

# Generic relationships among Molluginaceae inferred from a molecular phylogenetic analysis of the *matK* gene

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Genet. Mol. Res. 16 (2): gmr16029295

Received September 16, 2016

Accepted May 11, 2017

Published June 29, 2017

DOI <http://dx.doi.org/10.4238/gmr16029295>

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**ABSTRACT.** The family Molluginaceae (order Caryophyllales) is considered polyphyletic based on the photosynthetic pathway,  $C_4$  evolution, and phylogeny of the family. This inference was made based on photosynthetic, anatomical, and molecular datasets. The generic circumscription of this family has greatly been changed owing to the placement of several of its genera into the Caryophyllaceae, Microteaceae, Lophiocarpaceae, and Limeaceae families. However, the generic relationships are largely unknown. By virtue of high substitution rates within the species and the ability to resolve the phylogenetic position of morphologically very closely related species and species complexes, the *matK* gene has emerged as one of the potential chloroplast DNA molecular markers in plant molecular phylogenetics and DNA barcoding studies. We herein used molecular phylogenetic analyses of *matK* gene sequences using maximum parsimony and maximum likelihood analyses to infer the generic

relationships among currently recognized genera circumscribed under the family Molluginaceae. The resulting phylogenetic tree confirmed the polyphyly of the family Molluginaceae. The genus *Hypertelis* was found at the base of the Molluginaceae clade. The genus *Glinus* was close to *Glischrothamnus* and *Mollugo*, *Suessenguthiella* was close to *Coelanthum* and *Pharnaceum*, whereas *Polpoda* grouped with *Adenogramma* and *Psammotropha*. The present study constitutes a robust investigation of the molecular phylogenetic relationships among members of the family Molluginaceae. Future study should combine by combined analyses of morphological characters and multiple nuclear and chloroplast DNA sequences with a more comprehensive taxon sampling of the family Molluginaceae.

**Key words:** Molluginaceae; matK; Phylogeny; Generic relationships

## INTRODUCTION

The order Caryophyllales comprises about 30 families, recognized under the order Caryophyllales in the core eudicot clade of angiosperm phylogeny (Angiosperm Phylogeny Group, 2009). Most of the families in this order were given taxonomic rank of family during the last two decades (Sukhorukov et al., 2015). On the basis of presence of anthocyanin and other morphological characters, the family Molluginaceae was segregated from Aizoaceae (Endress and Bittrich, 1993). Results of molecular phylogenetic analyses revealed that the family Molluginaceae is polyphyletic (Cuénoud et al., 2002; Angiosperm Phylogeny Group, 2009). Several genera previously treated under the family Molluginaceae have therefore been shifted to other families. For example, the genus *Corbichonia* is now in the family Lophiocarpaceae, *Limeum* in Limeaceae, and *Corrigiola* with *Telephium* are in Caryophyllaceae (Endress and Bittrich, 1993; Cuénoud et al., 2002; Arakaki et al., 2011; Brockington et al., 2011; Christin et al., 2011; Christenhusz et al., 2014). As per the taxonomic treatment of Angiosperm Phylogeny Group (2009), the family Molluginaceae comprises ca. 90 species under ca. 10 genera (viz., *Adenogramma* Reichenbach, *Coelanthum* E. Meyer, *Glinus* L., *Glischrothamnus* Pilg, *Hypertelis* E. Meyer, *Mollugo* L., *Pharnaceum* L., *Polpoda* C. Presl, *Psammotropha* Ecklund & Zeyher, and *Suessenguthiella* Friedrich). These species are found in tropical to warm temperate regions, especially in southern Africa and northeast Brazil.

The polyphyly of the family Molluginaceae was established on the basis of photosynthetic, anatomical, and molecular datasets. This changed the generic circumscription greatly owing to the placement of several of its genera into family Caryophyllaceae, Microteaceae, Lophiocarpaceae, and Limeaceae (Christenhusz et al., 2014). The relationships among the genera in this family based on molecular phylogenetic analyses are largely lacking (Cuénoud et al., 2002, Angiosperm Phylogeny Group, 2009; Lee et al., 2013; Christenhusz et al., 2014). The maturaseK (matK) 1500 bp long chloroplast-encoded, rapidly evolving gene nested between the 5' and 3' exons of trnK, tRNA-lysine in the large single copy region of the chloroplast genome (Sugita et al., 1985). The rate of nucleotide substitution in the matK is six times higher than the amino acid substitution rate, and three times higher than that of the large subunit of Rubisco (Johnson and Soltis, 1994; Olmstead and Palmer, 1994). The high nucleotide and amino acid substitution rates of the matK provide sound phylogenetic signals

for resolving evolutionary relationships among flowering plants also at lower taxonomic levels (Hilu and Liang, 1997; Soltis and Soltis, 1998; Hilu et al., 2003) and plant DNA barcoding (Ajmal Ali et al., 2014). The present study aimed to analyze matK sequences of representative genera of the family Molluginaceae in order to infer the generic relationships.

