



Article

Back to Linnaeus: Proper Botanical Naming of the Tetraploid Indian *Acorus* (Acoraceae), an Important Medicinal Plant

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Abstract: The basal monocot genus *Acorus* comprises essential oil-producing plants widely used in traditional medicine in various countries, including India. *Acorus calamus* sensu lato is a polyploid complex where the essential oil composition, to some extent, depends on the ploidy level. The literature recognizes diploids (in temperate Asia and North America), triploids (Asian in origin, naturalized elsewhere) and tetraploids (temperate to tropical Asia) at the rank of varieties of *A. calamus*. We show that the current use of the name *A. calamus* var. *angustatus* for the tetraploids is not properly justified. The earliest name based on the Asian material is *A. calamus* var. *verus* published in 1753 by Linnaeus. We justify the use of the Linnaean variety for tetraploids by selecting an epitype based on the material cultivated in Peninsular India, for which direct chromosome counts are provided. The name *A. verus* is available if the tetraploid cytotype is recognized at the species rank. We support earlier data on the importance of leaf anatomy for cytotype diagnostics in *Acorus*, but also show the limitations of the use of this approach. The growth pattern of the tropical Indian tetraploid material is discussed, and the evergreen nature of the accession studied here is documented. The exact chromosome number of the tetraploid *Acorus* requires further clarification. All metaphase plates examined here showed at least 44 chromosomes, but plates apparently showing more than 44 chromosomes were found as well. They may be explained by technical difficulties in counting chromosomes in *Acorus*. Alternatively, our data may indicate the occurrence of aneuploid mixoploidy.

Keywords: aneuploidy; *Acorus calamus*; *Acorus verus*; leaf anatomy; chromosome numbers; diploid; essential oil; polyploidy; sweet flag; tetraploid; triploid



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1. Introduction

The basal monocot genus *Acorus* comprises essential oil-producing plants native to Asia and North America [1–9] and is widely used in traditional medicine in various countries, including India [10–16]. *Acorus calamus* sensu lato (sweet flag) is a polyploid complex where the essential oil composition depends on the ploidy level [1,17–21]. The literature recognizes diploids (in temperate Asia and N. America), triploids (Asian in origin, naturalized elsewhere) and tetraploids (temperate to tropical Asia) at the rank of varieties of *A. calamus* [1,22,23].

Proper botanical naming is important for all taxa, but it is especially significant for useful plants such as members of the genus *Acorus*. Our recent study has disentangled confusions created by the widespread incorrect use of the name *Acorus tatarinowii* [24].

We were prompted to write this paper by the fact that the name currently used for the tetraploid *Acorus* (*A. calamus* var. *angustatus* Besser, Flora 17(Beibl.): 30 (1834)), as it turned out, was not validly published in the publication cited in the literature [23,25]. Indeed, Besser [26] provided no more than the following statement on the page 30 of his publication in 1834: “*Acorus triqueter* est *A. Calamus* var. *angusta*”. Besser provided no description or diagnosis of this taxon but a mere reference to “*Acorus triqueter*” in a list of phanerogams and ferns collected in the Baikal Lake area, which he reprinted from Turczaninow [27]. The species name *A. triqueter* Turcz. ex Schott was validated many years later, in 1860 [28], and at the time of Besser’s work it was a *nomen nudum*. These problems were already highlighted by Wein [29]. It is the publication of Engler [25] where the name *A. calamus* var. *angustatus* was actually published. Even though Engler [25] formally provided a morphological description for his *A. calamus* var. *angustatus*, the listed characters showed no difference from another recognized variety, *A. calamus* var. *verus* L. In addition, the character that allows distinguishing cytotypes of *Acorus* (leaf anatomy [1,6]) was yet unknown at the time of Engler’s work.

While accepting the name *A. calamus* var. *angustatus* for all tetraploid members of the group, Röst [1] noticed the occurrence of temperate and (sub)tropical tetraploids. Rhizomes of tropical plants yield the so-called Indian oil of Calamus, which is characterized by very large amounts of β -asarone [1]. Furthermore, Röst [1], pointed out that if, for practical reasons, separate classification of tropical tetraploids becomes desirable, the name *A. calamus* var. *verus* L. is available for this β -asarone-rich ecotype. The problem is that the description of Linnaeus [30] provides no information for the identification of the ploidy level of plants he assigned to *A. calamus* var. *verus*. Moreover, the lectotype selected for this name is an illustration, and the leaf anatomy of the type specimen cannot be investigated. The variety is described from India, where all ploidy levels of *Acorus* are known [19].

The aim of the present paper is to resolve the issue of the correct naming of the tetraploid Indian *Acorus*. We show that there is no way of maintaining *A. calamus* var. *angustatus* for naming any taxon and select an epitype of *A. calamus* var. *verus* that provides a clear link between this name and tetraploid plants. We use indirect data from leaf anatomy as well as a direct chromosome count to infer the ploidy level.

2. Materials and Methods

2.1. Plant Material

Material cultivated outdoors in the Lead Botanical Garden of Shivaji University, Kolhapur, India and propagated vegetatively has been observed over decades. The rare events of flowering have been documented. The same accession has been moved to Moscow and Barnaul, Russia and cultivated there indoors since 2019 and 2020, respectively.

