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***Lygodium japonicum* (Lygodiaceae) Is Represented by a Tetraploid Cytotype in Florida**

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ABSTRACT.—Invasive species are one of the largest threats to vulnerable ecological communities and biodiversity today and are economic burdens across the globe. It is therefore crucial that we understand the origins and the driving forces that promote the establishment, persistence, and spread of these taxa. Polyploidy, or whole genome duplication, has been suggested as a possible factor facilitating the success of invasive taxa, yet is an understudied aspect in invasion biology. Although ferns are often neglected in invasive species inventories, several fern families are over-represented as naturalized and invasive taxa including the vining ferns in the family Lygodiaceae. The Japanese climbing fern, *Lygodium japonicum*, is native to eastern Asia, and since its introduction in the early 1900s through the ornamental plant trade, it has rapidly spread throughout the southeastern United States, creating dense thickets that smother native plants and disrupt agricultural pine logging. While previous chromosome counts of *L. japonicum* suggest that both diploid and tetraploid cytotypes occur in its native range, there are no data for populations in the invaded range to date. Using chromosome counts, flow cytometry, and spore size measurements, we assessed the ploidy of invasive populations of *L. japonicum* in the state of Florida. We found that *L. japonicum* is represented by a tetraploid cytotype throughout Florida. Our study is the first to examine the ploidy of invasive *L. japonicum* populations, although additional work will be needed to determine if this species is tetraploid throughout its invaded range.

KEY WORDS: polyploidy, invasive species, cytology, fern, *Lygodium*, chromosomes

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Polyploidy has long been considered a possible driver of invasive species' success (Baker and Stebbins, 1965). Multiple genomes may mask deleterious alleles and increase genetic diversity in the form of fixed heterozygosity, and compared to their diploid progenitors, polyploids may show altered morphology, increased ecological tolerance, and/or niche differentiation (reviewed by te Beest *et al.*, 2012). Variations in ploidal levels between native and invaded ranges have been observed in multiple angiosperms including *Senecio inaequidens* DC. (Asteraceae; Lafuma *et al.*, 2003), *Solidago gigantea* Aiton (Asteraceae; Schlaepfer *et al.*, 2008), and in Cactaceae (Lopes *et al.*, 2021). However, similar cytological patterns have yet to be explored in invasive ferns despite the prevalence of polyploidy (Wood *et al.*, 2009; Pelosi *et al.*, 2022) and invasive species (Jones *et al.*, 2019) in the clade.

Lygodium japonicum (Thunb.) Sw. is a highly invasive vining fern that has colonized much of the southeastern United States. Native to eastern Asia, records of *L. japonicum* in North America suggest an introduction occurred as early as the 1880s (Pemberton and Ferriter, 1998). In its native range, chromosome counts of *L. japonicum* have found both diploid ($2n=58$; Roy and Manton, 1964; Nakato, 1990) and tetraploid cytotypes ($n=58$; Manton and Sledge, 1954; Mitui, 1965; Mitui, 1968). The specimen examined by Roy and Manton (1964) was from southern China, while the specimens analyzed by Mitui (1965), Mitui 1968, and Nakato (1990) were from Japan, and the Manton and Sledge (1954) sample was from an unidentified location. Compared to the genome size of *L. microphyllum* (Cav.) R.Br., a known diploid ($1C=5.56$ pg, Kuo and Li, 2019), the only published $1C$ genome size estimate of 11.66pg for *L. japonicum* would make it a tetraploid (Hanson and Leitch, 2002). However, the specimen measured by Hanson and Leitch (2002) does not have an associated location, and the ploidal level of this species in its invaded range is therefore unknown. Here we used a combination of flow cytometry, spore size measurements, and chromosome counts to determine whether *L. japonicum* is represented by single or multiple cytotypes in the invaded range in Florida.

