



## *Lacomucinaea*, a new monotypic genus in Thesiaceae (Santalales)

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### Abstract

A new monotypic genus from southern Africa is described based on *Thesium lineatum*. *Lacomucinaea lineata* has a number of vegetative and floral morphological features that differ from *Thesium* and other members of Thesiaceae. An apparently unique feature of the plant is the presence of succulent, fusiform, terete leaves that are caducous, eventually leaving a persistent petiolar stub. The stem surface shows striations formed by cortical fibers inside raised ridges. Anatomically, this type of primary phloem fiber bundle also occurs in *Osyridicarpos*. A molecular phylogenetic analysis using nuclear ribosomal ITS and the chloroplast *trnLF* spacer for representatives of all genera in Thesiaceae showed that *Lacomucinaea* is sister to *Osyridicarpos*, further supporting this relationship suggested by stem anatomy characters. Recognizing this taxon as a distinct genus results in the genus *Thesium* being monophyletic. A key to all genera in Thesiaceae is provided.

**Key words:** parasitic plant; Santalaceae; flora of South Africa; taxonomy; *Thesium*

### Introduction

The largest genus in the sandalwood order (Santalales) is the root hemiparasite *Thesium* Linnaeus (1753: 207). Found in Africa, Europe, Asia, Australia, South America and North America (by introduction), *Thesium* is most diverse in South Africa where at least half the ca. 350 species are found. Although traditionally classified in Santalaceae, a molecular phylogenetic study (Der & Nickrent, 2008) identified six clades that were later classified at the family rank (Nickrent *et al.* 2010). Thus, *Thesium* was included in Thesiaceae along with five other genera: *Buckleya* Torrey (1843:170) is a genus of shrubs with five species disjunct in the eastern USA and eastern Asia. *Kunkeliella* Stearn (1972: 17), is composed of at least four species endemic to the Canary Islands. *Osyridicarpos* Candolle (1857a: 635) is widespread from tropical Africa to South Africa and is considered monospecific (Stauffer, 1961). Finally, *Thesidium* Sonder (1857: 364) is a small group of ca. nine species of South Africa, segregated from *Thesium* based mainly on the monoecious sexual state. Apart from *Kunkeliella*, these generic concepts follow those presented in Pilger (1935). That classification placed *Buckleya* in tribe Osyrideae and *Osyridicarpos*, *Thesidium* and *Thesium* in Thesieae along with *Arjona* Commerson ex Cavanilles (1797: 57) and *Quinchamalium* Molina (1782: 151), the latter two shown by Der & Nickrent (2008) to be in Schoepfiaceae.

A general review of the taxonomic history of *Thesium* was presented in Moore *et al.* (2010). Briefly, the first infrageneric classification was by Reichenbach (1828) who recognized three sections: *Thesium* (of Europe), *Thesiosyris* (of South Africa), and *Frisea* (of South Africa). Alphonse de Candolle (Candolle, 1857a, b) recognized 112 species of *Thesium* worldwide. This classification contained six sections: *Euthesium*, *Aetheothesium*, *Discothesium*, *Frisea*, *Chrysothesium* and *Psilothesium* (the South American species), losing his former section *Hagnothesium* by recognizing *Thesidium* as distinct (following Sonder 1857). The most comprehensive treatment of *Thesium* worldwide was that proposed by Hendrych (1972). Hendrych segregated two genera from *Thesium*, *Chrysothesium* (Jaubert & Spach) Hendrych (1994: 319; formerly section *Chrysothesium*) to accommodate three species from Turkey and one from Central Asia (Hendrych, 1994), and *Austroamericium* Hendrych (1963: 126) which included three species from Venezuela and Brazil. Following the molecular work by Moore *et al.* (2010), and based on the concept that genera should be monophyletic (Backlund & Bremer, 1998), Forest & Manning (2013) formally included *Thesidium* in *Thesium* sect. *Hagnothesium*. Also, based on the topology of the molecular tree in Der & Nickrent (2008), they included *Kunkeliella* in *Thesium* sect. *Kunkeliella*.

In this paper, we present the results of our molecular phylogenetic investigations of Thesiaceae. The main focus of this study is *Thesium lineatum* Linnaeus f. (1782: 162) of southern Africa, a taxon not sampled by Moore *et al.* (2010). Our phylogenetic results, as well as morphological character differences between this species, *Osyridicarpos* and *Thesium*, motivated us to recognize it as a new genus, here named *Lacomucinaea*.

## Materials and Methods

### Sampling

A total of 46 accessions in 41 species of Thesiaceae were included in the molecular study (Table 1). This sampling includes all the generic diversity in the family as well as a wide sampling within *Thesium* intended to span all the major clades (sections) in the genus. 33 of the ITS and 29 of the *trnLF* sequences used here were obtained from Genbank, most of which were derived from the study by Moore *et al.* (2010). Sequences from that work were selected if they were representative of a major clade and were properly identified (based on comparison of sequences bearing the same taxon name from our unpublished data). New sequences generated for this study include 13 ITS and 11 *trnLF*. *Lacomucinaea* was observed, photographed, and collected during field work by the authors in November 2007.