2.2. Leaf Anatomy

Röst [1] revealed that the size of air canals in leaf aerenchyma allows precise distinguishing of diploids, triploids and tetraploids of *A. calamus*, at least in a common garden experiment. Unfortunately, the protocol description was not precise enough to allow generating data directly compatible to those provided by Röst [1]. In an earlier study of Siberian *Acorus*, we provided such a clear protocol and documented differences between diploids and triploids from Altai Krai, Russia [6]. These data are used here for comparisons.

For sectioning, dry material of leaf lamina was rehydrated in hot water for two days and transferred into 70% ethanol. Free-hand cross-sections were made and observed in glycerol with Olympus SZX-4 microscope. Our protocol includes the use of the following two metrics [6]:

- (1) The number of air canals per 0.62 mm² of cross-section. We counted the number of the canals in each leaf in two circular fields of view situated in each leaf side within the secondary midrib as close as possible to the leaf margin, but avoiding the presence of chlorenchyma and peripheral vascular bundles. The number of canals per area was calculated as the total of completely visible canals plus $\frac{1}{2}$ of the total of incompletely

visible canals. The mean of the canal numbers in the two fields was calculated and used as a metric.

- (2) Mean number of cells in the septa between the air canals visible in cross-section of the leaf blade. Cells belonging to a septum were defined as those that are exposed into two adjacent canals. Cells situated at junctions of three or more septa and therefore exposed into more than two air canals (corner cells) were not counted. For each examined leaf, cell number was counted in 20 randomly selected septa in the fields of view described above, and then mean cell number per septum was calculated.

2.3. Chromosome Numbers

Direct chromosome counts were performed for living material cultivated in Kolhapur and Barnaul. In Kolhapur, the healthy root tips about 1 cm in length were excised at 8:00 a.m. and then treated with saturated solution of Paradichlorobenzene (PDB) for 3 h at 10 ± 2 °C in refrigerator. The roots were hydrolysed in 1 N HCL by warming on lamp. Then, the root-tips were washed with distilled water. The extreme tips of the roots were squashed in 2% propionic orcein. The somatic plates were observed and photographed with an Axio Imager A2 microscope (Carl Zeiss AG, Oberkochen, Germany). In Barnaul, fresh roots 3–6 cm long were used. The best results were obtained when the roots were placed in the pretreatment solution at 1:00–2:00 p.m. Roots were pretreated in 0.002 M 8-hydroxyquinoline for 2–3 h and then stained with 1% aceto-haematoxylin according to Smirnov [31]. After that, stained root tips cut off and squashed in a drop of 45% acetic acid. The metaphase plates were observed and photographed with an Axio Imager A1 microscope (Carl Zeiss AG, Oberkochen, Germany).

2.4. Nomenclatural Analysis

Protologues of all names discussed here were analysed and typification procedures were performed according to the rules of plant nomenclature [32].

3. Results and Discussion

3.1. Nomenclatural Status and Taxonomic Identity of *Acorus calamus* var. *angustatus* Engl.

The Engler's concept of *A. calamus* var. *angustatus* can be partly visualized from a list of specimens provided in his account [25]. The list includes specimens from Siberia, northern and eastern China and Japan. As soon as Engler [25] made a direct link to the list of plants collected in the Baikal region in Siberia [26] and cited Turczaninow's specimen from Dauria, the Central Siberian material may be most appropriate for the typification of *A. calamus* var. *angustatus*. Chromosome numbers of *Acorus* have been extensively studied in the regions of Central Siberia adjacent to Baikal (Irkutsk Oblast and Buryatia), and only the diploid cytotype was found [33–36]. However, the name *A. calamus* var. *angustatus*, incorrectly credited to Besser, has been ultimately selected for naming tetraploid rather than diploid members of the group of *A. calamus* [1,22]. This decision was in agreement with the data on the occurrence of the tetraploids in Japan [1].

In the belief that *A. calamus* var. *angustatus* had been validly published already by Besser [26], Engler [25] included a list of later synonyms into its protologue. Among those synonyms, Engler listed an earlier varietal name, *A. calamus* var. *spurius* (Schott) Engl., which he validly published in 1879 [37] as based on *A. spurius* Schott, described from Japan. This inclusion made the new varietal name, unknowingly validated for the first time by Engler, superfluous and illegitimate. According to the rules of botanical nomenclature [32], the name *A. calamus* var. *angustatus* is ultimately based on the type of *A. spurius* and is not available for use because of its illegitimate status, despite its common current acceptance in major online databases [23,38,39]. The situation is further complicated by the fact that specimens from digitized herbaria worldwide currently identified as *Acorus tatarinowii* Schott are automatically re-classified as *A. calamus* var. *angustatus* in GBIF. The name *A. tatarinowii* is a synonym of *A. calamus* sensu lato, but extensive herbarium

collections from SE Asia identified as *A. tatarinowii* (except the type collection) actually belong to *A. gramineus* [24].

This nomenclatural conclusion raises a question regarding the actual taxonomic identity of the species name *A. spurius* Schott. Its protologue was published in a paper based on herbarium specimens revised by Schott at Rijksherbarium in Leiden (now the National Herbarium of the Netherlands, Naturalis Biodiversity Center) [40], and provided the species provenance as follows: “Hab. Iaponia: Bürger”. There is a specimen in Leiden (L0325912, image seen) collected by Heinrich Bürger in Nagasaki, Japan during 1829–1832 [41] and identified by Schott as *A. spurius*. It is the only relevant specimen in that collection and therefore a holotype. The specimen contains two inflorescences at anthesis. The ploidy level of the specimen can be inferred through destructive sampling via studies of pollen stainability and spatha anatomy [42], but we had no opportunity of such studies for the type of *A. spurius*. Based on the spatha morphology, we predict that this specimen is likely tetraploid. Indeed, its spatha resembles that of some specimens inferred as tetraploids (e.g., the type collection of *A. griffithii* Schott [42]) in being widened towards the tip, where the secondary midvein becomes inconspicuous. The spatha tip of L0325912 is asymmetric, a condition typical of tetraploid accessions of the *A. calamus* group [1,22].

