Campylopus schmidii surveys 2016

Siuslaw National Forest: Heceta Dunes, Cape Perpetua and South Fork Jetty Umpqua Dunes

Report to ISSSSP March 2018

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Introduction

Campylopus schmidii is currently designated as Sensitive by the USDA Forest Service Region 6. It has been reported on moist, low nutrient habitat along the Oregon coast at the Heceta Dunes on both BLM and USFS parcels, an area east of the Darlingtonia wayside, and from Cape Perpetua. The first Oregon report is from 1933. It is assumed that spores of female plants were transported on air currents from Hawaii or possibly Asia. It has also been suggested that humans brought spores during migration events.

A species that looks very similar to *C. schmidii* is *Campylopus introflexus*, an invasive species first recorded from the west coast of North America in 1975. It occupies widely varying habitats including that of *C. schmidii.* This species is rapidly expanding its range on the coast, in the Willamette Valley and sites as far east as the west slope of the Cascade mountains in Oregon. *Campylopus introflexus* should be easy to identify because it has awns that are reflexed at 90 degrees when dry. However, some have few reflexed awns and these only on lower parts of the stems, making differentiation from other *Campylopus* species difficult.

Concerns that non-native vascular plant establishment, competition from *Campylopus introflexus*, rapidly changing dune conditions, and disturbance from off-road vehicles (OHV) may be having deleterious effects on *C. schmidii* populations prompted a revisit of sites documented by David Wagner, Scot Loring and John Christy.

Methods

In August 2016 we surveyed the previously reported sites and expanded the search area using the intuitive controlled method (Fig. 1). The surveyors were bryologists Amanda Hardman, Daphne Stone and David Kofranek. Areas we searched were Siuslaw Forest land north of Heceta Dunes ACEC and Cape Perpetua; the South Jetty of the Siuslaw River in the Oregon Dunes National Recreation Area; and close to the historical site on private land and Siuslaw National Forest land near Darlingtonia Wayside. Most of our collections consisted of material that lacked reflexed awns, since we were searching only for *C. schmidii*. We collected small samples of material with reflexed awns that we believed to be *C. introflexus* in order to make microscopic comparisons to plants with straight awns.

For microscopic examination, individual leaves were mounted and examined with a compound microscope. For examination of the costa, thin slices of the costa were made from various positions on the leaf, and examined with a compound microscope.

DNA extractions were conducted with MPBio DNA fastprep spin kit. Due to high concentrations of salts on the specimens, modifications were made to the extraction process. Prior to DNA extraction, specimens were soaked for 10 minute in 500ul molecular grade water with gentle agitation then rinsed. This process was repeated two times for each specimen. During DNA extraction, the ethanol wash incubation time was increased to 5 minutes. The ethanol wash aids in further desalting and removal of inhibiting detergents and impurities from the DNA extract. PCR amplification was performed with Thermo-Scientific Dream Taq Green reaction mix per the manufacturer's