### Molecular methods

Genomic DNA was obtained using two methods. The first was a standard 2X CTAB protocol (Nickrent 1994, 1997) and the second used a cell disrupter with ceramic beads (BIO101/ThermoSavant FastPrep FP120). The protocol is given in the DNeasy 96 Plant Kit (QIAGEN, Valencia, California, USA). The latter method proved to be particularly effective in yielding amplifiable DNA from herbarium specimens. PCR amplification of the ITS region was accomplished using the following two primers: 18S 1830for (5'-AAC AAG GTT TCC GTA GGT GA-3') and 26S 40rev (5'-TCC TCC GCT TAT TGA TAT GC-3') with standard reaction mix and cycling conditions (Nickrent *et al.*, 2004). The *trnLF* region was amplified using the primers published by Taberlet *et al.* (1991). The amplification products were cleaned in two different ways. For products generated and sequenced at SIUC and at Real Jardín Botánico de Madrid, either the QIAquick PCR purification kit (QIAGEN, Valencia, California, USA) or the E.Z.N.A. Clean kit (Omega Biotech, Doraville, Georgia, USA) was used. In-house cycle sequencing reactions were conducted in a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, California, USA) with the BigDye terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), using the above primers. Cycle sequencing reactions were purified using either an ethanol/sodium acetate precipitation method or with ExoSAP-IT® (USB Corporation, Cleveland, OH) generally following the manufacturer's instructions. At SIUC sequencing was conducted on an AB 3730S capillary DNA analyzer (Applied Biosystems). Finally, some of the sequences were generated by Macrogen Inc. (South Korea).

### Phylogenetic analyses

Seqencher® (Gene Codes Corp. version 4.2) was used to edit the electropherograms and to assemble contiguous sequences. The sequences were then imported into Se-AL v2.0a11 (Rambaut, 2007) and manually aligned. This alignment is available as Supplementary File S1. Gaps were treated as missing data for both ITS and *trnLF*. Three datasets were produced: ITS, *trnLF*, and a file where both ITS and *trnLF* were concatenated. Each dataset was exported as NEXUS files and imported into PAUP\* (Swofford, 2002) where maximum parsimony (MP) analyses were conducted. Heuristic searches were conducted with random addition sequence, holding one tree at each taxon addition step, and with tree-bisection-reconnection (TBR) branch swapping. Bootstrap analysis (BS) used 100 pseudo-replicates. Maximum likelihood trees were generated using rapid bootstrapping of 100 pseudo-replicates performed in RAxML v 7.0.4 (Stamatakis, 2006) under the GTR+I+G model. Bayesian phylogenetic inferences were performed using MrBayes v.3.2.2 (Ronquist *et al.*, 2012) run on the CIPRES Science Gateway (Miller *et al.*, 2010). The program MrModeltest v.2.3 (Nylander, 2004) was used to determine the model of sequence evolution for each dataset by the Akaike Information Criterion (AIC). The model selected for the three datasets was the General Time Reversible with a proportion of invariable sites and rate variation among nucleotides following a discrete gamma distribution (GTR+I+G). Two runs with eight simultaneous chains for each starting from random trees were carried out for two million generations, and sampled every 1000 generations. Of the 40002 trees obtained, the first 25% were discarded as burn-in; the 50% majority-rule consensus tree and the Bayesian posterior probabilities (PP) were obtained in MrBayes from the remaining 30002 trees.

TABLE 1. Accession information for all species used in this study. Genbank numbers in **bold** are for new sequences reported here.

Taxon name	ITS Genbank	<i>trnL</i> F Genbank	Country	DNA Extraction no. (DLN)	Collector	Herbarium	Date Collection
<i>Buckleya distichophylla</i>	AF291901	EF464484	USA	-	Boufford <i>et al.</i> 5/4/98	A	4 May 1998
<i>Buckleya graebneriana</i>	AF291905	--	China	-	Boufford <i>et al.</i> 26520	A	--
<i>Buckleya henryi</i>	AF291906	--	China	-	Boufford <i>et al.</i> 26105	A	--
<i>Buckleya lanceolata</i>	AF291904	GU294669	Japan	-	Yonekura <i>et al.</i> 95537	A	--
<i>Osyridicarpus schimperianus</i>	<b>KP318955</b>	<b>KP318968</b>	South Africa	4110	Nickrent 4110	SIU	16 Nov. 1996
<i>Lacomucinaea lineata</i>	<b>KP318956</b>	<b>KP318969</b>	South Africa	4413	Bagnidze <i>et al.</i> 1011	MO	17 May 1997
<i>Lacomucinaea lineata</i>	<b>KP318957</b>	--	South Africa	4725	Coppejans EC 1016.	BR	21 Aug. 1971
<i>Lacomucinaea lineata</i>	<b>KP318958</b>	--	South Africa	4726	Goldblatt 6526	BR	5 Feb. 1982
<i>Lacomucinaea lineata</i>	<b>KP318959</b>	--	South Africa	4800	Bamps 9427	BR	13 Feb. 2000
<i>Lacomucinaea lineata</i>	<b>KP318960</b>	<b>KP318970</b>	South Africa	5509	Mucina 020906/12	MA-878942	2 Sept. 2006
<i>Thesium (Kunkeliella) retamoides</i>	<b>KP318961</b>	<b>KP318971</b>	Spain (Canary Islands)	5559	Hernández s.n.	MA-655421	29 May 1999
<i>Thesium (Kunkeliella) subsucculentum</i>	<b>KP318962</b>	<b>KP318972</b>	Spain (Canary Islands)	4374	Santos Guerra	TFMC	23 Jan. 2002
<i>Thesium mauritanicum</i>	<b>KP318966</b>	--	Morocco	4844	Aedo <i>et al.</i> CA4296	MA-593174	26 June 1997
<i>Thesium mauritanicum</i>	<b>KP318967</b>	<b>KP318976</b>	Morocco	5193	Castroviejo <i>et al.</i> SC17994	MA-745841	8 July 2006
<i>Thesium (Thesidium) fragile</i>	GU256783	GU294681	South Africa	--	Verboom 912	BOL	--
<i>Thesium (Thesidium) leptostachyum</i>	GU256784	GU294607	South Africa	--	Forest 1	NBG	--
<i>Thesium (Thesidium) microcarpum</i>	GU256786	GU294609	South Africa	--	Verboom 1150	BOL	--
<i>Thesium (Chrysothesium) stelleroides</i>	<b>KP318963</b>	<b>KP318973</b>	Turkey	4848	Muñoz Garmedia <i>et al.</i> 4525.	MA-688669	3 Jul. 2001
<i>Thesium (Chrysothesium) minkwitzianum</i>	<b>KP318964</b>	<b>KP318974</b>	Uzbekistan	5112	Krassovskaya & Levichev 18	LE	8 Jul. 1976
<i>Thesium (Chrysothesium) cilicicum</i>	<b>KP318965</b>	<b>KP318975</b>	Turkey	4838	Muñoz Garmedia <i>et al.</i> 4566	MA-688649	3 Jul. 2001
<i>Thesium bergeri</i>	GU256778	GU294600	Greece	--	Brummit & Powell 18740	--	--
<i>Thesium impressum</i>	GU256776	GU294598	Turkey	--	Davis 23148	K	--
<i>Thesium alpinum</i>	GU256777	GU294599	Bulgaria	--	BK 80311	BOL	--
<i>Thesium chinense</i>	GU256781	<b>KP318977</b>	Russia	5153	Neczaeva s.n.	LE	25 June 1972
<i>Thesium humifusum</i>	GU256780	GU294604	Spain	--	Chase 1881	K	--

