Cytogeography of *Callisia* section *Cuthbertia* (Commelinaceae)

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Abstract

Determining the distribution of cytotypes across the geographic distribution of polyploid complexes can provide valuable information about the evolution of biodiversity. Here, the phytogeography of cytotypes in *Callisia* section *Cuthbertia* (Small, 1903) Hunt, 1986 is investigated. A total of 436 voucher specimens was georeferenced; 133 new specimens were collected. Based on flow cytometry data, DNA content of all cytotypes in *Callisia* section *Cuthbertia* was estimated. Utilizing chromosome counts and flow cytometric analysis, cytotype distribution maps were generated. Two disjunct groups of populations of diploid *Callisia graminea* (Small, 1903) Tucker, 1989 were discovered; tetraploid *C. graminea* ranges broadly from the coastal plain of North Carolina through central Florida. One hexaploid *C. graminea* individual was recorded in South Carolina, and numerous individuals of hexaploid *C. graminea* were found in central Florida. Diploid *C. ornata* (Small, 1933) Tucker, 1989 occurs in eastern Florida; previously unknown tetraploid and hexaploid populations of *C. ornata* were discovered in western and central Florida, respectively. Diploid *C. rosea* (Ventenat, 1800) Hunt, 1986 occurs in Georgia and the Carolinas, with populations occurring on both sides of the Fall Line. The cytotype and species distributions in *Callisia* are complex, and these results provide hypotheses, to be tested with morphological and molecular data, about the origins of the polyploid cytotypes.

Keywords

chromosome counts, cytotypes, endemic, Florida scrub vegetation, flow cytometry, genome size, polyploidy, sandhill vegetation, Southeastern United States

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**Introduction**

Polyploidy (whole-genome duplication) is a speciation mechanism that is a major evolutionary force; in fact, all angiosperms have undergone at least one ancient polyploidy event (Jiao et al. 2011, *Amborella Genome Project* 2013), and polyploidy has been a key driver of angiosperm diversity (De Bodt et al. 2005, Soltis et al. 2009, Soltis and Soltis 2009, Soltis and Soltis 2016, Tank et al. 2015).

Polyploids are classified in two major categories: allopolyploids and autopolyploids. Allopolyploids are by far the more studied form and arise via hybridization between species, whereas autopolyploids originate from the multiplication of genomes within a single species. An autopolyploid is frequently considered as a cytotype within a species along with its diploid progenitor, as in *Galax urceolata* (Poiret, 1804) Brummitt, 1972 (Baldwin 1941, Stebbins 1950), *Chamerion angustifolium* (Linnaeus, 1753) Holub, 1972 (Mosquin 1967), *Heuchera grossulariifolia* Rydberg, 1900 (Wolf et al. 1990), and *Vaccinium corymbosum* Linnaeus, 1753 (Camp 1945, Krebs and Hancock 1989). However, autotetraploids are occasionally recognized as species distinct from their diploid parent, such as *Zea perennis* (Hitchcock, 1922) Reeves & Mangelsdorf, 1942 (Ilits et al. 1979, Tiffin and Gaut 2001) and *Tolmiea menziesii* Torrey & Gray, 1840 (Judd et al. 2007). Lumping diploid progenitors with their multiple derivative cytotypes into a single species may mask evolutionary lineages and grossly underestimate biodiversity (Soltis et al. 2007).

To gain a better assessment of biodiversity and to guide conservation efforts for species of interest, data on both evolutionary and life-history characteristics are needed. *Callisia* section *Cuthbertia* (Commelinaceae) from the southeastern U.S.A. comprises a polyploid complex, with species of conservation concern, but the extent of polyploidy and the geographic distribution of cytotype diversity are unknown.

*Callisia* Loefling, 1758 is one of 39 genera in subfamily Commelinoideae (Burns et al. 2011) and is placed in tribe Tradescantieae subtribe Tradescantiinae. *Callisia* comprises approximately 23 species in six sections (*Hadrodemas* (Moore, 1963) Hunt, 1986, *Cuthbertia* (Small, 1903) Hunt, 1986, *Lauia* Hunt, 1986, *Brachyphylla* Hunt, 1986, *Leptocallisia* Bentham & Hooker, 1883, and *Callisia*) (Hunt 1986, Tucker 1989). Of these sections, *Cuthbertia* is endemic to the U.S.A., and *Brachyphylla*, *Leptocallisia*, and *Callisia* also have members that occur in the U.S.A. (Tucker 1989). The remaining two sections (*Lauia* and *Hadrodemas*) occur in Central America, South America, and the Caribbean. In recent phylogenetic analyses, *Callisia* is not monophyletic (Bergamo 2003, Burns et al. 2011), although, significantly, section *Cuthbertia* is monophyletic in all analyses (Bergamo 2003, Burns et al. 2011, Hertweck and Pires 2014).

*Callisia* section *Cuthbertia* consists of three morphologically distinct species (*C. graminea*, *C. ornata*, and *C. rosea*) that are endemic to the southeastern U.S.A. and have a base chromosome number of \(x = 6\) (Giles 1942, 1943). *Callisia graminea* (Small, 1903) Tucker, 1989, the grassleaf roseling, occurs from the southern border of Virginia through central Florida. Giles (1942, 1943) reported three ploidal levels (\(2x\), \(4x\), and \(6x\)) for this species and encountered a single triploid individual in Hoke County, NC. Based on cytological criteria, the tetraploid was interpreted as an autopolyploid derivative of diploid *C. graminea* (Giles 1942, 1943). The nature of polyploidy in hexa-
ploid *C. graminea* is not clear. Within *C. graminea*, two forms have been described: *C. graminea* forma *graminea* has pink flowers with anthocyanin pigments, and *C. graminea* forma *leucantha* (Lakela, 1972) Tucker, 1989 has white flowers and was described from two diploid cuttings (Lakela 1972). *Callisia ornata* (Small, 1903) Tucker, 1989 (Florida scrub roseling), a diploid (Giles unpublished), is endemic to central to southern Florida. *Callisia rosea* (Ventenat, 1800) Hunt, 1986 (Piedmont roseling) is a diploid (Anderson and Sax 1936), with a distribution from North Carolina to Georgia.

Although earlier studies (e.g., Giles 1942, 1943) provided the general pattern of species distributions and cytotypic diversity, the extent of cytotypic variation within and among species has not been examined in detail. Additional sampling of both populations and species is required to understand the extent and distribution of cytological variation in this clade. In this study, numerous new field collections were made, and known populations of *Callisia section Cuthbertia* were revisited; with the use of both traditional chromosome counts and flow cytometry, the ploidy of samples spanning the entire range of *Callisia section Cuthbertia* was investigated. Distribution maps of cytotypes and species were generated based on the cytological data obtained here, enabling future studies of phylogeny and polyploid origins in *Callisia section Cuthbertia*.