## MATERIAL AND METHODS

### Data collection

The chloroplast gene matK is one of the most variable coding genes of angiosperms (Sugita et al., 1985; Johnson and Soltis, 1994; Olmstead and Palmer, 1994). matK has very high evolutionary rate, and thus finds its application in plant DNA barcoding (Ajmal Ali et al., 2014; Kar et al., 2015) and molecular phylogenetic reconstructions at both lower and higher taxonomic levels (Hilu and Liang, 1997; Soltis and Soltis, 1998; Hilu et al., 2003). Therefore, the DNA sequence of the entire coding region of matK of 10 species belonging to 10 different genera recognized under Molluginaceae as per treatment of angiosperm phylogeny (Angiosperm Phylogeny Group, 2009) were retrieved from GenBank, National Centre for Biotechnology Information (NCBI) (Table 1). To compare the Molluginaceae matK sequences, the sequences of *Acanthocalycium spiniflorum* (family Cactaceae) and *Portulaca oleracea* (family Portulacaceae) were also retrieved from GenBank. The family Molluginaceae is supported as the sister group of Portulacaceae (Nyffeler et al., 2008; Nyffeler and Egli, 2010). Therefore, the matK sequence of *Alluaudia procera* (family Didiereaceae) was used as outgroup in the molecular phylogenetic analyses. The matK sequence of outgroup were also retrieved from NCBI (Table 1).

**Table 1.** The GenBank accession No. and span of matK gene sequences for all taxa included in the molecular phylogenetic analyses to infer the generic relationships among members of the family Molluginaceae.

Group		Taxon	GenBank accession No.	matK span
Ingroup				
Family				
Molluginaceae	1.	<i>Adenogramma teretifolia</i> (Thunb.) Adamson	FN825691	609-2159
	2.	<i>Coelanthum parviflorum</i> Fenzl	FN825759	1428-2194
	3.	<i>Glinus oppositifolius</i> (L.) Aug.DC.	FN825696	642-2177
	4.	<i>Glischrothamnus ulei</i> Pilg.	FN825699	593-1614
	5.	<i>Hypertelis spergulacea</i> E. Mey. ex Fenzl	FN825701	638-2197
	6.	<i>Mollugo verticillata</i> L.	JQ844142	623-2167
	7.	<i>Pharnaceum lanuginosum</i> J.C. Manning & Goldblatt	FN825752	605-2152
	8.	<i>Polpoda capensis</i> C. Presl	FN825753	632-2179
	9.	<i>Psammotropha quadrangularis</i> (L. f.) Fenzl	FN825755	611-2161
	10.	<i>Suessenguthiella scleranthoides</i> Friedr.	FN825756	636-2183
Cactaceae	11.	<i>Acanthocalycium spiniflorum</i> (K.Schum.) Backeb.	HM041644	614-2131
Portulacaceae	12.	<i>Portulaca oleracea</i> L.	HQ620836	1-1521
Outgroup				
Didiereaceae	13.	<i>Alluaudia procera</i> (Drake) Drake	HQ620842	598-2127

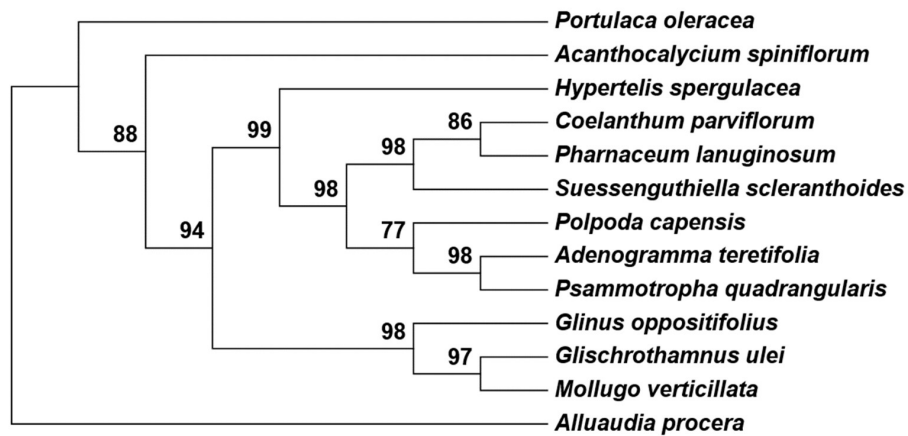
### Molecular phylogenetic analyses

The FASTA format of matK sequences of taxa included in the molecular phylogenetic analyses was aligned using ClustalX 1.81 (Thompson et al., 1997). Gaps in the sequence alignment were considered as missing data in the analyses. The generated aligned sequence data matrix was saved in nexus format. The data were then imported to MEGA5 (Tamura

