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**COLIMA (TIGRIDIEAE: IRIDACEAE), A NEW GENUS FROM WESTERN MEXICO
AND A NEW SPECIES: *COLIMA TUITENSIS* FROM JALISCO**

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ABSTRACT

Based on cytological, palynological, and DNA evidence, we propose the elevation of *Nemastylis* subgenus *Colima* to the generic status to include *Nemastylis convoluta*. The new genus is characterized by its united filaments, free anthers with longitudinal dehiscence, style divided into three branches with six arms at right angles, apical stigmatic surface, absence of nectaries, lack of mucro between the style arms and bisulcate pollen grains. A preliminary study based on morphological variation and DNA sequences variation suggests a close phylogenetic relationship between *Colima* and the genera included within the subtribe Tigridiinae. However the relationships of *Colima* at the generic level are not fully resolved by the molecular analysis. Finally, we describe and illustrate a new species of *Colima* from El Tuito, Jalisco. This new species is characterized by its winged stems, pedicels, ovaries and fruits. In addition, the tepals are creamy yellow, oblanceolate with acuminate apex and a smaller androgynocael apparatus than that of *Colima convoluta*.

Keywords: *Colima*, Iridaceae, *Nemastylis*, new genus, new species, Mexico.

RESUMEN

Con base en información citológica, palinológica y secuencias de ADN se propone la elevación de *Nemastylis* subgénero *Colima* al nivel de género para incluir en éste a *Nemastylis convoluta*. Este nuevo género se caracteriza por presentar filamentos unidos, anteras libres con dehiscencia longitudinal, estilo dividido en tres brazos con seis ramas dispuestas en ángulo recto, superficie estigmática apical, falta de nectarios, ausencia de mucrón entre las ramas del estilo y polen bisulcado. Un estudio preliminar basado en la variación de caracteres morfológicos y de secuencias de ADN, sugiere una cercana relación filogenética entre *Colima* y los géneros que integran la subtribu Tigridiinae. Sin embargo, las relaciones de *Colima* a nivel genérico no están completamente resueltas por el análisis molecular. Por último, se describe e ilustra una nueva especie del género *Colima* de El Tuito, Jalisco. En esta última los tallos, pedicelos, ovarios y frutos son alados. Además, los tépalos son de color amarillo-crema, oblanceolados con ápices acuminados y el androgineceo es más pequeño que en *Colima convoluta*.

Palabras clave: *Colima*, Iridaceae, México, *Nemastylis*, nuevo género, nueva especie.

INTRODUCTION

The tribe Tigridieae (Iridaceae) is strictly a New World group with centers of diversity in Mexico and temperate and Andean South America. Tigridieae comprises bulb forming plants with plicate leaves and a basic chromosome number of $n = 7$. Typical characteristics of this tribe are the elaborate structure of the anthers and distinctive style arms, which contrast with its vegetative uniformity. Based on differences of the style arms and stamens, more than 40 genera have been described, many of them monotypic (Rodríguez, 1999). The tribe is taxonomically difficult and phylogenetically poorly understood. The generic boundaries, species affiliations and phylogenetic relationships vary from one author to another (Goldblatt & Henrich, 1991; Goldblatt & Howard, 1992; Espejo & Lopez-Ferrari 1996a, 1996b; Ravenna, 1968, 1977). Thus, cladistic analysis of both morphology and molecules are needed to establish a reliable phylogeny.

Goldblatt (1982) proposed a division of Tigridiae into subtribes Cipurinae and Tigridiinae. Cipurinae possesses monosulcate pollen grains and a gametophytic number of $n = 7$. Conversely, bisulcate pollen grains and a gametophytic number of $n = 14$ characterize the subtribe Tigridiinae. However, some exceptions have been detected in cytological and palynological studies (Goldblatt & Howard, 1992; Goldblatt & Le Thomas, 1992; Kenton & Heywood, 1984; Rodríguez, 1999; Rudall & Wheeler, 1988). Moreover, the pollen morphology and the chromosome number of some species remain unknown. Thus the placement of some species within either subtribe is difficult. This highlights the necessity of a taxonomic re-evaluation, reflecting as far as possible, natural relationships within Tigridieae.

