



# Re-evaluation of generic characters for *Blinkworthia* (Convolvulaceae) based on morphology and reproductive organ development

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## Abstract

*Blinkworthia*, a tropical Asian genus of Convolvulaceae, was first characterized as an erect shrub with solitary flowers, three involucre bracts, and indehiscent, one-seeded berries. These three characters are diagnostic traits long used to distinguish *Blinkworthia* from other genera in the family. *Blinkworthia lycioides* was selected as a representative species for investigation of both morphology and ontogeny of the reproductive parts, while *B. convolvuloides* was only investigated morphologically. Nine stages based upon floral and fruit sizes were collected and prepared by the paraffin method for the developmental study. Examination of the developmental anatomy proved that some of the morphological characters have been misunderstood. In fact, none of the generic characters historically used to define the genus *Blinkworthia* are unique: the same character states are present in some species of *Argyreia*. This highlights that on morphological grounds it is not tenable to maintain *Blinkworthia* distinct from *Argyreia*. Pending the results from our ongoing molecular study of the genera in tribe Ipomoeae a firm decision can be taken regarding the generic circumscription.

**Keywords** *Blinkworthia convolvuloides* · *Blinkworthia lycioides* · Floral development · Fruit development · Morphogenesis · Taxonomic delimitation

## Introduction

*Blinkworthia* Choisy is a small genus belonging to the tribe Ipomoeae of the morning glory family (Stefanović et al. 2003) and is distributed in China, Myanmar, and Thailand (Fang and Staples 1995; Staples 2010). Choisy (1834),

working with herbarium specimens only, used four characters to define his new genus *Blinkworthia*: shrubby habit; the presence of an involucre of three bracts; distinctive corolla morphology; one-seeded berry. Later, Collett and Hemsley (1890), who studied living plants in Myanmar, revised the generic definition and used the following characters to distinguish *Blinkworthia* from the related genera *Argyreia* Lour., *Lettsomia* Roxb., and *Rivea* Choisy: solitary flowers; a two-celled ovary; and the non-twining habit. Collett and Hemsley (1890) also pointed out, and illustrated, that at flowering time the ovary is completely enclosed in the cylindrical nectary disc and that the berry is not always one-seeded and may contain up to four seeds. Hallier's (1893) investigation was the first comprehensive examination of the morphology, anatomy, and palynology for the whole family Convolvulaceae; he synthesized all available evidence in a new classification of tribal relationships founded on clear generic delimitation. Hallier reported bifacial leaf structure for *Blinkworthia* and otherwise repeated Choisy's morphological description. Hallier used the indehiscent fruit type as an important character to define the tribe Argyreieae, including the genera *Argyreia*, *Blinkworthia*, and *Rivea*. However,

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Hallier did not include fruit anatomy in his examination of generic characters and tribal delimitation.

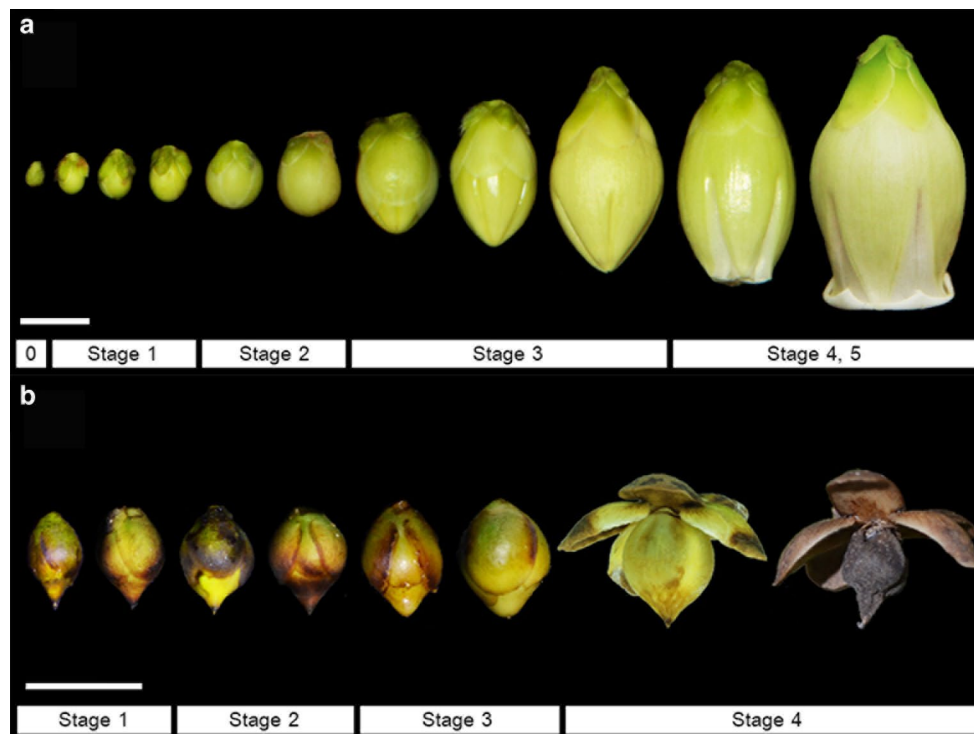
Today *Blinkworthia* consists of two species: *B. lycioides* Choisy and *B. convolvuloides* Prain. A third species, *B. discostigma* Hand.-Mazz., is treated as a synonym of *B. convolvuloides* Prain (Fang and Staples 1995; Kress et al. 2003; Staples 2010). The genus is closely related to *Argyreia* Lour. and probably sister to it based on the shared characters of indehiscent fruit with leathery pericarp (Wilkin 1999; Stefanović et al. 2003; Staples 2010). However, the only molecular phylogeny did not include *Blinkworthia* in the analysis (Stefanović et al. 2002); therefore, the relationships of this genus are still ambiguous. Several previous publications used only morphological features of the flowers and fruits for classification (Hallier 1893; Austin 1998; Wilkin 1999; Stefanović et al. 2003). Furthermore no investigation has been done for floral and fruit development to explore the nature and ontogenesis for the characters that have been used up until now to assert generic status of *Blinkworthia*. Therefore, selected characters used for recognition of this genus were critically examined for the first time to test their validity for generic delimitation of *Blinkworthia* as defined in the literature. *Blinkworthia lycioides* was selected as a representative species for detailed morphological and anatomical research because it is more widespread and readily available than *B. convolvuloides*. However, the morphology

of *B. convolvuloides* was also investigated for a broader understanding of the generic concept. Field observations for both species, followed up with laboratory studies of floral anatomy and fruit ontogeny for *B. lycioides*, were carried out to provide an enhanced morphological description and a critical evaluation of the generic circumscription.

## Materials and methods

### Plant materials and field observation

Flowers and fruits of *B. lycioides* were collected in different developmental stages between August and December then categorized into nine stages for analysis based upon floral and fruit sizes defined by ratio of length to width (Fig. 1). Field observations of the two species were recorded in their natural habitats in three provinces of Thailand and three locations in Myanmar. Voucher specimens were collected and deposited in the Bangkok Herbarium, Thailand (BK), the Forest Herbarium of Thailand (BKF), the Queen Sirikit Botanic Garden, Thailand (QBG), the Forest Research Institute of Yezin, Myanmar (RAF), and the Kochi Prefectural Makino Botanical Garden, Japan (MBK) (Table 1).



**Fig. 1** *Blinkworthia lycioides* the developmental series of flower buds **a** in five stages and fruits **b** in four stages. Scale bars = 1 cm. Vouchers: P. Rattanakrajang and P. Traiperm 5, 6, 14, 49

**Table 1** List of materials used in morphological and anatomical analyses

Species	Locality	Collector and number
<i>B. lycioides</i> Choisy	Myanmar, Shan State, Taunggyi	Y. Baba, W. Tanming, J. Peeraman and P. Rattanakrajang 104920
	Thailand, Chiang Mai, Mae Rim	P. Rattanakrajang and P. Traiper 6
	Thailand, Chiang Mai, Muang	P. Rattanakrajang and P. Traiper 14
	Thailand, Kanchanaburi, Sri Sawat	P. Rattanakrajang and P. Traiper 5
	Thailand, Ratchaburi, Suan Pueng	P. Rattanakrajang and P. Traiper 49
<i>B. convolvuloides</i> Prain	Thailand, Phetchaburi, Cha Am	P. Rattanakrajang and P. Traiper 2
	China, Yunnan	Li-Jianwu 1011
	Myanmar, Mandalay, Patheingyi	P. Rattanakrajang and M. Phy 116
	Myanmar, Mandalay, Pyin Oo Lwin	P. Rattanakrajang and M. Phy 117
	Myanmar, Mandalay	B. Pe 6909

## Light and stereo microscopy

Samples were collected and immediately fixed in 70% ethyl alcohol. Morphology was examined by stereomicroscope. Anatomical samples were prepared using the paraffin method (Johansen 1940). The flowers and fruits were dehydrated through a concentration series of tertiary butyl alcohol (TBA). Infiltration was applied with paraffin oil, and then the samples were embedded in paraffin wax. The embedded materials were sectioned in transverse and longitudinal planes at 20 µm thickness by a Leica SM2000 R sliding microtome. The sections were then cleared and stained with 1% Safranin O and 1% Fast Green, mounted in DePeX mounting media, observed and digitally photographed with an Olympus BX43 microscope with a camera attachment DP21. Terminology used for floral and fruit descriptions follows Evert (2006), and Kajita and Nishino (2009).

