

INTRODUCTION OF *GLOSSOSTIGMA* (PHRYMACEAE) TO NORTH AMERICA: A TAXONOMIC AND ECOLOGICAL OVERVIEW¹

DONALD H. LES,^{2,4} ROBERT S. CAPERS,³ AND NICHOLAS P. TIPPERY²

²Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269-3043 USA; and

³Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504 USA

Field surveys in eastern North America confirm the naturalization of *Glossostigma* plants at 19 localities in four states: Connecticut, New Jersey, Pennsylvania, and Rhode Island. DNA sequence analysis of individuals from 14 sampled populations identifies these nonindigenous plants as *Glossostigma cleistanthum*, a species native to Australia and New Zealand. These results correct prior misidentifications of North American plants as *G. diandrum*. The earliest North American record of *G. cleistanthum* (1992) is from a Ramsar tidal wetland in Connecticut. Morphological analyses demonstrate that *G. cleistanthum* differs from *G. diandrum* by its longer leaves and ability to produce both cleistogamous and chasmogamous flowers in response to ecological conditions. *Glossostigma cleistanthum* has a high reproductive potential and spreads rapidly within and between both artificial and natural habitats. A survey of more than 100 lakes indicated that *G. cleistanthum* occurs most often in waters with high clarity and low pH, alkalinity, conductivity, and phosphorous. Because of its affinity for oligotrophic conditions, this species is a particular threat to pristine natural aquatic communities, which often contain imperiled plants.

Key words: Australian flora; *Glossostigma*; invasive; nonindigenous; North America; Phrymaceae; phylogenetics; taxonomy.

Invasive plants profoundly impact species, communities, and ecosystems and represent regional and national threats to rare native plants in the United States (Wilcove et al., 1998; Farnsworth, 2004). They reduce the diversity of plant communities by interfering with colonization (Meiners et al., 2001; Yurkonis et al., 2005) and also affect productivity and ecosystem functions (Vitousek et al., 1996; Christian and Wilson, 1999). Predictably, the problems created by invasive plants are likely to increase with international trade (OTA, 1993). Invasions of nonindigenous aquatic plants also have been facilitated by internet trafficking and mail-order businesses, which contribute to their spread by subverting laws intended to prevent their introduction (Kay and Hoyle, 2001; Maki and Galatowitsch, 2003).

Despite decades of research, it remains uncertain why some nonindigenous species are more damaging ecologically than others (Meiners et al., 2001) and whether some communities are more vulnerable to invasion (Lonsdale, 1999; D'Antonio and Levine, 1999; Stohlgren et al., 1999; Herben et al., 2004; Gross et al., 2005). Invasive species often establish and become widespread before they are perceived as a problem (Crooks and Soulé, 1999). Therefore, studies are particularly useful when conducted early in the history of an introduction because they may be able to distinguish those species that will become invasive from those that will not (Blossey, 1999; Byers et al., 2002). The correct identification of a nonindigenous species often is difficult but essential for studying their invasive potential. Misidentifications can thwart efforts to elucidate

ecological characteristics, possibly leading to the implementation of unsuitable management practices.

Recently introduced to North America, *Glossostigma* Wight & Arn. (Phrymaceae) is a freshwater genus of seven or eight aquatic species indigenous to small water bodies in Australia, East Africa, India, and New Zealand (Cook, 1996a; USDA, 1998; Lamont and Fitzgerald, 2001; Beardsley and Barker, 2005). Systematic investigations (Beardsley and Barker, 2005) have resolved *Glossostigma* as monophyletic and place the genus in a clade with *Elacholoma* and *Mimulus prostratus*. *Peplidium* and several other *Mimulus* species comprise their sister group. These genera recently have been transferred from Scrophulariaceae to the newly redefined family Phrymaceae (Beardsley and Olmstead, 2002; Beardsley and Barker, 2005), the taxonomic disposition that is followed here.

Glossostigma species are minute annuals or perennials that occur submersed in shallow water or emergent on exposed sandy to muddy shorelines. Although fairly small in stature, plants can attain high densities and often form extensive mats on the substrate, hence their common name of mud mat. Due primarily to their small size, mud mats generally are not regarded as a nuisance within their native range; however, species can grow vigorously under appropriate conditions (Coffey and Clayton, 1988; Sainty and Jacobs, 1981; Cook, 1996b). Although the genus is viewed as ecologically benign, *Glossostigma* (especially *G. elatinoides*) is a popular aquarium plant (Mühlberg, 1982) because of its ability to form a lush, carpet-like growth (Windeløv, 1998; Kasselman, 2003). Little is known regarding the distribution, ecology or invasive potential of *Glossostigma* in North America.

The earliest record of *Glossostigma* in North America is a 1992 specimen from Hamburg Cove, Connecticut, which initially was misidentified as *Elatine* (Appendix). Soon afterwards, plants were collected in central New Jersey (NJ) and eastern Pennsylvania (PA), and eventually were identified as *G. diandrum* based on the determination of Pennsylvania specimens sent to W. R. Barker (State Herbarium of South Australia), an authority on the genus (A. Rhoads, Morris Arboretum; D. Snyder, New Jersey Department of Environ-

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⁴ Author for correspondence (e-mail: les@uconn.edu)

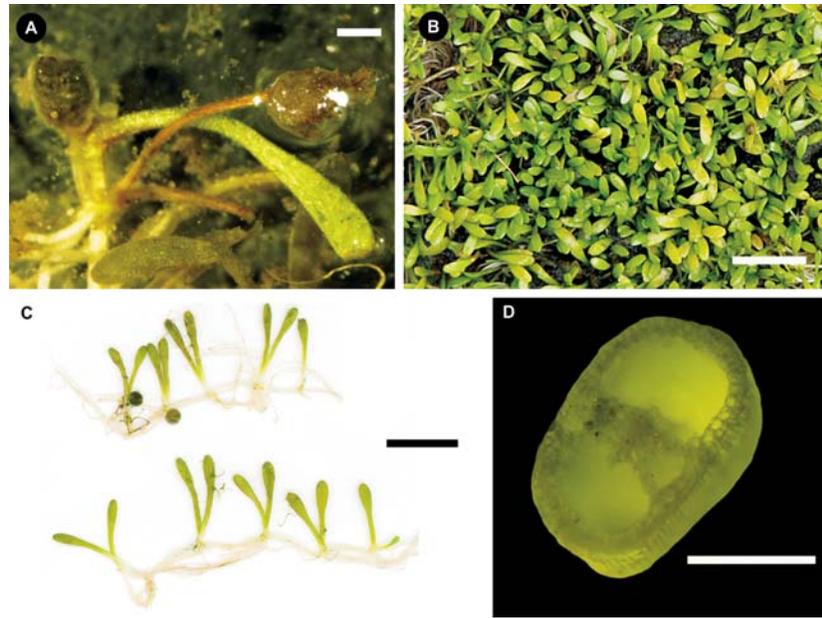


Fig. 1. Characteristics of *Glossostigma cleistanthum* in North America. (A) Individual plant of *G. cleistanthum* from Alexander Lake, Connecticut showing a nearly sessile, cleistogamous flower (left) and stalked, chasmogamous flower (right). Bar = 1 mm. (B) A dense patch of *G. cleistanthum* at Alexander Lake, Connecticut observed in 2005. Bar = 1 cm. (C) Submersed habit of *G. cleistanthum* showing long leaves, long rhizome internodes, and sessile (cleistogamous) fruits. Bar = 1 cm. (D) Light micrograph of cross section of a *G. cleistanthum* leaf showing the pair of large, longitudinal lacunae present in the lower half of blade. The air chambers allow dislodged plants (with attached fruits) to float and disperse. Bar = 0.5 mm.

mental Protection; personal observations). Subsequently, additional *Glossostigma* collections were made in Connecticut (CT), New Jersey, and Rhode Island (RI) (Appendix), and by the mid- to late 1990s, it became clear that the genus was spreading widely and rapidly throughout the eastern United States.

However, questions regarding the identification of the introduced *Glossostigma* plants arose. Connecticut plants possessed extremely short-stalked, cleistogamous flowers whereas NJ and PA material reportedly possessed long-stalked chasmogamous flowers. At first, the cleistogamous material was viewed as an environmental form of *G. diandrum* given that several references (e.g., Cook et al., 1974; Cook, 1996b; Johnson and Brooke, 1998) indicated that both flower types were produced by this species. Indeed, we observed individual plants that possessed both long-stalked chasmogamous flowers as well as nearly sessile, spherical, cleistogamous flowers (Fig. 1a). Furthermore, a “pest alert” brochure (USDA, 1998) illustrated “*Glossostigma diandrum*” with small, cleistogamous flowers, indicating that their stalks could be “short or as long as the leaves.”

Barker (1992b) distinguished cleistogamous-flowered *Glossostigma* plants as *G. cleistanthum*. Although some authors (e.g., Johnson and Brooke, 1998) maintained cautiously that the cleistogamous, submerged forms of *G. diandrum* “may belong to a distinct species,” a recent study incorporating molecular data (Beardsley and Barker, 2005) provided compelling evidence for the distinctness of *G. diandrum* and *G. cleistanthum*. Consequently, a reevaluation of North American plants is necessary, especially the status of those populations where both cleistogamous and chasmogamous flowers have been observed.

The life histories of some *Glossostigma* species are

understood inadequately. Cook (1996b) described *G. diandrum* as an “annual or when in permanent water perhaps perennial.” Barker (1992a) referred to *G. diandrum* as “possibly annual.” Yet, observations that NJ material, identified as *G. diandrum*, remained green throughout the winter (D. Snyder, NJ DEP; personal observation), convincingly indicated a perennial habit. *Glossostigma elatinooides* is described as a perennial, and *G. cleistanthum* is considered to be an “ephemeral or short-lived perennial” (Barker, 1992a).