Nemastylis is a genus of five species grouped in the subtribe Cipurinae, and according to Foster (1945), is an "agglomeration of discordant elements". The only shared characteristic of its members is the androgynoeical apparatus, in which the primary style-branches oppose the anthers and outer segments of the perianth. Furthermore, the style-branches divide into two filiform arms, which straddle the opposing anthers and project beyond them. Thus, between adjoining anthers there are two closely parallel projecting style-arms originating from different style-branches.

The geographic distribution of *Nemastylis* is restricted to the United States, Mexico and Guatemala. Four species grow in the United States: *Nemastylis floridana* Small, *N. geminiflora* Nutt., *N. nutallii* Pickering and *N. tenuis* (Herb.) Baker. *Nemastylis tenuis* is widely distributed in Mexico and extends south to Guatemala. Finally, *N. convoluta* Ravenna is endemic to the state of Colima, México (Ravenna, 1968; Molseed & Cruden, 1968; McVaugh, 1989; Espejo-Serna & López-Ferrari, 1996a, b).

MOLECULAR, CYTOLOGICAL AND PALYNOLOGICAL INCONSISTENCIES

A cladistic analysis of morphological data, sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and three intergenic spacers of cpDNA, plus the combination of all these data, demonstrated the monophyly of Tigridieae (Rodríguez, 1999). The same study also showed *Nemastylis* to be polyphyletic, with *N. convoluta* nested within Tigridiinae and *N. tenuis* within Cipurinae.

The results of Rodríguez (1999) led us to search for more evidence to test the affiliation of *N. convoluta* to Tigridiinae. Thus, a palynological and cytological study was carried out on botanical material collected at the type locality. We found *N. convoluta* to have a sporophytic chromosome number of $2n = 28$ and bisulcate pollen grains (Rodríguez et al., 2001). In contrast, *N. tenuis* has a sporophytic chromosome number of $2n = 14$ (Molseed, 1970; Goldblatt, 1982) and monosulcate pollen grains (Rodríguez et al., 2001). Likewise, *N. geminiflora* possesses monosulcate pollen grains (Rudall & Wheeler, 1988) and a chromosome number of $2n = 56$, a shared characteristic with *N. floridana* (Goldblatt, 1982); these species could be octoploids (Goldblatt, pers. com.). The pollen grains of *N. floridana* and *N. nuttallii* are monosulcate as well (A. Rodríguez & Ortiz-Catedral, unpubl. data) however the chromosome number for *N. nuttallii* remains unknown. Unfortunately, none of these three species were included in the study of Rodríguez (1999). Further studies of these members are necessary to better understand this genus.

TAXONOMIC TREATMENT

The available evidence supports a phylogenetic relationship between *Nemastylis convoluta* and the subtribe Tigridiinae, instead of subtribe Cipurinae. However, the floral structure of *N. convoluta* does not correspond strictly to any of the genera recognized in Tigridiinae (Table 1). Even the molecular data do not resolve the sister group relationship of this taxon. Therefore, we propose the elevation of subgenus *Colima* to generic status within Tigridiinae.

Colima (Ravenna) Aaron Rodr. & L. Ortiz-Catedral, stat. nov.

Nemastylis subgen. *Colima* Ravenna, Bonplandia 2: 282. 1968.

Plants robust, glabrous, up to 43 cm high; flowering stem arising from a short-necked bulb; 1-2 branched, secondary branch if present arising from the base of cauline leaf, bearing an inflorescence at the top; bulb scales deep brown purple; cataphylls often persistent and conspicuous, papery ivory colored; stem winged or not; basal leaves 1-2, glabrous, linear-lanceolate, attenuate, plicate; second leaf, if present, about as long as the first one or smaller; cauline leaf solitary; both basal and cauline leaves surpassing the floral stem; inflorescence a single and

Table 1. Morphological comparison between *Nemastylis convoluta* and Tigridiinae genera.

