SUBSPECIES CLASSIFICATION OF *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE)

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ABSTRACT

Hydrilla verticillata is a globally distributed aquatic plant and one of the most noxious aquatic weeds in the U.S.A. Phylogenetic evidence has shown that plants in different geographic regions are largely isolated, and there are morphological features that can distinguish the five major phylogenetic groups. *Hydrilla* plants in the U.S.A. have been identified as monoecious or dioecious biotypes, with each biotype having distinctive morphology and molecular sequence data. However, the recent discovery of a third invasive biotype in the northeastern U.S.A. has prompted the need for precise nomenclature that can refer to *Hydrilla* plants in the native and non-native range of this widespread species. New subspecific names and combinations are proposed in order to enable effective communication about the five major phylogenetic variants of *Hydrilla verticillata*. Two subspecies names are made as new combinations of existing species names: **Hydrilla verticillata** subsp. **angustifolia** and **Hydrilla verticillata** subsp. **lithuanica,** and two subspecies names are newly established: **Hydrilla verticillata** subsp. **australis** and **Hydrilla verticillata** subsp. **peregrina.**

RESUMEN

Hydrilla verticillata es una planta acuática de distribución mundial y una de las malezas acuáticas más nocivas en los EE. UU. La evidencia filogenética ha demostrado que las plantas en las regiones geográficas diferentes están en gran medida aisladas y hay características morfológicas que pueden distinguir los cinco grupos filogenéticos principales. Las plantas de *Hydrilla* en los EE. UU. se han identificado como biotipos monoicos o dioicos, y cada biotipo tiene una morfología distintiva y datos de secuencia molecular. Sin embargo, el reciente descubrimiento de un tercer biotipo invasivo en el noreste de los EE. UU. ha planteado la necesidad de una nomenclatura precisa que pueda referirse a las plantas *Hydrilla* en el rango nativo y no nativo de esta especie extendida. Se proponen nuevos nombres y combinaciones con el rango de subespecie, para permitir una comunicación eficaz sobre las cinco principales variantes filogenéticas de *Hydrilla verticillata*. Se crean dos nombres de subespecies como nuevas combinaciones de nombres de especies existentes: **Hydrilla verticillata** subsp. **angustifolia** y **Hydrilla verticillata** subsp. **lithuanica,** y se han establecido nuevamente dos nombres de nuevas subespecies: **Hydrilla verticillata** subsp. **australis** y **Hydrilla verticillata** subsp. **peregrina.**

Key Words: *Hydrilla*, morphometrics, phylogenetics, taxonomy, water-thyme

INTRODUCTION

Hydrilla verticillata (L.f.) Royle (hydrilla or water-thyme) is an aquatic monocot species native to Australia, Eurasia, and central Africa, which has received significant attention as a noxious weed in North America and elsewhere (Jacono et al. 2020; Benoit et al. 2019). The genus *Hydrilla* Rich. is currently regarded as monotypic, although a variety of species and infraspecific taxa have been named (Cook & Lüönd 1982). In the most comprehensive morphological study to date, Cook & Lüönd (1982) determined that variation in morphology, chromosome number, and physiology could not be parsed cleanly among taxonomic groups, and therefore they recommended that such variation be regarded as intraspecific.

Hydrilla has become a widespread invasive weed in the U.S.A., where three distinct variants exist. For almost 50 years, two 'biotypes' have been recognized, and studies have documented their differences in leaf morphology (Benoit et al. 2019), ecology (Steward 1993; Owens et al. 2012; True-Meadows et al. 2016), and reproductive status. Plants in more northern localities typically are the monoecious biotype, whereas southern plants tend to be the dioecious biotype, the latter existing as only pistillate (female) plants in the invasive range (Cook & Lüönd 1982; Haller 1982; Steward et al. 1984; Langeland 1996; Madeira et al. 1997, 2000; True-Meadows et al. 2016; Jacono et al. 2020). A third biotype was identified in 2020 with currently unknown

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reproductive status, but with more numerous leaves per node than other U.S.A. hydrilla plants, and these plants are referred to as the 'Connecticut River' biotype (Tippery et al. 2020).

Molecular data have confirmed that the invasive hydrilla biotypes belong to distinct phylogenetic lineages. Data from the uniparentally inherited plastid *trnL-F* region served as the first diagnostic DNA sequence markers and also provided the first evidence linking North American plants with potential source localities in the native range (Madeira et al. 2007; Zhu et al. 2015). Further investigation of nuclear DNA sequence data from the internal transcribed spacer region (ITS) and the phytoene desaturase gene (*PDS*) revealed instances of natural hybridization among the lineages that had been identified by plastid data (Benoit et al. 2019; Tippery et al. 2020). Nonetheless, the basic phylogenetic relationships that were resolved using nuclear DNA sequences were consistent with those on the plastid phylogeny. Moreover, even after accounting for hybridization, a broad geographic pattern remained. Five phylogenetic lineages were largely separated in the native *Hydrilla* range, and these could be distinguished readily by sequencing the plastid *trnL-F* region (Fig. 1).

Morphologically, hydrilla specimens rarely exhibit reproductive characteristics, so a limited number of features can be discerned from the vegetative portions of plants. Measurements of leaf length and width have been useful for discriminating among some of the phylogenetic groups, including the monoecious and dioecious biotypes in the U.S.A. (Benoit et al. 2019). In the invasive range, the presence of abaxial midrib prickles (sometimes referred to as 'spines'; Cook & Lüönd 1982; Benoit et al. 2019) was shown to be a reliable feature for identifying the dioecious biotype (Benoit et al. 2019), and the Connecticut River biotype could be distinguished from other North American biotypes by regularly having six or more leaves per node (Tippery et al. 2020). Combined with the generally isolated geographic distributions of the three biotypes in North America, these morphological features are useful for initial field identification. Nonetheless, in all portions of the native and adventive range, confident identifications of hydrilla specimens rely on genetic confirmation via DNA sequencing.

With the discovery of the third hydrilla biotype in North America, there is a greater need to refer to these plants using stable, regulated taxonomic names. In order to facilitate effective communication about native and non-native *Hydrilla* plants worldwide and to promote stable taxonomic categories for classifying invasive plants, this paper provides subspecies designations for *H. verticillata*. The subspecific rank was chosen to enable the continued usage of a single species name worldwide, and also to acknowledge a background level of interbreeding among lineages that is consistent with the subspecific level of classification.

