四、*Ludwigia pseudoalata* sp. nov. (Onagraceae Section *Microcarpium*), a New Species of the Southeastern United States

ABSTRACT. A new species, *Ludwigia pseudoalata* (Onagraceae), of the Section *Microcrapium* was described based on the collections from the Southeastern United States. Phylogenetically the new taxon is closely related to *L. alata*. The aquatic species is characterized by having nearly smooth or slightly ridged stems, and sepals that are often longer than capsules. Reciprocal monophyly of sisters *L. pseudoalata* and *L. alata* was recovered and significantly supported. Molecular dating suggested that the two species may have split some 1.08 MYA.

Key words: *Ludwigia pseudoalata*, new species, Onagraceae, systematics

1.Introduction

Ludwigia, a genus of the Onagraceae, consists of about 81 species in 23 diverse sections (Raven, 1963; Ramamoorthy and Zardini, 1987; Zardini and Raven, 1992), and is marked as monophyletic characterized by several synapomorphies, e.g., absence of a floral tube (Raven, 1979; Baum *et al.*, 1994). It was considered as one of the earliest surviving offspring within the Onagraceae (Peng 1988). *Ludwigia* is primarily a tropical and subtropical genus; and most North American species are restricted to the southeast Atlantic coastal plain and Gulf coastal plain (Peng, 1988). The genus is characterized by the absence of a floral tube, and has a chromosome number of x = 8 (Hoch, 1993). *Ludwigia* can be divided into 23 sections. Of them, the section *Microcarpium* is a polyploid complex of 14 species, and is characterized by regular fruit dehiscence (Zardini and Raven, 1992).

Compared to other genera, the distribution of *Ludwigia* is rather unusual in having a broad range in tropical and temperate wetlands of both North and South Hemispheres. North America is a diversity center for three of the sections (Raven, 1963), i.e., *Microcarpium* Munz (14 species), *Dantia* (DC.) Munz (5 species), and *Ludwigia* (4 species), which are

mainly distributed on the Coastal Plain of the southeastern North America (Peng, 1989). These three sections differ primarily in capsule morphology, habit, and phyllotaxy (Munz, 1944, 1965; Raven, 1963a; Peng, 1989). Of them, the section *Microcarpium* can be distinguished from other sections based on capsules regularly dehiscent by the separation of the disk . Biosystematic and taxonomic studies of section *Microcarpium* (Peng, 1988, 1989) have addressed the evolutionary relationships among its 14 species.

In the study, we described a new species that belongs to the section *Microcrapium* and is closely affined to *L. alata* Elliott. Morphologically, *Ludwigia alata* has been confused with *L. lanceolata* Elliott (Raven and Tai, 1979; Peng, 1989), owing to their similarity in being glabrous, apetalous, and most notably, in having winged, obpyramidal fruits, a unique character not occurring in other species of section *Microcarpium* (Peng, 1989). Of the 15 taxa in section *Microcarpium*, only *L. alata* and *L. lanceolata* have winged capsules, and they are glabrous (Peng, 1989). *L. pseudoalata* and *L. alata*, phylogeny and reciprocal monophyly were tested based on nuclear ribosomal ITS sequences.

2. Materials and methods

2.1. Species examined

Species of *Ludwigia* section *Microcarpium* were examined in this study (Table 1). This sampling scheme represents the taxonomy of *Ludwigia alata* complex. In total, four individuals of *L. pseudoalata*, four individuals of *L. alata*, and samples of six other species in section *Microcarpium*, *L. microcarpa* Michaux, *L. linearis* Walter, *L. polycarpa* Short and Peter, *L. curtisii* Chapman, *L. ravenii* Peng, *L. sphaerocarpa* Elliott and *L. lanceolata* Elliott, were included. Population samples of each species were collected from the field. Young leaves were collected and dried with silica gel. All samples were stored at -70℃ until they were processed. All vouchers were deposited in TAIE.

