

A Review of New Results in
Nigritella (Orchidaceae)

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SPRAWOZDANIA Z POSIEDZEŃ KOMISJI NAUKOWYCH

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Prof. Herwig Teppner (Austria) przedstawił własną pracę pt. *A review of new results in Nigritella (Orchidaceae)*.

The state of knowledge of the taxonomy of *Nigritella* at the beginning of our studies was one species with three subspecies (Moore, 1980: 332–333 in Flora Europaea). My own interest in *Nigritella* began with my first encounter with a pink flowered population in the Seekar of the Koralpe (southeastern border of the Alps) in 1970, which could not be determined at that time (later *N. lithopolitana* Ravnik, 1978). Furthermore, some first spot-checks showed that *Nigritella* could reveal some diversity in chromosome numbers and embryology; these were the facts and expectations that led me to the *Nigritella* studies. The resulting success would not have been possible without the collaboration with the chemist Dr Erich Klein (Purgstall near Graz), who planned, sponsored and organized the larger field trips, searched for much of the taxonomic literature, procured the loan of herbarium specimens, fixed some material and also got some additional material from other orchid friends.

This paper will present a short review of the genus together with some hitherto unpublished results.

At present, *Nigritella* contains c. 12 species. Following is a possible simplified review based on an artificial order (all measurements refer to the lowermost flowers in the inflorescence). Rare deviations are not considered at all. For details of variability see the relevant papers of Teppner and co-workers. The names of authors and citations can be found in Teppner & Klein, 1998:

- the*
1. Flowers dark, red-brown to brown-red, buds, and also under certain light circumstances the flowers, appear nearly black (but there are individuals with deviating colours, up to whitish, in some populations of *N. rhellicani* of the Central Alps and especially in the Bernina region, in Trentino, South Tyrol and North Tyrol). 2
 1. – Flower colour more or less brilliant red, pink or whitish. 6
 2. At least lower bracts of the inflorescence usually with a distinct peg-seam (marginal epidermal cells elongated to cylindrical papillae of c. 0.05–0.1 mm length; 'Stiftchensaum' in German). 3
 2. – Lower bracts of the inflorescence usually without papillae, smooth or sometimes only some marginal epidermal cells a little elongated (margin humpbacked or some papillae with up to 0.05 mm length). 4
 3. Lips widely open, transsection of the lip-constriction more or less semicircular. – Diploid. Jura, Alps, Ligurian Apennines, Balkan Peninsula up to N. Greece. *N. rhellicani*

3. – Lips largely closed, entrance narrow, margins of the lip approximated behind the column or margins touching or a little overlapping. – Diploid. Cantabrian Mountains, Pyrenees *N. gabasiana*
4. Length of the lip 7.9 – 12 mm, of the lateral sepals 7.2–10.5 mm, of the median sepal 7.0–10.2 mm, of the spur 0.8–1.1 mm (the largest flowers within the species). – Triploid. Scandinavia. *N. nigra* subsp. *nigra*
4. – Perigon parts in the middle a little shorter, spur usually 1.0–1.4 mm long. 5
5. Length of the lateral sepals usually 6.8–10 mm, spur usually 1.0–1.4 mm – Tetraploid. Pyrenees, Masif Central in France, Western W. Alps, Jura (Teppner & Klein, 1998: 222) *N. nigra* subsp. *iberica*
5. – Length of the lateral sepals usually 6.1–8.5 (9.0) mm, spur usually 1.0–1.2 (1.3) mm (the smallest flowers within the species). – Tetraploid. Eastern Alps. *N. nigra* subsp. *austriaca*
6. Flowers small, lip 4.5–5.4 mm (rarely up to 5.9 mm; the smallest flowers in the genus), spur 1.0–1.1 mm (rarely up to 1.5 mm) long. – Diploid. Eastern Carpathians in Ukraine and Romania. *N. carpatica*
6. – Flowers larger, lip at least 6 mm long, spur usually around 1.3–1.5 mm. 7
7. Distal part of the lip rolled up, lip appearing closed (the opening at the very tip of the lip excepted), petals and median sepal adjoining the lip even at the height of anthesis. – Tetraploid. N. E. Limestone Alps [Styrian Salzkammergut, Eisenerzer Alpen (?)]. *N. archiducis-joannis*.
7. – Distal part of the lip open, margins more or less spreading, petals and median sepal more or less diverging. 8
8. Tips of the perigon parts light-coloured, whitish, perigon dark pink towards the base. – Tetraploid. N. E. Limestone Alps (Salzkammergut) and Grazer Bergland. *N. stiriaca*
8. – The tips are the darkest coloured parts of the perigon. 9
9. Belly-like, inflated, basal part of the lip c. 2.7–3.5 mm wide. 10
9. – Belly-like, inflated, basal part of the lip c. 2–2.5 mm wide. 11
10. Flowers prevalently pink to whitish, perigon parts recurved, strongly diverging. – Tetraploid. N. Limestone Alps (from Bavaria to Lower Austria and Styria), Grazer Bergland and Central Apennines. *N. widderi*
10. – Flowers prevalently red, a minority of individuals with pink to whitish flowers, perigon parts more or less straightly stretched forward. – Pentaploid. S. Alps (Brenta Group). *N. buschmanniae*
11. Colour of the perigon in open flowers predominantly pink to pinkish white. 12
11. – Colour of the perigon in open flowers predominantly red (individuals with completely whitish pink flowers are extremely rare). 13
12. Lip 6–8.5 mm in length, spur 0.8–1.2 mm, margins of the distal part of the lip usually distinctly bent outwards, reddish component of the colour a clear red. – Diploid. W. Alps (Alpes Maritimes and Cottian Alps). *N. corneliana*
12. – Lip 6–7.5 mm, spur 1.2–1.4 mm in length, margins of the lip usually not bent outwards, the reddish colour with a tinge of lilac. – Diploid. S. E. Alps. *N. lithopolitana*
13. Entrance to the lip usually narrow, constricted, perigon brilliant red. – Tetraploid. Eastern Alps to Carpathians. *N. miniata*
13. – Entrance to the lip usually widely open (as in *N. rhellicani*), perigon red, but not so brilliant, with a tinge of lilac. – Tetraploid. S. Alps. *N. dolomitensis*

