Looking for Missing Swans

by Günter Gerlach* and Oscar Alejandro Pérez Escobar**

Phylogenetics of *Cycnoches* (Catasetinae: Orchidaceae)

Abstract: THE GENUS *CYCNOCHES* ENCOMPASSES AROUND 34 SPECIES DISTRIBUTED FROM SOUTHERN MEXICO to eastern Brazil and Bolivia. One of the most interesting traits of *Cycnoches* is the marked and variable sexual dimorphism present across its species, which in turn define its two infrageneric sections: sect. *Cycnoches* and sect. *Heteranthae*. In spite of the taxonomic revisions published so far and research on physiology, phytochemistry and anatomy carried out to date, the phylogenetic position of *Cycnoches* within Catasetinae is conflicting. More importantly, its relationships at species level as well as its biogeographic history remain elusive. Thus, the main goals of this research project are to reconstruct the phylogenetic relationships of *Cycnoches* (based on molecular, morphological and phytochemical datasets), as well as to infer its biogeographic history and the evolutionary pattern of change of its reproductive characters. Preliminary results of molecular phylogenetic studies confirm the monophyly (all the species of the genus descend from a single ancestor) of *Cycnoches*, as stated in previous phylogenetic reconstructions within Catasetinae. However, due to incomplete taxon sampling, phylogenetic relationships within *Cycnoches* still remain unresolved. Plant material (namely pseudobulbs, leave tissue, flowers in liquid) are urgently needed in order to continue with this research project, mostly from the members of sect. *Heteranthae.*

Key words: Orchidaceae, *Cycnoches*, molecular phylogenetics.

The genus *Cycnoches* (Catasetinae: Orchidaceae) is certainly one of the most outstanding ones among orchids because of its remarkable trait of production of functionally unisexual, dimorphic flowers. With approximately 34 species (Carr 2012; see Table 1), it ranges from Southern Mexico to Bolivia and northern Brazil with a diversity maximum in Panamá and Colombia. *Cycnoches* are commonly known as "swan orchids," their name acquired from the similitude of the slender curved column of male flowers with the neck of a swan. Traditionally the genus has been divided into the sections *Cycnoches* and *Heteranthae* (Rolfe 1909), based on the similarity of the male and female flowers of the species. Plants of *Cycnoches* usually grow epiphytically in lowland tropical wet forests, from sea level up to 5,200 feet (1,600 m). They are easily recognized by the following set of characters: (1) sexually dimorphic flowers; (2) column of the male flower elongated, slender, curved; (3) female flowers (from species of sect. *Cycnoches* and *Heteranthae*) usually white–greenish, with a short, stout column and an entire lip; (4) male flowers large with an entire lip and a prominent, undivided callus at the base of the lip (in sect. *Cycnoches*), or small, with the margin of the lip divided into finger like processes (in sect. *Heteranthae*).

[1] *Cycnoches dianae* **(sect.** *Heteranthae***) male flowers. Panama. Photograph by Günter Gerlach.**

Despite several investigations on the taxonomy (see Rolfe 1909, Schlechter 1916, Allen 1952 Carr 2012), physiology (Gregg 1982), phytochemistry (Gregg 1983) and phylogenetics (Chase and Pippen 1990, Romero 1990, Pridgeon and Chase 1998, Romero et al. 2009) of swan orchids that have been carried out to date, the species number considered as valid is still unclear. More importantly, the phylogenetic relationships at species level still remain elusive, and the phylogenetic

Table 1. Species of *Cycnoches* sect. *Cycnoches* and their distribution. The validity of names presented here follows the World Checklist of Selected Plant Families. http://apps.kew.org/wcsp/qsearch.do;jsessionid=7BB38E42DE2E3C293A40634900FE2A1C

Cyc. chlorochilon Klotzsch = *ventricosum* var. *chlorochilon* (Klotzsch) P.H. Allen	Eastern Panama, Colombia, Venezuela
Cyc. farnsworthianum D.E. Benn. & Christenson	Peru
Cyc. haagii Barb. Rodr. = *versicolor* Rchb.f.	Surinam, Venezuela, Colombia, Ecuador, Brazil, Peru, Bolivia
Cyc. lehmannii Rchb.f.	Ecuador, Peru
Cyc. loddigesii Lindl. = *cucullata* Lindl. = *loddigesii* var. *leucochilum* Hook.	Surinam, Venezuela, Colombia
Cyc. lusiae G. Romero & Garay	Venezuela
Cyc. ventricosum Bateman	Southern Mexico, Guatemala, Belize, Honduras, Nicaragua
Cyc. warszewiczii Rchb.f. = *tonduzii* Schltr. = *ventricosum* var. *warscewiczii* (Klotzsch) H.P. Allen	Southern Nicaragua, Costa Rica, Panama

Table 2. Species of *Cycnoches* sect. *Heteranthae* and their distribution. Species marked with asterisks belong to the *Cycnoches egertonianum* complex.

