



## Morphological strategies of *Cabomba* (Cabombaceae), a genus of aquatic plants

Carla Teixeira de Lima<sup>1,2</sup>, Francisco de Assis Ribeiro dos Santos<sup>1</sup> and Ana Maria Giuliatti<sup>1</sup>

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### ABSTRACT

This paper describes the vegetative and reproductive morphology of Cabombaceae species in Brazil, discussing its strategies for survival in an aquatic habitat. Through studies in the field, in cultivation and in the herbarium, we examined the following species: *Cabomba aquatica*, *C. caroliniana*, *C. furcata* and *C. haynesii*. In cultivation, only *C. aquatica*, *C. furcata* and *C. haynesii* produced flowers. In those three species, the flowers opened during the day on two consecutive days, submerging during the night, although the stigmata of the first two were receptive only on the first day, their anthers dehiscing on the following day. The flowers of *C. haynesii* remained unreceptive on the first day of anthesis, the stigmata and anthers maturing only on the second day, at different times. Fruit developed when the flowers were submerged. The data provided here are useful for the identification of these species, as well as laying the groundwork for future taxonomic and ecological studies.

**Key words:** Brazil, morphology, Nymphaeales, protogyny

## Introduction

The family Cabombaceae is included in the order Nymphaeales and within one of the oldest angiosperm lineages (Borsh *et al.* 2008). This placement is based on morphological characters, especially floral characters, as well as on molecular data (Cook 1996; Bosch *et al.* 2008; Taylor *et al.* 2008).

Cabombaceae includes two genera: *Brasenia* Schreb., represented solely by *B. schreberi* Gmel., which has not been recorded for Brazil (Souza & Lorenzi 2008); and *Cabomba* Aubl., represented by five species—*Cabomba aquatica* Aubl., *C. caroliniana* A. Gray, *C. furcata* Schult. & Schult.f., *C. haynesii* Wiersema and *C. palaeformis* Fassett—all of which occur in South America (Fassett 1953; Wiersema 1989; Ørgaard 1991; Williamson & Schneider 1994). Souza & Lorenzi (2008) recognized three of those species in Brazil, but they do not mention which. Amaral (2010) cited four of the five *Cabomba* species for Brazil, the exception being *C. palaeformis*. Lima *et al.* (2012) also cited those four species for the state of Bahia, Brazil. The species of *Cabomba* are quite similar and difficult to separate.

The present study aims to present morphological descriptions of the vegetative and reproductive parts of *Cabomba* species. We also discuss the taxonomic characters that aid in the identification of those species and analyze reproductive strategies based on studies of floral biology.

## Material and methods

The specimens of *Cabomba aquatica*, *C. caroliniana*, *C. furcata* and *C. haynesii* studied here were collected in the state of Bahia, from areas of *caatinga* (shrublands) and from fragments of Atlantic Forest. Voucher specimens (Table 1) were deposited in the Herbarium of the (Bahia) State University of Feira de Santana (code, HUEFS). To complete the descriptions and aid in the accurate identification of the species, we also analyzed specimens deposited in other herbaria. The herbarium collections examined in Brazil included those of the Alexandre Leal Costa Herbarium of the Federal University of Bahia (code, ALCB); the Herbarium of the Center for Cacao Research (code, CEPEC); the Herbarium of the National Institute for Amazonian Research (code, INPA); the Bradeanum Herbarium (code, HB); the Herbarium of the Federal University of Recôncavo da Bahia (code, HURB); the Herbarium of the Federal University of Vale do São Francisco (code, HVASF); the Herbarium of the Botanical Institute of São Paulo (code, SP); the Herbarium of the University of São Paulo Department of Botany (code, SPF); the Herbarium of the University of Brasília (code, UB); and the Herbarium of the Federal University of (the state of) Goiás. We also examined specimens in the collections of the Berlin-Dahlem Botanical Garden and Museum, in Berlin, Germany (code, B); the Natural History Museum, in London, England (code, BM); the

<sup>1</sup> Universidade Estadual de Feira de Santana, Programa de Pós-Graduação em Botânica, Departamento de Ciências Biológicas, Bahia, Brazil

<sup>2</sup> Author for correspondence: carlabotanica@yahoo.com.br

**Table 1.** Material used in the vegetative morphological and reproductive analysis of the *Cabomba* taxa studied.

Species	Collector (accession no.)	Collection site
<i>Cabomba aquatica</i> Aubl.	C. T. Lima (252)*	Conde, Rio Cramai
	Guedes, M. L. & Lima L.S. (10153)	Conde
	N. G. Jesus (871)	Alagoinhas
<i>C. caroliniana</i> A. Gray	R. M. Harley & A.M Giulietti <i>et al.</i> (54519)	São Francisco do Conde, Praia do Conde
	C. T. Lima & L.S.P. Lima (214, 340, 386)	Andaraí, Pantanal de Marimbus
<i>C. furcata</i> Schult. & Schult.f.	C. T. Lima & S.G. de Lima (217, 298, 301, 302, 382)*	Mata de São João
	R. M. Harley <i>et al.</i> (53781)	Formosa do Rio Preto
	C. T. Lima & S.G. Lima (210, 335)*	Piritiba
	C. T. Lima & S.G. de Lima (266)	Miguel Calmon
<i>C. haynesii</i> Wiersema	C. T. Lima & S.G. de Lima (369)*	Candeias
	C. T. Lima <i>et al.</i> (381)*	Conde, Rio Cramai
	F. França <i>et al.</i> (5001)	Candeias

\*Materials analyzed in the floral biology studies.

Royal Botanic Gardens, in Kew, England (code, K); the State Herbarium of Munich, in Munich, Germany (code, M); and the Herbarium of the National Museum of Natural History, in Paris, France (code, P).

The four species of *Cabomba* were cultivated in the municipality of Feira de Santana, Bahia. Some plants were cultivated in 1000-L polyethylene tanks, whereas others were cultivated in a 70 × 50 × 50 cm aquarium, which allowed better visualization of the plants. During the study period, three species flowered in cultivation, the exception being *C. caroliniana*. The illustrations were made with or without the use of a still camera attached to the microscope. We also analyzed the trichomes on the stems.

