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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 distintiva 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, Gaillardiiinae, *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 Rodríguez, 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 (Berzaín 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 μ L volumes with 0.5 μ L of 5 mM primer for both primers, 5–20 ng of DNA template, 0.1 μ L of GoTaq (Promega, Madison, WI, USA), 6.25 μ L of Failsafe Premix B (Epicentre, Madison, WI, USA), and 4.7 μ L 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 nucle-

otides 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 μ L reactions containing 10 U of Exonuclease I (Affymetrix, ThermoFisher Scientific, Waltham, MA, USA), 2 U of shrimp alkaline phosphatase (Affymetrix), 8 μ L of PCR product, and 8.5 μ L 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 |
|---|------------------------------|----------------------------------|---------------------------------|--------------------------|
| <i>Amblyolepis setigera</i> DC. | United States: Texas | Michael J. Moore et al. 1017 | OC | MT557590 |
| <i>Baileya multiradiata</i> Harv. & A.Gray ex A. Gray | United States: New Mexico | Michael J. Moore 365 | OC | MT557591 |
| <i>Baileya pauciradiata</i> Harv. & A.Gray ex A.Gray | United States: Arizona | H. David Hammond 11346 | TEX/LL | MT557592 |
| <i>Baileya pleniradiata</i> Harv. & A.Gray ex A.Gray | United States: California | Sarah J. De Groot et al. 4837 | TEX/LL | MT557593 |
| <i>Balduina angustifolia</i> (Pursh) B.L.Rob. | United States: Florida | Lucas C. Majure 4111 | OC | MT557594 |
| <i>Balduina</i> cf. <i>angustifolia</i> (Pursh) B.L.Rob. | United States: Florida | Michael J. Moore 3351 | OC | MT557595 |
| <i>Balduina uniflora</i> Nutt. | United States: Alabama | Lucas C. Majure et al. 4162 | OC | MT557596 |
| <i>Gaillardia aestivalis</i> (Walter) H.Rock var. <i>austrotexana</i> B.L.Turner | United States: Texas | Michael J. Moore et al. 967-7 | OC | MT557597 |
| <i>Gaillardia comosa</i> A.Gray | Mexico: San Luis Potosi | Michael J. Moore et al. 1245 | OC | MT557598 |
| <i>Gaillardia multiceps</i> Greene | United States: New Mexico | Michael J. Moore et al. 680-2 | OC | MT557599 |
| <i>Gaillardia pinnatifida</i> Torr. | United States: Utah | Michael J. Moore et al. 801 | OC | MT557600 |
| <i>Gaillardia pulchella</i> Foug. var. <i>pulchella</i> | United States: Texas | Michael J. Moore et al. 1050 | OC | MT557601 |
| <i>Gaillardia suavis</i> (A.Gray & Engelm.) Britton & Rusby | United States: Texas | Michael J. Moore et al. 990 | OC | MT557602 |
| <i>Gaillardia turneri</i> Averett & A.M.Powell | Mexico: Chihuahua | Michael J. Moore et al. 1589 | OC | MT557603 |
| <i>Helenium</i> sp. | United States: Texas | Michael J. Moore 1804 | OC | MT557632 |
| <i>Helenium amarum</i> (Raf.) H.Rock | | | | KF607067 |
| <i>Helenium amarum</i> (Raf.) H.Rock var. <i>amarum</i> | United States: Texas | Michael J. Moore 1060 | OC | MT557604 |

TABLE 1. Continued.

