

Cytotaxonomical considerations on *Epipactis robatschiana* (Orchidaceae), new species from Calabria (S Italy)

G. BARTOLO¹,*, S. D'EMERICO², S. PULVIRENTI¹, M.C. TERRASI¹ and S. STUTO¹

¹ Dipartimento di Botanica, Università di Catania, Via A. Longo, 19. I-95125 Catania, Italy.

² Dipartimento di Biologia e Patologia Vegetale, Università di Bari, Via Orabona, 4. I-70125 Bari, Italy.

Abstract - *Epipactis robatschiana* is a new species, described from Calabria (S Italy), where it grows in underwood of acidophilous pine-forests and beech-forests. This species is diploid with chromosome number $2n=2x=38$. The karyotype is asymmetrical and consists of 4 large and 15 small chromosome pairs. C-banding patterns of this new species is quite similar to those of the other species of *E. muelleri* group, but it differs in the distribution of some heterochromatin bands. Its ecology and relationships with allied species are discussed too.

Key words: Orchidaceae; *Epipactis robatschiana*; heterochromatin banding; karyology; taxonomy.

INTRODUCTION

In the frame of a cytotaxonomical investigation on the genus *Epipactis* in Calabria, the populations belonging to the cycle of *Epipactis muelleri* Godfrey are examined. According to literature data (BONGIORNI and GRÜNANGER 1993; CLAESSENEs and KLEINEN 1994; DELFORGE 2001; ROBATSCH 1983, 1995), this group is represented by *E. muelleri* Godfrey s. str., *E. placentina* Bongiorno and Grünanger, *E. peitzii* H. Neumann and Wucherpfening, *E. komoricensis* Mered'a fil., species distributed in Central and Western Europe, and morphologically differentiated by flowers autogamous with rostellum and clinandrium absent, stigma surface subhorizontal, anther crumbling pollinia falling directly on to the stigma, on which is leant.

In particular, the examined Calabrian populations differ from the above-mentioned species in many of morphological features regarding mainly

the flower pieces. Therefore, it is here described as a species new to science, which we are pleased to dedicate to Karl Robatsch, author of several taxonomical contributions regarding the genus *Epipactis*.

MATERIALS AND METHODS

The investigated material was collected in some localities of Southern Calabria (Serre Calabre and Aspromonte), and used for studying the flower morphology and karyology.

Mitotic and meiotic chromosomes were prepared from immature ovaries, pre-treated with 0.3% colchicine at 20°C for 2h. For Feulgen staining, they were fixed for 5 min in a 5:1:1:1 mixture (volume ratio) of absolute ethanol, chloroform, glacial acetic acid, and formalin. Hydrolysis was made at 20°C in 5.5 N HCl for 20 min (BATTAGLIA 1957a, b) and, finally, stained in freshly prepared Schiff's reagent.

For C-banding, immature ovaries were fixed in ethanol/glacial acetic acid (3 : 1 v/v) and stored in the deep freezer for at least 24 h. Subsequently, they were