In the floral biology studies, we recorded the following stages: transition from flower bud to flower in anthesis; flower color; timing of flower opening and closing. For ca. 10 flowers, we counted the pollen grains per anther and multiplied that by the number of stamina per flower. During the two days of anthesis, stigma receptivity was evaluated daily with the (10%) hydrogen peroxide test. Pollen grain viability was tested with neutral red at 1%. The presence or absence of osmophores was determined by submerging 10 flowers of each species in neutral red (1%) for 10 min, with subsequent washing in acetic acid at 5%. To determine whether light was required for flower opening, we covered the tank (blocking all light) between 3:00 a.m. and 10:00 a.m. on three randomly selected days.

## Results and discussion

### *Habit and growth form*

Species of *Cabomba* are perennial aquatic herbs occurring in fresh standing water or in water with slight currents in lakes or rivers. They propagate mainly by detachment of portions of the stem from the mother plant, with adventitious roots produced in the nodes. The stem is comprised

of a horizontal basal portion and an ascendant portion that reaches the surface during flower production (Fig. 1), with branching in several directions, similar to stoloniferous stems. Some authors, including Ørgaard (1991), Wiersema (1989), Williamson & Schneider (1994) and Feres & Amaral (2003), have reported the occurrence of a rhizome type of stem in *Cabomba*. However, we noted that part of the stem runs parallel to the ground, with roots and a continuous ascendant portion, and the stem therefore cannot be characterized as a rhizome or rhizomatous. Francisco & Barreto (2007) refuted the occurrence of rhizomes in the genus, explaining that there are no differences between aerial parts and underground parts of the stem. Those authors observed that the basal part of the stem sheds its leaves and produces roots when the water level decreases, reporting that when the water level rises, the basal part of the stem changes to a horizontal position begins to produce new branches from each node.

In *Cabomba* species, the stem has a monopodial growth. It is flexible and erect, with prominent nodes and internodes. In *C. furcata*, the stem is slightly flattened at the base, cylindrical in the intermediate section and slightly triangular at the apical portion; however, in the three other *Cabomba* species studied here, the stem is cylindrical (Fig. 2). According to Ørgaard (1991), the stem is fragile, breaks easily and decomposes rapidly, features that were observed in all of the *Cabomba* species evaluated here, the stem of *C. furcata* being found to be the most brittle. This is probably due to the presence of large quantities of lacunae that were much larger in size than those observed in the three other species, which hindered the collection of complete herbarium specimens.

In all of the *Cabomba* species studied here, the stems showed fine, longitudinal striations, which were red in *C. aquatica*, *C. furcata* and *C. haynesii*, whereas they were green in *C. caroliniana*. According to Ørgaard (1991) the reddish color of the vegetative parts of species of *Cabomba* is directly related to light intensity, and if the plant is removed



Figure 1. Habit of four species of the genus *Cabomba* (Cabombaceae): A) *C. aquatica*, B) *C. caroliniana*, C) *C. furcata* and D) *C. haynesii*.



to an environment with low light intensity, the older, reddish branches die and all the young branches turn green. However, in the present study, even when the species were cultivated in a low-light environment, their coloration was the same as that observed in the field.

We found that all four of the *Cabomba* species evaluated presented a pilose stem with multicellular (two- to three-celled) uniseriate trichomes (Fig. 2). These trichomes are believed to be closely related to the release of a layer of mucilage that covers all submerged parts of the plant.

The nodes are prominent as a result of the annular-shaped thickening. In *C. aquatica*, *C. furcata* and *C. haynesii* the nodes are reddish colored, due to the presence of red striations and papillae that also occur at the base of the petiole. In *C. caroliniana* the papillae are green.

The aerial stem may be branched or not, with one or more branches per node. In *C. furcata*, subsequent internodes are rotated 180° and three leaves grow from each node.

### Leaves

The leaves of *Cabomba* are floating or submerged. The floating leaves are temporary and produced solely at the apical parts of the stem, usually accompanying flower production. The first floating leaf often appears at the same node as the apical submerged leaf. The morphology of the floating leaves has generated controversy regarding their role. Ørgaard (1991), Wiersema (1989), Williamson & Schneider (1994) and Feres & Amaral (2003) all suggested that the floating leaves aid the flowers in floating. However, Francisco & Barreto (2007) regard the floating leaves as bracts. Nevertheless, given that the function of these leaves is to help the solitary flowers to float and that we often observed flowers in the axils of the submerged leaves, we prefer to maintain the term floating leaves.

The shape of the blades of the floating leaves is constant and is therefore a useful taxonomic character at the species level. The shape of the leaves is broad-elliptic to orbicular in *C. aquatica*; narrow-elliptic in *C. furcata*; and linear in *C. caroliniana* and *C. haynesii*. In *C. furcata*, *C. caroliniana* and *C. haynesii* there are two branches (arms) of different sizes with an acute apex (Fig. 2J-M). Feres & Amaral (2003) and Francisco & Barreto (2007) described the base of the laminae of the floating leaves of *C. caroliniana* and *C. furcata* as sagittate. However, considering that the leaf is peltate, with the petiole inserted in the median section of the dorsal side of the leaf and lacking a well-defined base, we describe the leaves of *Cabomba* as presenting two arms each, arising from the insertion of the petiole, and the terminations as extremities, resembling the branching of the T-shaped trichomes or Malpighiaceae trichomes. The width and length of the arms are characters that help distinguish the various species. In the key to species of *Cabomba*, Wiersema (1989) and Lima *et al.* (2012) used the width of the floating leaves to separate *C. caroliniana* from *C. haynesii*.