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TABLE 1. (Continued)

Taxon name	ITS Genbank	<i>trnL</i> F Genbank	Country	DNA Extraction no. (DLN)	Collector	Herbarium	Date Collection
<i>Thesium spinulosum</i>	GU256811	<b>KP318978</b>	South Africa	5424	García <i>et al.</i> 5424	MA-869529	3 Dec. 2007
<i>Thesium (Austroamericium) brasiliense*</i>	GU256824	GU294639	Brazil	--	Santos <i>et al.</i> 236	K	--
<i>Thesium asterias</i>	GU256857	GU294664	South Africa	--	Trinder-Smith 432	BOL	--
<i>Thesium capituliflorum</i>	GU256848	GU294655	South Africa	--	Moore 165	BOL	--
<i>Thesium cupressoides</i>	GU256859	GU294666	South Africa	--	Verboom 1026	BOL	--
<i>Thesium euphorbioides</i>	GU256791	GU294614	South Africa	--	Forest 953	NBG	--
<i>Thesium frisea</i>	GU256826	GU294640	South Africa	--	Forest CP3	NBG	--
<i>Thesium gracile</i>	GU256860	GU294667	South Africa	--	Verboom 1054a	BOL	--
<i>Thesium juncifolium</i>	GU256846	GU294653	South Africa	--	Moore 62	BOL	--
<i>Thesium leptocaulis</i>	GU256804	GU294624	South Africa	--	Forest 768	NBG	--
<i>Thesium lopollense</i>	GU256823	GU294638	South Africa	--	ANGOLA 1959	BOL	--
<i>Thesium paniculatum</i>	GU256821	GU294636	Angola	--	Moore 23	BOL	--
<i>Thesium pinifolium</i>	GU256800	GU294620	South Africa	--	Moore 43	BOL	--
<i>Thesium pseudovirgatum</i>	GU256835	GU294648	South Africa	--	Britton 1904/084	BOL	--
<i>Thesium pycnanthum</i>	GU256797	GU294617	South Africa	--	Britton 1904/082	BOL	--
<i>Thesium radicans</i>	GU256779	GU294601	Saudi Arabia	--	Collenette 8173	K	11 May 1987
<i>Thesium schweinfurthii</i>	GU256782	GU294605	Zaire	--	Malaisse 13645	K	--
<i>Thesium spicatum</i>	GU256853	GU294660	South Africa	--	Verboom 1300	BOL	--
<i>Thesium strictum</i>	GU256813	GU294622	South Africa	--	Forest 668	NBG	--
<i>Thesium triflorum</i>	GU256788	GU294611	South Africa	--	Moore 128	BOL	--
<i>Thesium viridifolium</i>	GU256793	GU294616	South Africa	--	Forest 680	NBG	--

\* Plant name for ITS sequence in Genbank given as *Austroamericium tepuiense*, but the specimen was collected in southern Brazil. *Thesium tepuiense* is endemic to the Tepui region of Venezuela, Roraima (N Brazil) and Guyana. This sequence is here tentatively identified as *T. brasiliense* until verification.

### Distribution map

393 records were downloaded from GBIF that were identified as *Thesium lineatum*. These herbarium records were not individually verified; however, we assume misidentifications are relatively rare given that this species is easily recognized. Among these, 172 contained latitude/longitude coordinates and 124 of these had unique locations. These unique locations were plotted on a map of southern Africa using Google My Maps. The generalized vegetation map of South Africa, Lesotho and Swaziland was modified from the bioregions map from Mucina & Rutherford (2006, Fig. 3.2 p. 33). The biome map of Namibia was obtained from the web site Digital Atlas of Namibia, map SFB 389 'ACACIA' subproject E1, University of Cologne and the color coding changed to correspond with those of South Africa. The biome map was then used as a layer in Adobe Photoshop and the dot map overlaid upon this layer.

### Stem anatomy

Herbarium samples of *Lacomucinaea* as well as *Osyridicarpos* and eight species of *Thesium* were boiled in a weak detergent solution and then transferred to 65% ethanol with 5% glycerin. The stems were hand sectioned, stained briefly in methylene blue, and rinsed. The sections were then observed and photographed using both stereo and compound microscopes. Photos of stem sections can be seen in Supplementary File S2.

