

## *Tamia* (Iridaceae), a Synonym of *Calydorea*: Cytological and Morphological Evidence

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**Abstract**—The monotypic genus *Tamia* was described by Ravenna in 2001 based on specimens of *Calydorea pallens* from Bolivia and West-Central Argentina (excluding Córdoba and San Luis). At the same time, Ravenna described *Calydorea undulata* as a new species to accommodate the excluded specimens. He used floral morphology to segregate *Tamia* from *Calydorea*. He described *Calydorea* as having the anthers twisted/circinate after dehiscence, whereas in *Tamia*, the anthers are straight. Similarly, in *Tamia* the upper third of the anther is adnate to the style arms while in *Calydorea* the anthers are free from the style branches. We here evaluate the validity of *Tamia* and *C. undulata* based on morphological and cytological approaches. An examination of living plants of both taxa showed the anthers to be straight during dehiscence and twisted when the pollen was exposed, likewise the stamens were completely free from style branches in both taxa. Both have a base chromosome number of  $x = 7$  (*C. undulata* diploid,  $2n = 14$ ; *T. pallens* tetraploid,  $2n = 28$ ). The karyotype formula for *C. undulata* was  $5m + 2sm + 7m + 7sm$  for *T. pallens*. The karyotype is bimodal in *C. undulata* and moderately asymmetrical in *T. pallens*. These chromosomal differences and differences in petal shape (the outer are flat for both taxa while the inner are geniculate in *T. pallens* but flat with undulate margins in *C. undulata*) and flower color (pale lilac with dark violet dots in *T. pallens* and violet-blue with violet stripes in *C. undulata*) suggest that these taxa are distinct species of *Calydorea*, where a polyploid series based on  $x = 7$  is known. The divergence of their karyotypes is within the observed chromosomal variability of genera in Iridaceae. Thus, we conclude that *Tamia* should be regarded as a synonym of *Calydorea*, with the return of its species to *Calydorea pallens*, because the floral differences between them are not enough to merit generic segregation. *Calydorea undulata* is nonetheless a valid species.

**Keywords**—floral morphology, Iridoideae, karyotypes, somatic chromosomes, taxonomy, *Tigridieae*.

Iridaceae (with ca. 65 genera and 1850 species) are a large cosmopolitan family of perennial evergreen or deciduous herbs, rarely annuals (Goldblatt et al. 1998; Rudall et al. 2003). It includes several important horticultural genera such as *Crocus* L., *Freesia* Klatt, *Gladiolus* L., and *Iris* L. Although they are distributed worldwide, the family has a marked concentration in the southern hemisphere (Goldblatt 1990).

There is good evidence that Iridaceae is monophyletic and defined by several synapomorphies including the presence of isobilateral unifacial leaves, styloid crystals, and flowers with only three stamens (Goldblatt et al. 1984, 1998; Goldblatt 2001; Rudall et al. 2003). There are four major lineages recognized as subfamilies: Isophysidoideae, Nivenioideae, Iridoideae, and Ixioidae. Isophysidoideae is monotypic and is considered to occupy a basal or near basal position in the family because of its superior ovary (Goldblatt 1979, 1990; Chase et al. 1995). The remaining subfamilies have inferior ovaries (Cronquist 1988; Goldblatt 1990; Chase et al. 1995; Goldblatt and Takei 1997).

Members of Iridoideae, the most widespread and diverse subfamily, fall into several large groups that have been circumscribed at the tribal level (Goldblatt 1990). The largely New World Sisyrinchieae (one species occurs on central Pacific islands), together with two strictly New World tribes, Trimezieae and Tigridieae, and the predominantly Old World Irideae (with some species of *Iris* in N. America) are a monophyletic lineage defined by molecular and morphological synapomorphies (Goldblatt 1990; Goldblatt and Rudall 1992; Goldblatt et al. 1998; Reeves et al. 2001). The tribe Tigridieae has centers of diversity in temperate and Andean South America and Mexico, and is further subdivided into the subtribes Tigridiinae and Cipurinae (Goldblatt 1990).