## Results

### Gross morphology and anatomy

In their natural habitats, *B. lycioides* formed an erect shrub habit (Fig. 2a, c), however, twining plants were found sometimes (Fig. 2b, d). Conversely, *B. convolvuloides* was typically a twiner (Fig. 2f) but we also once observed a plant with an erect shrub habit (Fig. 2e). In both species, flowering branches were pubescent and carried from 2 to 20 solitary flowers, which emerged from the leaf axils.

The flower was typically solitary (Fig. 3a), rarely paired (Fig. 3h), actinomorphic (Fig. 3c), pentamerous, hypogynous, homogamous, and pedunculate. Each flower was subtended by four elliptic bracts with strigose hairs abaxially in *B. lycioides* (Fig. 3a, h), but three to four leaf-like bracts with strigose hairs abaxially in *B. convolvuloides* (Fig. 3b, g); these bracts were typically arranged in a cruciate pattern.

The leathery calyx had five free sepals with quincuncial aestivation that enclosed the floral bud (Fig. 4b). The transverse section of the sepals revealed that the glabrous epidermis had epidermal cells with thick walls (Fig. 4d). The adaxial epidermal cells were larger than abaxial epidermal cells. Parenchyma cell sizes in the mesophyll layer were also different between the two sides. The adaxial parenchyma was smaller than the abaxial side. Vascular bundles were located closer to the adaxial surface of the lamina, where the intercellular spaces exist. Laticiferous tissues were found in the centre of the sepals (Fig. 4d). The developmental stages from flowers to young fruits were enclosed by the persistent calyx. However, the final stage of fruit development was not enfolded by the leathery calyx (Fig. 1b), which reflexed away from the fruit as maturation proceeds.

The actinomorphic, waxy corolla was urceolate in shape. The colour was greenish white outside and pinkish dotted inside (Fig. 3a, c, h). The corolla tube was connate and separated at the apex into five entire lobes (Fig. 3c). The corolla lobes had contortiplicate aestivation in floral bud stage (Fig. 4c). Petals had a similar structure in transverse section to the sepals, but differed in that the adaxial epidermal cells of the petals were narrowly oblong in shape instead of polygonal (Fig. 4d). Intercellular spaces and laticiferous tissues were found in the mesophyll layer of the petals (Fig. 4d), as well as druse crystals (Fig. 4e).

Within the flower, there were five fertile stamens attached to the base of the corolla tube. Trichome-like staminal secretory glands were found at the base of the filaments (Fig. 4g, h). Each anther was ovoid, dithecate, had four pollen sacs (tetrasporangiate) and was dorsally attached to the filament. Two pollen sacs were separated by a narrow septum and dehisced longitudinally. Placentoids consisting of parenchymal bulges of the septa intruded into each pollen sac. The anther wall was comprised of epidermis and fibrous endothecium. The pollen grains were globose, spiniferous, and pantoporate (Fig. 4f).





**Fig. 2** Growth habits of *Blinkworthia* spp. in their natural habitats: **a** *Blinkworthia lycioides*, shrub (voucher: Rattanakrajang and Traiperm 6); **b** *B. lycioides*, climber (P. Rattanakrajang and P. Traiperm 14); **c** *B. lycioides*, shrub (Y. Baba et al. 104920); **d** *B. lycioides*, climber (Y.

Baba et al. 104920); **e** *B. convolvuloides*, shrub (P. Rattanakrajang and M. Phyo 117); **f** *B. convolvuloides*, climber (P. Rattanakrajang and M. Phyo 116)

The pistil comprised two carpels, fully fused except for the distal part of the stigma; the pistil was included within the corolla (Fig. 3c). The style was filiform with two capitate stigmas (Fig. 3c). The syncarpous ovary was completely bilocular. The septum between the two locules extended for the full length of the superior ovary. Each carpel generally contained two fertile anatropous ovules, borne on a fleshy basal placenta. The floral nectary of *B. lycioides* was light yellow at anthesis. The nectary was developed as a disc at the base of the ovary (Fig. 3f). Initially this disc engulfed most of the ovary but during fruit development the disc stopped enlarging and did not enclose the entire fruit.

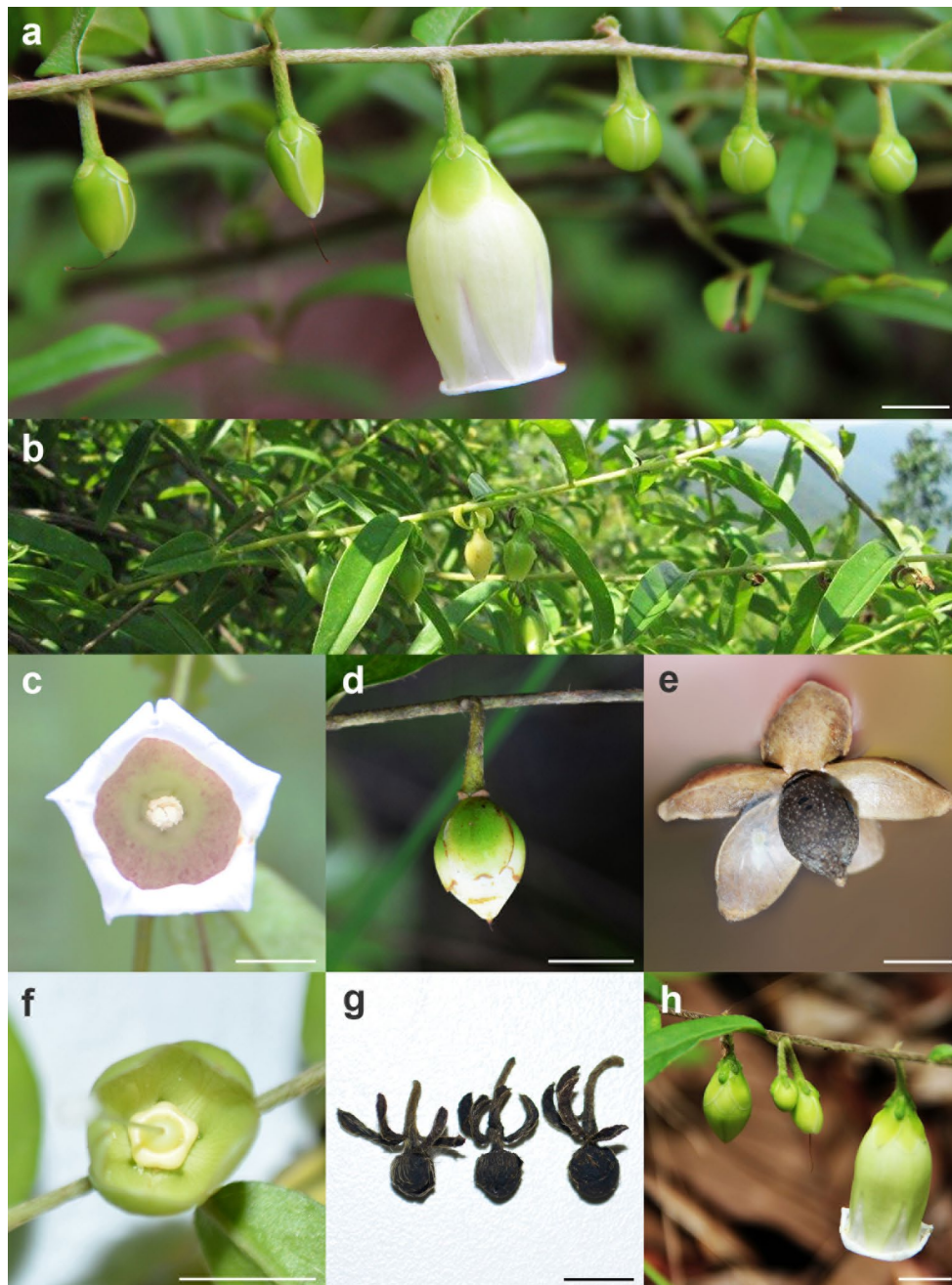
The fruit was a berry, glabrous, and light green colour in the immature stage. The fruits were enclosed by a slightly accrescent and woody calyx, whereas when ripe the fruits turned dark brown, dry, presented white spots on the surface, and the five free sepals opened out and reflexed away from the fruit (Fig. 3e). The fruit apex was apiculate (Figs. 1b, 3e). The nectary disc formed a narrow annular collar around the base of the fruit (Figs. 3f, 13b, d).

