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ON THE INTERRELATIONSHIPS OF CERTAIN SPECIES OF PETUNIA III. THE POSITION OF P. LINEARIS AND P. CALYCINA

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SUMMARY

Two species of *Petunia*, *P. linearis* and *P. calycina* were grown from seed collected in the wild. Their chromosome number (2n = 18) and karotype morphology have been studied. Both species seem to be related to *P. parviflora*, which found support in peroxidase isozyme data. The three 2n = 18 species differ considerably from the *Petunia* species with 2n = 14 chromosomes.

1. INTRODUCTION

In his review of the genus *Petunia*, FRIES (1911) gives rather a broad definition of the genus. In a former contribution, the species *P. axillaris* and *P. integrifolia* have been investigated and have been found to be closely related (WIJSMAN 1982, 1983). They can be hybridized, produce viable and fertile F1-hybrids, and have 14 chromosomes. By contrast, *P. parviflora* has 18 chromosomes, and cannot be hybridized with either of the former two (FERGUSON & COOLIDGE 1932; SINK & POWER 1978; own observations).

When we got seeds from other species from South America it seemed of interest to see whether the plants grown up from these seeds could be hybridized to *Petunia hybrida* or to one of its parental species as well as to compare their chromosome number and morphology. The two species *P. linearis* (Hook.) Paxt. and *P. calycina* Sendtn. have very seldom been mentioned in the literature and deserve fuller treatment.

2. NAMES, MATERIAL, METHODS

2.1 List of names

The sources of the relevant names used in the text are given below. For a full synonymy we refer to FRIES (1911).

Petunia Jussieu, Ann. Mus. Hist. Nat. 2 (1803) 215–216, Pl. XLVII.
Type of genus: *P. parviflora* Jussieu, l.c.
P. linearis (Hook.) Paxt., Mag. Bot. 2 (1836) 219.
Salpiglossis linearis Hook., Bot. Mag. 58 (1831) t. 3113;

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extension in Hooker, Bot. Mag. 60 (1833) t. 3256.
P. calycina Sendtn., in Martius, Flora brasiliensis X (1846) 173.
P. parviflora Jussieu, l.c.

2.2 Herbarium material

The following herbaria were visited for study of their *Petunia* material: Kew (K), British Museum (BM), Leiden (L), Utrecht (U). In addition material from München (M) and photographs from Genève (G) were received.

P. linearis is rare in collections. The type (K, no further data on the label) could be inspected as well as a sheet leg. Tweedie from (banks on the R.) Uruguay, which may come from the same source as the type, since Tweedie collected the seeds for the type described by HOOKER (1833). Further material: Lorentz, anno 1878, Concepcion del Uruguay, Entrerios, Argentina (K, M); Pedersen 12678, Est. Yuqueri, Concepcion, Corrientes, Argentina, 1980 (herb. Wijsman).

P. calycina material from Rio Grande do Sul, Brazil, has been inspected in K and U; as well as material mentioned by Fries (Malme 100, anno 1892, M). In addition: Pedersen 12605, Sao Lourenco do Sul, Rio Grande do Sul, Brazil, 1979 (herb. Wijsman). The Sello type was in Berlin, and has been lost; but a photograph from G has been inspected.

P. parviflora is plentiful in the herbaria. The type has been figured by JUSSIEU (1803). The type of the synonym *P. ovalifolia* Miers (K) was inspected as well.

2.3 Living material

The material consisted of inbread lines of the Institute of Genetics, Amsterdam, as well as of plants grown from seeds collected in the wild.

P. linearis:	S11 (1980), not yet inbred, Corrientes, voucher specimen Pedersen 12768.
P. calycina:	plants E3065 (1981), Rio Grande do Sul, voucher specimen Pedersen 12605.
P. parviflora:	S4, from Royal Botanic Gardens, Kew, inbred since 1960.
P. axillaris:	S2, from seed collected in Uruguay, inbred since 1958.
P. integrifolia:	S12, from seed collected near Porto Alegre, Rio Grande do Sul, Brazil, inbred since 1980 (ssp. integrifolia)
	S9, kindly sent by Drs Cornu and Maizonnier (Dijon, France), inbred since 1977 (ssp. inflata).

