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## ***Nigritella widderi* (Orchidaceae – Orchideae) in the Apennines**

By

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With 10 Figures

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### Summary

ROSSI W., CAPINERI R., TEPPNER H. & KLEIN E. 1987. *Nigritella widderi* (*Orchidaceae* – *Orchideae*) in the Apennines. – *Phyton* (Austria) 27 (1): 129–138, 10 figures. – English with German summary.

The *Nigritella* of Central Italy was identified as conspecific with *N. widderi* TEPPNER & KLEIN from the Northeastern Alps. It occurs in mountains of the region Abruzzo and in adjacent parts of neighbouring regions. Distribution in Italy, karyology (chromosome number  $2n = 80$ ) and embryology (apomixis, nucellar embryos) are discussed.

### Zusammenfassung

ROSSI W., CAPINERI R., TEPPNER H. & KLEIN E. 1987. *Nigritella widderi* (*Orchidaceae* – *Orchideae*) im Apennin. – *Phyton* (Austria) 27 (1): 129–138, 10 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die *Nigritella* Zentral-Italiens wird zu der aus den nordöstlichen Alpen beschriebenen *N. widderi* TEPPNER & KLEIN gestellt. Sie kommt im Apennin in der Region Abruzzo und in angrenzenden Teilen benachbarter Regionen vor. Die Verbreitung in Italien, Karyologie (Chromosomenzahl  $2n = 80$ ) und Embryologie (Apomixis, Nuzellarembryonen) werden diskutiert.

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On several mountains in the Central Apennines a *Nigritella* with pink coloured flowers occurs. Although known for a long time this plant has never been studied in detail. We present here the results of our research during the last years.

### Methods

Measurements of flower parts: fresh flowers were dissected and the flower parts stretched between slide and cover glass. Then measurements were made with the aid of an ocular micrometer in a stereo microscope.

For the karyological studies developing ovules were pretreated with 8-hydroxyquinoline for 6–8 hours and thereafter fixed with CARNOY's fluid. Ovules were stained by the FEULGEN method and squashed.

In the embryological investigations KARPECHENKO's fixative was used for fixation, the ovaries were dehydrated, embedded in paraffin, sectioned (10 µm thick) and stained with haematoxylin according to HEIDENHAIN's procedure.

Abbreviations used for the herbaria are in accordance with the Index Herbariorum (Ed. 7). Additionally: PESA = Erbario Centro Ricerche Floristiche Marche, Pesaro. – PESO = Erbario Parco Nazionale D'Abruzzo, Pescasseroli.

### History

The first indication of the light-coloured *Nigritella* from Central Italy was recorded by TENORE 1831: 457–458: "*Orchis nigra* SW. et WILLD. B. flore roseo. In alpinis editioribus Aprutii: Monte Corno, Pizzo di Sivo (ORSINI)". The first mountain mentioned by TENORE is the highest one (2914 m) of the Gran Sasso d'Italia group and also of the whole Apennines; the second, which today is called Pizzo di Sevo belongs to the Monti della Laga group, at the border between the regions Abruzzo, Lazio and Marche.

The second record was made by BERTOLONI 1854: 575 who reports the variety " $\beta$  floribus roseis" of *Nigritella angustifolia* RICH. ". . . ex Piceno in monte Acuto a Volpara ab ORSINO" and from the Alps; thus he considers all *Nigritellas* with pink flowers as belonging to the same "variety". Monte Acuto is probably Macera della Morte in the Monti della Laga group.

There were no other discoveries for a long time but several authors quoted the afore mentioned records.

PAOLUCCI & CARDINALI 1895: 126 found this orchid at Monte Rotondo (2013 m) in the Sibillini Mts. GRANDE 1925: 70 recorded old specimens collected by ORSINI in the mountains of the Abruzzi (Gran Sasso, Pizzo di Sivo) and from Monte Vettore (2476 m) in the Sibillini group.