2.2 Phylogenetic analyses

Nucleotide sequences were aligned with the program CLUSTAL V (Higgins *et al.*, 1992)

and later adjusted visually. Phylogenetic trees of the nrITS sequences were reconstructed using maximum likelihood (ML). The general time reversible GTR + I + G model with 6 substitution categories was determined to be the most suitable model by Modeltest v3.6 (Posada and Crandall 1998) and was used for all subsequent nucleotide analyses. ML trees based on nucleotide sequences were inferred using PHYML v2.4.5 (Guindon and Gascuel 2003), and bootstrap consensus values were calculated with 500 replicates. Maximally parsimonious (MP) trees were also sought with PAUP heuristic search strategies (Swofford, 2002). The length of the shortest trees was obtained by initiating at least 500 searches, each using random addition starting trees, with tree bisection-reconnection (TBR) branch swapping. The equally MP trees were then used as starting trees for TBR branch swapping. In all analyses, the maximum number of trees to be saved was set at 5000. Bootstrap values (Felsenstein, 1985) were calculated from 500 replicate analyses using a heuristic search strategy, simple addition sequence of the taxa, and TBR branch swapping.

We estimated the time since the new species split from its sister based on the genetic diversity of nrITS. To estimate the divergence between lineages, a well-supported rate of evolution is required. In seed plants, evolutionary rates were estimated at 3.9×10^{-9} (Lee *et al.*, 1996) substitutions per site per year for nr-DNA ITS. Bayesian estimates of the mutation rates and the ages of the most recent common ancestor (TMRCA) of the *Ludwigia* sequences were obtained using BEAST v. 1.3; available from http://evolve.zoo.ox.ac.uk/beast/ (Drummond *et al.*, 2006). Posterior estimates of the mutation rate and age of the TMRCA were obtained by Markov Chain Monte Carlo analysis, with samples drawn every 500 steps over a total of 1,000,000 steps. Adequate sampling and convergence to the stationary distribution were checked using TRACER v. 1.3 (Rambaut and Drummond, 2004). Posterior estimates of parameters were all found to be distinctly unimodal (although with wide 95% highest posterior densities), and all parameters appeared to be identifiable, despite the relatively low information content in the sequences and the small age range of the sequences.

3. **Results and Discussion**

3-1. Taxonomic treatment

Keys of *Ludwigia alata* complex

A1: Sepals greenish, about 1/2 as long as the capsule---------- *L. lanceolata*

A2: Sepals creamy whitish, nearly as long as the capsule

B1: Stems often distinctly ridged or winged; sepals nearly as long as the capsule

---------- *L. alata*

B2: Stems nearly smooth or slightly ridged; sepals often longer than the capsule

---------- *L. pseudoalata*

Ludwigia pseudoalata T.-W. Hsu, C.-I Peng and T. Y. Chiang *sp. nov.* plates. 14-15.

U.S.A. Florid: Gulf County: CR30A, Elev. ca. 5 m, 85°17'59"W, 29°41'52" N, *Hsu11256* (holotype: MO; isotypes: TAIE), CR30E, St. Joseph peninsula, Elev. ca. 5 m, 85°19'29"W, 29°41'05" N, *Hsu11257* (TAIE).

Herba perennis recta stolonibus, ubique glabra. Folia anguste lanceolato-elliptica, 3.5-7 cm longa, 0.3-0.5 cm lata. Flores axillares.Sepala utrinque virides, lato deltoidea, 2-3 mm longa, 1.5-2.1 mm lata, apice acuminato. Bracteolae 3-4 mm longae. Petala 0. Antherae 0.8 mm longae; filamenta 0.8 mm longa. Discus nectarii viridis. Stylus 0.8 mm longus. Capsula ovoidea.

植株直立，高約 50-70 cm，莖基部具走莖，全株光滑，莖圓柱形，有稜。走莖葉單葉互生，基部具點狀托葉 2，黑色，葉柄長 0.5cm，葉倒卵形至倒卵狀橢圓形，葉長 0.5-1 cm，寬 0.4-0.6cm，先端圓鈍或銳尖；基部漸狹下延；葉全緣；葉脈側脈約 2-3 對；直立莖葉單葉互生，基部具點狀托葉 2，黑色，葉柄長 0-0.5cm，葉長 3.5-7 cm，寬 0.3-0.5cm，披針狀橢圓形，深綠色，先端銳，基部漸狹下延，葉中肋表面平、背凸，側脈表面不明

