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Comparative Seedling Morphology in Three *Cymbidium* Species

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シュンラン属3種の幼植物の比較

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Seedling morphology was comparatively studied in three *Cymbidium* species; *Cymbidium pumilum* Rolfe (epiphytic), *C.goeringii* Reichb.f. (terrestrial) and *C.nipponicum* Makino (saprophytic). In *C.pumilum* embryos develop into obovoid protocorms on which 3–4 scaly leaves are formed prior to foliar leaves. In *C.goeringii* protocorms develop into rhizomes on which scaly leaves are formed until the foliage leaves are formed terminally. In *C.nipponicum* embryos also develop into rhizomes which are larger than *C.goeringii.* The rhizomes form sheath-like leaves instead of scaly ones, and not form foliage leaf.

Cymbidium species can be grouped into three types, epiphytic, terrestrial and saprophytic, according to their habitat. Embryos of epiphytic *Cymbidium* species develop into obovoid protocorms, which form foliage leaves (Bernard 1909, Ueda 1978). On the other hand, protocorms of terrestrial species develop into rhizomes and foliage leaves are formed several years after germination (Burgeff 1936, Ueda and Torikata 1968, Stoutamire 1974, Nagashima 1982b). Protocorms of saprophytic *Cymbidium* species also develop into rhizomes (Burgeff 1936, Mizuno et al. 1991). Detailed morphological studies have not been conducted on seedling development of the saprophytic species. There is also no comparative observation on the seedling development of these three *Cymbidium* types.

This paper describes comparative seedling morphology of *Cymbidium pumilum* (subtropical epiphyte), *C.goeringii* (temperate terrestrial) and *C.nipponicum* (temperate saprophyte).

Materials and methods

Seeds of *Cymbidium pumilum,* harvested on November 11, 1979, were sterilized in calcium hypochlorite solution (7g 100 ml^{-1}) and sown on the Hyponex culture medium (Kano 1963). Capsules of *C.goeringii* and *C.nipponicum* were collected on September 18, 1984 and September 14, 1989, respectively. They were dipped in 70%

ethanol for a few seconds and then placed in calcium hypochlorite solution for 10 min. before being opened. The immature seeds were sown on the Hyponex culture medium containing 2% peptone. The flasks were maintained at 22–25°C under 16 hr photoperiods of 2,000–3,000 Lux produced by cool white fluorescent tubes. Seedlings were taken for study weekly in *C.pumilum,* 235 and 2586 days after sowing in *C.goeringii,* and 345, 453 and 707 days in *C.nipponicum.* They were fixed with FAA containing 50% ethanol and observed under a dissecting microscope. The seedlings were then dehydrated in a *n*-butyl alcohol series, embedded in paraffin, cut at 6 μm and stained with Delafield's haematoxilin.

Results

Cymbidium pumilum – Seeds ca. 0.57 mm long, ca. 0.15 mm wide, consist of an ellipsoidal embryo, remaining suspensor and a transparent seed coat. Embryos ca. 0.17 mm long, ca. 0.1 mm wide, consist of undifferentiated cells. Meristem and vascular tissue are not differentiated (Fig. 1: A, H).