MATERIALS AND METHODS

A total of 43 *L. japonicum* individuals was collected from nine populations throughout its range in Florida (Fig. 1A). Leaf material was stored at 4°C prior to preparation for flow cytometry or used fresh. For sporophytes, the flow cytometry procedure followed the simplified two-step protocol published by Doležel, Greilhuber, and Suda (2007). The sample was mixed with leaf tissue of an internal standard (*Vicia faba* 'Inovec', $2C=26.9$ pg [Doležel, Sgorbati, and Lucretti, 1992] for DAPI and *Chlorophytum comosum*, $2C=24.1$ pg [Hornych *et al.*, 2019] for PI) and stained with either DAPI or PI. The stained sporophyte samples were then analyzed using Partec PA II (Sysmex, Münster, Germany) and Sysmex CyFlow Space (Sysmex, Münster, Germany) flow cytometers, for DAPI and PI-stained samples, respectively. A total of 17 sporophyte samples were analyzed via DAPI staining to test whether samples differ in their ploidy level and to estimate AT base percentage. Absolute genome size was calculated

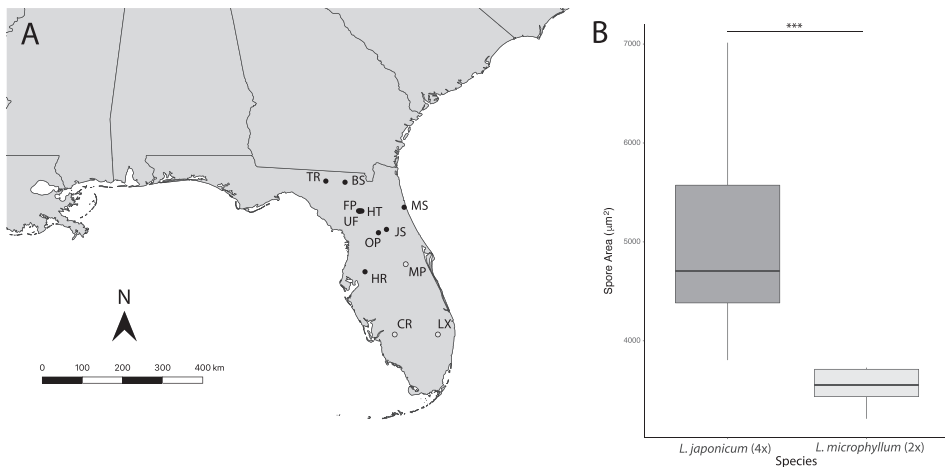


FIG. 1. A) Populations of *Lygodium japonicum* (black) and *L. microphyllum* (light gray) analyzed in this study. Population abbreviations for *L. japonicum* are as follows: BS – Big Shoals State Forest, FP – Forest Park, HR – Hillsborough River State Park, HT – Hawthorne Trail, JS – Juniper Springs, MS – Matanzas State Forest, OP – Ocklawaha Prairie Restoration Area, TR – Twin Rivers State Forest, UF – University of Florida Campus. Population abbreviations for *L. microphyllum* are as follows: CR – CREW Bird Rookery, LX – Loxahatchee National Wildlife Refuge, MP – Moss Park. B) Average spore area of *Lygodium japonicum* (4x, dark gray) and *L. microphyllum* (2x, light gray). *** significance level denotes $p < 0.001$.

for three sporophyte samples analyzed using the PI stain. The ratio of mean sample and standard fluorescence was calculated for all analyses. Consequently, the percentage of AT was estimated using the formula of Šmarda *et al.* (2008).

Spores were collected from fertile *L. japonicum* samples (21 of 43) and from *L. microphyllum* from three populations in Florida for comparison. Several spores from each specimen were sown on Bold's media (Bold, 1957) with Nitsch's micronutrients (Nitsch, 1951) and grown in a Percival growth chamber at 25°C on a 12:12 light:dark schedule. Up to three gametophytes from a specimen were pooled and used for genome size estimation with flow cytometry following a modified protocol from Roberts, Gladis, and Brumme (2009) and Loureiro *et al.* (2007); see Appendix 1 for a full protocol. Gametophyte samples were mixed with leaf tissue from an internal size standard (*Pisum sativum*, 2C=9.09pg [Doležel *et al.*, 1998]) and stained with PI. Stained samples were then analyzed using a BD Accuri C6 flow cytometer (Franklin Lakes, New Jersey, U.S.) at the University of Florida ICBR Cytometry Core Facility, RRID:SCR_019119. Although spores have been used previously to estimate genome size (*e.g.*, Kuo *et al.*, 2017), the number of spores isolated from each specimen was insufficient for flow cytometry. Furthermore, given that *Lygodium* gametophytes grow rapidly from spores (Lott *et al.*, 2003) and are mostly composed of a single cell layer (Takahashi *et al.*, 2015), these tissues are ideal for isolating nuclei for flow cytometry.

