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Phylogenetic Relationships Among Native and Naturalized *Hieracium* (Asteraceae) in Canada and the United States Based on Plastid DNA Sequences

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ABSTRACT. We used parsimony and maximum likelihood analyses of chloroplast DNA to examine the relationships of native North American *Hieracium* (hawkweeds; Asteraceae) with non-native *Hieracium* species being studied for classical biological control. Thirty-six taxa were included; these representing the three morphologically-based subgenera: *Pilosella* from Eurasia, the circumboreal *Hieracium*, and *Chionoracium* from North and South America. Results from the *trnT-F* and *petN-psbM* sequence data strongly support the morphologically based classifications. An exception is the placement of *H. canadense* within subgenus *Chionoracium*, rather than subgenus *Hieracium*, which may be due to chloroplast capture. Placement of the genus *Andryala* within *Hieracium* subgenus *Pilosella* is also supported. Additionally, species in subgenus *Pilosella* targeted for classical biological control are still supported as being in a separate subgenus from native North American taxa.

KEYWORDS: Biological control, chloroplast DNA, *Hieracium*, phylogeny.

Hieracium L. is a large genus of herbaceous perennials, commonly named hawkweeds, within the family Asteraceae. Estimates of the number of species worldwide range from 90 to 1,000 (Mabberley 1993) to nearly 5,000 (Shaw 1973) or 10,000 (Beaman 1990; the later two counts including mostly apomictic "microspecies"). Approximately 36 species are found within North America north of Mexico (Strother 2006), and about 13 of those are species introduced from Eurasia. Many of the introduced species are considered invasive in North America (Wilson et al. 2006).

The genus is typically divided into four subgenera: *Pilosella* (Hill) S.F. Gray, *Hieracium*, *Stenotheca* Fries., and *Mandonia* Arv.-Touv. (Zahn 1921–1923). Subgenus *Pilosella*, which contains Eurasian and northwest African species, is at times considered a separate genus (*Pilosella* Hill.; e.g., Sell 1987). Subgenus *Hieracium* has a circumboreal distribution, while subgenus *Stenotheca* is found in the Americas and possibly eastern Asia (Sell 1987). Subgenus *Mandonia* is restricted to South America. Garland (1990) pointed out that subgenus *Chionoracium* Schultz-Bip. has priority over subgenera *Stenotheca* and *Mandonia*, and that name will be used here onward. Thus, the vast majority of native North American *Hieracium* are from subgenus *Chionoracium*, while only two species of subgenus *Hieracium* have native ranges that include North America: *H. umbellatum* L. (narrow leaved hawkweed) and *H. canadense* Michx. (Canada hawkweed).

Non-native, invasive hawkweeds in North America belong to two subgenera, *Pilosella* and *Hieracium*. Although invasive hawkweeds have been widely established in the northeastern and midwestern United States since the mid-19th century (Voss and Bohlke 1978), their presence in the west is relatively recent (Wilson et al. 1997). The most widespread invasive species is *H. caespitosum* (meadow hawkweed). First recorded west of the continental divide in 1969 in Pend Orielle County, Washington, *H. caespitosum* is now distributed from upslope habitats in the Rocky Mountains of central Colorado to as far north as 58° latitude in British Columbia and 60° latitude in Alaska (L.M.W., unpubl. data). Expansion of the weed's range is estimated to be about 16% per year (Wilson and Callihan 1999).

Hieracium caespitosum and other invasive hawkweeds are found in a range of habitat types. In agricultural lands they invade permanent pastures, hayfields, open meadows, and abandoned farmland where the soil is coarse-textured, well-drained, and moderately low in organic matter (Wilson et al. 1997). Invasive *Hieracium* species can also be found in relatively undisturbed mountain meadows and clearings in forest zones, at elevations ranging from 450 m to over 1,500 m, with the largest infestations found around 1,000 m (Wilson et al. 1997). In natural areas, invasive hawkweeds threaten wildlife habitat, recreation areas, and natural biodiversity. They can quickly out-compete native vegetation (Makepeace 1985) and establish site dominance in as few as five years, reaching a density of 3,400 rosettes/m^2 (Reader 1978). For these reasons, there is ongoing research investigat-

ing potential biological control agents for invasive *Hieracium* species in subgenus *Pilosella*.

