

## **Methods of floral analysis in the Brazilian *Orchidaceae***

**Metodika květních rozborů brazilských druhů čeledi *Orchidaceae***

**Ruy José Válka Alves**

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The present paper is an introduction to the floral analysis method used in the determination of Brazilian orchids. It consists of projecting the diaphanized floral structures, namely column, sepals and petals, by means of an optical device and tracing the outlines and internal structures. The resulting sketch is very constant for the species and can be used in a similar way to human dactyloscopy, to identify the species. The multiple uses of this method are discussed and conventions are proposed for the graphic representation.

*Rua Marquesa de Santos 22 | 1004, Laranjeiras Rio de Janeiro, Brazil. (Until 12/1990: Bolestavova 26, 140 00, Praha, Czechoslovakia)*

### **DEFINITION AND ORIGIN OF FLORAL ANALYSIS**

The discovery of floral analysis cannot be attributed to a sole author and any date for it would be only relatively precise. Naturally it developed as a consequence of the growing perfection of optical devices and their use in biological sciences, specifically morphology and anatomy. HOEHNE (1940–1954) already took the first step towards floral analysis by drawing and describing the flattened-out labellum of most of the plants he studied. The authors who truly demonstrated the power of this method in orchid taxonomy in Brazil were PABST et DUNGS (1975, 1977). Besides over 200 botanical papers Guido Frederico Joao Pabst published what I believe to be the first simplified monograph on Brazilian orchids. Both volumes include floral analyses of most of the until then known species, organized according to their floromorphological affinity.

Floral analysis in the orchid family may be defined as the graphic representation (draft) of the flattened-out petals, sepals, and labellum with their significant internal and external structures. All further specifications are to be considered, conventions serving specific purposes.

The floral analysis method is a tool which enables us to distinguish tropical orchids in a simplified manner. The great diversity of tropical orchid species along with their high similarity among the related groups calls for a realistic method of determination which would certainly not be achieved by mere use of dichotomic keys. Floral analysis may thus be compared to the dactyloscopy used for human beings.

The technique may most commonly be applied in taxonomy as a sound system for differentiating characteristics, in the study of population variabi-

lity in floral structure for single species, or for mere complementation of morphological and anatomical studies (ALVES 1990). Though I found no record of floral analysis being used in other plant families it seems plausible in many of them, needing only experimental confirmation.

#### METHODS OF FLORAL ANALYSIS IN ORCHIDS

The basic procedure in floral analysis of orchids consists of the following steps:

1. Identification of the flowering plant to the genus if possible
2. Diaphanization of flowers
3. Dissection of flowers
4. Preparation of slides
5. Illustration of the observed structures

The method described here considers field conditions, hence the lack of most sophisticated laboratory instruments. The final results tend to be similar and equally precise as when lab equipment is used.

#### Materials

1. fresh or liquid-preserved flowers of the orchid, 2. heat source for boiling (burner), 3. two beakers or equivalent, 4. razor blade or scalpel, 5. two pairs of fine tipped tweezers, 6. transparent adhesive tape, 7. transparent acetate or film cleared with HClO solution, 9. concentrated ethyl alcohol, 10. distilled water or tapwater, 11. frames for projector slides, 12. slide projector, 13. clean white A4 paper, 14. thin pencil with eraser, 15. citric acid, 16. ruler.

#### Procedures

For preparing the flowers boil them in water for 1 to 5 minutes. (This should leave them soft and translucent.) The necessary time depends on the species, and extracting the pigments may be complemented by boiling them in the HClO solution, the citric acid solution, or in most cases simply in the alcohol. Two to three flowers should be boiled of each plant to be analyzed. The HClO solution is useful for boiling flowers that turn black after fixation (such as species of *Zygopetalum* or *Koellensteinia*). Druses may be extracted by NaOH solution.

Using tweezers remove the flowers and put them into a container of cold water, holding them by the ovary to avoid damage of the useful structures.

Place a rectangle of the transparent acetate (the size of 135 film or  $6 \times 6$  cm) on a white surface on the table. For small flowers up to 2 cm in size hold the ovary with one pair of tweezers and with the other hold the superior sepal parallelly at the base (at its insertion with the ovary), and carefully pull it off.

