Taiwania 68(3): 359–367, **2023**
DOI: 10.6165/tai.2023.68.359

Development of sclerotic and winged seeds of *Cyrtosia septentrionalis* (Rchb. f.) Garay and *Erythrorchis altissima* (Blume) Blume (Orchidaceae)

Goro NISHIMURA[1,*], Koichi KABURAGI[2], Yung I LEE[3], Tomohisa YUKAWA[4]

1. *8-1 Shimizucho, Gyoda,, Saitama 361-0047, Japan.* ***2.*** *Former Technical Official, Tanegashima Medical Plants Experiment Station. Current address: 4167-2 Noma, Nakatanecho, Kumagegun, Kagoshima 891-3604, Japan.* ***3.*** *Department of Life Science, National Taiwan University. No. 1, Sec. 4, Roosevelt Rd., 10617 Taipei, Taiwan.* ***4.*** *National Museum of Nature and Science. Amakubo 4-1-1, Tsukuba, Ibaraki 305-005, Japan. [*]Corresponding author's email: DQL10721@nifty.ne.jp*

(Manuscript received 26 March 2023; Accepted 14 August 2023; Online published 4 September 2023)

ABSTRACT: Although most orchid species produce minute, light seeds with a one-layer thin seed coat, some in the subfamily Vanilloideae show seed coat with sclerotization and wing development. We investigated the processes of their development by analyzing the anatomy of ovules of two species in the Vanilloideae, *Cyrtosia septentrionalis* (Rchb. f.) Garay and *Erythrorchis altissima* (Blume) Blume, that produce seeds with, respectively, rudimentary and well-developed wings. In both species, lignified dark material accumulated in the cells of the outermost layer of the outer integument, forming a sclerotic seed coat. Accumulation started from the cell walls of the outer periclinal surface. *C. septentrionalis* formed a large embryo and a rudimentary wing and *E. altissima* formed a small embryo and a well-developed wing. Fully developed seeds of *C. septentrionalis* and *E. altissima* were dispersed by animal and wing, respectively. In *C. septentrionalis*, vascular-bundle-like cells extended from the tip of the funiculus to the chalaza. In *E. altissima*, they branched into two parts: one reached the chalaza and the other reached a group of cells adjacent to the embryo sac in the micropylar region. The group of cells structurally and functionally resembled hypostase, although hypostase is reported to be present in the chalazal region. A helical vessel was found in one ovule in *E. altissima.* These observations indicate that the specialized cells represent degenerated vascular bundle cells.

KEY WORDS: Anatomy, helical vessel, hypostase, Orchidaceae, sclerotic seed coat, vascular-bundle-like cells, winged seed.

INTRODUCTION

Although most orchid species produce minute, light seeds with a one-layer thin seed coat and an undifferentiated embryo, some in the subfamilies Apostasioideae, Vanilloideae, Cypripedioideae, and Epidendroideae produce seeds with a sclerotic seed coat. Among them, some in the Vanilloideae exhibit further specialization involving seed coat wing development.

The sclerotic seed coat has been observed in the genera *Apostasia* (Nishimura and Tamura, 1993) and *Neuwiedia* (Garay, 1960) in the subfamily Apostasioideae; *Cyrtosia* (Cameron and Chase, 1998, Yang and Lee, 2014), *Epistephium* (Barthlott and Zieglar, 1981; Cameron and Chase, 1998; Barthlott *et al*., 2014), *Erythrorchis* (Cameron and Chase, 1998, Barthlott *et al*., 2014), *Galeola* (Beer, 1863; Garay, 1960; Barthlott and Zieglar, 1981; Cameron and Chase, 1998), *Pseudovanilla* (Cameron and Chase, 1998), and *Vanilla* (Beer, 1863; Swamy, 1947; Garay, 1960; Kimura, 1971; Cameron and Chase, 1998; Nishimura and Yukawa, 2010; Barthlott *et al*., 2014; Alomia *et al*., 2016) in the subfamily Vanilloideae; *Selenipedium* (Garay, 1960; Barthlott *et al*., 2014) in the subfamily Cypripedioideae; and *Palmorchis* (Cameron and Chase, 1998), *Pogoniopsis* (Alves *et al*., 2019), which once classified among the Vanilloideae (Pansarin, 2016), in the subfamily Epidendroideae. The wing of the seed coat has been observed in the genera *Clematepistephium* (Cameron and Chase, 1998), *Cyrtosia* (Kimura, 1971; Cameron and Chase, 1998), *Epistephium* (Cameron and Chase, 1998), *Erythrorchis* (Cameron and Chase, 1998), *Galeola* (Beer, 1863; Garay, 1960; Cameron and Chase, 1998), and *Pseudovanilla* (Cameron and Chase, 1998) in the subfamily Vanilloideae. Both are present in the genera *Cyrtosia*, *Epistephium*, *Erythrorchis*, *Galeola*, and *Pseudovanilla* in the subfamily Vanilloideae.

The anatomy of seed coat sclerotization has been studied in *Apostasia nipponica* (Nishimura and Tamura, 1993), *Vanilla planifolia* (Nishimura and Yukawa, 2010), and *Cyrtosia taiwanica* (Yang and Lee, 2014; *Cyrtosia javanica* in that study has been renamed as *Cyrtosia taiwanica* in Lee et al., 2019). In *A. nipponica*, dark material starts to accumulate in the inner periclinal cell wall of the second cell layer of the outer integument. In *V. planifolia* and *C. javanica*, it starts to accumulate in the outer periclinal cell wall of the outermost cell layer of the outer integument. The anatomy of seed coat wings was observed in *Cyrtosia septentrionalis*, with the presentation of one sectional view of the developed wing, but the developmental process is not shown (Kimura, 1971). Anatomical analyses of seeds with both features have not yet been performed. Therefore, we investigated the processes of seed coat sclerotization and wing development by anatomical investigation of developing ovules of *C. septentrionalis* and *Erythrorchis altissima*, which produce, respectively, seeds with rudimentary and well-developed wings.