## Results

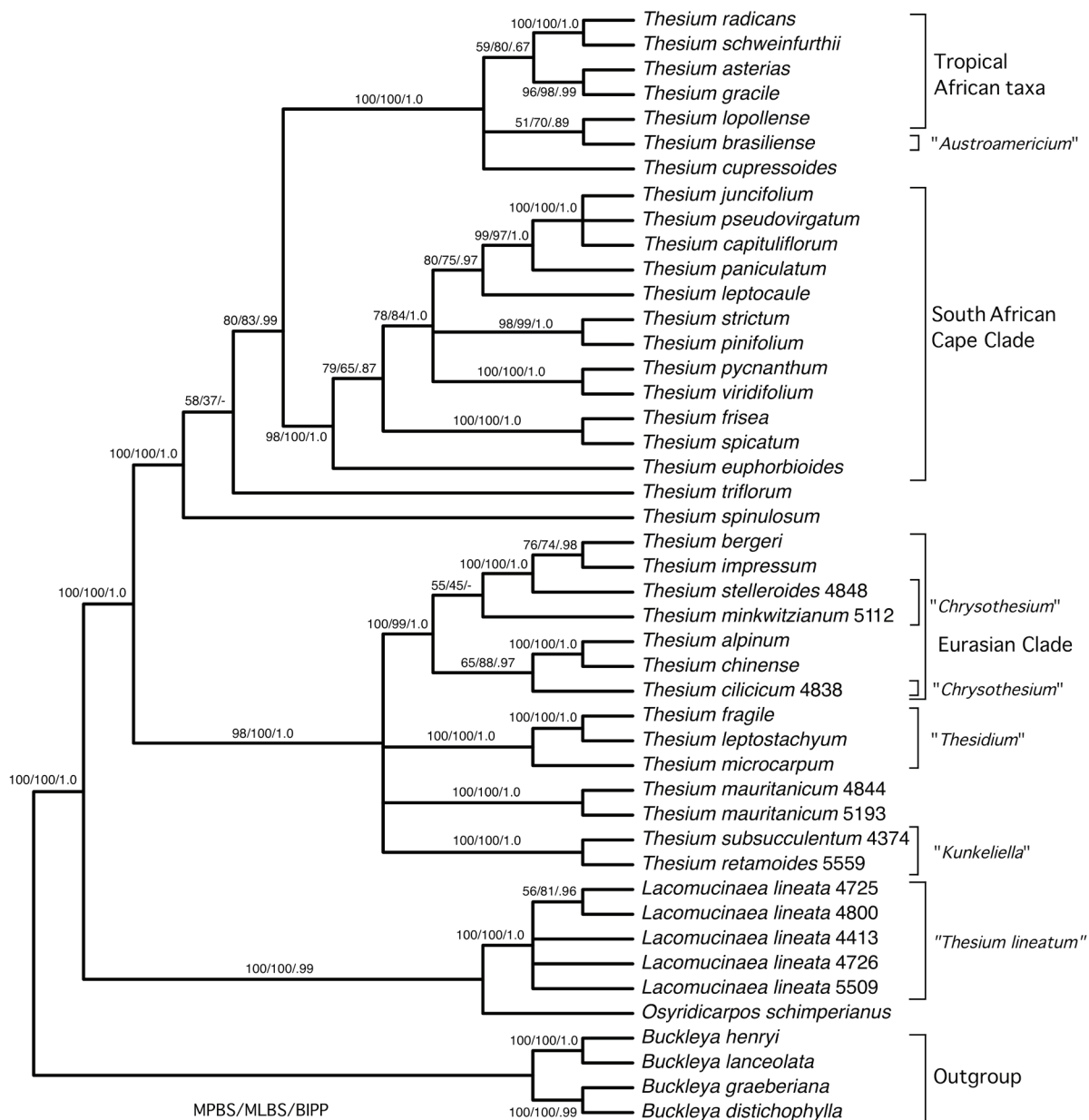
The characteristics of the ITS, *trnLF* and concatenated datasets are presented in Table 2. Although the alignment length for ITS was significantly shorter than that of *trnLF*, the number of parsimony informative sites as a percentage of the total length was higher (48% vs. 15%). The number of trees recovered for these two datasets also reflects the lower phylogenetic signal in the *trnLF* dataset vs. ITS. The ITS alignment shows a number of shared features between *Osyridicarpos* and *Lacomucinaea*, such as a gap (deletion) in ITS-1 (aligned positions 52–123) that is not seen in any other Thesiaceae. The already strong support for a relationship between these two taxa would have increased if gap coding had been employed.

TABLE 2. Characteristics of the different data sets

Dataset	No. Taxa	Alignment Length	PI*	Tree Length	MP	CI	RI	RC
ITS	45	759	366	1120	6	0.5763	0.8164	0.4964
<i>trnLF</i>	39	1170	179	482	468	0.717	0.8665	0.7047
Concatenated	45	1929	545	1619	84	0.6018	0.8219	0.5447
ITS + <i>trnLF</i>								

\*PI = Number parsimony informative sites, MP = Number maximum parsimony trees, CI = Consistency index minus uninformative sites, RI = Retention index, RC = Rescaled consistency index

The relationships among the 44 Thesiaceae taxa as shown in the ITS and *trnLF* trees were highly congruent, although less resolved in the latter. The individual gene trees (each for MP, ML, and BI) are presented in Supplementary File S3. The MP strict consensus tree resulting from the concatenated analyses (Fig. 1) shows at each node MP and ML BS values followed by BI posterior probabilities (PP). Rooting the tree with *Buckleya*, two strongly supported major clades are present: one containing *Osyridicarpos* and *Lacomucinaea* and another containing the remaining *Thesium* species. Within *Thesium*, two clades were resolved: the first containing *Kunkeliella* and *Thesidium* along with a Eurasian clade that includes *Chrysothesium*. Relationships among these secondary clades are poorly resolved in this analysis. The second strongly supported clade within *Thesium* includes all the remaining African species. *Thesium spinulosum* Candolle (1857a: 647) and *T. triflorum* Linnaeus. (1782: 162) are successively sister to the African clade. Two strongly supported clades exist among the African *Thesium*: the South African Cape clade and the Tropical African clade. Within the latter is the South American species *T. brasiliense* Candolle (1857a: 671) that had previously been recognized as a separate genus, *Austroamericium*.