| Taxon | Collection Locality | Collector Information | Herbarium Accession Information | GenBank Accession Number |
|---|------------------------------|---------------------------------------|---------------------------------|--------------------------|
| <i>Helenium amarum</i> (Raf.) H.Rock var. <i>badium</i> (A.Gray ex S. Watson) Waterf. | United States: Texas | Mark W. Bierner 92-24 | TEX/LL 412046 | MT557605 |
| <i>Helenium apterum</i> (S.F.Blake) Bierner | Mexico: Durango | T. Sultan Quedensley 10127 | TEX/LL | MT557606 |
| <i>Helenium arizonicum</i> S.F.Blake | | | | DQ391228 |
| <i>Helenium aromaticum</i> (Hook.) L.H.Bailey | Chile: Coquimbo | M. W. Bierner 51464 | TEX/LL 358831 | MT557607 |
| <i>Helenium atacamense</i> Cabrera | Chile: Antofagasta | M. O. Dillon et al. 5156 | TEX/LL 358835 | MT557608 |
| <i>Helenium autumnale</i> L. | United States: Texas | L. L. Hansen 5470 | TEX/LL 431512 | MT557609 |
| <i>Helenium autumnale</i> L. | | | | AH014025 |
| <i>Helenium autumnale</i> L. | | | | GU818553 |
| <i>Helenium autumnale</i> L. | | | | KF607068 |
| <i>Helenium bigelovii</i> A.Gray | United States: California | Betty H. Johnson 730 | TEX/LL | MT557610 |
| <i>Helenium bigelovii</i> A.Gray | United States: California | Kathy Harper s.n. | TEX/LL | MT557611 |
| <i>Helenium bigelovii</i> A.Gray | | | | AF229269 |
| <i>Helenium bigelovii</i> A.Gray | | | | AH013978 |
| <i>Helenium bigelovii</i> A.Gray | | | | DQ391229 |
| <i>Helenium bolanderi</i> A.Gray | United States: California | Andrew R. Moldenke et al. 24830 | TEX/LL | MT557612 |
| <i>Helenium brevifolium</i> (Nutt.) Alph.Wood | United States: Alabama | R. Kral 89261 | TEX/LL | MT557613 |
| <i>Helenium brevifolium</i> (Nutt.) Alph.Wood | | | | KF607069 |
| <i>Helenium campestre</i> Small | United States: Arkansas | R. Dale Thomas et al. 135512 | TEX/LL | MT557614 |
| <i>Helenium chihuahuense</i> 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 |
|---|---------------------------|-----------------------------------|---------------------------------|--------------------------|
| <i>Helenium donianum</i> (Hook. & Arn.) Cabrera var. <i>donianum</i> | Argentina: San Juan | Barbara E. Goodson et al. 1564 | TEX/LL | MT557616 |
| <i>Helenium donianum</i> (Hook. & Arn.) Cabrera var. <i>donianum</i> | Argentina: La Rioja | John D. Bacon et al. 1529 | TEX/LL 358836 | MT557617 |
| <i>Helenium drummondii</i> H.Rock | United States: Louisiana | Philip E. Hyatt 7280 | TEX/LL | MT557618 |
| <i>Helenium drummondii</i> H.Rock | United States: Texas | W. C. Holmes et al. 13175 | TEX/LL 445181 | MT557619 |