The submerged leaves are simple, palmate-partite; reniform to semi-circular in shape; membranous, crossed and opposite, except in *C. furcata*, in which they are crossed and 3-verticillate. The petiole is slightly pilose, mainly at the base, becoming glabrescent towards the apex. The lamina is palmate-partite, and has 3-7 primary segments, corresponding to 3-7 actinodromous primary veins. In *C. furcata*, there are usually three primary segments and in the other species five primary segments. The primary segments are subdivided by successive dichotomous divisions, and the central segment may present divisions of up to the fifth order, the last order originating at the terminal segment. Along the entire margin of the terminal segment, mainly at the apex, there are uniseriate trichomes that are unicellular or bicellular and generally caduceous (Fig. 2N-Y). Fassett (1953) used the morphology of the segments of the submerged leaves, together with perianth characters, to separate the species. Wiersema (1989) was the first author to give taxonomic importance to the phyllotaxy of submerged leaves. However, the crossed disposition has been reported only in species with opposite phyllotaxy, and here we found that the leaves of *C. furcata* are verticillate and crossed.

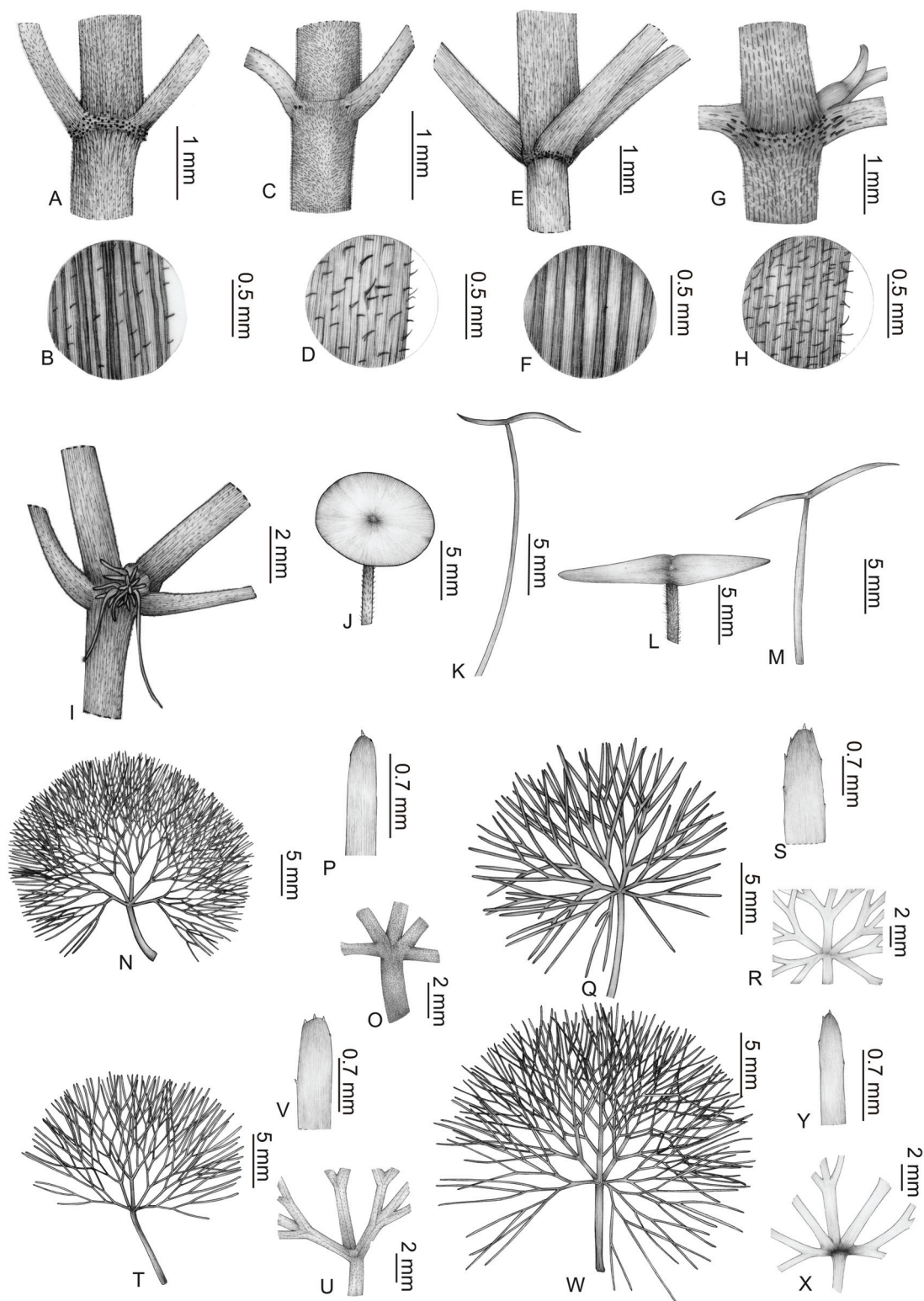
The coloration of the petioles and laminae of the submerged leaves is similar to that of the stem. The reddish coloration occurs at the base of the lamina, and is accompanied by small papillae and reddish striations. Striations also occur on the petioles in *C. aquatica*. In *C. furcata*, striations occur on the petiole and on all segments of the foliar lamina, producing an intense red coloration, which distinguishes this species from the all others in the genus.