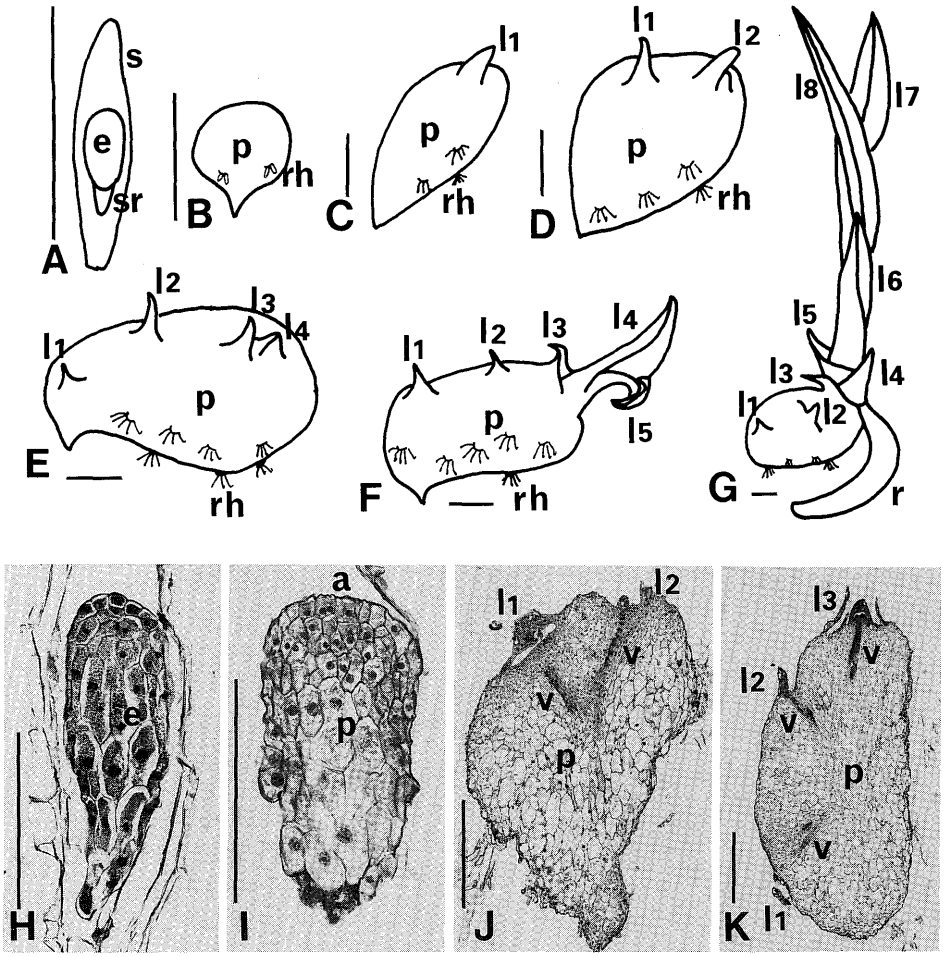
After sowing, the cells in the charazal region of the embryo divide and differentiate meristem (Fig. 1: I). The embryos swell and develop into pear-shaped, then obovoid protocorms (Fig. 1: B, C, D, E). Their tips become flatten (Fig. 1: B, I). Root hairs are formed at the lower surface of the protocorm. A leaf primordium is formed from the apical meristem (Fig. 1: C). The first three or four leaves are scaly (Fig. 1: D, E). A vascular bundle is differentiated in each of these leaves (Fig. 1: J, K). The fourth or fifth leaf becomes the first foliage leaf (Fig. 1: F, G). At this stage the length of the protocorms is ca. 2.6 mm. It takes about eight months until the first foliage leaf can be observed. Roots are formed from the chalazal end of the protocorm (Fig. 1: G).

Cymbidium goeringii – Seeds ca. 1.1 mm long, 0.15 mm wide consist of an ellipsoidal embryo, remaining suspensor and a transparent seed coat as in *C.pumilum.* Embryos ca. 0.16 mm long, 0.1 mm wide, consist of undifferentiated cells. Meristem and vascular tissue are not differentiated (Fig. 2: A, H).

After sowing, the embryos develop into pear-shaped protocorms, and the cells at the chalazal region become an apical meristem (Fig. 2: I). Root hairs are formed at the lower surface of the protocorm. Protocorm tips are flatten (Fig. 2: I). Protocorms of this species, unlike that of *C.pumilum,* do not remain obovoid, but elongate and become club-shaped (Fig. 2: B, C). The first leaf is formed from the apical meristem (Fig. 2: C, J), and is scaly as in *C.pumilum.* A vascular bundle is differentiated in it (Fig. 2: J, K). Following leaves, not only first three or four as seen in *C.pumilum,* remain scaly (Fig. 2: D, E). At this stage the protocorms develop into elongated rhizomes (Fig. 2: E). The diameter of the rhizomes is ca. 1.0 mm. Axillary buds develop and form branches which also form scaly leaves (Fig. 2: F). The rhizomes continue to elongate, and the foliage leaves are formed at the tip of the rhizome about 5 years after sowing (Fig. 2: G). Roots are formed near the basal part of the foliage leaves.