Cyc. aureum Lindl.	Costa Rica, Panama
Cyc. bennetii Dodson	Peru
Cyc. barthiorum G.F. Carr & Christenson	Southwestern Colombia
Cyc. brachydactylon Schltr.	Southwestern Colombia
Cyc. christensonii D.E. Benn.	Peru
Cyc. cooperi Rolfe = *pentadactylon* var. *cooperi* (Rolfe) Pabst	Brazil, Peru
Cyc. densiflorum Rolfe*	Middle east of Colombia
Cyc. dianae Rchb.f.* = *albidum* Kraenzl.	Panama, Colombia
Cyc. egertonianum Bateman* = *amparoanum* Schltr. = *pauciflora* Schltr. = *ventricosum* var. *egertonianum* Hook.	Southern Mexico, Guatemala, Honduras
Cyc. egertonianum var. viride Lindl* = *stelliferum* Lodd.	Southern Mexico, Guatemala, Honduras, El Salvador
Cyc. glanduliferum Rolfe*	Southern Mexico, Guatemala, Honduras
Cyc. guttulatum Schltr.*	Costa Rica, Panama, Nicaragua
Cyc. herrenhusanum Jenny & G. Romero	Colombia, Ecuador
Cyc. jarae Dodson & D.E. Benn.	Peru
Cyc. maculatum Lindl.	Venezuela
Cyc. manoelae V.P. Castro & Campacci	Brazil
Cyc. pachydactylon Schltr.*	Costa Rica, Panama, Nicaragua
Cyc. pentadactylon Lindl. = *amesianum* Sander = *espiritosantense* Brade ex Hoehne	Brazil, Peru
Cyc. peruvianum Rolfe = *thurstoniorum* Dodson	Ecuador, Peru
Cyc. powellii Schltr.*	Panama
Cyc. quatuorchristis D.E. Benn.	Peru
Cyc. rossianum Rolfe*	Costa Rica, Guatemala, El Salvador
Cyc. schmidtianum Christenson & G.F. Carr	Peru
Cyc. stenodactylon Schltr.*	Costa Rica, Panama, Nicaragua, El Salvador
Cyc. suarezii Dodson	Ecuador

position of *Cycnoches* within Catasetinae is conflicting. Most of the confusion with respect to the taxonomy of this genus is present within the species alliance called the *Cycnoches egertonianum* complex. The complex is composed of 10 very similar species (Romero and Gerlach unpubl.) mostly distributed in Central America and for which there are no type specimens or very poor herbarium material is known. Such confusion is mainly due to the highly variable characters used in the past by taxonomists for species delimitation such as flower color, shape and number of finger-like processes. Often these authors employed a single male or female flower for descriptions of new species. Careful investigations comparing flowers on a single inflorescence showed that even here the size and number of finger-like processes could be variable. So species delimitation with the use of these characters is questionable. To come to a well-founded taxonomy more individuals of each species covering the whole distribution area should be investigated.

Thus, the main objective of our interdisciplinary research project is to reconstruct the evolutionary relationships of *Cycnoches* at species and generic level, based on a combined data set of molecular, phytochemical (floral fragrance analysis) and morphological characters. We also aim to do a taxonomic revision of the genus with special emphasis on recircumscription of species based primarily on phytochemical characters, which will include descriptions and information about distribution and ecology as well as illustrations of each taxon. A well-resolved phylogeny of the evolutionary relationships of *Cycnoches* will allow us to infer the evolutionary trends in the reproductive systems within the genus, such as evolution of sexual dimorphism in *Cycnoches* and in the subtribe (i.e., evolution of different reproductive systems such as unisexuality in *Catasetum*, *Cycnoches*, and *Mormodes* and protandry [maturation of the anthers versus stigmas resolved in time] in *Dressleria*). Our research will shed light on the evolution of related genera with similar evolutionary trends and will form the basis for tackling other taxonomically problematic genera within Catasetinae (*Catasetum, Mormodes*).

As a result of our investigations, 46 sequences of *Cycnoches* and allied genera (i.e., *Catasetum, Clowesia, Dressleria* and *Mormodes*) have been newly generated. Our preliminary results support virtually 100% the monophyly of the subtribe Catasetinae as defined by Freudenstein et al. (2004) and Romero et al. (2009) (i.e.,

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[2] *Cycnoches maculatum* (sect. *Heteranthae*) female flowers. Venezuela.

[3] *Cycnoches ventricosum* (sect. *Cycnoches*) male flowers.

