Species delimitation of a moss clade in a global hotspot for bryophyte diversity

Resolving ambiguities in a clade of *Homalothecium*

Larke E. Reeber

H. megaptilum (bryophyteportal.org)

H. nuttallii (fenzenmosses.com)

Homalothecium characteristics

- Pleurocarpous moss
- Yellow-green, shiny
- On trees, rocks, rocky soil
- Long plicate leaves w/o awns
- Branches curled when dry, spreading when wet

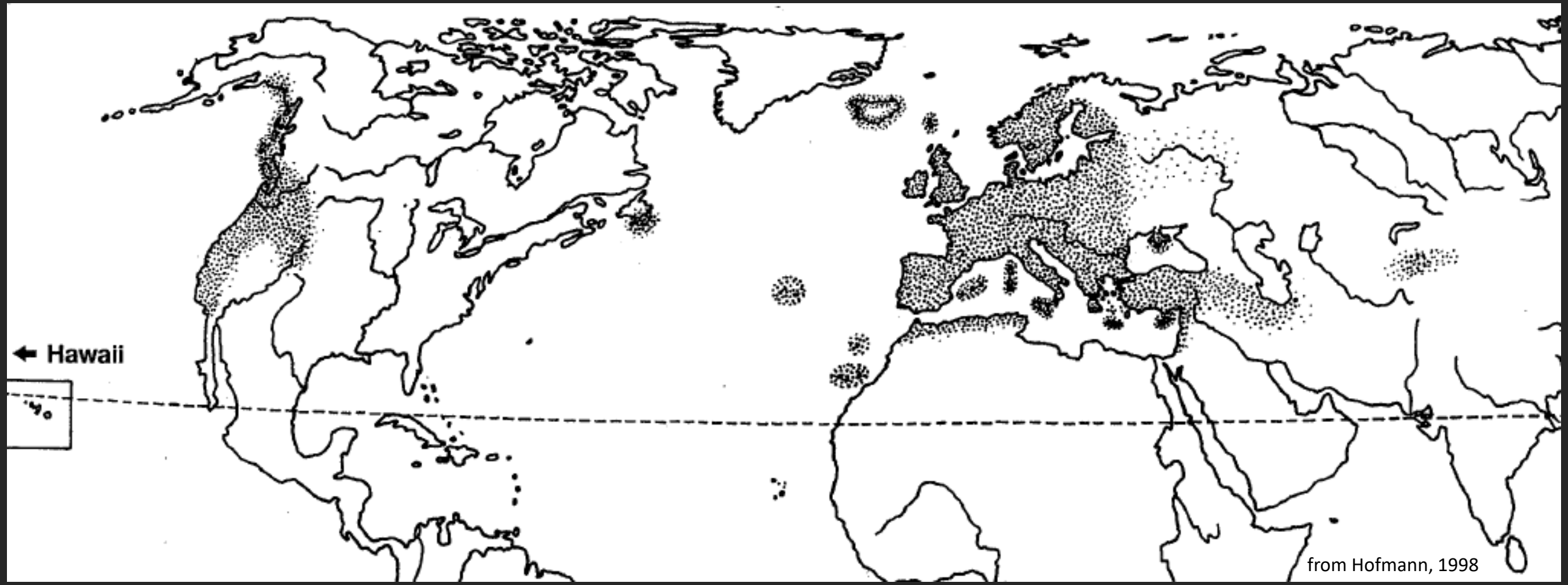
H. pinnatifidum (bryophyteportal.org)

H. fulgescens
(*blogs.ubc*.ca)

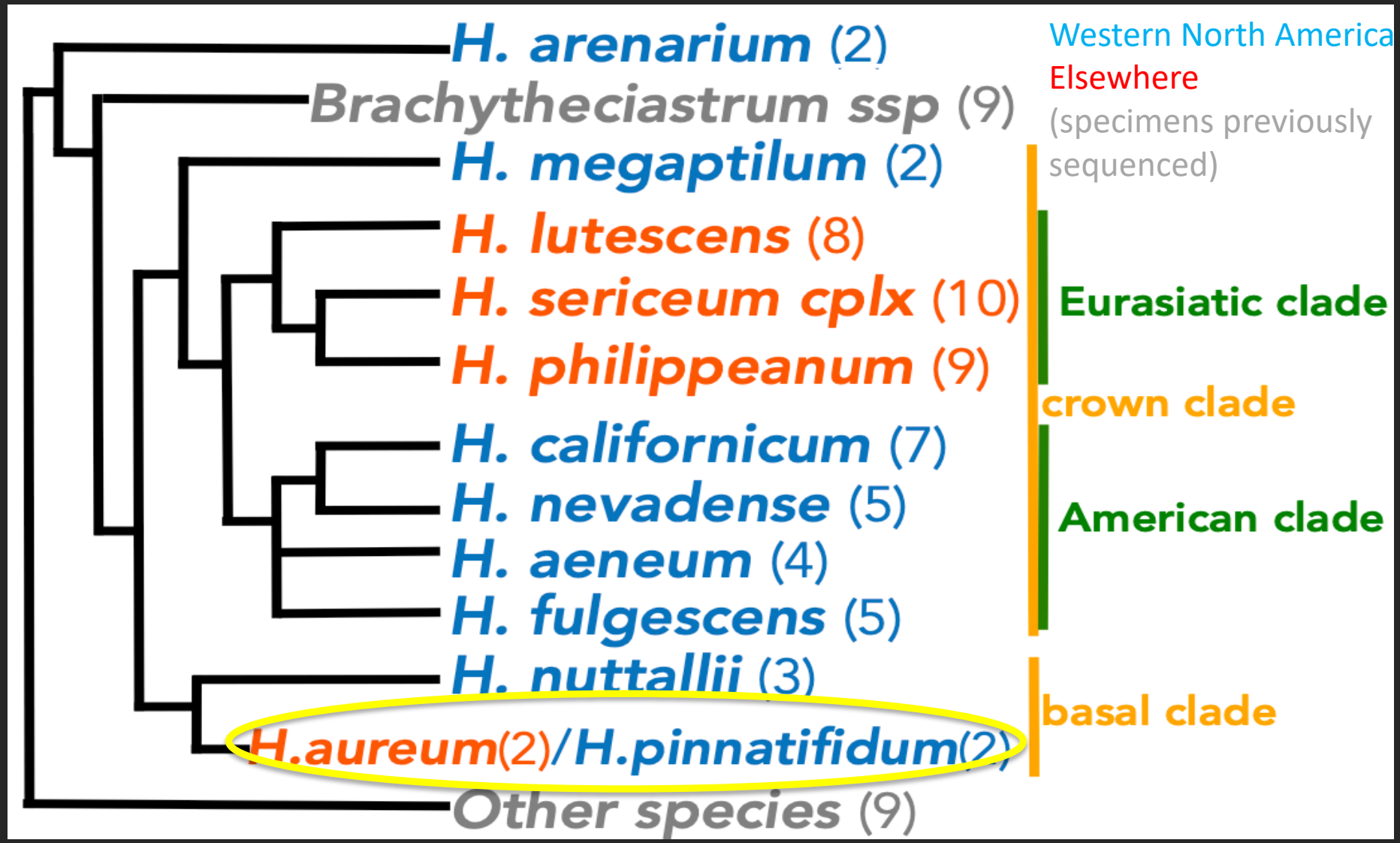
H. sericeum
(eol.com)

H. aureum
(portugal.inaturalist.org)

Homalothecium--Distribution

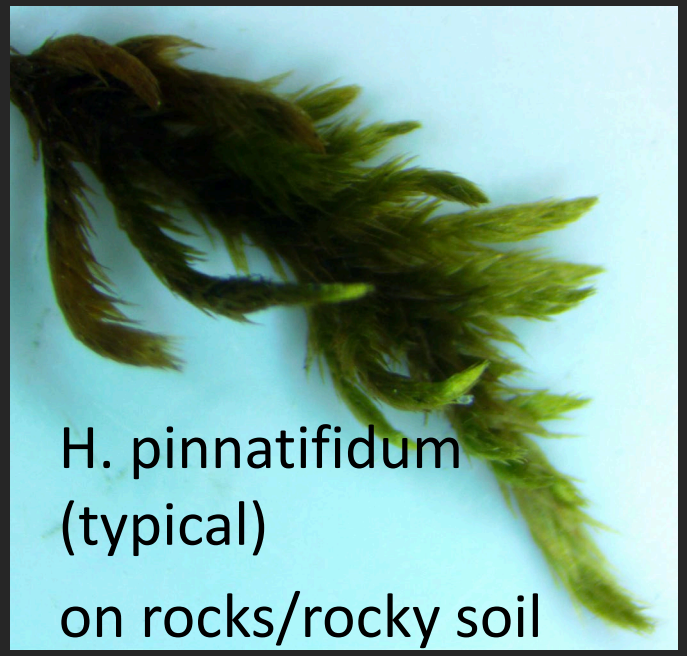


Homalothecium
Current Phylogeny



Phylogeny based on Hedenäs, et. al (2008); Hedenäs, Huttunen, Shevock, & Norris (2009); Hedenäs, et. al (2014); Sergio, et. al (2016); Huttunen, Hedenäs, Ignatov (2018)

Why this clade?



H. pinnatifidum
(typical)

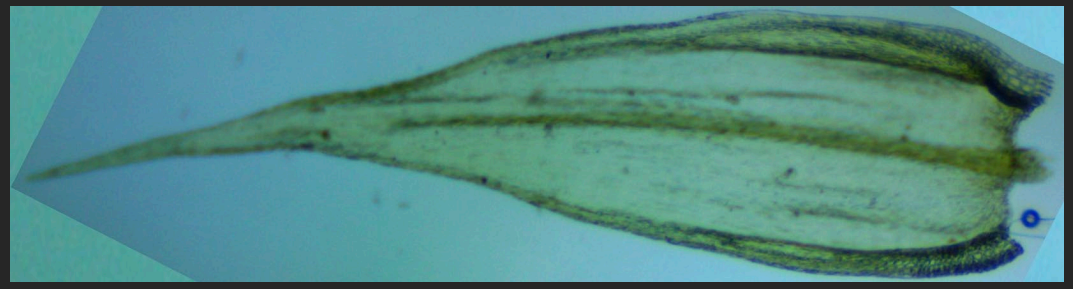
on rocks/rocky soil

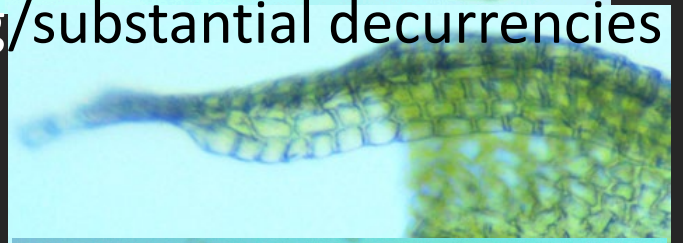
little/no decurrencies

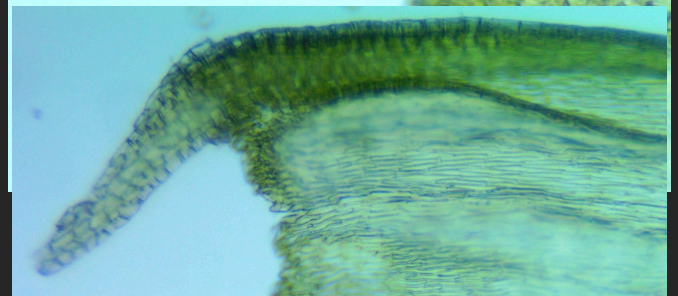
H. pinnatifidum
(atypical)

on leaf litter

long/substantial decurrencies







Also. . .
H. aureum/H. pinnatifidum

- Differing opinions
- Key diagnostic feature descriptions variable
- Number of samples in original molecular study small

Questions

- Is there more than one species within *H. pinnatifidum*?
- What is the *H. aureum* and *H. pinnatifidum* relationship?

Homalothecium--Hypotheses

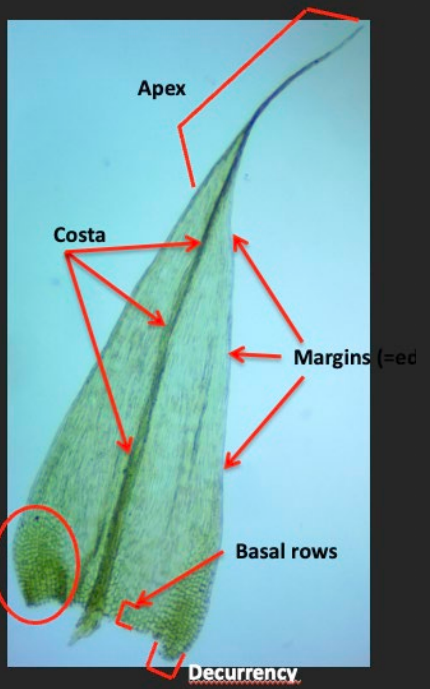
- Null Hypothesis: There is one species in this clade—*H. aureum.*
- Alternate Hypothesis: There are more than one species in this clade.

Methods Overview

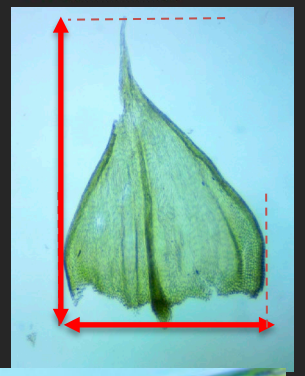
1. Look at LOTS of herbarium specimens of *H. pinnatifidum*.
   Define and classify into morphotypes.
2. Select samples for sequencing:
   Specimens of each *H. pinnatifidum* morphotype.
   Specimens of *H. aureum*
3. Extract DNA and sequence for following gene regions:
   ITS1-5.8-ITS2, *atpB-rbcL* and *rpl16* gene regions.
4. Analyze results, incorporating sequences from previous studies.

Morphotyping

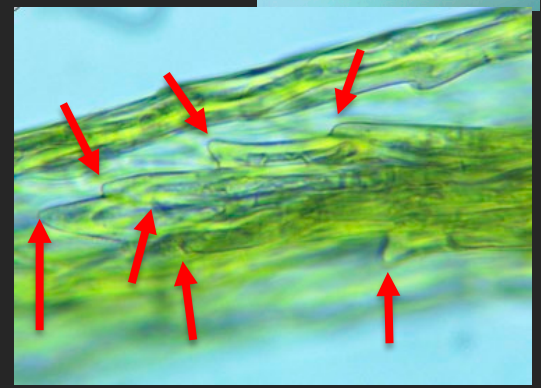
- Tracked 11 packet characteristics, 22 gametophytic characters and, when available, 16 sporophytic characters



➔Identified two unique morphotypes that did not fit current *H. pinnatifidum* taxonomic descriptions.



➔ Identified several other morphotypes that were intermediate between *H. pinnatifidum* and another *Homalothecium* species.

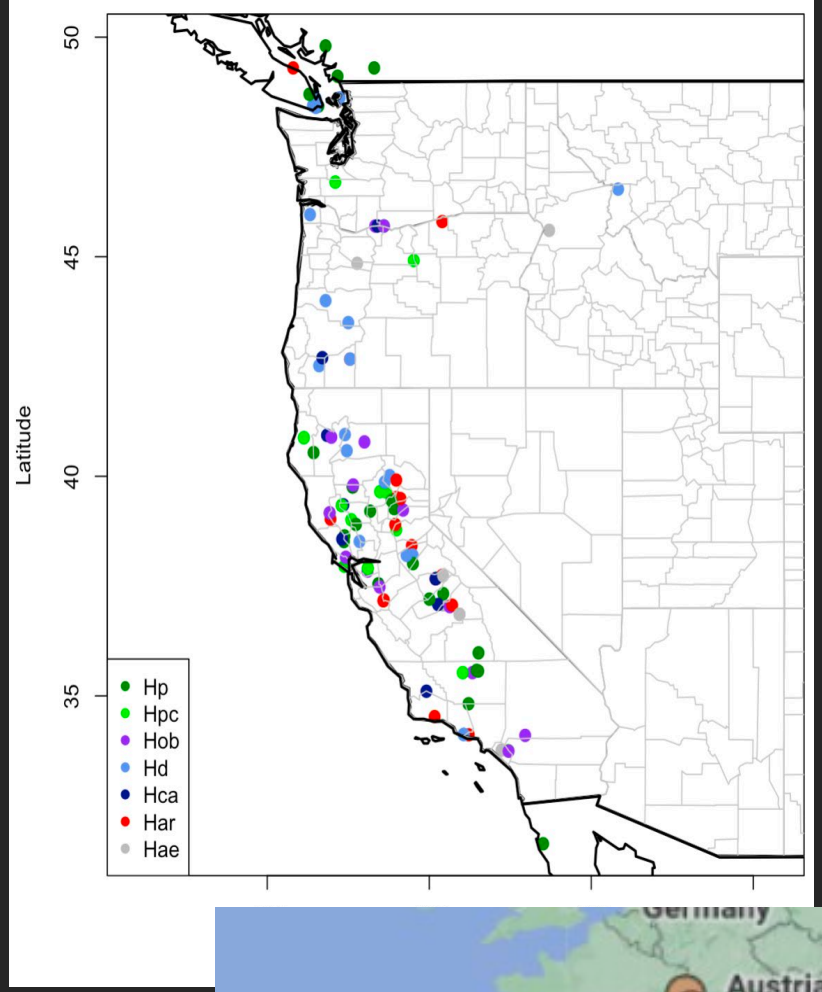


The numbers

- 446 specimens examined at packet level
- 211 examined microscopically; classified into morphotypes
- 72 specimens had DNA extracted and sequenced
  - 64 were *H. pinnatifidum:*
    - sampled each morphotype across geographic range
    - 12 paired specimens (same collector/ location/date, different morphotype)
  - 7 were *H. aureum*: across geographic range;
  - 1 was *H. nuttallii*

