

RESEARCH ARTICLE

Systematics Study on a Morphotype of *Allium tuberosum* Rottler ex Spreng. (Alliaceae) from Ladakh, India

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A morphotype of chinese chives (*Allium tuberosum* Rottler ex Spreng.) with pink mid-vein in white tepals on the abaxial surface growing in Leh, Ladakh (Jammu & Kashmir), India was studied on a comparative account with common type *A. tuberosum* (white tepals with green mid-vein) and a close wild relative, *A. ramosum* L. *Allium* subg. *Butomissa* (Salisb.) N. Friesen is discussed here with reference to taxa in India based on the study undertaken using observations from field, experimental, ecogeographical, herbarium and molecular data. A key is provided with some characters previously not included in the available diagnostic keys. Issues relating to use of terminology for plant characters are provided to facilitate field identification.

Key Words: *Allium ramosum*, *A. tuberosum*, Collection, Ecogeographic study, Morphotype, Subgenus *Butomissa*, Taxonomic key

Introduction

Allium subg. *Butomissa* (Salisb.) N. Friesen is a small group characterized by taxa having elongated cylindrical-conic bulb, reticulate or fibrous brown outer tunic and membranous, thin, inner tunic, stout rhizome and simple chromosome morphology (Friesen *et al.*, 2006; Li *et al.*, 2010). The taxa are distributed in the Himalayas and eastern to central Asia upto bordering eastern Mediterranean region. On the basis of morphology, chromosome and serological data, this subgenus was suggested to have affinity with subg. *Anguinum* and subg. *Melanocrommyum* whereas molecular data have supported the mid-position between subg. *Rhizirideum* and subg. *Cyathophora* (Li *et al.*, 2010).

Globally the subg. *Butomissa* is represented by two sections, *Butomissa* (Salisb.) Kamelin (*A. ramosum* L. and *A. tuberosum* Rottler ex Spreng.) and *Austromontana* (*A. oreoprasum* Schrenk and *A. gilgiticum* Wang & Tang) (Friesen *et al.*, 2006; Pandey *et al.*, 2017). Both the species of sect *Butomissa*— *A. ramosum* and *A. tuberosum* morphologically share characters and are debated for taxonomic distinctness (Blattner and Friesen, 2006). *A. tuberosum* is an important cultivated species

from East Asia (Blattner and Friesen, 2006) while *A. ramosum*, is a less-known cultivated vegetable in north-eastern China (Choi and Oh, 2011).

North China is considered to be the centre of domestication and diversity for *A. tuberosum* (Oyuntsetseg *et al.*, 2012); cytotypes of *A. tuberosum* are reported from Korea and north-eastern China (Friesen *et al.*, 2006; Seregin and Korniak, 2013). It was introduced mainly by Chinese into other parts of the world and presently cultivated in the temperate and tropical regions (Hanelt *et al.*, 1992).

In India, *A. tuberosum* has wider adaptability to diverse habitat in the area of occurrence; it is under sporadic cultivation in the Himalaya and the north-eastern region (Gohil, 1992; Negi and Pant, 1992; Pandey *et al.*, 2008; Pandey *et al.*, 2014). Occurrence as wild/semi-wild or as naturalized populations in India is doubtful (Gohil, 1992; Negi and Pant (1992); Sharma and Gohil, 2013; Pandey *et al.*, 2014). The second author has observed wild and naturalized populations of this taxon in Bhavnagar area in Kinnaur district (Himachal Pradesh). It has been reported as wild in China and in western-southern coastal areas of Korea and Chinese

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border near North Korea (Yang *et al.*, 1998; Xu and Kamelin, 2000; Blattner and Friesen, 2006; Choi and Oh, 2011).

