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Comparative karyological analysis of Indian *Amorphophallus* (Araceae)

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Running title: Karyotype studies in Indian *Amorphophallus*

Abstract

Comparative karyotypes of 22 accessions (14 species and six varieties) of Indian *Amorphophallus* (Araceae) are provided to deduce species interrelationships. Chromosome numbers for *A. hirsutus*, *A. nicolsonianus* and *A.* sp. are reported for the first time. Also, a new triploid cytotype of *A. konkanensis* was found. The diploid number $2n = 2x = 26$ was noted in 13 taxa whereas $2n = 2x = 28$ in three taxa. Six taxa exhibited $2n = 3x = 39$ chromosomes. The longest chromosome was recorded in *A. nicolsonianus* (9.70 µm) and the shortest in *A. paeoniifolius* var. *campanulatus* (3.26 µm). Amongst all the taxa, the karyotypes of *A. bonaccordensis* and *A. smithsonianus* are unusual as they possess subtelocentric chromosomes. Multivariate analysis of taxa based on six karyological parameters ($2n$, x, THL, M_{CA}, CV_{CL} and MCL) revealed three clusters. Species of different sections (*Amorphophallus*, *Conophallus* and *Rhaphiophallus*) grouped together indicating that the sections that were generated based on morphological similarity do not reflect the phylogeny of the genus. Our studies suggest the need for comprehensive phylogenetic analyses of Indian *Amorphophallus* to understand interspecific relationships. Furthermore, it is recommended that genomic in-situ hybridization (GISH) studies should be conducted to understand the origin and nature of triploids in the genus.

Keywords Aroids, Cytotaxonomy, Cytotype, Multivariate analysis, India

Introduction

The genus *Amorphophallus* Blume ex Decne. (Araceae Juss.) comprises some 200 species distributed throughout the paleotropics, from West Africa, the western border, eastward into Polynesia and southeastward to Australia (Sedayu et al. 2010). *Amorphophallus* belongs to the tribe Thomsonieae Blume that initially comprised two genera, *Amorphophallus* and *Pseudodracontium* N.E.Br. A phylogenetic study based on *matK* and *trnL* intron sequences on the tribe Thomsonieae has revealed that *Pseudodracontium* nests within *Amorphophallus*, making the latter paraphyletic to the former (Grob et al. 2002). Consequently, *Pseudodracontium* has been merged into *Amorphophallus*. This study sampled 46 *Amorphophallus* species, two *Pseudodracontium* species and six outgroups. However, only six Indian species (*A. commutatus* (Schott) Engl., *A. hirsutus* Teijsm. & Binn., *A. margaritifer* (Roxb.) Kunth, *A. napalensis* (Wall.) Bogner & Mayo, *A. paeoniifolius* and *A. smithsonianus* Sivad.) were included in this analysis. Recently, Claudel et al. (2017) conducted phylogenetic analysis of 157 species of *Amorphophallus* based on nuclear (*ITS1*) and plastid sequences (*matK* and *rbcL*). Consequently, four new subgenera were recognized, viz. *Afrophallus* Hett. & Claudel, *Amorphophallus*, *Metandrium* Stapf. and *Scutandrium* Hett. & Claudel. Some 11 Indian species were sampled in this study. These were included under subgenera *Amorphophallus*, *Metandrium* and *Scutandrium*. *Afrophallus* is the only subgenus that is restricted to tropical and subtropical Africa and Madagascar.

Amorphophallus is an economically important genus as the tubers are a good source of starch. *A. paeoniifolius* (Dennst.) Nicolson is cultivated in India, Sri Lanka and parts of Indonesia whereas *A. konjac* K.Koch is grown in China and Japan (PlantUse English Contributors 2017). In Japan, a traditional dish ("ito konnyaku") is prepared from the tubers of *A. konjac* (PlantUse English Contributors 2017). *A. konjac* also has great importance in traditional Chinese medicine. A gel prepared from the flour (obtained from the tuber) is utilized for detoxification, tumour-suppression, blood stasis alleviation and phlegm liquefaction (Chua et al. 2010).

In India, the genus has been revised by Jaleel et al. (2011, 2012 and 2014). It is represented by 24 taxa (20 species and four varieties), of which 13 species (*Amorphophallus bhandarensis* S.R.Yadav, Kahalkar & Bhuskute, *A. bognerianus* Sivad. & Jaleel, *A. bonaccordensis* Sivad. & Mohanan, *A. carnosus* Engl., *A. commutatus*, *A. hohenackeri* (Schott) Engl. & Gehrm., *A. konkanensis* Heet., S.R.Yadav & Patil, *A. longiconnectivus* Bogner, *A. longistylus* Kurz ex Hook.f., *A. margaritifer*, *A. mysorensis* E. Barnes & C.E.C. Fisch., *A. muelleri* Blume and *A. smithsonianus*) and three varieties (*A. commutatus* var.

anmodensis Sivad. & Jaleel, *A. commutatus* var. *anshiensis* Punekar, Lakshmin. & Sivad., and *A. commutatus* var**.** *wayanadensis* Sivad. & Jaleel) are endemic (modified after Jaleel et al. 2014).

