

RECENT ADVANCES ON EVOLUTION OF POLLINATION SYSTEMS AND REPRODUCTIVE BIOLOGY OF VANILLOIDEAE (ORCHIDACEAE)

EMERSON R. PANSARIN

Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Av. Bandeirantes 3900, 14040-901, Ribeirão Preto, SP, Brazil • epansarin@ffclrp.usp.br

ABSTRACT. Vanilloideae as currently circumscribed comprises nine genera and two tribes: Vanilleae and Pogonieae. The pantropical genus *Vanilla* has been frequently assumed to be natural on the basis of its climbing habit and lateral inflorescences. However, the inclusion of the rare *Dictyophyllaria dietschiana* in phylogenetic analyses makes the genus *Vanilla* paraphyletic. Within Pogonieae, phylogenetic analyses show that inclusion of *Pogoniopsis* turns the tribe paraphyletic. All analyses reveal that *Pogoniopsis* is closely related to members of Epidendroideae. Members of Pogonieae are pollinated by several groups of solitary and social bees, two pollination systems being recognized: reward-producing and deceptive. Molecular phylogeny suggests that the common ancestor to Pogonieae gave rise to two evolutionary lineages: one tropical with a condition of reward production; and one predominantly temperate-invading line with deceptive flowers. Reward-producing flowers characterize South and Central American clade (= *Cleistes*), while deceptive pollination is prominent in the clade including North American-Asiatic taxa plus Amazonian *Duckeella*. Species of “orchid bees” have been recorded as pollinators of the genus *Vanilla* (*V. planifolia* group and *V. pompona* group) in the Neotropics. In species of the *V. pompona* group, these bees are attracted by the fragrance of the flowers. Hummingbirds have been reported to pollinate some species of *Vanilla*. *Vanilla insignis*, *V. odorata* and *V. planifolia* are known to be pollinated through generalized food deception. Some species of *Vanilla* yield fruits through spontaneous self-pollination. This form of autogamy has been reported for *V. griffithii*, *V. palmarum*, *V. planifolia*, *V. savannarum* and *V. bicolor*. In Brazil, data on the pollination biology of *Vanilla* are scarce, but conclusive data are available for *V. edwallii*, which is pollinated by *Epicharis* (Apidae: Centridini). This species is rewardless, but male *Epicharis* are attracted to its flowers by their fragrance. Additionally, the Brazilian *V. dubia* and *E. sclerophyllum* are pollinated by bees. The mentum region of *V. dubia* and *V. edwallii* is dry, whereas that of *E. sclerophyllum* presents a small quantity of nectar. Flowers of *E. sclerophyllum* are scentless, while those of *V. dubia* are odoriferous. *Vanilla dubia* and *V. edwallii* are self-compatible and need a pollinator to yield fruits. In contrast, *Epistephium sclerophyllum* sets fruits through spontaneous self-pollination, but biotic pollination also occurs. Both species are primarily adapted to pollination by euglossine bees. Pollination by Euglossini seems to have evolved at least twice along the evolution of Vanilleae. Furthermore, shifts between rewarding and rewardless flowers and between autogamous and allogamous species have been reported among vanillas.

KEY WORDS: Flower reward, food deception, Pogonieae, pollination biology, reproduction, Vanilleae

Phylogenetic relationships within Vanilloideae. Since the first comprehensive study published by Darwin (1892), the pollination systems displayed by orchid flowers have inspired biologists over the centuries. Nowadays, several research groups have focused on understanding pollination biology and evolution of pollination systems of orchids based on the combination of phylogenetic hypothesis with morpho-anato-

mical studies and several kinds of ecological data (see Pansarin *et al.* 2012). In some orchid groups, however, these data are very difficult to be obtained since many species are not cultivable and investigations need to be made exclusively in the field. This is the case for Vanilloideae, a subfamily where data on the natural history of several genera, such as *Cleistes* for instance, must be collected exclusively in the natural environment.