	<i>Nemastylis convoluta</i>	<i>Alophia</i>	<i>Cobana</i>	<i>Fosteria</i>	<i>Sessilanthera</i>	<i>Tigridia</i>
Filaments	united	united	free	united	united	united or partially free
Anthers	free	coherent at base	free	free	free	free
Anther dehiscence	longitudinal	longitudinal	apical	apical	apical	longitudinal
Style	three branches bifid with style arms at right angles	three branches erect, bifid with ascending recurved style arms	three branches without subdivisions	six arms erect close together nearly parallel	three branches bifid with recurved style arms	three branches bifid with recurved style arms
Stigmatic surface	apical	apical	apical	all surface of the arms	apical	apical
Nectaries	absent	present	absent	present	absent	present
Mucro	absent	absent	absent	absent	present	present or absent
Pollen grains	bisulcate	monosulcate	bisulcate	bisulcate	bisulcate	bisulcate

terminal rhipidium, enclosed by spathes; rhipidia 1-2, secondary rhipidium if present at the top of a branch arising from the base of cauline leaf; flowers 2 to 7; pedicels about as long as the spathe, longer in fruit, erect, winged or not; ovary narrowly turbinate or clavate or triangular in front view, winged or not; perianth segments unequal, bright yellow to creamy yellow, outer tepals spreading to recurved, oblanceolate, acute or acuminate; inner tepals spreading, lanceolate or oblanceolate, acute or acuminate; nectaries absent; filaments united into a staminal column; anthers yellow; pollen grains bisulcate, ellipsoid; exine reticulate; style branches purplish to lavender, or whitish distally, cleft nearly to the base, without a mucro; fruit ellipsoid-clavate or triangular, winged or wingless; seed ovoid, chestnut colored; chromosome number $2n = 28$.

Colima convoluta (Ravenna) Aaron Rodr. & L. Ortiz-Catedral, comb. nov. (Fig. 1A, Fig. 1B, Fig. 1C)

Nemastylis convoluta Ravenna, Bonplandia 2: 282. Fig. 2A. Feb 1968. Type: Mexico. Colima: mountains with deciduous forest, 18 km on road from Colima to Manzanillo, *P. Ravenna* 395 (Holotype: in Ravenna's personal herbarium - n.v.).

Nemastylis mcvaughii Molseed & Cruden, Brittonia 20: 235. Fig 1. 1968. Type: Mexico. Colima: route 110, ca 17.5 km SSW of Cd. Colima, near km 238, disturbed and non-disturbed areas in tropical deciduous woodland, ca. 500 m, 9 Jul 1966, *E. W. Molseed* 449 (Holotype: UC M200434- n.v.; Isotypes: ICF- n.v., GH- n.v., MEXU, K- n.v., US- n.v.).

Nemastylis molseediana T. M. Howard, Plant Life 26: 16, nom. illegit. Pl. 2, facing p. 296; fig. 50. 1970.

Additional specimens examined: MEXICO. Colima: Mt. summit near pass, ca. 11 mi SSW of Cd. Colima on Manzanillo road, 500 m, 19 Jul 1957, *R. McVaugh* 15554 (MEXU, MICH, NY); municipio de Tecomán, 20 km NW de Colima sobre la carretera 54 D, 19°03' N, 104°11' W, 500 m, 14 Jul. 2001, *A. Rodríguez & L. Ortiz-Catedral* 2960 (ENCB, IBUG, IEB, MEXU).

Descriptions of *Nemastylis convoluta* and *N. mcvaughii* (here considered synonymous) are detailed regarding the vegetative and floral morphology, but do not include information about pollen grains and chromosome number. As mentioned above, this species has a sponophytic chromosome number of $2n = 28$. The pollen grains are $65 \times 42 \mu\text{m}$ in equatorial view.

Distribution. *Colima* is endemic to western Mexico in the states of Colima and Jalisco. The only known population of *C. convoluta* is at the type locality. It grows on calcareous hillsides SSW of the city of Colima in open and disturbed areas of tropical deciduous forest, dominated by *Neobuxbaumia mezcalensis* (Bravo) Backeberg, *Bursera* Jacq. ex L., *Cnidocolus* Pohl. and various Leguminosae. *Hechtia laevis* L. B. Smith is a notable ground cover. *Hymenocallis* Salisb. and *Bessera* Schult. f. are common in the area. *Colima convoluta* is abundant in this locality, growing in dense groups or scattered in the middle of spiny clumps of *Hechtia laevis* bearing leaves and flowers in sunny or semi-shaded spots. However open mining activities nearby could modify this habitat and *Colima convoluta* together with the associated species could be lost.

