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Evidence for Conspecificity of *Piper methysticum* Forst. f. and *Piper wichmannii* C. DC.

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Key Word Index—*Piper methysticum*; *Piper wichmannii*; Piperaceae; kavalactones; isozymes; cytology; genetic variation.

Abstract—Morphological, chemical, cytological and genetic evidence demonstrating the absence of taxonomic distinction between *Piper methysticum* and *Piper wichmannii* are reviewed. *Piper methysticum* is not a separate species, but rather a group of sterile cultivars selected from somatic mutants of *P. wichmannii*. As *P. methysticum* was described first (1786), it has priority and *P. wichmannii* (1910) is superfluous. A new subspecific classification is suggested that makes a distinction between the sterile cultivars (*P. methysticum var. methysticum*) and the wild populations (*P. methysticum var. Wichmannii*). Copyright © 1996 Published by Elsevier Science Ltd

Introduction

Kava, *Piper methysticum* Forst. f., is cultivated to make a psychoactive drink which is prepared by grinding and soaking the roots of this perennial shrub. It is an attractive cash-crop and dry roots are exported to the pharmaceutical industry that extracts active ingredients with physiological properties called kavalactones. It is also the only cultivated plant of economic importance with an area of distribution restricted entirely to the Pacific Islands, from Papua New Guinea to Hawaii.

Piper methysticum was first validly described by Forster (1786) who accompanied Cook's second voyage (1772–1775) as a botanist. There are a few botanical synonyms of *P. methysticum*, most of them merely listed without description, and hence of no botanical significance, or later than Forster's binomial of 1786. Another botanical species name has been applied to kava, *Piper wichmannii* C. DC., comprising the seed-producing wild forms (synonyms are *P. erectum* C. DC., *P. schlechteri* C. DC., and *P. arbuscula* Trelease). *Piper wichmannii* was first validly described by De Candolle (1910) when he was reviewing the Piperaceae family of Papua New Guinea. Both species, *P. methysticum* and *P. wichmannii*, have been shown to biosynthesize kavalactones and to include a range of chemotypes with various physiological properties (Lebot and Lévesque, 1989).

The aim of the present paper is to review morphological, chemical, cytological and genetic evidence, implying the absence of taxonomic distinction between these two binomials, and to suggest a new classification.

Materials and Methods

Herbaria specimens. The major world herbaria were either visited or invited to list their specimens of *Piper methysticum* and *P. wichmannii* (Kew, London, Leiden, Paris, Kuala Lumpur, Bogor, Brisbane, Sydney, Christchurch, Missouri and Harvard). Data from these specimens were compared with collections from smaller Pacific herbaria located in the Solomons, Vanuatu, Fiji, New Caledonia, Tahiti and Guam (Lebot, 1989).

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Ecogeographical survey of germplasm. A survey of the genetic resources of the plant species *Piper methysticum* and *Piper wichmannii* was conducted over the whole of Oceania and more than 300 accessions were collected from 42 Pacific islands. Morphological descriptors were used to describe accessions in the field and in germplasm collections and permitted a quick and easy differentiation of morphotypes (Lebot and Lévesque, 1989).

Chemical analysis. More than 250 accessions, originating from 51 islands of the Pacific and corresponding to about 121 different cultivars of *P. methysticum* and 25 wild forms and progenies of *P. wichmannii*, were analyzed for the chemical composition of their roots. Six major kavalactones were identified and quantified: 1 = demethoxy-yangonin (DMY, syn. = 5,6-Dehydrokavain), 2 = dihydrokavain (DHK), 3 = yangonin (Y), 4 = kavain (K), 5 = dihydromethysticin (DHM) and 6 = methysticin (M). The same type of roots were systematically selected for each plant. Extraction was performed on powdered dry roots which were placed in a Soxhlet apparatus for 6 h with chloroform. The extract was analysed by using HPLC (Lebot and Lévesque, 1996). Each extract's composition was coded in decreasing order of the proportion of each lactone present.

Isozyme electrophoresis. Leaf tissues of more than 300 accessions collected on 35 Pacific islands were analysed for isozyme variation in eight enzyme systems including aconitase (ACO), aldolase (ALD), diaphorase (DIA), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM). The buffer system was histidine citrate and samples were loaded onto starch gels and electrophoresed at 4°C during 6 h. The gels were scored for the presence or absence of electromorphs (Lebot *et al.*, 1991).