**FIGURE 1.** Phylogenetic tree of Thesiaceae obtained from the concatenated ITS + *trnLF* dataset. Numbers at nodes show maximum parsimony bootstrap / maximum likelihood bootstrap / Bayesian inference posterior probabilities. The labels along the right side of the tree indicate taxa that have been considered genera distinct from *Thesium*, but those in parentheses (“ ”) considered here synonyms.

## Discussion

### *Generic concepts in Thesiaceae*

The results of our analyses are generally congruent with those of Moore *et al.* (2010); however, that study did not include several key taxa such as *Osyridicarpos*, *Lacomucinaea*, *Kunkeliella*, *Chrysothesium* and *Thesium mauritanicum* Battandier (1889: 393). Given the topology of our tree (Fig. 1), assigning taxa to genera while maintaining monophyly could basically follow a “lumping” or “splitting” philosophy. We concur with Forest & Manning (2013), who avoided splitting the African from the Eurasian *Thesium*, and whose approach was therefore to lump *Thesidium* and *Kunkeliella* into a broadly defined *Thesium*. It should also be pointed out that the three members of “*Chrysothesium*” are not monophyletic and are included within a clade of other Eurasian *Thesium*. The species of *Chrysothesium* are morphologically very different to each other, sharing only the tubular flowers, a character also present in many other

species not included by Hendrych in his genus. For this reason, separate generic (or even sectional) status is not warranted for these three species. Moreover, *T. brasiliense* from South America is clearly related to a group of tropical African *Thesium*, thus it does not warrant separate generic status as *Austroamericium*.

Our analyses show that *Thesium lineatum* of southern Africa is sister to *Osyridicarpos* with strong support. If this taxon were to remain in *Thesium*, then *Osyridicarpos* should also be included in that genus to avoid paraphyly. A second approach would be to include *T. lineatum* in *Osyridicarpos*. A third approach, and the one we elect to follow here, is to name *T. lineatum* as a distinct genus. Although it shares a number of morphological features with *Thesium* and *Osyridicarpos*, there are distinct differences.

Stauffer (1961) concluded that *Osyridicarpos* was monotypic, with the only species being *O. schimperianus* Candolle (1857a: 635), and suggested it be maintained separate from *Thesium*. The taxon named *Osyridicarpos linearifolius* Engler (1900: 385) was found by Stauffer (1961) to be identical to *T. triflorum*. This species was included in our phylogeny (Fig. 1) and is distant from *O. schimperianus*. After examining many types and vouchers, Stauffer concluded that *Osyridicarpos* differed from *Thesium* in 1) the shape and constriction of the corolla tube, 2) an inconspicuous disk, 3) the presence of a conical projection at the apex of the placenta, 4) 4 or 5 ovules (vs. 3 or 4 in *Thesium*) and 5) the shape of the persistent corolla tube on the fruit. Stauffer (1961) admitted that *Osyridicarpos* was very closely related to *Thesium*, but justified keeping them separate because generally small differences were used to separate genera in Santalaceae. He was the first to recognize that the classification of genera into tribes Osyrideae and Thesieae as conceived by Bentham (1880) and Pilger (1935) was completely artificial. This conclusion was strongly supported by the molecular phylogenetic investigation of Santalaceae s.lat. by Der & Nickrent (2008).

When Stauffer (1961) discussed morphological differences between *Osyridicarpos* and *Thesium*, he used *T. triflorum* as his exemplar of the latter genus. Thus, the apparent differences did not encompass the range of morphologies seen among all *Thesium* species. From our examination of hundreds of *Thesium* specimens (see photographs on [www.phytoimages.siu.edu](http://www.phytoimages.siu.edu)), it appears the first four characters listed above are not diagnostic of the genera.

Genetic distances obtained from DNA sequence data have been used to address intergeneric differences in Santalales, for example in Cervantesiaceae (Rogers *et al.* 2008) and Aptandraceae (Ulloa *et al.* 2010). Using the ITS and trnLF data matrix, uncorrected “p” distances generated in PAUP\* (Swofford 2002) showed that *Buckleya* is the most distinctive member of Thesiaceae with average intergeneric distances ranging from 0.25 (*Osyridicarpos*) to 0.27 (*Lacomucinaea* and *Thesium*). The genetic distances also indicate that *Lacomucinaea* and *Osyridicarpos* are distinct from *Thesium* (values 0.16 and 0.13, respectively). This result provides support for maintaining these genera as distinct from *Thesium*. Among the four genera, the smallest distance (0.08) is seen between *Lacomucinaea* and *Osyridicarpos*. Considering only the genetic distance data, the argument could be made to classify these two monotypic taxa within one genus. We choose, however, to use a total evidence approach and consider the morphological differences as well.

Like *Buckleya*, *Lacomucinaea* is a true shrub with well developed woody stems. This habit is not seen in either *Thesium* or *Osyridicarpos*. In most species of *Thesium* the fruiting pedicel enlarges forming an elaiosome but this is not seen in *Lacomucinaea* and *Osyridicarpos*, likely reflecting dispersal by animals other than ants. Similar smooth, fleshy fruits lacking an elaiosome have evolved in *Thesium* Sect. *Kunkeliella*. Leaf morphology between *Lacomucinaea* and *Osyridicarpos* is quite different with the latter showing leaves with typical laminas and petioles. Although scale leaves form in some *Thesium* species via senescence, the succulent caducous leaves seen in *Lacomucinaea* are unique in the family.