It is important that the name *A. calamus* var. *angustatus* is illegitimate and therefore cannot be used for any taxon irrespective of the taxonomic identity of the name *A. spurius*. Furthermore, possible tetraploid nature of *A. spurius* does not violate the priority of the names *A. calamus* var. *verus* and *A. verus* for naming tetraploid plants.

3.2. Taxonomic Identity of *Acorus calamus* var. *verus* L.

Acorus calamus var. *verus* L., Sp. Pl. 1: 324 (1753). \equiv *Acorus verus* (L.) Burm.f., Fl. Ind.: 84 (1768). Lectotype (designated by Nicolson et al., Interpret. Van Rheedee’s Hort. Malab.: 274 (1988)): [icon] “Vaembu” in Rheedee, Hort. Malab. 11: 99, t. 48 (1692). Epitype (designated here): India, Maharashtra, Kolhapur city, Shivaji University, Lead Botanical Garden, Department of Botany, 28 Aug. 2019, D.D. Sokoloff, M.S. Nuraliev, A.G. Platonova, M.V. Remizowa, E.E. Severova, S.R. Yadav I-17 (MW0758480).

‘*Acorus verus*’ became a common designation for the Indian sweet flag soon after its discovery, although originally it was applied to the European *A. calamus* s.l. in order to distinguish it from other plants that passed in the literature as ‘*Calamus*’ [43]. Hermann [44] introduced this plant from Ceylon (now Sri Lanka) to the Botanical Garden in Leiden in 1675, where it was successfully cultivated. Hermann [44] knew his plant was from Ceylon and Malabar (now Kerala State, India) and considered it to differ from its European relatives solely in its slenderer rhizome. When Jan Commelin penned a diagnosis for the same plant, which he knew from a drawing produced for Hendrik Adriaan van Rheedee in Malabar, he used the same diagnostic character but added a feature of narrower leaves after having made a further comparison with European plants occurring in Holland [45]. In ‘*Flora Zeylanica*’ (1747), Linnaeus [46] applied the same diagnostic characters as used by Commelin [45] to distinguish the Indian plants. This concept persisted until Linnaeus eventually named the plant *A. calamus* var. *verus* L. in 1753 [30] and Burman raised this variety to the rank of species as *A. verus* (L.) Burm.f. in 1768 [47]. It should be noted that the early authors recognized differences in taste between the European and Indian *Acorus*, which may reflect variations in chemical compounds such as the occurrence of large amounts of β -asarone in the tetraploid Indian material [1].

Linnaeus [30] stated that *A. calamus* var. *verus* occurs in swampy riversides of India (“Habitat in Indiae fosis paludosis”). He used the same and only diagnostic character as in his earlier publication [46], which was limited to the slenderer rhizome. Of course, he did not mention any character that can be used to infer the ploidy level. There is no herbarium specimen linked to this varietal name, which seems to have been solely based on historical publications [44,45]. In the absence of herbarium collections, an illustration from Rheedee’s Hortus Malabaricus [45] was selected as the lectotype of *A. calamus* var. *verus* [48], but is most likely a holotype (the only element used by the author to establish a taxon).

The ploidy level of the material used by Rheede cannot be properly identified in the absence of herbarium specimens. Remarkably, the illustration published by Rheede [45] shows no reproductive organs. Flowers and fruits were not mentioned in his description of the Indian material, whereas the fact of abundant flowering of the European plants was mentioned [45]. Röst [1,49] noted that accessions of *A. calamus* s.l. differ with respect to their ability to produce flowers in common garden experiment. Some accessions failed to develop flowers despite many years of cultivation [1]. Out of eight samples of tropical tetraploids studied by Röst [49], only one has flowered. The sample that flowered was received from Phrae Hills, Thailand, which means the material was probably collected in a mountainous area [49]. It seems that tropical tetraploids tend to reproduce vegetatively without producing inflorescences, at least at low elevations. Observations by one of us (S.R.Y.) on *Acorus* plants cultivated at Shivaji University, Kolhapur, India showed that normally they do not produce inflorescences. Material from this collection was provided for cultivation in Russia, where it has been grown indoors in Moscow and Barnaul during five seasons (2019–2023). The plants extensively propagated vegetatively without commencing anthesis. Our direct chromosome counts documented their tetraploid nature (Figure 1). This accession, like the plants illustrated by Rheede [50], is derived from the material cultivated in peninsular India for medicinal purposes. As it has the same origin (southwestern tropical Indian cultivation) and agrees with the original material of Rheede in the lack of flowering, we believe that this accession is appropriate to select as an epitype of *A. calamus* var. *verus*. The epitype selected in the present paper contains material of the same individual plant, which was subsequently propagated vegetatively and cultivated in Russia for cytological and anatomical studies. With the epitype selected in the present paper, the use of the name *A. calamus* var. *verus* is particularly and unambiguously attached to tetraploid plants.

