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Systematics and Taxonomic Disposition of the Genus *Punica* L.

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ABSTRACT

The genus *Punica* belongs to a monogeneric family Punicaceae and includes two species *viz.*, *Punica granatum* L. and *P. protopunica* Balf. f. However, some authors also consider the ornamental dwarf pomegranate (*P. nana* Pers.) as a distinct species. The systematic and taxonomic circumscription of the genus *Punica* has been controversial in many floras. Earlier morphological studies considered the genus *Punica*, under Lythraceae. However, but based on distinct features like fruits with leathery pericarp, pulpy seeds with edible sarcotesta, ovule with multilayered outer integument and unicellular archesporium, it was found to differ from other typical Lythraceae genera and was therefore, included in a separate family, Punicaceae. The inclusion of the genus *Punica* in the order Myrtales is not questioned, but the family under which it should be placed is still a debatable question. Here, we discuss the taxonomic implications of the genus *Punica* in relation to different systems of classification that are already in place and nrDNA and CpDNA sequences that have been generated for the genus *Punica*, to understand the phylogenetic relationships with other taxa of the order Myrtales.

Keywords: phylogeny, pomegranates, Punicaceae

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INTRODUCTION

Linnaeus described the genus *Punica* for the first time in 1753. It is traditionally treated under the Punicaceae, a monogeneric family of two species i.e., *Punica granatum* and *Punica protopunica*. The species *P. protopunica*, so called Socotra pomegranate, is endemic to the island of Socotra, Democratic Republic of Yemen (Guarino *et al.* 1990). *Punica nana*, another form of *Punica granatum* is often treated as third species of *Punica* (Melgarejo and Martínez 1992).

Two subspecies of *P. granatum* have been distinguished on the basis of ovary colour, a stable character which is retained when they are grown from seeds. Subspecies *chlorocarpa* is found mainly in the Transcaucasus and subspecies *porphyrocarpa* mainly in central Asia (Anonymous 1969).

P. granatum, commonly known as 'pomegranate' has been used since the dawn of human civilization. Besides consumption as a raw fruit and for juice, pomegranate has tremendous medicinal potential and is used in traditional or herbal cures for many diseases like, cancer, diarrhea, diabetes, blood pressure, leprosy, dysentery, tapeworm infection, hemorrhage, bronchitis, gums bleeding, dyspepsia and throat inflammation (Aviram and Dornfeld 2001; Adams *et al*. 2006; Lansky and Newman 2007; Stover and Mercure 2007).

Botanical characteristics of the genus *Punica*

The plants are mostly shrubs or small trees about 5-10 m high, while few are dwarf (1-2 m). Stem is smooth with dark grey bark, often quadrangular if young, branches sometimes spiny. Leaves are opposite or subopposite, often crowded on short lateral shoots, short-petioled, simple, entire, exstipulate, 2-8 cm long, oblong or obovate, glossy, bright green, glabrous and eglandular. Inflorescences are terminal or axillary (Lawrence 1951; IBPGR 1986).

Flowers are actinomorphic, bisexual, terminal or axillary, solitary or few in clusters, with brightly coloured hypanthium; calyx is tubular, 5-8 lobed, persistent, fleshy, valvate; petals are 5-7, imbricate, brilliant orange-red, lanceolate, inserted between the calyx lobes, wrinkled; stamens are numerous, free, borne on calyx tube, filaments free, anthers dorsifixed, pollen grains aperturate and colporate; ovary is inferior, with several locules in two series, one above the other, placentation axile (in *P. protopunica*) or axile and parietal (in *P. granatum*, where the carpels become

