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REVIEW ARTICLE

Cytogenetic diversity and phylogeny of *Pancratium* (Amaryllidaceae): Taxonomic insights into a medicinal bulbous geophyte

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Abstract

The genus *Pancratium*, belonging to Amaryllidaceae, has widespread therapeutic utility mainly owing to presence of bioactive alkaloids. However, its taxonomical relationships remain inadequately explored, resulting in an ill-defined infra-generic classification. This necessitates further investigation using cytogenetic and molecular phylogenetic approaches, which can further clarify taxonomic relationships. Existing reports on such attributes are scattered across the literature, making the collation and consolidation of data a prerequisite for further analysis. The present review ascertained that cytogenetic characterization has been initiated in less than 50% of accepted *Pancratium* species, with no data for unplaced species. Most species exhibit a chromosome count of 2n=22 and n=11, suggesting widespread homoploidy, with dominance of asymmetric karyotype with x=11 as the most probable basic chromosome number. Nuclear genome size estimations are reported only in two species, with 2C values of 36.30 pg and 60.10 pg in *P. illyricum* and *P. maritimum*, respectively. Karyological studies have been done only in six species with mostly bimodal or graded asymmetric karyotypes and predominance of chromosomes with submedian to subterminal primary constrictions. Fluorochrome chromosome banding is reported only in *P. illyricum* and *P. maritimum,* both exhibiting CMA positive bands associated with nucleolar organizer regions identified by FISH. Thus, from the present review it is evident that the genus *Pancratium* exhibits widespread cytogenetic diversity with probable taxonomic utility but awaits the exploration of these traits in most species for further implications. Research on Mediterranean species using chloroplast non-coding DNA (cpDNA) sequences suggests the possible existence of a *P. maritimum* species complex. However, similar studies are limited in Asian species and entirely absent in Indian taxa, underscoring the need for further investigation. Correlation of such molecular phylogenetic analysis with karyological data and genome size can help in further elucidation of taxonomic relationships by establishing either distinctiveness or diversity of the established groups. However, such analysis is limited in literature and awaits further exploration.

Keywords: Amaryllidaceae; chromosome; cytogenetics; evolution; *Pancratium*; phylogeny

Introduction

Pancratium is a medicinally important genus belonging to the family Amaryllidaceae [subfamily Amaryllidoideae, tribe Pancratieae with 22 accepted species and 32 synonyms distributed exclusively around Europe, African Mediterranean region and Southern Asia (1-4). Apart from these, there are 18 unplaced newly discovered species comprising almost 25 % of total reported taxa, which require further studies for taxonomic clarification (4, 5). The taxonomy of *Pancratium* L. has been a subject of debate in the bulbous monocot systematics (6). The genus derives its name from the Greek word ‘pagkration’, meaning almighty, owing to the healing attributes of the taxon utilized in traditional medicine for ages (7, 8). Extracts from various plant organs, including bulbs, flowers and roots, along with isolated bioactive compounds, exhibit diverse therapeutic properties (9). Phytochemical investigation of *Pancratium* revealed the presence of more than fifty types of Amaryllidaceae alkaloids, mainly belonging to pancratistatin, lycorine, haemanthamine, galanthamine and the crinine groups, exhibiting significant anti-neoplastic, anti-microbial, anti-plasmodial and neuro-protective activities (2, 10-13). Antifungal and antiviral effects of these alkaloids (pancratistatin, lycorine, haemanthamine) are responsible for their use as antiseptic agents in traditional medicine and were recently reported to be effective against HIV and Dengue virus (8, 14). Apart from alkaloids, several other groups of phytochemicals of medicinal importance are reported, which include sterols, tannins, chlorides, sulfates, reducing sugars, flavonoids, resins, saponins and glycosides with traces of volatile oils in some species (15). Phytochemical extracts from several species of *Pancratium*

