diversity

MDPI

*Article*

# Inference of Ploidy Level in 19th-Century Historical Herbarium Specimens Reveals the Identity of Five *Acorus* Species Described by Schott

**Dmitry D. Sokoloff [1,*], Margarita V. Remizowa [1], Elena E. Severova [1] and Alexander N. Sennikov [2,*]**

[1] Department of Higher Plants, Biological Faculty, M.V. Lomonosov Moscow State University, 1, 12, Leninskie Gory, Moscow 119234, Russia; margarita.remizowa@gmail.com (M.V.R.); elena.severova@mail.ru (E.E.S.)
[2] Botanical Museum, Finnish Museum of Natural History, University of Helsinki, P.O. Box 7, FI-00014 Helsinki, Finland
* Correspondence: sokoloff-v@yandex.ru (D.D.S.); alexander.sennikov@helsinki.fi (A.N.S.)

**Abstract:** Heinrich Wilhelm Schott (1794–1865) was one of the pioneering researchers in the taxonomy of the species-rich monocot family Araceae. He described numerous new plant species in various genera, including *Acorus*, which is currently segregated as a monogeneric family and order occupying a position sister to the rest of the monocots. While describing his new species of *Acorus*, Schott mostly used characters that are currently considered of low, if any, taxonomic value. His descriptions lack some key characters including, for obvious reasons, chromosome numbers. Therefore, Schott's species concepts cannot be properly interpreted according to the current understanding of the taxonomic diversity of *Acorus*, even though his species names must be examined for implementation of the principle of nomenclatural priority. The only way of resolving the taxonomic identity of Schott's species names is through the identification of type specimens among historical herbarium collections, by inferring taxonomically significant characters that are missing in Schott's descriptions. On the basis of herbarium collections of the Komarov Botanical Institute, St. Petersburg (LE), we were able to infer ploidy levels of the materials used by Schott to describe *Acorus triqueter* (diploid, Siberia), *A. tatarinowii* (tetraploid, China), *A. nilaghirensis* (tetraploid, India), *A. griffithii* (tetraploid, Bhutan), and *A. commutatus* (tetraploid, Bhutan). Leaf anatomy and pollen stainability were used as cytotype markers. All five species belong to the polymorphic *Acorus calamus* complex that comprises important medicinal plants. Detailed historical and nomenclatural analyses of Schott's species names and herbarium collections are provided.

**Keywords:** Acoraceae; *Acorus calamus*; cytotype; diploid; leaf anatomy; pollen fertility; polyploidy; sweet flag; tetraploid; triploid



**Citation:** Sokoloff, D.D.; Remizowa, M.V.; Severova, E.E.; Sennikov, A.N. Inference of Ploidy Level in 19th-Century Historical Herbarium Specimens Reveals the Identity of Five *Acorus* Species Described by Schott. *Diversity* **2023**, *15*, 766. https://doi.org/10.3390/d15060766

Academic Editors: Yin-Long Qiu, Yang Liu and Mario A. Pagnotta

Received: 14 May 2023
Revised: 6 June 2023
Accepted: 10 June 2023
Published: 12 June 2023



## 1. Introduction

Heinrich Wilhelm Schott (1794–1865) was one of the pioneering researchers in the taxonomy of the species-rich monocot family Araceae. He described numerous new plant species in various genera, including *Acorus* [1–4], which is currently segregated as a monogeneric family and order occupying a position sister to the rest of the monocots [5–7]. In total, Schott recognized as many as 13 species of *Acorus* [2,3], of which ten were described or validated during his research. Many subsequent treatments [8–14] admitted only two species of *Acorus*, namely, *A. calamus* L. and *A. gramineus* Sol. ex Aiton, both described long before Schott in the 18th century [15,16], though this two-species taxonomic system has not been fully established because some experts accepted further species in the *A. calamus* group [17–22] or in the *A. gramineus* group [23–30]. Taxonomists that adhere to narrow species concepts may consider accepting at least some of Schott's species, but correct interpretation of his species names requires plenty of effort [31].

While describing his numerous new species of *Acorus*, Schott [1–3] mostly used morphological characters that are currently considered of low, if any, taxonomic value such as vegetative leaf length and width, and relative and absolute length of spadix and spatha. Schott paid attention to seed characters that are currently viewed as highly important to distinguish the *A. gramineus* group from the *A. calamus* group [11,12]. However, Schott had no ripe seeds in the material he studied to describe the majority of his species. For this reason, he described ovules rather than seeds, and such descriptions provided no easy-to-understand differences. Surprisingly, Schott did not mention the other key character distinguishing the groups of *A. gramineus* and *A. calamus*, namely, the presence or absence of a (false) midrib of the leaf blade.

As Schott worked long before the emergence of plant cytotaxonomy, he provided no data on ploidy levels. In contrast, the basic idea of classification of the *A. calamus* group developed during the 20th century implied recognizing diploids, triploids, and tetraploids as varieties [10,32,33] or species [17–22].

Thus, Schott's publications do not allow an easy interpretation of his species concepts according to the current understanding of the taxonomic diversity of *Acorus*, even though Schott's species names must be examined for implementation of the principle of nomenclatural priority [34]. The only way of resolving the taxonomic identity of Schott's species names is through the identification of type specimens among historical herbarium collections, by inferring characters missing in Schott's description from this material. Fortunately, earlier studies revealed clear correlations between plant fertility, leaf anatomy, and ploidy levels in *Acorus* [10,18,32,35–37]. The use of these characters should provide a link between cytotypes and Schott's species names.

In the present study, we use historical collections of the Komarov Botanical Institute, St. Petersburg (LE), to infer ploidy levels of the materials used by Schott to describe *A. triqueter* Turcz. ex Schott from Siberia, *A. tatarinowii* Schott from the neighborhoods of Beijing in China, *A. nilaghirensis* Schott from Peninsular India, and *A. griffithii* Schott from Bhutan. There is a consensus in the literature that *A. triqueter*, *A. nilaghirensis,* and *A. griffithii* belong to the *A. calamus* group [11,13,33]. Our earlier study disentangled controversies related to *A. tatarinowii* and revealed that despite the widespread erroneous use of this species name for a member of the *A. gramineus* group [23,24,27–30,38], its original description provided by Schott was based on material belonging to the *A. calamus* group [31].

The knowledge of chromosome numbers and therefore more precise taxonomic identification in the *A. calamus* group is of practical importance because *Acorus* is widely used in medicine for its essential oil and the ploidy level correlates with essential oil content and composition [39–43].

The question of the number of species and/or infraspecific taxa to be recognized within the *A. calamus* group remains controversial and requires detailed investigations involving molecular data. The present study is a step toward a comprehensive taxonomic revision of the group that will be presented elsewhere. Our tentative hypothesis is that diploid and tetraploid members of the *A. calamus* group belong to different taxonomic species. Their correct naming requires an extensive step-by-step inference of ploidy level in type material of various species names proposed for members of this group and currently considered synonyms of *A. calamus* s.l. In the present study, we disentangle problems of ploidy inference in type material of species names proposed by Schott using characters of leaf anatomy and pollen stainability.

## 2. Materials and Methods

### *2.1. Nomenclatural Analysis*

Protologues of all names discussed here were analyzed and typification procedures were performed for *A. triqueter*, *A. griffithii,* and *A. nilaghirensis* according to the rules of plant nomenclature [34]. The typification of *A. tatarinowii* was established and discussed in our earlier publication [31].

The original material was searched for in historical herbarium collections of the British Museum (BM), Royal Botanic Gardens, Kew (K), Muséum national d'Histoire naturelle, Paris (P) and Komarov Botanical Institute (LE). Herbarium specimens were examined *de visu* (LE) or as scanned images via the Internet (BM, K, P).

### *2.2. Ploidy Level*

Röst [10] studied leaf anatomy in four diploid, 22 triploid, and 14 tetraploid accessions of *A. calamus* (see [44] for a list of accessions used in [10]) and revealed that the size of air canals in leaf aerenchyma allows precise distinguishing of diploids, triploids, and tetraploids of *A. calamus* in a common garden experiment. Our follow-up studies [36,37] refined the protocol provided by Röst [10]. Leaf anatomy of specimens not cultivated in controlled conditions still provided information useful for inferring ploidy levels, though certain character overlapping between triploids and tetraploids was found [36,37]. Triploids, however, can be identified based on their sterility. Packer and Ringius [18] studied the stainability of pollen from as many as about 280 herbarium specimens of *Acorus* from Canada and provided convincing evidence that sterile triploids can be recognized based on their nonstaining pollen. Thompson [21] studied as many as 352 specimens from North America and came to the same conclusion.

The protocol for the present study and the interpretation of leaf anatomy were the same as in our earlier works [36,37]. 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 an Olympus SZX-4 microscope. Two metrics were used: (1) The number of air canals per 0.62 $mm^2$ of cross section. (2) Mean number of cells in the septa between the air canals visible in cross section of the leaf blade. See [36,37], for more details of the protocol.

Earlier studies of correlations between leaf anatomy and ploidy levels used cross sections made near the middle of the length of the leaf lamina [10,36,37]. During our work with historical specimens, we aimed at minimizing the damage from destructive sampling. We sampled fragments of leaf blades where the leaves were already broken in a specimen. In some instances, this resulted in sampling the distal part of the lamina. We believe that the structure of the aerenchyma maintains its diagnostic characters in the distal part of the lamina. For testing this hypothesis, leaf tip anatomy was studied in some non-historical specimens.

To study pollen stainability, flower fragments with anthers were sampled from herbarium specimens. Pollen grains were placed on a slide in a droplet of acetocarmine. The slide was kept at 80 °C for 1 min. Then, the slide was placed on wet filter paper in a Petri dish for 24 h. The slides were examined with a Nikon Eclipse Ci microscope.

