The Leafless *Vanilla* Species-Complex from the South-West Indian Ocean Region: A Taxonomic Puzzle and a Model for Orchid Evolution and Conservation Research

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Abstract: The pantropical genus *Vanilla* is a member of the Orchidaceae family, one of the largest and most diverse families of angiosperms in the world. It originated in America and differentiated in America, Africa, and Asia. About 132 species of *Vanilla* have now been accepted since the discovery of the genus. Within the *Vanilla* genus, leaflessness has appeared at least three independent times, in Africa, Asia, and America as an adaptation to drought conditions. The South-West Indian Ocean (SWIO) region, a biodiversity hotspot for orchids, is home to seven leafless species. Chloroplast markers failed to differentiate species within this recent SWIO clade. Morphological traits used for description are very similar between species and do not provide reliable identification. Moreover, some of the species have overlapping flowering periods and geographic distribution in Madagascar, increasing the probability of finding some sympatric species and, therefore, hybrids. Leafless *Vanilla* species from the SWIO islands are thus an excellent model for understanding the evolution of orchids. For their conservation, and to confirm the identity of these leafless species, an integrated approach with classical taxonomy using a large number of samples, intense fieldwork on biology and ecology, and molecular studies using variable markers is necessary.

Keywords: *Vanilla*; leafless; South West Indian Ocean; diversification; taxonomy; biology; ecology; diversity; ethnobotany

1. Introduction

With more than 24,000 species and 700 genera worldwide, Orchidaceae is one of the largest families of flowering plants [1,2], and originated around 83 Mya (Million years ago) [3,4]. Orchids are widely celebrated for their beautiful flowers, extraordinary diversity, and potential medicinal proprieties. For example, among the 494 terrestrial and epiphytic orchid species recorded in Southern Africa, 49 are used as traditional medicinal plants to treat a wide range of diseases [5]. The South-West Indian Ocean (SWIO) islands are one of the global hotspots for orchids. The Orchidaceae family is represented by 65 genera and over 1000 species in this region [6]. Madagascar is the richest, with approximately 862 species, of which more than 85% are endemics [7], and 57 genera [8]. The Orchidaceae genus *Vanilla* can be considered as a flagship genus in the SWIO region, because the genus is represented

there by wild, as well as cultivated, genetic resources. This region ranks first in terms of leafless *Vanilla* species richness [9,10]. It is also renown worldwide for its *Vanilla* fruit production, with the islands of Madagascar, Comoros and La Réunion being amongst the first world producers [11].

This paper provides an updated bibliographical review about the *Vanilla* genus. It is enriched by our own observations, and is focused on leafless species, particularly those indigenous and endemic to the SWIO islands (Madagascar, Comoros, Mayotte, Seychelles) and the East coast of Southern Africa. Some elements about the complexity of their taxonomy will be discussed.

2. The *Vanilla* Genus and Leafless Species

2.1. Botany

Vanilla plants are perennial vines characterized by thick and fleshy stems [12], a monopodial growth habit, aerial roots growing at each node, absence of pseudobulbs, fleshy fruits and wingless seeds [9]. The inflorescence is axillary [13]. The aerial roots are hairless, while the underground roots are hairy [14,15]. Leafless *Vanilla* species share most vegetative characteristics with leafy species, but they are photosynthetic plants with green, sometimes reddish to glaucous stems, distinguished by the absence of leaves or the modification of the leaves into scale-like structures [10,16]. Also, as opposed to leafy species, green stems of leafless species do not have a sclerenchyma band separating cortex from ground tissue [14]. Two types of roots were identified in leafless species from Madagascar: short, flattened or filiform roots used for attachment to a supporting tree, and long aerial roots up to 15 m exploring the soil for nutrition [15]. The same study demonstrated that populations in the north, where rainfall is higher, have thinner aerial roots with smaller xylem vessels than those in the arid south [15]. This is probably one of the developed strategy of succulent plants to face the scarcity or abundance of water in the soil [17]. Floral morphology is diverse but, in general, flowers are composed of three sepals, two petals, and a third large petal called labellum [10,12] forming a funnel surrounding the column [13]. Pollinia and stigma are separated by a membrane called the "rostellum". It usually prevents self-pollination within the same flower, although species are self-compatible [18,19]. The number of opened flowers per day varies from one to several, depending on the species. For Mexican leafy species, for example, it is generally one (e.g., *V. phaeantha*) to three (e.g., *V. calyculata*) [20]. In Madagascar, we observed that the number of opened flowers per inflorescence per day can go up to three in the leafless white-flower species (*V. madagascariensis*, *V. decaryana*, *V. bosseri*), whereas this number is on average one flower and rarely two in the yellow-flower populations of Montagne des Français (*V. humblotii*). This is also the case of *V. humblotii* from Mayotte, for which on average 1.84 flowers open per day and per individual [21]. Flowers usually open in succession and are short lived (one day) (e.g., in leafless *V. barbellata*, *V. claviculata*, *V. dilloniana* [22]) but can remain for two or three days for some species like the leafy species *V. inodora* [20]. Unfertilized flowers rapidly fall, otherwise they turn into fruits in the form of an elongated capsule, called a "pod".

2.2. Taxonomy

The genus *Vanilla* Plum. ex Miller forms a monophyletic group (34.6 Mya) (Figure 1) in the large Orchidaceae family, the Vanilloidae sub-family, the Vanilleae tribe and the Vanillinae sub-tribe [1,10,16,23].
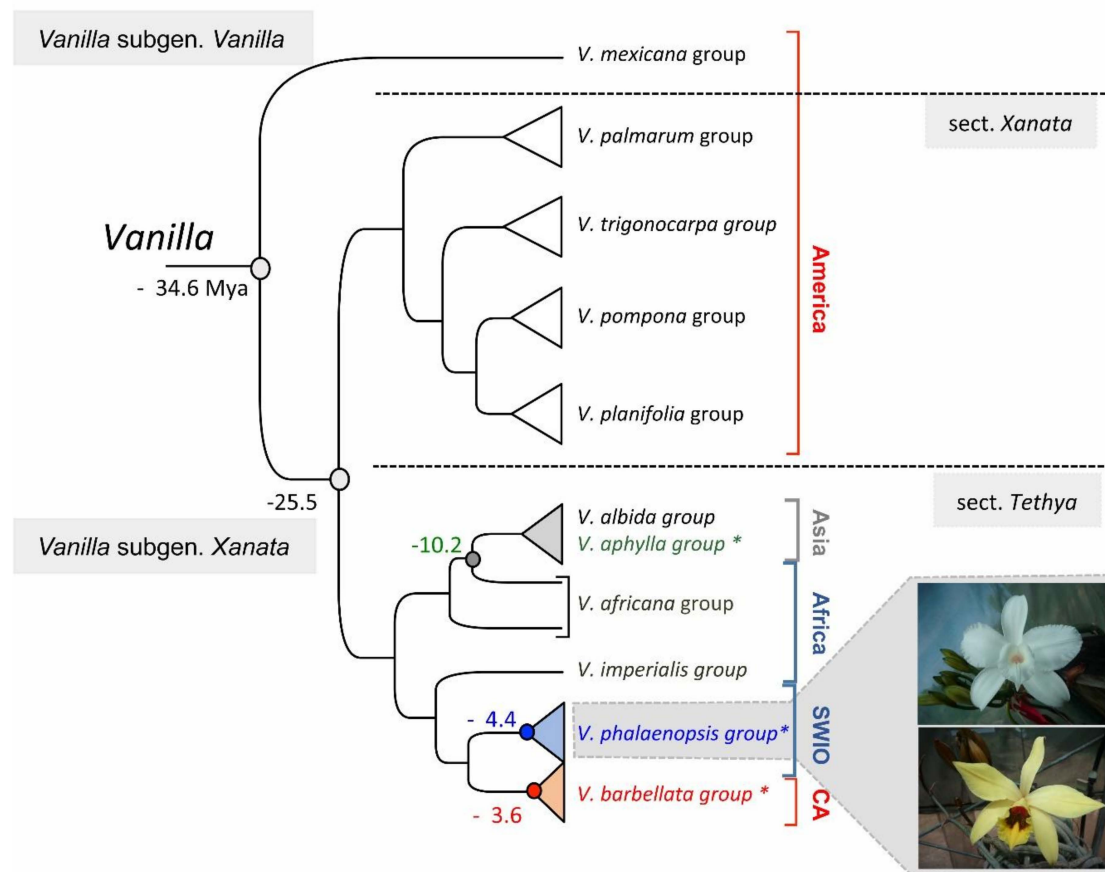
Figure 1. Representation of the (rbcL, psaB, psbB, psbC) phylogeny of Bouetard et al. in 2010 including species groups defined by Soto Arenas and Cribb in 2010, showing leafless *Vanilla* species names in color (green for Asia, blue for SWIO, red for Caribbean CA) [3,9]. Node dates in Mya correspond to datations obtained by Bouetard et al., with the datation of *Vanilla* node at −34.6 Mya estimated using 71 Mya as the minimum age for Vanilloideae published by Ramírez et al., 2007, based on fossil evidence of an orchid pollinarium (of *Meliorchis caribea*) attached to an extinct stingless bee (*Problebeia dominica*) recovered in Miocene amber [3,4].

