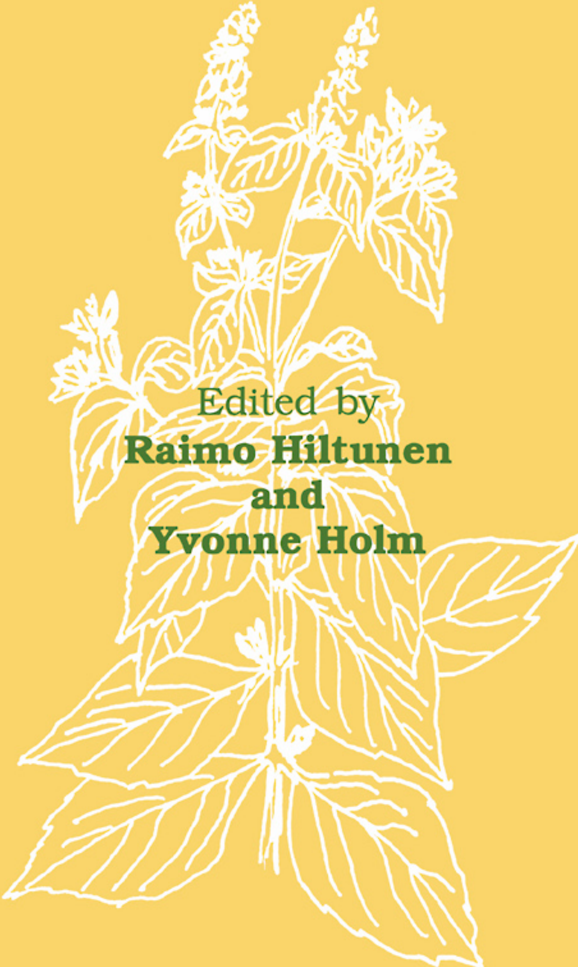
# BASIL

## The Genus *Ocimum*



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**Raimo Hiltunen**
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# BASIL

## The Genus *Ocimum*

*Edited by*

Raimo Hiltunen and Yvonne Holm
*Department of Pharmacy, University of Helsinki, Finland*

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# PREFACE TO THE SERIES

There is increasing interest in industry, academia and the health sciences in medicinal and aromatic plants. In passing from plant production to the eventual product used by the public, many sciences are involved. This series brings together information which is currently scattered through an ever increasing number of journals. Each volume gives an in-depth look at one plant genus, about which an area specialist has assembled information ranging from the production of the plant to market trends and quality control.

Many industries are involved such as forestry, agriculture, chemical, food, flavour, beverage, pharmaceutical, cosmetic and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts and spices for medicinal and aromatic purposes. All these commodities are traded world-wide. A dealer's market report for an item may say "Drought in the country of origin has forced up prices".

Natural products do not mean safe products and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants which are approved for use in medicine must not be used in cosmetic products.

The assessment of safe to use starts with the harvested plant material which has to comply with an official monograph. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxins, pesticide residue, as well as the required level of active principle. This analytical control is costly and tends to exclude small batches of plant material. Large scale contracted mechanised cultivation with designated seed or plantlets is now preferable.

Today, plant selection is not only for the yield of active principle, but for the plant's ability to overcome disease, climatic stress and the hazards caused by mankind. Such methods as in vitro fertilisation, meristem cultures and somatic embryogenesis are used. The transfer of sections of DNA is giving rise to controversy in the case of some enduses of the plant material.

Some suppliers of plant raw material are now able to certify that they are supplying organically-farmed medicinal plants, herbs and spices. The Economic Union directive (CVO/EU No 2092/91) details the specifications for the **obligatory** quality controls to be carried out at all stages of production and processing of organic products.