## Taxonomic Treatment

### *Lacomucinaea* Nickrent & M.A. García, *gen. nov.*

*Diagnosis:* Shrubs with glaucous branches and characteristic yellow-green longitudinal striations, solitary flowers and white drupes. It is distinguished from *Thesium* and *Osyridicarpos* by its early caducous leaves in which the senescence starts from the petiole, not from the apex.

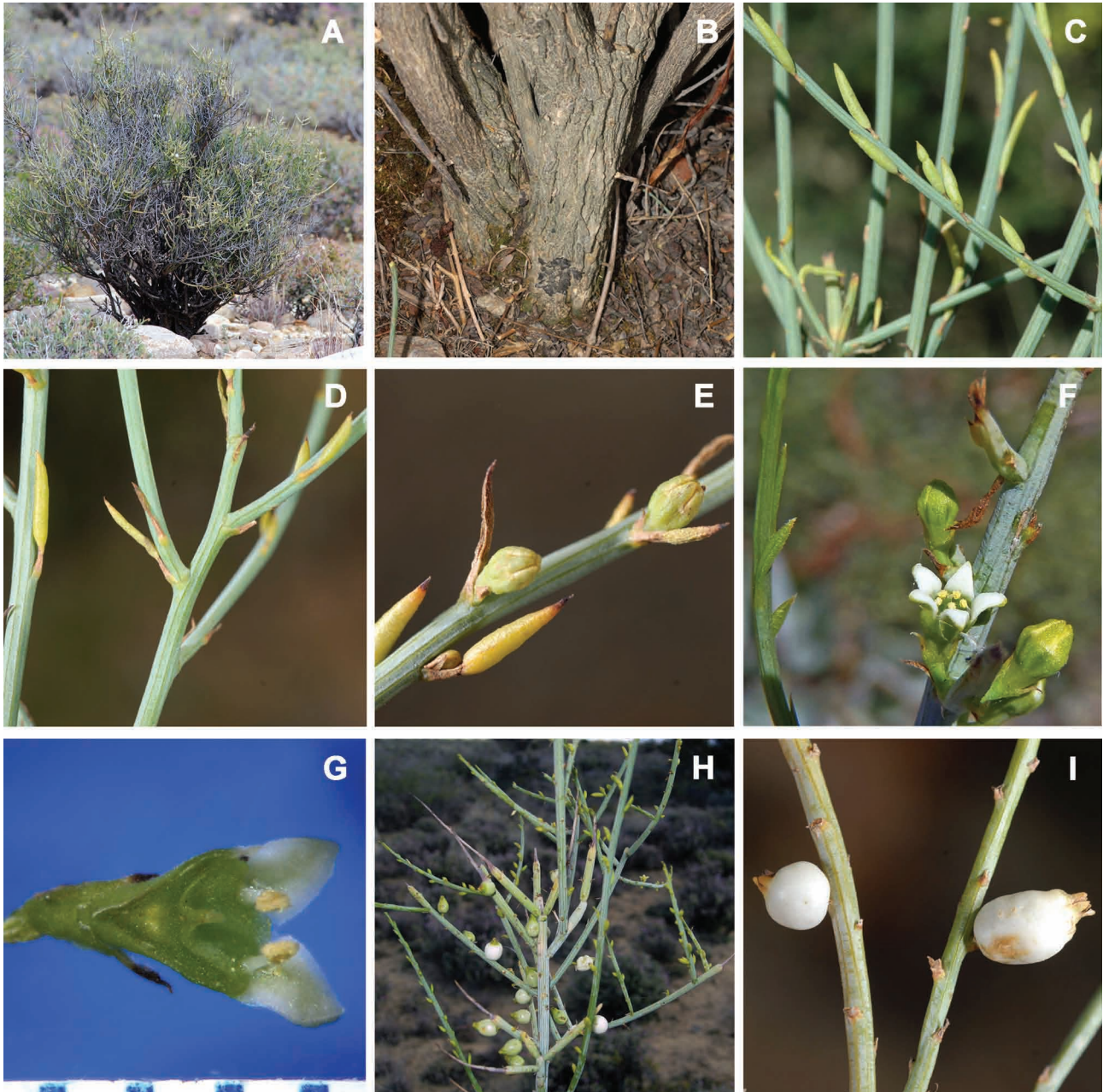
*Eponymy:* The name *Lacomucinaea* is in honor of Professor Ladislav (“Laco”) Mucina who was born 28 May 1956 in Piešťany, Slovakia (formerly Czechoslovakia). Dr. Mucina is a renowned expert on the South African vegetation and has a keen interest in the systematics of *Thesium* as well as numerous other plants.

*Lacomucinaea lineata* (Linnaeus f.) Nickrent & M.A.García, *comb. nov.* (Fig. 2).

Basionym: *Thesium lineatum* Linnaeus. (1782: 162). Type:—SOUTH AFRICA. “Habitat in Cap. Bonae spei.”, *C.P. Thunberg s.n.* (holotype UPS-THUNB 6035!).

*Thesium ephedroides* A.W. Hill (1910: 183). Type:—NAMIBIA. Kabub Mts., 1630 m, October 1906, *P.T. Range 65* (holotype B!).

*Thesium viscibaccatum* Dinter (1926: 452). Type:—NAMIBIA. Klinghardt Gebirge, 26 September 1922, *M.K. Dinter 4006* (holotype PRE!).