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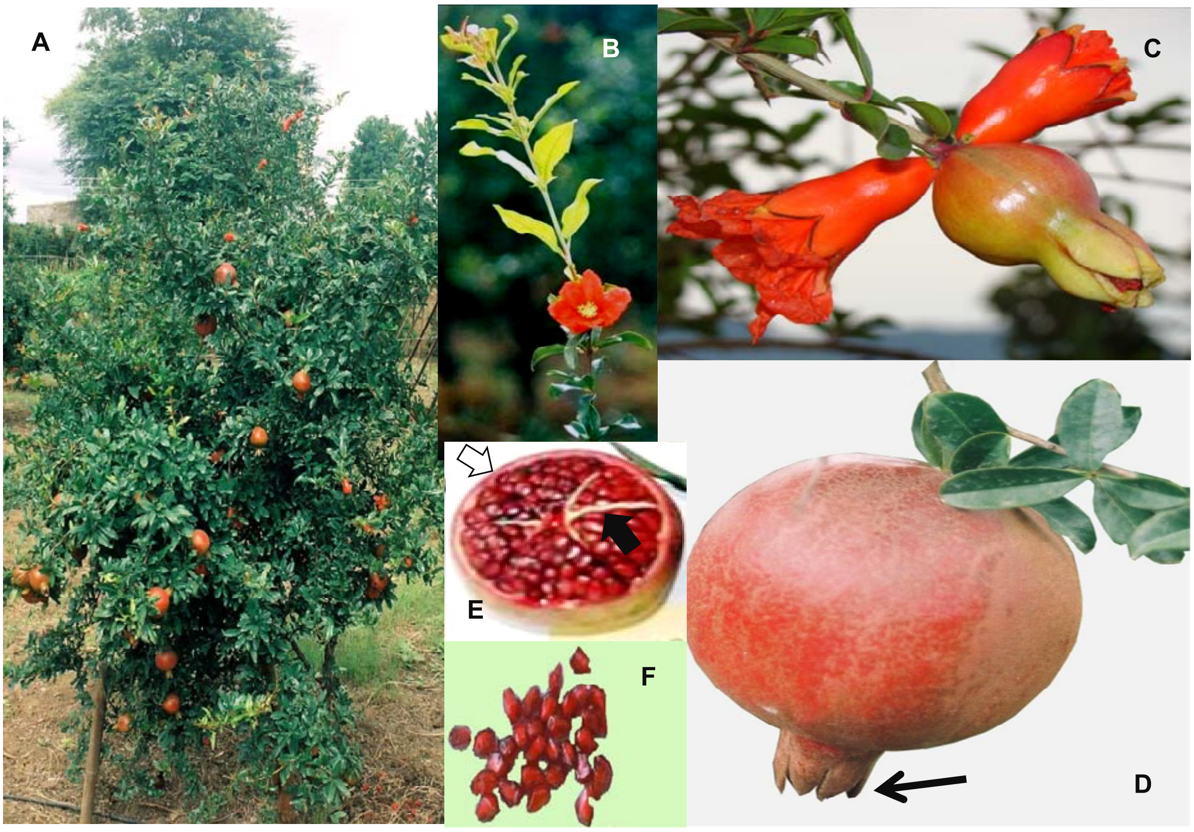

Fig. 1 Pomegranate growth habits. (**A**) Habit; (**B**) Flowering branch; (**C**) Mature flowers and fruit; (**D**) Mature fruit – arrow indicates to persistent calyx; (**E**) Cross-section of fruit – open arrow indicates to thick rind, solid arrow indicates to placentation; (**F**) Arils.

superposed in two or three layers by differential growth, the lower with axile placentation, the upper ostensibly parietal); ovules numerous, anatropous (Lawrence 1951; Watson and Dallwitz 1992).

Three types of flowers have been reported to occur on the same pomegranate plant, *viz.*, hermaphrodite, male flowers and intermediate forms. The calyx of the hermaphrodites is urceolate (pitcher-like) with a broad, well developed ovary. Male flowers are smaller, with a campanulate (bell-shaped) calyx and a rudimentary ovary. The intermediate forms exhibit various degrees of ovary degeneration. Fruits arising from such flowers drop early, or they are misshaped in maturity (Nath and Randhawa 1959c).

Heterostyly is common in pomegranate flowers. Long-styled perfect flowers are larger, have larger ovaries, and set more fruit than short style types, which are either intermediate or functionally male only. The proportion of these two flower types varies among cultivars and year to year (Martínez *et al*. 2000). Occasionally, "intermediate" flowers have styles that may equal the length of the long-styled flowers or are as short as the short-styled ones. Those with long styles occasionally become fertilized, but only rarely does such fruit mature and then it is malformed and defective. On the contrary, short-styled flowers are never fertilized and soon shed. The petals of these are a dull, pale rose, and the pollen is defective (Hodgson 1917). The long-styled flowers usually develop on old wood, whereas the short-styled flowers develop as new growth. The relative proportion of each is influenced by many factors. The best fruit is obtained from the early flowers, probably because they develop during more favourable meteorological conditions (Evreinoff 1953).