To compare spore sizes (a common proxy for ploidy in ferns, *e.g.*, Barrington, Paris, and Ranker, 1986; Schuettpetz, Pryer, and Windham, 2015) spores were fixed on slides and photographed at 400x magnification with an AmScope T340B-LED microscope and an AmScope MU1000-HS camera. Composite images were generated from multiple focus depths. At most ten spores per sporophyte were photographed. Spore length and width were measured following Barrington, Patel, and Southgate (2020). We further measured the area of the spore using the free-form polygon function in ImageJ (Schneider, Rasband, and Eliceiri, 2012). We used nested ANOVAs with populations nested within species to compare average spore length, width, and area between *L. japonicum* and *L. microphyllum* in R ver. 4.1.3 (R Core Team, 2022).

Sporophytes derived from sporophytic selfing (*sensu* Haufler *et al.*, 2016) were used for chromosome squashes. Actively dividing root tips were placed in 2mM 8-hydroxyquinoline for 4-6 hours at 4°C, fixed in Carnoy's solution (3:1 ethanol:glacial acetic acid) for 18-24 hours, and stored at room temperature in 70% ethanol until squashed. Tissues were transferred to 1M HCl at 60°C for 45-60 minutes depending on the size of the root, the root cap was removed to release the underlying cells which were macerated for about 30 seconds with a razor blade, and then squashed on a glass microscope slide with 1% aceto-orcein dye and 45% acetic acid. Squashes viewed with an AmScope Phase Contrast Kit-B/T 400/490/590/600 series lens and photographed at 1000x with the same scope and camera as above.

RESULTS AND DISCUSSION

We determined that *L. japonicum* is tetraploid in Florida, with an average 1C genome size of 14.38 ± 0.67 pg (mean \pm 1SD) and $48.0 \pm 2.69\%$ GC base content. The GC content of *L. japonicum* is notably high but ferns seem to have higher GC content compared to angiosperms (Šmarda *et al.*, 2019). We estimated the absolute genome size of 13 total samples from six populations: three sporophyte samples from three populations and ten gametophyte samples from three populations. The average genome size from sporophyte samples (13.28 ± 0.1 pg) was slightly lower than from gametophyte samples (14.72 ± 0.26 pg), which may be attributable to the use of different size standards and flow cytometers.

Compared to Hanson and Leitch (2002), our genome size estimates of *L. japonicum* are about 1.23 times larger than their reported size estimate of 1C=11.66pg, which could be explained by regional or methodological/technological differences. In both studies, the same method (flow cytometry) was used but with a different internal standard (Hanson and Leitch used *Allium cepa*, 2C=33.5pg). To infer the ploidy level of *L. japonicum*, we combined these flow cytometric data with chromosome counts. Compared to *L. microphyllum*, which is diploid with $2n=60$ (Fig. 2), the chromosome count of *L. japonicum* in this study is tetraploid with $2n=116$ (Fig. 2). Given that the 1C