Over time, various cytotaxonomic studies have been focused on Indian *Amorphophallus* (Asana & Sutaria 1939; Ramachandran 1977; Chauhan & Brandham 1985; Lekhak & Yadav 2012; Gholave et al. 2014; Shirly et al. 2014a, 2014b). The most recent and comprehensive study by Shirly et al. (2014a) deals with a karyomorphological investigation of 25 accessions of *Amorphophallus* comprising seven wild species and 18 morphotypes and wild relatives of *A. paeoniifolius*. A scheme of evolution of chromosome number is proposed depicting that the basic chromosome number $x = 7$ has led to the origin of diploid number $2n = 14$ and $2n = 13$. Shirly et al. (2014a) have also supposed that *A. dubius* may be the immediate ancestor of cultivated forms of *Amorphophallus*. Most of the above-mentioned cytotaxonomic investigations have focused on karyological features; however, none brings about the interspecific relationships within *Amorphophallus*. This paper aims to 1) present new chromosome data for the genus, and 2) perform a comparative analysis of karyological data (i.e. diploid number, chromosome morphology, mean chromosome length, and karyotype asymmetry) for 22 accessions (representing 14 *species* and six varieties) in order to elucidate interrelationships amongst the Indian taxa of *Amorphophallus'*.

Materials and methods

Plant collection

Plant samples (tubers) were collected form wild populations in peninsular India as well as from Andaman Nicobar Islands (Table 1). All the plants were grown in earthen pots and being maintained in the Botanic Garden of the Department of Botany, Shivaji University Kolhapur. One of the accessions collected from Bhandara district, Maharashtra state could not be assigned to any species. Hence, it was referred as *Amorphophallus* species (*A.* sp.). Voucher specimens were deposited in the herbarium of Department of Botany, Shivaji University, Kolhapur (SUK) (Table 1).

Chromosome preparation

Root tips from sprouting tubers were used for mitotic counts. Excised roots were pre-treated with aqueous saturated solution of *para*-dichlorobenzene at 8-10^{o}C for 6-7h. Further, pretreated roots were hydrolysed in 1 N HCl at 60 ^{o}C, washed in distilled water, stained and squashed with 2 % propionic-orcein. For each species at least 20 somatic plates from about five individuals were examined. Suitable metaphase plates were photographed under Leica 2000 DM compound microscope and Carl Zeiss Axio Imager A2 at 1000 x.

Karyotype and karyogram

Ten well-separated metaphase chromosome plates were selected for karyotype analysis by adopting the method of Levan et al. (1964). Chromosome morphology was determined by the centromeric index as: short arm × 100/total length of the chromosome. Homologous chromosomes were paired by length and centromeric index. The length of a chromosome was estimated from mean of the total length of the chromosome and standard deviation was calculated for mean values. Mean chromosome length (MCL) and the sum of lengths of all chromosomes of the complement (THL) were also calculated. Comparative karyograms were constructed for each species. The degree of karyotype asymmetry was determined by considering the categories of Stebbins (1971), and by measuring the Coefficient of Variation of Chromosome Length (CV_{CL}) and Mean Centromeric Asymmetry (M_{CA}) as proposed by Peruzzi & Eroğlu (2013).

Multivariate analysis

For comparing the karyotypes of the studied taxa, principal component analysis (PCA) was conducted using Past ver. 3.19 software (Hammer et al. 2001). Analysis was based on six parameters: chromosome number (2*n*), chromosome base number (*x*), THL, CV_{CL}, M_{CA} and MCL. A variance-covariance matrix was used.

Results

Chromosome numbers, karyotype characteristics and collection localities of the 22 studied accessions of *Amorphophallus* occurring in India are summarized in Table 1. Chromosome numbers for *A. hirsutus, A. nicolsonianus* and *A.* sp. were reported for the first time. Also, a new triploid cytotype of *A. konkanensis* was found. Figure 1 illustrates the mitotic metaphases while Figure 2 shows karyograms of diploid taxa. Mitotic observations showed that the taxa studied represent two ploidy levels, i.e. diploid (2*x*) and triploid (3*x*). The former included species with base number $x = 13$ ($2n = 26$) and 14 ($2n = 28$) while in the latter species with only $x = 13$ have been reported. Taxa with $x = 14$ fall in *Amorphophallus* section *Amorphophallus* whereas others with $x = 13$ represent *Conophallus* and *Rhaphiophallus*. MCL ranged from 3.26±0.56 µm (*A. paeoniifolius* var. *campanulatus*) to 9.70±2.17 µm (*A. nicolsonianus*). The largest chromosome (14.42 µm) was recorded in *A. nicolsonianus* and the shortest (2.30 µm) in *A. longiconnectivus*. THL varied from 45.64 µm (*A. paeoniifolius* var. *campanulatus*) to 285.15 µm (*A.* sp.). Karyotypes were either highly symmetrical (1a and 2a) or moderately symmetrical (2a, 2b and 3a). *A. bhandarensis*, *A. bulbifer*1, *A. commutatus* var. *anmodensis*, *A. commutatus* var. *anshiensis*, *A. hirsutus*, *A.*

konkanensis, A. mysorensis, A. paeoniifolius var. *paeoniifolius, A. sylvaticus* and *A.* species form the highly symmetric karyotype group. Moderately symmetric karyotypes included Stebbins 2a (*A. paeoniifolius* var. *campanulatus*), 2b (*A. bulbifer, A. commutatus* var. *commutatus, A. commutatus* var. *wayanadensis, A. nicolsonianus, A. muelleri, A. hohenackeri* and *A. smithsonianus*) and 3a categories (*A. bonaccordensis*). A prevalence of chromosomes with median (m) and submedian (sm) centromeres was observed in almost all analysed species except in the case of *A. bonaccordensis* and *A. smithsonianus,* which also possessed chromosomes with subtelocentric centromere (st). *A. bhandarensis, A. margaritifer* and *A. sylvaticus* possess a single type of chromosomes, i.e. all m type chromosomes. M_{CA} was found to be the lowest (8.0) for *A. bhandarensis* and *A. margaritifer* and the highest (32.0) for *A. bonaccordensis*. The lowest CV_{CL} was recorded for *A. bhandarensis* (12.0) and the highest for *A. hohenackeri* (30.0).