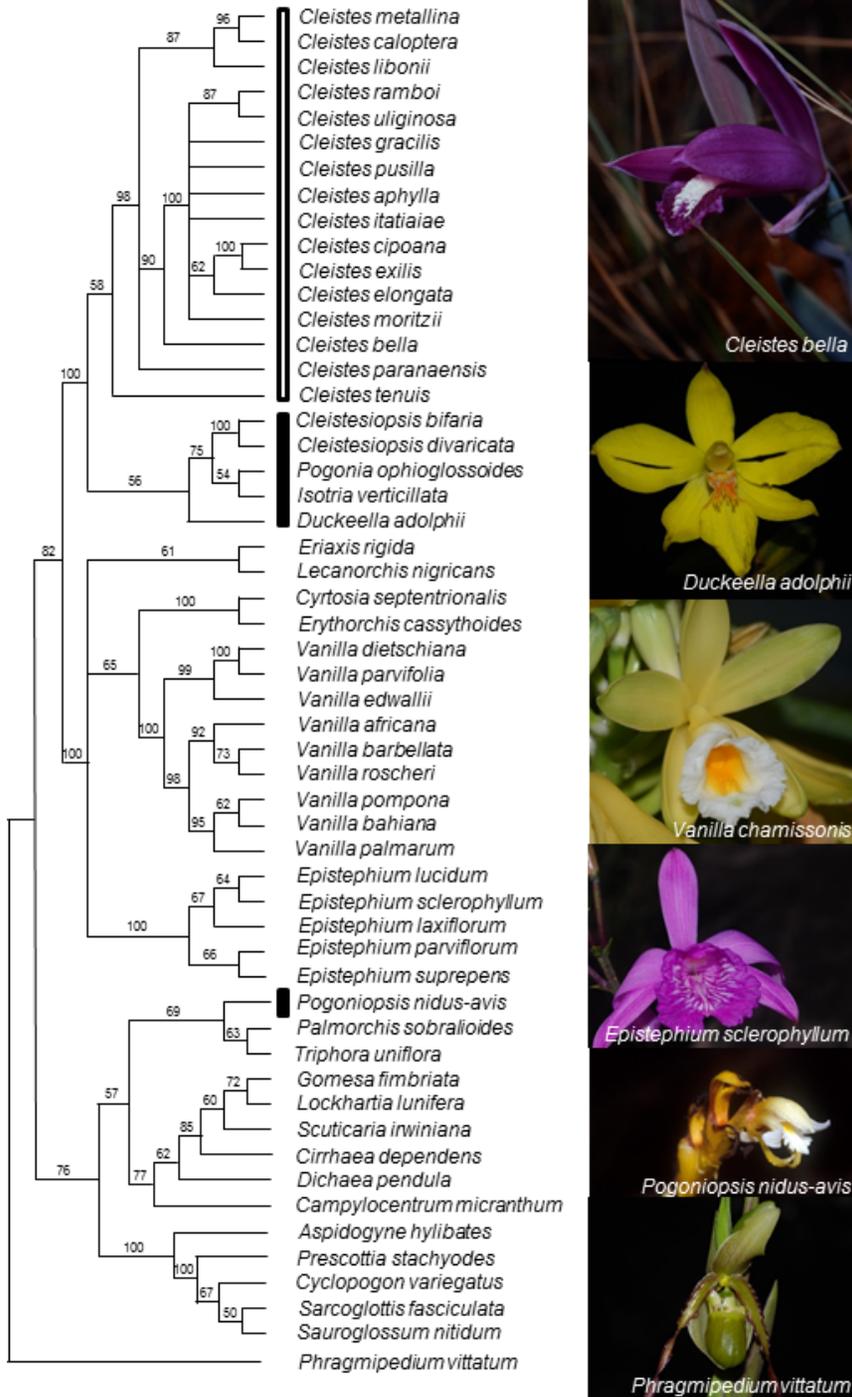


FIGURE 1. Maximum parsimony analyses based on combined 5.8S, 18S and 26S (nrDNA) regions of Pogonioideae (Orchidaceae, Vanilloideae). Bootstrap values >50 (MP) are given above branches. Vertical bars refer to tribe Pogonioideae. Empty bars = nectar-producing Pogonioideae (i.e., genus *Cleistes*). Full bars = rewardless Pogonioideae. Note that Vanilloideae and Pogonioideae, as currently circumscribed, are paraphyletic. Adapted from Pansarin *et al.* (2012).



FIGURE 2. *Vanilla dietschiana*. A. Habit. Note the stem with monopodial (pseudosympodial) growth; B. Flower. Note the green sepals and petals and the white tubular labellum with longitudinal keels on the median portion.

Vanilloideae has been considered as a monophyletic subfamily (Cameron, 2003, 2009). However, the inclusion of *Pogoniopsis* in the analysis turns Vanilloideae paraphyletic (Fig. 1; adapted from Pansarin *et al.* 2012). Vanilloideae is currently divided into the tribes Vanilleae and Pogonieae (Cameron 2003, 2009; Pansarin *et al.* 2012). Vanilleae is subcosmopolitan in distribution and comprises nine genera, namely *Clematepistephium*, *Cyrtosia*, *Epistephium*, *Eriaxis*, *Erythrorchis*, *Galeola*, *Lecanorchis*, *Pseudovanilla* and *Vanilla*. The genus *Vanilla* is pantropical and has been frequently assumed to be natural on the basis of its climbing habit and lateral inflorescences (Cameron & Molina 2006). However, the inclusion of the rare *Dictyophyllaria dietschiana* in the phylogenetic analyses makes the genus *Vanilla* paraphyletic (Pansarin 2010a, 2010b; Pansarin *et al.* 2012). Thus, *Dictyophyllaria* has been restored to *Vanilla* (Pansarin 2010a, 2010b; Pansarin *et al.* 2012). *Vanilla dietschiana* (Figs. 2A-B) is closely related to *V. edwallii* and *V. parvifolia* by floral and vegetative characters. *Vanilla dietschiana* is terrestrial, with monopodial (pseudosympodial) non-climbing habit. Its reduced leaves are pale green, reticulate-

veined and membranous, such as in *V. edwallii* and *V. parvifolia* (Pansarin 2010b).

The North American-Asiatic Pogonieae includes five genera: *Cleistis*, *Cleistesiopsis*, *Duckeella*, *Isotria* and *Pogonia* (Pansarin *et al.* 2008, 2012). The genus *Duckeella*, which is endemic to Amazonia, frequently emerges as sister to the rest of Pogonieae (Pansarin *et al.*, 2008, Pansarin *et al.* 2012). The genera *Isotria*, *Pogonia*, and *Cleistesiopsis* (Pansarin & Barros, 2008) form a temperate group, sister to the tropical *Cleistis*, a robustly supported South-Central American clade (*e.g.*, Pansarin *et al.* 2008, Pansarin *et al.* 2012).

Pogoniopsis, a myco-heterotrophic genus endemic to Brazilian forests, has been included in the tribe Pogonieae (Cameron, 2003, 2009), which has been assumed as monophyletic (Cameron & Chase 1999; Cameron *et al.* 1999). However, a phylogenetic analyses show that inclusion of *Pogoniopsis* turns the tribe paraphyletic (Fig. 1; Pansarin *et al.* 2012). All analyses reveal that *Pogoniopsis* is closely related to members of Epidendroideae (Fig. 1; Pansarin *et al.* 2012). *Pogoniopsis* have a sympodial habit with reduced leaves. The inflorescence is a terminal raceme (Fig. 3A). The flowers are whitish and the