Fascinating plant folklore and ethnopharmacology leads to medicinal potential. Examples are the muscle relaxants based on the arrow poison, curare, from species of *Chondrodendron,* and the antimalarials derived from species of *Cinchona* and *Artemisia.* The methods of detection of pharmacological activity have become increasingly reliable and specific, frequently involving enzymes in bioassays and avoiding the use of laboratory animals. By using bioassay linked fractionation of crude plant juices or extracts, compounds can be specifically targeted which, for example, inhibit blood platelet aggregation, or have antitumour, or antiviral, or any other required activity. With the assistance of robotic devices, all the members of a genus may be readily screened. However, the plant material must be **fully** authenticated by a specialist.

The medicinal traditions of ancient civilisations such as those of China and India have a large armamentarium of plants in their pharmacopoeias which are used throughout South East Asia. A similar situation exists in Africa and South America. Thus, a very high percentage of the world's population relies on medicinal and aromatic plants for their medicine. Western medicine is also responding. Already in Germany all medical practitioners have to pass an examination in phytotherapy before being allowed to practise. It is noticeable that throughout Europe and the USA, medical, pharmacy and health related schools are increasingly offering training in phytotherapy.

Multinational pharmaceutical companies have become less enamoured of the single compound magic bullet cure. The high costs of such ventures and the endless competition from me too compounds from rival companies often discourage the attempt. Independent phytomedicine companies have been very strong in Germany. However, by the end of 1995, eleven (almost all) had been acquired by the multinational pharmaceutical firms, acknowledging the lay public's growing demand for phytomedicines in the Western World.

The business of dietary supplements in the Western World has expanded from the Health Store to the pharmacy. Alternative medicine includes plant based products. Appropriate measures to ensure the quality, safety and efficacy of these either already exist or are being answered by greater legislative control by such bodies as the Food and Drug Administration of the USA and the recently created European Agency for the Evaluation of Medicinal Products, based in London.

In the USA, the Dietary Supplement and Health Education Act of 1994 recognised the class of phytotherapeutic agents derived from medicinal and aromatic plants. Furthermore, under public pressure, the US Congress set up an Office of Alternative Medicine and this office in 1994 assisted the filing of several Investigational New Drug (IND) applications, required for clinical trials of some Chinese herbal preparations. The significance of these applications was that each Chinese preparation involved several plants and yet was handled as a single IND. A demonstration of the contribution to efficacy, of each ingredient of each plant, was not required. This was a major step forward towards more sensible regulations in regard to phytomedicines.

My thanks are due to the staff of Harwood Academic Publishers who have made this series possible and especially to the volume editors and their chapter contributors for the authoritative information.

Roland Hardman

# PREFACE

Our interest in the plant named basil began in the early 1980s when the Division of Pharmacognosy at the University of Helsinki was involved in a cultivation project with aromatic plants. Basil is a tender herb, not very suitable for the Finnish climate. It could be successfully cultivated in greenhouse conditions though. Some years later Mr Galambosi brought seeds of different cultivars of *Ocimum basilicum,* cultivated the plants and made us analyse their essential oils. After that we were stuck in the fascinating world of basil oil.

When searching the literature for data on the chemical composition one could not help noticing the confusion in the nomenclature. Thus a need to clarify the taxonomy and nomenclature was born, and through Mr Galambosi we got into contact with Dr Paton, who accepted the task and now provides a delimitation of *Ocimum* from related genera and an infrageneric classification of *Ocimum.*

Chemotaxonomy has been used as a tool to separate the different species. However, it has not been very successful because there are a large number of subspecies, varieties, forms, cultivars and even some hybrids. These are not readily separated on the basis of the essential oil composition. In addition, the essential oil composition is known to vary depending on the cultivation methods, drying and isolation techniques used. The establishment of chemotypes within the species is not easily performed either. There is always one oil which will not fit into an already established group.