* Corresponding author: e-mail: bartolo@mbox.dipbot.unict.it

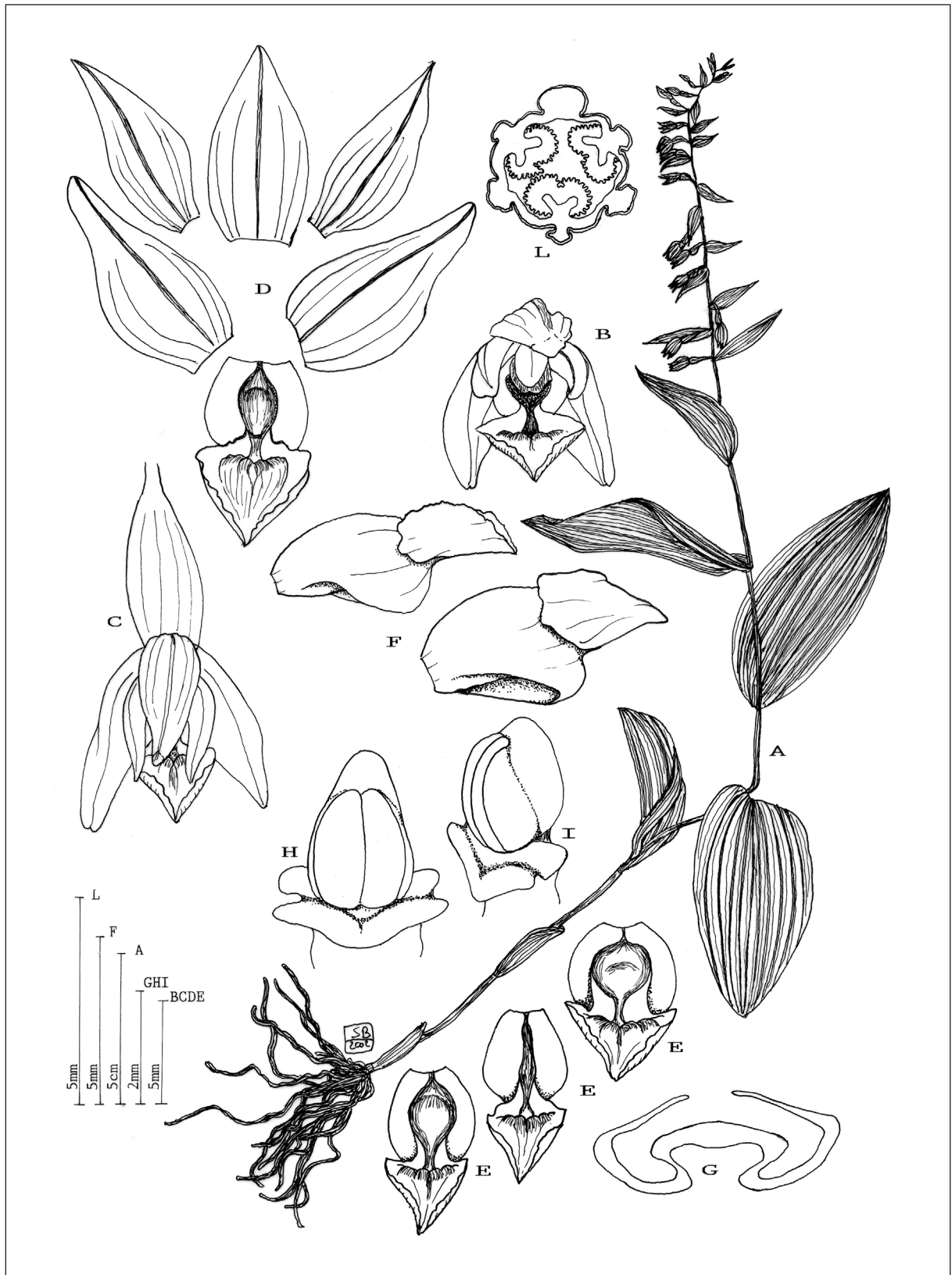


Fig. 1 – *Epipactis robertschiana* Bartolo, D'Emérico, Pulvirenti, Terrasi and Stuto. A: habit; B: flower frontal view; C: flower above view; D: flower pieces; E: labels frontal view; F: labels lateral view; G: section of hypochile; H: gynostemium frontal view; I: gynostemium lateral view.

squashed in 45% acetic acid; coverslips removed by the dry ice method and the preparations air-dried overnight. The slides were then immersed in 0.2 N HCl at 60° C for 4 min. After thorough rinsing they were incubated in 2xSSC at 60° for 1h, and then stained in 3-4% Giemsa (BDH) at pH 7 (D'EMERICO *et al.* 1999).

The nomenclature used for describing karyotype composition follows LEVAN *et al.* (1964). The karyotype was established measuring chromosomes on photographs. Chromosome pairs were identified and arranged basing on their length. Karyotype morphometric characters were evaluated by the indices A1 (degree of asymmetry in arm length within the chromosomes of the complement) and A2 (variation in chromosome lengths) (ROMERO ZARCO 1986).

C-banded karyotype was established on the basis of the previously assembled Feulgen ones, on the same individuals.

