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Research Article

Nitellopsis obtusa (Desv.) J. Groves, 1919 (Charophyta: Characeae): new records from southern Michigan, USA with notes on environmental parameters known to influence its distribution

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Abstract

Starry stonewort, *Nitellopsis obtusa* (Desv.) J. Groves, 1919, is deemed an invasive aquatic macroalga in North America where there is considerable concern about the impact of this species on lake ecology. During floristic inventories to characterize charophyte diversity in Michigan, we documented the occurrence of *N. obtusa* from seven inland lakes, confirming the expansion of this species into Cass and Van Buren Counties in the southwestern region of the state. We provide a description of the species along with morphological characters that distinguish it from other genera in the Characeae. Detailed voucher specimen information, including aquatic vascular plant associates, substrate type, pH, total alkalinity, and water depth, is presented for each collection site. Modes of dispersal and habitat parameters known to influence the distribution of *N. obtusa* are considered.

Key words: starry stonewort, charophytes, alkalinity, distribution, exotic species, zoochory

Introduction

Nitellopsis Hy, 1889, is one of six extant genera within the family Characeae. Members of the Characeae are submerged, macrophytic charalean green algae, commonly known as stoneworts and often referred to as charophytes (McCourt et al. 1996). Charophytes inhabit a wide variety of aquatic habitats (Wood 1965; Moore 1986; Martin et al. 2004) and play an integral role in community dynamics by providing food and refuge for invertebrates, fish, and waterfowl (e.g., Hutchinson 1975; Jeppeson et al. 1998; Van den Berg et al. 1998; Coops 2002). Historically, three extant species of *Nitellopsis* have been recognized: *N. bulbillifera* Donterberg, 1960, *N. sarcularis* Zaneveld, 1940, and *N. obtusa* (Desv.) J. Groves, 1919. *Nitellopsis bulbillifera*, originally collected from Argentina, and subsequently reported from New Mexico (Tindall et al. 1965), is likely conspecific with *Chara longifolia* C.B. Robinson, 1906 (see Proctor et al. 1967) and occurs in sporadic localities across the western United States and Canada (Mann et al. 1999). Garcia (1990) transferred Argentinian *N. bulbillifera* to the genus *Chara* Linnaeus, 1753, but as a novel combination *Chara bulbillifera* (Donterberg) Garcia, 1990. *Nitellopsis sarcularis* is only known from its type locality on the Indonesian island of Lombok (Zaneveld 1940). *Nitellopsis obtusa* is native to Eurasia where it is widely distributed from Europe to Japan (Wood 1965; Soulié-Märsche et al. 2002; Urbaniak 2003; Naz et al. 2010; Kato et al. 2014), but regionally red-listed as Near Threatened (NT) in Switzerland (Auderset Joye and Schwarzer 2012), Vulnerable (V) Finland (Koistinen 2010), Sweden (Johansson et al. 2010), and Britain (Stewart 2004), and Threatened I (CR+EN) in Japan (Japan Ministry of the Environment 2012).

Commonly known as starry stonewort, *Nitellopsis obtusa* was first reported in North America in 1978 when it was collected during a study of winter littoral