Chromosome counts. Cytological examination of *P. methysticum* cultivars and of *Piper wichmannii* (wild forms) originating from Papua New Guinea, Vanuatu, Fiji, Samoa, Hawaii, and Pohnpei was conducted to study possible variation. Counts were performed at mitosis on root tips and at meiosis on anthers (Lebot *et al.*, 1991).

Results

Area of distribution

A comprehensive bibliographical review and a study of herbarium specimens have allowed us accurately to identify the areas of distribution of *P. methysticum* and *P. wichmannii* (Lebot, 1989) (Fig. 1).

Piper methysticum specimens have been collected in Micronesia (Pohnpei, Palau and Guam); in Polynesia (Oahu, Molokai, Kauai, Maui, Hawaii, Nuku Hiva, Fatu Hiva, Uapou, Raiatea, Tahiti, Mangaia, Rarotonga, Aitutaki, Niue, Upolu, Savai'i, Tau, Tutuila, Tongatapu, Vava'u, Eua, Wallis, Futuna, and Alofi); and in Melanesia (Vanua Levu, Viti Levu, Vanua Balavu, Lakeba, Rewa, Tanna, Anatom and Pen-

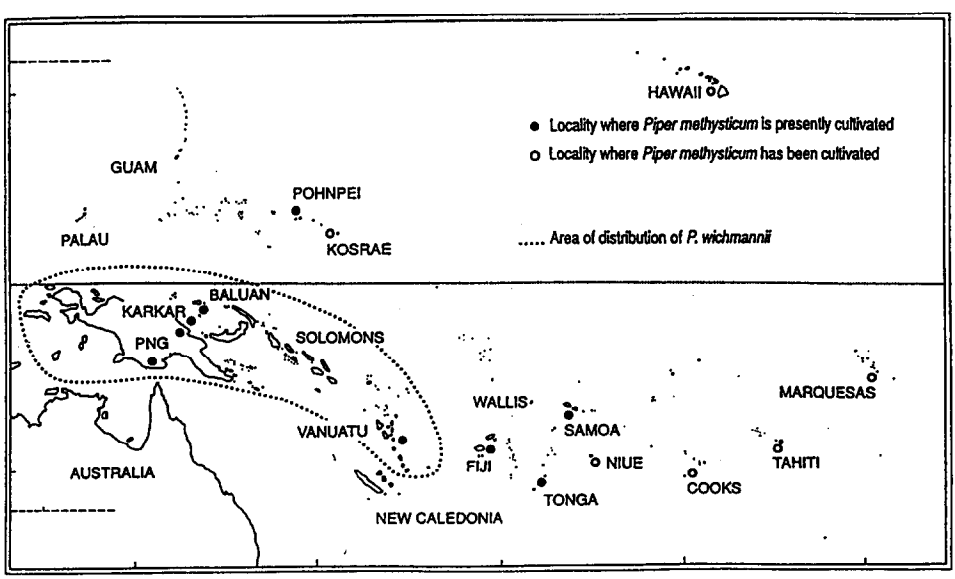


FIG. 1. AREA OF DISTRIBUTION OF *P. WICHMANNII* AND *P. METHYSTICUM*.

tecost). Only 13 specimens were seen from Papua New Guinea and three from Irian Jaya, on the southern border with Papua New Guinea. These were collected in Western Province, Lake Kutubu, Baluan, Karkar and Madang at the beginning of the century. The western edge of the distribution area for *Piper methysticum* is Irian Jaya, while the eastern boundary is the Marquesas. This species has never been collected in Indonesia, the Philippines or South America.

None of the collected specimens of *Piper methysticum* had seeds and female plants were uncommon. *Piper methysticum* is dioecious, producing male and female inflorescences on separate plants, but it does not reproduce sexually. When hand-pollinated, female inflorescences fall off before they produce fruit. Growers are unanimous in stating that no fruits or seeds have ever been seen on any *P. methysticum* plant. The information on specimens recorded by collectors demonstrates that, although kava has never been collected in undisturbed habitats, female plants, albeit rare, do occur in cultivation.