### Floral development

The floral development was studied at five arbitrary developmental stages defined by size of the floral structures (Fig. 1a). The first four stages represented the gynoecium and other floral organs as they increase in size up to the point of flower anthesis before fertilization and the last stage (fifth) was the flower after fertilization. In this research, we focused mainly on observations of the expansion and encirclement of the nectary disc through the five developmental stages.

At the first stage, the gynoecium was primarily cone shaped and topped with young stigmas (Fig. 5a, b). The centre of the filiform style consisted of a stylar canal located in between two median carpellary bundles (Fig. 5c). Two ovules inside the ovary locule slowly developed to the anatropous type (Fig. 5d). The floral nectary disc protruded from the receptacle and was nearly square in cross section at the base of the ovary (Fig. 5a, e).

For the second developmental stage, the protrusion of the nectary disc became apparent when the gynoecium was approximately 1.4–1.5 mm high, and topped with the



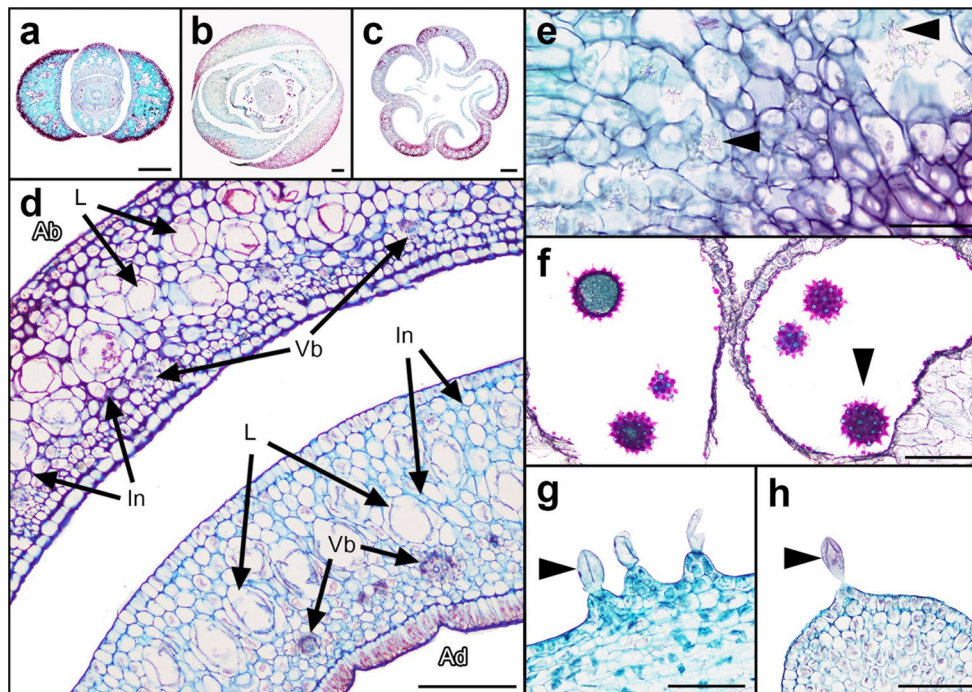
**Fig. 3** Flowers and fruits of *Blinkworthia* spp., principal characters: **a** *Blinkworthia lycioides*, fertile shoot with (from right to left) sequential, solitary floral buds, opened flower, and young fruits (voucher: *P. Rattanakrajang* and *P. Traiperm* 5); **b** *B. convolvuloides*, fertile shoot with young fruits (photo from K. Kothetnaingoo); **c** *B. lycioides*, view into centre of flower at anthesis, before fertilization (*P. Rattanakrajang* and *P. Traiperm* 6); **d** *B. lycioides*, lateral view of young fruit enclosed by calyx (*P. Rattanakrajang* and *P. Traiperm*

6); **e** *B. lycioides*, mature fruit displaying free, reflexed sepals (*P. Rattanakrajang* and *P. Traiperm* 6); **f** *B. lycioides*, view of pistil and calyx, after fertilization and corolla dehiscence (*P. Rattanakrajang* and *P. Traiperm* 6); **g** *B. convolvuloides*, immature fruits (dry) displaying four persistent, leaf-like bracts (*P. Rattanakrajang* and *M. Phyto* 117); **h** *B. lycioides*, fertile shoot with solitary and paired flower buds at adjacent axils (*P. Rattanakrajang* and *P. Traiperm* 2). Scale bars = 1 cm

filiform style which terminated with two distinct stigma lobes (Fig. 6a, b, c). The epidermis of the style consisted of cells with dense walls. The filiform style expanded and lengthened from the distal part of the ovary. The uneven

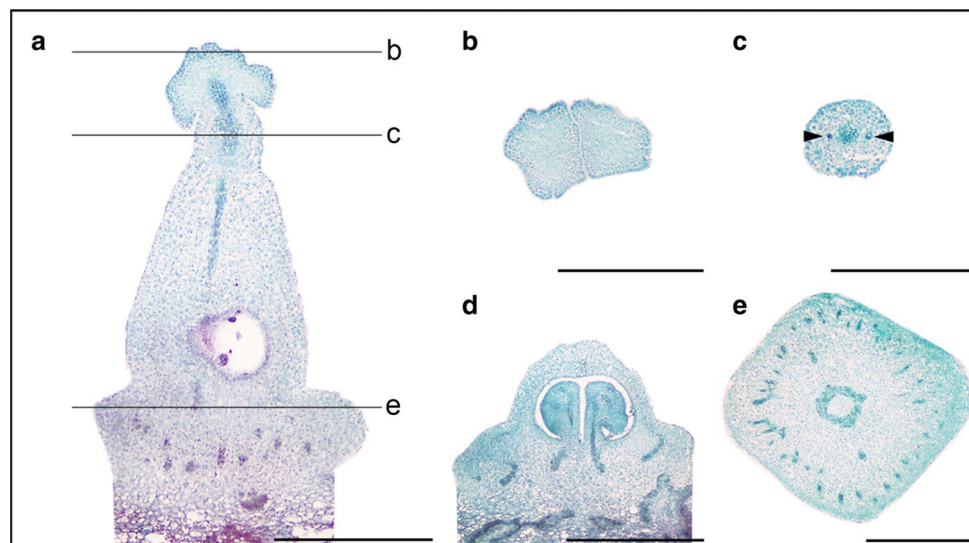
surface of the ovary was comprised of a single layer of epidermal cells (Fig. 6d, e). The ovary was partitioned into two locules by a complete septum inclusive of the transmitting tissue (two median carpellary bundles) (Fig. 6b, f, g). The





**Fig. 4** Floral anatomy of *Blinkworthia lycioides* (voucher: *P. Rattanakrajang* and *P. Traiperm 6*): **a** TS of early stage flower bud showing aestivation of bracts, scale = 500  $\mu$ m; **b** TS showing aestivation of calyx, scale = 500  $\mu$ m; **c** TS showing aestivation of corolla, scale = 500  $\mu$ m; **d** TS through the perianth structures showing epidermis, ground tissues, and vascular tissues, scale = 200  $\mu$ m; **e** LS

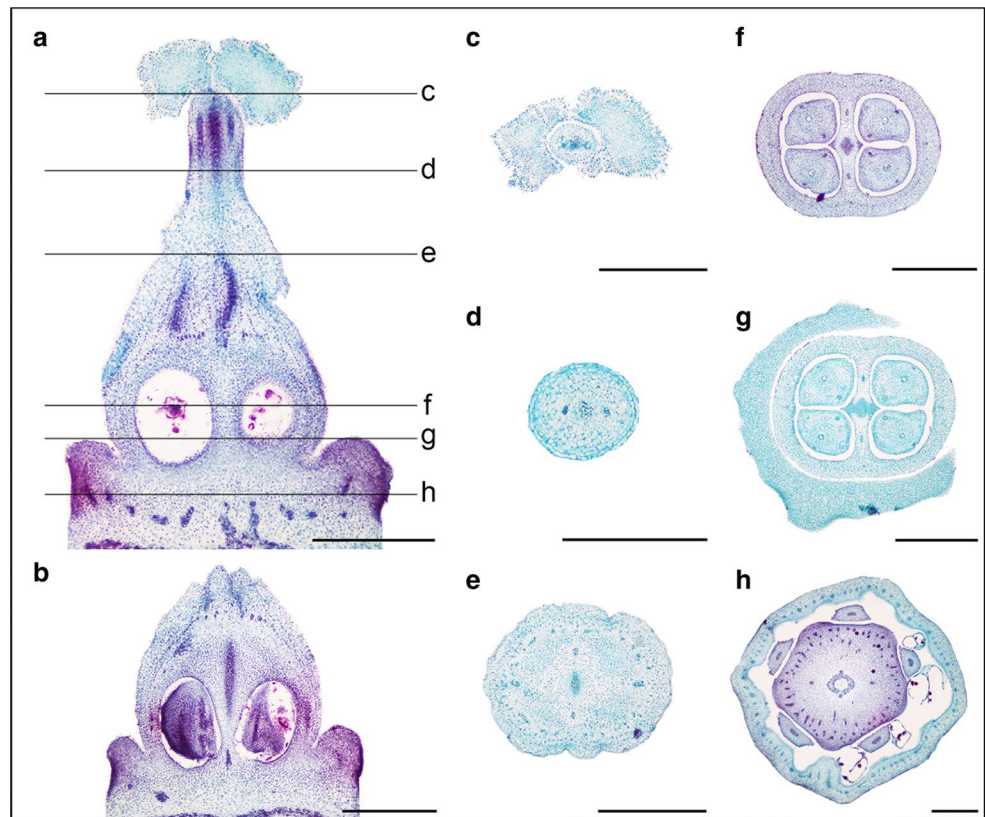
through the petal showing druse crystals (triangle), scale = 100  $\mu$ m; **f** TS of anther sacs showing the spiny pollen (triangle), scale = 100  $\mu$ m; **g**, **h** LS of filament base showing trichome-like, external secretory glands (triangle), scale = 100  $\mu$ m. *Ab* abaxial side, *Ad* adaxial side, *In* intercellular space, *L* laticifers, *LS* longitudinal section, *TS* transverse section, *Vb* vascular bundles