### Flower: bud and perianth

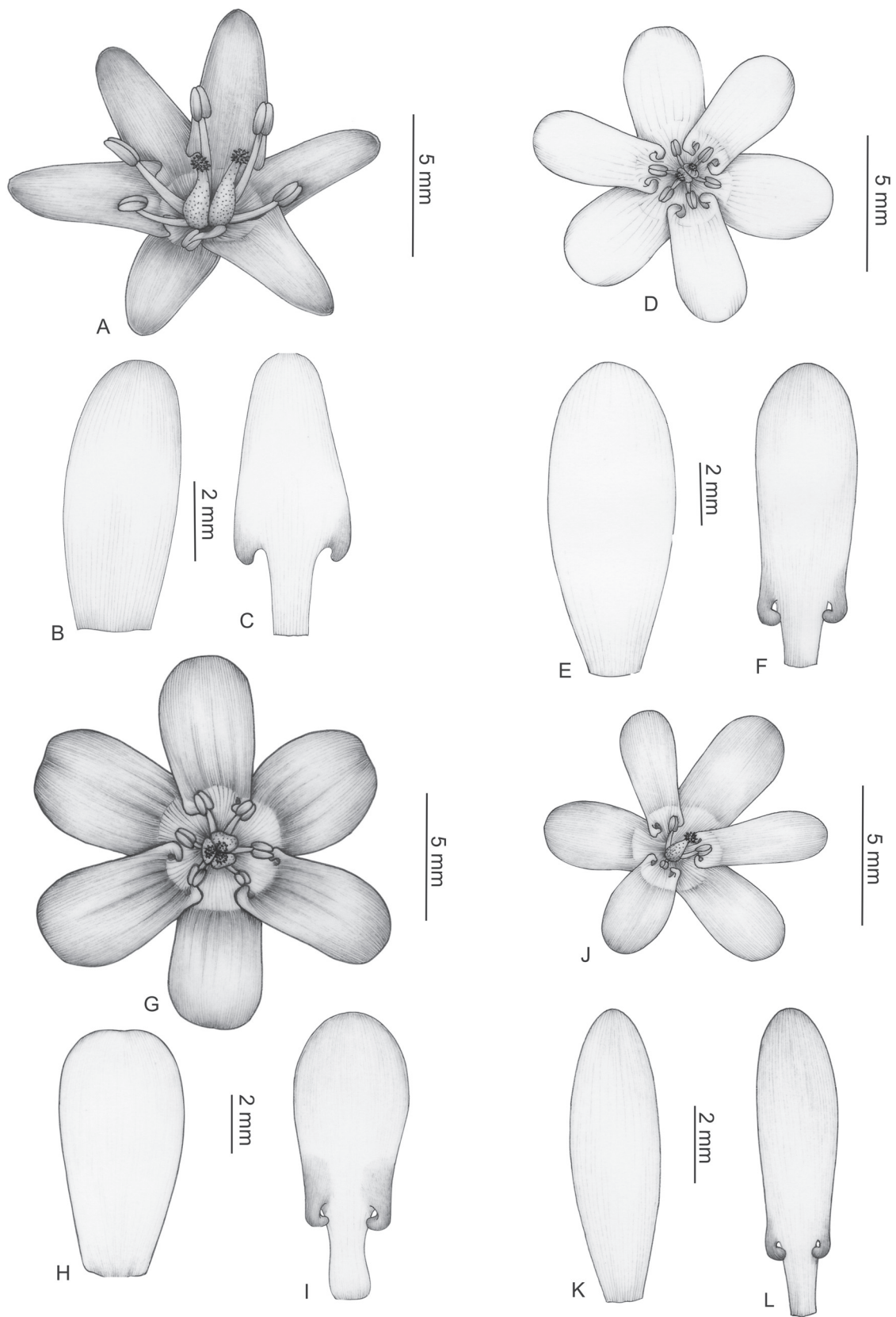
The buds of *Cabomba* develop underwater, isolated at the apex of the axillary peduncles. The peduncle elongates to allow the ovoid bud, at the beginning of anthesis, to remain submerged and floating with the help of the floating leaves and of the lacunae in the peduncle. After fertilization, the peduncle curves and the flower remains completely submerged throughout the development of the fruit.

The flowers are dichlamydeous, actinomorphic and trimerous; the perianth is marcescent (Fig. 3; Fig. 4A). In *C. aquatica* and *C. haynesii*, we observed fewer dimerous flowers per plant. Dimerism reduced the number of stamina in *C. aquatica* by half (to three), whereas it had no effect on the number of stamina in *C. haynesii*, which remained at three. Rataj (1977) used a specimen of *C. aquatica* with dimerous flowers, also with the number of stamina reduced to half, to describe *C. schwartzii* Rataj. The synonymization of this species was made by Ørgaard (1991), and it was confirmed through analysis of the type material (Schwartz 53905, INPA).

The perianth of *Cabomba* species is consistent in shape, number and coloration. The sepals are glabrous, petal-like and oblong with a truncate base, except in *C. furcata*, in



**Figure 2.** Morphology of four species of the genus *Cabomba* (Cabombaceae): A) stem, base of petiole in *C. aquatica*; B) detail of the stem with trichome in *C. aquatica*; C) stem, base of petiole in *C. caroliniana*; D) detail of the stem with trichome in *C. caroliniana*; E) stem, base of petiole in *C. furcata*; F) detail of the stem without trichome in *C. furcata*; G) stem, base of petiole in *C. haynesii*; H) detail of the stem with trichome in *C. haynesii*; I) stem; detail of node and root in *C. aquatica*; J) floating leaf in *C. aquatica*; K) floating leaf in *C. caroliniana*; L) floating leaf in *C. furcata*; M) floating leaf in *C. haynesii*; N) submerged leaf in *C. aquatica*; O) primary segment in *C. aquatica*; P) terminal segment in *C. aquatica*; Q) submerged leaf in *C. caroliniana*; R) primary segment in *C. caroliniana*; S) terminal segment in *C. caroliniana*; T) submerged leaf in *C. furcata*; U) primary segment in *C. furcata*; V) terminal segment in *C. furcata*; W) submerged leaf in *C. haynesii*; X) primary segment in *C. haynesii*; Y) terminal segment in *C. haynesii*.



**Figure 3.** Morphology of four species of the genus *Cabomba* (Cabombaceae): *C. aquatica*—A) Flowers, B) Sepal and C) Petal; *C. caroliniana*—D) Flowers, E) Sepal and F) Petal; *C. furcata*—G) Flowers H) Sepal and I) Petal; *C. haynesii*—J) Flowers; K) Sepal and L) Petal.



which they are obovate. The petals are ovate in *C. aquatica*; oblong in *C. caroliniana* and *C. haynesii*; and obovate in *C. furcata*. In all *Cabomba* species, the petals are unguiculate with an auriculate base, which includes a nectar gland (Fig. 3). The calyx and corolla display the same coloration: yellow in *C. aquatica*; lilac with a magenta tint in the main veins of *C. furcata*; white in *C. caroliniana*; and white with a light lilac hue in *C. haynesii*. Fassett (1953) used the shape of the sepals and petals to separate species within the genus *Cabomba*. However, Wiersema (1989) considered these characters to be of little taxonomically importance.

