Lankesteriana International Journal on Orchidology
ISSN: 1409-3871
lankesteriana@ucr.ac.cr
Universidad de Costa Rica
Costa Rica

Arévalo, Rafael; Cameron, Kenneth M.
MOLECULAR PHYLOGENETICS OF MORMOLYCA (ORCHIDACEAE: MAXILLARIINAE) BASED ON COMBINED MOLECULAR DATA SETS
Lankesteriana International Journal on Orchidology, vol. 13, núm. 1-2, agosto, 2013, pp. 1 -11
Universidad de Costa Rica
Cartago, Costa Rica

LANKESTERIANA 13(1–2): 1—11. 2013

MOLECULAR PHYLOGENETICS OF *MORMOLYCA* (ORCHIDACEAE: MAXILLARIINAE) BASED ON COMBINED MOLECULAR DATA SETS

Rafael Arévalo* & Kenneth M. Cameron

Department of Botany, University of Wisconsin-Madison, 430 Lincoln Drive,
Madison, WI 53706-1381, U.S.A.
*Author for correspondence: rafarev@gmail.com

Abstract. The Neotropical orchid genus *Mormolyca* Fenzl, as currently circumscribed, encompasses a diverse group of ca. 27species. Many of these were included traditionally in *Maxillaria* sect. *Rufescens*, when similarity of floral morphology was considered foremost in their classification rather than the evolutionary history of the taxa. In order to begin revising species delimitation and clarifying the evolution and biology of the genus, we present a phylogenetic hypothesis using sequence data from five plastid loci (*rpoC1*, *matK* gene and flanking *trnK* intron, *atpB-rbcL* intergenic spacer, and the 3' portion of *ycf1*) and the nuclear ribosomal internal and external transcribed spacers (ITS, ETS). Resulting trees using both Bayesian and parsimony inference are congruent with each other, and generally well resolved. Based on current level of sampling across Maxillariinae, these molecular data support the monophyly of *Mormolyca* and shed light on the interspecific phylogenetic patterns within the genus. These include an early divergent paraphyletic grade of *Mormolyca* species successively sister to a clade with at least two definable subclades within. The latter are characterized by two different flower morphologies that are likely related to their pollination systems. Although not all relationships within the genus are fully resolved or supported, these results offer a first glimpse into the phylogeny of a small group of epiphytic orchids characterized by an unusually high level of variable vegetative characters, floral fragrance profiles, and pollination systems.

Key words: Maxillariinae, *Mormolyca*, molecular phylogenetics, Bayesian inference, Orchidaceae onomy

Introduction

The orchid subtribe Maxillariinae (subfamily Epidendroideae: tribe Cymbidieae) is one of the most conspicuous and vegetatively diverse groups of Neotropical orchids (Dressler 1993; Whitten 2009). As a result, reconstructing evolutionary relationships among its more than 750 species historically has been challenging (Christenson 2002). However, molecular phylogenetic analyses published by Whitten *et al.* (2007) using DNA sequences from more than 600 specimens allowed for a new interpretation of the subtribe that has been useful for redefining particular genera (Whitten & Blanco 2011). Based on well-supported clades in the gene trees and defined by morphological synapomorphies, several genera of Maxillariinae were recircumscribed by Blanco *et al.* (2007). One genus in particular, *Mormolyca* Fenzl, changed significantly. Molecular phylogenetic analyses retrieved a strongly supported clade that included a paraphyletic *Mormolyca* s.s. sister to a clade composed of species from the previously recognized *Maxillaria rufescens* complex, with the genus *Chrysocycnis* embedded within it (Whitten *et al.* 2007). These now have been transferred into *Mormolyca* to achieve monophyly of the genus, thereby expanding it from six to ca. 27 species, and increasing the range of floral and vegetative diversity within the group. *Mormolyca*, therefore, represents another example of the way in which traditional orchid classification systems that have relied almost entirely on floral morphology do not always accurately reflect the evolutionary history of their taxa.

The genus *Mormolyca* as originally circumscribed by Garay and Wirth (1959) was differentiated from *Maxillaria* on the basis of morphological characters such as its long inflorescence, absence of a column foot, and moon-shaped viscidium. Species of the *Maxillaria rufescens* complex (a.k.a. the *Rufescens* complex) are vegetatively similar to *Mormolyca* s.s. in their shortly creeping rhizomes with unifoliate pseudobulbs subtended by papery bracts (Carnevali Fernández-Concha *et al.* 2001), but the inflorescences

are much shorter. On the opposite side of the spectrum, the two species previously placed in *Chrysocycnis* are characterized by their elongate, erect rhizomes between scattered unifoliate pseudobulbs (Sweet 1971).

Plants from both *Mormolyca* s.s. and the former concept of *Chrysocycnis* have flat open flowers with a tomentose, insect-like labellum and arcuate column (especially pronounced in *Chrysocycnis*). Given that flowers of *M. ringens* are know to be pollinated by male bees through a syndrome of deceit pseudocopulation (Singer *et al.* 2004), we expect many or all species of *Mormolyca* with similar insectiform flowers (Fig. 1 G, H, J & M), to be pollinated by sexual deceit—a pollination system that was made famous over the past century by studies of unrelated terrestrial orchids in Europe, Australia, and South Africa (Stoutamire 1974, 1983; Paulus & Gack 1990; Schiestl *et al.* 2003; Johnson & Morita 2006; van der Niet *et al.* 2011). In contrast, some of the *Mormolyca* species transferred from the *Rufescens* complex have semi-open flowers with a labellum pad of short, glandular trichomes (Fig. 1 A-C, E , K, L, N, O, Q, R), and exhibit a conspicuous diversity of pleasant floral scents (Christenson 2002; Flach *et al.* 2004; pers. obs.). These orchids are almost certainly not sexually deceptive but instead appear to offer rewards to their pollinators in the form of specialized, nutrient-rich trichomes (Davies *et al.* 2000; Davies & Turner 2004; Davies & Stpiczyńska 2012). The remainder of the species from the former *Rufescens* complex have either resin-secreting or resin-mimic flowers (Davies *et al.* 2012) with glossy labella (Fig 1. D, F, I, P), and a faint (sometimes absent), sweet, floral scent (personal obs.). These particular examples of highly specialized and varied floral forms indicate that pollinator-mediated selection probably played an important role in the diversification of *Mormolyca*. Thus, the newly expanded concept of the genus presents an especially appealing group to examine in greater detail from the perspective of taxonomy, systematics, and evolution.