**Fig. 5** *Blinkworthia lycioides* microtome sections of the first stage of floral development: **a** LS (medial) through the gynoecium, horizontal lines at b, c, and e indicate the levels of TS illustrated in the following figures; **b** TS through the distal part of pistil with two capitate stigma lobes; **c** TS through the style showing two median carpellary bundles

(triangle); **d** LS (tangential) through the gynoecium, note two ovules in locule; **e** TS through the proximal part of ovary, note shape of the nectary disc. All scale bars = 500  $\mu$ m. (voucher: *P. Rattanakrajang* and *P. Traiperm 6*). *LS* longitudinal section, *TS* transverse section

**Fig. 6** *Blinkworthia lycioides*, microtome sections of the second stage of floral development: **a** LS through the medial gynoecium, horizontal lines at c, d, e, f, g, and h indicate the levels of TS illustrated in the following figures; **b** LS (tangential) through the gynoecium, note one ovule per locule; **c** TS through the distal part of gynoecium at the level of the two capitate stigma lobes; **d** TS through the style with two median carpellary bundles; **e** TS through the distal part of ovary; **f** TS through the central part of ovary, note four ovules inside two locules; **g** TS through the lower part of ovary, note ovary encirclement by nectary disc; **h** TS through the proximal part of gynoecium, note pentagonal shape of nectary disc and positions of five filament bases inside corolla tube. All scale bars = 500  $\mu\text{m}$ . (voucher: *P. Rattanakrajang* and *P. Traiperm* 6). LS longitudinal section, TS transverse section



initial square plane of the nectary disc from the first stage transformed into a pentagonal plane, caused by pressure from the filament bases during development of the androecium (Fig. 6h).

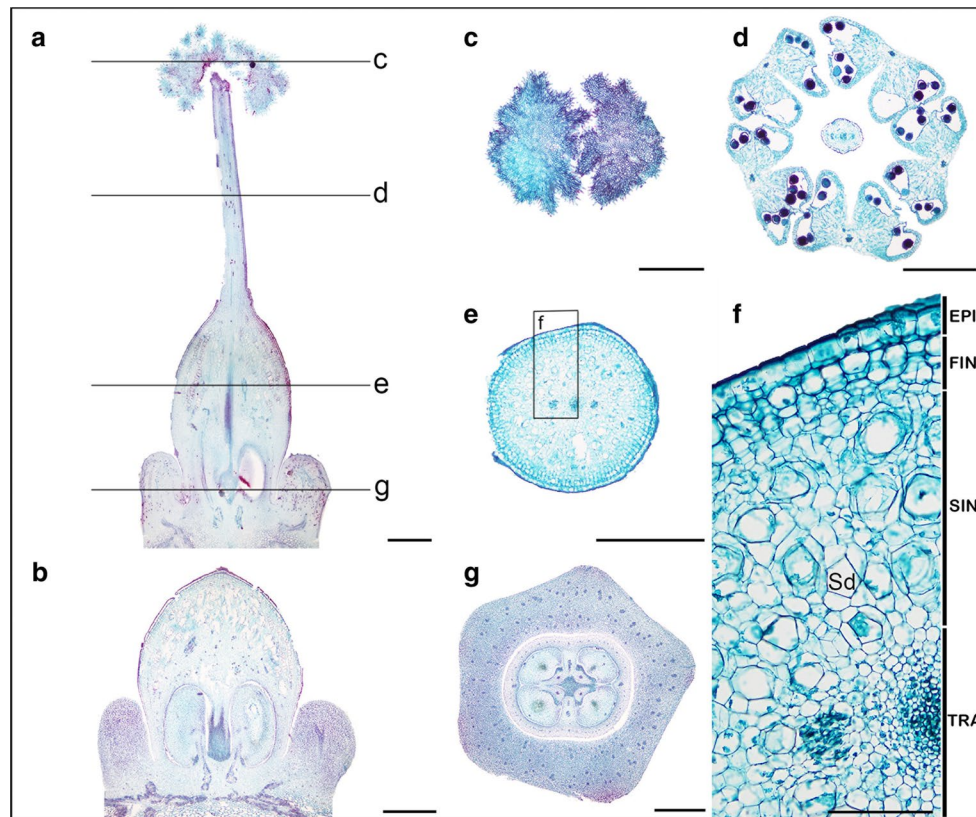
In the third stage of floral development, the syncarpous pistil had a superior ellipsoid ovary: the distal part of the ovary had elongated; the basal part of the ovary was constrained by the encirclement of the nectary disc. In the centre of the ovary, the basal placentas were fused at the septum base (Fig. 7a, b). The papillae on the stigmatic epidermis were inflated when the style extended approximately 6–7 mm in length (Fig. 7a, c). On the adaxial side of the corolla tube, transverse section at the level of the anthers showed vascular bundles of the epipetalous stamens. The spiny pollen grains inside the anther thecae were almost mature and ready for anther dehiscence (Fig. 7d). Layers inside the distal part of the ovary wall consisted of epidermis, internal tissue, and transmitting tissue, which will be transformed into exocarp, mesocarp, and endocarp, respectively. The outer epidermal layer of the ovary was covered by a thick cuticle layer. The internal tissue was pluriseriate and comprised varied sizes of fundamental cells and was differentiated in two zones. The first zone was composed of tightly ordered tangential parenchyma cells, while the presence of secretory ducts surrounded by epithelial cells revealed the second zone. Transmitting tissue showed two clusters of median carpellary bundles and a stylar canal (Fig. 7e, f).

The two ovules expanded to fill the available space in each locule. The pentagonal nectary disc had encircled the ovary (Fig. 7g).

The fourth developmental stage presented as the flower bud matured and anthesis took place. This stage was only slightly changed from the previous stage. The papillae on the epidermis of the stigmas were longer (Fig. 8c), and the style had lengthened (Fig. 8a). Most layers within the distal part of the ovary were similar to the previous stage. However, within the transmitting tissue appeared many lysigenous aerenchyma cells and intercellular spaces (Fig. 8e, f). The four ovules were mature (Fig. 8g), and the nectary disc had expanded to be higher than the top of the septum (Fig. 8a, b).

At the fifth or last developmental stage, the corolla had fallen off from the flower after anthesis. One ovule had been fertilized before the others and began developing (Fig. 9c, d); the other three ovules, if fertilized, will follow. All layers of the distal part of the ovary were similar to the former stage. This last stage clearly showed developmental phases of the secretory ducts from the initial phase to the lysigenous phase (Fig. 9e, phases numbered 1–7). Transverse section of the nectary disc showed the boundary of each layer, which comprised abaxial epidermis, ground tissue, and adaxial epidermis. Both sides of the epidermis were made up of a single layer of epidermal cells, but the abaxial epidermal cell walls were obviously thicker. The occurrence of secretory cells and intercellular spaces was observed within the





**Fig. 7** *Blinkworthia lycioides*, microtome sections of the third stage of floral development. **a** LS through the medial gynoeceum, horizontal lines at c, d, e, and g indicate the levels of TS illustrated in the following figures, scale = 500  $\mu$ m; **b** LS (tangential) through the gynoeceum, note one ovule per locule, scale = 500  $\mu$ m; **c** TS through the distal part of gynoeceum showing two capitata stigma lobes with fully expanded papillae, scale = 500  $\mu$ m; **d** TS through the style at level of androecium, note four pollen sacs in each of five anthers surrounding the style, scale = 500  $\mu$ m; **e** TS through the distal part of

ovary, scale = 500  $\mu$ m; **f** TS, close-up of figure e, note differentiation of ovary wall into four zones [epidermis (exocarp), first zone of internal tissue (mesocarp), second zone of internal tissue (mesocarp), and transmitting tissue (endocarp)], scale = 100  $\mu$ m; **g** TS through the centre part of ovary, note complete encirclement of ovary by the nectary disc, scale = 500  $\mu$ m. (voucher: P. Rattanakrajang and P. Traiperm 6). EPI epidermis, FIN first zone of internal tissue, LS longitudinal section, Sd secretory duct, SIN second zone of internal tissue, TRA transmitting tissue, TS transverse section

ground tissue (Fig. 9d, f). The centre and proximal part of the ovary was as large as the distal part of the ovary. The expanded length of the nectary disc was now more than one and a half times as long as the septum (Fig. 9a, b).

