Preliminary phylogenetic analysis of the fern genus Lomariopsis (Lomariopsidaceae)

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Abstract. A preliminary phylogenetic analysis is presented for *Lomariopsis* based on sequence data from the chloroplast intergenic spacer *trnL-trnF*. The analysis includes 27 (60%) of the approximately 45 species in the genus. A strict consensus of six most parsimonious trees supports two main clades—the *Sorbifolia*-group and the *Japurensis*-group—previously proposed based on heteroblastic leaf development. The *Sorbifolia*-group is entirely neotropical and includes all the Antillean species. The species in this clade had either smooth or crested spores, but the tree was ambiguous whether these spore types define two separate clades. The *Japurensis*-group consists of two clades, one primarily neotropical and the other entirely paleotropical. Within the neotropical clade nests a clade of two African species, which have long-spiny spores typical of the neotropical clade and unlike those found in the African-Madagascan clade. The occurrence of these two species in Africa is best explained by long-distance spore dispersal of their ancestral species from the neotropics to Africa. Within the paleotropical clade of the *Japurensis*-group, a clade of three African species is nested among seven species from Madagascar (all the species from that island). Within the genus as a whole, a derived character—the abortion of the rachis apex and its replacement by the distal lateral pinna assuming a terminal position—was found to have evolved separately in each of the four species with this kind of leaf apex. A scanning electron microcope study of the spores revealed five types, and a transformation series for these different types is proposed. Characters of spore morphology and heteroblastic leaf development agreed with many of the clades in the phylogenetic tree. This study represents the first phylogeny for the genus.

Key words: Ferns, biogeography, phylogeny, taxonomy, leaf development, spores.

Resumen. Se presenta un análisis filogenético preliminar para *Lomariopsis* basado en datos de secuencias del espaciador intergenético del cloroplasto *trnL-trnF*. El análisis incluye 27 (60%) de las aproximadamente 45 especies del género. Un consenso estricto de seis árboles más parsimoniosos soporta dos clados principales—el grupo *Sorbifolia* y el grupo *Japurensis*—previamente propuestos en análisis basados en el desarrollo heteroblástico de las hojas. El grupo *Sorbifolia* es completamente neotropical e incluye todas las especies de las Antillas. Las especies de este clado presentan esporas lisas o crestadas, sin embargo el árbol fue ambiguo con respecto a si este tipo de esporas define dos clados separados. El grupo *Japurensis* está compuesto por dos clados, uno principalmente neotropical y otro completamente paleotropical. Dentro del clado neotropical se encuentra anidado un clado de dos especies

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Africanas, que poseen un tipo de esporas con espinas largas que es típico del clado neotropical y diferente al encontrado en el clado compuesto por especies Africanas y de Madagascar. La mejor explicación para la presencia de estas dos especies en Africa es dispersión a largas distancias de esporas de sus especies ancestrales desde el neotrópico hacia África. Dentro del clado paleotropical del grupo *Japurensis*, un clado de tres especies africanas se encuentra anidado entre siete especies de Madagascar (la totalidad de las especies de esta isla). Para el género completo un carácter derivado, el aborto del ápice del raquis y su reemplazo por la pinna lateral distal asumiendo una posición terminal, se encontró que ha evolucionado por separado en cada una de las cuatro especies con esta clase de ápice de la hoja. Un estudio de microscopio electrónico de barrido de las esporas reveló cinco tipos, se propone una serie de transición para estos diferentes tipos. Los caracteres de la morfología de la espora y del desarrollo heteroblástico de la hoja proporcionaron soporte adicional para muchos de los clados en el árbol filogenético. Este estudio representa la primera filogenia del género.

Lomariopsis Fée is a pantropical fern genus with a few extensions into the temperate regions. It contains about 45 species, of which 15 occur in the American tropics (Moran, 2000), ten in Africa (Holttum, 1940), ten on the islands in the Indian Ocean (Holttum, 1938; Tardieu-Blot, 1960; Lorence, 1978), and ten in Asia, Malesia, Queensland, and the Pacific (Holttum, 1978). The species occur in wet forests, mostly below 1500 m.

The genus has a striking growth habit. Its stems are long-creeping and climb to heights of 10 meters on tree trunks. Periodically, a flush of fertile (spore-bearing) leaves is produced, and these are strongly dimorphic compared to the sterile leaves, being longer-petiolate, narrower, and more erect. The sori are acrostichoid; that is, they appear scattered across the undersurface of the pinnae, not arranged in discrete dots or lines as in most ferns. Soon after the spores are shed, the fertile leaves wilt, but the sterile leaves persist. Both sterile and fertile leaves are 1-pinnate with a terminal segment that resembles the lateral pinnae. The lateral pinnae are articulate to the rachis, but the articulations are apparently nonfunctional (the pinnae do not drop cleanly with age).

Lomariopsis has been studied floristically in the New and Old Worlds (Holttum, 1938, 1940, 1966, 1978; Moran, 2000), but little is known about its phylogeny. Hybridization in the genus is apparently rare, with only one example known (Moran & Watkins, 2004). Holttum (1938) suggested that there were two main clades in the genus based on heteroblastic leaf series (that is, the transformation in the morphology of the sequence of leaves from the earliest to a mature, spore-bearing one). He did not, however, name these groups. One group, informally called the *Sorbifolia*-group (Moran, 2000), has the early leaves in the series simple and entire, but when leaves are produced over 2 cm long, they are pinnate with dentate or crenate pinnae (Fig. 1A). In the second group, the *Japurensis*-group (Moran, 2000), the juvenile leaves remain simple and entire, resembling the terminal pinna of an adult leaf, until those in the sequence reach 15–20 cm long. At this length, the first lateral pinna is formed. This and subsequent pinnae are entire, not dentate. Gradually more and more pinnae are produced on the successively larger leaves until the adult stage is reached (Fig. 1B). Aside from Holttum's suggestion (1938) about two main clades based on heteroblastic leaf development, no other phylogenetic hypotheses have been proposed for the genus. *Lomariopsis* has no fossil record (Collinson, 2001; Skog, 2001).

The purpose of this paper is to assess the phylogeny of *Lomariopsis* using the chloroplast intergenic spacer *trnL-trnF*, and to use the resulting tree as a framework to examine the evolution of certain morphological characteristics within the genus, particularly spore wall structure.