RESULTS AND DISCUSSION

Epipactis robatschiana Bartolo, D'Emérico, Pulvirenti, Terrasi and Stuto, *sp. nov.*

TYPE - Italia, Bosco di Santa Maria, Serra San Bruno (CZ), 20 July 2002, Bartolo, Brullo and Stuto s.n. (holotype CAT; isotypes CAT, FI).

E. placentina Bongiorni and Grünanger affinis, foliis caulinis ellipticis vel ovato-lanceolatis, inferioribus rotundatis vel obtusis apice, 4-10 cm longis, 2-5cm latis, floribus cleistogamis, inapertis rare leviter apertis, ovario in sectione trasversale circolari, sepalis 9-10 mm longis, viridi-luteolis, hypochilio 4.5-5 mm longo, 4-5 mm lato, leviter nectarifero introrsum flexo, in centro invaginato, epichilio cordiforme, 2.5-3.5 mm longo, 3-5 mm lato, 4 callis basalibus ab ea diversa.

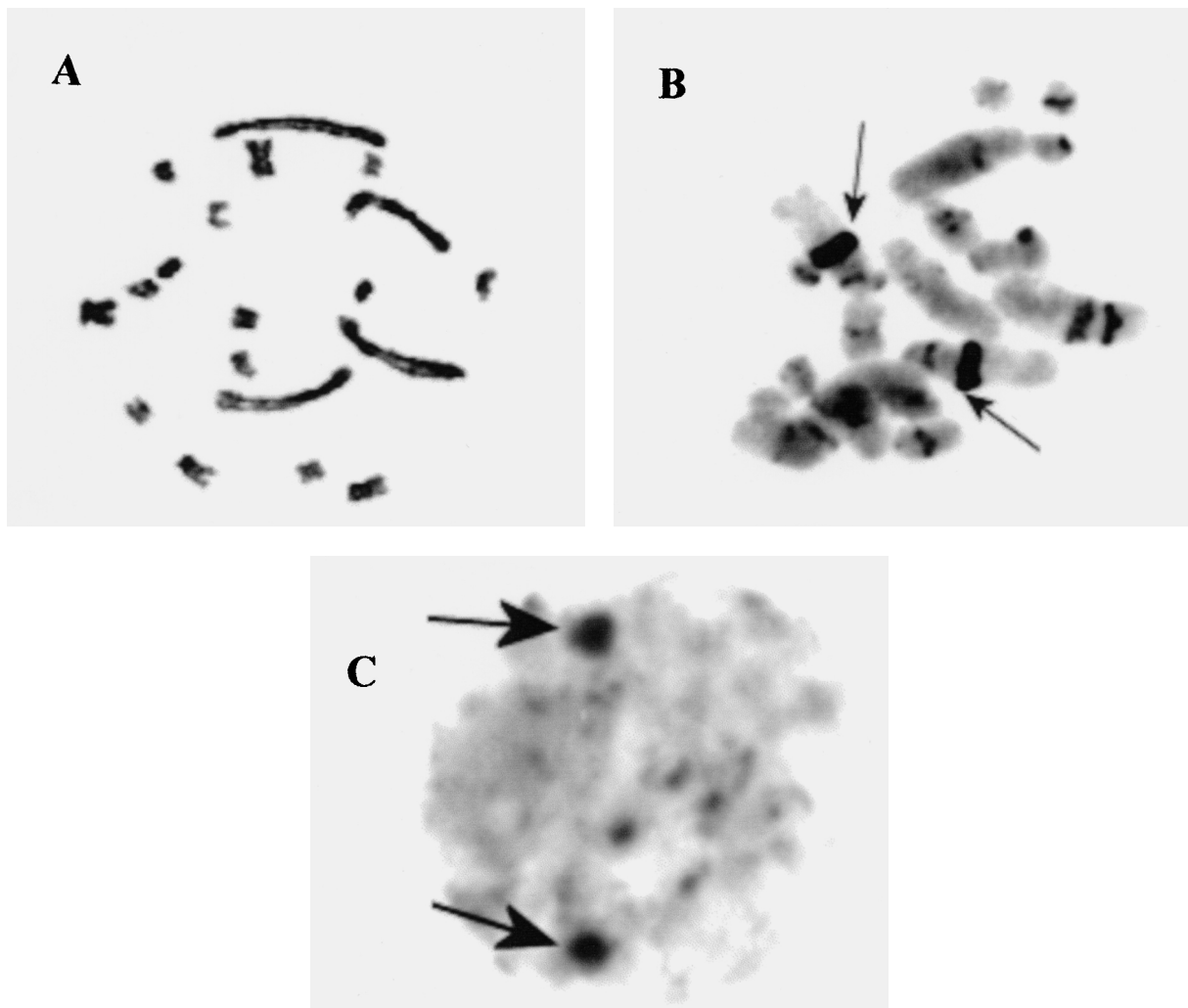


Fig. 2 – *Epipactis robatschiana*. Feulgen staining, meiotic pro-metaphase II (A); partial Giemsa C-banded mitotic metaphase showing conspicuous band in the large chromosome pair 3 (B); interphase nuclei exhibit two large chromocentres (C).

DESCRIPTION - Perennial herb, with branched rhizome. Stem (20) 25 – 40 (50) cm high, solitary, robust, erect, rigid, subglabrous to sparsely pubescent below, densely pubescent upwards, with 2-3 basal sheaths. Cauline leaves (3) 4 – 6 (7), rigid, elliptical to ovate-lanceolate, the lower ones rounded or obtuse at apex, 4-10 x 2-5 cm, the upper ones acute at apex, 4-10 x 1.5-3.5 cm.

Raceme more or less dense (7) 8-11 (12) cm long, (8) 13-20 (30) flowered; bracts lanceolate, the lower ones exceeding the flowers, the others equalling or shorter. Flowers obliquely pendent, cleistogamous, closed or sometimes slightly open. Ovary subclavate, slightly pedunculate, tuberculate, 7-10 mm long, in section circular with three very thickened and three less prominent ribs alternating.