The study undertaken on a morphotype of *A. tuberosum* collected from village Sumur of Nubra tehsil in Leh district (Jammu & Kashmir) having tepals with pink mid-vein on abaxial surface. It was compared with the type having green mid-vein on tepals, which is more common. Comparative study was also undertaken with *A. ramosum* and other taxa of this subg. occurring in India using selected material from field, experimental, herbarium and molecular evidences, and data from ecogeographical distribution to facilitate germplasm collection. Issues relating to usage of terminology for describing characters available in the existing literature were discussed. Also simplified identification key along with some more traits was provided for Indian taxa of subg. *Butomissa*.

Material and Methods

Study material of *A. tuberosum* (PMK 1707/Leh-2) was used for field, experimental and herbarium studies. Plants were grown at two locations – field gene bank (FGB) at ICAR-NBPGR (ICAR- National Bureau of Plant Genetic Resources) Regional Station, Bhowali, Uttarakhand (29°20' N, 79°30' E, altitude 1600 m in cold humid, sub-temperate climate) and ICAR-NBPGR, New Delhi (altitude 250m). For comparison, selected accessions of *A. tuberosum* having white tepals with green mid-vein (IC321643, IC353524, IC383446, NEH, NG3189, VDK/AK/25; KCB/AP/605, KP/AP/701, VDV/KSN/51, Leh-1) were used. Live material of *A. ramosum* (EC328498/PI-264799; coll. Russian Federation Collectors, White, George A, USDA-ARS) maintained at Bhowali and herbarium observations on *A. oreoprasum* collected from Leh, Ladak, J&K (MK134, 138; Khaltse, Hanupata) were used. The study was based on data from various resources including herbarium resources (a total of 32 specimens) of *A. tuberosum* and *A. ramosum* (41 specimens).

Data were recorded for vegetative (rhizome, bulb, leaf) and reproductive scape (perianth, stamen, pistil, fruit and seed) characters (measurements based on 10 samples). Micro-morphological characters of bulb, fibre and flower, and anatomy of leaf, scape and floral parts were observed in freshly harvested material using hand lens (10-40x magnification) and dissection microscope (Zeiss Stemi DV4). Floral morphology of taxa was done

with freshly harvested material and data were recorded for separated floral parts. For anatomical observations, transverse sections of leaf and scape were studied under the stereomicroscope. Seed morphology for comparison between *A. tuberosum* and *A. ramosum* with respect to characters—shape, colour and testa texture was studied using dissection microscope (10x magnification)

Herbarium specimens located at the Botanical Survey of India (BSI-CAL, ASSAM and BSD), Forest Research Institute (DD), Dehradun, National Botanical Research Institute Herbarium (LWG), Lucknow and National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi and e-herbaria of K, P, E, were used. Eco-geographical data were gathered from areas of collection and availability of taxon (mainly based on data from herbarium notes— DD, CAL, NHCP; K, P, E, PE, and global databases such as GBIF, Kew Catalogue and distributional map drawn using software (DIVA GIS7.5). Vouchers (HS23159a, b) of the experimental material were deposited in National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi.

Sequence of the ITS (Internal Transcribed Spacer) region amplified from the five accessions of *A. tuberosum* i.e. from the Leh region (Leh-1 and Leh-2), the north eastern Himalayas (NEH), western Himalayas (NG3189) and the accession maintained at the field gene bank in Bhowali (IC353524) and *A. ramosum* (EC328498).

Molecular study of two morphotypes of *A. tuberosum* (one with pink mid-vein and four with green mid-vein on tepal) was undertaken with sequence analyses of the Internal Transcribed Spacer region amplified from five accessions of *A. tuberosum* and one of *A. ramosum* maintained at the field gene bank in Bhowali, ICAR-NBPGR. Phylogenetic and molecular evolutionary analyses were conducted using Mega version 6 (Tamura *et al.*, 2013).