### Fruit development

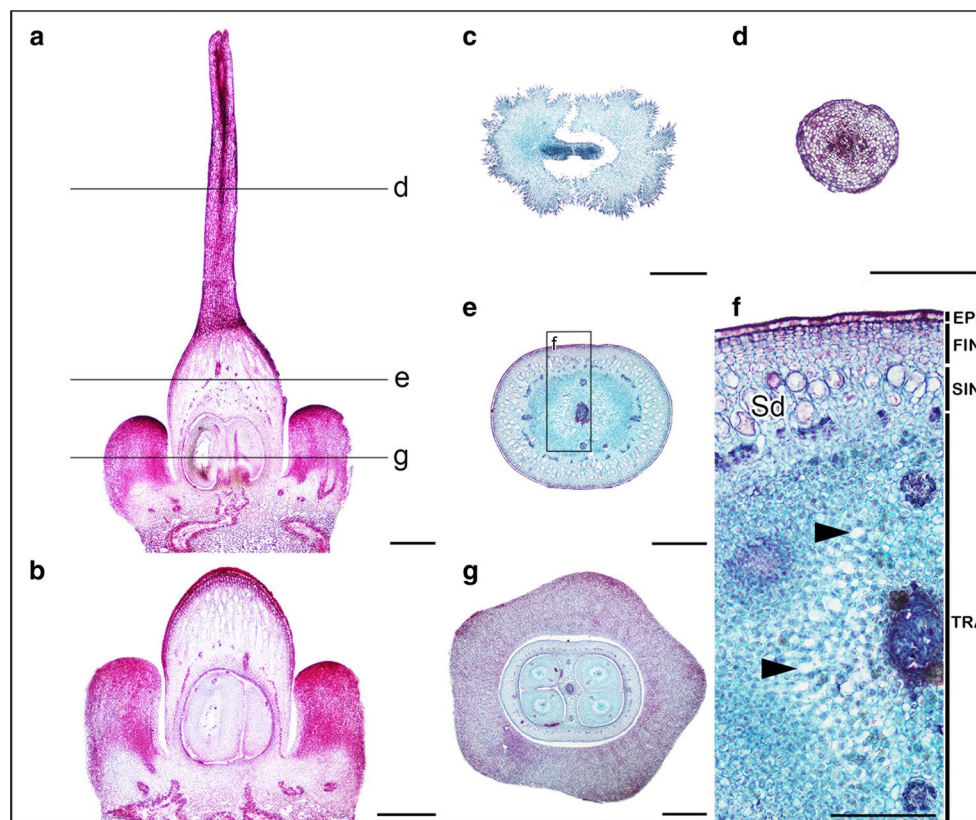
Four arbitrary developmental stages were studied with regard to anatomical and morphological structures, except the mature fruit stage which was studied only for its morphological features (Fig. 1b). The progression of nectary disc development was the principal focus studied in this sequence; the enclosing of the fruit by the calyx was also examined in the last fruit development stage.

At the first developmental stage (Fig. 10), the young fruit was subglobose with a narrow conical style, but lacked the stigma (Fig. 10a, b). The layers of cells between the distal and centre parts of the fruit were obviously different

(Fig. 10a). The structure of the distal part resembled the floral stages by having secretory ducts and druse crystals (Fig. 10c, e). The fruit pericarp was composed of exocarp, mesocarp, and endocarp. The abaxial side of the exocarp was covered by a single layer of epidermal cells with thickened walls. Mesocarp was differentiated into three zones. The outer zone was formed by up to ten cell layers, and there were regular rows of annular collenchyma cells in the uppermost part. Rounded parenchyma cells surrounding the carpellary bundles were found in the second zone. The third zone comprised approximately 7–8 thin-walled cell layers. The locular walls (e.g. the endocarp) were formed by adaxial, thick-walled epidermal cells (Fig. 10d, f). The fertilized ovules had developed to immature seeds. The nectary disc was still lengthening to encircle the midpoint of the fruit at this stage.

By the second stage of the fruit development (Fig. 11), the fruit had transformed into a subovoid shape with an





**Fig. 8** *Blinkworthia lycioides*, microtome sections of the fourth stage of floral development. **a** LS through the medial gynoeceum, horizontal lines at d, e, and g indicate the levels of TS illustrated in the following figures, scale = 500  $\mu$ m; **b** LS (tangential) through the gynoeceum, note one ovule per locule, scale = 500  $\mu$ m; **c** TS through the distal part of gynoeceum showing two capitate stigma lobes, scale = 500  $\mu$ m; **d** TS through the style, scale = 500  $\mu$ m; **e** TS through the distal part of ovary, scale = 500  $\mu$ m; **f** TS, close-up of figure e, note differentiation of ovary wall into four zones [epider-

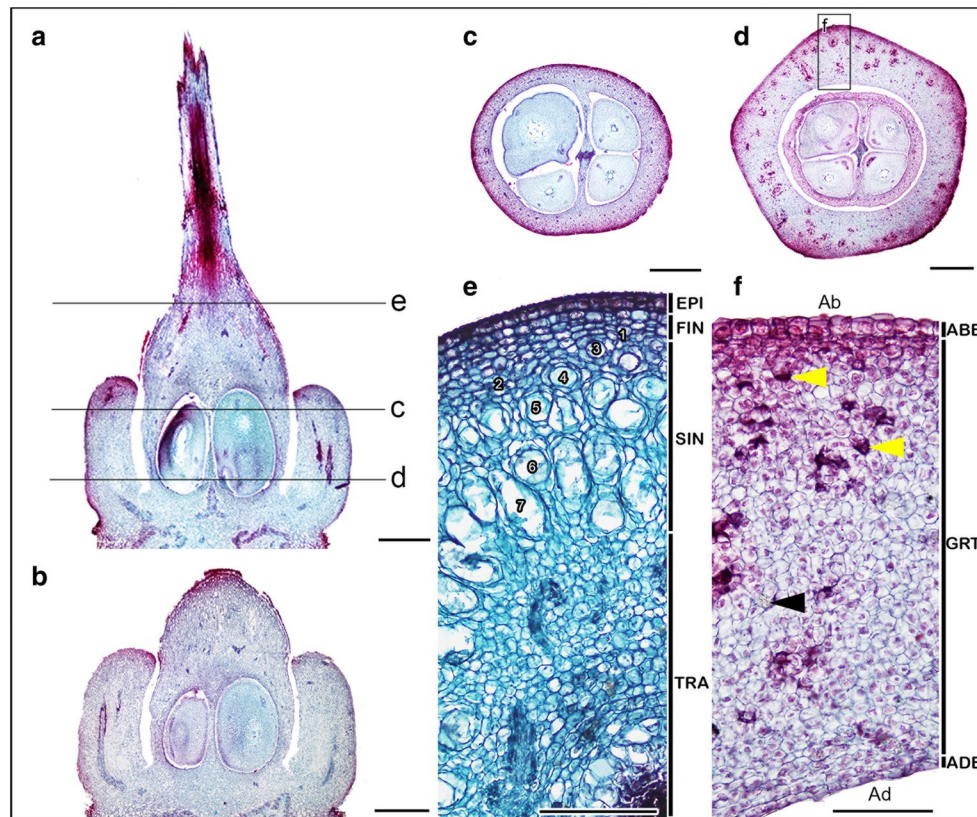
mis (exocarp), first zone of internal tissue (mesocarp), second zone of internal tissue (mesocarp), and transmitting tissue (endocarp) with lysigenous cells (triangle)], scale = 200  $\mu$ m; **g** TS through the centre part of ovary, note complete encirclement of ovary by the nectary disc, four ovules in two locules separated by a single septum, scale = 500  $\mu$ m. (voucher: *P. Rattanakrajang* and *P. Traiperm* 6). *EPI* epidermis, *FIN* first zone of internal tissue, *LS* longitudinal section, *Sd* secretory duct, *SIN* second zone of internal tissue, *TRA* transmitting tissue, *TS* transverse section

apical scar where the style dropped off. The distal part of the fruit had enlarged and was now larger than the centre part (Fig. 11a, b). The druse crystals were found in lysigenous intercellular spaces of the transmitting tissue (Fig. 11c, e). The pericarp resembled the previous stage (Fig. 11d, f). The sizes of the fertilized ovules and fruit had increased prominently. On the other hand, the nectary disc had started to senesce in this stage (Fig. 11a, b).

