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Source: Lundellia, 23(1) : 3-18

Published By: The Plant Resources Center, The University of Texas at Austin

URL: https://doi.org/10.25224/1097-993X-23.1.3

Taxonomy and Phylogeny of *Helenium scaposum* (Asteraceae, Helenieae, Gaillardiinae).

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Abstract: Phylogenetic analyses, biogeography, morphology, and ecology confirm that *Helenium scaposum* is a distinct species belonging to genus *Helenium*. Within *Helenium*, it appears that *H. scaposum* is most closely related to members of *Helenium* sect. *Leptopoda*. The morphological resemblance of *H. scaposum* to *H. drummondii, H. pinnatifidum*, and *H. vernale* justifies further study to better understand relationships among these species.

Resumen: Los análisis filogenéticos, biogeografía, morfología, y ecología confirman que *Helenium scaposum* es una especie distinctiva perteneciente al género *Helenium*. Dentro de *Helenium*, *H. scaposum* está más estrechamente relacionado con los miembros de la sección *Leptopoda* de *Helenium*. La semejanza morfológica de *H. scaposum* con *H. drummondii, H. pinnatifidum*, y *H. vernale* justifica estudios adicionales para entender mejor las relaciones entre estas especies.

Keywords: *Helenium scaposum*, Helenieae, Gaillardiinae, *Helenium* sect. *Leptopoda*, phylogenetics, biogeography, morphology, ecology, Cuba, endemics

Introduction

The first author became aware of *Helenium scaposum* Britton in the late 1960s while working on his dissertation project, a treatment of *Helenium* L. sect. *Tetrodus* (Cass.) DC. As this taxon was not part of his work at that time, Bierner placed it on his back-burner, and there it sat for some 50 years. Recently, Bierner decided to tie up some loose ends in his research by first dealing with *Helenium scaposum*.

Over the years, *Helenium scaposum* has been recognized as an endemic restricted to Isla de la Juventud, Cuba, formerly Isla de Pinos (e.g., Rock, 1957; Prede Rodríguez et al., 2000; Martínez Redondo and Herrera Oliver, 2003; Greuter and Rodriguez, 2016). It is restricted to the southern part of the western area known as the sabana arenosa and the western part of the northern region known as the sabana grande, where it grows in white sand associated with current or former pine forests (Balátová-Tuláčková and Capote, 1985). Puentes et al. (1985), using different terminology, noted that *H. scaposum* was restricted to the northeastern portion of Isla de la Juventud in the area they called Distrito Arenas Blancas. Herrera Oliver et al. (1999), in their evaluation of the state of conservation in the family Asteraceae in Cuba, classified *H. scaposum* as low-risk, conservation-dependent. Later it was placed on the Red List of Cuban vascular plants as being in danger and located in semi-natural savannas of pine forest regions (Berazaín Iturralde et al., 2005). The only chemical work we were able to find was that of Frias et al. (1972), who examined a number of taxa from Cuba for the presence of alkaloids and saponins; none were detected in *H. scaposum*.

Given the location of *Helenium scaposum* (Isla de la Juventud), its habit (scapiform), and certain morphological features such as its root system (numerous adventitious roots originating from a short rhizome), the first author had two major

questions: 1) is this plant actually a *Helenium*, and 2) if so, where does it fit into the genus? To aid in this quest, Bierner solicited the help of Bruce G. Baldwin, who produced the nuclear 18S–26S nrDNA internal transcribed spacer (ITS) region sequence and performed a preliminary phylogenetic analysis, and Michael J. Moore and his undergraduate students Helene Tiley, Flora Samis and Spencer Wight, who generated most of the remaining sequences in this study and performed a more detailed phylogenetic analysis.

Materials and Methods

A total of 60 *Helenium* accessions were included in phylogenetic analyses, representing 36 taxa in the genus (Table 1).

Total DNA of *Helenium scaposum* was extracted from a leaf fragment of *Greuter et al. 25969* (NY) using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA), with incubation for cell lysis of 1–2 h rather than 10 min, as indicated in the manufacturer's protocol. Total DNA of other newly sequenced *Helenium* taxa was isolated from field collected or herbarium specimens using either the Nucleon Phytopure kit (GE Healthcare Lifesciences, Pittsburgh, Pennsylvania, USA) or the CTAB method of Doyle and Doyle (1987), with the addition of 1% PVP-40.

For *H. scaposum,* the 18S–26S nrDNA ITS region was amplified by polymerase chain reaction (PCR) as indicated by Baldwin and Wessa (2000b) except for use of AccuPower PCR Premix (K-2016; Bioneer Corp., Chunbuk, Korea). For all other newly generated sequences, PCRs were performed in 12.5 μL volumes with 0.5 μL of 5 mM primer for both primers, 5–20 ng of DNA template, 0.1 μL of GoTaq (Promega, Madison, WI, USA), 6.25 μL of Failsafe Premix B (Epicentre, Madison, WI, USA), and 4.7 μL of sterile, deionized water. Reactions were run on a Bio-Rad PTC 200 thermocycler (Bio-Rad, Hercules, CA, USA) at Oberlin College. For *H. scaposum,* Exonuclease I and shrimp alkaline phosphatase were used to remove unincorporated nucleotides as directed in the PCR Product Pre-Sequencing Kit (70995, United States Biochemical Corp., Cleveland, Ohio, USA). For all other newly generated sequences, PCRs were cleaned in 16.5 μL reactions containing 10 U of Exonuclease I (Affymetrix, ThermoFisher Scientific, Waltham, MA, USA), 2 U of shrimp alkaline phosphatase (Affymetrix), 8 μL of PCR product, and 8.5 μL of sterile, deionized water. Sanger sequencing was conducted at the UC Berkeley DNA Sequencing Facility (Barker Hall) for *H. scaposum* and by Neogenomics (formerly SeqWright; Houston, TX, USA) using an ABI 3730xl automated sequencer (Applied Biosystems, ThermoFisher Scientific) for other taxa. The primer ITS5 (White et al., 1990) was used instead of ITS-I (Urbatsch et al., 2000) for sequencing. The resulting forward and reverse sequences for each reaction were trimmed and *de novo* assembled using default parameters of the Geneious assembler in Geneious version 7 (Biomatters, Auckland, New Zealand).