The first *Vanilla* subgeneric classification was essentially based on morphological descriptions and distinguished leafless species (Aphyllae section) with scale-like leaves, from leafy species (Foliosae section) which have conspicuous and persisting leaves [10,23]. The latter was divided into three sub-sections by Portères on the basis of labellum and leaf characteristics: Membranaceae, Papillosae, and Lamellosae [10,16]. Since the development of molecular approaches, which are playing an important role in plant taxonomy by allowing reliable phylogenetic-based classifications [24], phylogenetic markers (matK, rbcL, psaB, psbB, psbC, ITS) and chemical analyses have increasingly been used in *Vanilla* taxonomy [3,9,16,20,25]. A revision of the genus was done in 2010 by Soto Arenas and Cribb, who confirmed that *Vanilla* is monophyletic but noticed that the sections and subsections defined by Portères in 1954 are polyphyletic and incongruent with cladistic analysis [9]. Consequently, they proposed a new *Vanilla* classification based on phylogenetic and morphological reconstructions with two subgenera and 20 morphological groups [9]. The *Vanilla* subgenus is composed by species with membranaceous leaves, inflorescences poorly differentiated from the vegetative axis, a labellum without penicillar callus, a column united to the labellum only at the base, a sub-perpendicular anther and a concave stigma [9]. It is represented by *V. mexicana*, the type specimen for the *Vanilla* genus (Figure 1) [23].

The leafless species are now included in the other subgenus, *Xanata*, together with species with coriaceous to fleshy leaves [9]. Their inflorescences are well distinct from the vegetative axis,

they have a column joined to the labellum at least on half of its length, and a parallel anther [9]. This second subgenus is further divided into two sections: the *Xanata* section comprising mostly the leafy neotropical species (American) and divided into six groups, and the *Tethya* section including the leafy and leafless paleotropical species (African and Asian) and Caribbean leafless species [9,16]. The same year, Bouetard et al. proposed a phylogeographic classification based on chloroplast DNA sequencing which was congruent with the revision by Soto Arenas and Cribb (Figure 1) [3,9]. It also showed that leaflessness is polyphyletic and has appeared at least three independent times, in Africa, Asia and America as a convergent adaptation to drought conditions following coastal establishments and island colonization [3]. The leafless species all belong to the subgenus *Xanata* section *Tethya,* except *V. penicillata* which is the only species included in section *Xanata* [9]. Also, this chloroplast phylogeny showed the surprising result that the Caribbean leafless species originated from an African ancestor, via transoceanic dispersion to America, rather than from an American leafy species [3]. This molecular data contradicts previous suggestions made by Portères in 1954, that leafless *Vanilla* species from the three geographical distributions (SWIO, Asia and Caribbean) originated from leafy species from neighboring continents [10].

2.3. Evolution

Vanilla is a transoceanic genus, found only in tropical forests between the 27th north and south parallels, but absent in Australia (Figure 2).

According to Rolfe, the diversification of the genus happened in Brazil and Guiana [23]. However, based on floral morphology, Portères suggested a primary diversification center in Indo-Malaysia that has evolved into two groups (Madagascar, Mascarene Islands, Africa versus Asia and Western Pacific islands), with the American species resulting from the migration of Asian species during the Tertiary (65.5–2.5 Mya) [10]. But the recent molecular phylogeny rejected this theory of Portères and suggested a South American center of diversity [3]. In 2005, Cameron stated that the differentiation of the genus *Vanilla* would have taken place before the dissolution of Gondwana [26], about 129 Mya [3]. However, following a fossil-based tree dating in Orchids [4], Bouetard et al. supported the alternative theory that *Vanilla* genus probably appeared 34 Mya (Figure 1), and its migration from the New World to the Old World occurred at the beginning of the Tertiary, about 25 Mya via a transoceanic dispersion, facilitated by the proximity of the continents and the presence of the series of islands of the mid-oceanic ridge of the Rio [3]. Three obvious cases of transoceanic migration events from Africa were also noted: (1) the dispersal of the genus from Africa to Asia; (2) from Africa to the SWIO islands and finally (3) from Africa to the Caribbean islands [3], the two last events concerning therefore the evolution of leafless groups in the SWIO and Caribbean islands.
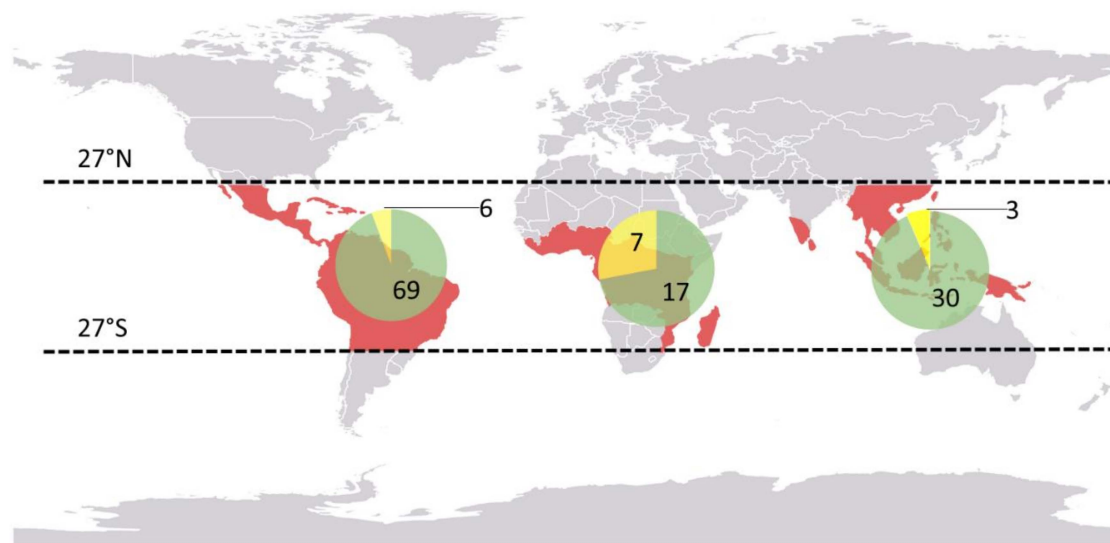
Figure 2. World distribution of *Vanilla* species: number of leafy *Vanilla* species (in green) and number of leafless species (in yellow) based on the World checklist of Selected Plant Families [27], the International Plant Name Index [28] and the database of Tropicos [29].

2.4. Ecology

Vanilla orchids are thermophilous [14]. These terrestrial, epiphytic or hemi-epiphytic plants are adapted to a variety of habitats [10,14,16,20], although they are scarce on mountain rain forest [14]. Lamellosae are found in fresh and wet places. They are mainly ombrophilous to mesophilous with various levels of heliophilia [10]. Papillosae species are also sciaphilous, living in rainforest formations, to heliophilous, sometimes growing in tropophilous and sub-xerophilous formations [10]. *V. organensis*, *V. palmarum*, *V. wrightii* and relatives without lamellae and with column fused to the labellum can be found in more mesophilic or even tropophilic formations in the area of Brazilian palm groves or wooded savannas [10]. Species with longitudinal lamellae and aromatic fruits are distributed in coastal or sublittoral forests [10]. However, some can be found in uncommon habitats like marshy forests (e.g., *V. arcuata* and *V. paludosa* [30]). While most species can climb to various substrates (trees and rocks), an epiphytic *Vanilla* (*V. palmarum*) is exclusively associated with nine species of palms in South America [31]. Some species can be found in different types of ecosystems like *V. inornata* [32]. There are species that thrive on particular substrates such as *V. martinezii* growing on an unusual peat-like soil [20], *V. planifolia* alliance in Central America restricted to calcareous or sedimentary substrate [14] and *V. insignis* found on seasonally dry volcanic areas [14]. *Vanilla* species are often associated with mycorrhizal fungi [15,16,33]. For example, studies in Puerto Rico, Cuba and Costa Rica recorded several species belonging to three genera of mycorrhizal fungi (*Ceratobasidium*, *Thanatephorus* and *Tulasnella*) in *V. potaei* and *V. planifolia* [33].