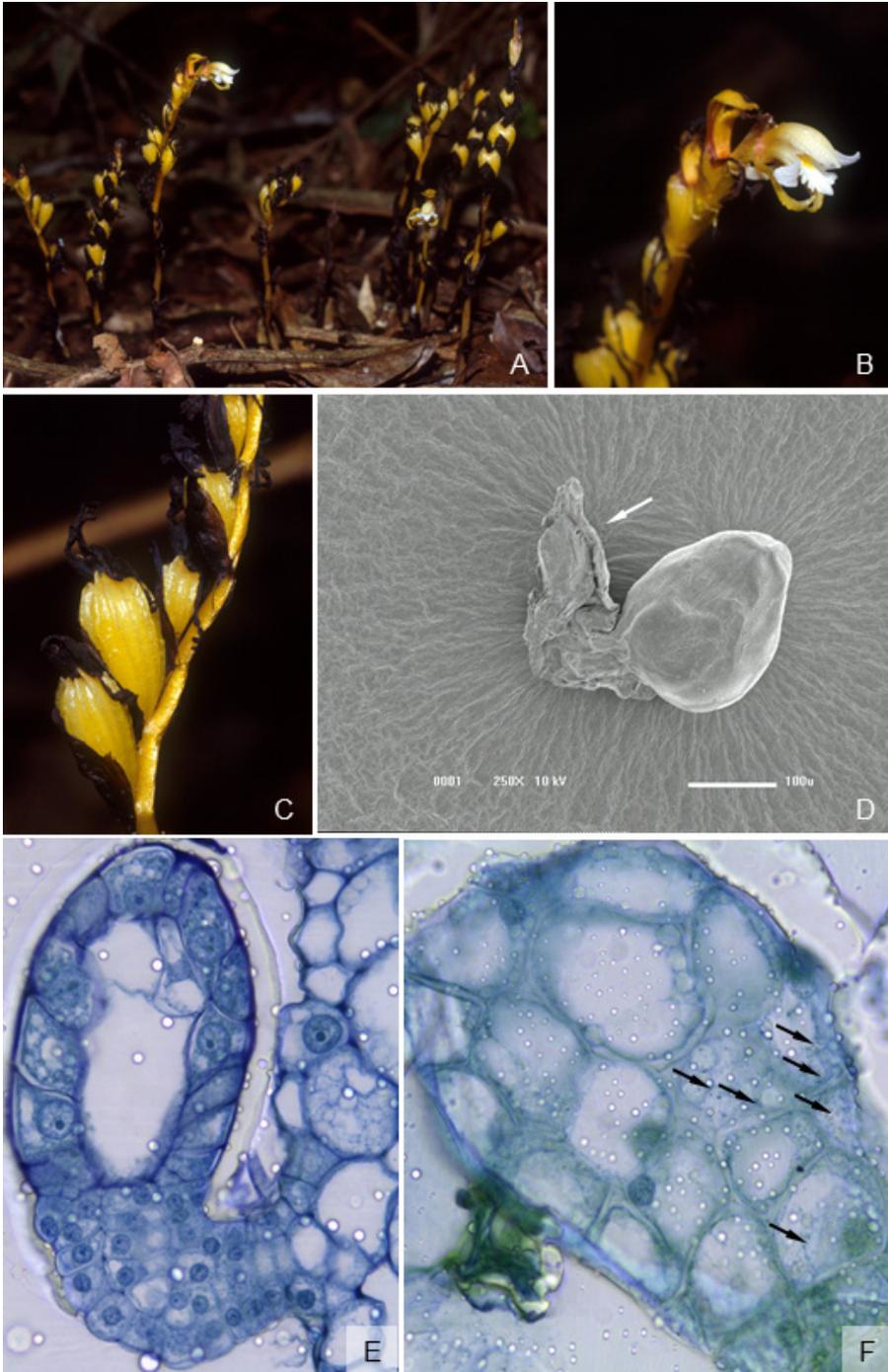


FIGURE 3. *Pogoniopsis nidus-avis*. A. Habit; B. Flower; C. Fruits (note the persistent perianth parts); D. Scanning Electron Microscopy (SEM) image of a sclerified seed. Note the remnant of the funiculus (arrow); E. Longitudinal cut of a seed stained with toluidine blue. Note the funiculus and the placenta; F. Longitudinal section of an ovule stained with toluidine blue showing the cells with large nuclei and densely stained cytoplasm. Note the granules, which are related to embryo nutrition (arrows).

perianth is persistent (Figs. 3A-B). The labellum is 3-lobed and presents clusters of yellow hairs on its mid portion. The lateral lobes are falcate and entire, while the apical lobe is spatulate and fringed. The pollen of *Pogoniopsis nidus-avis* is free and the anther is versatile. Furthermore, the flowers are characterized by the absence of the abscission zone between perianth and ovary (a characteristic common to members of Vanilloideae; Pansarin & Barros 2008, Pansarin *et al.* 2008, 2012), rewardless, flowers opening in succession, with fleshy and indehiscent fruits (Fig. 3C), and ovoid seeds with a crustose seed coat (Figs. 3C-F). The seeds of *Pogoniopsis* possess characteristics also found in *Vanilla* and *Palmorchis*. Longitudinal cuts of the seeds and ovules of *Pogoniopsis* reveal the cells possess large nuclei and densely stained cytoplasm. The cytoplasm possesses granules, related to embryo nutrition (E.R. Pansarin, unpubl. data; Figs. 3E-F).

Reproductive biology within Pogonieae. Floral biology and pollination systems of Pogonieae is currently well known, since all genera of this subtribe has been studied on this subject. Among the North American-Eastern Asiatic clade data are available for *Pogonia ophioglossoides* (Thien & Marcks 1972), *Pogonia japonica* (Matsui *et al.* 2001), *Isotria verticillata* and *I. medeoloides* (Mehrhoff 1983), in addition to *Cleistesiopsis divaricata* (syn. *Cleistis divaricata*) and *Cleistesiopsis bifaria* (syn. *Cleistis bifaria*; Gregg 1989, 1991a, 1991b). *Cleistesiopsis bifaria* and *C. divaricata* are pollinated by workers of *Bombus* and *Megachile* (Gregg 1989, 1991a, 1991b). *Isotria verticillata* is pollinated by small Halictidae, Anthophoridae and Andrenidae bees, while *I. medeoloides* is automatically self-pollinated (Mehrhoff 1983). *Pogonia ophioglossoides* is pollinated by queens and workers of bumblebees (Thien & Marcks 1972).