The ITS region alignment was created in Geneious using MAFFT (Katoh and Standley, 2016) with default parameters, and all sites with greater than 50% missing data were removed using Phyutility (Smith and Dunn, 2008). Maximum likelihood analyses were run using RAxML version 8.2 (Stamatakis 2014) using the GTRGAMMA model, 100 bootstrap replicates, and the following parameters: -T 2 -f a -x 12345 -p 12345. The resulting trees were visualized using FigTree version 1.4.4 (available at https://github.com/rambaut/figtree/releases).

Results and Discussion

The ITS region sequence prepared by Baldwin from the *Helenium scaposum* sample was initially included in a maximum likelihood analysis with other sequences in the ITS dataset of Baldwin et al. (2002), which confirmed its position in *Helenium* (Fig. 1). The sequence was then included in a maximum likelihood analysis with other sequences in an ITS region dataset produced by Moore et al., which also confirmed its position in *Helenium* (Fig. 2; Appendix 1).

TABLE 1. Voucher information and GenBank accession numbers for all accessions included in this study. Voucher and collection locality information are given only for newly submitted GenBank sequences. Herbarium codes follow *Index Herbariorum*, and numbers following herbarium codes are herbarium accession numbers. Specimens with no numbers after herbarium codes lack herbarium accession numbers (*s.n.*).

Taxon	Collection Locality	Collector Information	Herbarium Accession Information	GenBank Accession Number
Amblyolepis setigera DC.	United States: Texas	Michael J. Moore et al. 1017	OC	MT557590
Baileya multiradiata Harv. & A.Gray ex A. Gray	United States: New Mexico	Michael J. Moore 365	OC	MT557591
Baileya pauciradiata Harv. & A.Gray ex A.Gray	United States: Arizona	H. David Hammond 11346	TEX/LL	MT557592
Baileya pleniradiata Harv. & A.Gray ex A.Gray	United States: California	Sarah J. De Groot et al. 4837	TEX/LL	MT557593
Balduina angustifolia (Pursh) B.L.Rob.	United States: Florida	Lucas C. Majure 4111	OC	MT557594
Balduina cf. *angustifolia* (Pursh) B.L.Rob.	United States: Florida	Michael J. Moore 3351	OC	MT557595
Balduina uniflora Nutt.	United States: Alabama	Lucas C. Majure et al. 4162	OC	MT557596
Gaillardia aestivalis (Walter) H.Rock var. *austrotexana* B.L.Turner	United States: Texas	Michael J. Moore et al. 967-7	OC	MT557597
Gaillardia comosa A.Gray	Mexico: San Luis Potosi	Michael J. Moore et al. 1245	OC	MT557598
Gaillardia multiceps Greene	United States: New Mexico	Michael J. Moore et al. 680-2	OC	MT557599
Gaillardia pinnatifida Torr.	United States: Utah	Michael J. Moore et al. 801	OC	MT557600
Gaillardia pulchella Foug. var. *pulchella*	United States: Texas	Michael J. Moore et al. 1050	OC	MT557601
Gaillardia suavis (A.Gray & Engelm.) Britton & Rusby	United States: Texas	Michael J. Moore et al. 990	OC	MT557602
Gaillardia turneri Averett & A.M.Powell	Mexico: Chihuahua	Michael J. Moore et al. 1589	OC	MT557603
Helenium sp.	United States: Texas	Michael J. Moore 1804	OC	MT557632
Helenium amarum (Raf.) H.Rock				KF607067
Helenium amarum (Raf.) H.Rock var. *amarum*	United States: Texas	Michael J. Moore 1060	OC	MT557604

TABLE 1. Continued.

Taxon	Collection Locality	Collector Information	Herbarium Accession Information	GenBank Accession Number
Helenium amarum (Raf.) H.Rock var. *badium* (A.Gray ex S. Watson) Waterf.	United States: Texas	Mark W. Bierner 92-24	TEX/LL 412046	MT557605
Helenium apterum (S.F.Blake) Bierner	Mexico: Durango	T. Sultan Quedensley 10127	TEX/LL	MT557606
Helenium arizonicum S.F.Blake				DQ391228
Helenium aromaticum (Hook.) L.H.Bailey	Chile: Coquimbo	M. W. Bierner 51464	TEX/LL 358831	MT557607
Helenium atacamense Cabrera	Chile: Antofagasta	M. O. Dillon et al. 5156	TEX/LL 358835	MT557608
Helenium autumnale L.	United States: Texas	L. L. Hansen 5470	TEX/LL 431512	MT557609
Helenium autumnale L.				AH014025
Helenium autumnale L.				GU818553
Helenium autumnale L.				KF607068
Helenium bigelovii A.Gray	United States: California	Betty H. Johnson 730	TEX/LL	MT557610
Helenium bigelovii A.Gray	United States: California	Kathy Harper s.n.	TEX/LL	MT557611
Helenium bigelovii A.Gray				AF229269
Helenium bigelovii A.Gray				AH013978
Helenium bigelovii A.Gray				DQ391229
Helenium bolanderi A.Gray	United States: California	Andrew R. Moldenke et al. 24830	TEX/LL	MT557612
Helenium brevifolium (Nutt.) Alph.Wood	United States: Alabama	R. Kral 89261	TEX/LL	MT557613
Helenium brevifolium (Nutt.) Alph.Wood				KF607069
Helenium campestre Small	United States: Arkansas	R. Dale Thomas et al. 135512	TEX/LL	MT557614
Helenium chihuahuense Bierner	Mexico: Chihuahua	T. Lebgue et al. 3501	TEX/LL 057512	MT557615

TABLE 1. Continued.