**Materials and methods**

**Georeferencing**

To obtain locality data for *Callisia graminea*, *C. ornata*, and *C. rosea*, voucher specimens were examined from the following herbaria: GA, USCH, NCU, DUKE, US, AAH, FLAS, FSU, VSC, and SFU (codes follow Thiers 2016). The locality of each specimen was georeferenced by manually incorporating the label data into the web applications ACME mapper 2.1 (Poskanzer 2001) and/or GEOLocate (Rios and Bart 2010). Additional localities were obtained from the Master’s Thesis of A. Kelly (1991) and personal communications with members of the Florida Native Plant Society and photographers from Flickr.com. In all, 436 specimens were georeferenced from herbarium specimens and observation records. (See supplementary file 1: Table 1 for georeferenced data points.) The data points were used to produce a distribution map using ArcGIS 10.4 (ESRI 2016) and to locate known populations and contact zones of all three species and their cytotypes.

**Collecting of specimens**

The georeferenced data were used to relocate populations within the southeastern U.S.A.; additional localities were discovered by exploring similar habitats in protected areas and on private land. Collections on private land were made with permission of the land owners. Based on the georeferenced data, permits were obtained to collect in state parks, state forests, national parks, and protected areas of The Nature Conservancy and the U.S. Fish and Wildlife Service in Florida, Georgia, South Carolina, North Carolina, and Virginia (Table 1).
**Table 1.** Populations used in this study. Geographic location, ploidy, number of plants of each ploidy, total number of analyzed individuals, and voucher information for 133 populations of *Callisia graminea* (G), *C. ornata* (O), and *C. rosea* (R) from the southeastern United States. * indicates a new locality with voucher specimen.