PAGANELLI 1957: 9 reported his own finding at Monte Torrone (Sibillini) and, summarizing previous records, also quoted specimens collected by LEVIER in 1876 at "Monte Coccoello", a peak in the Magnola group (Abruzzo). FURRER & FURNARI 1960: 151 found this orchid again in the Gran Sasso Mts. (Passo della Portella e Valle delle Cornacchie). BAZZICHELLI & FURNARI 1968: 41 found it at Monte Petroso (2247 m) in the Monti della Meta

group, between Abruzzo and Lazio; they also report the finding by F. RASETTI near Forca d'Acero in Abruzzo.

The more recent records are those of TAMMARO 1978: 77 at Monte Sirente (2349 m) in Abruzzo, ROSSI & BASSANI 1982: 225 at La Meta Mt. (2241 m) between Lazio and Abruzzo and STEFFAN & STEFFAN 1984: 68 at Terminillo Mt. (2213 m) in Lazio. In PEDROTTI 1982: Tab. 3, 4 the *Nigritella* is included in the species lists of his Poo violaceae-Nardetum and Brachypodio-Festucetum spadiceae from Macera della Morte and Pizzo di Moscio (Monti della Laga).

All authors dealing with the *Nigritella* from Central Italy during the present century namend it *N. nigra*, giving little or no importance to its flower colour. Only DEL PRETE 1982: 266 and ROSSI & BASSANI 1982: 225 and 1985: 90 hesitate to include the Central Italian plants in *N. nigra*.

### Morphology and Taxonomy

In the book of ROSSI & BASSANI 1985: 91 a colour photo of the whole plant is included. The plants attain a height of 6–12 cm, on rare occasions up to 14 cm.

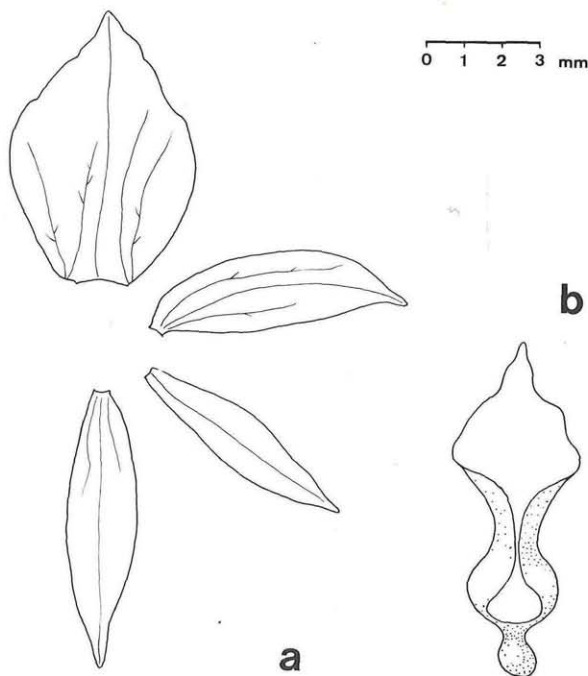


Fig. 1. *Nigritella widderi*. Flower from the middle of an inflorescence from M. Terminillo. – a floral parts stretched: lip, lateral sepal, petal, median sepal (from above). – b lip.

The basal part of the lip is distinctly bellied. Above this the lip is contracted and the edges are brought close together at the ventral side (Fig. 1 b). Therefore the lip is of the "*miniata* type" and very different from *N. nigra*.

Some measurements of flower parts taken from material from Mt. Terminillo (Fig. 1 a) and La Meta (no. 4 and 11 in the map, Fig. 2) provided the following results:

	maximum width		length
	basal fl.	apical fl.	
lateral sepals	2.3–2.7	1.8–2.1	7.5–4.7
median sepals	1.8–2.3	1.7–1.8	7.0–4.7
petals	1.6–1.8	1.4–1.7	6.7–4.7
lip (length without spur)	4.8–5.2	4.0–4.3	7.5–5.4
bellied portion of the lip	2.5–2.7	2.0–2.1	

The spur has a length of c. 1 mm or slightly more (0.9–1.3 mm).