Taxon	Collection Locality	Collector Information	Herbarium Accession Information	GenBank Accession Number
Helenium donianum (Hook. & Arn.) Cabrera var. *donianum*	Argentina: San Juan	Barbara E. Goodson et al. 1564	TEX/LL	MT557616
Helenium donianum (Hook. & Arn.) Cabrera var. *donianum*	Argentina: La Rioja	John D. Bacon et al. 1529	TEX/LL 358836	MT557617
Helenium drummondii H.Rock	United States: Louisiana	Philip E. Hyatt 7280	TEX/LL	MT557618
Helenium drummondii H.Rock	United States: Texas	W. C. Holmes et al. 13175	TEX/LL 445181	MT557619
Helenium elegans DC. var. *amphibolum* (A.Gray) Bierner	United States: Texas	Emily J. Lott et al. 4987	TEX/LL 210429	MT557620
Helenium elegans DC. var. *elegans*	United States: Texas	W. R. Carr et al. 14017	TEX/LL 005633	MT557621
Helenium flexuosum Raf.	United States: Texas	D. J. Rosen 4774	TEX/LL 433172	MT557622
Helenium flexuosum Raf.				AF295408 & AF295409
Helenium flexuosum Raf.				AH014033
Helenium flexuosum Raf.				KF607070
Helenium glaucum (Cav.) Stuntz	Chile: Colchagua	M. W. Bierner 51508	TEX/LL 358840	MT557623
Helenium laciniatum A.Gray	Mexico: Sonora	T. R. Van Devender et al. 2007-22	TEX/LL 205211	MT557624
Helenium linifolium Rydb.	United States: Texas	D. S. Seigler et al. 15504	TEX/LL 199704	MT557625
Helenium linifolium Rydb.	United States: Texas	Michael J. Moore 1687	OC	MT557626
Helenium mexicanum Kunth	Mexico: Queretaro	Jerzy Rzedowski 48894	TEX/LL 433654	MT557627
Helenium microcephalum DC. var. *microcephalum*	United States: Texas	Candice N. Cerda 368	TEX/LL 459878	MT557628
Helenium microcephalum DC. var. *microcephalum*	United States: New Mexico	John R. Crutchfield 2146	TEX/LL	MT557629

TABLE 1. Continued.

Taxon	Collection Locality	Collector Information	Herbarium Accession Information	GenBank Accession Number
Helenium microcephalum DC. var. *microcephalum*	United States: Texas	Michael J. Moore 1058	OC	MT557630
Helenium microcephalum DC. var. *ooclinium* (A.Gray) Bierner	United States: Texas	Emily J. Lott et al. 5229	TEX/LL 206753	MT557631
Helenium ovallense Bierner	Chile: Coquimbo	M. W. Bierner 52382	TEX/LL 358842	MT557633
Helenium pinnatifidum (Schwein. ex Nutt.) Rydb.	United States	Lucas C. Majure 4232	OC	MT557634
Helenium pinnatifidum (Schwein. ex Nutt.) Rydb.	United States: Florida	Steve L. Orzell et al. 19149	TEX/LL	MT557635
Helenium pinnatifidum (Schwein. ex Nutt.) Rydb.				KF607071
Helenium puberulum DC.	United States: California	Timothy S. Ross et al. 8163	TEX/LL	MT557636
Helenium quadridentatum Labill.	United States: Louisiana	R. Dale Thomas et al. 116493	TEX/LL	MT557637
Helenium quadridentatum Labill.				KF607073
Helenium radiatum (Less.) Bierner	Uruguay: Soriano	R. Irving et al. U-24A	TEX/LL	MT557638
Helenium scaposum Britton	Cuba: Isla de la Juventud	Greuter et al. 25969	NY	MT557639
Helenium scorzonerifolium (DC.) A. Gray	Mexico: Chiapas	Jacqueline A. Soule et al. 2317	TEX/LL 57712	MT557640
Helenium scorzonerifolium (DC.) A. Gray	Mexico: Chiapas	L. Alan Prather et al. 1111	TEX/LL 057719	MT557641
Helenium thurberi A.Gray	Mexico: Sonora	Elaine Joyal 2014	TEX/LL 057727	MT557642
Helenium thurberi A.Gray	United States: Arizona	Scott Sundberg 1279	TEX/LL	MT557643
Helenium urmenetae (Phil.) Cabrera var. *leguiffei* (Phil.) Bierner	Chile: Coquimbo	Mark W. Bierner 52388	TEX/LL	MT557644

TABLE 1. Continued.

Taxon	Collection Locality	Collector Information	Herbarium Accession Information	GenBank Accession Number
Helenium urmenetae (Phil.) Cabrera var. *urmenetae*	Chile: Coquimbo	M. O. Dillon et al. 4979	TEX/LL 358847	MT557645
Helenium vallenariense (Phil.) Bierner	Chile: Coquimbo	M. W. Bierner 51486	TEX/LL 358851	MT557646
Helenium vernale Walter	United States: Louisiana	T. L. Wendt et al. 7253	TEX/LL	MT557647
Helenium vernale Walter				KF607072
Helenium virginicum S.F.Blake				AH013988
Hymenoxys hoopesii (A.Gray) Bierner	United States: Colorado	Robert M. King et al. 13912	TEX/LL	MT557648
Hymenoxys integrifolia (Kunth) Bierner	Guatemala: Huehuetenango	M. Véliz 96.5749	TEX/LL	MT557649
Hymenoxys jamesii Bierner	United States: Arizona	Mark W. Bierner 91-84	TEX/LL	MT557650
Hymenoxys lemmonii (Greene) Cockerell	United States: Nevada	Ann Pinzl 10019	TEX/LL	MT557651
Hymenoxys odorata DC.	United States: New Mexico	Michael J. Moore et al. 3004	OC	MT557652
Hymenoxys richardsonii (Hook.) Cockerell var. *floribunda* (A.Gray) K.F.Parker	United States: Arizona	Michael J. Moore et al. 831	OC	MT557653
Hymenoxys subintegra Cockerell	United States: Arizona	Mark W. Bierner 92-34	TEX/LL	MT557654
Hymenoxys texana (J.M.Coult. & Rose) Cockerell	United States: Texas	David J. Rosen et al. 3987	TEX/LL 445217	MT557655
Marshallia caespitosa Nutt. ex DC. var. *signata* Beadle & F.E.Boynton	United States: Texas	Michael J. Moore 1057	OC	MT557656
Pelucha trifida S.Watson				AF229267
Plateilema palmeri (A.Gray) Cockerell	Mexico: Coahuila	George S. Hinton 27195	TEX/LL 60253	MT557657
Psathyrotes annua (Nutt.) A.Gray				AF229264
Psathyrotes ramosissima (Torr.) A.Gray	United States: California	Michael J. Moore et al. 3239	OC	MT557658
Psilostrophe cooperi (A.Gray) Greene	United States: California	Michael J. Moore et al. 3136	OC	MT557659