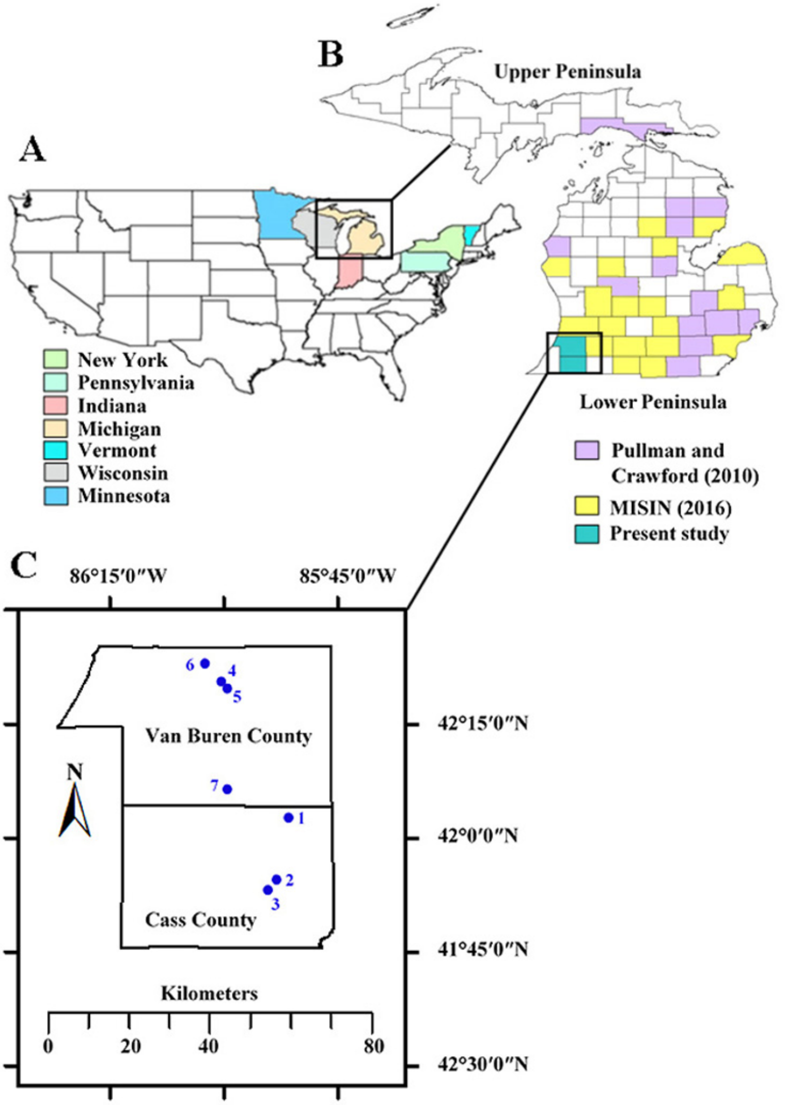

Figure 1. A. Distribution of *Nitellopsis obtusa* in the United States. B. Reported occurrences and extent of invasion of *N. obtusa* across Michigan. C. Geo-referenced localities of the first documented occurrences of *N. obtusa* in Cass and Van Buren Counties, MI; numbers represent site identification codes that correspond to those in Supplementary material Table S1.

vegetation in the St. Lawrence River near Goose Bay, Alexandria, New York (Geis et al. 1981). By 1983, it had extended its North American range into the Laurentian Great Lakes where its occurrence was documented from five locations along a 100 km stretch of the St. Claire-Detroit River system (Schloesser et al. 1986). Since its introduction, *N. obtusa* has spread across the Great Lakes region of the United States (Figure 1A) where it has been documented from 17 counties of New York (Sleith et al. 2015), 5 northern counties of Indiana (Eric Fischer, Indiana Department of Natural Resources, pers. comm.), 4 counties of Minnesota (K. Pennington, Minnesota Department of Natural Resources, pers. comm.) and Wisconsin (T. Plude, Wisconsin Department of Natural Resources, pers. comm.), and 2 counties of Pennsylvania (Pennsylvania Sea Grant 2016). In Michigan, the occurrence of *N. obtusa* was first confirmed in 2006, and by 2009 this species was observed in 26 lakes across 13 counties (Pullman and Crawford 2010). Based on sightings submitted to the Midwestern Invasive Species Information Network between 2012 and 2016, but excluding those presented herein, *N. obtusa* has been reported from an additional 19 counties across Michigan (MISIN 2016), bringing the total to 32 counties (Figure 1B). In addition, it has recently been confirmed from Orleans County in northern Vermont (A. Bove, Vermont Department of Environmental Conservation, pers. comm.) and in Presqu'ile Bay, Lake Ontario, Canada (Midwood et al. 2016).

In this paper, we document new site records of *Nitellopsis obtusa* collected from freshwater lakes in southern Michigan. We also present information on the morphological characters useful in distinguishing *N. obtusa* from other members in the Characeae. Notes on dispersal and habitat parameters known to be important in determining the distribution of this species both in its native European range and in North America are also discussed.

Methods

In August 2013, specimens of *Nitellopsis obtusa* were collected from several inland lakes in the Southern Peninsula of Michigan as part of a study to document charophyte diversity and their aquatic vascular plant associates. Charophytes and vascular macrophytes were collected either by hand or using a telescopic fan rake while wading in shallow water along the shores of public access sites. In deep water areas (i.e. > 1 m), charophyte sampling was carried out by tossing and retrieving a weighted, double-headed bow rake attached to 50 m of multi-filament polypropylene double braid dock line. Collection sites were geo-referenced with a Trimble® GeoXT™ handheld global positioning system receiver.