Fruit shape had changed to be larger and ovoid with the remnant of the style in the third stage of development (Fig. 12). Many large secretory ducts were found in the most distal part of the fruit (Fig. 12a–c). The longitudinal section presented the contraction of the nectary disc tissue (Fig. 12a, b, d). The pericarp showed some differences in comparison with the preceding stage. The exocarp remained similar to the previous stage. The mesocarp, however, revealed a new (fourth) zone: the first zone was reduced to 1–2 cell layers. The second zone consisted of secretory duct tissues. The

third zone included carpellary bundles, while the newly differentiated fourth zone comprised thin-walled parenchyma cells. The endocarp was composed of lignified and thick-walled epidermal cells (Fig. 12e, f). The nectary disc continued to decrease in size, and the shape of the upper disc became sharper and narrower than in the previous stages (Fig. 12b, d). The abaxial epidermis showed some disintegrated cells as well as the ground tissue layer presented more intercellular spaces from the lysis of parenchyma cells. In contrast, the adaxial epidermal cells still retained thickened cell walls (Fig. 12g, h).

In the fourth stage of development, the fruit was approximately 0.8–1.1 cm high and had attained its final form and structure. The calyx was reflexing away from the fruit in this stage (Fig. 13a, c). The nectary disc had become a thin sheath or collar and persisted at the base of fruit (Fig. 13b, d). The appearance of the pericarp had changed during fruit maturation: the colouration of the fleshy fruit had changed



**Fig. 9** *Blinkworthia lycioides*, microtome sections of the fifth stage of floral development, after anthesis. **a** LS through the medial gynoeceum, horizontal lines at c, d, and e indicate the levels of TS illustrated in the following figures, scale = 500  $\mu$ m; **b** LS (tangential) through the gynoeceum, note one ovule per locule, scale = 500  $\mu$ m; **c** TS through the distal part of ovary with septum, note only one ovule is developing, scale = 500  $\mu$ m; **d** TS through the centre of ovary showing complete encirclement of ovary by the nectary disc, scale = 500  $\mu$ m; **e** TS through the distal region of the ovary, note the successive phases in differentiation of secretory ducts (numbered 1–7

showing developmental phases of the secretory ducts from the initial phase to the lysigenous phase), scale = 200  $\mu$ m; **f** TS, close-up of figure **d**, note differentiation of nectary disc into two zones with crystalline aggregations (black triangle) and intercellular spaces (yellow triangles), scale = 100  $\mu$ m. (voucher: P. Rattanakrajang and P. Traiperm 6). Ab abaxial side, ABE abaxial epidermis, Ad adaxial side, ADE adaxial epidermis, EPI epidermis, FIN first zone of internal tissue, LS longitudinal section, SIN second zone of internal tissue, TRA transmitting tissue, TS transverse section

from light greenish to brownish in the dry, mature fruit (Figs. 1b stage 4, 13e, f).

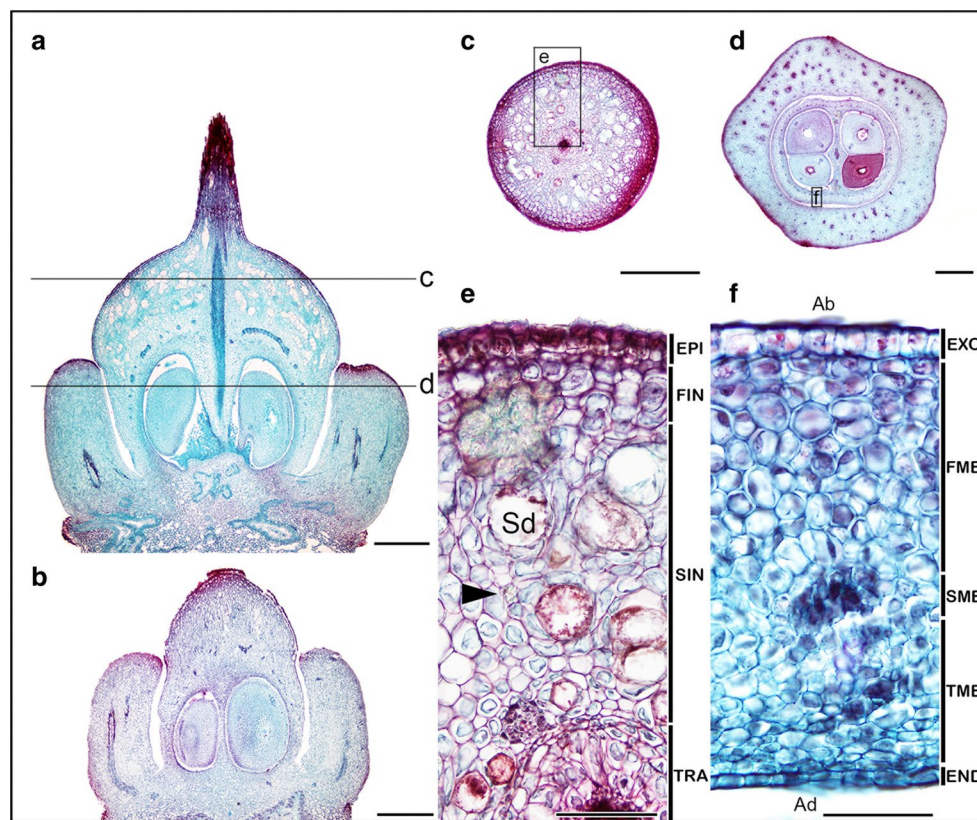
## Discussion and conclusions

Since the first publication of the protologue (Choisy 1834), the genus *Blinkworthia* has been morphologically distinguished from the closely related genus *Argyreia* by the involucre of bracts, the corolla shape, and the shrub habit. Moreover, the feature of three bracts was used to support this generic circumscription (Choisy 1845; Hallier 1893). Later, *B. convolvuloides* was included as the second member of the genus with five different specific characters: the climber habit; few branches; the flowers longer than the leaves; the ovate-oblong bracts; and the distinct pedicels (Prain 1894). Therefore, the shrub habit is no longer useful

as a generic character, and the position of the bracts varies with the species. Our observations confirm that the climber habit is found sometimes in *B. lycioides*. And they also show that *B. convolvuloides* can be an erect shrub in some cases.

The present study revealed that the bracts of *B. lycioides* and *B. convolvuloides* were always arranged in a cruciate pattern and usually 2–4 in number, which corresponded to the observations by Collett and Hemsley (1890), Gagnepain and Courchet (1915), and Prain (1894). However, this result did not agree with the concept that there are always three bracts as stated by Choisy (1845), and Hallier (1893); probably some bracts were missing from herbarium materials they examined. In addition, the morphological observations of *B. convolvuloides* showed that its bracts were positioned some distance below the calyx, attaching to the node between the peduncle and the pedicel. Hence, the idea of an involucre of





**Fig. 10** *Blinkworthia lycioides*, microtome sections of the first stage of fruit development. **a** LS (medial) through the young fruit, horizontal lines at **c** and **d** indicate the levels of TS illustrated in the following figures, scale = 500  $\mu$ m; **b** LS (tangential) through the fruit, scale = 500  $\mu$ m; **c** TS through the distal part of fruit, scale = 500  $\mu$ m; **d** TS through the middle part of the fruit, note four fertilized ovules expanded in two locules at upper level of nectary disc, scale = 500  $\mu$ m; **e** TS, close-up of figure **c**, differentiation of the distal part of pericarp into four zones recognized by the cell layers, note

druse crystal in SIN (triangle), scale = 200  $\mu$ m; **f** TS, close-up of figure **d**, note differentiation of pericarp into five zones and the development in each layer, scale = 50  $\mu$ m. (voucher: *P. Rattanakrajang* and *P. Traiperm 14*). *Ab* abaxial side, *Ad* adaxial side, *END* endocarp, *EPI* epidermis, *EXO* exocarp, *FIN* first zone of internal tissue, *FME* first zone of mesocarp, *LS* longitudinal section, *Sd* secretory duct, *SIN* second zone of internal tissue, *SME* second zone of mesocarp, *TRA* transmitting tissue, *TME* third zone of mesocarp, *TS* transverse section

bracts immediately subtending the calyx also proved unsatisfactory as a generic character.