Study samples

- REDO WNA MAP;
- ADD CORRECT *H. AUREUM* MAP



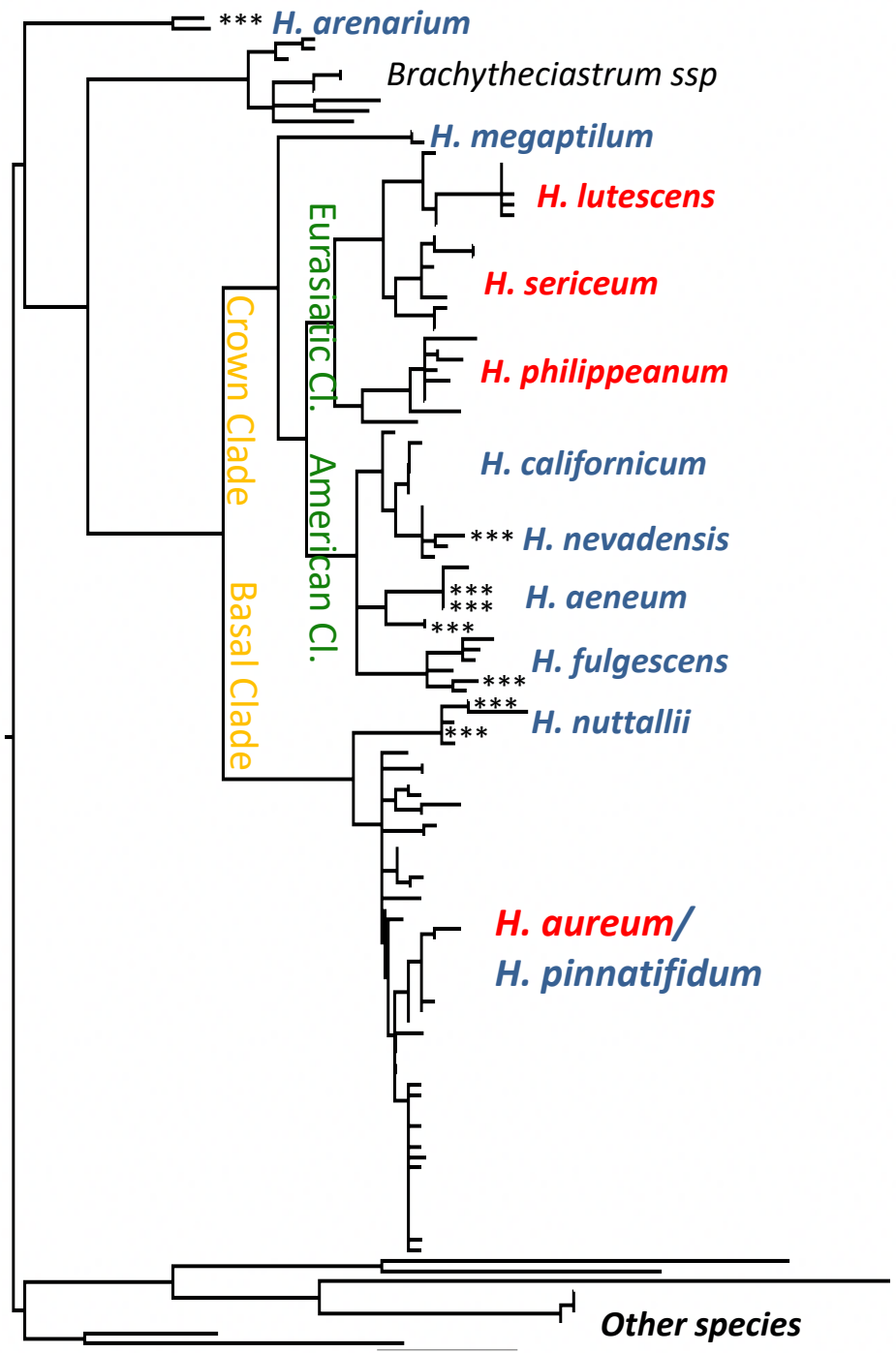
from Hofmann, 1998

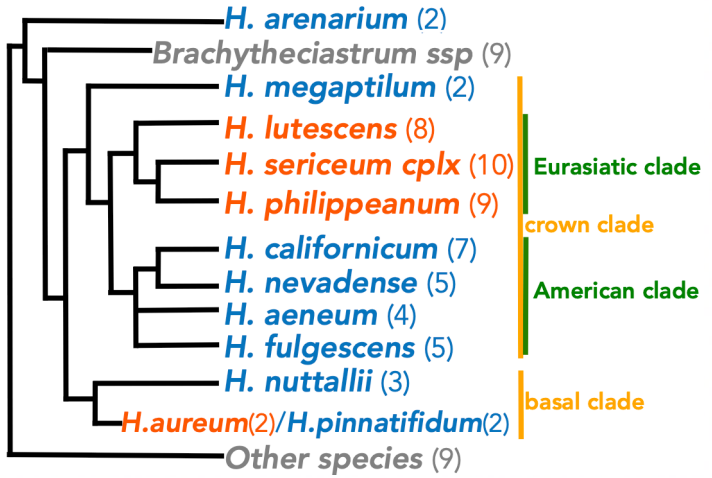
Genetic Analysis--Methodology

- All four genes concatenated—only specimens with >1 viable gene sequence
- Indels encoded per simple encoding method of Simons and Ochoterena (2000)
- Tree generated by RaxmL (ML, 100 bootstraps) with separate rate partitions for chloroplast (rpl16, atpB_rbcL) (GTR) ,nuclear genes (ITS1, ITS2)(GTR), and indels (BIN)
- FigTree used to visualize the trees

Gene region	rpl16	atpB-rbcL	ITS1	ITS2
Viable sequences	56	59	43	45
# of BP	730-779	399-713	237-405	421-437
Trimmed size				
Non-unique BP				
Phylogenetically useful BP				
Phylogenetically useful indels				

Add in trimmed size used, # of non-unique bp, # of phylogenetically useful bp, # of phylogenetically useful indels (should I do this, are these right descriptions, what should really be included?)





All sequences with >1 viable gene sequence

➔Recovered nearly identical overall phylogeny as previous papers, including basal clade with *H. nuttallii* sister to a *H. aureum/H. pinnatifidum* clade

➔Some (8), but not all, of the ”intermediate” morphotypes specimens are a better genetic match to a different *Homalothecium* species

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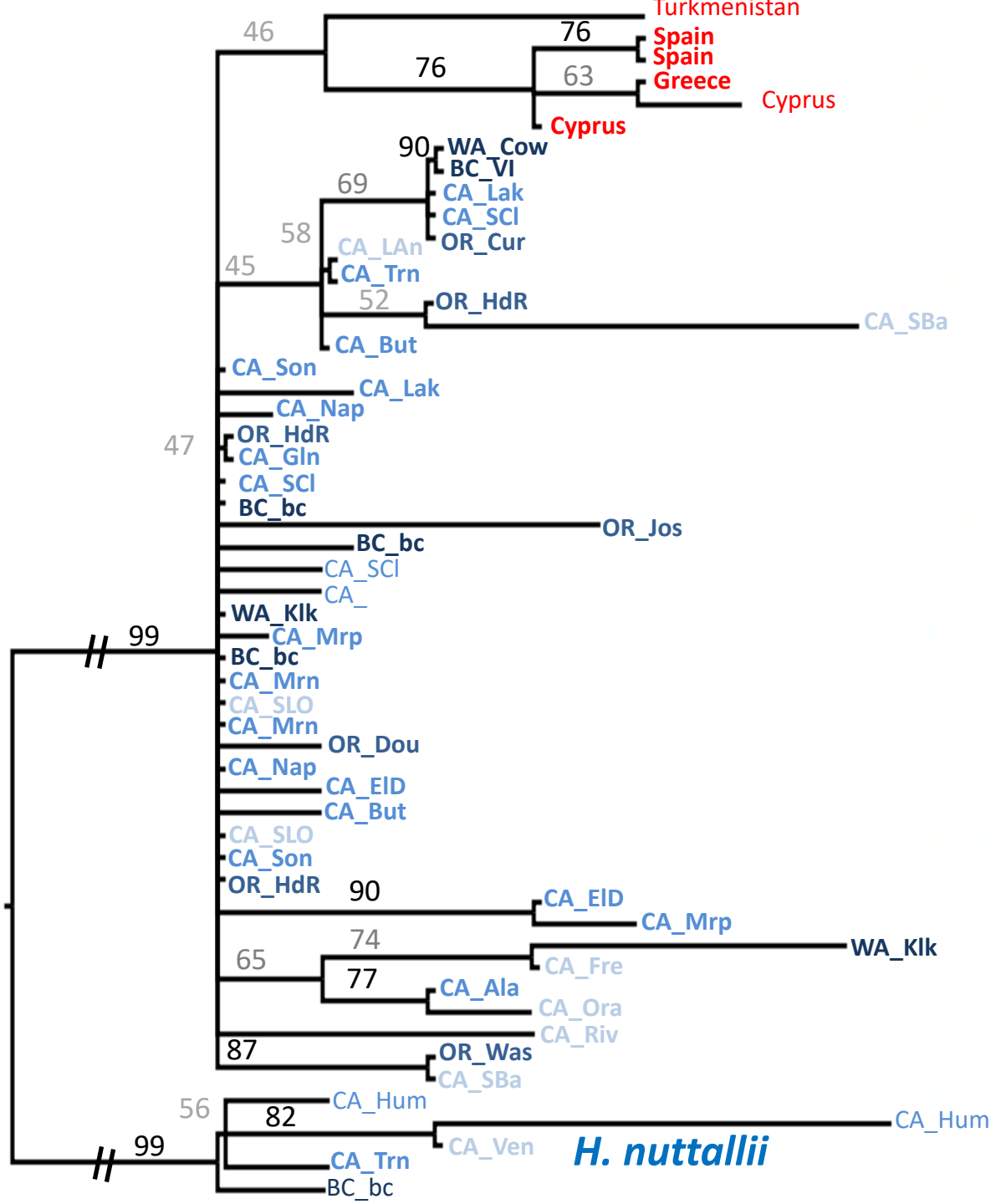


Basal Clade only

British Columbia, Washington, Montana
Oregon, Idaho
California—northern
California—southern
>75
60-75
45-59

➔*H. nuttallii* well supported monophyletic clade

➔Eurasiatic sequences (ie; *H. aureum*) form a very weak monophyletic clade

➔Western North American sequences (ie; *H. pinnatifidum*) do not form a clear monophyletic clade separate from Eurasiatic sequences

Implications

- No clade of any morphotype within *H. pinnatifidum*
- *H. aureum* in a clade but incompletely differentiated from *H. pinnatifidum*

Acknowledgements

- Funding:
  - Northern California Botanical Society
  - San Jose University Biological Sciences Dept.
  - Anonymous funder
- Advising:
  - Dr. Benjamin Carter
  - Dr. Tracy Misiewicz
  - Dr. Susan Lambrecht
  - James Shevock
- Herbaria Specimens from: CASC, UBC, SJSU, MO, CHSC
- General support: Jack Fendell, John McLaughlin, Charlotte Bony, Charlotte Miranda, Andy Frank

Add in herbaria logos

Basal clade only

Homalothecium
Questions??

Homalothecium
Questions??

Homalothecium
Backup

Preliminary
Results
--Haplo Networks

Methodology

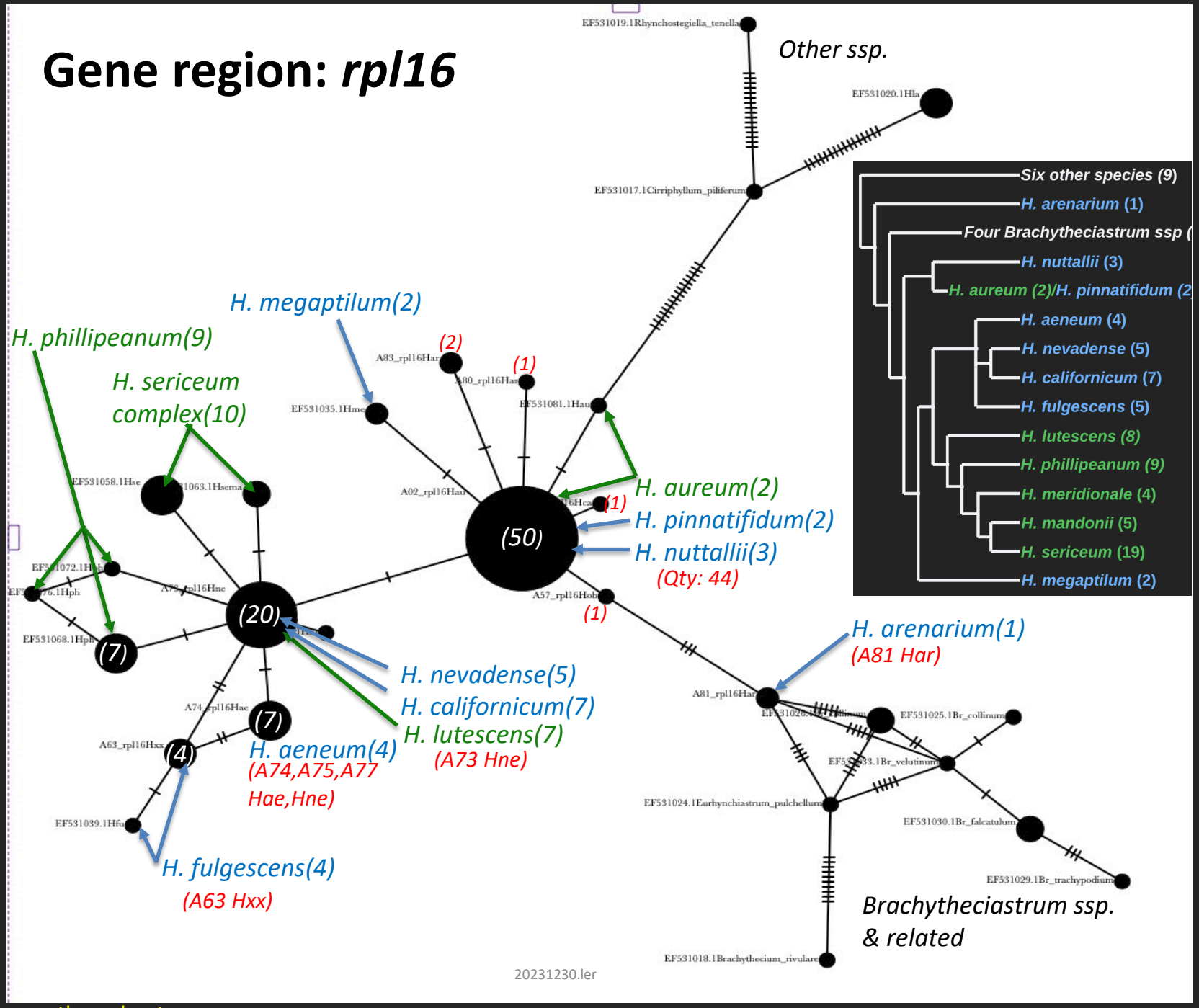
- Genious 2023.2.1: Build contigs, build and trim alignments, create Nexus output
- PopArt (2023): Build Haplonetworks

- Consensus sequence built from forward and reverse sequence of each specimen
  - Consensus sequence used for alignment (default settings)
  - If no consensus sequence, used higher quality of forward/reverse sequences
- Alignment building (for each gene) (default settings)
  - Start with consensus or best quality sequence for each specimen
  - After initial alignment, removed grossly misaligned sequences
  - Remove very poor quality/very incomplete sequences
  - Trimmed sequence ends so that most sequences were complete end to end
  - Exported as Nexus alignment
- Built Haplo network (PopArt)
  - Import Nexus alignment
  - Built Haplonetwork

Network notes for following pages

- Size of dot is number of specimens that had identical sequences
- Blue/Green names are GenBank accession species in that dot
- Red are *H. pinnatifidum* and *H. aureum* specimens that I sequenced that are in that dot(s) (Axx=specific specimen number, Hxx=morphotype)
- Number in parenthesis are quantities (in dot, of GB accessions, of specimens)
- Networks created in Popart
  - Hashmarks are number of nucleotide differences
  - Indels are not included as a difference
  - Nucleotides with ambiguity in any specimen are excluded in determination
- GenBank accessions: A few sequences were missing from GenBank (i.e.; the accession numbers of some phylogenetic tips were not listed in the paper); Affects *H. sericeum* complex
- "H. sericeum complex" includes *H. sericeum s.str.*, *H. mandonii*, and *H. meriodionales*

Gene region: *rpl16*

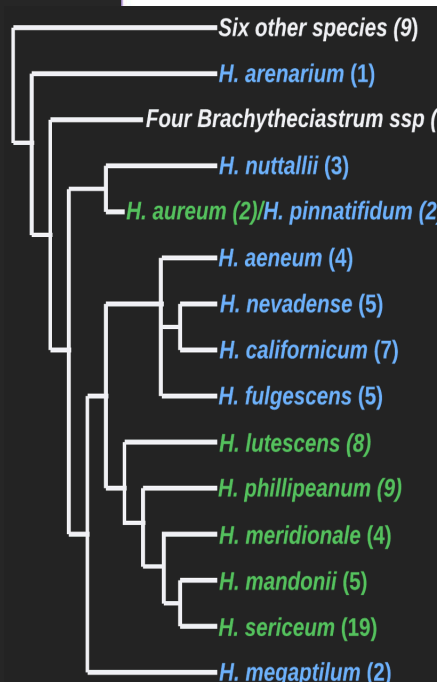


Fix mini-phylogeny throughout

Implications

- I still need to improve my identification skills.
  - A63, A73, A74, A75, A77, A81 probably misidentified
- Only differentiation between *H. nuttallii* and *H. pinnatifidum/H. aureum* (an indel) is not picked up by PopArt
- *None of the morphotypes are differentiated with rpl 16*
- ➔*No surprises*

atpB-rbcL



Other ssp

EF530951.1_Rhynchostegiella_tenella

EF530949.1Cirriphyllum_piliferum

EF530948.1Floribundaria_floribunda

EF530952.1Hla

Brachytheciastrum ssp

EF530950.1_Brachythecium_rivulare

EF530960.1B_collinum

EF530957.1B_collinum

EF530962.1B_trachypodium

EF530956.1Eurhynchiastrum_pulchellum

EF530966.1Har

A81atpB_rbcLHar

H. arenarium

A81Har

A41atpB_rbcLHca

A4xHca

H. aureum(2)

H. pinnatifidum(2)

A1xHpc(3),A2xHre(2),
A3xHd(2),A4xHca(3),
A6xHnu(1),A6xHxx(1),
A7xHae(2),A87Har(1)