PCA was carried out on 22 accessions of *Amorphophallus* for analyzing the karyological relationships among the investigated taxa (Figure 3). Cumulative variance explained by the first two (PC1 and PC2) components approaches to 73.26% of the total information. The formation of PC1 was due to $CV_{CL,}$ MCL, $M_{CA,}$ (positive values) and 2*n*, *x*, THL (negative values). PC2 was described by 2*n*, MCL, CV_{CL}, THL (positive values) and $M_{CA,}$ *x* (negative values). The projection of the taxa on the two axes revealed three clusters. The first cluster (Cluster I) comprised six triploid taxa (*A. bulbifer, A. longiconnectivus, A. konkanensis*1, *A. margaritifer, A. muelleri* and *A.* species). This cluster is limited to the positive values of PC1 and PC2. Second cluster (Cluster II) showing positive values of PC1 corresponded to diploid taxa (*A. bonaccordensis, A. hohenackeri, A. nicolsonianus, A. smithsonianus* and all the varieties of *A. commutatus*) with MCL more than 5 µm. The third cluster (Cluster III) was associated with negative values of PC1 and PC2. It was represented by *A. bhandarensis, A. bulbifer*1, *A. hirsutus, A. konkanensis, A. mysorensis, A. paeoniifolius* var. *campanulatus, A. paeoniifolius* var. *paeoniifolius* and *A. sylvaticus*. These taxa are diploid and have MCL lower than 5 µm except for *A. mysorensis*.

Discussion

The present analysis revealed that the diploid number 2*n* = 2*x* = 26, occurring in 13 taxa, was the prevalent. This is followed by 2*n* = 3*x* = 39 found in six taxa and 2*n* = 2*x* = 28 in three taxa. In India, the diploid number 2*n* = 28 is restricted to the species belonging to the section *Amorphophallus*. Additionally, *A. longituberosus* Engl. & Gehrm. and *A. prainii* Hook.f. occurring in the West Malaysia and Thailand also exhibit this number. Chauhan & Brandham (1985) split *Amorphophallus* into two major chromosomal groups; a smaller one having 2*n* =

28 and a much larger group with 2*n* = 26 or triploid 2*n* = 39 chromosomes. They observed that the former group has species with small chromosomes with low DNA values and the latter with much greater variation in DNA amount. The THL was higher (> 130 µm) for triploids as they possess an additional set of chromosomes. As THL values reflect MCL, larger the MCL higher are the THL values. For instance, amongst triploids, *Amorphophallus* species with highest THL (258.15µm) also has the largest MCL value (6.62 ± 1.23µm). Shirly et al. (2014a) have also obtained the maximum (303.64 µm) total length of the diploid chromosome complement (TCL) in *A. bulbifer,* which is triploid with 2*n* = 3*x* = 39. The values of TCL were on the higher side when compared with the THL values in the present investigation. The reason is that the TCL of Shirly et al. (2014a) concerns diploid chromosome complement in contrast to the haploid complement THL is associated with. Additionally, the somatic metaphases scored by Shirly et al. (2014a) clearly depict the incomplete condensation of chromatin material. Primary constrictions are hardly visible in these preparations and chromosomes still appear thread-like. Consequently, there is an overall increase in the chromosome length, MCL and THL. Amongst the diploids, the maximum THL value (120 µm) was observed in *A. nicolsonianus* that has chromosomes with the largest MCL value (9.70 ± 2.17µm). Lower M_{CA} values of 8.0 (*A. bhandarensis* and *A. margaritifer*) and 9.0 (*A. sylvaticus*) were due to uniformity in chromosome morphology. Maximum CV_{CL} (30) for *A. hohenackeri* was on account of the wide range (4.98–13.91 µm) of its chromosome length. Similarly, uniformity in the length of the chromosome complement (3.42 – 5.07 µm) in *A. bhandarensis* is responsible for its low CV_{CL} (12).

Haploid karyotype formulae (except for triploids) examined across the taxa reveal that except for two species, viz. *A. bonaccordensis* and *A. smithsonianus* that have an additional chromosome type (chromosomes with subterminal region centromeres), the remaining species have two types of chromosomes, one with median region and others with submedian region centromeres. Shirly et al. (2014a) have considered *A. bonaccordensis* and *A. smithsonianus* more evolved as far as karyotype asymmetry is concerned. However, they report subterminal chromosomes only in *A. bonaccordensis*. Furthermore, based on karyotype data and ISSR analysis (involving 17 ISSR primers), Shirly et al. (2014b) are of the opinion that *A. bonaccordensis* has probably originated from *A. hohenackeri* through chromosomal rearrangements and high mutation of the SSR loci. The karyotype data generated in the present study do not provide any substantial information either to corroborate or to disagree with the findings of Shirly et al. (2014b). Hence, phylogenetic data are needed to shed light on the genetic proximity of these two species.