*Ocimum sanctum* or Holy Basil is a sacred medicinal plant in India, where it is used for many different ailments in ethnomedicine. Some activities have been confirmed by pharmacological studies. *O.gratissimum, O.viride* and *O.suave* are native plants in different parts of Africa and are used in traditional medicine, mostly as expectorants. The essential oils of these species also exhibit large antimicrobial spectra. An important activity is the insect repelling effect of many *Ocimum* oils, which can be utilized in warm countries. The composition of the fixed oil of *Ocimum* seeds has been studied lately and it was found to have anti-inflammatory activity. In spite of all the pharmacological activities of *Ocimum* essential oils the main use of the plants is as aromatic plants and spices.

This book is intended to cover the present knowledge of all aspects of the cultivation, composition and use of *Ocimum* plants. There certainly are areas which need further exploitation, such as the enantiomeric composition of the essential oils. We hope this book will fulfil your expectations regarding its contents.

Raimo Hiltunen
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# 1.
# *OCIMUM:* AN OVERVIEW OF CLASSIFICATION AND RELATIONSHIPS

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SUMMARY The taxonomy and nomenclature of *Ocimum* are in a state of confusion. The aim of this paper is to clearly delimit *Ocimum* from related genera, provide an up-to-date infrageneric classification which can be used as a framework for understanding relationships within the genus, provide a list of recognised species with their correct names and common synonyms and a key to their identification. In all 64 species are recognised. A parsimony analysis was carried out which suggests *Ocimum* is a monophyletic group if segregate genera such as *Becium* and *Erythrochlamys* are included within it. An account of the morphological features of *Ocimum* is provided and the economic uses of the genus are briefly discussed in the context of the infrageneric classification.

## INTRODUCTION

*Ocimum* L. is a member of the Labiatae family. The typical characteristics of this family are a square stem, opposite and decussate leaves with many gland dots. The flowers are strongly zygomorphic with two distinct lips. Many of the family, particularly subfamily Nepetoideae, to which *Ocimum* belongs, are strongly aromatic due to essential oils which consist of monoterpenes, sesquiterpenes and phenylpropanoids.

*Ocimum,* unlike other economically important herbs in the Labiatae such as *Rosmarinus, Thymus* and *Salvia,* belongs in tribe *Ocimeae* which has declinate stamens. That is the stamens lie over the lower (anterior) lip of the corolla rather than ascending under the upper (posterior) lip. The *Ocimeae* are essentially a tropical tribe and *Ocimum* occurs naturally in tropical America, Africa and Asia. Unlike several other economic Labiatae, *Ocimum* requires warmth for growth and should be protected from frost.

*Ocimum* is an important economic and medicinal herb, and yet its taxonomy and nomenclature are in a bit of a muddle. Taxonomy underpins all plant science: we must know the correct name for a plant if we are to communicate information about its uses and relationships. It is important that each species has only one correct name to avoid confusion and aid clarity. *The International Code of Botanical Nomenclature* (Greuter *et al.,* 1994) ensures that names are attached by a standard set of rules. In the literature concerning *Ocimum,* particularly the industrial and economic papers, these rules have frequently not been applied and the same species is often referred to by more than one name.

The circumscription of *Ocimum* itself is also problematic. Estimates of species number vary from 30 (Paton 1992) to 160 (Pushpangadan & Bradu 1995). These differences are due partly to taxonomic reasons, for example, recent revisions such as Paton (1992) have reduced species number by placing some species in synonomy; partly for geographic reasons, in that many species are African and much Indian literature is written without knowledge of the literature pertinent to the African species; and partly because the generic description of *Ocimum* itself has changed. The aim of this paper is to discuss the delimitation of the genus and provide an infrageneric classification of the genus which can be used as a framework for future studies. A list of recognised species is provided which includes information on their distribution, habitats and types in order to help workers use the correct name for a particular species. Some of the more commonly found synonyms are also included under their correct name. A key to the species is provided and references to further information about the species are given.