Pollination data regarding South-Central American clade (*i.e.*, genus *Cleistis*) are available to several species, including *C. libonii* (syn. *Cleistis macrantha*; Fig. 4A), *C. aphylla* (Fig. 4B), *C. exilis* (Fig. 4C), *C. pusilla*, *C. paranaensis* (Fig. 4D), *C. bella* (Fig. 4E) and *C. rosea* (Fig. 4F) (Pansarin 2003; Pansarin *et al.* 2012). The species are pollinated by solitary or social bees, except *Cleistis libonii* (Fig. 4A), which is co-pollinated by hermit hummingbirds (Pansarin 2003). According to Pansarin *et al.* (2012), the reproductive

phenology, the flowering strategies and the pollination mechanisms are similar among Brazilian *Cleistis*. In the spring (from October to November), each plant produces one unbranched aerial stem, with one erect and terminal raceme. Flowering generally occurs two or three months later, in the rainy season, from late December to early April. During a single flowering period, the species of *Cleistis* generally have three or four blooming peaks, each showing a precise synchronism in which all mature buds of most individuals within a population open simultaneously in the early morning. Flowering synchronism, as observed in all studied species of *Cleistis*, also has been recorded for basal Epidendroids, such as *Triphora trianthophora* (Medley 1979) and *Psilochilus modestus* (Pansarin & Amaral 2008).

The flowers of *Cleistis* possess two nectar glands at the base of the labellum, and a longitudinal and central crest with clusters of yellow or white colored hairs or papillae on the upper third, which act as a nectar guide. The column is parallel-disposed to the lip and presents a terminal versatile anther with two yellow clusters of free monads. The stigmatic surface is generally flat and possesses small and hyaline papillae. All studied species of *Cleistis* offer nectar as reward, which is produced by two nectar glands located on the base of the labellum. These glands are densely vascularized and the nectar is secreted along the nectary surface (Pansarin *et al.* 2012).

Duckeella adolphii possess lateral inflorescences with yellow flowers. The perianth parts are membranous. The basal portion of the labellum possesses a central crest covered by multicellular papillae. The elaiophor-like papillae are constituted by non-secretory cells.

Species of *Cleistis* and *D. adolphii* are dependent on pollen vectors for fruit setting. Coherent with the similarity among the floral morphology of the *Cleistis* flowers, the pollination mechanism is similar among *Cleistis*. The bees land on the lip apex and probe for nectar at its base. While the bees leave the flower, the anther is disarticulated with the scutellum and free pollen monads are placed on the scutum. Afterwards, the anther returns to the original position. In *Duckeella adolphii* the bees land directly on the central crest of the labellum, *i.e.*, on the elaiophor-like calluses (Pansarin 2003; Pansarin *et al.* 2012). Although the



presence of nectaries has been recorded at the base of the lip of *Cleistes* (Pansarin 2003, Pansarin *et al.* 2012), *Cleistesiopsis divaricata* and *Isotria verticillata* are pollinated by food deception, although both species bear nectar guides (Mehrhoff 1983; Gregg 1991a) and floral glands have been reported at the lip base of *Isotria verticillata* (Correll 1950). A hair-like cluster on the lip simulates pollen and attracts pollinators by deceit to the flowers of *Pogonia* (Dressler 1993) and *Cleistesiopsis bifaria* (Gregg 1989).

Like the North American genera *Cleistesiopsis*, *Pogonia* and *Isotria verticillata* (Thien & Marcks 1972, Mehrhoff 1983, Gregg 1989, 1991a), the South American *Cleistes* are pollinated exclusively by bees (Pansarin 2003, Pansarin *et al.* 2012). Visitation only on the first day of flower opening, even in species with longer lasting flowers, is probably related to the accumulation of the total volume of nectar at this time, no nectar production taking place afterwards. An exception is *C. libonii* whose flowers were visited also on the second day of anthesis (Pansarin 2003).

In Pogonieae the pollen is free and the pollen mass from a single flower, deposited on the scutum of a bee, may pollinate several flowers (Gregg 1991a, 1991b). Furthermore, some authors agree that flowering synchronism combined with the production of short-lived flowers may favor cross-pollination within a population (Medley 1979, Pansarin & Amaral 2008). According to Catling and Catling (1991), this kind of synchrony is an important strategy favoring cross-pollination in plants with one or few-flowered inflorescences. In fact, when all flowers in a population open simultaneously (*i.e.*, gregarious plants) the floral display for pollinator attraction is maximized (Pansarin *et al.* 2012).

The genus *Isotria* with floral glands at the lip base (Correll 1950, Cameron 2003) appears as sister to the remaining North American and Asiatic Pogonieae in some phylogenetic analyses based on chloroplast DNA (Pansarin *et al.* 2008). In the Amazonian *Duckeella*

adolphii and in all other North American and Asiatic species (*Pogonia*, *Cleistesiopsis divaricata* and *C. bifaria*), floral glands are absent (see Thien & Marcks 1972, Gregg, 1989, 1991a, 1991b, Matsui *et al.* 2001). It has been suggested that in Orchidaceae deceptive mechanisms evolved from reward pollination systems (Dafni 1984, Ackerman 1986, Nilsson 1992). In the case of Pogonieae, reward production could be tested on the basis of molecular phylogeny. Our data suggest that ancestors of Pogonieae gave rise to two lineages, one of them spreading into tropical America, eventually originating the extant Neotropical *Cleistes* species with nectariferous flowers, and another one predominantly North American-Asiatic, but including the Amazonian *Duckeella*, pollinated through deceptive mechanisms (Fig. 1).