The relationship between *Mormolyca* s.s. and the *Rufescens* complex was initially suggested by analyses of anatomical and morphological characters (Atwood & Mora de Retana 1999; Holtzmeier *et al.* 1998), and subsequently confirmed by phylogenetic analyses of molecular data (Dathe & Dietrich 2006; Whitten *et al.* 2007). However, our knowledge of the evolutionary relationships within the group is still quite limited, as more species need to be incorporated into analyses. These include a handful of recently discovered species (Bogarin & Pupulin 2010; R. Arevalo and G. Carnevali unpubl. data). Not only is the genus now defined by vegetative rather than floral synapomorphies, but chemical characters related to pollination systems may also be useful for clarifying species boundaries. To understand patterns of diversification within the genus and in order to revise species delimitations that will ultimately lead to a stable classification of these orchids, a thoroughly sampled and well-supported phylogenetic framework is required. In this study we increase the dimensions of previously published molecular data sets by increasing taxon and gene samplings. We also implement alternative methods of molecular phylogenetic analysis (maximum parsimony and Bayesian inference) in an attempt to reconstruct a fully resolved and highly supported molecular phylogeny of *Mormolyca* that can be used in subsequent evolutionary studies and taxonomic revision of the genus.

Materials and methods

Taxon sampling — From the *ca.* 27 species estimated to be in the new broad concept of *Mormolyca* (Blanco *et al.* 2007), we sampled 23 species/morphospecies (Table 1). In addition to the 17 used by Whitten *et al.* (2007), we increased the number of samples and species by targeting missing taxa from areas poorly represented in the original matrices, namely Colombia, Ecuador, and Peru. Our outgroup taxa comprise 36 species from 16 other genera of Maxillariinae (Whitten *et al.* 2007), including three recently described species new to science (Arévalo *et al.* 2013). A few specimens could not be identified unequivocally to species; they may represent new species or are elements of highly variable species complexes. These are identified with either the species modifier "c.f." or the name of its putative closest relative or with the abbreviation "sp. nov." after the genus, respectively. Even though our sampling of *Mormolyca* s.l. is still 26% incomplete, we are confident that we have included enough representatives of the genus to begin making assertions about the evolutionary relationships among the taxa.

DNA extraction, amplification, and sequencing — Once specimens were obtained, plant tissue was

Table 1. Voucher information for taxa used in this study. Herbarium acronyms: COL = Universidad Nacional de Colombia, Bogotá D.C., Colombia; CR = Museo Nacional de Costa Rica, San José, Costa Rica; FLAS = University of Florida; JBL = Jardín Botánico Lankester, Universidad de Costa Rica, Cartago, Costa Rica; M = Botanische Staatssammlung München, München, Germany; SEL = Marie Selby Botanical Gardens, Sarasota, FL, U.S.A,; QCA = Pontificia Universidad Católica del Ecuador, Quito, Ecuador; SP = Instituto de Botânica, São Paulo, Brazil; UEC = Universidade Estadual de Campinas, Brazil; WIS = University of Wisconsin, Madison, U.S.A.Figure 3. Majority-rule consensus of 7500 trees obtained from Bayesian analysis (GTR + gamma model of evolution) of combined plastid and nuclear DNA regions. Numbers above branches are bootstrap percentages; numbers below branches are Bayesian posterior probabilities. Colored branches indicate the groups discussed in the text. Letter codes following the taxon name, where present, represent the country of provenance: CLM=Colombia, COS=Costa Rica, ECU=Ecuador, HON=Honduras, PAN=Panama, PER=Peru, PUE=Puerto Rico.

	Taxon	**Collector and number**	**Source**	**Herbarium**
1	*Brasiliorchis picta* (Hook.) R.B.Singer, S.Koehler & Carnevali	*Whitten 2755*	Brazil, cult.	FLAS
2	*Brasiliorchis schunkeana* (Campacci & Kautsky) R.B.Singer, S.Koehler & Carnevali	*Whitten 1992*	Brazil, cult.	FLAS
3	*Bifrenaria tetragona* (Lindl.) Schltr.	*Whitten 93156*	Brazil	FLAS
4	*Camaridium carinulatum* (Rchb.f.) M.A.Blanco	*Arévalo 932*	Colombia	COL
5	*Camaridium ochroleucum* Lindl.	*Gerlach 2003–3648*	Brazil	M
6	*Christensonella ferdinandiana* (Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	*Koehler 0089*	Brazil	SP
7	*Christensonella nardoides* (Kraenzl.) Szlach.	*Whitten 2502*	Ecuador, cult. Ecuagenera	FLAS
8	*Cryptocentrum peruvianum* (Cogn.) C.Schweinf.	*Whitten 2322*	Ecuador	FLAS
9	*Cryptocentrum* sp.	*Arévalo 931*	Colombia	COL
10	*Cyrtidiorchis alata* (Ruiz & Pav.) Rauschert	*Whitten 2932*	Ecuador, cult.	FLAS
11	*Cyrtidiorchis rhomboglossa* (F.Lehm. & Kraenzl.) Rauschert	*Giraldo 17*	Colombia	COL
12	*Eriopsis biloba* Lindl.	*Whitten 3153*	Ecuador	QCA
13	*Heterotaxis villosa* (Barb.Rodr.) F.Barros	*Arévalo 902*	Colombia	COL
14	*Heterotaxis violaceopunctata* (Rchb.f.) F.Barros	*Whitten 2294*	Brazil, cult.	FLAS
15	*Inti bicallosa* (Rchb.f.) M.A.Blanco	*Whitten 2636,*	Panama	FLAS
16	*Inti chartacifolia* (Ames & C.Schweinf.) M.A.Blanco	*Whitten 2752*	cult.	FLAS
17	*Mapinguari auyantepuiensis* (Foldats) Carnevali & R.B.Singer	*Whitten 2347*	Ecuador	FLAS
18	*Mapinguari longipetiolatus* (Ames & C.Schweinf.) Carnevali & R.B.Singer	*Atwood & Whitten 5075*	Costa Rica	SEL
19	*Maxillaria farinosa* Arévalo & Christenson, *sp. nov.*	*Arévalo 734*	Colombia	COL
20	*Maxillaria splendens* Poepp. & Endl.	*Koehler 0144*	Brazil, cult.	UEC
21	*Maxillaria tenebrifolia* Arévalo & Christenson, *sp. nov.*	*Arévalo 454*	Colombia	COL
22	*Maxillariella procurrens* (Lindl.) M.A.Blanco & Carnevali	*Whitten 2397*	Ecuador, cult.	FLAS
23	*Mormolyca* cf. *acutifolia* (Lindl.) M.A.Blanco	1: *Arévalo 1071*	Colombia, cult. Colomborquideas	WIS
		2: *Giraldo 44*	Colombia	COL
24	*Mormolyca* cf. *aureoglobula* (Christenson) M.A.Blanco	*Arévalo 1069*	Colombia, cult. Orquídeas del Valle	WIS
25	*Mormolyca chacoensis* (Dodson) M.A.Blanco	*Arévalo 947*	Perú, cult., Agroriente Viveros	COL