2.4 Methods

Chromosome counts from root tips, as well as peroxidase staining after electrophoresis of extracts of leaf or flower were according to VAN DEN BERG & WIJSMAN 1981. C-banded chromosome preparations of root tip meristems were obtained according to the BSG technique of DIETRICH et al. (1981).

3. RESULTS

3.1 Morphology *(Figs. 1, 2, 3)*

P. linearis (fig. 1) has narrow, linear leaves (32 × 5 mm). The flowers have a diameter of 27 mm; the colour of the limb is between HCC629 and 630 (rhodamine purple and cyclamen purple, respectively) with a bright yellow tube. The tube

is rather wide, though not yet to a degree as in *P. integrifolia.* The style is curved and bent down at the end and the stigma grooved so as to look nearly bilabiate (as in *P. axillaris*, though infinitely smaller). The coloured illustrations from 1833 (HOOKER), and 1837 (LINDLEY) render the species carefully (PAXTON's illustration (1836) has the flowers much too dark).

P. calycina (figs. 2 and *3)* has ovate leaves (12 × 29 mm); the long stems lie flat (though Fries makes mention of other varieties with erect stems). The flowers are very weakly coloured when fully expanded though in the tube dark veins are present, slightly treading out into the tube; the diameter is about 30 mm. When young the colour is HCC532 (petunia purple, light greyish). In the morning the flowers are slightly closed, to open after noon. The stigma is broad but not grooved. FRIES (1911) has synonymized *P. ovalifolia* Miers with *P. calycina*; the illustration of the former species in MIERS (1850) closely resembles our material, though the latter differs in having glabrous leaves. However, polymorphism for the latter character has been mentioned and described by Fries. Moreover, in the type (K) the leaves are glabrous as well.

P. parviflora (fig. 1) has a low creeping habit with tiny flowers (length 10 mm) and small narrow leaves (16 × 3 mm). Their colour resembles that of *P. linearis* to a remarkable degree. In our inbred line they are virtually not opening though FRIES (1911) illustrates an open flower. The style is slightly bent and the stigma is broad but not grooved.

All three species have strongly didynamous stamina and yellow pollen. In all three species at least in the lower part of the branches at the basis of the leaves young sprouts look like stipules. This is a habit also present in, e.g., *P. caesia* Sendtn. (chromosome number as yet unknown), but absent from the 2n = 14 species (*P. axillaris* and *integrifolia*).

3.2 Distribution

P. linearis and *P. calycina* are some of the geographically very restricted *Petunia* species. FRIES (1911) only described material of *P. linearis* from Concepcion del Uruguay (Entrerios, Argentina), from where the types may also come; Corrientes, Argentina (Concepcion, Bella Vista). As to *P. calycina*, Fries only mentions Rio Grande do Sul, Brazil, and Misiones, Argentina, which is based on five collections. The present study adds one collection from Rio Grande do Sul. Neither species has been mentioned in the flora of Santa Catharina (SMITH & DOWNS 1966). By contrast, *P. parviflora* is widespread, and common in herbaria.

3.3 Karotype morphology

Squash preparations of root tip meristems revealed chromosome numbers of 2n = 18 for the species *Petunia linearis, P. parviflora* and *P. calycina.* Of the former species, sufficient metaphase plates were obtained for a detailed study of its karyotype. A representative example is given in *fig. 4.* Length varies from 3.5 to 2.0 μm in completely contracted metaphase chromosomes, whereas the centromeric index (s/l+s) lies between 0.5 and 0.3, indicating median to submedian centromere positions.