MATERIALS AND METHODS

Herbarium specimen images were obtained from the respective websites of several herbaria (Tippery 2023). If geographic coordinates (latitude and longitude) were provided in an electronic format, these were used, otherwise they were newly obtained by locating points on Google Maps (https://maps.google.com/) using locality descriptions. In the event that only broad geographic areas were cited (e.g., country or province), the approximate midpoint for each geographic area was used. Specimens with geographic coordinates were plotted onto maps provided by the geoBoundaries global database of political administrative boundaries database (Runfola et al. 2020) using the *ggplot2* ver. 3.4.2 package (Wickham 2016) in R ver. 4.3.0 (R Core Team 2023). Latitude and longitude coordinates were 'jittered' to make multiple points at the same coordinates more visible, by adding a randomly generated, normally distributed number (mean = 0, standard deviation = 1) to both latitude and longitude.

Phylogenetic data.—Previously published DNA sequences for the plastid *trnL-F* were obtained from GenBank (Madeira et al. 2007; Benoit et al. 2019; Tippery et al. 2020). Representative sequences were obtained for the plants in each previously defined plastid clade (Madeira et al. 2007; Zhu et al. 2015). Sequences were aligned and analyzed following Benoit et al. (2019).

Morphological data.—In addition to the counts and measurements reported previously (Benoit et al. 2019), additional morphological data were obtained from electronic images of herbarium specimens, using the method described by Tippery et al. (2021). The following data were obtained: leaf length and width, leaf number

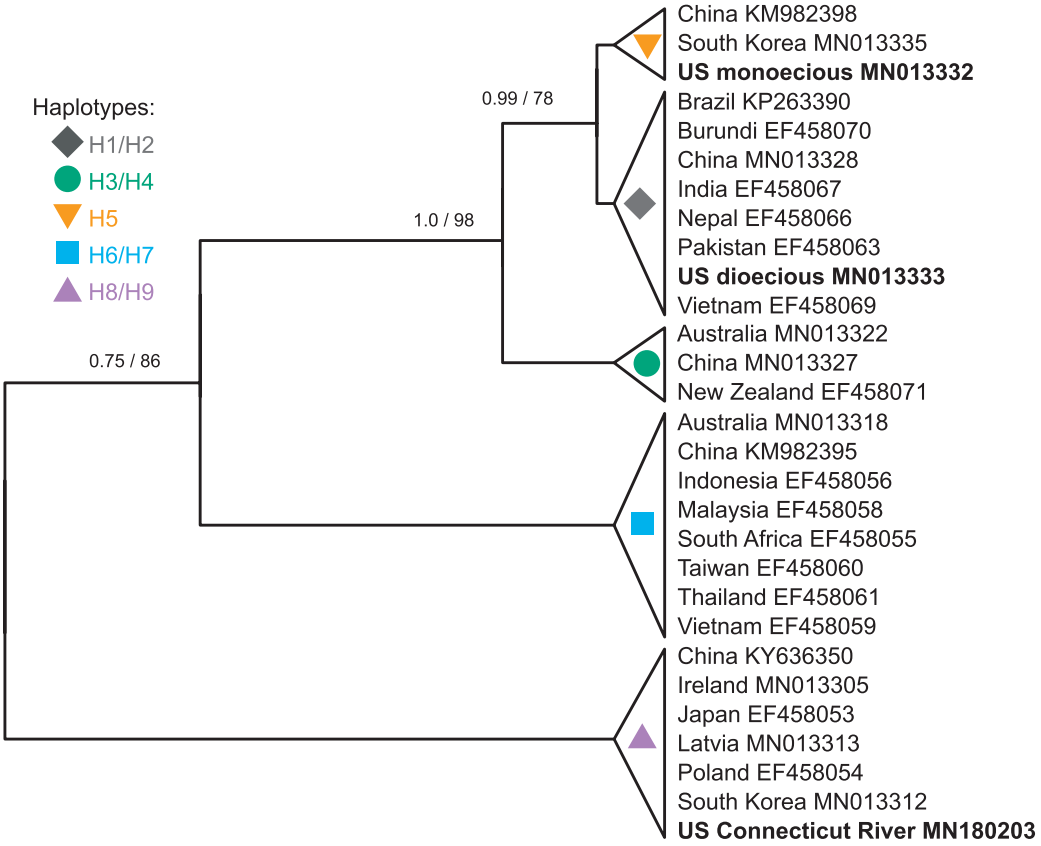

Fig. 1. Phylogeny of *Hydrilla* constructed using the plastid *trnL-F* region. Representative sequences from separate countries are followed by GenBank accession numbers. Sequences and methods were reported previously (Madeira et al. 2007; Benoit et al. 2019; Tippery et al. 2020). Internal node labels show Bayesian posterior probability and maximum likelihood bootstrap percentage. Haplotype identities are included for reference to previous publications (Zhu et al. 2015; Benoit et al. 2019; Tippery et al. 2020).

per node, and number of marginal prickles (teeth) per leaf (i.e., the total number obtained by counting both sides of the leaf). Leaf length:width ratio was calculated by dividing the length by width for each leaf.

Five morphometric categories (morphogroups) were established, corresponding to the major clades on the *trnL-F* phylogeny (Fig. 1). Morphogroups were identified using the haplotype numbers that were established by Zhu et al. (2015) and referenced by Benoit et al. (2019). The morphogroups were initially populated with specimens that had corresponding DNA sequence data (Madeira et al. 2007; Benoit et al. 2019; Tippery et al. 2020). Prior molecular data also enabled the inference of general geographic regions where a particular morphogroup was more likely to be found. For example, the molecular signature of dioecious invasive plants in the southern U.S.A. was also recovered from plants sampled in India (Benoit et al. 2019), thus specimens from these regions were initially assigned to the same morphogroup. The establishment of populated morphogroups enabled the inference of morphological characters that could distinguish the specimens in each morphogroup, and these criteria (along with geographic information) were used to assign the remaining specimens to morphogroups.

Morphological data were plotted in R (R Core Team 2023) using the package *ggplot2* ver. 3.4.2 (Wickham 2016). The morphological data for leaf length, leaf width, leaf length:width ratio, and number of leaves per

node were evaluated in a principal components analysis (PCA) using the *prcomp* function in R (R Core Team 2023), with values scaled to have unit variance.

