



Seedlings of *Neuwiedia* (Orchidaceae subfamily Apostasioideae) have typical orchidaceous mycotrophic protocorms

Kristiansen, Kim Anker; Rasmussen, Finn N.; Rasmussen, Hanne Nina

Published in:
American Journal of Botany

Publication date:
2001

Citation for published version (APA):
Kristiansen, K. A., Rasmussen, F. N., & Rasmussen, H. N. (2001). Seedlings of *Neuwiedia* (Orchidaceae subfamily Apostasioideae) have typical orchidaceous mycotrophic protocorms. *American Journal of Botany*, 88, 956-959.

BRIEF COMMUNICATION

SEEDLINGS OF *NEUWIEDIA* (ORCHIDACEAE SUBFAMILY APOSTASIOIDEAE) HAVE TYPICAL ORCHIDACEOUS MYCOTROPHIC protocORMS¹

KIM A. KRISTIANSEN,² FINN N. RASMUSSEN, AND HANNE N. RASMUSSEN³

Botanical Institute, University of Copenhagen, Gothersgade 140, DK-1123 Copenhagen K, Denmark

Naturally occurring seedlings of *Neuwiedia veratrifolia* were found in three localities in Sabah, Borneo, Malaysia. Seedlings consisted of an irregular oblong protocorm and a terminal leafy rooted shoot. Protocorms contained mycotrophic tissue of the kind typical of orchid mycorrhiza (tolypohagy). This finding demonstrates an important synapomorphy between *Neuwiedia* and other orchids and strongly supports the monophyly of Orchidaceae in the broad sense, including apostasioid orchids.

Key words: germination; mycorrhiza; *Neuwiedia*; orchid; Orchidaceae; protocorm; seedling.

The apostasioid orchids—*Neuwiedia* and *Apostasia*—have been regarded as either closest relatives to orchids or the most basal orchids by taxonomists and orchid experts since the first species of *Apostasia* was discovered (see Stern, Cheadle, and Thorsch [1993] for a thorough review of the classification history of the group). Few taxonomists (e.g., Hutchinson, 1973) have placed apostasioids outside an orchidaceous group at family or ordinal level. On the other hand, highly contrasting views on whether the group should be included in Orchidaceae in a broad sense or recognized as a separate family have been advanced. Reveal and Hoogland (1991) went as far as creating a separate family comprising only *Neuwiedia*, citing evidence for this view by Burns-Balogh and Funk (1986).

Orchidaceae in the strict sense are one of the most distinct of all plant families, easily recognized on floral characters. However, it has often been pointed out that the holomyco-

trophic protocorm stage, enabled by a presumed change of fungal symbiont from Zygomycetes to Basidiomycetes, may be the most important event in the evolution of orchids (Benzing, 1981; F. N. Rasmussen, 1994, 2000; H. N. Rasmussen, 1995). Benzing (1987, p. 50) wondered why it was not yet established whether apostasioids have this fundamental orchid characteristic. Siebe (1903), in his anatomical work on *Apostasia* and *Neuwiedia*, did not mention any mycorrhizal infections. This could be because he was working with fragments of herbarium specimens that may have been poorly preserved. The first notion of mycorrhiza in apostasioids seems to be by Stern, Cheadle, and Thorsch (1993) who mentioned the “occurrence of coils of fungal hyphae in some cortical cells of some species” and depicted infected cortical root cells of *Apostasia wallichii*. Stern and Warcup (1994) reported and illustrated infections by septate hyphae in root-tubercles of this species, but protocorms have so far not been observed from this group of orchids. The present paper describes the protocorm and seedling mycorrhiza of *Neuwiedia veratrifolia* Blume.

MATERIALS AND METHODS

Seedlings still retaining a protocorm from naturally occurring *Neuwiedia veratrifolia* plants were collected at three different localities (Table 1). Vouchers of adult plants were not collected owing to the scarcity of material at the sites, in particular at the natural population near Sin Tuong Tuong.

Root and protocorm material was washed free of soil, photographed, and fixed in FAA (24.5% formaldehyde, 98–100% glacial acetic acid, and 70% ethanol mixed 5:8:87) or GA-PFA: 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.0. The material was fixed over-

→

Figs. 1–6. *Neuwiedia veratrifolia* seedling and protocorm. 1. Seedling. Protocorm (large arrow) and the leafy shoot. Two young roots (small arrows) grow out from scale leaf bases. Scale bar = 10 mm. 2. Detail showing protocorm with ring pattern of hairy warts (arrows). Scale bar = 10 mm. 3. Transverse section of protocorm through row of hairy warts. Outer cortex with living infection (small arrows) and inner cortex with digestion cells in which the pelotons have collapsed (large arrows). Glycol methacrylate (GMA), stained in PAS (periodic acid-Schiff's reagent)–aniline blue black. Scale bar = 1 mm. 4. Infection in protocorm showing septate hyphae apparently with dolipores (arrows). GMA, stained in PAS–aniline blue black. Scale bar = 10 μm. 5. Hyphae in peloton are

view as advanced in the monograph by de Vogel (1969) and in recent taxonomic treatments by Dressler (1993) and Pridgeon (1999).