The flower colour is pink, darker in the upper flowers, lighter, almost whitish in the lower ones. In the individual flower the tips are more intensely coloured than the base. The rostellum fold protrudes very distinctly (in the side view of the rostellum).

Considering all this it is clear that the Apenninian plants are closely related to *N. widderi* TEPPER & KLEIN 1985 b from the Northeastern Calcareous Alps in Austria.

Only in one distinctive characteristic, in the width of the bellied basal part of the lip are there some differences. The Apenninian plants show in the basal flowers 2.5–2.7 mm, whereas this part in plants from the Alps measures (2.5–)2.7–3.2 mm; on average the bellied part is narrower in Italian plants, but there is a large overlapping in the measurements so that a distinction is not possible.

As shown later, Apenninian plants have also the same chromosome number and similar embryological features as *N. widderi* from the Alps.

### Distribution

All the 15 Italian locations of *Nigritella widderi* known up to now lie in the Central Apennines in the region Abruzzo and in the neighbouring parts of the regions Marche and Lazio. They fall into 11 of the U.T.M. grid squares (see Fig. 2). From 11 localities we have seen herbarium specimens, four are known to us from literature only. In the list of localities (numbering as in Fig. 2) the herbarium specimens and the references we have considered are indicated.

## 1) Marche, Monti Sibillini:

Monte Rotondo, prateria pseudoalpina, suolo calcereo, 1960–2000 m; 19. VII. 1954; V. MARCHESONI (CAME). (Ref.: PAOLUCCI & CARDINALI, 1895: 126).

## 2a) Marche, Monti Sibillini:

Monte Vettore. Reported by GRANDE 1925: 70 who quoted specimens collected by ORSINI. These specimens were in the Naples herbaria, a portion of which, including many Monocotyledons, was burnt by allied soldiers at the end of the 2<sup>nd</sup> World War to warm themselves up.

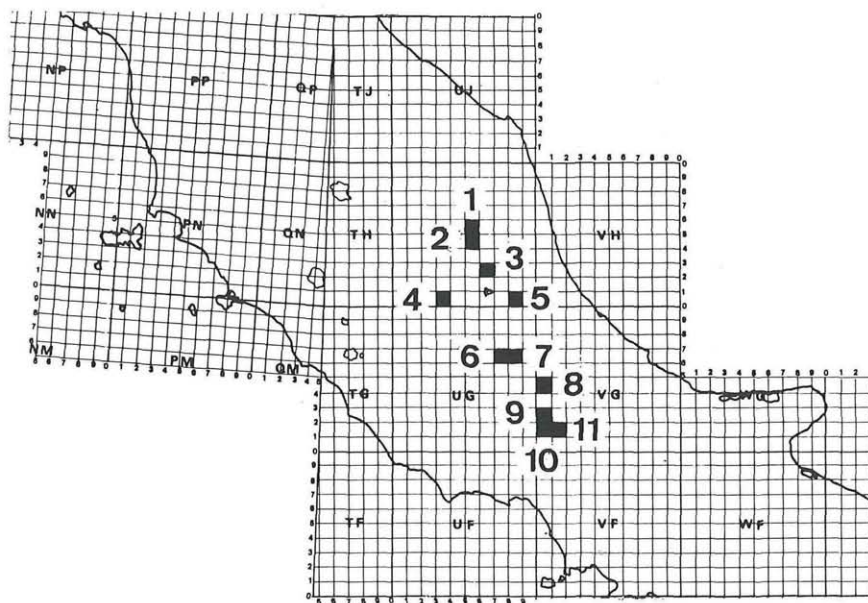


Fig. 2. Distribution of *Nigritella widderi* in the Central Apennines. U.T.M. grid squares with locations of *Nigritella* marked in black. 1 M. Rotondo, 2 M. Vettore and M. Torrione, 3 Macera della Morte (= M. Acuto), Pizzo di Sevo, Pizzo di Moscio and Pizetto, 4 M. Terminillo, 5 Gran Sasso (= M. Corno), 6 Monti della Magnola, 7 M. Sirente, 8 Serra Cappella, 9 Forca d'Acero, 10 M. Petroso, 11 La Meta.