Result and Discussion

Field, Experimental and Herbarium Study

Morphological Study

Wild population of new morphotype was observed along the boulders in disturbed habitat in village Sumur [3,092m; Nubra, Leh district (Jammu & Kashmir)] by the second author during exploration in September 2014. Plants appeared to be self sown with thick keeled leaves, tough reticulate fibres on rhizome, flower with tepals having pink mid-line on abaxial surface and dark



Fig. 1. *Allium tuberosum* (top- left-right) a variant (with pink mid-vein on tepals and fibrous roots); *A. tuberosum* (second row) a normal type (with green mid-vein on tepals and fibrous roots); *A. ramosum* and roots (third row); inflorescence of *A. oreoprasum* (live, opened) (photo credit: PS Mehta, ICAR-NBPGR Bhowali)

pink anther lobes. Plants were taller (0.9-1.0 m vs 0.6 m) and robust when compared to populations studied by the authors in their previous work (Pandey *et al.*, 2014) (Fig. 1). On critical examination of the plant parts, new morphotype was identified as *A. tuberosum* (PMK 1707; IC612066). In floristic and herbarium records *A. tuberosum* has been described variously for flower characters—white and pink flower, tepal stripped with pink etc. (Baker, 1892; Murti, 2001; Das Gupta, 2006; CAL507916, 23/7/54, Stainton, Sykes and Williams 1982). In the same area of Ladakh region (Dha, Hanu) in Leh, the second author also observed plant populations of *A. tuberosum* with tepals having green mid-vein growing in rocky crevices.

Comparative Study

Allium tuberosum (PMK1707) was used for comparative study with *A. tuberosum* (green mid-line) and *A. ramosum* (Table 1).

Micromorphology

Comparison of seed micromorphological characters of *A. tuberosum* and *A. ramosum* using scanning electron microscopy also supported similarity in testa ornamentation as revealed by Oyuntsetseg *et al.* (2012).

Seed micromorphology has been suggested as an important tool for taxonomic delineation of taxa in *Allium* (Ling *et al.*, 2000; Ferhat *et al.*, 2012). Friesen *et al.* (2006) reported seed testa ornamentation of sect. *Austromontana* (*A. oreoprasum*) to be differing in verrucose periclinal walls similar to that of the sect. *Butomissa* (*A. ramosum* and *A. tuberosum*). Seed morphological characterization revealed that seed shape and colour within subg. was more or less similar but minute differences were noted in the seed outline and testa ornamentation across subgenus. Comparative data for testa characters of *A. tuberosum* and *A. ramosum*, were captured under scanning electron microscope (Figure 2).

Ecogeographical Study

A. tuberosum with pink mid-vein on tepal showed variation in plant characters with respect to ecogeographical distribution, plant height, robustness of plant parts- stem, leaves, thicker fibre, colour of tepal as noted during field study. Plants of new morphotype were very tall (1.0m), robust with thicker fibre as compared to the green mid-vein type plant (0.5-0.75m); subterranean parts had more fibre in wild/semi-wild population as compared to the plants in disturbed habitats. Pandey *et al.* (2014) have studied the populations of *A. tuberosum* across

Table 1. Comparison of characters in *Allium tuberosum* (pink mid-vein and green mid-vein) and *A. ramosum*