Some author argue that nectar can be energetically expensive and resources for reward production could be allocated more usefully for other functions capable of increasing fitness in species pollinated through deception (Boyden 1982, Ackerman 1986). The main problem with this hypothesis is that in many orchids, fitness is pollination limited, rather than resource limited (Calvo & Horvitz 1990, Calvo 1993). Furthermore, although few studies have been performed on this subject (see Pyke 1991), the rate of nectar production tends to be lower in short-lived than in long-lived flowers (Johnson & Nilsson 1999). Coherently, flowers in nectar-producing tropical *Cleistes* are shorter lasting than in the deceptive *Isotria*, *Pogonia*, *Cleistesiopsis divaricata* and *C. bifaria* (Thien & Marcks 1972, Mehrhoff 1983, Gregg, 1989, 1991b). On the other hand, in comparison with mechanisms involving nectar production, rewardlessness may reduce the visitation frequency, consequently lowering the reproductive success (Dafni 1984, Ackerman 1986, Johnson & Nilsson 1999, Johnson, 2000). Another consequence of deceptive mechanisms may be the reduction of natural fruit set and thus fruits are formed through geitonogamy

Left, FIGURE 4. A-F. Species of Pogonieae and its pollinators. A. *Bombus atratus* probing for nectar at the lip base of *Cleistes libonii*. The detail shows the bee leaving a flower. Note some pollen on its scutum; B. *Dialictus* sp. leaving a flower of *C. aphylla*. Note the pollen on its scutum. The detail shows the bee probing for nectar at the lip base; C. *Augochlora* sp. leaving a flower of *C. exilis*. Note the pollen deposited on its scutum. The detail shows the bee probing for nectar at the lip base; D. Flower of *C. paranaensis*; E. Flower of *C. bella* in diagonal view showing the white hairs on the central labellar crest; F. *Xylocopa* sp. with its scutum covered with a pollen load leaving a flower of *C. rosea*. The detail is a *Xylocopa* sp. probing for nectar at the lip base.

(Ackerman 1986, Johnson & Nilsson 1999, Johnson 2000; Smithson 2002, Pansarin & Pansarin 2014a). These claims are supported by comparing plants from reward-producing and deceptive Pogonieae clades (Pansarin *et al.* 2012). North-American Pogonieae have low reproductive success (Thien & Marcks, 1972, Mehrhoff 1983, Gregg 1989, 1991b), comparing to Brazilian species (E.R. Pansarin, unpubl. data). In certain years, fruit set of some species of *Cleistes* (e.g., *C. rosea*, *C. libonii*, *C. metallina*, and *C. ramboi*) reached nearly 100% (E.R. Pansarin, unpubl. data). It is worth mentioning that production of one to few short-lived flowers by each inflorescence of Central and South American *Cleistes* and the precise flowering synchronization tend to reduce the occurrence of self-pollination and geitonogamy (Pansarin *et al.* 2012). Furthermore, orchids with soft pollen (such as occurs in all Pogonieae species; Cameron & Chase 1999, Pansarin *et al.* 2008) apparently are not prone to fruit set through geitonogamous self- (Johnson & Nilsson 1999). Self-pollination resulting from geitonogamy is considered a mechanism of fitness reduction because of depression by inbreeding and pollen loss.

Reproductive biology within Vanilleae. Data on reproductive biology available on Vanilleae are scarce. Studies on pollination biology within Vanilleae have been performed predominantly on species of *Vanilla* (e.g., Lubinsky *et al.*, 2006, 2010; Pansarin & Pansarin, 2014b; Pansarin *et al.*, 2014), which is the most diverse and economically important genus within Vanilloideae (Correll, 1953). The pollination system of *Epistephium* has been investigated more recently (Pansarin & Pansarin, 2014b). Within Vanilleae, euglossine bees have been recorded as pollinators of species of *Vanilla* (i.e., *V. planifolia* group and *V. pompona* group) in the Neotropics (e.g., Lubinsky *et al.*, 2006). Euglossine bees are also the main pollinators of *Epistephium sclerophyllum* and *V. dubia* in Brazil

(Pansarin *et al.*, 2014). In species of the *V. pompona* group, these bees are attracted by the fragrance of the flowers. They then display the typical behavior of pollination by male euglossine bees. Hummingbirds have been reported to pollinate some species of *Vanilla* (Bouriquet, 1954), while bats have been assumed to pollinate *V. chamissonis*, although this affirmation has been contested (Fleming *et al.*, 2009).

Vanilla insignis, *V. odorata* and *V. planifolia* are known to be pollinated through food deception (Soto Arenas *et al.*, unpubl. data). Due to low fruit set in natural populations, cultivated plants of *V. planifolia* are self-pollinated by hand to increase the production of fruits (Soto Arenas 1999). Some species of *Vanilla* yield fruits through spontaneous self-pollination (van der Pijl & Dodson 1966). This form of autogamy has been reported for *V. griffithii*, *V. palmarum*, populations of *V. planifolia*, and *V. savannarum* (see Pridgeon *et al.* 2003). Furthermore, flowers of a population of *V. bicolor* occurring in the Peruvian Amazon are obligatorily autogamous, producing fruits by cleistogamy (Van Dam *et al.* 2010).

Another Brazilian species that has been studied is *V. edwallii* (Fig. 5A), which is pollinated by *Epicharis* (Apidae: Centridini). *Vanilla edwallii* is rewardless, but male *Epicharis* are attracted to its flowers through the fragrance production (Pansarin *et al.* 2014). Additionally, Ruschi (1986) reports that *V. chamissonis* is visited by hummingbirds. However, in another study on this species, no pollinators were recorded, although fruits were observed in natural conditions (Reis *et al.* 2011). The flower morphology of *Vanilla* is very diverse, suggesting that different biotic vectors can act as pollinators of its species. Most species of *Vanilla* have tubular flowers where pollinators must enter through the tube formed by perianth and column (Dressler 1981). As occurs in *Vanilla*, the labellum margins of *Epistephium* are adnate to the column, resulting in a tubular flower (Dressler 1993).

Right, FIGURE 5. Species of Vanilleae and its pollinators. A. Flower of *Vanilla edwallii*. The detail shows a male *Epicharis* with pollen of *V. edwallii* on scutellum (arrow); B. Flower of *V. dubia*. The detail shows the cluster of penicillate hairs on the labellum (arrow); C. Labellum of *Epistephium sclerophyllum* in longitudinal section showing a *Bombus* (posed). Note its scutum just below the stigma and the articulated anther (arrow). The detail shows the cluster of hairs on the mid portion of the labellum (arrow); D. Labellum of *Epistephium sclerophyllum* in longitudinal section showing a bumblebee (posed). Note anther is disarticulated when the bee leaves the flower (arrow); E. *Plebeia droryana* collecting pollen directly from the anther. Note the corbiculae with pollen of the studied species; F. *Iridopelma* spider, capturing a pollinator (euglossine bee *Eufriesea nigrohirta*) on a flower of *E. sclerophyllum*.