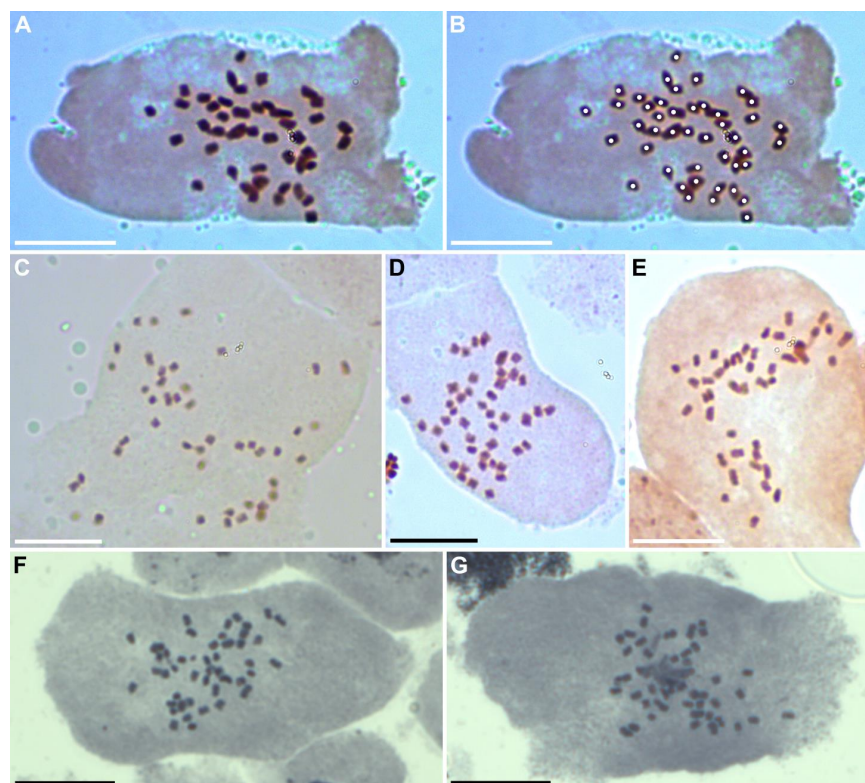


Figure 1. Identification of ploidy level in the epitype of *Acorus calamus* var. *verus* selected in the present study. All chromosome counts were made in root tips of plants from the same vegetative clone grown at Shivaji University, Kolhapur (A–E) and the South-Siberian Botanical Garden, Barnaul (F,G). Material from this clone was used to prepare the epitype of *A. calamus* var. *verus*. Scale bars = 10 μ m. At least 44 chromosomes can be counted in each metaphase plate illustrated here. (B) is the same image as (A) with our interpretation of 44 chromosomes indicated by white dots.

3.3. Chromosome Number of the Epitype Accession of *Acorus calamus* var. *verus*: A Possibility of Aneuploidy and Mixoploidy

The chromosome number $2n = 24$ is well-documented in diploid members of the *Acorus calamus* complex [6,8,18,33–36,49,51]; reports of less than 24 chromosomes are rare and mostly based on early publications (reviewed in [51]). Therefore, the basic chromosome number could be interpreted as $x = 12$. Triploids and tetraploids are expected to possess 36 and 48 chromosomes, respectively. Most counts of triploids indeed show 36 chromosomes (reviewed in [8,51]), but there are reports of 33–36 chromosomes [6,52,53]. For tetraploids, chromosome numbers $2n = 44$ [3,51,54–56], $2n = 45$ [57] and $2n = 48$ [19,22,49,51,55,58] are reported.

All metaphase plates examined here clearly showed at least 44 chromosomes (Figure 1). Therefore, we have no doubts about the tetraploid nature of our accession.

Precise counting of all chromosomes was sometimes problematic (Figure 1E–G). One can count 45 or apparently up to 48 chromosomes in some of our images (Figure 1), but such counts are not unequivocal. Problems appeared because of possible overlapping of chromosomes. At the same time, occasional separation of chromosome arms during squashing of the samples cannot be ruled out. All chromosome numbers that could be inferred from our images lie within the range of earlier reports for tetraploid accessions of *Acorus*, i.e., 44–48.

Our results resemble those of Wulf and Fritz [55] who studied a variegated accession of the tetraploid *Acorus* and found $2n = 48$ in some cells from the root tips, but there were also cases in which only a smaller number of chromosomes, but at least $2n = 44$, could be clearly identified. Such results can indicate the actual occurrence of cells with different chromosome numbers within a root (aneuploid mixoploidy). The term mixoploidy describes the co-occurrence of cells with different chromosome numbers in the same plant individual. The term aneuploidy describes differences in chromosome numbers caused by the gain or loss of some chromosomes rather than differences in the number of genomes. An alternative explanation, preferred by Wulf and Fritz [55], implies that the apparent differences in chromosome numbers can be an artifact caused by the aggregation of chromosomes. Wulf and Fritz [55] considered the second option more likely. In our view, the exact chromosome number of the tetraploid *Acorus* requires further clarification. Our recent study revealed that flow cytometry is a useful tool in resolving the issue of aneuploidy in *Acorus* [6]. Kumar and Singh [59] reported the widespread occurrence of aneuploidy in Indian *Acorus*, but not all images provided by the authors allow convincing counting of chromosomes. In contrast, Mittal et al. [19] studied as many as 50 Indian accessions of *Acorus* and found no evidence of aneuploidy. All 33 examined tetraploid accessions were reported as possessing 48 chromosomes, but photographs of metaphase plates were not provided [19]. Ogra et al. [21] studied 14 accessions of Indian *Acorus calamus* s.l. and reported only diploids with $2n = 24$ and tetraploids with $2n = 36$, but the images of metaphase plates provided in this paper, in our view, allow more than one interpretation of the precise chromosome number. In general, this analysis of published data and our own experience highlight difficulties in precise photographic documentation of the presence or absence of aneuploidy in *Acorus* under routine methods of microscopy.