## *Ocimum* and Related Genera

Briquet (1897) divided Tribe *Ocimeae* into three sub tribes. *Ocimum* belongs in Sub tribe *Ociminae* characterised by a small, flat lower (anterior) corolla lip with the stamens and style extending over it and up towards the upper (posterior) lip of the corolla. Ryding (1992) divided subtribe *Ociminae* into three informal groups. *Ocimum* was placed in the '*Ocimum*-group' along with *Becium* Lindl., *Erythrochlamys* Gürke, *Hemizygia* (Benth.) Briq., *Syncolostemon* E.Mey. ex Benth. and *Catoferia* (Benth.) Benth. *Orthosiphon* Benth. subgen. *Nautochilus* (Bremek.) Codd must be added to this group as it shares several characters found in *Ocimum* (Paton 1992).

*Becium* described by Lindley in 1842 contains 35 species, found in Africa, Madagascar and Arabia with one species reaching India. As generic concepts are currently applied, *e.g.* Sebald (1988, 1989), *Becium* can be distinguished from *Ocimum* by having a gland which exudes nectar at the base of the cymes in the inflorescence (Figure 1.1) and by having elongated anthers with parallel thecae rather than orbicular or reniform anthers with diverging thecae. *Becium* usually also has truncate lateral lobes of the calyx, but these are lacking in *B.irvinei* (J.K.Morton) Sebald. When the New World species currently placed in *Ocimum* are examined, this distinction is blurred with several species having a gland at the base of the cymes and divergent anther thecae.

*Erythrochlamys* was described in 1894 by Gürke and contains two species found in NE Tropical Africa. It has traditionally been separated from *Ocimum* by having an expanded upper lip of the calyx (Baker 1900). However, this character is also seen in *O.cirdnatum* A.J.Paton (Paton 1992) and *O.transamazonicum* Pereira (Pereira 1972). The character has already been recognized as poor evidence for generic separation with species formerly placed in *Erytbrocblamys* by Hedge and Miller (1977) due to the presence of an enlarged upper lip, now being placed in either *Ocimum* (Paton 1992) or *Endostemon* (Paton 1994) on account of similarities in other characters. *E.fruticosus* has appendiculate stamens like many species of *Ocimum*. This again throws doubt on the generic delimitation of *Erythrochlamys*.

Of the other currently recognized genera in the *Ocimum*-group, *Hemizygia* containing 32 species and *Syncolostemon* with 10 species are mainly southern African, but there is one species of *Hemizygia* in Madagascar and one in India. The genera merge into one another and good accounts can be found in Codd (1985). They differ from *Ocimum* by having fused anterior anther filaments. *Orthosiphon* subgenus *Nautochilus* contains 5 species and is found in Southern Africa (Codd 1964). This taxon shares many of the characters of *Ocimum lamiifolium* in particular. *Catoferia* includes 4 species and is found in Tropical America