*Calydorea* Herb. is a genus of ca. 25 species among the bulbous and plicate-leaved members of subtribe Cipurinae. Members of this genus have subequal tepals, free filaments,

and short slender style branches (Goldblatt and Henrich 1991; Goldblatt et al. 1998; Roitman and Castillo 2005). Among the Argentinean species, *C. pallens* Griseb., a species of small stature with fugacious but attractive lilac flowers, has been the subject of some taxonomic controversy. Based on this species, Ravenna (2001) published the monotypic genus *Tamia* with two diagnostic features, anthers that are straight after dehiscence and stamens where the upper third of the anther was adnate to the style branches. Included in *Tamia* were specimens from Bolivia and West-Central Argentina (provinces of Jujuy, Salta, Tucumán, La Rioja, Santiago del Estero, and Mendoza). Specimens from Córdoba and San Luis provinces that were previously reported as *C. pallens* were excluded. To accommodate them, Ravenna described a new species, *Calydorea undulata* (Ravenna 2001). According to Ravenna, these two taxa have different geographic distributions in adjacent provinces. They were described as both occupying the same type of habitat (sandy places) at overlapping elevations (between 800–2800 m a.s.l. for *T. pallens* and between 800–1000 m a.s.l. for *C. undulata*). We here evaluate their taxonomic status on both morphological and cytological grounds to test their validity.

### MATERIALS AND METHODS

Live populations were studied in Argentina, Córdoba Province, Punilla Department, Capilla del Monte, near El Zapato, 15 February 2004 (*C. undulata*: BAA 25455, *T. pallens*: BAA 25456), where both taxa cooccurred. The voucher specimens were deposited in the herbarium Gaspar Xuáres of the University of Buenos Aires (BAA). In addition to live material, nine other specimens including the isotype of *C. pallens* at Museo Botánico de Córdoba Herbarium (CORD) and specimens that Ravenna would have assigned to *T. pallens* from La Rioja, Mendoza and Salta provinces were also examined (Appendix 1; herbarium acronyms follow *Index Herbariorum*).

At least 20 fresh flowers per species were observed to describe the morphology in plants under culture (J. O. Hall Garden facilities, Facultad de Agronomía, Universidad de Buenos Aires, 34°35' S, 58°30' W) and in the natural populations previously cited above. Additionally, measure-

ments were taken from liquid-preserved flowers for specimens from the Córdoba populations just mentioned.

Somatic chromosomes were examined from primary roots from germinating seeds and adventitious roots from growing bulbs. When seed germination did not occur within 15 d, 500–1000 ppm gibberellic acid ( $GA_3$ ) was applied to break dormancy. Root tips were pretreated with 2 mM 8-hydroxyquinoline for 4 hr at room temperature ( $\pm 20^\circ C$ ). Roots were then fixed in a freshly made ethanol:acetic acid (3:1) mixture at room temperature for a minimum of 12 hr, hydrolyzed in 5N HCl for 45 min, and stained in Feulgen for 2–2.5 hr (Feulgen and Rossenbeck 1924). Squashes were made in a drop of 45% carmine acetic acid. Slides were made permanent by freezing them with liquid carbon dioxide (Bowen 1956), removing their coverslip, and mounting them in Euparal.

A total of 20 individuals and 100 cells per species were analyzed under a phase contrast microscope. Ten slides illustrating cells in metaphase for each species were photographed and photographs were used to measure: short arm length (s), long arm length (l), and total chromosome length (c) for each chromosome pair. The arm ratio ( $r = l/s$ ) was calculated and utilized to classify the chromosomes after Levan et al. (1964): metacentric ( $m$ ) ( $r = 1.00$ – $1.69$ ) or submetacentric ( $sm$ ) ( $r = 1.70$ – $2.99$ ). In addition, the centromeric index (Levan et al. 1964) was calculated as a percentage of the length of the short arm out of the total length of the chromosome ( $I = 100 s/c$ ). Satellites were classified according to Battaglia (1955) and satellite lengths were added to the length of the corresponding chromosome arms. In addition, average chromosome length (C) and haploid karyotype length (HKL), based on mean chromosome lengths for each species, were calculated. Karyograms were constructed by organizing the chromosomes into groups according to their arm ratio, ordering them by decreasing length within each category, and finally numbering them consecutively. Arm ratio and chromosome length were taken into account in recognizing homologues. Idiograms were based on the mean values for each species. Karyotype asymmetry was estimated using Stebbins' classification (1971) and Romero Zarco indices (1986) of  $A_1$  (intrachromosomal asymmetry index, which indicates the length difference between the chromosome arms), and  $A_2$  (interchromosomal asymmetry index, which indicates the size variation among the chromosomes).

