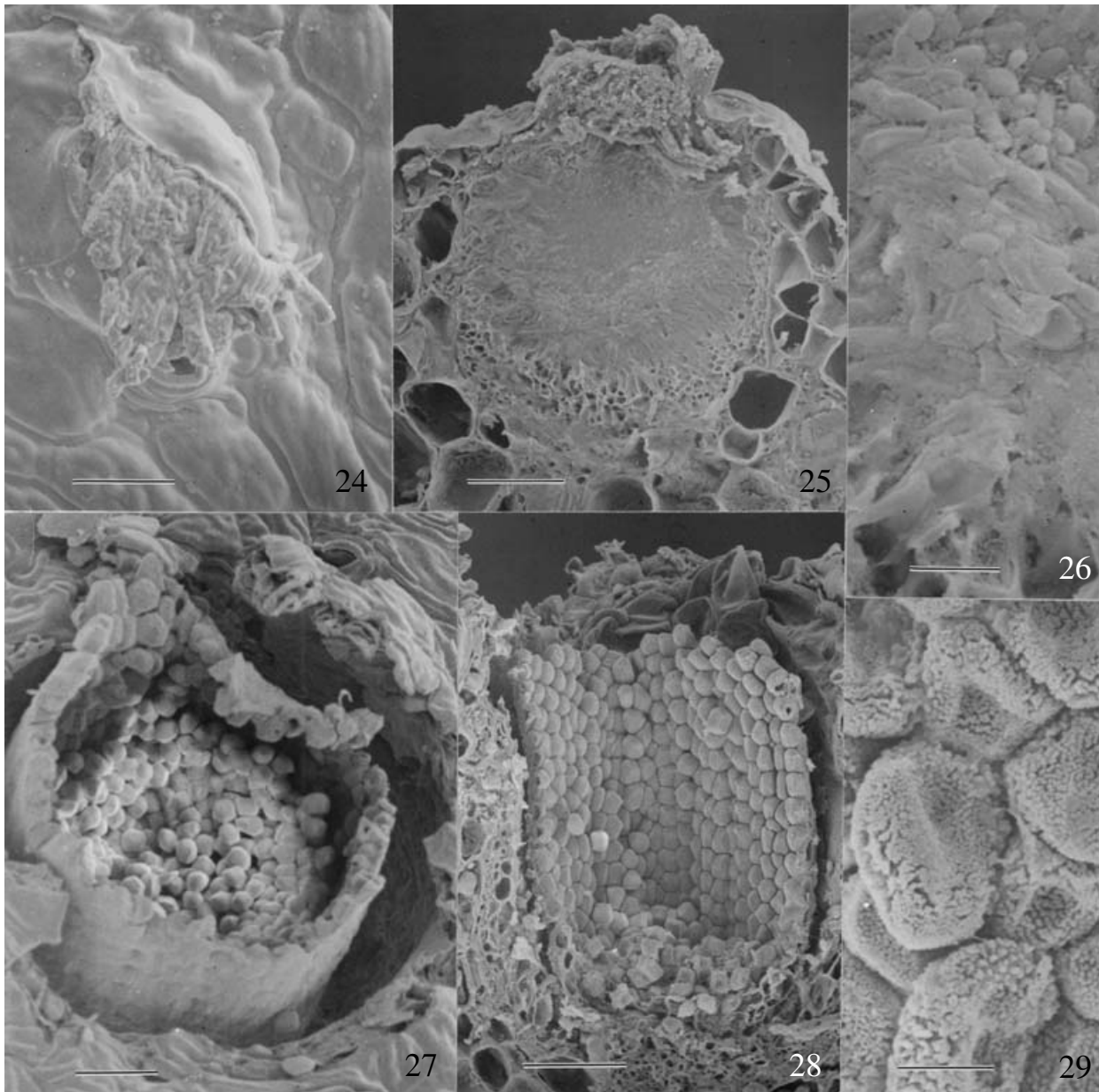
Notes. The host was originally identified as *M. muricata*. Since the original report the concept of this plant species has changed, and this epithet is now applied to a



Figs 18–23. *Endophyllum elytopappi*. **Fig. 18.** Aecidioid telia on a witches' broom 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 apex and germ pore. **Fig. 23.** Two aecidioid teliospores. Bar = 10 μ m.

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 known from the type locality of *A. metalasiae* (Karis 1989). However, as the type specimen has no flowers 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 flexuous hyphae, pycniospores and honeydew 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 (honeydew). **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 μm , 25 = 40 μm , 26 = 7 μm , 27 = 50 μm , 28 = 68 μm , 29 = 10 μm .