Studies on the secretory tissues of Vanilleae are very scarce. In the rewardless *V. edwallii*, the only secretory tissues are osmophores located on labellum (Pansarin *et al.* 2014). In *V. edwallii*, the labellar trichomes produce a heterogeneous substance formed by mucilage and volatile compounds in the mentum region, which is associated with pollinator attraction (Pansarin *et al.* 2014). In this species the fragrance is produced by multicellular epidermal papillae in the median portion of labellum, between the lateral lobes and the entrance of the mentum region (Pansarin *et al.* 2014). In *Vanilla dubia*, the trichomes at the labellum base also secrete volatile substances, which are associated with pollinator attraction (Pansarin & Pansarin 2014b). Besides the oil droplets responsible for the odor of *Vanilla dubia*, starch grains were also observed within the labellar trichomes (Pansarin & Pansarin 2014b). Starch is a polysaccharide stored by plant cells as an energy source (González 1999). In contrast to that recorded for *V. edwallii* (Pansarin *et al.* 2014), in *V. dubia*, starch grains are exclusively present on the labellum trichomes (Pansarin & Pansarin 2014b). Starch grains were already reported in association with osmophores in other Orchidaceae (Davies & Turner 2004), but it is commonly associated with the production of mucilage in different plant organs, including orchids (Leitão & Cortelazzo 2008).

Vanilla dubia is included in the *Vanilla planifolia* group, which includes vanillas with greenish flowers and a concave labellum with small papillae. The South American taxa of this group present a penicillate callus at the median portion of their labellum (Fig. 5B; Soto Arenas & Cribb 2010). Within this group, in addition to *V. dubia*, food deception has also been reported in *V. insignis*, *V. odorata* and *V. planifolia* (Soto Arenas *et al.*, unpubl. data). Pollination by male euglossine bees also has been reported for species of *Vanilla hostmanii* group: *V. cribbiana*, *V. dressleri* *V. hameri*; *Vanilla pompona* group: *V. pompona*, and *Vanilla trigonocarpa* group: *V. trigonocarpa* (Soto Arenas 1999, Pridgeon *et al.* 2003, Lubinsky *et al.* 2006, Soto Arenas & Dressler 2010), although the mechanisms involving fragrance collection are still unknown (Rodolphe *et al.* 2011). In *V. edwallii*, although fragrance is related to attracting male *Epicharis* (Apidae: Centridini) patrolling the flowers and maintaining them nearby, it is not a harvestable resource. *Vanilla edwallii* is a species

of the *Vanilla parvifolia* group, which includes the reticulate-veined vanillas (Soto Arenas & Cribb 2010). *Vanilla inodora* (*Vanilla mexicana* group) seems to be pollinated by *Xylocopa* spp., although no evidence of pollination by these carpenter bees has been presented (Soto Arenas & Dressler 2010). An unidentified species of *Xylocopa* was also recorded on *V. edwallii*, but it only acted as a floral visitor, perforating the base of the labellum in search for nectar (Pansarin *et al.* 2014).

The nectaries of *Epistephium sclerophyllum* do not present differentiated structures, as occurs in genus *Cleistes*, whose nectar is produced by nectar glands (Pansarin 2003, Pansarin *et al.* 2012). Rather, they are flat and composed by a single-layered epidermis with rectangular cells and three or four layers of (rectangular) parenchymal cells, supplied by vascular bundles, like in other orchids (Stpiczyńska & Davies 2006). The similarity between the epidermal cells and the underlying parenchyma results in a nectary with a homogeneous pattern.

As recorded for *Vanilla edwallii* (Pansarin *et al.* 2014), Centridini bees (male *Centris*) were also observed patrolling the open flowers of *Epistephium sclerophyllum* waiting for females to mate. However, the flowers of *E. sclerophyllum* produce a minimal quantity of diluted nectar, which is probed by several bee species (Fig. 5C-F). In addition to nectar, pollen was directly collected from anthers by Meliponini bees (Fig. 5E; Pansarin & Pansarin 2014b). Since, in most Orchidaceae, pollen is aggregated and forms indivisible pollinia (*e.g.*, Dressler 1993), the offering of pollen as a resource is rare among orchids. The flowers of *Epistephium sclerophyllum* are scentless and bee attraction seems to be essentially visual, while the fragrance of the flowers of *Vanilla dubia* attracts male euglossine. Despite the presence of osmophores, the bees entering into the labellar tube appeared to be interested in nectar, not fragrances (Figs. 5C-D; Pansarin & Pansarin 2014b). The pollination mechanism in *Epistephium* is similar to that recorded in *Cleistes* (Pansarin 2003, Pansarin *et al.* 2012, Pansarin & Pansarin 2014b). The bees land on the labellum apex and probe for nectar at its base (Fig. 5C). As the bees leave the flower, the anther is disarticulated with the scutellum and free pollen monads placed on the thorax (Fig. 5D). Afterwards, the anther returns to the original position (Fig. 5C). Although pollination by other

bee groups can occur (*E. sclerophyllum*; Figs. 5C-F), the flowers of both studied species are adapted to pollination by euglossines (Fig. 5F). Within Vanilloideae, data are still inconclusive, but *Epistephium*, which, as recorded here for *E. sclerophyllum*, has nectariferous flowers, emerges as a sister to the rest of tribe. Within the monophyletic genus *Vanilla*, although more data on pollination biology are still needed, deceptive and rewarding species have been recorded (see Pansarin *et al.* 2014), suggesting that shifts between rewarding and rewardless species have happened more than once during the evolution of a genus (Fig. 1; Pansarin *et al.* 2012). This is reinforced by our recent observations, in the Amazonian forest, on the pollination biology of *Vanilla palmarum*, whose flowers produce nectar and are visited by hummingbirds (E.R. Pansarin and A.W.C. Ferreira, unpubl. data).