are reported to have inhibitory activity on the enzyme acetylcholinesterase (AChE), thus exhibiting a potential for being a remedy for Alzheimer's disease, similar to other Amaryllidaceae species (16-18). This group of bulbous geophytes thus exhibits promising potential for the discovery of new drugs, but proper taxonomic characterization is a prerequisite for progress of research in this field. Despite genus-level characterization, species delimitation remains unclear, with many new species identified recently (19, 20). Apart from the 22 globally accepted species, there are 18 unplaced taxa within the genus *Pancratium*, which include either newly reported species or those with convoluted relationships. These species of the genus *Pancratium* still awaiting taxonomic clarification include *P. bhramarambae* Sadas., *P. bovei* Steud., *P. chlidanthus* Steud., *P. clavatum* L. f. ex Savage, *P. elphinstonii* Van Houtte ex Bosse, *P. guadelupense* Hort. ex M. Roem., *P. guatemalense* Standl. And Steyerm., *P. longifolium* Jacques, *P. maritimum var. aureum* Pynaert., *P. narbonense* L., *P. ornithogaloides* L.f.ex Savage, *P. plicatum* Livinst. ex Steud., *P. quitoense* (Herb.) Schult. and Schult. f., *P. tagliabue* Colla, *P. telanganense* Sadas., *P. trichromum* Cerv., *P. tristylum* De Rijk. and *P. venkaiahii* R. Prameela, et al. (4, 5, 19). This necessitates elaborate taxonomic analysis supported by multi-faceted data.

Though taxonomic classification of the genus *Pancratium* is mainly based on floral morphology (Fig. 1), those attributes are insufficient for delimitation of most bulbous geophytes. This is because of the presence of morphological convergence, environmental plasticity and continuous variations among phenotypic characters in bulbous geophytes. Thus, correlation of morpho-taxonomic data from other fields like comparative cytogenetics or genomics is necessary and reported to resolve ambiguities for allied taxa (21-27). Such detailed characterization, based on precise chromosomal attributes, has been useful in refining species classification, particularly for taxonomically debated members of bulbous geophytes. For example, in the genus *Drimia*, a bulbous geophyte belonging to the allied family Asparagaceae, comparative karyo-morphometric analysis based on fluorescent chromosome banding and genome size estimations successfully differentiated homoploid species that were previously considered conspecific with *D. indica* (22-23). Such precise knowledge of the cytogenetic architecture can potentially help to assess the genetic diversity, provide additional taxonomic data and serve as background information for further genomic analysis. Parameters significant for elaborate cytogenetic analysis include chromosome counts (zygotic and gametic), nuclear DNA content, karyo-morphology, meiotic behavior and sequence-specific characterization by fluorochrome banding and *in situ* hybridization. Comparative cytogenetic data for *Pancratium* remain scarce, with over 50% of species lacking even basic chromosome counts (Table 1) and most studies relying on conventional staining methods (Table 2). Comprehensive cytogenetic analysis, incorporating genome size estimation, meiotic studies and molecular cytogenetic techniques (banding, *in situ* hybridization), is largely absent. Karyotype analysis has been reported in only six species, while fluorochrome banding and fluorescence in situ hybridization (FISH) have been conducted in just two species (Table 2).

Fig. 1. Flower of *Pancratium longiflorum.*

The phylogenetic relationships and molecular systematics of bulbous geophytes, particularly within the broader monocot lineage, remain controversial (22, 24). Traditional taxonomic methods have limitations due to morphological convergence, environmental plasticity and cryptic speciation, which can be solved using DNA sequencing tools that provide precise, objective and reproducible data for phylogenetic studies. DNA sequence analysis, particularly of nuclear ribosomal DNA (rDNA) internal transcribed spacer sequences (ITS1 and ITS2) and cpDNA non-coding regions, has proven effective in resolving interspecies relationships in plant phylogenetics (25-30). Despite sequence variability the secondary structure of ITS1 and ITS2 (stem-loop or hair-pin structure) is highly conserved across related taxa. Therefore, the study of higher order secondary structures of rDNA ITS1 and ITS2 can provide supplementary information regarding the evolutionary circumscription of a taxon, but is completely lacking for the genus. The folding pattern of rRNA is a prerequisite for its functionality and remains conserved in spite of primary sequence divergence and thus can be a marker for species delimitation (27, 31). Compensatory base changes which alter the sequence of this region could restore the higher order structure of the stem, resulting in processing of pre-rRNA (27). It is already well established that rRNA structure is highly conserved throughout evolution, as most of the folding is functionally important despite primary sequence divergence. Different structural parameters of the rDNA ITS regions, viz. geometrical features, bond energies and base composition, are being used to study the phylogenetic relationships of various species.