#### Flower: androecium

In *Cabomba* species, the androecium has a variable number of stamina: typically six in *C. furcata* and *C. aquatica*; four or six in *C. caroliniana*; and typically three in *C. haynesii*. The filaments are cylindrical and of different sizes according to the day on which the flower opens (Fig. 4B-E). This condition is the result of the gradual growth of the stamen during the two days of anthesis (Williamson & Schneider 1994; Taylor *et al.* 2008). The anthers present a strong yellow coloration and are bithecate (tetrasporangiate) and basifixed, with latrorse dehiscence.

#### Flower: gynoecium

The gynoecium is apocarpous (one to three carpels), cyclic and yellow, with strigose trichomes nearly 0.1 mm in length on the ovary and up to the midpoint of the style. The ovary has one to three anatropous ovules, with laminar placentation. The stigmata are capitate, with long and delicate papillae, are whitish and are shiny when receptive (Fig. 4). After fertilization, the stigma becomes blackened and persistent at the apex of the fruit. The number of carpels varies among the species of *Cabomba*. Hoehne (1948), Casper (1878) and Ørgaard (1991) described *Cabomba* gynoecia with up to four carpels.

#### Floral biology

We found that, in cultivation, *Cabomba aquatica* and *C. haynesii* flowered throughout the year, whereas *C. furcata* flowered mainly from August to May (in Brazil). These observations are in accordance with the flowering period in nature (see Lima *et al.* 2012). Although *C. caroliniana* never flowered in cultivation, it flowers throughout the year in its natural habitat.

In *Cabomba aquatica*, *C. furcata* and *C. haynesii*, the flowers opened during the day on two consecutive days, submerging during the night. However, the stigmata of the first two species were receptive only on the first day and the anthers dehisce on the following day. The flowers of *C. haynesii* remained unreceptive on the first day of anthesis, the stigmata and anthers maturing only on the second day (at different times).

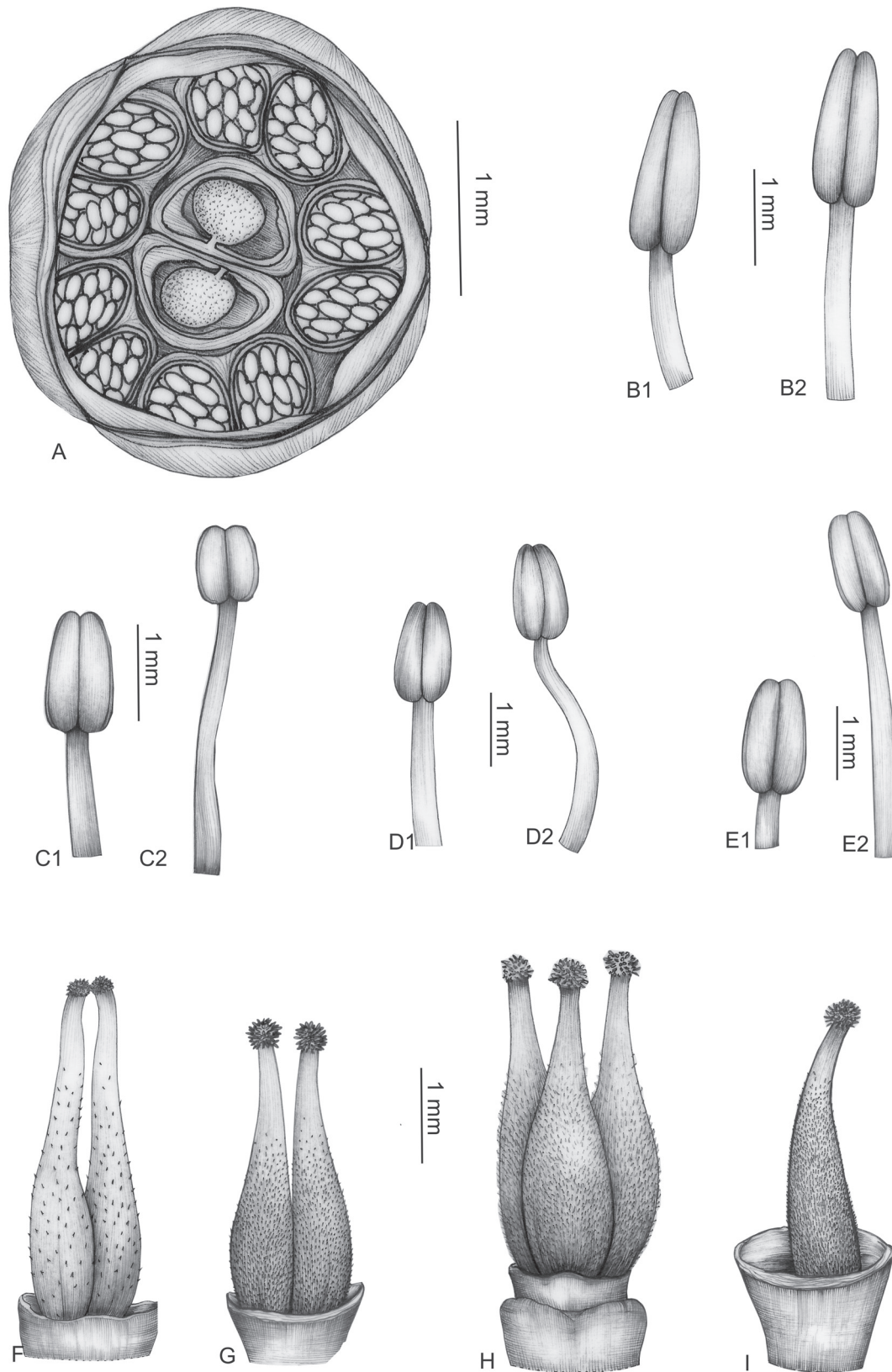
Over the two days of anthesis, the diameter of the flowers was 7.0-10.5 mm in *Cabomba aquatica*, 15.0-18.0 mm in *C. furcata* and ca. 10 mm in *C. haynesii*. On the first day, the floral peduncle remained 1-2 cm above the water surface, lifting the bud, in all three species. The flowers began anthesis at ca. 9:30 a.m. in *C. aquatica*; and at 11:00 a.m. in *C. furcata* (Fig. 5A). In both of those species, the indehiscent anthers were at the level of the ovary. However, the stigmata were already receptive, and the flowers can therefore be categorized as protogynous. The flowers of *C. aquatica* and *C. furcata* closed at 1:30 p.m. and 4:00 p.m., respectively, submerging thereafter (Fig. 5B). In *C. haynesii*, the flowers were in anthesis at ca. 11:20 a.m., although the anthers were indehiscent. Unlike those of the other species, the stigmata of *C. haynesii* were not receptive but already had a plumose appearance, which was maintained throughout the period of flower anthesis, and closed at 1:50 p.m., submerging thereafter (Fig. 5A and 5B). During the winter months (May to August), particularly on cold, rainy days, the flowers opened after 12:00 p.m. and closed at 4:00 p.m.