顯，下表面可見 4-8 對；花葉腋，單花、白色，近無梗；苞片 2，白色，生於子房基部，披針形，長約至花萼裂片先端，長 3-4 mm，寬約 1 mm；花萼 4 裂，裂片白色，三角形，長 2-3 mm，寬 1.5-2.1 mm，萼筒極淺；缺花瓣；雄蕊 4 枚，光滑，花絲長約 0.8mm，花藥 2 室，長約 0.8mm，基生，縱裂開口朝近軸面；雌蕊子房下位，上部具一圓盤狀花盤，具 4 稜，花柱長約 0.8mm，柱頭圓球形；花後、苞片與萼片皆宿存，近白色，花盤 4 稜，稍增大。種子橢圓球形，多數

Herbaceous, erect, ca. 50-70cm tall, the base of stem bears stolons, glabrous the whole plant, stem terete, ridged. Stolons leaves simple, alternate, bears 2 punctate stipules at base, black. Petiole 0.5cm long. Blades obovate to obovate-elliptic, 0.5-1cm long x 0.4-0.6cm wide. Rounded or acute at apex; attenuate and decurrent at base; margin entire. Lateral veins ca. 2-3 pairs. Stem leaves simple, alternate, bears 2 punctate stipules at base, black. Petiole 0-0.5cm long. Blades 3.5-7cm x 0.3-0.5cm wide, lanceolate-elliptic, deep green. Acute at apex, attenuate and decurrent at base. Midrib leveled adaxially, elevated abaxially; lateral veins obscure adaxially, distinctly 4-8 pairs abaxially. Inflorescences axillary, solitary, white, subsessile. Bracts 2, white, lanceolate, borne on the base of ovary, ca. levels with the apex of calyx lobe, 3-4mm long x ca. 1mm wide. Calyx lobes 4, white, deltoid, 2-3mm long x 1.5-2.1mm wide; calyx tube shallow. Petals lacking. Stamens 4, glabrous; filaments ca. 0.8mm long; anthers 2, ca. 0.8mm long, basifixed, longitudinal dehiscing toward adaxial side. Ovary epigynous, upper part bears a circular disk, 4 ridged. Style ca. 0.8mm long; stigma capitate. Bracts and calyx lobes persistent after anthesis, subwhite; disk 4-ridged, slightly enlarged. Seeds ellipsoid, many.

Taxonomic Notes:

All species of *Ludwigia* section *Microcarpium* grow at wetlands, including habitats of alluvial ground or in shallow water of ponds, lakes, rivers, streams, lagoons, sloughs, backwaters, swales, wet meadows or prairies, open swamp forests, drainages, and irrigation ditches (Peng, 1989).