3.4. Leaf Anatomy and Ploidy Level of the Epitype Accession

Our results on leaf blade anatomy (Figures 2 and 3A,B) show that the structure of the aerenchyma of the Indian tetraploid accession approaches the extreme figures found in Siberian triploids [6]. Therefore, though the difference between diploids and tetraploids in leaf anatomy is strong, distinguishing tetraploids from triploids using leaf anatomy alone may be problematic. Röst [1] concluded that leaf anatomy allows precise identification of ploidy level in a common garden experiment. Apparently, the leaf anatomy characters depend to some extent on growth conditions. This creates certain limitations for the use of leaf anatomy in the identification of the ploidy level.

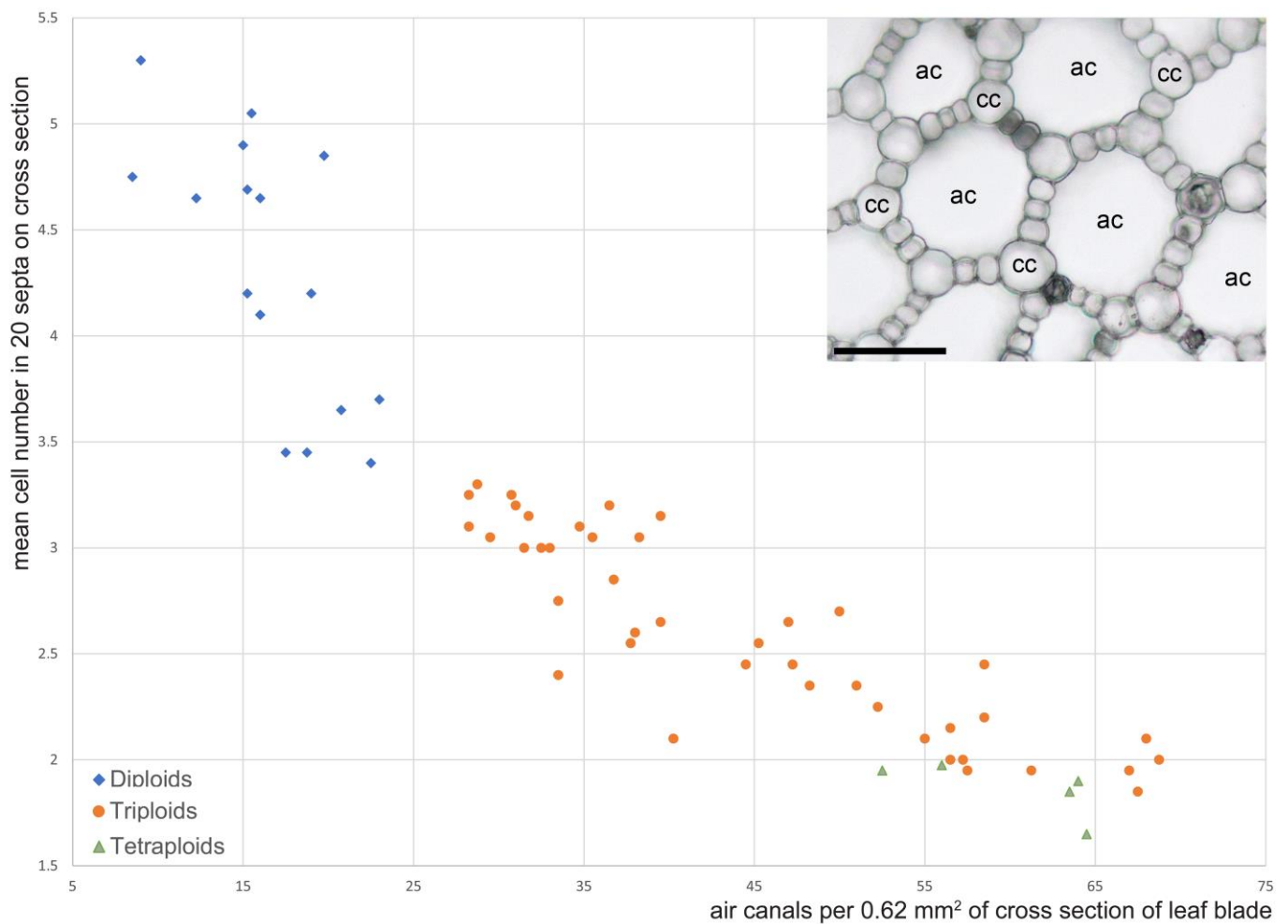


Figure 2. Variation in leaf blade aerenchyma in the *A. calamus* group. Data on diploids (diamonds) and triploids (circles) are taken from [6]. They are based on Siberian material with directly identified ploidy levels. Triangles are leaves of the tetraploid plant used to prepare the epitype of *A. calamus* var. *verus*. Inset: leaf blade aerenchyma of the individual used to prepare the epitype of *A. calamus* var. *verus*. See Figure 3A,B, for the location of the aerenchyma in the leaf blade. ac, air canals; cc, corner cells. Scale bar = 100 µm.