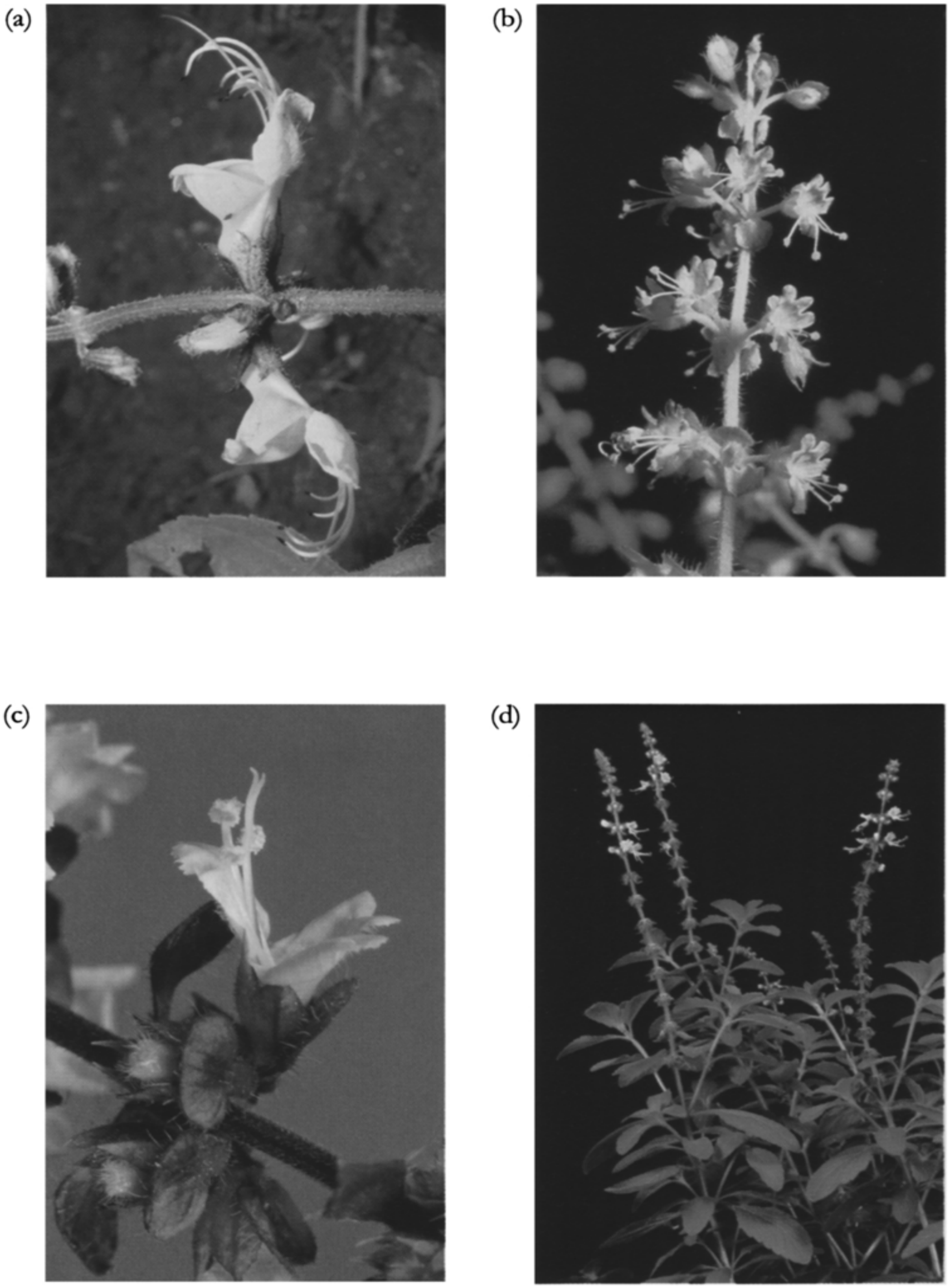

**Figure 1.1** (a) *Ocimum fimbriatum* showing the bowl-like gland at the base of the cyme; (b) *O. tenuiflorum;* (c) *O.basilicum;* (d) *O.kilimandscharicum.*

(Ramamoorthy 1986). The genus looks very different from the other species of the *Ocimum* group in having the cyme branches fused to the inflorescence axis, rather than spreading and free, rounded rather than subulate style branches and a bent rather than straight embryo.