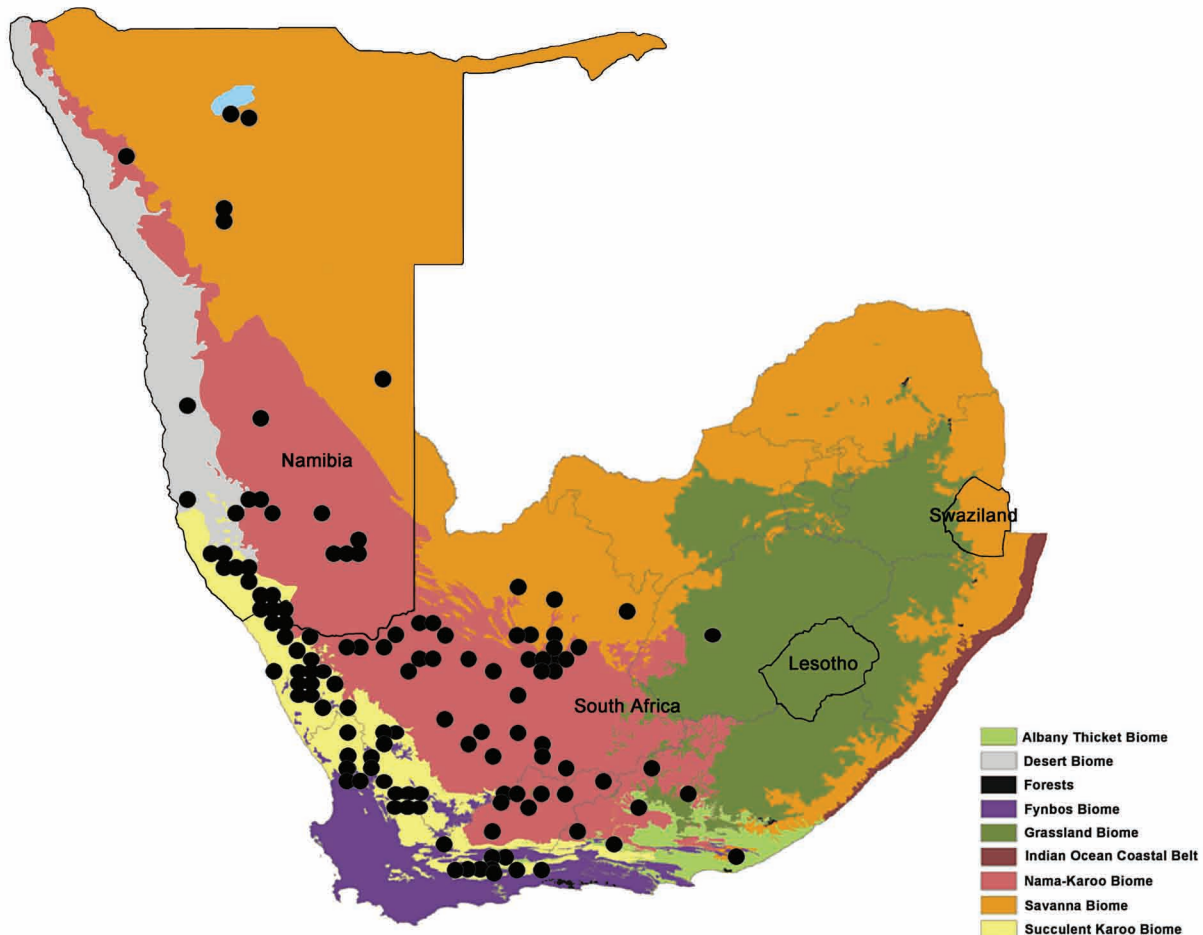


**FIGURE 2.** *Lacomucinaea lineata* vegetative and reproductive features. A. Plant habit. B. Base of stem showing bark features. C. Young branches bearing succulent leaves with petioles beginning to senesce. D. Older branches showing further senescence of leaves which eventually yellow and abscise leaving a peg-like petiolar stub. E. The flowers, here in bud stage, are borne singly in the axils of leaves. F. Inflorescences with flower buds and a flower in anthesis (photo by Nicky van Berkel, used with permission). G. Flower in longitudinal section, showing the two subtending bracteoles and the lobed hypanthial disk. H. Shoot with young and mature fruits. I. Closer view of fruits showing smooth surface and persistent corolla lobes at apex.

Additional specimens examined: SOUTH AFRICA: Barrydale, hellig van de Warmwaterberg. Klipperige bodem, 21 Aug. 1971, *E. Coppejans EC1016* (BR); Van Rhynsdorp DA, SW trending rocky slopes above Wiedouw River, 5 Feb. 1982, *P. Goldblatt 6526* (BR); Along R354, ca. 30 km north of Matjiesfontein, 29 Oct. 1996, *D. L. Nickrent et al.*



4088.5 (SIU); Prieska District. About 3 km north west of Prieska, 3 Apr. 2000, *P. Balkwill & L. McDade 11765* (MO); Namaqualand, Bulletrap (N of Springbok), 3–4 km on road to Nigramoep, 2 Sept. 2006, *L. Mucina 020906/12* (MA, NBG); 5.34 air km E of Middelplaas, 25 Nov. 2007, *M. A. García et al. DLN 5388* (MA, SIU, NBG); Hills above rest area along R 341, ca. 3.6 air km S of Klaarstroom at Peraboom Drif, 27 Nov. 2007, *M. A. García et al. DLN 5397* (MA, SIU, NBG). NAMIBIA: Lüderitz District. Farm Süd-Witpütz, *Lavranos & Pehlemann 21691* (MO).



**FIGURE 3.** Distribution of *Lacomucinaea lineata* obtained from 124 unique locations present on the GBIF herbarium record database. See Materials and Methods for sources of the biome maps for South Africa, Lesotho, Namibia, and Swaziland.