Phenology

The flowering habit of the pomegranate varies with the climatic conditions. In tropical climate, it flowers almost throughout the year whereas in subtropics once a year. In subtropical climates of the northern hemisphere, flowering occurs from the last week of March till the second week of May (Singh *et al*. 1978). In the Central Valley of California, pomegranate blooms from early May to November, with most flowering from mid May to early June (Stover and Mercure 2007). In subtropical Central and Western India, there are 3 distinct flowering seasons, *viz.*, *ambia bahar* (February-March), *mrig bahar* (June-July, coinciding with the break of monsoon) and *hasta bahar* (September-October) (Patil and Karale 1990).

Wild pomegranate flowers from the middle of April to the end of May in the temperate climate of Himachal Pradesh, Jammu and Kashmir and Uttarakhand, and two off-season blooms of much less intensity also appear during July and November (Parmar and Kaushal 1982; Rana *et al.* 2003). *Punica protopunica* flowers and fruits from December and January through to the summer and it bear smaller and less palatable fruits than *P. granatum* (Miller 2004).

The fruit

Pomegranate fruits are berry, globose or somewhat flattened, 5-12 cm in diameter, crowned by thick tubular calyx; pericarp (rind) smooth, coriaceous, woody, from brownish-yellow to red when ripe; mesocarp (albedo) spongy, divided in several chambers by a horizontal diaphragm and vertical septal membranes made of papery tissue, each chamber being filled by many seeds crowded on thick, spongy placentae; arils do not attach to septal membranes (Lawrence 1951; Purseglove 1968; Anonymous 1969). The seeds are surrounded by the juicy arils, which comprised the edible portion of the fruit (Watson and Dallwitz 1992). The aril juice sac is composed of many epidermal cells and colour of the arils range from deep red to virtually colourless according to different cultivars, whereas the enclosed seed varies in content of sclerenchyma tissue, which affects seed softness (**Fig. 1A-F**). The number of locules and arils varies, but may be as high as 1300 per fruit (Stover and Mercure 2007). The fruit has a prominent calyx, which is maintained to maturity and is a distinctive feature of the pomegranate fruit. The husk is comprised of two parts: the pericarp, which provides a cuticle layer and fibrous mat; and the mesocarp (known also as the albedo), which is the spongy tissue and inner fruit wall where the arils attach. Septal membranes are the papery tissue that further compartmentalizes groups of arils, but arils do not attach to this tissue. Fruits ripen about 6 to 7 months after flowering (Morton 1987). Pomegranate fruits are ripe when they have developed a distinctive colour and make a metallic sound when tapped.

Pollination

There are divergent opinions about the pollination in pomegranates. Pomegranate can be self-pollinated or cross-pollinated by insects (Morton 1987). Cross-pollination in pomegranate was reported by Gammie and Patwardhan (1929), and Nalawadi *et al.* (1973). Pross (1938) considered pomegranate to be a self-pollinated crop on the basis of the studies in four pomegranate cultivars grown in central Asia. However, Jalikop and Kumar (1990) and Karale *et al.* (1993) demonstrated that it is capable of both open and self pollination. The size and fertility of the pollen vary with the cultivar and season. There is very little wind dispersal of pollen (Morton 1987). Little is known about the pollinators in pomegranates. Nath and Randhawa (1959d) reported that pomegranate is both self- and cross-pollinated and the principal insect pollinators are black ants (*Camponotus* sp.), honey bees (*Apis* sp.) and lemon butterfly (*Papilio demoleus* Linn.). According to Melgarejo *et al.* (2000), pollination in pomegranate is primarily affected by insects or hummingbirds. Beetles belonging to the genera *Cetonia* and *Trichodes* were reported to affect both cross- as well as self-pollination in pomegranate, while devouring the flowers (McGregor 1976).