## Taxonomic History of *Ocimum*

*Ocimum* was described by Linnaeus in 1753 who listed 5 species. Bentham (1832) recognized just under 40 species and divided *Ocimum* into 3 sections: *Ocimum* [*Ocymodon* Benth.] with appendiculate posterior stamens; *Hierocymum* Benth. with hairs at the base of the posterior stamens and *Gymnocymum* Benth. with glabrous posterior stamens. The latter two sections contained a few species which are now placed in *Endostemon* N.E.Br., a genus Bentham did not recognize, or *Hemizygia.* Bentham (1848) then subdivided section *Ocimum [Ocymodon]* into three subsections on the basis of calyx morphology. In subsect. *Ocimum* (*Basilica* sensu Briquet 1897) the throat of the fruiting calyx is open and bearded (Figure 1.2 e-f); in subsect. *Gratissima* the throat is closed by the median lobes of the lower lip being pressed against the under surface of the upper lip (Figure 1.2 c-d, g-h); subsection *Hiantia* Benth., with truncate lateral calyx lobes, only included species which are sometimes placed in *Becium* (Sebald 1988, 1989; Paton 1995; see below). Bentham (1848) also added Sect. *Hemizygia* Benth. which Briquet (1897) considered to be a separate genus on account of the fused anterior stamens. Paton (1992) in his revision of African species of *Ocimum* recognized around 30 species and used Bentham's (1848) infrageneric classification of *Ocimum,* with sect. *Hemizygia* and subsect. *Hiantia* removed, preferring to consider the later as the separate genus *Becium*. This classification is supported by nutlet characters which Bentham did not consider and by analysis of pollen morphology (Harley *et al.,* 1992). However, this classification is not entirely without problems, as pointed out (Paton 1992) *Ocimum drdnatum* A.J.Paton does not fit neatly into the existing categories. *Ocimum lamiifolium* is also anomalous, appearing to have a close relationship to *Orthosiphon* subgenus *Nautochilus* (Bremek.) Codd.

Pushpangadan (1974; Pushpangadan & Bradu 1995; Sobti & Pushpangadan 1979) formulated a different infrageneric classification. The *'Basilicum'* group contains herbaceous annuals or sometimes perennials with black, ellipsoid, strongly mucilaginous seeds and with a basic chromosome number of n=12, whereas the *'Sanctum'* group are perennial shrubs with brown globose non-mucilaginous or weakly mucilaginous seeds and a basic chromosome number of n=8. The *Basilicum* group contains only section *Ocimum* subsection *Ocimum*. The remainder of the genus must be placed in the *Sanctum* group. This classification is commonly used in the economic and industrial literature, *e.g.* Darrah (1980), Pushpangadan and Bradu (1995) whereas Bentham's system is the basis for that used in taxonomic literature.

There are several problems with Pushpangadan's infrageneric classification. It does not adequately convey the variation within the genus. New World and Old World species which differ considerably in morphology are placed together within the *Sanctum* group. However, within the *Sanctum* group there are several distinct groups of species with similar attributes and this information is lost. Pushpangadan's system does not comply with the *International Code of Botanical Nomenclature* and thus should not be used as a standard.

The most recent study of New World species of *Ocimum* is that of Epling (1936) who recognizes 12 species 4 of which are pantropical, widely cultivated species. Epling does not refer to the infrageneric classification of the genus.

