



# A cladistic analysis of the subtribe Castillejinae (Scrophulariaceae-tribe Pedicularae)<sup>1</sup>

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**Abstract.** The subtribe Castillejinae, comprising six hemiparasitic genera and distributed primarily in western North and South America, is a monophyletic group defined by possessing anthers with unequal anther-sacs unequally attached to the filament. The phylogenetic relationships of the subtribe are investigated, using 26 characters from morphology and cytology. Seven genera, three subgenera, and three sections are considered to be terminal for the subtribe. Polarity of the characters is based on outgroup comparison. Three equally parsimonious trees and a strict consensus tree were generated, each with 35 steps (homoplasy 34.3%) and a consistent index of 0.743. This analysis strongly supports Chuang and Heckard's placement of the species previously assigned to the genus *Orthocarpus* into three genera, namely *Orthocarpus*, *Triphysaria*, and *Castilleja*, and relegation of the monotypic genus *Gentrya* to subgeneric status in *Castilleja*. This cladistic analysis supports most of our previously proposed classification and phylogenetic arrangement of the subtribe.

**Key words:** Cladistic analysis; Consensus tree; Consistent index; Homoplasy; Monophyletic group; Outgroup; Parsimonious tree; Phylogenetic relationships.

## Introduction

The subtribe Castillejinae (Scrophulariaceae-tribe Pedicularae) consists of about 250 herbaceous hemiparasitic species in six genera, and is distributed primarily in western North and South America. The subtribe is defined by possessing anthers with unequal anther-sacs unequally attached, i.e., the larger upper sac attached by its middle to the filament, the smaller by its apex. The genus *Ophiocephalus* lacks this feature, but exhibits sufficient morphological resemblances to be retained in the subtribe (Breedlove and Heckard, 1970; Chuang and Heckard, 1991). In a recent generic

treatment (Chuang and Heckard, 1991), we proposed to re-distribute the species assigned to the genus *Orthocarpus* (Keck, 1927) among three distinct genera: 1) *Orthocarpus*, a close relative of *Cordylanthus*, restricted to the type section and subgenus; 2) sections *Castillejooides* and *Cordylanthoides* of subg. *Orthocarpus* joined to the *Castilleja pilosa* group of the genus *Castilleja*; 3) subg. *Triphysaria* raised to distinct generic status. The monotypic genus *Gentrya* is reduced to subgeneric status in *Castilleja*. The large and expanded genus *Castilleja* is subdivided into three subgenera: *Colacus* (with the three sections *Oncorhynchus*, *Pilosae*, and *Pallescentes*); *Gentrya*; and *Castilleja*, which comprises the majority of species in the genus. The justification for such drastic generic realignment in this subtribe is discussed in detail elsewhere (Chuang and Heckard, 1991).

Numerical methods that attempt to develop phylogenetic hypotheses are termed cladistics, as opposed to phenetic methods, which determine relationship on the basis of overall similarity without *a priori*

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evolutionary assumptions. Theoretical aspects of cladistic methods have been summarized by Estabrook (1978) and Wiley (1981) and their application in plant systematics reviewed by Bremer and Wanntorp (1978), Funk and Stuessy (1978), and Humphries and Funk (1984). Of the available computer-assisted cladistic techniques, the two most commonly used are parsimony methods and character-compatibility analysis. Parsimony methods imply the construction of cladograms by minimizing the necessary number of changes in character states; character-compatibility methods construct cladograms consistent with a maximum number of characters (=largest cliques) without implying parallelism or reversal of character states (Nordal and Duncan, 1984).

So far as we are aware, there are only three studies in the family Scrophulariaceae utilizing cladistic approaches for generating presumed phylogeny at tribal, generic, and specific levels. These include tribe Veroniceae (subfamily Rhinanthoideae) by Hong (1984), subtribe Maurandyinae by Elisens (1985), and the new world genus *Antirrhinum* by Thompson (1988), the latter two taxa belonging to tribe Antirrhineae of subfamily Scrophularioideae (=Antirrhinoideae). The objectives of the present study are: 1) to test the presumed monophyletic nature of subtribe Castillejinae; 2) to compare the similarities and differences among the cladograms generated and our previously proposed hypotheses of taxonomic relationship; 3) to suggest modifications, if any proved necessary, of earlier classification schemes in subtribe Castillejinae.