A06atpB_rbcLHau

A30xHau

A12atpB_rbcLHpc

(18)

A16atpB_rbcLHpc

A1xHpc

EF530969.1Hau

A36atpB_rbcLHd

A3xHd

H. nuttallii(3)

A0xHau(4),A1xHpc(3),
A3xHd(2),A4xHca(2),
A5xHob(1),A6xHnu(1),
A7xHae(2),A8xHar(2)

A02atpB_rbcLHau

(20)

A5xHob(2),
A7xHae(1)
A8xHar(1)

A50atpB_rbcLHob

(4)

A73atpB_rbcLHne

(6)

H. nevadense(5)

A73Hne

A11atpB_rbcLHpc

(10)

A1xHpc(4),A3xHd(2),
A4xHca(1),A5xHob(2),
A8xHar(2)

A39atpB_rbcLHd

A3xHd

H. sericeum complex(10)

H. lutescens(8)

H. californicum(7)

H.phillipeanum(1)

A04Hau

A04atpB_rbcLHau

(27)

EF531015.1Hme

H. megaptilum(2)

A63atpB_rbcLHxx

(6)

H. fulgescens(5)

A63Hxx

A77atpB_rbcLHne

H. aeneum(1)

A77Hne

A74atpB_rbcLHae

(6)

H. aeneum(3)

H. phillipeanum(1)

A74Hae,A75Hne

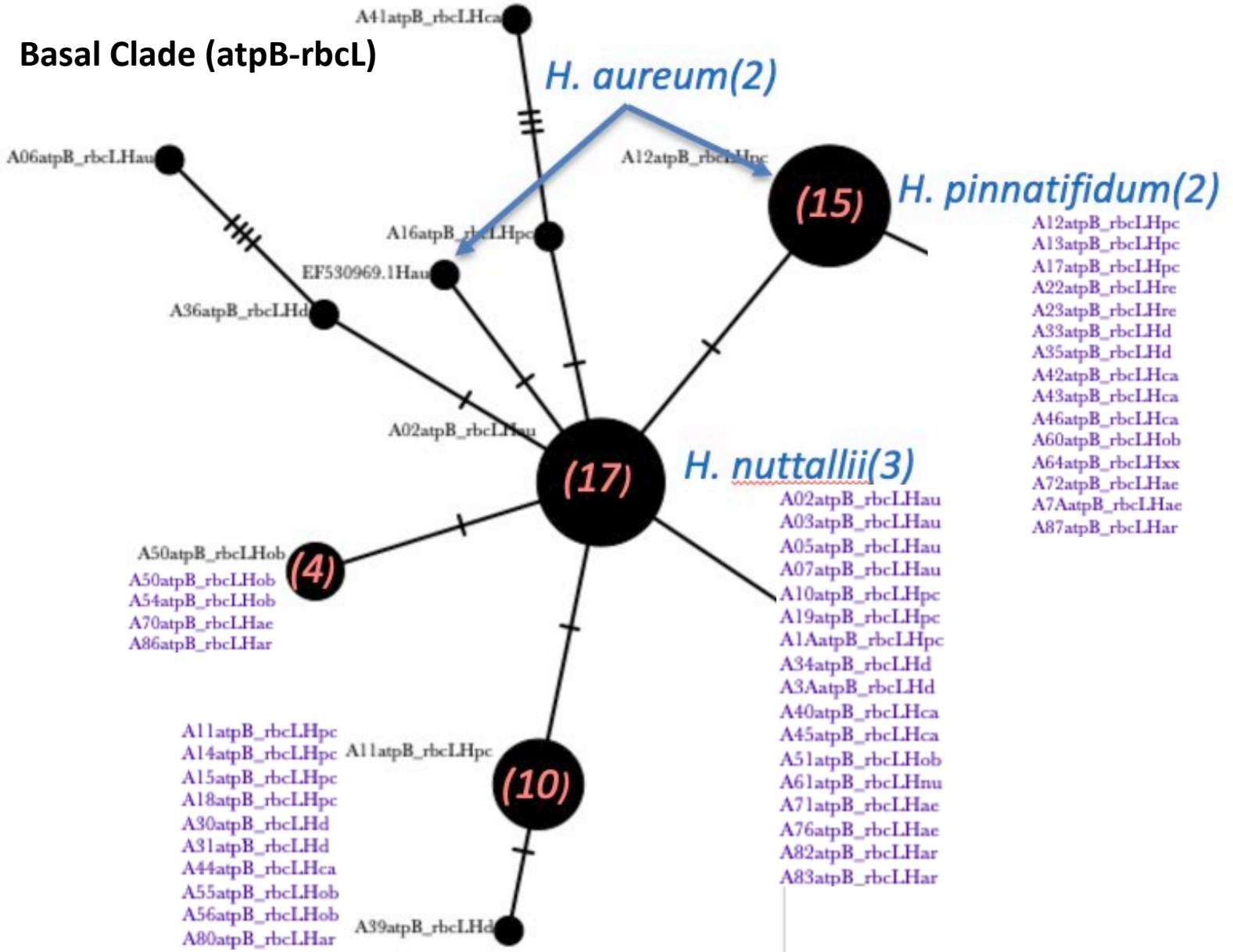
EF531002.1Hph

(6)

EF530995.1Hph

H. phillipeanum(7)

Basal Clade (atpB-rbcL)



Implications

- A63, A73, A74, A75, A77, A81 misidentified
- atpB_rbcL does differentiate between *H. pinnatifidum/ H. aureum* and *H. nuttallii*
- None of the morphotypes correspond to the network nodes
- But something may be going on. . .

Investigation

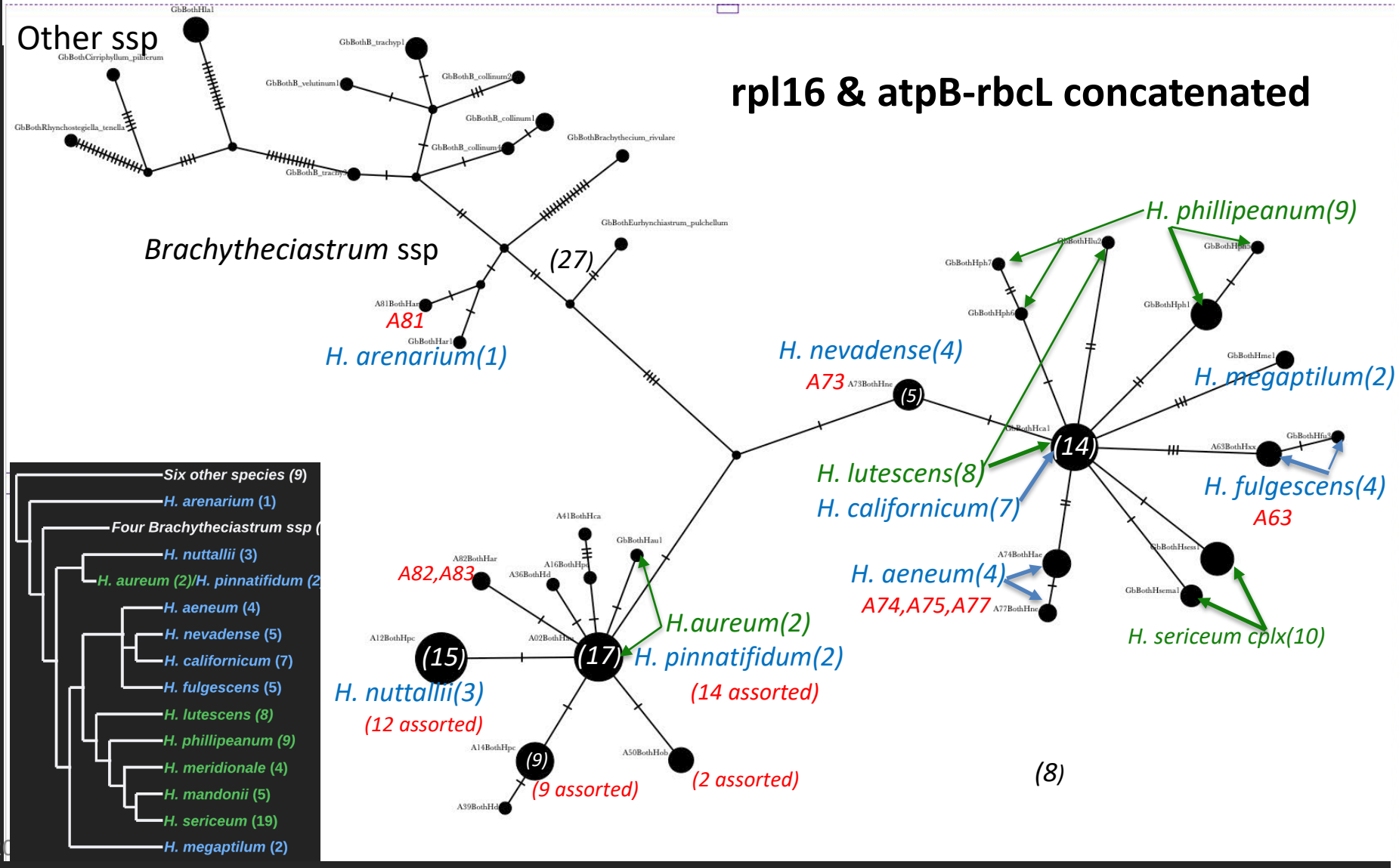
→A microscopic exam of samples within each group yielded no identifiable distinguishing feature that matched groupings.

Investigation

→Two nucleotides (positions 282/386) drove the groupings. Simple chi-squared test indicated these results may not be significant. [Did I do this right?]

Combination	All	GT	GC	AT	p-value
Sequences	59	29	17	13	
Proportion	1.00	0.491525	0.288136	0.220339	
Hpi (Prop.)	47	18 (0.383)	16 (0.340)	13 (0.277)	0.3250
Hau (Prop.)	7	6 (0.857)	1 (0.143)	0 (0.0)	0.1381
Hnu (Prop.)	5	5 (1.00)	0 (0.0)	0 (0.0)	0.0753

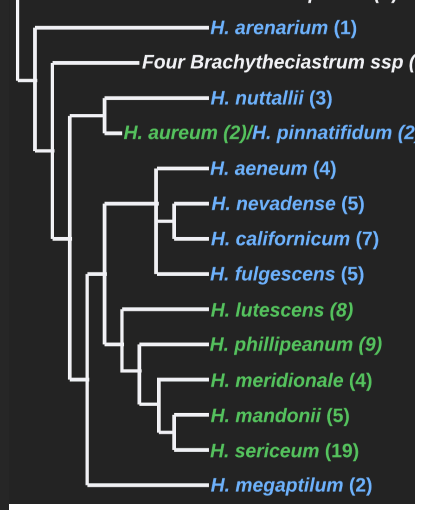
The chi-square approximations may be incorrect because of the small sample sizes for *H. aureum* and *H. nuttallii*, but the preliminary conclusion is that these differences are not statistically significant.


Other ssp
GbBothHla1
GbBothCirriphyllum_pilifera
GbBothB_trachyp1
GbBothB_velutinum1
GbBothB_collinum2
GbBothRhynchostegiella_tenella
GbBothB_collinum1
GbBothB_collinum4
GbBothBrachythecium_rivulare
GbBothB_trachy
GbBothEurhynchiastrum_pulchellum
Brachytheciastrum ssp
(27)
A81BothHar
A81
GbBothHar1
H. arenarium(1)
rpl16 & atpB-rbcL concatenated
H. phillipeanum(9)
GbBothHph7
GbBothHll2
GbBothHph1
GbBothHme1
H. megaptilum(2)
H. nevadense(4)
A73
A73BothHne
(5)
GbBothHca1
(14)
A63BothHxx
H. fulgescens(4)
A63
H. lutescens(8)
H. californicum(7)
A74BothHac
H. aeneum(4)
A74,A75,A77
A77BothHne
GbBothHsess1
GbBothHsema1
H. sericeum cplx(10)
A41BothHca
GbBothHau1
A82BothHar
A82,A83
A36BothHd
A16BothHp
H.aureum(2)
H. pinnatifidum(2)
(14 assorted)
A12BothHpc
(15)
A02BothHa
(17)
H. nuttallii(3)
(12 assorted)
A14BothHpc
(9)
(9 assorted)
A50BothHob
(2 assorted)
A39BothHd
(8)
Six other species (9)
H. arenarium (1)
Four Brachytheciastrum ssp (
H. nuttallii (3)
H. aureum (2)/H. pinnatifidum (2
H. aeneum (4)
H. nevadense (5)
H. californicum (7)
H. fulgescens (5)
H. lutescens (8)
H. phillipeanum (9)
H. meridionale (4)
H. mandonii (5)
H. sericeum (19)
H. megaptilum (2)

Implications

- *H. aureum/H. pinnatifidum/H. nuttallii* nodes are essentially unchanged

➔Likely due to the PopArt not using any indels or nucleotides with any ambiguity codes


H. arenarium (1)
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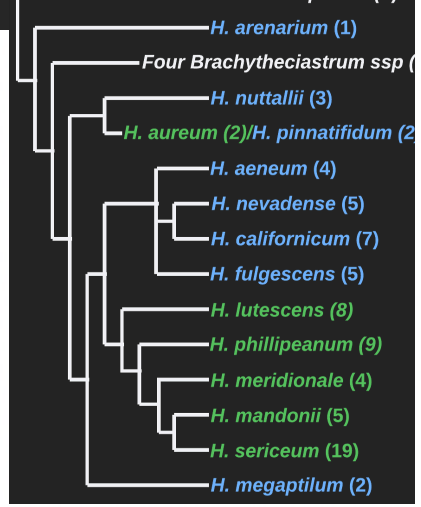
H. nuttallii (3)
H. aureum (2)/H. pinnatifidum (2
H. aeneum (4)
H. nevadense (5)
H. californicum (7)
H. fulgescens (5)
H. lutescens (8)
H. phillipeanum (9)
H. meridionale (4)
H. mandonii (5)
H. sericeum (19)
H. megaptilum (2)

ITS1

A34Hd_ITS1
A64Hxx_ITS1
H. pinnatifidum(2)
(23 assorted)
A30Hd_ITS1
A42Hca_ITS1
(25)
EF617569.1Hme
H. megaptilum(2)
(2)
A70Hae_ITS1
A03Hau_ITS1
(11)
EF617587.1Hph
EF617586.1Hph
(8)
(2)
A50Hob_ITS1
EF617581.1Hph
A61Hnu_ITS1
H.aureum(2)
A02,A03,A05,A07
(5 assorted)
H. phillipeanum(7)
H. sericeum cplx(1)
H. nuttallii(2)
A51,A61
(4)
DQ336906.1Hsess
EF558541.1Hlu
(15)
EF617571.1Hae
Brachytheciastrum ssp
EF617552.1VB_collinum
DQ200071.1B_collinum
(6)
(21)
H. lutescens(8)
H. sericeum cplx(7)
H. aeneum(4)
H.californicum(7)
H.fulgescens(5)
H.nevadense(4)
A63
DQ200074.1B_trachypodium
EF617575.1Hne
EF558537.1Floribundaria_floribunda
DQ200076.1Brachythecium_rivulare
EF558539.1Hla
EF617554.1Har
DQ200081.1Cirriphyllum_piliferum
Other ssp
DQ200107.1Rhynchostegiella_tenella
DQ200084.1Eurhynchiastrum_pulchellum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H. nuttallii (3)
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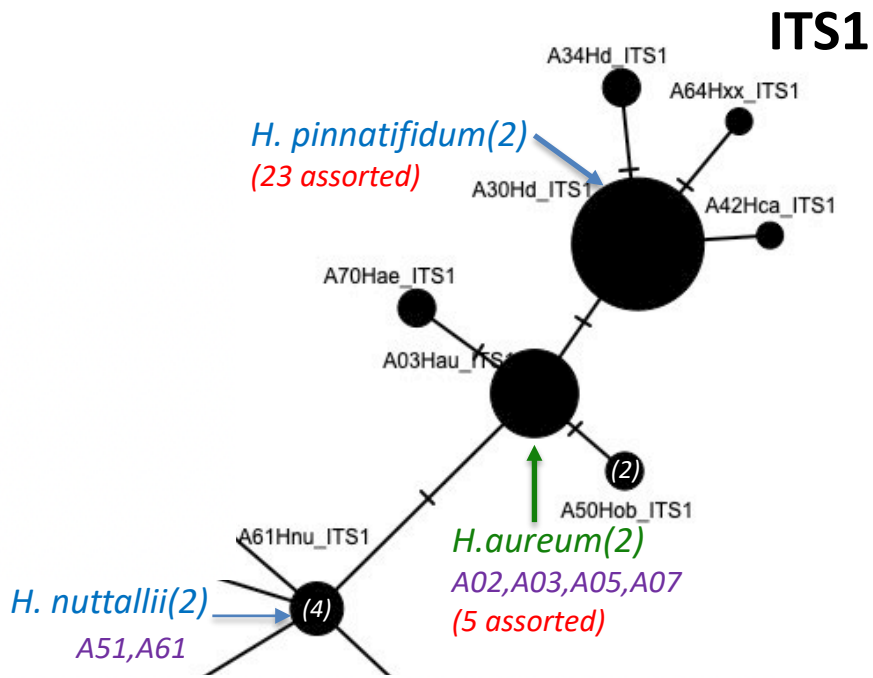
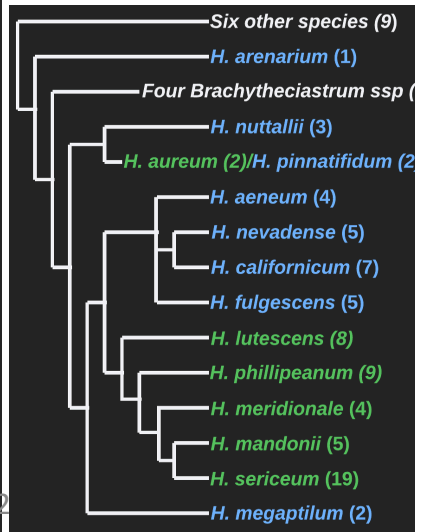