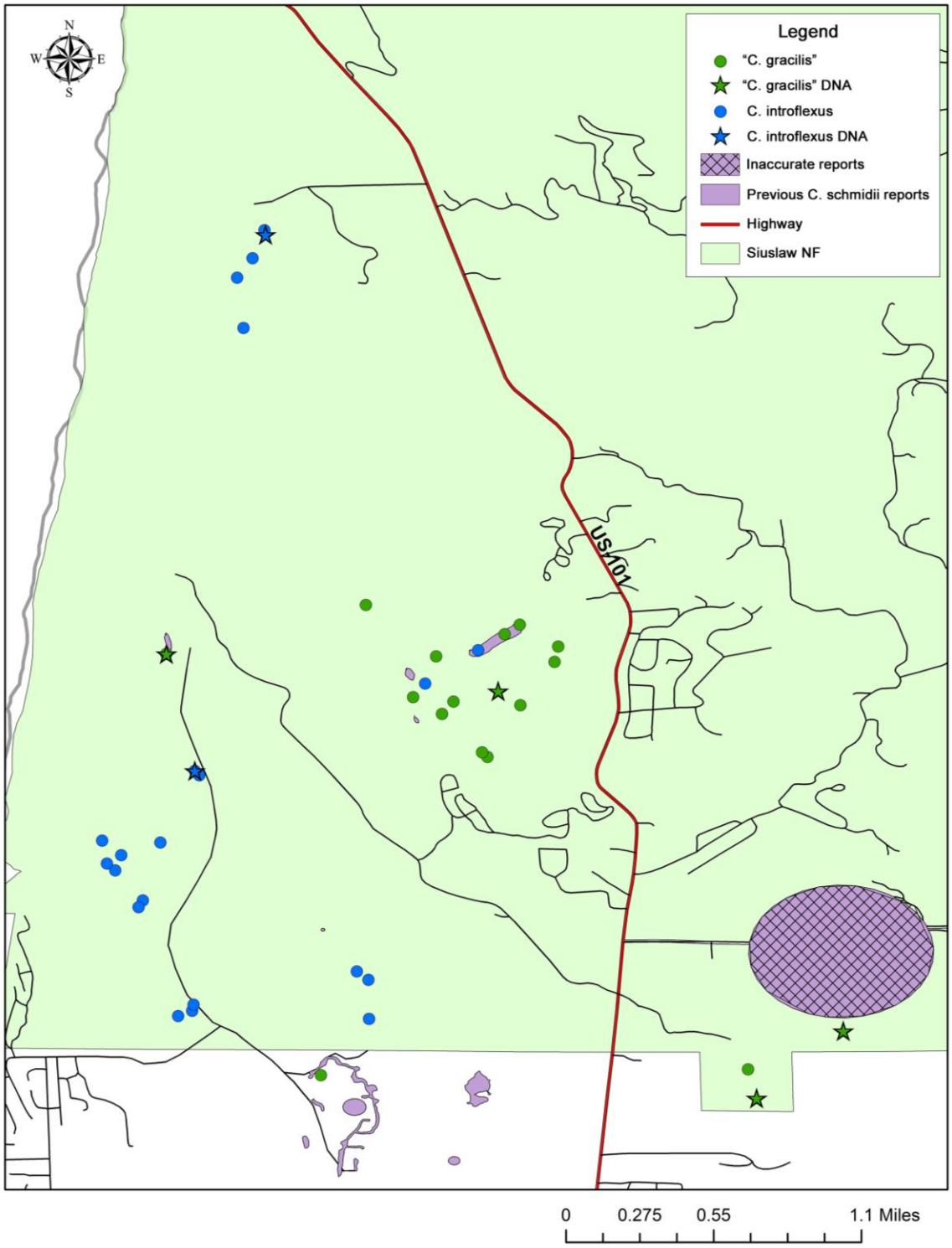

Figure 1. Sites of collected and identified *Campylopus* (Appendix 1). Green and blue stars show the sequenced samples. The map area is north of Florence, OR.

instructions. We used a single locus for PCR amplification: the ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') universal primer (White et al. 1990).. The PCR reaction chemistry is set up as follows: 15 µl of Green Taq, 12.8 µl DI water, 1.2 µl of primer solution, and 1 µl of DNA extract, multiplied by the number of samples (McCune et al. 2010). Due to poor PCR amplification results, a 10:1 dilution was made with 1 µl of pure DNA extract and 10 µl of molecular grade H_2O. Thermal cycle protocol begins with a hot start at 3 minutes at 94 °C, then 40 cycles at 1 minute at 94 °C, 1 minute at 52 °C, 2 minutes at 72 °C, with one extension cycle for 10 minutes at 72 °C, then a holding temperature of 4 °C for infinity. Due to poor DNA amplification, several PCR temperature gradients were performed to isolate an appropriate annealing temperature. The protocol was 3 minutes at 94 °C, then 40 cycles at 1 minute at 94 °C, 1 minute at 46-56 °C, 2 minutes at 72 °C, with one extension cycle for 10 minutes at 72 °C, then a holding temperature of 4 °C. The product was transferred to a 1% gel with the addition of Gel Red dye; the gel was placed in a electrophoresis apparatus in a 1% TAE buffer solution and ran for approximately 45 min at 40 volts. The PCR product was prepped for sequence with Affy-Metrix EXOSAP-IT two step kit. The protocol was 3 µl of PCR product to 1 µl of EXOSAP-IT. The thermal cycle protocol was 15 minutes at 87 °C and 15 minutes at 37 °C. Sequence chemistry was 8.8 µl of nuclease free DI water, 1.2 µl of sequence primer, and 1 µl of Exo-Saped PCR product.