Table 1. *Continued*.

26	*Mormolyca culebrica* Bogarín & Pupulin	*Whitten 2650*	Pánama, cult.	FLAS
27	*Mormolyca dressleriana* (Carnevali & J.T.Atwood) M.A.Blanco	1: *Arévalo 1066*	Pánama, cult.	WIS
		2. *Arévalo 1065*	Costa Rica, cult.	WIS
28	*Mormolyca fumea* Bogarín & Pupulin	*Bogarín 5729*	Costa Rica	CR
29	*Mormolyca gracilipes* (Schltr.) Garay & Wirth	*Arévalo 1061*	Colombia, cult., Orquídeas del Valle	WIS
30	*Mormolyca hedwigiae* (Hamer & Dodson) M.A.Blanco	1: *Koehler 0314*	Guatemala, cult.	ESA
		2: *Arévalo 1065*	Honduras, cult.	WIS
31	*Mormolyca moralesii* (Carnevali & J.T.Atwood) M.A.Blanco	1: *Bogarín 3826*	Costa Rica	JBL
		2: Bogarín 4139	Costa Rica	JBL
32	*Mormolyca peruviana* C.Schweinf.	*Whitten 2497*	Ecuador, cult.	FLAS
33	*Mormolyca polyphylla* Garay & Wirth	*Arévalo 950*	Ecuador, cult. Ecuagenera	COL
34	*Mormolyca pudica* (Carnevali & J.L.Tapia) M.A.Blanco	*Arévalo 1068*	Puerto Rico	WIS
35	*Mormolyca richii* (Dodson) M.A.Blanco	1: *Whitten 2362*	Ecuador, cult.	FLAS
		2: *Arévalo 1064*	Ecuador, cult.	WIS
36	*Mormolyca ringens* (Lindl.) Gentil	*Arévalo 1062*	Colombia, cult. Orquídeas del Valle	WIS
37	*Mormolyca rufescens* (Lindl.) M.A.Blanco	1: *Arévalo 1073*	U.S.A., cult. Marie Selby Botanical Gardens	WIS
		2: *Arévalo 941*	Perú, cult., Agroriente Viveros	WIS
		3: *Arévalo 1076*	U.S.A., cult. Marie Selby Botanical Gardens	WIS
		4: *Arévalo 1075*	U.S.A., cult. Marie Selby Botanical Gardens	WIS
		5: *Arévalo 942*	Perú, cult., Agroriente Viveros	COL
		6: *Arévalo 943*	Perú, cult., Agroriente Viveros	COL
38	*Mormolyca sanantonioensis* (Christenson) M.A.Blanco	*Arévalo 1070*	Colombia, cult. Orquídeas del Valle	WIS
39	*Mormolyca schlimii* (Linden & Rchb.f.) M.A.Blanco	*Giraldo 763*	Colombia	COL
40	*Mormolyca schweinfurthiana* Garay & Wirth	*Arévalo 956*	Ecuador, cult. Ecuagenera	COL
41	*Mormolyca suareziorum* (Dodson) M.A.Blanco	*Arévalo 945*	Perú, cult., Agroriente Viveros	COL
42	*Mormolyca* cf. *suareziorum* (Dodson) M.A.Blanco	*Whitten 2758*	Ecuador, cult.	FLAS
43	*Mormolyca* cf. *tenuibulba* (Christenson) M.A.Blanco	1: *Arévalo 878*	Colombia	COL
		2: *Arévalo 951*	Ecuador, cult., Ecuagenera	COL
		3: *Arévalo 1072*	Colombia, cult. Colomborquideas	WIS
44	*Mormolyca* sp. nov. A	*Arévalo 1063*	U.S.A., cult., Marie Selby Botanical Gardens	WIS
45	*Mormolyca* sp. nov. B	*Blanco 3108*	Ecuador, cult.	FLAS
46	*Mormolyca* sp. nov. C	1: *Arévalo 1074*	Colombia, cult. Colomborquídeas	WIS
		2: *Arévalo 939*	Perú, cult., Agroriente Viveros	COL
		3: *Arévalo 953*	Perú, cult., Agroriente Viveros	COL

Table 1. *Continued*.