## 3. Results and Discussion

### *3.1. Acorus triqueter Is a Diploid from Siberia*

*Acorus triqueter* Turcz. ex Schott, Prodr. Syst. Aroid.: 578 (1860).

Type: Russia. Buryat Republic: "Dahuria, Tunka", 1830, *N.S. Turczaninow s.n.* (lectotype designated here by D.D.S. and A.N.S.: LE 01082852!; isolectotypes LE 01082856!, LE 01082857!, LE 01082866!, LE 01082870!).

Turczaninow [45] recorded *Acorus calamus* s.l. from three localities in Russia: "prope salinas Selenginenses" (salt marshes along the Selenga River in Buryat Republic), "prope Tunka" (village along the Tunka River in Buryat Republic; 51.74° N, 102.55° E) and "in Dahuria" (west of Argun River, now in Transbaikalian Krai). According to the collections traced at LE and the routes of Turczaninow [46], the cited localities correspond to Turczaninow's collection years and may be interpreted as the course of Selenga between Selenginsk and Kiakhta (1829), Tunka River (1830), and a large area marked by four populated places: Chita, Nerchinsk, Borzya, and Aksha (1831). Historically, all these collection areas belonged to Dahuria, a large territory in southern Siberia situated east of Lake Baikal. In historical literature, the numerous Turczaninov collections were often referred to as having originated

from "Dahuria", without any further specification, which makes the deciphering of such references difficult in practice.

In the protologue of *A. triqueter*, Schott [2] cited herbarium specimens from the Imperial Botanical Garden in Saint Petersburg, which were collected by Turczaninow in "Dahuria". The specimens of *A. triqueter* currently kept at LE originated from the Botanical Museum of the Imperial Academy of Sciences in Saint Petersburg and the personal collection of Turczaninow, which was originally kept at the Imperial Kharkov University but then partly transferred (Siberian plants) to the Botanical Museum in Saint Petersburg [46]. It seems that the specimens from the Botanical Museum originally belonged, at least partly, to the Botanical Garden, judging from their former possession by C.A. von Meyer, vice-director of the Garden, and were subsequently transferred to the Museum because of the continuous exchange of their collections.

The collections of Turczaninow seen by Schott [2] originally belonged to the Botanical Garden, in agreement with his indication in the protologue of *A. triqueter*. This former possession was confirmed by the facts that the specimens were mounted on the paper used in the Garden at that time to mount the collection of C.F. von Ledebour, and the label of one of these specimens was copied by F. von Herder, then Assistant Curator of the Garden. Altogether, we traced three herbarium sheets of *A. triqueter* that were examined by Schott and labeled by him with this species name, which constitute the original material suitable for lectotypification.

Among the specimens of *A. triqueter* examined by Schott, one specimen (LE 01082852) was collected in "Dahuria, Tunka" by Turczaninow in 1830; its duplicate (LE 01082866) was curatorially separated by Herder when the collection was mounted. Further duplicates of the same collection (LE 01082856, LE 01082857, LE 01082870) were not seen by Schott. The specimens collected by Turczaninow in 1829 and 1831, which are currently preserved at LE, were not sent to Schott for examination.

One of the duplicates of the specimens collected by Turczaninow in 1830 (LE 01082856) is accompanied by another label dated 1829 (LE 01169540), which does not correspond to any herbarium material. This label was apparently misadded to the specimen when the collections were mounted after a long period of being kept loose in folders with other unmounted specimens.

One more specimen of the original material (LE 01082872), which is accompanied by the original annotation slip by Schott but does not bear a label of Turczaninow's collections, comes from "Nertschinsk". This locality practically excludes Turczaninow, who rarely mentioned it in connection with his collections, but corresponds to the specimens collected around Nerchinsk Town (now in Transbaikalian Krai) by M.S. Zenzinov (contemporary German spelling Sensinow), which were received by the Botanical Garden in 1854 and widely cited in botanical literature afterward [45].

Since it was the specimens collected by Turczaninow in "Dahuria" (i.e., Tunka) that were cited in the protologue of *A. triqueter*, these specimens are syntypes and thus eligible for lectotypification. The specimen from Nerchinsk presumably collected by Zenzinov is part of the original material but has no priority in lectotypification because it was not mentioned in the protologue.

The lectotype (Figure 1B) and isolectotypes of *A. triqueter* bear mature fruits with ripe seeds. Therefore, these plants were fertile. This observation rejects the possibility of the triploid chromosome number of this material because triploid representatives of the group of *A. calamus* are known to be fully sterile with no reports of even occasional seed development [35,36,47,48].


**Figure 1.** Lectotype of *Acorus triqueter* Turcz. ex Schott (LE 01082852) and its leaf anatomy: (**A**) General view of the specimen. (**B**) Detail showing ripe fruits. (**C**) Detail of the leaf lamina seen from the side possessing two large vascular bundles of the secondary midrib. (**D**) Detail of the leaf lamina seen from the side possessing only one vascular bundle of the secondary midrib. (**E**) Cross sections of the sampled leaf lamina. (**F**) Detail of aerenchyma in the leaf lamina; a small vascular bundle is visible. ac, air canal; adw, adaxial wing of the leaf lamina; cc, corner cell; lb, large vascular bundle of the secondary midrib. Scale bars: 5 cm (**A**), 1 cm (**B**–**D**), 2 mm (**E**), 0.1 mm (**F**). Image source (**A**,**B**) Virtual herbarium of Komarov Botanical Institute RAS (http://rr.herbariumle.ru/01082852; © Komarov Botanical Institute).

We studied the leaf anatomy in the lectotype (Figure 1E,F) and an isolectotype (LE 01082856, Figure 2A,B). The leaf sampled from the lectotype had a thin adaxial wing that was almost as long as the rest of the cross section in the vertical (i.e., dorsoventral) dimension as seen in the cross section of the lamina (Figure 1E). There was no pronounced abaxial wing. The thick part of the leaf had a large vascular bundle on one side and two large vascular bundles on the other side (Figure 1E). These large bundles formed what can be identified as a secondary midrib. In agreement with these anatomical observations, leaf laminas observed in surface views in the herbarium specimen possessed either one (Figure 1D) or two (Figure 1C) large bundles situated closer to the abaxial side of the ensiform leaf. These two conditions apparently correspond to the difference between the left and right sides of a leaf. Note that the boundary between the thick part of the lamina and the thin adaxial wing cannot be traced in surface view in dry material (Figure 1C,D). The leaf sampled from the isolectotype had a large vascular bundle on either side of the thick part of the lamina and possessed a well-pronounced adaxial wing and a less pronounced abaxial wing (Figure 2A).

In both the lectotype (Figure 1E,F) and isolectotype (Figure 2A,B) of *A. triqueter*, the thick part of the lamina was filled by aerenchyma with wide air canals. The septa between the canals as seen on cross sections were composed of one to nine cells. Here, and below, we follow our earlier approach [36,37] and do not include so-called corner cells in our counts, which can be defined as those that are exposed to more than two air canals and are situated at intersections of septa (Figure 1F).

Our quantitative data on leaf aerenchyma (Table 1) clearly suggest that Turczaninow's collection from Dahuria is composed of diploid plants. In both metrics of leaf anatomy used here, both samples that we examined from Turczaninow's collection fall into the area of our diagram that is occupied by diploid Siberian samples whose ploidy level is properly documented [36]. The difference in the shape of cross sections of the two leaf laminas studied here (Figures 1E and 2A) nicely falls within the range of the variation found in Siberian diploids in our earlier study [36].

**Table 1.** Pollen stainability, fruit development, leaf anatomy, and inferred ploidy levels in the studied accessions of *Acorus calamus* s.l. from historical herbarium collections.

| Specimen ID | Schott's Species Name | Nomencla-Tural Status | Number of Stain-ing/Nonstaining Pollen Grains Counted (% of Fertile) | Fruits with Seeds | Number of air Canals Per 0.62 $mm^2$ of the Cross Section of Leaf Blade (Mean of Two Fields of View) | Mean Cell Number in 20 Septa in Cross Section (Corner Cells not Counted) | Inferred Ploidy Level |
|---|---|---|---|---|---|---|---|
| LE 01082852 | *A. triqueter* | lectotype | n/a | present | 9.75 | 5.4 | diploid |
| LE 01082856 | *A. triqueter* | isolectotype | n/a | present | 22.5 | 4.5 | diploid |
| LE 01182327 | *A. tatarinowii* | isotype | 101/3 (97%) | absent | 107.25 | 1.6 | tetraploid |
| LE 01182325 | *A. tatarinowii* | isotype | 112/1 (99%) | absent | 134.5 | 1.9 | tetraploid |
| LE 01082877 | *A. griffithii* | isotype | 50/1 (98%) | absent | n/a | 1.2 | tetraploid |
| LE 01082855 | *A. nilaghirensis* | isolectotype | 14/1 (93%) | absent | n/a | 1.2 | tetraploid |

The fertile nature of Turczaninow's plants agrees with our conclusion on their diploid chromosome number. *Acorus calamus* s.l. is common in the area of Turczaninow's fieldwork in Central Siberia (Irkutsk Oblast and Buryat Republic). Chromosome numbers have been extensively studied for these territories, and only the diploid cytotype was found [49–52].

Another species name, *A. cochinchinensis* Schott, was accepted as correct for Asiatic diploid plants of the *A. calamus* group in some cytotaxonomic works [17,20]. This name must be rejected because its type-bringing synonym, *Orontium cochinchinense* Lour. [53], was

superfluous and therefore illegitimate due to the nomenclatural inclusion of a previously published species name, *Orontium japonicum* Thunb.

