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Underestimated regional species diversity in the Cape Floristic Region revealed by phylogenetic analysis of the *Erica abietina/E. viscaria* clade (Ericaceae)

M. D. PIRIE[1,2*], E. G. H. OLIVER[3], B. GEHRKE[2], L. HERINGER[2†], A. MUGRABI DE KUPPLER[4], N. C. LE MAITRE[1] and D. U. BELLSTEDT[1]

[1]*Department of Biochemistry, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
[2]*Institut für Organismische und Molekulare Evolutionsbiologie, Johannes Gutenberg-Universität, Anselm-Franz-von-Bentzelweg 9a, 55099 Mainz, Germany*
[3]*Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
[4]*INRES Pflanzenzüchtung, Rheinische Friedrich-Wilhelms-Universität Bonn, Katzenburgweg 5, 53115 Bonn, Germany*

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As one of the largest genera of flowering plants, the richness of species in *Erica* (Ericaceae) is all the more remarkable because > 80% of the > 800 species are endemic to the smallest floral kingdom, the Cape Floristic Region (CFR) of South Africa. In the CFR, pockets of narrowly endemic taxa appear in close juxtaposition with their widespread and variable relatives. The taxonomic challenges of Cape *Erica* are epitomized by the complex Cape '*abietina/viscaria* clade', currently comprising at least 25 species. We reassess species boundaries and patterns of regional endemism in this clade using a phylogenetic tree inferred from multiple nuclear ribosomal and plastid DNA sequences. We show that the seven currently recognized subspecies of *E. abietina* represent at least three independent, morphologically distinct lineages with non-overlapping geographical distributions. We resurrect the name *E. grandiflora* to include *E. abietina* subsp. *aurantiaca* and subsp. *perfoliosa* and we provide a new name for *E. abietina* subsp. *petraea* (*E. situshiemalis*). This means that *E. abietina* is now an additional endemic species for the Cape Peninsula, including the natural World Heritage Site, Table Mountain National Park.

ADDITIONAL KEYWORDS: biodiversity hotspot – Cape Floristic Region – Cape Peninsula – endemism – external transcribed spacer – phylogeny – species delimitation – Table Mountain National Park.

INTRODUCTION

Some of the greatest challenges in modern systematics are presented by species-rich, rapidly evolved clades. The sheer numbers of species in large genera such as *Astragalus* L. (2000–3000 species; Wojciechowski, Sanderson & Hu, 1999), *Carex* L. (*c*. 2000 species; Global Carex Group, 2015) and *Senecio* L. (*c.* 1000 species; Pelser *et al.*, 2007) present enormous challenges to monographers (Frodin, 2004) and such groups often include species complexes that cannot be resolved using traditional techniques. *Erica* L. (Ericaceae) is a case in point. The 860 species estimated by Oliver (2000) placed it 21st in Frodin's list of large genera (Frodin, 2004). The vast majority of *Erica* spp. are restricted to the botanically diverse Cape Floristic Region (CFR) of South Africa (Linder, 2003), where they diversified rapidly within the last *c*. 10–15 My (Pirie *et al.*, 2016). Dulfer (1964, 1965) published the last revision of *Erica*, but this was based almost exclusively on a small number of herbarium records and the 605 species that were treated have been followed by the description of a considerable number of new species in South Africa and the inclusion in *Erica* of 83 species from former 'minor genera', including *Phillipia* Klotzsch and *Blaeria* L. (Oliver, 2000). A more recent

*Corresponding author. E-mail: pirie@uni-mainz.de
†Current address: Rheinische Friedrich-Wilhelms-Universität Bonn, Germany.

overview of the vast diversity of *Erica* spp. has been provided in subsequent editions of an electronic identification aid (Oliver & Forshaw, 2012; Volk *et al.*, 2005), but a modern taxonomic revision of the genus as a whole is still lacking.

In one of a series of papers contributing to the taxonomy and systematics of Cape *Erica* spp. (e.g. Oliver, 1976, 1989, 2000; Oliver & Oliver, 2003), Oliver & Oliver (2002) began the enormous task of systematically revising the genus. They proceeded in numerical order following the system of Guthrie & Bolus (1905) based on the sections of Bentham (1839). As is typical for complex patterns of Cape botanical diversity, the species revised by Oliver & Oliver (2002) included a number of taxonomically challenging complexes that are morphologically variable across their geographical distributions. Within some species, they documented breaks in morphological variation that coincided with geographical discontinuities. One example is *E. plukenetii* L. (in which they recognized five subspecies), which shows striking differences, particularly in floral morphology corresponding to three differing pollination ecotypes found in different regions of the CFR (Van der Niet *et al.*, 2014). Two further examples are *E. abietina* L. (seven subspecies) and *E. viscaria* L. (six subspecies), which also show great variation in floral morphology across their distributions. Both species have long-tubed flowers generally typical of bird pollination (Rebelo, Siegfried & Oliver, 1985), but within each there is significant variation in both size and colour (Fig. 1), with the associated potential importance for reproductive isolation and the speciation process (Rebelo & Siegfried, 1985).

Molecular phylogenetic tools offer the means to test higher level groupings of species, to identify morphologically cryptic taxa and to delimit species (Doyle, 1997; Fujita *et al.*, 2012). Global analyses of independent molecular data for *Erica* are now available (Pirie, Oliver & Bellstedt, 2011; Pirie *et al.*, 2016) and as anticipated by Oliver & Oliver (2002), the species treated in their work proved to represent a mixture of both closely and more distantly related clades. This reflects parallel evolution of floral morphological characters (Pirie *et al.*, 2011), on which the classification of Guthrie & Bolus (1905) was based.

In the case of *E. plukenetii*, molecular phylogenetic analyses confirmed the monophyly of the species as delimited by Oliver & Oliver (2002), despite its high morphological variability (Pirie *et al.*, 2016; Van der Niet *et al.*, 2014). The status of *E. abietina* and *E. viscaria* has yet to be tested with equivalent data. They belong to an '*abietina/viscaria* clade', identified on the basis of nuclear ribosomal internal transcribed spacer (ITS) (Pirie *et al.*, 2011) and combined ITS and plastid sequence data (Pirie *et al.*, 2016). This clade currently represents 25 species, including 13 of the 24 classified in section *Pleurocallis* (species 12–35 in Oliver & Oliver, 2002), one of several (non-monophyletic) sections characterized by long tubular flowers.

Four of the seven subspecies of *E. abietina* feature on the IUCN Red List of Endangered Plants (IUCN, 2016; Raimondo *et al.*, 2009) and their taxonomic status (and that of the other taxa in the *abietina/viscaria* clade) has a direct influence on regional assessments of species diversity and endemism and hence for setting optimal conservation priorities. *Erica abietina* subsp. *perfoliosa* E.G.H.Oliv. & I.M.Oliv. is classified as Vulnerable and *E. abietina* subsp. *petraea* E.G.H.Oliv. & I.M.Oliv. has been assessed as Endangered (Raimondo *et al.*, 2009). These and the much more common and widespread *E. abietina* subsp. *aurantiaca* E.G.H.Oliv. & I.M.Oliv. are found on the mainland in the Cape Fold Mountains of South Africa. However, most subspecies of *E. abietina* are restricted to the Cape Peninsula, on the south-western tip of the African continent (Fig. 2), where they fall within the protection of the natural World Heritage Site, Table Mountain National Park. Two of these Cape Peninsula endemic subspecies also feature on the red list: *E. abietina* subsp. *constantiana* E.G.H.Oliv. & I.M.Oliv. as Rare and *E. abietina* subsp. *diabolis* E.G.H.Oliv. & I.M.Oliv. as Critically Endangered (Raimondo *et al.*, 2009).

Table Mountain is close to Cape Town and is threatened by anthropogenic influences including habitat destruction, rampant spread of alien vegetation and extreme suppression of the natural fire regime (Trinder-Smith, Cowling & Linder, 1996; Van Wilgen, Forsyth & Prins, 2012). The last of these has a particularly negative impact on floral elements, such as most *Erica* spp., that depend on post-fire regeneration from seed (Kraaij & Van Wilgen, 2014). Table Mountain and the adjacent, largely transformed, vegetation of the Cape Flats also house an endemic subspecies of *E. viscaria* (*E. viscaria* subsp. *viscaria*), but most of the diversity of *E. viscaria* is found in other regions of the south-western CFR, particularly the Hottentots Holland Mountains and the Kogelberg Biosphere Reserve on the opposite side of False Bay (Fig. 2). In contrast to Table Mountain, in many other areas of the CFR such as these, human intervention has resulted in increased rather than decreased frequency of fires (Kraaij & Van Wilgen, 2014). This phenomenon also impacts post-fire reseeding as it restricts the natural succession of fynbos vegetation to its initial stages prior to replenishment of the seed bank (Rebelo *et al.*, 2006). In both cases, an accurate representation of species diversity and distribution is needed in order to inform policy-makers and press for appropriate conservation management.