## RESULTS

**Flower Morphology**—Flowers of *Calydorea undulata* and *Tamia pallens* have unequal tepals: the outer are flat while the inner are shorter and geniculate in *T. pallens* (Fig. 1 A),

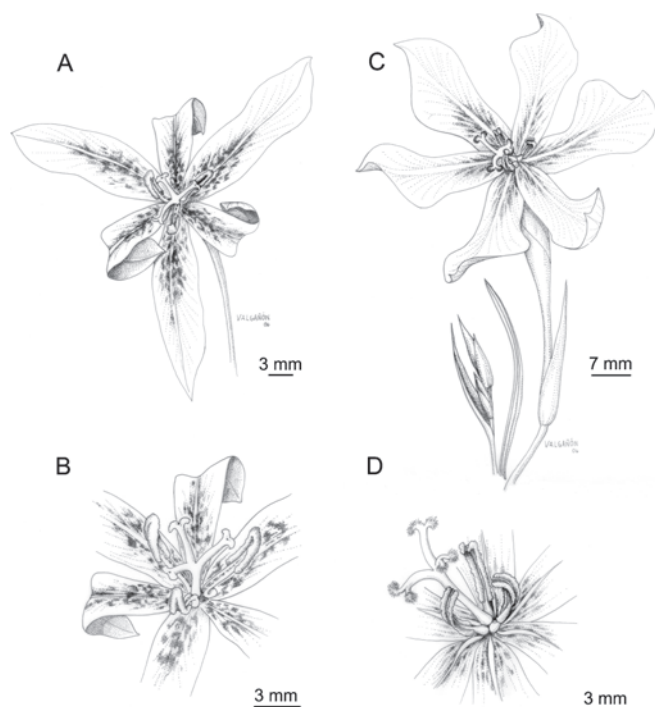


FIG. 1. Flower structure. A–B. *Tamia pallens*. C–D. *Calydorea undulata*. A, C. Lateral views; B, D. Detail of stamens and style branches.

whereas both kind of tepals are flat with undulate margins in *C. undulata* (Fig. 1 C). Flower color is pale lilac with dark violet dots in *T. pallens* and violet-blue with violet stripes in *C. undulata*. Filament lengths can also be used to distinguish them: 3–4 mm long in *T. pallens* and 1–2 mm long in *C. undulata*. Stamens and style branches have about the same length in *T. pallens* (Fig. 1 B), but in *C. undulata* style branches are longer than the stamens (Fig. 1 D).

The presence of twisted or circinate anthers (*Calydorea*) vs. straight anthers (*Tamia*) after dehiscence, and anthers free from style branches (*Calydorea*) vs. proximal portion of the anther adnate tightly to the styles (*Tamia*), were mentioned by Ravenna (2001) as distinguishing features. However, these features were not found to be diagnostic. Orientation of the anthers in living plants of both taxa was straight before dehiscence, and twisted after the pollen is exposed. Flowers of both taxa last from 4–6 hrs and, depending on the developmental stage when herbarium specimens are prepared, the anthers may look either straight or twisted. Stamens are completely free from style branches in both taxa (Fig. 1). Although anthers and style branches are almost opposite, there is no evidence of contact or fusion between whorls.

Habit, overall plant morphology, and flowering phenology (November–January) were similar in both taxa in the natural populations studied. The flowers of both taxa were frequently visited by halictid bees and syrphid flies, but no hybrids have been detected in the area.

**Cytology**—The chromosomes of both taxa were relatively large (Table 1; Fig. 2) with similar average lengths (Table 2). Chromosome numbers indicated that the base chromosome number for both is  $x = 7$  (Table 2) and that *C. undulata* is a diploid with  $2n = 14$  (Fig. 2A), whereas *T. pallens* is a tetraploid with  $2n = 28$  (Fig. 2B). Accordingly, the HKL of *T. pallens* was approximately twice that of *C. undulata* (Table 2).