## Materials and Methods

### *Taxa Used in the Analysis*

One genus (designated as the outgroup taxon) and ten taxa of subtribe Castillejinae at the generic, sub-generic, and sectional levels (ingroup taxa) were used as evolutionary units (EUs) for this study. These include the genera *Schwalbea* (outgroup), *Ophiocephalus*, *Clevelandia*, *Triphysaria*, *Orthocarpus*, *Cordylanthus*, and *Castilleja*; the last genus includes the three subgenera *Colacus* (with the three sections *Oncorhynchus*, *Pilosae*, and *Pallescentes*), *Gentrya*, and *Castilleja*.

The genus *Schwalbea* (two species) of the eastern United States was selected as an outgroup because it is customarily regarded as relatively primitive in tribe

Pediculareae (Pennell, 1935). *Ophiocephalus* is a monotypic genus endemic to San Pedro Martir, Baja California, Mexico. *Clevelandia*, also a monotypic genus, is endemic to the southern Sierra de la Giganta southward into the Cape Region, Baja California, Mexico. *Triphysaria* (five species) is distributed primarily in California, with the most widespread species, *T. pusilla*, extending north to British Columbia, Canada. *Orthocarpus* (nine species) is widely distributed in western North America, with the most widespread species, *O. luteus*, extending from California eastward to northwestern Minnesota, and to Ontario in Canada. *Cordylanthus* (18 species) is widely distributed in diverse habitats in the western United States and adjacent Mexico. *Castilleja*, the largest genus of the subtribe, comprises over 200 perennial and annual species and occurs chiefly in western North America. This genus has been treated recently as comprising the three subgenera *Castilleja*, *Gentrya*, and *Colacus*, the last including the three sections *Oncorhynchus*, *Pilosae*, and *Pallescentes* (Chuang and Heckard, 1991).

### *Characters*

The characters used for analysis are based on a detailed morphological study of vegetative organs, flowers, fruit and seeds, as well as exhaustive investigation of chromosome number (Chuang and Heckard, 1991 for subtribe Castillejinae; Pennell, 1935; Kondo, 1972, and Kondo *et al.*, 1978 for genus *Schwalbea*).

Twenty-six characters were selected and assigned plesiomorphic (primitive=0) and apomorphic (derived=1) states (Table 1). Some of these characters were used previously in suggesting possible phylogenetic relationships in the subtribe (Chuang and Heckard, 1991). By and large, the 26 characters used were fairly consistent for each EU; when inconsistency occurred, the most common condition was coded for that EU. The basic data matrix containing the character states for all EUs is shown in Table 2.

Character polarity was determined by outgroup comparison (Hennig, 1966; Watrous and Wheeler, 1981), using the genus *Schwalbea* as the outgroup. This genus is an excellent choice for an outgroup because it possesses many plesiomorphic states. The tribe Pediculareae (=Euphrasieae, Rhinanthae), a large group of some 30 genera, stands at the apex of evolution in the Rhinanthoid subfamily of Scrophulariaceae (Pennell, 1935). *Schwalbea* is the only genus in North America that

**Table 1.** Characters and their states used in cladistic analysis for genera, subgenera (including only genus *Castilleja*), and sections (including only subg. *Colacus* of genus *Castilleja*) in subtribe Castillejinae. A zero represents the plesiomorphic (primitive) state and one represents the apomorphic (derived) state.

1. Growth duration: 0=perennial; 1=annual
2. Leaf arrangement: 0=opposite; 1=alternate
3. Inflorescence types: 0=raceme, spiciform raceme to elongate or short spike; 1=variable ranging from spike, single-flowered to spiciform or glomerate clusters
4. Flower: 0=subtended by 2 bractlets; 1=subtended by 1 bract
5. Calyx: 0=5-lobed; 1=4-lobed
6. Calyx form: 0=tubular; 1=not tubular, single piece of spathe-like structure
7. Calyx incision: 0=subequally cleft; 1=unequally cleft
8. Corolla form: 0=slightly zygomorphic; 1=strongly zygomorphic
9. Corolla shape: 0=not dorsi-ventrally compressed; 1=usually strongly dorsi-ventrally compressed
10. Upper and lower corolla-lips: 0=subequal; 1=unequal with lower lip highly reduced, triplicate
11. Upper corolla-lip: 0=not forming a beak; 1=forming distinct beak or hood

13. Lower corolla-lip: 0=not inflated; 1=inflated to form a mono- or a tri-saccate pouch
14. Tip of lower corolla-lip: 0=not toothed; 1=3-toothed
15. Style: 0=straight, not enlarged near apex; 1=bent and enlarged near apex
16. Stigma shape: 0=enlarged, capitate to slightly bilobed; 1=not enlarged
17. Stigma position: 0=protruding beyond the tip of upper corolla-lip; 1=not protruding beyond the tip of upper corolla-lip
18. Stigma position: 0=enclined in corolla; 1=surpassing corolla

retains such primitive features as septicidal dehiscence of the capsule, a five-lobed calyx, and possession of two bractlets beneath the calyx (Pennell, 1935). Pennell (1935) further pointed out that *Schwalbea* was probably a member of the ancient Miocene flora once dispersed over the North Temperate zone, and that it now represents the survival of that element in eastern North America.