Piper wichmannii specimens have been collected from Papua New Guinea, the Solomons, and Vanuatu where it is common at elevations around 800 m. This species has not been collected anywhere else in the Pacific and Asia. Observed inflorescence of *P. wichmannii* showed a very good fruit set on crowded spikes. Fruits are very small but not easily dispersed by the wind. They remain on the mature inflorescence until it falls to the ground. Bats, which have been observed eating the long (up to 30 cm) inflorescence of *P. wichmannii*, could be responsible for its dispersal in the forest and from island to island.

Morphological evidence

Forster (1786) described *Piper methysticum* as follows: "Pepper, cordate, acuminate, and multiveined leaves with axillary, leafy, very short, pedunculate, and very broad spikes." *Piper methysticum* is a shrubby plant measuring from 1 to over 4 m in height. It is a hardy slow-growing perennial, generally resembling other Piperaceae. When it reaches maturity, the plant takes the form of a bouquet of ligneous stems clustered together at their base. Cultivars of *P. methysticum* exhibit tremendous variation of qualitative traits (e.g. pigmentation of stem internodes and leaves) and about 117 distinct morphotypes have been identified using seven morphological descriptors (Lebot and Lévesque, 1989).

Piper wichmannii is also a shrub similar to *Piper methysticum* in growth patterns and morphological features. The inflorescences are as long as the leaves, with peduncles shorter than the petioles (" ... male flowers 2-staminate; stamens 0.5 mm long; anthers reniform, dehiscing apically; filaments short, broad, and stout. Female flowers sessile; stigmas 3-fid, subsessile; bracts round, peltate, long pedicillate. Fruits sessile, somewhat obconical, free at maturity" (Chew, 1972)). No significant difference in either the male or the female flowers has been found between *Piper methysticum* and *Piper wichmannii* specimens.

Chew (1972) stated that *P. wichmannii* and *P. methysticum* are dioecious, but our field observations have revealed that monoecious plants also exist for the latter species, suggesting that the same phenomenon could occur for *P. wichmannii*. The major morphological difference between these two entities is the length of the inflorescence, which for *P. wichmannii* is as long as the lamina. Variability in the inflorescence length for cultivars of *P. methysticum* is also observed (from 6 up to 20 cm), but is always shorter than the lamina. Usually any particular form, wild or cultivated, is assumed to belong to the botanical species *P. wichmannii* when the spadix is as long as the lamina and the plant is erect with few stems.

There are minor differences between the two taxa in root characteristics. The tissue of *P. wichmannii* is noticeably harder than that of *P. methysticum*, and the proportion of woody elements is higher. The woody elements of *P. wichmannii* are

scattered around lumened tracheids and the parenchymatic tissue occupies a comparatively small area. Bark of *Piper wichmannii* possesses large, connected bands of brachysclereids, but the bark parenchyma of *Piper methysticum* cultivars contains nearly separate brachysclereids. *P. methysticum* rootstock is characterized by extraordinarily wide medullary ray segments.

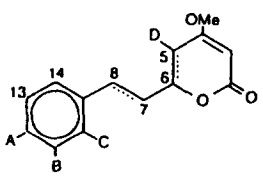
In fact, morphological differences between *P. wichmannii* and *P. methysticum* (e.g. pigmentation of stem internodes, leaf coloring or pubescence on lamina, woody elements of the roots) are no more significant than those existing between different cultivars of *P. methysticum*.

Chemical evidence

Apart from *P. methysticum* and *P. wichmannii*, the only *Piper* species that produces similar compounds is *P. sanctum*, from which one minor kavalactone, 5-methoxy-5,6-dihydromethysticin, has been isolated (Sengupta and Ray, 1987).

The six major kavalactones (Fig. 2) represent over 96% of the lipid extract. The extent of chemical variation existing within *P. methysticum* and *P. wichmannii* is presented in Table 1. When different cultivars of *P. methysticum* are planted together and uprooted from the same plot, they exhibit considerable chemical variation between cultivars (Lebot and Lévesque, 1989, 1996). These results indicate that the variability in chemical composition is controlled by genotype rather than by external factors.