Macrosatellites were present in both taxa (Figs. 2, 3) and were frequently seen (75–100% of the examined metaphases). In *C. undulata*, macrosatellites were located at the distal end of the short arm of chromosome pairs #2 and #5 (Fig. 3A; Table 1), whereas in *T. pallens* one of the satellites was attached to the distal end of the long arm of pair #5 and the other one to the distal end of the short arm of pair #7 (Fig. 3B; Table 1). The karyotypes showed that both taxa have only  $m$  and  $sm$  chromosomes, though in different proportions (Tables 1, 2; Fig. 3). Karyotypes were moderately asymmetrical according to the  $A_1$  and  $A_2$  indices obtained (Table 2) and Stebbins' classification (1971), where both taxa fell into the 2B category. In particular, *C. undulata* had a bimodal karyotype composed of two large chromosome pairs (#1 and #6) and five small ones (#2–5; Fig. 3A), whereas in *T. pallens* chromosome size changed gradually among the different pairs (Fig. 3B).

## DISCUSSION

The flowers of *Tamia pallens* and *Calydorea undulata* have subequal tepals, free stamens and slender style branches that are subopposite to the stamens. The diagnostic features used by Ravenna (2001) to distinguish the two genera (orientation of the anthers and adnation of anthers and style branches) were found not to be valid. Thus, following the criteria of Goldblatt and Henrich (1991) both taxa have to be included within *Calydorea*. This genus was erected by Herbert (1843) as a South American genus, characterized by subequal tepals

TABLE 1. Chromosome data of *Calydorea undulata* and *Tamia pallens*. s = short arm; l = long arm; c = total length, r = arm ratio; i = centromeric index; sat = satellite. Lengths of chromosomes in  $\mu\text{m}$ . Nomenclature follows Levan et al. (1964).

Pair	Chromosome Length: Mean $\pm$ s.d.					Nomenclature
	s	l	c	r	i	
<i>Calydorea undulata</i>						
1	2.7 $\pm$ 0.49	3.97 $\pm$ 0.83	6.68 $\pm$ 1.26	1.46	40.57	m
2	1.92 $\pm$ 0.3	2.45 $\pm$ 0.36	4.37 $\pm$ 0.6	1.28	43.94	m sat
3	1.76 $\pm$ 0.44	2.4 $\pm$ 0.46	4.16 $\pm$ 0.86	1.36	42.31	m
4	1.55 $\pm$ 0.3	2.18 $\pm$ 0.40	3.74 $\pm$ 0.66	1.41	41.44	m
5	1.54 $\pm$ 0.19	1.79 $\pm$ 0.20	3.34 $\pm$ 0.31	1.16	46.11	m sat
6	2.52 $\pm$ 0.66	4.5 $\pm$ 0.95	7.02 $\pm$ 1.55	1.78	35.89	sm
7	1.22 $\pm$ 0.25	2.6 $\pm$ 0.5	3.82 $\pm$ 0.66	2.13	31.93	sm
<i>Tamia pallens</i>						
1	3.04 $\pm$ 0.57	4.21 $\pm$ 0.73	7.25 $\pm$ 1.1	1.38	41.93	m
2	2.38 $\pm$ 0.27	3.41 $\pm$ 0.42	5.79 $\pm$ 0.64	1.43	41.1	m
3	2.06 $\pm$ 0.16	2.97 $\pm$ 0.40	5.04 $\pm$ 0.44	1.44	40.87	M
4	1.81 $\pm$ 0.28	2.33 $\pm$ 0.36	4.14 $\pm$ 0.52	1.28	43.72	m
5	1.56 $\pm$ 0.27	2.08 $\pm$ 0.22	3.66 $\pm$ 0.38	1.33	42.68	m sat
6	1.61 $\pm$ 0.23	1.97 $\pm$ 0.3	3.58 $\pm$ 0.46	1.22	44.97	m
7	1.31 $\pm$ 0.18	1.67 $\pm$ 0.18	2.98 $\pm$ 0.25	1.27	43.96	m sat
8	2.21 $\pm$ 0.39	4.11 $\pm$ 0.57	6.32 $\pm$ 0.88	1.86	34.97	sm
9	1.82 $\pm$ 0.44	3.46 $\pm$ 0.58	5.28 $\pm$ 0.88	1.9	34.47	sm
10	1.45 $\pm$ 0.22	3.21 $\pm$ 0.71	4.66 $\pm$ 0.88	2.21	31.16	sm
11	1.43 $\pm$ 0.19	2.88 $\pm$ 0.48	4.32 $\pm$ 0.62	2.01	33.11	sm
12	1.25 $\pm$ 0.22	2.42 $\pm$ 0.31	3.67 $\pm$ 0.51	1.94	34.06	sm
13	1.1 $\pm$ 0.11	2.03 $\pm$ 0.18	3.13 $\pm$ 0.25	1.84	35.14	sm
14	0.86 $\pm$ 0.16	1.80 $\pm$ 0.41	2.66 $\pm$ 0.44	2.09	32.33	sm