Sanger sequencing was performed by Eurofins, raw sequence data was received in zip files and uploaded to the sequence program Geneious R10 (2011-2017). All ITS sequences were assembled using De Novo method and trimmed manually. Once trimmed and aligned, a consensus sequence was generated and was ready for downstream applications. Sequences were loaded into Genbank and compared against the sequence database. Samples DS 9566, AH6214, AH6216, and AH6203 blasted to *Campylopus gracilis* at approximately 98-99% identity. Samples DS9559 and DS 9568 blasted to *Campylopus introflexus* at approximately 99% identity.

Results and Discussion

Morphology

The genus *Campylopus* in Flora of North America was written by the late Jan-Peter Frahm. In his key, the only character used to distinguish *C. introflexus* is awns, reflexed at 90°. Reviewing the descriptions of *C. introflexus* and *C. schmidii*, only 5 vegetative characters other than reflexed awns appear to be different between the two species (Table 1). Unfortunately Frahm did not specifically compare the two apparently similar species, although he did mention their similarity. Besides the reflexed awns, presence of sporophytes can be used to differentiate between the two species, since sporophytes have not been found on *C. schmidii* in North America. Although sporophytes are a clear indication of *C. introflexus*, individual plants of *C. introflexus* with sporophytes are often found growing in a patch of *Campylopus* without reflexed awns. This makes it difficult to determine what species is present, making field or laboratory identification more difficult.

Table 1. Comparison of characters differing between *Campylopus introflexus* and *C. schmidii*, taken from Flora of North America Vol. 27.

	C. introflexus	*C. schmidii*
chlorophyllose laminal cells	shortly rectangular to oblique	oval to narrow or elongate oval
alar cells	absent or thin-walled hyaline to reddish inflated cells	hardly differentiated
costa lamellae (ribs)	1-2 cells high	ribbed (no mention of rib height)
basal laminal cells	extending higher at margins and forming a V-shaped area	no mention of extending up margin, but illustration from FNA shows a slightly V-shaped wide group of hyaline cells
tomentum on stem	present or almost absent	no mention of tomentum

All of the specimens that did not have obviously reflexed awns or capsules were examined by making cross-sections of leaves and mounting whole leaves. The five characters shown in Table 1 were examined. Although each of these characters varied quite a lot, no specimens were found that were clearly not *C. introflexus*.

The results of these examinations are as follows:

1. The chlorophyllous leaf lamina cells were mainly oval to irregularly lumpy oval. In a few cases some lower cells had clearly square corners, not rounded. The majority of the cells were oval or irregular, with rounded corners.

2. Alar cells varied from reddish to pale green. The cell walls were nearly always thick and were in most cases inflated in a small alar patch on some but not all leaves from one stem. In many cases, the cells were approximately twice as wide as adjacent cells and not hugely inflated. However, the description of the alar cells of *C. schmidii* says "hardly differentiated" and we felt that these cells, even though not exactly thin-walled and inflated, were clearly different.

3. The lamellae on the back of the costa were clear on all specimens, but most sections from any group of leaves sectioned had lamellae only 1 cell high. These cells varied from having an even, smooth cell wall, to having a large papilla on top, to having minute papillae or possibly cuticular ridges that showed up as small crenulations along the top of the distal lamellar cell. Very few definitely 2-celled lamellae were seen.

4. The V-shaped patch of hyaline cells varied in shape from long and narrowly V-shaped to wide with a narrow extension of hyaline cells along the margin. Wider hyaline patches usually were on leaves that were wider and near the top of the stem, apparently perigonial or perichaetal bracts.