On the second day of anthesis, the floral peduncle remained 2-3 cm above the surface of the water (Fig. 6A). In *C. aquatica*, anthesis began at ca. 8:00 a.m.; first- and second-day flowers completed anthesis approximately at the same time (1:30 p.m.) and submerged thereafter. On rainy days, those events were delayed by ca. 30 min. In *C. furcata*, second-day flowers opened later (at ca. 10:40 a.m.), although first- and second-day flowers both closed at 4:00 p.m. In *C. haynesii*, second-day flowers also opened later and closed later, at ca. 10:20 a.m. and 2:40 p.m., respectively (Fig. 5C and 5D). During the winter months, there was also a delay in the timing of second-day anthesis of the flowers of *C. haynesii*, which were not fully open until 11:30 a.m.

When individuals of *Cabomba furcata* were deprived of light until 10:00 a.m., the beginning of the anthesis was delayed by ca. 4 h. We observed no significant change in the timing of flower opening in *C. haynesii* after exposure to the same light/dark cycle. It is likely that the flowers of *C. furcata* open in response to light, whereas the opening of *C. haynesii* flowers is triggered by temperature, given that the timing of the latter was altered on rainy days and on days that were particularly cold.

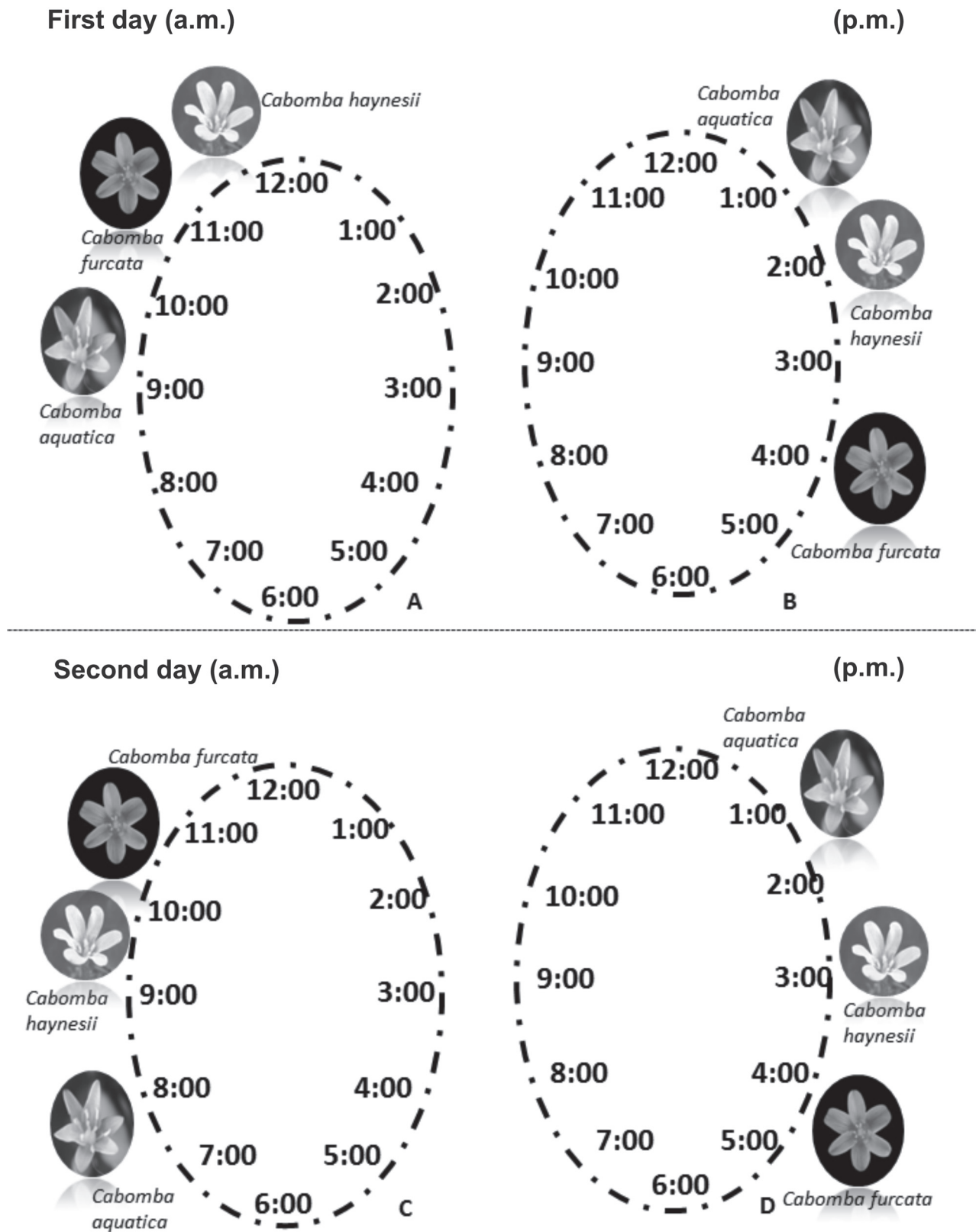
First- and second-day flowers of the three species studied here open synchronously. According to Dafni *et al.* (2005), this behavior plays an important role in attracting floral visitors, because of the visual aspects as well as the concentration of resources.