Aphyllae is the only group that contains species adapted to hot, xeric, full sun habitats [10,14,16]. They grow on coastal bushes (e.g., *V. roscheri* and *V. decaryana*), in very dry tropophilous forests (e.g., *V. madagascariensis*), on sandy soils (e.g., *V. perrieri*, *V. bosseri*, *V. aphylla*), on rocks (e.g., *V. humblotii*, *V. phalaenopsis* and *V. marmoreisense*), on coral reefs (*V. roscheri*) but also in mangrove swamps (e.g., *V. barbellata* and *V. dilloniana*) or in humid forests (*V. madagascariensis*) (Figure 3A,B) [8,10,34–36].

Figure 3. Ecology and usages of leafless *Vanilla* species: (**A**) *V. phalaenopsis* individual growing on a rock in Seychelles, (**B**) *V. decaryana* plant in thorny bushes in the south of Madagascar (Antandroka) and (**C**) leafless *Vanilla* stems for sale on the Tuléar market (Madagascar). (Photographies A, C: Pascale Besse, B: Cathucia F. Andriamihaja).

Leafless species are exclusively found on islands or on nearby littoral environment [10]. According to our observations in several populations in Madagascar, they are all hemi-epiphytes characterized by early germination and early stage in the soil, a root system exploring the ground and a long stem climbing along a support.

2.5. Species Diversity and Distribution

In the Vanilloideae sub-family, the *Vanilla* genus is the most species-rich [16]. However, the diversity of the *Vanilla* Plumier ex Miller genus is very complex. Indeed, as will be discussed later, *Vanilla* species delimitation is confused due to interspecific hybridization (e.g., *V. barbellata* × *V. claviculata* [22]) and phenotypic plasticity [18,37]. Furthermore, numerous synonymies still exist between species (Table 1) [9,10,27]. This diversity is constantly in revision thanks to new discoveries. In the last decade, for example, 28 novel species have been described [25,27,32,35,36,38–40]. The first extensive classification of the genus carried out by Rolfe in 1896, listed 50 species with six aromatic species [23]. Portères, in 1954, reported 110 species (18 aromatic) [10], this was reduced to 90 species by Cameron and Chase in 1999 [41]. The number of valid species was extended to 106 (35 aromatic) in 2010 [9,14]. Altogether, 231 *Vanilla* species, subspecies, varieties and synonymies are listed in the International Plant Names Index website [28] and 240 are registered in the Tropicos website [29]. The current world checklist of selected plant families (WCSPF) records 230 *Vanilla* names, of which 130 are accepted [27].

By adding the species *V. bosseri* discovered by Allorge-Boiteau in 2013 [35] and the last described species *V. armoriquensis* [40] to the list of 130 accepted species in WCSPF [27], the present number of *Vanilla* species would rise to 132. Most of these species are endemic to tropical America (75) (including *V. armoriquensis* found in Peru in 2020), where the Amazon Basin constitute the center of diversity [3,9,14], 24 occur in Africa (including *V. bosseri*), and 33 are distributed in Asia, New Guinea and the Pacific islands (Figure 2). Based on Rolfe's sections 116 *Vanilla* species among these 132 are leafy, the remaining species being leafless.

After the discovery of the first leafless species in 1799 (*V. claviculata*), 18 species have been recorded by Portères in 1854 [10]. This number is reduced to 16 at the present time due to several synonymies (Table 1). Leafless species are mainly distributed on islands and nearby coastal regions: along the east African coast, SWIO islands, southeast Asia and Caribbean islands [9,10], except *V. penicillata* which is found in South America (Table 1) [9].

Table 1. List of the 16 leafless *Vanilla* species accepted by Govaerts et al. [27]. Synonyms are in brackets. *V. bosseri* was not present in the accepted list of Govaerts et al., but is listed as an accepted name in Tropicos [29]. Morphological species groups are according to Soto Arenas and Cribb [9].

Species	Year	Continent	Group	Location
V. aphylla Blume. *(V. calopogon)*	1825	Asia	*V. aphylla*	Java, Malaysia, Philippines, Thailand
V. barbellata Rchb.f. *(V. articulata, V. bakeri, V. parishii)*	1865	America	*V. barbellata*	Caribbean Islands
V. bosseri L. Allorge. *(V. montagnacii)*	2013	Africa	*V. phalaenopsis*	Madagascar
V. claviculata Sw.	1799	America	*V. barbellata*	Caribbean Islands
V. decaryana H. Perrier.	1934	Africa	*V. phalaenopsis*	Madagascar
V. dilloniana Correll.	1946	America	*V. barbellata*	Caribbean Islands
V. humblotii Rchb.f.	1885	Africa	*V. phalaenopsis*	Comoros, Mayotte, Madagascar
V. madagascariensis Rolfe.	1896	Africa	*V. phalaenopsis*	Madagascar
V. marmoreisense Soto Calvo.	2019	America	*V. barbellata*	Cuba
V. penicillate Garay & Dunst.	1965	America	*V. penicillata*	Colombia, Suriname, Brazil
V. perrieri Schltr.	1924	Africa	*V. phalaenopsis*	Madagascar
V. phalaenopsis Rchb.f.	1867	Africa	*V. phalaenopsis*	Seychelles
V. poitaei Rchb.f. *(V. correllii, V. eggersii)*	1876	America	*V. barbellata*	Saint Domingue
V. roscheri Rchb.f.	1876	Africa	*V. phalaenopsis*	Tanzania, Kenya, Mozambique, South Africa
V. walkeriae Wight	1845	Asia	*V. phalaenopsis*	India, Sri Lanka
V. wightii Lindl. *(V. wightiana)*	1845	Asia	*V. aphylla*	India, Sri Lanka

2.6. Reproduction Biology

In most cases, *Vanilla* species combine vegetative and sexual reproduction, except for *V. mexicana*, *V. bicolor* and *V. palmarum* for which artificial clonal propagation was reported to be unsuccessful or difficult [18,37,42]. The vegetative reproduction is possible as soon as an individual or a sufficient fragment of stem cutting can take root in the soil [37], sprout vegetative buds [42] and climb to a support to create a new individual. This clonal propagation constitutes the predominant reproductive, colonization and survival strategy. Studies on leafless species also revealed their ability for clonal reproduction in natural habitats. In Puerto Rico, Nielsen and Siegismund used polymorphic enzymes to analyse genetic diversity [22,43]. The results showed a high clonality rate for *V. dilloniana* (38%) [22], a moderate rate for *V. barbellata* (25%) [22] and a low rate for *V. claviculata* (6%) [43]. In *V. humblotii* in Mayotte, microsatellite analyses showed a moderate clonality rate (12.5%), with an average clonal patch size of 4.6 (±2.7) m, and a phalanx distribution [21].

The sexual reproduction, on the other hand, can occur only during the flowering period and only when the individual has attained maturity [20]. Also, it is common that some populations of a species fail to bloom for one or more successive years, as it was reported for *V. hartii* in tropical America [20]. There are, however, species that bloom throughout the year or during several months like *V. inornata* [32], *V. rivasii* [44] or *V. bicolor* [42]. Although some *Vanilla* species have an unusual high natural fruit set (>40%) (Figure 4), which suggests self-pollination [20,42,45,46], most are pollinator-dependent due to the presence of the rostellum membrane separating the female and male parts of the flower [10,19,21,23]. Some mechanisms were reviewed by Gigant et al., to explain self-pollination: it may be caused by either stigmatic leak and/or the presence of a dehydrated or reduced rostellum, or agamospermy (e.g., *V. bicolor* and *V. palmarum*), but some spontaneous self-pollinations remain unexplained (e.g., *V. planifolia* and *V. chamissonis*) [18]. In addition to these mechanisms, Householder et al. also noted the absence of a rostellum in *V. guianensis* in Peru, facilitating pollen transfer between the two sexual organs [42].