In the present study, we evaluate North American *Glossostigma* using comparative molecular analyses to clarify the taxonomic status of the plants that have been introduced to the various portions of this region. We present a chronology of the introduction of the genus that is based upon herbarium specimen vouchers and provide an emended morphological description of the observed material. Although ecological affinities of *Glossostigma* have been studied in Australia and New Zealand, species do not always occur in the same environments when they invade new locations. Therefore, we also characterized a number of ecological factors associated with North American occurrences of these plants. Finally, we give an overview of the potential ecological impact of this introduction based upon our analysis of sites where this species has been introduced. Ultimately, our goal was to assess the invasive potential of this relatively recently introduced aquatic plant.

MATERIALS AND METHODS

A list of all known North American *Glossostigma* localities was compiled from information provided by personal communications with Ann Rhoads (Morris Arboretum), David Snyder (NJ DEP), Floyd Yoder (U.S. Dept. of Agriculture [USDA]) and Colette Jacono (USDA), as well as from herbarium

records at the University of Connecticut herbarium (CONN) and our own field observations in CT, NJ, and RI. This information was organized chronologically to provide an approximate timeline for the introduction of *Glossostigma* plants into North America (Appendix). We were able to visit a total of 13 of the 19 known North American localities of *Glossostigma* where we recorded field observations and acquired plant material for DNA and morphological analyses (Appendix). Additionally, plant material from the PA site was collected and sent to us by A. Rhoads (Morris Arboretum).

Molecular analysis—We obtained DNA sequences of the nrITS (ITS) region for 20 *Glossostigma* accessions (Appendix). These accessions included specimens from 14 of 19 known North American localities of *Glossostigma* and from one specimen collected previously by D. H. Les in Australia. The entire ITS region was amplified using the ITS4 and ITS5 primers (Baldwin, 1992) and sequenced using ITS4. We also retrieved from GenBank previously published ITS sequences for *Glossostigma cleistanthum* (AY943120), *G. diandrum* (AY943121), *G. drummondii* (AF478937), *G. elatinoides* (AY943119), *Glossostigma* sp. (AY943122), *Elacholoma* sp. (AY943118) and *Mimulus prostratus* (AY943125) for comparative analyses.

Similarly, we obtained ETS sequences for the same *Glossostigma* accessions from which the ITS data were derived (Appendix). First we amplified the entire intergenic spacer region (IGS) using modifications of the universal primers (Baldwin and Markos, 1998), which were truncated to achieve a lower melting temperature (primer sequences: 26S-IGS: 5'-CCAATAGGGAACGTGAGCTG-3'; 18S-IGS: 5'-GAGACAAGCATATGACTACTGG-3'). We sequenced two North American accessions (Chase Reservoir, CT; Alexander Lake, CT) using the 18S-IGS primer to read through the same location as the ETS-B primer designed for *Mimulus* (Beardsley and Olmstead, 2002). This step allowed us to develop an amplification primer that was highly specific to *Glossostigma* (ETS-Glos: 5'-GTAAGGTGCATGAGTGGTG-3'). We then used the primers 18S-IGS and ETS-Glos to amplify the remainder of the *Glossostigma* accessions and sequenced the resulting amplicons using the 18S-IGS primer. We also retrieved ETS sequences from GenBank for the following taxa: *G. cleistanthum* (AY943092), *G. diandrum* (AY943093; AY943094), *G. drummondii* (AF478970), *G. elatinoides* (AY943091), *Glossostigma* sp. (AY943095; AY943098), *G. trichodes* (AY943096; AY943097), *Elacholoma* sp. (AY943090), and *Mimulus prostratus* (AY943099).

Sequences were aligned manually using MacClade 4 (Maddison and Maddison, 2000) and were analyzed for polymorphisms and variable sites using Sequencher (Gene Codes Corp., Ann Arbor, Michigan, USA). Although we obtained longer reads for some ITS sequences (described later), we restricted our analysis to an alignment of 598 contiguous positions to minimize the amount of missing data from among the various sequence accessions. Similarly, the ETS sequences were truncated to 453 sites to minimize missing data. All newly generated sequences have been deposited in the GenBank database under accession numbers DQ371397–DQ371430.

Relative pairwise sequence divergence for ITS and ETS (separately and combined) was estimated as uncorrected (p) distances using PAUP*, version 4.0b10 (Swofford, 2000). We partitioned the ITS and ETS data and conducted a partition-homogeneity test (500 replicates) using PAUP* (Swofford, 2000) to evaluate whether these data sets differed from random pairwise partitions of the data. Observing no significant difference by this test (see Results, *Molecular analysis*) we combined the ITS and ETS data (1051 nucleotide positions) for subsequent analyses.

The aligned, combined sequences were analyzed phylogenetically by maximum parsimony as implemented in PAUP* (Swofford, 2000) (exhaustive search; random taxon addition; TBR; characters unordered, weighted equally). Insertion-deletions (“indels”) were treated as missing data. Because they were identified as comprising the sister clade to *Glossostigma* in previous analyses (Beardsley and Barker, 2005), we used the sequences of *Elacholoma* sp. and *Mimulus prostratus* as outgroups and employed outgroup rooting.

When relative p -distances indicated that all North American accessions of *Glossostigma* were identical for ITS and ETS (see Results, *Molecular analysis*), we facilitated the analyses and acquisition of tree statistics (skewness [g_1]; minimum tree length; number of trees; consistency index [CI]; consistency index excluding uninformative sites [CI_{exc}]; retention index [RI]) by excluding all but one North American sequence (Lake Galena, PA) from our runs. Because only a single tree resulted, assigned branch lengths were obtained using the “describe trees” option to output a phylogram. Internal (“nodal”) support for tree nodes was provided by computing bootstrap values using the “branch and bound” search algorithm and 10 000 replicates. Although culling the identical data reduced the number of sequences analyzed to 11, trees were

redrawn to include the previously excluded, identical sequences depicted as a polytomy, a method that enabled us to represent the results obtained for all accessions in one diagram.

Morphological analysis—Leaf and internode lengths were measured on plants from 10 localities representing all four North American states (CT, NJ, PA, RI) where *Glossostigma* is known to occur. For comparison, we also measured leaves and internodes on nine Australian specimens of *G. diandrum* and *G. cleistanthum* from material at CANB. To reduce developmental variability, all measurements were restricted to mature leaves in exclusion of any terminally occurring leaves. Means and standard errors were calculated for all measurements. Potential correlation between leaf and internode lengths was evaluated by computing Pearson correlation coefficients with the level of significance ($P < 0.05$) determined using a Bonferroni distribution as implemented by the SYSTAT software package (SYSTAT, 2005). The presence of chasmogamous (CH) and/or cleistogamous (CL) flowers was noted for each accession analyzed. Differences in mean leaf and rhizome length between North American plants bearing chasmogamous flowers and those having only cleistogamous flowers were evaluated statistically using t tests as implemented with SYSTAT (2005). A one-way ANOVA (SYSTAT, 2005) was conducted to test whether leaf length differed significantly between the North American *Glossostigma* material and specimens of *G. diandrum* or *G. cleistanthum* originating from Australia. Three analyses were conducted to compare (1) North American plants (both emerged and submersed) with Australian material of *G. cleistanthum*, (2) North American plants (both emerged and submersed) with Australian material of *G. diandrum*, and (3) North American plants (emerged only) with Australian material of *G. diandrum* (emerged plants).

Ecological analysis—Distribution maps were plotted to depict the native Australian ranges of *G. cleistanthum* and *G. diandrum* using the mapping function provided at the “Australia’s Virtual Herbarium” website (<http://www.flora.sa.gov.au/cgi-bin/avh.cgi>). For North America, the positions of observed *Glossostigma* patches were recorded using a Trimble Geo XT high-accuracy global positioning system (GPS) running TerraSync software (Trimble, 2005) to provide baseline georeference data for mapping and future monitoring purposes. We recorded the occurrence of every observable *Glossostigma* patch for all Connecticut localities by conducting comprehensive field surveys. Although lakes were not surveyed as extensively at other localities (NJ, PA, RI), we obtained GPS data for at least some reference patches and evaluated the relative abundance of patches based on a survey of roughly comparable lengths of shoreline (c. 50–75 m) at all sites except PA. We georeferenced the PA site from information provided by voucher labels using the GNIS database (USDA, 2005). Georeference data were used to map all of the known North American localities using ArcView mapping software (ESRI, 2004). A detailed map showing the distribution of patches was made for the Mansfield Hollow, CT site, which had been surveyed comprehensively using GPS.

The density of plants per patch was estimated both quantitatively and qualitatively. In determining our estimates, each leaf pair (i.e., node) was considered as a separate individual following Chapman et al. (1971). During surveys of each North American site visited, *Glossostigma* density was estimated in representative patches and assigned visually to one of three categories: high, medium or low. Digital photographs were taken of plants within 0.25 m² quadrat frames placed in several patches representing each of these categories. The number of individuals then was counted on each photograph and used to compute representative values of plant density per square meter.

For one locality (Alexander Lake, CT), the average number of flowers per node and mean number of seeds per fruit were calculated and used along with density data to estimate frequency of cleistogamous flowers in the population and overall plant reproductive potential. Preliminary germination rates were evaluated by placing mature seeds (taken from this same population) in Petri dishes lined with damp filter paper, which were kept in a seed incubator at 15°C (16h/8h light/dark regime) for 30 days.