Place the sepal on the acetate slide as indicated in Fig. 1 (C). The same step should be repeated for the labellum and right petal and sepal (those located to the right in a frontal viewing of the flower). These structures should be placed as indicated in Fig. 2 (A—D). In plants where a spur (calcar or stigmatiferous process) is present, such as *Habenaria*, this should be included as in Fig. 2 (C). Larger flowers or those with connate parts should be dissected with a razor blade (Fig. 2 I). The excessive water in the structures on the acetate should be removed by pressing an absorbent paper on them from

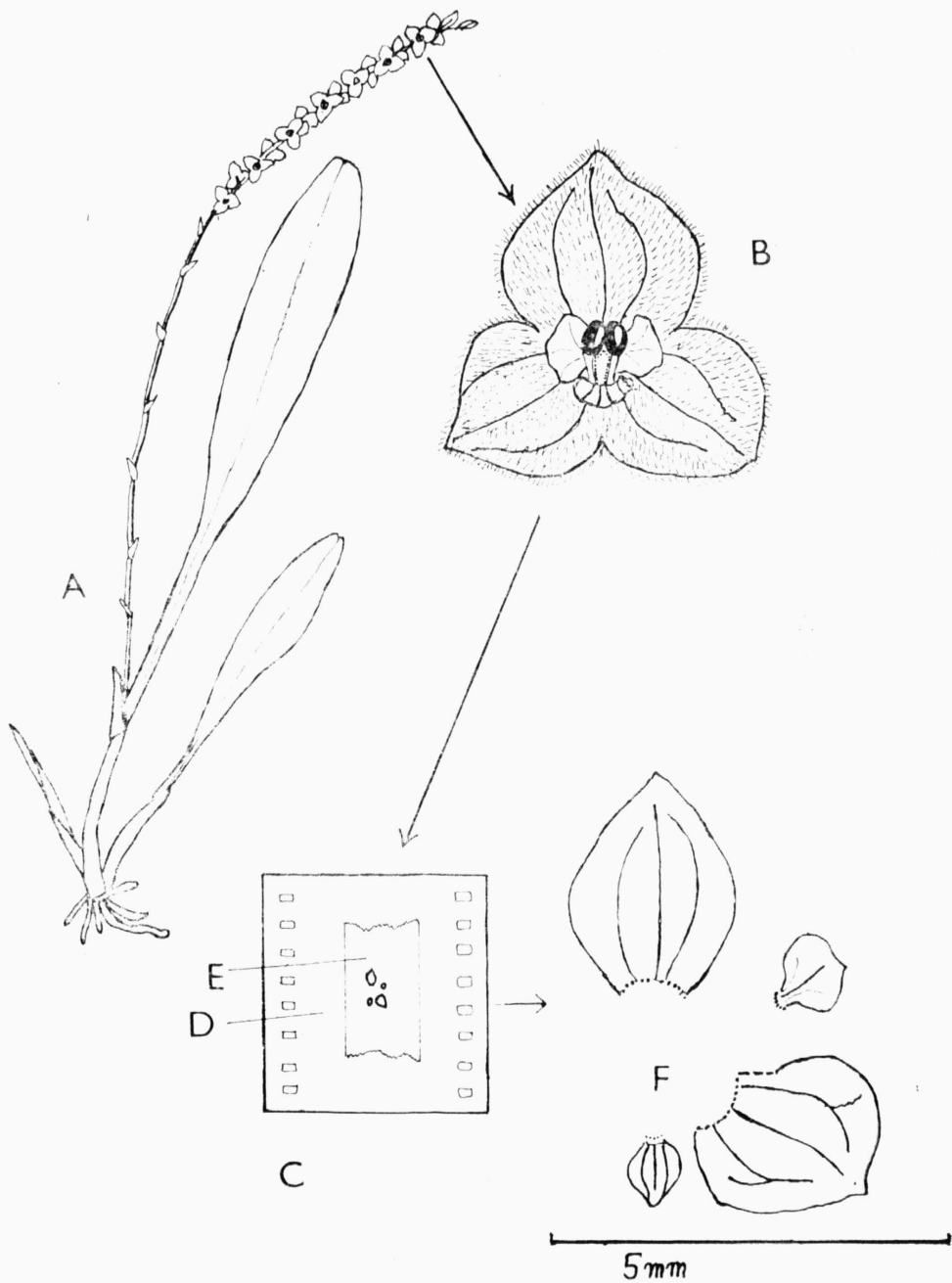


Fig. 1. — *Stelis puberula* B. Rodr., A — Whole flowering plant, B — enlarged flower in natural position, C — mounted slide for projection, D — acetate slide, E — adhesive tape cover slip, F — resulting floral analysis.

above. The parts should be held onto the surface with tweezers to avoid curling by uneven drying.

