

Les Mehrhoff Botanical Research Award Report
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“Targeted surveys for *Salicornia maritima* in downeast Maine”



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Executive Summary

Salicornia maritima Wolff & Jeffries (Amaranthaceae) is a halophytic annual restricted to the upper latitudes of the east coast of North America. Historic collections and surveys have placed *S. maritima* in the Maine flora, where it has been recorded from Hancock, Knox, Lincoln, and Washington Counties. Due to a complicated taxonomic history, obscure defining morphological characteristics, and the difficulty in creating voucher specimens for this succulent species, these records of occurrence in New England have mostly been disqualified by present-day experts, with field study deemed necessary to confirm population occurrences.

In order to clarify the status of *S. maritima* in the New England flora, surveys of ten sites in Maine and New Brunswick were completed in late summer and early fall of 2023. Subsequent morphological study of field and herbarium specimens failed to definitively identify occurrences of *S. maritima*, though some individual specimens displayed characteristics attributed to both *S. maritima* and the common *S. depressa*. Further field research, paired with laboratory methods such as flow cytometry, is recommended in order to provide more definitive insight into this cryptic taxon.

Introduction

The genus *Salicornia* has a complicated taxonomic history, due to population inbreeding and phenotypic plasticity, which results in subtle to nearly invisible morphological differentiations between species (Kadereit et al., 2007). In the Northeast region of North America, there have been at least three and no more than five species of *Salicornia* identified since the implementation of Europogenic botanical methods in the United States (Wolff & Jeffries, 1987). In New England, the status of some species of *Salicornia* is unclear, due to the previously described difficulties in identification in both field and laboratory settings. The relatively recently described *Salicornia maritima* Wolff & Jeffries is one such species. As a taxonomic descendent of *Salicornia europaea* L., *Salicornia europaea* var. *prostrata* (Pall.) Fernald, *Salicornia prostrata* Pall., *Salicornia herbacea* (L.) L.—all of which have been identified on New England voucher specimens from Maine since the 1830's—its status in the New England flora has been, in effect, “grandfathered” in, but not effectively confirmed through field study (A. Haines, pers. comm.).

As a result, its status in the state of Maine is unclear, and it currently holds a SH (State Historic) ranking in Maine (Maine Natural Areas Program, 2021). In Canada, where the species was first described, *S. maritima* is listed as S5 (Secure) in New Brunswick, S4 (Apparently Secure) in Prince Edward Island and Ontario, and S3 (Vulnerable) in Quebec (NatureServe Explorer, 2016). However, present-day Canadian natural heritage staff do not feel confident in their correct identification of this taxon (S. Blaney, pers. comm.)

At present and as delineated by the *Flora Novae-Angliae*, the New England flora contains four separate taxa within the genus *Salicornia*: *ambigua*, *biglovii*, *depressa*, and *maritima* (Haines 2011). *Salicornia ambigua* is easily separated from the others by its perennial habit (as such, it is often described in a separate genus, *Sarcoconia* A.J. Scott), and *Salicornia biglovii* has greatly thickened inflorescences, flowers concealed by bracts, and prominent mucros on the leaf apices (Haines 2011). *Salicornia depressa* and *S. maritima* are much more difficult to tell apart. There are three known dichotomous key sources available that provide characteristics for separating the two species: the original description paper for *Salicornia maritima*, Wolff & Jeffries 1987; Roland's *Flora of Nova Scotia*, 1998; and Haines' *Flora-Novae-Angliae*, 2011 (Table 1).

Table 1: Comparison of dichotomous keys that include *S. maritima*, and the characteristics separating it from *S. depressa* or the synonymous *S. europaea*.

Source	<i>S. depressa</i> Standl. / <i>S. europaea</i> L.	<i>S. maritima</i> Wolff & Jeffries
Wolff & Jeffries 1987	Terminal spike and branches cylindrical to extremely tapered, fertile segments tubular in shape, (5-)7-23(-25) fertile segments in the terminal spike, profuse exertion of stamens, scarious border 0.3-0.4 mm wide, tetraploid	Terminal spike and branches swollen and rounded at tip, fertile segments distinctly widest from the midsegment to the top, 2-10(-14) fertile segments in the terminal spike, exertion of stamens rare, scarious border of less than 0.3 mm wide, diploid
Roland 1998	Terminal axis cylindrical to extremely tapered; fertile segments tubular; stamens profusely exerted (protruding)	Terminal axis and branches swollen and round at tip; fertile segments widest from mid segment to top; stamens rarely exerted
Haines 2011	Inflorescences cylindric to long-tapering, the terminal with (5-)7-23(-25) fertile segments; scarious margin of leaves 0.3-0.4 mm wide; flowers with exerted stamens; fertile segments cylindric	Inflorescences swollen and rounded near apex, the terminal with (3-)5-10(-14) fertile segments; scarious margin of leaves narrower than 0.3 mm; flowers not exerting stamens, or sometimes, but then after dehiscence; fertile segments widened in the apical portion