Figure 1. A–D, Cape Peninsula subspecies of *Erica abietina*: A, subsp. *abietina* (corolla dark red); B, subsp. *atrorosea* (*MP1029*) (corolla rose to deep rose); C, subsp. *diabolis* (*MP1015*) (corolla rose-pink); and D, subsp. *constantiana* (corolla pale to deeper rose-pink). E, *E. nevillei* (*MP1056*) (corolla red). F, *E. quadrisulcata* (*MP1031*) (corolla orange above, yellow underneath). G–I, extra-Cape Peninsula subspecies of *E. abietina*: G, subsp. *aurantiaca* (*E. grandiflora* subsp. *grandiflora*; *MP514*) (corolla orange to orange-red); H, subsp. *perfoliosa* (*E. grandiflora* subsp. *perfoliosa*; *MP1071*) (corolla pure yellow); and I, subsp. *petraea* (*E. situshiemalis*) (corolla pure yellow). Photographs: A, D, I: E.G.H.O.; B, C, E–H: M.D.P.

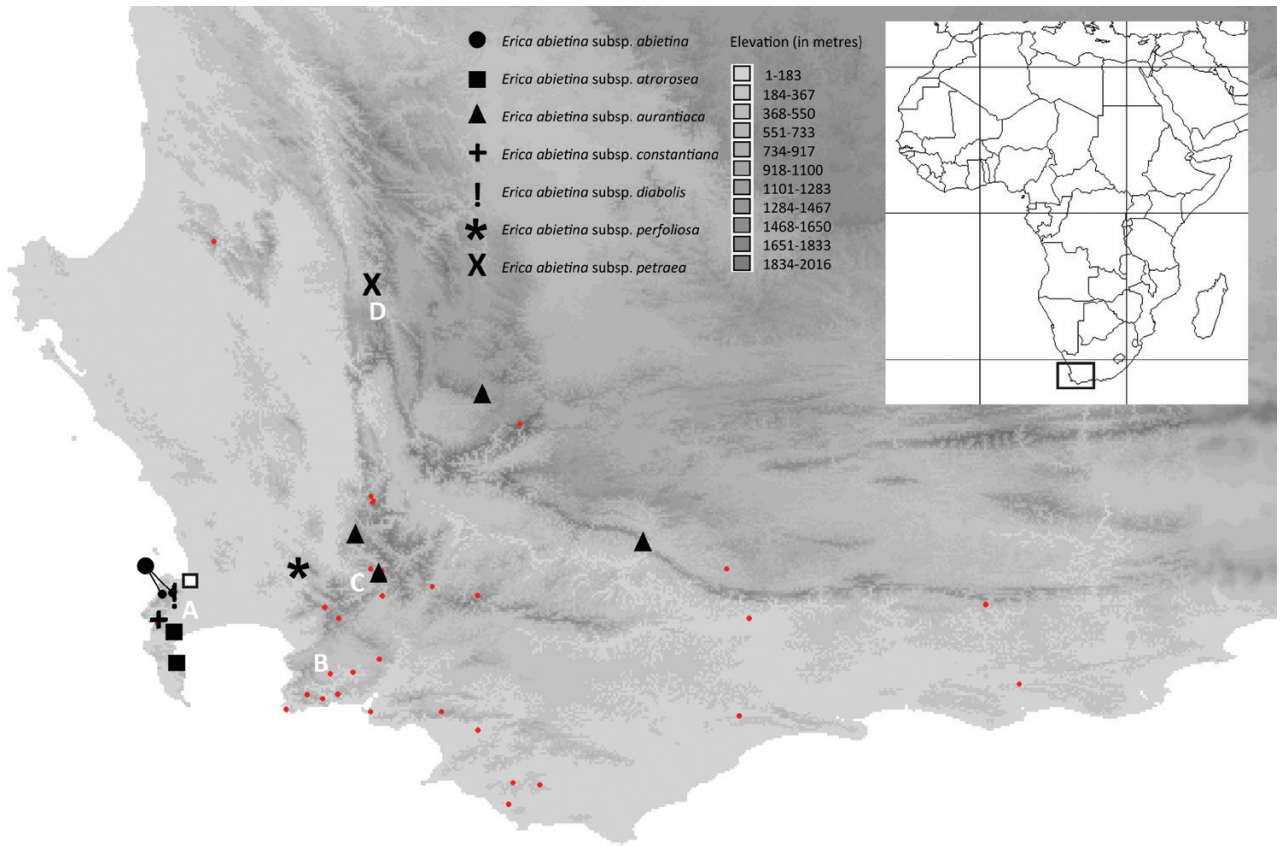

Figure 2. Distribution across the Cape Floristic Region (inset map indicates south-western position on the African continent) of ingroup samples included in phylogenetic analyses here. The subspecies of *Erica abietina sensu* Oliver & Oliver (2002) are represented with larger symbols as indicated and other taxa with smaller dots. A, Cape Peninsula (including the Table Mountain National Park); B, Kogelberg Biosphere reserve; C, Hottentots Holland Nature Reserve; and D, Groot Winterhoek Wilderness Area; the white square indicates central Cape Town.

As part of a renewed attempt to address taxonomic uncertainty in *Erica* systematically, we aim in this work to use molecular data to reassess species boundaries and patterns of regional endemism in the *abietina/viscaria* clade, with particular focus on *E. abietina*. We test the monophyly of the species using independent nuclear and plastid DNA sequence datasets and implement the taxonomic consequences of the results.

MATERIAL AND METHODS

Taxon and molecular sampling

We based taxon sampling on the results of Pirie et al. (2011, 2016), together representing *c.* 60% of Cape *Erica* spp. from numerous localities across the CFR, further informed by our ongoing sampling efforts that at the time of writing cover *c.* 70% of known Cape species diversity (data not shown). In total, we sampled 64 accessions representing three outgroup and 25 ingroup species. The ingroup included all seven subspecies of *E. abietina* (including three not previously analysed), both subspecies of *E. regia* Bartl., four of the six subspecies of *E. viscaria* and 22 other species (including *E. latiflora* L.Bolus; not previously analysed), with multiple accessions of several taxa to test their monophyly. Of section *Pleurocallis* (in which 13 of the ingroup species are classified), we have yet to sample *E. globulifera* Dulfer, *E. tenax* L.Bolus, *E. porteri* Compton and *E. onosmiflora* Salisb. Given the polyphyly of the section and lack of unambiguous synapomorphies for the *abietina/viscaria* clade, we cannot assume that these belong to the clade or that they are the only members of that clade that we have not sampled. Our sampling probably roughly reflects our overall sampling of *Erica* spp. (i.e. *c.* 70%) and, given our broad coverage of localities across the CFR, we are likely to be missing predominantly rarer species and narrow regional endemics. The outgroups were *E. alopecurus* Harv., a more distant outgroup representative of the extra-CFR African clade (Pirie *et al.*, 2011), and *E. pycnantha* Benth. and *E. corifolia* L.,

both representatives of the *articularis* clade that is not part of the *abietina/viscaria* clade (Pirie *et al.*, 2011) and is its probable sister group (Pirie *et al.*, 2016). Accession details are presented in Table 1 and the geographical distribution of ingroup samples is illustrated in Figure 2.

To assess the consistency of our phylogenetic hypothesis given the potential for gene tree conflict at and below the species level, we collected sequence data representing two independent gene trees: that of the plastid genome (plastid DNA) and that of one nuclear encoded marker, the *18S/5.8S/26S* nuclear ribosomal (nrDNA) gene region. We selected some individual markers (nrDNA ITS; plastid encoded *trnT-trnL-trnF-ndhJ* intron/intergenic spacer and *trnK-matK* spacer regions) based on their variability shown in previous work on *Erica* (Van der Niet *et al.*, 2014; Mugrabi de Kuppler *et al.*, 2015) and designed new primers to additionally sequence up to *c.* 800 bases of the nrDNA external transcribed spacer (ETS) region in order to obtain a more resolved nrDNA gene tree. Some ITS sequences were taken from Pirie *et al.* (2011) and some ITS and plastid sequences were taken from Pirie *et al.* (2016). All other data were generated newly for this study (Table 1).

DNA extraction, PCR amplification and cycle sequencing

We used two different laboratory protocols: (1) direct amplification (without DNA isolation) was performed using the method of Bellstedt *et al.* (2010), in particular when only nrDNA markers were to be sequenced; and (2) DNA isolation (followed by separate PCR) was performed using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). In both cases, leaf material was ground using a Qiagen Tissuelyser (Retsch GmbH, Haan, Germany).

Primer design for ETS was initially accomplished by amplification and sequencing using primers *18S*-ETS (Baldwin & Markos, 1998) with STT-ETS (Li, Zhang & Alexander, 2001) and designing *Erica*-specific primers based on the resulting partial success. We subsequently performed long-range PCR using primers *18S*-ETS with *26S*-IGS (Baldwin & Markos, 1998) in order to design further primers for amplification and sequencing of longer fragments. These primers are documented in Table 2 (with the others used here) and in an aligned ETS matrix presented in Supporting Information Appendix S1. Amplification and sequencing of ETS for *Erica* accessions was performed using primer 18S_ETS in combination with either ETS_Erica1 (yielding a product of *c.* 600 bp) or Erica_813R (*c.* 800 bp product), with reagents and protocol as for 18S-ETS/STT-ETS below.