| <i>Helenium elegans</i> DC. var. <i>amphibolum</i> (A.Gray) Bierner | United States: Texas | Emily J. Lott et al. 4987 | TEX/LL 210429 | MT557620 |
| <i>Helenium elegans</i> DC. var. <i>elegans</i> | United States: Texas | W. R. Carr et al. 14017 | TEX/LL 005633 | MT557621 |
| <i>Helenium flexuosum</i> Raf. | United States: Texas | D. J. Rosen 4774 | TEX/LL 433172 | MT557622 |
| <i>Helenium flexuosum</i> Raf. | | | | AF295408 & AF295409 |
| <i>Helenium flexuosum</i> Raf. | | | | AH014033 |
| <i>Helenium flexuosum</i> Raf. | | | | KF607070 |
| <i>Helenium glaucum</i> (Cav.) Stuntz | Chile: Colchagua | M. W. Bierner 51508 | TEX/LL 358840 | MT557623 |
| <i>Helenium laciniatum</i> A.Gray | Mexico: Sonora | T. R. Van Devender et al. 2007-22 | TEX/LL 205211 | MT557624 |
| <i>Helenium linifolium</i> Rydb. | United States: Texas | D. S. Seigler et al. 15504 | TEX/LL 199704 | MT557625 |
| <i>Helenium linifolium</i> Rydb. | United States: Texas | Michael J. Moore 1687 | OC | MT557626 |
| <i>Helenium mexicanum</i> Kunth | Mexico: Queretaro | Jerzy Rzedowski 48894 | TEX/LL 433654 | MT557627 |
| <i>Helenium microcephalum</i> DC. var. <i>microcephalum</i> | United States: Texas | Candice N. Cerda 368 | TEX/LL 459878 | MT557628 |
| <i>Helenium microcephalum</i> DC. var. <i>microcephalum</i> | 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 |
|--|------------------------------|---------------------------------------|---------------------------------|--------------------------|
| <i>Helenium microcephalum</i> DC. var. <i>microcephalum</i> | United States: Texas | Michael J. Moore 1058 | OC | MT557630 |
| <i>Helenium microcephalum</i> DC. var. <i>ooclinium</i> (A.Gray) Bierner | United States: Texas | Emily J. Lott et al. 5229 | TEX/LL 206753 | MT557631 |
| <i>Helenium ovallense</i> Bierner | Chile: Coquimbo | M. W. Bierner 52382 | TEX/LL 358842 | MT557633 |
| <i>Helenium pinnatifidum</i> (Schwein. ex Nutt.) Rydb. | United States | Lucas C. Majure 4232 | OC | MT557634 |
| <i>Helenium pinnatifidum</i> (Schwein. ex Nutt.) Rydb. | United States: Florida | Steve L. Orzell et al. 19149 | TEX/LL | MT557635 |
| <i>Helenium pinnatifidum</i> (Schwein. ex Nutt.) Rydb. | | | | KF607071 |
| <i>Helenium puberulum</i> DC. | United States: California | Timothy S. Ross et al. 8163 | TEX/LL | MT557636 |
| <i>Helenium quadridentatum</i> Labill. | United States: Louisiana | R. Dale Thomas et al. 116493 | TEX/LL | MT557637 |
| <i>Helenium quadridentatum</i> Labill. | | | | KF607073 |
| <i>Helenium radiatum</i> (Less.) Bierner | Uruguay: Soriano | R. Irving et al. U- 24A | TEX/LL | MT557638 |