Because of their broad ecological amplitudes, and the wide range of habitats they occupy, invasive *Hieracium* species occur in proximity to many native *Hieracium* species. This increases the ecological risk of introducing biological control agents, because closely related, non-target species have a higher probability of being affected by a biological control agent than more distantly related species (Wapshere 1974). In a meta-analysis, Pemberton (2000) found that 40 out of 41 non-target, native species that were attacked by biological control insects were very closely related (congeneric) to the target weeds for which the biological control agents were introduced. This compares with non-target effects found in only one out of 24 biological control programs on weeds without close relatives (no native congeners). Other factors beyond evolutionary relationship, such as similarities in chemistry, habit, or phenology, can also influence non-target effects, but when determining the potential for non-target effects, taxonomic relationships among target and native species should be considered.

Recent molecular research and earlier morphological comparisons have suggested that the presently accepted infrageneric *Hieracium* classification may not reflect evolutionary relationships in the genus. Fehrer et al. (2003), analyzing chloroplast DNA sequences, found that the genus *Andryala* L., which is native to the Mediterranean region, was nested within *Hieracium* subgenus *Pilosella* (with which it shares achene morphology). Sell (1987) noted that subgenera *Hieracium* and *Chionoracium* can be distinguished by the arrangement and morphology of involucral bracts (a graduated series vs. an inner row of long bracts and an outer row of lax, short bracts, respectively). Sell also suggested that, because of overall similar appearance and involucral bracts, subgenus *Chionoracium* be placed within the genus *Crepis* L., though subgenus *Chionoracium* does have chromosome number and pappi that are similar to subgenus *Hieracium*. Synonymies also exist between the two genera (e.g., *H. fendleri* = *C. ambigua* A. Gray). These results and suggestions prompted us to further examine relationships within *Hieracium*.

The goals of this research are to use chloroplast DNA sequences from a majority of the native and naturalized North American *Hieracium* (1) to test the current morphologically-based infrageneric classification and (2) to examine the relationships of native North American *Hieracium* with those non-native species being proposed for classical biological control.

Materials and Methods

The 36 *Hieracium* collections included taxa from subgenera *Pilosella* ($n = 7$), *Hieracium* ($n = 8$), and *Chionoracium* ($n = 21$). Among *Chionoracium* were the majority of species native to North America north of Mexico. Among *Pilosella* were the majority of invasive species naturalized in the United States and Canada. Tissue was collected from field collections or from greenhouse plants at the University of Idaho, Moscow, or was obtained from herbarium specimens (Appendix 1). Outgroup DNA sequences (*Taraxacum* F.H. Wigg., *Crepis*, *Hypochaeris* L., and *Andryala*) were obtained from GenBank. Morphological data were collected from a combination of other treatments (Strother 2006; Beaman 1990) as well as the specimens used in this study. In a few cases our specimens did not have mature seed for morphological analysis.

Genomic DNAs were isolated using a modified CTAB method (Hillis et al. 1996). Multiple nuclear and chloroplast loci used in previous interspecific systematic studies were screened for adequate variation within *Hieracium*, including the ITS regions of nrDNA (Baldwin et al. 1995), the fourth intron of phosphoenolpyruvate carboxylase (*PepC*; Gaskin and Schaal 2002), *trnS-trnG* (Hamilton 1999), *trnG-trnR* (Doyle et al. 1992), *trnT-trnF* (Taberlet et al. 1991), and *petN-psbM* (Lee and Wen 2004). Of all loci, only the *trnT-trnF* and *petN-psbM* regions could be consistently amplified and showed adequate variation for this study. The *trnT-trnF* region has been used to understand species relationships in multiple plant families, including the Asteraceae (e.g. Pelser et al. 2003; McKown et al. 2005). The *petN-psbM* region has been shown to be amplifiable across a range of families and was useful in understanding species relationships with *Panax* (Araliaceae; Lee and Wen 2004). PCR amplification of the chloroplast *trnT - trnL* intergenic spacer utilized the primer pair "a" and "b" of Taberlet et al. (1991). For the *trnL* intron, and the *trnL-trnF* intergenic spacer we used primer pair "c" and "f" of Taberlet et al. (1991). The *trnT-trnL* intergenic spacer, *trnL* intron, and the *trnL-trnF* intergenic spacer are adjacent loci. Amplification of the *petN-psbM* intergenic spacer utilized the primer pair "petN1" and "psbM2R" of Lee and Wen (2004). The thermal cycling programs for these three fragments were as follows: one cycle of 95°C (2 min); 30 cycles of 95°C (1 min), 52°C (1 min), 72°C (2 min); and then 32°C (5 min). A 30 µl reaction containing 3 µl of genomic extract, 1× NH_4 PCR buffer (Bioline USA, Inc., Boston, Mass.), 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM of each primer, and 0.75 units of Biolase DNA Polymerase (Bioline) was performed for each primer pair for each individual. PCR products were purified using QIAquick PCR Purification kit (Qiagen Corp., Valencia, Calif.) prior to sequencing in a CEQ 2000XL automated sequencer (Beckman Coulter, Inc., Fullerton, Calif.) using standard protocols including the LFR-1 method of injection time and voltage. Sequences, deposited in GenBank (Appendix 1), were aligned manually using Se-Al (Rambaut 1996). The aligned matrix was deposited in TreeBase (study number S1607).