Pollinators visit flowers for various reasons, such as collecting nectar, oil, pollen, stigmatic exudates or floral scents [47–49]. In *Vanilla*, floral odor seems to play an important role in attracting pollinators as in the case of *V. trigonocarpa* or *V. pompona* in America [14,20,42]. Other strategies are also used by orchid species to attract pollinators such as deceptive systems [50], as described for *V. planifolia*,

V. odorata, and *V. insignis* in America [18]. In orchids, bee-pollination also requires a morphological association between floral characteristics and the insect traits [47,51–53].
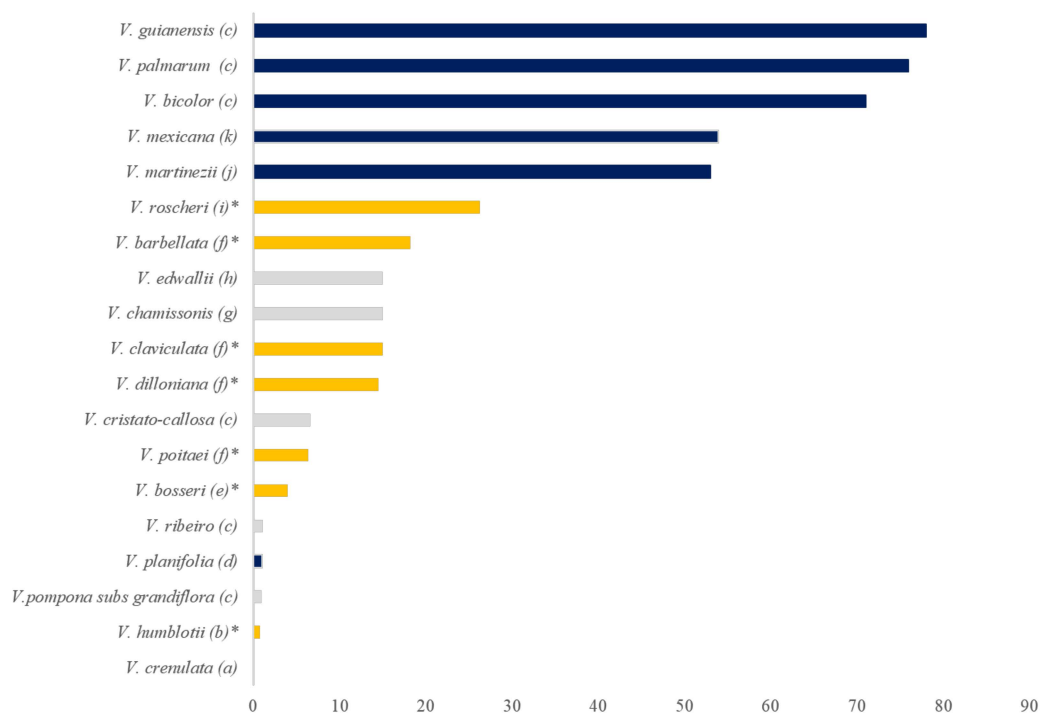

Figure 4. Rate of natural fruit set (%) of *Vanilla* species according to (**a**) Soto Arenas and Cameron [14], (**b**) Gigant et al. [21], (**c**) Householder et al. [42], (**d**) Ackerman et al. [54], (**e**) Petersson [53], (**f**) Tremblay et al. [45], (**g**) Macedo [55], (**h**) Pansarin, Aguiar, and Pansarin, 2014, (**i**) Gigant et al., 2014, (**j**) Soto Arenas and Dressler, 2010, (**k**) Gigant et al. [46]. Species that can self-pollinate spontaneously are in dark blue, leafless *Vanilla* species are in orange and indicated by *, and the grey bars represent the other species.

In most cases in America, euglossine bee species were proved to be the main *Vanilla* pollinators (Table 2) [14,42,56], probably alongside with other species such as *Trigona* bees, *Xylocopa* bees [42], birds (e.g., *V. abundiflora* and *V. griffithii* [20]) and even bat species (e.g., *V. insignis* [20]).

With regards to the leafless *Vanilla* species, the low natural fruit set of six studied species (<30%) testifies their dependence on pollinators (Figure 4). Soto Arenas and Cameron suggested that species in Puerto Rico would probably be pollinated by bees of the genus *Centris*, although it was also reported that they could be pollinated by euglossine bees [45]. In Africa, three leafless species have been studied for their reproduction biology, and the results show that they are most probably pollinated by female Xylocopinae (Allodapine) bees, although these bees might also act as pollen thieves [19,21,53]. Different Allodapine bee species were observed to move *V. bosseri* and *V. roscheri* pollen, but Meliponae and Anthophorine might be the true pollinators, respectively (Table 2). The presence of a rostellum that covers the entire stigmatic surface prevents self-pollination in *V. roscheri* [19]. Despite this, Sibaya populations (South Africa) have a high natural fruit set of up to 26.3% [19]. This can be explained by the large number of visiting insects recorded (about 11), of which three Apidae have been observed to move pollen probably for brood provisioning (Table 2) [19]. The observed natural fruit set of *V. humblotii* was very low (0.62 to 1.2%) in natural populations from Mayotte [21], possibly due to insufficient pollinators attributed to the highly fragmented landscape. Studies conducted on populations of *V. bosseri* in the CNRFEREF forest in Madagascar showed that this taxon is bee-pollinated, allogamous but self-compatible (like the two previous species) with a low flowering rate and a low natural fruit set of 3.96% [53]. Although several bee species belonging to four orders (*Hymenoptera*, *Lepidoptera*, *Orthoptera*, *Diptera*) as well as one Primate species

(*Prophithecus verreauxi*) were recorded visiting flowers [53], this low natural fruit set indicates a limited pollination. Indeed, only three bee species were found to carry pollen similar to the one of *V. bosseri* (Table 2) [53]. The author hypothesized that the pollinators may instead act as pollen thieves and reduce *V. bosseri* reproductive success [53].

Table 2. Potential pollinators of some *Vanilla* species. Leafless species are highlighted in bold.

Vanilla Species	Potential Pollinators	References
	America	
V. insignis	*Eulaema polychrome*	[20]
V. planifolia	*Euglossa spp.*	[20]
V. pompona subs grandiflora	*Eulaema spp.*	[42,56]
V. cribbiana	*Eulaema sp.*	[20]
V. pompona	*Eulaema cingulata*	[57]
V. trigonocarpa	*Euglossa asarophora*	[20]
V. edwallii	*Epicharis affinis*	[52]
	Asia	
V. siamensis	*Thrinchostoma sp.*	[58]
V. cf kaniensis	*Aegilopa sp.*	[14]
	Africa	
V. bosseri	*Macrogalea ellioti, Liotrigona mahafalya, Liotrigona madecassa*	[53]
V. roscheri	*Allodapula variegate, Allodapa rufogastra,* Anthophorini	[19]
V. humblotii	*Allodape obscuripennis, Nectarinia coquerelli*	[21]

2.7. Vanilla: A Taxonomic Complex Group

Since 1703, when the first three species were described by Plumier, the taxonomy of the genus has always been considered to be difficult [23].

Indeed, the botanical identification of *Vanilla* species is mainly based on the description of flowers which are often ephemeral, variable within species due to phenotypic plasticity (e.g., *V. barbellata*, *V. bakeri*, *V. parishii* [10]), or similar between cryptic species [20,59]. Sometimes, no type specimen or botanical collections were recorded (e.g., *V. guianensis* [42]). Besides, the number of individuals used for species description was often limited due to scarcity of flowers [9]. Vegetative characteristics also show considerable intra-specific variations and inter-specific similarities between related species [34,35,37].

Hybridization is also one of the factors that can blur the boundaries between species, leading to a mismatch between morphology and genetics. Prezygotic and postzygotic barriers can generally prevent or limit hybridization [60]. In the Orchid family, prezygotic barriers include differences in floral morphology, and phenological and ecological isolation [51,57,61]. Postmating barriers result from incompatibility mechanisms, hybrid inviability or sterility, as reported for some deceptive orchids [62,63]. In the *Vanilla* genus, differences in flowering period and pollinator specificity are probably the two main factors that can limit gene exchange between sympatric species [10,20,22,56]. On the other hand, postmating barriers were not observed following artificial crossing experiments between closely related species (e.g., *V. planifolia* × *V. pompona* [37]) or highly differentiated species (e.g., *V. aphylla* × *V. planifolia* [59,64]). Many *Vanilla* species occur in sympatry (Table 3), and cases of natural interspecific hybrids have been reported [22,43,65].