Taxonomy and nomenclature.—After assigning specimens to morphogroups and determining the approximate geographic boundaries for morphogroups, the type specimens for previously described taxa were evaluated against the morphological data for all available specimens. If the type specimens for a particular taxon aligned with the geographic distribution and morphometric data for an existing morphogroup, then the morphogroup was equated with that taxon.

RESULTS

A total of 1,070 herbarium specimens, representing 51 different countries, were evaluated for this study (Fig. 1). Morphological data were collected from 294 specimens. Although specimens could not always be assigned confidently to morphogroups, some broad patterns enabled morphogroup assignment. Specifically, plants in the H6/H7 morphogroup generally had longer and thinner leaves, whereas H1/H2 plants tended to have shorter and wider leaves (Fig. 3A–C). Plants in the H6/H7 and H8/H9 morphogroups had the largest number of leaves per node (Fig. 3D), and the largest number of marginal prickles per leaf belonged to plants in the H6/H7 morphogroup. The PCA plot shows relatively little separation between morphogroups, using the morphological characters that were examined (Fig. 4). The greatest separation (i.e., PC1 axis) was influenced jointly by leaf length and length:width ratio, with plants in morphogroup H6/H7 having the slenderest leaves and morphogroup H1/H2 having the stoutest leaves. The PC2 axis mostly followed leaf number. Together, the first two principal components explained 79.1% of the variance (PC1: 54.7%, PC2: 24.4%).

Specimens belonging to the H1/H2 morphogroup were found in Africa and eastern Eurasia, H3/H4 specimens were found from Southeast Asia to Australasia, H5 specimens were found in the Korean peninsula and adjacent areas, H6/H7 specimens were found from Southeast Asia to Australasia, and H8/H9 specimens were found in northern Eurasia (Fig. 2). A small number of anomalous specimens were placed in different morphogroups than the other specimens in their geographic region, for example plants in Central America and Africa with the H6/H7 morphology, plants in southern Africa with the H8/H9 morphology, and plants in the United Kingdom with the H5 morphology (Fig. 2).

DISCUSSION

Although there is considerable variation among hydrilla specimens worldwide, most specimens can be placed reliably into morphogroups. The relative geographic isolation of morphogroups and their phylogenetic distinctness also support the inference that they are independent evolutionary units, albeit with some overlapping morphological variation and occasional interbreeding (Benoit et al. 2019). The taxonomic designation of subspecies is appropriate for segregating such evolutionary units (Hamilton & Reichard 1992) and has the additional benefit of retaining the current species name that has been used for scientific communication and legislation.

Identifying morphogroups.—Much of the interest for identifying infraspecific groups in hydrilla has been driven by researchers and land managers in the U.S.A., where three distinct strains of the species exist (Jacono et al. 2020; Tippery et al. 2020). Benoit et al. (2019) reported that almost all specimens with prickles on the abaxial surface of the leaf midvein belonged to the H1/H2 plastid genotype, and this information has facilitated the morphological identification of the corresponding U.S.A. dioecious biotype. In the present study, additional morphological data were evaluated for plants across the global hydrilla range, with the result that morphogroups can be differentiated with greater confidence. Flowers and fruits are rarely observed on hydrilla specimens, and thus these features were excluded from consideration in the present study. Nonetheless, vegetative features such as leaf dimensions, leaf number, and leaf marginal prickle number are informative for identifying specimens. In the future it would be valuable to evaluate the morphogroups in the context of both vegetative and reproductive features. Moreover, an increased effort to document morpholo-