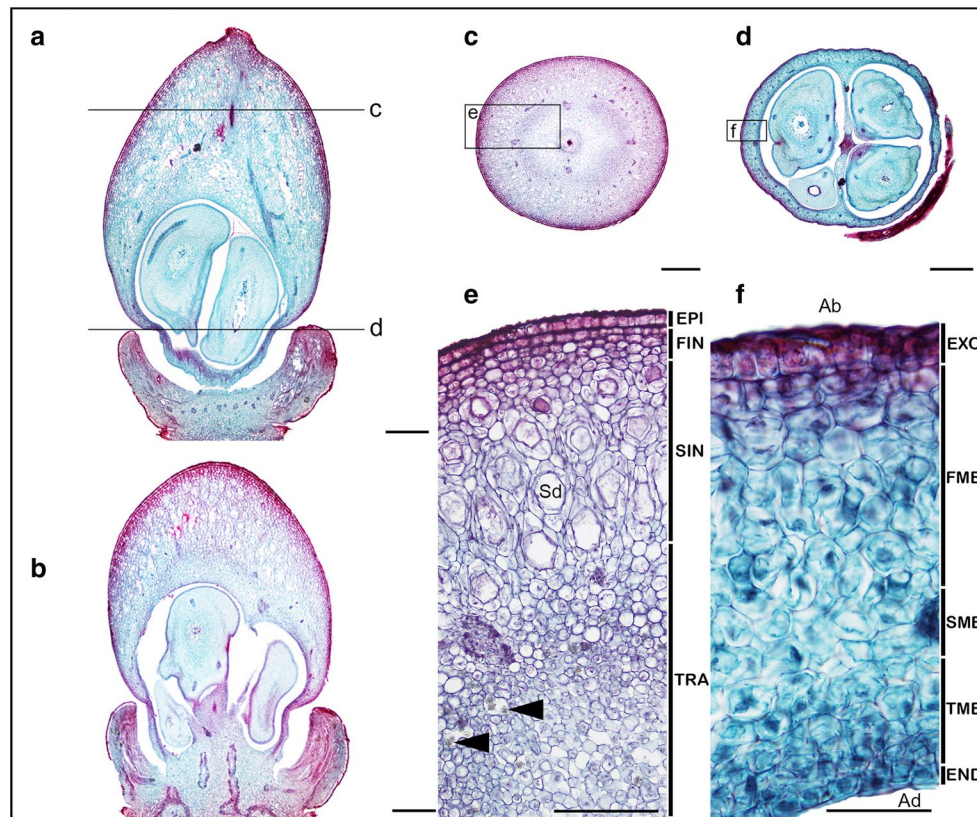
The urceolate or tubular corolla shape was found in both species of *Blinkworthia* (Choisy 1834; Prain 1894), but this character has been shown to occur in several *Argyreia* species, for example, *A. collinsae* (Craib) Na Songkhla and Traiperm (Khunwasi et al. 2005) and *A. leucantha* Traiperm and Staples (Staples and Traiperm 2008).

Our present investigation showed that the flower of *B. lycioides* has a two-celled syncarpous gynoecium. Its ovary contained two locules separated from each other by a complete septum. The locules occupied less than half of the ovary height with solid tissues above that. Our observations corresponded with those in a previous study on two Indomalaysian and four Madagascan *Argyreia* species (Deroin 1993). This two-celled ovary character coincided with the results reported by Collett and Hemsley (1890). It contradicted the study by Wilkin (1999), which described

four locules in the ovary separated by thick septa, and placed *Argyreia*, *Rivea*, and *Blinkworthia* into the same clade. However, Fig. 3 in Wilkin's treatment (Wilkin 1999: 862) shows that *B. lycioides* has equivocal data in terms of ovary locule number.

An ovary included in the cylindrical nectary disc was described for *B. lycioides* by Collett and Hemsley (1890). Fahn (1952, 1953) called this the ovarian type or discoid type nectary because it surrounded the base of the ovary. The architecture and location of the nectary disc in *B. lycioides* were similar to some species of *Ipomoea* L. (Deroin 1999; Galetto and Bernardello 2004), *Stictocardia* Hallier f. and *Turbina* Raf. (Deroin 1999), which is a conventional feature in this family (Cronquist 1981). In this study, we investigated the anatomical changes that occurred during the development of the nectary disc. Interestingly, the nectary disc was circular and just attached at the base of fruit. This result showed that the pericarp was





**Fig. 11** *Blinkworthia lycioides*, microtome sections of the second stage of fruit development. **a** LS through the medial portion of a fruit, horizontal lines at c and d indicate the levels of TS illustrated in the following figures, scale = 500  $\mu$ m; **b** LS through the quarter part of fruit, scale = 500  $\mu$ m; **c** TS through the distal part of fruit, scale = 500  $\mu$ m; **d** TS through the pericarp of lower part of fruit, note fertilized ovules expanded in locules, portion of nectary disc at lower right quadrant, scale = 500  $\mu$ m; **e** TS, close-up of figure c, note differentiation of the distal part of pericarp into four zones and the devel-

opment in each layer showing druse crystals inside the intercellular spaces (triangles), scale = 200  $\mu$ m; **f** TS, close-up of figure d, note differentiation of pericarp into five zones and the development in each layer, scale = 50  $\mu$ m. (voucher: P. Rattanakrajang and P. Traiperm 14). Ab abaxial side, Ad adaxial side, END endocarp, EPI epidermis, EXO exocarp, FIN first zone of internal tissue, FME first zone of mesocarp, LS longitudinal section, Sd secretory duct, SIN second zone of internal tissue, SME second zone of mesocarp, TRA transmitting tissue TME third zone of mesocarp, TS transverse section

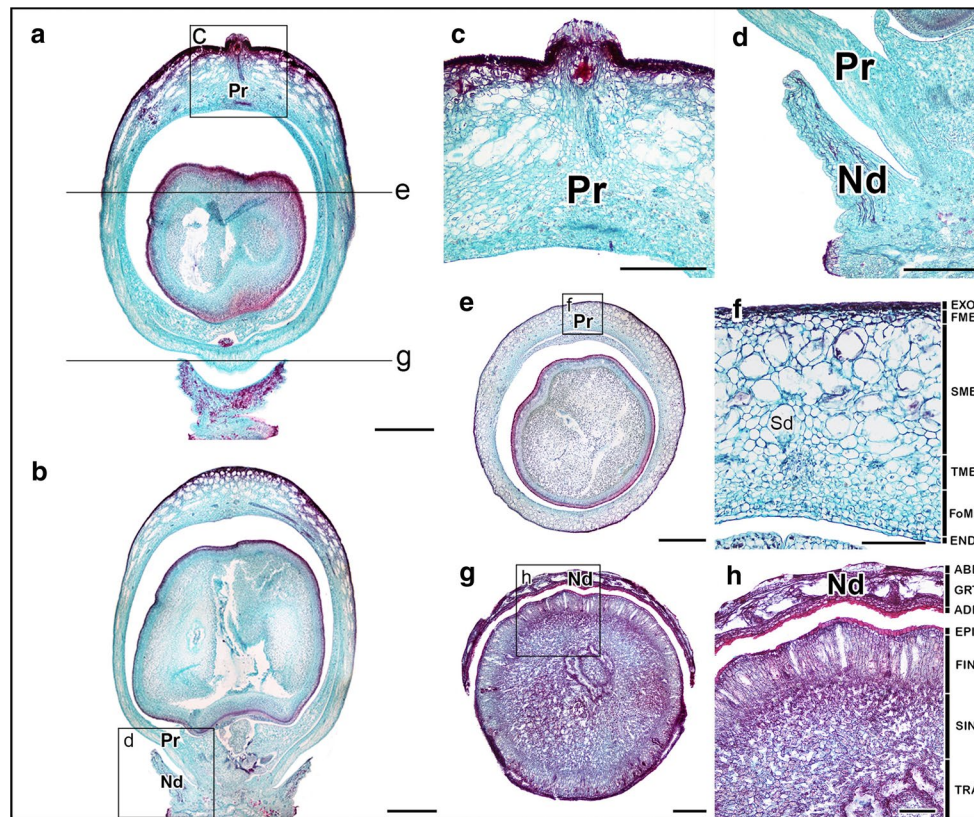
not engulfed by the nectary disc, which was completely at odds with what Staples (2010) described.

Fruit type and dehiscence mechanism have been consistently used characters applied to generic delimitation and to the tribal classification of the Convolvulaceae (Hallier 1893; Stefanović et al. 2003). Previous descriptions stated that the fruit of *Blinkworthia* was indehiscent, berry-like (baccate), and covered by a leathery pericarp, which corresponded with observations made in this study. We investigated the anatomical changes in each ovary layer for all developmental stages from flower bud to mature fruit. Fruits of *B. lycioides* were covered by a pericarp which consisted of three anatomically distinct layers: exocarp, mesocarp, and endocarp, following Fahn's (1990) terminology. The epidermis of the ovary transformed into the fruit exocarp. The internal ovary wall tissue became the fruit mesocarp. And the locule wall turned into the endocarp. Choisy (1834, 1845) described the fruit as one-seeded and baccate (berry-like) and used this as

a generic character. Hallier (1893) repeated this assertion. However, Collett and Hemsley (1890) had already pointed out that the fruit was certainly baccate, but was sometimes four-seeded. Later authors (Prain 1894; Fang and Staples 1995; Staples 2010) agreed with this and our study concurred: *Blinkworthia* fruits may have 1–4 seeds.