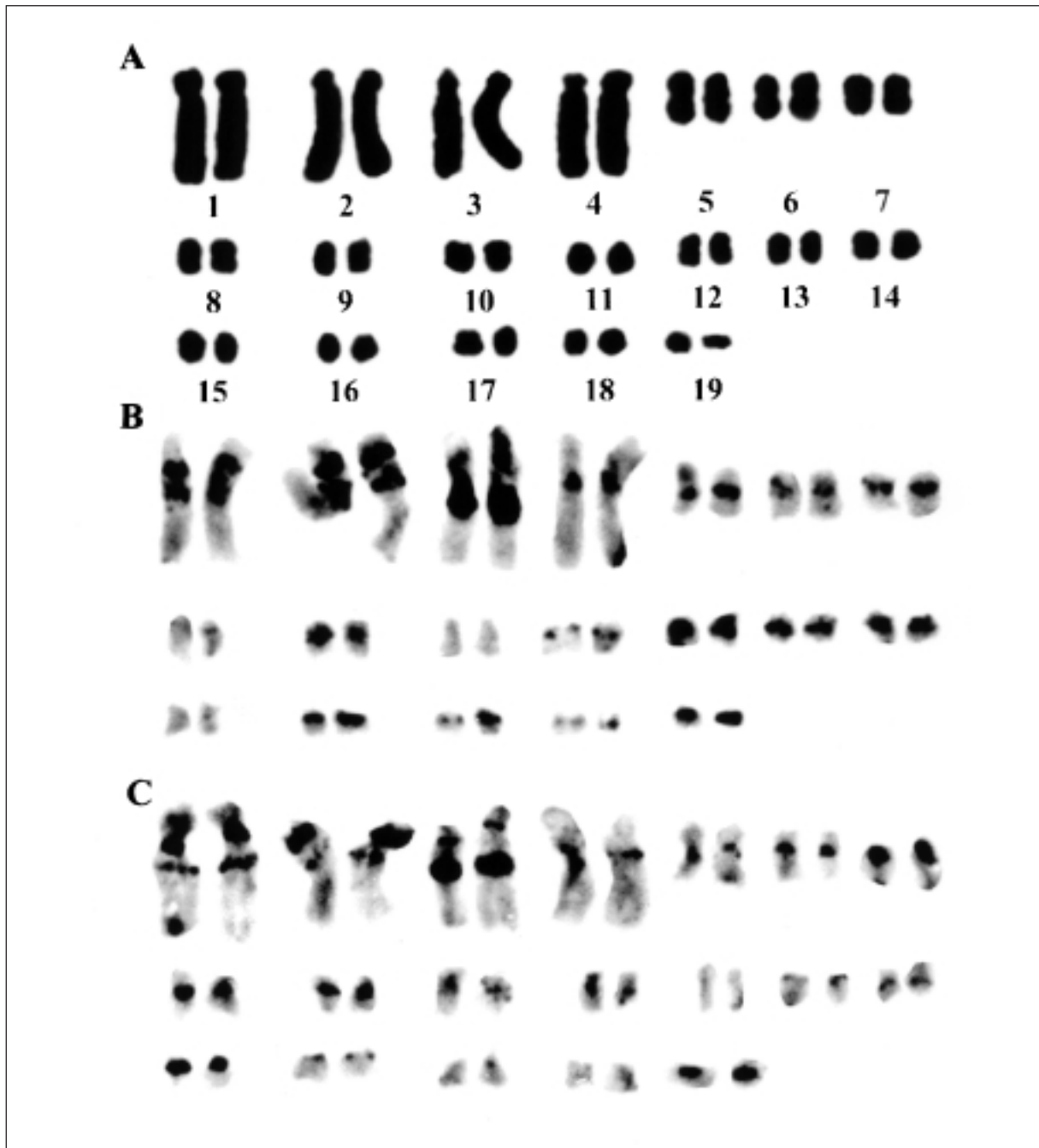


Fig. 3 – Diploid Feulgen karyotype of *Epipactis robatschiana* (A); Giemsa C-banded karyotype of *Epipactis robatschiana* (B) and *Epipactis placentina* (C).

Sepals ovate to ovate-lanceolate, green-yellowish, 9-10 x 3-5 mm, 5-veined. Petals ovate to ovate-lanceolate, green, tinged with pink in the upper part, 8-10 x 3.5-4.5 mm, 5-veined. Labellum 7-8 mm long; hypochile lightly nectarial, 4.5-5 x 4-5, pinkish outside, brown-reddish inside, invaginate at the centre, lateral margins tinged with purplish, bend inwards; epichile cordate, 2.5-3.5 x 3-5 mm, pink to white-pinkish, reflexed at the margin, with 4 rugose basal protuberances and apex never deflexed. Anther sessile, rounded at apex, leant above the stigma. Clinandrium inconspicuous. Rostellum lacking. Stigma quadrangular, subhorizontal (Fig. 1).

DISTRIBUTION and ECOLOGY - *E. robatschiana* is an orophilous species linked to underwood of *Fagus sylvatica* L. or *Pinus nigra* Arnold ssp. *calabrica* (Land.) E. Murray forests. It seems circumscribed to some mountain places of S Calabria (Serre Calabre and Aspromonte), where it grows on siliceous substrata, together with other orchids, as *Epipactis microphylla* (Ehr.) Swartz, *E. meridionalis* Baumann and Lorenz, *E. belle-*

borine (L.) Crantz s. l., *Neottia nidus-avis* (L.) L.C.M. Richard, and *Limodorum brulloi* Bartolo and Pulvirenti.

E. robatschiana is a clearly cleistogamous species, flowering in late July.

KARYOLOGY - All the investigated individuals of *E. robatschiana* showed a diploid chromosome number of $2n = 38$. Meiotic studies revealed 19 bivalents in E.M.C.s at metaphase I and $n=19$ chromosomes at pro-metaphase II (Fig. 2A). The karyotype is asymmetrical of the "bimodal" type (STEBBINS 1971) with an intrachromosomal index A1 equal to $0.34 (\pm 0.02)$ and an interchromosomal index A2 of $0.66 (\pm 0.02)$ (ROMERO ZARCO 1986). It consists of 8 large and 30 small chromosomes; the former chromosomes in the complement are subtelocentric (Fig. 3A). The karyological formula is resulted $2n=2x=38: 6M+18m+6sm+8st$.