Trait	<i>A. tuberosum</i> (pink mid-vein)	<i>A. tuberosum</i> (green mid-vein)	<i>A. ramosum</i>
Plant odour	Onion like	Onion like	Garlic like
Leaves	Linear, keeled, margin smooth	Linear, flat, margin smooth	Linear, keeled, margin smooth
Scape (cm)	50-70, two angled	25-60, two angled	25-60, terete, obscurely angled
Spathe	2-3 partite, persistent	2-3 partite, persistent	1-2 partite, persistent
Flower	Stellate	Stellate	Campanulate
Tepal colour	White with pink mid-vein abaxially	White with pale green mid-vein abaxially	White, tinged with pale red, pale red mid-vein
Tepal shape, size	Outer: oblong-lanceolate, 4-7×1.8-3mm Inner: ovate-elliptical, 4-7×2.1-3.5mm. shorter than outer, margin entire	Outer: oblong-lanceolate, 4-7×1.8-3mm Inner: ovate-elliptical, 4-6×2.1-3.5mm. shorter than outer, margin entire	outer oblong, slightly narrower than inner, 4.5-11×2-2.9 mm; inner: oblong-obovate (4.5-9×1.8-3.1mm)
Tepal apex	Acute-acuminate	Acute- acuminate	Acuminate, reflexed
Umbel	Hemispheric-subglobose, lax	Hemispheric-subglobose, lax	Hemispheric-subglobose, sub-erect (not lax)
Stamens	Six, subequal; stamens 4/5th the length of the tepals;	Six, subequal; stamens 4/5th the length of the tepals;	Six, shorter, half the length of the tepals
Anthers	Dark pink, inner ones slightly wider at base than outer at base	Green-yellow, inner ones slightly wider than outer at base	Mauvish, inner ones slightly wider than outer at base
Ovule (no.)/locule	Two	Two	Two-four
Capsules	Spreading	Spreading	Erect-suberect
Seed colour	Black	Black	Darkest brown
Seed shape, size	Irregularly rhomboidal-oval, rough; 3.8-4.1×2.8-3.1 mm	Irregularly rhomboidal-oval; rough; 3.2 × 2.5 mm	Oval, fringed margin smooth, shiny; 4.0-4.5 × 2.5-3.2 mm
Flowering period	August-September	August-September	June-August