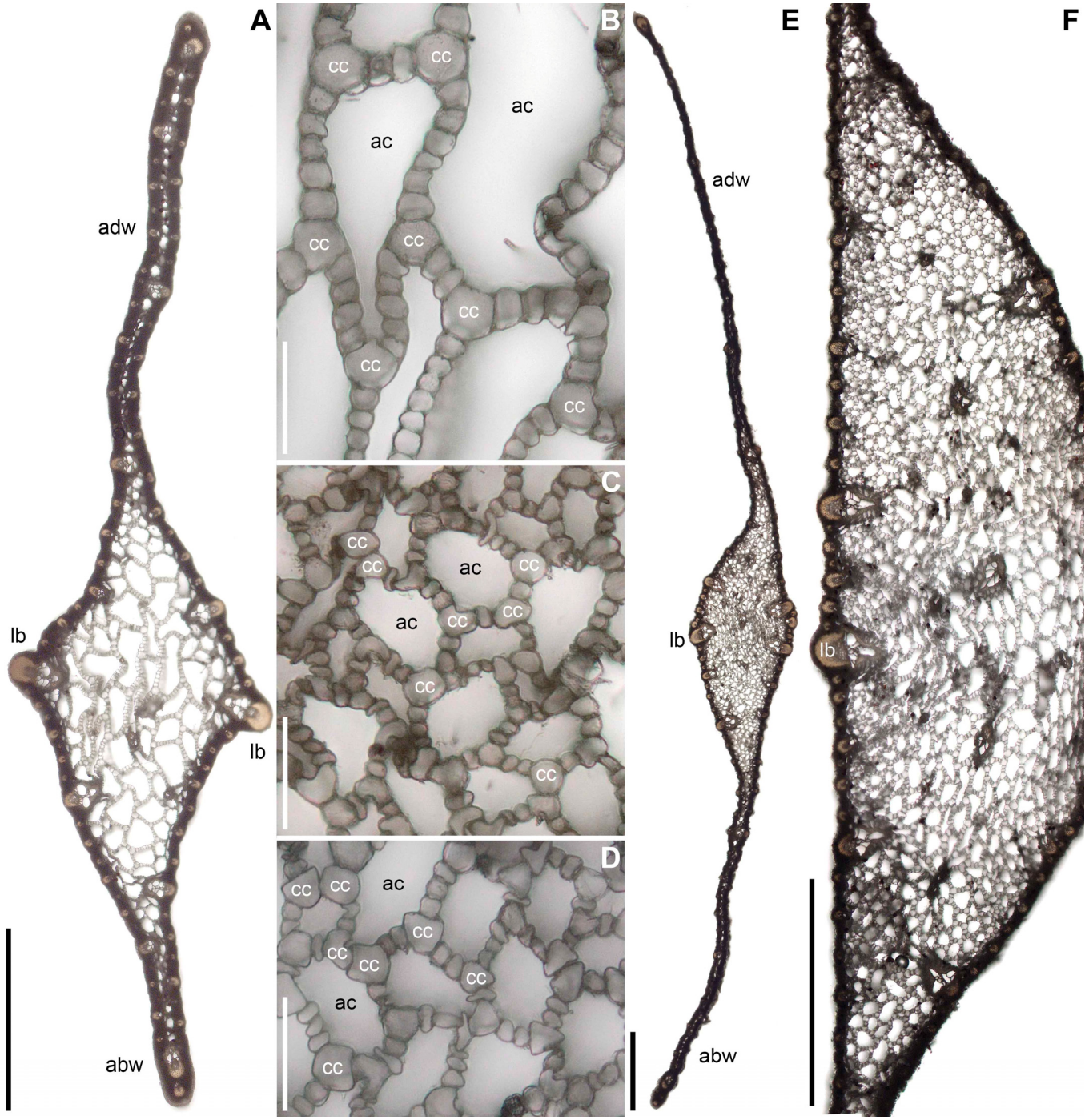


**Figure 2.** Leaf lamina anatomy in an isolectotype of *Acorus triqueter* Turcz. ex Schott and isotypes of *A. tatarinowii* Schott: (**A**,**B**) Isolectotype of *Acorus triqueter* (LE 01082856). (**C**,**E**) Isotype of *A. tatarinowii* (LE 01182325). (**D**,**F**) Isotype of *A. tatarinowii* (LE 01182327). (**A**,**E**) Entire cross sections. (**B**–**D**) Details of aerenchyma. (**F**) Central part of the cross section. abw, abaxial wing of the leaf lamina; ac, air canal; adw, adaxial wing of the leaf lamina; cc, corner cell; lb, large bundle of the secondary midrib. Scale bars: 1 mm (**A**,**E**,**F**), 0.1 mm (**B**–**D**).

Some earlier studies recognized tetraploids of the *A. calamus* group as a segregate species and used the name *A. triqueter* for plants at this ploidy level [17,19,20]. Our study

does not support this view because the type material of *A. triqueter* is inferred here to be diploid.

### 3.2. *Acorus tatarinowii* Is a Tetraploid from N China

*Acorus tatarinowii* Schott, Oesterr. Bot. Z. 9: 101 (1859).

Type [31]: China. Beijing, Changping District: "in a small river near hot springs in Tangshan [Xiaotangshan]", 1840s, *A.A. Tatarinov s.n.* (holotype LE 01182329!; isotypes LE 01182325!, LE 01182326!, LE 01182327!).

The type collection of *Acorus tatarinowii* has been nomenclaturally evaluated in our previous study [31], on which our present work is based. Despite some earlier views on the identity of *A. tatarinowii* as a member of the *A. gramineus* group (summarized in [30]), the type collection belongs to the *A. calamus* group [31].

We studied leaf anatomy and pollen stainability of two isotypes (LE 01182325 and LE 01182327). The pollen was well staining, indicating the fertile nature of the plants (Table 1).

The leaf fragment taken from LE 01182325 allowed complete cross sections to be produced (Figure 2E). The sampled leaf had a thin adaxial wing and a thin abaxial wing. Both wings were well separated from the thick central part of the leaf. The adaxial wing was longer than the abaxial wing in the vertical (i.e., dorsoventral) dimension as seen in the cross section of the lamina (Figure 2E). The abaxial wing was almost as long in the dorsoventral dimension as the thick central part of the leaf (Figure 2E). The thick part of the leaf had a large vascular bundle on one side and two large vascular bundles on the other side (Figure 2E). Several densely spaced smaller bundles were visible on the side of the two larger bundles between them. All these bundles formed what can be interpreted as a well-pronounced secondary midrib. The boundary between the thick part of the lamina and the two wings cannot be traced in surface view in dry material, but the secondary midrib can be easily recognized (not shown here for LE 01182325, but illustrated for the holotype in [31]). The leaf fragment taken from LE 01182327 allowed our investigation of the thick part of the lamina only (Figure 2F). Its structure is similar to that in LE 01182325.

The occurrence of two thin and well-delimited wings of the leaf lamina (adaxial and abaxial) is typical for tetraploid accessions of the *A. calamus* group [10], though some diploid accessions from Siberia also approach this condition [36].

The leaf aerenchyma of both sampled isotypes (Figure 2C,D) was similar in the mean cell number per septa to that in the tetraploid Indian accession, for which direct chromosome counts are available (Figure 3). The number of air spaces per area was even greater than in the tetraploid Indian accession (Figure 3).

According to Röst [10], diploids have less than 19 air canals per 0.62 $mm^2$ of the cross section of the lamina, whereas tetraploids have 77 to ca. 140 canals per 0.62 $mm^2$ of the cross section. We do not know whether Röst [10] counted air canals that are only partially visible on the observed area of 0.62 $mm^2$, but it is clear that our data on the isotypes of *A. tatarinowii* fit the figures for tetraploids provided by Röst [10] even better than the data on the tetraploid Indian accession. In general, the nearly twofold difference in the canal number per area between the Indian sample and the isotypes of *A. tatarinowii* agrees with the degree of variation found by Röst [10] for tetraploids.

To summarize, the shape of the cross section of the lamina does not contradict, and the characters of the leaf aerenchyma strongly suggest, the tetraploid chromosome number of the type material of *A. tatarinowii*.

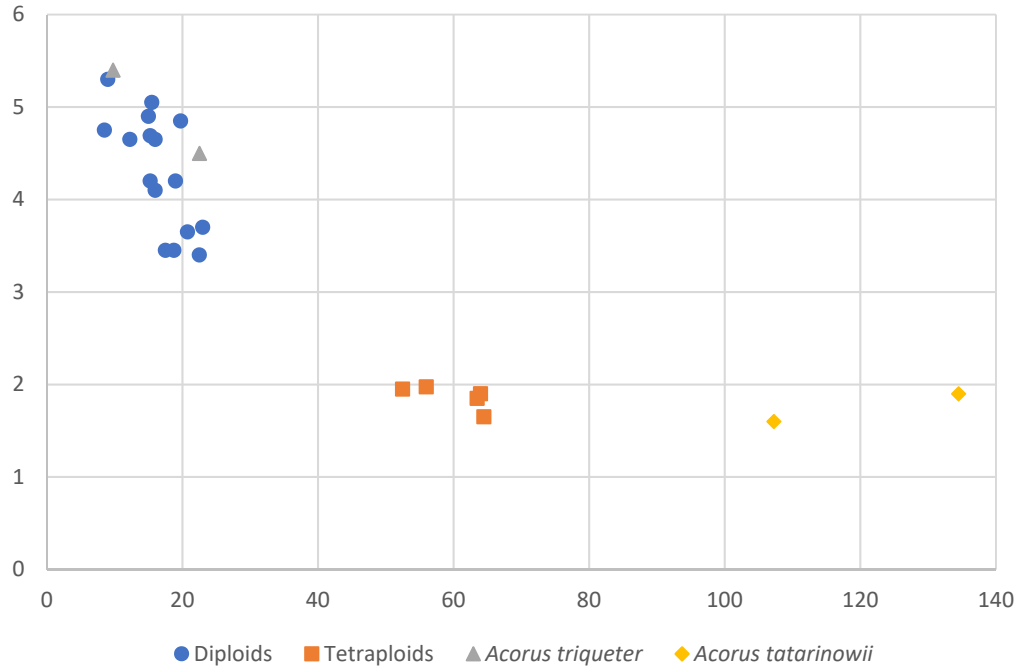


**Figure 3.** Inference of ploidy levels in the type material of *Acorus triqueter* and *A. tatarinowii* using leaf anatomy. Vertical axis: mean cell number in 20 septa in the cross section. Horizontal axis: air canals per 0.62 $mm^2$ of the cross section of leaf blade. Circles represent data on a Siberian diploid sample with direct chromosome count 2n = 24 (taken from [36]). Squares represent data on an Indian tetraploid sample with direct chromosome counts 2n = 44–48 [37]. Triangles and diamonds are data based on specimens from the type collections of *A. triqueter* and *A. tatarinowii*, respectively.