Variability in chemical composition is presented in Table 2. Chemotype variability is not due to the geographic origin of the cultivars as most clones have been widely distributed (Lebot *et al.*, 1992) throughout the Pacific Islands. Variation between plants of the same cultivar is limited. These results show that kavalactone composition is very homogeneous within the clone and that chemotype is consistent. Kavalactone content is highest after 18 months on average, and this



	A	B	C	D	C5-C6	C7-C8
. Kavain (4)					–	=
. 7,8-Dihydrokavain (2)					–	–
. 5,6-Dehydrokavain (1)					=	=
. Yangonin (3)	OMe				=	=
. 5,6,7,8-Tetrahydroyangonin	OMe				–	–
. Methysticin (6)	$O\text{-}CH_2\text{-}O$				–	=
. Dihydromethysticin (5)	$O\text{-}CH_2\text{-}O$				–	–
. 5,6-Dehydromethysticin	$O\text{-}CH_2\text{-}O$				=	=
. 5,6-Dihydroyangonin	OMe				–	=
. 7,8-Dihydroyangonin	OMe				=	–
. 10-Methoxy-yangonin	OMe		OMe		=	=
. 11-Methoxy-yangonin	OMe	OMe			=	=
. 11-Hydroxy-yangonin	OMe	HO			=	=
. Hydroxykavain				OH	–	=
. 11-Methoxy-12-hydroxy-dehydrokavain	OH	OMe			=	=

FIG. 2. MOLECULAR STRUCTURE OF KAVALACTONES.

TABLE 1. CHEMICAL VARIATION WITHIN *P. METHYSTICUM* AND *P. WICHMANNII*

Kavalactones:	DMY (1)	DHK (2)	Y (3)	K (4)	DHM (5)	M (6)	KL%
P. methysticum ($n=121$)							
Mean	07.98	28.93	10.29	20.26	14.07	18.14	11.31
STD	02.69	08.60	03.49	06.66	04.95	05.32	03.76
CV%	33.76	29.75	34.00	32.87	35.21	29.36	33.26
P. wichmannii ($n=25$)							
Mean	19.49	18.94	07.28	05.19	31.84	16.95	08.41
STD	11.11	09.04	02.84	03.35	13.94	07.16	03.00
CV%	57.03	47.72	38.17	64.44	43.79	42.26	35.75

DMY = demethoxy-yangonin (1), DHK = dihydrokavain (2), Y = yangonin (3), K = kavain (4), DHM = dihydromethysticin (5), M = methysticin (6), KL% = total kavalactones content, expressed in percentage of dry matter yield.

TABLE 2. CHEMOTYPES IDENTIFIED WITHIN *P. METHYSTICUM* AND *P. WICHMANNII* (coded in decreasing order of the proportion of the six major kavalactones in the extract)

Piper wichmannii						*Piper methysticum*					
1	5	6	2	3	4	2	1	4	6	5	3
1	6	5	3	2	4	2	5	4	6	3	1
2	5	6	1	3	4	2	5	6	1	3	4
2	1	5	6	3	4	2	4	5	6	1	3
5	2	1	6	3	4	2	4	6	5	3	1
5	2	6	4	3	1	2	6	5	4	3	1
						4	2	6	3	5	1
						6	2	5	3	4	1
						6	4	2	5	3	1
						6	4	1	3	2	5
						6	4	3	2	5	1

1 = demethoxy-yangonin, 2 = dihydrokavain, 3 = yangonin, 4 = kavain, 5 = dihydromethysticin, 6 = methysticin.

content remains stable during the subsequent growth of the plant, although the rootstock biomass continues to increase over time (a shrub can live up to 15–20 years). A range of chemotypes exist in the wild forms and in the cultivars. A few wild forms of *P. wichmannii* produce chemotypes identical to cultivars of *P. methysticum* (chemotype 256134 in Table 2). The domestication process of kava is in fact a clonal selection of chemotypes. Drinkers do not appreciate a high percentage of DHK (2) and DHM (5), but chemotypes with a high percentage of K (4) and a low percentage of DHM (5) produce a pleasant and desirable physiological effect.

Cytological evidence

About 130 mitotic chromosome counts were obtained for *P. methysticum* and *P. wichmannii*. No obvious variation in chromosome numbers was apparent between *P. methysticum* clones representing different morphotypes and chemotypes or between monoecious and dioecious plants. Chromosome counts obtained from pollen mother cells of *P. methysticum* showed about 65 bivalents. Although tetrad formation appeared normal, cotton blue staining revealed poorly formed pollen grains.