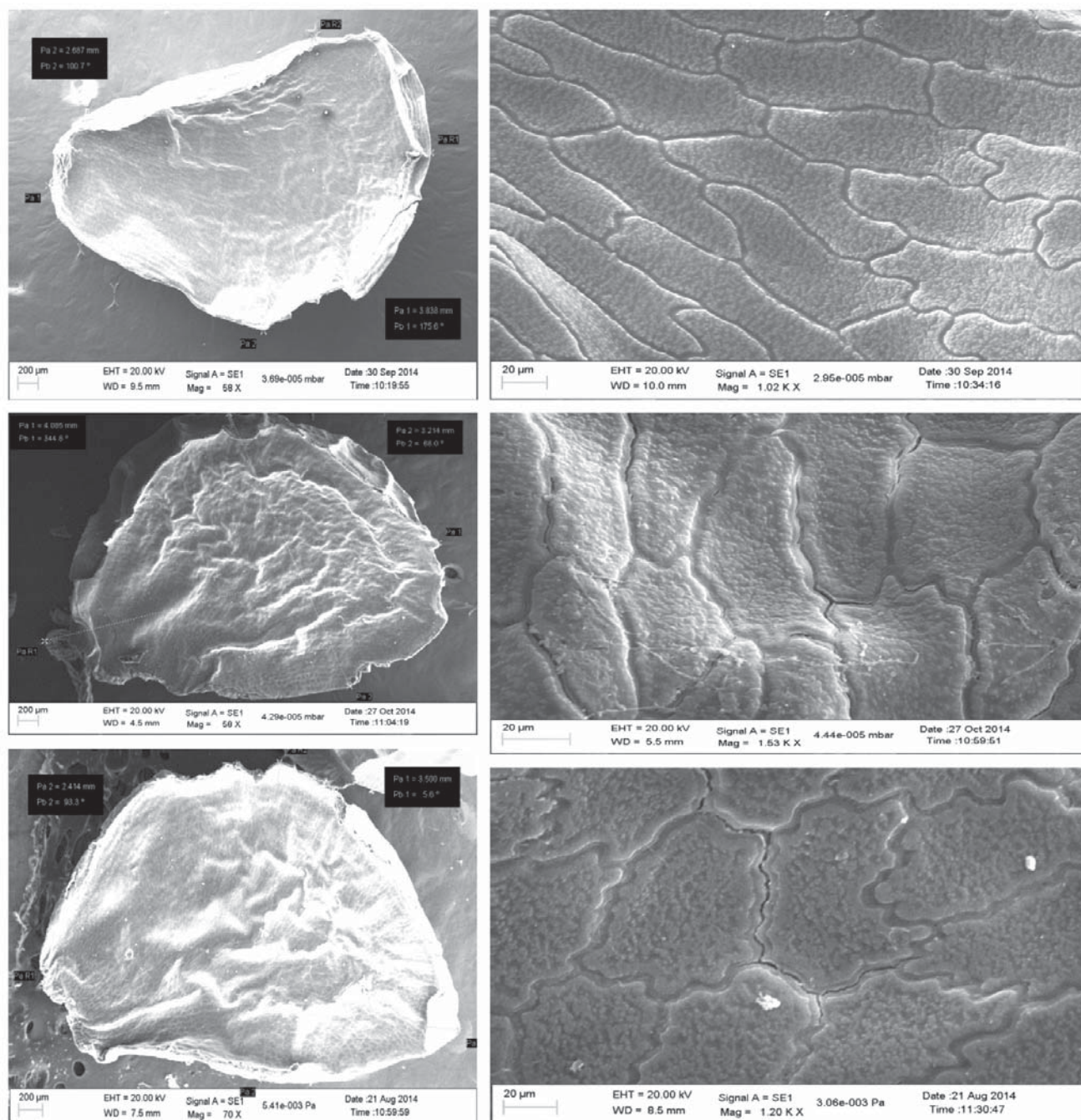


Fig. 2. Seed micro-morphology, general view and testa ornamentation: *A. tuberosum* (top row- pink mid-vein), *A. tuberosum* (middle row- green mid-vein); and *A. ramosum* (bottom row)

distribution range. In this study the morphotype with pink mid-vein on tepal was recorded as less-common type for India.

On the basis of available evidences and data in the present study the authors suggested that the pink-vein on tepal is very distinct from *A. ramosum* where the latter species does not occur in India though Murti (2001)

opined its doubtful occurrence in the Indian Himalaya. Phytogeographically, *A. ramosum* occurs in steppes of Siberia, Mongolia and northern China, whereas *A. tuberosum* is native to South-western China (Oyuntsetseg *et al.*, 2012). Sect. *Austromontana* is distributed in the mountainous region from eastern to central Asia upto bordering eastern Mediterranean region. *Allium*

oreoprasum Schrenk occurs in sunny slopes and stony river banks in Leh region, Ladakh Himalaya, J&K (1200-2700m). *A. gilgiticum* is rare endemic and reported only from its type locality in Gilgit, North Pakistan (Gohil, 1992).

Ecogeographical distribution map drawn using software (DIVAGIS7.5) showed that concentration of species diversity in Sect. *Austromontana* was more towards western parts of the Himalayas extending in higher altitudes of west Asia, whereas sect. *Butomissa* was distributed towards eastern part of Asia-northeastern China and Mongolia (Fig. 3). *Allium tuberosum* was wide spread from western to eastern parts, especially of North China to southern parts continued with its Indian boundaries. *A. ramosum* was confined to the northern part of eastern Asia. To our understanding, none of the available data indicated its occurrence in Indian region.

Molecular Studies

Molecular study using sequence analyses of ITS (Internal Transcribed Spacer) region revealed that two morphotypes of *A. tuberosum* were similar whereas they were very distinct from *A. ramosum* (Fig. 4). Zeder

(2006) reported the distinction between *A. tuberosum* and *A. ramosum* based on data from RAPD and origin of the former species.

Phylogenetic and molecular evolutionary analysis conducted using Mega version 6 (Tamura *et al.*, 2013) showed that accessions of *A. tuberosum* (Leh-1 and Leh-2) were distinct from other accessions of *A. tuberosum* and *A. ramosum* (Fig. 4). The accession Leh-2 showed 95.4 per cent similarity to four accessions of *A. tuberosum* (Leh-1, IC353524, NG3189, NEH) used in the study and is distant from *A. ramosum* by 15.9 per cent based on the analysis of 285bp ITS region amplified from all these accessions. Leh-2 is pink-flowered *A. tuberosum* whereas Leh-1 was *A. tuberosum* (green mid-vein) collected from the same region.