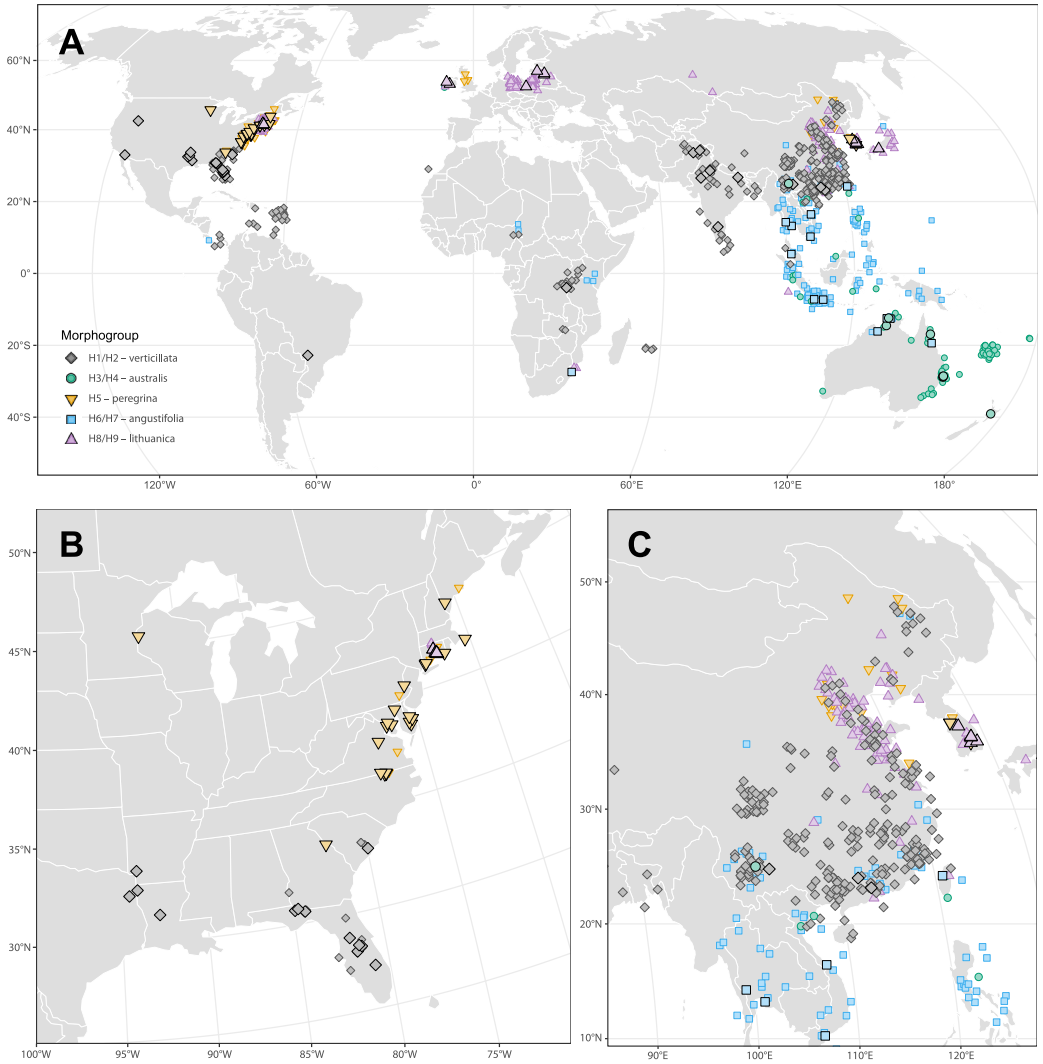

Fig. 2. Global distribution of *Hydrilla* morphogroups. Points are colored by the morphogroup designations that were determined in this publication. Points with black outlines indicate localities where plants were included in phylogenetic analyses (Madeira et al. 2007; Zhu et al. 2015; Benoit et al. 2019; Tippery et al. 2020). Points are randomly 'jittered' (standard deviation = 1 degree latitude or longitude) to facilitate viewing of multiple points that occupy the same or similar coordinates. A. World map. B. Map showing the portion of the U.S.A. where samples are most dense. C. Map of eastern Eurasia showing the adjacent and overlapping ranges of several morphogroups.

gical variation in the context of environmental conditions potentially could determine which aspects of variation have environmental versus genetic causes.

Plants in the H6/H7 morphogroup are perhaps the most distinctive, with a large number of leaves per node, and leaves that are narrower and longer than plants in other morphogroups. The relative geographic isolation of this morphogroup, with specimens mostly collected in Malesia, also help to identify these plants. The H1/H2 plants also are distinctive, with shorter and broader leaves, providing the greatest contrast to plants in the H6/H7 morphogroup. H1/H2 plants occupy the widest geographic range, from Africa to eastern Eurasia, and this morphogroup was the most commonly encountered. In the U.S.A. the presence of abaxial

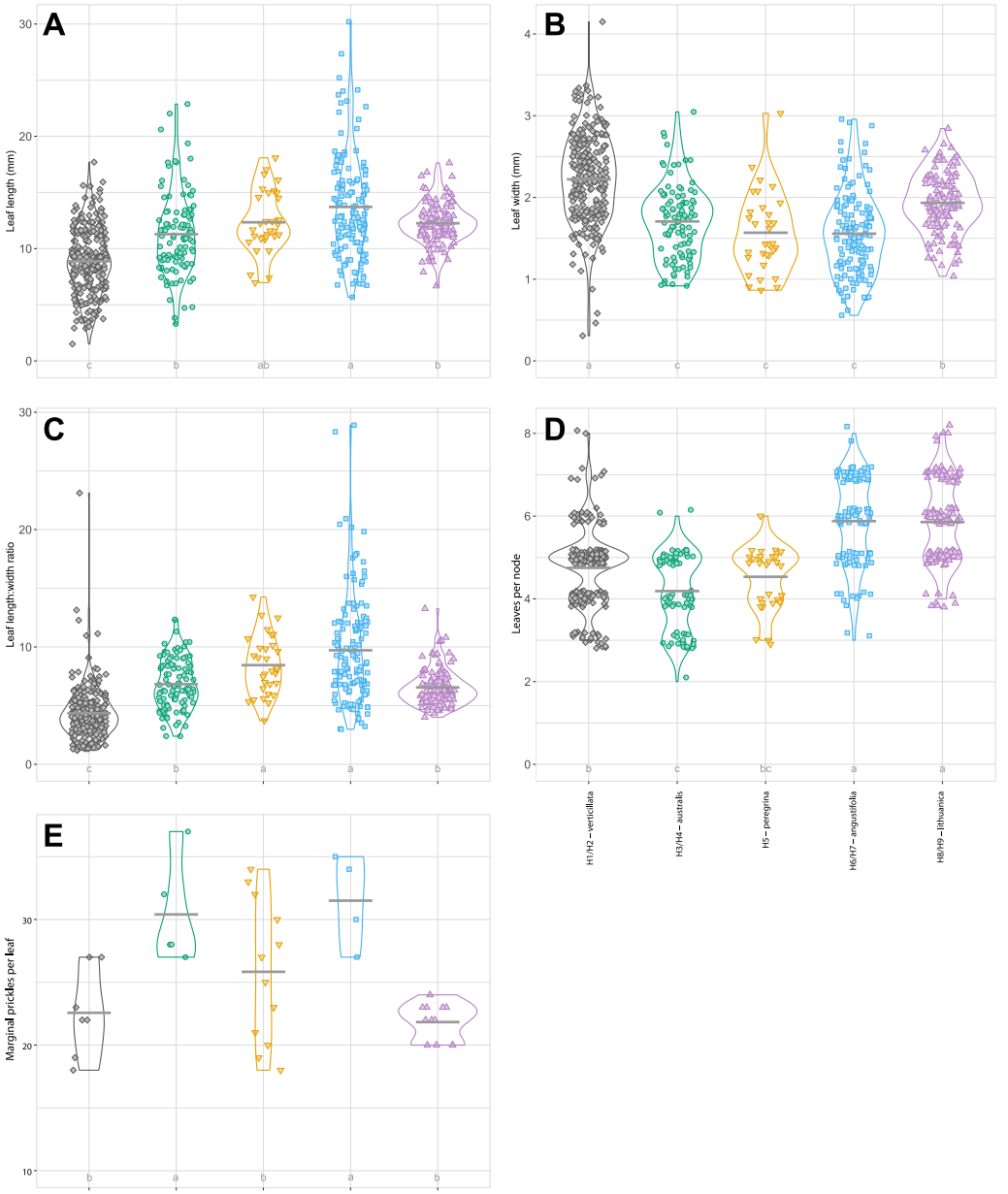

Fig. 3. Summary distributions of morphometric data. Points are colored by the morphogroup designations that were determined in this publication. Points are 'jittered' on the x-axis to facilitate viewing of multiple similar measurements. **A.** Leaf length. **B.** Leaf width. **C.** Leaf length:width ratio. **D.** Leaf number per node (whorl). For this plot, points are also jittered on the y-axis. **E.** Marginal prickle number per leaf.