The overall shape of the cross section of the leaf lamina in our accession shows features indicated as characteristic of tetraploid accessions of *Acorus calamus* by Röst [1]. Namely, the secondary midrib of the ensiform leaf blade is clearly pronounced and sharp, whereas the abaxial and adaxial portions of the leaf blade are both wide and thin (Figure 3A).

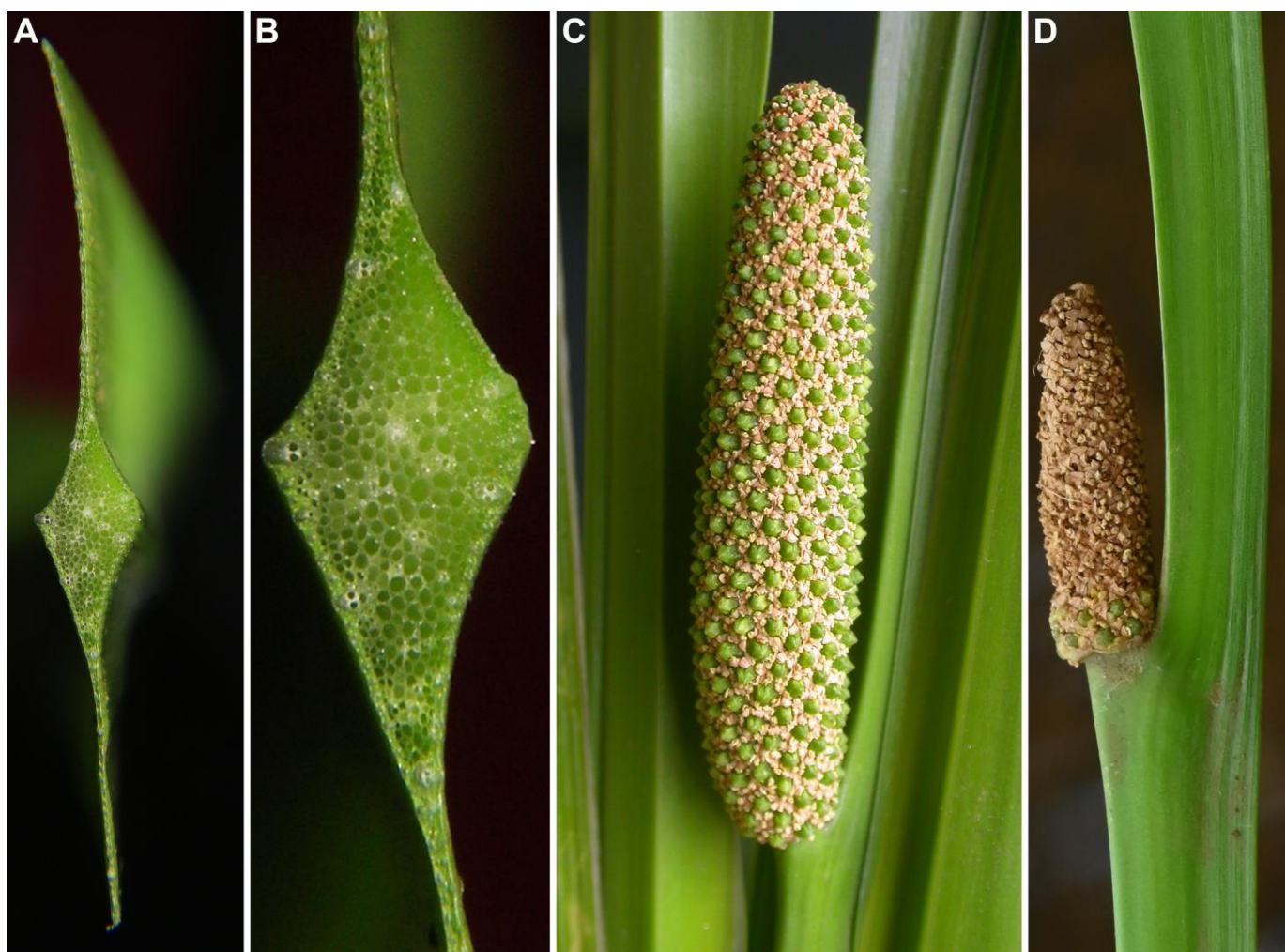


Figure 3. (A,B) Leaf blade section of the plant used to prepare the epitype specimen of *Acorus calamus* var. *verus*, Shivaji University, Kolhapur, August 2019. Images: M.S. Nuraliev. The leaf blade of *Acorus* is ensiform, i.e., flattened in the vertical plane (A), with both left and right surface being morphologically abaxial. There is a well-defined area of the secondary midrib (B) with a thin peripheral layer of chlorenchyma interrupted by vascular bundles and the inner area composed by aerenchyma with numerous air canals. (C,D) Inflorescences of the same accession on rare occasions of flowering in Kolhapur. Images: S.R. Yadav. (C) An inflorescence produced in 2004. (D) An inflorescence produced in 2023; note the absence of well-developed gynoecia, except in the two lowermost whorls of flowers.