Karyotypes of taxa having chromosomes with absolute dominance of or only median region centromeres (m) were highly symmetric (1a and 1b). Species with moderately symmetric karyotypes (2a, 2b and 3a) usually showed m and sm chromosomes. *A. bonaccordensis* and *A. smithsonianus* had st chromosomes in addition to m and sm types. These species have a more evolved karyotype (Shirly et al. 2014b). Shirly et al. (2014b) reported st chromosomes in *A. bonaccordensis* and *A. commutatus*.

For triploid taxa, all the 39 chromosomes can be arranged in descending order of their length as they could not be assembled into groups of three. Hence, it is difficult to ascertain their auto or allopolyploid nature. This necessitates meiotic analyses of the triploids. Additionally, in-situ hybridization tools such as genomic in-situ hybridization (GISH) can be used to understand the origin of triploids and, more in general, their phylogenetic relationships (Castiglione and Cremonini 2012). For instance, Bisht & Mukai (2001) used GISH to identify the donors of the allotetraploid ($2n = 4x = 36$) *Eleusine africana* Kenn.-O'Byrne, a wild species of Finger Millet. Interestingly, all the triploids set viable seeds, with the only exception of *A. bulbifer* and *A. muelleri*, which multiply through bulbils. This could be due to apomixis, as it has been reported for both triploids (*A. bulbifer* and *A. muelleri*) and diploids (*A. kiusianus* (Makino) Makino) (Hetterscheid & Ittenbach 1996). However, apomixis in *A. konkanensis*, *A. longiconnectivus*, *A. margaritifer* and *A.* species needs to be confirmed. A thorough investigation of these triploids from cytogenetical and embryological point of view may clarify if apomixis is operating.

Indian species of *Amorphophallus* have been grouped into three sections, viz. *Amorphophallus*, *Conophallus* and *Rhaphiophallus* (Jaleel et al. 2011, 2012, and 2014). In the present investigation, section *Amorphophallus* is represented by only three taxa. *Conophallus* and *Rhaphiophallus* consist of seven and ten taxa, respectively. Multivariate analysis (PCA) of karyological parameters groups the studied taxa into three clusters. However, this clustering does not reflect their morphological proximity as none of the three clusters comprises species solely from a single section. For instance, the first cluster comprising triploids and the second cluster of diploids have species belonging to both *Conophallus* and *Rhaphiophallus*. The third cluster represents species from all the three sections, i.e. *Amorphophallus*, *Conophallus* and *Rhaphiophallus*. This indicates that the sectional classification by Engler (1911) does not fit the karyological evidences provided here. Apart from the Indian section *Rhaphiophallus*, which is still in use, the original sectional classification of Engler (1911) has been abandoned (Hetterscheid et al. 1994). Grob et al. (2002) and Lekhak &Yadav (2012) suggested that section *Rhaphiophallus* may be

polyphyletic. Phylogenetic study based on *rbcL* sequences by Gholave et al. (2016) also supports the study of Grob et al. (2002). Phylogenetic trees constructed based on Maximum likelihood (ML) and Bayesian (BI) analyses of *rbcL* sequences showed the polyphyletic nature of section *Rhaphiophallus*. Seven species of the section *Rhaphiophallus* formed a monophyletic group but the remaining three species namely, *A. bonaccordensis*, *A. hohenackeri* and *A. smithsonianus* take an isolated position in a tree. Karyological data generated in the present study also predict that *A. bonaccordensis* and *A. smithsonianus* are very different from the rest of the species in possessing st chromosomes. A strict consensus tree based on chloroplast (*matK*) and intron (*trnL*) sequences placed *A. commutatus*, *A. hirsutus* and *A. paeoniifolius* in the same clade whereas *A. margaritifer* and *A. smithsonianus* did not form a common group (Grob et al. 2002). The former group represents species from section *Conophallus* and *Amorphophallus* whereas the latter belong to the same section, i.e. *Rhaphiophallus*. The grouping of *A. commutatus* with *A. hirsutus* and *A. paeoniifolius* does not hold true from cytogenetics point of view. *A. commutatus* is diploid with $2n = 2x = 26$ chromosomes whereas both *A. hirsutus* and *A. paeoniifolius* are diploid with $2n = 2x = 28$ chromosomes. In fact diploid number of $2n = 2x = 28$ has so far not been reported outside section *Amorphophallus* as far as Indian taxa are concerned. *A. smithsonianus* was not assigned to any of the five clades and occupied an isolated position along with *A. rhizomatosus* (Grob et al. 2002). Similarly, the clade occupied by *A. napalensis* did not have any other Indian species although *A. napalensis* is a member of section *Amorphophallus*.

Recently, Claudel et al. (2017) have also found the section *Rhaphiophallus* to be polyphyletic, consisting of two independent groups**.** *A. hohenackeri* and *A. smithsonianus* nested in the subgenus *Scutandrium* whereas *A. sylvaticus*, *A. konkanensis*, *A. margaritifer* and *A. longiconnectivus* in subgenus *Metandrium*. This study included only 11 species found in India. Therefore, a wider sampling is needed to understand the interrelationships amongst the Indian taxa.

Karyological parameters allowed us to recognize three groups of taxa with no regard to their morphological similarity. Diploid and triploid cytotypes of a species show great divergence and do not group together. Cluster first and second comprise diploid taxa. Species of the second cluster have longer chromosomes and hence MCL value more than 5 µm. All the varieties of *A. commutatus* occupy close position on account of their similar karyotype features. *A. bonaccordensis*, *A. hohenackeri*, *A. smithsonianus* and *A. nicolsonianus* were placed distantly within the Cluster II. Although *A. bonaccordensis* and *A. smithsonianus* both have st chromosomes, the number varies. *A. smithsonianus* with five st chromosomes has a

more asymmetric karyotype than *A. bonaccordensis* and hence both are placed apart. *A. hohenackeri* and *A. nicolsonianus* are separated in the cluster on account of their large chromosomes and higher $CV_{CL.}$ Cluster III consists of taxa with $2n = 26$ and $2n = 28$. These are species with chromosomes less than 5 µm and highly symmetric karyotypes (1a or 2a category).