Key to *Endophyllum* spp. with vesicle-like modified basidiospores occurring on taxa in Asteraceae

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

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

Locality	Host ^a	PREM no.	l × w (min.–max.) ^b	l × w (95% conf. lim.) ^c
Lions Head, Cape Town (holotype)	<i>C.m.m.</i>	6587	19–28 × 15–21	22–24 × 17–19
Gydo Pass, Ceres	<i>C.m.m.</i>	57891	18–26 × 15–22	22–23 × 18–19
Houw Hoek Pass, Bot River	<i>C.m.m.</i>	57895	19–24 × 16–21	21–22 × 18–19
Kirstenbosch, Cape Town	<i>C.m.m.</i>	57893	18–25 × 16–20	21–22 × 17–18
Simon's Town	<i>C.m.m.</i>	56799	23–39 × 18–34	30–32 × 25–27
Paarl	<i>C.m.m.</i>	56801	20–27 × 15–24	22–23 × 19–20
De Hoop Nature Reserve	<i>C.m.m.</i>	56808	22–30 × 18–23	24–25 × 20–21
Grahamstown	<i>C.m.p.</i>	56815	19–29 × 14–20	21–22 × 17–18
Heidelberg	<i>C.m.p.</i>	56812	17–27 × 16–22	21–22 × 18–19
Jeffrey's Bay	<i>C.m.p.</i>	56816	19–27 × 14–22	21–22 × 17–18
Heidelberg	<i>C.m.p.</i>	57333	18–31 × 14–20	21–22 × 17–18
Sedgefield	<i>C.m.p.</i>	56813	18–29 × 16–21	22–23 × 17–18
Cathedral Peak	<i>C.m.c.</i>	56794	22–29 × 16–25	24–25 × 19–20
Calitzdorp	<i>C.m.s.</i>	56823	20–27 × 16–22	23–24 × 19–20
Stilbaai	<i>C.sp.</i>	56822	18–24 × 14–22	21–22 × 18–19
Koeberg Nature Reserve	<i>C.i.</i>	56795	20–28 × 16–24	24–25 × 20–20
Stellenbosch	<i>O.c.</i>	57903	19–25 × 14–19	21–23 × 16–18
Swartberg Pass	<i>O.p.</i>	57900	18–26 × 15–23	20–22 × 17–18

^a *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*.

^b Minimum and maximum range of length and width of 50 spores from each locality.

^c 95% confidence limits of the average length and width of 50 spores from each locality.

RESULTS

Morphology

In all specimens studied, fresh aecidioid 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 (Hiratsuka & Cummins 1963), being defined as subepidermal, with determinate growth and a strongly convex hymenium (Figs 24–26). Pycnia were not observed on *E. elytropappi*, but may well be produced. The aecidioid telia (Figs 27–28) and aecidioid 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 aecidioid 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 warts (verruculae), and with no pore plugs or refractile granules. The verruculae in these four species are all uniformly small (Fig. 29). There was some variation in aecidioid teliospore dimensions from different specimens collected throughout the known range of *E. osteospermi* (Table 2). This variation was, however, not associated with geographic location. Aecidioid teliospores from drier localities were slightly smaller than those from wetter localities. These types of pycnia, aecidioid telia and aecidioid teliospore morphologies are typical of the *Pucciniaceae* (Cummins & Hiratsuka 2003).

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

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* (Doidge 1950). Morris (1982) recorded populations on *C. monilifera* ssp. *monilifera* from the Cape Peninsula (Constantia), and in the Stellenbosch, Franschhoek and Villiersdorp districts. Unfortunately no voucher specimens from these localities were preserved. Furthermore, these records are all in the winter rainfall areas in the western part of the Western Cape Province, South Africa. The above specimens listed