Although studies on vanilloid species pollinated by biotic vectors are scarce (exceptions in Lubinsky *et al.* 2006, 2010, Pansarin & Pansarin 2014b, Pansarin *et al.* 2014), spontaneous self-pollination has been recorded for some taxa, as *V. bicolor* (Van Dam *et al.* 2010), *Vanilla griffithii*, *V. palmarum* and *V. savannarum* (Pridgeon *et al.* 2003). Probably this is also the case with *Epistephium sclerophyllum*, whose stigmatic surface is not clearly separated from the anther (Pansarin & Pansarin 2014b). In *E. sclerophyllum* despite the formation of fruits through autogamy, bees act as pollinators, thus contributing to the possible formation of fruits through cross-pollination.

ACKNOWLEDGEMENTS. The author thanks Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (grants 00/11015-9 and 07/07445-7), and Conselho Nacional do Desenvolvimento Científico e Tecnológico (grant 454490/2015-1) for funds provision.

LITERATURE CITED

- Ackerman, J. D. (1986). Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* 1, 108–113.
- Bouriquet, G. (1954). Culture. In: G. Bouriquet (Ed.). *Le vanillier et la vanille dans le monde* (pp. 430–458). Paris: Paul Lechevalier.
- Boyden, T. C. (1982). The pollination biology of *Calypso bulbosa* var. *americana* (Orchidaceae): initial deception of bumblebee visitors. *Oecologia* 55, 178–184.
- Calvo, R. N. & Horvitz, C. C. (1990). Pollinator limitation, cost of reproduction, and fitness in plants, a transition matrix demographic approach. *The American Naturalist* 136, 499–516.
- Cameron, K. M. (2003). Vanilloideae. In: A. Pridgeon, P. Cribb, M. W. Chase & F. Rasmussen (Eds.). *Genera Orchidacearum* (pp. 281–334). Oxford: Oxford University Press.
- Cameron, K. M. (2009). On the value of nuclear and mitochondrial gene sequences for reconstructing the phylogeny of vanilloid orchids (Vanilloideae, Orchidaceae). *Annals of Botany* 104, 377–385.
- Cameron, K. M. & Chase, M. W. (1999). Phylogenetic relationships of Pogoniinae (Vanilloideae, Orchidaceae): an herbaceous example of the eastern North America – eastern Asia phylogeographic disjunction. *Journal of Plant Research* 112, 317–329.
- Cameron, K. M., Chase, M. W., Whitten, W. M., Kores, P. J., Jarrell, D. C., Albert, V. A., Yukawa, T., Hills, H. G. & Goldman, D. H. (1999). A phylogenetic analysis of the Orchidaceae: evidence from *rbcL* nucleotide sequences. *American Journal of Botany* 86, 208–224.
- Cameron, K. & Molina, M. C. (2006). Photosystem II gene sequences of *psbB* and *psbC* clarify the phylogenetic position of *Vanilla* (Vanilloideae, Orchidaceae). *Cladistics* 22, 239–248.
- Catling, P. M. & Catling, V. R. (1991). A synopsis of breeding systems and pollination in North American orchids. *Lindleyana* 6, 187–210.
- Correll, D. S. (1950). *Native orchids of North America*. Waltham: Chronica Botanica.
- Correll, D. S. (1953). *Vanilla*- its history, cultivation and economic importance. *Economic Botany* 7, 291–358.
- Dafni, A. (1984). Mimicry and deception in pollination. *Annual Review of Ecology and Systematics* 15, 259–278.
- Darwin, C. (1892). *The various contrivances by which orchids are fertilized by insects*. 2ND ed. rev. (1st ed. 1862). New York: Appleton.
- Dressler, R. L. (1981). *The orchids: natural history and classification*. Cambridge: Harvard University Press.
- Dressler, R. L. (1993). *Phylogeny and Classification of the Orchid Family*. Portland: Dioscorides Press.
- Fleming, T. H., Geiselman, C. & Kress, W. J. (2009). The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany* 104, 1017–1043.
- Davies, K. L. & Turner, M. P. (2004). Morphology of floral papillae in *Maxillaria Ruiz & Pav.* (Orchidaceae). *Annals of Botany* 93, 75–86.
- González, M. L. (1999). Polisacáridos. In: A. M. Del Fresno (Ed.). *Farmacognosia General* (pp. 335). Madrid: Ed. Síntesis.
- Gregg, K. B. (1989). Reproductive biology of the orchid *Cleistes divaricata* (L.) Ames var. *bifaria* Fernald growing in a West Virginia meadow. *Castañea* 54, 57–78.