Substrate composition (by visual inspection) and associated species were recorded for each site visited. For CT, we analyzed differences in environmental conditions that existed between five lakes where *Glossostigma* occurred and 102 lakes (surveyed during June–October 2004 and 2005) where it does not occur (Connecticut Agricultural Experiment Station, New Haven Connecticut, 2005; unpublished data from the Invasive Aquatic Plant Survey Program). We used SYSTAT (2005) to perform chi-square analyses and Mann–Whitney nonparametric tests for comparing differences in maximum depth,

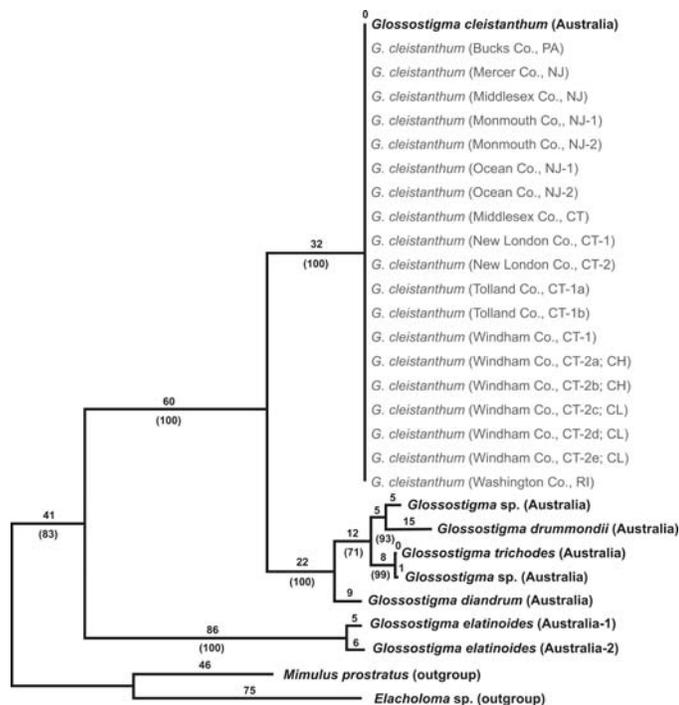


Fig. 2. Maximum parsimony cladogram constructed from combined nrITS/ETS DNA sequence data depicting phylogenetic relationships among Australian species of *Glossostigma* and their close allies (boldface type) together with 19 accessions of North American plants (lightface type) that were introduced to four states: Connecticut (CT), New Jersey (NJ), Pennsylvania (PA) and Rhode Island (RI). Numbers above branches represent assigned branch lengths; numbers below represent bootstrap values. These results clearly place all sampled North American material within *G. cleistanthum* but distinctly apart from the closely related *G. diandrum*. North American accessions include their county and state of origin. Numbers indicate different sites within the same state and county; lowercase letters indicate multiple accessions from within a site. CH = chasmogamous flowers present; CL = cleistogamous flowers present on material sequenced. The newly reported sequence for *G. elatinoides* (GenBank DQ371401) is represented by the accession labeled "Australia-2."

Secchi depth, temperature, pH, dissolved oxygen, conductivity, alkalinity, and total phosphorus in two water samples: one taken from a depth of 0.5 m (surface) and a second taken just off the bottom in the deepest part of each lake (bottom). For the chi-square test, lakes were divided into the following categories, which were delimited using the median values for all lakes combined: maximum depth (m) [<4 , ≥ 4]; Secchi depth (m) [<2 , ≥ 2]; surface temperature ($^{\circ}\text{C}$) [<25.2 , ≥ 25.2]; bottom temperature ($^{\circ}\text{C}$) [<19.0 , ≥ 19.0]; surface pH [<6.5 , ≥ 6.5]; bottom pH [<6.1 , ≥ 6.1]; surface dissolved oxygen (mg/L) [<7.3 , ≥ 7.3]; bottom dissolved oxygen (mg/L) [<0.63 , ≥ 0.63]; surface conductivity ($\mu\text{S}/\text{m}^2$) [<113 , ≥ 113]; bottom conductivity ($\mu\text{S}/\text{m}^2$) [<124 , ≥ 124]; surface alkalinity (mg CaCO_3/L) [<21 , ≥ 21]; bottom alkalinity (mg CaCO_3/L) [<25.5 , ≥ 25.5]; surface total P (ppb) [<20 , ≥ 20]; bottom total P (ppb) [<48 , ≥ 48]. Total P data were not available for one lake where *Glossostigma* occurred.

RESULTS

Molecular analysis—We obtained an ITS alignment of 598 nucleotide positions for representative *Glossostigma* plants collected at 14 of 19 (74%) of the known North American localities. The sequences uniformly yielded chromatograms with no evidence of site polymorphisms. Based on this alignment, the degree of sequence divergence (uncorrected p -

distances) ranged from 14.3% (*Elacholoma* vs. *G. elatinoides*) to 0% (*G. cleistanthum* vs. all North American accessions). The highest ITS sequence divergence observed within *Glossostigma* occurred between *G. elatinoides* and *G. drummondii* ($p = 13.3\%$). The p -distances indicated that the ITS sequences of the 14 North America populations surveyed were identical to each other ($p = 0\%$) and to a previously published sequence of *Glossostigma cleistanthum* from Australia (GenBank accession AY943120). The ITS sequences of North American *Glossostigma* plants differed considerably ($p = 3.6\%$) from those reported for *G. diandrum* (GenBank accession AY943121), the species to which North American material had been assigned previously.

For ETS we analyzed a region of 453 contiguous, aligned sites. Again, there was no evidence of any site polymorphisms in the sequence chromatograms. Sequence divergence (p -distance) in ETS ranged from 24.7% (*Glossostigma* sp. [GenBank accession AY943098] vs. *G. elatinoides* [GenBank accession DQ371401] to 0% (*G. cleistanthum* [GenBank accession AY943092] vs. all North American accessions). The ETS nucleotide sequences of all 14 North American populations were identical to each other ($p = 0\%$) as well as to a previously published sequence of *G. cleistanthum* from Australia (GenBank accession AY943092). However, aside from their nucleotide identity, the North American *Glossostigma* ETS sequences did lack a single nucleotide gap that occurred in the published sequence of *G. cleistanthum* from Australia (GenBank accession AY943092). This single gap was the only difference detected between North American *Glossostigma* accessions and *G. cleistanthum* from examination of 1051 aligned sites of combined ETS/ITS sequence data. In contrast, the ETS sequence of North American *Glossostigma* accessions differed considerably ($p = 9.4\%$) from those reported for *G. diandrum* (GenBank accessions AY943093, AY943094) the species name originally assigned to the plants. A specimen of *G. elatinoides* collected from the Australian Capital Territory (ACT) [GenBank accessions DQ371401, DQ371416] was similar ($p = 1.8\%$, 0.5% , respectively) to the ETS and ITS sequences reported previously for *G. elatinoides* (GenBank accessions AY943091, AY943119).

A partition homogeneity test indicated that the ETS and ITS data showed no statistically significant incongruence ($p = 1.000$), thus providing the rationale to combine them in the phylogenetic analyses. Parsimony analysis of the combined ETS/ITS data yielded a single minimum-length tree (428 steps) with low homoplasy ($\text{CI} = 0.883$; $\text{CI}_{\text{exc}} = 0.841$; $\text{RI} = 0.875$) and a high level of phylogenetic signal ($g_1 = -1.46$). Internal (bootstrap) support for nodes was moderate to high, ranging from 71–100% (Fig. 2). The maximum parsimony tree clearly showed that all *Glossostigma* material originating from North America was associated with native material of *G. cleistanthum* collected in Australia, while remaining quite distinct from *G. diandrum* (Fig. 2). The North American specimens formed a strongly supported clade (bootstrap = 100%) that included the Australian specimen of *G. cleistanthum*, whereas *G. diandrum* occurred in a sister clade that also was strongly supported (100% bootstrap; Fig. 2). Conclusively, the ETS and ITS sequence data indicated that all North American *Glossostigma* plants should be recognized taxonomically under the name *G. cleistanthum*.

Morphological analysis—Leaves on submersed (and cleistogamous) plants of North American *Glossostigma* were

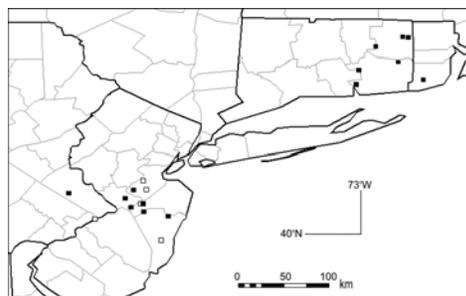


Fig. 3. Map showing the location of 19 sites where *Glossostigma* has been found in North America (see Appendix). Closed squares indicate populations surveyed for molecular analysis; open squares indicate populations not surveyed.

significantly longer (7.0–57.0 mm; mean ± SE: 20.8 ± 0.48 mm) than those on emergent (and chasmogamous) plants (4.5–11.0 mm; 7.5 ± 0.13 mm) compared across all populations (*t* test, *N* = 470, 105, *t* = -26.810; *P* < 0.0001). The three populations having the shortest leaves (Alexander Lake, CT; Lake Galena, PA; Meadowbrook Pond, RI) also were those with plants producing chasmogamous flowers. Leaves on six specimens of Australian *G. diandrum* were significantly shorter (5.3 ± 0.16 mm) than those of the North American plants, whether we analyzed both emergent and submersed individuals (18.4 ± 0.44 mm; ANOVA, *F*_{1,821} = 314.83; *P* < 0.0001), or only the emersed individuals (7.5 ± 0.13 mm; ANOVA, *F*_{1,246} = 101.70; *P* < 0.0001). Leaf lengths of three Australian specimens of *G. cleistanthum* fell within the range of North American plants but on average were shorter (ANOVA, *F*_{1,551} = 45.92; *P* < 0.0001). Leaf length varied considerably among *G. cleistanthum* populations, both within North America and Australia (ANOVA, *F*_{11,541} = 261.53; *P* < 0.0001).