3.5. Flowering and Growth Pattern of the Tetraploid Indian *Acorus*

Flowering was not observed during the cultivation of our Indian accession in Moscow. Over decades of observations, flowering was observed only in 2004 and 2023 in Kolhapur (Figure 3C,D). Each time, only one inflorescence was found. The inflorescence developed in 2023 had no properly developed gynoecia in most flowers. No fruit formation was recorded in Kolhapur.

Röst [1] noticed that (sub)tropical tetraploids can be distinguished by keeping leaves green for a much longer period in autumn and starting regrowth earlier in spring in common garden experiments with temperate accessions. Orga et al. [21] reported differences in the timing of autumn leaf senescence and spring leaf emergence among representatives of different cytotypes of *Acorus* in India.

The tetraploid Indian accession studied here is evergreen (Figures 4 and 5). Plants of *A. calamus* s.l. have two clearly defined generations of leaves per year [5]. Detailed observations on our tetraploid Indian accession cultivated in Moscow showed that plants

maintain the leaves of the late summer generation during the whole winter. These old leaves exhibited only partial senescence (e.g., drying of the distal parts of some leaves). Short leaves were formed between the two main generations of long leaves. These were visible on shoot tips during the winter and early spring. An image taken in early April in Moscow (Figure 5A) shows that the long leaves of the previous generation are still green. An image taken in mid-April (Figure 5B) shows the emergence of the spring generation of long leaves and only partial senescence of the old generation of long leaves. Therefore, our accession fits very well the tendency described for tropical accessions in the literature [1]: instead of showing a late autumn senescence, it is merely evergreen. In contrast, temperate accessions of *Acorus calamus* s.l. showed a complete autumn senescence of long leaves in outdoor (Figure 5D) as well as indoor conditions (Figure 5E) in Moscow.



Figure 4. (A) Living plants used to prepare the epitype specimen of *Acorus calamus* var. *verus*, Shivaji University, Kolhapur, August 2019. Image: M.S. Nuraliev. (B,C) The same accession in April 2023. Images: S.R. Yadav. og, old generation leaf; ng, new generation leaf; sl, short leaf formed between the two generations of large leaves.