The diploids are *N. carpatica*, *N. rhellicani*, *N. gabasiana*, *N. corneliana* and *N. lithopolitana*. We include *N. cenisia* Foelsche & Gerbaud in *N. rhellicani*, because the investigation of allozymes showed no differences and Kalteisen (2001) was not able

to separate the two in the field. This was also my opinion concerning material from Mt. Cenis collected by Klein & Drescher in 1995.

It is remarkable that all the diploids possess a seed coat structure of the *N. rhellicani* type (Fig. 1). This means, that the testa cells at the chalazal end of the seed are more or less isodiametric or up to twice as long as wide, with straight or a little curved anticlinal walls. The cells in the micropylar part of the seeds are elongated; one longitudinal row on the flanks of the seed contains c. 6–10 cells. The anticlinal walls of the testa cells are thick [(3–)4–9 μm]. Variability is high, but only *N. corneliana* (Fig. 2) seems to be a little deviating in having shorter, more rounded seeds with only c. 5–7(–8) cells in a longitudinal row. Single *corneliana*-like seeds may occur sometimes in *N. rhellicani* at a low percentage but in the westernmost part of its distribution area a higher percentage or dominance was observed.

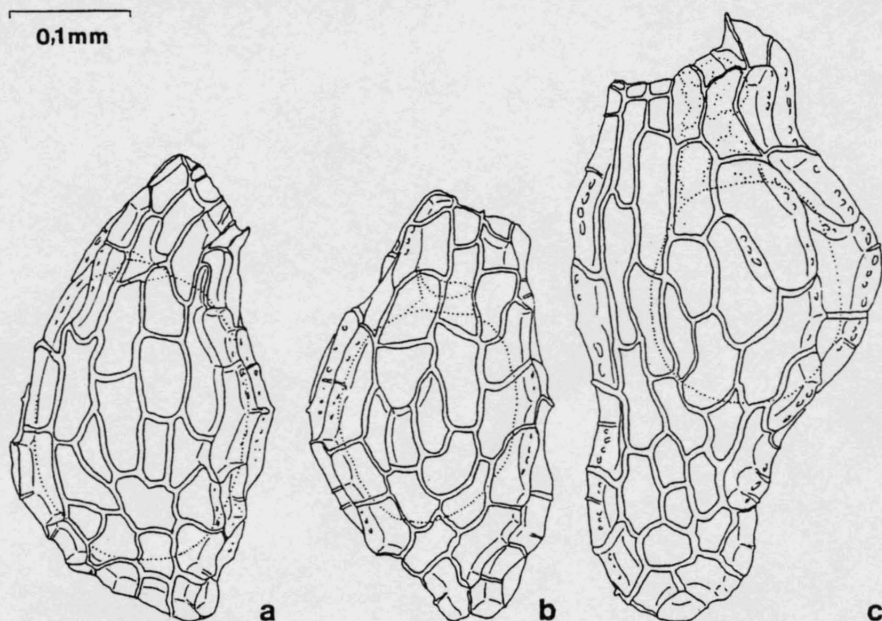


Fig. 1. Seeds of *Nigritella lithopolitana*, Carinthia, Obir, leg. Teppner & Karl, 1995. – Dotted line: embryo, suspensor remains included

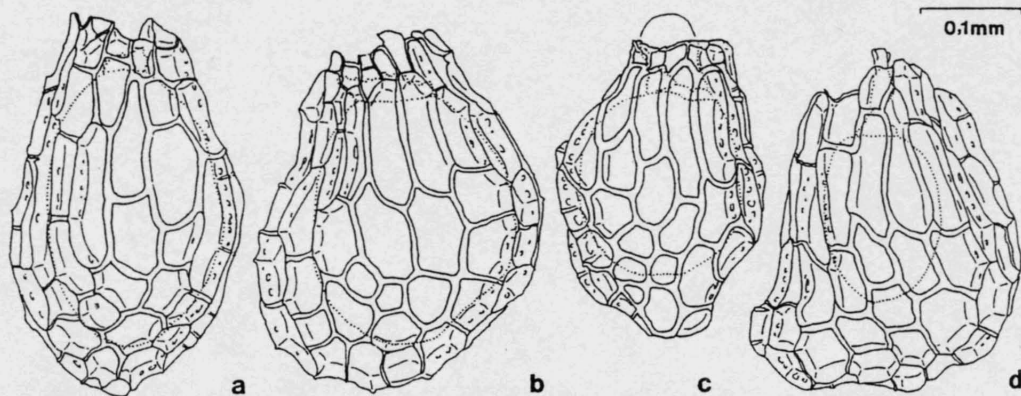


Fig. 2. Seeds of *Nigritella corneliana*, Italy, Cottian Alps, Mte. Nebin, leg. Karl, 1993

All polyploid *Nigritellas* show seeds of the *N. miniata* type (Fig. 3, 4); in the shorter cells in the chalazal half or two thirds of the testa the anticlinal walls are wavy and therefore meshing with the neighbouring cells. The cells in the micropylar

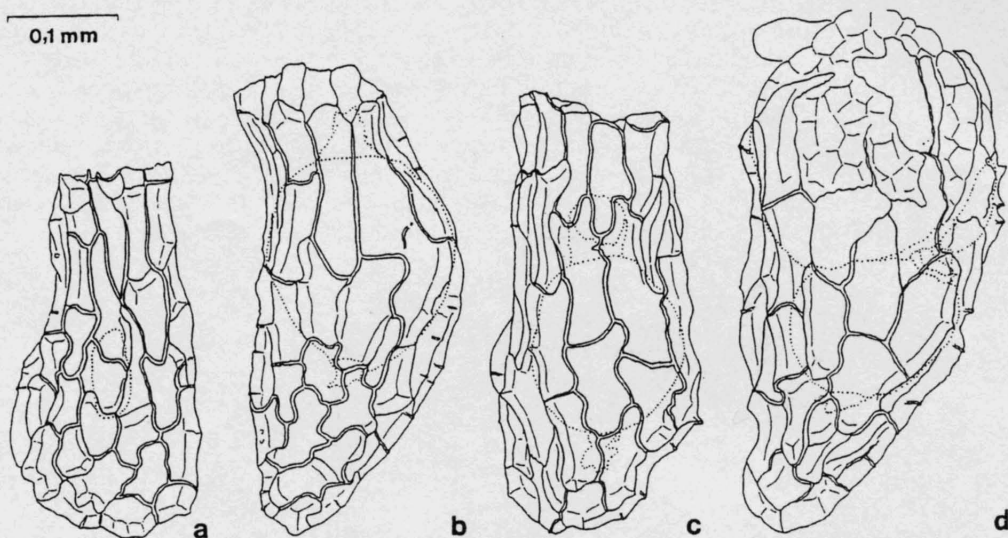


Fig. 3. Seeds of *Nigritella miniata*, Carinthia, Dobratsch, leg. Teppner & Karl, 1998. – „a” with an aborted embryo. In „b”, and „c” one embryo with two suspensors each. In „d” two embryos and seed coat ruptured