## Description

Shrub to 2 m tall, densely branched, compact or spreading; bark on basal stems furrowed and ridged, grey to brown; young branches sometimes succulent, glabrous, glaucous, with yellow-green longitudinal striations that branch dichotomously and also anastomose. These striations later, following periderm growth, form the ridges on the bark of older branches. Shorter branches becoming spinose with age, with dry, tapering, pungent apices. Leaves alternate, simple, ciliolate when young, sparsely distributed on younger growth, petiole ca. 1/5 to 1/6 length of leaf, blade succulent, initially somewhat fusiform, terete, tapering more at the distal end, apex acute, 2–5 × 1 mm, caducous, progressively senescing on developmentally older stems, petiole and leaf apex senescing first, eventually followed by the middle portion of the leaf; leaf abscising leaving a persistent brown petiolar stub or sometimes as a withered whole leaf; lateral branches subtended by one main bract and two transverse ones above. Flowers solitary, peduncle 1.5 mm long, subtended by a bract, at apex with two transversely oriented (with respect to bract), triangular to sublanceolate

bracteoles, initially green, becoming brown and squamate, apex acuminate, senescing, margin ciliolate. Flower pedicel 1 mm long, merging gradually into the receptacle. Calyx not differentiated (fused to ovary and hypanthial cup), ca. 0.6 mm high, bearing a slightly raised, elliptic gland at lobe junctions. Corolla actinomorphic, (4)5-merous, 2.5–3.0 mm wide, corolla tube not present, lobes valvate, triangular, 1–1.5 mm long, uncinata, outer surface glabrous, inner surface without apical trichomes (beard), white. Stamens 5, antipetalous, inserted at junction of disk and corolla, at anthesis reaching only midway to corolla lobes, anther and filament each 0.4–0.5 mm long, anther subbasifixed, dithecal, tetrasporangiate, introrse, connected to corolla by post-staminal trichomes. Pollen heteropolar, tricolpate (see Fig. 4a in Stearn 1972). Disk lining hypanthium, at rim with distinct rounded lobes alternating with corolla lobes. Style filiform, ca. 0.8 mm long, stigma small, 2-lobed, lobes globose, positioned at ca. the height of the anthers. Ovary inferior, unilocular, placentation free-central with 3 pendulous ovules, placenta contorted with a peg-shaped projection at its apex. Fruit a drupe, pedunculate with persistent, scarious subtending bracteoles, white at maturity, globose to ellipsoid, 5–8 mm long, surface smooth, no reticulum visible, crowned by persistent, scarious, brown corolla lobes. Seed enclosed in a stony endocarp.

*Lacomucinaea* has a number of morphological features that are generally considered xeric adaptations. The glaucous stems are photosynthetic and, particularly on shorter axes, may become shoot spines such as those seen in *Ulex* Linnaeus (1753: 741; Fabaceae). Both its leaves and distal stems are succulent. The caducous nature of its leaves, not seen in any other species in Thesiaceae, are reminiscent of this syndrome in succulents such as *Opuntia subulata* Engelmann (1883: 627; Cactaceae) and some members of *Euphorbia* Linnaeus (1753: 450; Euphorbiaceae). The striations that inspired the specific name of this plant are formed by cortical fibers that form long, raised ridges on the stems. Anatomically, these are primary phloem fiber bundles (not collenchyma as stated in Hendrych 1972), similar to the bast fibers seen in stems of flax (*Linum usitatissimum* Linnaeus (1753: 277; Linaceae)). For *Lacomucinaea* and *Osyridicarpos*, these bundles are located in the cortex near the epidermis with a cushion of collenchyma in between. This arrangement is different from that seen in many South African *Thesium* with decurrent leaves where the primary phloem fiber bundles are located deep in the cortex near the vascular cambium. (Supplementary File S2).

*Lacomucinaea* is widely distributed in southern Africa ranging from central South Africa to Namibia (Fig. 3). It has not been reported for Botswana (Setshogo, 2005) but might be expected in the savanna biome in the southwestern part of that country. Examination of its distribution across biomes and bioregions of South Africa (Mucina & Rutherford, 2006) shows it is most abundant in the Succulent Karoo and Nama-Karoo biomes. This plant is nearly absent in the Fynbos biome, with the exception of the Hantam Plateau Dolerite Renosterveld, part of the Granite and Dolerite Renosterveld Bioregion (Mucina & Rutherford 2006). It is also known from the Great Fish Noorsveld vegetation unit of the Albany Thicket biome near Grahamstown.

### Key to the Genera of Thesiaceae

1. Leaves opposite to subopposite; leafy sepals prominent on carpellate flowers ..... *Buckleya*
- Leaves (or in squamate taxa, scales) alternate (rarely subopposite, e.g. *Thesium imbricatum*); sepals absent (rarely present and vestigial in a few taxa, e.g. *T. wilczekianum* and *T. libericum*), not leafy ..... 2
2. Subshrubs, herbaceous perennials, herbaceous annuals; leaves without a distinct petiole (sessile); fruiting pedicel often swollen forming an elaiosome; fruit surface often with prominent venation (exceptions to latter two in *Thesium* Sect. *Kunkeliella*) ..... *Thesium*
- Compact shrubs or scrambling subshrubs; leaves with distinct petioles; fruiting pedicel not swollen into an elaiosome; fruit surface smooth without prominent venation ..... 3
3. Scrambling subshrubs; leaf blades flat, lamina broad, not succulent and caducous; petiole > 4 mm long; placenta twisted its entire length ..... *Osyridicarpos*
- Compact shrubs; leaf blades terete, lamina fusiform, succulent and caducous; petiole < 4 mm long; placenta twisted in upper half ..... *Lacomucinaea*

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