| Population | Locality | Geographic coordinates | Ploidy / Number of plants | Voucher no. |
|------------|----------|------------------------|---------------------------|-------------|
| Callisia graminea (Small) G. Tucker |
| G-1* | Gainesville Regional Airport | FL Alachua 29°42.01'N 082°15.72'W | 1 3 | 307 |
| G-2 | Jct. Tower Rd. and SW 8 Ave | FL Alachua 29°38.63'N 082°25.24'W | 1 4 | 223 |
| G-3 | Morningside Nature Center | FL Alachua 29°39.56'N 082°16.45'W | 1 1 | 234 |
| G-4 | Jct. Hwy 200 and CR. 491 | FL Citrus 28°58.51'N 082°21.84'W | 1 2 | 229 |
| G-5* | Along Rod Rd. | FL Clay 30°01.52'N 081°51.95'W | 1 1 | 225 |
| G-6 | Golden Branch Head State Park | FL Clay 29°50.75'N 081°57.04'W | 1 2 | 309 |
| G-7 | Silver Sand Lake Rd. | FL Clay 29°47.49'N 081°58.32'W | 1 4 | 311 |
| G-8* | Tate Hell State Forest along New River | FL Franklin 29°52.42'N 084°41.79'W | 1 4 | 306 |
| G-9* | Richloam State Forest/Dark Stretch Rd. | FL Hernando 28°29.10'N 082°08.87'W | 1 6 | 349 |
| G-10* | Edwards Rd., Lady Lake | FL Lake 28°54.12'N 081°53.40'W | 1 3 | 235 |
| G-11* | Lake Griffin State Park | FL Lake 28°52.31'N 081°53.41'W | 1 3 | 236 |
| G-12* | Seminole State Forest along Co. Rd. 42 | FL Lake 29°00.82'N 081°31.05'W | 1 1 | 345 |
| G-13* | Seminole State Forest | FL Lake 28°49.31'N 081°28.01'W | 1 1 | 362 |
| G-14* | Lake Norris Rd. | FL Lake 28°49.89'N 081°32.41'W | 1 1 | 363 |
| G-15* | ATV trail at Ocala National Forest | FL Marion 29°21.76'N 081°44.21'W | 1 1 | 230 |
| G-16 | Silver River State Park | FL Marion 29°12.15'N 082°02.77'W | 1 4 | 348 |
| G-17* | Along Mason Rd. | FL Putnam 29°42.50'N 082°00.77'W | 1 2 | 224 |
| G-18* | Ordway Biological Center H1 & H2 area | FL Putnam 29°41.70'N 081°57.87'W | 1 2 | 302 |
| G-19* | Etoniah Creek State Forest | FL Putnam 29°46.43'N 081°51.91'W | 1 3 | 308 |
| G-20 | Dunns Creek State Park entrance Sisco Rd. | FL Putnam 29°31.84'N 081°35.34'W | 1 4 | 310 |
| G-21* | Wakala State Forest | FL Putnam 29°28.24'N 081°39.37'W | 1 2 | 360a |
| G-22 | Along State Rd. 46 | GA Bulloch 32°20.94'N 081°50.57'W | 1 3 | 242 |
| G-23 | Jct. Hwy 185 and Turkey Ridge Dr. | GA Charlton 30°24.76'N 082°11.70'W | 1 2 | 317 |
| Population | Locality | State | County | Latitude   | Longitude   | Ploidy / Number of plants | Geographic coordinates |
|------------|----------|-------|--------|------------|-------------|---------------------------|------------------------|
| G-24*      | General Coffee State Park | GA     | Coffee | 31°31.50'N | 082°46.33'W | 2x 4x 6x                  | N 1 3 318              |
| G-25       | N. Connector Rd./206 Jct. 135 | GA     | Coffee | 31°32.27'N | 082°48.75'W | 1 3                       | N 3 319                |
| G-26*      | George Smith State Park      | GA     | Emanuel| 32°32.64'N | 082°07.32'W | 1 6                       | N 1 241                |
| G-27*      | Ochicoo Preserve, Halls Bridge Rd. | GA     | Emanuel | 32°31.73'N | 082°27.38'W | 1 4                       | N 1 320                |
| G-28       | Fort Stewart                  | GA     | Evans  | 32°06.92'N | 081°47.10'W | 1 4                       | N 1 243                |
| G-29*      | Conway CT./Interstate Parkway | GA     | Richmond | 33°29.24'N | 082°06.12'W | 1 1                       | N 1 322                |
| G-30       | Fort Gordon                   | GA     | Richmond | 33°23.33'N | 082°14.56'W |                           | N 239                  |
| G-31*      | Singletary Lake State Park   | NC     | Bladen | 34°35.41'N | 078°26.87'W | 1 3                       | N 1 263                |
| G-32*      | Jones Lake State Park         | NC     | Bladen | 34°42.11'N | 078°37.22'W | 1 3                       | N 1 268                |
| G-33*      | Jones Lake State Park         | NC     | Bladen | 34°42.11'N | 078°37.22'W | 1 3                       | N 1 268                |
| G-34*      | Along NC 242 near Jones Lake State Park | NC     | Bladen | 34°42.00'N | 078°36.35'W | 1 2                       | N 1 270                |
| G-35*      | Along NC 242 N. of Jones Lake State Park | NC     | Bladen | 34°45.40'N | 078°36.56'W | 1 5                       | N 1 271                |
| G-36*      | White Lake, along NC 741, Barnes Food Co. | NC     | Bladen | 34°39.41'N | 078°30.17'W | 1 5                       | N 1 272                |
| G-37*      | Jones Lake State Park, campsite | NC     | Bladen | 34°40.79'N | 078°35.99'W |                           | N 274                  |
| G-38*      | Along Burney Rd. underneath powerline | NC     | Bladen | 34°44.38'N | 078°43.68'W | 1 4                       | N 1 334                |
| G-39*      | River Rd., underneath powerline | NC     | Bladen | 34°46.18'N | 078°47.24'W | 1 3                       | N 1 335                |
| G-40       | Bay Tree Lake State Park/undeveloped | NC     | Bladen | 34°40.22'N | 078°25.66'W | 1 6                       | N 1 261                |
| G-41       | Along Hwy 41 close to Bay Tree Lake State Park | NC     | Bladen | 34°41.21'N | 078°25.26'W | 1 3                       | N 1 262                |
| G-42       | Along Hwy 11 towards Delco under powerline | NC     | Bladen | 34°24.61'N | 078°15.60'W | 1 4                       | N 1 266                |
| G-43       | Along Jessup Pond              | NC     | Bladen | 34°51.72'N | 078°43.76'W |                           | N 275                  |
| G-44       | Lake Waccamaw State Park.      | NC     | Columbus | 34°16.73'N | 078°27.89'W |                           | N 267                  |
| G-45*      | Mack Simmons Rd.              | NC     | Cumberland | 34°54.45'N | 078°44.20'W |                           | N 276                  |
| G-46*      | Along NC 210, Jct. with Sidney Bullard Rd. | NC     | Cumberland | 34°58.69'N | 078°43.84'W | 1 4                       | N 1 278                |
| G-47*      | Ft. Bragg/John Mill Rd.       | NC     | Cumberland | 35°10.70'N | 079°05.39'W | 1 3                       | N 1 341                |
| G-48*      | Ft. Bragg/NE. training/Mc Closkey Rd. | NC     | Cumberland | 35°09.84'N | 078°56.97'W | 1 3                       | N 1 342                |
| G-49       | Cedar Creek Rd., Tatum farm    | NC     | Cumberland | 34°56.32'N | 078°44.58'W | 1 1                       | N 1 277                |
| Population | Locality | State | County | Geographic coordinates | Ploidy / Number of plants | Voucher no. |
|------------|----------|-------|--------|-----------------------|---------------------------|------------|
| G-50       | Along Dunns Rd./NC 301 | NC    | Cumberland | 35°06.42’N 078°46.52’W | 2x | 279 |
| G-51       | Open Area along NC 24 | NC    | Harnett | 35°15.61’N 079°02.47’W | 1 | 3 | 284 |
| G-52       | Along Rockfish Rd. | NC    | Hoke | 34°59.32’N 079°05.82’W | 1 | 3 | 286 |
| G-53       | In open area along Red Springs Rd. | NC    | Hoke | 34°52.38’N 079°12.17’W | 1 | 4 | 287 |
| G-54*      | Weymouth Sandhill Nature Preserve | NC    | Moore | 35°08.95’N 079°22.10’W | 1 | 3 | 288 |
| G-55       | Along Riverview Dr. | NC    | Moore | 35°11.48’N 079°10.94’W | 1 | 3 | 285 |
| G-56       | Along NC 11/ Hwy 53 | NC    | Pender | 34°29.72’N 078°11.49’W | 1 | 3 | 264 |
| G-57       | Along NC 11/ Hwy 53 | NC    | Pender | 34°29.72’N 078°11.49’W | 1 | 1 | 265 |
| G-58*      | Grey Woods Rd. | NC    | Richmond | 34°57.52’N 079°38.47’W | 1 | 3 | 297 |
| G-59*      | Sandhills Game Land | NC    | Richmond | 35°01.83’N 079°36.70’W | 1 | 2 | 336 |
| G-60*      | Sandhills Game Land/442/Ledbetter Rd. | NC    | Richmond | 35°03.62’N 079°38.09’W | 1 | 3 | 337 |
| G-61*      | Sandhills Game Land | NC    | Richmond | 34°58.61’N 079°30.42’W | 1 | 2 | 338 |
| G-62*      | Sandhills Game Land SR 1331, 15/501 | NC    | Richmond | 34°58.50’N 079°26.93’W | 1 | 2 | 339 |
| G-63*      | Sandhills Game Land, Aberdeen Rd./Hill Creek Rd. | NC    | Richmond | 34°59.49’N 079°26.76’W | 1 | 3 | 340 |
| G-64       | Sandhills Game Land along McDonald Church Rd. | NC    | Richmond | 35°01.24’N 079°37.18’W | 1 | 2 | 290 |
| G-65       | NC Hwy 177 | NC    | Richmond | 34°50.41’N 079°45.54’W | 1 | 1 | 295 |
| G-66       | Along Saint Stevens Church Rd. | NC    | Richmond | 34°49.82’N 079°50.55’W | 1 | 1 | 296 |
| G-67       | NC 242, 0.3 mi N. of Cumberland Co. line | NC    | Sampson | 34°53.35’N 078°31.28’W | 1 | 3 | 273 |
| G-68       | Along Spiveys Corner Hwy. | NC    | Sampson | 35°10.72’N 078°28.65’W | 1 | 2 | 280 |
| G-69       | Edge camp Mackall along Aberdeen Rd. | NC    | Scotland | 35°00.84’N 079°26.70’W | 1 | 2 | 289 |
| G-70       | Along 1328, Hoffman Rd./Butler Rd. | NC    | Scotland | 34°59.14’N 079°31.99’W | 1 | 2 | 291 |
| G-71       | Along Peach Orchard Rd. under powerline | NC    | Scotland | 34°55.77’N 079°23.86’W | 1 | 3 | 292 |
| G-72       | Along US 401 and forest edge | NC    | Scotland | 34°50.49’N 079°23.98’W | 1 | 1 | 293 |
| G-73       | Along forest edge of Hamlet Rd. | NC    | Scotland | 34°48.01’N 079°38.03’W | 1 | 2 | 294 |
| G-74       | Along Piney Grove Church Rd. | NC    | Wayne | 35°17.32’N 077°50.92’W | 1 | 1 | 281 |
| G-75*      | Aiken State Park | SC    | Aiken | 33°32.55’N 081°28.92’W | 1 | 4 | 324 |
| Population | Locality | State | County | Latitude  | Longitude  | 2x | 4x | 6x | N  | Voucher no. |
|------------|----------|-------|--------|-----------|------------|----|----|----|----|------------|
| G-76*      | Parcel at Jct. Hwy 283 & US 1/Columbia Hwy N | SC    | Aiken  | 33°36.11'N | 081°41.04'W | 5  |    |    |    | G-76*      |
| G-77       | Aiken Gopher Tortoise Heritage Preserve | SC    | Aiken  | 33°30.00'N | 081°24.52'W | 1  |    |    |    | 231        |
| G-78*      | Carolina Sandhills National Wildlife Refuge | SC    | Chesterfield | 34°31.46'N | 080°13.63'W | 1  | 3  |    | 331 |            |
| G-79*      | Sandhill State Forest | SC    | Chesterfield | 34°33.37'N | 080°03.84'W | 1  | 3  |    | 332 |            |
| G-80*      | H. Cooperblack Jr. Memorial trail/James Rd. | SC    | Chesterfield | 34°34.03'N | 079°55.75'W | 1  | 2  |    | 333 |            |
| G-81       | Along Hwy 102 | SC    | Chesterfield | 34°38.30'N | 080°05.22'W | 1  | 5  |    | 249 |            |
| G-82       | Teals mill Rd./Cheraw State Park | SC    | Chesterfield | 34°37.25'N | 080°16.92'W | 1  | 3  |    | 250 |            |
| G-83       | W. Old Camden Rd. | SC    | Chesterfield | 34°22.28'N | 080°17.44'W | 1  | 2  |    | 252 |            |
| G-84       | US 1 | SC    | Chesterfield | 34°26.17'N | 080°21.27'W | 1  | 3  |    | 258 |            |
| G-85       | Along Old Stagecoach Rd. | SC    | Chesterfield | 34°22.99'N | 080°23.29'W | 1  | 1  |    | 255 |            |
| G-86       | Along Old Georgetown Rd. E. | SC    | Chesterfield | 34°22.04'N | 080°25.92'W | 1  | 4  |    | 330 |            |
| G-87       | Co. Rd. S. 18-137 | SC    | Dorchester | 34°38.30'N | 080°23.11'W | 1  | 4  |    | 248 |            |
| G-88       | Tillman Sand Ridge Heritage Preserve, Sandhill Rd. | SC    | Jasper | 32°29.69'N | 081°11.55'W | 1  |    |    | 247 |            |
| G-89*      | Along Jefferson Davis Hwy/US 1 | SC    | Kershaw | 34°18.73'N | 080°32.49'W | 1  | 3  |    | 256 |            |
| G-90*      | Goodale State Park | SC    | Kershaw | 34°17.42'N | 080°31.55'W | 1  | 3  |    | 329 |            |
| G-91*      | Jefferson Davis Hwy/US 1 | SC    | Kershaw | 34°22.04'N | 080°25.92'W | 1  | 4  |    | 330 |            |
| G-92*      | Lee State Park | SC    | Lee | 34°11.81'N | 080°11.36'W | 1  | 3  |    | 251 |            |
| G-93       | Shealy's Pond Heritage Preserve | SC    | Lexington | 34°51.82'N | 081°14.19'W | 1  |    |    | 232 |            |
| G-94       | Peachtree Rock Preserve | SC    | Lexington | 33°49.71'N | 081°12.11'W | 1  |    |    | 233 |            |
| G-95*      | Ft. Jackson, Area 26 B firebreak 16 | SC    | Richland | 34°00.85'N | 080°47.40'W | 1  | 2  |    | 257 |            |
| G-96*      | Ft. Jackson, Area 34 B near Chauers Pond Rd. | SC    | Richland | 34°02.36'N | 080°43.30'W | 1  | 3  |    | 258 |            |
| G-97*      | Ft. Jackson, Area 11 E. of Wildcat Rd. | SC    | Richland | 34°05.06'N | 080°50.61'W | 1  | 2  |    | 259 |            |
| G-98       | Ft. Jackson, S. edge of pond of Westons Recreation | SC    | Richland | 33°59.96'N | 080°50.03'W | 1  |    |    | 260 |            |
| G-99       | Sesquicentennial State Park | SC    | Richland | 34°05.82'N | 080°54.57'W | 1  | 3  |    | 326 |            |
| G-100*     | Sesquicentennial State Park | SC    | Richland | 34°04.92'N | 080°54.38'W | 1  | 4  |    | 327 |            |
| G-101      | Faunas Rd. | SC    | Richland | 34°08.34'N | 081°02.33'W | 1  |    |    | 328 |            |
### Geographic Coordinates