There are few reports on phylogenetic relationships of the American (32), Mediterranean (3) and West African (8, 33) members of *Pancratium* with scanty study on Asian species

Table 1. Summary of chromosome number and genome size reports in the genus *Pancratium*

Sl. name	Species Name	Taxonomic Status	Synonyms	Chromosome number		Genome size (pg/2C)*	Reference
				Zygotic (2n)	Gametic (n)		
Species with reports on chromosome number and genome size							
1.	*Pancratium canariense* [Ker-Gawl.]	Accepted	*Pancratium teneriffae* Willd., *Bollaea canariensis* Parl.	22	11	-	(35, 52)
2.	*Pancratium illyricum* L.	Accepted	*Pancratium stellar* Salisb., *Halmyra stellaris* Parl., *Zouchia illyrica* Raf., *Almyra illyrica* Salisb., *Almyra stellaris* Salisb.	22, 44	-	36.30	(36, 46, 51, 53, 59, 62)
3.	*Pancratium longiflorum* Roxb. ex Ker-Gawl.	Accepted	*Pancratium cambayense* Herb	22	-	-	(37, 40, 47)
4.	*Pancratium maritimum* L.	Accepted	*Hymenocallis caroliniana* (L.) Herb., *Hymenocallis lacera* Salisb., *Hymenocallis maritima* M.Roem, *Hymenocallis ruizii* M.Roem, *Pancratium carolinianum* L., *Pancratium angustifolium* M.Roem, *Pancratium aegyptiacum* M.Roem, *Pancratium barcinonense* Sennen, *Pancratium angustifolium* Lojac., *Pancratium abchasicum* Regel, *Pancratium mirennae* Mattei., *Pancratium linosae* Soldano &F.Conti, *Scilla parva* Garsault	20, 22, 28	-	60.1, 49.66	(36, 40, 45, 53, 54, 59-62)
5.	*Pancratium sickenbergeri* Asch. & Schweinf. ex Boiss.	Accepted	*Pancratium sickenbergeri var. desertorum* Sickenb., *Pancratium sickenbergeri var. littorale* Sickenb.	22	-	-	(14)
6.	*Pancratium tenuifolium* Hochst.	Accepted	*Pancratium chapmanni* Harv., *Pancratium hirtum* A.Chev.,	22	-	-	(39, 40, 58)
7.	*Pancratium trianthum* Herb.	Accepted	*Pancratium saharae* Coss. ex Batt. & Trab., *Pancratium trianthum var. saharae* (Coss. ex Batt. & Trab.) Maire, *Pancratium saharae var. chatinianum* Batt., *Pancratium trianthum var. chatinianum* (Batt.) Maire & Weiller	22, 66	-	-	(39, 40, 41)
8.	*Pancratium triflorum* Roxb	Accepted	*Pancratium malabathricum* Herb.	22, 33	-	-	(40, 42)
9.	*Pancratium verecundum* Aiton	Accepted	-	44, 55	11	-	(40, 43)
10.	*Pancratium zeylanicum* L.	Accepted	*Pancratium uniflorum* Stokes, *Pancratium tiaraeflorum* Salisb.	22, 48, 90, 80-100	-	-	(40, 44, 48, 56, 57)
Species lacking reports on chromosome number and genome size							
11.	*Pancratium arabicum* Sickenb.	Accepted	-				
12.	*Pancratium biflorum* Roxb	Accepted	-				
13.	*Pancratium centrale* (A.Chev.) Traub.	Accepted	*Mizonia centralis* A.Chev. & A.Chev.				
14.	*Pancratium donaldi* Blatt.	Accepted	-				
15.	*Pancratium foetidum* Pomel.	Accepted	*Pancratium collinum* Coss. & Durieu ex Coss., *Pancratium foetidum var. rifanum* Maire, *Pancratium foetidum var. tunetanum* Batt., *Pancratium foetidum var. oranense* Batt, *Pancratium foetidum var. saldense* Batt, *Pancratium foetidum var. brachysiphon* Maire		**No reports**		
16.	*Pancratium landesii* Traub	Accepted	-				
17.	*Pancratium maximum* Forssk.	Accepted	*Mizonia maxima* (Forssk.) *A.Chev.*				
18.	*Pancratium nairii* Sasikala & Reema Kumari	Accepted	-				
19.	*Pancratium parvicoronatum* Geerinck	Accepted	-				
20.	*Pancratium parvum* Dalzell	Accepted	*Pancratium parvum var. malabaricum* Baker, *Pancratium parvum subsp. malabaricum* (Baker) Traub				
21.	*Pancratium sanctae-mariae* Blatt. & Hallb.	Accepted	-				
22.	*Pancratium tortuosum* Herb.	Accepted	*Pancratium tortifolium* Boiss.				