Of the characteristics listed in Table 1, only the scarious leaf margin characteristic is reliable and measurable at all times of year; the characteristics describing inflorescence shape and apex form rely on a subjective analysis best informed by comparative study, and the extent of stamen exertion is only visible during a brief period in an individual's life cycle, which may not occur at the same time across regional populations. Stamen filaments may be observable for some time, but appear to quickly wither on some specimens (Appendix A, Photo 5), and per Haines 2011, may be exerted for both *S. depressa* and *S. maritima* after dehiscence. Furthermore, the succulent nature of *Salicornia* creates difficulties in producing useful herbarium voucher specimens; as the plant dries, many morphological features are shrunk or desiccated, and characteristics that are visible on fresh specimens may be lost (Kadereit et al., 2012).

The original species description for *S. maritima* Wolff & Jeffries identifies chromosomal differences between this taxon and *S. europaea* (Wolff & Jeffries 1987).¹ *S. maritima* is described as diploid, and *S. europaea* is described as tetraploid. There are currently no reliable field techniques for assessing ploidy level, and ploidy may only be determined through laboratory methods such as flow cytometry (requiring specialized lab equipment), or microscopic analysis of stomae, stoma guard cells, or pollen grain size (requiring specialized materials and microscopy techniques) (Kadereit et al., 2012).

¹ *Salicornia europaea* L. is currently synonymous with both *S. depressa* Standl. and *S. maritima* Wolff & Jeffries (ITIS 2023). For the purposes of this study, the tetraploid *S. europaea* referenced in Wolff & Jeffries 1987 and Roland 1998 will be treated as *S. depressa* Standl. For a thorough taxonomic history of this complex, see Wolff & Jeffries 1987.

Therefore, a comprehensive field study across *Salicornia* populations in Maine and New Brunswick must focus on the scarious leaf margin width for specimen identification as the most practical and reliable field characteristic, with anecdotal observation of fertile segment form and thickening used as a confirming characteristic.

Methods

Survey sites in Maine and New Brunswick were selected based on overlay of mapped *Spartina* Saltmarsh habitat with conserved lands (i.e., land trust preserves and state or province properties), and were selected to provide the greatest possible geographic range for this study (Fig. 1).