Cytology

The chromosome number in several cultivars of pomegranates has been reported differently by various workers. There are divergent reports on the chromosome numbers ($n = 8, 9$; $2n = 18, 19$) of *P. granatum* (Darlington and Wylie 1955). Nath and Randhawa (1959a) found $2n = 16$ for 6 Indian cultivars, except for the ornamental '*Double Flower*' cultivar, which had $2n = 18$. Tetraploid with $2n = 32$ was obtained from the cultivar '*GB-1*' ($2n = 16$) by air-layering (IBPGR 1986). The diploid chromosome ($2n = 16$) of pomegranate contains 1.4 pg (= 1412 Mbp) of DNA (Ohri 2002; Bennett and Leitch 2005).

Masoud *et al.* (2005) also reported $n = 8$ chromosomes in all the pomegranate cultivars, but significant variability in their chiasma frequency, chromosome pairing and segregation was recorded among the cultivars indicating their genomic difference. B-chromosomes and the formation of a low number of quadrivalents have also reported in some cultivars of pomegranates (Masoud *et al.* 2005; Sheidai and Noormohammadi 2005). Significant increase or decrease in the number of chiasmata was observed by Sheidai (2007) in the Iranian pomegranate cultivars where B-chromosomes are present leading to a change in genetic recombination. We also carried out cytological studies and found $n=8$ chromosomes (**Fig. 2**) in wild pomegranates.

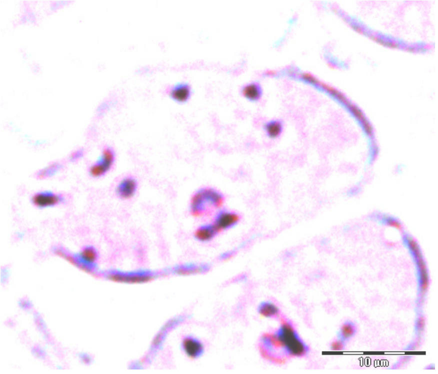

Fig. 2 Haploid chromosome number in the wild pomegranates.

Distinguishing features of pomegranate

The genus *Punica* L. is characterized by several easily distinguishable morphological features, such as the fruit with leathery pericarp and the pulpy seeds with edible sarcotesta (Dahlgren and Thorne 1984). Besides, the ovules of *Punica*, with their thick, multilayered outer integument and unicellular archesporium, differ from those of Lythraceae *sensu stricto* (Huang and Shi 2002). The union of the ovary with the receptacle of the thalamus that forms a peculiar type of fruit, especially termed as "balusta" is also a very distinctive character of *Punica* (Nath and Randhawa 1959b).

SYSTEMATICS OF THE GENUS *PUNICA*

The genus *Punica* with its distinctive characteristics is somewhat isolated in the order Myrtales. However, the inclusion of *Punica* in the order Myrtales is not questioned, but the family under which it should be, is long been a debatable question. It has been placed under the family Punicaceae, Lythraceae and Myrtaceae by different biologists at different times. Different systems of classification that treated *Punica* differently, has been given in **Table 1**.

Morphological approach

The genus *Punica* was initially included under the family Myrtaceae (Baillon 1880); however, Bentham and Hooker (1865) objected the inclusion of *Punica* under Myrtaceae on the ground that it does not have dotted glands on the leaves and infra-marginal veins, its calyx is valvate, and also it does not have aromatic principles in the green parts. They included *Punica* as an anomalous genus in the family Lythraceae. Metcalfe and Chalk (1950) also justified the inclusion of *Punica* in Lythraceae due to its similarity in the anatomical characters, especially the occurrence of intraxylary phloem. However, based on the gross morphological attributes like union of the ovary with the receptacle of the thalamus and unique morphology of the fruit (balusta), the genus *Punica* was suggested by several biologists to include in a separate family, Punicaceae (Hutchinson 1926; Warming and Potter 1932; Rendle 1938; Gundersen 1950; Lawrence 1951, Nath and Randhawa 1959b).

The distinct features of *Punica*, such as fruits with leathery pericarp, pulpy seeds with edible sarcotesta, ovules with thick multilayered outer integument and unicellular archesporium, differ from those of Lythraceae *sensu stricto* and therefore it was considered under a separate family Punicaceae in the classification system of Engler (Melchior 1964), Takhtajan (1980) and Cronquist (1981). But the wood anatomy (Bridgewater and Baas 1978; Graham *et al.* 1993), chromosome data (Tobe *et al.* 1986), and pollen morphology (Patel *et al.* 1984; Graham *et al.* 1990) suggested the inclusion in, or at least a close relationship with, Lythraceae *sensu lato*. However, the combination of all these features leads Tobe and Raven (1983) to suggest *Punica* as being a distinct archaic offshoot within Lythraceae *sensu lato*.