| <i>Helenium scaposum</i> Britton | Cuba: Isla de la Juventud | Greuter et al. 25969 | NY | MT557639 |
| <i>Helenium scorzonerifolium</i> (DC.) A. Gray | Mexico: Chiapas | Jacqueline A. Soule et al. 2317 | TEX/LL 57712 | MT557640 |
| <i>Helenium scorzonerifolium</i> (DC.) A. Gray | Mexico: Chiapas | L. Alan Prather et al. 1111 | TEX/LL 057719 | MT557641 |
| <i>Helenium thurberi</i> A.Gray | Mexico: Sonora | Elaine Joyal 2014 | TEX/LL 057727 | MT557642 |
| <i>Helenium thurberi</i> A.Gray | United States: Arizona | Scott Sundberg 1279 | TEX/LL | MT557643 |
| <i>Helenium urmenetae</i> (Phil.) Cabrera var. <i>leguiffei</i> (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 |
|--|------------------------------|------------------------------|---------------------------------|--------------------------|
| <i>Helenium urmenetae</i> (Phil.) Cabrera var. <i>urmenetae</i> | Chile: Coquimbo | M. O. Dillon et al. 4979 | TEX/LL 358847 | MT557645 |
| <i>Helenium vallenariense</i> (Phil.) Bierner | Chile: Coquimbo | M. W. Bierner 51486 | TEX/LL 358851 | MT557646 |
| <i>Helenium vernale</i> Walter | United States: Louisiana | T. L. Wendt et al. 7253 | TEX/LL | MT557647 |
| <i>Helenium vernale</i> Walter | | | | KF607072 |
| <i>Helenium virginicum</i> S.F.Blake | | | | AH013988 |
| <i>Hymenoxys hoopesii</i> (A.Gray) Bierner | United States: Colorado | Robert M. King et al. 13912 | TEX/LL | MT557648 |
| <i>Hymenoxys integrifolia</i> (Kunth) Bierner | Guatemala: Huehuetenango | M. Véliz 96.5749 | TEX/LL | MT557649 |
| <i>Hymenoxys jamesii</i> Bierner | United States: Arizona | Mark W. Bierner 91-84 | TEX/LL | MT557650 |
| <i>Hymenoxys lemmonii</i> (Greene) Cockerell | United States: Nevada | Ann Pinzl 10019 | TEX/LL | MT557651 |
| <i>Hymenoxys odorata</i> DC. | United States: New Mexico | Michael J. Moore et al. 3004 | OC | MT557652 |
| <i>Hymenoxys richardsonii</i> (Hook.) Cockerell var. <i>floribunda</i> (A.Gray) K.F.Parker | United States: Arizona | Michael J. Moore et al. 831 | OC | MT557653 |
| <i>Hymenoxys subintegra</i> Cockerell | United States: Arizona | Mark W. Bierner 92-34 | TEX/LL | MT557654 |
| <i>Hymenoxys texana</i> (J.M.Coult. & Rose) Cockerell | United States: Texas | David J. Rosen et al. 3987 | TEX/LL 445217 | MT557655 |
| <i>Marshallia caespitosa</i> Nutt. ex DC. var. <i>signata</i> Beadle & F.E.Boynton | United States: Texas | Michael J. Moore 1057 | OC | MT557656 |
| <i>Pelucha trifida</i> S.Watson | | | | AF229267 |
| <i>Plateilema palmeri</i> (A.Gray) Cockerell | Mexico: Coahuila | George S. Hinton 27195 | TEX/LL 60253 | MT557657 |
| <i>Psathyrotes annua</i> (Nutt.) A.Gray | | | | AF229264 |