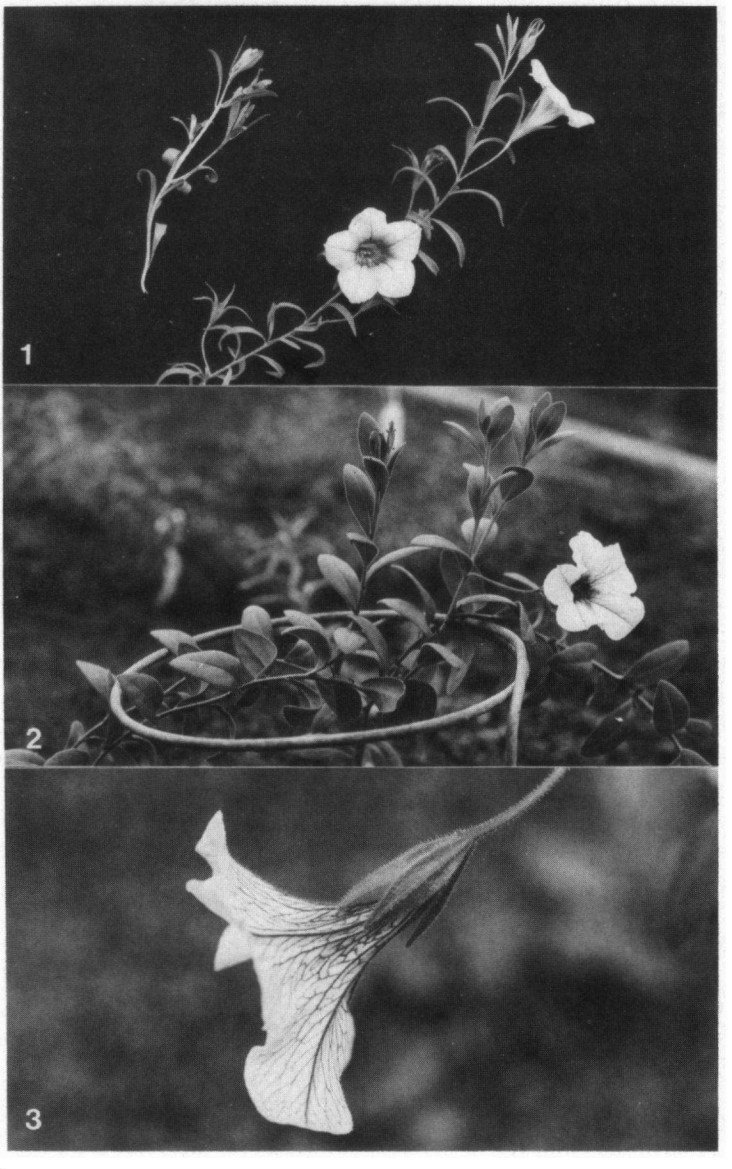

PLATE I.
Fig. 1. Right: *Petunia linearis*, top of a flowering plant; Left: *Petunia parviflora*. The mature flower at the left side did not expand further.
Fig. 2. *Petunia calycina*, general habit (natural position: prostrate).
Fig. 3. *Petunia calycina*, corolla and calyx of a fully expanded flower.

In some of the metaphase plates, one or two chromosomes with microsatellites were observed. These NO-chromosomes could be disposed as the seventh or

eight pair in the karyotype with the chromosomes arranged in order of decreasing length.

The karyogram of *P. linearis* was compared to the karyotype of *P. hybrida* (data derived from SMITH et al. 1973). Size and location of the constitutive heterochromatin in metaphase chromosomes were studied at the hand of C-banded root tip preparations of *P. linearis*. Though mitotic activity was low and the results of the C-banding procedure were rather poor, we obtained evidence for the occurrence of obvious telomeric C-bands at the short arm of two chromosomes, which are assumed to correspond to the nucleolar organizing regions of the satellite chromosomes, and telomeric bands both at the short and the long arm of most other chromosomes. Centromeric bands were very small or absent in all cases. An example of C-banded chromosomes is given in *fig. 5*.

3.4 Crosses. Interspecific hybridization

The chromosome number 2n = 18 suggested that the three species *P. linearis, P. calycina,* and *P. parviflora*, are more closely related to each other than to the 2n = 14 species *P. axillaris* s.l., *P. integrifolia* s.l., and their offspring, *P. hybrida*. However, efforts to hybridize the 2n = 18 species have failed, either to 2n = 14 species or to 2n = 18 species. However, there is one exception. The cross *P. linearis* × *P. calycina* yielded seeds, and the reciprocal cross as well. The young plants from these seeds have an intermediate habit between the two parents, but leaning to *P. calycina* (leaves more oblong). The supposed hybrids will be studied further.

As to self-propagation, our inbred line S4 (*P. parviflora*) has been grown from seed for 20 years without artificial pollination or noticeable contamination (by *P. hybrida*), and is obviously self-fertile. In *P. linearis* we observed self-incompatibility. Seeds from compatible combinations up to now only occasionally germinated. However, spraying the seeds after sowing with 2 ml gibberellic acid (GA3, 100 mg/l) per pot, greatly improved germination. Plants of *P. calycina* were self-sterile, and we have not found a compatible combination because too few plants were involved.