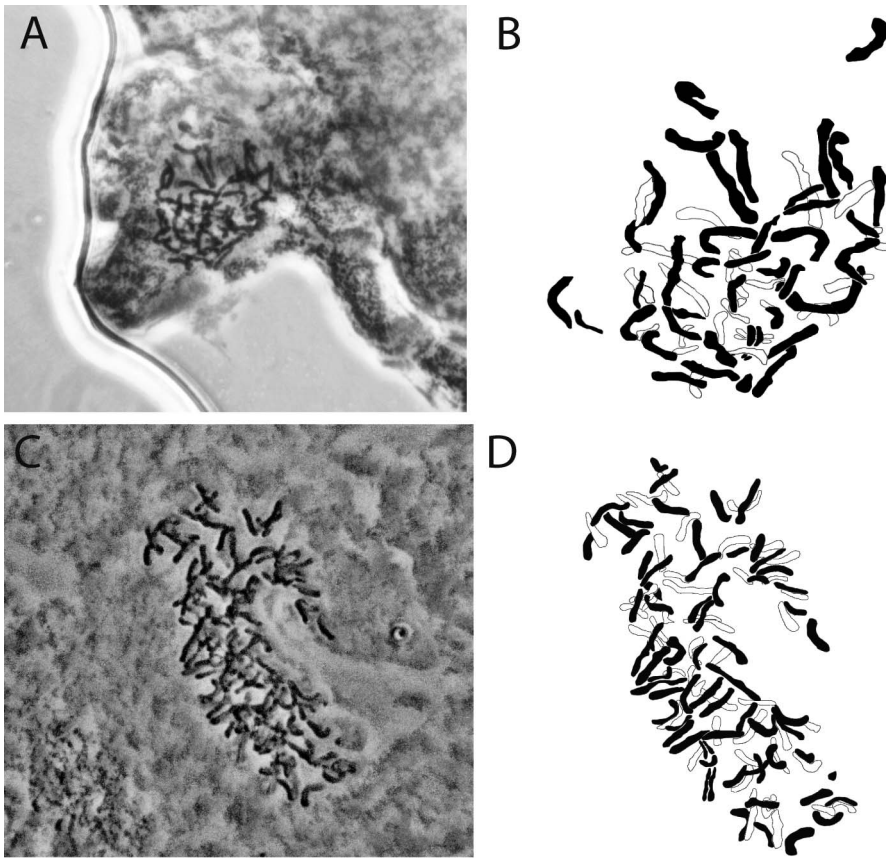


FIG. 2. Mitotic chromosome squashes from root tips of *Lygodium microphyllum* (A, B) and *L. japonicum* (C, D). Explanatory diagrams are provided in B and D, with white outlined chromosomes underlying black chromosomes, to enhance clarity.

genome size of diploid *L. microphyllum* is 6.20pg, the genome size estimates of *L. japonicum* of $1C = 14.38\text{pg}$ correspond to a tetraploid cytotype.

Although chromosome counts are the most definitive way to assess ploidy, there are drawbacks and difficulties with generating these data. Flow cytometry has therefore been an important supplement for inferring ploidy levels in plants (Doležel, Greilhuber, and Suda, 2007). Chromosome counts are time- and labor-intensive and require actively dividing material, such as root tips for mitotic counts or spore mother cells for meiotic counts. In contrast, several samples can be run in a few hours on a flow cytometer and a variety of tissues can be stored for up to months for use in genome size estimation. Particular difficulties with generating chromosome counts in *Lygodium* included hardness of the root cap, “sticky” chromosomes (e.g., Walker, 1966), the relatively high number of chromosomes resulting in a high degree of overlap, and initial overstaining of the cytoplasm. By integrating chromosome

TABLE 1. Chromosome numbers, ploidy, genome size, and average spore measurements of two invasive *Lygodium* species in Florida. *Genome size from Clark *et al.* (2016); †Genome size from Kuo and Li (2019).

Species	Approximate 2n Chromosome Number	Ploidy	Genome Size (1C, pg) Prior Study / This Study	Spore Length (μm)	Spore Width (μm)	Spore Area (μm^2)
<i>L. japonicum</i>	116	4x	11.66* / 14.38	70.7	78.3	4993
<i>L. microphyllum</i>	60	2x	5.56 [†] / 6.20	58.2	64.0	3538

counts and flow cytometry, we were able to expand our ability to infer cytotypes of samples across its range in Florida.

The average spore lengths of *L. japonicum* (70.7 μm) and *L. microphyllum* (58.2 μm , Table 1) are similar to measurements reported by Hanks (1998) based on Scanning Electron Micrographs of herbarium material, with ranges of 70-79 μm and 62-73 μm for *L. japonicum* and *L. microphyllum*, respectively. There was a larger variation in spore measurements in *L. japonicum* (ranges in length: 62.0-85.2 μm , width: 68.8-95.4 μm , area: 3805-7014 μm^2) compared to *L. microphyllum* (ranges in length: 56.6-60.6 μm , width: 61.3-65.8 μm , area: 3212-3729 μm^2 , Fig. 1B). This difference in variation may be due to larger sample sizes for *L. japonicum*, which was the focus of our study. There was a significant difference in spore area between species ($F_{1,19} = 16.297$, $p < 0.001$, Fig. 1B), but not between populations within species ($F_{7,19} = 0.369$, $p > 0.05$). We also found a significant difference in spore length ($F_{1,19} = 25.985$, $p < 0.001$) and spore width ($F_{1,19} = 22.814$, $p < 0.001$) between species, but not within populations within species ($F_{7,19} = 0.629$, $p > 0.05$; $F_{7,19} = 0.603$, $p > 0.05$ for length and width, respectively). Interestingly, Hanks (1998) did not find a correlation between spore size and ploidal level in *Lygodium*, although this correlation is supported in several fern genera with both monolete and trilete spores (e.g., *Adiantum* and *Polystichum*; Barrington, Patel, and Southgate, 2020) and in the present study. Unlike Barrington, Patel, and Southgate (2020) and this study, Hanks (1998) took measurements along the longest axis of each tetrahedral spore, which may account for differences in spore sizes. Spores of *Lygodium* spp. also vary in ornamentation, which may impact these measurements. Furthermore, spore measurements by Hanks (1998) were not made on the same samples used for ploidal estimates, rather, ploidy was determined based on literature searches. Future phylogenetic and cytological studies across the genus will be necessary to verify if spore size is a viable proxy for ploidal level in *Lygodium*.