Within submersed North American populations, mean leaf length varied from a low of 12.0 ± 0.54 mm at Alexander Lake, CT to 26.7 ± 0.60 mm at Lake Carasaljo, NJ, and 45.4

± 1.04 mm for one group of plants with exceptionally long leaves at Hayward Lake, CT. The length differences were significant in an analysis across nine populations (four in NJ, five in CT) treating Hayward Lake’s long-leaved plants as a separate population from other Hayward Lake plants (ANOVA, *F*_{8,461} = 308.43; *P* < 0.0001).

Rhizome internode length of North American *G. cleistanthum* varied from 1–10 mm (mean ± SE: 4.0 ± 0.27 mm) among chasmogamous specimens and from 1–18 mm (mean ± SE: 6.42 ± 0.15 mm) among cleistogamous specimens. Mean leaf length of North American plants correlated highly with mean rhizome length (*r* = 0.74; *P* = 0.009), indicating that short-leaved plants occurred in more tightly clumped habits than did longer-leaved plants. A similar trend was not evident in Australian material of *G. diandrum* (*r* = 0.47; *P* = 0.200).

Chasmogamous flowers occurred only on plants collected in emersed condition, whereas submersed plants produced only cleistogamous flowers. A mixture of both chasmogamous and cleistogamous flowers was observed on some emersed specimens from Alexander Lake, CT, Galena Lake, PA, and Meadowbrook Pond, RI (Fig. 1a).

Ecological analysis—Our compilation of records identified a total of 19 known *Glossostigma* localities in North America: 11 sites in NJ, six sites in CT and one site each in PA and RI (Appendix). Mapped georeference data indicate that *Glossostigma* populations occur in two major concentrations throughout the northeastern United States, one centered in E Connecticut/W Rhode Island and the other in E Pennsylvania and central New Jersey (Fig. 3). Patch distributions ranged from relatively localized to widespread within six intensively surveyed CT populations (Table 1). For the remainder of lakes visited, we observed similar variation in plant distributions ranging from few, localized occurrences (Meadowbrook Pond, RI; Mercer Lake, NJ; Pete Sensi Park, NJ) to moderately distributed patches (McCormack Lake, NJ) to widespread occurrences of plants (Lake Carasaljo, NJ; Prospertown Lake, NJ; Rising Sun Lake, NJ). The occurrence of *Glossostigma*

TABLE 1. Compositional overview of 14 North American *Glossostigma* populations.

Location	Distribution	Patch #	Patch size	Patch density	Flowers	Substrate	Max depth (cm)
Alexander Lake, CT	widespread	59	small–large	medium–high	CH, CL	sand	>400
Chase Reservoir, CT	local	4	small–large	medium–high	CL	sand, silt	200
Hamburg Cove, CT	local	3	small–medium	low–medium	CL	sand, sand/silt	50 ^a
Hayward Lake, CT	widespread	32	small–large	low–high	CL	sand	150
Hopeville Pond, CT	local	11	small–large	low	CL	sand	50
Mansfield Hollow, CT	widespread	91	small–large	low–medium	CL	sand, sand/silt	50
Lake Carasaljo, NJ	local	n/a	small	medium–high	CL	sand/gravel	50
McCormack Lake, NJ	local	n/a	small	low	CL	sand	20
Mercer Lake, NJ	local	n/a	small	low	CL	sand	20
Pete Sensi Park, NJ	local	n/a	small	low–medium	CL	sand, cobble	15
Prospertown Lake, NJ	widespread	n/a	medium–large	medium–high	CL	sand	30
Rising Sun Lake, NJ	widespread	n/a	medium–large	medium–high	CL	sand/silt	50
Lake Galena, PA	local	n/a	medium–large	medium–high	CH, CL	sand, clay, gravel	n/a
Meadow Brook Pond, RI	local	n/a	small	low	CH, CL	sand	>20

Notes: Distributional categories were approximated as local: restricted to one or a few areas of the lake; widespread: occurring widely around a lake. Patch number (where provided) indicates the total number of patches present in the lake as a result of detailed field surveys made in 2005. Patch size was approximated as small: area <2 m²; medium: area = 2–50 m²; large: area >50 m². Patch densities were determined visually, but correspond roughly to the following values as determined by subsample analyses (see Materials and Methods, *Ecological analysis*): high: >15 000 plants/m²; medium: 1000–15 000 plants/m²; low: <1000 plants/m². CH = chasmogamous flowers present, CL = cleistogamous flowers present. Sediment types were determined visually. Maximum (max) depth was determined by comprehensive site surveys for all CT sites in 2005; for all other sites it represents the depth observed at collection sites, which is not necessarily the maximum possible depth of occurrence at the site. n/a = data not available.

^a This area is tidal; depth would be approximately 150 cm at high tide.

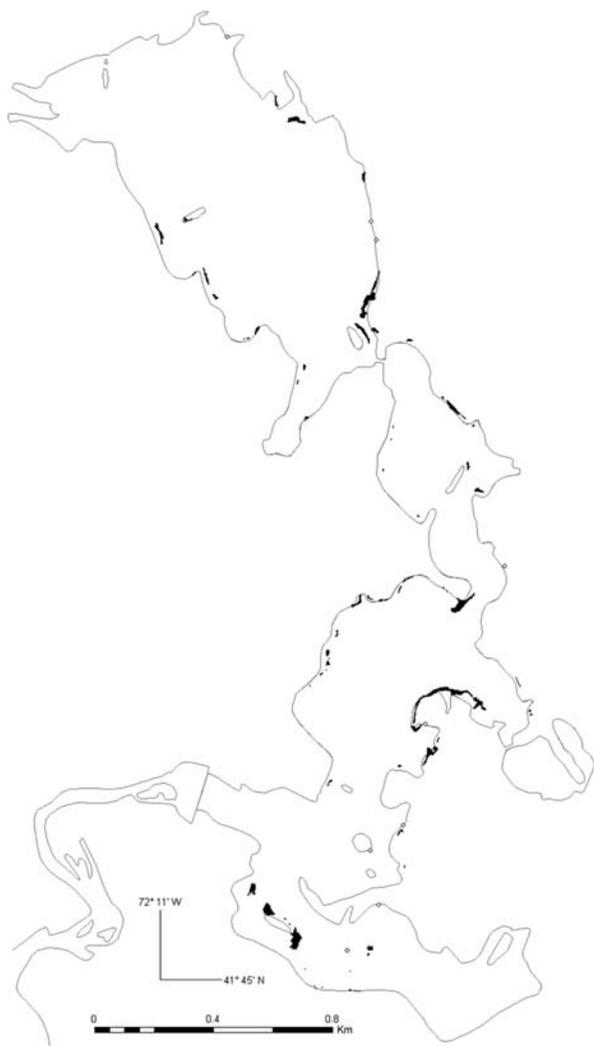


Fig. 4. Map showing locations of *Glossostigma cleistanthum* patches in Mansfield Hollow Reservoir, Connecticut based on results of a detailed GPS survey of the entire lake in 2005. Unknown to this site prior to 2003, *G. cleistanthum* now is estimated to cover nearly 12 000 m² of habitat and to occupy more than 1.3% of the littoral zone of the lake less than 1.4 m in depth (see Results, *Ecological analysis*). Open symbols represent patches less than 1 m². Uppermost locality is near the site where the species was first observed in 2003.

plants in Lake Galena, PA has been described as “uncommon in sparse patches” (*Jacono 198 & H. D. Jacono s.n.*; FLAS) (Table 1).

Patch densities estimated from digital photographs ranged from 464 plants/m² (Mansfield Hollow, CT) to 25 300 plants/m² (Alexander Lake, CT; Fig. 1b). These data were used to quantify the visually determined density categories approximately as: high, >15 000 plants/m²; medium, 1000–15 000 plants/m²; low, <1000 plants/m². Patch density estimates for all sites are summarized in Table 1.

At one site (Mansfield Hollow, CT), *Glossostigma* occurred in 91 discrete patches (Table 1; Fig. 4) to cover a total area of 11 894 m². The patches ranged in size from <1 to 1573 m². Of all patches, 21% were <1 m² in area, 31% were 1–10 m², 23% were 10–100 m², 21% were 100–1000 m² and 4% were >1000

m². Using bathymetry data for the lake, we estimated that the total area occupied by *Glossostigma* in the Mansfield Hollow reservoir represented 1.3% of the lake bottom where the water was <1.4 m deep and 0.8% of the lake bottom where the water was <2.3 m deep (Fig. 4).

Glossostigma grew in association with 29 aquatic species (Table 2). Two taxa (*Eleocharis acicularis*, *Elatine* spp. [*E. americana* or *E. minima*]) were common associates (observed in nine and eight of the 14 sites, respectively), whereas *Gratiola aurea* was an associate only in CT populations and *Ludwigia palustris* only in NJ populations (Table 2).

Inspection of many submerged plants showed cleistogamous flowers to be extremely abundant. On plants collected from Alexander Lake, CT (September, 2005), flowers were produced at 49 of 68 nodes (72%); however, not all flowers produced fruits. Of 603 nodes examined, only 15 (2.5%) had produced mature fruits. The number of seeds in each fruit ranged from 12–73 ($N = 22$ fruits; mean \pm SE: 36.7 ± 3.83). Using these figures, we concluded that *Glossostigma* populations such as the one at Alexander Lake have an extremely high reproductive potential with a square meter of dense plants having the capacity to produce more than 18 000 flowers, 600 fruits and 23 000 seeds on average each year. Seed germination (without cold stratification) was low after 30 days with only 3 of 219 seeds (1.4%) germinating. However, even if the actual germination rate typically is this low, then the denser patches in populations such as Alexander Lake, CT potentially could yield over 300 new individuals (genets) per meter of plants, depending on their establishment success.