***pg**= Picogram; **2C**= diploid C-value

Table 2. Reports on karyology and molecular cytogenetic analysis in *Pancratium* species

Species	Cytological attributes			
	Karyotype*	Fluorochrome banding	FISH	References
Pancratium illyricum L	Asymmetric karyotype (12Sm+10St) with nTCL= 86.1 µm with chromosome size range of 5-11 µm	Fluorochrome banding using DAPI and CMA showed the presence of CMA positive bands associated with nucleolar chromosomes	-	(36, 46, 59, 62)
Pancratium longiflorum Roxb. ex Ker-Gawl.	Symmetric karyotype (6M+6Sm+10St) with 3 pairs of chromosomes having NOR and nTCL= 188 µm	-	-	(37, 40, 47)
Pancratium maritimum L.	Asymmetric karyotype (12Sm+10St) with nTCL= 120.3 µm with chromosome size range of 6.8-17.6 µm	Fluorochrome banding using DAPI and CMA showed the presence of CMA positive bands associated with nucleolar chromosomes	-	(36, 40, 45, 59-62)
Pancratium tenuifolium Hochst. (Synonym: *Pancratium hirtum* A.Chev.)	Assymetric karyotypes with variable complements among populations, but predominance of chromosomes with submedian to terminal primary constrictions, nTCL varied from 216.5-439.5µm among populations.	-	-	(39, 40, 58)
Pancratium triflorum Roxb.	Asymmetric karyotype [8 Long (6m+2Sm) + 8 Medium (2Sm+2St+2t+2St.sat) + 6 Short (4t+2m)] with nTCL=219.9 µm and chromosome size range 13.4-33 µm. A pair of medium-sized chromosome with secondary constriction on short arm	-	-	(40, 42)
Pancratium zeylanicum L.	Asymmetric karyotype (4 Long +12 Medium +6 Short) with nTCL= 98 µm with chromosome size range of 5.5-14 µm	-	-	(40, 44, 48, 55-57)

nTCL= Total haploid chromatin length; **M**= Median primary constriction; **Sm**= Sub median primary constriction; **St**= Sub terminal primary constriction; **t**= Terminal primary constriction; **sat**= Satellite; **NOR**= Nucleolar organizer region; **µm**= Micrometer; **DAPI**= 4′,6-diamidino-2-phenylindole; **CMA**= Chromomycin A3

(34). The analysis of non-coding nucleotide sequences has assisted the understanding of evolutionary circumscription at all levels of the taxonomic hierarchy in plant phylogenetics. However, there are no phylogenetic studies on Indian species of *Pancratium*, which is a basic criterion for holistic evolutionary analysis (3).

A comprehensive comparative cytogenetic and genomic analysis of *Pancratium* is essential to assess interspecific variation and karyo-evolutionary trends. However, for cytogenetic comparison of *Pancratium* species compilation of existing reports is necessary since such collective reviews are outdated and not all inclusive. An explicit review integrating molecular phylogenetic data to clarify taxonomic relationships is also absent from the literature. This review aims to consolidate existing knowledge on karyo-evolutionary processes and their cytotaxonomic implications in *Pancratium* phylogeny. Additionally, it seeks to explore patterns of cytogenetic variation in relation to molecular phylogeny. Such an analysis can identify key knowledge gaps and provide a foundation for future taxonomic classification and conservation efforts.