| <i>Psathyrotes ramosissima</i> (Torr.) A.Gray | United States: California | Michael J. Moore et al. 3239 | OC | MT557658 |
| <i>Psilostrophe cooperi</i> (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 |
|---|---------------------------|-------------------------|---------------------------------|--------------------------|
| <i>Tetranneuris acaulis</i> (Pursh) Greene | | | | AF229282 |
| <i>Tetranneuris linearifolia</i> (Hook.) Greene | United States: Texas | Michael J. Moore 1111 | OC | MT557660 |
| <i>Trichoptilium incisum</i> (A.Gray) A.Gray | United States: California | LeRoy Gross et al. 1766 | TEX/LL | MT557661 |

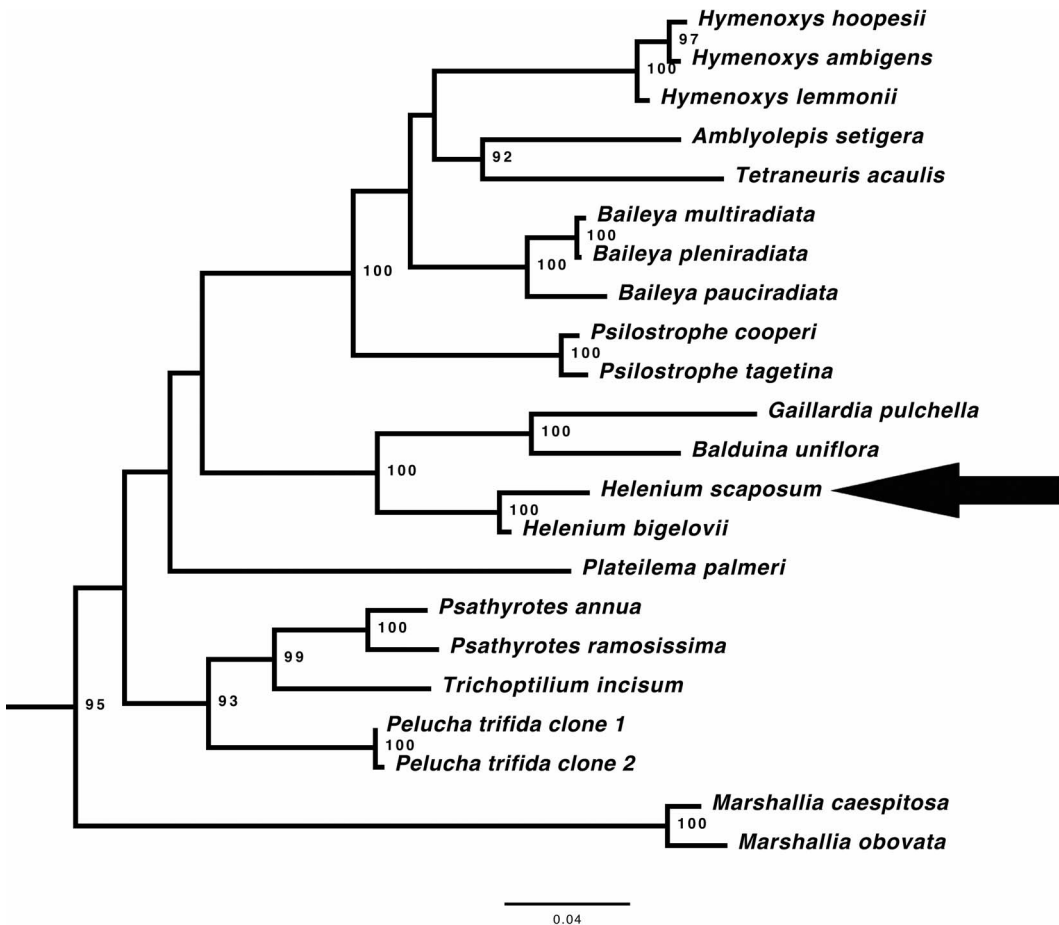


FIG. 1. Results of initial maximum likelihood phylogenetic analysis including *Helenium scaposum* and taxa across the Heliantheae alliance sampled by Baldwin et al. (2002), showing only the clade corresponding to tribe Helenieae. Bootstrap clade support values above 90% are shown at nodes. Note that 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., *Tetranuris* 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 tetra-neuranae 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 Siguaná, Pinelands (type labels); “White sand pine-lands, west-central districts. Type from near Siguaná” (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 cylindrical to cylindrical-campanulate, 5-lobed, 2.5–3.0 × 0.7–1.0 mm, sparsely to moderately pubescent, sparsely glandular. Cypselsae 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*.

| | <i>H. scaposum</i> | <i>H. drummondii</i> | <i>H. pinnatifidum</i> | <i>H. vemale</i> | <i>H. campestre</i> | <i>H. brevifolium</i> | <i>H. flexuosum</i> |
|-----------------------------------|--|--|--|--|----------------------------|--|--------------------------------|
| 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 | NA | Moderate to strong | Weak | Weak | Moderate to strong | Weak to moderate | Strong |
| Decurrence | Moderate to dense | Sparse | Moderate to dense | Usually glabrous | Moderate to dense | Sparse to moderate | Sparse to moderate |
| Peduncle (Distal) | 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 Pubescence | 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 |
| Involute 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 | Sparse to moderate | Sparse to moderate | Moderate | Glabrous or sparse | Moderate to dense | Sparse to moderate | Moderate to dense |
| Pubescence | 8-13 | 13-30 | 13-34 | 13-30 | 9-15 | 9-24 | 8-13 |
| Ray Floret Number | 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 (mm) | 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.