According to Jose and Sharma (1985) and Okada (1986), the genus *Piper* is a homogeneous group with a basic number of $x=13$. Consequently, the accessions of *P. methysticum* and *P. wichmannii* examined are all decaploids with $2n=10x=130$ chromosomes. Despite vegetative propagation, there is uniformity

in the chromosome numbers of *P. methysticum* cultivars and the ploidy level is identical in sterile cultivars of *P. methysticum* and wild forms of *P. wichmannii*. However, polyploidy alone cannot be considered as the only explanation for the sterility observed in *P. methysticum* cultivars because wild forms of *P. wichmannii* are also decaploids and fertile in the wild.

Genetic evidence

Isozymes are proteins synthesized by genes; isozyme polymorphism, or dissimilarity between individuals, serves as a measure of genetic diversity. The isozyme technique has proved suitable for identifying duplicates in germplasm collections, for ascertaining the genetic fingerprints of cultivars, and for clarifying phylogenic relationships. A total of 53 different electromorphs were identified including 5 for ACO, 2 for ALD, 6 for DIA, 3 for IDH, 16 for MDH, 5 for ME, 5 for PGI and 11 for PGM. No 'species-specific' electromorph was identified in either *P. methysticum* or *P. wichmannii*.

All the enzyme systems were polymorphic in *P. wichmannii* accessions and a total of eight different zymotypes was observed, but plant populations at any particular site were monomorphic with regard to isozymes. Although *P. wichmannii* is apparently dioecious, it shows remarkably little genetic variation. The progeny of two collections for example (approximately 120 individuals) from Western Province, Papua New Guinea, were monomorphic for most of the enzymes studied. At any particular collection site plant population was genetically uniform. If *P. wichmannii* is dioecious in the wild, then the progeny should be segregating at least for male and female types. The very limited variation observed in more than 120 seedlings suggests that apomixis or self pollination occurs but further evidence is needed to confirm this hypothesis.

Among *P. methysticum* cultivars, there was less variation in isozyme banding patterns. Only four of the eight enzyme systems (ACO, DIA, MDH, PGM) were polymorphic, and only three zymotypes were observed. There are several possible explanations for the absence of variability at the isozyme level. *Piper methysticum* might be a group of sterile clones resulting from human selection of somatic mutants. We have shown (Lebot and Lévesque, 1996) that only a few genes are responsible for the morphological and chemical variation observed and none of these are linked with loci controlling isozyme markers.

Cluster and principal component analyses (Fig. 3) conducted on data obtained from the banding patterns (absence/presence of electromorphs) indicates that the *P. wichmannii* accessions originating from the Western Province of Papua New Guinea are genetically very different from *P. methysticum*. This observation suggests that these *P. wichmannii* populations are unlikely to be the wild progenitors of the cultivated *P. methysticum*. The closest *P. wichmannii* zymotype (9) is found in Vanuatu and presents the same zymotype as cultivars of *P. methysticum* from Vanuatu and Southern Papua New Guinea. This suggests that *P. methysticum* (zymotypes 9 and 10) could have been domesticated in Vanuatu from *P. wichmannii* (zymotype 9). Zymotype 9 appears in accessions of both taxa, which appear to represent a single species.

Discussion

Piper methysticum and *P. wichmannii* are cultivated and wild forms, respectively, of the same species based upon convincing morphological, chemical, cytological and genetic grounds. The presence and number of related *Piper* spp. indicate that the area of origin of *P. methysticum* is somewhere in Melanesia. The botanical evidence enables us to specify that the area of domestication of *P. methysticum* cultivars is within the areas of distribution of *P. wichmannii* wild forms.