Our results provide a better understanding of chromosome diversity and species interrelationships in Indian *Amorphophallus*. Besides confirming the earlier chromosome reports, new chromosome numbers and karyotypes were provided for species of peninsular India and Andaman. Chromosome data are now lacking only for four Indian species: *A. bognerianus, A. carnosus, A. kachinensis* Engl. & Gehrm., *A. longistylus* Kurz ex Hook.f. The former two species fall under sect. *Conophallus* whereas the latter in sect. *Amorphophallus*.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Asana JJ, Sutaria RN. 1939. On the number of chromosomes of some Indian Araceae. J. Univ. Bombay. 7: 58–64.

Bisht MS, Mukai Y. 2001. Indentification of genome donors to the wild species of Finger Millet, *Eleusine africana* by genomic in situ hybridization. Breed. Sci. 51: 263–269.

Castiglione MR, Cremonini R. 2012. A fascinating island: 2*n* = 4. Plant Biosyst. 146: 711–726.

Chauhan KPS, Brandham PE. 1985. Chromosome and DNA variation in *Amorphophallus* (Araceae). Kew Bull. 40: 745–758.

Chua M, Baldwin TC, Hocking TJ, Chan K. 2010. Traditional uses and potential health benefits of *Amorphophallus konjac* K. Koch ex N.E.Br. J Ethnopharmacol. 128: 268–278

Claudel C, Buerki S, Chatrou LW, Antonelli A, Alvarez N, Hetterscheid W. 2017. Large-scale phylogenetic analysis of *Amorphophallus* (Araceae) derived from nuclear and plastid sequences reveals new subgeneric delineation. Bot. J. Linn. Soc. 184: 32–45

Engler A. 1911. Araceae–Lasioideae. In: Engler, A. (ed.), Das Pflanzenreich48 (IV. 23 C). Wilhelm Engelmann, Leipzig, pp. 1-130.

Gholave AR, Jadhav JP, Yadav SR. 2014. Cytotaxonomical studies on three varieties of *Amorphophallus commutatus* (Araceae) endemic to the Western Ghats. Cytologia. 79: 359–363.

Gholave AR, Pawar KD, Yadav SR, Bapat VA, Jadhav. J.P. 2016. Reconstruction of molecular phylogeny of closely related *Amorphophallus* species of India using plastid DNA marker and fingerprinting approaches. Physiol. Mol. Biol. Plants. 23: 155–167.

Grob GBJ, Gravendeel B, Eurlings MCM, Hetterscheid WLA. 2002. Phlyogeny of the tribe Thomsonieae (Araceae) based on chloroplast, *matK* and *trnL* intron sequences. Syst. Bot. 27: 453–467.

Hammer Ø, Harper, DAT, Ryan PD. 2001. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol. Electron. 4(1), art. 4: 9pp.

Hetterscheid W, Ittenbach S. 1996. Everything you always wanted to know about *Amorphophallus*, but were afraid to stick your nose into!!!!! Aroideana 19: 7–131.

Hetterscheid WLA, Yadav SR, Patil KS. 1994. Notes on the genus Amorphophallus (Araceae)-V. *Amorphophallus konkanensis*, a new species from India, and taxonomic reflections on *Amorphophallus* section *Rhaphiophallus*. Blumea. 39: 289–294.

Jaleel A, Sivadasan M, Alfarhan A, Thomas J, Alatar A. 2011. A taxonomic revision of *Amorphophallus* Blume ex Decne. Sect. *Rhaphiophallus* (Schott) Engl. Araceae in India. Bangladesh J. Plant Taxon. 18: 1–26.

Jaleel A, Sivadasan M, Alfarhan A, Thomas J, Alatar A. 2012. A taxonomic revision of *Amorphophallus* Blume ex Decne. Sect. *Conophallus* (Scott) Engl. (Araceae) in India. Bangladesh J. Plant Taxon. 19: 135–153.

Jaleel A, Sivadasan M, Alfarhan A, Thomas J, Alatar A. 2014. A taxonomic revision of *Amorphophallus* Blume ex Decne. Sect. *Amorphophallus* (Araceae) in India. Bangladesh J. Plant Taxon. 21: 105–120.

Lekhak MM, Yadav SR. 2012. Cytotaxonomy of the species of *Amorphophallus* sect. *Rhaphiophallus* (Schott) Engl. (Araceae) of the Indian subcontinent. Nucleus. 54:169–176.

Levan A, Fredge K, Sandberg A. 1964. Nomenclature for centromeric positions on chromosomes. Hereditas. 52: 201–220.

Peruzzi L, Eroğlu HE. 2013. Karyotype asymmetry: again, how to measure and what to measure? Comp. Cytogenet. 7: 1–9.

PlantUse English contributors, "*Amorphophallus* (PROSEA)," PlantUse English, https://uses.plantnetproject.org/e/index.php?title=*Amorphophallus*_(PROSEA)&oldid=327578 (11 February 2019).

Ramachandran K. 1977. Karyological studies on four south Indian species of *Amorphophallus*. Cytologia. 42: 645–652.