| | <i>H. scaposum</i> | <i>H. drummondii</i> | <i>H. pinnatifidum</i> | <i>H. vernale</i> | <i>H. campestre</i> | <i>H. brevifolium</i> | <i>H. flexuosum</i> |
|---------------------|--|---|---|---|---|---|---|
| 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 | <i>n</i> = 16 | <i>n</i> = 16, 17 | <i>n</i> = 17 | <i>n</i> = 14 | <i>n</i> = 13, 14 | <i>n</i> = 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); Sigüanea region, rays bright yellow, white-sand sabanas, 18 Mar 1954, *Killip 43706* (F, NY, US); Sabanas al noreste de la pista del Aeropuerto de Sigüanea, 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).

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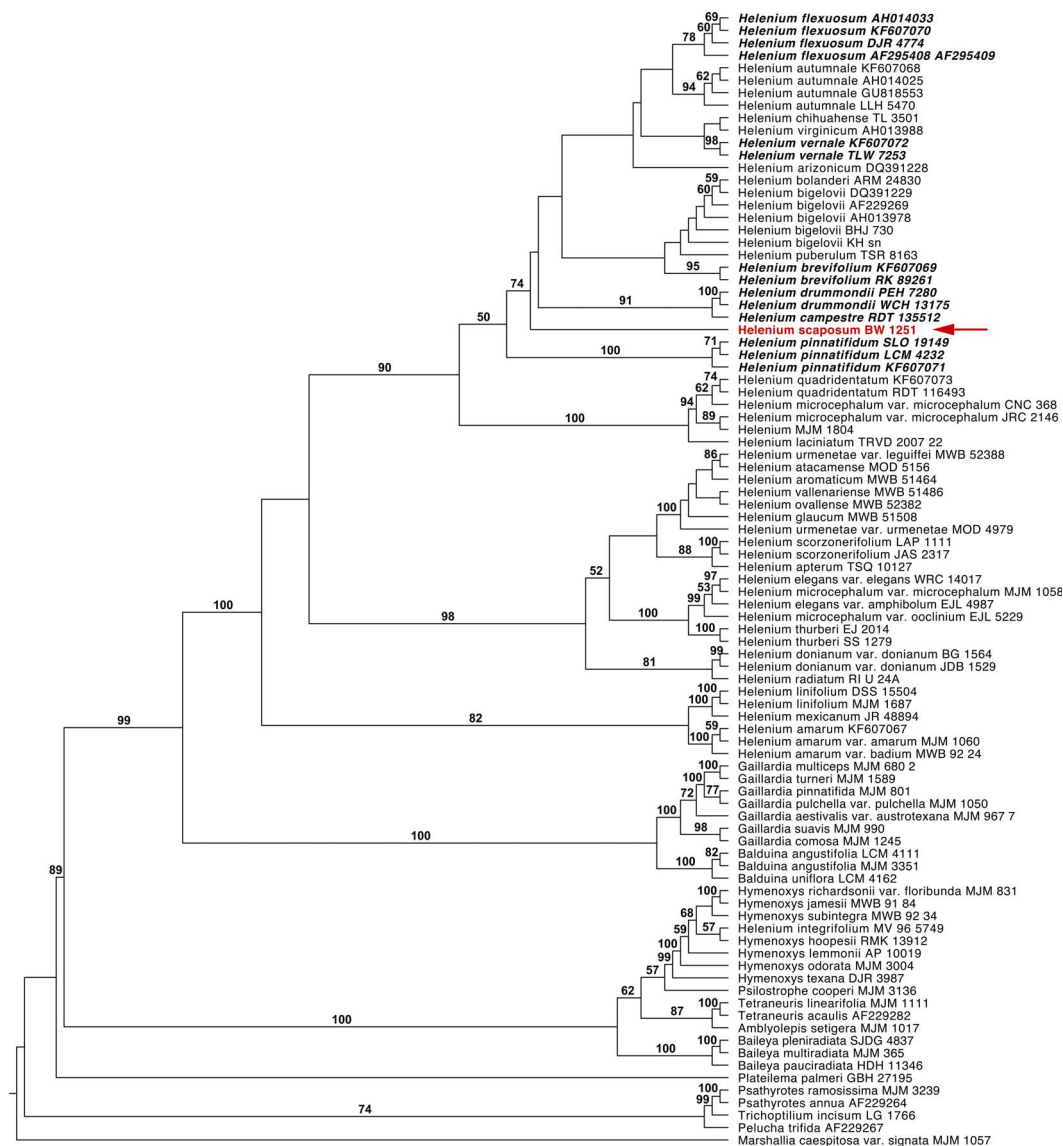
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APPENDIX 1. Cladogram showing results of a maximum likelihood analysis of *Helenium* and outgroup species in Helenieae. Taxa in *Helenium* sect. *Leptopoda* are in bold italics, and *H. scaposum* is highlighted in red and by the red arrow. Bootstrap 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 |
|--|--|
| <i>Helenium</i> L. sect. <i>Amarum</i> Bierner | <i>H. amarum</i> (Raf.) H. Rock var. <i>amarum</i> <i>H. amarum</i> (Raf.) H. Rock var. <i>badium</i> (A. Gray ex S. Watson) Waterf. |
| <i>Helenium</i> L. sect. <i>Hecubaea</i> (DC.) A. Gray | <i>H. apterum</i> (S.F. Blake) Bierner <i>H. scorzoneraefolium</i> (DC.) A. Gray |
| <i>Helenium</i> L. sect. <i>Helenium</i> | <i>H. autumnale</i> L. <i>H. virginicum</i> S.F. Blake |