L. alata 生長於矮林下、高草地內或林緣水溝，屬稍遮陰環境；走莖有稜或稍具 wing，植株稍肉質，直立莖具明顯 wing，苞片稍長於子房，果實為圓錐體形。

L. pseudoalata 生長於開闊潮濕草地，強光照射下環境；走莖無 wing，植株不具肉質，直立嫩莖具不明顯 wing，老莖無 wing，苞片遠長於子房，可長至萼片頂端，果實為圓

錐體形。

L. lanceolata 之生長環境與 *L. pseudoalata* 接近，未開花時外型與 *L. pseudoalata* 非常接近，但果實方形

3-2. Phylogenetic analyses and molecular dating

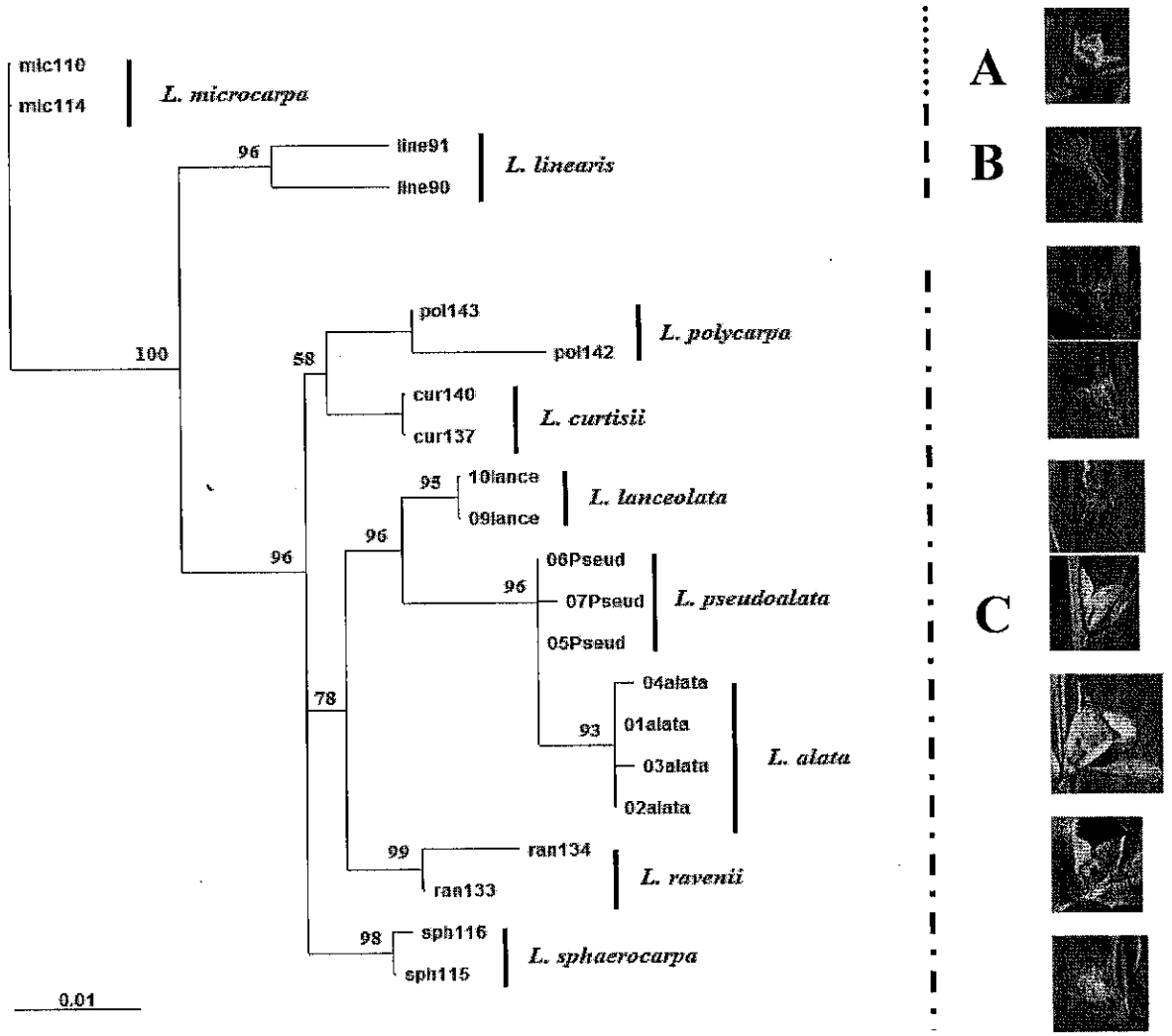
We obtained nucleotide sequences of nrDNA ITS from samples of *L. alata*, and *L. pseudoalata*, and six other species of section *Microcarpium*, *L. microcarpa*, *L. linearis*, *L. polycarpa*, *L. curtisii*, *L. ravenii*, *L. sphaerocarpa* and *L. lanceolata*. The sequence length of ITS region of nrDNA varied from 659 to 667 bp. In total, 669 bp of the consensus length were aligned among sequences. In total, 17 sites were variable. All of them were phylogenetically informative.

The phylogeny of the nrITS region was reconstructed based on a maximum-likelihood (ML) method. Three clades, A-C, were identified based on the ITS region. *L. microcarpa* and *L. linearis* separately shaped the clade A and B, and other species constructed the clade C. *L. alata* has been confused with *L. lanceolata*, owing to their similarity of morphological characters. The molecular evident also showed they were closely related. The reciprocal monophyly of *L. alata* and *L. pseudoalata* was also recovered and supported with high bootstrap values at each node (Fig. 4). The phylogeny of the ITS region was also reconstructed by the maximum-likelihood (MP) analysis. The MP analyses recovered phylogenetic trees with consistent topologies of ML phylogeny. Morphologically, Smooth or slightly ridged Stems and sepals that are often longer than the capsule in *L. pseudoalata* could be distinguish from *L. alata.* Both molecular and morphological evidence indicated distinctness between the two species (Fig. 1), both of which can be distinguished from other species by sepals creamy whitish, and nearly as long as the capsule.