Our study is the first to examine the possibility of ploidal variation in *L. japonicum* in its introduced range. The difference in the ploidal level between native and invaded range can be important in determining the origin(s) of the invasion when compared to the cytotype distribution in the native range. Whether *L. japonicum* is tetraploid throughout its invaded range is unknown and further investigation is needed to determine how and if cytotype variation is present in both native and invaded ranges. Despite its ecological and

economic impacts, the mode of polyploidization (allo- or autopolyploidy) has yet to be explored for *L. japonicum*, which can impact patterns of inheritance and genetic variation (Parisod, Holdregger, and Brochmann, 2010). These and other questions remain unanswered yet are critical to understanding the evolution, tolerance, and persistence of this invasive fern.

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APPENDIX 1.

Flow cytometry protocol with modifications based on Roberts, Gladis, and Brumme (2009) and Loureiro *et al.* (2007).

1. Place leaf material in petri dish. The amount of tissue is group specific and rather subjective. Estimate by eyeballing “the size of a pinky nail” – aim for 0.25g fresh weight tissue. **Note:** This may be increased to improve reads, if necessary. If desired, include standard tissue to be co-chopped. A smaller amount of standard (relative to sample) may be used to prevent the standard signal from swamping out the sample.
2. Place the petri dish with the samples on a cold brick, which will serve as a cold chopping surface, or in a dish of ice.
3. Add 1000 μ l cold lysis woody plant buffer (Loureiro *et al.*, 2007) to the petri dish and chop tissue with a single-edged razor blade for 60 seconds. Retain the pipette tip from this step to mix the sample later. **Note:** Again, the amount of chopping (fine vs. course) may be adjusted depending on the tissue. Finer chopping may result in more noise. Use a new razor blade for each sample. Do not over-chop!
4. Swirl the chopped material in the lysis buffer for approximately 20-30 seconds until a green tint appears on the liquid.
5. Remove the end of the retained pipette tip with the razor blade and use the larger opening to mix the material by pipetting up and down a couple of times.
6. Filter 800 μ l of suspension through Falcon[®] 5 mL Round Bottom Polystyrene Test Tube with 35 μ m nylon mesh strainer cap and keep on ice.
7. Transfer 500 μ l of filtrate to a new 1.5mL Eppendorf and add 2.5 μ l of RNase A (1-10 mg/mL). Incubate for 10 minutes.
8. Add 6 μ l PI stock solution for 500 μ l of filtrate (for an estimated final concentration of 50 μ g/mL) and cover tubes with foil to protect from light. Incubate on ice and analyze within 10 minutes for fresh tissue or keep on ice and protect from light; dry tissue may require incubation for 20-30 min.
9. Run on Accuri C6 flow cytometer (Galbraith, 2009).

APPENDIX 2. Collection and voucher information on specimens used in this study. Specimens used in this study have been deposited in the University of Florida Herbarium (FLAS). See Fig. 1 for population abbreviations.