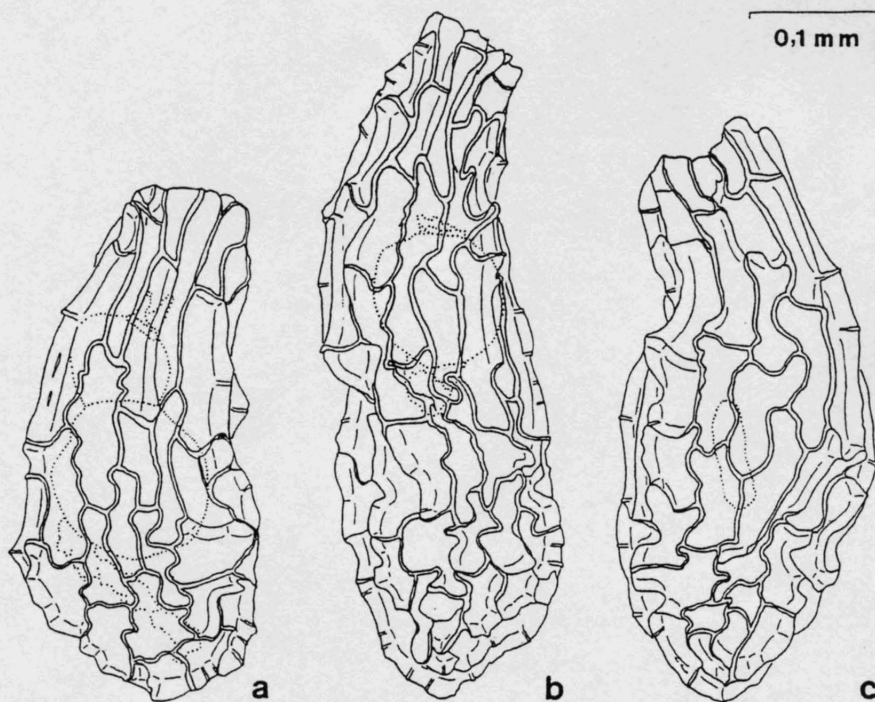


Fig. 4. Seeds of *Nigritella widderi*, Styria, Hohe Veitsch, leg. Deutsch, 1996. – In „a” two embryos, the upper one aborted. In „b” one embryo with two suspensors and in „c” embryo aborted

part are highly elongated and one longitudinal row of cells on the flanks of the seeds consists usually of (4) 5–6 (–7) testa cells. The walls are usually distinctly thinner (2–5 μm , rarely in single seeds up to 6 μm) than in the preceeding type. In my opinion, it is highly improbable that the *miniata* testa type originated at the triploid or tetraploid level. For morphological reasons, it is more plausible to assume that in an early phase of evolution (in the Tertiary or early Quaternary) in *Nigritella* existed diploids with *miniata* type seeds and other main characteristics of *N. nigra* which are now extinct.

Another interesting point in *Nigritella* is its embryology. The diploids are sexual with *Polygonum* type development of the embryo sac but with the characteristic reduction in the chalazal half of the ES in orchids, which do not initiate endosperm: usually one pentaploid nucleus is formed by fusion of the four chalazal nuclei and the micropylar polar nucleus. Consequently only one sperm nucleus is used up in the fertilization process.

Polyploid *Nigritellas* reproduce apomictically through adventitious embryony in the form of nucellar embryos. In the buds, before meiosis in the EMCs, one or more cells of the nucellus enlarge, the chromatin in the nuclei decondense and these cells get transformed to embryogenic cells (=embryocytes). A summary of the embryological results is given in Teppner (1996). Little progress has been made since, because of the experimental difficulties in orchids. Artificial crosses would be helpful to estimate the inheritance of important species characteristics and of the origin of apomixis as well. One of my pupils, G. Deutsch (2001), was able to produce hybrids within the genus and to grow many of them by symbiotic culture, first *in vitro* and then *ex vitro* to an age of two years old. Finally, only c. twenty individuals survived for more than four years, but not even one has flowered till now. In a following study (Deutsch, 2002), he could show some evidence of apomixis (formation of embryogenic cells) in certain diploid individuals of spontaneous hybrids of *Gymnadenia conopsea* \times *N. rhellicani*, but the true proof for the further development and establishment of such apomictic hybrid embryos is still lacking. For the understanding of population structure and phylogeny, it would be important to know if partial sexuality occurs in polyploids. It has been proved, that in tetraploid \times diploid-crosses within *Nigritella* triploid embryos originate in the gynoeceia at a low percentage (Teppner, 1996). But the first small random samples from populations for allozyme studies showed no signs of partial sexuality (Hedren & al., 2000: 259). Thus, if it occurs successfully in nature it cannot be very abundant.