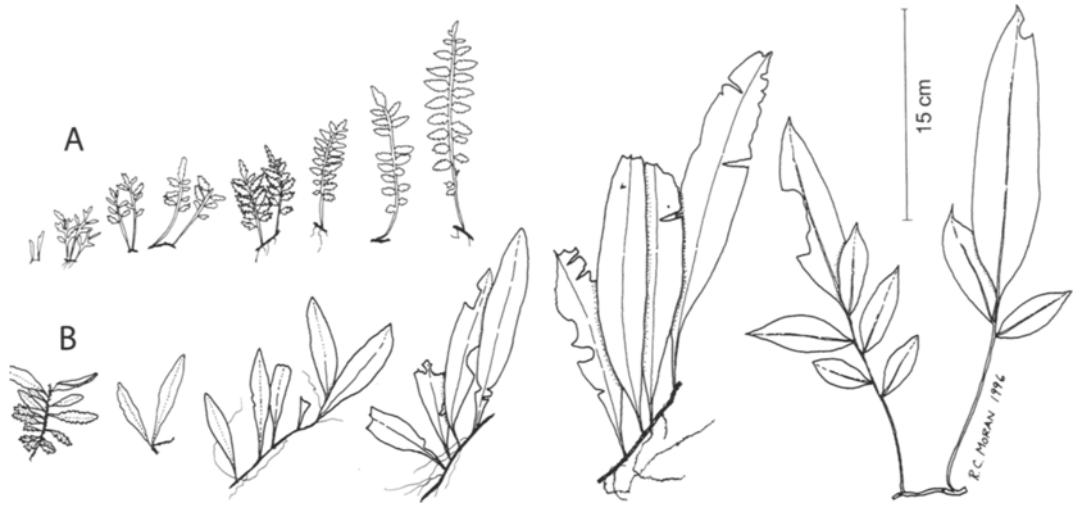

FIG. 1. Heteroblastic leaf series of *Lomariopsis*. **A.** *Sorbifolia*-group (*L. vestita*). **B.** *Japurensis*-group (*L. nigropaleata*).

Methods

Study of Spores

Spores were obtained from herbarium specimens at MO, NY, and P of 33 species of *Lomariopsis*. With dissecting needles the spores were transferred from the specimens to aluminum scanning electron microscope (SEM) stubs coated with a carbon or asphalt adhesive. The stubs were then coated with gold-palladium in a sputter coater for 30 to 40 seconds and imaged digitally with an SEM. Images of all species examined were posted at the public website www.plantsystematics.org.

Taxon Sampling for Molecular Phylogeny

Material was collected in the wild or from herbarium specimens at NY and P. For the outgroup we used *Elaphoglossum amygdalifolium* (Mett. ex Kuhn) H. Christ, which is sister to the rest of the species in its genus (Rouhan et al., 2004). *Elaphoglossum* has been believed related to *Lomariopsis* (along with *Bolbitis*, *Teratophyllum*) because both have dimorphic sterile and fertile fronds, acrostichoid sori, and creeping rhizomes with an elongated (in cross section), root-bearing ventral vascular bundle (Holttum, 1978; Kramer et al., 1990). Voucher information and GenBank accession numbers are reported in Table I.

For the analysis, 27 species were available. These constitute 60% of the species in the genus. When several specimens were available for a species, they were included in the analysis to assess the monophyly of that species. Thus, 51 specimens were included in the ingroup and analyzed. All neotropical species were included except *L. sorbifolia* and *L. underwoodii*. All seven species in Madagascar (Tardieu-Blot, 1960) were included in the analysis, and for Africa, six out of the ten species were included. Asia was represented by only one species (DNA extractions from herbarium material of other Asian species proved unsuccessful).

Morphological data were not added to the matrix. This will be done after our monographic study of the genus worldwide is completed.

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from either field-collected, silica gel-dried fronds or herbarium specimens when fresh material

TABLE I

HERBARIUM VOUCHER INFORMATION AND GENBANK ACCESSION NUMBER FOR SPECIES USED FOR THE MOLECULAR PHYLOGENETIC STUDY OF LOMARIOPSIS. DNA WAS EXTRACTED FROM HERBARIUM LAMINAE FOR SPECIMENS MARKED WITH *; OTHERWISE, DNA WAS EXTRACTED FROM SILICA-DRIED LAMINA MATERIAL

Species	Country, collector (herbarium)	GenBank accession number (*trnL-trnF* sequences)
Outgroup		
E. amygdalifolium (Mett. ex Kuhn) H. Christ	Costa Rica, *Herrera 2063* (CR, INB, NY, USJ)	AY534845
Lomariopsis		
L. amydrophlebia (Sloss. ex Maxon) Holttum	Puerto Rico, *Woodburg s.n.*, in 1966 (NY)*	DQ396555
L. amydrophlebia (Sloss. ex Maxon) Holttum	Puerto Rico, *Woodburg s.n.*, in 1977 (NY)*	DQ396554
L. boivinii Holttum	Madagascar, *Boivin 1947/2* (P)*	DQ396557
L. boivinii Holttum	Madagascar, *Rakotondrainibe 1769* (P)*	DQ396556
L. cordata (Bonap.) Alston	Madagascar, *Rakotondrainibe 1771* (P)*	DQ396558
L. crassifolia Holttum	Madagascar, *Humblot 442* (P)*	DQ396559
L. guineensis (Underw.) Alston	Sierra Leone, *Fay & Fay s.n.*, in 1985 (NY)*	DQ396560
L. hederacea Alston	Cameroon, *Raynal 9954* (P)*	DQ396561
L. jamaicensis (Underw.) Holttum	Jamaica, Maxon & Killip 1463 (NY)*	DQ396562
L. japurensis (Mart.) J. Sm.	Bolivia, *Sundue 708* (LPB, NY, USZ)*	DQ396563
L. japurensis (Mart.) J. Sm.	Bolivia, *Jimenez 2016* (LPB, NY)*	DQ396568
L. japurensis (Mart.) J. Sm.	Costa Rica, *Moran 6381*(CR, INB, NY)	DQ396567
L. japurensis (Mart.) J. Sm.	Ecuador, *Moran 6021* (NY, QCA, QCNE)	DQ396566
L. japurensis (Mart.) J. Sm.	Ecuador, *Moran 6061* (NY, QCA, QCNE)	DQ396565
L. japurensis (Mart.) J. Sm.	Peru, *Bell 88180* (NY)*	DQ396564
L. kunzeana (Underw.) Holttum	United States, *Peck s.n.* (NY)	DQ396569
L. kunzeana (Underw.) Holttum	Haiti, *Zanoni 28649* (EHH, NY)*	DQ396570
L. latipinna Stolze	Ecuador, *Moran 6027* (NY, QCA, QCNE, TUR)	DQ396571
L. lineata (C. Presl) Holttum	Thailand, *Larsen 45851* (AAU, NY)*	DQ396572
L. longicaudata (Bonap.) Holttum	Madagascar, *Janssen 2493* (P)	DQ396574
L. longicaudata (Bonap.) Holttum	Madagascar, *Rakotondrainibe 6191* (P)*	DQ396573
L. madagascarica (Bonap.) Alston	Madagascar, *Decary 18186* (P)*	DQ396576
L. madagascarica (Bonap.) Alston	Madagascar, *Kessler 12786* (NY)	DQ396575
L. mannii (Underw.) Alston	Democratic Republic of Congo (Zaire), *Kassner s.n.*, in 1914 (P)*	DQ396577
L. marginata (Schrad.) Kuhn	Brazil, *Amorim 1920* (CEPEC, NY)*	AY540045
L. marginata (Schrad.) Kuhn	Brazil, *Labiak 104* (NY, UPCB)*	DQ396578
L. marginata (Schrad.) Kuhn	Brazil, *Pires et al. 50316* (NY)*	DQ396579
L. maxonii (Underw.) Holttum	Costa Rica, *Smith 1660* (CR, NY, UC)*	DQ396581
L. maxonii (Underw.) Holttum	Costa Rica, *Moran 4172* (CR, NY, UC)	DQ396580
L. muriculata Holttum	Democratic Republic of Congo (Zaire), *Vanderyst* 12996 (P)*	DQ396582
L. nigropaleata Holttum	Bolivia, *Jimenez 1949* (LPB, NY)*	DQ396583
L. nigropaleata Holttum	Ecuador, *Moran 6053* (P)	DQ396584
L. palustris (Hook.) Mett. ex Kuhn	Sierra Leone, *Fay 1124* (NY)*	DQ396585
L. pervillei (Mett.) Kuhn	Madagascar, *Rakotondrainibe 6626* (P)	DQ396586
L. pervillei (Mett.) Kuhn	Madagascar, *Rakotondrainibe 6623* (P)*	DQ396587
L. pollicina Willem. ex Kuhn	Madagascar, *Kessler 12785* (GOET, NY)	DQ396589
L. pollicina Willem. ex Kuhn	Comoros, *Rakotondrainibe 6707* (P)	DQ396588
L. prieuriana Fée	Panama, *Moran 5080* (MO, NY, PMA)*	DQ396590
L. prieuriana Fée	Venezuela, *Cortez 475* (NY, VEN)	DQ396591
L. recurvata Fée	Mexico, *Rivera 1343* (NY)*	DQ396592
L. recurvata Fée	Mexico, *Hernández 2286* (NY)*	DQ396593
L. rossii Holttum	Liberia, *Fay 1237* (NY)*	DQ396594
L. salicifolia (Kunze) Lellinger	Ecuador, *Moran 6022* (AAU, NY, QCA, QCNE, TUR)	DQ396597
L. salicifolia (Kunze) Lellinger	Ecuador, *Moran 6956* (NY, QCA, QCNE)	DQ396596