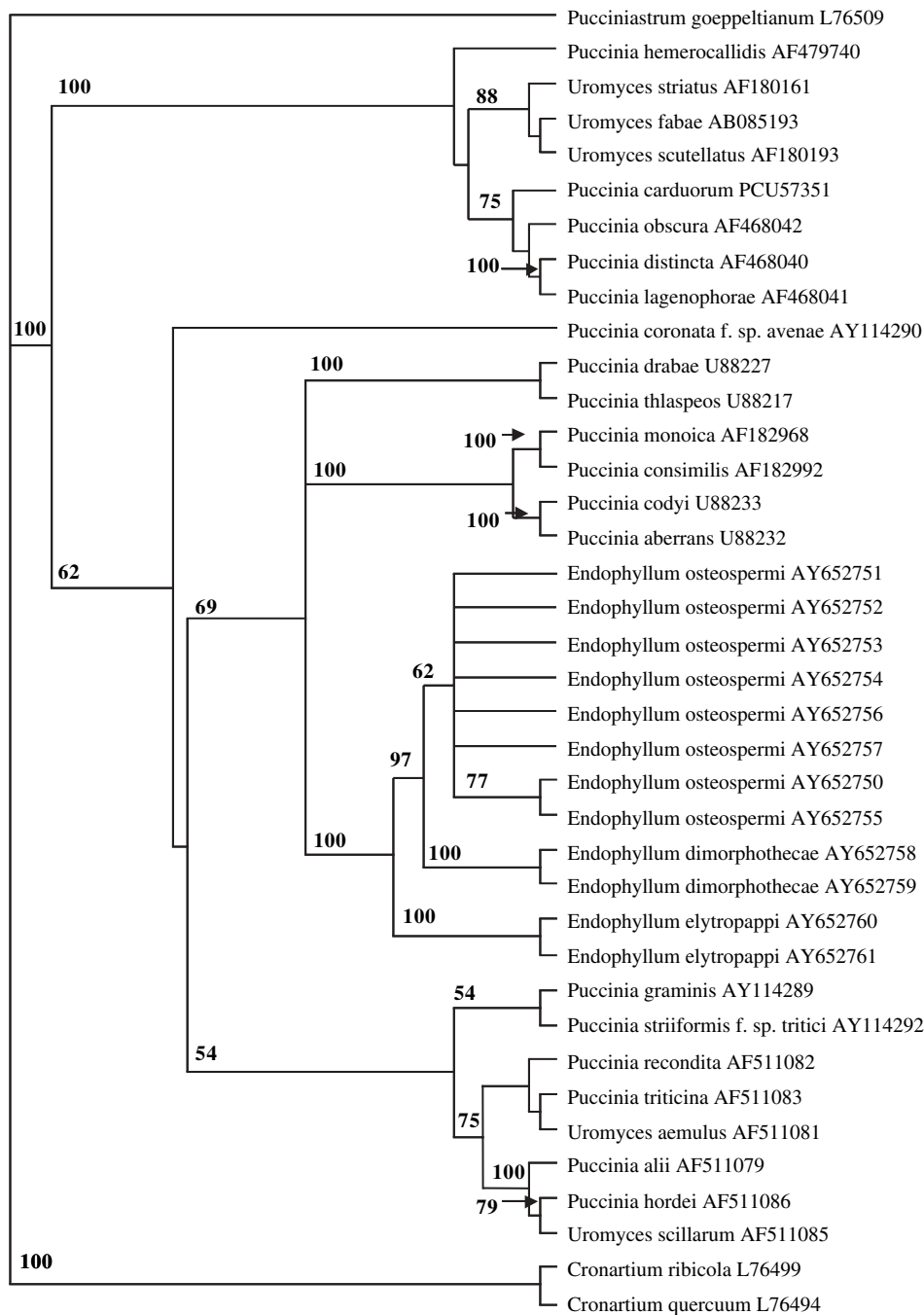


Fig. 30. Strict consensus of 167 most-parsimonious trees of the ITS1-5.8S-ITS2 rDNA region of *Endophyllum dimorphothecae*, *E. elytropappi*, and *E. oteospermi*. Bootstrap support values (1000 replicates) are shown at the nodes.

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

In addition to *Chrysanthemoides* species, *E. oteospermi* was 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. oteospermi*, as witches' brooms were only found on

plants occurring amongst bushes of *C. monilifera* on which *E. oteospermi* was abundant (Wood & Nordenstam 2004). The accidioid telia were not observed to mature and open on *O. potbergense*, neither were witches' brooms found on plants further away from infected *C. monilifera*. A similar situation occurred in *O. ciliatum* at one locality (2 km S of Simon's Town), but at another locality no infected *C. monilifera* plants were found near infected *O. ciliatum* plants (Jonkershoek Nature Reserve). At both these sites the accidioid telia matured and opened normally. Where infected *O. polygaloides* plants were found, no infected *C. monilifera* plants were found nearby (at least