TABLE 1. Continued.

Taxon	Collection Locality	Collector Information	Herbarium Accession Information	GenBank Accession Number
Tetraneuris acaulis (Pursh) Greene				AF229282
Tetraneuris linearifolia (Hook.) Greene	United States: Texas	Michael J. Moore 1111	OC	MT557660
Trichoptilium incisum (A.Gray) A.Gray	United States: California	LeRoy Gross et al. 1766	TEX/LL	MT557661

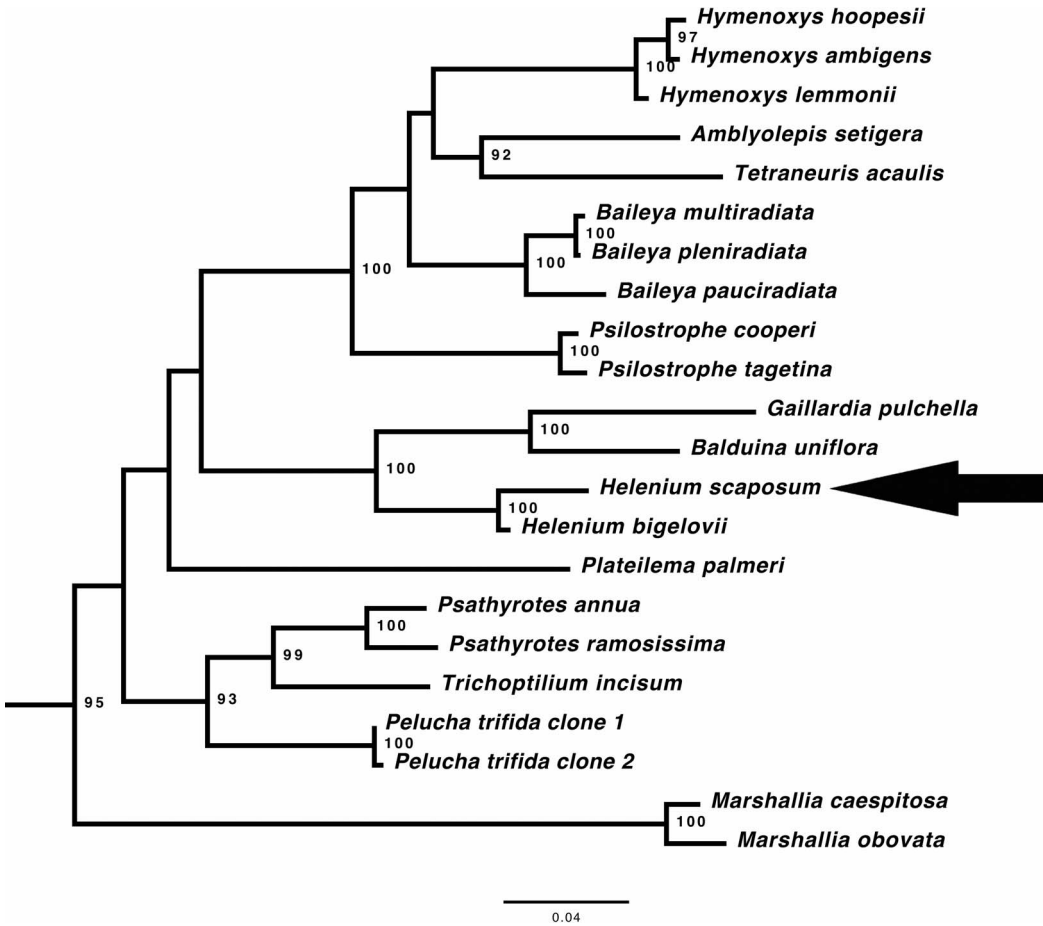

FIG. 1. Results of initial maximum likelihood phylogenetic analysis including *Helenium scaposum* and taxa across the Heliantheae alliance sampled by Baldwin et al. (2002), showing only the clade corresponding to tribe Helenieae. Bootstrap clade support values above 90% are shown at nodes. Note that the samples of *Helenium scaposum* (denoted by arrow) and the only other included species of *Helenium* (*H. bigelovii*) constitute a robust clade.