The reagents for PCR amplification were as follows and the thermocycler settings are reported in Table 2. For PCR with primers 18S-ETS with STT-ETS per 25-μL reaction we included 2.5 μL Sigma 10× buffer (Sigma-Aldrich, St Louis, MO, USA); 2.0 μL 25 mM $MgCl_2$, 1.0 μL DMSO, 0.25 μL 4 μg/μL BSA, 0.5 μL 10 mM dNTPs, 0.25 μL each of 20 μM forward and reverse primers, 0.1 μL 5 U/μL Sigma Taq and 1.0 μL template DNA. For long-range PCR using 18S-ETS with 26S-IGS we used the Phusion-High-Fidelity Polymerase Kit (New England Biolabs GmbH, Frankfurt am Main, Germany), including per 50-μL reaction 10.0 μL Phusion 5× buffer, 1.5 μL DMSO, 0.5 μL 10 mM dNTPs, 0.5 μL 4 μg/μL BSA, 0.5 μL each of 20 μM forward and reverse primers, 0.5 μL Phusion DNA polymerase and 2.0 μL template DNA. For PCR of plastid markers and ITS sequences we included per 25-μL reaction 2.5 μL 10× buffer, 2.0 μL 25 mM $MgCl_2$, 1.0 μL 5 mM dNTPs, 0.25 μL 4 μg/μL BSA, 1 μL DMSO (ITS only), 0.1 μL Taq polymerase, 0.25 μL each of 20 μM solutions of the two primers and 1 μL DNA template.

For direct sequencing, PCR products were treated in the original PCR tube by addition of a 10-μL solution including 0.025 μL of 20 units/μL exonuclease I (Fermentas Life Sciences, Burlington, ON, Canada), 0.25 μL 1 unit/μL shrimp alkaline phosphatase (Promega, Madison, WI, USA) and incubation (in a thermocycler) at 37 °C for 30 min and at 95 °C for 5 min. Then 1 μL of the resulting product was used for cycle-sequencing with the primers reported in Table 2, using Applied Biosystems (Foster City, CA, USA) Big Dye terminator kits according to the manufacturer's instructions. Cycle-sequencing products were analysed using an automatic sequencer 3130XL Genetic Analyzer (Applied Biosystems).

If direct sequencing resulted in ITS or ETS sequences with polymorphic sites, the corresponding amplicons were cloned using the pGEM-T Easy Vector Systems (Promega) to test whether the phylogenetic signals of the underlying copies differed. PCR and sequencing of clones was performed using the same primers and protocols as above.

Alignment and phylogenetic analyses

Sequences were aligned by eye in Mesquite (Maddison & Maddison, 2015). We performed preliminary phylogenetic analyses of markers separately under parsimony using PAUP* and under maximum likelihood (ML) using RAxML (as below), to identify any differences within the plastid and nrDNA datasets that would indicate experimental error and to assess the phylogenetic signal of cloned ETS sequences. On confirming the monophyly of clones from single individuals (and identifying any exceptions), single sequences with congruent phylogenetic signal were arbitrarily

Table 1. Accessions details of vouchers for DNA samples, including GenBank accession numbers. Vouchers were lodged at NBG, and all were collected in the Western Cape, Republic of South Africa (RSA) with the exception of *E. alopecurus* (Free State; RSA). GenBank accession codes KY110749-KY110860 were newly generated for this work

Taxon	Locality	Voucher	Terminal	ITS	ETS	*trnL-F-ndhJ*	*trnT-L*	*matK*
Erica abietina L. subsp. *abietina*	Cultivated: KBG	*Hitchcock, A, 156/94*	abietina_abi_ ANA	KP737517	KY110749	KP737382	KY110839	KP737746
	Cape Peninsula, Devil's Peak	*Pirie, MD, 1013*	abietina_abi_ MP1013	HQ858885	KY110750	KU832577	KU831802	
	Cape Peninsula, Table Mt.	*Pirie, MD, 933*	abietina_abi_ MP933	HQ858886				
Erica abietina L. subsp. *atrorosea* E.G.H.Oliv. & I.M.Oliv.	Cape Peninsula, COGH. NR	*Pirie, MD, 1029*	abietina_atr_ MP1029	KY110798	KY110751		KY110840	
	Cape Peninsula, Silvermine	*Pirie, MD, 950*	abietina_atr_ MP950	HQ858888				
Erica abietina L. subsp. *aurantiaca* E.G.H.Oliv. & I.M.Oliv. (=*E. grandiflora* L.f. subsp. *grandiflora*)	Du Toit's Pass	*Pirie, MD, 1320*	abietina_aur_ MP1320	KY110800	KY110752	KY110817	KY110841	
	Franschhoek	*Pirie, MD, 499*	abietina_aur_ MP499	HQ858889	KY110753	KY110818	KY110842	
	Theronsberg Pass	*Pirie, MD, 514*	abietina_aur_ MP514	HQ858890	KY110754	KY110819	KY110843	
	Near Montagu	*Pirie, MD, 717*	abietina_aur_ MP717	KY110799	KY110755	KY110820	KY110844	
Erica abietina L. subsp. *constantiana* E.G.H.Oliv. & I.M.Oliv.	Cape Peninsula; Chapman's Peak	*Merry, C, 6*	abietina_con_ CM6	KU832330		KU832578	KU831803	
Erica abietina L. subsp. *diabolis* E.G.H.Oliv. & I.M.Oliv.	Cape Peninsula	*Merry, C, 11*	abietina_dia_ CM11	KU832331		KU832579	KU831804	
	Cape Peninsula, Devil's Peak	*Pirie, MD, 1015*	abietina_dia_ MP1015	HQ858887	KY110756	KY110821	KY110845	
Erica abietina L. subsp. *perfoliosa* E.G.H. Oliv. & I.M.Oliv. (=*E. grandiflora* L.f. subsp. *perfoliosa* E.G.H.Oliv. & Pirie)	Stellenbosch, Jonkershoek valley	*Pirie, MD, 1071*	abietina_per_ MP1071	KY110801	KY110757	KY110822	KY110846	
Erica abietina L. subsp. *petraea* E.G.H.Oliv. & I.M.Oliv. (=*E. situshiemalis* E.G.H.Oliv. & Pirie)	Porterville area, Groot Winterhoek	*Oliver, EGH, s.n.*	abietina_pet_ EO	KY110802	KY110758	KY110823	KY110847	

Table 1. *Continued*

Taxon	Locality	Voucher	Terminal	ITS	ETS	*trnL-F-ndh*J	*trnT-L*	*matK*
	Porterville area, Groot Winterhoek	*Oliver, EGH, 11449*	abietina_pet_ EO11449	KY110803	KY110759			
Erica alopecurus Harv.	Free Sate; Golden Gate National Park	*Pirie, MD, 630*	alopecurus_ MP630	HQ858902	KY110760	KU832593	KU831817	KU831609
Erica axilliflora Bartl.	Carrruthurs Hill	*Pirie, MD, 916*	axilliflora_ MP916	HQ858929	KY110761	KU832619	KU831841	
Erica collina Guthrie & Bolus	Western Cape	*Oliver, EGH, 12613*	collina_ EO12613	KU832372		KU832680	KU831896	
Erica corifolia L.	Kogelberg B.R., Kogelberg trail	*Pirie, MD, 823*	corifolia_ MP823	HQ858988	KY110762	KU832690	KU831905	KU831633
Erica cruenta Soland.	Near Kogelberg B.R.	*Pirie, MD, 745*	cruenta_MP745	HQ858991	KY110763	KU832693	KU831907	
Erica cruenta Soland.	Rooihoogte, N of Villiersdorp	*Pirie, MD, 999*	cruenta_MP999	KY110804	KY110764	KY110824	KY110848	
Erica doliiformis Salisb.	Franschhoek	*Pirie, MD, 710*	doliiformis_ MP710	HQ859017	KY110765	KY110825	KY110849	
	Limietberg NR, Bain's Kloof	*Pirie, MD, 797*	doliiformis_ MP797	HQ859016		KU832723	KU831938	
Erica elimensis L.Bolus	Cordale, Diepgat	*Oliver, EGH, 12843*	elimensis_ EO12843	KU832399		KU832730		
Erica filamentosa Andrews	Swellendam, Bontebok N.P.	*Oliver, EGH, 12728*	filamentosa_ EO12728	KU832405	KY110766	KU832753	KU831966	
Erica hibbertii Andrews	Franschhoek Pass area, Purgatory/ Amandel River	*Oliver, EGH, 11952*	hibbertii_ EO11952	HQ859083				
	Boland/Overberg border	*Pirie, MD, 982*	hibbertii_ MP982	KU832432	KY110767	KU832806	KU832017	
Erica latiflora L.Bolus	Elgin basin	*Oliver, EGH, 12865*	latiflora_ EO12865	KY110805	KY110768	KY110826		
Erica nematophylla Guthrie & Bolus	Riversdale District, Langeberg	*Oliver, EGH, 12747*	nematophylla_ EO12747	KU832483	KY110769	KU832907	KU832112	
Erica nevillei L.Bolus	Cultivated: KBG	*Hitchcock, A, 86/04*	nevillei_ANA	KY110806	KY110770	KY110827	KY110850	
	Cape Peninsula, Noordhoek Piek trail	*Pirie, MD, 1056*	nevillei_ MP1056	KU832485	KY110771	KU832910	KU832115	
Erica parilis Salisb.	Cultivated: KBG	*Hitchcock, A, 97/04*	parilis_ANA	KY110807	KY110772	KY110828	KY110851	
	Hex River Mts, Matroosberg	*Pirie, MD, 751*	parilis_MP751	HQ859177	KY110773	KU832932	KU832137	
Erica phillipsii L.Bolus	Piketberg	*Pirie, MD, 1357*	phillipsii_ MP1357	KY110808	KY110774	KY110829	KY110852	
	Limietberg NR, Bain's Kloof	*Pirie, MD, 794*	phillipsii_ MP794	HQ859199	KY110775	KU832957	KU832161	
Erica pinea Thunb.	Franschhoek	*Pirie, MD, 693*	pinea_MP693	KY110809	KY110776	KY110830	KY110853	
	Limietberg NR, Bain's Kloof	*Pirie, MD, 789*	pinea_MP789	HQ859204	KY110777	KU832963	KU832166	
Erica pycnantha Benth.	Kogelberg B.R., Perdeberg	*Pirie, MD, 1011*	pycnantha_ MP1011	KU832513	KY110778	KU832984	KU832190	
Erica quadrisulcata L.Bolus	Cultivated: KBG	*SANBI, 543/87*	quadrisulcata_ ANA	KY110810	KY110779	KY110831	KY110854	
	Cape Peninsula, COGH. NR	*Pirie, MD, 1031*	quadrisulcata_ MP1031	KU832515	KY110780	KU832987	KU832193	