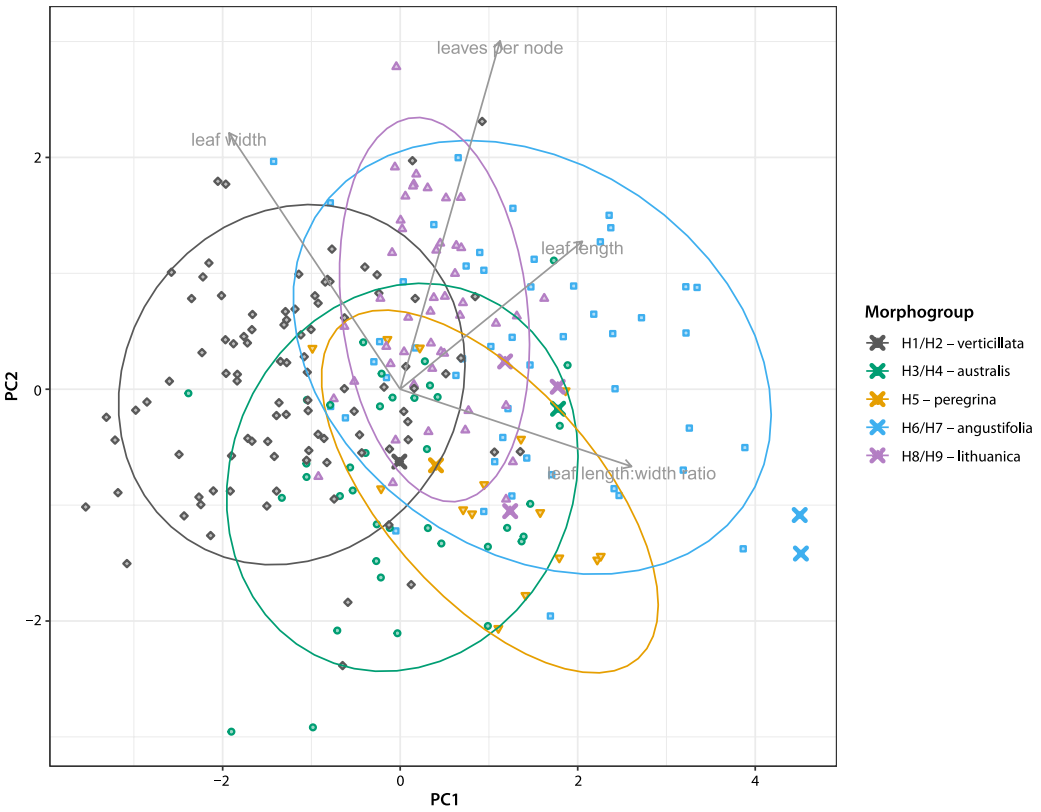

Fig. 4. Principal components plot for morphometric data. Points are colored by the morphogroup designations that were determined in this publication. The directions and relative sizes of the loading vectors (eigenvectors) for the various morphometric characters are shown as gray arrows. Points marked with an '×' indicate type specimens for the respective morphogroups.

midvein prickles is a helpful determinant for the H1/H2 morphogroup, and this feature was not observed in other morphogroups, although it should be noted that this feature is difficult to observe on herbarium specimen images unless the image has extraordinarily high resolution. Plants in the H8/H9 morphogroup are noteworthy for having more than five leaves per node on average, and they are distinct from the H6/H7 plants through their leaf shape and size. The remaining two morphogroups, H3/H4 and H5, are difficult to distinguish from each other on morphology alone, although the data do show a tendency toward more marginal prickles and stouter leaves (Fig. 3). The geographic ranges for these morphogroups do not overlap (H5 specimens are found north of 30°N latitude, whereas H3/H4 specimens are more southern), so it also will be useful to consider the locality of specimens when assigning specimens to morphogroups.

The morphogroup designations in this paper should be taken as working hypotheses, and the application of DNA sequence data will continue to serve as a more definitive method for identifying specimens. Also, it should be noted that the data for this study were obtained by studying a limited number of features in dried herbarium specimens, and the morphogroups should be evaluated independently by studying a broad array of morphological characters in both dried and freshly collected plants. A small number of specimens in this study were observed to have anomalous morphologies that did not match the predominant morphogroup for their particular region, and this result may indicate environmental variation, incorrectly applied identification criteria, or genetic variation that has not been accounted for. Some of these specimens also could represent

introduced plants from other morphogroups or instances of hybridization. In any case, the appropriate molecular evidence should be applied when identifying anomalous specimens.

Assigning names to morphogroups.—There are several species names available in *Hydrilla*, and the type material and geographic distributions of these were evaluated in light of the available molecular and morphometric data. The type species of the genus is *Hydrilla ovalifolia* Rich., *nom. illeg.* (= *H. verticillata*), for which the type specimen was collected in India. The morphology of the type specimen best matches plants in the H1/H2 morphogroup, which are prevalent in India (Fig. 2). Two names are considered synonymous with *H. verticillata*: *H. polysperma* Blatt. and *H. wrightii* Planch., both of which also were described from specimens collected in India. Next, the species *H. angustifolia* Hassk. and *H. najadifolia* Zoll. & Moritzi were both named from material collected in Indonesia, and the characteristically narrow leaves of plants in the H6/H7 morphogroup and the predominance of that morphogroup in Malesia help to equate these species with morphogroup H6/H7. Similarly, the type specimen for *H. lithuanica* (Besser) Dandy, collected in Lithuania, matches the morphology of H8/H9 morphogroup specimens and the geographic distribution of this morphogroup in northern Eurasia. These three previously named species also are represented by DNA sequences that were obtained from material collected in the vicinity of their respective type specimens, thus increasing the confidence in morphogroup assignment beyond simply evaluating morphological similarity. Two of the morphogroups (H3/H4 and H5) are not represented by any currently designated *Hydrilla* species, and if they are to be recognized as independent taxa they will need to be provided with new names.

Thus far no subspecies designations have been made in the genus *Hydrilla*. Several varietal names were established for *H. verticillata* by Caspary (1858), but the names are minimally useful because several disparate specimens were cited for each variety, and in several cases a single specimen apparently belongs to more than one variety (Cook & Lüönd 1982). For example, the protologue for *Hydrilla verticillata* var. *inconsistens* Casp. cites different leaves on the same stem as belonging to different varieties (Caspary 1858; Cook & Lüönd 1982). However, as validly published names, the taxa described by Caspary (1858) must be provided with lectotypes and equated with the subspecies categories proposed herein. Fortunately, the majority of varietal names proposed by Caspary (1858) cite specimens that are attributable to (and in some cases homotypic with) either *H. verticillata* or *H. lithuanica*, as referenced in the taxonomic section below. Lectotypes for these varieties are selected that conform optimally to both the original intent of the taxonomic name and the current understanding of subspecies morphology and geography.