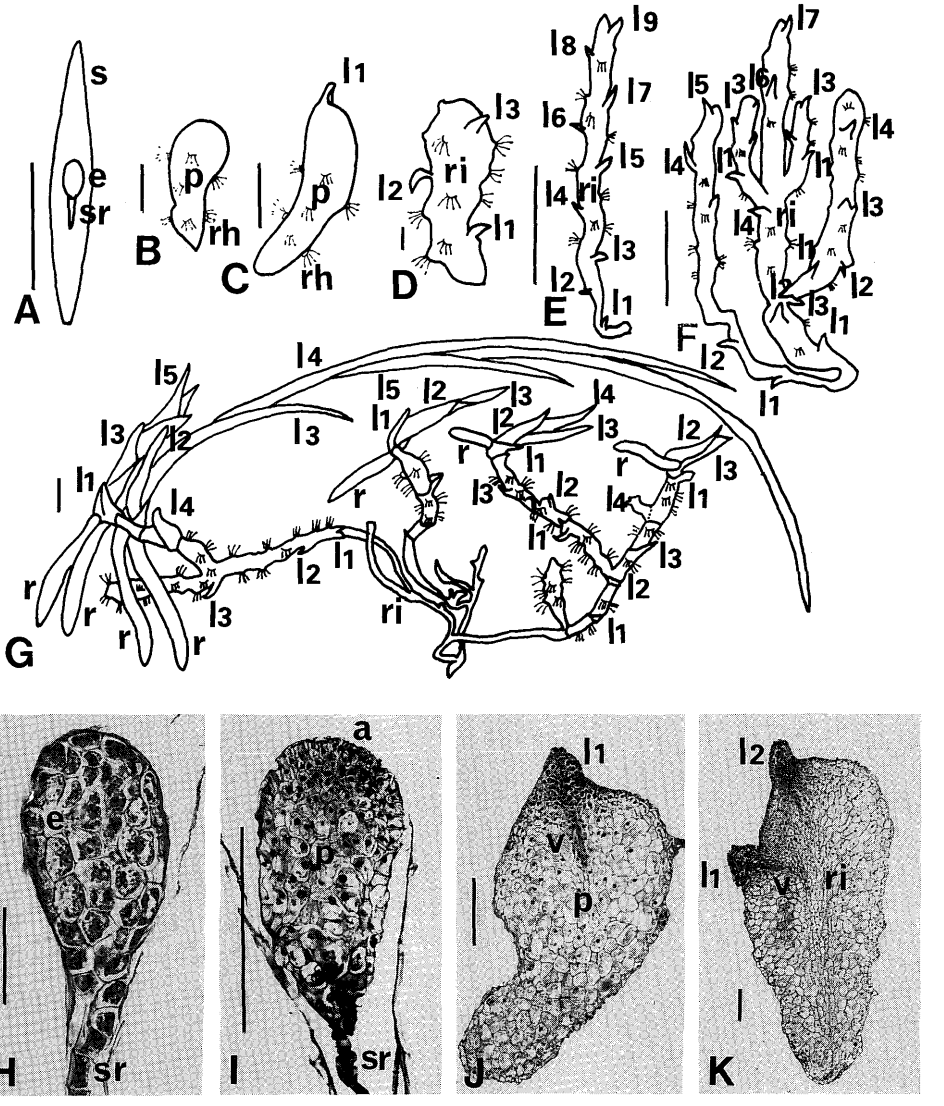
Cymbidium nipponicum – Seeds ca. 0.9 mm long, 0.14 mm wide consist of an ellipsoidal embryo, remaining suspensor and a transparent seed coat as in two other species. Embryos ca. 0.13 mm long, ca. 0.1 mm wide, consist of an ellipsoidal mass of undifferentiated cells. Meristem and vascular tissues are not differentiated (Fig. 3: A, I).

After sowing, the embryos develop into pear-shaped protocorms (Fig. 3: B) and differentiate an apical meristem at the charazal region (Fig. 3: J). Root hairs are formed at the lower surface of the protocorms. Protocorm tips are flatten (Fig. 3: B).



Figs. 1. A–K. Seedling development in *Cymbidium pumilum*. A: Seed. B: Seedling, 20 days old. C: Seedling, 65 days old. First leaf can be seen. D: Seedling, 115 days old. Second and third leaf can be seen. E: Seedling, 160 days old. Fourth leaf can be seen. F: Seedling, 249 days old. The first foliage leaf can be seen. G: Seedling, 303 days old. 1_1–1_3 are scaly and 1_4–1_8 are foliage. Root can be seen. H–K: Longisections of seedlings. H: Embryo. I: Seedling, 20 days old with a differentiated meristem. J: Seedling 160 days old showing two buds on the protocorm. K: Seedling, 249 days old showing an apical shoot and two axially buds with a scaly leaf.
a = apical meristem; e = embryo; 1_{1-8} = leaf; p = protocorm; r = root; rh = root hairs; s = seed coat; sr = suspensor remains; v = vascular bundle.
Bars indicate 0.5 mm in A–G, I–K and 0.1 mm in H.