Table 1. *Continued*

Taxon	Locality	Voucher	Terminal	ITS	ETS	*trnL-F-ndhJ*	*trnT-L*	*matK*
Erica regia Bartl. subsp. *mariae* (Guthrie & Bolus) E.G.H.Oliv. & I.M.Oliv.	Riversdale Distr., between Melkhoutfontein and Gouritsmond	*Gehrke, B, 580*	regia_mar_BG580	HQ859235				
	De Hoop NR	*Pirie, MD, 930*	regia_mar_MP930	HQ859236	KY110781	KU832995	KU832201	
Erica regia Bartl. subsp. *regia*	Geelrug (cult.: KBG)	*SANBI, 1613/70*	regia_reg_ANA	KU832518	KY110782	KU832996	KU832202	KU831696
	Viljoenshof to Elim	*Oliver, EGH, 11404*	regia_reg_EO11404	HQ859238				
	Elim area, Waterford	*Oliver, EGH, 12275*	regia_reg_EO12275	HQ859239				
	Viljoenshof to Elim	*Pirie, MD, 922*	regia_reg_MP922	HQ859237	KY110783	KU832997	KU832203	
Erica stokoei L.Bolus	Cultivated: KBG	*Unknown, 62/83*	stokoei_ANA	KY110811	KY110784	KY110832	KY110855	
	Kogelberg B.R., Kogelberg trail	*Pirie, MD, 825*	stokoei_MP825	HQ859285	KY110785	KU833050	KU832256	
Erica thomae L.Bolus	Kogelberg B.R., Kogelberg trail	*Pirie, MD, 807*	thomae_MP807	HQ859297	KY110786	KU833068	KU832272	KU831712
Erica vestita Thunb.	Cultivated: KBG	*SANBI, 176/05*	vestita_ANA	KU832567	KY110787	KU833111	KU832313	KU831720
	Riviersonderend Mts, Jonaskop	*Bytebier, B, 2667*	vestita_BB2667	HQ859327				
	Langeberg Mts	*Muasya, AM, 4468*	vestita_CS4468	HQ859328				
	Klein River Mts; Glengart/ Morning Star	*Oliver, EGH, 12702*	vestita_EO12702	KU832568	KY110788	KU833112	KU832314	
	Langeberg Mts; Marloth NR	*Pirie, MD, 1079*	vestita_MP1079	KY110812	KY110789	KY110833	KY110856	
	Pearly Beach; Heidehof	*Pirie, MD, 910*	vestita_MP910	HQ859329				
Erica viscaria L. subsp. *longifolia* (Bauer) E.G.H.Oliv. & I.M.Oliv.	Cultivated: KBG	*Hitchcock, A, 91/04*	viscaria_lon_ANA	KY110814	KY110790	KY110838	KY110857	
	Hottentots Holland NR	*Pirie, MD, 1270*	viscaria_lon_MP1270	KY110813	KY110791	KY110835	KY110859	
	Franschhoek	*Pirie, MD, 504*	viscaria_lon_MP504	HQ859331	KY110792	KY110834	KY110858	
	Stellenbosch, Jonkershoek	*Pirie, MD, 678*	viscaria_lon_MP678	HQ859332		KU833115	KU832317	
Erica viscaria L. subsp. *macrosepala* E.G.H.Oliv. & I.M.Oliv.	Babylon's Tower	*Pirie, MD, 682*	viscaria_mac_MP682	KY110815	KY110793	KY110836	KY110860	
	Kogelberg B.R., Kogelberg trail	*Pirie, MD, 808*	viscaria_mac_MP808	HQ859333	KY110794	KU833116	KU832318	
Erica viscaria L. subsp. *pendula* E.G.H.Oliv. & I.M.Oliv.	Bot River, Highlands road	*Oliver, EGH, 12466*	viscaria_pen_EO12466	HQ859334	KY110795	KU833117	KU832319	

Table 1. *Continued*

Taxon	Locality	Voucher	Terminal	ITS	ETS	*trnL-F-ndhJ*	*trnT-L*	*matK*
	Houw Hoek	*Pirie, MD, 603*	viscaria_pen_MP603	KY110816	KY110796	KY110837		
Erica viscaria L. subsp. *viscaria*	Cape Peninsula	*Villiers, MJ de, 4*	viscaria_vis_MdV4	HQ859335	KY110797	KU833118	KU832320	

Table 2. Primers used for PCR and sequencing

Marker	Primers	Thermocycler protocol
ETS (general)	18S-ETS (Baldwin & Markos, 1998)/ STT-ETS (Li *et al.*, 2001)	97 °C 2 min; 40 cycles (97 °C 10 s/55 °C 30 s/72 °C 25 s); 72 °C 7 min
ETS (long range)	18S-ETS / 26S-IGS (Baldwin & Markos, 1998)	98 °C 45 s; 30 cycles (98 °C 10 s/72 °C 4 min); 72 °C 10 min
ETS (*Erica*-specific)	18S_ETS/<=GGCAAGCACCGTTTA GCATGAACA=ETS_Erica1; <=GCCCGTGGCATCACTTTCCAACG =ETS_Erica_813R (this study)	97 °C 2 min; 40 cycles (97 °C 10 s/55 °C 30 s/72 °C 25 s); 72 °C 7 min
ITS	AB101 (Douzery *et al.*, 1999)/8P (Möller & Cronk, 1997); ITS17se/ ITS26se (Sun *et al.*, 1994)	94 °C 1 min; 35 cycles (94 °C 1 min/55 °C 1 min/72 °C 2 min); 72 °C 4 min
trnT-L	a/b (Taberlet *et al.*, 1991)	80 °C 5 min; 30 or 35 cycles (95 °C 1 min/50 °C 1 min/ ramp 0.38 °C/s to 65 °C/65 °C 4 min); 5 min 65 °C
trnL-ndhJ	c/f (Taberlet *et al.*, 1991); e (Taberlet *et al.*, 1991)/ndhJ (Shaw *et al.*, 2007)	As above
trnK-matK	matK6 (Shaw *et al.*, 2005)/ matK79R (Mugrabi de Kuppler *et al.*, 2015)	As above

retained for combined analyses. Individual markers were imported into SequenceMatrix (Vaidya, Lohman & Meier, 2011), which was used to export concatenated matrices (nrDNA, plastid DNA and all) for further analyses (TreeBase study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S18985).

For each of the three concatenated matrices the best fitting data partitioning strategies (given models implemented in RAxML and MrBayes as below) were selected with PartitionFinder (Lanfear *et al.*, 2012), using a heuristic search strategy ('greedy') and comparison of fit by means of the Bayesian information criterion. Genes, introns and spacer regions within those markers were specified as potential data partitions, with plastid DNA genes combined in a single partition because the data were missing for many of the taxa (see Results). Analyses were restricted to 44 taxa for which a minimum of 1 kb of sequence data had been generated.