In addition to visual attractants, flowers of *Cabomba* species display two auriculate nectar glands at the base of the petals (Fig 3C, 3F, 3I and 3L), providing nectar and pollen grains as rewards for possible pollinators. Within the ANITA grade, the production of nectar has been detected in four families (Cabombaceae, Nymphaeaceae, Schisan-



**Figure 4.** Morphology of four species of the genus *Cabomba* (Cabombaceae): A) transverse section of first-day flower; stamens of first-day flowers—B1) *Cabomba aquatica*, C1) *C. caroliniana*, D1) *C. furcata* and E1) *C. haynesii*; stamens of second-day flowers—B2) *C. aquatica*, C2) *C. caroliniana*, D2) *C. furcata* and E2) *C. haynesii*; gynoecium—F) *C. aquatica*, G) *C. caroliniana*, H) *C. furcata* and I) *C. haynesii*.





**Figure 5.** Timing of anthesis of *Cabomba aquatica*, *C. furcata* and *C. haynesii*: A) flower opening on the first day; B) flower closing on the first day; C) flower opening on the second day; D) flower closing on the second day.

draceae and Illiciaceae), providing compelling evidence of cross-pollination (Thien *et al.* 2009).

Between the closing of first-day flowers and the opening of second-day flowers, the filaments grew and the stamina increased in length (doubling in some cases), allowing the anthers to be at the same level as the stigma. In *Cabomba aquatica*, anther dehiscence occurred approximately one hour after the opening of the first-day flowers. In the other two species, that delay was only ca. 20 min. Shortly before the pollen grains were released, the filaments were positioned at an angle of ca. 45° relative to the carpels, and the position of the anthers was parallel to the water surface, turned towards the perianth. Typically, some anthers remain indehiscent. *Cabomba aquatica* and *C. furcata* produce ca. 250 pollen grains per anther, whereas *C. haynesii* produces only ca. 160 grains per anther, which is considered a small quantity of pollen (Cruden 1977). The pollen grains are released as thick massulae. Pollen viability is ca. 98% in *C. aquatica*, 97% in *C. furcata* and 100% in *C. haynesii*.

The gynoecium of *Cabomba aquatica* is typically composed of two carpels, rarely one or three; that of *C. furcata* has three carpels, rarely two or only one; and that of *C. haynesii* typically has only one carpel. According to Caspary (1878), Hoehne (1948) and Ørgaard (1991), the number of carpels in *Cabomba* is not consistent among the species, varying from one to four. In the present study, the number of carpels ranged from one to three among the species studied. On the first and second day of anthesis, the style was slightly curved towards the perianth. On the first day of anthesis in *C. aquatica* and *C. furcata*, both of which are protogynous, the stigmata of each carpel remained curved towards the perianth and ca. 2 mm apart, the stigma papillae being elongated and with a shiny appearance (Fig. 6). On the second day of anthesis, the carpels of those two species were closer together, and the papillae begin to wilt rapidly just after the beginning of anthesis on the second day, before anther dehiscence (Fig. 6D, 6F and 6H).

Schneider & Jeter (1982), Osborn & Schneider (1988) and Osborn *et al.* (1991) also categorized species of *Cabomba* as protogynous. However, in the present study, the stigmata of *C. haynesii* were receptive only on the second day of anthesis, despite displaying extended papillae and a shiny appearance on both days of anthesis, being slightly larger on the second day. The need for flowers to open on two consecutive days might be related to the fact that the flowers of *C. haynesii* are always small and isolated, and anthesis over a period of two days could therefore increase the visible area and attract a greater number of floral visitors. Given that we identified no osmophores in any of the species evaluated, we can assume that the visual features constituted the only attractant for floral visitors.

In *Cabomba aquatica*, each carpel produces one ovule, multiple ovules (two or three) are rarely observed, and the pollen-ovule ratio is of ca. 1000:1. In *C. furcata*, each carpel produces two ovules and the pollen-ovule ratio is ca. 300:1.

In *C. haynesii*, each carpel produces two ovules and the pollen-ovule ratio is ca. 240:1. According to Cruden (1977), *C. aquatica* is facultatively xenogamous, *C. furcata* is facultatively autogamous or facultatively xenogamous and *C. haynesii* is facultatively autogamous. However, in the present study, we observed no fruit development, which indicates that *Cabomba* species require a vector for pollination.

Pollen grains that shed in massulae cannot be transported by wind or water (Thanikaimoni 1986). Together with protogyny and the absence of a pollinator, this could explain the absence of self-pollination in *Cabomba* species. Pollen grains presented as massulae in *C. haynesii* would also explain why self-pollination does not occur even when stigmata are receptive and the anthers dehisce on the same day in the absence of a pollinator.