Maximum Parsimony (MP) analysis of the data set was performed using PAUP* v. 4.0b8 (Swofford 2000). The heuristic MP search employed 500 random taxon addition sequences and the tree-bisection-reconnection (TBR) branch-swapping algorithm. All characters were weighed equally and insertion/deletion events, no matter what their length, were treated as one mutation, as in Simmons and Ochoterena (2000). Measurements included tree length, branch length, consistency index (CI), retention index (RI), and decay analysis (Bremer 1988). A 5,000 replicate fast stepwise-addition bootstrap analysis was conducted to assess clade support. Concordance of the *trnT-trnF* and the *petN-psbM*

regions was evaluated with the partition-homogeneity test implemented in PAUP*, using 500 random repartitions.

Templeton tests were performed by making alternate topologies in MacClade 4.0 (Maddison and Maddison 2000), using these as constraints in PAUP* heuristic searches, then comparing the original and constrained topologies. The range of resultant P values from the Wilcoxon's signed rank test (Rolhf and Sokal 1995) was used to determine the statistical significance of the difference in length between the original and alternative topological hypotheses (significance at $P < 0.05$ in a one-tailed test).

Maximum Likelihood (ML) analysis was conducted using PHYML, a hill-climbing algorithm that adjusts tree topology and branch lengths simultaneously (Guindon and Gascuel 2003). Proportion of invariable sites was estimated, and six substitution rate categories were used. The starting tree was built with the neighbor-joining method, and the program was set to optimize topology, branch lengths and rate parameters. Branch support was obtained using 500 bootstrap replications. Modeltest 3.7 (Posada and Crandall 1998) was used to select the preferred model of sequence evolution for the combined data set.

Results

The degapped DNA sequence lengths in *Hieracium* varied from 516–570 bp (*trnT-trnL* intergenic spacer); 440–448 bp (*trnL* intron); 338–356 (*trnL-trnF* intergenic spacer); and 561–583 (*petN-psbM* intergenic spacer). The most variable region for *Hieracium* species was the *trnT-trnL* intergenic spacer (6.9% variable sites), followed by *petN-psbM* intergenic spacer (5.9%), *trnL-trnF* intergenic spacer (5.8%), and *trnL* intron (4.0%). Insertion/deletion events within *Hieracium* included the following sizes (in bp): 1, 1, 1, 1, 2, 2, 3, 3, 3, 4, 4, 6, 7, 8, 8, 12, 13, 15, 16, 21, 38, and 48. A poly-A stretch (10–20 bp) from the center of the *trnT-trnL* intergenic spacer was removed from the analysis because the exact number of repeats was difficult to determine due to sequencing error in that specific region. Results of the partition-homogeneity test ($P = 0.18400$) indicated that the data from the *trnT-trnF* and the *petN-psbM* regions reflect the same underlying phylogeny; therefore these datasets were combined for phylogenetic analyses. The combined, aligned sequence length, including outgroups, was 2066 bp, with 238 variable and 97 (4.7%) parsimony informative characters. Excluding outgroups, there were 116 variable and 77 (3.7%) parsimony informative characters. For *Hieracium*, 592 (0.8%) of the 74376 data matrix cells were scored as missing data.