Rodríguez et al. (1996) reported *Nemastylis convoluta* from the vicinity of El Tuito, Jalisco. However, detailed analysis of the material collected in 1996, fresh material from the same locality collected in July 2001, and a comparison with fresh material from the type locality of *C. convoluta* led us to conclude that this is an undescribed species. We propose its treatment as follows.

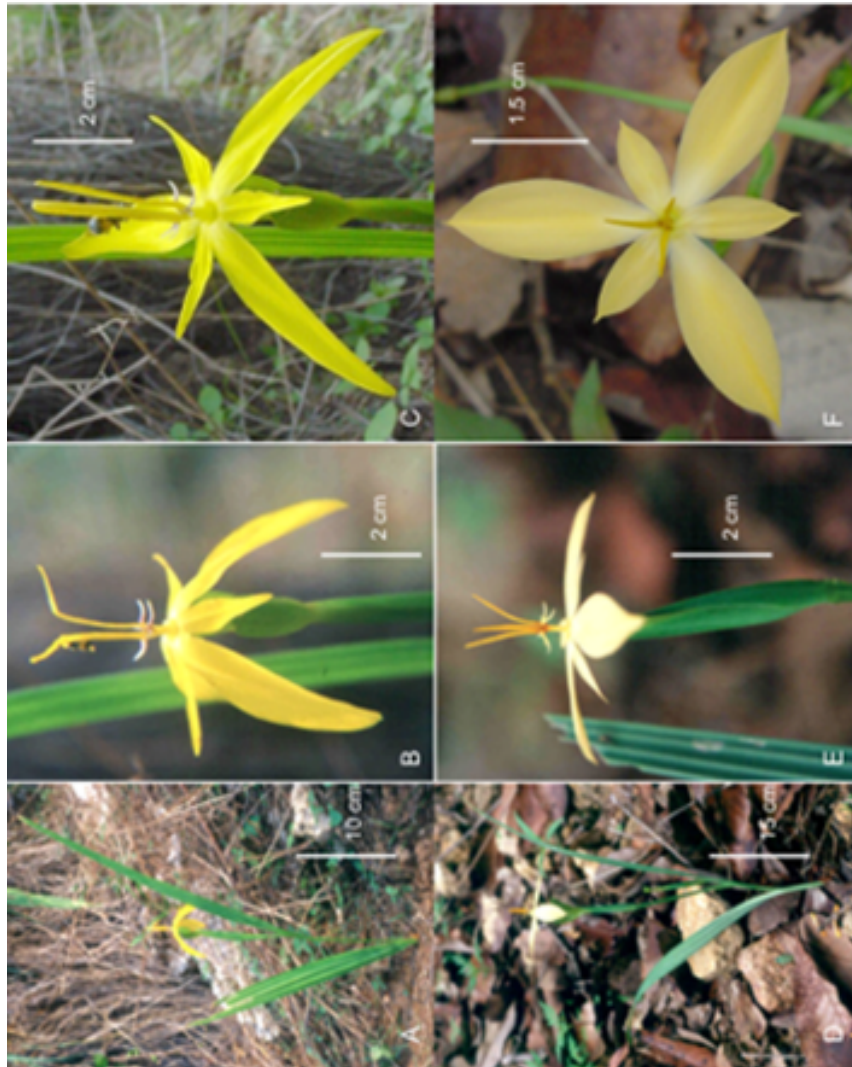


Fig. 1. A-C *Colima convoluta*. A. habit; B. flower, lateral view; C. flower, frontal view; D-F *Colima tuitensis*. D. habit; E. flower, lateral view; F. flower, frontal view. Photos by A. Rodríguez.

Colima tuitensis Aaron Rodr. & L. Ortiz-Catedral, sp. nov. (Fig. 1D, Fig.1E, Fig.1 F; Fig. 2)

Type: Mexico: Jalisco: municipio de Cabo Corrientes, km 10 sobre la brecha El Tuito-Chacala, 22°02'23" N, 105°23'24" W, 500 m, 30 Jul. 1995, *R. Ramírez-Delgado* & *J. A. Pérez de la Rosa* 3390 (Holotype: IBUG; Isotypes: IEB, ENCB, MEXU, UAMIZ).

