The taxonomic status of the two subspecies has varied several times, depending on the researcher. Based on differences in chromosome number and morphological characteristics (e.g., flower size and plant pubescence), Ludwigia uruguayensis (Cambess.) H. Hara, was separated into two species: L. grandiflora and L. hexapetala (Hook. & Arn.) Zardini, H. Y. Gu & P. H. Raven (Raven & Tai 1979, Greuter & Raus 1987, Zardini et al. 1991a). Nesom & Kartesz (2000) treated these as subspecies, L. grandiflora subsp. grandiflora and L. grandiflora subsp. hexapetala, of a single species because they differ only in quantitative, intergrading morphological characters. Ward (2012) treated them as two varieties: L. grandiflora var. grandiflora and L. grandiflora var. hexapetala.
Moreover, in a study of the invasive population in Britain, Armitage et al. (2013) recommended that the taxa be treated as subspecies because they differ in chromosome number, but lack clear morphological differences.

Considering the above, we treat the taxa as two subspecies within a single species. Species of Ludwigia are morphologically very similar (Ghahramanzadeh et al. 2013). Their identification is difficult (Dandelot et al. 2005b), and identification is especially difficult in the absence of flowers (Ghahramanzadeh et al. 2013). For the recognition of subspecies of L. grandiflora, floral characteristics and the pubescence of the stems and leaves have been regarded as significant (Zardini et al. 1991a, Nesom & Kartesz 2000). Additionally, the subspecies differ in chromosome number, which was used to support their distinctiveness (Suyama et al. 2008, Kadono & Okamoto 2018).

Ludwigia grandiflora has been used as an ornamental (Ruaux et al. 2009), and naturalized populations have been recorded in the USA (Oka-da et al. 2009) and in Europe (Dandelot et al. 2005a, Nehring & Kolthoff 2011, Stiers et al. 2014). The invasiveness of L. grandiflora has ecological impacts on native aquatic vegetation and detritus communities (Stiers et al. 2011). In Japan, L. grandiflora subsp. grandiflora has been recorded as being in Wakayama (Naito & Hieda 2014) and Hyogo prefectures (Suyama et al. 2008), and in Lake Biwa in Shiga prefecture (Kadono 2014). Ludwigia grandiflora subsp. hexapetala has been recorded as being in Kagoshima Prefecture (Kadono 2014, Kadono & Okamoto 2018). Because L. grandiflora spreads rapidly, its dense floating stems in some parts of Lake Biwa cover the water surface (Fig. 1) and cause many problems, such as choking of the water channels, blocking of sunlight, and interfering with boat navigation and fishing. As a result of these conditions, the local government in Shiga Prefecture began management activities in 2013. Ludwigia grandiflora was subsequently included in the List of Regulated Living Organisms in 2014 under Japan’s Invasive Alien Species Act (Ministry of the Environment, Government of Japan 2018).

In previous reports, Ludwigia grandiflora in Lake Biwa was described as L. grandiflora subsp. grandiflora (Kadono 2014) or L. grandiflora sensu lato (Uyemura et al. 2015). Ludwigia grandiflora is a plastic species, but from our preliminary morphological observations we concluded that L. grandiflora in Lake Biwa was not L. grandiflora subsp. grandiflora. Misidentification of alien species can have serious consequences (Pyšek & Richardson 2010). As the basis for the management of L. grandiflora in Lake Biwa, its correct identification is needed.

In this study, we focused on several morphological characters to determine the identity of Ludwigia grandiflora in Lake Biwa. To support our morphological observations, we also determined the chromosome number. On the basis of the findings, we discuss L. grandiflora in Lake Biwa and its infraspecific identity.

**Materials and Methods**

**Measurement and observation of morphological characters**

Ludwigia grandiflora was first recorded in Akanoi Bay in southern Lake Biwa in 2009 (Council to Manage Aquatic Invasive Alien Plants in Lake Biwa 2014). We measured six floral characters: length of the sepal, petal, style, short filament, long filament and ovary (Fig. 2) and evaluated the pubescence of the stems in plants from the population of L. grandiflora established on the eastern coast of the Karasuma Peninsula near the locality where it was first recorded. All measurements and observations were made using living materials from 20 emergent stems. The measurements and observations were then compared with description in the articles using living materials (Zardini et al. 1991a) and herbarium specimens (Nesom & Kartesz 2000) and type specimens examined in K and digital images on the websites E, K and P (Appendix 1). We used ImageJ v.1.52a (Schneider et al. 2012), to measure the characteristics of flowers in the digital images. Characters partially covered by other...
Fig. 1. *Ludwigia grandiflora* in Lake Biwa. A: Dense mats of flowering plants (Shiga Pref., Karasuma Peninsula, Lake Biwa, June 8, 2014). B: Monospecific stand of flowering plants (Shiga Pref., Karasuma Peninsula, Lake Biwa, June 1, 2015). Photographs by S. Hieda.

Fig. 2. Diagram showing the six floral characters measured in *Ludwigia grandiflora*. A, Sepal length; B, petal length; C, style length; D, short filament length; E, long filament length; F, ovary length.

plant parts were inferred from visible ones.