Key to Subgenus *Butomissa*

Taxonomic identity of *A. tuberosum* and *A. ramosum* has always remained confusing due to gross morphological similarity of plant parts (Zeder, 2006; Sharma and Gohil, 2013). Previous studies based on marked differentiation of floral parts and life history in *A. tuberosum* and *A. ramosum* with regard to floral traits (stellate/campanulate flowers; narrow-ovate tepals with

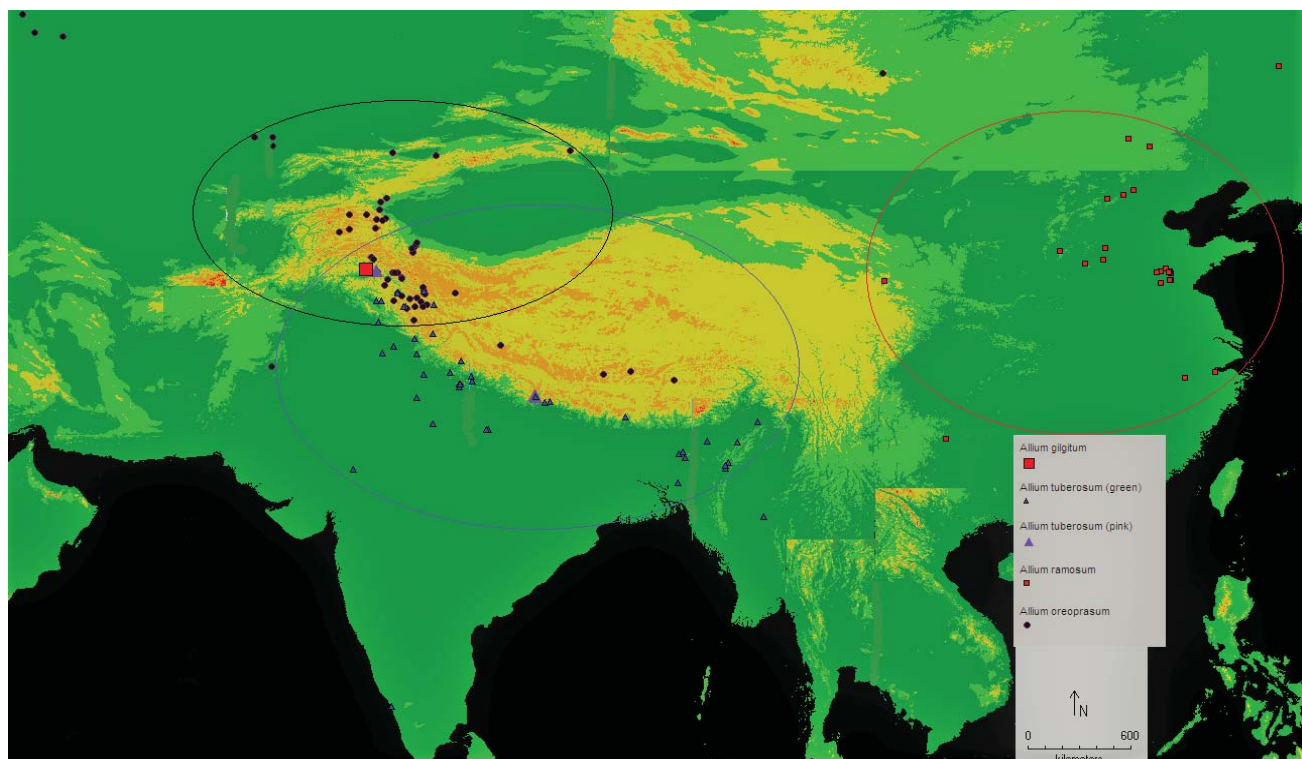


Fig. 3. Areas of occurrence of taxa in *Allium* subg. *Butomissa*: *Allium tuberosum* tepal with pink mid-vein and the common types with green mid-vein on tepal used in the study; other taxa - *A. ramosum*, *A. oreoprasum* and *A. gilgiticum* (herbarium and other databases)