Attach the parts of the flower to the acetate by means of transparent adhesive tape (substituting a cover slip). The drying is completed by heat inside the slide projector itself permitting the slides to last many years. For effective drying puncture the tape slightly before application, or allow the tape to overlap.

Mount the slide into a frame and set the slide projector. Project the image on a wall and select the desired image size by moving the projector closer to or further from the wall. Focus.

Tape an A4 sheet of paper on the wall in front of the projector. Trace the outlines of all desired structures with a soft pencil. A scale should be included in this step by measuring amplification size (a practical manner is to tape a 1 cm long strip of paper to the finished slide and just trace its image with a ruler. This strip should be placed horizontally under the lip.)

At this point the floral analysis is completed. For publications tracing on parchment using indian ink is sometimes required. Nevertheless the drafting should follow some homogeneous method permitting other researchers to interpret the structures. For this purpose some conventions partly based on the practice commonly used in most published analyses are proposed here.

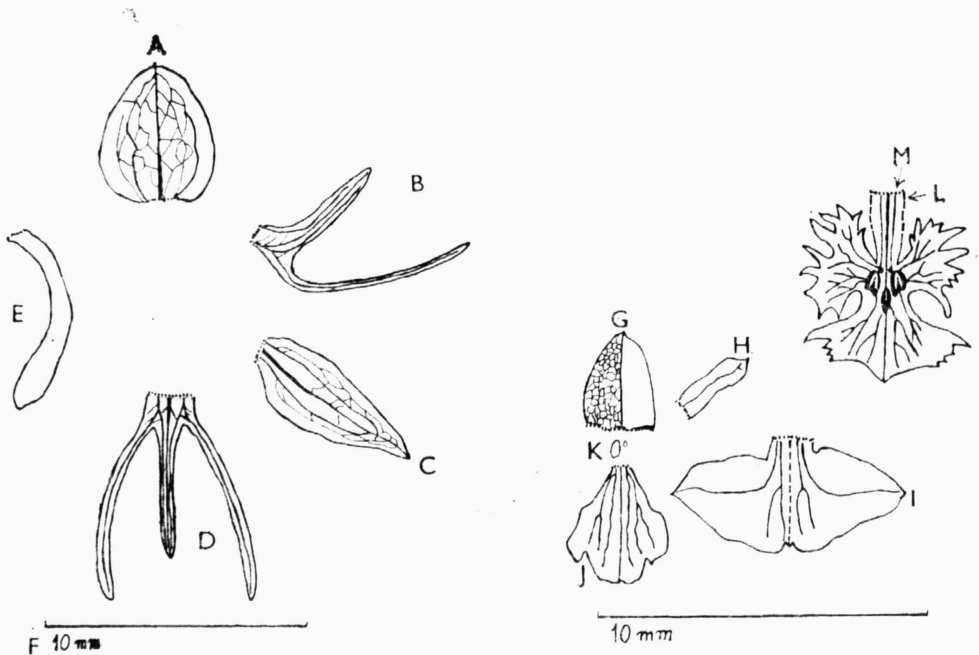


Fig. 2 - A-E: *Habenaria repens* NUTT., A - superior sepal, B - right petal, C - right sepal, D - labellum, E - spur, F - scale line, G-K: *Polystachya concreta* (JACQ.) GARAY et SWEET, analysis in uncommon position due to synsepalum, G - superior sepal (truly inferior due to nonresupination), showing anastomosed venation, H - right petal, I - synsepalum (median dashed line shows connation zone), J - labellum, K - degree value for nonresupination, L - M: *Epidendrum denticulatum* agg., L - dashed line indicating separation of connate structures (lip and column), M - dotted line indicating cut at base of structure (labellum)

PROPOSED CONVENTIONS FOR FLORAL ANALYSES IN ORCHIDS

1. Resupinate flowers should be represented with the lip pointed downward as in the natural position. Nonresupinate flowers should be drawn in the same position but an indication should be included in the center of the drawing as zero degrees (Fig. 2 K). The flowers with double resupination are to be indicated as 360 degrees as in Fig. 3 (A).

2. Outlines are represented by a full line when expressing natural contour as in Fig. 1 (3).

3. Outlines should be dotted when indicating cuts at the natural base of structures (Fig. 1—3).

4. Outlines are represented by dashed lines when indicating incisions separating naturally connate structures (Fig. 2 I)

5. Vascular traces are indicated by normal lining of corresponding width.

6. Callus and glandular protuberances are full black spots when of small size, or shaded to indicate their three dimensional appearance as in Fig. 3 (B).