In North America, *Glossostigma* occurs predominantly on sand (100% of sites surveyed), with silt and gravel making up additional substrate components in 42% and 17% of sites respectively (Table 1). The maximum depth at which *Glossostigma* occurred correlated positively with Secchi depth ($r = 0.91$, $P = 0.032$). A Mann–Whitney test, which determines whether measured values of variables differed between lakes with and without *G. cleistanthum*, indicated significant differences in pH, conductivity, and total (bottom) phosphorous (all lower in lakes where this species occurred) (Table 3). Similarly, a chi-square test determined that *G. cleistanthum* was overrepresented in lakes with greater Secchi depth and lower pH, conductivity, alkalinity, and total (bottom) phosphorous (Table 3). Thus, in North America, *G. cleistanthum* tends to occur in clear, acidic, oligotrophic lakes with low alkalinity, conductivity, and phosphorous.

DISCUSSION

Taxonomic implications—Correct species identification is essential for evaluating the introduction of any nonindigenous organism. This task is complicated by the fact that newly introduced species (and even some of their genera, families, etc.) are absent from local identification manuals and may require monographs with more global coverage for species identifications. Additionally, aquatic plants are relatively scarce (fewer than 2% of all angiosperm species) and often occur in groups that are poorly known taxonomically or whose taxonomy remains incompletely understood.

The introduction of *Glossostigma* to North America epitomizes these problems. The genus is native entirely to the Old World and never has been monographed. Although Willis (1970) called for “a competent revision of the whole

TABLE 2. Plant species associated with *Glossostigma cleistanthum* in North America as determined by field surveys and herbarium specimen records.

Species	Location of population											
	Connecticut						New Jersey					
	AL	CR	HC	HL	HP	MH	LC	LM	ML	PL	PS	RS
<i>Brasenia schreberi</i>										+		
<i>Cabomba caroliniana</i>							+			+		+
<i>Callitriche</i> sp.											+	
<i>Ceratophyllum demersum</i>												+
<i>C. echinatum</i>							+					
<i>Crassula aquatica</i>			+									
<i>Eleocharis acicularis</i>	+			+	+	+		+	+	+	+	+
Elatine spp.	+	+		+	+	+	+	+				+
<i>Eriocaulon aquaticum</i>	+			+								
<i>E. parkeri</i>			+									
<i>Gratiola aurea</i>	+	+		+	+	+						
<i>Heteranthera multiflora</i>												+
Isoetes sp.		+		+								
<i>Lilaeopsis chinensis</i>			+									
<i>Limosella subulata</i>			+									
<i>Ludwigia palustris</i>							+	+	+		+	+
Myriophyllum heterophyllum							+			+		
<i>M. humile</i>						+						
<i>M. tenellum</i>								+				
<i>Nymphaoides cordata</i>				+								
<i>Potamogeton bicupulatus</i>					+	+						
<i>P. epiphydrus</i>				+	+		+					
Riccia fluitans											+	
<i>Sagittaria</i> sp.			+		+	+						
<i>Sparganium</i> sp.				+								
<i>Utricularia gibba</i>						+						
<i>U. striata</i>							+					
<i>U. vulgaris</i>						+	+			+		
<i>Vallisneria americana</i>							+					

Notes: Names in boldface type represent genera commonly associated with *Glossostigma* in its native range (see Discussion, *Introduction and ecology*). Abbreviations: AL = Alexander Lake, CR = Chase Reservoir, HC = Hamburg Cove, HL = Hayward Lake, HP = Hopeville Pond, MH = Mansfield Hollow, LC = Lake Carasaljo, LM = Lake McCormack, ML = Mercer Lake, PL = Prospertown Lake, PS = Pete Sensi Park, RS = Rising Sun Lake (see Table 1). Boxes delimit those species that occur commonly in or that are restricted to either Connecticut and/or New Jersey sites.

genus” some 35 years ago, the taxonomy remains in flux with new species still being named and some taxa (e.g., “*Glossostigma* sp. B”) that yet await a definitive taxonomic disposition (Barker, 1982, 1992b; Beardsley and Barker, 2005). Generally, *Glossostigma* species are quite similar; they are minute, simple morphologically, and have few characters useful for identification. Beardsley and Barker (2005) attributed 6–8 *Glossostigma* species to Australia; however, the genus is not included in *Aquatic Plants of Australia* or its supplement (Aston, 1973, 1977), and no comprehensive taxonomic treatment of the genus exists for the country. Rather, sets of different species have been treated in various regional floras of the continent (e.g., Black, 1948; Barker, 1981, 1992a; Sainty and Jacobs, 1981), which have specified the few characters used taxonomically—number of calyx lobes (3 or 4), number of stamens (2 or 4), pedicel length, flower type (cleistogamous or chasmogamous).

In combination, these factors made it extremely difficult to identify *Glossostigma* when it was first encountered in North America (see TAXACOM listserv archives for 30 Aug 1995, message 12). The determination of North American material (from PA specimens) eventually was made by W. R. Barker, an authority on Australian Scrophulariaceae (and *Glossostigma* in particular), who identified the plants as *G. diandrum* (A. Rhoads, Morris Arboretum, personal observation).

However, we found that the published descriptions of *G. diandrum* were inconsistent with our field observations of North American plants. Primarily, the morphologically similar species *G. diandrum* and *G. cleistanthum* (both with 3 calyx lobes and 2 stamens) supposedly were distinguishable by their flower type, i.e., the former having long-pedicelled (2–15 mm) chasmogamous flowers and the latter bearing short-pedicelled (0–0.5 mm) cleistogamous flowers (Barker, 1992a, b). Yet, we found that short-pedicelled, cleistogamous flowers occurred on nearly every North American specimen of *Glossostigma*, an observation that directly contradicted the description of *G. diandrum*.

We also considered the possibility that both *G. diandrum* and *G. cleistanthum* had been introduced to North America given that some populations included plants with chasmogamous flowers. This possibility gained credibility when we established that plants with chasmogamous flowers also possessed significantly shorter leaves (4.5–11 mm) and shorter rhizome internodes (1–10 mm) than did those with cleistogamous flowers (7–57 mm and 1–18 mm, respectively). These observations were consistent with reports that leaves of *G. diandrum* are much shorter (2–15 mm) than those of *G. cleistanthum* (4–45 mm) despite some overlap in length at the lower end of the range (Barker, 1981). Furthermore, at one site (Alexander Lake, CT), a sharp line of demarcation separated the longer-leaved cleistogamous plants (in deeper water) from the

TABLE 3. Comparison of habitat variables in Connecticut lakes where *Glossostigma cleistanthum* occurs compared to 105 surveyed lakes where it does not occur.

Variable	With <i>Glossostigma</i>	Without <i>Glossostigma</i>	<i>t</i>	<i>P</i>	χ^2	<i>P</i>
Maximum depth	2.6	4.0	0.61	ns	1.11	ns
Secchi depth	2.5	1.9	1.376	ns	4.86	<0.05
Temperature (surface)	24.6	25.3	0.211	ns	3.17	ns
Temperature (bottom)	18.2	19.1	0.160	ns	1.35	ns
pH (surface)	5.9	6.4	2.27	<0.05	4.18	<0.02
pH (bottom)	5.5	6.2	2.12	<0.05	1.82	ns
Dissolved O ₂ (surface)	7.2	7.7	0.60	ns	0.81	ns
Dissolved O ₂ (bottom)	3.8	0.6	0.69	ns	3.42	ns
Conductivity (surface)	68	116	2.11	<0.05	7.69	<0.01
Conductivity (bottom)	70	128	2.24	<0.05	8.53	<0.01
Alkalinity (surface)	11.5	22.5	1.92	ns	7.39	<0.01
Alkalinity (bottom)	11.3	27.8	1.96	ns	6.08	<0.02
Total P (surface)	16	21	0.69	ns	2.81	ns
Total P (bottom)	20	50	1.72	<0.05	5.58	<0.02

Notes: Shown are the median values and results of a Mann–Whitney test (*t*) and χ^2 analysis of categories (see Materials and Methods, *Ecological analysis*). Variables showing significant differences ($P < 0.05$) are highlighted in bold. ns = not significant. These results indicate that *G. cleistanthum* occurs mainly in clear, acidic, oligotrophic lakes of low alkalinity and conductivity.

short-leaved chasmogamous plants (in shallow water or exposed substrates), which indicated that the two, morphologically distinct taxa also seemed to occupy different ecological niches.

However, this hypothesis was not substantiated by further morphological analyses. First, our measurements of Australian specimens of *G. diandrum* indicated that the species possessed leaves that were significantly shorter (2–10 mm) than cleistogamous-flowered North American plants, and most also were shorter than even the chasmogamous-flowered plants. Furthermore, the North American specimens fell well within the range of leaf length observed for Australian material of *G. cleistanthum* but outside the range of *G. diandrum*, even when only emergent plants (which would be most similar to *G. diandrum*) were compared. Adding to the uncertainty were comments by Barker (1992b) who reported that *G. diandrum* (typically with pedicellate chasmogamous flowers) could produce cleistogamous flowers “in rare instances,” but noted that they were stalked and occurred on plants with “normal” (chasmogamous) flowers. He also remarked that pedicels of the cleistogamous flowers in *G. cleistanthum* were “usually 0–0.5 mm long” but also could be “rarely 5–9 mm long” (Barker, 1992b). Thus, it would be extremely difficult to identify with certainty any *Glossostigma* plant that possessed both sessile and pedicellate flowers (as in several of our cases), especially if plants were in fruiting condition where other floral details would be lacking. Observing that a number of North American plants possessed both pedicellate, chasmogamous, and nearly sessile, cleistogamous flowers (Fig. 1a) in addition to those numerous individuals strictly bearing sessile, cleistogamous flowers, it became evident that flower type was unreliable taxonomically and that identification of North American plants as *G. diandrum* could not be validated solely using the morphological data available.