MP analysis on the combined data set recovered one island of 104 trees with length of 287 steps (CI = 0.8990, RI = 0.9375). One of the 104 most parsimonious trees is shown in Fig. 1.

Using the Akaike Information Criterion (AIC: Akaike 1974) model selection framework, Modeltest 3.7 determined that the GTR + G model of evolution was the best fit for the combined data set (AIC = 4512.089). Base frequencies were: A = 0.37, C = 0.15, G = 0.16, T = 0.32; and a gamma shape parameter of 0.585. Substitution rates were A–C = 0.72, A–G = 0.62, A–T = 0.25, C–G = 0.54, C–T = 0.84, G–T = 1.0 (fixed). The ML result (loglk = −4,120.064) is shown in Fig. 2.

Discussion

The chloroplast loci used in this study give ample variation (3.7% parsimony informative characters) to estimate the relationships of subgenera and most species in *Hieracium*. Polytomies exist in the most parsimonious trees, however, and the addition of more loci, or the substitution of more quickly evolving loci, may enable recovery of a completely resolved tree.

The inclusion of *Andryala* within *Hieracium* subgenus *Pilosella* is supported by our results. This had been shown earlier by Fehrer et al. (2003), but has not yet been addressed taxonomically.

The placement of *Chionoracium* within *Crepis*, suggested by Sell (1987), was not supported by our analysis. An earlier molecular study of Cichorieae by Samuel et al. (2003) included *Crepis aurea*, *H. murorum* and *H. bifidum* (both in subgenus *Hieracium*), and did not show a sister taxon relationship for these three taxa. We further tested this by adding all available *Crepis trnT-L* and *trnL-F* intergenic spacer data from GenBank (four species, none of which are native to North America: *C. aurea* AF528396, *C. tectorum* U82026-27, *C. viscidula* AF528397, and *C. crocea* AJ240842). In the MP analysis (phylogeny not shown), these *Crepis* formed a monophyletic group (87% bootstrap) outside of *Hieracium* s.l. *Crepis* is a morphologically variable genus of some 200 species from the northern hemisphere, South America, and South Africa (Mabberley 1993) that has been split by some authors into several genera (Sell 1987). Of future interest would be testing the relationship of *Hieracium* s.l. to *Crepis* that are native to North America.

Subgenus *Chionoracium* forms a strongly supported monophyletic group (MP and ML bootstraps of 94% and 99%, respectively), except for the insertion of *H. canadense*. This species can be found in northern continental U.S.A. and Canada and has previously been considered a synonym of *H. umbellatum* (e.g., Strother 2006) or a subspecies of *H. umbellatum* (Guppy 1978). Our data indicate that the chloroplast of *H. canadense* is set deeply within subgenus *Chionoracium*. To confirm our results for this species, we analyzed another *H. canadense* specimen (*L. Wilson, 16 July 2003*, Idaho), and found the same DNA sequence result. To take *H. canadense* out of subgenus *Chionoracium* and in-