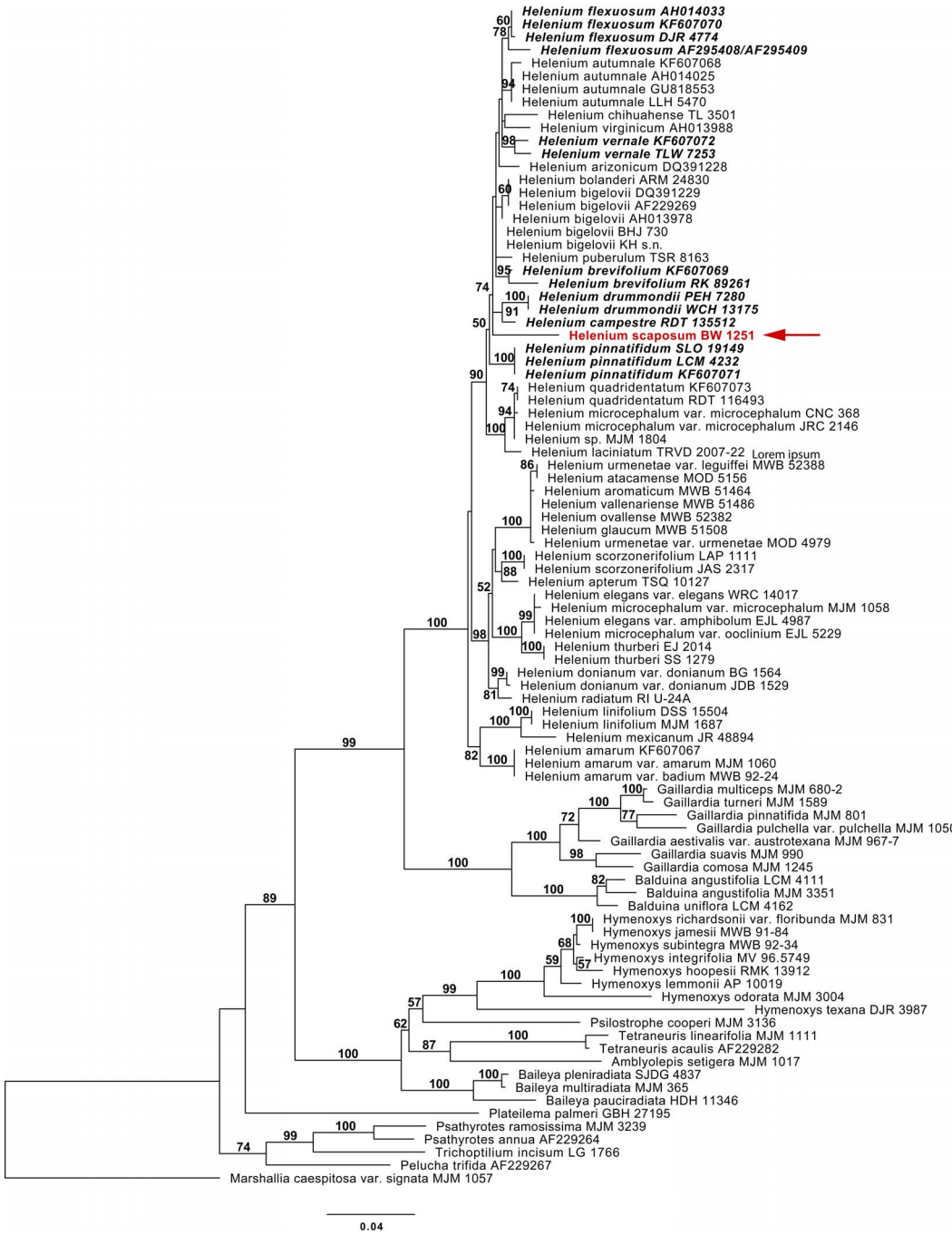

FIG. 2. Phylogram showing results of a maximum likelihood analysis of *Helenium* and outgroup species in Helenieae. Taxa in *Helenium* sect. *Leptopoda* are in bold italics, and *H. scaposum* is highlighted in red and by the red arrow. Bootstrap clade support values at or above 50% are shown at nodes.

The analysis of Baldwin clearly answered question 1; this plant belongs in *Helenium*. The *Helenium scaposum* sequence associated with *Helenium* in a data set that included representatives of the closely related genera *Balduina* Nutt. and *Gaillardia* Foug. The data set also included representatives of all other Helenieae genera: *Amblyolepis* DC., *Baileya* Harv. & A. Gray ex Torr., *Hymenoxys* Cass., *Marshallia* Schreb., *Pelucha* S. Watson, *Plateilema* (A. Gray) Cockerell, *Psathyrotes* (Nutt.) A. Gray, *Psilostrophe* DC., *Tetraneuris* Greene, and *Trichoptilium* A. Gray (see Baldwin and Wessa, 2000a, and Baldwin et al., 2002).

Question 2 — where does *Helenium scaposum* fit into the genus — has been answered to some extent by the analysis of Moore et al. Baldwin's data set included only one *Helenium* sequence other than *H. scaposum* (that of *H. bigelovii* Torr. & A. Gray), while the data set of Moore et al. included sequences of 36 *Helenium* taxa plus multiple populations of many of the taxa (Table 1). Furthermore, the Moore et al. data set included representatives of all of the currently recognized sections of *Helenium* (Appendix 2). The North American sections are sect. *Amarum* Bierner, sect. *Hecubaea* (DC.) A. Gray, sect. *Helenium*, sect. *Leptopoda* (Nutt.) Wood, and sect. *Tetrodus* (Cass.) DC. (Bierner, 1972). The South American sections are sect. *Cephalophora* (Cav.) Hoff., and sect. *Actinea* (Juss.) Bierner (Bierner, 1978, 1987).

To add some perspective, Rock (1957) in his work on *Helenium* stated, "At this time, I would like to emphasize the apparent relationship of *H. scaposum* to the tetraneuranae of Rydberg and suggest that the most likely affinity of this species is with *Plateilema Palmeri* (A. Gray) Cockerell." In fact, the only conspicuous morphological characteristic that might indicate such a close relationship between *H. scaposum* and *P. palmeri* is that both have scapiform capitulescences. Furthermore, the analyses of both Baldwin and Moore et al. (this study) indicate that *H. scaposum* and *P. palmeri* are well separated phylogenetically (Figs. 1 and 2; Appendix 1). In addition, a close relationship of these two taxa is ecologically and biogeographically doubtful; *H. scaposum* is confined to white sand associated with current or former pine forests on Isla de la Juventud, Cuba, and *P. palmeri* is found on fine clay-loam soils of Chihuahuan desert scrub/grasslands from Brewster County, Texas, to central Coahuila and southern Nuevo León, Mexico (Jackson et al., 2015; Jackson, 2017). Judging from biogeography alone, it seems most likely that *H. scaposum* fits into the "widespread, disjunct to western Cuba" pattern of Sorrie and Weakley (2001), and is more likely to be related to members of *Helenium* from the southeastern United States. In fact, Sorrie and Weakley (2001) state, "Western Cuba includes the provinces of Habana, Isla de Pinos (Isla de Juventud), Matanzas, and Pinar del Rio. Nearly all of the plants listed here are found as disjuncts to Cuba only. A number of the 'widespread' taxa are confined in the United States to the Florida peninsula." While *H. scaposum* was not among the taxa listed by Sorrie and Weakley (2001), it is endemic to Isla de la Juventud, and two members of *Helenium* from the southeastern United States, *H. pinnatifidum* (Nutt.) Rydb. and *H. flexuosum* Raf., have populations that extend into southern Florida.