5. All specimens examined had tomentum to varying degrees.

The morphological investigation appeared to indicate that none of our collections were different from *C. introflexus*, except for the lack of recurved awns and sporophytes. We noted that new growth at the tip of some shoots had straight awns, while recurved awns only appeared low on the stems in the previous year's growth. If recurved awns are not always present on every plant, there appears to be no way to distinguish between the two species. Our observations led us to wonder if what we have been calling *C. schmidii* for over 60 years is actually *C. introflexus* without recurved awns.

Genetics

To test this theory we decided to investigate the species genetically. First attempts to sequence the DNA from collections that were made in 2016 along with specimens collected by Marty Stein in 2006 and John Christy in 2008 failed. On a second attempt, we found that rinsing salt from the specimens before extraction improved our results. Six collections resulted in 98-99% matches to ITS sequences on GenBank. Of these, all specimens with reflexed awns were identified as *C. introflexus*. To our surprise, all sequenced specimens with straight tips were identified as *C. gracilis*, and none were at all close genetically to *C. schmidii* (Table 2). Unfortunately, the one specimen we had available that was identified by Frahm (Christy 1456) did not provide useable DNA.

Table 2. Locations and collection numbers of successfully sequenced specimens.

Campylopus, successful sequences							
coll.	**coll. number**	**ITS1&4, 98-99%**	**leaf tips**	**State**	**County**	**UTM E**	**UTM N**
Daphne Stone	9559	introflexus	some leaves bent	OR	Lane	0410161	4878799
Daphne Stone	9566	gracilis	straight	OR	Lane	0410048	4879504
Daphne Stone	9568	introflexus	bent	OR	Lane	0410510	4882020
Amanda Hardman	6214	gracilis	straight	OR	Lane	411477	4879261
Amanda Hardman	6216	gracilis	straight	OR	Lane	0412900	4877185
Amanda Hardman	6203	gracilis	straight	OR	Lane	410468	4881895

Campylopus gracilis is known in North America only from British Columbia. This species has a very wide costa that occupies 3/4 or more of the leaf width and has conspicuous hyaline alar cells. Our specimens of "*C. schmidii*" have neither of these easily observed characters.

In conclusion, what we have been calling *C. schmidii* since 1933 is not *C. schmidii* nor is it closely related to that species. Instead it is a species closely related to, but definitely not, *C. gracilis*. Our identification of *C. introflexus*, the species quickly invading the Pacific Northwest, is correct, matching published sequences of this species well. In the following paragraphs what we previously called "*C. schmidii*" will now be called "*Campylopus* sp. 1"

Conclusions

This project leaves the main question of our study unanswered: what species do we have on the coastal dunes that appears to be rare? The answer will be revealed when DNA sequences of this species can be compared extensively with other *Campylopus* species world-wide (which are currently lacking in Genbank), and morphological characters are identified that distinguish it from other species. It is possible that it is an undescribed species.

Population Stability

Population stability is difficult to assess when it is not always possible to positively identify the species in the field. However it appears that most populations of *Campylopus* sp. 1 on Forest Service land have not decreased since previous surveys. Many new patches were found in the area south of Alder Dune and north of Sutton Creek.

The population reported from east of the Darlingtonia Wayside is either gone or not relocated. The location given was on private land that is now a muddy horse pasture. It is possible that the population exists on other private land that we were not able to access. Also, since the site is a fairly old report, the location may be inaccurate.

No *Campylopus* sp. 1 was found at the previously reported site on the upper trail at Cape Perpetua. *Campylopus subulatus* was located in several spots, mostly on or near rock. It is unclear if Loring mistakenly identified this species as *Campylopus* sp. 1 or if a population of the target species has disappeared.

At South Jetty, the location of Christy's 1991 site yielded no *Campylopus* sp. 1. Because the location may have been somewhat inaccurate, a large area was searched, including an area approximately 100 ft. east of the road, as described by Christy as the location. *Campylopus introflexus* was abundant but no *Campylopus* sp. 1was seen.