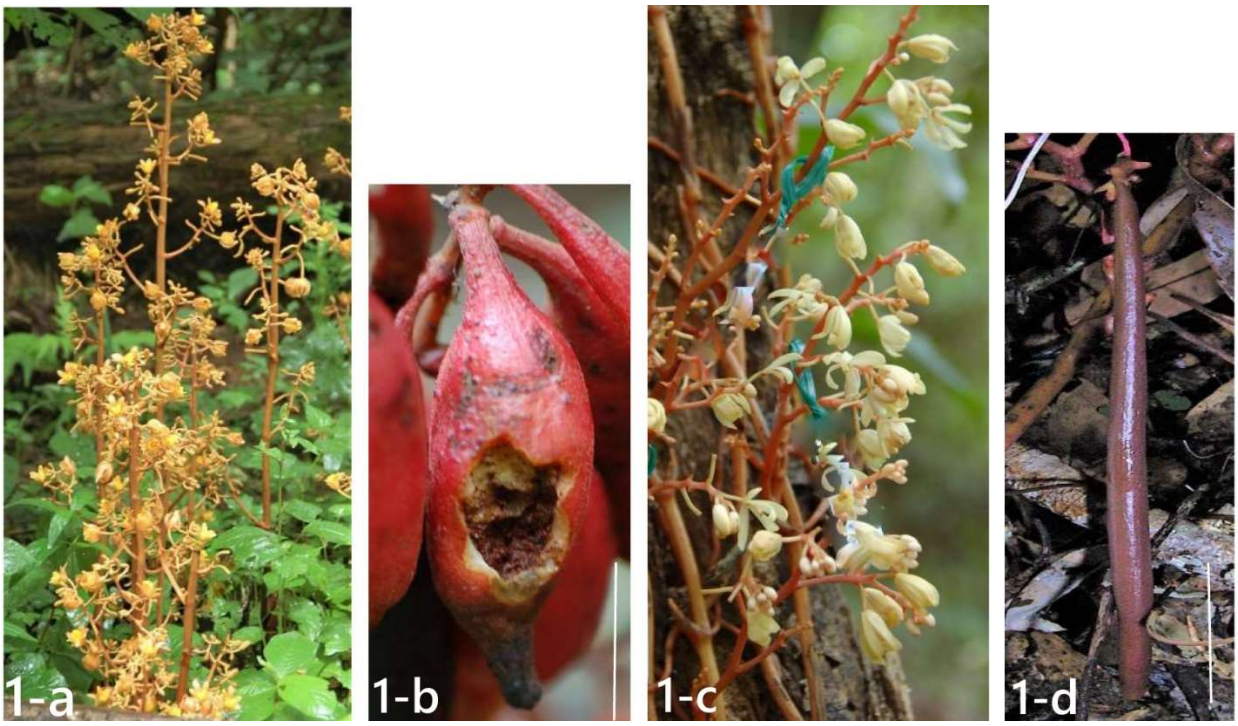

Fig. 1. Flowers and fruits of ***Cyrtosia septentrionalis*** and ***Erythrorchis altissima***. **a.** Flowers of ***C. septentrionalis***. **b.** Berry eaten by animals at 119 days after pollination (DAP). **c.** Flowers of *E. altissima*. **d.** Capsule at 119 DAP. Bars in **b** and **d**, 3 cm.

MATERIALS AND METHODS

Plant materials

Cyrtosia septentrionalis and Erythrorchis altissima are closely related mycoheterotrophic species occurring in forest. Both belong to the subfamily Vanilloideae but differ greatly in stem and fruit morphologies: *C. septentrionalis* has an erect stem and bears berries (Figs. 1a, b), while *E. altissima* has a climbing stem and bears capsules (Figs. 1c, d). Here, *C. septentrionalis* flowered from early to mid-July in 2011 in Minami Takao, Honshu Tokyo, Japan. The first day of flowering was recorded as pollination day based on our observation that flowers started closing on the second day of flowering, and also based on the observation by Suetsugu (2013) that column morphology investigation showed that in *C. septentrionalis,* autonomous self-pollination usually occurs within 1 day of the start of flowering. *E. altissima* flowered in mid-June 2009 and in mid-July 2010 on Tanegashima Island, Ryukyu Islands, Japan. Flowers were pollinated both manually and naturally. Fruits of both species were collected weekly from the days before pollination to 119 days after pollination (DAP). The ways of seed dispersal were comparatively observed in the two species.

Histological observation

Collected fruits were fixed in FAA solution containing 50% ethanol and dehydrated through a butanol series. To observe the sclerotization of the seed coat, we cut developing seeds longitudinally, and to observe wing development, we cut seeds transversely through the center of the wing. Samples were embedded in paraffin, cut into 6-µm slices on a microtome, and stained with Delafield's haematoxylin or safranin.

Measurements

Seeds were collected from berries and capsules at 119 DAP, We recorded the length and weight of the seeds, and the length, width, and thickness of the sclerotic seed coat, which emvelops the embryo. We also collected seeds from the mature dry berries of *C. septentrionalis* and from the split capsules of *E. altissima* and weighed them. Photographs were taken and lengths were measured under a transmission electron microscope (Olympus Cx23) or a stereoscopic microscope (Micronet Santa 2) and weights were measured with an ultra-micro balance (Sartorius Cubis MSA6.6S-000-DF Micro Balance).

RESULTS

Seed structures in *Cyrtosia septentrionalis* and *Erythrorchis altissima*

The berries of *C. septentrionalis* had feeding signs of animals at 119 DAP (Fig. 1b). The seeds had fully developed on this day. On the other hand, in *E. altissima* the seeds fully developed and detached from the placenta before the capsule split at 119 DAP (Fig.1d). We confirmed that the capsules were split and released the seeds in the air at 147 DAP. Seeds of *C. septentrionalis* have a large embryo and a rudimentary wing (Fig. 2a).

Table 1. Seed length, width, weight, embryo length, width, and thickness of sclerotic seed coat in ***Cyrtosia septentrionalis*** and ***Erythrorchis altissima***.