Which brings us back to question 2 — where does *Helenium scaposum* fit into the genus *Helenium*? While it seems clear from Figure 2 that *H. scaposum* is a distinctive taxon, as indicated by its relatively long branch length (i.e., it has several unique base changes), and is associated with a large clade including, among other taxa, members of *Helenium* sect. *Leptopoda* (highlighted in the tree), the relationships within this large clade are generally poorly supported, making it impossible based on this phylogenetic analysis to make any strong claim as to a sister-taxon relationship. Having said that, a second look at biogeography and a first look at morphology and ecology seem to be warranted.

All of the taxa of *Helenium* sect. *Leptopoda* (Appendix 2) have distributions that fit within the "widespread, disjunct to

western Cuba" pattern of Sorrie and Weakley (2001); however, only *H. flexuosum* and *H. pinnatifidum* have distributions that extend to far southern Florida (see Rock 1957, p. 139). With regard to habitat preference, *H. scaposum* grows in white sand associated with current or former pine forests, and the taxa of *H.* sect. *Leptopoda* grow in various types of sandy soils often at the edges of pine forests (Rock, 1957; Table 2), which also is in accord with the "widespread, disjunct to western Cuba" pattern of Sorrie and Weakley (2001). Morphologically, *H. scaposum*, while very diminutive compared to the taxa of *H.* sect. *Leptopoda*, seems to have more in common with *H. drummondii* H. Rock, *H. pinnatifidum*, and *H. vernale* Walter (Table 2), the three of which, according to Rock (1957), form a subgroup within sect. *Leptopoda*. Even though *H. flexuosum* has a distribution that extends into southern Florida, it seems on morphological grounds (Table 2) to be a poor candidate as a sister-taxon to *H. scaposum*. Knowing the chromosome number of *H. scaposum* could be very important for establishing a sister-taxon relationship; hence, a visit to Isla de la Juventud may be in Bierner's not-too-distant future. In the meantime, we believe that the most likely association of *H. scaposum* within *Helenium* is with taxa of *H.* sect. *Leptopoda*, in particular *H. drummondii, H. pinnatifidum*, and *H. vernale*.

We recognize that the phylogenetic tree produced by Moore et al. (Fig. 2 and Appendix 1) provides an opportunity to comment on sectional delimitations (Appendix 2) and relationships in *Helenium*. However, that is outside of the scope of this paper and will be the subject of future studies.

Taxonomic Treatment

Helenium scaposum Britton, Bull. Torrey Bot. Club 43: 469. 1916. Type: CUBA. Isle of Pines (now Isla de la Juventud), Vicinity of Siguanea, Pinelands (type labels); "White sand pine-lands, west-central districts. Type from near Siguanea" (protologue), 15 Feb–06 Mar 1916, *N. L. Britton, E. G. Britton, Percy Wilson 14346* (Holotype: NY #126645!; Isotypes: CM #211017!, F #459430!, GH #00008778!, S #S-R-1007 – as database image!, US #793094!).

Herbs, perennials. Root systems composed of numerous adventitious roots originating from a short rhizome. Aerial stems 1 per plant, erect, unbranched. Leaves all basal, tightly clustered; usually petiolate; blades prominently veined with distinct midribs and distinct lateral veins, spatulate to oblanceolate to linear-oblanceolate, apices acute to rounded, margins undulate to undulate-dentate, sometimes entire, adaxial faces glabrous, abaxial faces glabrous or sparsely to moderately pubescent, very strongly gland-dotted. Heads 1 per plant, radiate. Peduncles 3–12 cm, sparsely to moderately pubescent proximally, moderately to densely pubescent distally, expanded apically. Involucres hemispheric to subglobose, 5–8(–10) × (6–)8–10 mm. Phyllaries persistent, usually 8 in each of 2 series, outer phyllaries usually proximally connate, spreading to erect in fruit, adaxial faces sparsely to moderately pubescent, sparsely to moderately glandular. Receptacles hemispheric to sub-hemispheric; paleae none. Ray florets 8–13; corollas yellow, laminae fan-shaped, 3-lobed, 6.0–9.0 × 3.5–4.5 mm, adaxial faces glabrous, usually eglandular, abaxial faces sparsely to moderately pubescent, sparsely to moderately glandular. Disc florets 50–100+; corollas yellow, throats cylindric to cylindric-campanulate, 5-lobed, 2.5–3.0 × 0.7–1.0 mm, sparsely to moderately pubescent, sparsely glandular. Cypselae obpyramidal to narrowly so, sparsely to moderately pubescent, sparsely glandular, 1.5–2 × 0.8–1.2 mm; pappus scales 6–8, elliptic to obovate, 0.6–0.8 × 0.3–0.4 mm, apices lacerate.

Distribution and Habitat. Plants endemic to the western part of Isla de la Juventud, Cuba. Growing in white sand associated with current or former pine forests; elevation ca 2–9 m.

Flowering and Fruiting. February through May.

TABLE 2. A morphological and ecological comparison of *Helenium scaposum* with the taxa of *Helenium* sect. *Leptopoda*.