Bayesian estimates of the mutation rates and the age of the most recent common ancestor (TMRCA) of the nrITS sequences were calculated using BEAST v. 1.3. Accordingly, *L. pseudoalata* and *L. alata* split some 1.08 MYA, while both species and *L. lanceoalata*

could be traced back to a common ancestor about 2.47 MYA. Besides, populations of *L. pseudoalata* coalesced about 0.43 MYA.

In the study, both morphological and molecular evidence suggests that *L. pseudoalata* and *L. alata* are the most closely related. Speciation of this newly described species may have occurred for more than one million years.



圖六、以Maximum-likelihood方法所構築的水丁香屬小果組ITS親緣關係圖。

Table 4. Samples of *Ludwigia* taxa used for molecular analyses

No.	Species	Locality	Voucher
01	*L. alata*	USA, Florida, Citrus Co., CR490A	Hsu 11281
02	*L. alata*	USA, Florida, Hernando Co., CR 550	Hsu 11283

03	*L. alata*	USA, Florida, Putnum Co., CR309, Welaka State Forest	Hsu 11350
04	*L. alata*	USA, Florida, Volusia Co., CR415	Hsu 11346
05	*L. pseudoalata*	USA, Florida, Gulf Co., CR30A	Hsu 11256
06	*L. pseudoalata*	USA, Florida, Gulf Co., CR30A	Hsu 11256
07	*L. pseudoalata*	USA, Florida, Gulf Co., CR30E, St. Joseph peninsula	Hsu 11257
08	*L. pseudoalata*	USA, Florida, Gulf Co., CR30E, St. Joseph peninsula	Hsu 11257
09	*L. lanceolata*	USA, Florida, Clay Co.	Peng 19376
10	*L. lanceolata*	USA, Florida, Walton Co., CR20	Hsu 11230
90	*L. linearis*	USA, Alabama, highway 55 and CR24	Hsu 11217
91	*L. linearis*	USA, Florida, Walton Co., CR20	Hsu 11228
110	*L. microcarpa*	USA, Florida, Citrus Co., CR490A	Hsu 11278
114	*L. microcarpa*	USA, Florida, Sarasota Co., CR 72	Hsu 11294
115	*L. sphaerocarpa*	USA, Florida, Levy Co., CR 24	Hsu 11277
116	*L. sphaerocarpa*	USA, Florida, Lafaytte Co., CR51	Hsu 11270
133	*L. ranvenii*	USA, Virginia, Western Rd., Grassfield	Hsu11379
134	*L. ranvenii*	USA, Virginia, Western Rd., Grassfield	Hsu11379
137	*L. curtissii*	USA, Florida, Okechobee Co., CR68	Hsu 11333
140	*L. curtissii*	USA, Florida,Gladeo Co., CR74	Hsu 11316
142	*L. polycarpa*	USA, Missouri, Frankin Co.	Hsu 11373
143	*L. polycarpa*	USA, Indiana, Jackson Co.	Hsu 11385

五、 物種野外現況

水丁香屬小果組所也種類皆生育於低海拔溼地，因棲息地的干擾、人類開發的破壞與外來種的入侵對許多原生的水丁香屬植物族群受到極大的生存威脅，例如 *Ludwigia ravenii* 當初發表時的分佈地(Peng, 1984)，在 2003 年的野外採樣時皆未再發現，最後也僅出現在一處砍伐溼地，而也僅有少數植株；*L. alata* 與 *L. lanceolata* 族群變異極大，以前紀錄的採集點大多數皆已消失，僅存的採集點族群數量也極為稀少；*L. polycarpa* 於密蘇里州的

族群幾近絕滅，北部的新英格蘭已也歸類為瀕絕物種；其他受威脅名錄詳如表四。

表五、水丁香屬小果組目前野外現況

物種	野外現況	物種	野外現況
L. alata	受威脅	*L. pilosa*	
L. curtissii (*L. simpsonii*)		*L. polycarpa*	受威脅
L. glandulosa		*L. pseudoalata*	受威脅
L. glandulosa ssp. *brachycarpa*	受威脅	*L. ravenii*	瀕絕
L. lanceolata	受威脅	*L. sphaerocarpa*	
L. linearis		*L. stricta*	瀕絕*
L. linifolia	受威脅	*L. suffruticosa*	
L. microcarpa			

* Leadlay E. A. and P. S. Wyse 1989.

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