Caulis alatus usque 43 cm altus, in rhipidium 4.5 cm longum terminatus; ramus secundarius praesens ex folio caulino exortus, in rhipidium, 3.8 cm longum terminatus; flores 4 vel 8, dilute flavi; pedicelli filiformes alati, in fructu dilatati; ovarium triquetrum, alatum, 0.4 cm longum per anthesin; tepala dimorpha, dilute flava, exteriora oblanceolata, acuminata, 2-3.4 cm longa, 1 cm lata; tepala interiora oblanceolata, acuminata, 1.6 cm longa, 0.5 cm lata; filamenta connata, 0.5-0.6 cm longa; antherae luteae, 1.2-1.7 cm longae; pollinis granula bisulcata, 90 x 44 µm ab aequatore visa, elliptica; stylus et fila aequilongi; styli rami ad basim flavi, apicibus albis 0.4-0.5 cm longi; capsula triquetra, 1.1-1.5 cm longa; semina ovoidea, castanea, 0.2 cm longa; chromosomatum numerus $2n = 28$; Julii florescet.

Plant glabrous, up to 43 cm high; bulb wide ovoid to subglobose, 4 cm long, 1.5-2 cm wide, bulb coats deep brown purple; cataphyll 3-10 cm long, 0.6-0.8 cm wide, papery ivory colored; basal leaves 1-2, glabrous, plicate, linear-lanceolate; the first basal leaf 23-40 cm long, 0.6-1 cm wide; secondary leaf if present, about as long as the first or shorter, narrowly linear; cauline leaf solitary, 15-33 cm long, 0.5-1.1 cm wide; both, basal and cauline leaves surpassing the flowering stem; stem winged, up to 43 cm long, with a rhipidium at the top, enclosed by spathes, 4.5 cm long; secondary rhipidium present, at the top of the secondary branch, 3.8 cm long; flowers 4 to 8, creamy yellow; pedicels filiform, winged, longer in fruit; ovary triangular in cross-view, winged, 0.4 cm long at anthesis; tepals unequal, outer tepals oblanceolate, acuminate, 2-3.4 cm long, 1 cm wide; inner tepals oblanceolate, acuminate, 1.6 cm long, 0.5 cm wide; filament column 0.5-0.6 cm long; anthers yellow 1.2-1.7 cm long; pollen grains 90 x 44 µm in equatorial view; style and filaments equal in length; style branches yellow at base, with white apex, 0.4-0.5 cm long; fruit a triangular capsule, 1.1-1.5 cm long; seed ovoid, chestnut, 0.2 cm long; chromosome number $2n = 28$. Blooming in July.

Additional specimens examined: Mexico. Jalisco: municipio de Cabo Corrientes 15.5 km al W de El Tuito sobre la brecha El Tuito-Chacala, 3 km N de Zicatán, 20°19'32" N, 105°26'18" W, 445 m, 26 Jul. 1999, *R. Ramírez* & *A. Rodríguez* 5917a (IBUG); 15 Jul. 2001, *A. Rodríguez* & *L. Ortiz-Catedral* 2961 (IBUG, MEXU).

Colima tuitensis is easily distinguished from *C. convoluta* in having winged stems, pedicels, ovaries and fruits. The tepals are creamy yellow, oblanceolate with