#### Phylogenetic Analysis

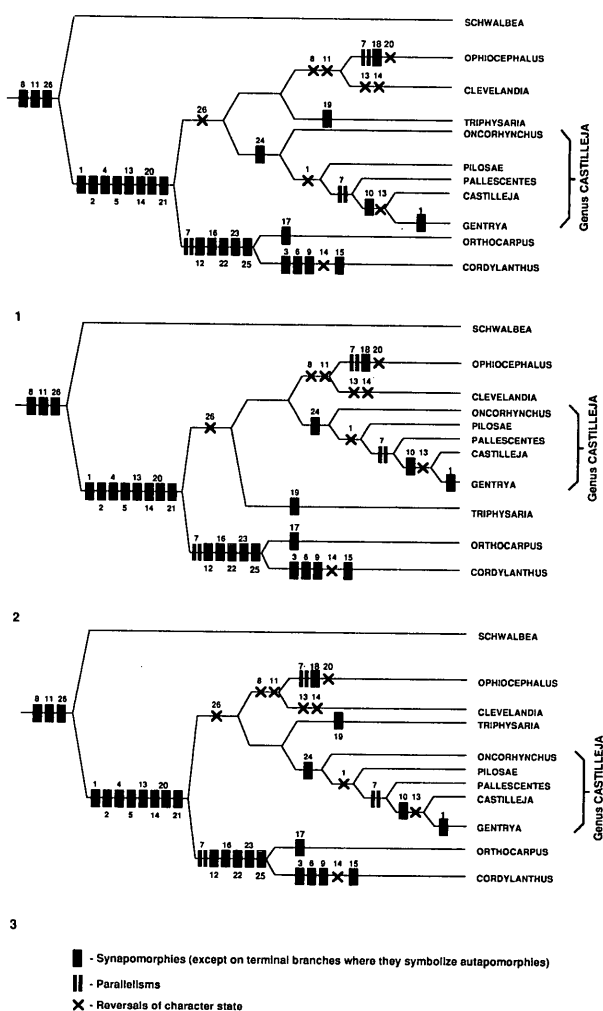
The basic data matrix (Table 2) was used to perform cladistic analyses, using the Wagner parsimony algorithm of PAUP (Phylogenetic Analysis Using Parsimony) version 3.0, developed by Dr. David Swofford at the Illinois Natural History Survey. We have attempted to find multiple equally parsimonious trees and the most parsimonious tree using MULPARS and SWAP=GLOBAL options, and a consensus tree for a set of equally parsimonious trees using the CONTREE program. The same basic data matrix was also subjected to compatibility analysis using the CLIQUE program of PHYLIP (Phylogeny Inference Package) version 3.1, developed by Dr. Joseph Felsenstein at the University of Washington.