Table 3. Sympatric species recorded. Species for which hybridization was observed are highlighted in bold.

Sympatric Species	Location	References
Leafy Species		
V. hartii & *V. cribbiana*	Mexico	[20]
V. hartii & *V. insignis*	Mexico	[20]
V. hartii & *V. odorata*	Mexico	[20]
V. hartii & *V. inodora*	Mexico	[20]
V. rivasii & *V. dressleri*	Colombia	[44]
V. javieri & *V. appendiculata*	Colombia	[66]
V. yanesha & *V. mexicana*	Peru	[38]
Leafless Species		
V. barbellata & **V. claviculata**	Puerto Rico	[43]
V. barbellata & *V. dilloniana*	Puerto Rico	[22]
V. dilloniana & *V. claviculata*	Puerto Rico	[22]
V. madagascariensis & **V. perrieri**	Madagascar	[8] [1]
V. madagascariensis & *V. bosseri*	Madagascar	[1]

[1] Personal observation.

Thanks to an integrative taxonomic approach using ITS markers, the hybrid status of *V.* × *tahitiensis*, described as a new species in 1933, was recently proven [16,65]. It is a hybrid between *V. planifolia* and *V. odorata*, although it is still unclear where the hybridization took place. A study in Puerto Rico demonstrated the occurrence of hybrids between sympatric leafless species [22,43]. *V. dilloniana*, *V. claviculata* and *V. barbelatta* are three leafless species endemic to the Caribbean islands [22]. They share similar vegetative traits but can be easily discriminated from each other by their labellum [9,10,22]. The three species grow in sympatry in the western/southwestern part of the Susua Forest Reserve (Puerto Rico). Populations of the two species, *V. barbellata* and *V. claviculata*, presented the same alleles but differed in allelic frequencies [22,43]. Genetic differentiation within *V. barbellata* and *V. claviculata* species (mean 14.1%) was low compared to the one between them (23.7%), which allowed individuals to be correctly assigned to their respective species, except for one locality (Susua Reserve Forest) where both species co-exist [22]. Indeed, a complementary study demonstrated the existence of some hybrid individuals in this locality, with intermediate morphological characteristics between the two original taxa, and a high heterozygosity [43]. Furthermore, cross-pollination experiments were successful between *V. barbellata* and *V. claviculata* with high seed quality [43].

The mixed reproductive system (vegetative and sexual) of *Vanilla* can lead to a complex mixture of sexual reproduction and uniparental lineages [67]. Furthermore, polyploidization is frequent in *Vanilla*, especially in African species, increasing intraspecific diversity and making species delimitation even more difficult [37,68,69]. As a result of the three characteristics previously developed, i.e., (a) uniparental reproduction (clonal reproduction or self-fertilization), (b) interspecific hybridization between sympatric species, and (c) polyploidization capacity, the *Vanilla* genus gathers the criteria defined by Ennos, French, and Hollingsworth in 2005 to designate "Taxonomically Complex Groups" (TCGs) [67].

2.8. Ethnobotanic and Commercial Importance

The first use of *Vanilla* plants dates back to the time of the Maya civilization where it was called "sisbic" [20,70]. *Vanilla* was probably used by Mayas to treat skin wounds and bites at least since the 14th century, and the Mayas were also the first to develop the methods for treating fruits [70]. Then, other uses of *Vanilla* were also reported, such as vermicide in Mexico and Cuba, and to flavor rum by Chiapas at the beginning of the 19th century [20]. Roots of *V. grandifolia* and *V. ovalis* serve as a material for making fishing nets and baskets [14]. *Vanilla* species that produce aromatic fruits belong exclusively to the *Vanilla* subgenus *Xanata* sect. *Xanata* [9,71]. Three *Vanilla* species are cultivated and commercialized: *V. planifolia* worldwide especially in the SWIO region, *V.* × *tahitiensis* in the Pacific Ocean region and *V. pompona* in central America, the French Caribbean and Guiana [14,68].

But *V. planifolia* represents the most economically important species. It was first introduced in Europe in 1510 and used for medicinal and flavoring purposes [23]. The discovery of the manual pollination technique by Charles Morren in 1836 and the simplification of the technique in Reunion island by Edmond Albius in 1841 has made the culture of commercial *Vanilla* plants easy and possible worldwide [72].

Although only *V. planifolia* is of major economic interest on the global scale, the other species represent an important gene pool for the future breeding programs of commercial varieties. Indeed, other species have interesting traits that *V. planifolia* may not always have, such as high fruit set [42], self-pollination [20,42,46], resistance to diseases [59,64,73], medicinal proprieties [10,23,70,74] and adaptation to drought [10,23].

The leafless group comprises *Vanilla* species with a high potential for the future. In addition to their drought adaptation, they were shown to be resistant to *Fusarium*, one of the most destructive diseases of the *V. planifolia* plantations [75]. For example, four leafless *Vanilla* species from the SWIO region (*V. phalaenopsis*, *V. humblotii*, *V. madagascariensis*, *V. roscheri*) and *V. aphylla* from Asia were resistant or only slightly sensitive to *Fusarium oxysporum* f.sp. *radices-vanillae* after in vitro inoculation tests [59,64]. Also, leafless species are valued in a variety of traditional uses and for their medicinal properties. Chemical analyses realized on *V. dilloniana* stems showed antimicrobial and antiproliferative activities [74]. In India, local people use the stem of *V. walkeriae* as fodder for cows having new calves to increase their milking capacity [76] and to treat fever in cattle [77]. *V. decaryana* is used in the south of Madagascar for its aphrodisiac proprieties (Figure 3C) [10]. Traditional uses of leafless *Vanilla* plants are dominated by the knowledge of ethnic groups in southern Madagascar. Stems are used as a "tonic" for men but also in "post-partum" and child cares [78]. Ethnobotanical surveys have also revealed that the sister species *V. madagascariensis* is widely used in Malagasy cosmopolitan cities to treat certain diseases and for its aphrodisiac properties [79–81]. Also, *V. roscheri* serves as a charm to protect homes in South Africa [5].

Taking into consideration these interesting specificities, leafless species constitute an important genetic pool for *Vanilla* breeding programs especially in the present context of global climate change.

2.9. Conservation Issues

As for all wildlife species, wild *Vanilla* populations are in great danger due to anthropogenic activities. According to the IUCN red list, eight *Vanilla* species (*V. hartii*, *V. inodora*, *V. insignis*, *V. odorata*, *V. phaeantha*, *V. planifolia*, *V. pompona*, *V. somai*) are listed as endangered, and one species (*V. cribbiana*) is categorized as critically endangered [82]. Some species have a very limited distribution (e.g., *V. denshikoira* in Colombia [39]) and are critically threatened by agricultural practices and deforestation (e.g., *V. paulista* and *V. dietschiana* in Brazil) [83–85]. Populations even consist, in extreme cases (e.g., *V. javieri* in Colombia), of a single known individual with a generally low fruit set [66]. In Malaysia, *V. montana* has been indicated as a rare species with the type specimen as the only known plant [9], and recently it has been reported to be a lost taxa [86]. Wild *V. madagascariensis* are also sometimes over-exploited to be used in traditional medicine [79].

Habitat destruction can also lead to the loss of genetic diversity or reduce the pollinator availability, which can impact the reproduction strategy of species. This was demonstrated for two leafless species from the SWIO: *V. roscheri* in South Africa and *V. humblotii* in Mayotte, respectively [19,21,87].

A study using microsatellite markers demonstrated a total absence of genetic diversity, i.e., a generalized homozygosity and monomorphic loci in populations of *V. roscheri* near lake Sibaya in South Africa [19]. Two scenarios have been put forward to explain this worrying situation. First, habitat fragmentation could have led to population bottleneck and isolation, which resulted in inbreeding [19]. Alternatively, the Sibaya populations could be the result of clonal reproduction of a single migrant from Northern regions [19]. Although widely distributed, this species is considered as endangered and rare in several countries. For example, it has been classified as a flagship species, the second rarest endemic and threatened species in the St Lucia Wetland Park and an endangered

species in South Africa [5,88]. *V. roscheri* is also one of the traditional African plants [5]. Given this lack of genetic diversity, anthropogenic pressures and potential uses, in situ and ex situ conservation efforts of *V. roscheri* in South Africa should be implemented urgently. Indeed, loss of genetic diversity is synonymous with an inability to evolve in response to environmental changes, and is often associated with inbreeding and reduction in reproductive fitness [89]. Thus, the current status of natural populations in other sites throughout the distribution range of *V. roscheri* should be monitored in order to prevent genetic erosion and preserve the species.