ITS1
A34Hd_ITS1
A64Hxx_ITS1
H. pinnatifidum(2)
(23 assorted)
A30Hd_ITS1
A42Hca_ITS1
A70Hae_ITS1
A03Hau_ITS
(2)
A50Hob_ITS1
A61Hnu_ITS1
H.aureum(2)
A02,A03,A05,A07
(5 assorted)
H. nuttallii(2)
A51,A61
(4)

Implications

- *H. nuttallii* separate from *H. pinnatifidum/ H. aureum*
- *A51, A61 also misidentified*
- *H. pinnatifidum* incompletely separated from *H. aureum* (all *H. aureum* together, bu t also includes some *H. pinnatifidum*)

ITS2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H. aureum (2)/H. pinnatifidum (2
H. aeneum (4)
H. nevadense (5)
H. californicum (7)
H. fulgescens (5)
H. lutescens (8)
H. phillipeanum (9)
H. meridionale (4)
H. mandonii (5)
H. sericeum (19)
H. megaptilum (2)

H. nuttallii(2)
A51,A61
AF403589.1Hnu
A51Hob
EF617591.1Hsess
AF395635.1Eurhynchium_pulchellum
EF617558.1Hlu
Other ssp
AF403651.1Brachythecium_rivulare
H. lutescens(7)
H. sericeum cplx (8)
EF617561.1Hlu
DQ200957.1B_trachypodium
AF403667.1B_velutinum
AY166440.1B_collinum
A05Hau
Brachytheciastrum ssp
H.aureum(2), H. pinnatifidum(2)
H. aeneum(3), H.californicum(7),
H. fulgescens(4), H. nevadense(5),
H. phillipeanum(1), (42 assorted)
A50Hob
AF403662.1B_falcatulum
(4)
EF617569.1Hme
H. megaptilum(2)
AF403647.1Hph
EF617554.1Har
H. arenarium(1)
EF617572.1Hae
H. phillipeanum(8)
H. fulgescens(1)
H. aeneum(1)
A63
EF617549.1Hla
AF403616.2Hla
Other ssp
AF395633.1Rhynchostegiella_tenella
AF403580.1Floribundaria_floribunda
AF403608.1Cirriphyllum_piliferum
(8)

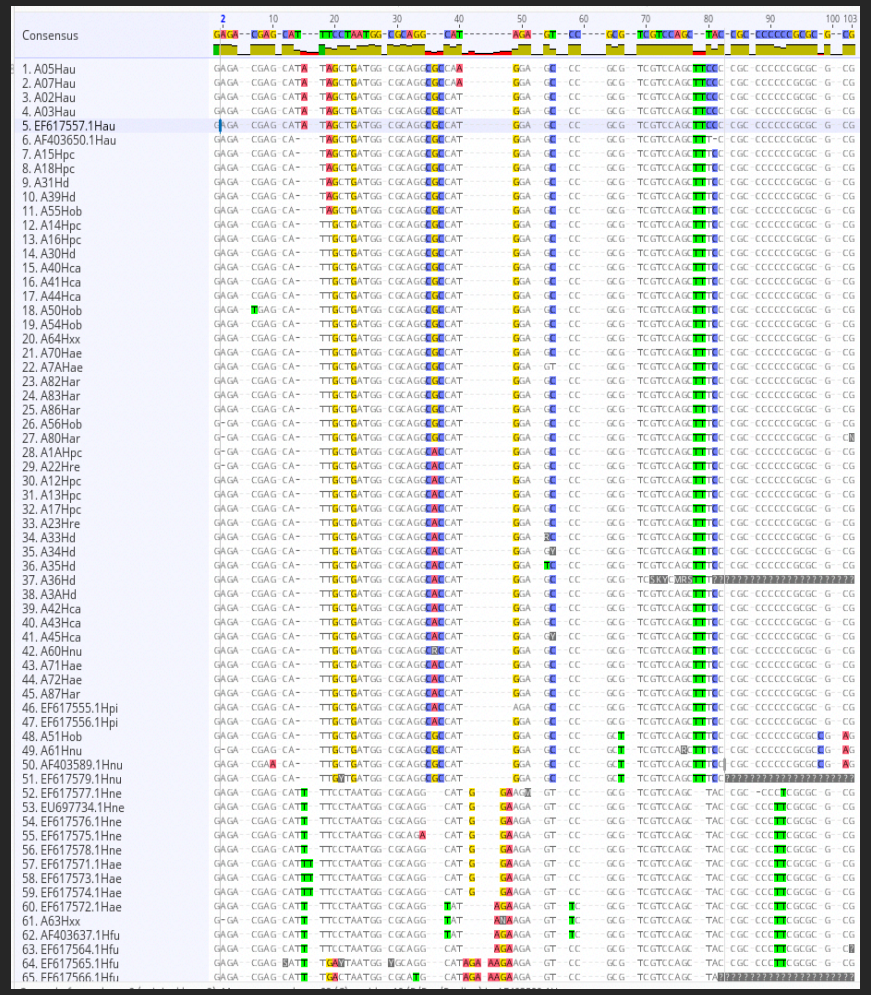
Implications

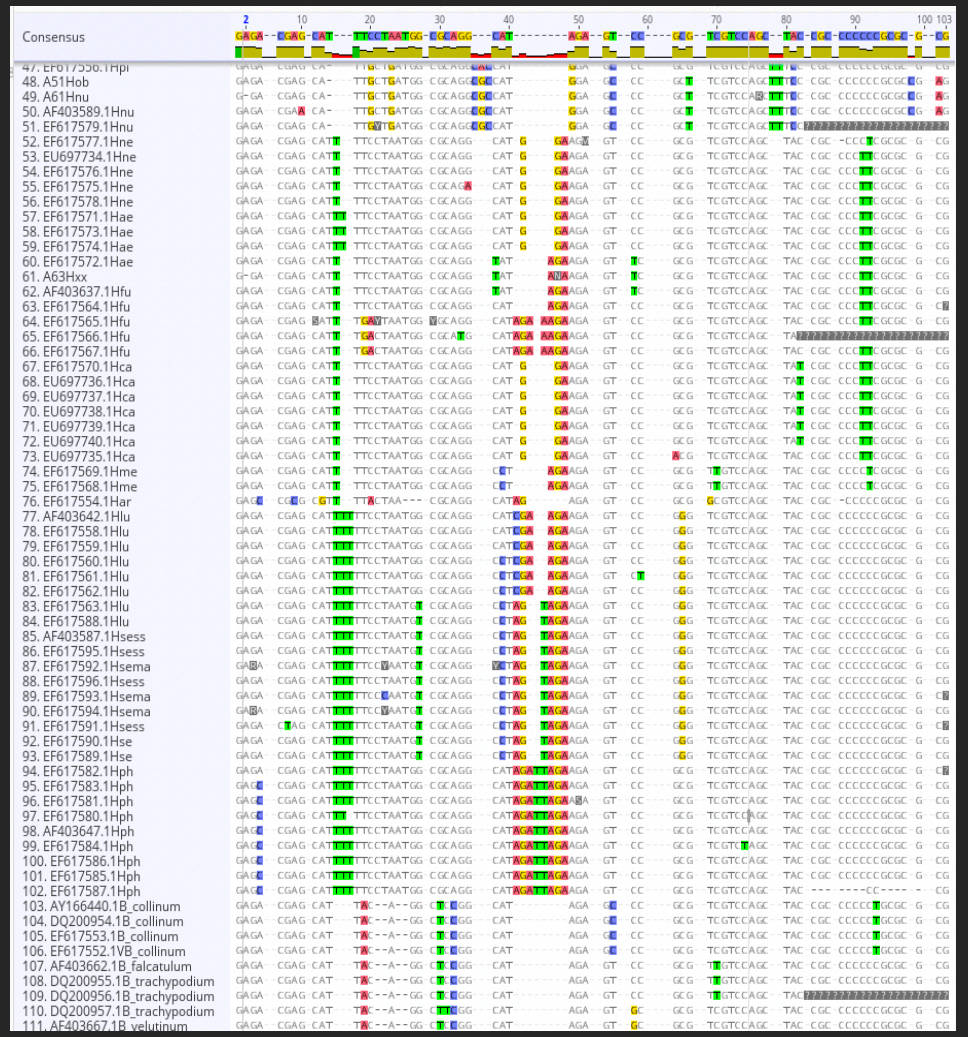
- *H. nuttallii* separate from *H. pinnatifidum/ H. aureum*
- *H.aureum/H. pinnatifidum* not even completely separated from most other *Homalothecium* ssp

➔Why?

Why a big blob?

➔Due to non-use of indels and nucleotides with ambiguity codes



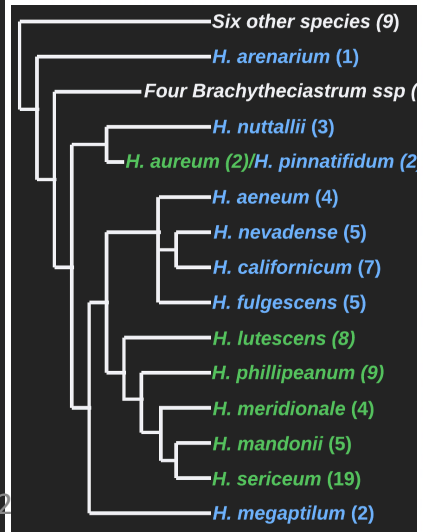


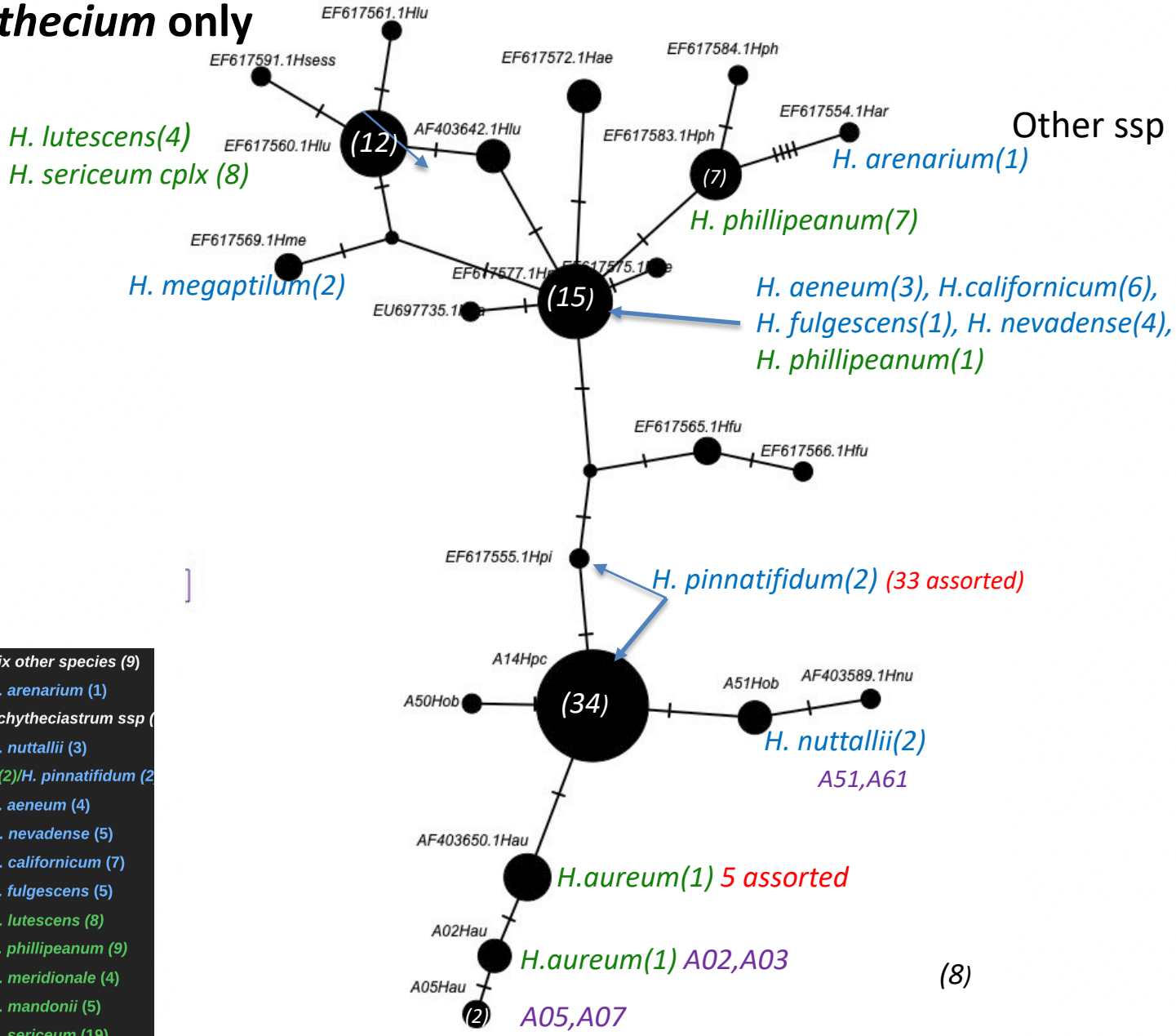
ITS2 sequences with most if undifferentiated nucleotides removed

➔almost none of the differences visible above are used

ITS2

Homolathecium only





H. lutescens(4)
H. sericeum cplx (8)

Other ssp

H. arenarium(1)

H. phillipeanum(7)

H. megaptilum(2)

H. aeneum(3), H.californicum(6),
H. fulgescens(1), H. nevadense(4),
H. phillipeanum(1)

H. pinnatifidum(2) *(33 assorted)*

H. nuttallii(2)
A51,A61

H.aureum(1) *5 assorted*

H.aureum(1) A02,A03

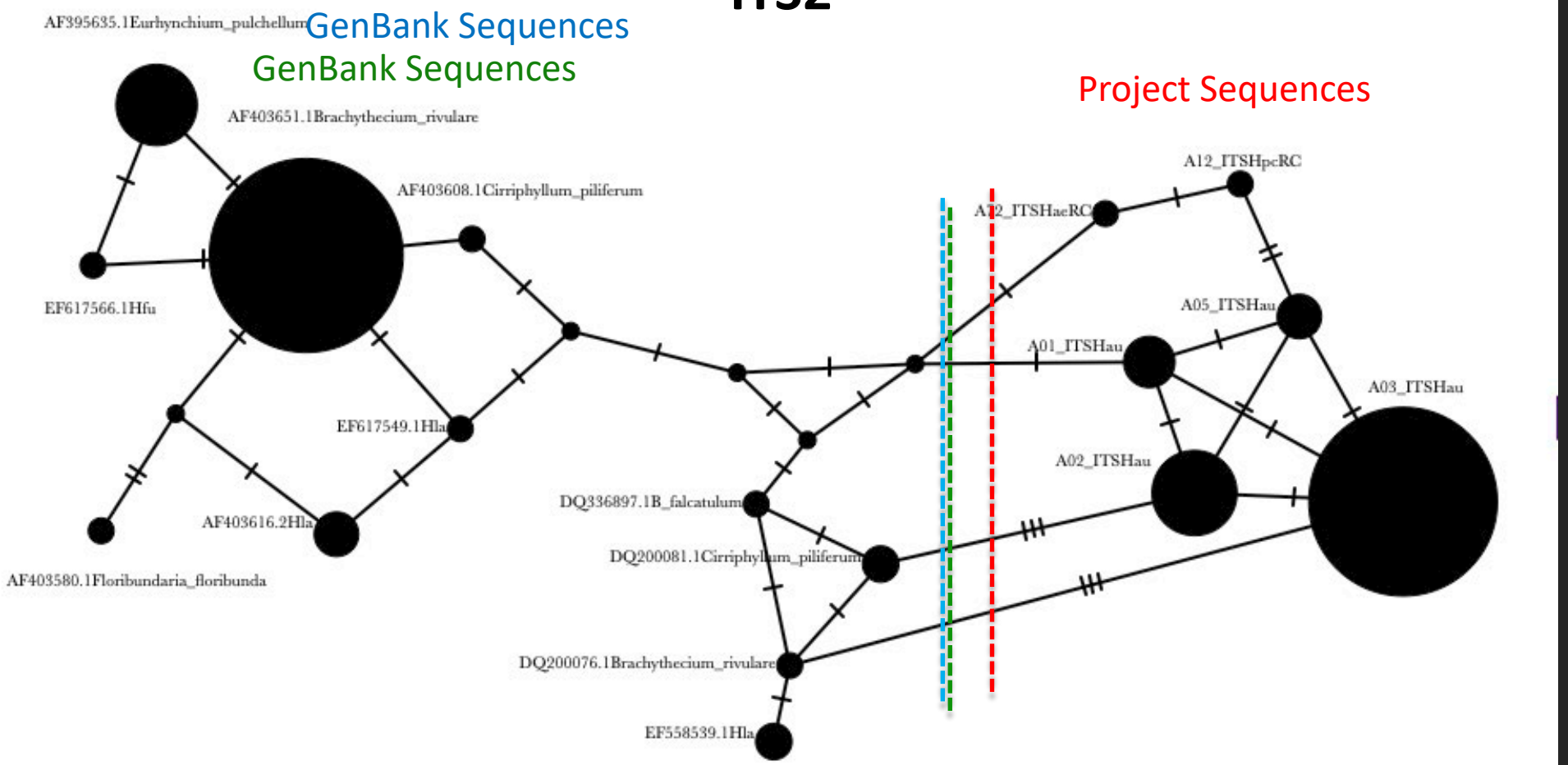
(8)