Threats

The main threat to *Campylopus* sp. 1 remains OHV use of the dunes. It is clear that dune buggies disturb patches of *Campylopus*, as seen in Fig. 2, taken at Heceta Dunes ACEC just south of our study area. Counteracting this type of disturbance is the slow but steady encroachment of forest onto low-lying sandy areas adjacent to dunes. The encroachment of vascular vegetation is closing some paths to OHV use. In these areas, *Campylopus* sp. 1 appears stable and undisturbed in large patches (Fig. 3). It is able to survive in partial shade under the pine forest. However if succession continues these populations may be shaded out.

Fig. 2. *Campylopus* sp. 1 population around young trees on margin of open dune on Heceta Dunes ACEC. ATV tracks fracture the even carpet. Photo by Daphne Stone.

Fig. 3. Large patches of DNA-confirmed *Campylopus* sp.1 persist in areas on forest edges and along paths. Photos by Amanda Hardman.

Habitat improvement

Much of the habitat for *Campylopus* sp.1 remains stable. Although trees are invading areas that were once very open deflation plains, this natural succession continues to provide habitat for *Campylopus* sp.1 along its margins. Several improvements could be made.

1. Close population sites to ORV use. This is difficult to monitor and enforce so it may not be successful.

2. Remove Scot's broom and gorse from deflation plains. Scot's broom was planted along the coast to stabilize dunes, and has spread throughout the area. This species threatens to cover deflation plains and other low-lying areas. Gorse is also spreading slowly into this dune complex.

3. Remove *Ammophila arenaria* from low-lying areas where *Campylopus* sp.1 is found. *Ammophila* is mostly found on loose sand, not low-lying more stable sand that is the habitat for *Campylopus* sp.1.

References

Frahm, J-P. 2007. Campylopus *in*: Flora of North America Vol. 27. Oxford University Press, New York. Pp. 366-376.

Acknowledgements

We appreciate the ISSSSP funding of this project as well as the gracious patience on their part while waiting for our report.

Appendix 1. Campylopus collection sites (NAD 1983).

Species	LAT	LONG
C. introflexus	44.061585	-124.109205
C. introflexus	44.043858	-124.121764
C. introflexus	44.056614	-124.121379
C. introflexus	44.051469	-124.125912
C. introflexus DNA confirmed	44.056851	-124.121617
C. introflexus	44.051831	-124.126353
C. introflexus	44.053077	-124.12661
C. introflexus	44.086136	-124.117866
C. introflexus	44.063391	-124.106352
C. introflexus	44.084619	-124.118505
C. introflexus	44.083564	-124.119335
C. introflexus	44.043426	-124.112226
C. introflexus	44.043426	-124.112226
C. introflexus	44.080846	-124.118996
C. introflexus	44.045537	-124.112258
C. introflexus	44.045993	-124.112883
C. introflexus	44.043574	-124.122513
C. introflexus	44.049841	-124.124418
C. introflexus	44.049466	-124.12465
C. introflexus	44.044192	-124.121686
C. introflexus	44.052286	-124.125583
C. introflexus	44.052975	-124.123478
C. sp 1	44.064767	-124.104105
C. sp 1	44.06426	-124.104921
C. sp 1	44.04037	-124.114819
C. sp 1	44.065841	-124.112395
C. sp 1	44.060851	-124.109856
C. sp 1	44.059939	-124.108292
C. sp 1	44.060606	-124.107677
C. sp 1	44.063058	-124.108608
C. sp 1	44.063591	-124.102031
C. sp 1	44.062758	-124.102226
C. sp 1 DNA confirmed	44.063186	-124.123154
C. sp 1	44.060411	-124.104073
C. sp 1	44.057607	-124.105847
C. sp 1	44.057861	-124.106135
C. sp 1 DNA confirmed	44.061171	-124.105269
C. sp 1	44.040687	-124.091801
C. sp 1 DNA confirmed	44.042766	-124.086667
C. sp 1 DNA confirmed	44.039119	-124.091343