### Generic delimitation in *Blinkworthia*

These studies of floral and fruit development demonstrated that the characters used historically to define *Blinkworthia* as a genus distinct from *Argyreia* were not an adequate justification for maintaining two genera (Table 2). Although previous authors (Hallier 1893; Austin 1998; Wilkin 1999) maintained the status quo (two genera), the present study contributed more detailed analysis concerning the morphology and anatomy of flower and fruit and established a



**Fig. 12** *Blinkworthia lycioides*, microtome sections of the third stage of fruit development. **a** LS through the medial portion of a fruit, horizontal lines at e and g indicate the levels of TS illustrated in the following figures, scale = 500  $\mu$ m; **b** LS through the quarter part of fruit, scale = 500  $\mu$ m; **c** close-up of figure a, note remnant of style and the distal part of pericarp, scale = 500  $\mu$ m; **d** LS through the proximal part of fruit, note nectary disc, scale = 500  $\mu$ m; **e** TS through the middle region of fruit, at level of distal portion of seed, scale = 500  $\mu$ m; **f** close-up of figure e, note pericarp, showing component cell layers, scale = 500  $\mu$ m; **g** TS through the proximal

part of fruit, note partial nectary disc around upper hemisphere, scale = 200  $\mu$ m; **h** close-up of figure g, note pericarp and nectary disc, scale = 50  $\mu$ m. (voucher: *P. Rattanakrajang* and *P. Traiperm 14*). ABE abaxial epidermis, ADE adaxial epidermis, END endocarp, EPI epidermis, EXO exocarp, FIN first zone of internal tissue, FoME fourth zone of mesocarp, FME first zone of mesocarp, GRT ground tissue, LS longitudinal section, Nd nectary disc, Pr pericarp, Sd secretory duct, SIN second zone of internal tissue, SME second zone of mesocarp, TRA transmitting tissue, TME third zone of mesocarp, TS transverse section

fundamental baseline for making careful comparisons that previously was lacking.

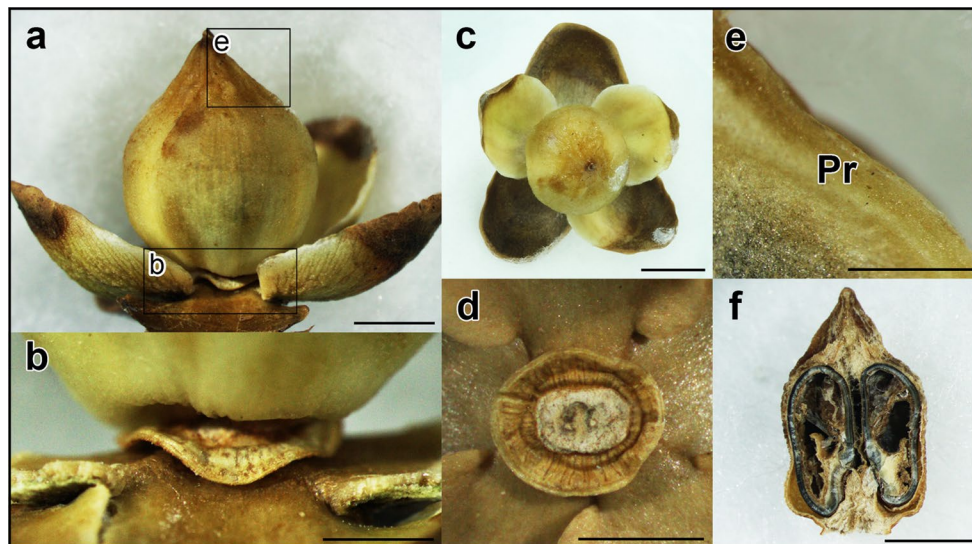
*Blinkworthia* was first circumscribed as a genus based on a combination of these characters: shrubby habit; the presence of an involucre of bracts; and distinctive corolla morphology (Choisy 1834). The genus was later interpreted to be closely related to *Argyreia* Lour., *Lettsomia* Roxb., and *Rivea* Choisy, but could be separated from all three by the non-twining habit with solitary flowers, ovary enclosed by the nectary disc, and a two-celled ovary that gave rise to an indehiscent fruit with up to four seeds (Collett and Hemsley 1890).

All the characters used previously to define the genus were investigated in this study. The first character of erect, shrubby habit has also been found in some *Argyreia*, for instance *A. wallichii* Choisy (Staples et al. 2015), and *A. cuneata* (Willd.) Ker Gawl. (Clarke 1883). Moreover, this

study revealed that twining habit occurred in *Blinkworthia*: *B. convolvuloides* was usually a twiner, sometimes erect, whereas *B. lycioides* twined when conditions are suitable, i.e. lack of light or drought stress (Fig. 2b, d, f). Thus, plant habit appeared to be a plastic character that changed dependent on environmental conditions and was not a reliable generic character. The flower was reported to be solitary (Fig. 3a). This character was found not only in *Blinkworthia*, but it also appeared in some species of related genera in the tribe Ipomoeae, such as in *Argyreia siamensis* (Craib) Staples, which commonly has a solitary flower with four cruciate bracts on the pedicel (Staples 2010). Further confounding the matter, two flowers per axil were sometimes found in *Blinkworthia* (Fig. 3h).

The inclusion of the ovary inside the nectary disc was described by Collett and Hemsley (1890). Staples (2010) wrongly interpreted this and assumed the fruit was engulfed





**Fig. 13** *Blinkworthia lycioides*, the fourth stage of fruit development in enlarged macroscopic views from stereomicroscopy. **a** Lateral view of whole dried fruit, calyx partly reflexed, nectary disc visible at base of fruit, scale = 2.5 mm; **b** close-up of figure **a**, note nectary disc reduced to undulate collar around fruit base, scale = 2.5 mm; **c**

top view of whole fruit and calyx, scale = 5 mm; **d** top view of nectary disc after fruit has been removed, scale = 2.5 mm; **e** LS through distal part of fruit pericarp, scale = 1.0 mm; **f** LS through medial dry fruit, note colour of fruit, scale = 2.5 mm. (voucher: *P. Rattanakrajang* and *P. Traiperm 14*). *LS* longitudinal section, *Pr* pericarp

**Table 2** Comparison of morphological characters between *Blinkworthia* and the closely related genus, *Argyreia*

Character	<i>Blinkworthia</i> Choisy	<i>Argyreia</i> Lour.
Habit	Erect shrub or climber	Climber or prostrate (rarely erect shrub)
Inflorescence type	Flower solitary (rarely paired)	Cymose, panicle or capitate (rarely solitary)
Bract number	4 (rarely 5)	2–3 (4 in <i>A. siamensis</i> )
Bract position	Involucre or below calyx	Below calyx, at junction of peduncle/pedicel
Corolla shape	Urceolate or tubular	Funnelform, campanulate, tubular or urceolate (rare)
Flower orientation	Pendulous	Erect, ascending or pendulous
Nectary disc	Partly enclosing ovary	Annular, pentagonal or partly enclosing base of ovary
Locules per ovary	2 locules	2 or 4 locules
Ovule number per locule	2 ovules	1 or 2 ovules
Fruit type	Berry, indehiscent	Berry, indehiscent
Seeds number per fruit	4 or fewer	4 or fewer, or only 1

and covered by the persistent nectary disc. However, the results from study of flower/fruit development in this investigation showed that the fruit in *B. lycioides* was not enclosed by the nectary disc. It only surrounded the ovary base and stopped enlarging so that it does not enclose the entire fruit during fruit development. Furthermore, the last stage of fruit development revealed that the fruiting calyx was reflexed from the ripe berry (Figs. 3e, 13a, c). On the contrary, Choisy (1834) believed that the fruits were covered by the leathery calyx.

This study has shown that none of these characters can be used absolutely to define the genus *Blinkworthia*, because some of the same character states occurred in species assigned to other genera. Simultaneously, our

knowledge of the purported sister genus, *Argyreia*, particularly the full diversity of character states in this species-rich genus, has provided fresh context for evaluating generic delimitation questions and culminated in the first comprehensive enumeration of the *Argyreia* species (Staples and Traiperm 2017). Our study concluded that the generic definition for *Blinkworthia* was weak. No taxonomic changes were proposed here, pending more detailed study of the second species assigned to the genus using flower and fruit samples recently collected, as well as comparable studies of floral and fruit ontogenesis in species of *Argyreia*. More information from other techniques, such as molecular genetics of the genera in the tribe Ipomoeae



(Sumanon et al. in prep), is needed before a firm decision can be taken regarding the generic circumscription.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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