TABLE I
(CONTINUED)

Species	Country, collector (herbarium)	GenBank accession number (*trnL-trnF* sequences)
L. salicifolia (Kunze) Lellinger	Ecuador, *Moran 6129* (NY, QCA, QCNE)	DQ396595
L. sp.	Madagascar, *Janssen 2527* (P)	DQ396602
L. sp.	Madagascar, *Janssen 2444* (P)	DQ396601
L. sp.	Madagascar, *Rouhan 318* (P)	DQ396603
L. vestita E. Fourn.	Costa Rica, *Folsom 9011* (NY)*	DQ396599
L. vestita E. Fourn.	Costa Rica, *Moran 6382* (CR, INB, NY, USJ)	DQ396598
L. wrightii Mett. ex D. C. Eaton	Cuba, *Underwood 948* (NY)*	DQ396600

was unavailable (Table I). For field-collected material, we tried to avoid contamination by collecting young fronds free of epiphyllous bryophytes and fungal or insect parasites. For silica gel-dried samples, total DNA was extracted from approximately 1 cm^2 of leaf tissue using a modified CTAB protocol of Doyle and Doyle (1987; see Struwe et al., 1998). For herbarium material, the DNEasy Plant Mini kit Qiagen (Valencia, CA) was used following the manufacturer's protocol but with a proteinase K digestion during the lysis step: 30 μL of proteinase K were added per tube and tubes were incubated on a tipping plate at 42°C for 24 hrs.

The polymerase chain reaction (PCR) was used to amplify the chloroplast spacer region *trnL-trnF*. The region was amplified and sequenced using universal primers "e" (5'-GGT TCA AGT CCC TCT ATC CC-3'), and "f" (5'-ATT TGA ACT GGT GAC ACG AG-3') designed by Taberlet et al. (1991). PCR amplifications were typically prepared in 25 μL reactions using 0.1 μL to 0.75 μL of non-diluted genomic DNA, 2.5μL of 10x Taq Buffer with 15 mM $MgCl_2$ (1.5 mM $MgCl_2$ final), 2.5 μL of dNTPs (250 μM final of each), 5 μL of 5 M betaine solution (Q-solution), 2.5 μL of 2.5 μg/μL BSA solution, 1 μL of each primer at 10 μM, 1 U Qiagen Inc. Taq DNA Polymerase, and purified water to volume. A typical amplification program began with one initial denaturation step for 5 min at 94°C, then 35 cycles of 1 min at 94°C, 30 s at 50°C, 1 min at 72°C, followed by an extension period of 7 min at 72°C, and was performed on a DNA Engine DYAD™ Peltier Thermal Cycler. The resulting PCR products were checked on a 1% agarose gel with ethidium bromide and purified using QIAquick PCR purification kit (Qiagen). They were directly sequenced using the amplification primers with a Perkin Elmer ABI 377XL automated sequencer. Forward and reverse sequences obtained were edited and assembled using Sequencher (version 4; Gene Codes Corporation, Ann Arbor, Michigan). Thus, new complete sequences for *trnL-trnF* were obtained for 27 species (Table I).

SEQUENCE ALIGNMENT, INDEL CODING, AND PHYLOGENETIC ANALYSES

Automatic alignments were first generated using ClustalX (Thompson et al., 1997), and the resulting alignments were examined and improved manually with the MUST package (Philippe, 1993), followed by preparation of formatted files for analyses. Short indels found during the alignment were treated as missing data.

The data matrices obtained were analyzed under the maximum parsimony procedure using PAUP* version 4.0b10 (Swofford, 2002) on a PC Athlon 1.2 GHz.

The maximum parsimony (MP) heuristic search analyses were conducted using 1000 random-addition sequence replicates, tree bisection-reconnection (TBR) branch swapping, and the MulTrees option on. Non parametric bootstrap analysis (Felsenstein, 1985) was used to evaluate the robustness of each node (noted BS for Bootstrap Score), using 1000 replicates of similar heuristic searches (but with one random addition sequence per bootstrap replicate). Because all characters were equally sampled, uninformative characters can have a significant effect on robust-

ness that is neither logical nor desirable. Therefore, uninformative characters were removed before the bootstrap procedure (DeSalle et al., 2002).

Results

Sequence Variation

The length of the *trnL-trnF* intergenic spacer-sequences ranged among the ingroup from 332 bp (*Lomariopsis crassifolia*) to 356 bp (*L. guineensis*), and in the outgroup *E. amygdalifolium* it was 322 bp long. The total length of aligned *trnL-trnF* sequences resulted in 373 bp because indels were inserted. Thus, out of the 373 aligned sites, 181 were variable, and 84 were phylogenetically informative. The percentage of variable sites was 52.9%, and the percentage of informative sites was 46.4%.