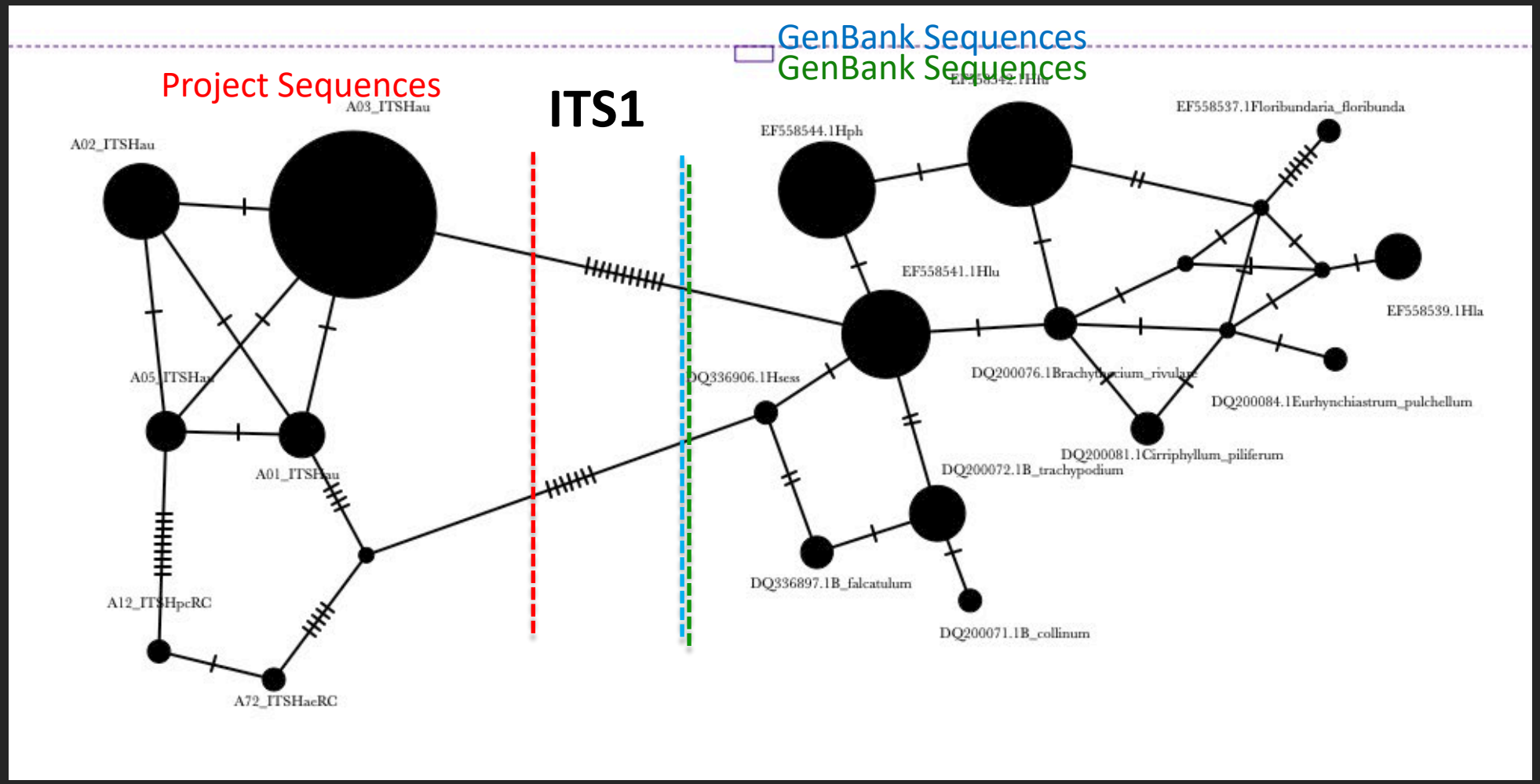
A05,A07

Implications

➔ *The avoidance of indels/ambiguities are making the use of the PopArt tool not terribly useful*

ITS2


AF395635.1Eurhynchium_pulchellum
GenBank Sequences
GenBank Sequences
Project Sequences
AF403651.1Brachythecium_rivulare
AF403608.1Cirriphyllum_piliferum
EF617566.1Hfu
EF617549.1Hla
AF403616.2Hla
AF403580.1Floribundaria_floribunda
DQ336897.1B_falcatulum
DQ200081.1Cirriphyllum_piliferum
DQ200076.1Brachythecium_rivulare
EF558539.1Hla
A12_ITSHpcRC
A12_ITSHaeRC
A05_ITSHau
A01_ITSHau
A03_ITSHau
A02_ITSHau


GenBank Sequences
GenBank Sequences
Project Sequences
ITS1
A03_ITSHau
A02_ITSHau
A05_ITSHau
A01_ITSHau
A12_ITSHpcRC
A72_ITSHaeRC
EF558544.1Hph
EF558537.1Floribundaria_floribunda
EF558541.1Hlu
EF558539.1Hla
DQ336906.1Hsess
DQ200076.1Brachythecium_rivulare
DQ200084.1Eurhynchiastrum_pulchellum
DQ200081.1Cirriphyllum_piliferum
DQ200072.1B_trachypodium
DQ336897.1B_falcatulum
DQ200071.1B_collinum

Info sent by email

A. I think I understand the surprise in atpB-rbcL network and I do not believe the single nucleotide difference causing it is informative.
The atpB-rbcL grouping of a large % of my specimens with *H. nuttallii* instead of with *H.pinnafidum/H.aureum* is due to a single nucleotide at position 429 in the aligned atpB-rbcL.This nucleotide is sometimes a C instead of a T. If you discard all the sequences (both mine and GenBank) that are not in this basal clade of *H. nuttallii, H.pinnatifidum, H. aureum* and look at the number of each of these three basal clade species that has a C instead of T, the differences between the three species are not significant. For this analysis, I am assuming that my *H. pinnatifidum* specimens are all *H. pinnatifidum* (so excluded the six that are clearly mis-identified).
H.pinnatifidum: 31.2% (15/48) [=15 samples had C in this position instead of T, out of 48=2 GenBank + 46 my sequences]
H.aurem: 12.5% (1/8) [=1 sample had a C in this position instead of T, out of 8=2 GenBank +6 my sequences]
H.nuttallii: 0.0% (0/3) [out of 3 GenBank sequences]

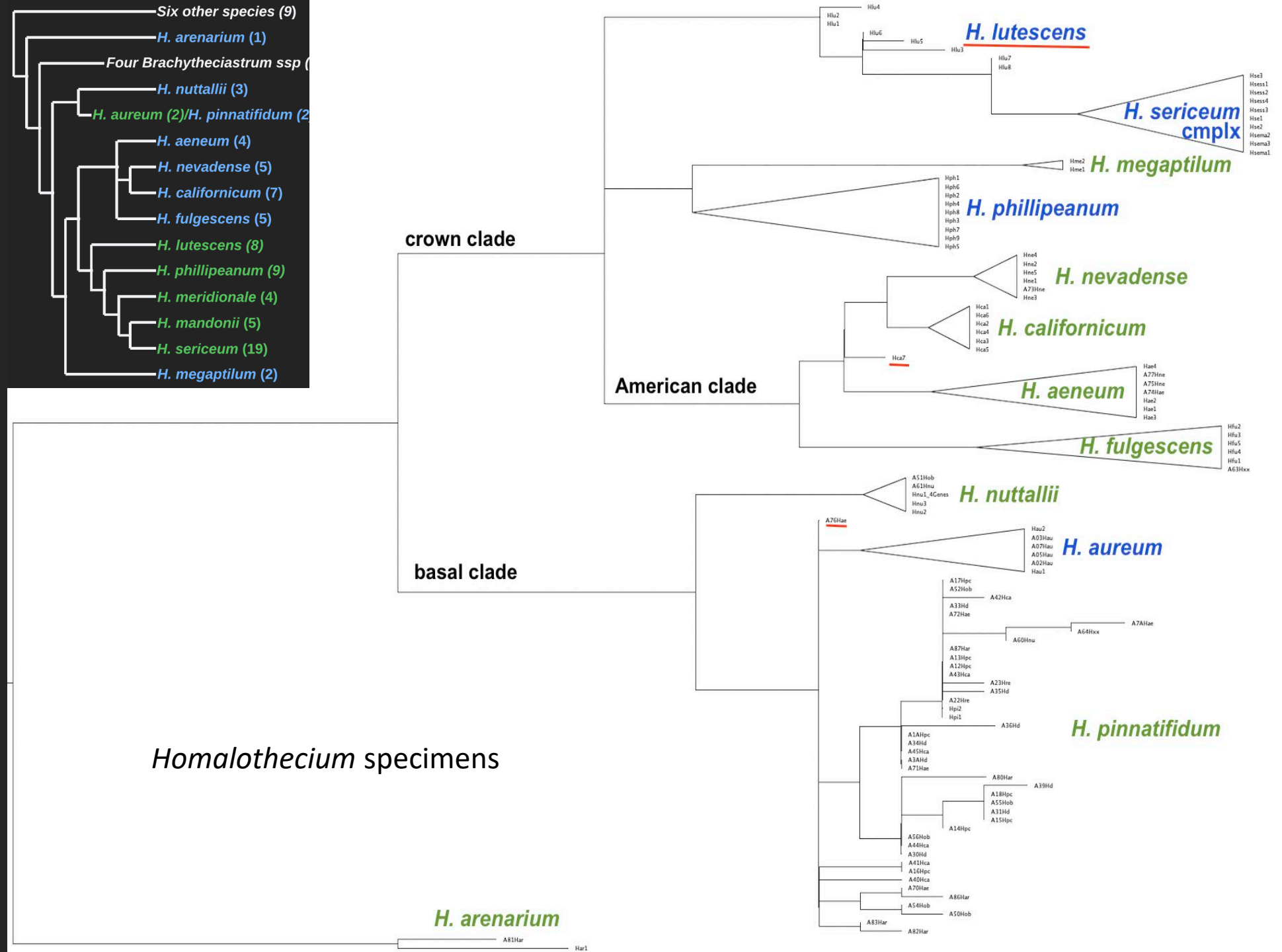
B: I think we should probably resequence both ITS1 and ITS2. If we only do one, I think it should be ITS2, since we are looking within *H.aureum/H.pinnatifidum*. Here is what I found (see attached for details):

Number of places in gene that differentiate between	rpl16	atpB-rbcL	ITS1	ITS2
Basal clade (*H. aureum, H. pinnatifidum, H. nuttallii*) from crown clade (rest of *Homalothecium* s.str.)	1	1	1	7
H. nuttallii from *H.aureum/H.pinnatifidum*	0	1	4	1
H. aureum from *H. pinnatifidum*	0	0	1	2

C. Since there is a leadtime, I think we should order primers and any other supplies needed now while we discuss options and decide the next best paths. The cost is relatively low and I don't mind paying for it.

D. Given the lack of/incompleteness of ITS sequences for *H. nuttallii,* if we decide to redo ITS1 and/or ITS2, I think I should find and prep a few additional *H. nuttallii* specimens for sequencing also. I don't think we need to redo the ITS sequences for the six specimens that are mis-identified. We should order now any supplies we need for that also.

See also GeneDifferences.docx for nucleotide differences and location among these genes for GenBank sequences

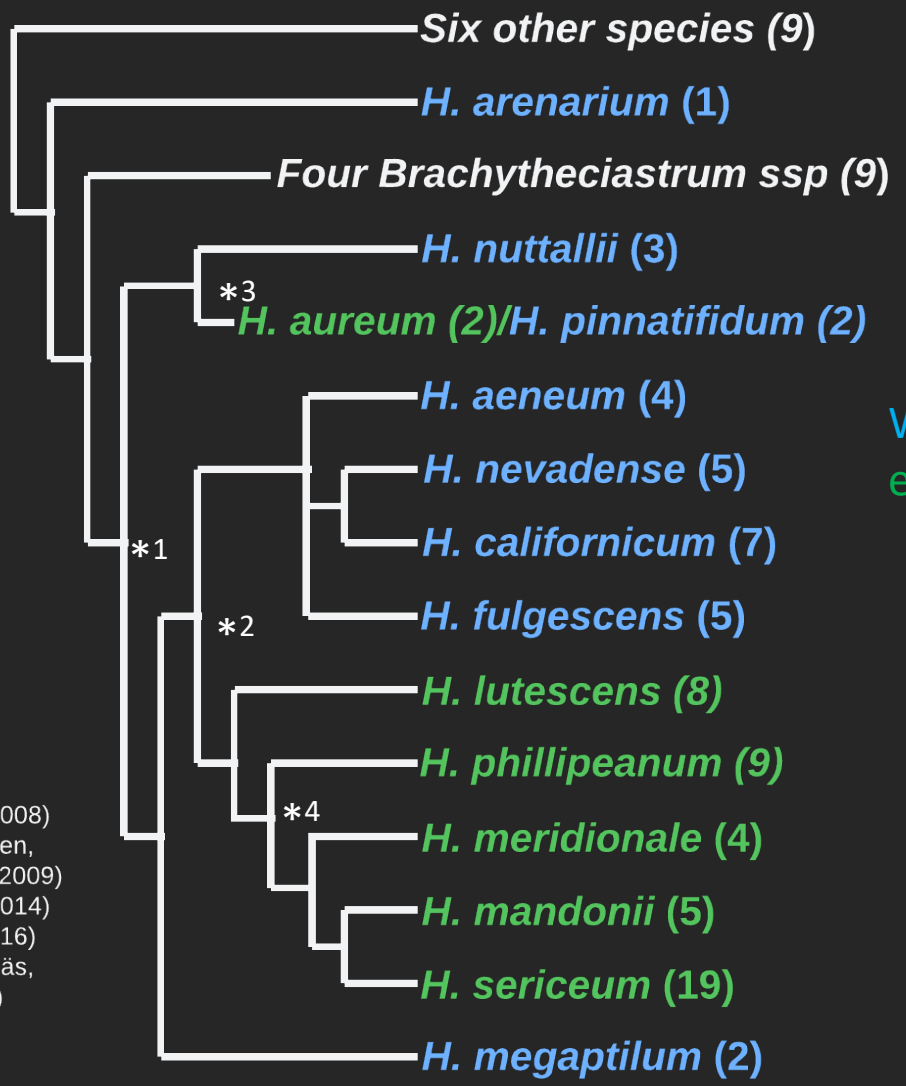


Homalothecium specimens

230427 Meeting

- To Do:
- Get list of specimens with both rpl16 and atpB-rbcL results in the three large nodes.
- Go through same specimens looking for morphological characters that might unite specimens in each node.

Homalothecium—Phylogeny ages



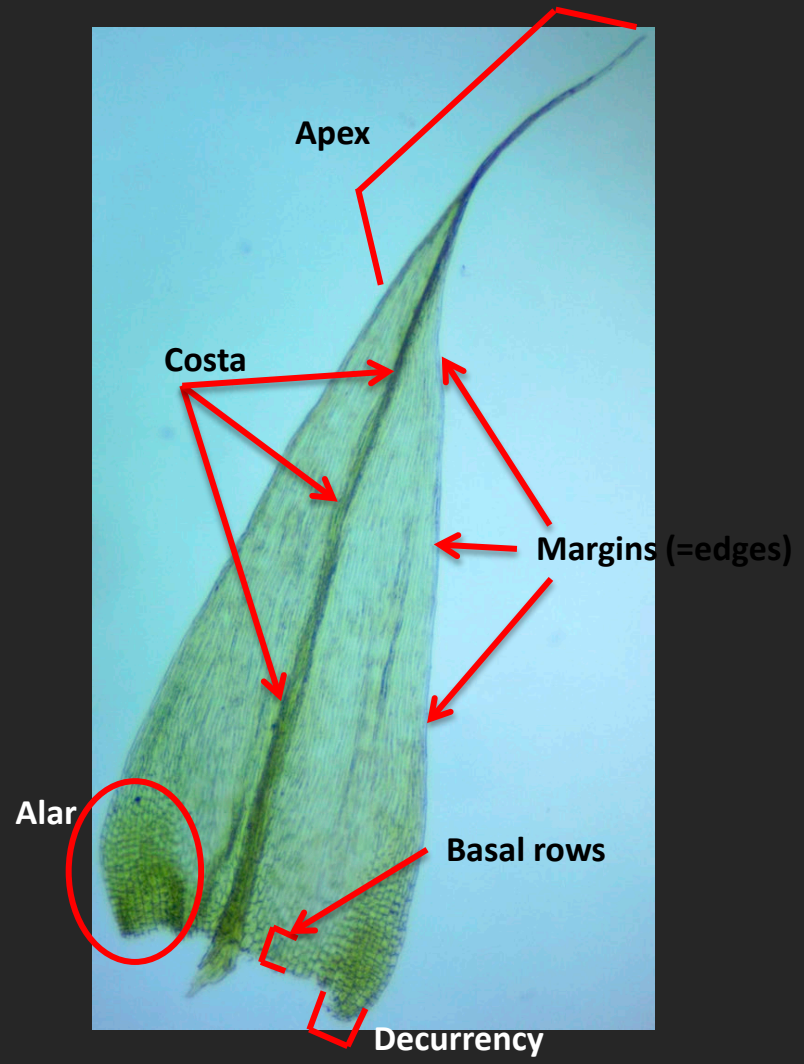
Western North America
elsewhere

Based on:
Hedenäs, et. al. (2008)
Hedenäs, Huttunen, Shevock, & Norris (2009)
Hedenäs, et. al. (2014)
Sergio, et. al. (2016)
Huttunen, Hedenäs, Ignatov (2018)