Somatic chromosome number in the genus *Pancratium*

Chromosome counts have been reported for ten out of the twenty-two accepted species of *Pancratium* (Table 1), accounting for less than fifty percent of the total species (4). Somatic chromosome number of 2n=22 chromosomes is reported in most species studied, viz. *P. canariense* (35), *P. Illyricum* (36), *P. longiflorum* (37), *P. maritimum* (36), *P. sickenbergeri* (38), *P. tenuifolium* (39, 40), *P. trianthum* (39 - 41), *P. triflorum* (42), *P. verecundum* (40, 43) and *P. zeylanicum* (44) indicating their probable homoploid nature. *P. maritimum* and *P. zeylanicum* showed variations in somatic chromosome number exhibiting 2n= 20, 22, 28 chromosomes and 2n= 22, 48, 90, 80–100 chromosomes respectively (40, 45). Polyploidy is reported in four species, namely *P. triflorum, P. illyricum, P. verecundum* and *P. trianthum* exhibiting 2n=33, 2n= 44, 2n= 44, 55 and 2n= 66 chromosomes respectively, in addition to 2n=22 chromosomes (40, 46). A basic chromosome number of x=11 chromosomes is proposed for the genus (3, 43, 47), thus species exhibiting 2n=22, 33, 44, 55 and 66 chromosomes are probable diploids, triploids, tetraploids, pentaploids and hexaploids, respectively. However, detailed karyological analysis is required for clarification of their autopolyploid or allopolyploid nature. Thus, in some studies, additional somatic chromosome counts have been sporadically reported in multiples of 9, 10, 12 and 23 (48–50) but these do not align with later reports (51), necessitating further validation. Somatic chromosomes count of 2n=22 chromosomes was observed in most species, indicating possible homoploid nature. Dysploidy was observed in *P. maritimum* (2n= 20, 22, 28) and *P. zeylanicum* (2n= 22, 48, 90, 80–100) as evident from change in chromosome number without involving entire genome set, indicating probable chromosome restructuring. Moreover, polyploidy was observed in a few species like *P. triflorum*, *P. illyricum*, *P. verecundum* and *P. trianthum* (Table 1) indicating the occurrence of genome duplication.

Gametic Chromosome number in the genus *Pancratium*

Gametic chromosome count of n=11 chromosomes is reported only in two species, *P. canariense* (52) and *P. verecundum* (43) and thus x=11 chromosomes seems the most probable basic chromosome number for the genus (Table 1). The basic chromosome number (x) should be determined based on a broader analysis of somatic (2n) and gametic (n) counts across multiple species. Since the majority of species have 2n = 22, x = 11 is likely, but further research on

meiotic behavior, chromosome pairing and ancestral karyotypes is essential to confirm the same. However, meiotic counts and detailed analysis of meiotic configurations and secondary associations are lacking for the rest of the species (Table 1). Further cytogenetic investigations are required to confirm meiotic stability and chromosome behavior across the genus. Moreover, homoploidy and polyploidy is a common occurrence in many of the species, hence detailed analysis of meiotic configurations is a prerequisite for further clarification of karyo-evolutionary trends.

Genome Size in the genus *Pancratium*

Nuclear DNA content has been estimated only in two species of *Pancratium* (f 1), thus, genome size estimation is lacking for more than ninety percent of reported species. According to available reports, *P. Illyricum* exhibited genome size of 36.30 pg/2C (53) while *P. maritimum* exhibited two reports on genome size of 49.66 pg/2C and 60.1 pg/2C (53, 54). Since the chromosome counts of these accessions are not reported, no comparison on ploidy level can be made. However, reports based on separate accessions indicate 2n=22 chromosomes is the most widespread somatic chromosome count for both species. Thus, it is evident that there is genome size variation between possibly homoploid species which therefore can serve as an important taxonomic parameter (22, 27). Such estimations on polyploid species are also necessary for study of genetic relationships.

Karyology in the genus *Pancratium*

Karyological investigations have been conducted on six species of *Pancratium* based on conventional methods (Table 2). A predominance of chromosomes with subterminal to terminal constrictions is evident in most species, with an asymmetric karyotype being the general trend. Among species karyologically investigated, only *P. longiflorum* exhibited a symmetrical karyotype (40, 47), whereas the other five species (*P. illyricum*, *P. maritimum, P. tenuifolium, P. triflorum* and *P. zeylanicum*) exhibited asymmetrical karyotype (36, 40, 55-57).