### *3.3. Acorus griffithii Is a Tetraploid from Bhutan*

*Acorus griffithii* Schott, Oesterr. Bot. Z. 8: 351 (1858).

Type: Bhutan. "Benka" (Tashigang Town, c. 27.339° N, 91.547° E), 1–4 February 1838, *W. Griffith 519* (Herb. East India Company 2626; Herb. late East India Company 5941) (holotype: K 000883788; isotypes K 000883697 (fragment with vegetative leaves and unfolded spatha), BM 000958483, LE 01082877!, P 02137244).

This species was described based on a single gathering cited in the protologue [1], which was kept in the personal herbarium of J.D. Hooker, then Director of Kew Botanic Gardens (subsequently incorporated into the herbarium collections of the Gardens). The cited specimens were collected by W. Griffith in "Bootan" (Bhutan).

William Griffith was known to contemporary scientists as a keen botanist and prolific botanical collector [54,55], who collected plant specimens as a researcher documenting the plant morphology and geography rather than as a plant hunter (which was a typical occupation of a traveling botanist in those times) [56,57]. He bequeathed his unpublished manuscripts and extensive herbarium collections to the East India Company, which was the employer of Griffith during his fieldwork. Specimens collected by Griffith were originally labeled by tiny field tickets (one per gathering), solely featuring their field numbers with the corresponding text in Griffith's diaries; sometimes field labels were added with locality data and observations.

Griffith observed and collected plants of *Acorus* in two areas during his journey to Bhutan during 1837–1838, which he treated as two localities under two collection numbers [58]. Based on his travelogue and observations in collections, we can conclude that he collected plants in the following places.

The first collection number of Griffith (519) is linked to the locality that he called "Benka" in the text ("Tassgong or Benkar" on the map) [58], which corresponds to present-day Tashigang (alternative spelling Trashigang) Town in Bhutan. Griffith arrived at this place on 1 February 1838 and proceeded further on 5 February [59].

The second collection number of Griffith (1254) is linked to two or even more localities [58] in present-day Bangladesh and India: "Nurtung in paludib[us] copiosa Sylhet etc." According to the diaries [59], Griffith stayed at "Nurtung" (present-day Nartiang Town in Meghalaya State, India) on 12 November 1837, where he collected some *Acorus* plants along the Myntang River. Earlier in 1837, during 15–21 September, he passed extensive jheels (seasonally shallowly flooded areas) of Sylhet District (present-day Bangladesh) from Habiganj Town up to the Surma River west of Chhatak Town, for at least 70 km, in which further plants of *Acorus* were collected.

Hooker [54] lamented on the great difficulties in deciphering Griffith's collections, which were distributed under distribution numbers but lacked the original collection numbers, which were the only link between the plants and their collection localities. To overcome these difficulties, we examined the specimens at K bearing the original collection numbers and inferred their collection data from the published travel diaries [59]. Specimens distributed under distribution numbers were linked to the original collection numbers using the features of plant morphology and preparation observed in the collections at K.

Specimens collected by Griffith during this expedition, which were numerous and rich in duplicates, were distributed in two steps. First, the specimens were distributed directly from the East India Company, in 1850 or shortly afterward [60,61]. When the East India Company was nationalized in 1858, its London headquarters, the East India House, was abandoned by the company, and the remaining herbarium collections were acquired by the Kew Botanic Gardens. These specimens were labeled and distributed by the Kew Gardens during 1863–1864 [55]. In both distributions, the specimens were renumbered curatorially; the distribution numbers were separate and did not correspond to the original field numbers of Griffith, whereas the original field tickets were retained only for the specimens kept at Kew.

The distribution numbers of the *Acorus* collection were 2626 (East India Company) and 5941 (Kew Botanic Gardens), of which the original specimens were deposited to K. Further duplicates were traced at BM (E.I.C. distribution), LE, and P (Kew distribution). One more duplicate from BM, lacking the original number but seemingly belonging to the E.I.C. distribution, was traced at P (P 02137244). All specimens are illustrated in Figure 4 and labeled as in Table 2.

Our comparative analysis of available herbarium specimens allowed us to recognize three kinds of plant material that can be interpreted as three different collections made by Griffith at three different places and seasons (Figure 4, Table 2). Collection 1 has plants at the male stage of anthesis that are less than 40 cm tall with leaves less than 1 cm wide and spadix less than 5 cm long. Young leaves are present in plants of Collection 1, whereas Collections 2 and 3 lack young leaves. Collection 2 includes plants c. 50 cm tall with leaves about as wide, and spadices about as short, as in Collection 1. Distal parts of the leaves are longitudinally split during collecting; apparently, the material was not immediately pressed. Collection 3 includes plants >70 cm tall with leaves more than 1 cm wide. The foliage appears to be senescent and the inflorescences are old, well postanthetic. The foliage is well pressed though discolored in contrast to Collection 2 where it is partly green, but poorly preserved.

The three collections recognized here may be variously mixed in the same specimen (Figure 4, Table 2).

The *Acorus* specimen at K bearing the original ticket 519 (K 000883697) includes two clearly different parts interpreted here as belonging to Collections 1 and 3. The specimen under the original number 1254 (K 002467072) is also a mixture of two kinds of plants interpreted here as Collections 2 and 3. The element of Collection 3 has no inflorescence in K 002467072, whereas the one in K 000883697 has no vegetative leaves, but these two elements are remarkably alike. There is a specimen in Paris (P 02137232) with the same composition of two elements as in K 002467072, though the element of Collection 3 is reproductive and incomplete here.


**Figure 4.** Specimens of the *Acorus calamus* group collected by W. Griffith in northern British India and dependencies and currently preserved in Herbaria K, LE, BM, and P. Plant material is interpreted as belonging to three different collections (c1, c2, c3, see Table 2). All specimens are at the same magnification. Image sources: Virtual herbarium of Komarov Botanical Institute RAS (http://rr.herbariumle.ru/01082877; © Komarov Botanical Institute), portals of the Natural History Museum, London (https://data.nhm.ac.uk/object/61b3d38a-8ea3-4bf5-abe6-f1ef0a5d3257; © Natural History Museum) and Muséum national d'Histoire naturelle, Paris (https://science.mnhn.fr/institution/mnhn/collection/p/item/p02137244, https://science.mnhn.fr/institution/mnhn/collection/p/item/p02137232; © Muséum national d'Histoire naturelle); the Kew Herbarium Catalogue (http://specimens.kew.org/herbarium/K000883788; http://specimens.kew.org/herbarium/K000883697; http://specimens.kew.org/herbarium/K002467072; © RBG Kew).

**Table 2.** Herbarium specimens of the *Acorus calamus* group collected by W. Griffith in northern British India and dependencies.

| **Herbarium Collection (See Labels in Figure 4)** | **c1** | **c2** | **c3** |
|---|---|---|---|
| Griffith's collection number | 519 | 1254 | 1254 |
| E.I.C. distribution number | 2626 | no distribution | no distribution |
| Kew distribution number | 5941 | 5941 | 5941 |
| herbarium specimens | K 000883788,<br>K 000883697 (pro parte),<br>BM 000958483,<br>LE 01082877,<br>P 02137244 | K 002467072 (pro parte),<br>P 02137232 (pro parte) | K 000883697 (pro parte),<br>K 002467072 (pro parte),<br>P 02137232 (pro parte) |
| collection date | 1–4 February 1838 | 15–21 September 1837 | 12 November 1837 |
| present-day country | Bhutan | Bangladesh | India |
| locality as printed on distribution labels | East Himalaya | East Bengal | East Bengal |
| locality as written on distribution labels | Bhotan | Khasia | Khasia |
| locality as in Griffith's travel diaries | Benka | Sylhet | Nurtung |
| locality in present-day terminology | Tashigang District: Tashigang Town | Sylhet Division: from Habiganj Town to Surma River west of Chhatak Town | Meghalaya State: West Jaintia Hills District, Nartiang Town |
| estimated coordinates | 27.339° N, 91.547° E | between 24.4° N, 91.4° E and 25° N, 91.62° E | 25.566° N, 92.21° E |
| estimated elevation | 700 m | 50 m | 1200 m |
| phenological phase | male stage of anthesis | postanthetic | postanthetic (one inflorescence abortive?) |
| characteristic features | plants less than 40 cm tall, leaves less than 1 cm wide, young leaves present, spadix < 5 cm long | plants c. 50 cm tall, leaves less than 1 cm wide, distal parts of leaves longitudinally split during herborization, young leaves absent, spadix < 5 cm long | plants >70 cm tall, leaves more than 1 cm wide, foliage appears to be senescent, spadix > 5 cm long |
| inferred ploidy level | tetraploid | pending destructive sampling | pending destructive sampling |
| nomenclatural status | type collection of *Acorus griffithii* | no type status | no type status |

Clearly, there was a certain mixture of specimens during their complex history of distribution. The simplest way of interpreting this mixture is the following. What we recognized as Collections 2 and 3 are parts of what Griffith numbered as 1254. Griffith collected plants of 1254 in two localities at different times, but this was not considered of any importance by himself or the curators. What we recognize as Collection 1 corresponds to Griffith's number 519. We found this collection accidentally mixed with specimens from the other area only once, in K 000883697. Our interpretation agrees with the occurrence of young foliage and anthetic inflorescences in Collection 1 because Griffith visited Benka in February, at the very beginning of the season. Collection 3 contains senescent plants and thus most likely corresponds to the material collected in November at Nartiang, whereas partly green plants were apparently collected in September in Sylhet (Table 2).