	H. scaposum	*H. drummondii*	*H. pinnatifidum*	*H. vernale*	*H. campestre*	*H. brevifolium*	*H. flexuosum*
Plant Height (cm)	3–12	20–60	30–80	30–80	40–100	20–100	30–100
Heads Per Plant	1	1(–3)	1(–3)	1(–3)	3–20	1–10	(1–)5–50(–80+)
Stem Leaves	No	Yes	Yes	Yes	Yes	Yes	Yes
Leaf Decurrence	NA	Moderate to strong	Weak	Weak	Moderate to strong	Weak to moderate	Strong
Peduncle Pubescence (Distal)	Moderate to dense	Sparse	Moderate to dense	Usually glabrous	Moderate to dense	Sparse to moderate	Sparse to moderate
Basal Leaf Pubescence	Glabrous or sparse to moderate	Glabrous	Glabrous or rarely sparse	Glabrous or rarely sparse	Moderate to dense	Glabrous or sparse	Glabrous or sparse to moderate
Basal Leaf Margins	Usually undulate to undulate–serrate, sometimes entire	Usually entire, sometimes undulate–serrate	Usually pinnatifid, sometimes undulate to undulate–serrate	Usually entire, sometimes undulate to undulate–serrate	Entire or undulate–serrate	Usually entire, sometimes undulate to undulate–serrate	Entire or serrate
Involucre H × W (mm)	5–8(–10) × (6–)8–10	10–18 × 15–25	12–20 × 15–30	10–17 × 15–27	9–17 × 10–20	10–15 × 10–20	8–17 × 9–17
Phyllary Pubescence	Sparse to moderate	Sparse to moderate	Moderate	Glabrous or sparse	Moderate to dense	Sparse to moderate	Moderate to dense
Ray Floret Number	8–13	13–30	13–34	13–30	9–15	9–24	8–13
Ray Floret L × W (mm)	6–9 × 3.5–4.5	14–25 × 4–10	15–22 × 5–8	15–21 × 5–10	20–30 × 8–15	12.5–21 × 4–8	10–20 × 5–10
Disc Floret Number	50–100+	250–650(–1000+)	250–650(–800+)	150–650(–800+)	150–500(–700+)	200–500(–800+)	25–500(–700+)
Disc Floret L (mm)	2.5–3.0	4.3–5.4	4–5.5	4.6–6	3–5	4–5.6	2.3–3.7
Disc Floret Lobes (number; color)	5; yellow	5; yellow	5; yellow	5; yellow	5; red–brown	5; red–brown	4(–5); red–brown

TABLE 2. Continued.

	H. scaposum	*H. drummondii*	*H. pinnatifidum*	*H. vernale*	*H. campestre*	*H. brevifolium*	*H. flexuosum*
Pappi	6–8; apices lacerate, not aristate	5–12; deeply lacerate, not aristate	8–11; entire to slightly lacerate, not aristate	Usually 8; entire or lacerate, not aristate	6–7; entire, not aristate	6–8; entire, not aristate	5–6; entire, aristate
Pappus Scale L (mm)	0.6–0.8	2–3.7	1.2–1.5	1.5–2	0.3–0.5	1–1.7	0.6–1(–1.7)
Chromosome Number	Unknown	$n = 16$	$n = 16, 17$	$n = 17$	$n = 14$	$n = 13, 14$	$n = 14$
Habitat	Associated with current or former pine forests	Most often associated with current or former pine forests	Most often associated with current or former pine forests	Most often associated with current or former pine forests	Most often associated with current or former pine forests	Most often associated with current or former pine forests	Often associated with current or former pine forests, but also in other wet areas
Habitat Substrate	Sand	Most often sandy soils	Most often sandy soils	Most often sandy soils	Most often sandy soils	Most often sandy soils	Most often sandy soils

Specimens Examined. CUBA. Isla de la Juventud: On white sand, pine barrens, Los Indios, 19 May 1910, *Jennings 429* (CM, GH, NY); Vicinity of Los Indios, white sand, 13 Feb 1916, *Britton, Britton & Wilson 15204* (NY); Los Indios: sur les sables blancs, 1-4 May 1944, *Marie-Victorin & Alain 16* (GH); Westport region, white-sand sabanas, 23 Apr 1956, *Killip 45658* (US); Savannas, Santa Bárbara, May 1940, *Bros. León & M. Victorin 17875* (NY); Siguanea region, rays bright yellow, white-sand sabanas, 18 Mar 1954, *Killip 43706* (F, NY, US); Sabanas al noreste de la pista del Aeropuerto de Siguanea, 2 m, arenas blancas, 21°38′53″N, 82°56′34″W, flores todas amarillas, 28 Feb 2002, *Greuter, Rankin & Pérez 25969* (NY – Note: Voucher for DNA studies).

Acknowledgments

We are grateful to the institutions cited above for loans of specimens. We are especially grateful to TEX/LL and NY for allowing us to remove leaf fragments. Bierner thanks George Yatskievych for assistance with loans and with collecting the leaf sample that was used by Baldwin for the *Helenium scaposum* ITS region sequence, and Jose L. Panero for his help with the Spanish resumen and the manuscript in general. Baldwin thanks Bridget Wessa for lab assistance. We thank the US National Science Foundation (DEB 1054539), the National Geographic Society, and Oberlin College for support.