## 2b) Marche, Monti Sibillini:

Monte Torrione, prat. pseudoalpina, suolo calcereo, 2000–2100 m; 5. VIII. 1955; V. MARCHESONI (CAME). (Ref.: PAGANELLI 1957: 9).

## 3a) Lazio/Abruzzo/Marche, Monti della Laga:

In Appenninis, in Monte Acuto [= Macera della Morte, 2073 m]; ORSINI (RO). (Ref.: BERTOLONI 1854: 575, PEDROTTI 1982: Table 3,4).

## 3b) Lazio/Abruzzo, Monti della Laga:

Pizzo di Sivo [= Pizzo di Sevo, 2422 m]; VII. [18] 29; ORSINI (RO; and another sheet). – Pizzo di Sivo; VII. [18] 32 SANGUINETTI (RO). – In cacum. herbos. Pizzo di Sivo; ORSINI (FI; and another sheet). (Ref.: TENORE 1831: 458, GRANDE 1925: 70).

3c) Marche/Abruzzo, Monti della Laga:

Pizzo di Moscio (Ref.: PEDROTTI 1982: Table 3).

3d) Lazio, Monti della Laga:

Monti della Laga: pascoli a *Nardus stricta* nel versante W del Pizzetto (RI), 2050–2100 m; suolo arenaceo; 3. VII. 1985; A. BRILLI-CATTARINI & L. GUBELLINI (PESA).

4) Lazio, Monti Reatini:

M. Terminillo, morene di fondo del circolo glaciale a Nord della vetta, 1900 m circa; 6. VII. 1983; M. & P. STEFFAN (FI). – M. Terminillo, vers. NE, m 2000 circa; 10. VII. 1983; W. ROSSI & G. SPAZIANI (RO). – Terminillo; VII. 1983; B. ANZALONE (RO). (Ref.: STEFFAN & STEFFAN 1984: 68).

5) Abruzzo, Gran Sasso d'Italia:

M. Corno, Erbario TENORE (NAP). (Ref. TENORE 1831: 458, GRANDE 1925: 70, FURRER & FURNARI 1960: 151).

6) Abruzzo, Monti della Magnola:

In pascuis alpinis prope cacumen montis Coccorello in Agro Marsico rarissima; 23. VII. 1876; E. LEVIER (FI). – In pascuis . . . Coccorello, supra Avezzano, rarissima; 2200 m; 23. VII. 1876; E. LEVIER (W). (Ref.: PAGANELLI 1937: 9). The "M. Coccorello" as indicated in Fo. 146 of the map from the "istituto geografico militare" (1: 100.000) has an altitude of 1786 m. Therefore most probably LEVIER has meant the main peak of the group (2220 m). We acknowledge the help of the Institute of Geography of the University of Graz in localizing the name "M. Coccorello".

7) Abruzzo, Monte Sirente:

Monte Sirente (TAMMARO 1978). It was not possible to look in the herbaria of the "Dipartimento di Scienze Ambientali" of the University of L'Aquila, because the botanical section is now moving to a new building and specimens have been packed for a long time. Prof. TAMMARO, however, personally confirms, the specimens from M. Sirente were collected by him and are in his herbarium.

8) Abruzzo, Monte Marsicano:

M. Cappella [= Serra Cappella] (Opi), 2100 m; 28. VI. 1985; B. PETRICCIONE (PESC).

9) Abruzzo, Monti della Meta.

SE Forca d'Acero, sella tra San Nicola e Colle Consolito. Specimens have never been collected. BAZZICHELLI & FUNARI 1968 report a finding of Prof. F. RASETTI, who can be fully trusted.

10) Abruzzo, Monti della Meta:

Monte Petroso, m 2050; 11. VII. 1961; G. BAZZICHELLI (RO). (Ref.: BAZZICHELLI & FURNARI 1958: 51).