	Cyrtosia septentrionalis	***Erythrorchis altissima***	***t-test***
seed			
length (mm)	0.85 (±0.015z)	1.65 (±0.031)	5.71E-19 **
width (mm)	0.55 (±0.01)	0.98 (±0.021)	4.91E-17 **
weight (μg)	28.0 (±0.14)	6.6 (±0.67)	4.7E-29 **
embryo within sclerotic seed coat			
length (mm)	0.54 (±0.019)	0.29 (±0.016)	4.8E-11. **
width (mm)	0.36 (±0.014)	0.23 (±0.004)	1.3E-8. **
thickness of the sclerotic seed coat (mm)	0.024 (±0.007)	0.023 (±0.004)	0.16, (p<.05) NS

z means ±SE (n=20); ** p<.001; NS: Not Statistically Significant

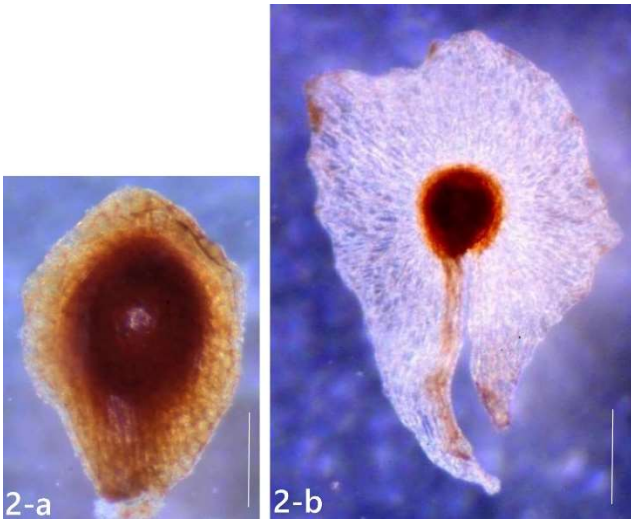

Fig. 2. Fully developed seeds at 119 DAP. **a.** ***Cyrtosia septentrionalis.*** **b.** ***Erythrorchis altissima***. Seeds are shown by same scale. Bars, 300 μm..

Seeds of *E. altissima* have a small embryo and a well-developed wing (Fig. 2b). Morphological measurements are shown in Table 1.

The seed length and width of *E. altissima* were respectively 1.94×(Seed length 1.65mm in *E. altissima* is 1.94 times 0.85mm in *C. septentrionalis*.) and 1.78× those of *C. septentrionalis*; the embryo length and width of *C. septentrionalis* were respectively 1.86× and 1.57× those of *E. altissima*; and the seed weight of *C. septentrionalis* was 4.2× that of *E. altissima*. The thickness of the sclerotic seed coat was almost the same in the two species (Table 1).

Seed coat sclerotization and wing development in *Cyrtosia septentrionalis*

(1) Seed coat sclerotization – The ovule primordium arose from the placenta before pollination (Fig. 3a), and the ovule elongated at the time of pollination (flowering) (Fig. 3b). The inner integument differentiated at 7 DAP (Fig. 3c) and the outer integument differentiated at 14 DAP (Fig. 3d). The inner integument stayed in two cell layers and later became compressed. The elongated outer integument enveloped the inner integument, and the outermost cell layer of the outer integument thickened. Two polar nuclei differentiated at 21 DAP (Fig. 3e). At the same day, embryo, two spherical polar nuclei and antipodal cells were observed in the magnified embryo sac at 21 DAP (Fig. 3f). The outer integument developed into four cell layers. Lignified dark material accumulated in the cells of the outermost layer from the cell walls of the outer periclinal surface. The dark material did not accumulate in the inner three layers at 28 DAP (Fig. 3g). Dark material continued to accumulate, and the embryo grew further. The antipodal cells increased in number at 35 DAP (Fig. 3h) and they degenerated. Dark material fully accumulated in the cells of the outermost layer of the outer integument, forming a sclerotic seed coat at 42 DAP (Fig. 3i). The sclerotic seed coat reached the maximum thickness at 119 DAP. The inner three cell layers of the outer integument became compressed (Fig. 3j). Lignified sclerotic seed coat was stained red with safranin (Fig. 3k).

(2) Wing development and differentiation of vascular-bundle-like cells – The outer integument developed into a wing at 21 DAP. The outermost cell layer did not thicken or accumulate dark material (Fig. 4a). The wing consisted of four or five layers of enlarged cubic or elliptical cells at 42 DAP. Long and narrow vascular-bundle-like cells extended from the tip of the funiculus to the chalaza (Fig. 4b, c), and the cell walls were lignified (Fig. 4d).

Seed coat sclerotization and wing development in *Erythrorchis altissima*

(1) Seed coat sclerotization – The ovule primordium arose from the placenta before pollination (Fig. 5a), and the ovule elongated at the time of pollination (flowering) (Fig. 5b). The inner integument differentiated at 7 DAP (Fig. 5c) and the outer integument differentiated at 14 DAP (Fig. 5d). The inner integument stayed in two cell layers (Fig. 5d) and later became compressed. The elongated outer integument enveloped the inner integument, and the outermost cell layer of the outer integument thickened at 35 DAP (Fig. 5e). At the same day, embryo, two polar nuclei and antipodal cells were