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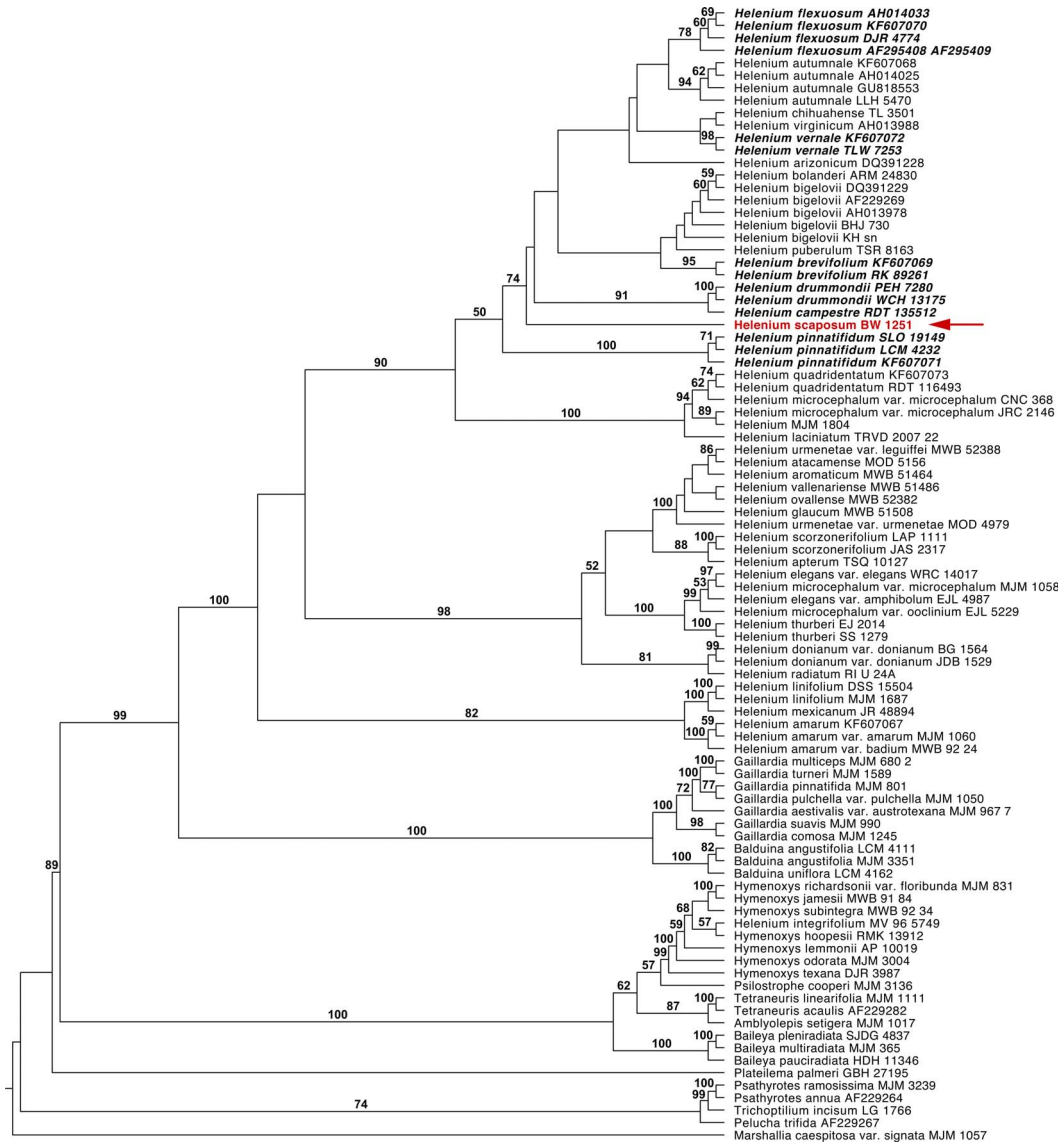

APPENDIX 1. Cladogram showing results of a maximum likelihood analysis of *Helenium* and outgroup species in Helenieae. Taxa in *Helenium* sect. *Leptopoda* are in bold italics, and *H. scaposum* is highlighted in red and by the red arrow. Bootstrap clade support values at or above 50% are shown at nodes.

APPENDIX 2. Sectional breakdown of *Helenium* (see Bierner, 1972, 1978, 1987).

Sections – North America	Taxa
Helenium L. sect. *Amarum* Bierner	*H. amarum* (Raf.) H. Rock var. *amarum*
	H. amarum (Raf.) H. Rock var. *badium* (A. Gray ex S. Watson) Waterf.
Helenium L. sect. *Hecubaea* (DC.) A. Gray	*H. apterum* (S.F. Blake) Bierner
	H. scorzoneraefolium (DC.) A. Gray
Helenium L. sect. *Helenium*	*H. autumnale* L.
	H. virginicum S.F. Blake
Helenium L. sect. *Leptopoda* (Nutt.) A. Wood	*H. brevifolium* (Nutt.) Alph. Wood
	H. campestre Small
	H. drummondii H. Rock
	H. flexuosum Raf.
	H. pinnatifidum (Schwein. ex Nutt.) Rydb.
	H. vernale Walter
Helenium L. sect. *Tetrodus* (Cass.) DC.	*H. arizonicum* S.F. Blake
	H. bigelovii A. Gray
	H. bolanderi A. Gray
	H. chihuahuense Bierner
	H. elegans DC. var. *elegans*
	H. elegans DC. var. *amphibolum* (A. Gray) Bierner
	H. laciniatum A. Gray
	H. linifolium Rydb.
	H. mexicanum Kunth
	H. microcephalum DC. var. *microcephalum*
	H. microcephalum DC. var *ooclinium* (A. Gray) Bierner
	H. puberulum DC.
	H. quadridentatum Labill.
	H. thurberi A. Gray
Sections – South America	Taxa
Helenium L. sect. *Actinia* (Juss.) Bierner	*H. donianum* (Hook. & Arn.) Cabrera var. *donianum*
	H. donianum (Hook. & Arn.) Cabrera var. *linearifolium* (Kuntze) Bierner
	H. radiatum (Less.) Bierner
Helenium L. sect. *Cephalophora* (Cav.) Hoffm.	*H. aromaticum* (Hook.) L.H. Bailey
	H. atacamense Cabrera
	H. glaucum (Cav.) Stuntz
	H. insulare (Phil.) Cabrera
	H. ovallense Bierner
	H. urmenetae (Phil.) Cabrera var. *urmenetae*
	H. urmenetae (Phil.) Cabrera var. *leguiffei* (Phil.) Bierner
	H. vallenariense (Phil.) Bierner