After C-banding, somatic metaphase chromosomes showed the presence of numerous centromeric heterochromatin. Pairs 1 and 2 showed a medium-large band on the short arms and an intercalary medium-large on long arm both prox-

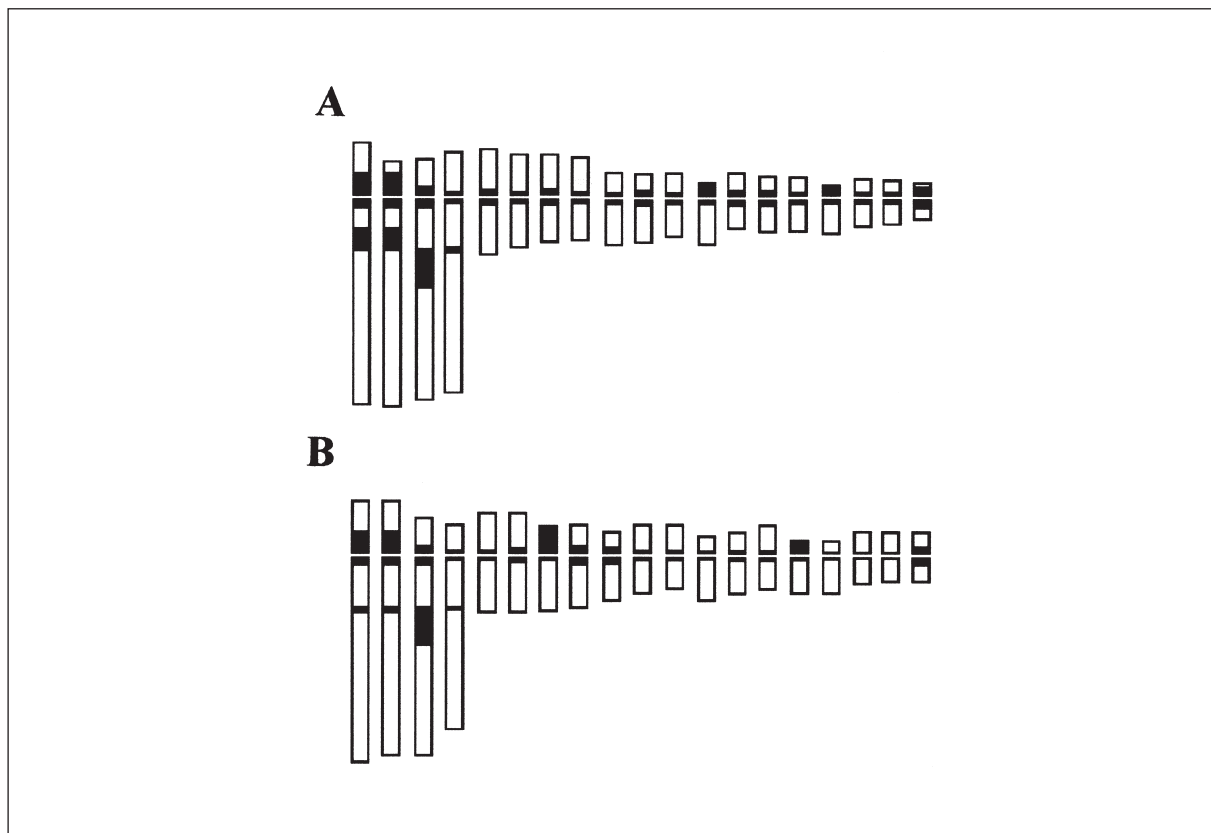


Fig. 4 – Idiograms showing basic chromosome sets and heterochromatin distribution of *Epipactis robatschiana* (A) and *Epipactis placentina* (B).