| Population | Locality | State | County | Latitude | Longitude |
|------------|----------|-------|--------|----------|-----------|
| O-1        | Forks of River Rd. | VA | Southampton | 36°33.85' N | 76°55.96' W |
| O-2        | Suffolk City, DCR | VA | VA | 36°33.77' N | 76°54.82' W |
| O-3        | Turkey Creek Sanctuary | FL | Brevard | 28°01.01' N | 80°36.18' W |
| O-4        | Wickham State Park | FL | Brevard | 28°09.64' N | 80°39.54' W |
| O-5        | Highland State Park | FL | Highlands | 27°28.85' N | 81°31.57' W |
| O-6        | Lake June in Winter Scrub State Park | FL | Highlands | 27°17.83' N | 81°25.14' W |
| O-7        | Little Manatee State Park/Muscotag Park | FL | Hillsborough | 27°40.08' N | 82°22.38' W |
| O-8        | Little Manatee State Park/Duval trail | FL | Hillsborough | 27°39.53' N | 82°22.38' W |
| O-9        | Seminole State Forest/Brandley Branch Rd. | FL | Lake | 28°52.94' N | 81°31.08' W |
| O-10       | Seminole State Forest/Campfire picnic area | FL | Lake | 28°28.85' N | 81°31.08' W |
| O-11       | Seminole State Forest/Wakulla trail | FL | Martin | 26°59.58' N | 80°08.83' W |
| O-12       | Lake Louise State Park/Primitive camping | FL | Lake | 28°27.17' N | 81°44.13' W |
| O-13       | Lake Kissimmee State Park/Nature trail picnic area | FL | Lake | 27°57.61' N | 80°12.77' W |
| O-14       | Lake Kissimmee State Park/Nature trail picnic area | FL | Lake | 27°57.59' N | 80°12.77' W |
| O-15       | Lake Kissimmee State Park/Main entrance | FL | Lake | 27°57.59' N | 80°12.77' W |
| O-16       | Lake Kissimmee State Park/Brickyard Rd. | FL | Polk | 27°56.34' N | 81°30.56' W |
| O-17       | Lake Kissimmee State Park/School Bus Rd. | FL | Polk | 27°56.34' N | 81°30.56' W |
| O-18       | Lake Kissimmee State Park/Brickyard Rd. | FL | Polk | 27°56.34' N | 81°30.56' W |
| O-19       | Lake George State Forest | FL | Polk | 27°56.34' N | 81°30.56' W |
| O-20       | Damas Creek State Park entrance-Sisco Rd. | FL | Polk | 27°56.34' N | 81°30.56' W |
| O-21       | Ocala Schoefer State Park along Legacy trail | FL | Putnam | 27°10.27' N | 80°09.58' W |
| O-22       | Ocala Schoefer State Park along Legacy trail | FL | Putnam | 27°10.27' N | 80°09.58' W |
| O-23       | Lake George State Forest | FL | Volusia | 27°11.84' N | 81°30.56' W |