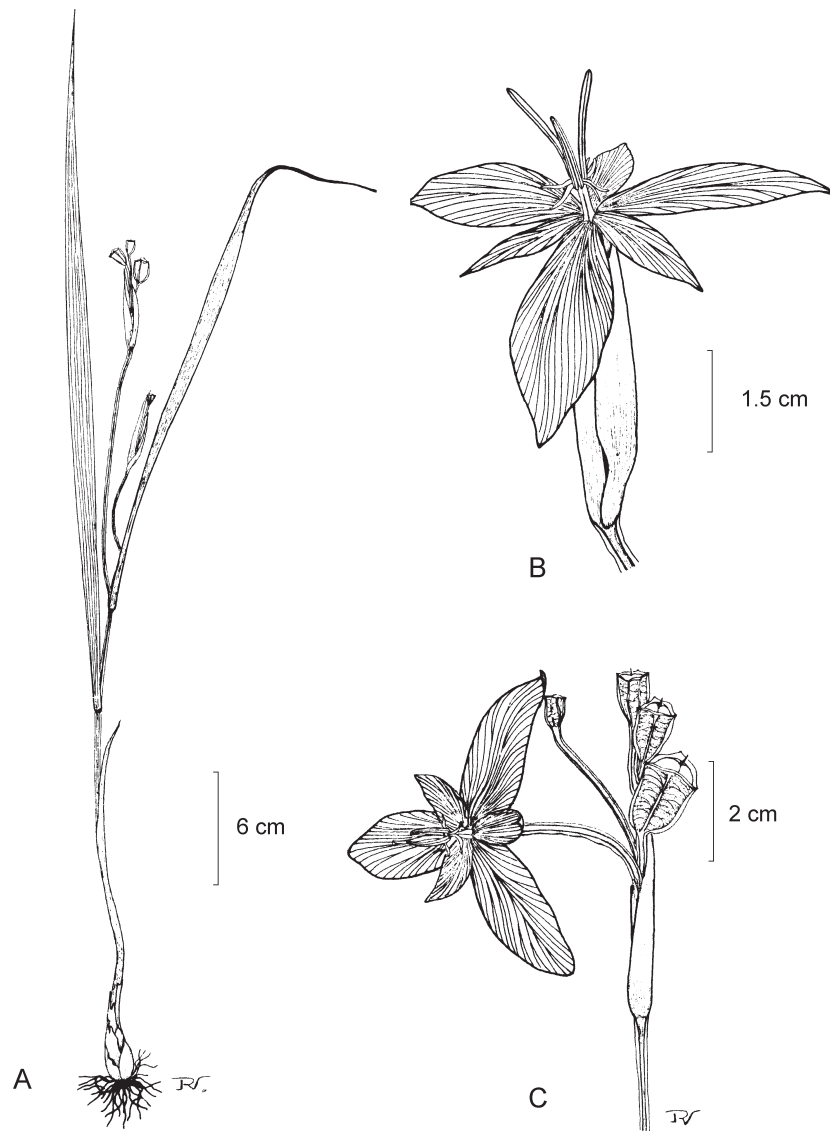


Fig. 2. *Colima tuitensis*. A. habit; A. Rodríguez & L. Ortiz-Catedral 2961 (IBUG, MEXU); B. flower, frontal view; R. Ramírez & A. Rodríguez 5917a (IBUG); C. flower and fruits; R. Ramírez & A. Rodríguez 5917a (IBUG). Illustration by Refugio Vazquez.

acuminate apex and a smaller androgynoecial apparatus than that of *C. convoluta*. In the latter species the stems, pedicels, ovary and fruits are wingless, the tepals are bright yellow and lanceolate with acute apex. Finally, both species differ in pollen size. In equatorial view, *C. convoluta* has pollen grains of 65 x 42 μm , whereas the pollen grains of *C. tuitensis* are 90 x 44 μm . Table 2 summarizes the distinguishing characteristics of both species.

Table 2. Comparison of distinguishing characters of *Colima convoluta* and *C. tuitensis*.

	<i>Colima convoluta</i>	<i>Colima tuitensis</i>
Color of tepals	bright yellow	creamy yellow
Stem and pedicels	wingless	winged
Ovary and fruits	ellipsoid-clavate, wingless	triangular, winged
Outer floral bract length	6 cm	4.3 cm
Inner floral bract length	5.5 cm	3.7-4.1 cm
Anthers length	2.2-2.6 cm	1.2-1.7 cm
Outer tepals length	3.6-4.1 cm	2-3.4 cm
Inner tepals length	1.8 cm	1.6 cm
Pollen grain size (equatorial view)	65 x 42 μm	90 x 44 μm

Distribution. *Colima tuitensis* is known only from the type locality in the tropical oak forest near the towns of El Tuito and Zicatán. It grows on shady cliffs close to the road forming small dense groups or scattered in the forest. The forest is dominated by *Quercus elliptica* Née and *Q. magnoliifolia* Née. *Agave schidigera* Lem. forms a closed groundcover near the road. Although the population is numerous, the construction of new roads and soil erosion during the rainy season, could extirpate this species.

Etymology. The name refers to El Tuito, a town close to the type locality.

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