**Chromosome count**

To determine chromosome numbers, we collected five stems from the population on the northern coast of Karasuma Peninsula and in Akanoi Bay. Since we obtained no accurate chromosome count for the population studied morphologically (eastern coast of Karasuma Peninsula), we observed the somatic chromosomes of the population (northern coast of Karasuma Peninsula and Akanoi Bay) near the eastern coast of Karasuma Peninsula in the southern part of Lake Biwa. After sampling, we cultivated the stems in the experimental greenhouse at the University of Shiga Prefecture. We collected root tips and treated them as follows, with slightly different treatment times for samples from Karasuma Peninsula and Akanoi Bay. Root tip samples were pre-treated in 2.2 mM 8-hydroxyquinoline solution at 25 °C for 14 h 15 min (Karasuma) or 13 h 15 min (Akanoi) and fixed in 3:1 ethanol:acetic acid at 25 °C for 1 h 30 min (Karasuma) or 2 h 10 min (Akanoi). After fixation, the samples were washed with tap water and then treated with 1 N HCl at 60 °C for 8 min (Karasuma) or 10 min (Akanoi). After fixation, the samples were stained with 2.0 % lacto-propionic orcein solution at room temperature for about 1 h. After the treatment, we prepared slides of the root tips by using the squash technique and observed somatic chromosomes under a compound microscope (model BX60, Olympus, Tokyo, Japan).

This study was conducted with permission to raise an invasive alien species under Japan’s Invasive Alien Species Act (registration number: 14000287).

**Results**

**Morphological characters**

Table 1 shows the values for the six floral characters and the degree of pubescence for the plants of *Ludwigia grandiflora* in Lake Biwa, along with values from previous taxonomic reports (Zardini *et al.* 1991a, Nesom & Kartesz 2000) and from type specimens. Two of the floral characters (petal length and style length) were within the range of values for *L. grandiflora* subsp. *hexapetala*. Three other floral characters (sepal length, short filament length and ovary length) were within the range of values for both subspecies; long filament length was not within the range of value for either subspecies. The pubescence of the stems corresponded most closely with the state reported for of *L. grandiflora* subsp. *hexapetala*.

**Chromosome number**

The somatic chromosome number for *Ludwigia grandiflora* from the two sites in Lake Biwa—Karasuma Peninsula and Akanoi Bay (Fig. 3)—were $2n = ca. 80$ (Table 1). This number is greater than in *L. grandiflora* subsp. *grandiflora*, (hexaploid, $2n = 48$) and the decaploid hybrid ($2n = 64$), and is almost the same as the number reported for *L. grandiflora* subsp. *hexapetala* (decaploid, $2n = 80$). Because of technical difficulties we were unable to determine the chromosome number precisely.

**Discussion**

Measurements of the morphological characters of *Ludwigia grandiflora* in Lake Biwa largely correspond to those of *L. grandiflora* subsp. *hexapetala*, indicating that *L. grandiflora* in Lake Biwa is *L. grandiflora* subsp. *hexapetala*. The results of our cytological studies support this identification. Our chromosome count on *L. grandiflora* in Lake Biwa, $2n = ca. 80$, is imprecise, but because *Ludwigia* species has not been shown to contain aneuploids (Raven & Tai 1979), it supports treating our result as $2n = 80$, a value greater than for the chromosome complement of *L. grandiflora* subsp. *grandiflora* (hexaploid, $2n = 48$) or for the octoploid hybrid ($2n = 64$). Our findings indicate that *L. grandiflora* in Lake Biwa is not *L. grandiflora* subsp. *grandiflora*, as formerly reported, but rather *L. grandiflora* subsp. *hexapetala*.

In Japan, *Ludwigia grandiflora* subsp. *grandiflora* is considered to have been introduced as a
plant to purify water (Suyama et al. 2008, Naito & Hieda 2014). By comparison, *Ludwigia grandiflora* subsp. *hexapetala* has been used as an ornamental plant.

Kadono & Okamoto (2018) recently speculated that the photograph in Kadono (1994) taken in Kanoya City, Kagoshima Prefecture, and identified as *L. peploides* (Kunth) P. H. Raven subsp. *stipulacea* (Ohwi) P. H. Raven is possibly *L. grandiflora* subsp. *hexapetala*. It is also possible that species of *Ludwigia* sect. *Jussiaea* in Japan have been misidentified at the species or subspecies level. As an example, *L. grandiflora* has been identified as the similar species *L. peploides* (diploid: 2n = 16). It might also have been misidentified as *L. peploides* subsp. *stipulacea*, a native, threatened subspecies. *Ludwigia grandiflora* subsp. *hexapetala* has also been cultivated as *L. peploides* subsp. *stipulacea* in the botanical garden, Sakuya Konohana Kan, in Osaka City. After we consulted with the director of the botanical garden, its cultivation was stopped. We also reported this situation to the Kinki Regional Environment Office, Ministry of the Environment, Japan. It is possible that *L. grandiflora* is still being cultivated in Japan because of misidentification. Clearly, genera with species known to be taxonomically difficult and registered as legally restricted should receive special attention.