spreading from the base, free filaments, and short slender style branches (Goldblatt et al. 1998). Several genera sharing the same flower structure but occurring outside of South America, are regarded as synonyms of *Calydorea* by Goldblatt and Henrich (1991), *Cardiostigma* Baker, *Salpingostylis* Small,

*Catila* Ravenna, and *Itysa* Ravenna. Thus, Goldblatt and Henrich expanded the geographical distribution of *Calydorea* to Central and North America. Our results suggest that the monotypic genus *Tamia* should also be regarded as a synonym of *Calydorea*, because it shares a basic flower structure with all members of *Calydorea*. In addition, both taxa have the same basic chromosome number ( $x = 7$ ) although differences occur in ploidy level and chromosome characteristics.

The chromosome number for *Tamia pallens* ( $2n = 28$ ) obtained during this study agrees with a previous chromosome count by Goldblatt and Takei (1997) (as *Calydorea pallens*). Our count of  $2n = 14$  is the first cytological report for *Calydorea undulata*. The ancestral base number in Tigridaeae appears to be  $x = 7$  (Goldblatt and Takei 1997). Thus, the chromosome numbers here reported agreed with the tribal pattern.

There are chromosome counts for about half of the Iridaceae species, including a good sampling of Nivenioideae, Iridoideae, and Ixiodeae (Goldblatt and Takei 1997). According to Goldblatt (1990),  $x = 10$  is most likely ancestral given the base numbers of the less specialized genera. Goldblatt and Takei (1997) have suggested an early burst of polyploidy in the evolution of the family or a paleopolyploid origin, because most generic ancestral base numbers in Iridaceae are relatively high (Grant 1981).

TABLE 2. Cytological characteristics of *Calydorea undulata* and *Tamia pallens*.  $2n$  = somatic chromosome number; HKL = total haploid karyotype length; C = mean chromosome length; r = mean arm ratio;  $A_1$  = mean intrachromosomal;  $A_2$  = interchromosomal asymmetry index. Measurements are in  $\mu\text{m}$ . An asterisk indicates that two chromosome pairs bear satellites.

Taxon	Ploidy level	$2n$	Karyotype formula	HKL	C	r	$A_1$	$A_2$
<i>C. undulata</i>	2x	14	5 m* + 2 sm	33.13	4.73	1.51	0.31	0.31
<i>T. pallens</i>	4x	28	7 m* + 7 sm	62.48	4.46	1.66	0.37	0.30

FIG. 2. Photomicrographs of mitotic metaphases. A. *Calydorea undulata* ( $2n = 14$ ). B. *Tamia pallens* ( $2n = 28$ ). Arrows indicate satellites.



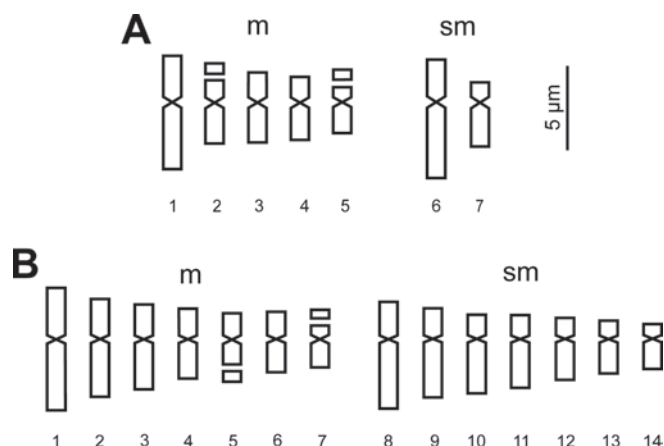


FIG. 3. Idiograms for karyotypes of both species. A. *Calydorea undulata*. B. *Tamia pallens*. m = metacentric chromosomes; sm = submetacentric chromosomes.