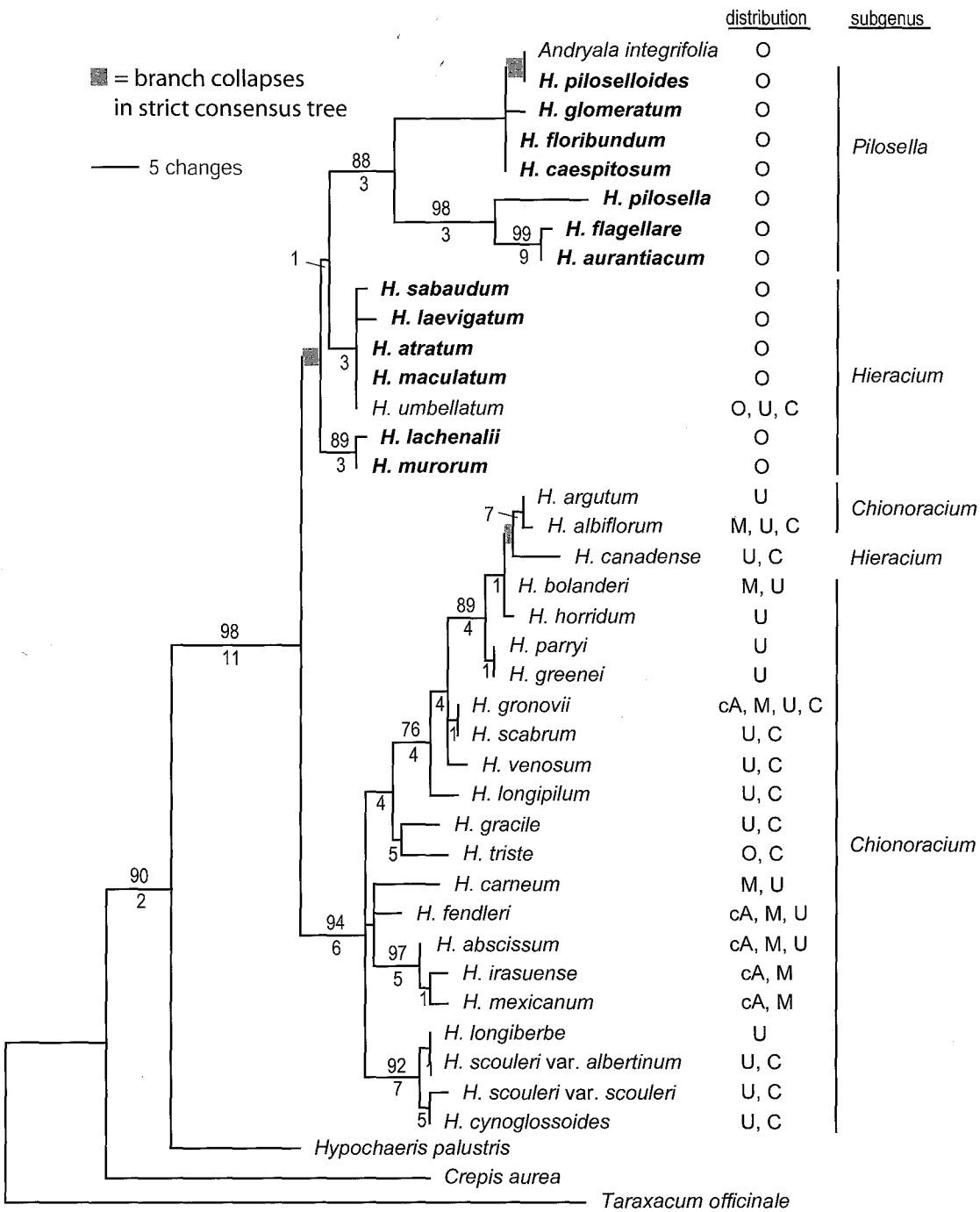

FIG. 1. One of the 104 most parsimonious trees of length 287 steps resulting from the analyses of 40 sequences of *Hieracium* and outgroups based on the combined *trnT-trnF* and *petN-psbM* data set. Bootstrap values (> 75%) are above each branch, with decay indices below. Branches that collapse in the strict consensus tree are indicated by thick grey lines. Native distribution is listed after species name, using the following code: O = Old World; cA = Central America; M = Mexico; U = continental U.S.A.; C = North America north of continental U.S.A. Taxa in bold type are considered invasive in North America (Wilson et al. 2006).

clude it in a monophyletic subgenus *Hieracium* would require a tree topology 310 steps in length (23 steps longer than the unconstrained most parsimonious tree topology). The null hypothesis of these two topologies being statistically similar is rejected ($P < 0.0001$ in a Templeton test). The anomalous placement of this species may be due to recent hybridization or chloroplast capture. There