A comprehensive cladistic study of relationships of the families within the order Myrtales was published by Johnson and Briggs (1984). This study was based on phenotypic (morphological, anatomical, palynological, and embryological) characters and provided strong support for a clade comprising Trapaceae, Onagraceae, Lythraceae *sensu lato* (including also *Duabanga* and *Sonneratia*), and Punicaceae, a second clade formed by Myrtaceae, Heteropyxidaceae, and Psiloxylaceae, and sister group relationships between Alzateaceae and Rhynchocalycaceae, and Penaeaceae and Oliniaceae, respectively.

Lythraceae *sensu lato* is a large family comprised of 31

Table 1 Taxonomic position of the genus *Punica* in different classification systems.

Bentham and Hooker's System (1862-83)	**Engler's System (Melchior 1964)**	**Takhtajan's System (1980)**	**Dahlgren's System (1980)**	**Cronquist's System (1981)**	**Young's System (Bedell and Reveal 1982)**	**Thorne's System (1983)**	**APG-II System (2003)**
Dicotyledons	Dicotyledoneae	Magnoliopsida	Dicotyledoneae	Magnoliopsida	Magnoliopsida	Dicotyledoneae	Magnoliopsida
Polypetalae	Archichlamydeae	Rosidae	Myrtiflorae	Rosidae	Rosidae	Myrtiflorae	Rosidae
Calyciflorae	Myrtiflorae	Myrtanae	Myrtales	Myrtales	Myrtanae	Myrtales	Myrtales
Myrtales	Myrtineae	Myrtales	Lythraceae	Punicaceae	Myrtales	Lythrineae	Lythraceae
Lythraceae	Punicaceae	Myrtineae	*Punica* L.	*Punica* L.	Lythraceae	Lythraceae	*Punica* L.
Punica L.	*Punica* L.	Punicaceae			*Punica* L.	*Punica* L.	
		Punica L.					

genera and 620 species, and comprises of four subfamilies, with one, Punicoideae, sometimes regarded as a satellite family of Lythraceae *sensu stricto*. The generalized morphology of the family, together with the very distinctive taxa, suggests that the family is of great age (Still 2006). Morphological plasticity and absence of synapomorphies in the morphological characters among the members of ancient family Lythraceae reflects an early pattern of rapid radiation in the family, and therefore the establishment of relationship amongst the genera under Lythraceae is difficult due to their remarkable range of morphological variation. However, the genus *Punica* was considered closest to *Lagerstroemia* (Graham *et al.* 1993).

Molecular approach

The use of DNA sequences in taxonomy dates back to 30 years when ribosomal RNA probes were developed for the identification and phylogenetics of eubacteria and archaebacteria (Fox *et al.* 1980). The term 'molecular taxonomy' was used by Scherer and Sontag (1986) for the first time. Molecular taxonomy/DNA taxonomy concerns the circumscription and delineation of species using evolutionary species concepts. The use of molecular data in the investigation of relationships within morphologically complex families has been a powerful approach for achieving well-delimited taxa (Fior *et al.* 2006; Pissard *et al.* 2008). Ideally, DNA-based cladistic analysis can provide an independent source of information capable of resolving relationships among morphologically intractable groups. With sufficient taxon sampling that includes representatives from all major subfamilial taxa, DNA-based topologies can be the starting point for reinterpretations on the development and evolution of intricate morphological characters. As a first step, morphological features may simply be optimized on molecular trees, with extensive studies on morphology as an inevitable second step (Schonenberger and Conti 2003).