3.5 Biochemical data

As to the pigments in the flower, *P. linearis* has malvidin aglycons in the flower limb, and petunidin in the yellow flower tube, but *P. calycina* has petunidin all over, the same distribution as in certain mutants (mf^-) of *Petunia hybrida* (WIERING & DE VLAMING, 1977). Both species contain quercetin flavonol.

Nearly the only plant of *P. linearis* germinating from untreated hybrid seed harvested in our greenhouse has an albino flower, suggesting the presence of an albino mutation in the wild parental population.

By starch gel electrophoresis the peroxidase isoenzymes have been investigated (*fig. 6*). *P. linearis* and *P. calycina* show a peroxidase pattern that has much in common.

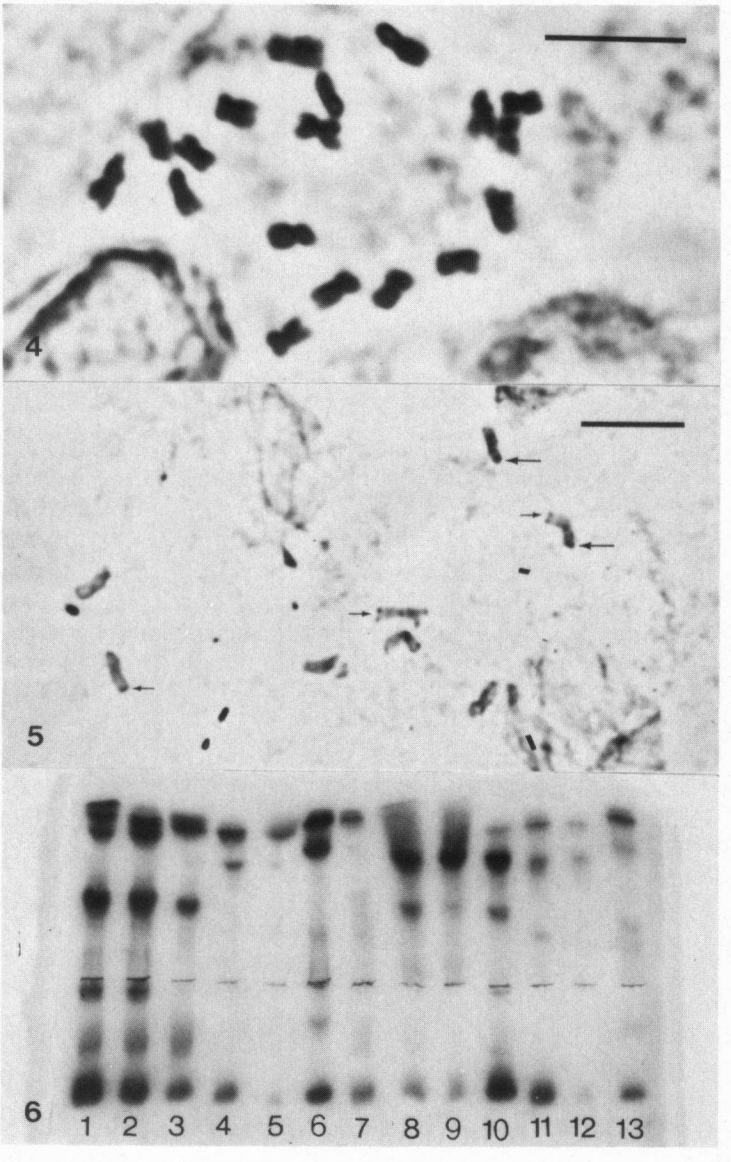

PLATE II.

Fig. 4. Karyogram of *P. linearis* (2n = 18). Orcein stained squash preparation. Bar equals 10 μm.

Fig. 5. Incomplete metaphase plate of *P. linearis* stained according to the C-banding technique. The large arrows indicate the C-bands of the nucleolar organizing region of the satellite chromosomes. The small arrows show distinct telomeric C-bands. Bar equals 10 μm.