Where possible, charophyte and vascular macrophyte samples were identified *in situ*, using a 20× Bausch & Lomb Hastings Triplet hand lens. Samples that required greater magnification for proper identification were sorted by genus, placed in Whirl-Paks® and transported to the Aquatic Plant Herbarium of Purdue University Northwest, Westville, Indiana for further examination. Charophyte identifications are based on descriptions in Wood (1965), Moore (1986), and Urbaniak (2003). Taxonomy and nomenclature of associated aquatic vascular plants (Appendix 1) follow that of the Flora of North America Editorial Committee (1993+) for the families: Araceae (Thompson 2000), Ceratophyllaceae (Les 1997), Haloragaceae (Scribailo and Alix 2014), Hydrocharitaceae (Haynes 2000a), Najadaceae (Haynes 2000b), Nymphaeaceae (Wiersema and Hellquist 1997); with revisions of infrageneric designations within the genus *Nuphar* by Padgett (2007), Potamogetonaceae (Haynes and Hellquist 2000), and Pontederiaceae (Horn 2002).

Specimens of *Nitellopsis obtusa* were prepared and deposited in The Friesner Herbarium (BUT) at Butler University, Indianapolis, Indiana. Duplicates of *N. obtusa* and associated aquatic macrophytes have been retained at Purdue University Northwest. Images of each voucher specimen of *N. obtusa* were captured with a Nikon D 80 digital camera. Bulbils and axial nodes were placed in distilled water and photographed using a Nikon SMZ-U microscope fitted with a DS-Fi2 camera head.

Figure 2. Dense sample of *Nitellopsis obtusa* collected by retrieving a weighted, double-headed bow rake from the public access dock at Upper Jeptha Lake, Van Buren County, MI. Photograph by M.S. Alix.

Substrate type, water depth (m), pH, and total alkalinity (ppm $CaCO_3$) were determined *in situ*. Substrate type was based on visual inspection of the sediments within the population area. Water depth was measured by lowering a braided polyester line, weighted on one end and calibrated in 0.1 m increments. Subsurface pH was measured using an IQ Model 150 multi-parameter meter with a stainless steel ion-selective field effect transistor (ISFET) probe. A LaMotte alkalinity test kit (model DR-A) was used to determine total alkalinity.

Results

Nitellopsis obtusa was collected from seven inland lakes in Michigan (Figure 1C, Supplementary material Table S1). These specimens represent the first documented occurrences of this species from Cass and Van Buren Counties in the southern portion of the Lower Peninsula. We emphasize "documented" because most reports of this species in Michigan are based on observation rather than voucher specimens. The population discovered at Donnell Lake, Cass County is an estimated 145 km west of the nearest Michigan site reported by Pullman and Crawford (2010) and approximately 18 km north of the closest known population in Indiana, in Simonton Lake, Elkhart County.

The populations of *Nitellopsis obtusa* reported herein consist of largely monospecific localized dense stands near the public access sites (i.e. boat launches) associated with the lakes.

These sites contain robust individuals typically anchored in marl or sand, where a single rake cast and retrieval often resulted in tines completely filled with thalli (Figure 2). Five of the populations are associated

Figure 3. *Nitellopsis obtusa* collected from Fish Lake, Cass County, MI (scale = 5 cm). Photograph by R.W. Scribailo.

with mesotrophic lakes, whereas the remaining two are found in lakes classified as eutrophic (Table S1). The beds of *N. obtusa* are associated with alkaline surface waters, ranging from 152 to 184 ppm $CaCO_3$ (Table S1), suggesting that these are hard-water lakes. Measurements of pH vary between 7.8 and 8.4 (Table S1). The most frequently encountered aquatic vascular plants associated with *N. obtusa* were *Ceratophyllum demersum* (Linnaeus, 1753) and *Najas flexilis* (Willdenow) Rostkovius and W. L. E. Schmidt, 1824, whereas *Chara contraria* (A. Braun ex Kützing, 1845) was the most closely-associated charophyte.