Karyotypes are either bimodal or decrease gradually with chromosomes distinguished into three categories: long (L), medium (M) and short (S) types, where the difference between short and long chromosomes of a complement is more than two-fold for most species. Among species with asymmetrical karyotype, *P. illyricum* and *P. maritimum* exhibited similar karyo-morphology, both with 6 pairs of chromosomes with submedian primary constrictions and 5 pairs of chromosomes with subterminal primary constrictions. Population variation in karyological complement was observed in *P. tenuifolium* (39, 40, 58). Although both *P. triflorum* and *P. zeylanicum* exhibited graded asymmetric karyotypes, the former exhibited 4 pairs each of long and medium sized chromosomes with 3 pairs of short chromosomes, while the latter exhibited 2 pairs of long, 6 pairs of medium sized and 3 pairs of short chromosomes (Table 2). Thus, the karyological attributes exhibited significant diversity exploration of which might lead to the elucidation of probable karyo-evolutionary trends.

Nucleolar chromosomes were reported in two species of *Pancratium* studied (37, 40, 42, 47). Nucleolar Organizer Regions (NORs) were observed on three chromosome pairs in *P. longiflorum*, while one pair of medium-sized chromosomes with secondary constriction on short arm was reported in *P. triflorum* (Table 2). Distribution of NORs is an important karyo-evolutionary trait which is yet to be explored for the genus *Pancratium*. Comparative NOR mapping helps in understanding chromosomal rearrangements that occur over evolutionary time scales and thus are important markers for studying karyotype evolution and species divergence.

Total Haploid Chromatin Length (nTCL) varied drastically from 86.1 µm in *P. illyricum* to 439.5 µm in *P. tenuifolium*, although taxa studied were homoploids with 2n=22 chromosomes. Even for species like *P. triflorum* and *P. zeylanicum* that exhibited similar karyomorphology, total haploid chromatin length varied 1.5-fold that indicates difference in genome size among homoploids. Such genome size variation among homoploids is not uncommon due to repetitive DNA content differences. Thus, along with nuclear genome size, comparison of nTCL can be useful for comparative cytogenetic analysis. It can also be concluded that the genus exhibits significant cytogenetic diversity utilizable as taxonomic characters.

Molecular cytogenetic analysis in the genus *Pancratium*

Detailed molecular cytogenetic studies by fluorochrome chromosome banding and FISH are yet to be initiated for more than 90 % of the species of the genus (Table 2). To date, only one report on detailed cytogenetic analysis in two species of *Pancratium* (*P. illyricum* and *P. maritimum*) using fluorochrome banding with Chromomycin A (CMA) and 4′,6-diamidino-2-phenylindole (DAPI) stains along with *in situ* hybridization with rDNA probes (59). Fluorochrome banding using DAPI and CMA showed the presence of CMA positive bands associated with nucleolar chromosomes in both *P. illyricum* and *P. maritimum*, indicative of the association of GC rich heterochromatin with NOR. This is indicative of probable taxonomic implications of molecular cytogenetic studies following detailed analysis of comparative banding patterns across multiple species. Fluorochrome chromosome banding enabled the identification neither of NOR in both species, previously unrecognizable by conventional staining methods. Such detailed cytogenetic studies for other species of *Pancratium* are a prerequisite for extensive analysis of molecular architecture of chromosomes and elucidation of probable karyo-evolutionary trends. Analysis of such comparative banding patterns across multiple species; and correlation of such data with chromosome number, genome size, or phylogenetic divergence; provide possible links to evolutionary rearrangements (22, 27).