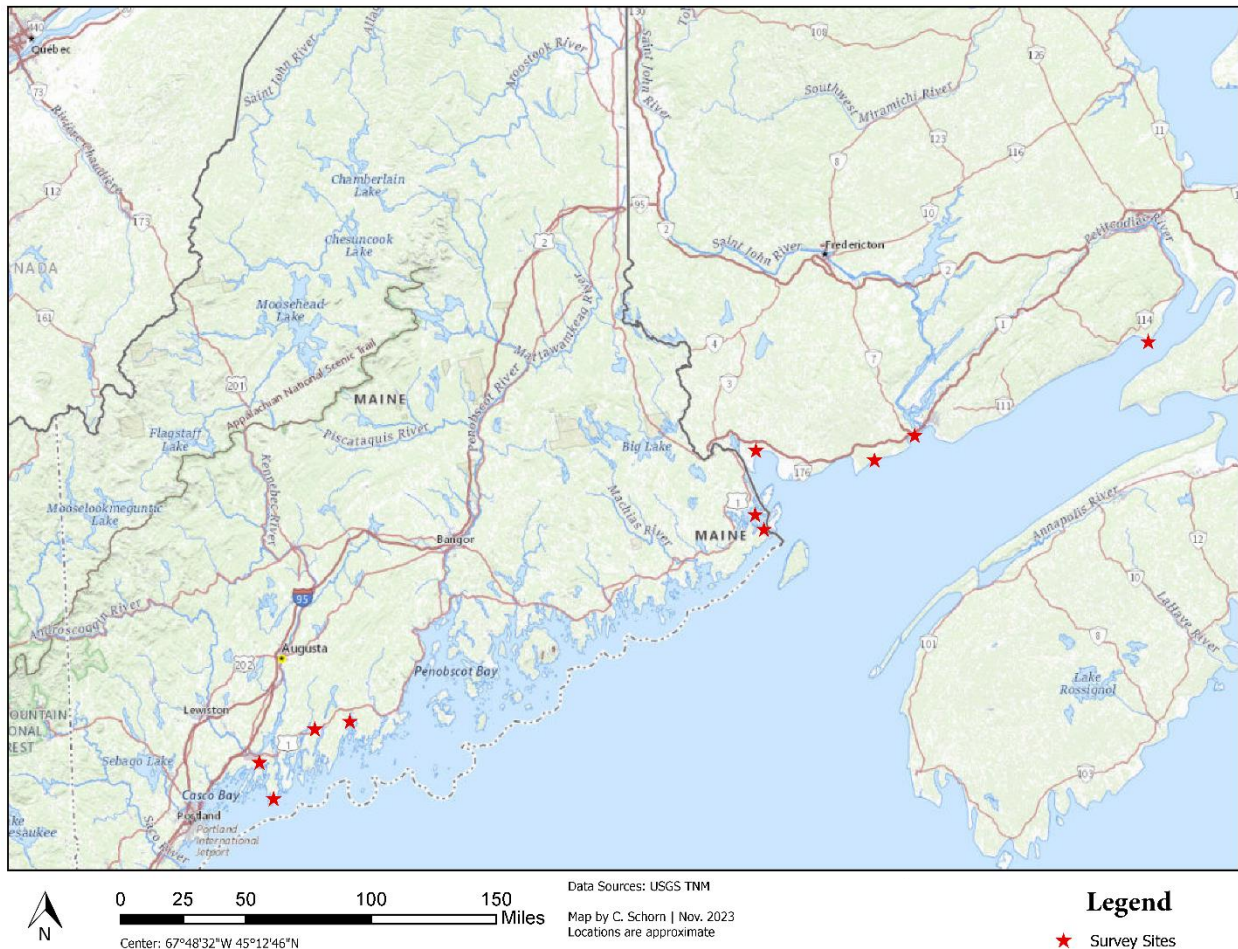


Fig. 1: Map of selected survey sites.

Field survey paths were selected to cover the greatest extent of the accessible saltmarsh habitat. Where *Salicornia* spp. were observed, photographs were taken of the general field setting and gestalt. No more than three specimens (as defined by genets) were taken from any one site for microscopy and/or voucher preparation, and only after landowner permission had been granted.

Microscopy was performed on the same day as the specimen collection with an Amscope Trinocular Stereo Zoom (7x-45x). A millimeter scale ruler was placed adjacent to specimen material, and

photographs taken with a Samsung Galaxy A32 mounted to the eyepiece. Photographs were analyzed using ImageJ Version 1.54d; scale was set to 1 mm using the scale ruler visible in the photograph, and all visible and in-focus scarios leaf margins in each photograph were measured to two decimal places.

Findings

Results

From 3 August to 9 October, 2023, a total of ten sites were visited in Maine and New Brunswick (Table 2). From those sites, a total of 468 photographs were taken of *Salicornia* spp. habitat, gestalt, and specimens. Three voucher specimens were collected and will be deposited in the NEBS herbarium. Of the 468 photographs, a total of 114 were usable for imaging analysis of the scarios leaf margin width, representing an estimated 14 separate plant genets. Of these 14 genets, two had an average ($n=8$, $n=6$, respectively) scarios leaf margin width of less than 0.3 mm, and twelve had an average scarios leaf margin width above 0.3 mm. However, these two specimens did not display thickened fertile segments that were widened in the apical portion (Appendix A, Photo 7). Conversely, specimens that did ostensibly display thickened fertile segments had leaf margins wider than 0.3 mm, corresponding with *S. depressa* (Appendix A, Photo 8).

Table 2: Survey sites.

Site ID	Date Surveyed	Town	State/Province	<i>Salicornia</i> Observed
1	3 August 2023	Lubec	Washington Co., Maine	Yes
2	3 August 2023	Lubec	Washington Co., Maine	Yes
3	26 August 2023	Bocabec	New Brunswick, Canada	Yes
4	26 August 2023	Chance Harbour	New Brunswick, Canada	Yes
5	26 August 2023	St. John	New Brunswick, Canada	Yes
6	27 August 2023	Fundy Albert	New Brunswick, Canada	Yes
7	16 September 2023	Newcastle	Lincoln Co., Maine	No
8	24 September 2023	Phippsburg	Sagadahoc Co., Maine	Yes
9	7 October 2023	Waldoboro	Lincoln Co., Maine	Yes
10	9 October 2023	Brunswick	Cumberland Co., Maine	Yes