| <i>Helenium</i> L. sect. <i>Leptopoda</i> (Nutt.) A. Wood | <i>H. brevifolium</i> (Nutt.) Alph. Wood <i>H. campestre</i> Small <i>H. drummondii</i> H. Rock <i>H. flexuosum</i> Raf. <i>H. pinnatifidum</i> (Schwein. ex Nutt.) Rydb. <i>H. vernale</i> Walter |
| <i>Helenium</i> L. sect. <i>Tetrodus</i> (Cass.) DC. | <i>H. arizonicum</i> S.F. Blake <i>H. bigelovii</i> A. Gray <i>H. bolanderi</i> A. Gray <i>H. chihuahuense</i> Bierner <i>H. elegans</i> DC. var. <i>elegans</i> <i>H. elegans</i> DC. var. <i>amphibolum</i> (A. Gray) Bierner <i>H. laciniatum</i> A. Gray <i>H. linifolium</i> Rydb. <i>H. mexicanum</i> Kunth <i>H. microcephalum</i> DC. var. <i>microcephalum</i> <i>H. microcephalum</i> DC. var. <i>ooclinium</i> (A. Gray) Bierner <i>H. puberulum</i> DC. <i>H. quadridentatum</i> Labill. <i>H. thurberi</i> A. Gray |
| Sections – South America | Taxa |
| <i>Helenium</i> L. sect. <i>Actinia</i> (Juss.) Bierner | <i>H. donianum</i> (Hook. & Arn.) Cabrera var. <i>donianum</i> <i>H. donianum</i> (Hook. & Arn.) Cabrera var. <i>linearifolium</i> (Kuntze) Bierner <i>H. radiatum</i> (Less.) Bierner |
| <i>Helenium</i> L. sect. <i>Cephalophora</i> (Cav.) Hoffm. | <i>H. aromaticum</i> (Hook.) L.H. Bailey <i>H. atacamense</i> Cabrera <i>H. glaucum</i> (Cav.) Stuntz <i>H. insulare</i> (Phil.) Cabrera <i>H. ovalense</i> Bierner <i>H. urmenetae</i> (Phil.) Cabrera var. <i>urmenetae</i> <i>H. urmenetae</i> (Phil.) Cabrera var. <i>leguiffei</i> (Phil.) Bierner <i>H. vallenariense</i> (Phil.) Bierner |