The provenance of the distributed plants stated on their printed labels of the Kew distribution ("East Himalaya" for Griffith 519; "East Bengal" for Griffith 1254) agrees very well with our interpretation.

With a complete history of Griffith's collections of *Acorus* at hand, we can return to the question of the typification of *A. griffithii*. There are two specimens of *A. griffithii* kept in the personal herbarium of Hooker (now at K). The first specimen (K 000883788) consists of three vegetative and two generative plant fragments mounted together and accompanied by an identification slip written by Schott and a distribution number of 'Herb. East India Company' (specimen distribution dated 1850). This specimen was collected in present-day Bhutan ("Benka"). The second specimen (K 000883697), which is accompanied by a distribution label from 'Herbarium of the late East India Company' (specimen distribution dated 1863–1864) and an original ticket of Griffith, was not available to Hooker prior to the publication of the protologue of *A. griffithii* and was therefore not examined by Schott. This means that only a single specimen of *A. griffithii* was studied by Schott, which is necessarily a holotype. Other specimens of the same collection (K 000883697 pro parte, BM 000958483, LE 01082877, and P 02137244) are duplicates and have the nomenclatural status of isotypes.

The type collection of *A. griffithii* (Figures 4 and 5A) has relatively short leaves (up to 40 cm) compared to many other available collections of the *A. calamus* group from various counties, including Bhutan. According to "Flora of Bhutan", *A. calamus* s.l. has leaves 37–80 cm long [62]. The difference can be explained by the fact that Griffith collected his material in early February, whereas most other collections of *A. calamus* s.l. were made during the summer or spring. Our observations on an Indian accession of tetraploid *Acorus* revealed that it has a shorter generation of leaves during the winter [37].

On a global scale, members of the *A. calamus* group are mostly summer-green plants. This character is even used to distinguish this group from *A. gramineus* in "Flora of Japan" [14]. There are, however, differences in the timing of autumn leaf senescence and spring leaf emergence among representatives of different cytotypes of the *A. calamus* group [10,63]. Röst [10] demonstrated that (sub)tropical tetraploids can be distinguished by their keeping leaves green for a much longer period in autumn and starting regrowth earlier in spring in common garden experiments with temperate diploid, triploid, and tetraploid accessions. Moreover, the tetraploid Indian *A. calamus* s.l. is at least sometimes truly evergreen [37]. This growth pattern, to our knowledge, was not reported for diploids and triploids. Therefore, the fact that Griffith collected plants with green leaves in early February already suggests that the plants were tetraploids.

*Acorus calamus* s.l. normally grows at elevations of 610–2800 m and flowers from April to July in Bhutan [62]. Assuming that Griffith's material was most likely collected in the river valley, it comes from an elevation of about 700 m, near the lower margin of its altitudinal range. These local climatic conditions should support the occurrence of a (sub)tropical evergreen plant. The fact that the plants had inflorescences (at the second, male stage of anthesis) in early February is remarkable compared to other observations made in Bhutan. More data should be collected on the timing of anthesis of the tropical tetraploid *Acorus*. Such reports are scattered because the plants seldom produce inflorescences in the tropics [37].

We studied the leaf anatomy and pollen stainability of the isotype of *A. griffithii* kept at Saint Petersburg (LE 01082877). The pollen was well staining, indicating the fertile nature of the plants (Table 1).

Leaves of LE 01082877 have a well-pronounced secondary midrib along most of the length of the lamina (Figure 5C), but the midrib becomes inconspicuous toward the leaf apex (Figure 5B). The leaf apex tends to be S-shaped (Figure 5A,B), a feature that is typical of tetraploids of the *A. calamus* group [10,32].


**Figure 5.** Isotype of *Acorus griffithii* Schott (LE 01082877) and its leaf anatomy: (**A**) General view of the specimen. (**B**) Detail of the leaf tip. (**C**) Middle part of the leaf lamina with a single large vascular bundle of the secondary midrib. (**D**) Cross section of the leaf. (**E**,**F**) Details of the cross section. ac, air canals; lb, large bundle of the secondary midrib; arrowhead, the place of sampling for leaf anatomy. Scale bars: 5 cm (**A**), 5 mm (**B**,**C**), 1 mm (**D**), 0.1 mm (**E**), 0.5 mm (**F**). Image source (**A**–**C**) Virtual herbarium of Komarov Botanical Institute RAS (http://rr.herbariumle.ru/01082877; © Komarov Botanical Institute).

The leaf fragment of LE 01082877 studied here (see arrowhead in Figure 5A) anatomically was almost completely composed of thin adaxial and abaxial wings (Figure 5D). A large bundle of the secondary midrib was present on only one side of the leaf (Figure 5D).

These features agree with our observations of the external morphology of the intact leaf tip (Figure 5B). Only a limited amount of aerenchyma was present in the central part of the cross section of the lamina (Figure 5E,F). It is not possible to count the number of air canals per 0.62 $mm^2$ of the cross section because this area exceeds the width of the strip of aerenchyma. The septa as observed in cross sections are mostly composed of 1–2 cells (mean: 1.2), which is lower than the figures found in a tetraploid Indian accession with direct chromosome counts and in the isotypes of *A. tatarinowii* (Table 1, Figure 3). Since tetraploids differ from diploids in having fewer cells per septum, we conclude that Griffith's material belongs to tetraploids.

To ensure that the features of aerenchyma remain stable in distal parts of the leaf lamina in both diploids and tetraploids, leaf tip sections of confirmed diploids and tetraploids were examined. They convinced us of the stability of the character (Figure 6).

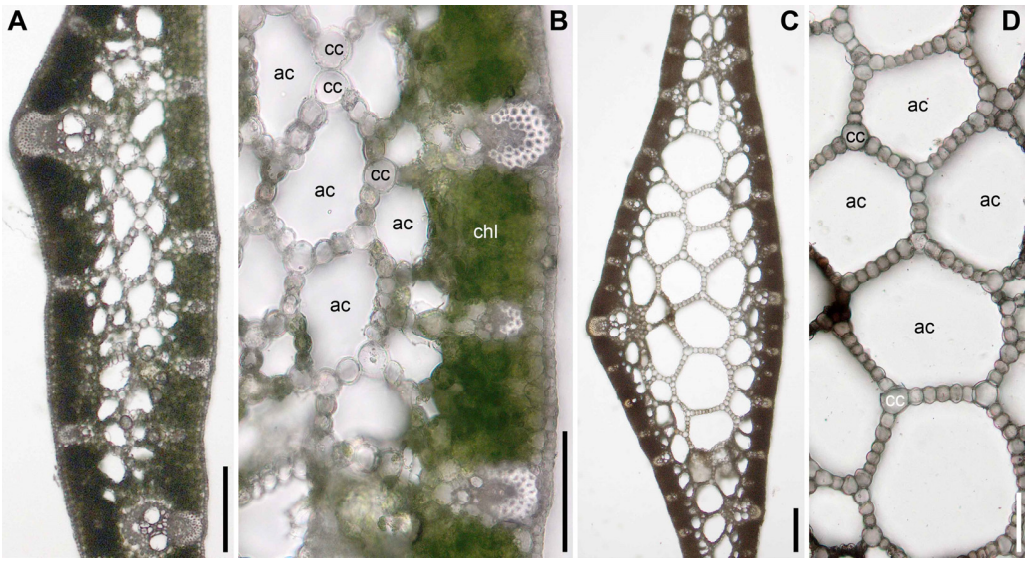


**Figure 6.** Anatomy of distal parts of the leaf lamina in specimens of the *Acorus calamus* group with precisely identified ploidy level. These images show that the structure of leaf aerenchyma maintains features characteristic of diploids and tetraploids up to the distal part of the lamina. Diploids, compared to tetraploids, have wider air cavities divided by septa with more numerous cells visible in the cross section: (**A**,**B**) Tetraploid from India (voucher: MW0758480). (**C**,**D**) Diploid from Siberia (voucher: MW0955200). Scale bars: 0.2 mm (**A**,**C**), 0.1 mm (**B**,**D**).

### *3.4. Acorus commutatus Is Another Name for A. griffithii*

*Acorus commutatus* Schott, Prodr. Syst. Aroid.: 578 (1860).

Type: (icon) Griffith, Ic. Pl. Asiat. 3: pl. CLXII (1851) (holotype). Epitype (designated here by D.D.S. and A.N.S.): Bhutan. "Benka" (Tashigang Town, c. 27.339° N, 91.547° E), 1–4 February 1838, *W. Griffith 519* (Herb. East India Company 2626; Herb. late East India Company 5941) (K 000883788; isoepitypes K 000883697 (Collection 1), BM 000958483, LE 01082877!, P 02137244).

While traveling through northern British India and its dependencies, Griffith produced numerous analytical drawings of various plants, in which he especially studied the structure of floral parts, including the anatomy of ovaries. These drawings, collected into four dedicated volumes, each accompanied by a separate volume of textual comments, were published posthumously by the Government of Bengal on behalf of the East India Company [54].

The drawings were considered to be "executed in the same bold and rather rude, but faithful, manner, for which the elder Richard was distinguished" ([54], p. 447). The original drawings were lithographed for publication by local copyists [54], who may also have introduced distortions when the pencil drawings were insufficiently clear in detail. Due

to the lack of supervision from the author of the drawings, this process may have led to significant inaccuracies in the minute features of the plant portraits.