Genes utilized in phylogenetics and systematics differs in their ability to provide support for relationships at different taxonomic levels. The length of a gene sequence and the number of base pairs analyzed may affect its utility in inferring phylogenetic relationships. The choice of an appropriate gene or a set of genes is fundamental to success in phylogenetic analysis. The genes present in single copies, or if in multiple copies then these are homogeneous, of size range 500-1000 base pairs, containing both conserved as well as variable regions, and preferably lacking many introns especially of long sequences are generally considered to be ideally suited for such primary sequence-based molecular phylogenetics (Ranade 2003). The genes like *rbc*L, *atp*B, *mat*K, *trn*L, *trn*F and rDNA regions were used to resolve relationships among different plant taxa at the genus to family or higher level (Soltis *et al.* 1990; Conti *et al.* 1993; Soltis *et al.* 1993, 1997; Hershkovitz and Zimmer 1999; Savolainen *et al.* 2000; Soltis *et al.* 2001a; Hilu *et al.* 2003; Berry *et al.* 2005; Guo and Ge 2005; Huang *et al.* 2005; Wang *et al.* 2007; Saini *et al.* 2008; Zeng *et al.* 2008; Gulbitti-Onarici *et al.* 2009). Soltis *et al.* (1993) found that *rbc*L sequence data may have limited ability to resolve generic-level differences in some taxonomic groups. Utilization of *mat*K sequences has been effective for inferring differences in lower taxa (genus or below) (Johnson and Soltis 1994, 1995; Soltis *et al.* 1996, 2001b). Nuclear DNA regions, such as 18S and 26S rDNA, have shown tremendous potential for inferring phylogenies at taxonomic levels below the genus, and have been used in many different organisms from plants to bacteria (White *et al.* 1990; Nickrent and Soltis 1995; Soltis *et al.* 1997, 2001a).

In some instances sequences from two or more genes have been utilized to deduce phylogenetic relationships and the discrimination abilities compared to validate the use of the genes. Nickrent and Soltis (1995) used *rbc*L and nuclear 18S rDNA sequences to compare angiosperm phylogeny and determined that sequences from either were efficient to distinguish differences among flowering plants at higher taxonomic levels. In a similar study, Savolainen *et al.* (2000) used sequences from both *atp*B and *rbc*L to investigate phylogeny among a wide array of flowering plants, concluding that, with some discrepancies, phylogenetic trees derived from the two genes were congruent. A comprehensive study of six gene sequences was conducted by Soltis *et al.* (2001a) to elucidate relationships within Saxifragaceae. Trees generated from nuclear sequences agreed closely with those derived from plastid sequences and a phylogenetic classification of the family was resolved.

Conti *et al.* (1993) did the phylogenetic analysis of seven tribes of Onagraceae using *rbc*L sequence data. In this study, they included the families Lythraceae, Punicaceae and Trapaceae as out-groups. Phylogenetic analysis of *rbc*L sequence data produced a single most parsimonious tree that defined three strongly supported monophyletic groups within the family: all tribes except Jussiaeae; Onagreae and Epilobieae; and, most interestingly, Fuchsieae and Circaeeae. The data also indicated an apparent slowdown in the rate of *rbc*L sequence divergence in the woody *Fuchsia* lineage relative to the herbaceous *Circaea* lineage. The placement of monogeneric tribes Lopezieac and Hauyeae were not strongly supported. In the most parsimonious tree resulting from analysis of *rbc*L sequences of nine taxa of Onagraceae and three out-group taxa (*Punica*, *Lythrum* and *Trapa*) of different families, Punicaceae showed sister relationships to Onagraceae and Lythraceae, but more closer to Onagraceae with 98% bootstrap support.

The first comprehensive cladistic analysis of the circumscription of Myrtales and their relationships with other rosid families was carried out by Conti *et al.* (1996). In this analysis, 80 *rbc*L sequences representing 36 taxa from families traditionally included in Myrtales and 44 taxa from other Rosidae were considered with an objective to determine the relationships of some controversial families, and to identify the most likely sister groups of Myrtales. Based on *rbc*L sequence analysis, they reviewed the families that are to be under the Myrtales and suggested to include the family Vochysiaceae, and to exclude the families Thymelaeaceae, Lecythidaceae, Haloragaceae and Gunneraceae from the order Myrtales.