Phylogenetic Results

Analysis of the *trnL-trnF* matrix yielded six MP trees of 303 steps (Fig. 2), with CI = .70 (excluding uninformative characters), and RI = .92. In the strict consensus tree (Fig. 2), most of the relationships were well resolved at the specific level, but at a lower taxonomic level the *trnL-trnF* data was not variable enough to resolve relationships between different specimens of the same species. The strict consensus tree shows that all the *Lomariopsis* species comprise two large clades (Fig. 2). The *Sorbifolia* clade received low support (BS = 50%), but the *Japurensis* clade received a higher bootstrap value (BS = 74%). The *Sorbifolia* clade contains exclusively neotropical species, whereas the *Japurensis* clade contains two subclades, one being mainly neotropical with two nested African species, and the other being exclusively paleotropical.

Discussion

The monophyly of *Lomariopsis* cannot be assessed from this study because more outgroups need to be included in the analysis. The genus, however, appears monophyletic based on morphology. Its presumed synapomorphies are the notched ventral vascular bundle of the rhizome, the dark sclerenchyma sheaths surrounding each vascular bundle, articulate pinnae, and imparipinnate leaves (Moran, 2000).

Within *Lomariopsis*, two main clades were formed by the *Japurensis-* and *Sorbifolia*-groups (Fig. 2). This agrees with the two distinct types of heteroblastic leaf development that characterize each group (Fig. 1). Branch support for the *Sorbifolia*-group, however, was weak (BS = 50%, Fig. 2), whereas that for the *Japurensis*-group was moderately strong (BS = 74%).

The *Sorbifolia*-group is entirely neotropical, and all the species in the Antilles belong to it (Moran, 2000). The Antillean species in the analysis come out in two clades, one formed by *L. kunzeana* and *L. jamaicensis*, and the other by *L. amydrophlebia* and *L. wrightii* (BS = 78 and 99%, respectively). Although these two clades do not form a monophyletic group, branch support for their separation is weak (BS = 56%; Fig. 2). Both clades have smooth spores with varying amounts of spherical deposits (Fig. 3). The other species in the *Sorbifolia* group (*L. maxonii, L. vestita, L. recurvata,* and *L. salicifolia*) occur in Central and South America. These species have crested spores with tall thin wings (Fig. 4).

The *Japurensis*-group occurs in the neotropics and paleotropics, forming monophyletic clades in both regions. Within the neotropical clade (*L. marginata* through *L. japurensis*, Fig. 2) there nests a subclade composed of two African species, *L. guineensis* and *L. palustris*. This nested relationship is probably the result of long-distance spore dispersal from the neotropics to Africa, not continental drift (see Moran and Smith (2001) for arguments supporting this view in other ferns with similar disjunct distributions). Both African species have spores with extremely long spines (Fig. 5). This spore type is not found in other African taxa but is present in the neotropical *L. prieuriana*.

The sister relationship of *L. japurensis* and *L. latipinna* (Fig. 2) agrees with spore type. Both have smooth spores with none or only sparse spherical deposits, as is shown for *L. latipinna* in Fig. 5.

With the exception of *L. guineensis* and *L. palustris*, the paleotropical species formed a monophyletic group (Fig. 2). *Lomariopsis*

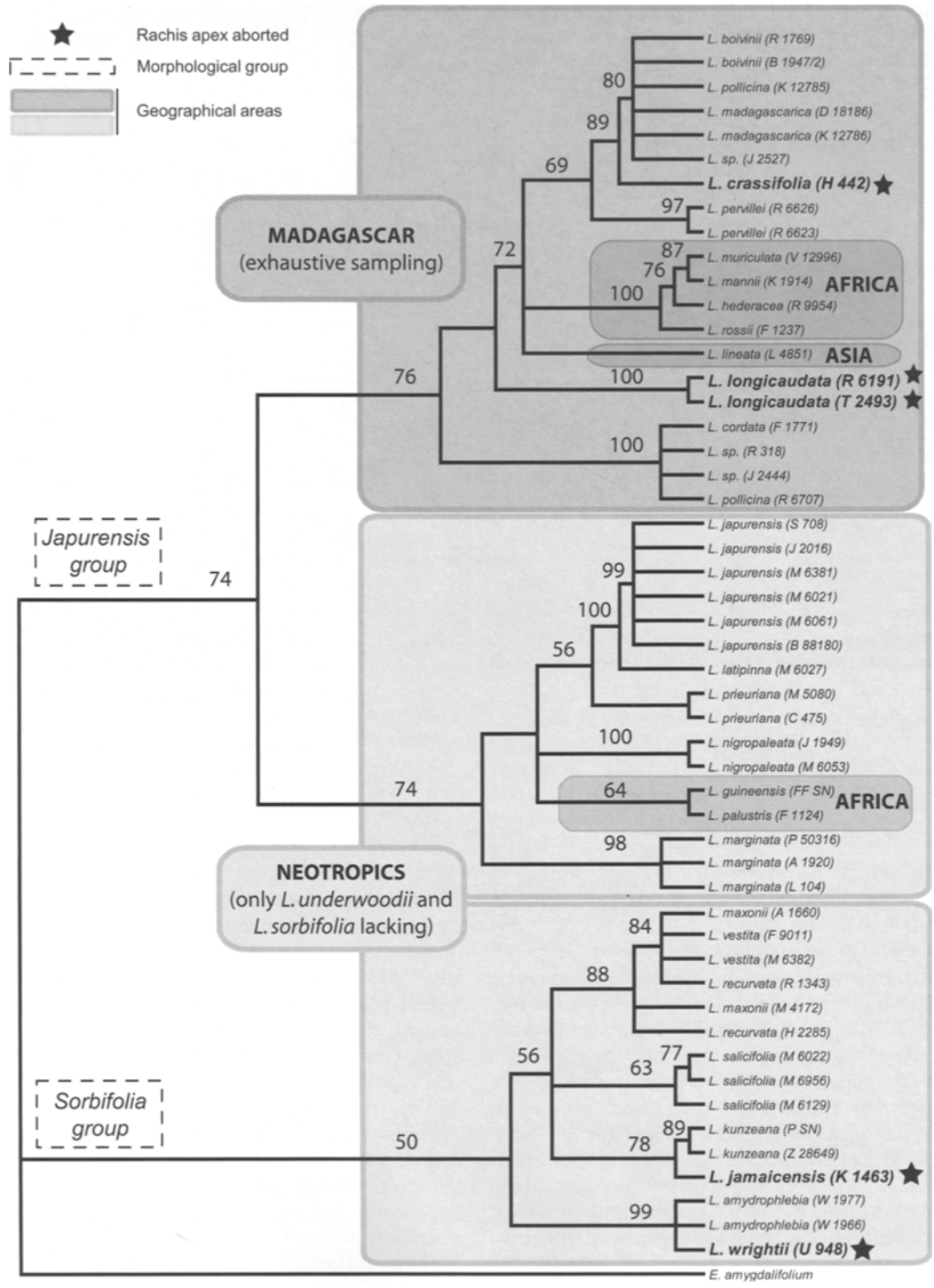

FIG. 2. *Lomariopsis* phylogeny. Strict consensus of six trees based on *trnL-trnF* spacer (analysis included a heuristic search for the most parsimonious trees with 1000 random-sequence addition replicates). Tree was rooted with *Elaphoglossum amygdalifolium*. Numbers above the branches are bootstrap support values ≥ 50%. Those species marked with a star have an aborted rachis apex (Fig. 8).