7. Marginal hairs present on the parts of certain species are to be symbolically represented as in Fig. 1 (B).

8. All floral analyses should be provided with a scale line running horizontally under the labellum (Fig. 2 F).

Other characteristics of the flower are usually left to verbal description by most authors. The column may be drawn additionally where it plays an important taxonomic role, which applies to most of the terrestrial orchids in PABST et DUNGS (1975, 1977). Some of the drawings in that book published

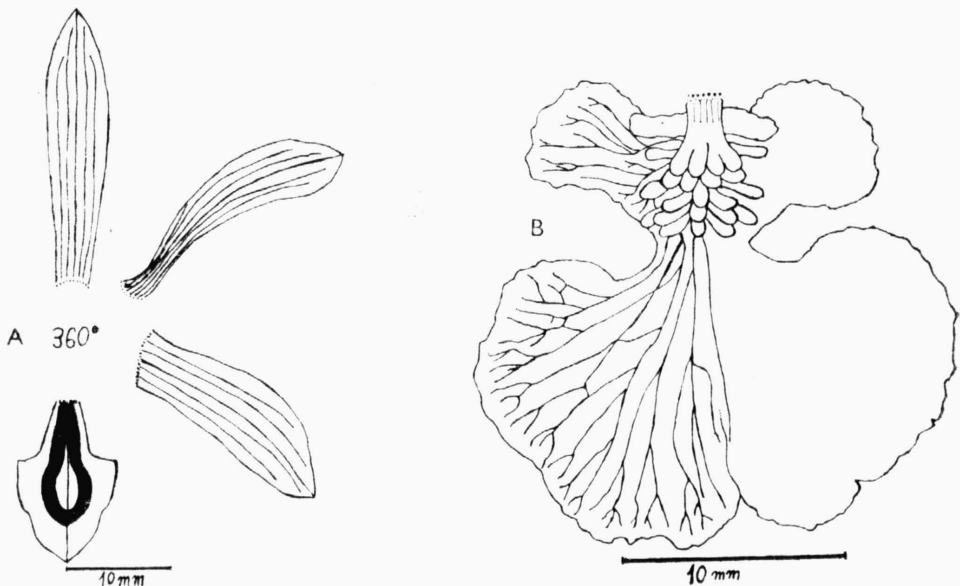


Fig. 3 — A — *Anacheilium vespa* (VELL.) PABST ex DRESSLER, showing at center indication for double resupination, B — *Oncidium blanchetii* REICHENBACH f., shows three dimensionally calli on the labellum isthmus and the fleshy portion on the basal margin.