*[1]7.9Mya
*[2]5.7Mya
*[3]4.0Mya
*[4]2.9MYa

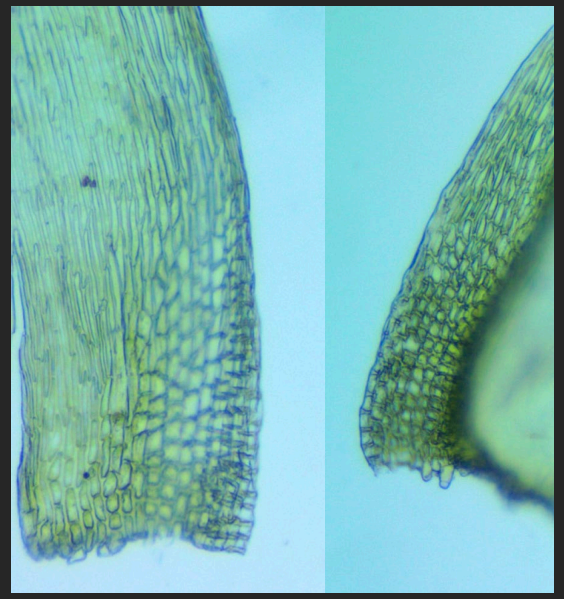
MOSS LEAF TERMINOLOGY

- Shape: overall shape of leaf
- Margin: edge of leaf
- Costa: leaf mid-rib
- Alar region: area of distinct cells in corners of leaf
- Decurrencies: cells of leaf that attach to a stem below
- Basal rows: rows of distinct cells at base of leaf
- Apex: Distal part of leaf

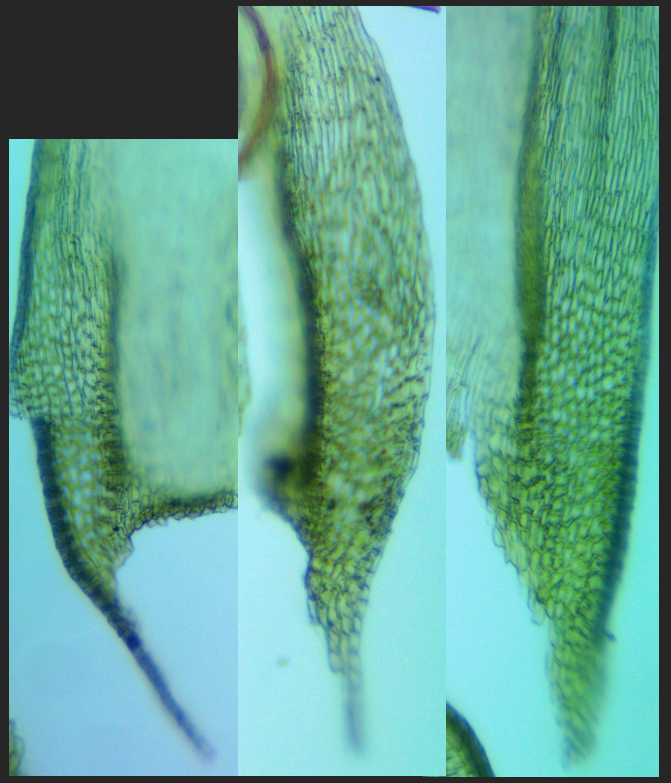


Tracked many characters

- Leaf decurrencies--# of cells (length x width)



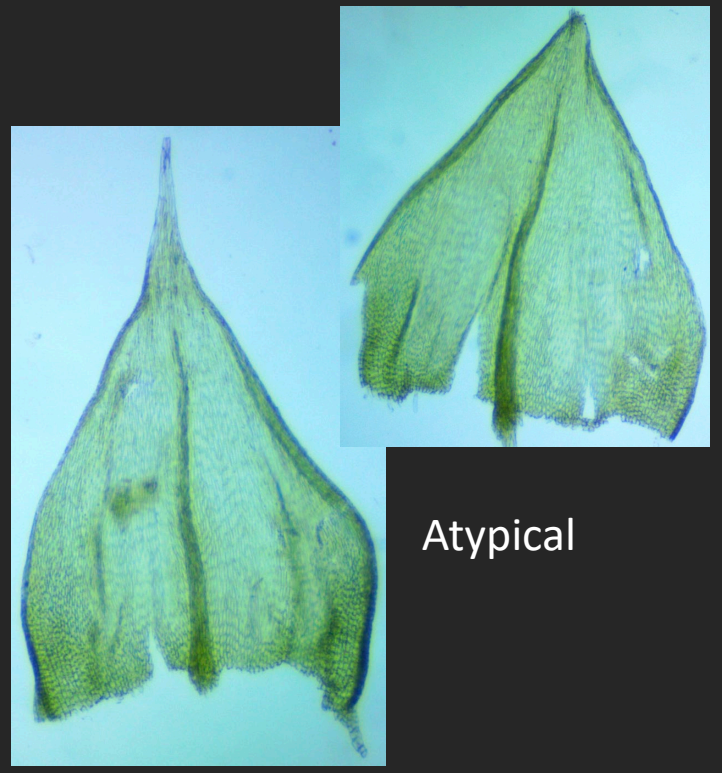
Typical



Atypical

Leaf Shape

Typical



Atypical

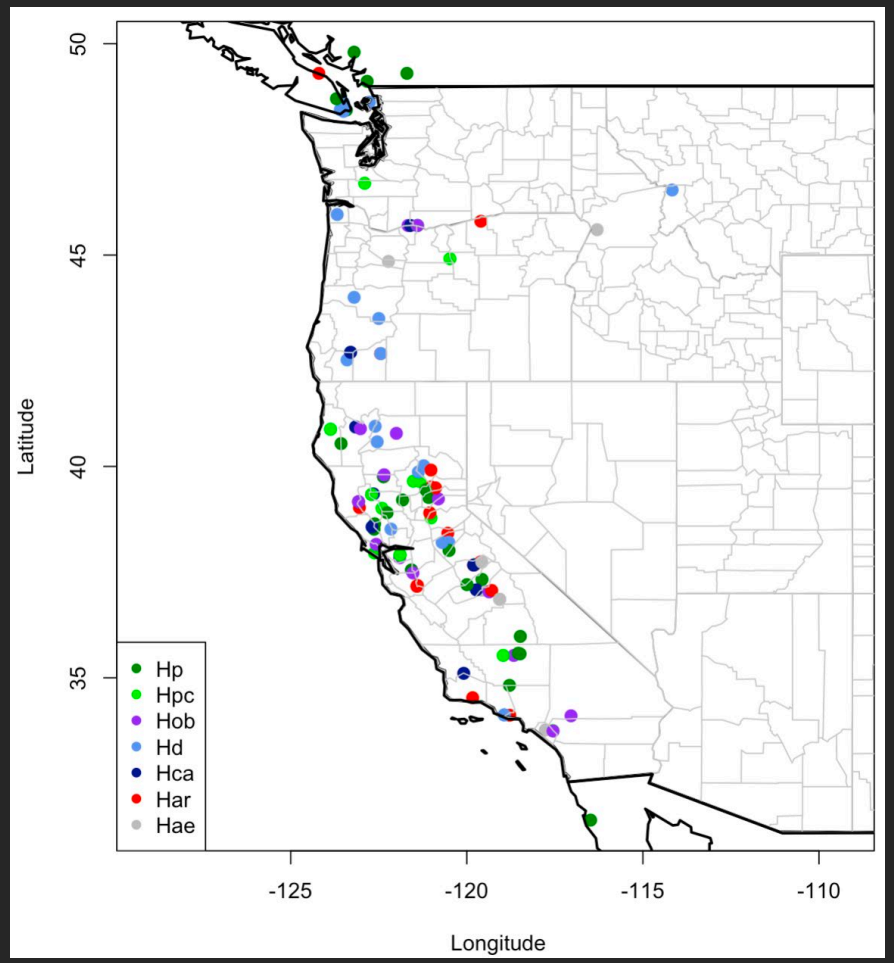
Other characters being tracked. . .

- Substrate
- Leaf length/width
- Leaf margins
- Mid-lamina cell length
- Leaf basal rows

- Leaf alar cell characteristics
- Costa spines
- Sporophyte characters
  - Operculum: conic/rostrate/rostellate/ length
  - Urn: amount of curvature length
  - Seta: rough/smooth

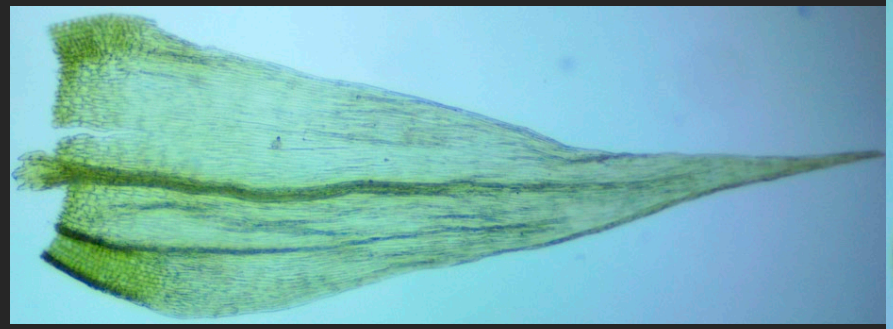
H. pinnatifidum morphotypes

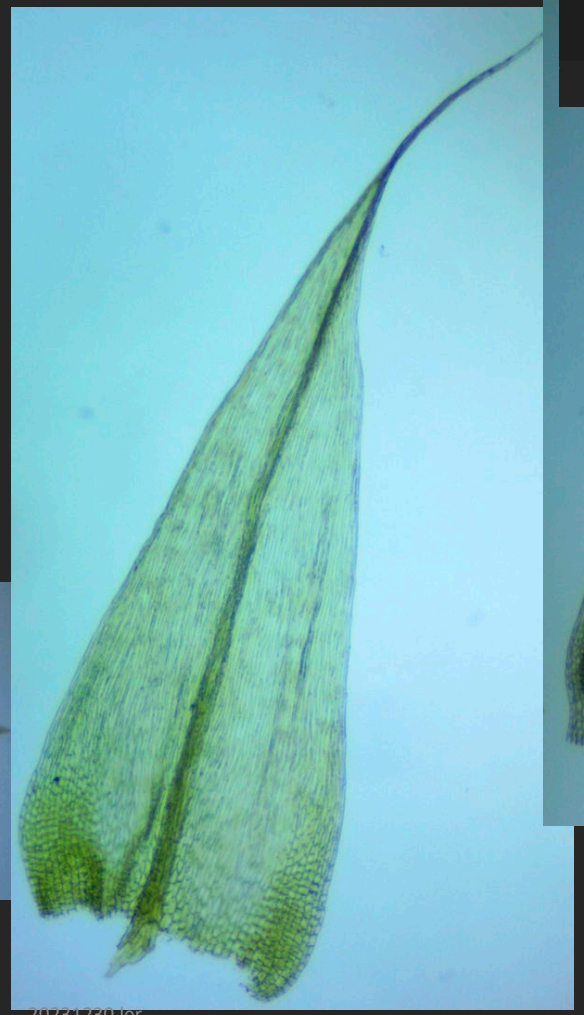
- *H. pinnatifidum* specimens were classified into seven morphotypes
- Samples of each morphotype were selected for sequencing

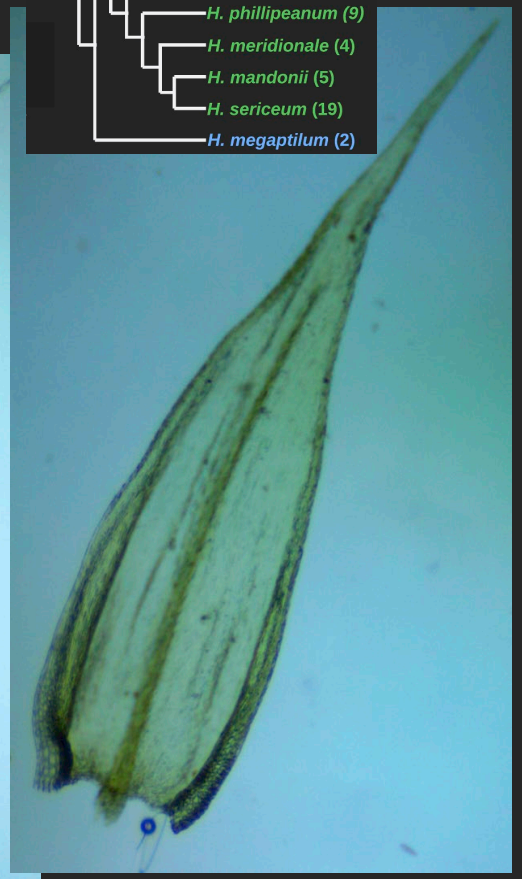


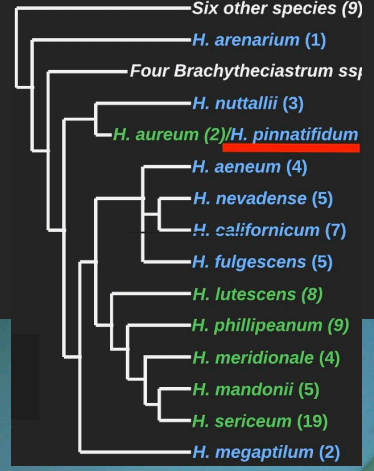
H. pinnatifidum (classic) [Hp,Hpc]A1x (10)

- Shape
- Margins
- Decurrencies
- Costa



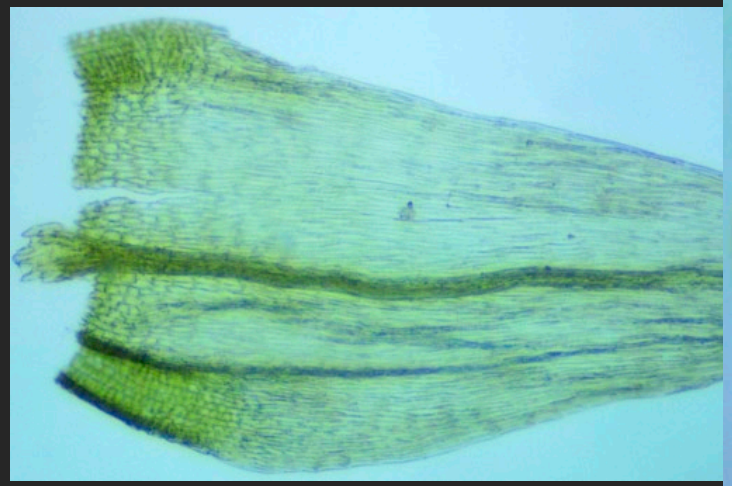


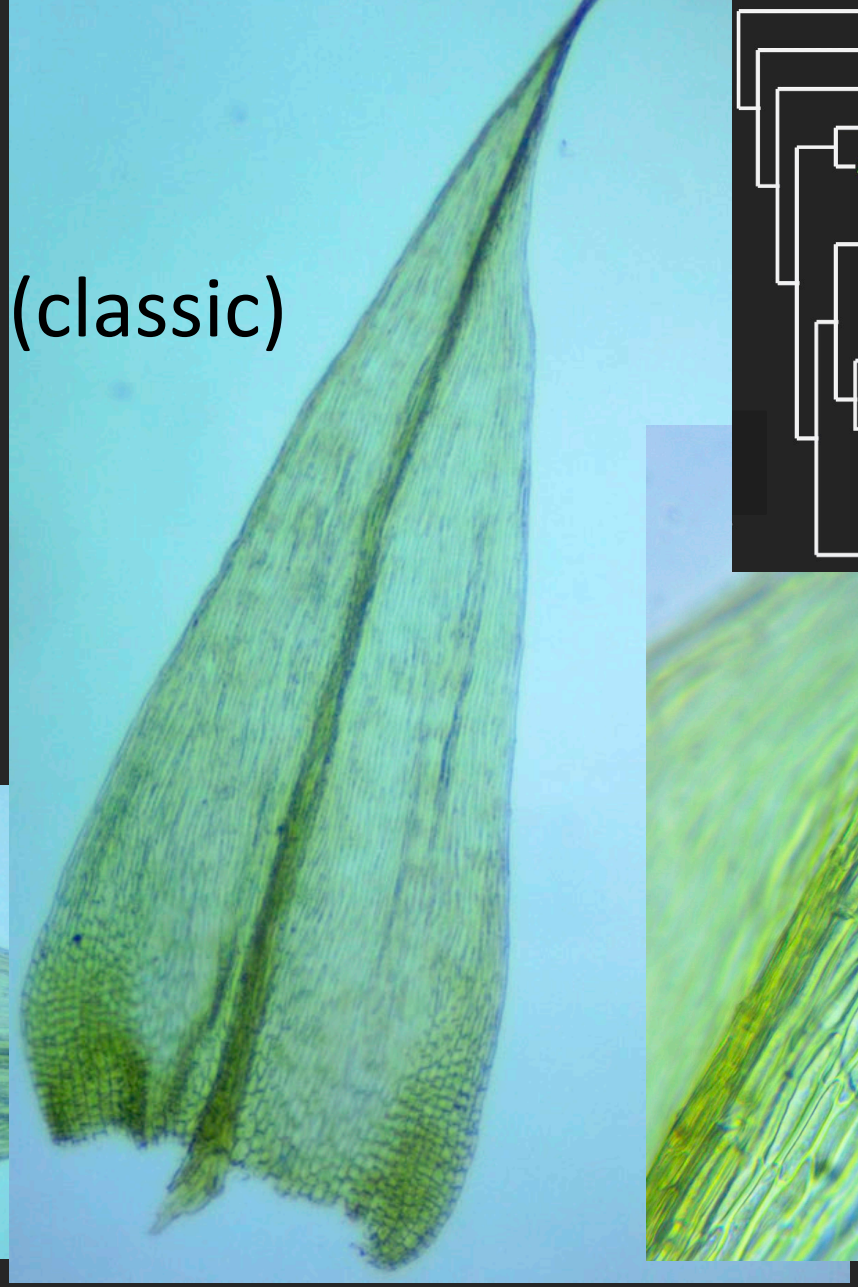


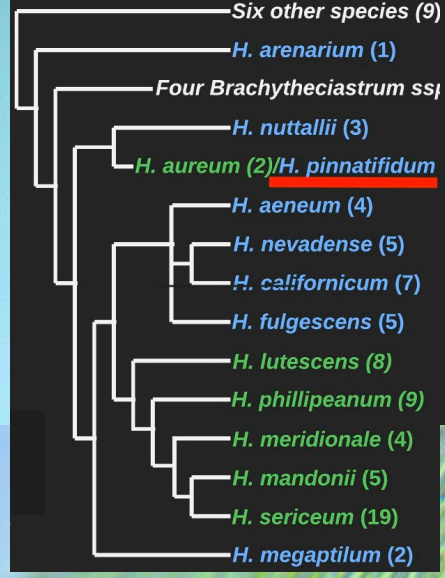


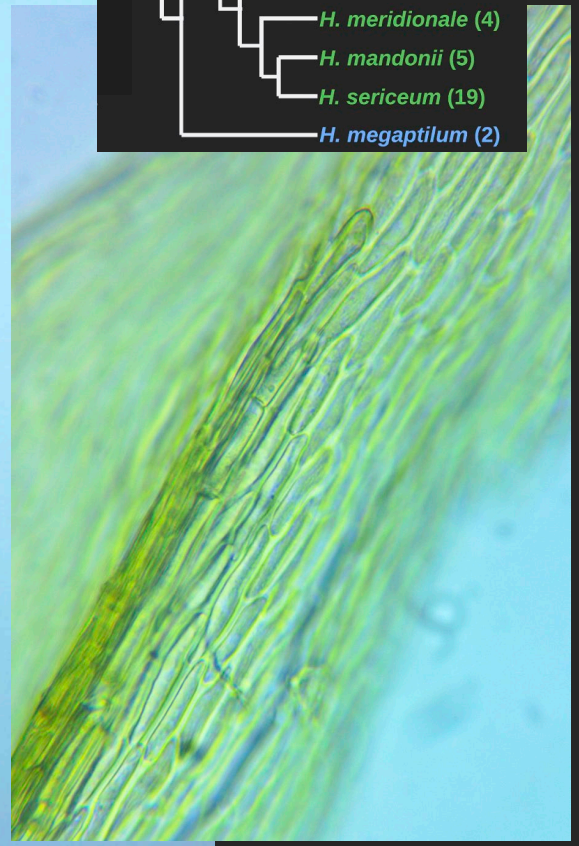
H. pinnatifidum (classic) [Hpc, Hp]