Summary.—The evidence presented in this paper corroborates the conclusion drawn by Cook & Lüönd (1982) that the morphological variation in *Hydrilla* cannot easily be parsed among different species. However, there is enough morphological, biogeographic, and phylogenetic evidence to support the establishment of subspecies, for the purpose of referring to meaningful biological units. In the invasive range, the subspecies already are being monitored and managed as separate entities, and the names provided herein will facilitate communication about their ecological and morphological differences. Additionally, with the acknowledgment that two or more subspecies exist in parts of the native range, botanists can investigate other aspects of their biological differences that might become useful for studying hydrilla in the future.

TAXONOMIC TREATMENT

Hydrilla verticillata (L.f) Royle subsp. **verticillata** Ill. Bot. Nat. Hist. Himal. 1:376. 1839. *Serpicula verticillata* L.f., Suppl. Pl. 416. 1781. *Hydrilla ovalifolia* Rich., Mem. Inst. Par. xii. II. 76. t. 2. 1811 [nom. invalid. - homotypic]. *Vallisneria verticillata* (L.f.) Roxb., Hort. Bengal. 71. 1814. *Udora verticillata* (L.f.) Spreng., Syst. Veg., ed. 16 [Sprengel] 1:170. 1824. *Hydrilla roxburghii* Steud., Nomencl. Bot. [Steudel], ed. 2. 1:780. 1840 [nom. invalid. - homotypic]. *Hydrilla dentata* Casp., Bot. Zeitung (Berlin) 12:56. 1854 [nom. invalid. - homotypic]. *Hydrilla verticillata* var. *roxburghii* Casp., Jahrb. Wiss. Bot. 1:494. 1858. *Elodea verticillata* (L.f.) F.Muell., Key Syst. Vict. Pl. i. 423. 1888. TYPE: INDIA: *König s.n.* (LECTOTYPE, designated by Cook & Lüönd (1982:486): LINN [Savage Cat. No. 1106-1]; ISOLECTOTYPES: BM, C [C10012873], C [C10012874], C [C10012875], C [C10012876], C [C10012877], C [C10012878]).

Hottonia serrata Willd., Sp. Pl., ed. 4 [Willdenow] 1(2):814. 1798. TYPE: INDIA: *Klein s.n.* (**LECTOTYPE, designated here:** B [B-W-17363-01-0]).

Hydrilla verticillata var. *brevifolia* Casp., Jahrb. Wiss. Bot. 1:495. 1858. TYPE: INDIA: Kanaor inférieur, *Royle s.n. 9/10/1816* (**LECTOTYPE, designated here:** P [P0213215]).

Hydrilla verticillata var. *inconsistens* Casp., Jahrb. Wiss. Bot. 1:496. 1858. TYPE: INDIA: *Klein s.n.* (**LECTOTYPE, designated here:** B [B-W-17363-01-0]).

Hydrilla verticillata var. *tenuis* Casp., Jahrb. Wiss. Bot. 1:495. 1858. TYPE: INDIA: Iheels, S of Calcutta, *Thompson s.n.* (**LECTOTYPE, designated here:** L [L.1203233]).

Hydrilla wightii Planch., Ann. Sci. Nat., Bot. sér. 3, 11:79. 1849. TYPE: INDIA: Hb. Wight [Wall. cat. no. 5048.C] (HOLOTYPE: K [K001104605]; ISOTYPE: BM).

Hydrilla polysperma Blatt., J. Proc. Asiat. Soc. Bengal 26:356. 1931. TYPE: INDIA: Rajasthan, Mount Abu, Naki Talao, Oct 1916, *Hallberg & Blatter 11189* (HOLOTYPE: BLAT).

This subspecies corresponds to the phylogenetic clade H1/H2 (Zhu et al. 2015; Benoit et al. 2019), and also to the dioecious invasive biotype in the U.S.A. (Zhu et al. 2015; Benoit et al. 2019). Plants can be identified by stout leaves that are generally less than five times longer than wide.

Common name.—Whorled hydrilla.

Distribution.—Thus far all hydrilla plants collected in India and adjacent countries are identified as this subspecies. Its native range also extends eastward in Eurasia into China, and westward into Africa. Invasive plants belonging to this subspecies are fairly widespread in North and South America (Madeira et al. 2007; Zhu et al. 2015; Benoit et al. 2019).

Specimens examined. **AFGHANISTAN:** Laghman, *Podlech 19887* (W). **BANGLADESH:** Dhaka, *Hooker & Thomson* (U). **BURUNDI.** Bujumbura, *Reekmans 7687* (BR). **CAMEROON:** Lacs Mokolō, Ghesquière 5009 (BR). **CHINA:** Anhui, *East China Workstation 3588* (PE); Beijing, *Wetland Plant Collection Team 10-011* (PE); Fujian, *Chung 2568* (AU); Guangdong, *Liang 61407* (IBSC); Guangxi, *Xing'an Collection Team 16* (IBK, IBSC); Guizhou, *Yang 93-444* (GZTM); Hainan, *Hou 72822* (IBK, IBSC, PE); Heilongjiang, *Wang 1210* (PE); Henan, *Henan Forestry Department 258* (PE); Hubei, *Farmland Weed Investigation Team 224* (PE); Hunan, *Chen 1154* (IBK, IBSC); Jiangsu, *Liu 1096* (IBSC); Jiangxi, *Yi 9805* (JJF); Jilin, *Zhang 2720* (IFP); Liaoning, *Zhu 545* (IFP); Shaanxi, *Fu 5488* (PE); Shandong, *Hou 201007012* (QFNU); Sichuan, *Liou & Wang 524* (PE); Yunnan, *Wang 78892* (PE); Zhejiang, *Zhou 2233* (IBSC). **DEMOCRATIC REPUBLIC OF THE CONGO:** Kivu, *Kinet 176* (BR). **INDIA:** Karnataka, *Pieterse s.n.* (CONN); Kashmir, *Liou 5844* (PE); Rajasthan, *Pieterse s.n.* (CONN). **NEPAL:** *Pieterse s.n.* (CONN). **SRI LANKA:** *Thwaites 2310* (BM, P). **TANZANIA:** *Tanner 1651* (BR). **UGANDA:** *Greenway 8820* (BR). **U.S.A. Florida:** *Benoit 14* (CONN). **Georgia:** *Benoit 22* (CONN). **South Carolina:** *Benoit 25* (CONN). **VIETNAM:** Tonkin, *Balansa 4558* (P). **ZAMBIA:** Kafue River, *Munkonge s.n.* (L).