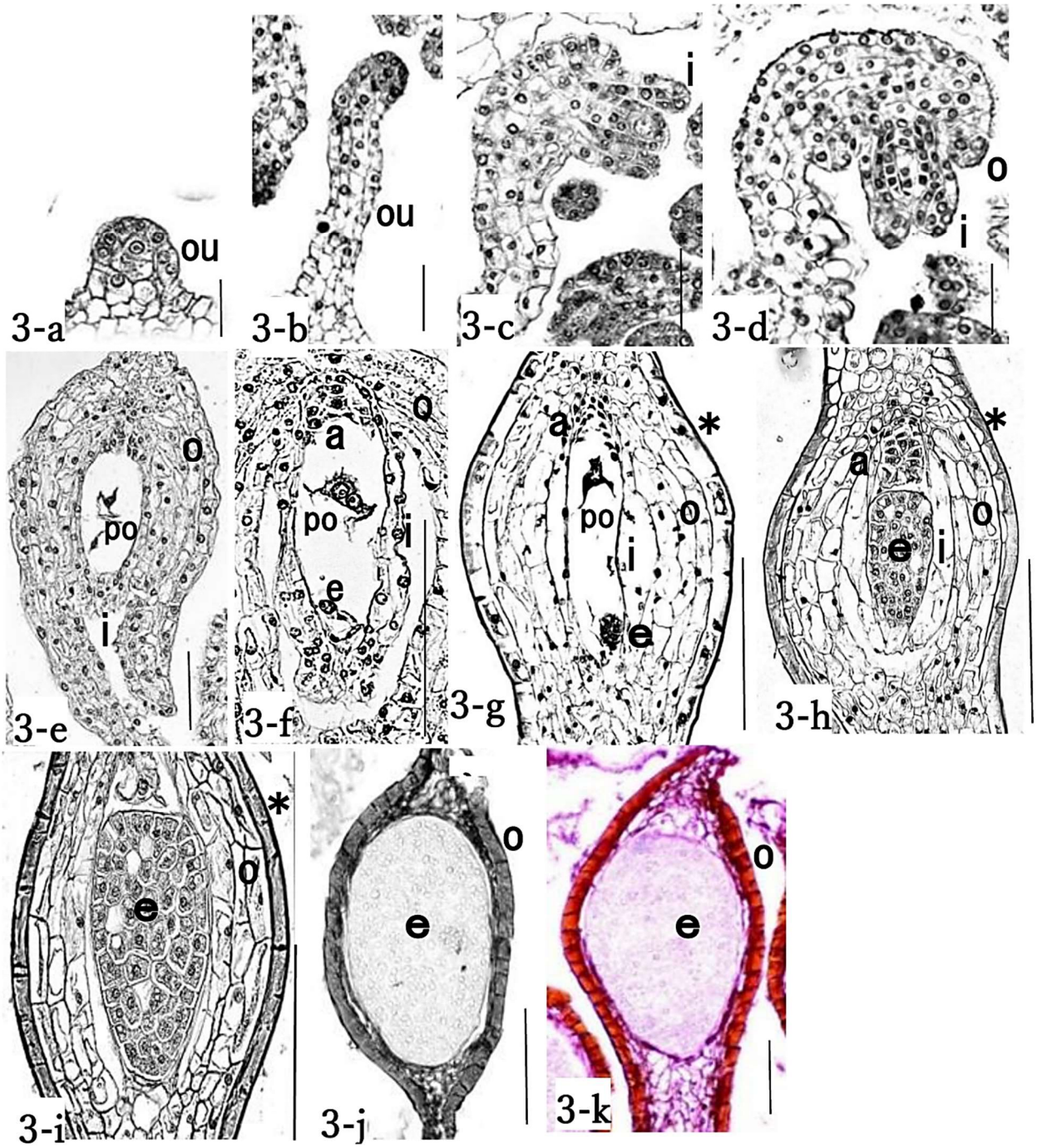

Fig. 3. Ovule development and seed coat sclerotization in ***Cyrtosia septentrionalis***. **a.** Ovule primordium rises from the placenta before pollination (flowering). **b.** Elongated ovule at the time of pollination. **c.** Inner integument differentiation at 7 DAP. **d.** Outer integument differentiation at 14 DAP. **e.** Outermost cell layer of the outer integument thickens at 21 DAP. **f.** Embryo, two spherical polar nuclei and antipodal cells are observed in the embryo sac at 21 DAP. **g.** Dark material starts to accumulate in the cells of the outermost layer from the outer periclinal cell wall at 28 DAP. **h.** Dark material accumulates further and antipodal cells increase in number at 35 DAP. **i.** Dark material fully accumulates in the cells of the outermost cell layer of the outer integument at 42 DAP. **j.** Fully developed seed at 119 DAP. **k.** Lignified sclerotic seed coat is stained red with safranin. **a,** antipodal cells; **e**, embryo; **i**, inner integument; **o**, outer integument; **ou**, ovule; **po**, polar nuclei: ∗, accumulated dark material. Bars, 20 μm in **a**, 50 μm in **b**–**d,** 200 μm in **e**–**k**.