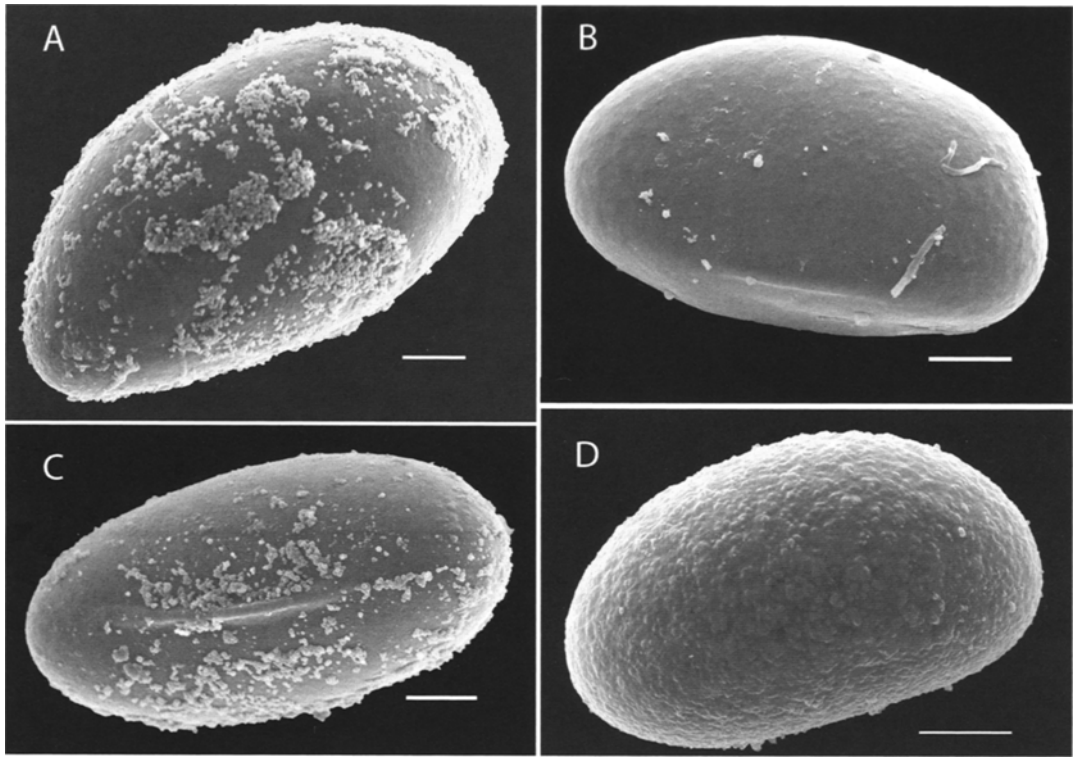

FIG. 3. Smooth spores with various amounts of spherical deposits, from Greater Antillean species of *Lomariopsis* belonging to the *Sorbifolia*-group. **A.** *L. underwoodii* (Jamaica, *Purdie s.n.,* in 1863, NY). **B.** *L. kunzeana* (Haiti, *Holdridge 2131,* NY). **C.** *L. wrightii* (Cuba, *Shafer 4438,* NY). **D.** *L. jamaicensis* (Jamaica, *Butler s.n.,* June 1900, NY). Scale bars = 10 micrometers.

cordata – *L. pollicina,* both endemic to Madagascar, formed a strongly supported clade (BS = 100) sister to the rest of this group. These two species have spores with spines formed from a build-up of granular deposits (Fig. 6). This spore type also characterizes *L. pervillei,* which belongs to another clade (Fig. 2), suggesting that the evolution of this spore type has been homoplastic. *Lomariopsis lineata,* the sole Asian species in the analysis, belongs to a clade formed with one Madagascan subclade and one continental African subclade. Because of unresolved relationships, the phylogeny is ambiguous as to which region the Asian species is most related to.

In the paleotropical clade, four African species (*L. muriculata, L. mannii, L. hederacea,* and *L. rossii*) formed a strongly supported monophyletic group (BS = 100%). Three of the species have lacy spores (we have not examined the spores of *L. mannii*), a characteristic that occurs outside the clade in some species from Madagascar (Fig. 7). *Lomariopsis congoensis* also has this spore type (SEM photomicrograph by Alice Tryon, attached to the specimen *Cervoni s.n.* at P, from Cameroon) and presumably belongs to this clade.

The remaining clade, *L. boivinii* – *L. pervillei* (Fig. 2), is endemic to Madagascar and the Comoro Islands. The spores in this clade vary, being smooth in *L. crassifolia* (but apparently without spherical deposits as in species from the Greater Antilles), short-spiny in *L. pervillei* (Fig. 6E, F), and lacy in *L. boivinii* and *L. madagascarica* (Fig. 7C, D, E).

Two species in the paleotropical clade (*L. crassifolia* and *L. longicaudata*) have an unusual leaf apex that represents an apomorphic