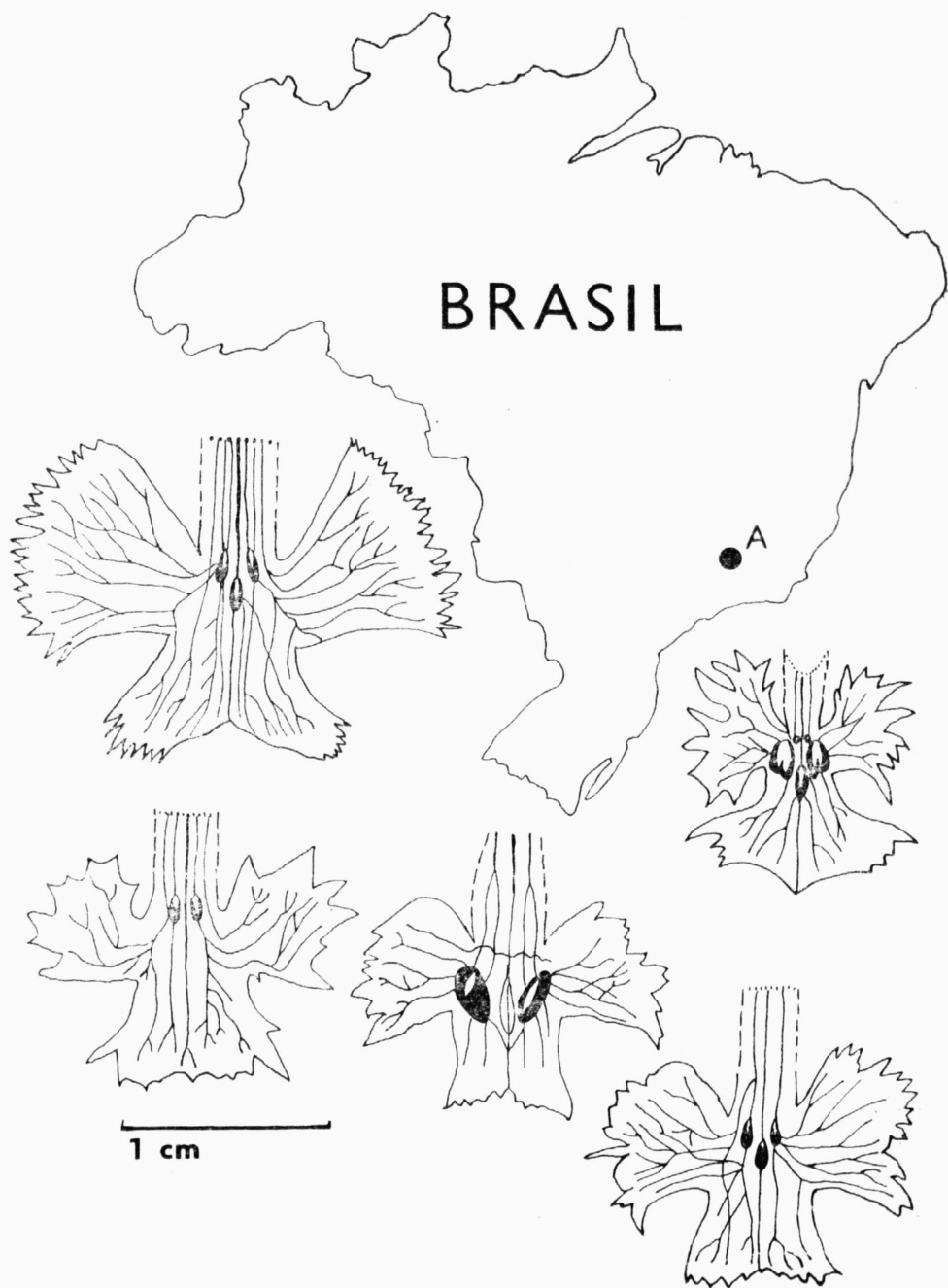


Fig. 4. — Approximate location (A) of a  $2 \times 2$  km area comparing lip structure in the *Epidendrum denticulatum* alliance (only the lips are shown).

as analyses are truly only sketches of the floral structures in natural positions (not flattened out), as for example *Epidendrum ellipticum* on page 307. Until the methods become unified, such drawings may contribute to the general misunderstanding of certain taxonomic groups.

PABST et DUNGS (1975, 1977) illustrated in this manner all Brazilian genera hitherto recognized.

In the world literature available I have found very few papers using floral analysis in orchid description, all material being available from South America and Mexico, for example HAGSATER (1981) and BRAGA (1980).

I have experience of about 3500 floral analyses so far and the determination efficiency has been of about 90 % for known taxa. Problems with the determination of such genera as *Eltroplectris* result usually from insistence on clinical dissection patterns, while these cases call for a more flexible interpretation on their connate structures. Genera like *Stanhopea*, *Gongora*, and *Coryanthes* are also difficult to delineate with the floral analysis method, but they are so exceptional with their morphology and relatively few in number, allowing verbal descriptive techniques. Even in their case I have successfully distinguished among species encountered by means of floral analysis.

The main use of floral analysis for the future is no doubt that of simplifying the determination work in taxonomy. With very small chromosomes the determination of the family is too time consuming by caryotype. In most cases live material necessary for this is not available. Hopefully this method may be of use in unifying the descriptions of most authors working with taxonomy in the *Orchidaceae* and hence in elucidating this complex part of science.

Additionally comparing the variations in flowers of a single species can be reached or at least supplemented by this method. I undertook many studies of small sites, mapping the apparently related taxa, as indicated in Fig. 4.

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#### SOUHRN

Práce je úvodem do metodiky květních rozborů, která byla užita při determinaci brazilských druhů čeledi *Orchidaceae*. Je probána mnohostranná použitelnost, pracovní postup, a navržena konvence grafického znázornění. Metoda se osvědčila u orchidejí vzhledem k jednotnosti jejich květů na úrovni druhů a variability na úrovni rodů. Zjednodušuje determinaci druhů a činí ji spolehlivější ve srovnání s klasickými srovnávacími metodami i metodami genetickými, jejichž užití je pro obtížnost stanovení karyotypu u této čeledi nepoužitelné.

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