Hydrilla verticillata subsp. **angustifolia** (Hassk.) Tippery, **comb. et stat. nov.** BASIONYM: *Hydrilla angustifolia* Hassk., Pl. Jav. Rar. 117. 1848. TYPE: INDONESIA: "prope Weltevreden, prope Buitenzorg et ad pedem montis ignivomi Gedeh" (TYPE: L [not found]).

Hydrilla najadifolia Zoll. & Moritzi, Syst. Verz. Zoll. 91. 1846. *Hydrilla verticillata* var. *longifolia* Casp., Jahrb. Wiss. Bot. 1:497. 1858. TYPE: INDONESIA: *Zollinger 125* (**LECTOTYPE, designated here:** P [P02131176]; ISOLECTOTYPES: BM [BM000958574], BR [BR0000006885328], G [G00164628], G [G00164629], G [G00164630], L [L.0050334], LE [LE00000355], MPU [MPU018828], MPU [MPU018829], P [P02131159]).

Hydrospondylus submersus Hassk., Flora 25(2, Beibl.):33. 1842. TYPE: INDONESIA: "in stagnantibus prope et intra hortorum Tjipannas copiose" (TYPE: L [not found]).

This subspecies corresponds to the phylogenetic clade H6/H7 (Zhu et al. 2015; Benoit et al. 2019). Plants can be identified by having slender leaves that are generally more than ten times longer than wide, with 30 or more marginal prickles per leaf.

Common name.—Narrow-leaved hydrilla.

Distribution.—The native range of this subspecies includes Southeast Asia and Malesia, as well as some plants in Australia.

Specimens examined. **AUSTRALIA:** Northern Territory, *Jacobs 9903* (NSW); Western Australia, *Jacobs 9876* (NSW). **BRUNEI:** *Van Niel 3748* (L). **CAMBODIA:** *Godefroy 97* (P). **CHINA:** Fujian, *Yan 153* (AU); Guangdong, *Chen 8418* (IBK, PE); Yunnan, *Wang 73993* (PE). **INDONESIA:** Java, *Zollinger 125* (BM, BR, G, L, MPU, P); Sulawesi, *Otto 4* (L); Sumatra, *Meijer 7125* (L). **LAOS:** Vientiane, *Vongsay 76* (P). **MALAYSIA:** Kedah, *Imin FRI 70066* (L). **PAPUA NEW GUINEA:** Simbu, *Sterley 80-594* (L). **PHILIPPINES:** Luzon, *Santos 57-130* (L). **THAILAND:** *Larsen 1568* (L). **VIETNAM:** Tonkin, *Balansa 4132* (P).

Hydrilla verticillata subsp. **australis** Tippery, **subsp. nov.** TYPE: AUSTRALIA. NEW SOUTH WALES: Alstonville, Bullwinkle Park, Maguires Creek, *S.W.L. Jacobs 9839* (HOLOTYPE: NSW [NSW594307]; ISOTYPE: CONN [CONN00109654]).

These plants are distinguished from most other *Hydrilla* subspecies by having 5 or fewer leaves per node, leaves with a length:width ratio < 10, > 18 marginal prickles each, and lacking abaxial midvein prickles. They are most similar to *Hydrilla verticillata* subsp. *peregrina* but are distinguished by having leaves with > 25 marginal prickles and a length:width ratio < 10. This subspecies corresponds to the phylogenetic clade H3/H4 (Zhu et al. 2015; Benoit et al. 2019).

Etymology.—The name 'australis' refers to the fact that this subspecies occupies the southernmost portions of the *H. verticillata* range.

Common name.—Southern hydrilla.

Distribution.—The native range of this species includes Australasia, with some plants also found in Southeast Asia and Malesia.

Specimens examined. **AUSTRALIA:** New South Wales: *Jacobs 9839* (CONN, NSW); Northern Territory, *Jacobs 9898* (CONN, NSW); Queensland, *Orchard 4763* (L); Western Australia, *Marchant s.n.* (L). **INDONESIA:** Sulawesi, *Vriese s.n.* (L); Sumatra, *Afriastini 827* (P). **NEW CALEDONIA:** *Mackee 196* (P). **VIETNAM:** Tonkin, *Bon 1097* (P).

Hydrilla verticillata subsp. **lithuanica** (Besser ex Rchb.) Tippery, **comb. et stat. nov.** Basionym: *Udora lithuanica* Besser ex Rchb., Fl. Germ. Excurs. 139. 1830. *Hydora lithuanica* (Besser ex Rchb.) Andrz. ex Besser, Flora 15(2, Beibl.):13. 1832. *Hydrilla verticillata* var. *crispa* Casp., Jahrb. Wiss. Bot. 1:496. 1858. *Hydrilla lithuanica* (Besser ex Rchb.) Dandy, Clapham, Tutin & E.F. Warb. Fl. Brit. Isles 1183. 1952. Type: LITHUANIA. Vilnius, Lake Szuriuta (Szwinta), *Besser s.n. s.d.* (lectotype, designated by Cook & Lüönd (1982:486): W; isolectotypes: BM, K [K000912636], K [K000912637], L, P [P02130598], P [P06899751]).

Udora pomeranica Rchb., Icon. Fl. Germ. Helv. (H.G.L. Reichenbach) 7:31. 1845. *Anacharis pomeranica* (Rchb.) Peterm., Deutschl. Fl. 529. t. 82. fig. 650. 1849. *Hydrilla dentata* var. *pomeranica* (Rchb.) Casp., Bot. Zeitung (Berlin) 12:56. 1854. *Hydrilla verticillata* var. *gracilis* Casp., Jahrb. Wiss. Bot. 1:495. 1858. Type: POLAND: Szczecin (Stettin), Dammscher See, *Schramm, Fl. Germ. Exsicc. 2142* (**lectotype, designated here:** L [L0700381]; isolectotypes: G, K [K000912634], L [L0700382], P [P06899753]).