#### Results and Discussion

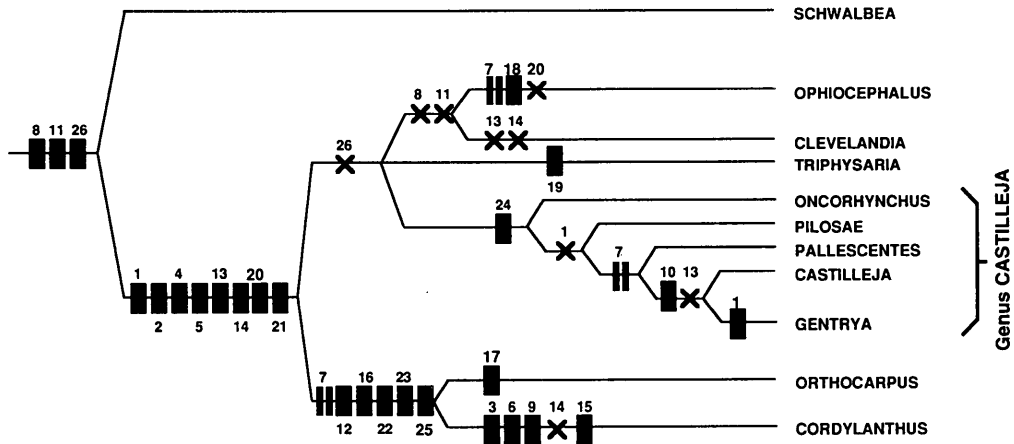
Three very similar equally parsimonious trees (Figs. 1, 2, 3) were generated in this analysis, each with 35 character state changes and a consistency index of 0.743, but differing in the placement of the genus *Triphysaria*. A strict consensus tree (Rohlf, 1982), summarizing the shared information from the three trees, is shown in Fig. 4. Among the 35 character state changes in the cladograms, three are parallelisms and nine are reversals, for a total homoplasy of 34.3%. Only one largest clique of 21 characters was obtained in character compatibility analysis using the CLIQUE program. It included characters 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, and 26. The characters not compatible with the largest clique are: growth duration (1), calyx incision (7), shape of lower corolla-lip (13), toothling at tip of lower corolla-lip (14), and anther-sac shape and position (20). The cladogram generated in this analysis is almost identical to that of the consensus tree produced by the PAUP program.

The subtribe *Castillejinae* is characterized by the combination of features including: alternate leaves (2),

flower subtended by a single bract (4), calyx 4-lobed (5), lower corolla-lip inflated (13), tip of lower corolla-lip 3-toothed (14), anther-sacs unequal in size and unequally attached to the filament (20), and loculicidal dehiscence of the capsule (21). It is believed to be monophyletic. There are two major clades in this subtribe. The first clade, which comprises the two genera *Cordylanthus* and *Orthocarpus*, is defined by six shared derived characters (synapomorphies), i.e. calyx unequally cleft (character 7), tip of upper corolla-lip closed (12), stigma unexpanded (16), ovules campylotropous (22), hilum of seeds lateral (23), and seeds 4-30 per capsule

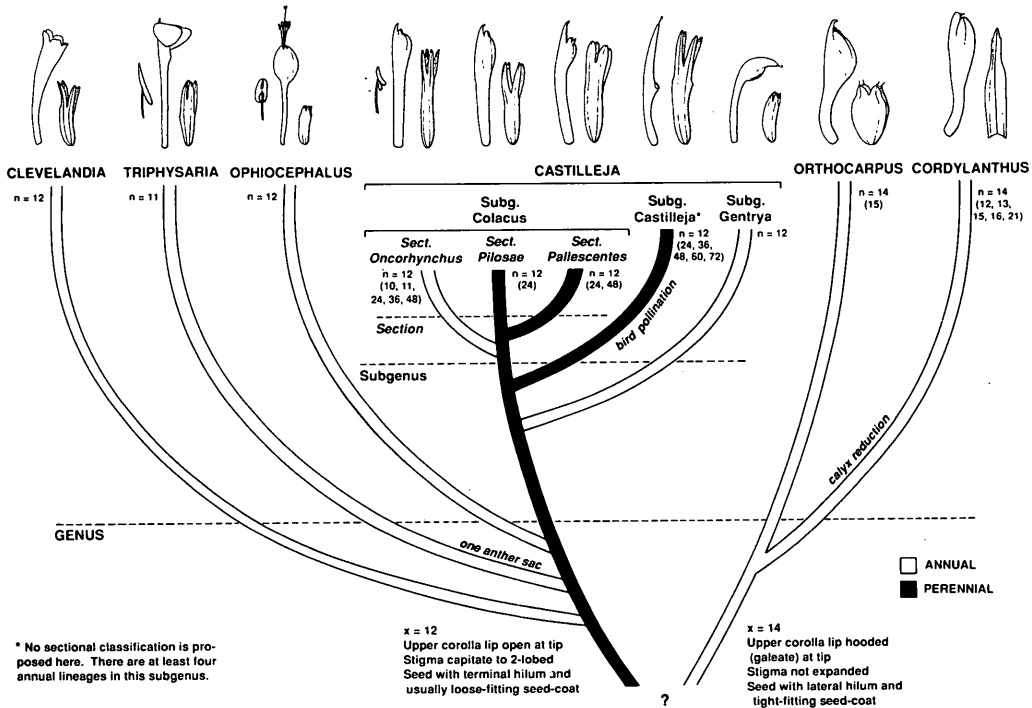


Figs. 1-3. Cladograms of subtribe Castillejinae, resulting from PAUP analyses. Fig. 1. One of the three most equally parsimonious trees. Fig. 2. One of the three most equally parsimonious trees. Fig. 3. One of the three most equally parsimonious trees.