imal to the centromere, whereas pair 3 showed a very large band on the long arm (Fig. 3B, 4A). Interphase nuclei exhibited two characteristics large chromocentres equivalent to the number and size of bands detected on pair 3 (Fig. 2B, 2C). Two small chromosome pairs are characterized by heterochromatic short arm. The banding pattern of *E. robatschiana* appears to be very similar to *E. placentina* (D'EMERICO *et al.* 1999), mainly for the occurrence of similar conspicuous bands in some chromosomes (Fig. 3C, 4B). In fact, both complements of *E. robatschiana* and *E. placentina* revealed a similar distribution of heterochromatin bands in four large (pairs 1-4) and in some small chromosome pairs. However, there are significant differences in amount and distribution of heterochromatin in two chromosome pairs. These differences concern the pairs 1 and 2 of *E. robatschiana*, which have a large segment of intercalary band on the long arm, while in *E. placentina* it is very thin. In addition, in *E. robatschiana* the heterochromatin content of the karyotype-length amounts to 34.77%, while in *E. placentina* it is 26.53%.

The major amount of heterochromatin in *E. robatschiana* suggests a structural rearrangements in the chromosome complement, testifying an origin likely more recent of this species (STERGIANOU 1989; VOSA 1997; D'EMERICO *et al.* 2000).

RELATIONSHIPS - *Epipactis robatschiana* shows close relations with *E. placentina* to which it has been previously referred (ROBATSCH 1995, BARTOLO *et al.* 1996, BERNARDO and PUNTILLO 2002). Remarkable morphological differences allow to distinguish very well the two species. In particular, *E. robatschiana* shows cauline leaves elliptical to ovate-lanceolate, the lower ones rounded or obtuse at apex, 4-10 x 2-5 cm, flowers cleistogamous, normally closed or sometimes lightly open, ovary in section circular, sepals green-yellowish, 9-10 mm long, petals green-pinkish, hypochile 4.5-5 x 4-5, invaginate at the centre, with margin bend inwards, epichile pink to white-pinkish, cordate, 2.5-3.5 x 3-5 mm, with 4 rugose basal protuberances, while *E. placentina* is characterized by cauline leaves ovate to ovate-lanceolate, the lower ones acute at apex, 2.5-6.5 x 2-3.5 cm, flowers never cleistogamous and always open, ovary in section subtriangular, sepals green-reddish at margin, 7-8 mm long, petals pink-reddish, hypochile 2.5-4.5 x 3.4-4.3 mm, cup-shaped, concave, with erect margin, epichile pink-reddish, triangular, 3-4 x 3-4 mm, with 2 basal protuberances.

Besides, as already emphasized, the two species show significant differences also in the chromosome complement; regarding the shape of some chromosomes and the distribution of heterochromatin.

As concerns the other species of *E. muelleri* group, *E. robatschiana* is quite similar to *E. muelleri*, *E. komoricensis*, and *E. peitzii*, mainly for the pale colour of the flowers (green to white-pinkish), but clearly differs for the size and shape of the leaves and flower pieces. In particular, *E. robatschiana* seems to have closer relations with *E. komoricensis* (MERED'A 1996, 1998), since the latter, due to its flowers sometimes closed, is occasionally cleistogamous.

However, *E. komoricensis* differs in some morphological features, as cauline leaves normally narrowly lanceolate, 1-3.5 cm wide and acute at apex, sepals 10.5-12.5 mm long, petals yellow-greenish, 9-11 x 4-5 mm, hypochile cup-shaped, concave with erect margin, epichile triangular to cordate, flat at margin and curved dorsally, 3.5-4.3 mm long. Besides, *E. komoricensis* occurs in the calcicolous beech-forest.

EXAMINED SPECIMENS - Italia, Bosco di Santa Maria, Serra San Bruno (CZ), 18.VII.1997, Bartolo, Brullo, Scelsi and Spampinato s.n. (CAT); *ibid.*, 18.VII.1998, Bartolo, Brullo, Giusso and Pulvirenti s.n. (CAT); Strada tra Serra San Bruno e Ferdinandea, 25.VII.2001, Bartolo, Brullo, Giusso and Terrasi s.n. (CAT); Aspromonte, Caserma Forestale presso Gambarie (RC), 24.VII.2001, Bartolo, Brullo, Giusso and Terrasi s.n. (CAT); *ibid.*, Monte Basilicò (RC), 19.VII.2002, Bartolo, Brullo, and Stuto s.n. (CAT).

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