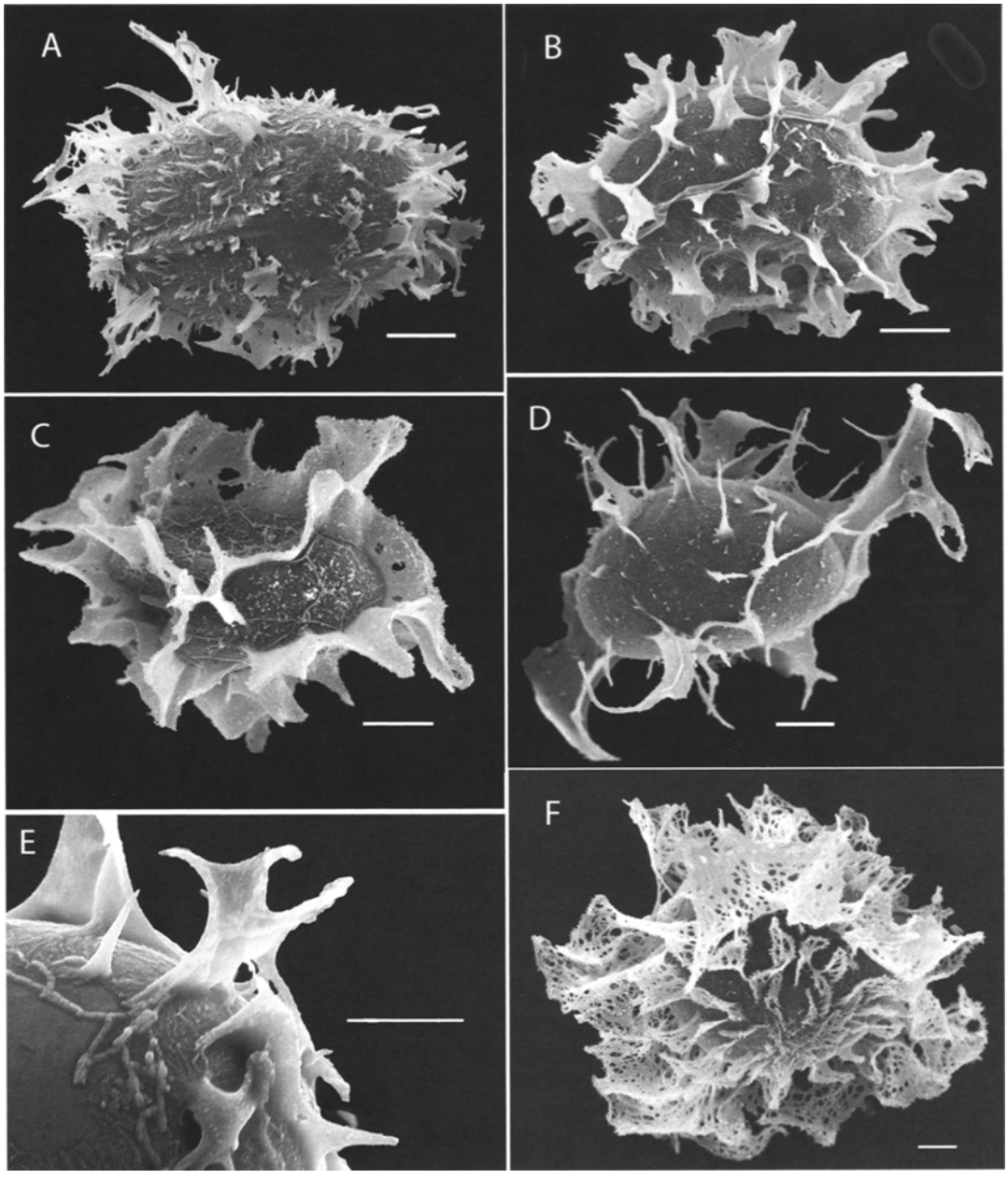

FIG. 4. Cristate spores from neotropical species of *Lomariopsis* belonging to the *Sorbifolia*-group. **A.** *L. sorbifolia* (Lesser Antilles, *Boldingh 453*, NY). **B.** *L. recurvata* (Mexico, *Rzedowski 20373*, NY). **C.** *L. salicifolia* (Colombia, *Fonnegra 2915*, NY). **D, E.** *L. vestita* (Mexico, *Wendt 3822*, NY). **F.** *L. maxonii* (Panama, *Croat 66514*, NY). Scale bars = 10 micrometers.

condition. The rachis tip aborts and is represented by a small nubbin (Fig. 8). It is pushed aside and replaced by the distal, lateral pinna, which turns upright and assumes a terminal position. This "terminal" pinna articulates to the rachis just like the lateral ones—thus betraying its lateral origin (when the leaf apex does not abort, the rachis apex differentiates into a terminal pinna and therefore no articulation is present). This character

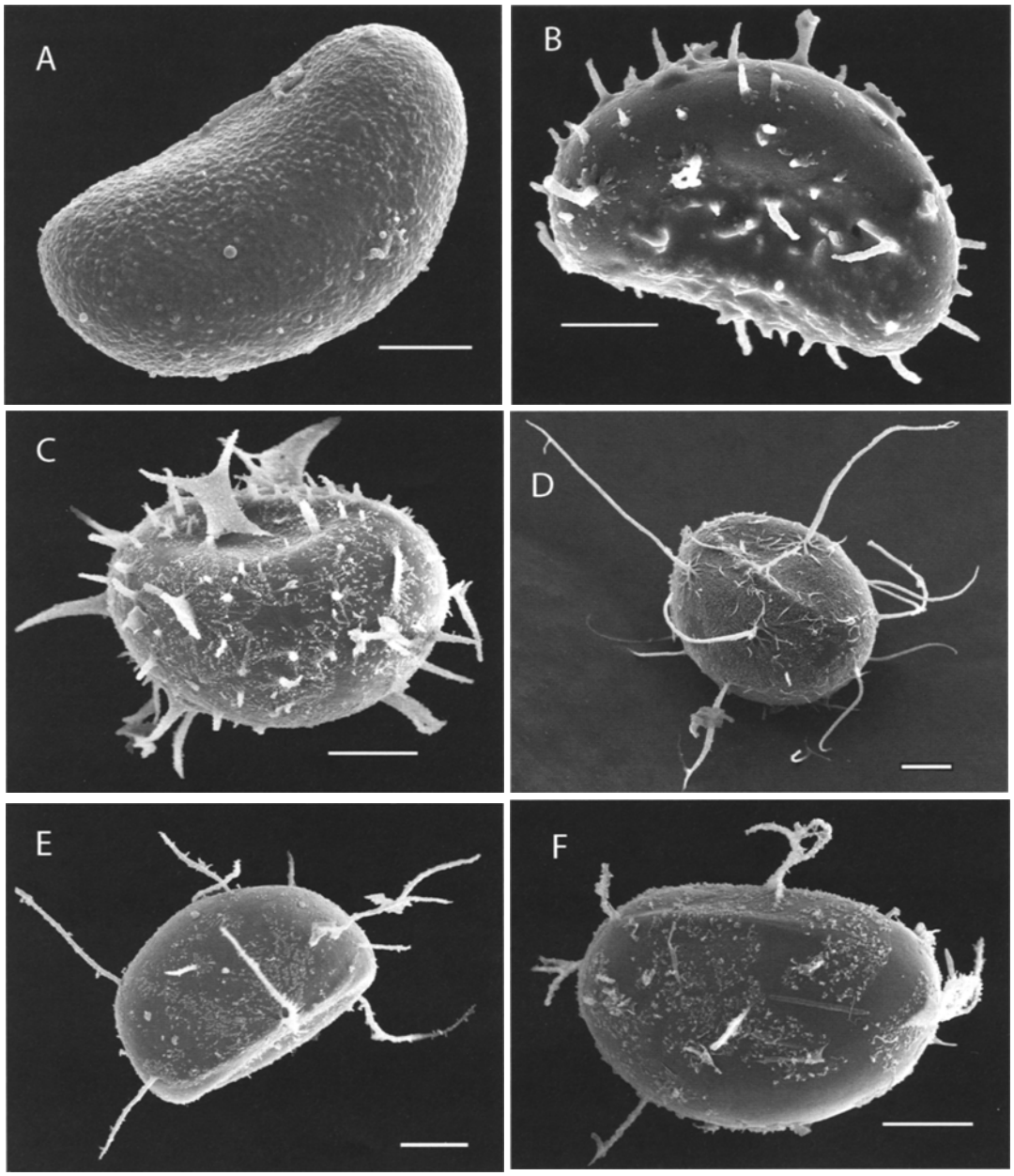

FIG. 5. Spores of *Lomariopsis* species belonging to the *Japurensis*-group. **A.** *L. latipinna* (Ecuador, *Moran 6027*, NY). **B.** *L. marginata* (Brazil, *Handro 2192*, NY). **C.** *L. nigropaleata* (Ecuador, *Holm-Nielson 4278*, NY). **D.** *L. prieuriana* (French Guiana, *de Granville 8473*, NY). **E.** *L. guineensis* (Sierra Leone, *Fay 1147*, NY). **F.** *L. palustris* (Sierra Leone, *Fay 1140*, MO). Scale bars = 10 micrometers.