Fortunately, a genetic means to facilitate species identification was provided by the publication of DNA sequence data for all known *Glossostigma* taxa occurring in Australia (Beardsley and Barker, 2005). Because most of the taxa were sufficiently divergent for the loci surveyed (nrITS, nrETS, *trnL*) that data set enabled species determinations to be made using a comparative sequencing approach. Thus, by analyzing the

DNA sequences of *Glossostigma* material collected from the introduced, North American populations and comparing them to the sequences obtained for the native Australian populations, we were able to achieve an identification that was based on data that were independent from their morphology.

We found that the DNA sequences of all North American populations surveyed, including those plants with cleistogamous flowers, chasmogamous flowers, or mixtures of both floral types, matched the sequence reported for *G. cleistanthum* while differing considerably from those reported for *G. diandrum* (Fig. 2). Although *G. cleistanthum* originally had been regarded as a variant of *G. diandrum* (e.g., Johnson and Brooke, 1998), a relatively high level of DNA sequence divergence (see Results, *Molecular analysis*) and their distinct cladistic relationships (Fig. 2) support the recognition of these taxa as separate species. In contrast, multiple accessions of *G. diandrum*, *G. trichodes* (Beardsley and Barker, 2005), and *G. elatinooides* (see Results, *Molecular analysis*) resolved as clades with relatively minor sequence divergence between accessions. Furthermore, the complete identity of DNA sequences obtained from the two loci (ETS, ITS) surveyed across all North American accessions enabled us to conclude that only one species had been introduced to the continent and that it was *G. cleistanthum*.

We have a high level of confidence that the DNA sequence in GenBank was obtained from authentic material of *G. cleistanthum* because the DNA voucher (Barker 4597, AD) not only was collected by the author of *G. cleistanthum* but also was listed by him among the “specimens examined” in the protologue (Barker, 1992b; Beardsley and Barker, 2005). Moreover, based on prior taxonomic concepts, it would have been far more likely to misidentify a chasmogamous-flowered accession of *G. cleistanthum* as *G. diandrum* (as occurred originally with the North American material), than to identify a cleistogamous-flowered plant as any species but *G. cleistanthum*.

Rather than representing taxonomic distinctions, the close association of leaf and rhizome internode length with flower type as described (see Results, *Morphological analysis*) must reflect differential phenotypic responses of *G. cleistanthum*

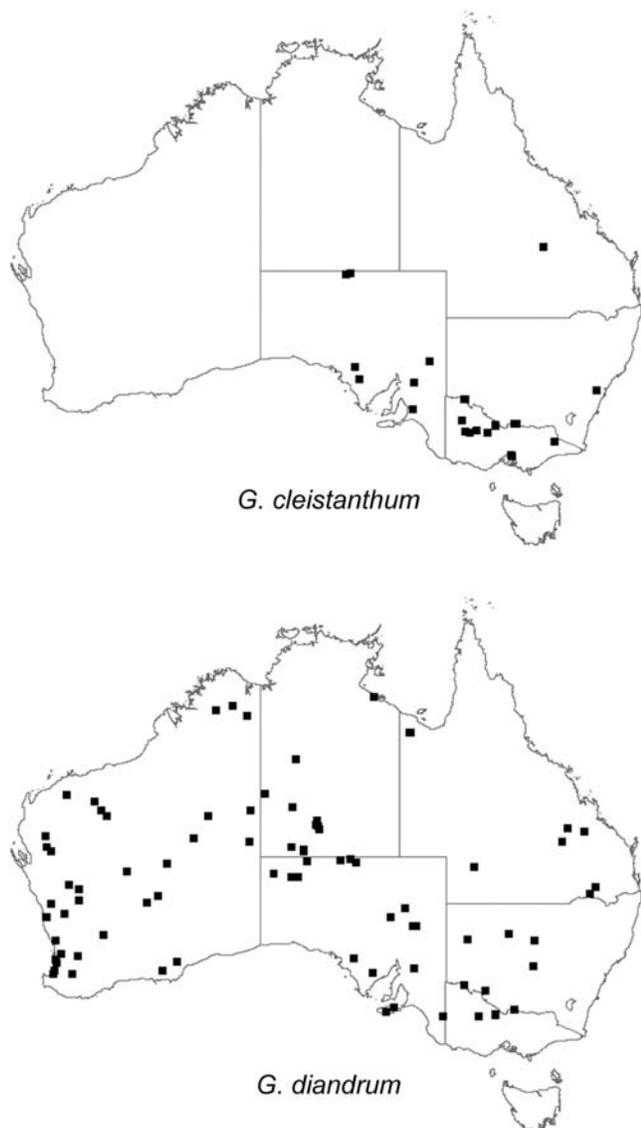


Fig. 5. Maps of presumed native distribution of *Glossostigma cleistanthum* (top) and *G. diandrum* (bottom) in Australia (mapped using "Australia's Virtual Herbarium" website; see Materials and Methods, *Ecological analysis*). *Glossostigma cleistanthum* occurs mainly in temperate (southeastern) portions of the Australian continent, whereas *G. diandrum* is widespread in much of the tropics. Some specimens plotted on the maps may be misidentified (see Discussion, *Taxonomic implications*).

plants when grown under submersed vs. emerged conditions. Our field observations indicated that North American plants growing permanently submersed in deeper water possessed only cleistogamous flowers, whereas plants growing on saturated but non-inundated substrates produced chasmogamous flowers. Plants with mixtures of chasmogamous and cleistogamous flowers were found in intermediate sites that were likely to experience alternating conditions of exposure and (shallow) inundation.

The short leaves and internodes of chasmogamous-flowered plants produce a highly clumped habit, whereas submersed plants display longer leaves along an elongate rhizome (Fig.

1c). We also observed (at the Alexander Lake site) that chasmogamous plants were annual and died back entirely in the autumn, while the submersed, cleistogamous plants remained green and viable (we observed healthy, flowering [cleistogamous] plants beneath the ice in Alexander Lake, CT on 26 December 2005). Thus, it appears that duration (i.e., annual or perennial habit) also is associated with the different floral forms observed in *G. cleistanthum*, at least in the North American populations. Australian material of *G. diandrum* that we examined did not have this correlation. That species consistently produced short leaves along a fairly wide range of rhizome lengths. Based on these results, a revised morphological description of North American *G. cleistanthum* is given:

Diminutive, aquatic, creeping, herbaceous, glabrous, emerged annuals or submersed perennials (at depths of 0.1–4.0 m), reproducing vegetatively by rhizome fragmentation; rhizomes slender, the internodes short (1–10 mm) on emerged plants or elongate (1–18 mm) on submersed plants, each rhizome node producing a cluster of 3–6 (normally 5) adventitious roots and an opposite pair of small, erect, spatulate leaves; leaves containing 2 parallel, longitudinal lacunae basally (Fig. 1d), the blades 4.5–11 mm long when emerged and 7–57 mm long when submersed (80% were 9–25 mm); flowers cleistogamous and short-pedicelled (0–2 mm) when submersed or chasmogamous and longer-pedicelled (>4 mm) when emerged; calyx 3-lobed, urceolate and 1.5–3.0 mm (cleistogams); corolla rudimentary (cleistogams) or exserted, 5-lobed, bilabiate, whitish (chasmogams); stamens 2; capsules 1.4–1.8 mm with 12–73 seeds (cleistogams); seeds reticulate, 0.4–0.5 mm, flattened bilaterally.

Although leaf length has not been used taxonomically in the genus, our analyses indicate that *G. cleistanthum* can be distinguished from *G. diandrum* by its greater leaf length, which typically averages more than 10 mm, whereas leaves of *G. diandrum* seldom reach 8 mm and typically average about 5 mm. An examination of herbarium material of *G. diandrum* found that its leaves lacked the lacunal spaces observed in *G. cleistanthum*. Whether this feature remains consistent among the different ecological forms of both species requires further study.

Because it is evident that the degree of morphological variability in *G. cleistanthum* has been underestimated, a thorough reevaluation of Australian *Glossostigma* material using molecular methods is recommended given the likelihood that some specimens of *G. diandrum* and *G. cleistanthum* have been identified incorrectly. In particular, *G. diandrum* is distributed widely in tropical climates but also extends into temperate regions where it essentially overlaps with the more temperate distribution of *G. cleistanthum* (Fig. 5). Some records in temperate Australia are completely superimposed, indicating either close sympatry of the two species or misidentifications of the mapped specimens. Similarly, several records of *G. cleistanthum* in tropical/subtropical localities are disjunct and anomalous with the other temperate records for the species, yet they overlap with records for *G. diandrum*, again indicating possible misidentifications (Fig. 5). In any case, the native Australian range of *G. cleistanthum* is predominantly (if not entirely) temperate and provides one indication why the species has established and naturalized so readily in temperate portions of North America.

Introduction and ecology—The most plausible means of introduction for *Glossostigma cleistanthum* into North America

is through the careless disposal of plants imported as aquarium ornamentals. Although *G. elatinooides* is preferred as an ornamental aquarium plant (Kasselmann, 2003), the difficult identification of species in this genus would almost insure that other taxa inadvertently are supplied to the aquarium trade as well. Indeed, Barker (1992b) noted several cultivated collections of *G. cleistanthum* in Australia, one "from aquarium tanks." More than 75% of all nonindigenous aquatic plants in the New England region have been introduced as escapes from cultivation with nearly all persisting since their initial introduction (Les and Mehrhoff, 1999; Les, 2002). Similarly, the prolonged persistence of *G. cleistanthum* is indicated by the fact that plants were still present at every known site of the species that we visited, including the earliest (1992) locality known for all of North America (Appendix).