Griffith's drawing of *Acorus* [64] shows that it was devised by a highly qualified botanist who paid attention to a lot of non-trivial details such as the variation in the flower groundplan. At the same time, the drawing includes some inaccuracies that may be explained by the unsupervised work of a copyist. For example, it is difficult to figure out what leaf represents a spatha in the illustration (even though Griffith provided a fully adequate discussion on spatha in the text [65]). The spadix appears to be stalked in the figure, which is never the case in *Acorus*. In panel 1 of the drawing, the central and right-hand tepals are shown alternately overlapping each other, in an unrealistic way, being apparently misunderstood by the copyist.

Griffith illustrated pollen grains in their dehydrated and hydrated state. At first glance, the images of hydrated grains are unrealistic because they appear to show two apertures. Such grains are unknown in *Acorus*, and a dehydrated grain is shown as clearly monosulcate. The two aperture-like slits shown in the hydrated grains are in the same sector of the grain rather than in two opposite sectors as can be expected in real grains with two apertures. We should conclude that Griffith (or the copyist?) had mistaken the aperture margins of fully hydrated pollen grains for two individual apertures. In our view, this interpretation is rather plausible. If so, the pollen protoplast should have been viable in most pollen grains. Otherwise, Griffith, with his detailed interest in variation, would have illustrated defective pollen grains, too. Therefore, we have evidence that the pollen was fertile, which agrees with our conclusion that the chromosome number of *Griffith 519* was tetraploid (see above).

The text accompanying the drawing [65] says that Griffith observed *Acorus* plants in "the Khasiya and Sub-Himalayan ranges". The first locality seemingly denotes the Garo-Khasi-Jaintia Range rather than the Khasi Hills proper, and means Nartiang Town, now in Meghalaya State, India, whereas the second locality corresponds to the collection of *A. griffithii* from Tashigang Town in Bhutan. These localities and the history of their herbarium collections are detailed above under *A. griffithii*.

The drawing portrays a plant in full blossom, with rather short inflorescences and spathae. As shown above, Griffith made only one herbarium collection of *Acorus* that was in flower (Table 2), i.e., the specimens from Tashigang, Bhutan. This collection also agrees with the drawing in morphology and, therefore, is its obvious source.

Being unaware of the identity of the *Acorus* specimens collected by Griffith from Bhutan and the illustration made by Griffith from the same plants, Schott (1860) established a new species of *Acorus* (*A. commutatus* Schott) solely based on this illustration (unlike for other species, he omitted to mention the dried or live plants examined in this particular case, and stated that he derived his species description from the published illustration). In doing so, he produced two species names based on the same plant collection but on its different physical elements, i.e., herbarium specimen and illustration. This fact establishes the taxonomic identity of these two species but does not make their names illegitimate because their nomenclatural types were formally different. To ensure the nomenclatural identity of these species names in addition to their taxonomic identity, and because of the practical impossibility of inferring a ploidy level from the type illustration, we designate the holotype of *A. griffithii* as an epitype of *A. commutatus*.

### *3.5. Acorus nilaghirensis Is a Tetraploid from the Peninsular India*

*Acorus nilaghirensis* Schott, Oesterr. Bot. Z. 9: 101 (1859). Type: India. Tamil Nadu State: "Khoondas. In montibus Nilagiri" (Kundah River, Nilgiri Mts., c. 11.284 N, 76.643 E), April 1851, *F. Metz* (Hohenacker, Plantae Indiae orientalis sect. V (M. Nilagiri) no. 1314) (lectotype designated here by D.D.S. and A.N.S.: LE 01082855!; isolectotype LE 01082858!).

This species was described by Schott [2] based on a single collection from "India orientalis (Nilagiri montes)", which he examined in the specimens distributed as "Hohenacker pl. Ind. orient."

These specimens were collected by Johan Friedrich Metz (1819–1886), a Christian (Protestant) missionary at Kethi, a station of the Basel Mission Society near Udhagamandalam (British spelling Ootacamund) Town in Nilgiris District, Tamil Nadu State, India [66,67]. The sampled area was situated in the Nilgiri Mts., Western Ghats (Sahyadri) Mountain Range, and also at Mangalore Town [68], whereas the *Acorus* specimen was collected at "Khoondas" (Kundah River) in the Nilgiri Mts. Metz sampled plant specimens for commercial distribution by R.F. Hohenacker on behalf of the Botanical Travel Society in Esslingen [69], which was a major institution for plant hunting and distribution in the 19th century [70]. The *Acorus* specimen was seemingly collected in 1851 because it was reported as being dispatched from India to Europe in December of that year within the second set of the Nilgiri collections of Metz [71]. It was distributed in 1853 in the fifth installment of Indian plants collected by Metz [72].

The Indian exsiccatae collected by Metz and edited by Hohenacker were widely distributed in Europe, and many further duplicates of the type collection of *A. nilaghirensis* are expected elsewhere. For example, a set of these plants was purchased for the British Museum in London [73].

Schott [2] made no mention in the protologue of *A. nilaghirensis*, in which collection he examined the type specimen or specimens. His article contained descriptions of a number of new species of aroid plants, which were based almost exclusively on the collections received on loan from the Imperial Botanical Garden in Saint Petersburg [2,3].

Specimens from the Nilgiri Mts. distributed by Hohenacker were accessioned by the Imperial Botanical Garden in Saint Petersburg in 1856 [74]; a specimen of *Acorus* from these collections was sent on loan to Schott shortly thereafter [31]. The Herbarium of the Botanical Garden was a predecessor of the Komarov Botanical Institute (LE), in which we traced two specimens of the type collection. One specimen, previously possessed by the Botanical Garden, as evident from its mounting paper sheet, bears an identification label written by Schott and is undoubtedly part of the original material of *A. nilaghirensis*. The second specimen was originally kept at the Botanical Museum of the Imperial Academy of Sciences in Saint Petersburg and was not available to Schott [31].

In his monograph [3], Schott regularly mentioned places in which he examined herbarium collections but only in those cases when a single herbarium institution was involved. In the entry for *A. nilaghirensis*, Schott stated that he had seen dried specimens but a particular institution was not specified, meaning that he examined the material not only from Saint Petersburg but also elsewhere.

Noteworthy, in the entry of *Rhaphiophallus hohenackeri* Schott, he cited another collection from India made by Metz, which he received directly from Hohenacker. In our opinion, this mention hints at the other source of collections for *A. nilaghirensis*.

Indian specimens collected by Metz were distributed in six installments and numbered sequentially by Hohenacker according to their preparation for distribution. The first installment, with plants collected near Mangalore, was originally published (offered for sale) in 1847 [75]. Since then, the sale of Indian plants had been repeatedly advertised by Hohenacker, with the greatest number of exsiccatae offered in 1858 [69]. The specimens of *R. hohenackeri* revised by Schott were distributed with printed labels under the heading of 1847 but numbered 2164b, thus indicating their very late actual arrival to Esslingen and distribution (according to our observations, the practice of supplementing previously published commercial exsiccatae by further herbarium specimens was quite common in the 19th century). This collection evidences that Schott received a set of aroid plants collected by Metz directly from Hohenacker, around 1857, and the lack of mention of a single herbarium institution for *A. nilaghirensis* indicates that a specimen of this plant was also included in Hohenacker's parcel.

As *A. nilaghirensis* was based on more than one specimen, lectotypification of this species name may be needed. The personal herbarium of Schott, in which a specimen of *A. nilaghirensis* was presumably kept, was completely destroyed in 1945 at the end of the Second World War [76]. For this reason, we designate a specimen in Saint Petersburg,

which was examined and annotated by Schott prior to the publication of the protologue of *A. nilaghirensis*, as a lectotype of this species name.

Our data on pollen fertility and leaf anatomy (Table 1, Figure 7) are similar to those of the isotype of *A. griffithii*, thus indicating that *A. nilaghirensis* was described based on tetraploid material, too.


**Figure 7.** Isolectotype of *Acorus nilaghirensis* Schott (LE 01082855) and its leaf anatomy: (**A**) General view of the specimen. (**B**) Cross section of the leaf. (**C**) Detail of the cross section showing leaf aerenchyma. (**D**) Middle part of the leaf lamina with a single large vascular bundle of the secondary midrib. ac, air canal; cc, corner cell; lb, large vascular bundle. Scale bars: 5 cm (**A**), 1 mm (**B**), 0.1 mm (**C**), 1 cm (**D**). Image source (**A**,**D**) Virtual herbarium of Komarov Botanical Institute RAS (http://rr.herbariumle.ru/01082855; © Komarov Botanical Institute).

The type collection of *A. nilaghirensis* consists of reproductive parts of the shoots including a relatively long scape (c. 30 cm) and spatha (apparently, at least up to 50 cm). The distal part of the spatha was lost in all three plant fragments available in the two LE specimens. Small fragments were sampled near broken leaf tips. The leaf fragments were almost completely composed of thin adaxial and abaxial wings (Figure 7B; only one of the two fragments is illustrated). A large bundle of the secondary midrib was present on only one side of the leaf. Only a limited amount of aerenchyma was present in the central part of the cross section of the lamina (Figure 7C). The septa as visible in cross sections are mostly composed of 1–2 cells (mean: 1.2), which is lower than the figures found in the tetraploid Indian accession with direct chromosome counts and in the isotypes of *A. tatarinowii* (Table 1, Figure 3).

## 4. Conclusions

Our study allowed the inference of ploidy levels in historical herbarium specimens that can be precisely linked to species names proposed by Schott in the basal monocot genus *Acorus*. Our data fit well with earlier results on the geographical distribution for cytotypes within the group of *A. calamus* s.l. Future studies should focus on the search for potential diagnostic characters of hexaploid members of the group. We did not consider the possibility of the hexaploidy nature of any specimen discussed here because of their origin. As far as is known from detailed cytotaxonomic studies, hexaploids are localized in Yunnan [77] and Kashmir [42], areas that are far from the origin of the plants discussed here. Cytotype distribution is known to correlate with altitudinal range in South Asia, with diploids and hexaploids occurring at high elevations [42], much above the positions of all localities in China, Bhutan, and India discussed in the present paper.