### Ploidy / Number of plants

- **Callisia ornata** (Small) G. Tucker
- **O-1**
- **O-2**
- **O-3**
- **O-4**
- **O-5**
- **O-6**
- **O-7**
- **O-8**
- **O-9**
- **O-10**
- **O-11**
- **O-12**
- **O-13**
- **O-14**
- **O-15**
- **O-16**
- **O-17**
- **O-18**
- **O-19**
- **O-20**
- **O-21**
- **O-22**
- **O-23**
Cytogeography of Callisia section Cuthbertia (Commelinaceae).

| Population | Locality                          | State | County | Latitude     | Longitude    | Ploidy | Number of plants | Voucher no. |
|------------|-----------------------------------|-------|--------|--------------|--------------|--------|------------------|-------------|
| O-24*      | Deland                            | FL    | Volusia| 29°00.11'N  | 081°13.25'W | 1      | 1                | 365         |
|            |                                   |       |        |              |              |        |                  |             |
| Callisia rosea (Vent.) D.R. Hunt |
| R-1        | Along Chert Quarry Rd.            | SC    | Allendale| 33°02.28'N  | 081°28.26'W | 1      | 3                | 245         |
| R-2*       | Heggie's Rock Preserve            | GA    | Colombia| 33°32.34'N  | 082°15.09'W | 1      | 3                | 321         |
| R-3*       | Lake Russel State Park            | GA    | Elbert | 34°09.60'N  | 082°44.42'W | 1      | 3                | 237         |
| R-4*       | Bobbie Brown State Park           | GA    | Elbert | 33°58.35'N  | 082°34.64'W | 1      | 3                | 238         |
| R-5*       | Elijah Clarke State Park          | GA    | Lincoln| 33°51.22'N  | 082°24.02'W | 1      | 3                | 323         |
| R-6        | Fort Gordon                       | GA    | Richmond| 33°23.49'N  | 082°14.54'W | 1      | 3                | 240         |
| R-7        | Fort Stewart                      | GA    | Tattnall| 32°02.54'N  | 081°48.84'W | 1      | 4                | 244         |
Mature individuals were sampled in the summers of 2012, 2013, 2014, and 2015. Only known localities with collection years between 1970 and 2012 were visited, unless the locality was in a protected area. This approach was used to increase the chances of finding intact populations but meant that we were unable to resample all of Giles’s (1942, 1943) locations. Voucher specimens were deposited at the University of Florida Herbarium (FLAS); collection numbers are provided in Table 1.

Population localities were surveyed for individuals with different growth habit and habitat; we then collected across that diversity. Contact zones between species, based on the georeferenced localities, were more intensively surveyed by searching for distinct morphological variation (habit, leaf, and flower) to increase the probability of encountering mixed cytotypes. Two to six live plants were collected per locality. Plants were removed with 15 cm of soil circumference to increase the survival rate and placed in plastic bags. At the Department of Biology, University of Florida greenhouse, plants were then potted in a soil mixture of 1:1 sand and potting soil (Pro-Mix) and were kept under natural light. During the period from December–March, the individuals of putative diploid *C. graminea* and *C. rosea* were given a four-month dormancy treatment at 4°C to mimic their natural habitat.

**Chromosome counts**

Two individuals per cytotype of *C. graminea* were used as a control for flow cytometry analysis by counting chromosome numbers using established methods (see below). Previous studies of members of Commelinaceae found that cell division in root tips occurs at high frequency during late morning to early afternoon (Faden and Suda 1980). After a series of hourly collections, 2:00 pm was determined to be the optimal time for collecting root tips of *C. graminea*, *C. ornata*, and *C. rosea*.

Root tips were placed in 2 mM 8-hydroxyquinoline following Soltis (1980) for 24 hours at 4°C and then fixed in a 3:1 absolute ethanol-glacial acetic acid solution for 24 hours. Root tips were then placed in 70% ethanol and stored until needed at 4°C. Digestion of the root tips and spreading of the chromosomes on slides were performed following the methods of Kato et al. (2011). Chromosomes were stained with DAPI and visualized using a Zeiss Axio Imager M2 microscope (Carl Zeiss Microscopy LLC, Thornwood, NY, U.S.A.).

**Flow cytometry**

Preparation of all samples for flow cytometry followed Roberts et al. (2009), in which each sample consisted of approximately 1 cm² of fresh leaf tissue of *Callisia*; 0.5 cm² dried leaf tissue of *Vicia faba* (26.9 pg) was used as an internal standard (Dolezel et al. 2007). Samples were finely chopped with a sharp single-edged razor blade in a petri dish for 2 min in 1 ml of cold lysis buffer (0.1 M citric acid, 0.5% v/v Triton X-100, 1% w/v PVP–40 in distilled water) (Hanson et al. 2005, Mavrodiev et al. 2015).
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After 20–30 sec of incubation on a cold brick that served as a cold chopping surface, each sample was further treated and measured based on the methods of Mavrodiev et al. (2015) on an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, U.S.A). In all, the ploidy of 300 samples was assessed in batches of 28 samples.

For the estimation of genome size, three plants of the same accession were analyzed using the Flow Cytometry Kaluza Analysis Software 1.3 (Beckman Coulter Life Sciences 2016). The relative DNA content was calculated using the ratio of the mean fluorescent peak of the sample to the mean fluorescent peak of the internal standard, multiplied by the genome size of the standard, *Vicia faba* (Dolezel et al. 2007).

**Results**

**Georeferencing and collecting**

All GPS points obtained here were incorporated into a map with ARCGIS 10.4 (ESRI 2016) (Figure 1). The results show that *Callisia graminea* ranges from North Carolina to central Florida with an isolated population in southern Virginia. *Callisia rosea* occurs predominantly in South Carolina and Georgia, and *C. ornata* is found in central to southern Florida. Specimens were collected at 133 localities, of which 61 were known from the 436 georeferenced localities and 72 were newly discovered populations. A list of these localities is provided in Table 1, indicating the geographic origin, ploidal level with corresponding number of plants, total number of analyzed individuals, and voucher information for each sample. Illustrations of the habits of diploid *C. graminea*, *C. ornata*, and *C. rosea* are provided in Figure 2.

**Chromosome counts**

Chromosome numbers were obtained for three individuals per cytotype in *C. graminea*, confirming the presence of $2n = 2x = 12$ (diploids; Figure 3a), $2n = 4x = 24$ (tetraploids; Figure 3b), and $2n = 6x = 36$ (hexaploids; Figure 3c). The diploid and tetraploid counts were obtained for plants from known locations for which previous counts were available (Giles 1942, Kelly 1991). The hexaploids were discovered while counting spreads of putatively tetraploid *C. graminea* from Lake County, FL (Table 1). These $2x$, $4x$, and $6x$ individuals of *C. graminea* were then used as references in subsequent analyses using flow cytometry.