The presence of *G. cleistanthum* in North America (not included by Les and Mehrhoff, 1999) further strengthens the premise that one new invasive aquatic plant species has been added to the flora of the northeastern United States approximately every decade since 1850 (Les, 2002). Such introductions invariably will continue unless the importation, sale, and cultivation of potentially invasive aquatic plants somehow are curtailed (Les and Mehrhoff, 1999; Les, 2002).

Once introduced, dispersal of *Glossostigma* species is facilitated by seeds carried in the mud adhering to waterfowl feet (Cook, 1996b; Lamont and Fitzgerald, 2001). Various ducks and geese frequent many of the sites where we observed *G. cleistanthum* and undoubtedly are involved in its dispersal. Seed production in *G. cleistanthum* occurs via chasmogamous or cleistogamous flowers, depending mainly on whether plants are submersed or emergent. Chasmogamous flowers occur only on emergent plants. These reportedly are insect pollinated (Cook, 1996b) and adapted for cross-pollination. Flowers possess a tongue-shaped stigma (hence the generic name), which covers the mouth of the corolla while situating the receptive surface to the exterior (Barker, 1982). When stimulated tactilely, the stigma springs open, coming to lie against the corolla as it exposes the anthers; after a short time, the stigma reflexes to its original position (Johnson and Brooke, 1998; Beardsley and Olmstead, 2002).

The significance of chasmogamous pollination deserves further study as *Glossostigma* flowers typically are found having few or undeveloped pollen grains (Argue, 1986). Because of its very low pollen to ovule ratio ($P/O = 5.0$) and cleistogamous flowers, Barker (1982) concluded that *G. cleistanthum* was obligately autogamous. Although Barker apparently was unaware that *G. cleistanthum* also can produce numerous chasmogamous flowers on occasion, they probably are of little consequence to the breeding system given that even the closely related and chasmogamous *G. diandrum* also appears to be highly autogamous (Barker, 1982).

Introduced North American populations of *G. cleistanthum* produce far fewer chasmogamous flowers than cleistogamous flowers. Seeds produced by chasmogamous *Glossostigma* flowers are dispersed narrowly by wind and rain by means of a "splash-cup" method (Barker, 1982). However, with dense patches of cleistogamous-flowered plants capable of yielding upwards of 23 000 seeds/m² (see Results, *Ecological analysis*), the opportunity for dispersal of *G. cleistanthum* would be quite high if the seeds were released on exposed shorelines where waterfowl browse. Although Barker (1992b) indicated that the short-stalked fruits of *G. cleistanthum* typically are pushed down into the mud, we observed that most seed production in

North American populations occurs underwater from submersed cleistogamous flowers that remain above the substrate or sink immediately when detached from plants. However, we also found that whole plants with attached cleistogamous flowers will float readily on the surface if they are dislodged from the substrate (e.g., as a result of waterfowl foraging).

Flotation is due to buoyancy conferred by longitudinal air-space lacunae in the bases of the small leaves (Fig. 1d). In such instances, the fruits can be carried for some distance in a lake until they are deposited along the banks as the dislodged plantlets eventually wash ashore. Along the drift line on mud flats in one site (Mansfield Hollow Reservoir, CT), we observed young *Glossostigma* plants whose small rhizomes radiated in all directions from a central point, a pattern that would result from a lodged fruit where several seeds had germinated simultaneously. Seedbank studies in New Zealand wetlands found that *G. cleistanthum* often dominated areas where inundation was nearly permanent. In these areas, submersed plants died during occasional periods of exposure while most wetland plants were intolerant of the nearly continuous inundation (Casanova and Brock, 2000). A final consideration regarding dispersal potential of *G. cleistanthum* is its ability to perennate when submersed; thus, vegetative reproduction by rhizome elongation also occurs at a local scale.

In any case, an efficient dispersal mechanism for *G. cleistanthum* is indicated by the widespread distribution of plants (19 known sites spanning a distance of nearly 400 km), which has been achieved in just over a decade. Undoubtedly, many more sites will become known as more purposeful surveys are undertaken for the plant. Potential sites for *G. cleistanthum* can be predicted using the rather consistent habitat features that characterize the known North American populations. Most North American sites are lacustrine shores of small ponds to large lakes, although one site (Hamburg Cove, CT) is a tidal freshwater riverine shoreline. In all cases, substrates range from pure sand to various combinations of sand with clay, silt, and occasionally gravel. Lakes with sandy shorelines are most likely to support this species and provide habitat similar to that in New Zealand where *Glossostigma* forms dense mats when growing on sandy substrates or on gravel with sandy interstices (Brown, 1979).

In New Zealand, *Glossostigma* is common in lakes where water levels vary (Riis and Hawes, 2002). Because of its ability to survive under submersed or emergent conditions, sites with a gently sloping shoreline and fluctuating water levels provide ideal habitat. Tolerance to these conditions explains why *G. cleistanthum* has adapted well to the daily tidal fluctuations in Hamburg Cove, a freshwater wetland on the Connecticut River.

Comparison of water variables for Connecticut lakes where *G. cleistanthum* does and does not occur provides some insight into the ecological affinities of this introduced species and assists in identifying potential sites for new invasions. We found *G. cleistanthum* to occur in clear, acidic water of low alkalinity, conductivity, and nutrients (Table 3), i.e., essentially oligotrophic conditions. Although we did not survey the water chemistry of lakes in NJ, PA, or RI, many of those sites appeared to possess similar water characteristics, at least by visual inspection. Certainly, the association of *G. cleistanthum* with oligotrophic habitats presents a different situation than the many cases where invasive aquatic weeds occur in eutrophic or polluted sites. Even though a number of *G. cleistanthum* sites (especially in NJ) are disturbed or artificial habitats (e.g., sand pits and reservoirs), it is evident that *G. cleistanthum* also has

the potential to invade more pristine areas, which often support rare or imperiled species.

In North America, *G. cleistanthum* occurs frequently with other small plants of sandy sediments such as *Elatine minima*, *Limosella aquatica*, *Eriocaulon aquaticum*, and *Isoetes* spp. In New Zealand, *Glossostigma* species occupy similar communities and commonly are associated with many of the same genera (e.g., *Elatine*, *Isoetes*, *Lilaeopsis*, *Limosella*, *Myriophyllum*) as *G. cleistanthum* in North America (Chapman et al., 1971; Carter, 1973; Patterson, 1978; Wells et al., 1998; Riis and Hawes, 2002). Among the 29 species observed to associate with *G. cleistanthum*, the most consistent were *Eleocharis acicularis* and *Elatine* spp. (i.e., *E. minima* or *E. americana*) (Table 2). *Gratiola aurea*, another plant with both submersed and emergent life forms, co-occurred in nearly all of the New England sites but was not seen in any of the NJ sites; conversely, *Ludwigia palustris* (also amphibious), co-occurred in most NJ sites, but not in any of the New England sites (Table 2). The unusual exclusive distributions of *Gratiola aurea* and *Ludwigia palustris* are difficult to explain given that both species are widespread throughout eastern North America, including both New England and NJ.

***Glossostigma cleistanthum*: invasive or benign?**—The present study of *G. cleistanthum* in North America has provided sufficient information to evaluate the invasive potential of this species. According to Les and Mehrhoff (1999), invasive species are (1) nonindigenous, (2) capable of establishment (i.e., naturalized), and (3) capable of spreading significantly within natural communities. The first two criteria are easiest to demonstrate. There is no question that *G. cleistanthum* is nonindigenous to North America, given that even the genus was unknown to the New World until 1992. Also, there can be little doubt that *G. cleistanthum* has naturalized in North America as evidenced by its spread to at least 19 known sites spanning several hundred kilometers in the eastern United States and its persistence at some localities for more than 14 years (Appendix).

However, it is not as easy to demonstrate whether a species has the ability to spread “significantly” in a natural community, given the ambiguity associated with these terms. First, it is difficult to establish precise qualifications for “natural” communities because even the most pristine landscapes seldom are devoid of cultural perturbation. *Glossostigma cleistanthum* mainly has colonized anthropogenic lakes such as abandoned sand quarries (e.g., McCormack Lake, NJ) and reservoirs (e.g., Mansfield Hollow, CT); however, it also has colonized natural communities such as Nellie’s Pond, NJ, which is recognized as an environmentally sensitive site by the New Jersey Department of Environmental Protection (UTU, 1998), and Hamburg Cove, CT, a wetland of international significance within the Lower Connecticut River (Ramsar, 2005). Two additional Connecticut sites (Alexander Lake, Hayward Lake) also are natural water bodies (Jacobs and O’Donnell, 2002). Several other sites (e.g., Assunpink Lake, McCormack Lake, Rising Sun Lake) may not represent natural communities per se, but currently are designated as state wildlife refuges.

The potential impact on imperiled species is another concern because invasive species have been identified as a leading cause of extinction (Mooney and Cleland, 2001). Indeed, several species associated with *G. cleistanthum* (*Ceratophyllum echinatum*, *Crassula aquatica*, *Elatine* spp., *Eriocaulon parkeri*, *Heteranthera multiflora*, *Lilaeopsis chinensis*, *Limo-*

sella subulata, *Myriophyllum tenellum*, *Utricularia gibba*) currently are listed as imperiled taxa in CT and/or NJ (CT DEP, 2005; NJ DEP, 2005).

Due to the brief time that has elapsed since its introduction (14 years), it is not possible to appraise the rapidity of spread for *G. cleistanthum* using specimen accumulation curves as done for other aquatic plants by Les and Mehrhoff (1999). However, within its first 10–20 years of introduction, *G. cleistanthum* already has been recorded from two to three times as many localities as was *Cabomba caroliniana*, an aquatic plant that has become notoriously invasive in southern New England (Les and Mehrhoff, 1999). This observation indicates that *G. cleistanthum* has the potential to spread rapidly throughout the Northeast and elsewhere if suitable habitats exist.