47	*Nitidobulbon nasutum* (Rchb.f.) Ojeda & Carnevali	*Whitten 1869*	Ecuador	FLAS
48	*Nitidobulbon proboscideum* (Rchb.f.) Ojeda & Carnevali	*Atwood & Whitten 5056*	Venezuela	SEL
49	*Ornithidium montezumae* Arévalo & Christenson	*Arévalo 674*	Colombia	COL
50	*Ornithidium* cf. *semiscabrum* Lindl.	*Arévalo 588*	Colombia	COL
51	*Ornithidium aggregatum* (Kunth) Rchb.f.	*Arévalo 623*	Colombia	COL
52	*Pityphyllum saragurense* (Dodson) Whitten	*Whitten 3084*	Ecuador, cult.	QCA
53	*Pityphyllum antioquiense* Schltr.	*Whitten 2473*	Ecuador, cult.	FLAS
54	*Rhetinantha acuminata* (Lindl.) M.A.Blanco	*Whitten 2698*	Ecuador	FLAS
55	*Rhetinantha notylioglossa* (Rchb.f.) M.A.Blanco	*Koehler 0033*	Brazil	UEC
56	*Sauvetrea alpestris* (Lindl.) Szlach.	*Whitten 2551*	Ecuador, cult.	FLAS
57	*Sauvetrea laevilabris* (Lindl.) M.A.Blanco	*Whitten 2358*	Ecuador, cult.	FLAS
58	*Trigonidium egertonianum* Bateman ex Lindl.	*Arévalo 1060*	cult.	WIS
59	*Trigonidium obtusum* Lindl.	*Whitten 2997*	cult.	FLAS

preserved in silica gel, and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.). Living specimens were documented with detailed photographs prior to being pressed for herbarium vouchers. When possible, flowers were collected and preserved in FAA for micro-morphological assessment.

For each specimen we attempted to sequence the plastid *matK* gene and flanking *trnK* intron, the *atpB-rbcL* intergenic spacer, the *rpoC1* gene, and the downstream (3') portions of *ycf1*, along with the nuclear ribosomal ITS and ETS regions. DNA amplification and sequencing was carried out following published primers and methods, with modifications when necessary (matK+*trnK*, *atpB-rbcL*, *rpoC1* and ITS from Whitten *et al.* 2007; *ycf1* from Neubig *et al.* 2009; ETS from Monteiro *et al.* 2010). Electropherograms were assembled and edited using Geneious Pro 5.0.3 (Drummond *et al.* 2010); alignments were generated using MUSCLE (Edgar 2004) and adjusted by eye using MacClade (Maddison & Maddison 2005).

Data analysis–Phylogenetic inference methods included maximum parsimony (MP) and Bayesian inference (BI). We performed combined analysis with ITS + ETS (nrDNA), all four plastid regions (cpDNA), and finally with all regions (total DNA). MP analyses were performed in PAUP* 4.10b (Swofford 2002) with Fitch parsimony as the optimality criterion (unordered characters, equal weights; Fitch 1971), ACCTRAN optimization, and gaps treated as missing data. The heuristic search strategy consisted of 5000 random-addition replicates of branch swapping by subtree-pruning-regrafting (SPR), saving multiple trees (MULTREES), and holding five trees at each step. The resulting trees were then used as starting trees for tree-bisection-reconnection swapping (TBR). Levels of support were estimated from 1000 bootstrap replicates, using TBR swapping for five random-addition replicates per bootstrap replicate. Parsimony ratchet search strategy for finding shortest trees was also performed with the program PAUPRat (Sikes & Lewis 2001). The software package MRBAYES v3.2.1 (Ronquist *et al.* 2012) was used for BI analyses. Tree searches were performed assuming single and multiple models of sequence evolution for each partition, following the "Akaike information criterion" as implemented in JModeltest (Posada 2008). For each analysis, Markov chain Monte Carlo (MCMC) searches were made for 10 million generations, sampling every 1000 generations, with a burn-in of 25% and chains heated to 0.07 (increasing the frequency of data swapping between chains).

Results

Statistics associated with MP analyses of cpDNA, nrDNA, and total DNA data sets are summarized in Table 2. Analysis of both cpDNA and nrDNA data sets strongly support the monophyly of *Mormolyca*,

TABLE 2. Features of DNA data sets used in this study. CI = consistency index; RI = retention index. Percentages calculated in relation to aligned length.

Data set	No. of ingroup taxa	Aligned length	No. of variable characters	No. of parsimony-informative characters	No. of most parsimonious trees	Tree length	CI	RI
nrDNA	38	1368	546 (39.9%)	301 (22%)	126,660	1129	0.637	0.716
cpDNA	38	7242	1254 (17.3%)	521 (7.2%)	88,329	2155	0.650	0.706
totalDNA	38	8610	1801 (20.9%)	822 (9.5%)	8353	3373	0.629	0.688

although relationships within the genus differ in the absence of strongly supported clades in the nrDNA analysis (trees not shown). By comparison, the total DNA matrix offered the highest level of resolution among all genera and within *Mormolyca*, as assessed by bootstrap support. The strict consensus tree from MP analysis of the total DNA data set is presented in Fig. 2. For BI analysis, the model-based estimate based on the cpDNA regions produced a tree with higher levels of support when compared to the nrDNA tree (trees not shown). Consensus trees from BI and MP were similar, but the Bayesian tree was more highly resolved within *Mormolyca*. This is the tree upon which our discussion will follow (Fig. 3).

Placement of the recently described species (*Maxillaria farinosa*, *M. tenebrifolia*, and *Ornithidium montezumae*) within their respective lineage in the overall tree confirm their assigned genera (Fig. 2). *Mormolyca* s.l. forms a monophyletic group with strong support. Taxa within the genus can be divided into three clusters (showed with color branches on tree) for the sake of further discussion: a grade of early divergent species and two clades (Fig. 2).

Mormolyca s.s. species (green branches on tree) are the earliest extant lineages to diverge from the genus, represented here by *M. polyphyla*, *M. peruviana*, *M. schweinfurthiana*, *M. gracilipes*, and *M. ringens*, ending with *M. schlimii*, which is sister to the single large clade containing all members of the former *Rufescens* complex (Fig. 2). Topographically, this clade, consists of clade I (purple branches) with weak support (BP 69%, PP 1.0) but fully resolved, and clade II (orange branches) that is not fully resolved.