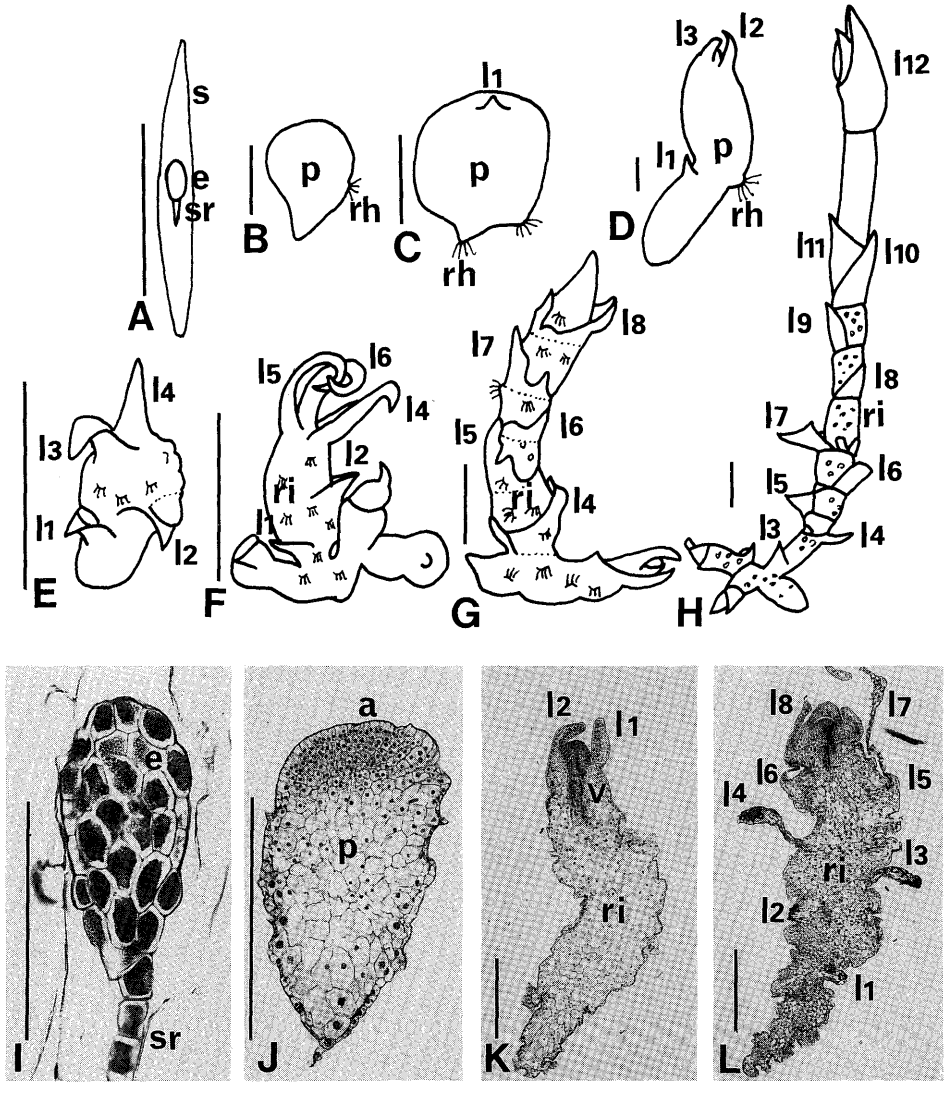
The first leaf, formed from the apical meristem, is scaly (Fig. 3: C, D). A vascular bundle is differentiated in it. As the protocorms develop, they become club-shaped (Fig. 3: D) and then elongate into rhizomes (Fig. 3: K, L). The second and third leaves also remain scaly (Fig. 3: E, F). The leaves above the fourth are longer than those first to third forming a sheath-like structure with entirely amplexicaular base unlike in *C.goeringii* (Fig. 3: G). Branches are formed at the basal region of the rhizomes (Fig. 3: F, G, H). The rhizomes grow larger than the ones in *C.goeringii*. Their diameter is ca. 2.0 mm. Neither foliage leaves nor roots are formed (Fig. 3: H).



Figs. 2. A–K. Seedling development in *Cymbidium goeringii*. H–K and H–L are 235 days, and E–G are 2586 days (about 7 years) after sowing. A: Seed. B: Club-shaped protocorm. C: Seedling with the first leaf. D: Seedling with several leaves. E: Elongated rhizome with scaly leaves. F: Branched rhizomes. G: Seedling with foliage leaves and roots. H–K: Longisection of seedlings. H: Embryo. I: Seedling with an apical meristem. J: Seedling with the first leaf and a vascular bundle. K: Seedling with two scaly leaves.
a = apical meristem; e = embryo; l_{1-9} = leaf; p = protocorm; r = root; rh = root hairs; ri = rhizome; s = seed coat; sr = suspensor remains; v = vascular bundle
Bars indicate 0.5 mm in A–D, I–K, 5.0 mm in E–G and 0.1 mm in H.