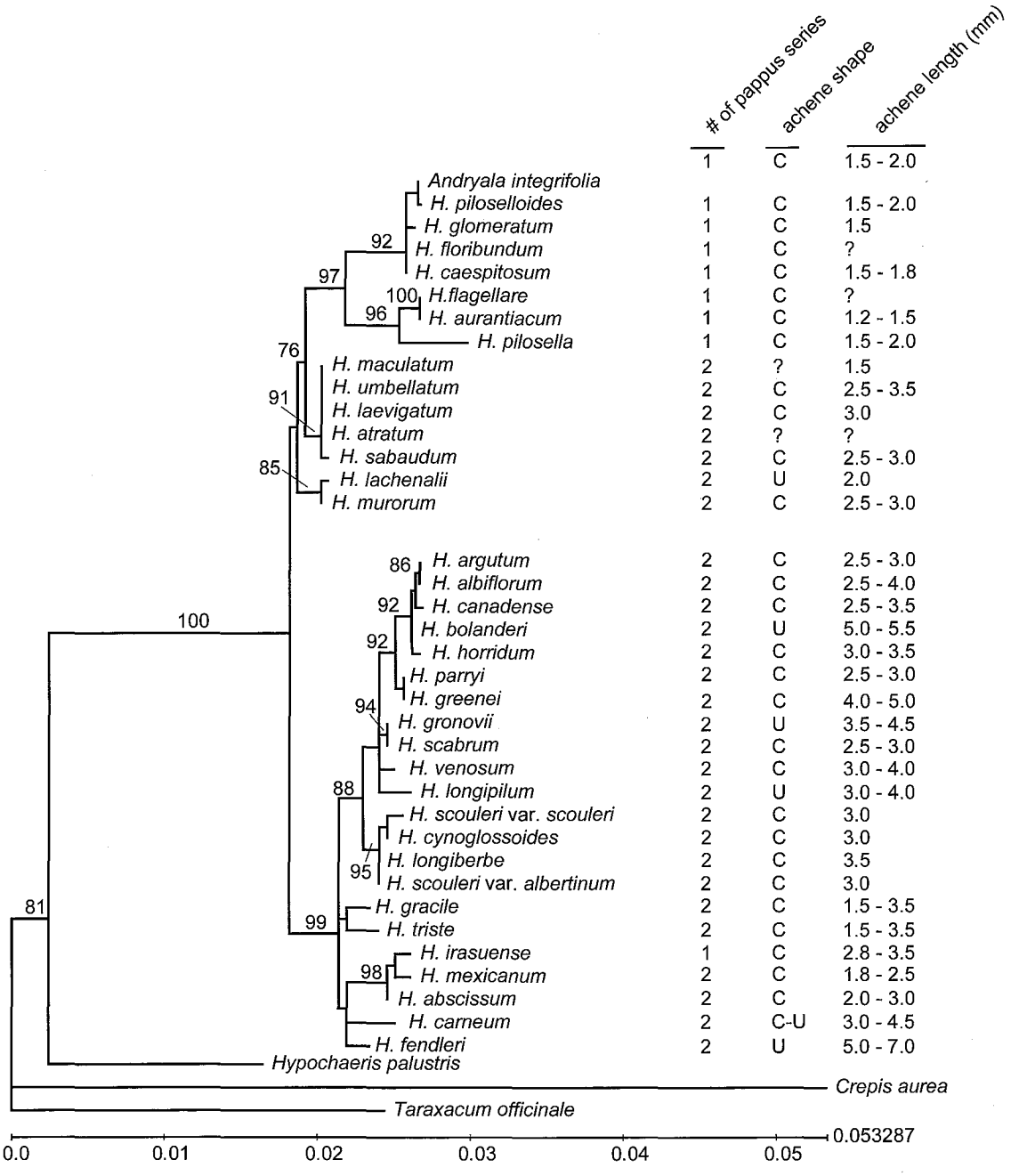

FIG. 2. Maximum likelihood tree of *Hieracium* and outgroups based on the combined *trnT-trnF* and *petN-psbM* data sets. Bootstrap values (> 75%) are above each branch. Number of pappus series, achene shape (C = columnar, U = urceoloate), and achene length (mm) are also presented.

is support for the existence of both of these processes in the genus (Fehrer et al. 2003; Trewick et al. 2004). Multiple hybrid combinations are recognized within the genus, including combinations of species within subgenus *Pilosella*, within subgenus *Chionoracium*, within subgenus *Hieracium*, and between *H. canadense* and *H. scabrum* (USDA 2005). Additionally, *H. canadense* and taxa from subgenus *Chionoracium* can be found in close proximity and intermediate forms do exist (L.M.W. pers. obs.). A more in-depth analysis including multiple samples of *H. canadense* and any in-

termediate forms, along with data from a co-dominant, variable molecular marker, would be useful in clarifying this result.