Sedayu A, Eurlings MCM, Gravendeel B, Hetterscheid, WLA. 2010. Morphological character evolution of *Amorphophallus* (Araceae) based on a combined phylogenetic analysis of *trnL, rbcL* and *Leafy* second intron sequences. Bot. Stud. 51: 473–490.

Shirly RA, Suhara Beevy SB, Siril EA. 2014a. Cytotaxonomic investigations to assess diversity and evolution in *Amorphophallus* Blume ex Decne. (Araceae). Nucleus 57: 189–201

Shirly RA, Suhara Beevy SB, Siril EA. 2014b. Chromosomal translocations in the evolution of *Amorphophallus bonaccordensis* from *A. hohenackeri*. Flora. 209: 632–640.

Stebbins GL 1971. Chromosomal evolution in higher plants. London: Edward Arnold.

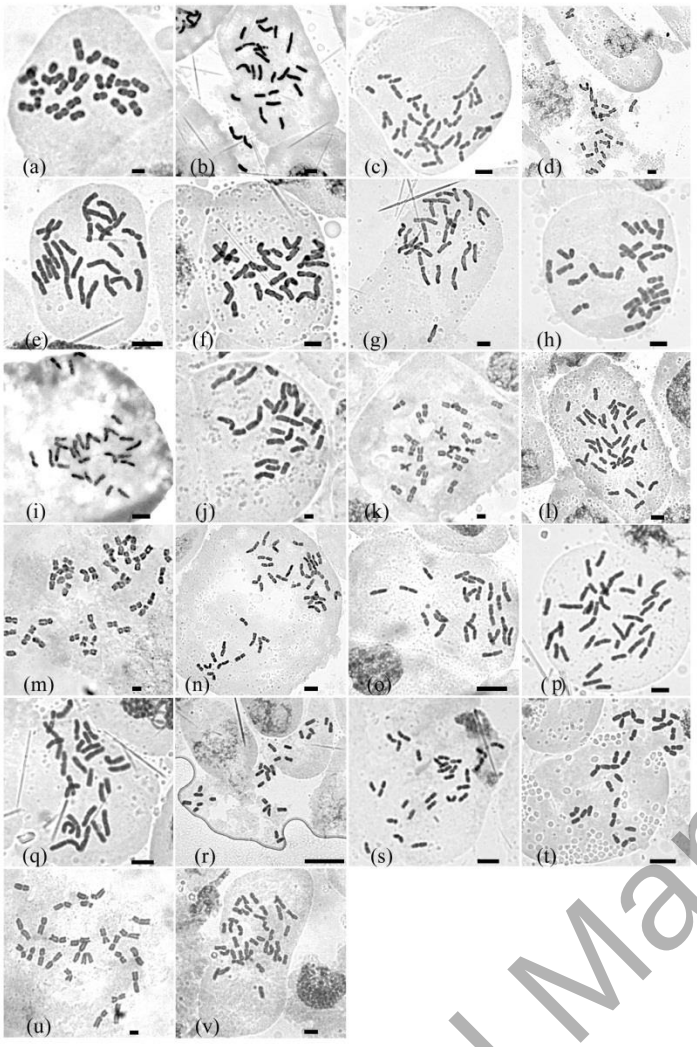

Figure 1. Mitotic metaphase chromosomes of *Amorphophallus* species: (a) *A. bhandarensis* (2*n* = 2*x* = 26); (b) *A. bonaccordensis* (2*n* = 2*x* = 26); (c) *A. bulbifer* (2*n* = 3*x* = 39); (d) *A. bulbifer*1 (2*n* = 2*x* = 26); (e) *A. commutatus* var. *anmodensis* (2*n* = 2*x* = 26); (f) *A. commutatus* var. *anshiensis* (2*n* = 2*x* = 26); (g) *A. commutatus* var. *commutatus* (2*n* = 2*x* = 26); (h) *A. commutatus* var. *wayanadensis* (2*n* = 2*x* = 26); (i) *A. hirsutus* (2*n* = 2*x* = 26); (j) *A. hohenackeri* (2*n* = 2*x* = 26); (k) *A. konkanensis* (2*n* = 2*x* = 26); (l) *A. konkanensis*1 (2*n* = 3*x* = 39); (m) *A. longiconnectivus* (2*n* = 3*x* = 39), n. *A. margaritifer* (2*n* = 3*x* = 39), o. *A. mysorensis* (2*n* = 2*x* = 26); (p) *A. muelleri* (2*n* = 3*x* = 39); (q) *A. nicolsonianus* (2*n* = 2*x* = 26); (r) *A. paeoniifolius* var. *campanulatus* (2*n* = 2*x* = 26); (s) *A. paeoniifolius* var. *paeoniifolius* (2*n* = 2*x* = 26); (t) *A. smithsonianus* (2*n* = 2*x* = 26): (u) *A. sylvaticus* (2*n* = 2*x* = 26); (v) *A.* sp. (2*n* = 3*x* = 39). Bars: 5 µm.