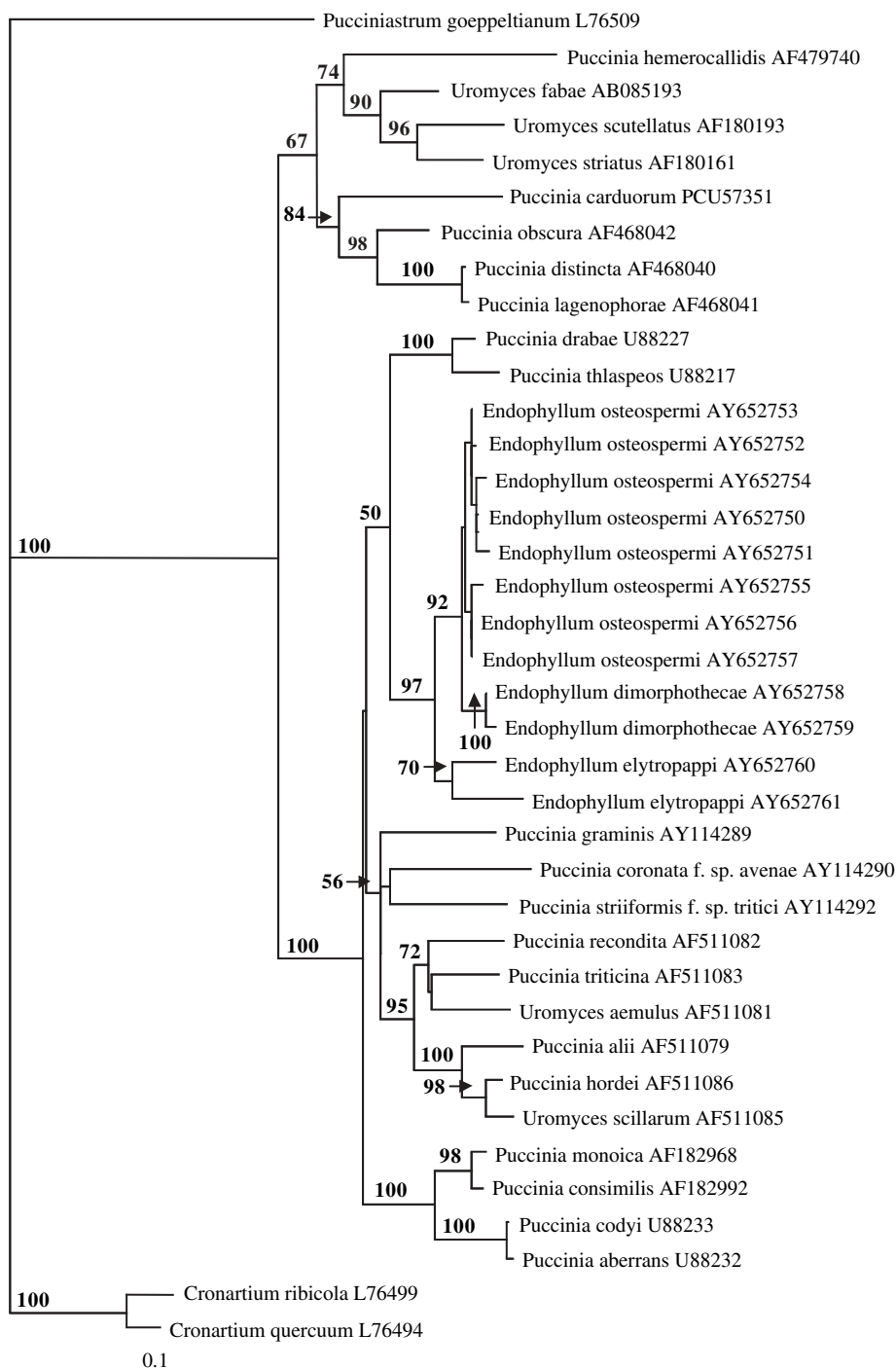


Fig. 31. Neighbour-joining tree of the ITS1–5.8S–ITS2 rDNA region of *Endophyllum dimorphothecae*, *E. elytropappi*, and *E. oteospermi*. Bootstrap support values (1000 replicates) are shown at the nodes.

within 1 km). 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 was 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 (fleshy exocarp 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 re-examined (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 was therefore described as a new species, *E. dimorphothecae*. This

host plant is ecologically distinct from *C. monilifera*, occurring in areas much drier than those where *C. monilifera* grows (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 were hosts of *Endophyllum* species, were host to *E. elytopappi* (*Elytropappus* and *Stoebe* spp.) or *E. macowanianum* (*Conyza scabrida*) (Wood 2005). The only other species of rust fungus found in South Africa that caused witches' brooms on asteraceous hosts was 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 (Lion's Head, Cape Town) from which the type of *E. osteospermi* also originated. The latter species, however, was found to still be present in that area, as is the host plant (*Metalasia densa*). *Aecidium metalasiae* is morphologically very similar to *E. elytopappi*, but is readily distinguished by its thicker, aecidioid teliospore walls, and is therefore maintained as a separate species. However, it may be only an aberrant specimen of *E. elytopappi*. 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 koa* (syn. *Atelocauda koa*; Gardner 1981, Chen, Gardner & Webb 1996, Walker 2001). Vesicle-like 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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