Molecular phylogenetic studies in the genus *Pancratium*

The family Amaryllidaceae has been reported as monophyletic based on a study of Eurasian species using the plastid *ndhF* sequence. Preliminary rDNA ITS sequence analysis suggested the placement of *Pancratium* forms a basal lineage within Amaryllidaceae along with genera such as *Hannonia and Lapiedra* (3, 6). A separate study resolved the taxonomic relationships of twenty-eight Mediterranean *Pancratium* accessions by reconstructing a phylogenetic tree

using plastid DNA markers: the *trnL-trnF* region, *ndhF* and *rbcL* gene sequences. According to this study, *P. maritimum*, *P. linosae* and *P. arabicum* formed the terminal monophyletic clade and the latter two species were concluded to fall under the morphological variability of *P. maritimum*, although considered separate previously (3). Also, *P. hirtum* and *P. trianthum* were found to have identical sequences and formed a sister clade to *P. tortuosum*, while *P. hirtum* is otherwise considered a synonym of *P. tenuifolium* (4). These reports indicate contradictory relationships that need clarification and reanalysis based on taxonomic consensus. RAPD (Random Amplified Polymorphic DNA) analysis of six *Pancratium* species (33) showed the presence of distinct polymorphism between *P. arabicum* and *P. maritimum*, while *P. tortuosum* was placed distant from all other species. However, RAPD is a low-resolution marker system prone to reproducibility issues and is not robust for resolving phylogenetic relationships. It can indicate genetic variability but should not be relied upon solely for resolving taxonomic status.

The phenotypic and genetic variability of the Central Mediterranean species of the genus, including *P. maritimum* was also established using the sequences of three plastid DNA regions, viz. *rbc*L, *mat*K and *trn*H-*psb*A (60) and this study considered *P. linosae* identical to *P. maritimum.*

In another phylogenetic study using rDNA ITS sequence of six *Pancratium* species, close relationships among *P. tortuosum* and *P. tenuifolium* were established (34). A study demonstrated the monophyletic origin of ten Senegalese species of *Pancratium* based on *rbc*L sequences (8). However, phylogenetic studies including Asian species of *Pancratium* are still inadequate, especially evolutionary relationships among the Indian members of *Pancratium* are poorly understood. Therefore, to address this issue, it is necessary to conduct an explicit phylogenetic study based on rDNA ITS and cpDNA non-coding sequence complexity, including representatives from broader geographical zones.

Conclusion

The medicinally valued genus *Pancratium*, belonging to Amaryllidaceae awaits taxonomic elucidation of infra-generic relationships with 22 accepted species, 35 species relegated to synonymy and 18 species with unplaced taxonomic status owing to their relatively recent discovery and thus lacking elaborate character study. Also, being a bulbous geophyte with continuous variation and limited discriminating parameters, the study of additional genetic characters like chromosome count, meiotic behavior, nuclear genome size, karyo-morphology, fluorochrome banding, in situ hybridization and molecular phylogenetic traits becomes necessary for further analysis. Review of literature showed that cytogenetic characterization has been initiated in less than 50 % of accepted species and is completely lacking in unplaced species. From the consolidation of available data, the following conclusions can be implicated:

- Majority of species have 2n = 22 chromosomes and x = 11 is likely the basic chromosome number, but needs further confirmation by examining meiotic behavior, chromosome pairing and ancestral karyotypes.
- Prevalence of homoploidy and polyploidy observed among species studied, while detailed analysis of meiotic configurations is a prerequisite for further clarification of karyo-evolutionary trends.
- Predominance of asymmetric karyotype reported among species studied with variation in nucleolar organizing regions, indicating chromosomal rearrangements that occur over evolutionary time.
- Variation in total chromatin length and genome size observed among homoploid species studied implicating utility as taxonomic marker.
- Studies on fluorochrome banding analysis suggest the co-localization of CMA positive bands on nucleolar chromosomes and variation in distribution of GC rich heterochromatin among taxa, indicative of probable taxonomic implications.
- The monophylly of the genus *Pancratium* is reported with Eurasian species predominantly, however, the molecular phylogeny among the Asian species, particularly Indian taxa, is still ill defined.

To summarize, the genus *Pancratium* shows widespread cytogenetic diversity among studied taxa, indicating that exploration of these characters is a prerequisite for elucidation of taxonomic relationships. However, both cytogenetic and phylogenetic studies are yet to be initiated in majority of taxa. In view of this lacuna, an unambiguous comprehensive cytogenetic and molecular phylogenetic study is essential.

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Authors' contributions

SN and PSS conceived of the study, reviewed the literature and drafted the manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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