Discussion

This study confirms the inclusion of species from the *Maxillaria rufescens* complex and *Chrysocycnis* species within *Mormolyca* (Blanco *et al.* 2007, Whitten *et al.* 2012). In general, as now defined, *Mormolyca* s.l. can be recognized by the following combination of characters: unifoliate pseudobulbs subtended by a non-foliaceous (papery) sheaths (except for *M. polyphylla,* which has elongated pseudobulbs subtended by more than one sheath and up to three apical leaves), the single-flowered, erect inflorescence produced from the older parts of the rhizome (rather than from the terminal growth), perianth parts that lack fibers, and the clavate, arcuate column (Blanco *et al.* 2007). Distribution of the genus ranges from southern Mexico in the north to Bolivia and northern Brazil in the south, with plants typically found in forests at elevations from sea level to 1900 m.a.s.l. (based on gathered data from herbarium specimens). One taxon, *M. pudica*, is apparently restricted to the Greater Antilles (Carnevali Fernandez-Concha *et al.* 2001). A weak geographic pattern can be detected in the phylogenetic reconstruction, with most of the early-diverging species restricted to the southern part of the range, (i.e., from Peru, Ecuador, and Colombia), whereas the more derived clades have a more northern distribution, reaching all the way to southern Mexico.

The consecutively early-diverging and paraphyletic taxa are represented here by most species of *Mormolyca* s.s. and *M. schlimii*. All are morphologically similar, particularly in their flowers (i.e. absence of a column foot , the insectiform labellum, and the prominent arcuate column). Unfortunately, our study did not include *M. aurorae* or *M. fuchsii*, which are known only from the type specimens, and *M. lehmanii,* for which we were unable to obtain samples. We would expect these species to be positioned in this part of the tree as well. The appearance of *M. gracilipes* and *M. schweinfurthiana* as sister species may reflect a misidentification of one of the samples and/or poor alpha-taxonomy. These two species are similar in form, and the two names are often incorrectly applied. We

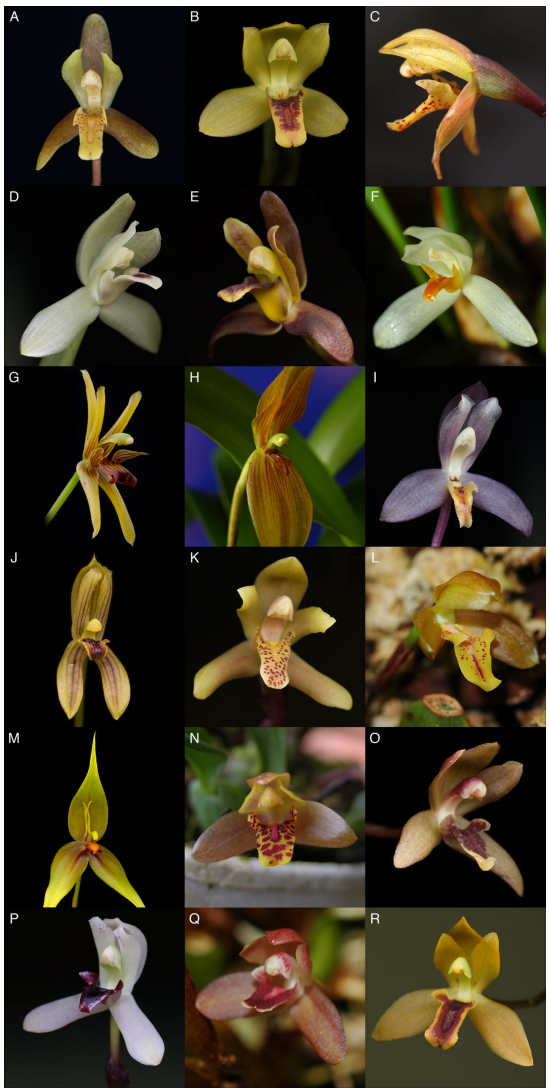

Figure 1. A. *Mormolyca acutifolia;* B. *M. aureoglobula;* C. *M.* cf. *chacoensis;* D. *M. culebrica;* E. *M. dressleriana;* F. *M. hedwigiae;* G. *M. peruviana;* H. *M. polyphylla;* I. *M. richii;* J. *M. ringens;* K. *M. rufescens;* L. *M. sanantonioensis;* M. *M. schweinfurthiana;* N. *M. suareziorum;* O. *M. tenuibulba;* P. *M.* sp. nov. A; Q. *M.* sp. nov. B; R. *M.* sp. nov. C. All photos by R. Arévalo, except J & M by D. Bogarín.

suspect that they may, in fact, be describing the same species. However, more material including the type material needs to be examined to make a conclusive decision regarding their taxonomy.

Looking at the species distribution within the larger clade that groups the entire *Rufescens* complex, patterns seem to emerge. Although there is only weak bootstrap support (BS 69%, PP 1.0) for this group, species with a glossy labellum cluster together. Some of these secrete small quantities of resin on the labellum and are thought to mimic taxa that produce lipoidal rewards (Davies *et al.* 2012; Arévalo, unpubl. data). This clade includes one of the new species that was found as a result of this study (*Mormolyca* sp. nov. A), which is sister to the rest of the clade consisting of *M. richii*, *M. hedwigiae*, *M. culebrica*, and *M. fumea.* The latter, which is not recognized by the World Checklist of Selected Plant Families (Govaerts *et al.* 2011) and considered a synonym of *M. aureoglobula*, appears as a distinct branch sister to *M. culebrica* in our analyses. This result indicates that it probably does deserve to be treated as a distinct species.

The remaining *Mormolyca* species from the former *Rufescens* complex, characterized by their semi-open, fragrant flowers with a labellum pad of short, glandular trichomes (Flach *et al.* 2004; pers. obs.), also cluster together. Species delimitation in this group has been difficult historically, with nearly every species of the complex included at one point either in a broad concept of either *Mormolyca rufescens* (Lindl.) Blanco, or *M. acutifolia* (Lindl.) Blanco. Large- flowered specimens with conspicuous fragrances are usually associated with *M. rufescens*, whereas all small-flowered entities are usually considered to be related to *M. acutifolia*. A factor contributing to this taxonomic confusion is the fact that *M. acutifolia* (Lindl.) Blanco is poorly defined. Other than the type specimen itself, there is only a vague description from Lindley (1839) and a single drawing of the flower labellum found with the holotype.