To infer gene tree topologies and clade support, analyses of nrDNA and plastid DNA matrices were performed under parsimony, using PAUP* (Swofford, 2003), maximum likelihood (ML) using RAxML (Stamatakis, 2006) and Bayesian inference using MrBayes 3.2 (Ronquist *et al.*, 2012). To find the shortest trees, we employed a heuristic search strategy in PAUP* involving 1000 random addition sequences with TBR branch swapping, saving a maximum of 50 trees in each replicate. To assess parsimony clade support, we performed 10 000 bootstrap replicates starting each with a single random addition sequence and implementing TBR branch swapping, saving a single tree per replicate (following Müller, 2005). Partitioned RAxML analyses were performed including bootstrapping on CIPRES (Stamatakis, Hoover & Rougemont, 2008). Bootstrapping was halted automatically following the majority-rule 'autoMRE' criterion and bootstrap support (BS) was presented on the best scoring ML tree. Two independent MrBayes runs of 10 million generations each were performed, sampling every 1000 generations, implementing the partitions and substitution models as selected using PartitionFinder. Convergence

was assessed (using the potential scale reduction factor, PSRF) and post-burnin tree samples were summarized (using the sumt command) in MrBayes.

Conflict between nrDNA and plastid DNA gene trees was assessed by comparing nodes subject to 70% or higher BS and/or 0.95 posterior probability (PP) and visualized by means of a tanglegram of strict consensus trees generated using Dendroscope 3 (Huson & Scornavacca, 2012). Where gene tree conflict was identified, the taxa with conflicting phylogenetic signals were removed prior to concatenated analyses under parsimony, ML and Bayesian inference, as above. Taxa that were represented by < 1 kbof sequence data (i.e. in some cases only ITS sequences were available from Pirie *et al.* 2011; Supporting Information Appendix S2) were also excluded.

RESULTS

A comparison of the variability of the main molecular markers used is presented in Table 3. The nrDNA markers yielded at least double the number of potentially parsimony-informative characters per PCR amplicon/sequence compared to the plastid DNA markers, and the newly developed ETS protocol yielded 20% more potentially informative and 85% more variable, uninformative characters than ITS.

No polymorphisms were apparent in electropherograms resulting from direct sequencing of ITS PCR products. In contrast, up to four polymorphic sites per sequence were apparent in the ETS electropherograms of five samples: *E. hibbertii* Andrews sample MP982, *E. abietina* subsp. *petraea* sample EO, *E. abietina* subsp. *aurantiaca* samples MP514 and MP717, and *E. viscaria* subsp. *macrosepala* E.G.H.Oliv. & I.M.Oliv. sample MP682. The corresponding PCR products were therefore cloned. Preliminary analyses showed generally consistent phylogenetic signal (no conflict supported by ≥ 70% BS) between individual plastid DNA markers and between nrDNA ITS and ETS. Two clones of *E. abietina* subsp. *aurantiaca* (one each of samples MP717 and MP514) did not exhibit this within-locus tree consistency. These differed in phylogenetic signal both from other ETS clones and from ITS sequences of the same sample, implying a sister-group relationship to the '*abietina* clade', as opposed to being nested in the '*viscaria* clade' (as defined below; Supporting Information Appendix S3). Single arbitrarily chosen ETS clones per sample were retained for combined analyses, excluding the incongruent clones of MP717 and MP514. The best fitting substitution models and partitioning strategies inferred using PartitionFinder given models available under RAxML and MrBayes for the plastid DNA, nrDNA and all concatenated data are reported in Table 4.

Combination of individual plastid DNA and nrDNA markers resulted in better resolved plastid DNA and nrDNA gene trees; these are compared in the tanglegram in Figure 3, with BS from PAUP* and RAxML and PP clade support values from MrBayes analyses. Most nodes subject to ≥ 70% BS and/or 0.95 PP were consistent between gene trees, with the exception of those subtending five samples representing *E. cruenta* Soland., *E. stokoei* L.Bolus and *E. collina* Guthrie & Bolus (which nrDNA placed outside the ingroup) and one of *E. phillipsii* L.Bolus (conflict within the ingroup; Fig. 3). Clade support differed somewhat according to the different methods, but both plastid DNA and nrDNA trees feature a Cape Peninsula endemic '*abietina* clade', comprising *E. abietina* subsp. *abietina*, *E. abietina* subsp. *atrorosea* E.G.H.Oliv. & I.M.Oliv., *E. abietina* subsp. *constantiana*, *E. abietina* subsp. *diabolis*, *E. quadrisulcata* L.Bolus and *E. nevillei* L.Bolus, to the exclusion of accessions of *E. abietina* subsp. *petraea*, *E. abietina* subsp. *aurantiaca* and *E. abietina* subsp. *perfoliosa*. Both genes trees show the last two subspecies to be more closely related to accessions of *E. viscaria* and other species, which in the nrDNA tree are grouped in a larger '*viscaria* clade' (Fig. 3).

The phylogenetic hypothesis based on concatenated plastid DNA and nrDNA sequence data (excluding the above taxa with conflicting phylogenetic signals) is presented in Figure 4. The topology is consistent with both

Table 3. Variability and potentially informative characters of molecular markers (excluding the generally less variable *trnL* intron and *trnK-matK* spacer/gene, for which fewer accessions were sequenced) across a directly comparable subset of 44 taxa

Marker	Aligned	Constant	Parsimony-uninformative	Potentially parsimony-informative
ITS	905 (plus 3 indels)	832	41	35
ETS	825 (plus 2 indels)	709	76	42
trnT-trnL	1328 (1070 included, plus 4 indels)	1006	52	16
trnL-trnF-ndhJ spacers	1000 (plus 4 indels)	953	39	12

Table 4. Best fitting partitions and substitution models

Matrix/method	RAxML	MrBayes
plastid DNA	GTR+G (unpartitioned)	(1) F81+G: *trnTL* (2) F81+G: plastid DNA genes, *trnF-ndhJ*, *trnK-matK*, *trnL* intron, *trnL-trnF*
nrDNA	(1) GTR+G: 18S, ITS1, 5.8S, ITS2, 28S	(1) JC: 18S, 5.8S (2) K80+G: ITS1, ITS2
	(2) GTR+G: ETS	(3) HKY+G: 28S, ETS
plastid DNA + nrDNA	(1) GTR+G: 18S, 28S, 5.8S, ITS1, ITS2	(1) JC: 18S, 5.8S (2) K80+G: ITS1, ITS2 (3) HKY+G: 28S, ETS
	(2) GTR+G: ETS	(4) GTR+G: *trnTL*
	(3) GTR+G: plastid DNA genes, *trnF-ndhJ*, *trnK-matK*, *trnL* intron, *trnL-trnF*, *trnTL*	(5) F81+G: plastid DNA genes, *trnF-ndhJ*, *trnK-matK*, *trnL* intron, *trnL-trnF*

individual gene trees (minus the conflicting elements). There is significant (≥ 70% BS; ≥ 0.95 PP) or strong support (depending on the phylogenetic method) for monophyly of the *abietina/viscaria* clade. A sister-group relationship of *E. parilis* Salisb. with the rest of the clade is supported under Bayesian inference; the clade otherwise comprises a basal polytomy also including *E. thomae* L.Bolus; *E. abietina* subsp. *petraea*; the '*abietina* clade'; *E. phillipsii* and *E. doliiformis* Salisb. (two smaller-flowered species that according to the likelihood-based methods represent a clade sister to the *abietina*-clade), and the *viscaria*-clade (comprising the remaining species). The Cape Peninsula subspecies of *E. abietina* are a monophyletic group nested in the *abietina* clade.

DISCUSSION

Phylogenetic approaches using DNA sequence data are an invaluable tool for addressing the challenges involved in revising large genera (Williams *et al.*, 2014). Species boundaries can in principle be tested using a modest number of molecular markers and standard phylogenetic inference methods, even in a complex group such as the *E. abietina/viscaria* clade. When particular populations prove to be only distantly related to their putative conspecifics (confirmed by data from independent linkage groups), this supports their recognition as distinct species. However, given coalescent stochasticity within populations and hybridization between them, lower-level taxa (particularly at and below the species level) might not be expected to be monophyletic according to all (or even any) given molecular markers (Doyle, 1992; Maddison, 1997). In the face of species paraphyly without clear gene tree congruence, coalescence-based species delimitation approaches based on multiple independent markers (Maddison, 1997; Fujita *et al.*, 2012; Naciri & Linder, 2015) would be a necessary basis for any taxonomic conclusions. Both scenarios are apparent in our results. There are distantly related clades representing geographically disjunct and morphologically distinct populations of taxa (currently recognized under *E. abietina*). There is also evidence of putative lineage sorting artefacts and/or past reticulation in the form of limited gene tree conflict (Fig. 3; Supporting Information Appendix S3) and paraphyly of taxa, particularly in the poorly resolved *viscaria* clade (Fig. 4).