The management of *Ludwigia grandiflora* is a serious challenge, but Kamigawara & Hieda (2018) have reported cases of local eradication of the invading *L. grandiflora* in England. They show that eradication is possible through suitable management during the early stages of introduction of *L. grandiflora*. Hieda (2018) also observed, and warned, that *L. grandiflora* subsp. *hexapetala* in Lake Biwa is resistant to desiccation; plant fragments can regenerate after drying naturally. Still living fragments can act as propagules—a capability that requires more attention.

In this paper, we have presented evidence that *Ludwigia grandiflora* in Lake Biwa is not *L. grandiflora* subsp. *grandiflora*, but rather *L. grandiflora* subsp. *hexapetala*. This re-identification has implications for management because, in contrast to the management of *L. grandiflora* subsp. *grandiflora*, the life history characteristics of *L. grandiflora* subsp. *hexapetala* (e.g. reproductive system, seed viability, and growth conditions) have been studied (Dandelot et al. 2005b, Reaux et al. 2009, Thouvenot et al. 2013). The information already available may be important in the management of *L. grandiflora* subsp. *hexapetala* in Lake Biwa. We thank Dr. N. Miura and Dr. J. Miyamoto for technical instructions regarding chromosome counting, Dr. Y. Kadono, Mr. S. Fujii, Mr. M. Kuribayashi and Ms. S. Mori for advice on the identification of *Ludwigia grandiflora* in Lake Biwa, Dr. K. Nakai and the Kinki Regional Envi-

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**Table 1. Comparison of morphological and cytological characters of *Ludwigia grandiflora* subsp. *grandiflora*, *L. grandiflora* subsp. *hexapetala* and *L. grandiflora* in Lake Biwa.**

<table>
<thead>
<tr>
<th>Character</th>
<th>*L. g. subsp. <em>grandiflora</em> (^1)</th>
<th>*L. g. subsp. <em>hexapetala</em> (^1)</th>
<th><em>L. g. in Lake Biwa</em> (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepal length</td>
<td>0.6–1.8 cm</td>
<td>0.8–1.9 cm</td>
<td>1.7±0.1 cm</td>
</tr>
<tr>
<td>Petal length</td>
<td>1.2–2.6 cm</td>
<td>1.5–3.0 cm</td>
<td>2.9±0.1 cm</td>
</tr>
<tr>
<td>Style length</td>
<td>4.7–8.2 mm</td>
<td>5.8–10.0 mm</td>
<td>9.2±0.7 mm</td>
</tr>
<tr>
<td>Short filament length</td>
<td>2.3–5.3 mm</td>
<td>1.6–5.2 mm</td>
<td>5.2±0.5 mm</td>
</tr>
<tr>
<td>Long filament length</td>
<td>3.7–6.4 mm</td>
<td>3.1–7.5 mm</td>
<td>8.2±0.6 mm</td>
</tr>
<tr>
<td>Ovary length</td>
<td>6.0–12.0 mm</td>
<td>5.0–15.0 mm</td>
<td>11.6±1.1 mm</td>
</tr>
<tr>
<td>Pubescence of stems</td>
<td>Villous to densely villous</td>
<td>Glabrous to densely villous</td>
<td>Glabrous to densely villous</td>
</tr>
<tr>
<td>Chromosome number</td>
<td>2n = 48 (rarely 2n = 96)</td>
<td>2n = 80</td>
<td>2n = ca. 80</td>
</tr>
</tbody>
</table>

\(^1\): Maximum range is shown based on measurements from Nesom & Kartesz (2000) and Zardini et al. (1991a) and our measurements and observations of type specimens at K and of digital images of specimens at E, K and P.

\(^2\): Measurements of morphological characters in this study. Each indicates the mean ± SD (n = 20).
vironment Office (Ministry of the Environment, Government of Japan), for guidance on the application for permission to raise invasive alien species under Japan’s Invasive Alien Species Act, Dr. K. Shutoh and Mr. S. Nemoto for advice on the examination of specimens, Ms. S. M. Frisby (K) for facilitating the examination of herbarium specimens, Dr. S. Hosoi-Tanabe for technical instructions regarding the use of the Olympus BX60 microscope, and students at the University of Shiga Prefecture for their help with this study.

References


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APPENDIX I. Information on type specimens (see Zardini et al. 1991a).

<table>
<thead>
<tr>
<th><strong>Ludwigia grandiflora</strong> (Michx.) Greuter &amp; Burdet subsp. grandiflora</th>
<th><strong>Ludwigia grandiflora</strong> (Michx.) Greuter &amp; Burdet subsp. hexapetala (Hook. &amp; Arn.) G. L. Nesom &amp; Kartesz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type: <strong>USA.</strong> Georgia, Chatham Co., Savannah, 1785–1796, A. Michaux s.n. (holo- P, n.v.; iso- P, digital image!: P01819435; P01819436)</td>
<td>Type: <strong>URUGUAY.</strong> in marshes, 1832. J. Tweedie s.n. (holo- K; K, digital image!: K000533256; iso- E, digital image!: E00285563; E00285564)</td>
</tr>
</tbody>
</table>