Species	Sample ID	State	County	Population	Latitude	Longitude	Date	Voucher Specimen	Notes
<i>Lygodium japonicum</i>	JAP122	Florida	Alachua County	UF	29.640076	-82.3513	20 March 2021	J.A. Pelosi 122	https://www.inaturalist.org/observations/60110161
<i>Lygodium japonicum</i>	JAP123	Florida	Alachua County	UF	29.6441415	-82.3467	20 March 2021	J.A. Pelosi 123	https://www.inaturalist.org/observations/70577635
<i>Lygodium japonicum</i>	JAP124	Florida	Alachua County	UF	29.63081	-82.3702	20 March 2021	J.A. Pelosi 124	
<i>Lygodium japonicum</i>	JAP125	Florida	Alachua County	HT	29.636748	-82.3142	20 March 2021	J.A. Pelosi 125	
<i>Lygodium japonicum</i>	JAP126	Florida	Alachua County	HT	29.636748	-82.3141	20 March 2021	J.A. Pelosi 126	
<i>Lygodium japonicum</i>	JAP127	Florida	Alachua County	HT	29.636748	-82.3141	20 March 2021	J.A. Pelosi 127	
<i>Lygodium japonicum</i>	JAP128	Florida	Alachua County	FP	29.638976	-82.3938	20 March 2021	J.A. Pelosi 128	
<i>Lygodium japonicum</i>	JAP129	Florida	Alachua County	FP	29.638976	-82.3938	20 March 2021	J.A. Pelosi 129	
<i>Lygodium japonicum</i>	JAP130	Florida	Alachua County	FP	29.638976	-82.3938	20 March 2021	J.A. Pelosi 130	
<i>Lygodium japonicum</i>	JAP131	Florida	Alachua County	FP	29.638976	-82.3938	20 March 2021	J.A. Pelosi 131	https://www.inaturalist.org/observations/72295785
<i>Lygodium japonicum</i>	JAP132	Florida	Suwannee County	TR	30.369736	-83.1976	28 March 2021	J.A. Pelosi 132	https://www.inaturalist.org/observations/72295834
<i>Lygodium japonicum</i>	JAP133	Florida	Suwannee County	TR	30.369736	-83.1976	28 March 2021	J.A. Pelosi 133	https://www.inaturalist.org/observations/72295936
<i>Lygodium japonicum</i>	JAP134	Florida	Hamilton County	BS	30.34202	-82.7273	28 March 2021	J.A. Pelosi 134	https://www.inaturalist.org/observations/72295998
<i>Lygodium japonicum</i>	JAP135	Florida	Hamilton County	BS	30.34202	-82.7273	28 March 2021	J.A. Pelosi 135	
<i>Lygodium japonicum</i>	JAP136	Florida	Hamilton County	BS	30.34202	-82.7273	28 March 2021	J.A. Pelosi 136	

APPENDIX 2. Continued.

Species	Sample ID	State	County	Population	Latitude	Longitude	Date	Voucher Specimen	Notes
<i>Lygodium japonicum</i>	JAP137	Florida	Hamilton County	BS	30.34202	-82.7273	28 March 2021	J.A. Pelosi 137	https://www.inaturalist.org/observations/72296349
<i>Lygodium japonicum</i>	JAP138	Florida	Hamilton County	BS	30.34202	-82.7273	28 March 2021	J.A. Pelosi 138	https://www.inaturalist.org/observations/72296410
<i>Lygodium japonicum</i>	JAP139	Florida	Hamilton County	BS	30.34202	-82.7273	28 March 2021	J.A. Pelosi 139	
<i>Lygodium japonicum</i>	JAP140	Florida	Hamilton County	BS	30.34202	-82.7273	28 March 2021	J.A. Pelosi 140	
<i>Lygodium japonicum</i>	JAP141	Florida	St. John's County	MS	29.728336	-81.2776	28 March 2021	J.A. Pelosi 141	
<i>Lygodium japonicum</i>	JAP142	Florida	St. John's County	MS	29.728336	-81.2776	28 March 2021	J.A. Pelosi 142	
<i>Lygodium japonicum</i>	JAP143	Florida	St. John's County	MS	29.728336	-81.2776	28 March 2021	J.A. Pelosi 143	
<i>Lygodium japonicum</i>	JAP144	Florida	St. John's County	MS	29.728336	-81.2776	28 March 2021	J.A. Pelosi 144	
<i>Lygodium japonicum</i>	JAP145	Florida	Hillsborough County	HR	28.147602	-82.2373	17 April 2021	J.A. Pelosi 145	
<i>Lygodium japonicum</i>	JAP146	Florida	Hillsborough County	HR	28.147602	-82.2373	17 April 2021	J.A. Pelosi 146	
<i>Lygodium japonicum</i>	JAP147	Florida	Hillsborough County	HR	28.147602	-82.2373	17 April 2021	J.A. Pelosi 147	
<i>Lygodium japonicum</i>	JAP148	Florida	Hillsborough County	HR	28.147602	-82.2373	17 April 2021	J.A. Pelosi 148	
<i>Lygodium japonicum</i>	JAP149	Florida	Marion County	OP	29.104598	81.9077	17 April 2021	J.A. Pelosi 149	https://www.inaturalist.org/observations/52078474
<i>Lygodium japonicum</i>	JAP150	Florida	Marion County	OP	29.104598	81.9077	17 April 2021	J.A. Pelosi 150	
<i>Lygodium japonicum</i>	JAP151	Florida	Marion County	OP	29.104598	81.9077	17 April 2021	J.A. Pelosi 151	

APPENDIX 2. Continued.