also occurs in two species from the Greater Antilles: *L. jamaicensis* and *L. wrightii*. Because the four species appear independently in different parts of the tree, the leaf apex character is best interpreted as homoplastic. The adaptive significance of this characteristic, if any, is unknown.

On the basis of morphology, Moran and Smith (2001) proposed two sister species relationships between neotropical and African

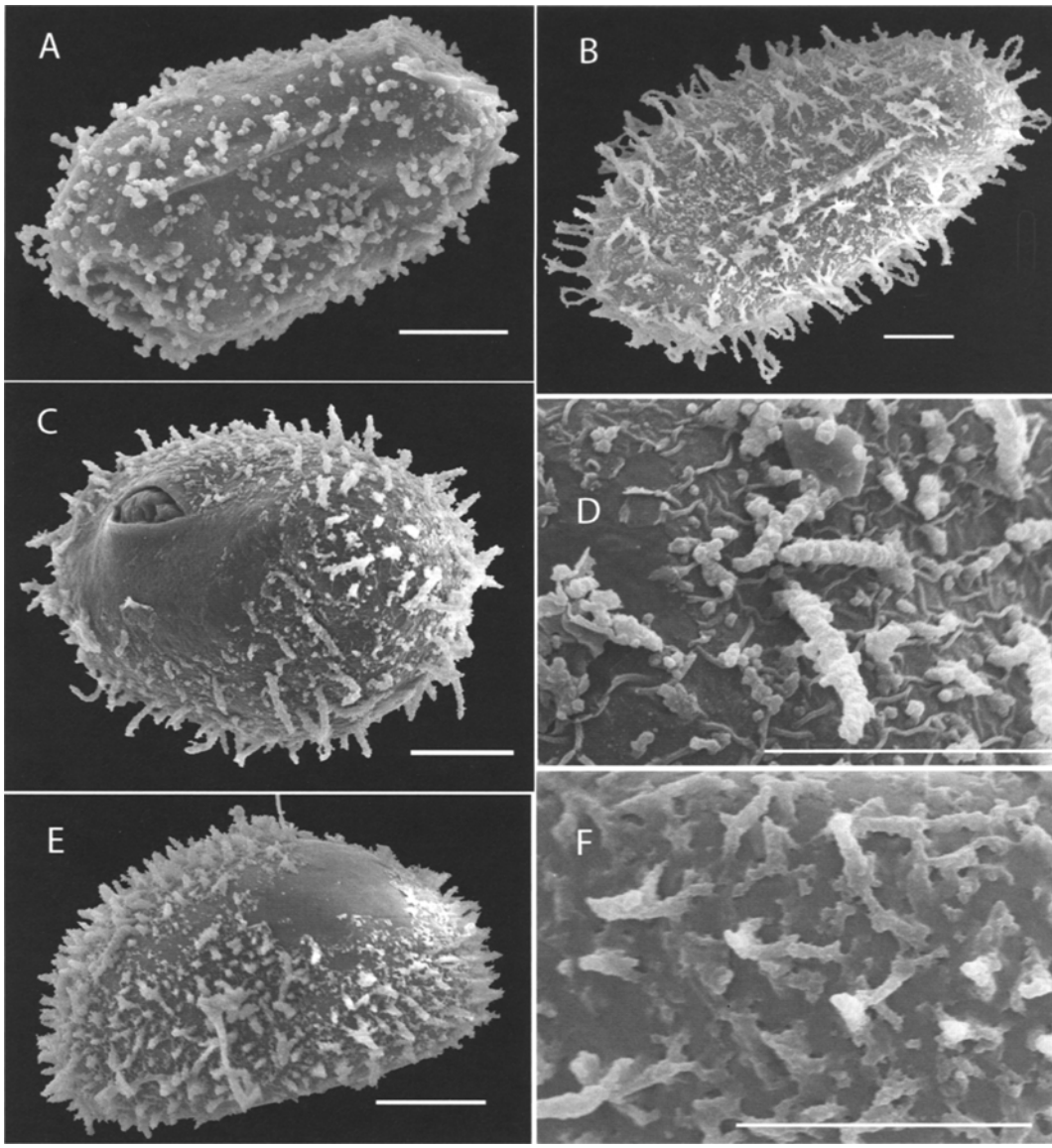

FIG. 6. Spiny spores from African species of *Lomariopsis* belonging to the *Japurensis*-group. **A.** *L. cordata* (Madagascar, *Leeuwenburg et al. 14285*, P). **B.** *L. pollicina* Madagascar, *Rakotondrainibe 4364* (P) or Madagascar, *Raharimala & Rakotondrainibe 1937*, P). **C, D.** *L. warneckei* (Tanzania, *Schlieben 2084*, P). **E, F.** *L. pervillei* (Comoro Islands, *Marie 42*, P). Scale bars = 10 micrometers.

species of *Lomariopsis*; namely, between *L. salicifolia* (as *L. fendleri*, a synonym) and *L. warneckei*, and between *L. vestita* and *L. decrescens*. Unfortunately, neither of these relationships could be tested in this study because herbarium specimens of the two African species did not yield extractable DNA. Also, herbarium specimens of the two African species did not yield spores in good condition to allow comparison with those of their postulated neotropical sister species.