Individuals of *Nitellopsis obtusa* have a moderately stout green to greyish thallus comprised of a central axis up to 1 mm in diameter, several whorls of branchlets, 4–8 branchlets per node, and branchlets often having 2–3 segments (Figure 3). Gametangia were absent from all specimens of *N. obtusa*, excluding those collected from Upper Jeptha Lake, which possess

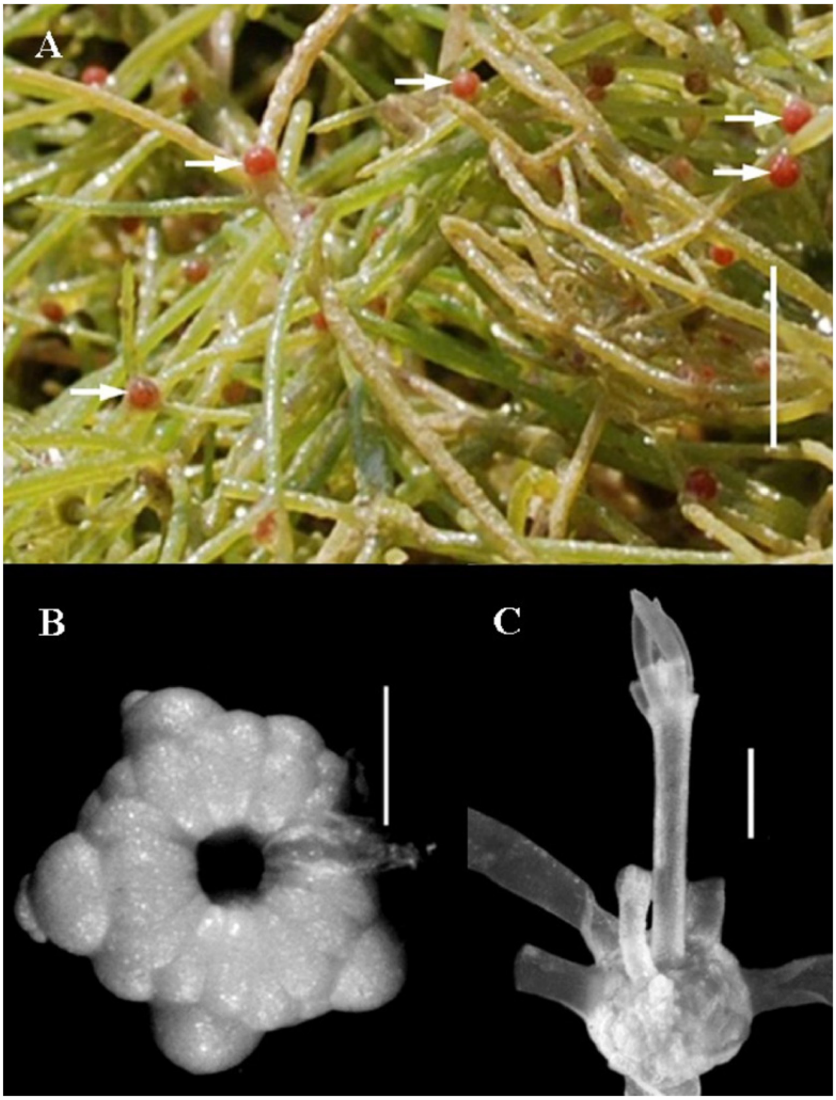

Figure 4. Morphology of *Nitellopsis obtusa* collected from Upper Jeptha Lake, Van Buren County, MI. **A**. Thalli with white arrows indicate antheridia (scale = 1 cm). **B**. Stellate bulbil (scale = 1 mm). **C**. Axial nodal cells with germinating shoots (scale = 1 mm). Photomicrographs by M.S. Alix.

antheridia (Figure 4A). Thus, no individuals with female reproductive structures (i.e. oogonia) were observed or collected. Particularly in the absence of gametangia, several authors (e.g., Wood 1965; Moore 1986; Urbaniak 2003) suggest the most diagnostically useful character to distinguish *N. obtusa* from other charophytes is the presence of white, multicellular, stellate bulbils on the former species (Figure 4B), which typically occur as modified whorls of branchlets at proximal nodes (Figure 4C). In the absence of bulbils, *N. obtusa*, which displays the typical characean growth form (Wood 1965), can easily be mistaken for members in the genera *Chara*, *Nitella*, and *Tolypella*. The possibility of misidentification emphasizes the importance of documenting occurrences with voucher specimens. When bulbils are absent, *N. obtusa* can be distinguished from *Chara* species by the presence of stipulodes around axial nodes, which the former species does not produce. In addition, *N. obtusa* does not possess spine cells and the main axis and branchlets are ecorticate, giving the thallus a smooth appearance when incrustations of lime are absent. These features are also shared with all species of *Nitella* and *Tolypella*, but unlike taxa in these genera, *N. obtusa* does not have divided branchlets.