In Mayotte, *V. humblotii* is represented by nine populations spread over less than 20 km^2 [87]. It is part of the protected species list cited in a Prefectoral decree (Arrêté n°361/DEAL/SEPR/2018), establishing the list of protected plant species and regulating the use of endangered plant species in the French department of Mayotte. According to the report carried out jointly by the IUCN (International Union for Conservation of Nature), the CBNM (Conservatoire Botanique National de Mascarin), the CBNF (Fédération des Conservatoires Botaniques Nationaux) and the MNHN (Muséum National d' Histoire Naturelle), *V. humblotii* is classified as "Vulnerable" in Mayotte due to competition with invasive species, clearing and burning carried out for banana, cassava and yam crops. A conservation strategy is being implemented [87]. A study using microsatellite markers revealed that, surprisingly, the genetic diversity of *V. humblotii* in Mayotte is relatively high. It is probably a remnant diversity, maintained almost exclusively by clonal reproduction [21], given the low fruiting rate and the lack of pollinators observed. Nevertheless, the very high anthropogenic pressure on *V. humblotii* populations in Mayotte could lead to a bottleneck, reducing genetic diversity, increasing inbreeding and putting populations in danger.

Given the lack of information or alarming status of some natural populations, the implementation of in situ and ex situ conservation actions is urgent for *Vanilla* species, as proposed by Flanagan and Mosquera-Espinosa for *Vanilla* species in Colombia [90].

3. The Leafless *Vanilla* Clade from the SWIO Region: A Taxonomic Puzzle

SWIO leafless *Vanilla* species are an interesting model for evolutionary studies of the genus *Vanilla* as they represent a recent monophyletic group [3,91] evolving in a mosaic of environments [92,93], with a high potentiality for breeding programs due to drought adaptation capacity [10,23], disease resistance [64,73] and medicinal properties [10,79–81].

The SWIO islands are home for nine *Vanilla* species including two Malagasy leafy species belonging to the Papillosae sub-section of Portères and *Tethya* section of Soto Arenas and Cribb, and seven leafless species [8–10,34,35]. After describing the distribution and botanical differences between these leafless species, we will discuss the importance of this clade for evolutionary studies.

3.1. Distribution

Among the seven leafless species from the SWIO, *V. humblotii* is the only one that has been observed in several countries: Comoros, Mayotte and Madagascar [8,10,34], although it was described as endemic to the Comoros Archipelago (Figure 5) [10]. This species grows on rocks in the north of Madagascar and in the Comoros, whereas the closely related species *V. perrieri* is found in dry forests with sandy soils in the west and south of Madagascar (Figure 5) [8,10,34]. Three other species are indigenous to Madagascar, of which *V. madagascariensis* is the most widely distributed (Figure 5) [8,10]. Indeed, it occurs in the dry forests along the west coast and was also observed in the northeastern rainforests of Madagascar [8,29]. Given its large distribution, *V. madagascariensis* lives in sympatry with the other Malagasy leafless *Vanilla* species (Table 3, Figure 5) [8]. *V. decaryana* grows mainly in dry deciduous shrubs among Didieraceae in the south of Madagascar [10]. Finally, *V. bosseri* was observed in several localities in the west of Madagascar [35,53]. *V. roscheri* is found along the coast and islands of East Africa. The species has been observed mainly in Ethiopia, Somalia, Kenya, Tanzania, Zanzibar, Pemba Islands, Mozambique and South Africa [5,9,10,19,94]. *V. phalaenopsis* is described as endemic to Seychelles (Figure 5) [9,10].
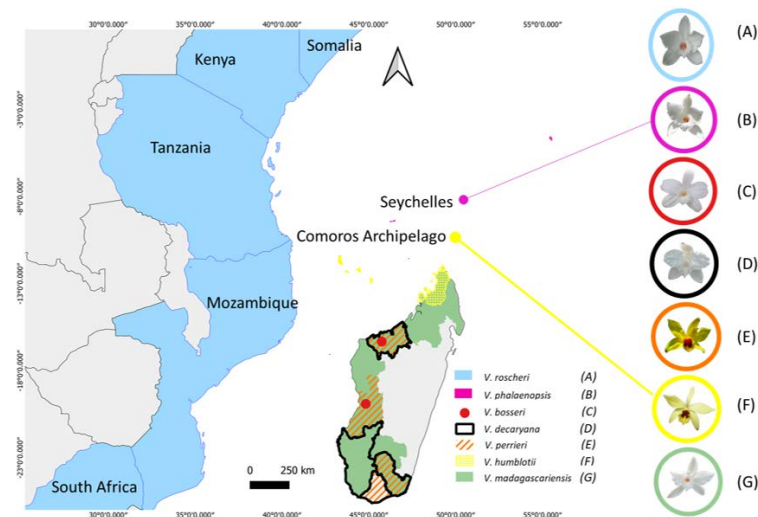
Figure 5. Distribution area of the seven leafless *Vanilla* species from the SWIO region according to Allorge-Boiteau [34], Allorge-Boiteau [35], Cribb and Hermans [8], Chinsamy, Finnie, and Van Staden [5], Luke [94], Portères [10]. Species are numbered from A to G. Images of their flowers are shown: photographies by (**A**) Rodolphe Gigant near Sibaya Lake (South Africa), (**B**) Thierry Pailler in Seychelles, (**C**) Cathucia F. Andriamihaja in Marosely (Madagascar), (**D**) Hoby Nomenjanahary in Mangily (Madagascar). (**E**) Cathucia F. Andriamihaja in Befandrama (Madagascar), (**F**) Johnson G. Andrianantenaina in Montagne des Français Park (Madagascar), and (**G**) Alemao Botomanga in Maromandia (Madagascar).

3.2. Botanical Description

Initially, Portères described seven leafless species in the SWIO [10], but one was invalidated by Soto Arenas and Cribb in 2010 in the absence of a Latin description and type specimen [9], and another one was recently added [35]. Seen for the first time in 2012 by Lucile Allorge-Boiteau in the Namoroka reserve [35], *V. bosseri* is one of the leafless species with white flowers endemic to Madagascar. Allorge-Boiteau describes it as a very fragrant species [35]. This species was also observed in the Morondava region by Bosser who identified it as *V. montagnacii* [35]. However, the latter was invalidated and Portère's description indicated the presence of a median hair in the basal position, which is not observed in *V. bosseri* [10,35].