Species	Sample ID	State	County	Population	Latitude	Longitude	Date	Voucher Specimen	Notes
<i>Lygodium japonicum</i>	JAP152	Florida	Marion County	OP	29.104598	81.9077	17 April 2021	J.A. Pelosi 152	
<i>Lygodium japonicum</i>	JAP153	Florida	Marion County	OP	29.104598	81.9077	17 April 2021	J.A. Pelosi 153	
<i>Lygodium japonicum</i>	JAP155	Florida	Marion County	JS	29.185114	-81.7109	17 April 2021	J.A. Pelosi 155	
<i>Lygodium japonicum</i>	JAP156	Florida	Marion County	JS	29.185114	-81.7109	17 April 2021	J.A. Pelosi 156	
<i>Lygodium japonicum</i>	JAP157	Florida	Marion County	JS	29.185114	-81.7109	17 April 2021	J.A. Pelosi 157	
<i>Lygodium japonicum</i>	JAP182	Florida	Alachua County	HT	29.636748	-82.3141	5 March 2022	J.A. Pelosi 125	
<i>Lygodium japonicum</i>	JAP183	Florida	Alachua County	HT	29.636748	-82.3141	5 March 2022	J.A. Pelosi 125	
<i>Lygodium japonicum</i>	JAP184	Florida	Alachua County	HT	29.636748	-82.3141	5 March 2022	J.A. Pelosi 125	
<i>Lygodium japonicum</i>	JAP185	Florida	Alachua County	HT	29.636748	-82.3141	5 March 2022	J.A. Pelosi 125	
<i>Lygodium japonicum</i>	JAP186	Florida	Alachua County	HT	29.636748	-82.3141	5 March 2022	J.A. Pelosi 125	
<i>Lygodium japonicum</i>	JAP187	Florida	Alachua County	HT	29.636748	-82.3141	5 March 2022	J.A. Pelosi 125	
<i>Lygodium japonicum</i>	JAP188	Florida	Alachua County	UF	29.63081	-82.3702	5 March 2022	J.A. Pelosi 124	
<i>Lygodium japonicum</i>	JAP189	Florida	Alachua County	UF	29.63081	-82.3702	5 March 2022	J.A. Pelosi 124	
<i>Lygodium japonicum</i>	JAP190	Florida	Orange County	MP	28.36655	-81.194625	5 May 2022	J.A. Pelosi 193	https://www.inaturalist.org/observations/117208234
<i>Lygodium microphyllum</i>	OWCF1	Florida	County	LX	26.499859	-80.3146	26 May 2021	J.A. Pelosi 159	

APPENDIX 2. Continued.

Species	Sample ID	State	County	Population	Latitude	Longitude	Date	Voucher Specimen	Notes
<i>Lygodium microphyllum</i>	OWCF2	Florida	Palm Beach County	LX	26.499859	-80.3146	26 May 2021	J.A. Pelosi 159	
<i>Lygodium microphyllum</i>	OWCF4	Florida	Palm Beach County	LX	26.499859	-80.3146	26 May 2021	J.A. Pelosi 159	
<i>Lygodium microphyllum</i>	OWCF5	Florida	Palm Beach County	LX	26.499859	-80.3146	26 May 2021	J.A. Pelosi 159	
<i>Lygodium microphyllum</i>	CREW1	Florida	Collier County	CR	26.33847	-81.6198	9 March 2022		https://www.inaturalist.org/observations/108275233
<i>Lygodium microphyllum</i>	CREW2	Florida	Collier County	CR	26.33847	-81.6198	9 March 2022		https://www.inaturalist.org/observations/108275233
<i>Lygodium microphyllum</i>	CREW3	Florida	Collier County	CR	26.33847	-81.6198	9 March 2022		https://www.inaturalist.org/observations/108275233

DATA: Specimens used in this study have been deposited in the University of Florida Herbarium (FLAS). Flow cytometry data, spore images and measurements, and code are available at <https://github.com/jessiepelosi/LyJapCyto>.