11) Lazio, Monti della Meta:

La Meta, pianoro cacuminale, versante laziale; 16. VII. 1981; W. ROSSI (RO). – La Meta, pianoro cacuminale, 2200 m circa; 29. 6. 1985; W. ROSSI (GZU). – La Meta, i Piscurri, 1850 m circa; 4. VII. 1985; C. SULLI (PESC). (Ref.: ROSSI & BASSANI 1982: 225).

The altitudinal range varies from 1800 to 2200 m above sea level. On labels of ORSINI specimens is sometimes written "In cacumine . . . Pizzo di Sivo" but probably it does not mean the specimens were collected at the

very top of the mountain (2422 m). During recent studies in the same area *Nigritella* has been observed between 1830 and 2073 m (PEDROTTI 1982).

*Nigritella nigra* (L.) REICHENB. f. do not occur in Central Italy; the southernmost site lies in the Ligurian Apennines.

### Karyology

Chromosomes of *N. widderi* were counted on mitotic metaphase plates in ovules (material from M. Terminillo, fixed by CAPINERI & ROSSI and KLEIN & ROSSI). The chromosome number of  $2n = 80$  (Fig. 3) is the same as in plants from the Alps (TEPPNER & KLEIN 1985 b).

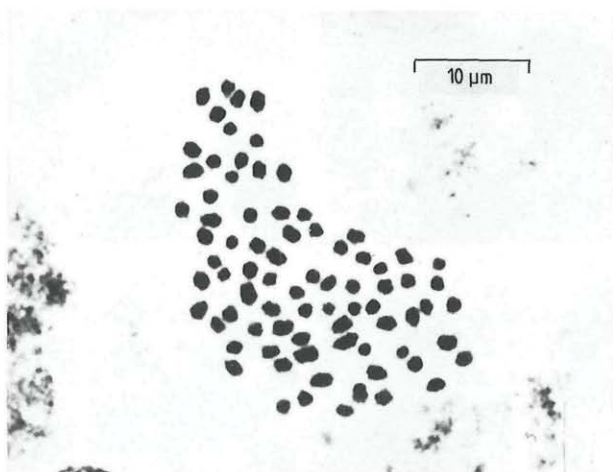


Fig. 3. *Nigritella widderi*, ovule, mitotic metaphase plate with  $2n = 80$  chromosomes.

### Embryology

The material for investigation was also collected on M. Terminillo. Before meiosis, during meiosis or at tetrad stage at the top of the nucellus, initial cells of the adventive embryos develop. The embryo sac mothercells may degenerate very early before meiosis. But more abundant EMCs enter meiosis (megasporeogenesis) and megagametogenesis. While the three upper megaspores degenerate, the chalazal one develops further. The female gametophyte reaches the twonucleate (Fig. 4, 6) or tetranucleate (Fig. 7) stages; occasionally further division takes place (Fig. 5). Up to now this division was not observed in *N. widderi* from the Alps (TEPPNER & KLEIN 1985b), but this is the same stage as seen in *N. nigra* (TEPPNER & KLEIN 1985a: Fig. 19b, chalazal nuclei still in metaphase); it usually leads here to a