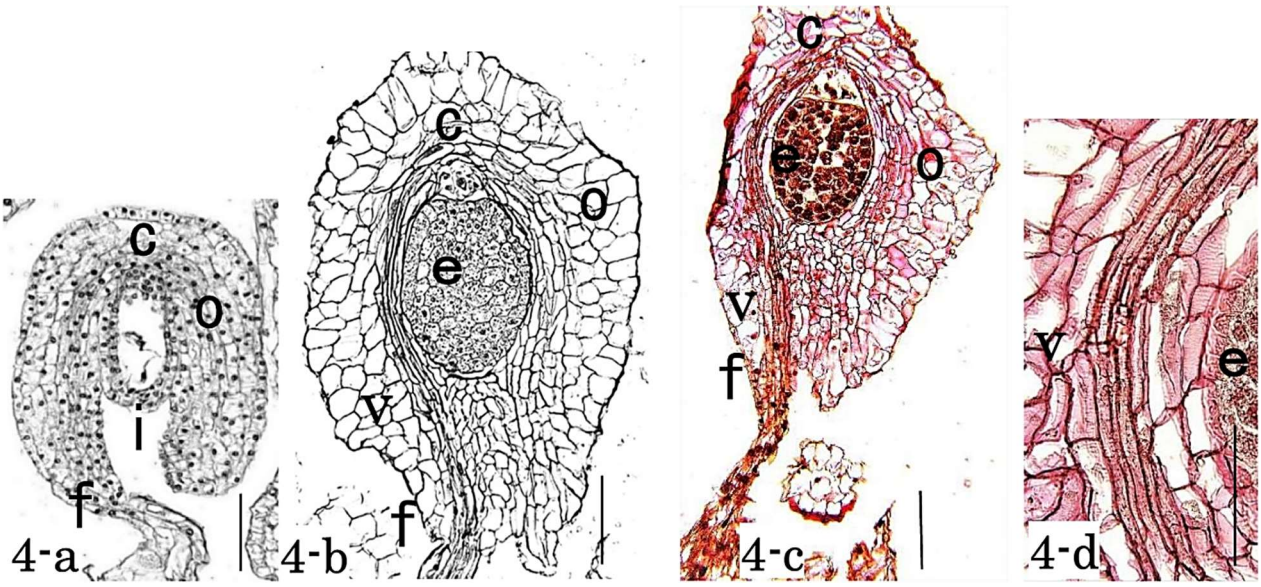

Fig. 4. Wing development and vascular-bundle-like cells differentiation in ***Cyrtosia septentrionalis***. **a.** Outer integument grows to form a wing. Outermost cell layer does not thicken nor accumulate dark material at 21 DAP. **b, c.** Wing consists of 4 or 5 layers of large cubic or ellipsoidal cells. Vascular-bundle-like cells extend from the tip of the funiclus to the chalaza at 56 DAP. **d.** Each vascular-bundle-like cells is long and narrow, and cell walls are lignified at 56 DAP. **c,** chalaza; **e**, embryo; **f**, funiculus; **i**, inner integument; **o,** outer integument (wing); **v,** vascular-bundle-like cells. Bar, 50 μm in **a**, 100 μm in **d**, 200 μm in **b c.**

observed in the magnified embryo sac at 35 DAP (Fig. 5f). The antipodal cells increased in number and they degenerated. The outer integument developed into two or three cell layers. Lignified dark material accumulated in the cells of the outermost layer from the cell walls of the outer periclinal surface. Dark material did not accumulate in the inner layers at 42 PFD (Fig. 5g). A group of cells with thick cell walls having several periclinal ridges on them differentiated adjacent to the embryo sac in the micropylar region and developed into a hypostase-like tissue at 42 DAP (Fig. 5g). The cells of the outer integument formed protrusions from the micropylar and chalazal sides becoming a wing (Fig. 5h). Dark material fully accumulated in the cells of the outermost layer of the outer integument, forming a sclerotic seed coat at 63 DAP (Fig. 5i). The sclerotic seed coat reached maximum thickness and the seeds detached from the placenta at 119 DAP. The inner layers of the outer integument became compressed (Fig. 5j). Lignified sclerotic seed coat was stained red with safranin (Fig. 5k).

(2) Wing development and differentiation of vascular-bundle-like cells – The outer integument developed into a wing. The outermost cell layer did not thicken or accumulate dark material at 21 DAP (Fig. 6a). The wing consisted of four or five layers of enlarged cubic or ellipsoidal cells. There was a distinctive cleft in the funiculus region of the wing at 49 DAP (Fig. 6b). Vascular-bundle-like cells extended from the tip of the funiculus and branched into two: one branch reached the chalaza and the other reached a hypostase-like group of cells adjacent to the embryo sac in the micropylar region. The walls of the group of cells were thick and lignified and had several periclinal ridges at 49 DAP (Figs. 6c–e). A helical vessel differentiated in the funiculus region of the wing (Fig. 6f). In *E. altissima*, vascular bundles of the ovary did not enter into the placenta (Fig. 7a).

DISCUSSION

Cyrtosia septentrionalis berries were eaten by animals and the seeds may have been dispersed with feces as observed in this study, seed coat sclerotization in this species can be explained as an adaptation that allows its seeds to pass the digestive tract without being digested. In contrast, *Erythrorchis altissima* seed is dispersed with wind as we observe in the habitat, and we were unable to identify any adaptive advantage to seed coat sclerotization. The trait may simply have been inherited intact from a common ancestor of the two species. In both species, the seed wing develops by the enlargement of cubic or elliptical cells derived from the outer integument. Wing development in *E. altissima* could be an adaptation that allows windborne dispersal of its sclerotized seeds. The rudimentary seed with wing development in *C. septentrionalis* is presumably vestigial owing to the wing function's having been rendered obsolete by the evolutionary transition to bird-mediated seed dispersal. Although seed coat sclerotization and wing development in *C. septentrionalis* and *E. altissima* are likely the result of single evolutionary events, the observed trait diversity may be due to diversification of seed dispersal mode.

In both *C. septentrionalis* and *E. altissima*, the dark material accumulated from the outer periclinal cell wall of the outermost cell layer of the outer integument, as in