SPORE ANALYSIS

Related genera such as *Bolbitis*, *Lomagramma*, and *Teratophyllum* usually have

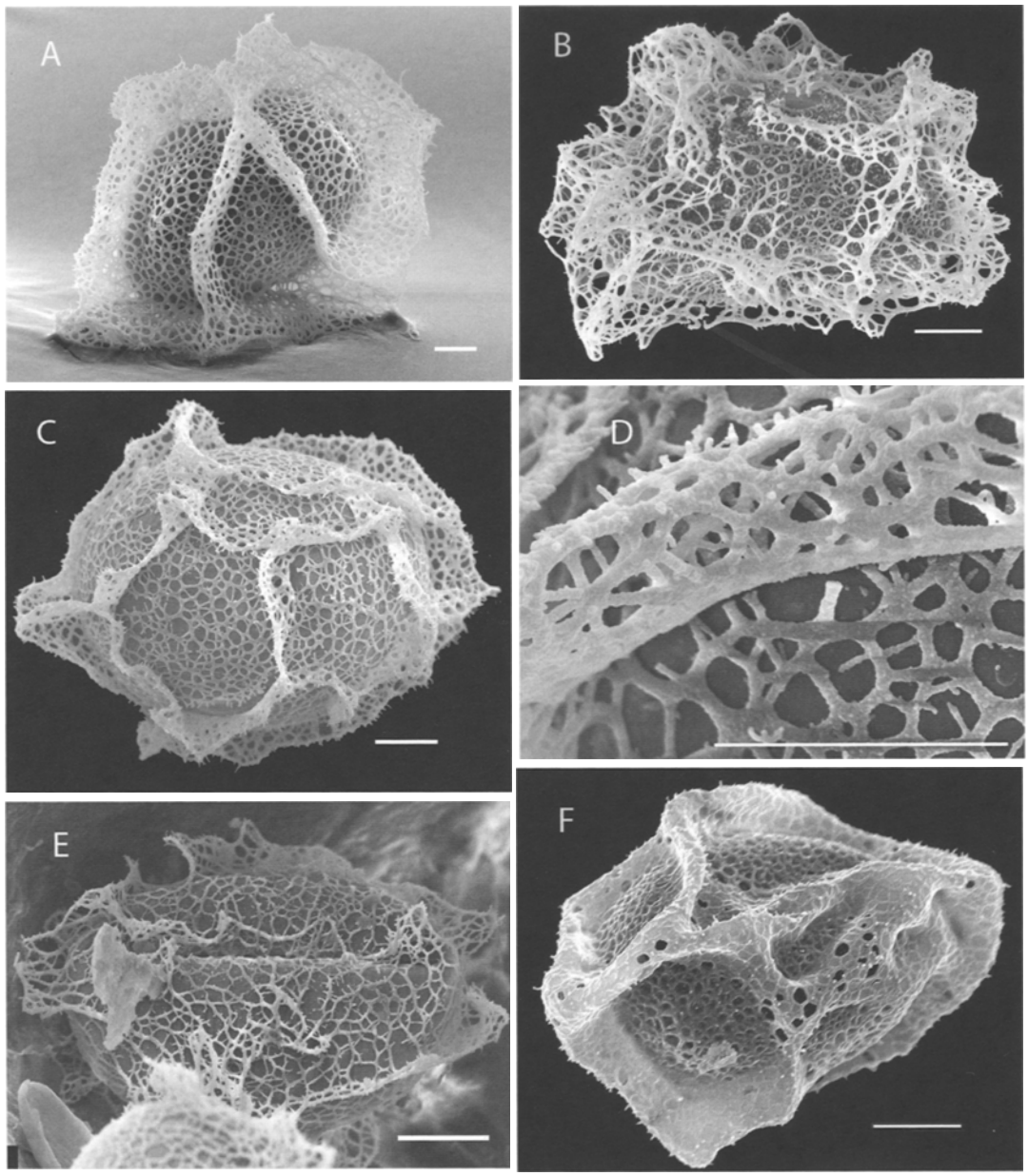

FIG. 7. Lacy spores from African and Madagascan species of *Lomariopsis* belonging to the *Japurensis*-group. **A.** *L. longicaudata* (Madagascar, *Aridy & Rahajasoa 107*, MO). **B.** *L. hederacea* (Gabon, *Reitsma & Reitsma 2959*, NY). **C, D.** *L. boivinii* (Madagascar, *Simpson 88/51*, MO). **E.** *L. madagascarica* (Madagascar, *Leeuwenbert 13900*, MO). **F.** *L. rossii* (Liberia, *Fay & Fay 1237*, NY). Scale bars = 10 micrometers.

spores with sharp wings or broad folds. This is widespread in other dryopteroid ferns (Tryon & Lugardon, 1991) and is therefore interpreted here as the ancestral state for *Lomariopsis*. Species with thin wings or crests occur in all of the three main clades of *Lomariopsis* (Fig. 2), and it appears that these crests have been modified or lost to produce the other spore types found in the genus. In some cases the crests have been lost, resulting

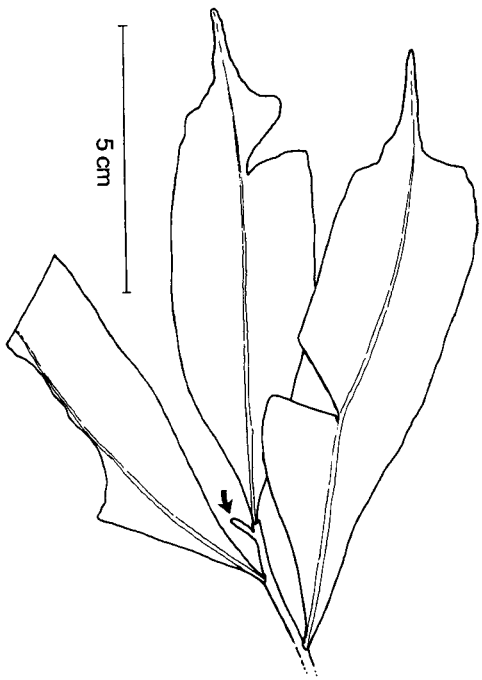

FIG. 8. Aborted leaf apex (arrow) of *Lomariopsis wrightii* (Cuba, *Underwood & Earle 948*, NY).

in smooth spores such as those seen in the two clades in the *Sorbifolia*-group from the Greater Antilles (Fig. 3) and in the neotropical *Japurensis*-group (Fig. 5; *L. japurensis* and *L. latipinna*). In other cases the crests have become divided and discontinuous into flattened appendages, and this can be seen in spores of the crested species of the *Sorbifolia*-group (Fig. 4) and in those of the neotropical *Japurensis*-group (Fig. 5). Sometimes the flattened appendages become further narrowed into spines. Transitions between the two can be seen in Figs. 4D, E and 5C. Presumably, the long spines in *L. prieuriana, L. guineensis,* and *L. palustris* evolved this way.

A separate modification of the crests occurs in the paleotropical clade in the African and Madagascan species (Fig. 7). Here the crests become lacy or net-like by fenestration of the wings. From this type the short-spiny spores found in *L. cordata, L. pervillei*, and *L. pollicina* (Fig. 6) appear to have been derived by a reduction in the height and lateral extent of the fenestrated wings.

All results presented here are preliminary. To improve our understanding of the evolutionary relationships within *Lomariopsis,* several investigations are needed. Stronger phylogenetic hypotheses could be obtained by adding to the data matrix more DNA regions, morphological characters, and outgroups. There also remain 18 species (40% of the genus) to be included in the analysis. We intend to do this and report the results in a future paper.

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