Placement of various morphological character states on the phylogenies failed to reveal additional useful characters for distinguishing subgenera. For example, achene shape, when mapped on to our phylogeny, does not appear to discriminate between subgenera, as both columnar and urceolate forms are found in naturalized and native species of the New World. Potential for stoloniferous growth continues to separate subgenus *Pilosella* from subgenera *Hieracium* and *Chionoracium*, as does the single vs. double series of pappi (except for *H. irasuense*). Shorter achenes (≤ 2.5 mm), previously used to discriminate subgenus *Pilosella* (Sell 1987), can also be found in subgenera *Hieracium* and *Chionoracium* (e.g., 1.5 mm in *H. maculatum*, *Voss 16384* (MSC); 1.5–3.5 mm in *H. triste*, Strother 2006).

Potential conflicts for classical biological control would be if target species, such as *H. caespitosum* and *H. aurantiacum*, were most closely related to, or embedded within, New World native species clades. The close relationship would indicate a shorter coalescence time period between target and native species, and consequently less time for the evolution of host-specific organisms. Additionally, any gene flow between native and target species since introduction would complicate biological control efforts. The subgenus *Chionoracium* contains sexual, diploid species (Guppy 1978), presenting a potential risk of out-crossing with non-native, invasive species from the subgenera *Hieracium* and *Pilosella*. Both *Hieracium* and *Pilosella* contain many polyploid, apomictic species, suggesting that hybridization with other species would be rare, but the apomixis is facultative (Tutin et al. 1976), and obligately sexual plants exist (Gadella 1987). Interspecific hybrids, at least between species of *Pilosella*, can be common (e.g. Morgan-Richards et al. 2004), but at this time there are no known hybrids between invasive species of subgenus *Pilosella* and subgenus *Chionoracium*.

In conclusion, the current morphologically-based infrageneric classification of *Hieracium* is supported by our molecular analysis, with the exception of *H. canadense* being placed within subgenus *Chionoracium*. Our molecular results also support the earlier morphologically-based conclusion that the species targeted for biological control are in a separate lineage from native North American species.

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APPENDIX 1. *Hieracium* and outgroup taxa collection information, with voucher information and GenBank accession numbers (*trnT-trnL* intergenic spacer, *trnL* intron, *trnL-trnF* intergenic spacer, and *petN - psbM* intergenic spacer, in that order; "-" indicates that no DNA was sequenced for that region). For some plants DNA sequence data was acquired directly from GenBank.