Fig. 6. Electrophoretogram of peroxidase isoenzymes in several species of *Petunia*.
1–7: leaves, 8–13: flowers. Positions from left to right: 1, 2, 8, 9: *P. integrifolia* spp. *inflata*, line S9. 3, 10: *P. axillaris* spp. *axillaris*, line S2. 4, 11: *P. linearis*. 5, 12: *P. calycina*. 6, 13: *P. parviflora*. 7: somatic hybrid *P. parviflora* + *P. axillaris* spp. *parodii*.

3.6 Ecology

P. linearis is a common plant in Prov. Corrientes, Argentina, and was collected on rather low land, in open scrub, together with such species as *Copernicia alba, Bromelia balansae, Lithraea molleoides, Schinus* sp., *Sapium haematospermum, Gleditsia amorphoides, Cestrum strigillatum, Acacia bonariensis, Schistogyne decaisneana, Fagara hiemalis, Myricia* sp.?, *Eupatorium clematideum, Pterocaulon virgatum, Scoparia montevidensis*; there is practically no grass cover, a *Digitaria, Distichlis spicata,* and *Cynodon maritimum* may be seen here and there. The soil is a very heavy clay, with a thin cover of whitish sand.

P. calycina was found growing along the roadside in medium dry grassland with rather compact soil, accompanied by species as *Eryngium* spec., *Schlechtendalia luzuliflora,* and *Vernonia megapotamica.*

P. parviflora seems to be hygrophilous, and is restricted to clay. Because of its small size, it cannot compete with grass, and is restricted to bare patches, where it can thrive and survive, though flooded by up to 10 cm of water. The flowers open about noon, and close in the afternoon. The accompanying species are much the same as with *P. linearis*; the following can be added: *Atriplex montevidensis, Sesuvium portulacastrum, Scerophylax lorentzii, Diplachne uninervia, Sporobolus pyramidatus.*

DISCUSSION

We feel confident of the identification of the species mentioned. As to our *P. linearis* material, the flowers are slightly larger than in the Lorentz specimens, but the related species *P. heterophylla* Sendtn. is not involved, because no "heterophylly" to the same extent as in the type of *P. heterophylla* (photograph in G) is present.

Apparently, the three 2n = 18 species cannot be crossed to the 2n = 14 species, nor be intercrossed, with the exception of *P. linearis* and *P. calycina.* As to *P. linearis,* in 1837 HERBERT wrote: 'I cannot cross it with other sorts of Petunia. It will belong to at least a separate section of Petunia with linear leaves". So the answer has been given to BAILEY's (1896) question: "it would be interesting to know if Petunia intermedia [= P. linearis], which was introduced about the same time as P. violacea [= P. integrifolia], and which appears to be lost to cultivation, entered into any of these early hybrids [= P. hybrida]".

Propagating self-incompatible species like *P. integrifolia, P. linearis* and *P. calycina,* is difficult and in this light Bailey's remark about the loss of *P. linearis* to cultivation can be readily understood. By contrast, PAXTON (1836) wrote about the same species: "In 1833 flowers produced seeds very freely... we may expect erelong to find it one of the most conspicuous ornaments in our flower garden". Moreover, FRIES (1911) refers to herbarium material from cultivation "from several gardens". Could the self-propagational barrier be overcome, well might horticulture profit. The main problem, however, is that the seeds obtained are very refractory to natural germination.

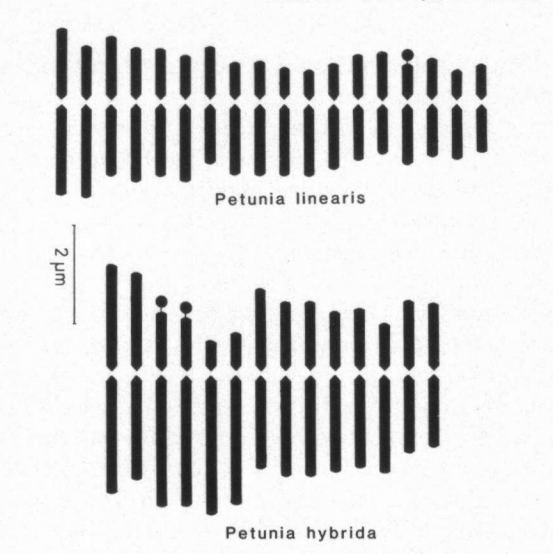

Fig. 7. Comparative schematic representation of karyograms of *P. linearis* and *P. hybrida*. In the corresponding metaphase of *P. linearis*, only one chromosome was observed with a satellite. The chromosomes are arranged in order of decreasing length. In *P. hybrida*, the chromosomes are arranged according to SMITH et al. (1973).