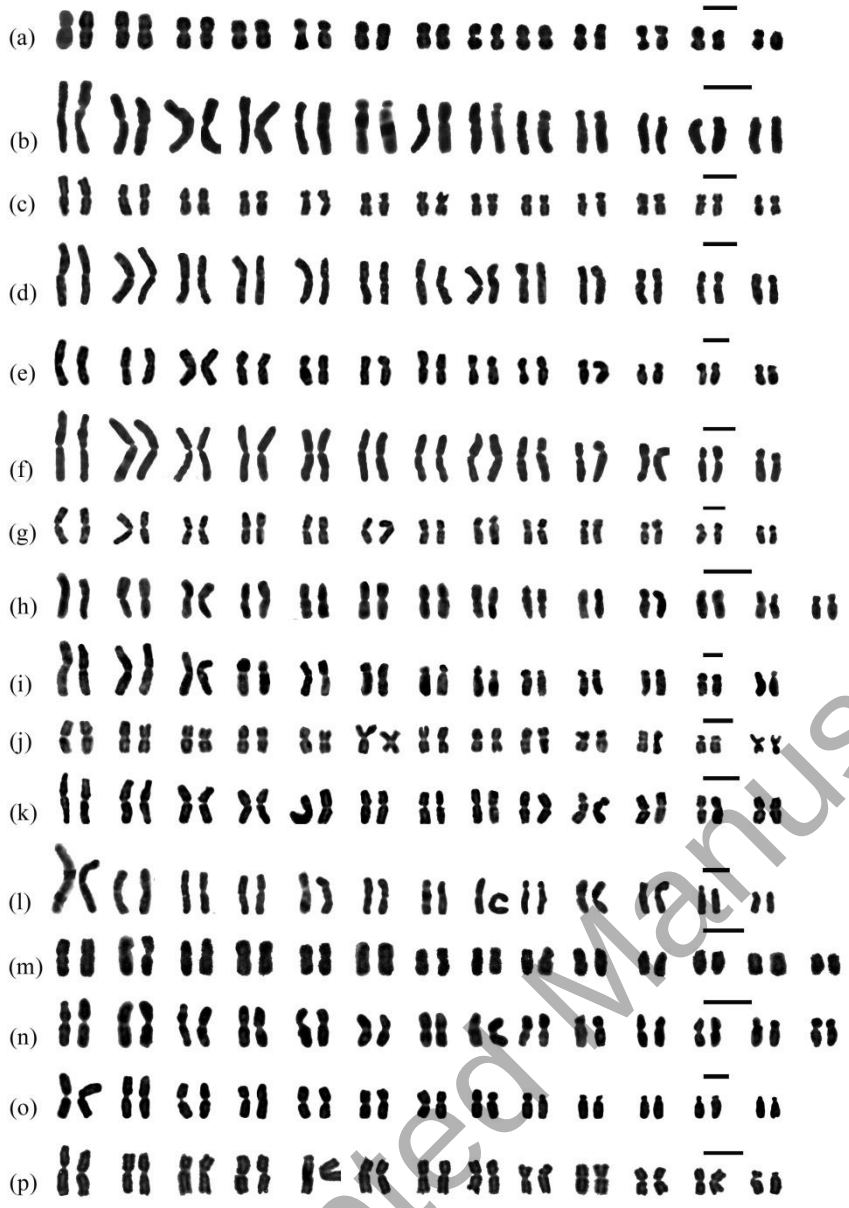

Figure 2. Karyograms of diploid *Amorphophallus* species (2*n* = 2*x* = 26): (a) *A. bhandarensis;* (b) *A. bonaccordensis*; (c) *A. bulbifer*1; (d) *A. commutatus* var. *anmodensis*; (e) *A. commutatus* var. *anshiensis*; (f) *A. commutatus* var. *commutatus*: (g) *A. commutatus* var. *wayanadensis*: (h) *A. hirsutus*; (i) *A. hohenackeri*; (j) *A. konkanensis*; (k) *A. mysorensis*; l) *A. nicolsonianus*; (m) *A. paeoniifolius* var. *campanulatus*; (n) *A. paeoniifolius* var. *paeoniifolius*; (o) *A. smithsonianus*; (p) *A. sylvaticus.* Bars: 5 µm.

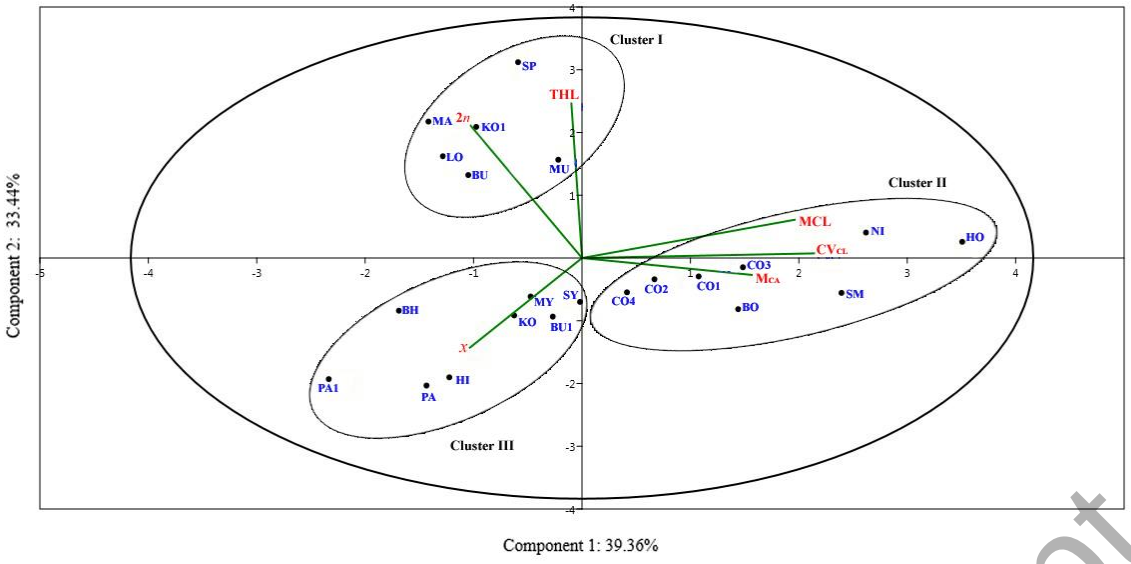

Figure 3. PCA biplot scattergram showing relation between variables and taxa included in the analysis. Taxa abbreviations are given in Table 1.