Six of the SWIO species form, together with *V. walkeriae* from Asia, the *V. phalaenopsis* morphological group according to Soto Arenas and Cribb's classification [9]. This group is characterized by a flabellate labellum with two rows of hairs beside the adaxial line, obtuse and very wide petals [9]. However, the last species discovered in this group, *V. bosseri*, was not included in the list. Although similar to the others, *V. bosseri* does not completely satisfy the description of the *V. phalaenopsis* group due to the absence of hairs on its labellum (Figure 6) [35].
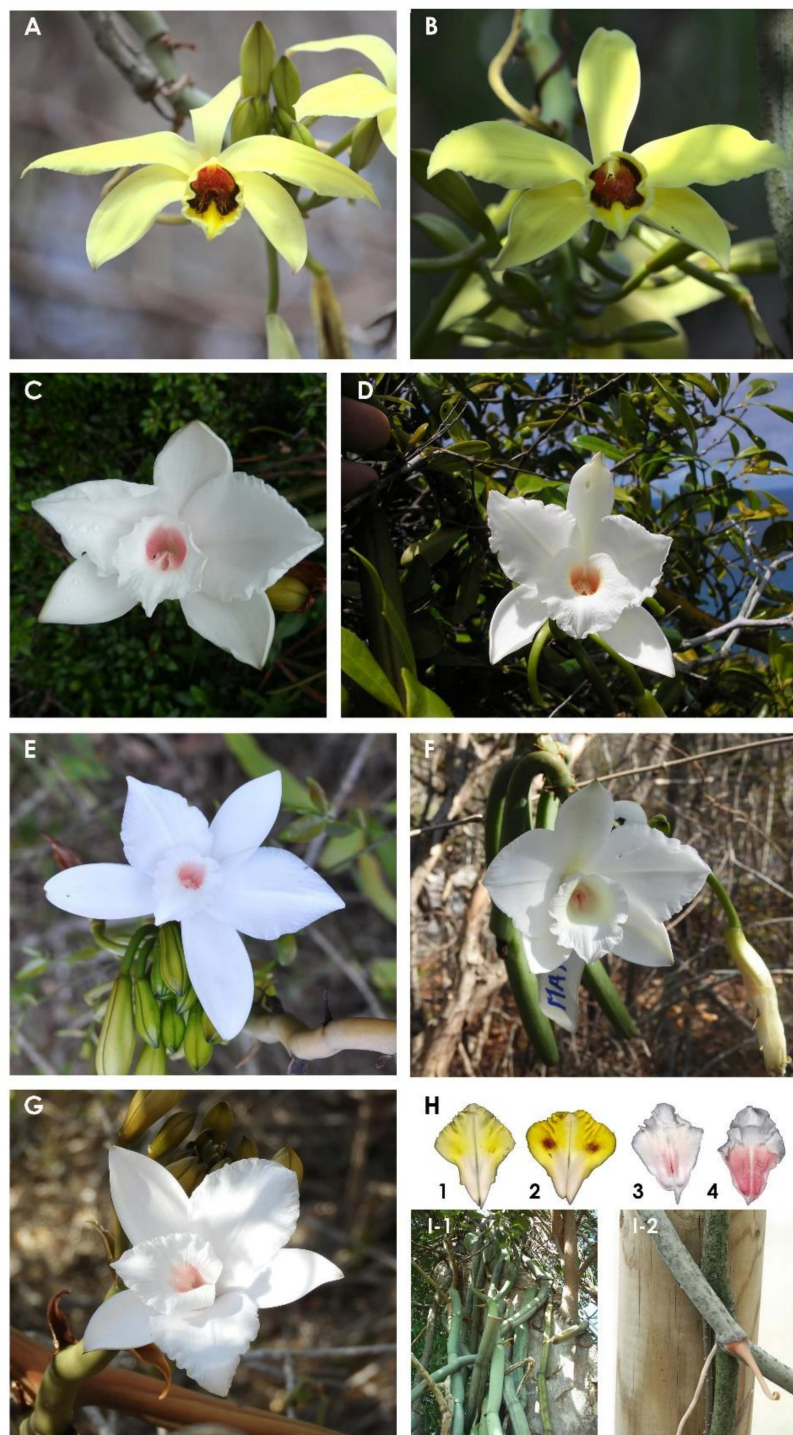
Figure 6. Morphological diversity of the seven leafless *Vanilla* species from the SWIO region. Flowers of (**A**): *V. humblotii* from Montagne des Français (Diego, Madagascar), (**B**): *V. perrieri* from Befandrama in the district of Analalava (Madagascar), (**C**): *V. roscheri* from Lake Sibaya in South Africa, (**D**): *V. phalaenopsis* from Seychelles, (**E**): *V. madagascariensis* from Maromandia in the district of Analalava (Madagascar), (**F**): *V. bosseri* from Marosely in the district of Port Bergé (Madagascar), (**G**): *V. decaryana* from Mangily in the district of Tuléar II (Madagascar). (**H**): scan of (1) the back side of *V. humblotii* labellum, (2) the back side of *V. perrieri* labellum, (3) the inside of *V. bosseri* labellum and (4) the inside of *V. madagascariensis* labellum. (**I**): Stems of (1) *V. bosseri* from Antsianitia (Madagascar), (2) *V. humblotii* CR0108 from the Comoros (BRC Vatel collection). (Photo A, B: Johnson G. Andrianantenaina, C: Rodolphe L. Gigant, D: Thierry Pailler, E: Alemao Botomanga, F,G,H: Cathucia F. Andriamihaja, I: Pascale Besse).

Nevertheless, the seven leafless *Vanilla* species from the SWIO region are very similar morphologically [9,10]. Following our own comparative observations in the SWIO region, the main discriminant morphological features between the various species were identified. The major distinctive features between the seven species are presented in Table 4 and flower images are shown in Figure 6.

Table 4. Leafless *Vanilla* species from the SWIO region: geographical distribution, flowering period and morphological characteristics of the flower and stem (C: color, LP: length of petal, LS: length of sepal, La: labellum, Fl: inflorescence, F: form, Tx: texture).

Distribution	Flowering Period	Flower	Stem
		V. bosseri L. Allorge, 2013	
Madagascar: Southwesten [a] Namoroka [a] Morondava [a]	September-November [b,c]	LS: 4.5 cm [a] C: white [a] La: pinkish throat with no pubescence and red protrusion between the crests [a,b]	C: grey in young stems and turns green in old stems [a] F: four longitudinal grooves [a] Tx: generally glabrous [a,c]
		V. decaryana H. Perrier, 1934	
Madagascar [d]: South [d] Southwestern [e] Andohahela [e]	January [d]	LP: 3 cm [d] LS: 2.5 cm [d] Fl: 20–40 flowers [d] La: pinkish throat with two pubescent lines [d]	C: greenish-brown [d] F: two longitudinal grooves [e] Tx: glabrous [c,d,e]
		V. humblotii Reichenbach f., 1885	
Comoros [d], Mayotte [d,g] Madagascar [f]: North [f] Ankarana [e] Bemaraha [e] Andohahela [e]	November-April [g]	C: canary-yellow [d,g] LP: 5.5–7 cm [d] LS: 5.5–6.5 cm [d] La: very pubescent with velvety red or dark maroon throat [d,h], papillosae [h] Fl: 40–50 flowers [g]	C: glaucous green [d] or bluish [e] F: one longitudinal groove [e] Tx: verrucose [c,d,e]
		V. madagascariensis Rolfe, 1896	
Madagascar [d]: North (Sava, Diana, Ankarana) [a] Isalo [a] Ivohibe [a]	June-October [a,d]	C: white [d] LP: 5.5–6.5 cm [a] LS: 5.5–6.5 cm [d] Fl: 10–20 flowers [a,d] La: pubescent with red throat [a]	C: glaucous green [d] F: one longitudinal grooves [e] Tx: glabrous [a,d,e]
		V. perrieri Schlechter, 1925	
Madagascar: Ankarafantsika [d] Western coast (Majunga, Morondava, Fort Dauphin, Ambovombe) [e]	November-December [c]	C: sharp yellow [d] LP: 5.5 cm [d] LS: 6.5 cm [d] La: pubescent with bright red or dark brownish throat [d,h] not papillosae [h], extension of the hair on the back [d]	C: dark green [e] F: two longitudinal grooves [e] Tx: glabrous [d,e], similar to *V. madagascariensis*, but sometimes verrucose [c]
		V. phalaenopsis Reichenbach f., 1868	
Seychelles [a]	April [i] July-November [j]	C: white [d] LP: 5.5–6 cm [d] LS: 5.5–6 cm [d] Fl: corymbiform [d] La: two pubescent lines and yellow-orange desk [d]	C: green [i] F: one longitudinal groove [d] Tx: glabrous, similar to *V. madagascariensis* [i]
		V. roscheri Reichenbach f., 1877	
East African: Mozambique [d] Zanzibar [d] Pemba Island [d] Dar-es-Salam [d] South Africa [k]	November-March [k]	C: white [d] LP: 7.5–8 cm [d] LS: 7.5–8 cm [d] Bud: brownish [d] La: two pubescent lines desk and pink throat [d,k]	C: reddish-brown [d] Tx: glabrous similar to *V. madagascariensis* [i]

[a] [35], [b] [53], [c] personal observation, [d] [10], [e] [34], [f] [8], [g] [21], [h] [9], [i] P. Besse, personal communication, [j] [6], [k] [19].

The stem texture and color are often different between species, with species showing glabrous or verrucose; grey, brown, red to green stems; with one to four longitudinal grooves [10,19,21,34,53]. But these characters are also highly variable within species. Based on flower color, species can be divided into two groups: species with yellow flowers and those with white flowers [8,10,34]. *V. humblotii* and *V. perrieri* compose the first group. The former has a verrucose stem and yellow canary

flowers, that allows to distinguish it from *V. perrieri* which exhibits a glabrous stem and sharp yellow flowers [10,34]. One of the particularities of *V. perrieri* is also the presence of an extension of the hairs outside the hulls on the back of the labellum (Figure 6) [10]. Five closely-related species compose the white-flower group, of which three are endemic to Madagascar [8–10,34,35]. All have a smooth stem and hairs on the inside of the labellum, except *V. bosseri* whose labellum is rather characterized by two pink crests and a short red protrusion between the crests (Figure 6) [34,35,53]. The five species also differ in the color of the inside of the labellum and flower size, *V. decaryana* being the one with the smallest flowers (Table 4) [9,10,34].