**Flow cytometry**

Ploidy was estimated via flow cytometry for 300 plants of *C. graminea* (representing 96 populations), *C. ornata* (from 23 populations), and *C. rosea* (from 7 populations). The results and the number of individuals analyzed per population are given in Table
Figure 1. Distribution map of Callisia section Cuthbertia. Distribution of Callisia graminea, C. ornata, and C. rosea based on georeferenced data. Multiple species occurring in sympatry are designated by superimposed symbols; these locations are further indicated by black lines that highlight the symbols.

1. Three distinct groups of fluorescence intensities were obtained from these analyses that were congruent with chromosome counts of diploid, tetraploid, and hexaploid C. graminea. Histograms for the cytotypes of C. graminea are shown in Figure 4. Results for 26 individuals (17%) of tetraploid C. graminea had a lower fluorescence intensity (sug-
suggesting a smaller genome size) than the remaining 83% of tetraploid \( C. \text{graminea} \). The ploidy of the former plants was verified by chromosome counts, and all were tetraploid.

The relative genome size of individuals of \( C. \text{rosea} \) was similar to that of diploid \( C. \text{graminea} (2n = 2x = 12) \) (see below), confirming that our samples of \( C. \text{rosea} \) are diploid, in agreement with the literature (Giles 1942). Most individuals of \( C. \text{ornata} (2n = 2x = 12) \) were also inferred to be diploid, as expected based on previous counts (Giles unpublished), but our analysis also revealed previously unknown tetraploid \( (2n = 4x = 24) \) and hexaploid populations \( (2n = 4x = 36) \) of \( C. \text{ornata} \). The latter were found in Seminole State Forest, FL, where they occur in sympatry with tetraploid individuals of \( C. \text{graminea} \). All polyploid levels were verified with chromosome counts; chromosome spreads are depicted in Figure 3.

Genome size (2C-value) of cytotypes in \( \text{Callisia section Cuthbertia} \) was estimated; data are presented in Table 2 along with previously calculated genome sizes by Hertweck (2011) and Jones and Kenton (1984).

**Distribution map** – Based on the flow cytometry data, the distribution of cytotypic variation among the 126 populations sampled \( [C. \text{graminea} (96 populations), C. \text{ornata} (23 populations), and C. \text{rosea} (7 populations)] \) was mapped (Figure 5). This map shows that diploid \( C. \text{graminea} \) is restricted to two disjunct areas: one in Franklin County, VA, and the second stretching along the Fall Line from North Carolina to South Carolina. Tetraploid \( C. \text{graminea} \) has a broader distribution that runs along the
Figure 3. Mitotic metaphase chromosome spreads from root tips. A diploid *Callisia graminea* (*2n = 2x = 12*) B tetraploid *C. graminea* (*2n = 4x = 24*) C hexaploid *C. graminea* (*2n = 6x = 36*) D diploid *C. ornata* (*2n = 2x = 12*) E tetraploid *C. ornata* (*2n = 4x = 24*) F hexaploid *C. ornata* (*2n = 6x = 36*) and G diploid *C. rosea* (*2n = 2x = 12*).

Table 2. Genome sizes (2C) of *Callisia* section *Cuthbertia* and their cytotypes and previously reported 2C-values. Voucher numbers apply only to the current study.

| Species     | Chromosomes | 2C value (pg) | Hertweck 2011 | Jones and Kenton 1984 |
|-------------|-------------|---------------|---------------|-----------------------|
| *C. graminea* 2x (IEM 342) | *2n = 12* | 41.75 ± 0.67 |               |                       |
| *C. graminea* 4x (IEM 251) | *2n = 24* | 78.55 ± 0.42 |               |                       |
| *C. graminea* 6x (IEM 236) | *2n = 36* | 122.86 ± 0.8 |               |                       |
| *C. ornata* 2x (IEM 353)   | *2n = 12* | 48.51 ± 1.09 |               |                       |
| *C. ornata* 4x (IEM 352)   | *2n = 24* | 87.99 ± 0.4  |               |                       |
| *C. ornata* 6x (IEM 349)   | *2n = 36* | 129.73 ± 0.56 |             |                       |
| *C. rosea* 2x (IEM 237)    | *2n = 12* | 43.70 ± 1.78 | 43.52         | 77.3                  |

coastal plain from North Carolina to central Florida. Hexaploid *C. graminea* occurs in Lake and Hernando Counties, FL, and one individual was found in Richland County, SC. In South Carolina, one hexaploid *C. graminea* individual was found growing sympatrically with multiple tetraploid *C. graminea* plants. Based on extensive collecting, our observations suggest that the tetraploid *C. graminea* samples from North Carolina
Cytogeography of Callisia section Cuthbertia (Commelinaceae).

Figure 4. Histograms of fluorescence intensity (FL2-A) of propidium iodide-stained nuclei. **A** diploid *C. graminea*, **B** tetraploid *C. graminea*, and **C** hexaploid *C. graminea*. *Vicia faba* was used as the internal standard.
Figure 5. Distribution of cytotypic variation in *C. allisia* section Cuthbertia. Diploid *C. graminea* (red circles) ranges from Virginia to North and South Carolina; tetraploid *C. graminea* (purple circles) occurs along the coastal plain from North Carolina to central Florida; hexaploid *C. graminea* (black plus signs) is restricted to central Florida. Diploid *C. ornata* (red squares) occurs in eastern and central Florida; tetraploid *C. ornata* (purple squares) is restricted to central and western peninsular Florida; hexaploid *C. ornata* (green plus signs) is restricted to central Florida. *Callisia rosea* (all diploid; green diamonds) occurs along the Georgia – South Carolina border. Localities with multiple cytotypes or taxa are indicated by black lines. Note: The black plus signs are the hexaploids of *C. graminea*, and the green plus signs are hexaploids of *C. ornata*. 
are the largest of this species, with clumps that exhibit a diameter of over 25 cm compared to plants in South Carolina, Georgia, and Florida, with a maximum diameter of 15 cm.

Diploid *C. ornata* occurs in eastern Florida (from Putnam through Martin Counties), and tetraploid *C. ornata* occurs in western Florida (Polk, Hillsborough, Highlands, and Lake Counties). Hexaploid *C. ornata* occurs in Lake and Volusia Counties in central Florida.

Diploid *C. rosea* occurs in the piedmont of Georgia and South Carolina with some scattered populations in the coastal plain.