This subspecies corresponds to the phylogenetic clade H8/H9 (Zhu et al. 2015; Benoit et al. 2019), and also to the 'Connecticut River' invasive biotype in the U.S.A. (Tippery et al. 2020). Plants can be identified by having often more than five leaves per node and leaves with 20–25 marginal prickles each.

Common name.—Northern hydrilla.

Distribution.—The native range of this species extends across Eurasia at latitudes north of 30°N. Invasive plants have been confirmed in the northeastern U.S.A. (Tippery et al. 2020).

Specimens examined. **CHINA:** Anhui, *Wild Plant Comprehensive Utilization Committee 125* (PE); Beijing, *Wild Plant Collection Team 4-159* (BJFC); Hebei, *Yan 7906* (PE); Jiangsu, *Deng et al. 3656* (PE); Liaoning, *Sato 2370* (PE); Shandong, *Hou et al. 160325* (QFNU). **JAPAN:** Akita, *Faurie 15788* (P); Ehime, *Faurie 11735* (P); Fukushima, *Savatier 2497* (P). **LITHUANIA:** Vilnius, *Besser s.n.* (BM, K, L, P, W). **POLAND:** West Pomerania, *Schramm s.n.* (WAG). **REPUBLIC OF KOREA:** Gyeonggi-do, *Na 90155-3* (CONN); Gyeongsangbuk-do, *Na 80277-4* (CONN). **U.S.A. Connecticut:** *Stebbins s.n.* (CAES, UWW).

Hydrilla verticillata subsp. **peregrina** Tippery, **subsp. nov.** Type: REPUBLIC OF KOREA (SOUTH KOREA): Seoul, Palang Reservoir, *Pemberton s.n.*, Oct 1989 (holotype: CONN [CONN00226721]).

These plants are distinguished from most other *Hydrilla* subspecies by having 5 or fewer leaves per node, leaves with a length:width ratio < 10, > 18 marginal prickles each, and lacking abaxial midvein prickles. They are most similar to *Hydrilla verticillata* subsp. *australis* but are distinguished by having leaves with < 35 marginal prickles and a length:width ratio > 5. This subspecies corresponds to the phylogenetic clade H5, and also to the monoecious invasive biotype in the U.S.A. (Madeira et al. 2007; Zhu et al. 2015; Benoit et al. 2019).

Etymology.—The name 'peregrina' (meaning 'wandering', 'traveling', or 'exotic') refers to the fact that this subspecies was first characterized from material in the invasive range, and only later were corresponding individuals found in the native range.

Common name.—Wandering hydrilla.

Distribution.—The native range of this species includes the Korean peninsula and adjacent regions in China. Invasive plants in more northern portions of the U.S.A. belong to this subspecies (Madeira et al. 2007; Zhu et al. 2015; Benoit et al. 2019).

Specimens examined. **CHINA:** Hebei, *Sato 6118* (PE); Heilongjiang, *Wang 738* (IFP); Liaoning, Zhao 2158 (IFP). **REPUBLIC OF KOREA:** Seoul, *Pemberton s.n.* (CONN). **U.S.A. Connecticut:** *Murray 05-092* (CONN). **Delaware:** *Benoit 54* (CONN). **District of Columbia:** *Benoit 48* (CONN). **Maine:** *Hahnel & McPhedran s.n.* (CONN). **Maryland:** *Benoit 49* (CONN). **Massachusetts:** *Mehrhoff 21583* (CONN). **North Carolina:** *Weiss 111* (CONN). **Pennsylvania:** *Benoit 58* (CONN). **Virginia:** *Weiss 110* (CONN). **Wisconsin:** *Netherland s.n.* (CONN).

SYNONYMS FOR SPECIES OUTSIDE OF *HYDRILLA*

Blyxa japonica (Miq.) Maxim. ex Asch. & Gürke, Nat. Pflanzenfam. [Engler & Prantl] II. i. 253. 1889. *Hydrilla japonica* Miq., Ann. Mus. Bot. Lugduno-Batavi 2:271. 1866. TYPE: JAPAN: *Siebold & Bürger s.n.* (LECTOTYPE, designated by Cook & Lüönd (1983:21): U; ISOTYPE: LE).

Hydrilla alternifolia Miq., Ill. Fl. Archip. Ind. 52. 1870. TYPE: INDONESIA: Borneo, *Korthals s.n.* (**LECTOTYPE,** first-step designated by Cook & Lüönd (1983:25), second-step **designated here:** L [L0050324]; ISOLECTOTYPES: L [L0581232], U [U0002501]).

Lagarosiphon muscoides Harv., J. Bot. (Hooker) 4:230. 1841. *Hydrilla muscoides* (Harv.) Planch., Ann. Sci. Nat., Bot. sér. 3, 11:79. 1849. TYPE: SOUTH AFRICA: Albany, *Zeyher s.n.* (LECTOTYPE, designated by Symoens & Triest (1983:463): TCD; ISOLECTOTYPE: P [P02074483]).

Hydrilla dregeana C. Presl, Abh. Königl. Böhm. Ges. Wiss. ser. 5, 3:542. 1845. TYPE: SOUTH AFRICA: Cape, Port Elizabeth, Swartkops River, *Drege 2276c* (**LECTOTYPE,** first-step designated by Obermeyer (1964:140), second-step **designated here:** K [K000346010]; ISOLECTOTYPES: BM, G [G00168252], K [K000346009], L, P [P02074478]), P [P02074485]).

KEY TO SUBSPECIES OF *HYDRILLA VERTICILLATA*

1. Leaf length:width ratio < 5, abaxial midvein prickles 0–6 ____ **H. v.** subsp. **verticillata**
1. Leaf length:width ratio > 5, abaxial midvein prickles absent or very rarely present.
   2. Leaves > 15 mm long, length:width ratio > 7, > 30 marginal prickles per leaf ____ **H. v.** subsp. **angustifolia**
   2. Leaves < 15 mm long, length:width ratio < 10, 18–40 marginal prickles per leaf.
      3. 5 or more leaves per node, < 25 marginal prickles per leaf ____ **H. v.** subsp. **lithuanica**
      3. 5 or fewer leaves per node, > 18 marginal prickles per leaf.
         4. Leaves with < 35 marginal prickles each and a length:width ratio > 5 ____ **H. v.** subsp. **peregrina**
         4. Leaves with > 25 marginal prickles each and a length:width ratio < 10 ____ **H. v.** subsp. **australis**

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