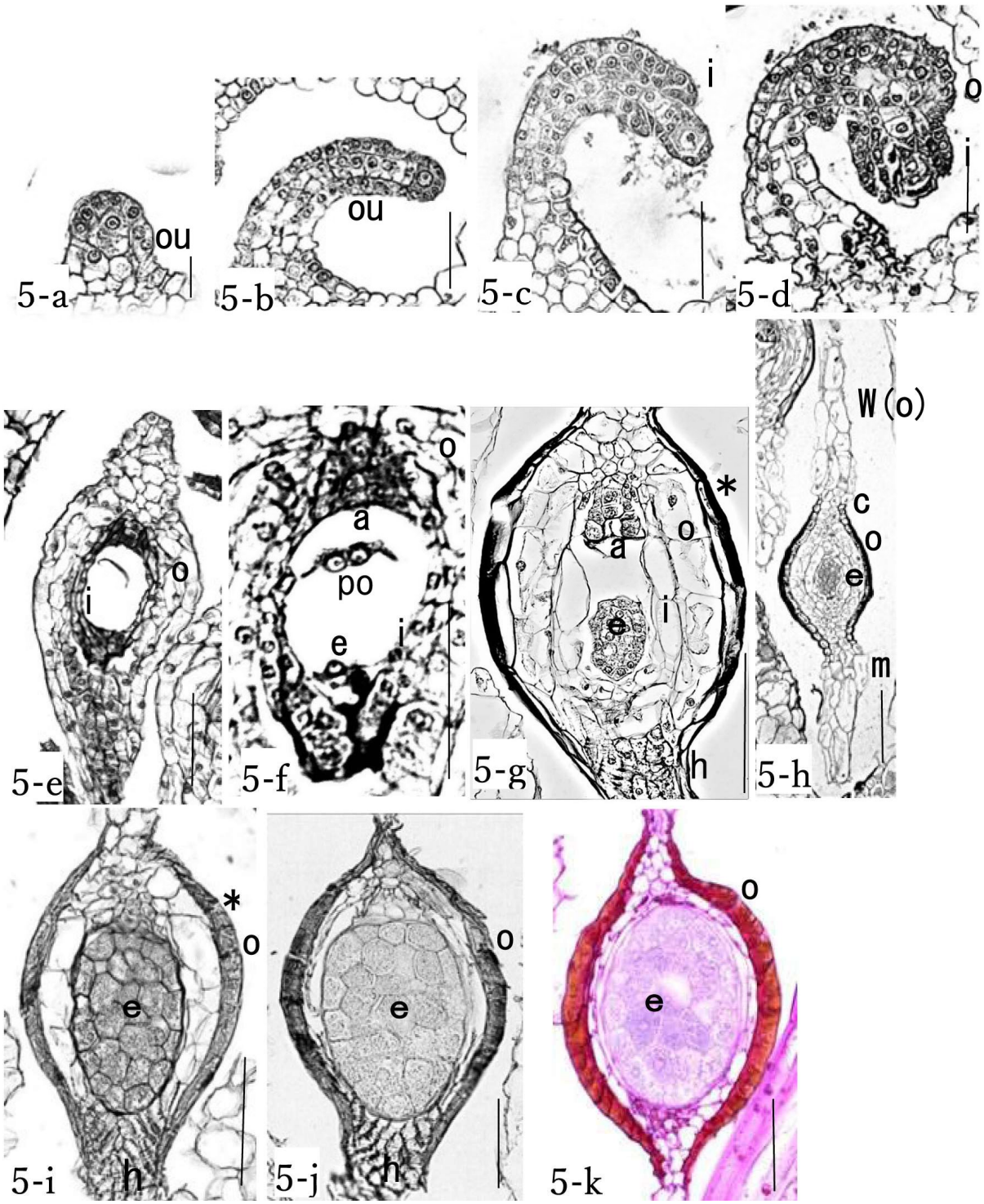

Fig. 5. Ovule development and seed coat sclerotization in ***Erythrorchis altissima***. **a.** Ovule primordium rises from the placenta before pollination (flowering). **b.** Elongated ovule at the time of pollination. **c.** Inner integument differentiation at 7 DAP. **d.** Outer integument differentiation at 14 DAP. **e.** Outermost cell layer of the outer integument thickens at 35 DAP. **f.** Embryo, two spherical polar nuclei and antipodal cells are seen in the embryo sac at 42 DAP. **g.** Dark material accumulates in the cells of the outermost layer from the outer periclinal cell wall. Antipodal cells increase in number. Hypostase-like cells with thick walls differentiate adjacent to the embryo sac in the micropylar region at 42 DAP. **h.** Outer integument cells form protrusions from both micropylar and chalazal sides to form a wing at 42 DAP. **i.** Dark material accumulates fully in the outer most cell layer. Outer integument consists of 2 or 3 cell layers at 63 DAP. **j** Fully developed seed at 119 DAP. **k.** Lignified sclerotic seed coat is stained red with safranin. **a,** antipodal cells; **c,** chalaza**; e,** embryo; **h**, hypostase-like group of cells **i,** inner integument; **m,** micropyle; **o,** outer integument; **ou,** ovule; **po**, polar nuclei; **w**, wing. ∗, accumulated dark material. Bars, 20 μm in **a**, 50 μm in **b**–**d,** 100 μm in **e**–**k**.