Discussing *E. abietina*, Oliver & Oliver (2002) documented the characteristics common to the species as they defined it (e.g. similar inflorescence structure, apiculate leaves, sepals with sessile glands on the margins), but emphasized its variability, particularly in the size, colour, indumentum and stickiness of the flowers, the degree of inclusion/exsertion of the stamens, the shape of the anthers, the length of the leaves and its habitat preferences. In particular, they noted the range of flower colours (red, orange-red, orange, deep pink, pink or yellow) stating, 'In the fresh state these colours are very distinctive, and would clearly lead one to use them as specific characters, but in dried material without colour notes, identification is nigh impossible and one has to resort to a few morphological characters'. Despite the wide colour variation across the clade as a whole, these distinctive colours are characteristic of lineages identified in our analyses: in particular, red to pink for the Cape Peninsula subspecies of *E. abietina* (Fig. 1A–D) as opposed to orange to orange-red for *E. abietina* subsp. *aurantiaca* (Fig. 1G; and *abietina* clade species *E. quadrisulcata* and *E. nevillei*; Fig. 1E, F) and yellow for *E. abietina* subspp. *perfoliosa* (Fig. 1H) and *petraea* (Fig. 1I). All but one subspecies of *E. abietina* were reported to exhibit sessile glands on the adaxial side of the calyx in the middle zone next to the margins, the notable exception being *E. abietina* subsp. *petraea*.

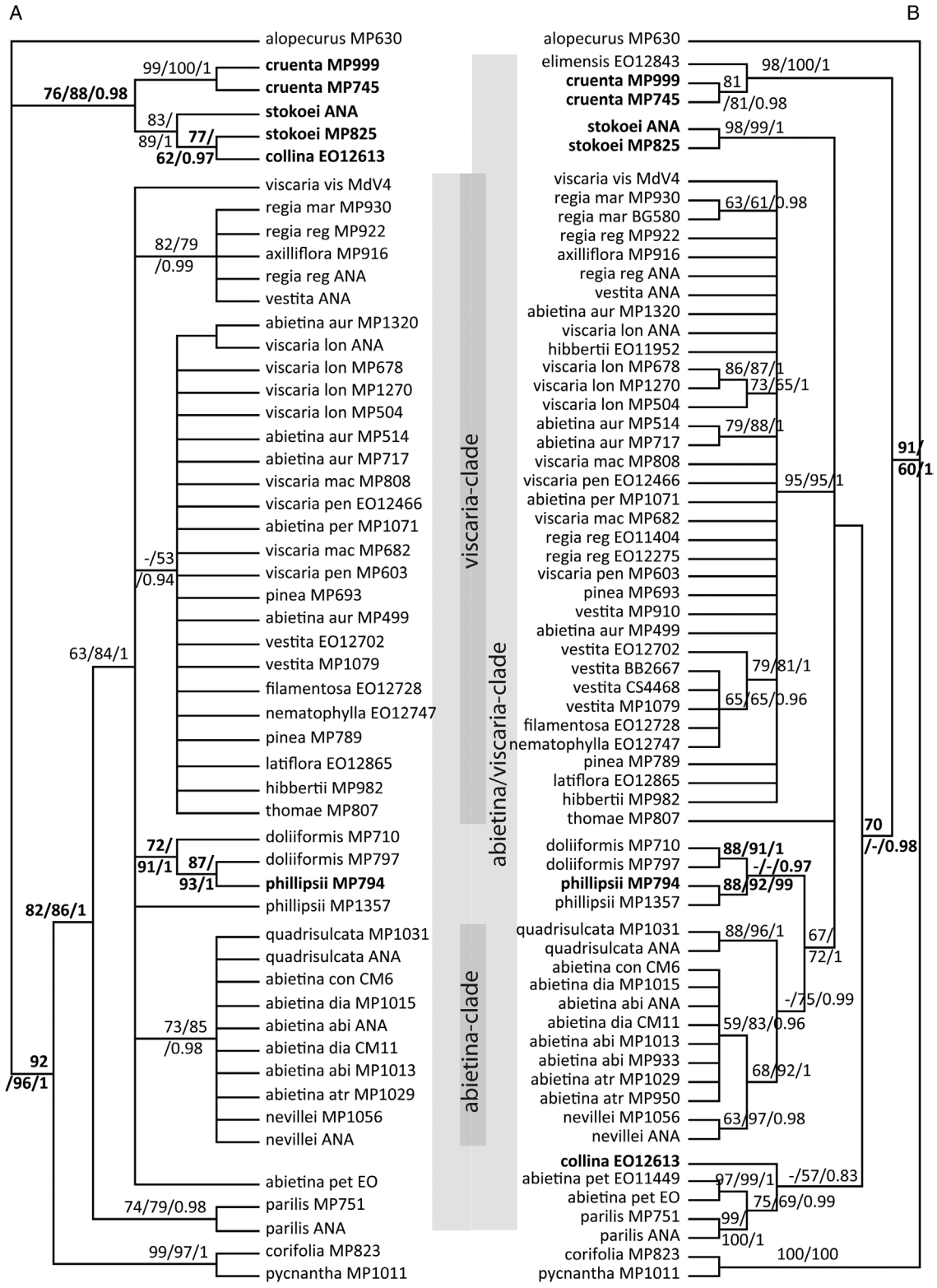

Figure 3. A tanglegram comparing (A) plastid DNA and (B) nrDNA gene trees with values above the branches representing bootstrap support (BS) under parsimony (using PAUP*) and maximum likelihood (ML; RAxML) and posterior probabilities (PP) from Bayesian inference (MrBayes; from left to right parsimony BS/ML BS/PP). Conflicting taxa and nodes are indicated in bold type.

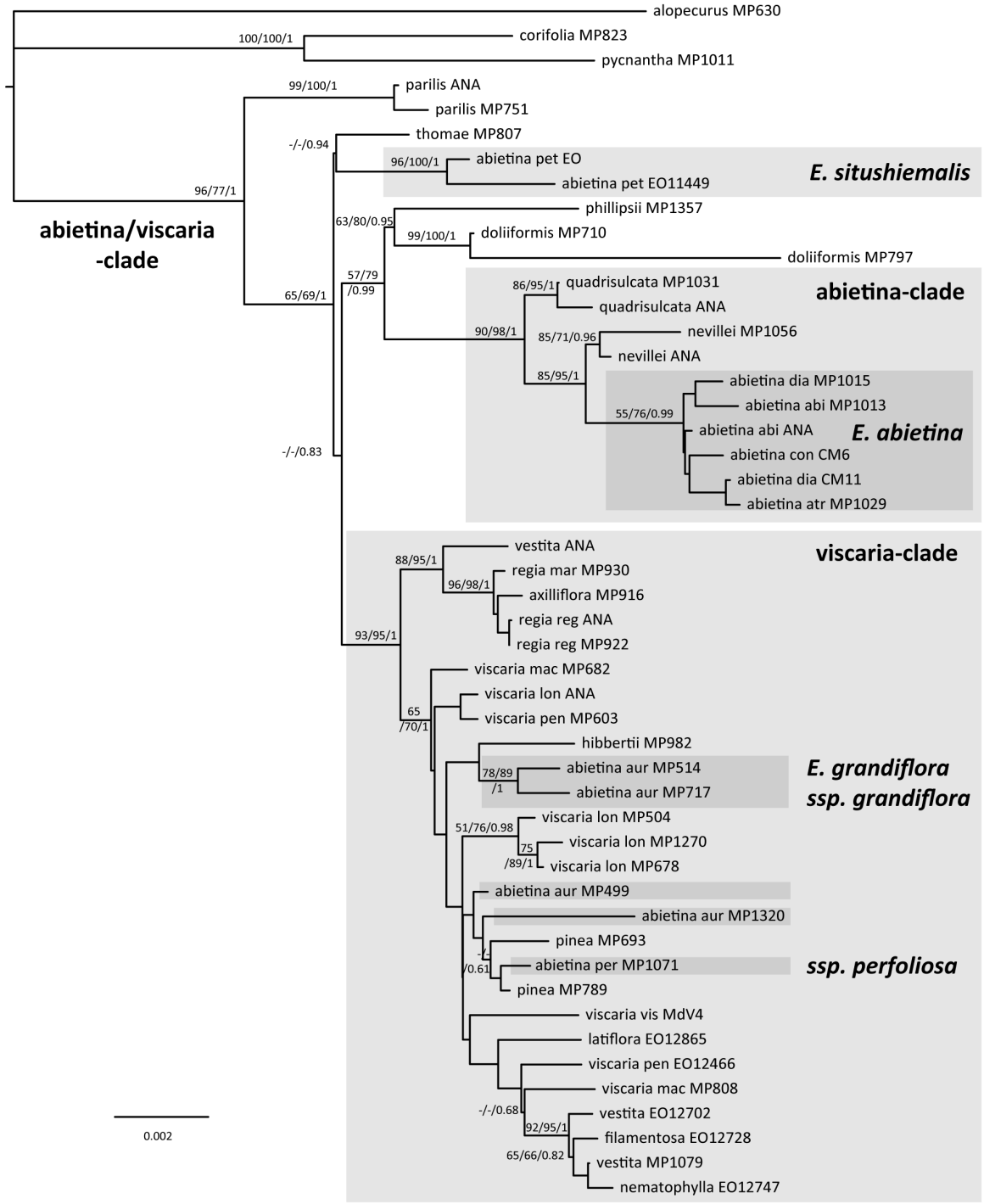

Figure 4. The phylogenetic hypothesis based on concatenated (congruent) plastid DNA and nrDNA sequence data. The tree is the best found under maximum likelihood (ML) using RAxML, with branch lengths in substitutions per site. Values above the branches represent bootstrap support (BS) under parsimony (using PAUP*) and ML (RAxML) and posterior probabilities (PP) from Bayesian inference (MrBayes; from left to right parsimony BS/ML BS/PP).