LITERATURE CITED

- BENZING, D. H. 1981. Why is Orchidaceae so large, its seeds so small and its seedlings mycotrophic? *Selbyana* 5: 241–242.
- . 1987. Major patterns and processes in orchid evolution: critical synthesis. In J. Arditti [ed.], *Orchid biology IV*, 34–77. Cornell University Press, Ithaca, New York, USA.
- BURGEFF, H. 1936. *Samenkeimung der Orchideen*. Gustav Fischer, Jena, Germany.
- BURNS-BALOGH, P., AND V. FUNK. 1986. A phylogenetic analysis of the Orchidaceae. *Smithsonian Contributions to Botany* 61: 1–79.
- CAMERON, K. M., M. W. CHASE, M. W. WHITTEN, P. J. KORES, D. C. JARRELL, V. A. ALBERT, T. YUKAWA, H. G. HILLS, AND D. H. GOLDMAN. 1999. A phylogenetic analysis of the Orchidaceae: evidence from *rbcL* nucleotide sequences. *American Journal of Botany* 86: 208–224.
- DE VOGEL, E. F. 1969. Monograph of the tribe Apostasioideae. *Blumea* 17: 313–350.
- DRESSLER, R. L. 1993. *Phylogeny and classification of the orchid family*. Cambridge University Press, Cambridge, UK.
- FREUDENSTEIN, J. V., AND F. N. RASMUSSEN. 1999. What does morphology tell us about orchid relationships? A cladistic analysis. *American Journal of Botany* 86: 225–248.
- HOLMGREN, P. K., N. H. HOLMGREN, AND L. C. BARNETT. 1990. *Index herbariorum*. Part I: The herbaria of the world, 8th ed. New York Botanical Garden, Bronx, New York, USA.
- HUTCHINSON, J. 1973. *The families of flowering plants*, 3rd ed. Clarendon Press, Oxford, UK.
- JUDD, W. S., W. L. STERN, AND V. I. CHEADLE. 1993. Phylogenetic position of *Apostasia* and *Neuwiedia* (Orchidaceae). *Botanical Journal of the Linnean Society* 113: 87–94.
- PRIDGEON, A. M. 1999. Apostasioideae. In A. M. Pridgeon, P. J. Cribb, M. W. Chase, and F. N. Rasmussen [eds.], *Genera orchidacearum*, vol. 1, 94–104. Oxford University Press, Oxford, UK.
- RASMUSSEN, F. N. 1994. Aspects of orchid phylogeny. In A. M. Pridgeon [ed.], *Proceedings of the 14th World Orchid Conference Glasgow 1993*, 52–60. Her Majesty's Stationary Office, Edinburgh, UK.
- . 2000. Ins and outs of orchid phylogeny. In K. Wilson and D. A. Morrison [eds.], *Monocots: systematics and evolution*, 426–431. CSIRO (Commonwealth Scientific and Industrial Research Organisation), Collingwood, Australia.
- RASMUSSEN, H. N. 1995. *Terrestrial orchids: from seeds to mycotrophic plant*. Cambridge University Press, Cambridge, UK.
- REVEAL, J. L., AND R. D. HOOGLAND. 1991. Validation of three family names in the Magnoliophyta. *Bulletin du Muséum national d'Histoire naturelle, 4e série. 13 section B, Adansonia: botanique, phytochimie* 1–2: 91–93.
- RUZIN, S. E. 1999. *Plant microtechnique and microscopy*. Oxford University Press, New York, New York, USA.
- SIEBE, M. 1903. *Ueber den anatomische Bau der Apostasiinae*. Hörning & Berkenbusch, Heidelberg, Germany.
- STERN, W. L., V. I. CHEADLE, AND J. THORSCH. 1993. Apostasiads, systematic anatomy, and the origins of Orchidaceae. *Botanical Journal of the Linnean Society* 111: 411–455.
- , AND J. H. WARCUP. 1994. Root tubercles in apostasiad orchids. *American Journal of Botany* 81: 1571–1575.
- VEYRET, Y. 1974. Development of the embryo and the young seedling stages of orchids. In C. Withner [ed.], *The orchids: scientific studies*, 223–265. Wiley, New York, New York, USA.