Discussion

In numerous species in Orchidaceae including *Arundina bambusifolia* (=*A.graminifolia;* Mitra 1971), *Calanthe discolor* (Nagashima 1982a), *Cattleya aurantiaca* (Nishimura 1981), *Cypripedium acaule* (Curtis 1943), *Paphiopedilum insigne* var. *sanderae* (Nagashima 1982b), *Encyclia vitellina, Habenaria rhodocheila, Neobenthamia*



Figs. 3. A–L. Seedling development in *Cymbidium nipponicum*. A–F and I–K are 345 days. G and L are 453 days, and H is 707 days after sowing. A: Seed. B: Pear-shaped protocorm. C: Globular protocorm with the first leaf. D: Seedling showing a club-shaped protocorm. E: Seedling showing three scaly leaves. F: Seedling showing leaves and branches. G: Seedling with an elongated rhizome with sheath-like leaves. H: Seedling with elongated rhizome. I–L: Longisection of seedlings. I: Embryo. J: Seedling with an apical meristem. K: Seedling showing elongated rhizome with two leaves. L: Seedling with several leaves. a = apical meristem; e = embryo; l_{1-12} = leaf; p = protocorm; rh = root hairs; ri = rhizome; s = seed coat; sr = suspensor remains; v = vascular bundle.
Bars indicate 0.5 mm in A–D and J–L. 3.0 mm in E–H and 0.1 mm in I.

gracilis, Polystachya vaginata, and *Spathoglottis affinis* (Nishimura unpublished), the embryo develops into a grobular protocorm and the first leaf, which is differentiated on the protocorm, becomes foliage. Among the *Cymbidium,* the epiphytic *C.pumilum* is rather unusual in having larger obovoid protocorm, ca. 2.6 mm in diameter, with several scaly leaves prior to the foliage leaves

on it. Similar seedling structure can be seen in the epiphytic *Cymbidium lowianum* (Bernard 1909), showing similarity to the above mentioned species.

The seedling structure in *C.goeringii* shows more unusual than *C.pumilum.* In this species the protocorm does not stay being obovoid, but develops into a slender rhizome. Scaly leaves are formed on the rhizomes until foliage leaves differentiate about 5 years after sowing. Similar seedling structure were observed in the terrestrial *Cymbidium lancifolium* (Burgeff 1936), and some other terrestrial genera including *Cremastra appendiculata, Eulophidium maculatum* and *Geodorum pictum* (Stoutamire 1974). The most unusual structure is seen in the saprophytic *C. nipponicum.* In this species the rhizome develops larger than that of *C.goeringii,* the diameter is 2.0 mm in *C.nipponicum* and 1.0 mm in *C.goeringii.* The leaves formed on the rhizome are sheath-like instead of scaly and foliage leaves are not formed. The similar seedling structure was observed in the saprophytic *Cymbidium macrorhizum* (Burgeff 1936).

The comparative observation in this study may indicate that in *Cymbidium* species the specialization of the seedling structure progresses in a geophilous direction from epiphytic to terrestrial and then to subterranean. In Orchidaceae terrestrial habit is generally considered to be ancestral and the epiphytic is derived (Schmit and Schmit 1977; Dressler 1980). The results of this report may offer some counterevidences to this currently accepted view.

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要 旨

シュンラン属に含まれる生態的に異なる3種，つまり着生種 *Cymbidium pumilum*，地生種 *C. goeringii*，腐生種 *C. nipponicum* について種子を無菌培地に播種し幼植物を比較観察した．着生

種 *C. pumilum* では発芽後，胚は洋ナシ状から倒卵形のプロトコームに発達し，プロトコーム上に葉が分化してくる．最初の3－4葉は鱗片状にとどまり，4－5葉目から普通葉となって展開する．プロトコームはシュンラン属以外の多くのラン科植物に見られるものより大きく発達する．次に，地生種 *C. goeringii* ではプロトコームは伸長を続け，棒状の根茎に発達する．根茎上の葉は鱗片状にとどまる．根茎は分枝を繰り返しつつ，伸長を続ける．やがて，根茎の先端に普通葉が形成される．最も特異な形態は腐生種 *C. nipponicum* で見られる．本種では根茎は *C. goeringii* より更に大きく発達し，葉は葉鞘状となり普通葉はまったく形成されない．

以上のように，シュンラン属で見られる3つの生態的に異なる種では，着生種が他の多くのラン科植物の幼植物の形態と最も似ており，地生種そして腐生種の順で形態的特殊化が進んでいることが明らかとなった．