Also, within this cluster we find *M. dressleriana* as sister to a group of species in which relationships and alpha-taxonomy are still imprecise. This group reunites what we are considering *M. acutifolia* along with some entities originally related to the species and now segregated (*i.e. M. pudica*, *M. aureoglobula*, *M. sanantonioensis*, *M. moralesii*). It also contains multiple individuals of *M. tenuibulba*, a species characterized by having ascending rhizomes with long pseudobulbs and coconut-scented flowers. Accessions of this species are not monophyletic, which we believe may be due to incomplete data sampling. A new morphologically distinct species

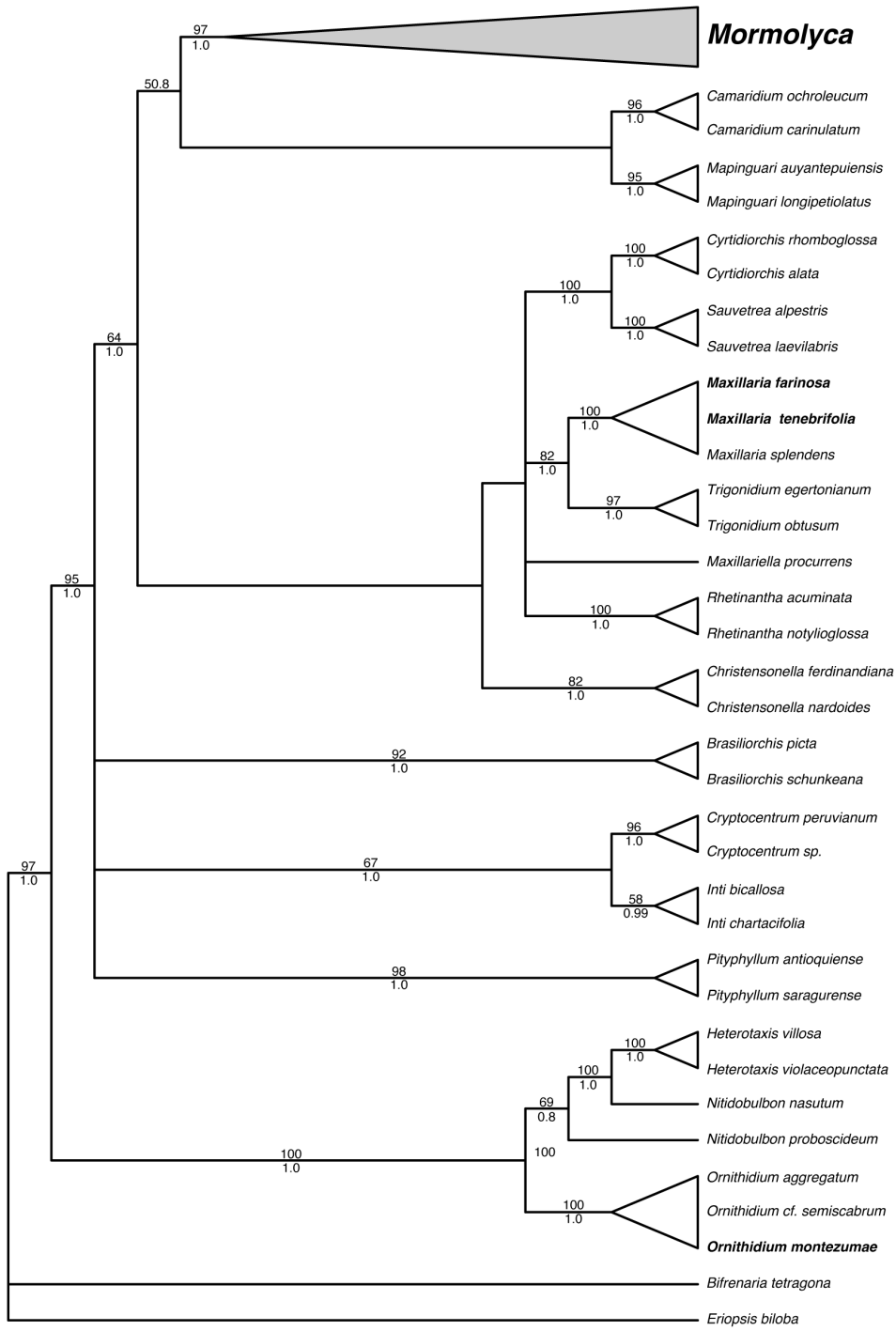

FIGURE 2. Strict consensus tree from the 8353 equally most parsimonious trees after maximum parsimony analysis of the combined (plastid and nuclear) data matrix. Numbers above branches are bootstrap percentages; numbers below branches are posterior probabilities for clades estimated by the proportion of occurrence in the tree set from Bayesian analysis. Taxa in bold correspond to species recently described by the authors.