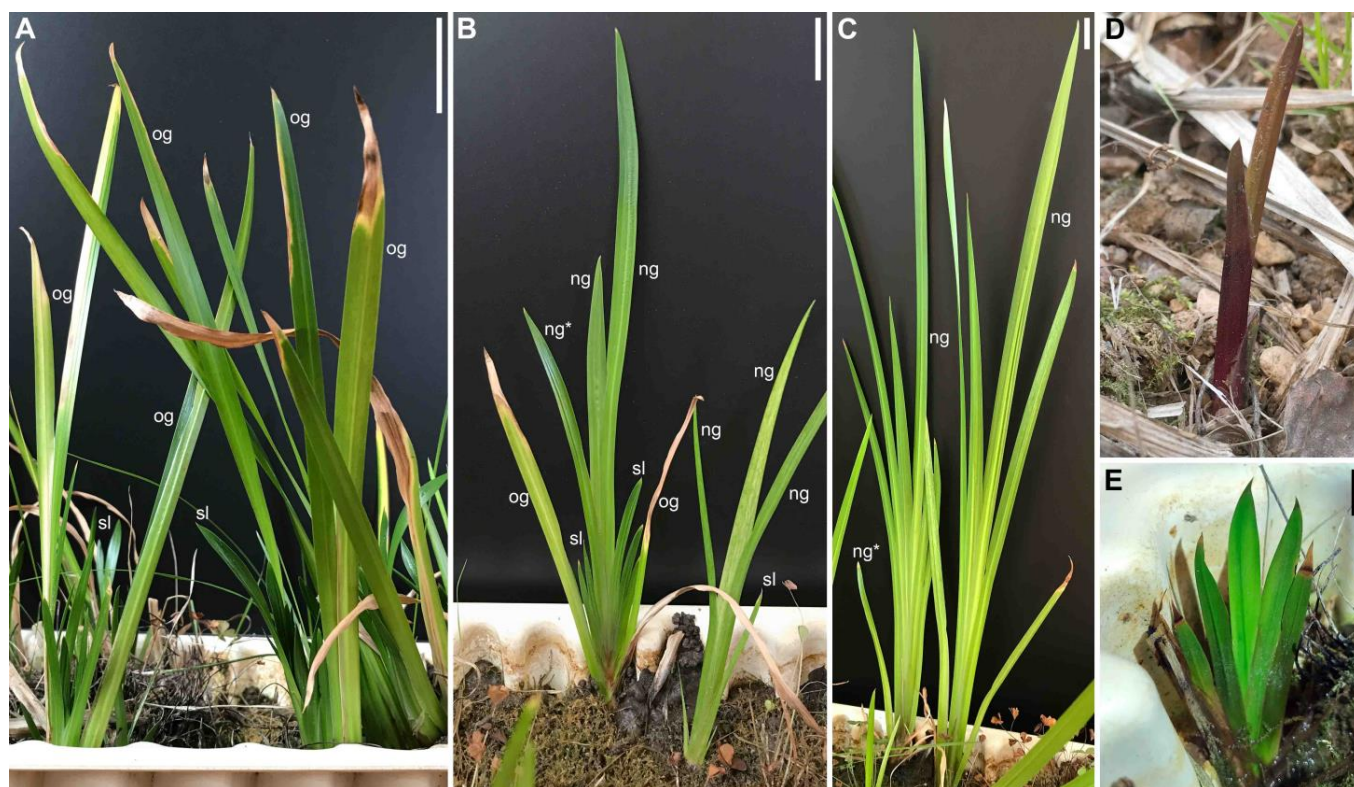


Figure 5. Growth patterns of temperate and tropical accessions of *Acorus calamus* s.l. (A,B) Living plants of the tropical accession used to prepare the epitype specimen of *Acorus calamus* var. *verus* in indoor cultivation in Moscow. (A) Image taken on 2 April 2023, before the emergence of the spring generation of leaves. (B) Image taken on 15 April 2023, after the emergence of the spring generation of leaves. (C) Image taken on 10 June 2023 showing fully developed leaves of the spring generation. (B,C) illustrate the same two shoots. og, old generation leaf; ng, new generation leaf; ng*, the first-formed new generation leaf of a shoot (the abbreviation is used to show the identity of leaves in images (B,C); sl, short leaf formed between the two generations of large leaves. (D,E) Accessions of *Acorus calamus* s.l. received from temperate regions and cultivated in Moscow; images taken on 15 April 2023. Note the absence of living leaves of the old generation. Only short leaves are visible. (D) Material collected in Vladivostok, Primorsky Krai, Russia (voucher: MW0963257) and cultivated outdoors. (E) Material collected near Alleisk, Altai Krai, Russia (voucher: MW0955192) and cultivated indoors in the same pot with the tropical Indian material (A–C). Scale bars = 3 cm (A–C), 1 cm (D,E). Images: D.D. Sokoloff.

4. Conclusions

Our study resolved nomenclatural issues related to naming tetraploid members of the *Acorus calamus* complex. Tetraploids (that only occur in Asia) should be named *A. calamus* var. *verus* L. if the taxon is recognized as a variety, or *A. verus* (L.) Burm.f. if the taxon is recognized as a species. Our preliminary data suggest that tetraploids, contrary to traditional views, should be recognized at the rank of a species. This conclusion will be discussed in detail in our forthcoming publication.

Diploid members of the complex occur in North America and Asia [1]. Names *A. calamus* var. *americanus* Raf. or *A. americanus* (Raf.) Raf. were suggested as having nomenclatural priority if both American and Asian diploids are recognized as a taxon, either at the rank of variety or as a species [1,8,9,23]. We shall discuss the American taxa described by Rafinesque elsewhere.

The names *A. calamus* var. *calamus* and *A. calamus* L. s.str. can be used for sterile triploids [1,23]. These issues are summarized in Table 1.

Table 1. A summary of cytotypes recognized in the *Acorus calamus* complex.

	Diploid	Triploid	Tetraploid	Hexaploid
Chromosome numbers	24, ?sometimes 22	36, sometimes 33–35	44–48	66, 72
Distribution	Asia, N America	Asia, naturalized in Europe and N America	Asia	Rare in Asia (Kashmir and Yunnan) [19,53]
Name with nomenclatural priority at the rank of variety	<i>Acorus calamus</i> var. <i>americanus</i> Raf. according to [1]	<i>Acorus calamus</i> var. <i>calamus</i>	<i>Acorus calamus</i> var. <i>verus</i> L.	Unknown
Name with nomenclatural priority at the rank of species	<i>Acorus americanus</i> (Raf.) Raf. according to [9]	<i>Acorus calamus</i> L. s.str.	<i>Acorus verus</i> (L.) Burm. f.	Unknown

We support earlier findings on the value of leaf structure for identification of ploidy levels in the *A. calamus* complex [1,6,21]. Röst [1] used material grown in common garden experiments under controlled conditions and was confident in distinguishing diploids, triploids and tetraploids using leaf anatomy. Our data agree with the idea that environmental conditions may affect leaf anatomy to some degree. Indeed, we found that the leaf characters of tetraploids closely approached the extreme figures found in triploids. On the other hand, it seems that tetraploids and diploids remain well-distinguishable from each other using leaf anatomy. We will present our results of testing this hypothesis using ample material in a forthcoming publication.

At least some tropical tetraploids of the *Acorus calamus* complex are evergreen plants. Therefore, the evergreen nature of *A. gramineus* can be used to distinguish it from *A. calamus* sensu lato in certain regions, such as Japan [60], but not on a general scale.

The exact chromosome number of the tetraploid *Acorus* requires further clarification. There are reports in the literature of 44 to 48 chromosomes, the latter number fitting the generally accepted basic chromosome number $x = 12$. All metaphase plates examined here showed at least 44 chromosomes. Plates apparently showing more than 44 chromosomes were found as well. They may be explained by technical difficulties in counting chromosomes in *Acorus*. Alternatively, our data may indicate the actual occurrence of different chromosome numbers in various cells within a root tip (aneuploid mixoploidy).

Author Contributions: D.D.S. and A.N.S. performed a nomenclatural analysis and wrote the draft text; D.D.S. and M.V.R. cultivated and investigated plant material in Moscow; M.V.S. cultivated plant material in Barnaul and performed chromosome counts; M.V.R. studied leaf anatomy; S.R.Y. made observations on plants cultivated in India and performed chromosome counts. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Digital image of the epitype specimen of *Acorus calamus* var. *verus* is available at <https://plant.depo.msu.ru/open/public/en/item/MW0758480> [61].

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