The (sterile) 2n = 32 somatic hybrid, *P. axillaris* spp. *parodii* + *P. parviflora*, as constructed by POWER et al. (1980) and kept in our greenhouses, has a remarkable semblance to *P. linearis* though the flowers are smaller (diameter 17 mm).

Chromosome counts revealed somatic chromosome numbers of 2n = 18 for *P. linearis*, *P. parviflora* and *P. calycina*. Analysis of their chromosome portraits showed rather homogeneous karyotypes with metacentric to submetacentric chromosomes varying in length from 3.5 to 2.0 µm (see idiogram of *P. linearis*, *fig. 7*). Comparison between the species made clear that chromosome morphology may differ only slightly. Most interesting is the finding that the karyotypes of these three species as a group differ in several respects from the 14-chromosomes species of the genus *Petunia*. Firstly, there is a significant difference of absolute chromosome length between both groups: the chromosomes of the 18-chromosome species are about half the length of *Petunia hybrida* (2n = 14)chromosomes (4–7 µm). There is no evidence for assuming variation in chromosome morphology due to alterations in the position of the centromeres. Comparison of the idiograms of species from both groups demonstrates that centric fusions probably did not play a role in the evolutionary divergence of *Petunia*.

Secondly, confirming data about absolute chromosome size differences between both groups came from WHITE & REES (1983) who found total DNA content in 2C nuclei of *P. parodii* (2n = 14) and *P. parviflora* to be 3.0 and 1.6 pg, respectively. One may speculate that the variation in nuclear DNA content can have an effect on the amount of highly repetitive sequences and, as a consequence, the size of the constitutive heterochromatin segments. DIETRICH et al.

(1981) have shown that the distribution of the C-band heterochromatin in *P. hybrida* is restricted to the centromere regions and to the NORs of the satellite chromosomes. This was confirmed by own data of pachytene chromomere distribution and that of BROWN (1966) and GOTTSCHALK (1953). In our study of C-banding patterns in *P. linearis*, we expected minor bands in the centromere regions only. However, our tentative results unequivocally demonstrate the presence of distinct bands at the telomeres. The significance of this banding pattern recommends C-banding analyses in *P. parviflora* and *P. calycina* and the study of pachytene chromomere patterns at all 18-chromosomes species. All together, chromosome morphology of these *Petunia* species differs in several respects from *P. hybrida* and related species. Additional karyotype data might give better insight into the phylogenetic relationship between these species and other representatives of the Solanaceae (cf. GOTTSCHALK 1953).

Differences at the biochemical level between *P. axillaris* spp. *parodii* and *P. parviflora* had already been mentioned. KUMAR et al. (1981) described differences in the "fraction 1 protein" (ribulose 1,5-bisphosphate carboxylase), small subunit, of which *parodii* shows two polypeptides, but *parviflora* three (different) polypeptides. At the chloroplast genome level, another difference between the 2n = 14 species and *P. parviflora* was detected, the latter having a small difference in the physical map in both inverted repeat regions (KUMAR et al. 1982).

The implication is that *P. parviflora* is only distantly related to the 2n = 14 species. From the present study we infer that *P. linearis* and *P. calycina* are more closely related to *P. parviflora*. The relationship is stressed by protein data. It would be interesting to interpret the (similar) peroxidase patterns of *P. linearis* and *P. calycina* in terms of the defined genes of 2n = 14 *Petunia* (WIJSMAN, 1983). Whether the second anodal band (from the front) is homologous to PRXb or to PRXf (expressing itself in leaves) cannot be said, but the combination of bands certainly is more than just a mutant pattern; it must be based on a combination of mutations, and therefore, a common pattern is an indication of shared descent.

We conclude that the 2n = 14 species related to *P. integrifolia* on the one hand, and the 2n = 18 species related to *P. linearis* on the other hand represent two species groups in an advanced degree of evolutionary divergence.

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