Discussion

Great concern currently exists over the invasion of *Nitellopsis obtusa* throughout Michigan as well as other regions of the United States. Excluding records from this study, the Midwest Invasive Species Information Network lists 175 reported sightings of *N. obtusa* in Michigan over the last four years (MISIN 2016), thus this species appears to be spreading across inland lakes of this state at an alarming rate. A fundamental question is whether the spread of this species has been the product of sexual or asexual reproduction. Pullman and Crawford (2010) suggest that both modes of reproduction have likely contributed to the rapid spread of this species by the intra- and inter-lake dispersal of propagules through activities associated with recreational boating as well as movements associated with waterfowl, shorebirds, and mammals. As *N. obtusa* is a dioecious species (Wood 1965; Moore 1986; Urbaniak 2003), sexual reproduction requires the presence of both sexes. Evidence indicates that all known populations of this species in North America, including those in the current study, are either comprised of males (Mann et al. 1999) or of individuals that have not yet been observed to produce gametangia. The absence of gametangia or of one sex is a common phenomenon reported for European and Japanese populations (Olsen 1944; Luther 1951a; Willén 1960; Kasaki 1962; Krause 1985, 1997; Blindow 2009). In some cases, well-studied beds of this species have not become fertile for decades (Blindow 2009), with fertility sometimes being associated with the establishment of individuals in shallow water zones (Krause 1985). Specimens of *N. obtusa* from this study were collected from beds at depths between 30 and 150 cm (Appendix 1), and samples collected from Upper Jeptha Lake, both sterile and those that once possessed antheridia, have been in culture at Purdue University Northwest for nearly four years without producing any gametangia. Thus, future monitoring of beds at the Michigan sites for the production of oogonia is warranted. Although it is possible that female individuals may be discovered, it appears that the spread of *N. obtusa* in North America has only occurred via the dispersal of vegetative propagules. This clonal regeneration strategy through fragmentation and bulbil production has been responsible for the spread and persistence of other well-documented populations of charophytes (Bociag and Rekowska 2012).

It is plausible that waterfowl-mediated zoochory has aided the spread of *Nitellopsis obtusa* via dispersal of vegetative fragments and/or propagules. Southern Michigan, for example, is located within the Mississippi Flyway and is associated with several major corridors for migratory waterfowl (Bellrose 1968). In *Chara aspera* (Willdenow, 1809) and *Chara hornemannii* (Wallman, 1853), two species distantly related to *N. obtusa*, 0% germination of bulbils occurred after the ingestion of bulbil-laced mud by domesticated mallards (Proctor 1962) suggesting the internal transport of propagules (endozoochory) is not as significant as external transport (epizoochory). However, no field study has yet assessed the impacts of environmental conditions, desiccation rate, and transit/retention time on the viability of vegetative propagules of *N. obtusa*, which in turn likely affect the success of its dispersal. Furthermore, most data, although anecdotal, indicate that human-mediated transport by recreational boating is the primary mechanism responsible for the spread of *N. obtusa* (Sleith et al. 2015; Midwood et al. 2016). The beds of *N. obtusa* reported herein were largely mono-specific, and localized to areas typically < 100 m^2 from public boat launches, suggesting recent introductions and dispersal to these lakes by watercraft and trailers carrying vegetative propagules.