- Alar cells
- Costa spine



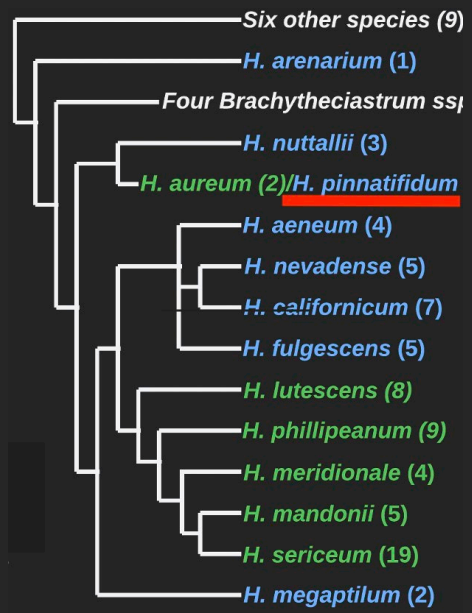


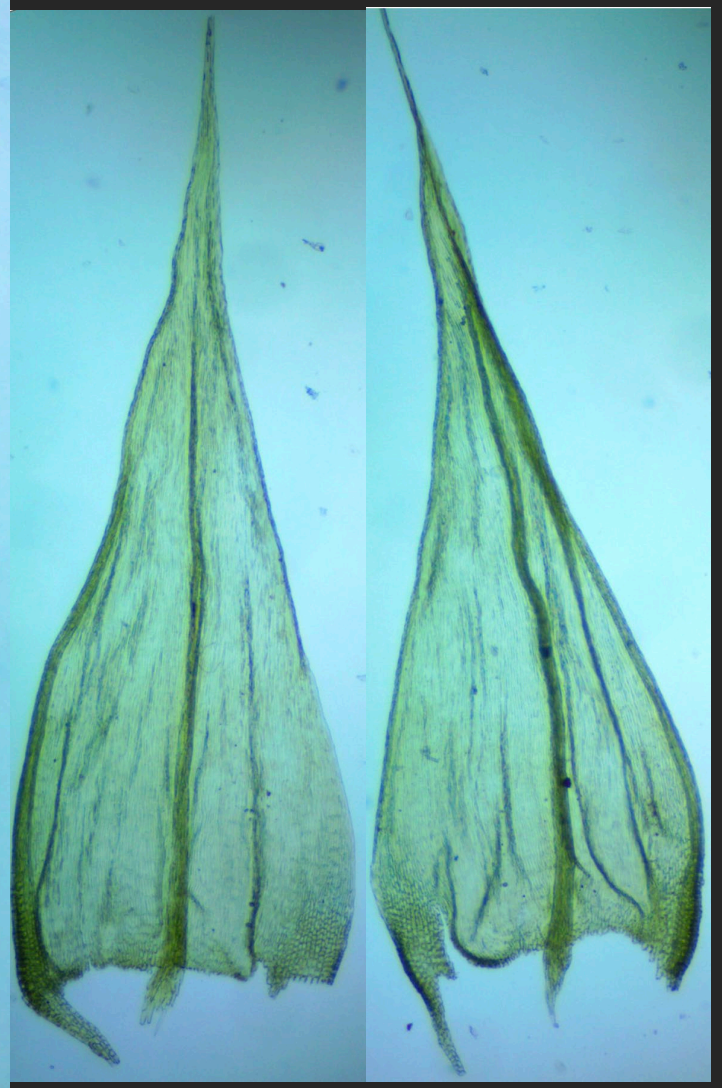




H. decurrentifolium [Hd] A3x (11)

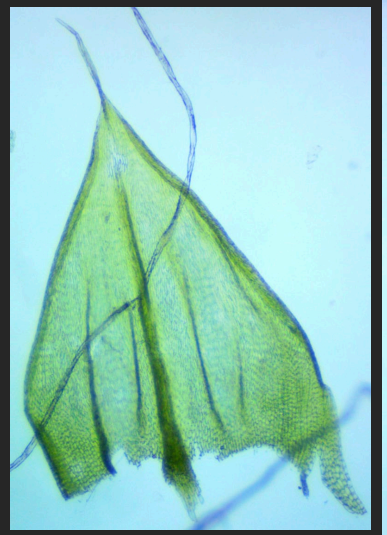
- Shape
- Costa
- Alar cells
- Margins
- Decurrencies

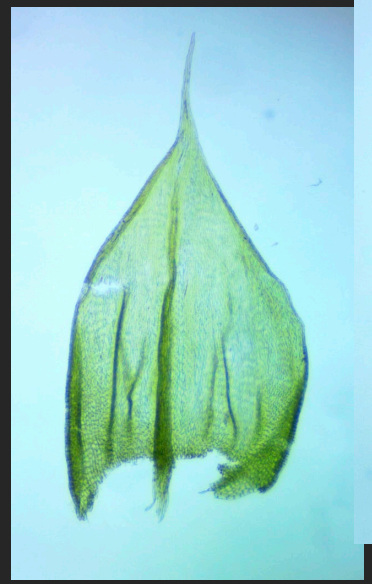


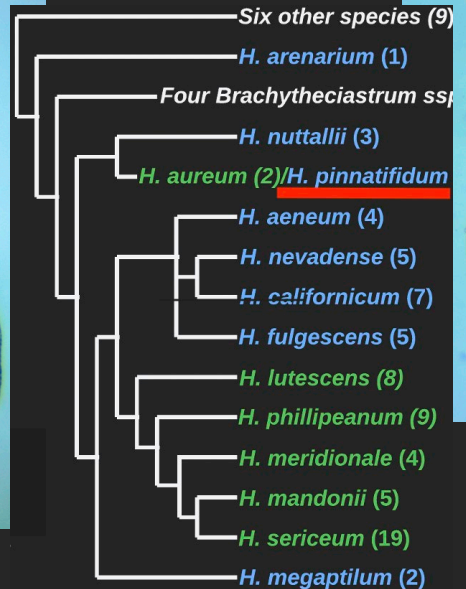


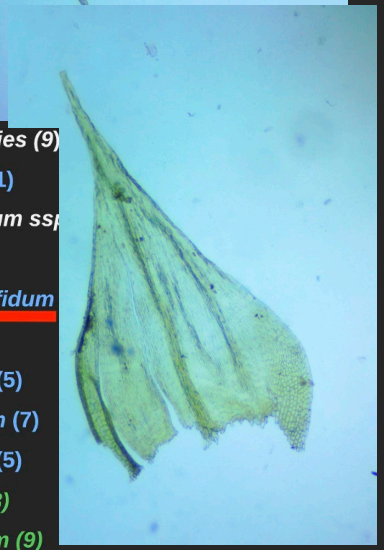
H. orbicularium [Hob] A5x (7)

- Shape
- Costa
- Alar cells
- Margins
- Decurrencies



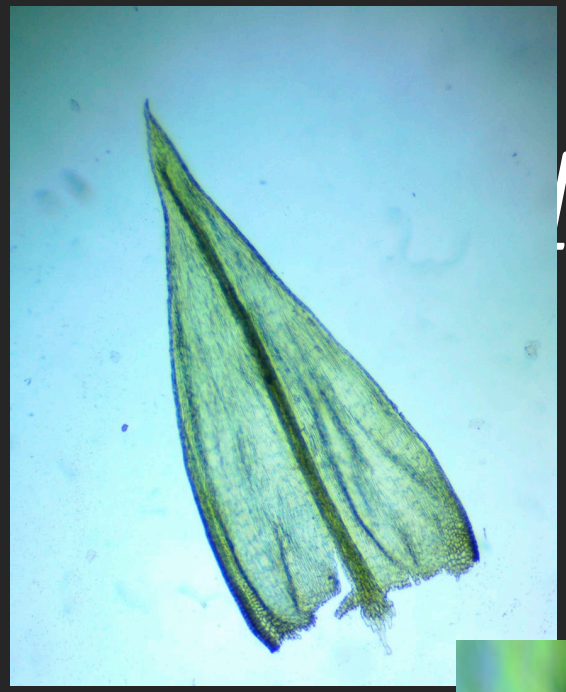


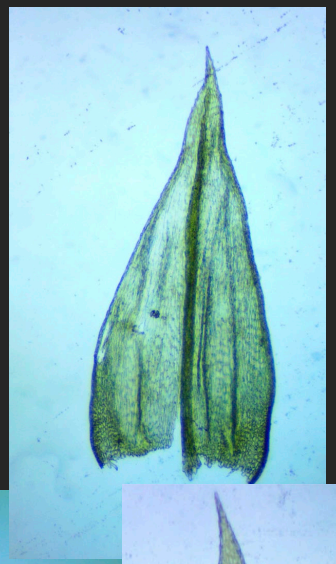


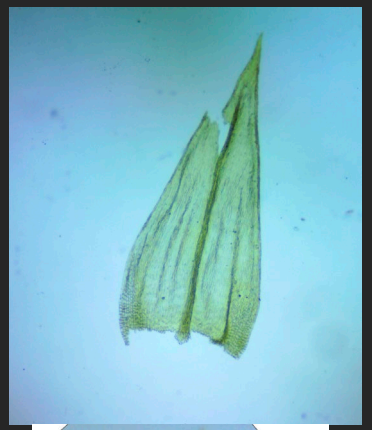


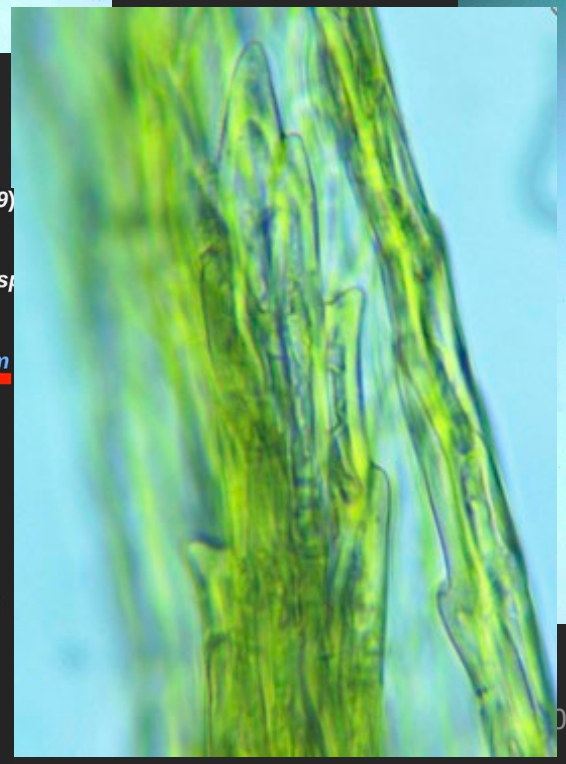
[Har] A8x (6)

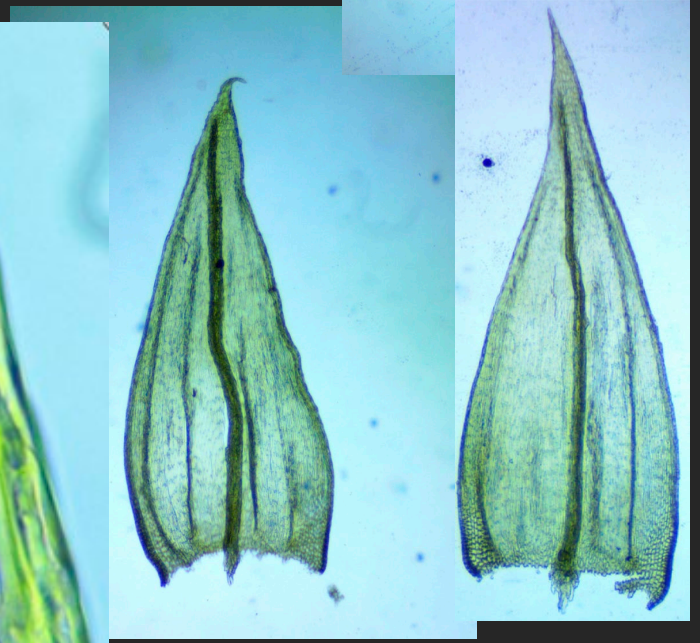
- Shape
- Costa
- Alar cells
- Margins
- Decurrencies

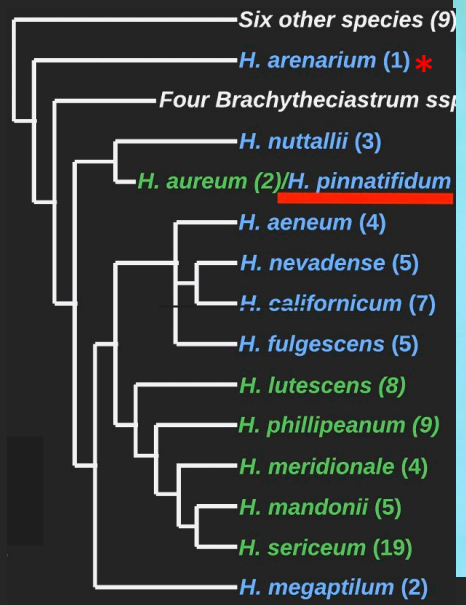












[Hre(4)]A2x

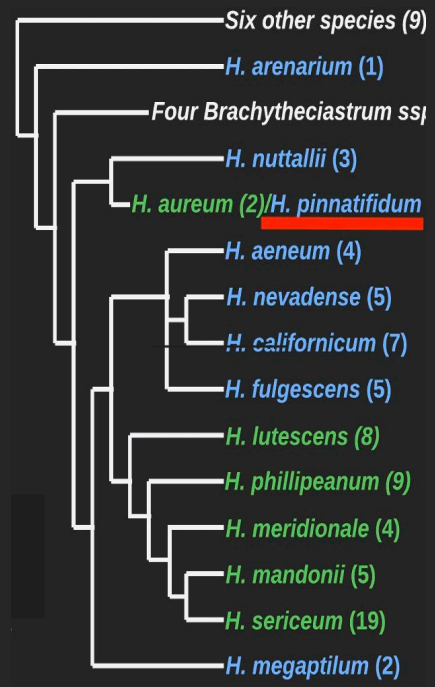
- Similar to Hpc/Hp
- Difference
  - Alar cells
    - orientation

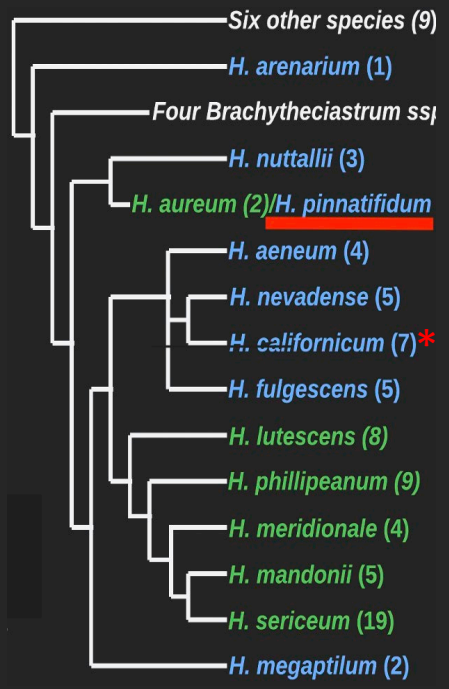
[Hca(5)]A4x

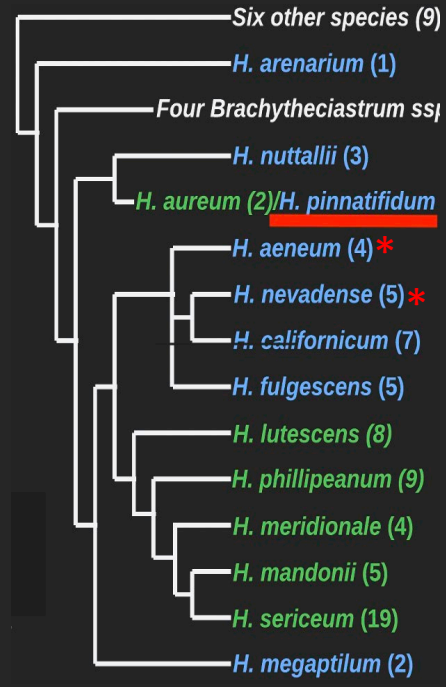
- Similar to Hd
- Differences:
  - Bigger