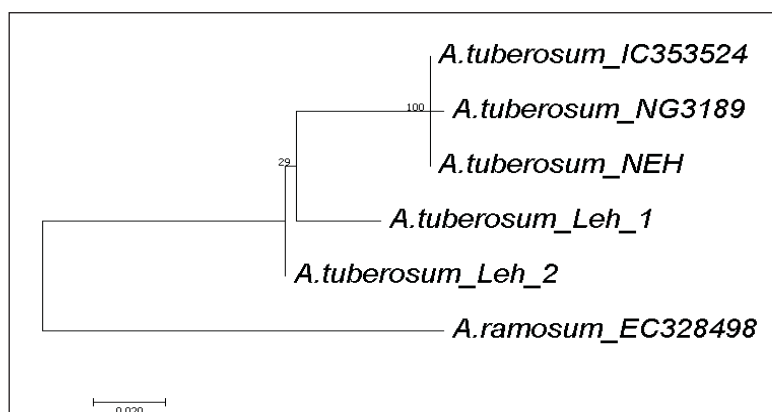


Fig. 4. Dendrogram based sequence analyses of the internal transcribed spacer region for two species of *A. tuberosum* and *A. ramosum*

green mid-vein/lanceolate-oblong tepals with reddish mid-vein; shorter tepals-5.6-6.9 mm/ 6-11 mm; stamens 4/5th the length of the tepals/stamens half the length of the tepals), ovaries with two vs two-four ovules/locule and phenology (August-October/June-August) advocated their status as separate species (Stearn, 1946; Hanelt, 1988; Sharma, 2005; Blattner and Friesen, 2006; Sharma and Gohil, 2013). *Allium odorum* L. is a synonym of *A. ramosum* L.

Among the most distinguishable features noted in this study for *A. ramosum* (comparison with *A. tuberosum*) were tepals margin wavy vs. entire, stamens length much shorter as compared to the tepal length and flower (campanulate), opening pattern-tepals never open wide as compared to that of widely opened tepals of *A. tuberosum* (widely open/stellate) (Fig. 1). The present study indicated that presence of pink mid-vein on tepal in *A. ramosum* is not a diagnostic trait as suggested by many workers (Stearn, 1946; Sharma, 2005; Sharma and Gohil, 2013). Some additional characters such as leaf anatomy, and orientation of tepals with respect to stamen, other tepal characters (tepal colour, margin and apex) have been suggested in field key for identification of taxa of the subg. *Butomissa*.

Field identification key for subg. *Butomissa* (Salisb.) Kamelin (modified from Friesen *et al.*, 2006) is provided below:

Bulb cylindric, tunic coarsely reticulate-subreticulate; perianth white or pink

Leaf blades flat, not keeled; flowers wide open on maturity, stellate, inner tepals ovate- elliptical, outer tepals oblong-lanceolate, inner tepals shorter than outer, margin entire, white with pale green or pink mid-vein

abaxially; ovules two per locule

----- *A. tuberosum*

Leaf blades angled (keeled); flowers never open widely, not stellate, inner and outer tepals elliptical, inner tepals longer than outer, margin wavy, pinkish with reddish mid-vein abaxially, ovules two-four per locule

----- *A. ramosum*

Bulb narrowly ovoid-cylindric, tunic reticulate or fibrous; perianth white-rosy purple

Bulbs ovoid-cylindric, tunic reticulate, leaves narrow, linear, 1-4mm wide, umbels few flowered, erect-suberect, tepals obovate-elliptic, acuminate apex with a reflexed point, pale pink-white with dark purple mid-line