Finally it would be interesting to speculate about the phylogeny of *Nigritella*. It is evident, that primary triploids originated as a first step in the evolution of polyploids. (autopolyploid or allopolyploid) That triploids can originate even in present-day populations has been proved by the discovery of one single autotriploid plant in a population of the diploid *N. carpatica* (Teppner & al., 1994: 180–181). The possibility of secondary triploids in the case of partial sexuality of apomictic tetraploids has been mentioned above. Furthermore, the existence of one such bridge-taxon, the allotriploid *Nigritella nigra* subsp. *nigra* in Scandinavia is a fact pointing in this direction. By backcrossing with diploids, tetraploids can be produced.

Much more insight into the phylogeny of *Nigritellas* was revealed by the allozyme studies carried out by Hedrén (Hedrén & al., 2000). Regrettably, at the time of collecting the material for this study, fresh leaves of only one diploid species (*N. carpatica*) were not available to us and *N. dolomitensis* had not been described yet; additional investigations of both are on the way. Only *N. widderi* from the Apenninian partial area is not available till now.

From the results of Hedrén & al., 2000: 255, Fig. 3, it can be seen, that out of the ten loci investigated, at three loci, the polyploids contain alleles, which are not known from the four diploids. Of course, *N. carpatica* has not been checked till now,

but it is unlikely, that it will contain all the three alleles; if so, additional, ancient, hypothetical diploids must be postulated for the evolution of polyploids. One group of polyploids is *N. nigra* with TPI1c (an allele of one gene for Triose-phosphate isomerase) including the triploid *N. nigra* subsp. *nigra* in Scandinavia and the two tetraploid subspecies *iberica* and *austriaca*; the latter two differing in the dose of the alleles at the PGM (Phosphoglucumutase)-locus. According to my own theory another triploid, different from subsp. *nigra*, played one parent to *N. nigra* subsp. *austriaca*. The second group with SKDa (Shikimate dehydrogenase) comprises *N. widderi*, *N. archiducis-joannis* and *N. buschmanniae*. The third group, only with alleles known from the diploids, contains *N. miniata* and *N. stiriaca*. It is possible, that on the basis of morphological characteristics *N. dolomitensis* could also be a member of this group. For *N. widderi* and *N. miniata* multiple origin seems to be probable.

From the historical and phytogeographical points of view, the genus *Nigritella* must be very old and a member of the tertiary matrix of the Alpine flora. Its phylogeny and distribution must have been remodelled repeatedly by tertiary and quaternary climatic changes. Some indications for this: the bicentric area of triploid *N. nigra* subsp. *nigra* in Scandinavia is often interpreted as influence of glaciation (Holmboe, 1936, e.g. Gjaerevoll, 1963); if so, the triploid must have been present in Scandinavia at least before the last glaciation. The tetraploid *N. nigra* subsp. *austriaca* occupies largely areas with some relic endemism; especially the Northeastern Limestone Alps are well known for the persistence of relics during the last glaciation (e.g. Pils, 1988, 1995; Dullinger & al., 2000); *N. nigra* subsp. *austriaca*, a tetraploid, grows in this area without the diploid *N. rhellicani*. The disjunct occurrence of *N. widderi* in the central Apennines is clearly not a recent event either. The older age of the disjunct *N. nigra* subsp. *nigra* and *N. widderi* is emphasized by the fact that in both at least one of the putative diploid progenitors seems to be extinct. And the history of the diploids must be naturally much older.

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Prof. Kazimierz Kochman przedstawił własną pracę pt. *Modulacja uwalniania gonadotropin przysadkowych przez kompleksy GnRH z metalami*.

Autor nie dostarczył streszczenia.

Prof. Z. Srebro przedstawił prace: dr Marioli Grabowskiej, dr Małgorzaty Schlegel-Zawadzkiej, dra Gabriela Nowaka i dra Mariusza Pappa pt. *Wykorzystanie aktywności osoczowej beta-hydroksylazy dopaminowej i jej związków*