The further distinctiveness of the Cape Peninsula species was also noted: 'The [Cape] Peninsula taxa tend to form a group having the apex of the corolla lobes a little more rounded, whereas the taxa from the mainland have more acute apices to the corolla lobes' (Oliver & Oliver, 2002).

Erica abietina sensu Oliver & Oliver (2002) thus comprises at least three morphologically distinct and geographically disjunct lineages that are more closely related to other lineages in the *E. abietina/viscaria* clade than they are to each other. In the taxonomic treatment that we present here, we therefore segregate *E. abietina* into three species. We redefine *E. abietina s.s.* as a Cape Peninsula endemic including four existing subspecies and we describe one new species for the distantly related Groot Winterhoek Mountains endemic *E. abietina* subsp. *petraea* (*E. situshiemalis* E.G.H.Oliv. & Pirie stat. & nom. nov.). We reinstate the previous name *E. grandiflora* L.f. for the widespread (outside the Cape Peninsula) and variable *E. abietina* subsp. *aurantiaca*, including in it, on exclusively morphological grounds (the combination of acute corolla lobes and adaxial sessile glands on the calyx), *E. abietina* subsp. *perfoliosa* as a second subspecies. In effect, the morphological justifications for these alternative species delimitations were presented in Oliver & Oliver (2002). The main difference between their taxonomy and that informed by our phylogenetic evidence lies in the relative importance of characters in terms of taxonomic rank.

However, the phylogenetic hypothesis for the *viscaria* clade, which includes *E. grandiflora*, is not easily translated into a classification. Resolution is limited in both independent gene trees and there is a lack of obvious species monophyly. Indeed, the widespread but easily recognized *E. vestita* is apparently paraphyletic, with two accessions strongly supported in a clade with the single accessions of *E. filamentosa* and *E. nematophylla* and the third accession in a separate subclade that also includes the two subspecies of *E. regia* and the single accession of *E. axilliflora*. These factors suggest that data representing multiple independent and meaningfully resolved gene trees will be needed to address uncertainty in species boundaries in this complex and that concatenation approaches as employed here may not be an appropriate means of analysing them (Pirie, 2015). Adding to this phylogenetic uncertainty, the possibility that the group harbours more taxonomic entities than have been recognized to date was mooted by Oliver & Oliver (2002), who wrote of *E. viscaria* subsp. *longifolia*: 'This is the most variable subspecies, which it may, on more detailed population studies coupled with molecular analyses, be possible to divide into more subspecific taxa'. Despite the clear need for further work on the *viscaria* clade, among its species only the name *E. viscaria* L. (Linnaeus & Dahlgren, 1770) is older than *E. grandiflora* L.f. (Linnaeus, 1782) and our results do not indicate that these two species are any more closely related to each other than they are to any other species in the complex. Hence we predict that our reinstatement of *E. grandiflora* for *E. abietina* subsp. *aurantiaca* is likely to be robust. By including *E. abietina* subsp. *perfoliosa* in *E. grandiflora* we seek to restrict taxonomic changes to the minimum necessary to address the polyphyly of *E. abietina sensu* Oliver & Oliver (2002).

Despite its small area (only 49 000 ha; Rebelo *et al.*, 2006), the Cape Peninsula is a region of high plant species endemism in general (Oliver, Linder & Rourke, 1983; Simmons & Cowling, 1996; Trinder-Smith *et al.*, 1996; Helme & Trinder-Smith, 2006) and one for *Erica* spp. and subspecies of *E. abietina* in particular (Oliver & Oliver, 2000). In the Cape Peninsula, Trinder-Smith *et al.* (1996) counted 2285 indigenous vascular plant species (representing according to them the greatest concentration of plant species per unit area in the CFR) and Helme & Trinder-Smith (2006) reported 158 endemic species and three endemic subspecies (7% of the flora). The most species-rich genus in the Cape Flora as a whole, *Erica*, is also represented by the greatest number of endemic species on the Cape Peninsula: 35 according to recent estimates (E. G. H. Oliver, unpubl. data), now including *E. abietina* as newly defined here.

In the *abietina/viscaria* clade our results show examples of putative diversification in the Cape Peninsula (the endemic Peninsula *abietina* clade) and independent origins of Cape Peninsula lineages (*E. viscaria* subsp. *viscaria*, also endemic to the Cape Peninsula but nested in the *viscaria* clade; Fig. 4). The Cape Peninsula can therefore be regarded as a local 'hotbed' of speciation and of the evolutionary process (Cowling & Pressey, 2001) and as a repository of phylogenetic diversity (Forest *et al.*, 2007) derived from the wider CFR. Both attributes serve to emphasize the conservation importance of the protected areas of the Cape Peninsula, most notably Table Mountain National Park. *Erica* is spectacularly diverse in the CFR biodiversity hotspot and our results suggest that its true richness may be even higher than currently recognized. Given the complex morphological variation typical of hyper-diverse groups such as Cape *Erica*, molecular tools offer invaluable insight into species boundaries and patterns of endemism. The implications of these results are that the status of geographically restricted and morphologically distinct populations of *Erica* in the CFR (as reflected in numerous taxa described at subspecific ranks) should be critically assessed in the context of the phylogenetic diversity of related species. By identifying more of the cryptic narrowly endemic species diversity that survive in the nature reserves and national parks of the remarkable CFR we hope to further highlight the importance of ongoing support for their protection and appropriate management.

Taxonomic treatment

We treat only the (former) taxa of *E. abietina*, with additional comparison to closely related species.

Characters distinguishing *E. abietina*, *E. grandiflora* and *E. situshiemalis* are indicated with bold type. Diagnostic features and distributions are adapted from Oliver & Oliver (2002) and Oliver & Forshaw (2012), of which the former can be referred to for additional voucher material, illustrations and notes and the latter for comparisons to more distantly related taxa. Conservation status follows Raimondo *et al.* (2009); http://redlist.sanbi.org/index.php (last accessed 7 November 2016).

Erica abietina L. Species plantarum edn 1,1: 355 (1753); Salter: 634 (1951); Dulfer: 37 (1965); Oliv. & Oliv. (2002). Lectotype: '*Erica Africana, Abietis folio longiore & tenuiore, floribus oblongis, saturate rubris*' in Seba, Locupl. Rer. Nat. Thes. 1; 31, t.21, f.1, 1734 (designated by Oliver: 497; 2007).

Diagnostic features: Corolla shortly obconical to tubular, reducing regularly towards the base, 8–26 mm long, dark red through to paler rose pink; **apex of the corolla lobes rounded**; **calyx with adaxial sessile glands**; anthers bilobed not distinctly bipartite; ovary emarginate, obovoid, covered with dense, short, retrorse hairs; leaves 8–14 mm long. Differs from species of the *abietina* clade in corolla colour (yellow, orange above in *E. quadrisulcata*) and form of corolla (lacking the four grooves at base of the tube in *E. quadrisulcata* and the basal restriction zone of eight grooves in *E. nevillei*); in inflorescence structure (lacking the spike-like arrangement at ends of branches in *E. nevillei*); and anther exsertion (included in *E. abietina*; exserted in *E. nevillei*).

Distribution: Restricted to mountains on the Cape Peninsula. 50–900 m.

Subsp. ***abietina***
Erica coccinea sensu PJ.Bergius: 92 (1767), non L.: 355 (1753); Benth.: 627 (1839); Guthrie & Bolus: 59 (1905). Type: without locality or collector (SBT).

Illustrations: Schumann & Kirsten: 46. t. 26 (1992): Oliver & Oliver: t. 9 (2000).

Diagnostic features: Corolla dark red, tubular, 18–26 mm long, spiculed to sparsely puberulous and slightly viscid; sepals subovate, narrowly acute to acuminate, sparsely pilose with adaxial sessile glands; anthers included to exserted; leaves 10–12 mm long.

Distribution: Upper rocky slopes and plateau of Table Mountain; 500–900 m. Figure 1A.

Conservation status: Classified as 'least concern'.

Subsp. ***atrorosea*** E.G.H.OIiv. & I.M.Oliv.: 50 (2002). Type: Western Cape. 3418 (Simonstown): Froggy Pond, (-AB), 14 June 1949, *Barker 5355* (NBG).

Erica purpurea Andrews: t. 50 (1795); Benth.: 627 (1839); Guthrie & Bolus: 58 (1905). Iconotype: Andrews: t. 50 (1795).