3.3. A Taxonomic Puzzle

When genetic differentiation is associated with consequent morphological differentiation, species delimitation is generally easy [95]. But in some cases, when species are highly differentiated morphologically but little divergent genetically (adaptive radiation), or when the morphological change is slow and does not reflect genetic divergence (nonadaptive radiation), species delimitation becomes a challenging task [95]. Often, adaptive radiation occurs among a related and recently diverged sympatric group [95–98], whereas nonadaptive radiation would be the result of prolonged geographical isolation of several populations of the same species, followed by subsequent slow speciation resulting from nonecological processes [98]. However, nonadaptive radiation can be followed by adaptive radiation when the environment of the two species becomes different [98]. Species delimitation is even harder when recent sympatric species can hybridize [97,99]. SWOI leafless *Vanilla* species are one of the most recently formed groups in the genus *Vanilla* (4.4 Mya) (Figure 1) [3]. Therefore, species divergence within the group has occurred recently. The observations we made on the vegetative and floral organs of individuals belonging to three species *V. roscheri*, *V. phalaenopsis* and *V. madagascariensis* present in the BRC (Biological resources center) Vatel (Reunion island) allowed us to confirm that they are closely related and difficult to differentiate. Flowers mainly show variations in size (Table 4), which is not always a sufficient criterion to discriminate species, especially in the *Vanilla* genus with high intra-specific variation [100]. Also, Cameron suggested that these three species could probably be morphological variations of a single species [101]. Given that these three species are supposedly endemic to three different geographical areas (East Africa, Madagascar, Seychelles), whose separation took place well beyond the formation of the leafless group (Figure 7), we suppose that they are probably separate populations of the same species that have recently diverged and are evolving via nonadaptive radiation.

On the other hand, the five described leafless species found in Madagascar can be differentiated by the size of the flower, and the color and the form of the labellum [8,34,35]. As previously discussed, these species are sympatric in several areas, and interspecific hybridization is possible between *Vanilla* species [18,43]. During our fieldwork in the north of Madagascar, we observed one individual with flowers showing intermediate colors between yellow and white, suggesting a possible hybridization between species. Unfortunately, we were unable to obtain images. The possible occurrence of five leafless species in Madagascar with some overlapping distribution and the possible hybridization raise numerous questions about the mechanisms of their evolution and speciation. For these Malagasy species, adaptive radiation and ecological speciation have probably occurred in the past via one of the five mechanisms reported in Madagascar by Vences et al. [92]. Concerning the remaining species, *V. humblotii*, supposed to be endemic from the Comoros archipelago, its presence in Madagascar and its great morphological similarity with the native Malagasy species, *V. perrieri*, suggest that they either form subspecies of the same species or are the result of colonization followed by differentiation long after the formation of the islands. Species movement between SWIO islands has already been demonstrated in various genera including birds (e.g., *Nectarinia*, *Hypsipetes* [102,103]), plants (e.g., genus of Coffeeae tribe [104]) and more especially orchids (e.g., *Bulbophyllum*, *Angraecum* [105–107]).

Figure 7. Map showing the date (in Mya) of separation and formation of the SWOI islands adapted from Warren et al., 2003 [102].

Complexes of closely related species endemic to different islands provide an interesting model to better understand the different stages of speciation. In fact, islands own several characteristics that make them an excellent natural laboratory, including the presence of strict boundaries, the isolation, the high endemicity, the small size relative to continent, the geographic history, and the environment heterogeneity providing natural ecological and evolutionary replicates [108]. This interest in islands goes back to Darwin, who used a variety of islands as a model to illustrate different patterns of species richness and evolution, of which the famous Galapagos islands largely studied until now [109]. All these interesting features are visible in the SWIO islands, which are a biodiversity hotspot [110]. At the scale of Madagascar, the oldest island of the SWIO region separated from Africa 130 Mya and from India 88 Mya (Figure 7), seven bioclimatic regions with unique vegetation are present, ranging from a humid tropical forest in the east to an arid biome in the far south (Figure 8) [93]. This Malagasy ecological contrast is the source of various speciations, particularly in the animal kingdom (e.g., lemur species [111,112]).
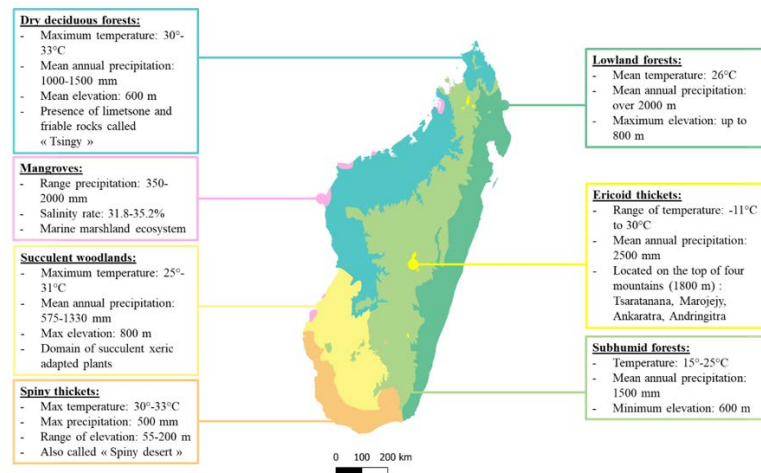
Figure 8. Map of the seven ecoregions of Madagascar adapted from WWF's ecoregion in Burgess et al. [93].

Moreover, SWIO islands have variable ages and different geographical histories (volcanic islands like Comoros vs. mainland islands such as Madagascar and Seychelles), allowing to test several speciation mechanism hypotheses. The presence of *Vanilla* sister-species, endemic to the African continent or to different islands (*V. roscheri*, *V. madagascariensis*, *V. phalaenopsis*) and the presence of sympatric *Vanilla* species in Madagascar offer a promising system for species diversification studies.

3.4. Conclusion and Prospects

Because of today's environmental crisis, biodiversity conservation has become a major global concern. Two kinds of strategies are generally recommended to achieve this goal: species-based conservation and conservation at the ecosystem scale [113]. Whatever the approach, efforts to catalogue and understand biodiversity form the basis for species and habitat effective conservation. However, correctly defining species belonging to taxonomically complex groups like *Vanilla*, where conventional classification fails, is not always evident. Yet, incorrect taxonomic classification could have important consequences for basic biological research and harm biodiversity conservation [114,115]. For example, *V. humblotii* is considered as endemic to the Comoros archipelago and is classified as endangered (EN) in Mayotte and therefore under protection status since 2006. However, this species has also been described as present in Madagascar, making its overall conservation status questionable. According to many authors, taxonomy often requires the comparison between several models based on different types of data and should incorporate molecular investigations [114,116,117]. In the case of the seven leafless *Vanilla* species native to the SWIO, classifications based on morphological characters in a very detailed manner [10,34,35], on a large sampling, as well as population genetics and phylogenetic analyses are needed. Chloroplast genes rbcL, psaB, psbB, and psbC have already been used for *Vanilla* phylogeny [3]. But these sequences are not variable enough to properly clarify the taxonomy in the recent SWIO clade [24,118]. As the available information on genetic structuring and phylogeny of these species is insufficient, the resolution of their taxonomy is problematic, which limits the development of conservation actions. Integrative taxonomy using phylogenetic analyses with more divergent regions of DNA such as ITS [24,118], genetic structuring analysis with highly polymorphic and rapidly evolving markers like microsatellites [97], completed by a morphological comparison of several individuals from the different species like what has been done for *Carappa spp.* [116] and *Coffea spp.* [119] will be a powerful approach to clarify their taxonomy. Such integrative approaches are currently becoming frequent in species delimitation [9,20,25,99,116] and mandatory for species complexes [97,99,116] or cryptic species [114,120,121]. These different markers associated with other environmental variables such as climatic or soil data would also help to understand the speciation processes at play [122–124].

Issues related to interspecific hybridization should also be addressed in taxonomic and phylogenetic studies [99] of these seven SWIO leafless species.

In addition, interspecific and intraspecific crossbreeding tests as done for *V. barbellata* and *V. claviculata* [43], *V. bosseri* [53], *V. humblotii* [21] and *V. roscheri* [19], the modeling of the geographical distribution and population genetic studies of the different species will greatly improve our knowledge about these interesting models and allow the implementation of effective and sustainable conservation programs.

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