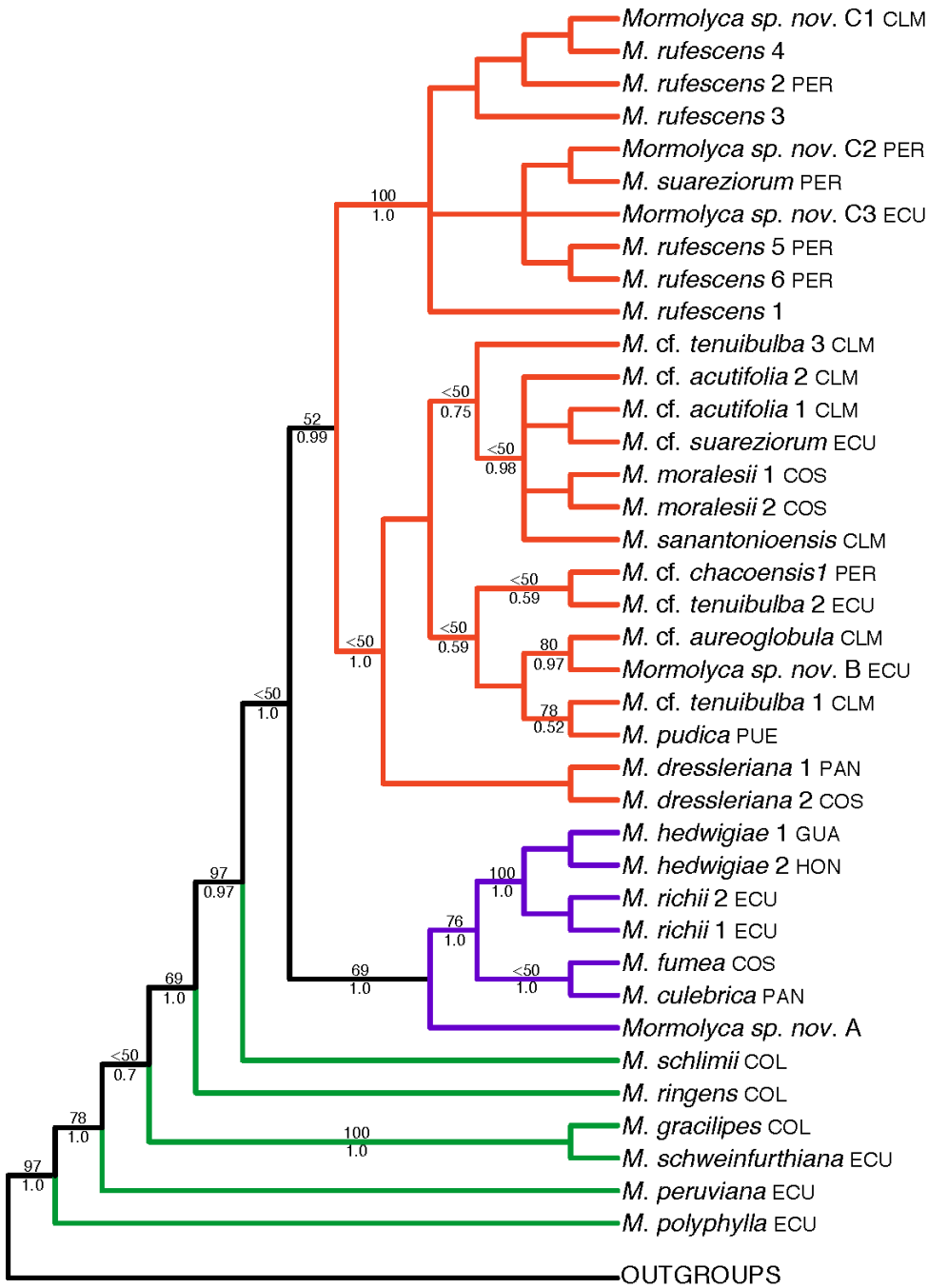

Figure 3. Majority-rule consensus of 7500 trees obtained from Bayesian analysis (GTR + gamma model of evolution) of combined plastid and nuclear DNA regions. Numbers above branches are bootstrap percentages; numbers below branches are Bayesian posterior probabilities. Colored branches indicate the groups discussed in the text. Letter codes following the taxon name, where present, represent the country of provenance: CLM=Colombia, COS=Costa Rica, ECU=Ecuador, HON=Honduras, PAN=Panama, PER=Peru, PUE=Puerto Rico.

of *Mormolyca* previously recognized (G. Carnevali, pers. comm. 2008), also appears here (*Mormolyca* sp. nov. B). In general, the topology recovered for this part of the tree indicates that species boundaries need to be better defined in this group. A revision is currently in progress.

Finally, we recovered a well-supported clade that includes all large-flowered plants associated with *M. rufescens*, as well as the small-flowered species *M. suareziorum*, along with accessions of what we are considering another new species of *Mormolyca* (*Mormolyca* sp. nov. C) from three different locations. We included multiple samples of *M. rufescens* that vary in flower color pattern, a character often used to segregate new species of *Mormolyca* (e.g. Christenson 2010), to test taxonomic hypotheses of reciprocal monophyly. Unfortunately, precise relationships among these accessions are unresolved in our analyses, and so issues of species delimitations will require further study.

In summary, despite our efforts to resolve phylogenetic relationships more fully within *Mormolyca*, more work still remains to be done. Species delimitation in this genus is difficult, but we feel strongly that variation in flower color should be reconsidered or even disregarded as a character used to segregate species. Given the importance of flower micro-morphology in their pollination systems, detailed morphological analyses are currently underway in the search for unambiguous synapomorphies. This is our first attempt to reconstruct a phylogeny of *Mormolyca,* and we are confident that the addition of genetic data from more variable loci such as the low-copy nuclear gene *PhyC* (Russell *et al.* 2010), as well as micro-morphological characters will help us achieve a better estimate of relationships within the genus. Evolutionary processes that underlie the patterns of variation and specialization exhibited by this group of plants remain to be investigated. To address these challenges and utilize *Mormolyca* as a model system for understanding orchid pollinator evolution within Neotropical epiphytic Orchidaceae, we expect eventually to couple these molecular phylogenetic data with floral morphology and patterns of volatile production to assess the role of evolving flower form and function in the process of orchid speciation.

Literature cited

Arévalo, R., K. Cameron & E. Christenson. 2013. Three new species of *Ornithidium* and *Maxillaria* (Maxillariinae) from Colombia: recent discoveries from the Cordillera Occidental of the Colombian Andes. Orchids 82: 44-48.

Atwood, J.T. & D.E. Mora de Retana. 1999. Flora Costaricensis family #39 Orchidaceae: tribe Maxillarieae: subtribes Maxillariinae and Oncidiinae. Fieldiana, Bot., n.s. 40: 1-182.

Blanco, M.A., G. Carnevalli, W.M. Whitten, R.B. Singer, S. Koehler, N.H. Williams, I. Ojeda, K.M. Neubig & L. Endara. 2007. Generic realignments in Maxillariinae (Orchidaceae). Lankesteriana 7: 515-537.

Bogarín, D. & F. Pupulin. 2010. Two new species of *Mormolyca* from Costa Rica and Panama. Orchid Dig. 74: 427-47.

Carnevali Fernández-Concha, G., J.L. Tapia Muñoz & M. Gómez-Juárez. 2001. A synopsis of the *Maxillaria rufescens* complex in Mexico, Central America, and the Greater Antilles. Brittonia 53: 454-465.

Christenson, E.A. 2002. *Maxillaria*, an overview. Pp. 279-290 *in:* Proceedings of the 16th World Orchid Conference (ed. J. Clark, W. Elliott, G. Tingley & J. Biro). Vancouver Orchid Society, Richmond, British Columbia, Canada.