----- *A. oreoprasum*

Bulbs cylindric, tunic fibrous, leaves broad, obtuse, 10-20mm wide, umbels many flowered, loosely arranged, tepals lanceolate, acute apex, rosy-purple with faded mid-line

----- *A. gilgiticum*

Botany of Allium tuberosum

The plant has clustered cylindrical bulbs arising from horizontal rhizome; membrane reticulate-sub-reticulate and dull-yellow brown. Leaves – linear, shorter than scape, flat, solid, margin smooth; scape – slender, covered with leaf sheaths at base. Spathe – two-three partite, persistent. Umbel – hemispheric-subglobose, lax, many flowered; flower – stellate, outer tepals oblong-lanceolate, inner tepals ovate-elliptical, shorter than outer, margin entire, white with pale green or pink mid-vein abaxially; filaments six, subequal with narrowly triangular base, as long as perianth segments, connate at base, adnate

to perianth segments, anther lobes yellow-dark pink; inner ones slightly wider at base than outer ones. Ovary-obconical-globose, minutely tuberculate, without concave nectaries at base. Capsules loose, spreading, seeds black, two per locule, about 1mm and irregularly rhomboidal.

Morphological Data—A Major Concern

The data recorded on plant characters during field, experimental, herbarium and ecogeographical studies needs special consideration for plant genetic resource and systematic study (Pandey *et al.*, 2017; Negi *et al.*, 2018). While studying the genus *Allium*, authors opined that the protologue description, data on available taxonomic literature and herbarium resources depicting characters are not clearly perceived. Data may vary with stage at which observations were recorded which may be misleading. The characters of *A. tuberosum*—tepals/flower colour, streaking white or pinkish white, flower red, white or pink, lilac, reddish-pink white with pink streak were recorded in the literature (Baker, 1892; Murthi, 2001; Dasgupta, 2006). Some new characters used from leaf anatomy, flower opening pattern, orientation of tepal and stamen, and tepal characters (tepals colour and mid-vein, margin, apex) have been included in field key for identification.

Leaf description for *A. ramosum* in protologue and other published literature has been given as hollow, fistulose which was not observed by the authors (Pandey *et al.*, 2018). Authors believe that the term fistulose or hollow as attributed by Choi and Bu (2011) does not fit into the fistulose character of leaf in subg. *Cepa*. Anatomical study carried out using live material of *A. ramosum* revealed that the hollow leaf was an unstable character and varies with stage and habitat which may be linked to different soil conditions. We therefore prefer to use the term 'keeled' (ref. Baker 1892) instead of 'fistulose' used for *A. ramosum* (only observed when dissected). Further, many characters with respect to morphology vs. temporal variation have not always been interpreted as habitat-linked characters.

Such variations were noted by the authors in other species of *Allium* (*A. chinense* G. Don.; Pandey *et al.*, 2016; 2018) growing under diverse ecological conditions. Criterion fixed by Kamelin who differentiated *A. tuberosum* and *A. ramosum* on the basis of fistulose leaves is therefore not agreeable to authors. Study on *A. gilgiticum* was undertaken only using the herbarium data

(K000844282; K000844283). However, availability of insufficient material of *A. oreoprasmum* and *A. gilgiticum* made it difficult to comment on all the characters.

The following points need special attention for recording data: a) habit and ecogeography- plant height, tunic and fibre characters with variation in altitudinal, temporal and soil conditions; b) bulb- shape with respect to stage at which studied or prepared as herbarium specimen; c) tunic-character distinction between fibrous, coriaceous and reticulate; and d) flower colour at maturity level.

Conclusion

The above study clearly identified *A. tuberosum* in India with distinct tepal characters as compared to a common morphotype and classified it to be very distinct type from the related taxon *A. ramosum* based on morphology of fibre, leaf, flower and seed testa characters. The study has addressed issues on taxonomy of the subgenus with respect to: 1) need to support ecogeographic and herbarium data; 2) limitations on availability of material for indepth experimental study; and 3) correct usage of terminology for taxon description.

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