[4] Maximum likelihood tree under the GTR + Γ model for Catasetinae and related subtribes obtained from concatenated nuclear ribosomal ETS and ITS loci. Specimens from taxa in bold were newly sequenced for this study. Clades enclosed in gray brackets correspond to members of the former Catasetinae *sensu* Dressler (1993). Clades enclosed in black brackets are genera included today in subtribe Catasetinae *sensu* Freudenstein et al. (2004). Bootstrap support values above 50% are shown above the branches.

[5] Male flowers of *Cycnoches* section *Heteranthae* (*Cycnoches peruvianum*). A. Side view of flower: 1) column; 2) lip; 3) ovary; 4) pedicel. B. Column: 1) non-functional stigmatic chamber; 2) rostellar band; 3) clinandrium: 3a) projections, 3b) filament. C. Pollinarium: 4) anther cap; 5) pollinia; 6) stipe; 7) viscidium. D. Lip: 8) claw; 9) hypochile; 10) basal calli; 11a-11d) dactylar processes; 12) apical calli; 13) epichile.

[6] Male flowers of *Cycnoches* section *Cycnoches* (*Cycnoches ventricosum*). A. plant habit. B. column: 1) non-functional stigmatic chamber; 2) rostellar band; 3) clinandrium: 3a) projections, 3b) filament. C. pollinarium: 4) pollinia; 5) stipe; 6) viscidium. D. Lip: 7) claw; 8) calli; 9) hypochile; 10) epichile.

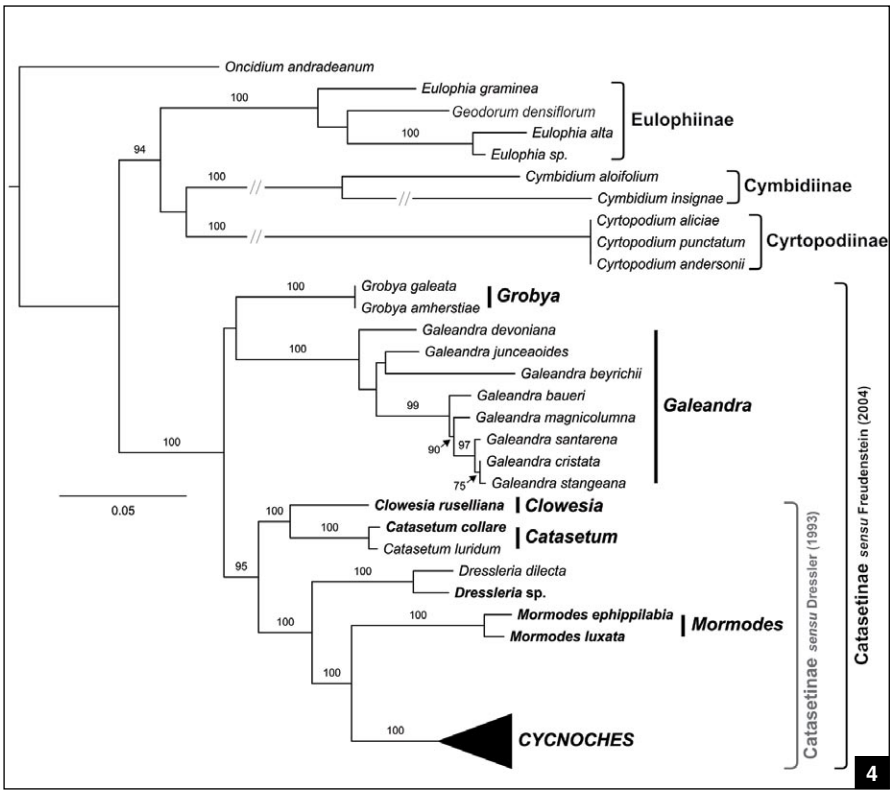
Grobya and *Galeandra* are to be included). More importantly, *Cycnoches* appears as a monophyletic group sister to the genus *Mormodes* in a strongly supported clade (100% bootstrap support). Our analyses have included for the first time in the taxa sampling material of one representative of *Grobya* (more will be included), which were not included in previous studies. Relationships within *Cycnoches* still remain unknown, however, due to an incomplete taxon sampling.

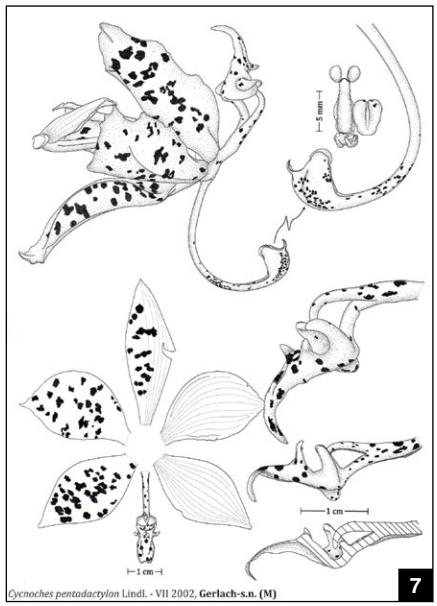
Such research projects demand a great deal of resources, including the vegetative material itself, which represents for us, at this very moment, the most critical and limiting resource. Therefore, the purpose of this short communication is to make an urgent call for cooperation and assistance, addressed to all orchid growers and lovers with any kind of interest in *Cycnoches*. Although in our preliminary work, nearly half of the species of the genus have been sampled for molecular and morphological studies, we still have a need for more material, namely flowers preserved in liquid, leaf tissue for molecular investigations and living plants (pseudobulbs) from several of the more rare species (mostly from Central America and Amazonia regions) that we don't have in cultivation. The material hereby requested is essential for the following:

1. DNA extraction and amplification: Leaf or floral tissue dried and preserved in silica gel is required for extraction of DNA and amplification of certain genes (in our project we are currently working with two nuclear and one chloroplast genes). A voucher specimen composed of at least a dried flower and a leaf should be coupled with the tissue employed for DNA isolation.

2. Analysis of floral fragrances and morphological studies: Plants for further cultivation are needed, in order to obtain flowers from which fragrances can be sampled with the use of an air pump and an adsorbent cartridge. Fragrances are further analyzed by means of gas chromatography, which may detect its most abundant chemical compounds. The flower will be later on preserved in liquid (solution of ethanol and glycerin) for analytical and morphological studies.

Your cooperation is very important to us. We want to include all the missing swan orchids in our investigations, to be as complete as possible in our research on the genus, so that we can continue with our project and thus solve the riddle that has surrounded its taxonomy for more than 200 years!





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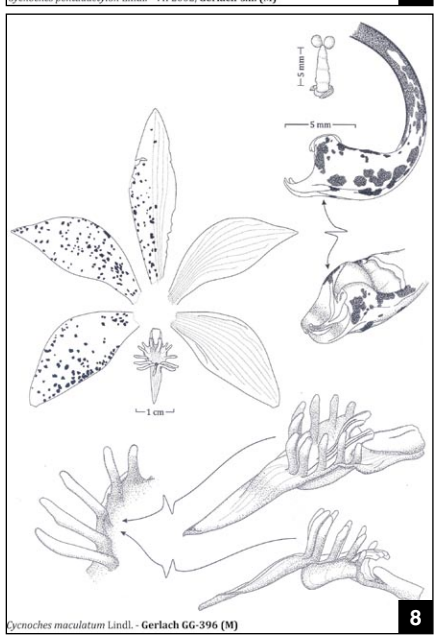

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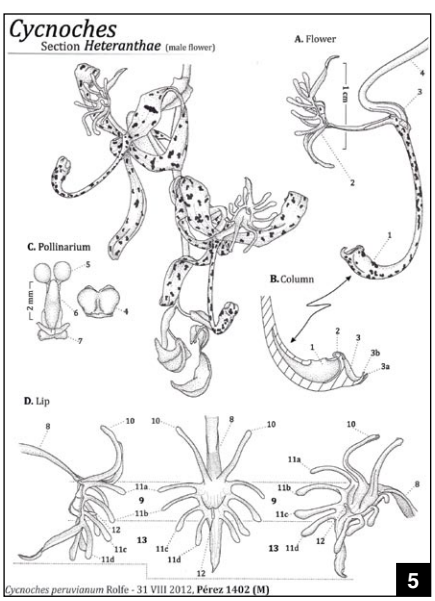
[7] Male flower of *Cycnoches pentadactylon*.

[8] Male flower of *Cycnoches maculatum*.



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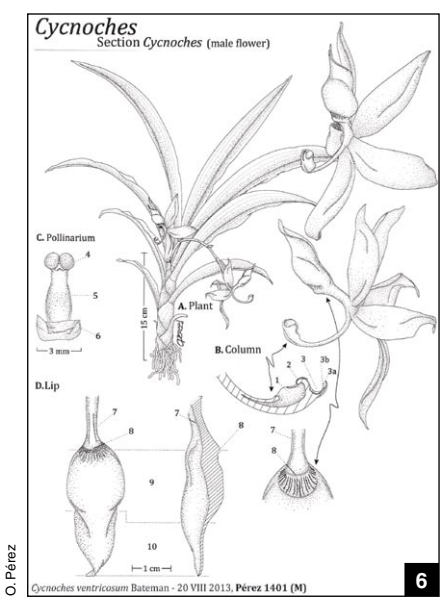

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* Botanischer Garten München, Menzinger Straße 61, D - 80 638, Munich, Germany; e-mail: gerlach@extern.lrz-muenchen.de

** Ludwig-Maximilians Universität, Menzinger Straße 67, D - 80 638, Munich, Germany; e-mail: oapereze@yahoo.com