In another study, Conti *et al.* (1997) used the *rbc*L sequences for interfamilial relationships analyses in Myrtales. Five major clades were identified in the *rbc*L consensus tree of a Melastomataceae lineage, a Myrtaceae lineage, Onagraceae, a Lythraceae lineage, and Combretaceae. The *rbc*L topology splitted into two major clades of Myrtales: Clade I comprised the Melastomataceae and Myrtaceae lineage; Clade II included Combretaceae sister to a subclade formed by the Lythraceae lineage and Onagraceae. The Lythraceae lineage including *Trapa*, *Duabanga* and *Punica* with a bootstrap support of 65% supported an expanded interpretation of familial boundaries for Lythraceae to include *Trapa*, *Duabanga* and *Punica*. However, the authors suggested to consider the *rbc*L tree with caution, because the clade that included *Trapa*, as well as *Duabanga*, were weakly supported and additional data from evolving genes might result in a slightly different topology within the Lythraceae lineage. They compared this *rbc*L data with that of the cladistics based on phenotypic data of Johnson and Briggs (1984). The *rbc*L tree was found agreeable with the results of previous morphological analyses by providing strong support for the monophyly of Onagraceae, Combretaceae and Melastomataceae *sensu stricto*. However, the *rbc*L tree deviated from previous morphological interpretation of familial boundaries by including *Trapa* in the Lythraceae lineage and Vochysiaceae in the Myrtaceae lineage.

Phylogenetic analysis of the family Sonneratiaceae and its relationship to Lythraceae based on ITS sequences of nuclear rDNA was carried out by Shi *et al*. (2000). Two traditional genera of Sonneratiaceae (*Duabanga* and *Sonneratia*), four genera of Lythraceae (*Cuphea*, *Heimia*, *Lawsonia* and *Lagerstroemia*) and two out-group genera, one each from Combretaceae and Myrtaceae, were considered in this study so as to determine the proper systematic placement of the Sonneratiaceae. Based on phylogenetic analysis of the ITS sequences, paraphyly of the traditional Lythraceae was shown with the genus *Lagerstroemia* nested within the Sonneratiaceae. Occurrence of Sonneratiaceae within the Lythraceae was supported with a high bootstrap value of 96% in the maximum parsimony tree. Therefore, the authors suggested the inclusion of Sonneratiaceae in the family Lythraceae irrespective of high sequence divergences (ranged from 16.07 to 33.58%) among the genera of these families. However, molecular analysis of ITS region was not in congruence with the Ko's taxonomic classification (1993) of genus *Sonneratia* into two sections, *Sonneratia* and *Pseudosonneratia*.

Huang and Shi (2002) investigated the phylogenetic relationships of Lythraceae *sensu lato* by parsimony and likelihood analysis of 85 accessions representing 23 species and 16 genera that had been assigned to the family at various times. The three different markers like the *rbc*L gene, the *psa*A-*ycf*3 spacer, and the nuclear ITS regions including 5.8S ribosomal gene were taken into consideration. The three data sets that were generated by three different markers were highly congruent on the basis of the partition homogeneity test in this study. Phylogenetic analysis based on the combined data strongly supported the monophyly of the Lythraceae *sensu lato*, in which the satellite genera like *Duabanga*, *Punica*, *Sonneratia* and *Trapa* were included, with Onagraceae and Combretaceae as out-groups. The subfamily Lythroideae was proposed as paraphyletic with the other four monotypic subfamilies (Duabangoideae, Punicoideae, Sonneratioiodeae and Tropoideae) nested within. Further, the analysis supported the sister relationship between *Sonneratia* and *Trapa* instead of *Duabanga* and *Sonneratia* (as was treated traditionally). In the combined maximum parsimony and quartet puzzling trees of three markers, *Punica* consistently appeared as sister to *Pemphis* with relatively high bootstrap support (85%, 78%), and *Punica* was considered within the family Lythraceae under the order Myrtales. Recently, Angiosperm Phylogeny Group (APG II 2003) in their revised and updated classification has considered Punicaceae under the family Lythraceae.

It has been observed that though the *Punica* appeared sporadically as an out-group in a few phylogenetic studies of some closely related families under the order Myrtales, but as such there is no comprehensive study on molecular systematics focusing on the phylogenetic relationship of the genus *Punica* with other related taxa of the order Myrtales. It is therefore of paramount significance to study both the species of the genus *Punica*, and its different cultivars grown in different parts of the world to establish its phylogeny and systematics.

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