Erica phylicifolia Salisb.: 364 (1802); Salter: 636 (1951); Dulfer: 36 (1965). Type: Sponte nascentem in Hottentots Holland. *I. Mulder s.n.* (K!).

Erica hesseana J.C.Wendl. ex Klotzsch: 634 (1835); Guthrie & Bolus: 61 (1905); Dulfer: 38 (1965). Type: Prom. b. sp.. *Hesse s.n.* (MEL!).

Illustration: Schumann & Kirsten: 45. t. 23 (1992).

Diagnostic features: Corolla rose to deep rose, tubular, 18–22 mm long, ± glabrous and somewhat sticky; sepals broadly lanceolate, shortly acuminate, sparsely puberulous with adaxial sessile glands; anthers included, occasionally manifest; leaves 8–14 mm long.

Distribution: Lower slopes of Table Mountain at Kirstenbosch along the mountains southwards to Cape Point; 50–400 m (not sympatric with subsp. *abietina*); the most widespread subspecies. Figure 1B.

Conservation status: Classified as 'least concern'.

Subsp. ***diabolis*** E.G.H.Oliv. & I.M.Oliv.: 51 (2002). Type: Western Cape, 3318 (Cape Town): saddle between Devil's Peak and Table Mountain, 2100 ft [640 m], (-CD), 25 August 1973, *Kirsten 422* (NBG).

Erica coccinea L. var. *echiiflora sensu* Bolus: 60 (Guthrie & Bolus, 1905) non *E. echiiflora* Andrews. *E. abietina* var. *echiiflora* (Bolus) Salter: 643 (1951); Dulfer: 37 (1965). Illustration: Schumann & Kirsten: 46, t. 27 (1992).

Diagnostic features: Corolla rose-pink, shortly obconical, 11–14 mm long, subglabrous, subviscid; sepals ovate, shortly acuminate, pilose; anthers included, situated about ⅔ way up tube; leaves 10–12 mm long.

Distribution: Only on the saddle between Devil's Peak and Table Mountain; 600–900 m. Figure 1C.

Conservation status: Classified as 'critically endangered', due largely to its limited extent.

Subsp. ***constantiana*** E.G.H.Oliv. & I.M.Oliv.: 51 (2002). Type: Western Cape, 3418 (Simonstown): Constantiaberg, middle N slopes, 620 m, (-AB), 21-09-1999, *E.G.H. & l.M.Oliver 11335* (NBG).

Erica conica Lodd.: t. 1179 (1824); Benth.: 664 (1839); Guthrie & Bolus: 60 (1905); Salter: 649 (1951); Dulfer: 37 (1965). Iconotype: Lodd.: t. 1179 (1824).

Illustrations: Schumann & Kirsten: 47, t. 28, 29 (1992); Oliver & Oliver: t. 10b (2000).

Diagnostic features: Corolla pale to deeper rose-pink; obconical, 8–11 mm long, glabrous, subviscid; sepals lanceolate-ovate, subacuminate; anthers always included, situated about ⅔ way up tube; leaves 8–14 mm long; very similar to subsp. *diabolis*.

Distribution: Mountains from Constantia Nek to Chapman's Peak; 350–600 m. Figure 1D.

Conservation status: Classified as 'rare'.

Erica grandiflora L.f., Supplementum plantarum: 223 (1782); Benth.: 628 (1839); Guthrie & Bolus: 57 (1905). Type: Caput bonae spei. *Thunberg s.n.* (UPS).

Diagnostic features: **Corolla orange-red, orange or yellow**, tubular, 10–34 mm long, lobes acute; calyx long-acuminate from ovate base, **with adaxial sessile glands**, anthers bipartite often with highly reduced appendages along edge of apex of the filament, leaves 16–42 mm long. Morphologically variable, but differs from species of the *viscaria* clade in corolla colour (contrasting with purple, pink or white in *E. viscaria* subsp. *pendula*, *E. latiflora*, *E. nematophylla*, *E. filamentosa*); hairiness of the sepals (as opposed to non-hairy in *E. vestita*, *E. viscaria* subspp. *pustulata* and *macrosepala*); hairiness of the ovary (as opposed to non-hairy in *E. hibbertii* and *E. pinea*); exsertion of the style (not exserted in *E. viscaria* subspp. *viscaria* and *gallorum*); and mode of regeneration (re-seeding as opposed to re-sprouting in the also highly morphologically variable *E. viscaria* subsp. *longifolia*).

Distribution: Widespread on the mainland in the CFR (see subsp. *grandiflora*), but not on the Cape Peninsula. *Erica grandiflora* was previously regarded as a synonym of *E. abietina* subsp. *aurantiaca*.

Subsp. ***grandiflora***

Erica abietina subsp. *aurantiaca* E.G.H.Oliv. & I.M.Oliv.: 51 (2002). Type: Western Cape, 3319 (Worcester): Fransch Hoek Pass, mtn slopes NE of top of pass, 2500 ft (760 m), (-CC), February 1966, *Chater in STE30037* (NBG. holotype BM. BOL. K. PRE).

Erica exsurgens Andrews: t. 22 (1796); Benth.: 627 (1839); Guthrie & Bolus: 57 (1905); Dulfer 35 (1965). *E. grandiflora* var. *exsurgens* E.G.H.Oliv.: 204 (1967). Iconotype: Andrews: t. 22 (1794–1802).

Illustrations: Baker & Oliver, t. 14 (1967); Schumann & Kirsten 44, t. 14 (1992).

Diagnostic features: Corolla orange to orange-red (10–)25–30[–34] mm long, glabrous, sometimes with a few hairs on lobes, sticky to non-sticky; sepals long acuminate from ovate base, with large area of adaxial sessile glands; anthers included to far exserted; leaves 16–20 mm long.

Distribution: On the mainland from the hills just north-east and east of Cape Town, inland to the Witteberg at Matjiesfontein, and south-east to the Langeberg near Ashton, but absent from the Cape Peninsula, open coastal hillslopes to rocky inland mountains, 80–1500 m. Figure 1G.

Conservation status: Classified as 'least concern'.

Subsp. ***perfoliosa*** (E.G.H.Oliv. & I.M.Oliv.) E.G.H.Oliv. & Pirie comb. nov.

Erica abietina subsp. *perfoliosa* E.G.H.Oliv. & I.M.Oliv.: 52 (2002). Type: Western Cape. 3318 (Cape Town): Stellenbosch, Jonkershoek Twins, SW slopes, 600 m, (-DD), 24 May 2001, *E.G.H. & l.M.Oliver 11912* (NBG, holotype; BM, BOL, K, MO, NY, P, PRE, S).

Illustration: Schumann & Kirsten: 44, t. 15 (1992).

Diagnostic features: Corolla pure yellow, 20–25 mm long, densely, finely hairy, non-sticky; sepals broadly elliptic and long acuminate, with adaxial non-sticky sessile glands; anthers included to manifest; leaves 20–30(–42) mm long.

Distribution: Only in the Jonkershoek Valley near Stellenbosch on the moister granitic slopes facing south and south-west; 250–640 m. Figure 1H.

Conservation status: Classified as 'vulnerable'.

Erica situshiemalis E.G.H.Oliv. & Pirie stat. & nom. nov.

E. abietina subsp. *petraea* E.G.H.OIiv. & I.M.Oliv.: 53 (2002), non *E. petraea* Benth.: 668 (1839). Type: Western Cape. 3319 (Worcester): Porterville area, Groot Winterhoek Mtns, Kliphuisvlakte, road to Groot Kliphuis, rock crevices in rocky outcrop, 1140 m, (-AA),

23 November 1999, *E.G.H. & l.M. Oliver 11440* (NBG, holotype; K, PRE). Etymology. Latin: *situs* = the place, site + *hiemalis* = of winter, wintery; from Winter + hoek = corner, place or region (Afrikaans).

Diagnostic features: **Corolla pure yellow**, ± 20 mm long, densely and finely hairy, non-sticky, lobes acute; sepals narrow lanceolate, acute, **without adaxial sessile glands**; anthers manifest to exserted. Differs from related species of the *abietina/viscaria* clade in corolla (tubular inflated to campanulate and 4—10 mm long in *E. parilis* Salisb.; urceolate, 3–5 mm long, pink, with large pink petaloid sepals in *E. collina* Guth. & Bol.).

Distribution: Rock ledges and crevices only, restricted to a few rocky outcrops on the mountains above Porterville; 1000–1100 m. Figure 1I.

Conservation status: Classified as 'endangered'. Known populations of *E. situshiemalis* are few and highly localized; some but not all fall within the protection of the Groot Winterhoek reserve. Those on the Porterville plateau outside the reserve are threatened by farming activities and invasion from alien vegetation combined with increased frequency of fire.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Aligned ETS matrix including cloned sequences and showing primer sites and the sequences used in their design.

Appendix S2. Overview of marker sampling and alignment lengths summarized using SequenceMatrix.

Appendix S3. ETS network and ITS tree, both representing strict consensus trees from the shortest trees recovered under parsimony.