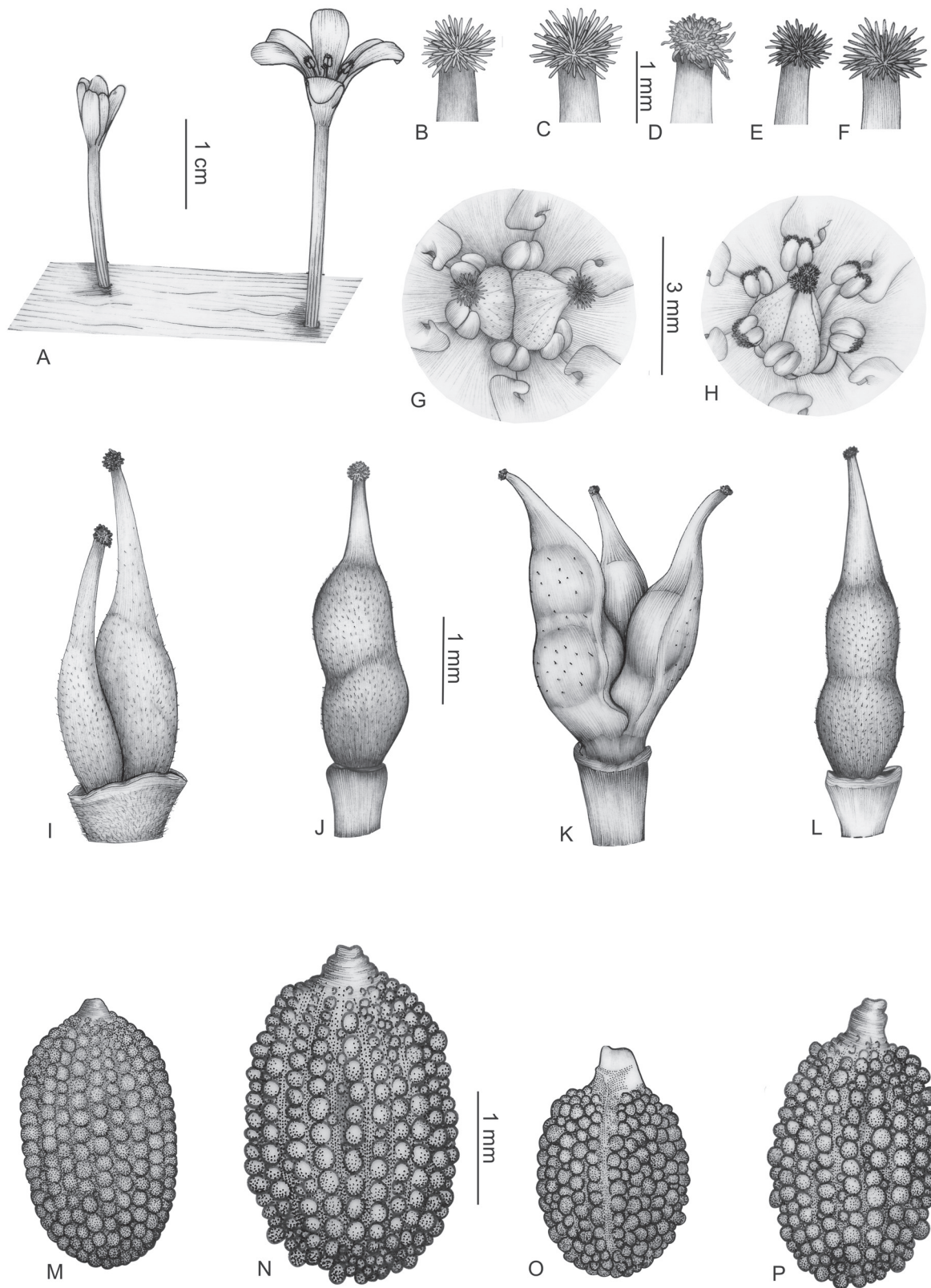
During fruiting, *Cabomba* species typically develop only one carpel in a follicle-like and indehiscent fruit. The pericarp of each fruit is coriaceous and yellowish, but the seed area is marked and slightly blackened (Fig. 6I-L). In *C. aquatica*, each fruit typically develops only one seed; in *C. furcata*, each fruit typically develops two seeds; and in *C. haynesii*, each fruit typically develops one or two seeds. The shape of the seed varies from oval in *C. aquatica* and *C. haynesii* to orbicular in *C. caroliniana* and *C. furcata*. Seed length also varies, from  $\leq 3$  mm in *C. caroliniana* to  $\leq 2$  mm (most  $< 2$  mm) in *C. furcata* (Fig. 6M-P). At maturity, the seeds display a tuberculate testa (with small tubercles arranged longitudinally) and are yellowish with several small red spots. The fruit is protected by a marcescent perianth; the anthocarp detaches from the peduncle and falls to the bottom of the aquatic environment to begin germination, developing a new seedling.

## Conclusions

Species of *Cabomba* can be distinguished from each other on the basis of their vegetative and reproductive morphology, which generally tends to be consistent between specimens observed in nature and those grown in cultivation. We propose some new terms and morphological characters to describe and distinguish among *Cabomba* species: “verticillate-crossed” to describe the leaves of *C. furcata*; orbicular leaves in *C. aquatica*; arms on the side of the laminae of the floating leaves of *C. furcata*, *C. caroliniana* and *C. haynesii*; pollen grains as massulae; and the anthocarp as the dispersal unit.

Protogyny is described as a characteristic feature of *Cabomba*. However, the absence of protogyny observed in *C. haynesii* signals the development of new reproductive strategies and possibly different interactions with pollinators.

There is a need for further studies involving molecular analyses and evaluating reproductive biology, in order to correlate the different reproductive strategies and evolutionary adaptations within the order Nymphaeales.



**Figure 6.** Aspects of floral biology in four species of the genus *Cabomba* (Cabombaceae). A) difference in peduncle length between first- and second-day flowers in *C. haynesii*; stigmata of first-day flowers—B) *C. aquatica*, C) *C. caroliniana* and E) *C. haynesii*. stigmata of second-day flowers—D) *C. furcata* and F) *C. haynesii*; G) position of stigmata on the first day in *C. furcata*; H) position of stigmata on the second day in *C. furcata*; fruit—I) *C. aquatica*, J) *C. caroliniana*, K) *C. furcata* and L) *C. haynesii*; Seeds—M) *C. aquatica*, N) *C. caroliniana*, O) *C. furcata* and P) *C. haynesii*.



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