The chromosomes of both taxa are large, a common feature in Iridoideae (Goldblatt 1971; Goldblatt et al. 1998). The moderately asymmetrical karyotype of *Tamia pallens* could be derived from a paleodiploid through polyploidy and chromosome rearrangements. Further studies, using banding techniques or analyzing meiotic pairing would be appropriate to elucidate this situation. On the other hand, *Calydorea undulata* exhibits a bimodal karyotype, similar to those reported in Iridoideae and Ixioidae (Kenton et al. 1990; Goldblatt and Takei 1997). Bimodality is common in the Tigridaeae and is also a conserved feature, despite variation in chromosome and karyotype size (Goldblatt 1982; Kenton et al. 1990). Goldblatt (1982) has suggested a basic karyotype in Tigridaeae comprised of two long and five shorter chromosome pairs, as established during this study for *C. undulata*. In most species of Tigridaeae, the longest pair is m, and the next longest are sm or st.

The different ploidy level and karyotype formula present in *T. pallens* and *C. undulata* provide evidence that Ravenna (2001) was correct in distinguishing these two taxa. Our data indicates that the two taxa should be recognized as two distinct species within *Calydorea*. Chromosome counts for *Calydorea* show a polyploid series based on  $x = 7$  (e.g. *C. amabilis*  $2n = 14$ , *C. nuda*  $2n = 14$ , *C. azurea*  $2n = 28$ , *C. xyphioides*  $2n = 42$ ; Goldblatt 1982; Goldblatt and Takei 1997). The differences in their karyotypes fall within the chromosomal variability within genera in Iridaceae (e.g. Winge 1959; Molseed 1970; Goldblatt 1982, 1991; Kenton et al. 1990).

In summary, our data does not support the recognition of the genus *Tamia* but does support the recognition of two species, *Calydorea pallens* and *C. undulata*. Both taxa cooccur in the geographical area studied, share the same pollinators which can pass from flowers of one to the flowers of the other, and bloom at the same time. However, hybrids are not known (spontaneous or experimentally produced) and may not be possible because of their different chromosome numbers and karyotypes.

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## APPENDIX. 1. Representative specimens examined.

*Calydorea undulata*—ARGENTINA. Córdoba: Dept. Punilla, Jan 1925, *W. Lossen* 37 (GH). Sierras de Córdoba; 1911, *C. Silva* 22070 (LP). Dept. Capital, La Calera, Dec 1887, *Spegazzini s.n.* (LPS 18968, LP).

*Calydorea pallens*—ARGENTINA. Catamarca: Dept. Ancasti, Sierra del Alto, Mal Paso, Jan 26, 1944, *Castellanos* 47688 (BA). La Rioja, Dept. Gral. Belgrano, Olta, Loma Colorada, Feb 1 1940, *Castellanos* 33521 (BA).

Ruta Nac. 38 entre Chamical y Chañar, Campo Exper. I.N.T.A., “Las Vizcacheras”, bajo la ochava del alambrado, Feb 9 1990, *Biurrum et al.* 3072 (CTES). Paraje La Llanura, camino municipal entre El Quebrachal y Tala Verde, Apr 3 1990, *Biurrum et al.* 3299 (CTES). Mendoza: Dep. La Paz, entre Desaguadero y Estancia Jarilla, *Ruiz Leal s.n.* (MEN). Santiago del Estero: Dept. Ojo de Agua, San Andrés de Báez, Dec 17 1944, *R. Maldonado-Bruzzone* 1566 (LP). Salta: Dept. Capital, Pasaje del Río Juramento, Feb 1873, *Lorentz et Hieronymus* 371 (isotype: CORD!).