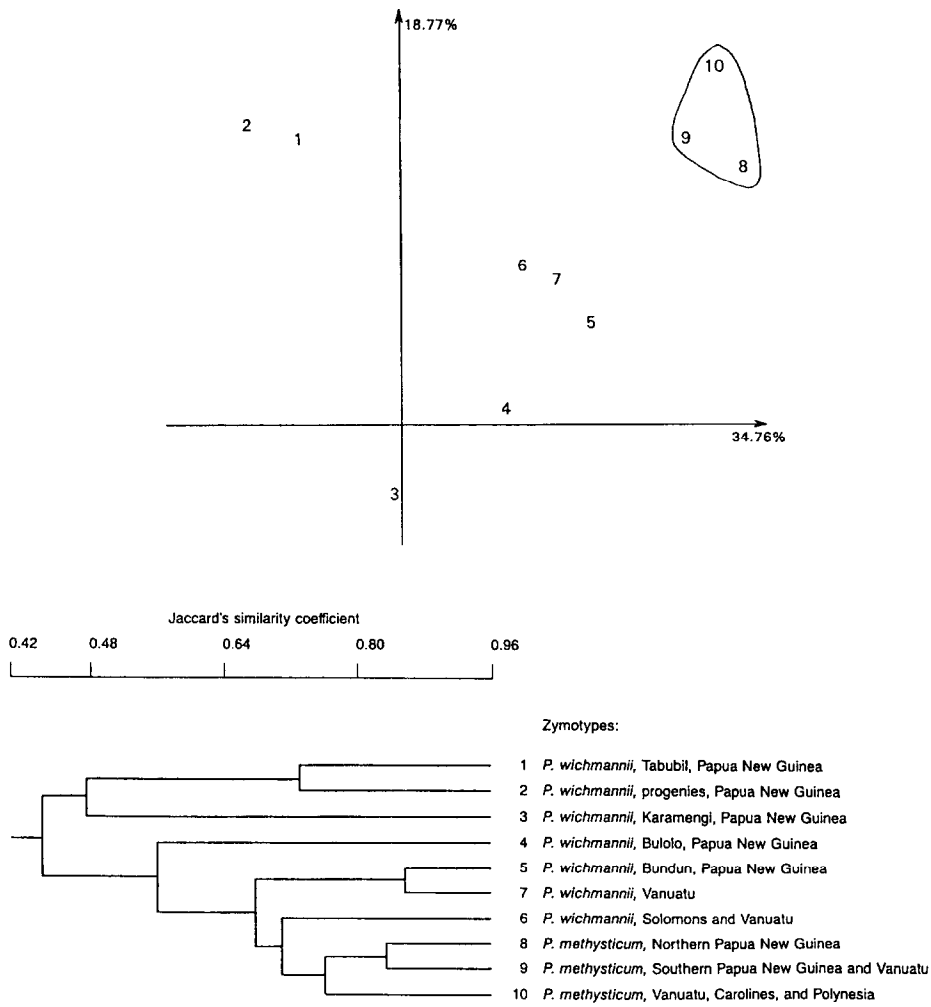


FIG. 3. DENDROGRAM (UPGMA) AND PRINCIPAL COMPONENTS ANALYSIS OF ZYMOTYPES.

Based on field experience, the length of the inflorescence and the proportion of woody elements in the roots are the only characteristics which allow differentiation between the two taxa. Specific level for *P. methysticum* and *P. wichmannii* is not supported by chromosome counts. These two forms of the same species are the only ones in the genus *Piper* from which major psychoactive kavalactones have been isolated. Wild plants of *P. wichmannii* in Melanesia appear to have at least partial fertility and show more isozyme variation which suggest outcrossing, but our isozyme study also supports the assumption that *P. wichmannii* and *P. methysticum* are conspecific.

Piper methysticum consists of sterile cultivars cloned ultimately from *P. wichmannii* in an on-going selection process. *Piper methysticum* being known only from gardens, should really not be considered as a 'species', but as a putative cultivar. *Piper methysticum* morphological and chemical variability is largely the result of human selection and cloning of somatic mutations in genetically similar, vegetatively propagated cultivars. *Piper methysticum* cultivars are Pacific domesticates that originated outside Southeast Asia and New Guinea; specifically, we suggest

that farmers in the northern islands of Vanuatu were the first to select and develop the species as a vegetatively reproduced root crop (Lebot *et al.*, 1992).

As *P. methysticum* was described first (1786), it has priority above C. De Candolle's *P. wichmannii* (1910). Because kava is such an important economic plant, subsuming *P. methysticum* within *P. wichmannii* would certainly create both conceptual and practical problems. As a distinction between wild and cultivated *P. methysticum* is possible and useful, we suggest the following classification:

P. methysticum Forst. f. *var. methysticum*: The sterile, cultivated form.
P. methysticum Forst. f. *var. Wichmannii* (DC) Lebot *stat. nov.*: The fertile, wild population.

The latter classification would not cause any conceptual or practical problems with regard to taxonomy and/or nomenclature, and yet it would allow ease of communication and differentiation of sterile cultivars and fertile wild populations.

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