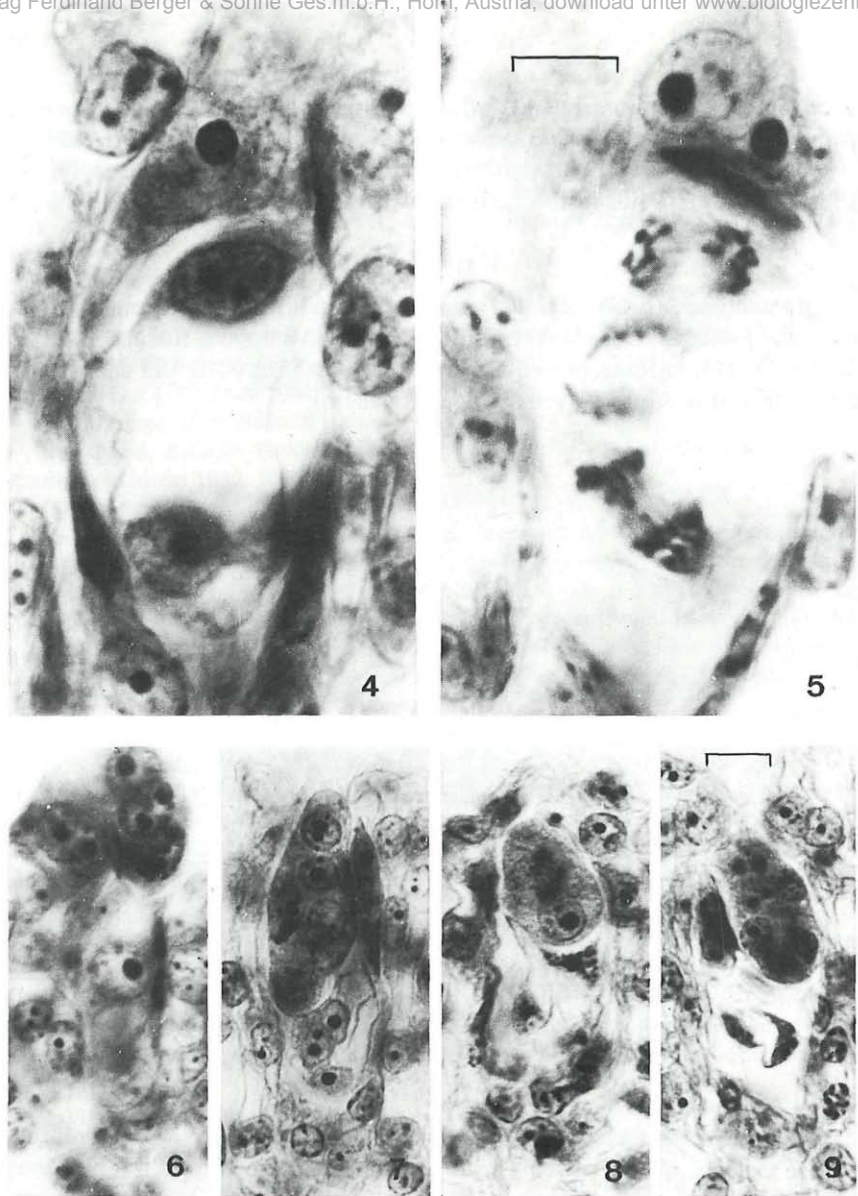


Fig. 4-9. *Nigritella widderi*. Development of femal gametophytes and nucellar embryos. - Fig. 4. Twonucleate embryo sac and one initial cell of nucellar embryo. - Fig. 5. Anaphase in the tetranucleate ES; the spindles of the two chalazal nuclei arranged closely together (the lower one nearly out of the optical plane). Two nucellar embryo initials present. - Fig. 6. Twonucleate ES, two nucellar embryos. - Fig. 7. Tetranucleate ES, two nucellar embryos. - Fig. 8. ES degenerating at the tetranucleate stage, one nucellar embryo. - Fig. 9. ES degenerating at the twonucleate stage, two nucellar embryos. - Scale bars: 10  $\mu$ m.



fournucleate embryo sac. In *N. widderi* only exceptionally and as an extremely rare event one of us (CAPINERI) has seen third divisions leading to the octonucleate stage of ES (Fig. 10), but cellularisation has not been observed. Accordingly, degeneration of the ES usually takes place at the two (Fig. 9) or tetranucleate (Fig. 8) stage.

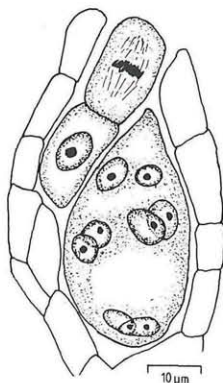


Fig. 10. *Nigritella widderi*, embryo sac with eight nuclei. — At the top: initials of nucellar embryos.

In the meantime at the top of the nucellus one or more initials develop into young nucellar embryos which are embedded by the growing integuments and take the place of the degenerating ES (Fig. 6–9).

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