The present study highlights the value of a synthesis between the history of botany and micromorphology in inferring plant biology. It disentangles issues of plant nomenclature and provides a step toward a global taxonomic revision of the basal monocot genus *Acorus,* which includes plants of high medicinal importance. Moreover, we further support the idea that herbarium collections provide an important resource of information on the actual and historical distribution of cytotypes in *Acorus* (e.g., [21]). This information is highly important for the purpose of conservation of genetic resources because the chemical composition of essential oils correlates with ploidy levels in *Acorus* [10,41–43,63].

**Author Contributions:** D.D.S. and A.N.S. performed a nomenclatural analysis and wrote the draft text; M.V.R. studied and interpreted leaf anatomy; E.E.S. studied and interpreted pollen stainability. All authors have read and agreed to the published version of the manuscript.

**Funding:** The work of A.N.S. received no external funding. The study of taxonomy, anatomy, and palynology (D.D.S., M.V.R., and E.E.S.) was supported by the Russian Science Foundation, project 19-14-00055-P.

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** High-quality images of all herbarium specimens discussed here are available through data portals of the Herbarium of the Komarov Botanical Institute (https://en.herbariumle.ru, accessed 27 April 2023), the Natural History Museum, London (https://data.nhm.ac.uk/, accessed 27 April 2023), and Muséum national d'Histoire naturelle, Paris (https://science.mnhn.fr/institution/mnhn/search, accessed 27 April 2023), as well as the Kew Herbarium Catalogue (http://apps.kew.org/herbcat, accessed 27 April 2023).

**Acknowledgments:** We are indebted to L.V. Averyanov, D.V. Geltman, A.E. Grabovskaya-Borodina, A.V. Leostrin, and L.V. Orlova for facilitating physical and digital access to collections of the Herbarium of the Komarov Botanical Institute, and to Lesley Walsingham and Maria Vorontsova for digitizing additional material of *Acorus* at Kew and access to these images. Open access funding provided by University of Helsinki.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Schott, H.W. Aroideen-Skizzen. *Oesterr. Bot. Z.* **1858**, *8*, 349–351. [CrossRef]
2. Schott, H.W. Aroideen-Skizzen. *Oesterr. Bot. Z.* **1859**, *9*, 98–101. [CrossRef]
3. Schott, H.G. *Prodromus Systematis Aroidearum*; Typis Congregationis Mechitharisticae: Vienna, Austria, 1860.
4. Schott, H.G.; Araceae, H.W. Pars altera. Nova genera et species, adiectis quibusdam adnotationibus. *Ann. Mus. Bot. Lugduno-Batavi* **1864**, *1*, 278–286.
5. Duvall, M.L.; Learn, G.H., Jr.; Eguiarte, L.E.; Clegg, M.T. Phylogenetic analysis of rbcL sequences identifies *Acorus calamus* as the primal extant monocotyledon. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 4641–4644. [CrossRef]
6. Bogner, J.; Mayo, S.J. Acoraceae. In *The Families and Genera of Vascular Plants*; Kubitzki, K., Ed.; Springer: Berlin, Germany, 1998; Volume 4, pp. 7–11.
7. The Angiosperm Phylogeny Group; Chase, M.W.; Christenhusz, M.J.M.; Fay, M.F.; Byng, J.W.; Judd, W.S.; Soltis, D.E.; Mabberley, D.J.; Sennikov, A.N.; Soltis, P.S.; et al. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* **2016**, *181*, 1–20. [CrossRef]
8. Engler, A. Araceae. In *Monographiae Phanerogamarum Prodromi Nunc Continuatio, Nunc Revisio*; Candolle, A., de Candolle, C., Eds.; G. Masson: Paris, France, 1879; Volume 2.
9. Engler, A. Araceae-Pothoideae. In *Das Pflanzenreich, 21 (IV. 23B)*; Engler, A., Ed.; Engelmann: Leipzig, Germany, 1905; pp. 1–330.
10. Röst, L.C.M. Biosystematic investigations with *Acorus* L. 4. Communication. A synthetic approach to the classification of the genus. *Planta Med.* **1979**, *37*, 289–307. [CrossRef]
11. Li, H.; Zhu, G.; Bogner, J. Acoraceae. In *Flora of China*; Wu, Z., Raven, P., Eds.; Science Press: Beijing, China; Missouri Botanical Garden Press: St. Louis, MI, USA, 2010; Volume 2, pp. 1–2.
12. Bogner, J. Acoraceae. In *Flora Malesiana*, 1st ed.; Noteboom, H.P., Ed.; Nationaal Herbarium Nederland: Leiden, The Netherlands, 2011; Volume 20, pp. 1–13.
13. Boyce, P. Acoraceae. In *Flora of Thailand*; Santisuk, T., Larsen, K., Eds.; The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation: Bangkok, Thailand, 2012; Volume 11, pp. 322–325.
14. Murata, J. Acoraceae. In *Flora of Japan*; Iwatsuki, K., Boufford, D.E., Ohba, H., Eds.; Kodansha: Tokyo, Japan, 2016; Volume IVb, p. 5.
15. Linnaeus, C. *Species Plantarum*; L. Salvius: Holmia, Sweden, 1753.
16. Aiton, W. *Hortus Kewensis, or a Catalogue of the Plants Cultivated in the Royal Botanic Garden at Kew*; George Nicol: London, UK, 1789; Volume 1.
17. Löve, Á.; Löve, D. Drug content and polyploidy in *Acorus*. *Proc. Genet. Soc. Can.* **1957**, *2*, 14–17.
18. Packer, J.G.; Ringius, G.S. The distribution and status of *Acorus* (Araceae) in Canada. *Can. J. Bot.* **1984**, *62*, 2248–2252. [CrossRef]
19. Löve, Á.; Löve, D.; Löve, Á. Chromosome Number Reports LXXXIX. *Taxon* **1985**, *34*, 729–730. [CrossRef]
20. Petersen, G. Cytology and systematics of Araceae. *Nord. J. Bot.* **1989**, *9*, 119–166. [CrossRef]
21. Thompson, S.A. Systematics and Biology of the Araceae and Acoraceae of Temperate North America. Ph.D. Thesis, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 1995.
22. Thompson, S.A. Acoraceae. In *Flora of North America North of Mexico*; Magnoliophyta: Alismatidae, Arecidae, Commelinidae (In Part), and Zingiberidae; Flora of North America Editorial Committee, Ed.; Oxford University Press: New York, NY, USA, 2000; Volume 22, pp. 124–127.
23. Larsen, K. Cytology of vascular plants III. A study of Thai aroids. *Dansk Bot. Ark.* **1969**, *27*, 39–59.
24. Li, H. Acorus. In *Flora Reipublicae Popularis Sinicae*; Wu, C., Li, H., Eds.; Science Press: Beijing, China, 1979; Volume 13, pp. 4–9. (In Chinese)
25. Wei, F.N.; Li, Y.K. A new spice, *Acorus macrospadiceus* from south China. *Guihaia* **1985**, *5*, 179–182. (In Chinese)
26. Zhu, Z. Some new taxa of *Acorus* (Araceae) from Sichuan. *Acta Bot. Bor.-Occ. Sinica* **1985**, *5*, 118–121. (In Chinese)
27. Ho, P.H. *An Illustrated Flora of Vietnam*; Nha Xuat Ban Tre: Ho Chi Minh, Vietnam, 2000; Volume 3, pp. 1–1020. (In Vietnamese)
28. Zhang, W. Pharmacognostic Study of *Acorus*—From a Phylogenetic Perspective. Ph.D. Thesis, Hong Kong Baptist University, Hong Kong, China, 2005.
29. Shu, H.; Zhang, S.; Lei, Q.; Zhou, J.; Ji, Y.; Luo, B.; Hong, L.; Li, F.; Liu, B.; Long, C. Ethnobotany of *Acorus* in China. *Acta Soc. Bot. Polon.* **2018**, *87*, 3585. [CrossRef]
30. Cheng, Z.; Shu, H.; Zhang, S.; Luo, B.; Gu, R.; Zhang, R.; Ji, Y.; Li, F.; Long, C. From folk taxonomy to species confirmation of *Acorus* (Acoraceae): Evidences based on phylogenetic and metabolomic analyses. *Front. Plant Sci.* **2020**, *11*, 965. [CrossRef]
31. Sokoloff, D.D.; Remizowa, M.V.; Nuraliev, M.S.; Averyanov, L.V.; Sennikov, A.N. The first genome from the basal monocot family has been misnamed: Taxonomic identity of *Acorus tatarinowii* (Acoraceae), a source of numerous chemical compounds of pharmaceutical importance. *Diversity* **2023**, *15*, 176. [CrossRef]
32. Wulff, H.D. Zur Zytologie, geographischen Verbreitung und Morphologie des Kalmus. *Arch. Pharm.* **1954**, *287*, 529–541. [CrossRef]
33. POWO. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. 2023. Available online: http://www.plantsoftheworldonline.org/ (accessed on 29 March 2023).