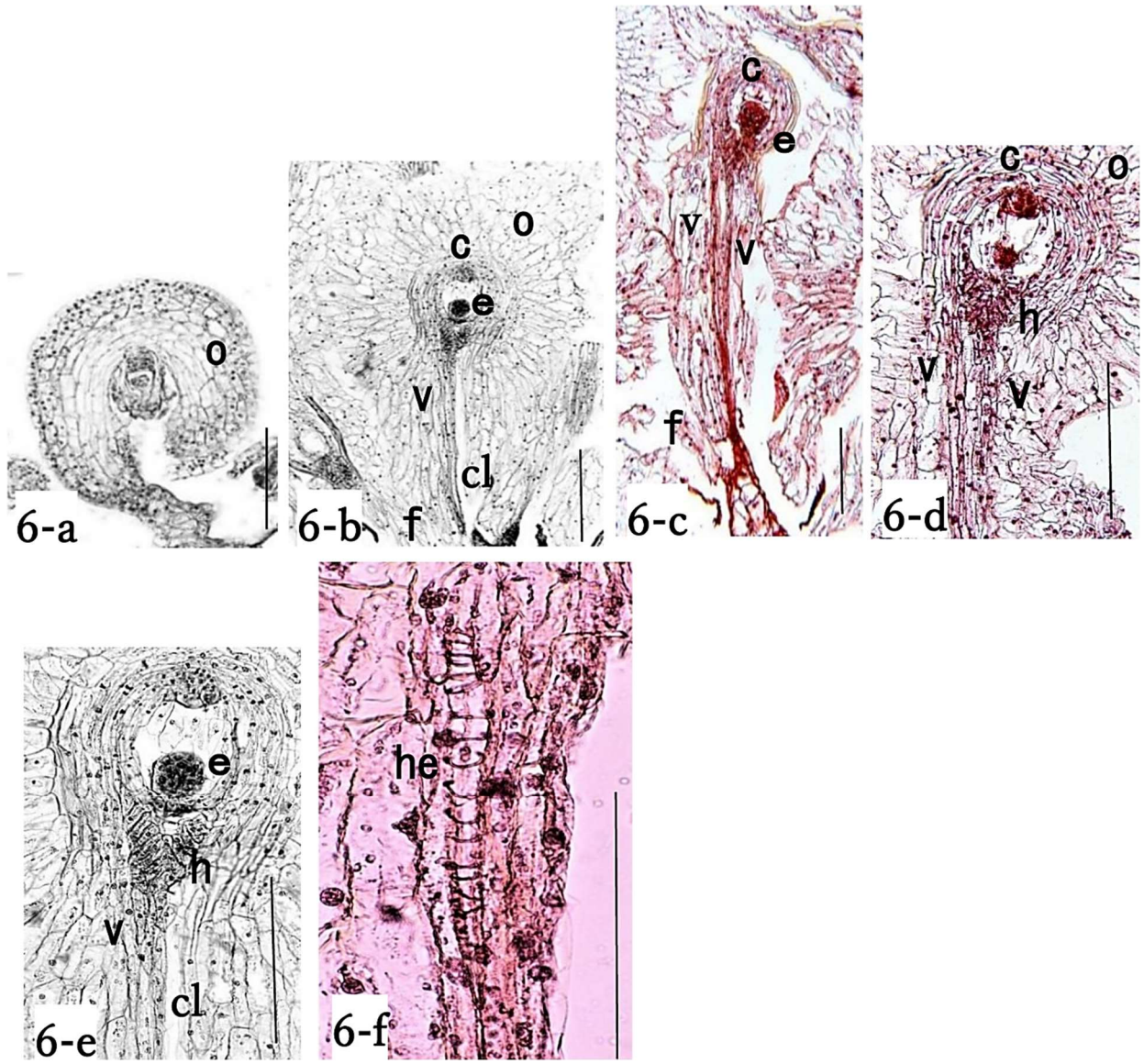

Fig. 6 Wing development and vascular-bundle-like cell differentiation in ***Erythrorchis altissima***. **a.** Outer integument grows to form a wing. Outermost cell layer does not thicken or accumulate dark material at 21 DAP. **b.** Wing consists of 4 or 5 layers of large cubic or ellipsoidal cells. There is a distinctive cleft in the wing in the funiculus region at 56 DAP. **c–e.** Vascular-bundle-like cells branch in two: one reaches the chalaza and the other reaches a hypostase-like group of cells adjacent to the embryo sac on the micropylar side. **c** and **d.** are at 49 DAP. **e.** is at 56 DAP which is magnified view of **b**. **f.** Helical vessel differentiates. **c,** chalaza; **cl**, cleft; **e,** embryo; **f,** funiculus**; h,** hypostase-like group of cells; **he,** helical vessel; **o**, outer integument; **v,** vascular bundle-like cells. Bars, 100 μm in **a,** 200 μm in **b-f.**

Vanilla planifolia (Nishimura and Yukawa, 2010) and *C. taiwanica* (Yang and Lee, 2014). On the other hand, in *Apostasia nipponica* (Apostasioideae), it accumulated from the inner periclinal cell wall of the second cell layer of the outer integument (Nishimura and Tamura, 1993). This is also the case in *Neuwiedia* (Apostasioideae) (Cameron and Chase, 1998). This type of dark material accumulation would be a shared character in the Apostasioideae. This anatomical difference suggests independent evolution of sclerotization in the Apostasioideae and Vanilleae.

Vascular-bundle-like cells extended from the tip of the funiculus to the chalaza in *C. septentrionalis* but branched into two parts in *E. altissima*. A helical vessel was found in *E altissima.* This observation indicate that the specialized cells represent degenerated vascular bundle cells. We have not seen any reports which proof the presence of vascular bundles or their vestiges in orchid seeds and as Withner *et al*. (1974) wrote: 'At no place is there any evidence of traces branching off to supply the placentae', vascular bundles of ovary did not enter into the placenta in *E. altissima (*Fig. 7) and also in *C. septentriomalis.* There is no direct connection between the vasculatures in ovule and ovary.