**Discussion**

**Georeferencing** – *Callisia section Cuthbertia* consists of three species native to the southeastern U.S.A., with three ploidal levels within *C. graminea* and *C. ornata* and diploids in *C. rosea*. The map of the geographic distribution (Figure 1) of all georeferenced voucher specimens depicts all specimens of *C. graminea*, *C. ornata*, and *C. rosea* without ploidal levels, collected from 1894 until present. *Callisia graminea* is the most widely distributed of all species in the genus, ranging from Virginia to Florida. *Callisia ornata* is restricted to Florida; although one specimen was recorded from Charleston County, GA, *C. ornata* was not found in Georgia in this study. *Callisia rosea* occurs mainly in Georgia and the Carolinas, but two herbarium specimens were found from Duval County and Highlands County, FL. The localities of these two herbarium specimens of *C. rosea* were vague, and *C. rosea* was not observed in Florida in this study.

**Flow cytometry and genome size** – Flow cytometry analysis of ploidal levels in 300 individuals from 126 populations together with 60 additional chromosome counts confirmed the presence of diploid, tetraploid, and hexaploid cytotypes of *C. graminea* and *C. ornata*. Significantly, tetraploid and hexaploid *C. ornata* were previously unknown. Our analysis also confirmed that *C. rosea* is diploid. However, Anderson and Sax (1936) and Ichikawa and Sparrow (1967) reported only tetraploids in *C. rosea*. This might be a misidentification of broad-leaved tetraploid *C. graminea* as *C. rosea*, as suggested by Giles (1942), who only detected diploids in *C. rosea*. Overall, three distinct fluorescent intensity peaks were seen in the histograms among the *C. graminea* and *C. ornata* cytotypes, with peaks for the tetraploids that are approximately twice the size of those of the diploids and for the hexaploids that are approximately three times those of the diploids. This general pattern of genome size increase in polyploids is to be expected relative to their diploid progenitors (Leitch and Bennett 2004).

It is interesting to note that 26 individuals (17%) of tetraploid *C. graminea* had a lower fluorescence intensity than the remaining 83%, suggesting a smaller genome size. The individuals with the smaller peak than that typical of other tetraploids were measured twice with the flow cytometer, and the results were consistent. The chromosome numbers
of these samples were verified by chromosome counts, and all were tetraploid \( (2n = 4x = 24) \). Reductions in genome size in polyploids are common (Leitch and Bennett 2004), and in this study two hypotheses are possible: genome downsizing or the occurrence of multiple origins from parents having different genome sizes. Because this variation in genome size occurs among individuals within populations and because the individuals are not clustered in a single geographic area, we suggest that this variation in DNA content might be a result of genome downsizing, but this hypothesis requires further testing.

Genome size can be used, with other methods, to hypothesize putative progenitors of polyploids (e.g. Eilam et al. 2010). In diploid *C. graminea* the estimated 2C-value is 41.75 pg; the value for tetraploid *C. graminea* is 78.55 pg. According to Giles (1942), multivalent chromosome pairing was observed in tetraploid *C. graminea*, suggesting autopolyploidy. If tetraploid *C. graminea* is of autopolyploid origin, the expected DNA content would be 83.47 pg, but the observed DNA content of tetraploid *C. graminea* is 4.95 pg lower than the expected 2C-value. Newly formed polyploids usually possess a DNA content equal to the sum of the 2C-values of their progenitors (Bennett et al. 2000, Eilam et al. 2010). Over time, however, genome downsizing in polyploids relative to their progenitors is expected (Leitch and Bennett 2004), which seems to be the case in tetraploid relative to diploid *C. graminea*.

Due to the rarity of hexaploid *C. graminea* in South Carolina, we only calculated the 2C-value of hexaploids that occur in Florida. Hexaploid *C. graminea* may be of allo- or autopolyploid origin. If from allopolyploid origin, the expected 2C-value would be 127.06 pg, with diploid *C. ornata* (48.51 pg) and tetraploid *C. graminea* (78.55 pg) as the progenitors. The observed genome size of hexaploid *C. graminea* is 122.86 pg, which is lower than the expected value, again consistent with genome downsizing. In the case of an autopolyploid origin with tetraploid *C. graminea* (78.55 pg) as parent, we would expect a genome size of 117.83 pg, which is approximately 5 pg less than the observed 2C-value. Genome size data do not conclusively elucidate the origins of hexaploid *C. graminea*; both allo- and autopolyploidy are possible, and its origin requires further testing. However, Giles (1942) noted multivalence formation, generally indicative of autopolyplody, in hexaploid *C. graminea*.

Tetraploid *C. ornata* has a 2C-value of 87.99 pg. It could be of autopolyploid origin with diploid *C. ornata* (48.51 pg) as the parent given that no other extant taxa are sympatric with it. However, the expected DNA content (97.02 pg) is at least 9 pg higher than observed; in contrast, when considering tetraploid *C. ornata* as a possible allopolyploid with tetraploid *C. graminea* (78.55 pg) and diploid *C. ornata* (48.51 pg) as parents (based on an unreduced gamete of the latter), the results (87.79 pg) are similar to the observed DNA content. These results therefore support allopolyploidy over autopolyploidy, yet further analyses are needed to clarify the origin of this cytotype.

Hexaploid *C. ornata* could be of allo- or autopolyploid origin. If allopolyploid, the expected genome size would be 127.06 pg with diploid *C. ornata* (48.51 pg) and tetraploid *C. graminea* (78.55 pg) as parents. The observed DNA content is 129.73 pg, which is slightly higher than the expected 2C-value. Alternatively, it could be an allohexaploid between tetraploid *C. ornata* (87.99 pg) and diploid *C. graminea* (41.75 pg), with an expected genome size of 129.74 pg, essentially identical to the observed
value. In the case of autopolyploidy, we calculated an expected 2C-value of 145.53 if the value is 3 times that of diploid *C. ornata* (48.51 pg), 136.5 pg if tetraploid (87.99 pg) and diploid (48.51 pg) *C. ornata* are considered the parents, and 131.99 pg if a reduced and unreduced gamete of tetraploid *C. ornata* yield the hexaploid. The latter case is closest to the observed value, suggesting either that hexaploid *C. ornata* is of allopolyploid origin, or if an autopolyploid, it arose via the third possible mechanism outlined above; these hypotheses require further investigation.

Based on the Plant DNA C-values Database, http://data.kew.org/cvalues/ (Bennett and Leitch 2012), recorded species of Commelinaceae have a minimum 2C-value of 5.16 pg for *Commelina erecta* L. 1753 and a maximum of 86.7 pg for *Tradescantia virginiana* L. 1753. The DNA content of hexaploid *C. graminea* and hexaploid *C. ornata* are currently the highest within Commelinaceae and Commelinales (Leitch et al. 2010) with 122.86 pg and 129.73 pg, respectively. Jones and Kenton (1984) reported that the 2C-value of *C. rosea* is 77.3 pg, with a chromosome count of 2n = 24, consistent with tetraploidy reported by Anderson and Sax (1936) and Ichikawa and Sparrow (1967); however, as noted above, Giles (1942) only detected diploids (2n = 12) for *C. rosea*, consistent with our results. The closest 2C-value to 77.3 pg is the 2C-value of tetraploid *C. graminea* with 78.55 pg and 2n = 24 chromosomes; tetraploid *C. graminea* plants with broad leaves may be misidentified as *C. rosea* (Giles 1942). A voucher specimen of *C. rosea* from Jones and Kenton (1984) was not reported, so we cannot assess if the plant material used for the DNA content analysis was identified correctly. A misidentification is likely since the genome size estimation of Hertweck (2011) is close to our values. Likewise, previous tetraploid counts (Anderson and Sax 1936, Ichikawa and Sparrow 1967, Jones and Kenton 1984) may also be for tetraploid *C. graminea* plants that were misidentified as *C. rosea*. Alternatively, there may be cryptic tetraploidy in *C. rosea* that we failed to detect, but given our extensive sampling, we do not believe this to be the case.