Andryala integrifolia L. — from GenBank (-, AY879119, AY879119, -). *Crepis aurea* (L.) Cass. — from GenBank (-, AF528396, AF528396, -). *H. abscissum* Less. — México, México, *Soule 1407* (MSC) (DQ451191, DQ460846, DQ460882, DQ460918). *H. albiflorum* Hook. — Freezeout Saddle, Idaho, USA, *Wilson s.n.* (ID) (DQ451179, DQ460833, DQ460869, DQ460905). *H. argutum* Nutt. — Monterey County, California, USA, *Thomas s.n.* (ID) (DQ451178, DQ460832, DQ460868, DQ460904). *H. atratum* Fries — Mt. Rainier, Washington, USA, *unknown* (WTU) (DQ451194, DQ460849, DQ460885, DQ460921). *H. aurantiacum* L. — from seed, *Gaskin 4131* (ID) (DQ451177, DQ460831, DQ460867, DQ460903). *H. bolanderi* Gray — Del Norte County, California, USA, *Roche & Korfhage R-2024* (ID) (DQ451187, DQ460842, DQ460878, DQ460914). *H. caespitosum* Dumort. — King County, Washington, USA, *Wilson s.n.* (ID) (DQ451180, DQ460834, DQ460870, DQ460906). *H. canadense* Michx. — Bonner County, Idaho, USA, *Wilson s.n.* (ID) (DQ451195, DQ460850, DQ460886, DQ460922). *H. carneum* Greene — Tucson, Arizona, USA, *Wilson s.n.* (ID) (DQ451189, DQ460844, DQ460880, DQ460916). *H. cynoglossoides* Arv. - Touv. — Sandpoint, Idaho, USA, *Wilson s.n.* (ID) (DQ451203, DQ460858, DQ460894, DQ460930). *H. fendleri* Sch. Bip. — Tucson, Arizona, USA, *Wilson s.n.* (ID) (DQ451190, DQ460845, DQ460881, DQ460917). *H. flagellare* Willd. — North Hampton, New Hampshire, *Littlefield s.n.* (ID) (DQ451176, DQ460830, DQ460866, DQ460902). *H. floribundum* Wimm. & Grab. — British Columbia, Canada, *Wilson s.n.* (ID) (DQ451173, DQ460827, DQ460863, DQ460899). *H. glomeratum* Froel. — British Columbia, Canada, *Wilson s.n* (ID) (DQ451172, DQ460826, DQ460862, DQ460898). *H. gracile* Hook. — Freezeout Saddle, Idaho, USA, *Wilson s.n.* (ID) (DQ451196, DQ460851, DQ460887, DQ460923). *H. greenei* A. Gray — Jackson County, Oregon, USA, *Roche and Korfhage R-2023* (ID) (-, DQ460839, DQ460875, DQ460911). *H. gronovii* L. — Kalamazoo County, Michigan, USA, *Wilson s.n.* (ID) (DQ451181, DQ460835, DQ460871, DQ460907). *H. horridum* Fr. — Mono County, California, USA, *unknown* (WTU) (DQ451197, DQ460852, DQ460888, DQ460924). *H. irasuense* Benth. — Chiapas, México, *Villasenor et al. s.n.* (MSC) (DQ451192, DQ460847, DQ460883, DQ460919). *H. lachenalii* C.C. Gmel. — Kalamazoo County, Michigan, USA, *Wilson s.n.* (ID) (DQ451198, DQ460853, DQ460889, DQ460925). *H. laevigatum* Willd. — King County, Washington, USA, *Wilson s.n.* (ID) (DQ451186, DQ460841, DQ460877, DQ460913). *H. longiberbe* Howell — Oneonta Gorge, Oregon, USA, *Wilson s.n* (ID) (DQ451199, DQ460854, DQ460890, DQ460926). *H. longipilum* Torr. ex Hook. — Adams County, Wisconsin, USA, *Cochrane 14234* (ID) (DQ451184, DQ460838, DQ460874, DQ460910). *H. maculatum* Schrank — Mackinac County, Michigan, USA, *Voss 16384* (MSC) (DQ451200, DQ460855, DQ460891, DQ460927). *H. mexicanum* Less. — México, México, *Soule 1393*(MSC) (DQ451193, DQ460848, DQ460884,

DQ460920). *H. murorum* L. — Pierce County, Washington, USA, *Wilson s.n.* (WTU) (DQ451201, DQ460856, DQ460892, DQ460928). *H. parryi* Zahn — Jackson County, Oregon, USA, *Roche & Korfhage R-2025*(ID) (DQ451183, DQ460837, DQ460873, DQ460909). *H. piloselloides* Vill. — Glacier National Park, Montana, USA, *Wilson s.n.* (ID) (DQ451174, DQ460828, DQ460864, DQ460900). *H. sabaudum* L. — King County, Washington, USA, *Wilson s.n.* (ID) (DQ451185, DQ460840, DQ460876, DQ460912). *H. scabrum* Michx. — Kalamazoo County, Michigan, USA, *Higman 910* (WTU) (DQ451182, DQ460836, DQ460872, DQ460908). *H. scouleri* Hook. var. *albertinum* (Farr) G.W. Douglas & G.A. Allen — Wasco County, Oregon, USA, *Wilson s.n.* (ID) (DQ451204, DQ460859, DQ460895, DQ460931). *H. scouleri* Hook. var. *scouleri* — Idaho County, Idaho, USA, *unknown* (ID) (DQ451202, DQ460857, DQ460893, DQ460929). *H. triste* Willd. ex Spreng. — Skagway, Alaska, USA, *unknown* (WTU) (DQ451205, DQ460860, DQ460896, DQ460932). *H. umbellatum* L. — Skamania County, Washington, USA, *Wilson s.n.* (ID) (DQ451206, DQ460861, DQ460897, DQ460933). *H. venosum* L. — North Carolina Botanical Garden, USA, *J. Randall s.n.* (ID) (DQ451188, DQ460843, DQ460879, DQ460915). *Hypochaeris palustris* (Phil.) Reiche — from GenBank (-, AY148097, AY148097, -). *Taraxacum officinale* F.H. Wigg. — Richland County, Montana, USA, *Gaskin 5040* (ID) (EF015610, EF015611, EF015611, DQ450882).