The high vagility of *G. cleistanthum* is indicated further by its high reproductive potential (described earlier) and proven record of rapid dispersal within lakes. Although the species was not observed in Mansfield Hollow Reservoir, CT until 2003, within 2 years it had spread to occupy 91 discrete patches covering nearly 12 000 m² of shoreline. In Mansfield Hollow, *G. cleistanthum* now occupies 1.3% of the prime littoral habitat (below 1.4 m depth) in the lake (Fig. 4). Furthermore, Mansfield Hollow is one site where *Glossostigma* plants occur in relatively low densities (Table 1), presumably as a consequence of their recent introduction. Some of our quantitative surveys, in lakes where the species undoubtedly has persisted for a longer duration, found extraordinarily high densities reaching as much as 25 000 plants/m². At some of the sites where such high densities have developed (Alexander Lake, CT; Rising Sun Lake, NJ), many portions of the lake bottom virtually appear as a solid green mat of *G. cleistanthum* that extends for many meters to the exclusion of all other species. From our field observations and survey data, it is readily apparent that once this species is introduced to a lake, its widespread dispersal throughout the prime littoral habitat is inevitable. Together, these observations indicate that *G. cleistanthum* is indeed an invasive species and must be regarded as a potentially serious threat to natural aquatic plant communities in North America.

Ironically, there appears to be little present concern over *Glossostigma* among natural resource management agencies. Because of its diminutive stature, the plant commonly is perceived as nonthreatening. Its low growth form is inconspicuous to lake residents, does not pose a threat to boating or other forms of water recreation, and its prolific seed production even provides food for waterfowl. However, one must distinguish between plants that are invasive (i.e., posing an ecological threat) and those that are “weeds,” i.e., species that interfere with management or appreciation of natural resources (Les and Mehrhoff, 1999). At least at present, *G. cleistanthum* does not appear to be a weed, but it most certainly is invasive.

Because *G. cleistanthum* grows mainly in low underwater mats, it is extremely difficult to detect until the patch density reaches a conspicuous level of infestation. As a consequence, the species is probably much more widely established than current records would indicate. We advise that surveys for *Glossostigma* should be undertaken in prospective new areas (e.g., eastern Long Island, New York), as well as more intensive field work in other portions of NJ, eastern PA, and southern New England where records currently exist, to obtain further distributional information for this invasive species.

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APPENDIX. Chronology of *Glossostigma* observations in North America. Fields occur in the following order for each entry: state, county, municipality, locality: voucher information. Each observation is separated by a semicolon. Dates in boldface type represent the earliest record for a site (other dates are later collections from the same sites). Voucher specimens are in italics, and the herbarium where they have been deposited is in parentheses.

CT, New London, Lyme, Hamburg Cove: **20 Oct 1992**, *Moorhead 92020043* (CONN)^a; NJ, Middlesex, Scotts Corner, McCormack Lake: John Fishback, **1993** (sight record reported by David Snyder); NJ, Middlesex, Scotts Corner, McCormack Lake: 13 Jul 1994, *Snyder 4601* (dbs, CHR, PH); NJ, Middlesex, Scotts Corner, McCormack Lake: 31 Aug 1994, *Schuyler 7943* (PH)^b; NJ, Middlesex, Scotts Corner, McCormack Lake: 5 Oct 1994, *Snyder 4753* (dbs, CHR, PH); PA, Bucks, Doylestown, Lake Galena: sight record by Ann Rhoads, **1994**; NJ, Ocean, Lahaway Creek, Prospertown Lake: **19 Feb 1995**, *Snyder 4765* (dbs); NJ, Ocean, Lakewood, Lake Carasaljo: **8 Aug 1995**, *Snyder 4932* (dbs); NJ, Mercer, Mercer Co. Park, Mercer Lake: **23 Aug 1995**, *Snyder 4987* (dbs, CHR, PH); PA, Bucks, Doylestown, Lake Galena: 28 Aug 1995, *A. Rhoads s.n.* (MOAR)^c; PA, Bucks, Doylestown, Lake Galena: 24 Aug 1996, *A. Rhoads s.n.* (MOAR); NJ, Monmouth, Roosevelt, Rising Sun Lake: sight record by David Snyder, **1996–1997**; NJ, Monmouth, Allentown, Pete Sensi Park: sight record by Floyd Yoder, **1997–2000** (pers. comm.); CT, New London, Lyme, Hamburg Cove: 15 Oct 1998, *Moorhead 4151* (CONN)^d; PA, Bucks, Doylestown, Lake Galena: 7 Jun 2000, *C. C. Jacono 198 & H. D. Jacono s.n.* (FLAS); NJ, Mercer, Mercer Co. Park, Mercer Lake: 21 Aug 2001, *Jacono 315 & H. D. Jacono s.n.* (FLAS); NJ, Monmouth, Allentown, Pete Sensi Park: 21 Aug 2001, *Jacono 314 & H. D. Jacono s.n.* (DOV, FLAS, Z); NJ, Monmouth, Roosevelt, Assunpink Lake: **23 Jul 2002**, *Snyder 6414A* (dbs); NJ, Burlington, Delanco, Nellie's Pond: **7 Aug 2002**, *Snyder 6448* (dbs); CT, New London, Griswold, Hopeville Pond: **21 Aug 2002**, *Mehrhoff 21853* (CONN)^d; RI, Washington, Richmond, Meadow Brook Pond: **12 Sep 2002**, *L. L. Gould, s.n.* (CONN)^d; CT, New London, Griswold, Hopeville Pond: 19 Sep 2002, *Mehrhoff 21921* (CONN)^d; CT, Tolland, Mansfield, Mansfield Hollow: **5 Sep 2003**, *Moody 320 & D. H. Les s.n.* (CONN)^d; CT, Windham, Killingly, Alexander Lake: **13 Jul 2005**, *Capers 849 &*

Russell s.n. (CONN)^e; CT, Windham, Killingly, Chase Reservoir: **8 Aug 2005**, *Capers 968 & Russell s.n.* (CONN)^e; CT, Middlesex, East Haddam, Hayward Lake: **8 Aug 2005**, *Selsky 158 & Bugbee 27* (CONN)^e; CT, Middlesex, East Haddam, Hayward Lake: 9 Aug 2005, *Selsky 173 & Bugbee 42* (CONN)^e; CT, Windham, Killingly, Chase Reservoir: 21 Aug 2005, *Auer et al., s.n.* (CONN)^e; CT, New London, Lyme, Hamburg Cove: 27 Aug 2005, *Capers 1087* (CONN)^e; NJ, Middlesex, Patrick's Corner, Farrington Lake: **31 Aug 2005**, *Snyder s.n.* (dbs); CT, Windham, Killingly, Alexander Lake: 18 Sep 2005, *Capers 1210* (CONN)^e; CT, Middlesex, East Haddam, Hayward Lake: 24 Sep 2005, *Capers 1258* (CONN)^e; CT, New London, Griswold, Hopeville Pond: 1 Oct 2005, *Capers 1292* (CONN)^e; CT, Tolland, Mansfield, Mansfield Hollow: 6 Oct 2005, *Capers 1298 & Les s.n.* (CONN)^d; PA, Bucks, Doylestown, Lake Galena: 16 Oct 2005, *A. Rhodes s.n.* (CONN, MOAR)^d; CT, Windham, Killingly, Alexander Lake: 17 Oct 2005, *Capers 1301 & Les s.n.* (CONN)^d; RI, Washington, Richmond, Meadow Brook Pond: 17 Oct 2005, *Capers 1302 & Les s.n.* (CONN)^d; CT, New London, Lyme, Hamburg Cove: 22 Oct 2005, *Capers 1306* (CONN)^d; NJ, Middlesex, Scotts Corner, McCormack Lake: 31 Oct 2005, *Capers 1319 & Les 687* (CONN)^e; NJ, Mercer, Mercer Co. Park, Mercer Lake: 31 Oct 2005, *Capers 1320 & Les 688* (CONN)^e; NJ, Monmouth, Allentown, Pete Sensi Park: 31 Oct 2005, *Capers 1321 & Les 689* (CONN)^e; NJ, Ocean, Lahaway Creek, Prospertown Lake: 31 Oct 2005, *Capers 1322 & Les 691* (CONN)^e; NJ, Monmouth, Roosevelt, Rising Sun Lake: 31 Oct 2005, *Capers 1323 & Les 698* (CONN)^e; NJ, Ocean, Lakewood, Lake Carasaljo: 31 Oct 2005, *Capers 1324 & Les 701* (CONN)^e; NJ, Ocean, Forked River Mountain, Sand pit: sight record, **2005** (Snyder, pers. comm.); NJ, Middlesex, Jamesburg, Lake Manalapan: sight record by Floyd Yoder, **2005** (pers. comm.); CT, Windham, Killingly, Alexander Lake: 26 Dec 2005, *Capers 1354* (CONN).

Notes: * = voucher for DNA sequence data (see Discussion, *Molecular analysis for GenBank accession numbers*); dbs = personal herbarium of David B. Snyder. CT = Connecticut, NJ = New Jersey, PA = Pennsylvania, RI = Rhode Island.

^a Identified originally as *Elatine triandra*; annotated as *G. cleistanthum* by D. H. Les on 3 Oct 2005.

^b Identified originally as *Limosella acaulis*; annotated as *G. diandrum* by A. E. Schuyler in 1995.

^c Duplicate material annotated as *G. diandrum* by W. R. Barker in 1995.

^d Identified originally as *G. diandrum*; annotated as *G. cleistanthum* by D. H. Les on 11 Oct 2005.

^e Identified originally as *Glossostigma* sp.; annotated as *G. cleistanthum* by R. S. Capers on 1 Dec 2005.