Author's Notes

Identification of *Salicornia* specimens in a field setting, using the taxonomic keys available, was difficult. A sub-millimeter ruler and a 20x hand lens are necessary to confirm the scarios leaf margin width, which is the only measurable characteristic that separates *S. maritima* from *S. depressa*. There was an incredibly wide plasticity of forms in *Salicornia* individuals, seeming to correlate most closely with the habitat the individuals were found in; individuals were most frequently found in or along the margins of open pannes—where they were thicker and more red-colored earlier in the season (Appendix A, Photos 13 & 14)—or growing within thatches of *Spartina patens*, where they grew in prostrate forms that were thinner, longer, and red-colored later in the season (Appendix A, Photos 10, 11, & 12). This differentiation in forms as a response to shade and plant density has been reported in other *Salicornia* species from Europe (Davy et al., 2001). The generalized form of *Salicornia* individuals seemed to change throughout the seasons as well; fertile segments naturally grow thicker and more red towards the end of the year, but this was not consistent across populations at any given time. Furthermore, anther exertion was only definitively observed in the very beginning of August, and not consistently across a population of otherwise very similar-looking individuals (Appendix A, Photo 2). Given the plasticity of forms within this complex, two characteristics given by Roland 1998 and Haines 2011—general form of

fertile segments, and exertion or non-exsertion of anthers—are subjective and/or constrictively time-limited.

The author of this study visited the Harvard University Herbarium on 2 June 2023, and reviewed all vouchered specimens identified as *Salicornia maritima* collected from New England and Canada. The difficulty of preparing *Salicornia* specimens was evident, and many key morphological characteristics were not discernable to the author's eye at the time (though future additional examination, after the lengthy review of fresh material this field season, may produce new insights). The use of the Harvard University Botany Libraries was useful for accessing resources such as Roland's Flora of Nova Scotia and older editions of Gray's Manual of Botany, and generated valuable research leads.

The possibility exists, however, that the author never observed any true *S. maritima* individuals at all. It may be that the characteristics listed in the available resources are extremely evident on *S. maritima* individuals, and that they are easily differentiated from *S. depressa* in a comparative analysis. In this case, the populations at the Canadian stations should be reviewed by other botanists, since the small population of *Salicornia* present did not illustrate all characteristics of *Salicornia maritima* (some scarious leaf widths were less than 0.3 mm wide, but fertile segments were not distinctively widened in the apical portion (Appendix A, Photo 7).), yet were identified as such in New Brunswick natural heritage records.

A population observed in July 2022 in Bass Harbor, Hancock Co., ME, was tentatively identified as *S. maritima* by Alice Palmer, on the basis of observed thickened apical portions of the fertile segments (A. Palmer, pers. comm.) Further exploration and imaging of this population can confirm a positive identification of *S. maritima*. Additionally, further research is being done by Palmer and the Brook Moyers Lab at the University of Massachusetts-Boston on *Salicornia depressa*, which may shed additional insight into this species complex or provide future opportunities for field survey of New England populations of *Salicornia* (A. Palmer, pers. comm.)

Although *Salicornia* was not observed at Site 7, a new occurrence of *Symphyotrichum subulatum* (S1 Endangered in Maine) was observed and will be reported.

Conclusions & Recommendations

To clarify the status of *Salicornia maritima* in the New England flora, further research is recommended. The present resources available for identification of *Salicornia maritima* are impractical in a field setting. Only one characteristic is measurable and usually preserved in dried material; all others either require subjective analysis of fresh material, serendipitous timing of phenological stage, or genetic testing. This is no criticism of the invaluable taxonomic research done to date, but is a problem inherent to this difficult genus, where genetically differentiated lineages may appear morphologically identical, or morphologically distinct accessions may have identical genotypes (Kadereit et al., 2012). One approach that may be fruitful is to perform a general collection of *Salicornia* specimens in New England and the Canadian maritime provinces for chromosomal analysis (e.g., flow cytometry) in a laboratory setting. Cross-referencing the subsequent ploidy level result—and more securely identifying the diploid *S. maritima* versus the tetraploid *S. depressa*—with an analysis of morphological features and thorough imaging of features may provide insight on how best to identify genetically distinct taxa within a *Salicornia* population of varied forms, and establish the presence or absence of *Salicornia maritima* in the New England flora.

It is recommended that botanists preparing voucher specimens of *Salicornia* include any germane field observations to their collection labels, such as anther presence, specific descriptions of fertile segment shape, or growth form. Printed photographs may also be useful for inclusion in voucher packets.

If the results of this study are purely a result of not having observed true *S. maritima* specimens, then future study should focus on targeting populations earlier in the year, when stamens may be more reliably exerted (or not). Survey efforts should seek to target as many populations as possible within a short window of time, in order to most accurately compare growth forms between populations within a given “snapshot” in phenological time. Additionally, there are many viable saltmarshes in Maine—especially in Hancock and Washington Counties—which were not surveyed in the course of this study, and which may yet harbor stations of *Salicornia maritima*.

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