Christenson, E.A. 2010. A showy new species of *Maxillaria* (Orchidaceae) from Bolivia. Richardiana 10: 94-98.

Dathe, S. & D.H. Dietrich. 2006. Comparative molecular and morphological studies in selected Maxillariinae orchids. Willdenowia 36: 89-102.

Davies, K.L. & M. Stpiczyńska. 2012. Comparative anatomy of resin-secreting and putative resin-mimic species of *Maxillaria* s.l. (Orchidaceae: Maxillariinae). Bot. J. Linn. Soc. 170: 405-435.

Davies, K.L. & M.P. Turner. 2004. Morphology of floral papillae in *Maxillaria* Ruiz & Pav. (Orchidaceae). Ann. Bot. 93: 75-86.

Davies, K. L., C. Winters & M.P. Turner. 2000. Pseudopollen: its structure and development in *Maxillaria* (Orchidaceae). Ann. Bot. 85: 887-895.

Dressler, R.L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press, Portland, Oregon.

Drummond, A.J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, J. Heled, M. Kearse, R. Moir, S. Stones-Havas, S. Sturrock, T. Thierer & A. Wilson. 2010. Geneious v5.1, Available from http://www.geneious.com.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-1797.

Fitch, W.M. 1971. Toward defining the course of evolution–minimum change for a specific tree topology. Syst. Zool. 20: 406-416.

Flach, A., R.C. Dondon, R.B. Singer, S. Koehler, E. Amaral Maria do Carmo & A.J. Marsaioli. 2004. The chemistry of pollination in selected Brazilian Maxillariinae orchids: floral rewards and fragrance. J. Chem. Ecol. 30: 1045-1056.

Garay, L.A. & M. Wirth. 1959. On the genera *Mormolyca* Fenzl and *Cyrtoglottis* Schltr. Canad. J. Bot. 37: 479-490.

Govaerts, R., J. Dransfield, S.F. Zona, D.R. Hodel & A. Henderson. 2011. World Checklist of Orchidaceae. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://apps.kew.org/wcsp/ Retrieved 2013-01-22.

Holtzmeier, M.A., W.L. Stern & W.S. Judd. 1998. Comparative anatomy and systematics of Senghas's cushion species of *Maxillaria* (Orchidaceae). Bot. J. Linn. Soc. 127: 43–82.

Huelsenbeck, J.P. & F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754-755.

Johnson, S.D. & S. Morita. 2006. Lying to Pinocchio: floral deception in an orchid pollinated by long-proboscid flies. Bot. J. Linn. Soc. 152: 271-278.

Lindley, J. 1839. *Maxillaria acutifolia*. *In* J. Lindley (ed.) , Edwards's Botanical Register, t. 92. London.

Maddison, D.R. & W.P. Maddison. 2005. MacClade 4: analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, Massachusetts.

Monteiro, S.H., A. Selbach-Schnadelbach, R.P. de Oliveira & C. van den Berg. 2010. Molecular phylogenetics of *Galeandra* (Orchidaceae: Catasetinae) based on plastid and nuclear DNA sequences. Syst. Bot. 35: 476-486.

Neubig, K., W.M. Whitten, B. Carlsward, M. Blanco, L. Endara, N. Williams & M. Moore. 2009. Phylogenetic utility of *ycf1* in orchids: a plastid gene more variable than *matK*. Pl. Syst. Evol. 277: 75-84.

Paulus, H.F. & C. Gack. 1990. Pollinators as prepollinating isolation factors: evolution and speciation in Ophrys (Orchidaceae). Israel J. Bot. 39: 43-79.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molec. Biol. Evol. 25: 253-256.

Ronquist, F. & J.P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Russell, A., R. Samuel, V. Klejna, M. Barfuss, B. Rupp & M. W. Chase. 2010. Reticulate evolution in diploid and tetraploid species of *Polystachya* (Orchidaceae) as shown by plastid DNA sequences and low-copy nuclear genes. Ann. Bot. 106: 37-56.

Schiestl, F.P., R. Peakall, J.G. Mant, F. Ibarra, C. Schulz, S. Franke & W. Francke. 2003. The chemistry of sexual deception in an orchid-wasp pollination system. Science 302: 437-438.

Sikes, D.S. & P.O. Lewis. 2001. Beta software, version 1. PAUPRat: PAUP* implementation of the parsimony ratchet. Distributed by the authors. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut.

Singer, R.B., A. Flach, S. Koehler, A.J. Marsaioli & M.C.E. Aamaral. 2004. Sexual mimicry in *Mormolyca ringens* (Lindl.) Schltr. (Orchidaceae: Maxillariinae). Ann. Bot. 93: 755-762.

Stoutamire, W.P. 1974. Australian terrestrial orchids, thynnid wasps and pseudocopulation. Amer. Orchid. Soc. Bull. 43: 13-18.

Stoutamire, W.P. 1983. Wasp-pollinated species of *Caladenia* (Orchidaceae) in south western Australia. Austral. J. Bot. 31: 383-394.

Sweet, H.R. 1971. Orquídeas Andinas poco conocidas V. *Chrysocycnis* Linden & Rchb.f.. Orquideología 6: 3-10.

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, Massachusetts.

van der Niet, T., D.M. Hansen & S.D. Johnson. 2011. Carrion mimicry in a South African orchid: flowers attract a narrow subset of the fly assemblage on animal carcasses. Ann. Bot. 107: 981-992.

Whitten, W.M. 2009. Subtribe Maxillariinae. Pp. 119-123 *in:* Genera orchidacearum Vol. 5. Epidendroideae (Part two) (ed. A.M. Pridgeon, P.J. Cribb, M.W. Chase & F.N. Rasmussen). Oxford University Press, Oxford.

Whitten, W.M. & M.A. Blanco. 2011. Defining generic limits in *Maxillaria*. A return to the orchidaceous mine. Orchids 80: 104-113.

Whitten, W.M., M.A. Blanco, N.H. Williams, S. Koehler, G. Carnevali, R.B. Singer, L. Endara & K.M. Neubig. 2007. Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based on combined molecular data sets. Amer. J. Bot. 94: 1860-1889.