- Gregg, K. B. (1991a). Reproductive strategy of *Cleistes divaricata* (Orchidaceae). *American Journal of Botany* 78, 350–360.
- Gregg, K. B. (1991b). Defrauding the deceitful orchid: pollen collection by pollinators of *Cleistes divaricata* and *C. bifaria*. *Lindleyana* 6, 214–220.
- Johnson, S. D. (2000). Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behavior. *Biological Journal of the Linnean Society* 71, 119–132.
- Johnson, S. D. & Nilsson, L. A. (1999). Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. *Ecology* 80, 2607–2619.
- Leitão, C. A. E. & Cortelazzo, A. L. (2008). Structural and histochemical characterization of the collectors of *Rodriguezia venusta* (Orchidaceae). *Australian Journal of Botany* 56, 161–165.
- Lubinsky, P., Van Dam, M. H. & Van Dam, A. R. (2006). Pollination of *Vanilla* and evolution in Orchidaceae. *Lindleyana* 75, 926–929.
- Lubinsky, P., Romero-González, G. A., Heredia, S. M. & Zabel, S. (2010). Origins and patterns of vanilla cultivation in tropical America (1500–1900): no support for an independent domestication of vanilla in South America. In: D. Havkin-Frenkel & F. C. Belanger (Eds.). *Handbook of vanilla science and technology* (pp. 117–138). Oxford: Wiley-Blackwell.
- Matsui, K., Ushimaru, T. & Fujita, N. (2001). Pollinator limitation in a deceptive orchid, *Pogonia japonica*, on a floating peat mat. *Plant Species Biology* 16, 231–235.
- Medley, M. E. (1979). *Some aspects of the life story of Triphora trianthophora* (Sw.) Rydb. (Three birds orchid) with reference to its pollination. MD Thesis. St Andrews: Andrews University.
- Mehrhoth, L. A. (1983). Pollination in the genus *Isotria* (Orchidaceae). *American Journal of Botany* 70, 1444–1453.
- Nilsson, L. A. (1992). Orchid pollination biology. *Trends in Ecology & Evolution* 7, 255–259.
- Pansarin, E. R. (2003). Biología floral de *Cleistes macrantha* (Barb. Rodr.) Schltr. (Orchidaceae: Vanilloideae: Pogoniinae). *Revista Brasileira de Botânica* 26, 73–80.
- Pansarin, E. R. (2010a). *Vanilla dietschiana*, returns from *Dictyophyllaria*. *Orchids* 79, 106–109.
- Pansarin, E. R. (2010b). Taxonomic notes on Vanilleae (Orchidaceae: Vanilloideae): *Vanilla dietschiana*, a rare south American taxon transferred from *Dictyophyllaria*. *Selbyana* 30, 198–202.
- Pansarin, E. R. & Amaral, M. C. E. (2008). Pollen and nectar as a reward in the basal epidendroid *Psilochilus modestus* (Orchidaceae: Triphoreae): A study of floral morphology, reproductive biology and pollination strategy. *Flora (Jena)* 203, 474–483.
- Pansarin, E. R. & Barros, F. (2008). Taxonomic notes on Pogonieae (Orchidaceae): *Cleisteslopsis*, a new genus segregated from *Cleistes*, and description of two new South American species, *Cleistes batistana* and *C. elongata*. *Kew Bulletin* 63, 441–448.
- Pansarin, E. R., Salatino, A. & Salatino, M. L. F. (2008). Phylogeny of South American Pogonieae (Orchidaceae, Vanilloideae) based on sequences of nuclear ribosomal (ITS) and chloroplast (*psaB*, *rbcL rps16*, and *trnL-F*) DNA with emphasis on *Cleistes* and discussion of biogeographic implications. *Organisms, Diversity and Evolution* 8, 171–181.
- Pansarin, E. R., Salatino, A., Pansarin, L. M. & Sazima M. (2012). Pollination systems in Pogonieae (Orchidaceae: Vanilloideae): A hypothesis of evolution among reward and rewardless flowers. *Flora (Jena)* 207, 849–861.
- Pansarin, E. R., Aguiar, J. M. R. B. V. & Pansarin, L. M. (2014). Floral biology and histochemical analysis of *Vanilla edwallii* Hoehne (Orchidaceae: Vanilloideae): an orchid pollinated by *Epicharis* (Apidae: Centridini). *Plant Species Biology* 29, 242–252.
- Pansarin E. R. & Pansarin L. M. (2014a). Reproductive biology of *Epidendrum tridactylum* (Orchidaceae: Epidendroideae): a reward-producing species and its deceptive flowers. *Plant Systematics and Evolution* 300, 321–328.
- Pansarin, E. R. & Pansarin, L. M. (2014b). Floral biology of two Vanilloideae (Orchidaceae) primarily adapted to pollination by euglossine bees. *Plant Biology (Stuttgart)* 16, 1104–1113.
- Pridgeon, A. M., Cribb, P. J., Chase, M. W. & Rasmussen, F. N. (2003). *Genera Orchidacearum, vol. 3, Orchidoideae (Part 2), Vanilloideae*. London: Oxford University Press.
- Pyke, G. H. (1991). What does it cost a plant to produce floral nectar? *Nature* 350, 58–59.
- Reis, C. A. M., Brondani, G. E. & Almeida, M. de (2011). Biología floral e propagação vegetativa de baunilha. *Scientia Agraria Paranaensis* 10, 69–82.
- Rodolphe, G., Séverine, B., Michel, G. & Pascale, B. (2011). Biodiversity and evolution in the *Vanilla* genus. In: O. Grillo & G. Venora (Eds.). *The dynamical processes of biodiversity - case studies of evolution and spatial distribution* (pp. 1–27). Rijeka: InTech.
- Ruschi, A. (1986). *Aves do Brasil*. Rio de Janeiro: Expressão e Cultura.
- Smithson, A. (2002). The consequences of rewardlessness in orchids: reward-supplementation experiments with *Anacamptis morio* (Orchidaceae). *American Journal of Botany* 89, 1579–1587.
- Soto Arenas, M. A. (1999). *Filogeografía y recursos genéticos de las vanillas de México*. México

- DF: Instituto Chinoin AC. Informe final SNIB-CONABIO proyecto No. J101.
- Soto Arenas, M. A. & Cribb, P. (2010). A new infrageneric classification and synopsis of the genus *Vanilla* Plum. ex Mill. (Orchidaceae: Vanillinae). *Lankesteriana* 9, 355-398.
- Soto Arenas, M. A. & Dressler, R. L. (2010). A revision of the Mexican and Central American species of *Vanilla* Plumier ex Miller with a characterization of their ITS region of the nuclear ribosomal DNA. *Lankesteriana* 9, 285-354.
- Stpiczyńska, M. & Davies, K. L. (2006). Nectary structure in *Symphoglossum sanguineum* (Rchb.f.) Schltr. (Orchidaceae). *Acta Agrobotanica* 59, 7-16.
- Thien, L. B. & Marcks, B. G. (1972). The floral biology of *Arethusa bulbosa*, *Calopogon tuberosus*, and *Pogonia ophioglossoides* (Orchidaceae). *Canadian Journal of Botany* 50, 2319-2325.
- Van Dam, A. R., Householder, J. E. & Lubinsky, P. (2010). *Vanilla bicolor* Lindl. (Orchidaceae) from the Peruvian Amazon: auto-fertilization in *Vanilla* and notes on floral phenology. *Genetic Resources and Crop Evolution* 57, 473-480.
- van der Pijl, L. & Dodson, C. H. (1966). *Orchid flowers, their pollination and evolution*. Florida: University of Miami Press.