Although *Nitellopsis obtusa* has invaded many inland lakes of Michigan, its degree of invasiveness in these habitats and impacts on ecological conditions are currently unclear. It has been suggested that *N. obtusa* is the most aggressive aquatic macrophyte in Michigan and displaces native aquatic plants, which in turn negatively impacts associated fauna (Pullman and Crawford 2010). However, with the exception of one study showing *N. obtusa* impacts on the biomass and species richness of other macrophytes (Brainard and Schulz 2017), supporting data are limited and subsequent citations appear to be a case of an "anecdotal citation chain" (e.g. Adrian-Kalchhauser et al. 2017). In addition, modelling based on temperature and precipitation predicts suitable climatic conditions for the establishment of this species within the Eastern Temperate Forest, Great Plains, and Intermountain West ecoregions, as well as marine coastal areas along the eastern seaboard of the United States (Escobar et al. 2016). To determine the extent of suitable habitat for the invasion of *N. obtusa* within the broader geographical range defined above, fine-scale parameters, such as pH, alkalinity, conductivity, water clarity, and salinity, known to be of importance in defining the ecological niche of aquatic macrophytes, need to be considered. *Nitellopsis obtusa* is typically associated with hard-water lakes of high alkalinity and elevated bicarbonate concentrations (e.g., Krause 1985; Simons and Nat 1996; Vestergaard and Sand-Jensen 2000; Soulié-Märsche et al. 2002; Baastrup-Spohr et al. 2013; Rey-Boissezon and Auderset Joye 2015; Torn et al. 2015). Within the Eastern Temperate Forest ecoregion, lakes of the Midwest are highly alkaline and offer strong invasive potential for the spread of *N. obtusa*. However, the Atlantic Coastal Plain, which is also part of this ecoregion, contains soft-water inland lakes (U.S. Geological Survey 2016), which likely will not provide suitable habitat. The beds of *N.* obtusa discovered during this study are associated with lakes having moderately alkaline (Table S1) and moderate to very hard (i.e. 130–220 ppm $CaCO_3$) surface waters (U.S. Geological Survey 2016).

Evaluating the tolerance limits of fine-scale habitat parameters is a critical step in refining predictive models and increasing their level of accuracy. For example, the mean and maximum values of salinity reported in Escobar et al. (2016) approach those of seawater and are substantially above values reported from experimental studies (e.g., Katsuhara and Tazawa 1986; Winter et al. 1999) and natural habitats (e.g., Olsen 1944; Luther 1951b; Simons and Nat 1996; Krause 1997; Doege et al. 2016) for this freshwater species. An examination of the dataset of Escobar et al. (2016) indicates that 186 of the 187 localities with reported salinities approaching seawater are coastal freshwater habitats within the Netherlands. Between 2007 and 2015, the two largest lakes in this region, Ijsselmeer and Markermeer, had salinity values ≤ 0.50 psu (Rijkswaterstaat 2017). The remaining locality is Cosmeston lake in Wales, which in 2007 when this species was first collected had an annual mean chloride concentration of 27.2 mg/L, corresponding to a salinity < 0.1 psu (Burgess et al. 2009). It is unclear why these localities have reported salinity values approaching seawater unless they were perceived to be marine habitats because of their close proximity to the ocean. Use of salinity values outside the tolerance limits of *N. obtusa* data leads to the erroneous prediction that suitable habitat for this species exists within coastal marine waters of North America and Eurasia.

Eutrophication is an additional factor which may impact the establishment of this species in the United States. Although *Nitellopsis obtusa* is predominantly found in oligo- to mesotrophic lakes in Europe (Krause 1985; Soulié-Märsche et al. 2002), it can tolerate eutrophic conditions (Kabus and Wiehle 2012; Korsch 2013). In the current study, only Fish and Saddle Lakes are classified as eutrophic (Table S1) while only one of 31 lakes having confirmed occurrences of *N. obtusa* from New York and Indiana is eutrophic (New York State Department of Environmental Conservation 2015; Indiana Department of Environmental Management 2016, Appendix 1). It is currently unknown how the reduced light climate of eutrophic lakes will affect the competitive ability of *N. obtusa* particularly in southern states where other highly aggressive invasive species are prevalent. Disturbance associated with the mechanical and chemical control of invasive aquatic macrophytes in eutrophic lakes may influence the outcome of competition by providing additional colonisable habitat for the establishment of *N. obtusa*. Similarly, *N. obtusa* became abundant in eutrophic lakes in northern Germany after the activity of cyprinids uprooted macrophytes, creating colonisable space (Trapp 1999).

Future studies of the invasive potential of *Nitellopsis obtusa* in North America should attempt to determine those parameters that characterize habitat most suitable for the establishment of this species. Aquatic ecologists need to better understand the life history characteristics of *N. obtusa*, particularly with regard to longevity and viability of bulbils, sexuality of populations, and the survival and growth potential of this species across a range of lake trophic conditions in order to create, prioritize, and implement sound management strategies. In addition, extended quantitative studies of species richness and abundance, food web dynamics, and water quality are needed to evaluate the long-term impacts of *N. obtusa* on the ecological integrity of North American lakes in which it has become established.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Summary of pH, alkalinity, and trophic state of lakes with *Nitellopsis obtusa*.

Appendix 1. Voucher specimen information.

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