**Distribution** – As shown in Figure 5, two isolated populations of diploid *C. graminea* were detected. One population is in Suffolk County, VA, and the other is in North and South Carolina. These two isolated populations may have been part of a once larger geographic range for diploid *C. graminea*, but due to heavy agricultural activities in this part of North Carolina, suitable habitats ranging from Johnston County to Northampton County were transformed to farmland (personal observation). This anthropogenic influence may have caused the separation of the two isolated groups of diploid *C. graminea*.

Tetraploid *C. graminea* ranges from the coastal plain of the Carolinas to central Florida, with additional populations in the Florida panhandle (Franklin County, FL). This cytotype is clearly more abundant than diploid *C. graminea*; it is usually found in xeric disturbed areas and exhibits a larger growth form than diploid *C. graminea*. These tetraploids were abundant in Bladen and southern Cumberland Counties, NC, which border the isolated locality of diploid *C. graminea* in North Carolina. These two areas (occupied by tetraploid and diploid plants, respectively) are separated by the city of Fayetteville, NC. Although diploid and tetraploid entities of *C. graminea* were reported to be geographically isolated (Bergamo 2003, Giles 1942, 1943, Kelly 1991), one tetraploid individual was found within a diploid population in Cheraw State Park,
SC; this individual is morphologically similar to the surrounding diploid *C. graminea*. This finding supports Giles’s (1942) hypothesis that tetraploid *C. graminea* is an autotetraploid because it occurs consistently with diploid *C. graminea*. This hypothesis requires testing with molecular data.

The Fall Line runs essentially east-west through Georgia and from southwest to northeast in the Carolinas. Diploid *C. rosea* occurs on both sides of the Fall Line from Georgia to North Carolina. In Fort Gordon (Richmond County, GA), diploid *C. rosea* occurs in sympathy with tetraploid *C. graminea*. Although these two species occur in sympathy, hybrids were not observed at the site.

Diploid *C. ornata* is endemic to Florida, and tetraploid individuals of *C. ornata* occur in western Florida. These individuals may be autoploidy, with diploid *C. ornata* as their progenitor. The distribution map in Figure 5 clearly supports the assumption of autoploidy, because there are no other *Callisia* species recorded in the region of diploid and tetraploid *C. ornata*. Morphologically, tetraploid *C. ornata* individuals show an increased axillary branching pattern, which is less common in diploid individuals. Axillary branching is a characteristic of *C. graminea*. Tetraploid *C. graminea* and diploid *C. ornata* are likely parents, through the union of one reduced gamete of tetraploid *C. graminea* and one unreduced gamete of diploid *C. ornata*.

In South Carolina, one hexaploid individual of *C. graminea* was found growing sympatrically with multiple tetraploid individuals of *C. graminea*. Hexaploid *C. graminea* in South Carolina appeared to be rare, and in 1942 only one individual was reported by Giles (1942). These rare hexaploid individuals may be allopolyploids, with diploid *C. rosea* and tetraploid *C. graminea* as their parents or autoploidy with tetraploid *C. graminea* as their progenitor. Regarding allopolyploidy, *C. rosea* was not found sympatrically with tetraploid *C. graminea* in South Carolina; however, from the map of georeferenced specimens (Figure 1), there is a significant overlap of distribution between tetraploid *C. graminea* and diploid *C. rosea* in the Carolinas. With regard to autoploidy, individuals may have resulted through the union of one reduced and one unreduced gamete of tetraploid *C. graminea* given that no other *Callisia* species were observed in the population.

In Lake and Hernando Counties, FL, hexaploid individuals exhibited intermediate morphological characteristics between *C. graminea* and *C. ornata*. Some populations had typical tetraploid *C. graminea* or diploid *C. ornata* characteristics (Figure 2). Two forms were distinguished based on habit: (1) hexaploid *C. graminea* and (2) hexaploid *C. ornata*. Hexaploid *C. graminea* and one of its possible progenitors, tetraploid *C. graminea*, grow in sympathy at the Seminole State Forest, and hexaploid *C. ornata* was found growing with tetraploid *C. graminea* at the entrance to Brantley Branch Rd. (Seminole State Forest). The co-occurrence of hexaploids and tetraploids suggests that the hexaploids may be of allopolyploid origin. Hexaploid *C. graminea* was also collected at Lake Griffin State Park, Edward Rd., Lady Lake, and Seminole State Forest, FL. In Dunns Creek State Park and Welaka State Forest, diploid *C. ornata* and
tetraploid *C. graminea* occur in sympathy; however, hexaploids were not found in these contact zones.

The rare hexaploid collected in South Carolina is most likely independently evolved from the hexaploids from Florida, and this entity from South Carolina could be either an allo- or autopolyploid. If allopolyploid, one likely parent, *C. rosea*, only occurs in Georgia and the Carolinas; if autopolyploid, the likely parent is tetraploid *C. graminea*. The hexaploid entities of Florida might be allopolyploid due to the intermediate morphological characters, with diploid *C. ornata* and tetraploid *C. graminea* as progenitors.

*Callisia graminea* forma *leucantha*, which was reported near Tampa, FL, was not found, but one white-flowered tetraploid individual of *C. graminea* was encountered among pink-flowered individuals in each of the following three locations: Sesquicentennial State Park, SC; Chesterfield Co., SC; and Tate’s Hell State Forest, FL. One white-flowered individual of diploid *C. rosea* was found in Heggie’s Rock Preserve, Appling, GA. White flowers reflect an absence of anthocyanins, which may result from mutations in any of the genes in the anthocyanin pathway or from lack of expression of potentially functional genes (Ho and Smith 2016, Rausher 2008). In *Callisia* section *Cuthbertia*, variation in flower color is common, but there is no association between color and ploidy within or among populations. Loss of anthocyanin pigments seems to occur sporadically within this complex.

Morphological and molecular analysis is an important next step in unraveling the complex relationships among cytotypes of *Callisia* section *Cuthbertia*. This work will allow us to reveal the parentage, evolutionary history, and the evolutionary role of all cytotypes within *Callisia* section *Cuthbertia*.

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Supplementary material I

Georeferenced data points
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