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- - Synapomorphies (except on terminal branches where they symbolize autapomorphies)
- ▬▬ - Parallelisms
- ✕ - Reversals of character state



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Figs. 4-5. Cladogram resulting from PAUP analyses and phylogenetic relationships based on less explicit methods. Fig. 4. A strict consensus tree from the three most equally parsimonious cladograms (length 35, consistency index = 0.743). Fig. 5. Postulated phylogenetic relationships within subtribe Castillejinae (from Chuang and Heckard, 1992, fig. 34), showing close similarity to those cladograms resulting from cladistic analyses.

(25). These two genera form a monophyletic group. The genus *Cordylanthus* possesses four unique, derived characters (autapomorphies; characters 3, 6, 9, 15) and is a sister group of the reconstituted genus *Orthocarpus* (Chuang and Heckard, 1991, 1992) which is defined by a single autapomorphy, i.e. stigma not protruding from the tip of the upper corolla-lip (17). The second clade includes the remaining genera *Castilleja*, *Clevelandia*, *Ophiocephalus*, and *Triphysaria*, and is defined by the reversal feature of chromosome number  $n=11$  or 12 (26). The relationships of these four genera are poorly understood and are not well differentiated by cladistic analysis. The three equally parsimonious cladograms generated show differences in placement of the genus *Triphysaria* and the consensus tree forms a trichotomy with three poorly resolved lineages in this clade. However, it is clear that the genera *Ophiocephalus* and *Clevelandia* form a monophyletic group in the first lineage based on the presence of two shared primitive characters (synplesiomorphies, reversals), i.e. slightly zygomorphic corolla (8) and a beakless upper corolla lip (11). The genus *Ophiocephalus* is defined by autapomorphic characters, i.e. unequally cleft calyx (7) and stamens surpassing corolla (18) and the primitive character (reversal) of anther-sacs of equal size equally attached to the filament (20). It is hypothesized to be a sister group of the genus *Clevelandia*, which is characterized by two primitive characters (reversals), i.e. uninflated lower corolla-lip (13) and entire tip of lower corolla-lip (14). The proposed sister group relationship of *Ophiocephalus* and *Clevelandia* is consistent with their restricted geographical distribution in Baja California, Mexico, and their shared primitive features indicated above. The genus *Triphysaria* forms an isolated lineage, differing from the other two only by the possession of a single anther-sac per stamen (19). The third lineage comprises the genus *Castilleja* (including its five infrageneric taxa) and is grouped together by the synapomorphy of loose-fitting seed-coat (24). Each infrageneric taxon appears to be well-defined by a combination of features including duration of growth (1), calyx incision (7), length of upper and lower corolla-lips (10), and degree of inflation of the lower corolla-lip (13).

The present cladistic analysis strongly substantiates our argument that *Orthocarpus* as previously circumscribed (Keck, 1927) was polyphyletic, consisting of three distinctive species groups (clades) that are no

closer to each other in overall phenetic similarity than they are to other members of related genera (Chuang and Heckard, 1991). Therefore our proposed revision (Chuang and Heckard, 1991) to distribute the members of previously circumscribed *Orthocarpus* into the three distinct genera, *Orthocarpus*, *Triphysaria*, and *Castilleja* is overwhelmingly supported by this analysis. It verifies a relegation of the genus *Gentrya* to subgeneric status in *Castilleja*. It also verifies that all of the six genera recognized in that treatment are monophyletic. The cladograms generated reveal intergeneric relationships closely resembling those previously arrived at by less explicit methods (Fig. 5; Chuang and Heckard, 1991).

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## 玄參科馬先蒿族火焰草亞族之系統分類研究

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火焰草亞族共有 6 屬，約 250 種半寄生草本植物，幾乎全部分佈在北美洲及南美洲。這亞族是單源發生 (monophyletic)，其最主要特徵是雄蕊有不同大小花藥，本文採用了 26 個特狀 (包括形態及細胞染色體) 進行這亞族之系統發生及親緣關係之研究。系譜 (cladistic) 分析結果得了 3 個相等的最簡約系統樹 (equally parsimonious trees) 及一致性的系統樹 (strict consensus tree)，每 1 系統樹有 35 步驟 (同型性 34.3%) 及配合指數 (consistent index) 0.743。這分析證實了 Chuang 及 Heckard 1991 年所重訂火焰草亞族分類及系統發生。