[Hae/Hne(11)]A7x

- Similar to Hpc/Hp
- Differences
  - Alar cells
    - shape/position
    - number
    - walls







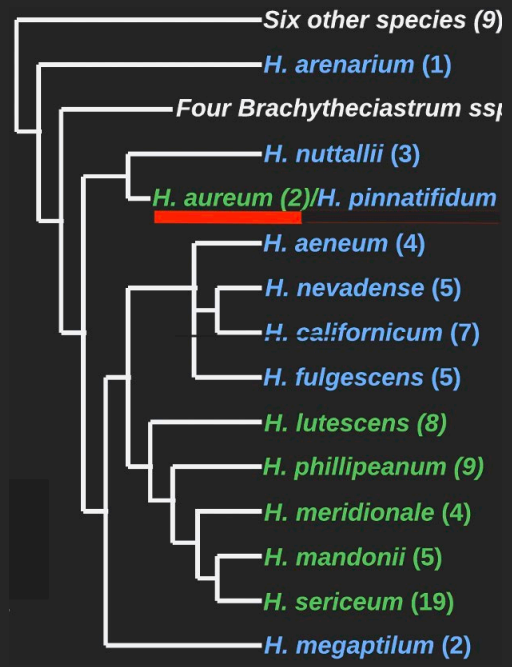
[Hau(7)]

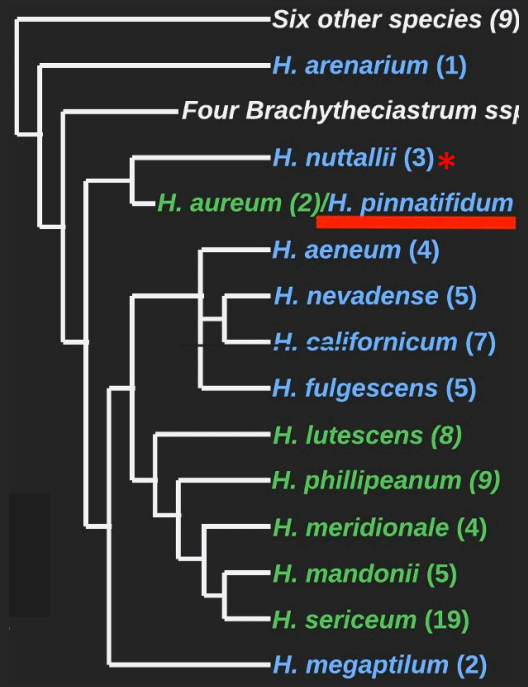
- Not North America
  - North Africa(2)
  - Southern Europe(4)
  - East Kazakhstan(1)
- (4)

[Hnu(3) / Hxx(2)]

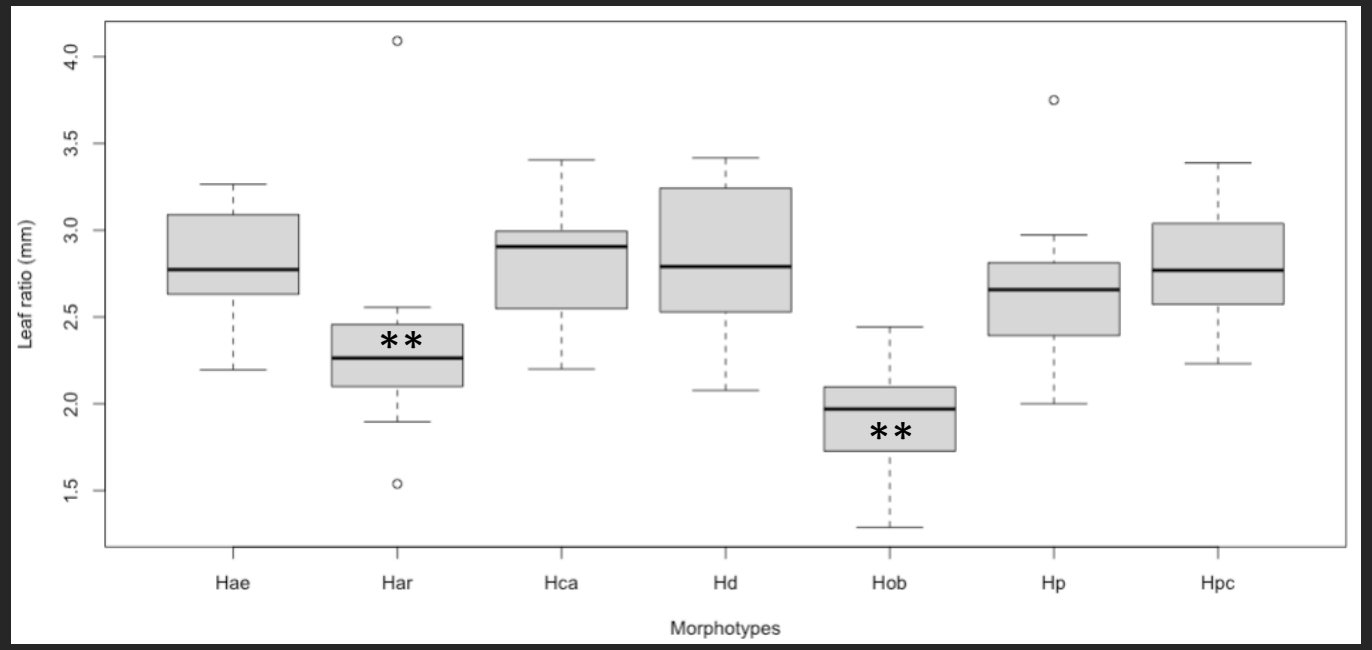
- 1 IDed as *H. nuttallii*
- *2 with a few very minute recurved margin teeth*

- *ID=H. pinnatifidum*
- *Did not fit another morphotype*





H. pinnatifidum morphotypes leaf length/width ratio



Specimens to collection table

Greec	*Ost-Aegaeis*		*1997*	*9*	*6*	*Ruprecht Dull*		*aui*	*Steinwuste m*	*UBC*		*80B7F7*	*Hau*	
Spain	*Ciudad Real*		*2006*	*6*	*14*	*Mateo, Ru*	*s.n.*	*aureum*		*MO*	*K. Cezón*	*urn:cat*	*Hau*	*1*
Spain	*Toledo*		*2004*	*5*	*20*	*Rafael He*	*s.n.*	*aureum*		*MO*	*K. Cezón*	*urn:cat*	*Hau*	*1*
Cypru	*Girne*		*2017*	*1*	*20*	*Shafer-Ve*	*37985*	*aui*	*lichter Kiefernwald*		*Schafer-Verwimp,*		*Hau*	
Greece			*2017*	*3*	*26*	*Blockeel,*	*46/123*	*aui*	*on stony bank*	*Privat*	*Blockeel, Tom*		*Hau*	
Cypru	*Girne*		*2017*	*1*	*21*	*Shafer-Ve*	*37992*	*aui*	*Barranco obe*	*MO*	*Schafer-Ve*	*A02*	*Hau*	
Greece			*2016*	*3*	*20*	*Blockeel,*	*45/184*	*aui*	*in turf on rock*	*Privat*	*Blockeel, T*	*A03*	*Hau*	
Spain	*Cuenca*		*2005*	*5*	*26*	*Cezón, Ka*	*K1518*	*aui*	*carretera hac*	*MO*	*K. Cezón*	*A05*	*Hau*	*1*
Spain	*Leon*		*2015*	*6*	*6*	*Cano, M.J.*	*8949*	*aui*	*embal: Suelo*	*MO*	*Cano, M. J.*	*A07*	*Hau*	
Canad	BC		2017	4	10	Lee, Olivia	3234	ful	Bluff below G	UBC		**A1A**	Hpc	
USA	CA	Glenn	1997	5	19	Toren, Da	5058	pin	Mixed Conife	CASC	Toren, Dav	**A34**	Hd	
Canad	BC		2002	3	6	Schofield,	119245	pin	; outcrop slop	UBC	Schofield, '	**A3A**	Hd	
USA	CA	Riverside	2001	3	25	Sargent, M	2795	pin	Chaparral, co	CASC	Toren, Dav	**A40**	Hca	
USA	OR	Hood River	2000	10	9	Schofield,	116717	pin	; boulder	UBC	Schofield, '	A45	Hca	
USA	CA	Ventura	2016	10	3	Carter, Be	9565	aureum		SJSU		A51	Hob	JS
USA	CA	Trinity	2009	7	2	Shevock, J	33067	pin	Mixed On tru	CASC	Shevock, Ja	A61	Hnu	
USA	CA	Santa Clara	2017	3	27	Carter, Be	9797	aureum	Growi	SJSU		A71	Hae?	JS
USA	CA	San Luis Obi	2007	5	4	Carter, Be	1829	pinnatifid	On sha	SJSU		A12	Hpc	JS
USA	CA	Marin	2009	3	7	Shevock, J	32609	pin	Dougla On me	CASC	Toren, Dav	A13	Hpc	
USA	WA	Klickitat	2002	4	12	Schofield,	119410	pin	; dry open slo	UBC	Schofield, '	A17	Hpc	
USA	CA	El Dorado	2017	6	3	Shevock, J	50283	pin	Mixed conifer	CASC	Toren, Dav	A22	Hre	
USA	OR	Douglas	1996	10	28	Wagner, D	8650	pin	Ceanothus sh	CASC		A23	Hre	
USA	CA	Napa	2007	11	10	Carter, Be	2073	pinnatifidum		SJSU	det. Carter	A33	Hd	JS
USA	CA	Mariposa	2006	11	11	Shevock, J	29269	pin	Foothi On soi	CASC	Shevock, Ja	A42	Hca	
USA	CA	Sonoma	1993	3	4	Bourell, M	5129	pin	Pseud On soi	CASC	Toren, Dav	A43	Hca	
USA	CA	Sonoma	1993	3	4	Bourell, M	5130	pin	in stre On roc	CASC	Toren, Dav	A60	Hnu	
USA	CA	Napa	2007	11	10	Carter, Be	2079	pinnatifidum		SJSU		A64	Hxx	JS
USA	CA	Marin	2007	12	2	Carter, Be	2153	pinnatifidum		SJSU	det. Carter	A72	Hae	JS
USA	CA	Lake	2012	3	29	Carter, Be	6809	pinnatifid	Groiw	SJSU		A7A	Hae?	JS
Canad	BC		1991	5	2	Schofield,	96242	pin	dry cliff shelf	UBC		A87	Har	
USA	CA	Butte	2015	1	7	Shevock, J	46119	pin	Riparian woo	CASC	Toren, Dav	A14	Hpc	
USA	OR	Curry	2018	3	27	Shevock, J	51351	pin	Mixed hardw	CASC	Shevock, Ja	A15	Hpc	
USA	WA	Cowlitz	2000	5	1	Schofield,	114208	pin	; shaded outc	UBC	Schofield, '	A18	Hpc	
USA	CA	Los Angeles	2012	1	27	Sagar, Tar	1651	pin	On north faci	CASC	Toren, Dav	A30	Hd	
USA	CA	Santa Clara	2017	3	27	Carter, Be	9792	aureum	Growi	SJSU		A31	Hd	JS
USA	CA	Trinity	2009	7	2	Shevock, J	33079	pin	Mixed On me	CASC	Shevock, Ja	A44	Hca	
USA	CA	Lake	2012	3	29	Carter, Be	6820	pinnatifid	Growi	SJSU	det. Carter	*A55*	*Hob*	JS
USA	OR	Hood River	2008	2	19	Schofield,	127752	pinnatifidum		UBC	Schofield, '	A56	Hob	
USA	CA	Santa Barba	2006	3	26	Kellman, K	5066	pin	in shee sandst	CASC	K. Kellman	A80	Hp/Har	
USA	CA	Orange	2011	5	10	Newell, Di	279	pin	Coasta On sid	CASC	Toren, Dav	A50	Hob	
USA	CA	Alameda	1988	3	18	Bourell, M	3446	pin	Dry creek nea	CASC	Toren, Dav	A54	Hob	
USA	CA	Fresno	1996	1	5	Shevock, J	12644	pin	In a fo On soi	CASC	Toren, Dav	A70	Hae	
USA	*WA*	*Klickitat*	*2002*	*4*	*12*	*Schofield,*	*119420*	*pin*	*; dry outcrop*	*UBC*	*Schofield,*	A86	Har	

USA	CA	Fresno	1996	1	5	Shevock, J	12644	pin	In a fo On soi	CASC	Toren, Dav	A70	Hae	
USA	**WA**	**Klickitat**	**2002**	**4**	**12**	**Schofield,**	**119420**	**pin**	**; dry outcrop**	**UBC**	**Schofield,**	A86	Har	
USA	OR	Wasco	2008	3	23	Shevock, J	31598	pin	Junipe On soi	CASC	Toren, Dav	A16	Hpc	
USA	OR	Josephine	1991	4	17	G.B. Stral	6424	pin	on moist soil	UBC	Schofield, '	A36	Hd	
Canad	BC	*Vancouver Is*	1996	6	24	Djan-Chek	2149	pin	gently sloping	UBC	Djan-Cheka	A39	Hd	
USA	CA	Santa Barba	2008	11	17	Shevock, J	32577	pin	Querc On soi	CASC	Toren, Dav	A41	Hca	
USA	CA	Riverside	1977	4	24	Harpel, Ju	17a	pin	splash On roc	CASC	Judy Harpe	A63	Hxx	
USA	CA	Mariposa	2006	10	21	Shevock, J	29175	pin	Querc On gra	CASC	Shevock, Ja	A82	Har	
USA	CA	El Dorado	2017	10	5	Shevock, J	50902	pin	Mixed conifer	CASC	Toren, Dav	A83	Har	
Kazak	***East Kazakhstan***		***1995***	***7***	***0***	***N. Bryant***	***2041***	***aureum***		***MO***		*A01*	*Hau*	
Algeri	***Batna***		***1985***	***4***	***9***	***Frahm, Ja***	***844515***	***aureum***		***MO***		*A04*	*Hau*	
Morrocco			***1997***	***3***	***17***	***Cano, M.J., Gallego, M.T.***		***aui***	***Roca caliza, q***	***MO***	***Jimenez, J.***	*A06*	*Hau*	
Mexic	Baja		2009	1	15	Carter, Be	3290	pinnatifidum		SJSU	***det. Carter***	A10	Hp	***ISU***
USA	CA	Santa Barba	2017	3	10	Shevock, J	50111	pin	Sandstone ro	CASC	Toren, Dav	A11	Hpc	
Canad	BC		2012	10	3	Golinski, K	7433	pin	Upper beach	UBC		A19	Hp	
USA	CA	Kern	1995	10	28	Shevock, J	12495	pin	In a pi On bas	CASC	Norris, Dar	A20	Hre	
USA	CA	Alameda	1988	3	18	Bourell, M	3446	pin	Dry creek nea	CASC	Toren, Dav	A21	Hre	
USA	CA	Marin	2007	12	2	Carter, Be	2168	pinnatifidum		SJSU	det. Carter	A32	Hd	JSU
USA	**CA**	**Butte**	**2015**	**1**	**7**	**Shevock, J**	**46117**	**pin**	**Riparian woo**	**CASC**	**Toren, Dav**	A35	Hd	
USA	OR	Hood River	2000	10	9	Schofield,	116734	pin	Grassy open	UBC	Schofield, '	A37	Hd	
USA	MT	Ravalli	1995	9	15	Merrifield	1312	pin	On shaded cli	CHSC		A38	Hd	CH
USA	WA	San Juan	1993	8	16	Harpel, Ju	9206	pinnatifid	on a r	CASC	Judy Harpe	A3B	Hd	
USA	CA	Yuba	2015	2	3	Janeway,	11606	pin	sloping On ricl	CASC		A46	Hca	
USA	CA	San Luis Obi	2007	5	4	Carter, Be	1834	pinnatifid	Along	SJSU	det. Carter	A52	Hob	JSU
USA	CA	Fresno	2009	3	26	Shevock, J	32677	pin	In rock Granit	CASC	Shevock, Ja	A53	Hob	
USA	WA	Klickitat	2002	4	12	Schofield,	119428	pin	; cliff face	UBC	Schofield, '	A57	Hob	
USA	WA	Jefferson	2022	8		Reeber, La	22080701	nut	in fron on roc	SJSU		A62	Hnu	
USA	CA	Trinity	2009	7	2	Shevock, J	33067	pin	Mixed On tru	CASC	Shevock, Ja	A73	Hne	
USA	ID	Idaho	1999	9	16	Schofield,	113743	pin	; dry shelf of	UBC		A74	Hae	
USA	ID	Idaho	1999	9	16	Schofield,	113759	pin	; exposed bou	UBC		A75	Hne	
USA	OR	Hood River	2000	10	9	Schofield,	116728	pin	Grassy open	UBC	Schofield, '	A76	Hae/Hr	
USA	OR	Clatsop	1995	7	25	Bourell, M	5354	pinnatifidum		CASC		A77	Hne	
USA	***WA***	***San Juan,Wa***	***1993***	***9***	***8***	***Harpel, Ju***	***9554***	***pin***	***on soil in the***	***UBC***	***Joya, Stev***	A78	Hae	
USA	WA	San Juan,Wa	1993	9	8	Harpel, Ju	9551	pin	on a sandstor	UBC		A79	Hae/Hr	
USA	CA	Alameda	1996	4	3	Whittemo	5455	pin	in part stony	CASC	A. T. W.	A81	Har	
USA	OR	Hood River	2000	10	9	Schofield,	116730	pin	Grassy open	UBC	Schofield, '	A85	Har	
USA	ID	Boundary	1978	6	19	Anderson,	22603	pin	Exposed rocks	CASC		urn:cat	Hae	