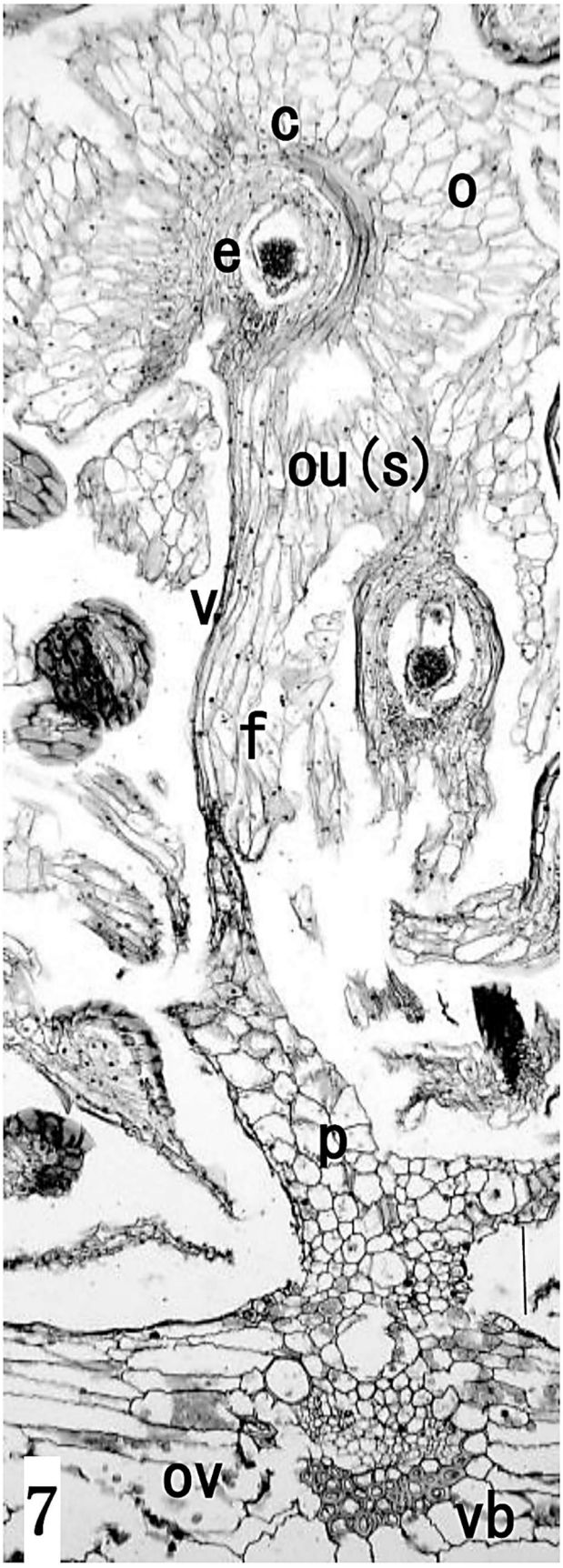

Fig. 7. Section at 49 DAP showing ovary, placenta and ovule in ***Erythrorchis altissima***. The vascular bundles of the ovary do not extend into the placenta. There is no direct connection between the vascular bundles in the ovary and the vascular-bundle-like cells in the ovule. **c,** chalaza**; e,** embryo; **f,** funiculus; **o,** outer integument; **ou**, ovule; **ov**, ovary; **p,** placenta; **s** seed, **v,** vascular-bundle-like cells; **vb,** vascular bundles in ovary. Bar, 200 μm.

As mentioned above, vascular-bundle-like cells branched in two in *E. altissima.* One branch reached the chalaza and the other reached a mass of cells adjacent to the embryo sac on the micropylar side. The cell walls of this cell group are lignified and have several periclinal ridges. The cell group seems to connect the vascular-bundle-like cells and the embryo sac to translocate nutrients from the former to the latter to feed the embryo. These structures and functions of the cell group resemble those of hypostase. Rudall (1997) described the hypostase as a well-defined group of cells immediately adjacent the embryo sac (i.e., subtending the antipodals), normally with thickened, refractive cell walls which are partially suberinized or lignified, found in many monocot taxa, and cited from Maheshwari (1950) and Tilton (1980) that many authors speculated that it is related to the translocation of nutrients to the megagametophyte and embryo. Although the mass of cells in *E. altissima* structurally and functionally resembles the hypostase, it is present on the opposite side of the embryo sac: the group of cells in the micropylar region but the hypostase in the chalazal region. Further work will be necessary to determine the function of the hypostase-like group of cells in the micropylar region of the ovule in *E. altissima*.

ACKNOWLEDGMENTS

We thank Mr. Jiro Arai, Mr. Yuji Sugita and Mr. Norimasa Hashimoto for their advice on the plant habitats, and Dr. Edward C. Yeung for his advice.

LITERATURE CITED

Alomia, Y.A., Munoz, E.A., Acosta-Rangel, M., Otero, J.T. 2016 Morphometric analysis of *Vanilla* seeds (Orchidaceae) by microscopic techniques. Lankesteriana **16(1)**: 21–26.

Alves, M.F., Pinheiro, F., Niedzwiedzki, M.P., Mayer, J.L.S. 2019 First record of ategomic ovules in Orchidaceae offers new insights into mycoheterotrophic plants. Front. Plant Sci. **10**: 1–11.

Barthlott, W., Grosse-Veldmann, B., Korotkova, N. 2014 Orchid Seed Diversity. A scanning electron microscopy survey. Englera 32

Barthlott, W., Ziegler, B. 1981 Mikromorphologie der Samenschalen als systematisches Merkmal bei Orchideen Berichte der Deutschen Botanischen Gesellschaft **94**: 267–273.

Beer, J.G. 1863 Beiträge zur Morphologie und Biologie der Familie der Orchideen. Carl Gerold's Sohn, Vienna.

Cameron, K.M., Chase, M.W. 1998 Seed morphology of Vanilloid orchids (Vanilloideae: Orchidaceae). Lindleyana **13**: 148–169.

Garay, L.A. 1960 On the origin of the Orchidaceae. Botanical Museum Leaflets, Harvard University **19(3)**:57–96.

Kimura, C. 1971 Embryological studies of *Galeora septentrionalis* Reichb. Fil. (Orchidaceae). Sci. Rep. Tohoku Imp. Univ., Ser. 4 Biol. **35**:253–258.

Lee, Y.I, Yu, S.K., Liao, P.C., Wang, K.H., Lin, T.P. 2019. Newly discovered native orchids of Taiwan (XIV). Taiwania **64(4)**: 339–346.

Maheshwari, F. 1950 An introduction to the embryology of Angiosperm. McGraw hill, New York.

Nishimura, G., Tamura, M. 1993 Seed coat formation in *Apostasia nipponica.* J. Jpn. Bot. **68**: 219–223.

Nishimura, G., Yukawa, T. 2010 Dark material accumulation and sclerotization during seed coat formation in *Vanilla planifolia* Jacks. Ex Andrews (Orchidaceae). Bull. Natl. Mus. Nat. Sci., Ser. B. **36**: 33–37.

Pansarin, E.R. 2016 Recent advances on evolution of pollination systems and reproductive biology of Vanilloideae (Orchidaceae). Lankesteriana **16(2)**: 255–267.

Rudall, P.J. 1997 The nucellus and chalaza in monocotyledons: Structure and systematic. Bot. Rev. **63(2)**: 140–181.

Suetsugu, K. 2013 Autogamous fruit set in a mycoheterotrophic orchid *Cyrtosia septentrionalis*. Plant Syst. Evol. **299(3)**: 481–486.

Swamy, B.G. 1947 On the life history of *Vanilla planifolia.* Bot. Gaz. **108(3)**: 449–456.

Tilton, V.R. 1980 Hypostase development in *Ornithogalum caudatum* (Liliaceae) and notes on other types of modifications in the chalaza of angiosperm ovules. Can. J. Bot. **58(19)**: 2059–2066.

Withner, C.L., Nelson, P.K., Wejksnora, P.J. 1974 The anatomy of orchids. In Withner, C.L., The Orchids. Scientific Studies. Wiley. P. 271.

Yang, C.K., Lee, Y.I 2014 The seed development of a mycoheterotrophic orchid, *Cyrtosia javanica* Blume. Bot. Stud. **55**: 44.