Table 1. Comparative karyotypes of 22 accessions (14 species and 6 varieties) of *Amorphophallus*. Coefficient of Variation of Chromosome Length (CV_{CL}), Mean Centromeric Asymmetry (M_{CA}), THL, total haploid chromosome length, MCL, mean chromosome length, St, type of asymmetry. *represents species endemic to India.

Taxon (abbreviated form)	2*n*	Ploidy	M_{CA}	CV_{CL}	THL (µm)	MCL±SD (µm)	St	Haploid karyotype formulae	Collection locality	Voucher specimen
Section *Amorphophallus*										
A. hirsutus (HI)	28	2*x*	16.0	19.0	50.35	3.59±0.67	1a	11m+3sm	Andaman Island	ARG-35
A. paeoniifolius var. *campanulatus* (PA)	28	2*x*	19.0	17.0	45.64	3.26±0.56	2a	10m+4sm	Botanical Garden, SUK, Maharashtra	ARG-34
A. paeoniifolius var. *paeoniifolius* (PA1)	28	2*x*	13.0	13.0	48.76	3.48±0.47	1a	14m	Botanical Garden, SUK, Maharashtra	ARG-37
Section *Conophallus*										
A. bulbifer (BU)	39	3*x*	20.0	17.0	138.33	3.55±0.56	2b	-	Jog falls, Shimoga District, Karnataka	ARG-18
*A. bulbifer*1 (BU1)	26	2*x*	17.0	18.0	51.86	3.99±0.72	1a	13m	Damchara, Cachar District, Assam	ARG-65
**A. commutatus* var. *anmodensis* (CO1)	26	2*x*	15.0	23.0	82.44	6.34±1.44	1b	11m+2sm	Anmod Ghat., Goa	ARG-21
**A. commutatus* var. *anshiensis* (CO2)	26	2*x*	16.0	20.0	81.51	6.26±1.24	1b	11m+2sm	Anshi National Park, Uttara Kannada District, Karnataka	ARG-22
A. commutatus var. *commutatus* (CO3)	26	2*x*	18.0	23.0	91.70	7.05±1.64	2b	8m+5sm	Jog falls, Shimoga District, Karnataka	ARG-19
**A. commutatus* var. *wayanadensis* (CO4)	26	2*x*	16.0	20.0	70.78	5.44±1.11	2b	11m+2sm	Mulshi, Pune District, Maharashtra	ARG-20
**A. nicolsonianus* (NI)	26	2*x*	22.9	23.0	120.85	9.70±2.17	2b	9m+4sm	Silent Valley, Agasthyamalai, Kerala	ARG-15
A. muelleri (MU)	39	3*x*	26.0	19.0	159.17	4.08±0.79	2b	-	Andaman Island	ARG-36
Section *Rhaphiophallus*										
**A. bhandarensis* (BH)	26	2*x*	8.0	12.0	53.40	4.10±0.48	1a	13m	Tumsar, Bhandara District, Maharashtra	ARG-38
**A. bonaccordensis* (BO)	26	2*x*	32.0	21.0	65.53	5.04±1.07	3a	5m+7sm+1st	Bonaccord estate, Agasthyamala hills, Thiruvananthapuram District, Kerala	ARG-50
A. hohenackeri (HO)	26	2*x*	25.0	30.0	114.58	8.81±2.61	2b	6m+7sm	Calicut University, Malappuram District, Kerala	ARG-16
**A. konkanensis* (KO)	26	2*x*	12.0	18.0	50.09	3.85±0.69	1a	12m+1sm	Belgaum District, Karnataka	ARG-12
**A. konkanensis*1 (KO1)	39	3*x*	13.0	18.0	186.28	4.77±0.89	2b	-	Gondia District, Maharashtra	ARG-24
**A. longiconnectivus* (LO)	39	3*x*	10.0	19.0	151.08	3.87±0.79	1b	-	Pipariya, Khandwa District, Madhya Pradesh	ARG-03
**A. margaritifer* (MA)	39	3*x*	8.0	17.0	188.79	4.84±0.89	1b	-	Parasnath , Giridih District, Jharkhand	ARG-08
**A. mysorensis* (MY)	26	2*x*	11.0	17.0	65.34	5.02±0.85	1a	13m	Billigirirangan Hills, Chamarajanagar District., Karnataka	ARG-33
**A. smithsonianus* (SM)	26	2*x*	31.0	26.0	77.05	5.92±1.52	2b	6m+2sm+5st	Bonaccord estate, Agasthyamala hills, Thiruvananthapuram District, Kerala	ARG-14
A. sylvaticus (SY)	26	2*x*	9.0	22.0	58.50	4.50±1.00	1b	13m	Vedanthangal Bird Sanctuary, Kancheepuram District, Tamil-Nadu.	ARG-40
A. sp. (SP)	39	3*x*	12.7	17.0	258.15	6.62±1.23	1b	-	Tumsar, Bhandara, Maharashtra	ARG-66