34. Turland, N.J.; Wiersema, J.H.; Barrie, F.R.; Greuter, W.; Hawksworth, D.L.; Herendeen, P.S.; Knapp, S.; Kusber, W.-H.; Li, D.-Z.; Marhold, K.; et al. (Eds.) International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code). In Proceedings of the Adopted by the Nineteenth International Botanical Congress, Shenzhen, China, 23–29 July 2017; Koeltz Botanical Books: Glashütten, Germany, 2018. [CrossRef]
35. Wulff, H.D. Über die Ursache der Sterilität des Kalmus (*Acorus calamus* L). *Planta* **1940**, *31*, 478–491. [CrossRef]
36. Sokoloff, D.D.; Skaptsov, M.V.; Vislobokov, N.A.; Smirnov, S.V.; Shmakov, A.I.; Remizowa, M.V. Morphological characterization of diploid and triploid *Acorus calamus* (Acoraceae) from southern Western Siberia, parthenocarpy in sterile plants and occurrence of aneuploidy. *Bot. J. Linn. Soc.* **2021**, *195*, 189–215. [CrossRef]
37. Sokoloff, D.D.; Remizowa, M.V.; Skaptsov, M.V.; Yadav, S.R.; Sennikov, A.N. Back to Linnaeus: Proper botanical naming of the tetraploid Indian *Acorus* (Acoraceae), an important medicinal plant. *Diversity*. *submitted*.
38. Shi, T.; Huneau, C.; Zhang, Y.; Li, Y.; Chen, J.; Salse, J.; Wang, Q. The slow-evolving *Acorus tatarinowii* genome sheds light on ancestral monocot evolution. *Nat. Plants* **2022**, *8*, 764–777. [CrossRef]
39. Wulff, H.D. Der Ölgehalt verschiedenchromosomiger Rassen vom Kalmus (*Acorus Calamus* L.). *Zeitschr. Naturforsch. A* **1946**, *1*, 600–603. [CrossRef]
40. Wulff, H.D. Ölgehalt und Chromosomenzahl des nordamerikanischen Kalmus (*Acorus calamus* L.). *Arch. Pharm.* **1950**, *283*, 155–161. [CrossRef]
41. Röst, L.C.M.; Bos, R. Biosystematic investigations with *Acorus* L. 3. Communication. Constituents of essential oils. *Planta Med.* **1979**, *36*, 350–361. [CrossRef]
42. Mittal, N.; Varshney, V.K.; Song, B.H.; Ginwal, H.S. High levels of diversity in the phytochemistry, ploidy and genetics of the medicinal plant *Acorus calamus* L. *Med. Aromat. Plants* **2015**, *S1*, 002. [CrossRef]
43. Ahlawat, A.; Katoch, M.; Rama, G.; Ahuja, A. Genetic diversity in *Acorus calamus* L. as revealed by RAPD markers and its relationship with β-asarone content and ploidy level. *Sci. Hortic.* **2010**, *124*, 294–297. [CrossRef]
44. Röst, L.C.M. Biosystematic investigations with *Acorus* L. (Araceae). 1. Communication. Cytotaxonomy. *Proc. K Ned. Akad. Wet.* **1978**, *81*, 428–441.
45. Turczaninow, N. Flora Baicalensi-Dahurica seu Descriptio plantarum in regionibus cis- et transbaicalensibus atque in Dahuria sponte nascentium. *Bull. Soc. Imp. Nat. Moscou* **1854**, *27*, 53–130.
46. Borodin, I. *Collectors and Collections in the Flora of Siberia*; Imperial Academy of Sciences: Saint-Petersburg, Russia, 1908. (In Russian)
47. Mücke, M. Über den Bau und die Entwicklung der Früchte und über die Herkunft von *Acorus calamus* L. *Bot. Zeit.* **1908**, *66*, 1–23.
48. Vinogradova, Y.K. Variability of *Acorus calamus* L. biological characteristics in natural and invasive populations. *Byull. Glavn. Bot. Sada* **2004**, *187*, 23–31. (In Russian)
49. Chepinoga, V.V.; Gnutikov, A.A.; Enushchenko, I.V.; Chepinoga, A.V. *Acorus calamus*. In:Marhold, K., Ed. IAPT/IOPB Chromosome Data 6. *Taxon* **2008**, *57*, 1267, E1. [CrossRef]
50. Krivenko, D.A.; Kotseruba, V.V.; Kazanovsky, S.G.; Verkhozina, A.V.; Chernova, O.D. *Acorus calamus*. In: Marhold, K., Ed IAPT/IOPB chromosome data 16. *Taxon* **2013**, *62*, 1357, E4.
51. Probatova, N.S.; Gnutikov, A.A.; Rudyka, E.G.; Chepinoga, V.V. Chromosome numbers of some plant species from Baikal Siberia. *Bot. Zhurn.* **2008**, *93*, 162–181.
52. Chepinoga, V.V. *Chromosome Numbers of Plant Species from Baikal Siberia*; Nauka: Novosibirsk, Russia, 2014.
53. de Loureiro, J. *Flora Cochinchinensis: Sistens Plantas in Regno Cochinchina Nascentes. Quibus Accedunt Aliæ Observatæ in Sinensi Imperio, Africa Orientali, Indiæque Locis Variis. Omnes Dispositæ Secundum Systema Sexuale Linnæanum*; Ulyssipone: Lisboa, Portugal, 1790.
54. Hooker, J.D. Works of the late William Griffith, Esq. F. L. S. & C. Posthumous papers, bequeathed to the Hon. East India Company, and printed by the order of the Government of Bengal. *Lond. J. Bot.* **1848**, *7*, 446–449.
55. Hooker, J.D. *Catalogue of the Plants Distributed at the Royal Gardens, Kew, under the Sanction of the Secretary of State for India, from the Herbaria of Griffith, Falconer, and Helfer*; J.E. Taylor: London, UK, 1865.
56. Lang, W.H. William Griffith, 1810–1845. In *Makers of British Botany: A Collection of Biographies by Living Botanists*; Oliver, F.W., Ed.; Cambridge University Press: Cambridge, UK, 1913; pp. 178–191.
57. Huxley, L. (Ed.) *Life and Letters of Sir Joseph Dalton Hooker*; J. Murray: London, UK, 1918; Volume 1.
58. Griffith, W. *Itinerary Notes of Plants Collected in the Khasyah and Bootan Mountains, 1837–1838, in Affghanisthan and Neighbouring Countries, 1839 to 1841*; J.F. Bellamy: Calcutta, UK, 1848.
59. Griffith, W. *Journals of Travels in Assam, Burma, Bootan, Affghanistan and the Neighbouring Countries*; Bishop's College Press: Calcutta, UK, 1847.
60. Hooker, J.D.; Thomson, T. *Introductory Essay to the Flora Indica*; W. Pamplin: London, UK, 1855.
61. Anonymous. Intended distribution of the Herbaria of the East India Company. *Bot. Gaz.* **1849**, *1*, 112.
62. Noltie, H.J. *Flora of Bhutan Including a Record of Plants from Sikkim and Darjeeling*; Royal Botanic Garden Edinburgh: Edinburgh, UK, 1994; Volume 3.
63. Ogra, R.K.; Mohanpuria, P.; Sharma, U.K.; Sharma, M.; Sinha, A.K.; Ahuja, P.S. Indian calamus (*Acorus calamus* L.): Not a tetraploid. *Curr. Sci.* **2009**, *97*, 1644–1647.
64. Griffith, W. *Icones Plantarum Asiaticarum*; C.A. Serrao: Calcutta, UK, 1851; Volume 3.
65. Griffith, W. *Notulae ad Plantas Asiaticas*; C.A. Serrao: Calcutta, UK, 1851; Volume 3.

66. Bliss, E.M. (Ed.) *The Encyclopaedia of Missions*; Funk & Wagnalls: New York, NY, USA, 1891; Volume 1.
67. Stewart, R.R. Missionaries and clergymen as botanists in India and Pakistan. *Taxon* **1982**, *31*, 57–64. [CrossRef]
68. Fürnrohr, A.E. Getrocknete Pflanzensammlungen: Plantarum Indiae orientalis Sect. V. *Flora* **1854**, *37*, 187–189.
69. Hohenacker, R.F. Verkäufiche Pflanzensammlungen. *Oesterr. Bot. Z.* **1859**, *9*, 1–4.
70. Wörz, A. *Der Esslinger Botanische Reiseverein 1825–1845: Eine Aktiengesellschaft zur Durchführung Naturkundlicher Sammelreisen*; Logos Verlag: Berlin, Germany, 2016.
71. Hohenacker, R.F. Anzeige: Sammlungen ostindischer Pflanzen aus Canara und den Nilgherries. *Flora* **1851**, *34*, 718–720.
72. Hohenacker, R.F. (Ed.) *Plantarum Indiae orientalis Sect. V. Cura Rev. Fr. Metz, Missionarii Evangelicae Societatis Basileensis, Pro Maximu Parte in Montibus Nilagiri Collectas, a DD. Bentham, Fenzl, Hochstetter, Meisner, Miquel, de Schlechtendal, C.H. Schultz Bip., Steudel aliisque Examinatas Edidit R.F. Hohenacker*; Esslinger Botanische Reiseverein: Esslingen, Kingdom of Württemberg, 1853.
73. British Museum. *The History of the Collections Contained in the Natural History Departments of the British Museum*; Trustees of the British Museum: London, UK, 1904; Volume 1.
74. Lipsky, V.I. Herbarium. In *Historical Account of the Imperial Botanical Garden in Saint-Petersburg for 1873–1898*; Fischer von Waldheim, A.A., Ed.; Imperial Botanical Garden: Saint-Petersburg, Russia, 1899; pp. 36–160. (In Russian)
75. Hohenacker, R.F. Anzeigen: Getrocknete Pflanzen aus Klein-Asien, Sibirien und Ost-Indien. *Flora* **1847**, *30*, 622.
76. Riedl, H. Heinrich Wilhelm Schott (1794–1865). *Taxon* **1965**, *14*, 209–213. [CrossRef]
77. Wang, H.; Li, W.L.; Gu, Z.J.; Chen, Y.Y. Cytological study on *Acorus* L. in southwestern China, with some cytogeographical notes on *A. calamus*. *Acta Bot. Sinica* **2001**, *43*, 354–358.