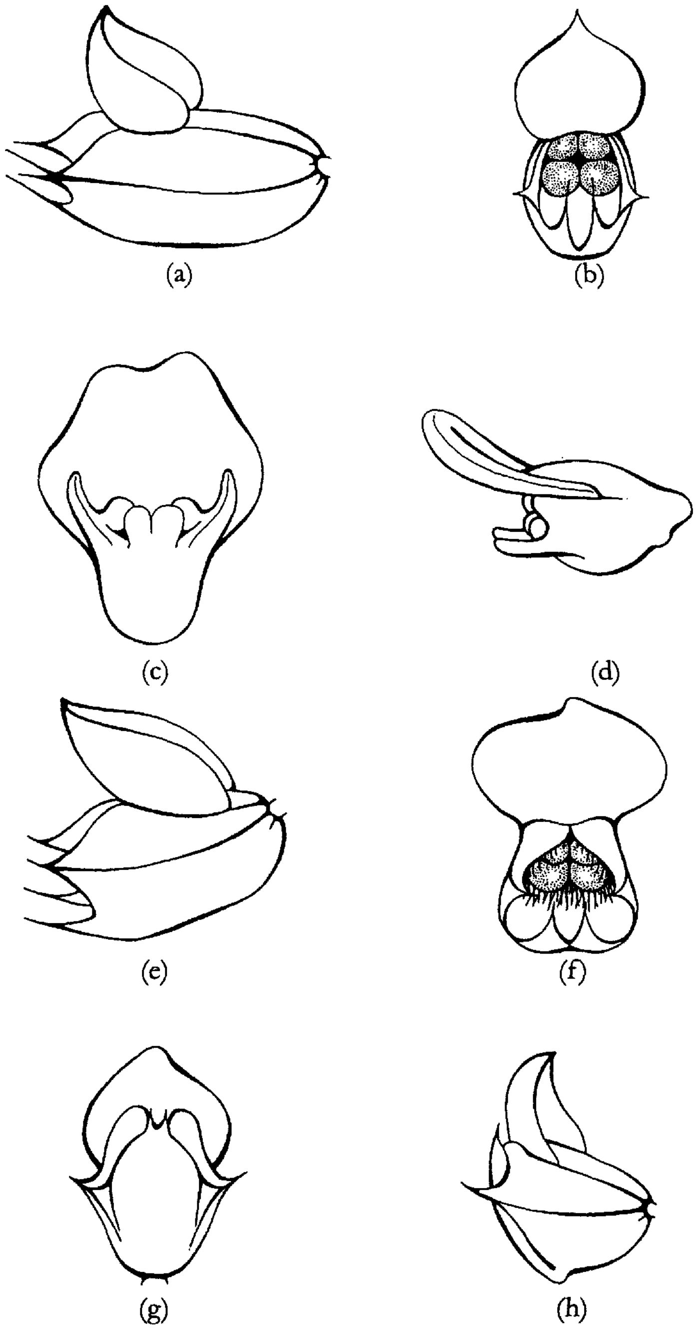


**Figure 1.2** Fruiting calyx morphology in *Ocimum*. (a-b) *Ocimum lamiifolium*. (a) side view; (b) front view. (c-d). *O.cufodontii*. (c) view from beneath; (d) side view, (e-f) *O.basilicum* (e) side view, (f) front view, (g-h) *O.gratissimum*. (g) view from beneath; (h) side view. Drawn by Emmanuel Papadopoulos. Reproduced from Paton (1992) by permission of RBG Kew.

## GENERIC AND INFRAGENERIC DELIMITATION OF *OCIMUM*

Currently there is no up-to-date infrageneric classification which adequately communicates the variation of *Ocimum* throughout its geographical range, nor clearly indicates how the genus should be delimited from its closest allies. A parsimony analysis of the genus and close relatives was carried out to try and determine the generic delimitation and infrageneric relationships and to produce a classification which could serve as a standard foundation for the further scientific and economic investigation of *Ocimum*. The parsimony analysis of the *Ocimum-group* of genera was carried out using PAUP version 3.1.1 (Swofford 1993) run on a Power MacIntosh 8100/80.

The choice of sample used for the parsimony analysis was based on a herbarium study of the whole of *Ocimum, Becium, Erythrochlamys* and *Orthosiphon* subgenus *Nautochilus*. Although this group contains some 64 species many of the species were similar when only conservative characters were examined. For example within subsect. *Ocimum,* Paton (1992) recognized 6 species. However, they differ only in habit and leaf shape, characters which are very variable phylogenetically. A similar situation applies in *Becium*. This may indicate that speciation within the currently recognized *Ocimum* and *Becium* in Africa has been fairly recent. In all, a representative sample of 20 taxa were chosen to represent the variation in *Ocimum, Becium, Erythrochlamys,* and *Orthosiphon* subgenus *Nautochilus*. All Bentham's (1848) sections and subsections of *Ocimum* were represented as follows:

1. Sect *Ocimum* subsect. *Ocimum*. This section contains 7 species and is represented in the analysis by *O.americanum*. The other species in the group only differ in leaf morphology and habit.
2. Sect. *Ocimum* subsect. *Gratissima.* This section contains 6 species and is represented in the analysis by *O.gratissimum, O.jamesii, O.cufodontii*.
3. Sect. *Ocimum* subsect. *Hiantia* (sometimes recognized as *Becium*). This group contains 35 species and is represented by *B.fimbriatum, B.grandiflorum, B.dhofarense* and *B.irvinei*. This represents one species from each of the infrageneric groups of *Becium* recognized by Sebald (1988, 1989). There is little morphological variation within Sebald's groups.
4. Sect. *Hierocymum.* Bentham recognised 11 species in this group, but some have been moved to *Endostemon* (Paton 1994) or *Platostoma* (Paton 1997). The group is represented in this study by *O.lamiifolium* Benth., *O.selloi* Benth., *O.nudicaule* Benth. and *O.tenuiflorum* L.
5. Sect. *Gymnocimum*. Bentham recognized 8 species in this group. Most have been moved to other genera such as *Fuerstia* (Paton 1995b), *Endostemon* (Paton 1994) or *Hemizygia* (Codd 1985). The group is represented in the analysis by *O.campechianum* Mill, and *O.ovatum* Benth.
6. *O.tircinatum* is also included in the sample.

*Erythrochlamys* is represented by both its species *E.fruticosus* and *E.spectabilis. Orthosiphon* subgen. *Nautochilus* includes 5 species (Codd 1985) and is represented by *O.labiatus* and *O.tubiformis. Syncolostemon, Irlemizygia* and *Catoferia* were also included in order to investigate the generic delimitation of *Ocimum*. *Orthosiphon* subgenus *Orthosiphon* from the *Orthosiphon* group (Ryding 1994) was chosen as the outgroup and the oligomorphic genera

*Syncolostemon, Hemizygia* and *Catoferia* were scored as single taxa. *Orthosiphon aristatus* (Blume) Miq. was added because of its resemblance to *Catoferia* (Hedge 1992).

## Morphology

Morphological variation within *Ocimum* and allies is summarized below. Characters used in the cladistic analysis can be found under the appropriate section. The number in parenthesis preceding the character refers to that character's position in the data matrix. The data matrix used for the analysis is provided in Appendix 1.

### *1.* *Habit*

Members of the genus can be annual herbs which lack a rhizome, possessing only a tap root (*e.g., O.basilicum* L.) or they can be suffrutices with a large or small perennating rhizome which can produce annual or short-lived stems (*e.g., O.obovatum* E.Mey. ex Benth.). Sometimes they are shrubs with quite woody stems (*e.g., O.forskolei* Benth.) or they can be much softer subshrubs *(e.g., O.tenuiflorum)*. The habit is very variable with closely related species having very different habits. For example, on evidence from other characters, the species of *Ocimum* sect. *Ocimum,* subsect. *Ocimum* (as defined by Paton 1992) are closely related, but annuals, shrubs and suffrutices occur within this group. The whole plants are often strongly aromatic, but some species have a very weak scent, *e.g., O.filamentosum* Forssk. Habit characters were not used in cladistic analysis.

### *2.* *Leaf scars*

The leaves can be petiolate or sessile, often toothed at the margin. Some species from arid regions, *e.g., O.cufodontii* (Lanza) Paton, *Erythrochlamys spectabilis* Gürke have deciduous leaves which leave behind conspicuous leaf scars.

(3) Leaf scars: absent (0); present (1).

### *3.* *Inflorescence*

The typical inflorescence in *Ocimum* is a thyrse composed of opposite 1–3-flowered cymes. Two opposing cymes are sometimes called a verticil and several verticils are usually borne on the inflorescence axis. Cymes are usually 3-flowered but rarely, *e.g.,* in *O.irvinei* J.K.Morton, they are reduced to a single flower. The cymes are subtended by bracts which sometimes abscise early, *e.g.,* sect. *Hiantia*. In these cases the bract scar often develops into a bowl-shaped, gland-like structure which serves as an auxiliary nectary and is frequently visited by ants (Figure 1.1a) (Vogel 1998). In *Catoferia* the cyme axis is fused to the inflorescence axis giving the appearance that the flowers arise several millimetres above the bract. In all other species studied the cyme was sessile and the flower pedicel arose from immediately above the bract.