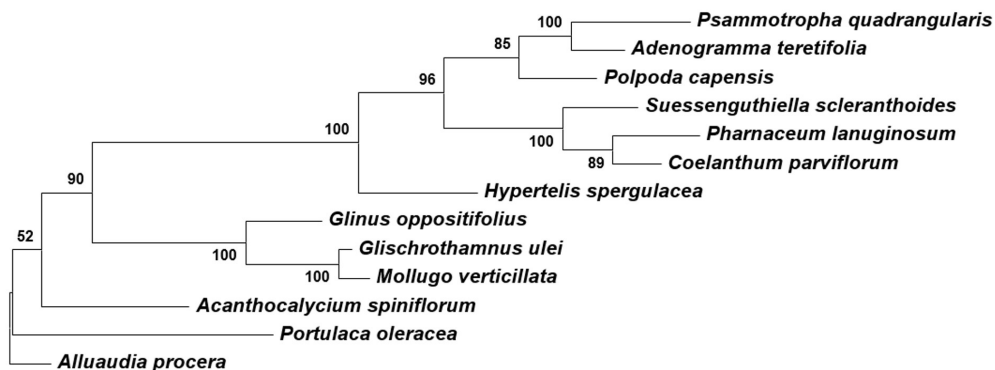
et al., 2011) and subsequently converted to MEGA format. We then performed maximum parsimony (MP) (Eck and Dayhoff, 1966; Felsenstein, 1985; Nei and Kumar, 2000) and maximum likelihood (ML) analysed in MEGA5 (Tamura et al., 2011).

## RESULTS AND DISCUSSION

The MP analysis of the matK sequences resulted in to three maximally parsimonious trees (MPTs) with a tree length of 138, a consistency index of 0.755, a retention index of 0.851, and the composite index of 0.703 for all sites and 0.348 for parsimony-informative sites (Figure 1). There were a total of 246 positions in the final dataset, of which 55 were parsimony informative. The ML analysis resulted in to tree with the highest log likelihood -1099.02 (Figure 2).



**Figure 1.** The bootstrap strict consensus of three maximally parsimonious trees of *Molluginaceae* based on molecular phylogenetic analysis of matK sequence. The bootstrap supports at the node more than 50% in 500 replicates have been shown above lines.



**Figure 2.** The maximum likelihood tree of *Molluginaceae* based on molecular phylogenetic analysis of matK sequences. The number at node indicates bootstrap supports.

The resulting bootstrap (BS) strict consensus MPT and maximum likelihood tree (MLT) rooted at outgroup *A. procera* confirmed the polyphyly of Molluginaceae [88% BS (MPT), 90% BS (MLT)]. The MPT revealed that the basal clade of *P. oleracea* (Portulacaceae) and the *A. spiniflorum* (Cactaceae) clade show proximity [88% BS (MPT), 52% BS (MLT)] with the genera of the family Molluginaceae, which were grouped together in the clade [94% BS (MPT), 90% BS (MLT)]. In the Molluginaceae clade, there were three distinct subclades. The family Molluginaceae is sometimes partially or completely treated along with Aizoaceae and Phytolaccaceae. However, Molluginaceae differs in its absence of betalains, presence of anthocyanins and that is typically has free sepals. The synapomorphies known for Molluginaceae are lacking (Endress and Bittrich, 1993). Thus, in the traditional sense, Molluginaceae is polyphyletic (Cuénoud et al., 2002; Angiosperm Phylogeny Group, 2009; Christenhusz et al., 2014).

The subclade I consisted of *M. verticillata*, *G. ulei*, *G. oppositifolius* [98% BS (MPT), 100% BS (MLT)]. The *G. oppositifolius* (generic synopsis: stipules absent) shows close relationship to *G. ulei* (generic synopsis: dioecious (sub) shrubs, ovules 7-8 per locule) and *M. verticillata* (generic synopsis: locules 3-5, ovules many per locule).

The subclade II consists of *H. spergulacea*, *C. parviflorus*, *P. lanuginosum*, *S. scleranthoides*, *P. capensis*, *A. teretifolia*, and *P. quadrangularis* [99% BS (MPT), 100% BS (MLT)]. Among the subclade II, *H. spergulacea* makes the subclade polyphyletic with the rest of the six taxa of the subclade i.e. *P. quadrangularis*, *A. teretifolia*, *P. capensis*, *S. scleranthoides*, *P. lanuginosum*, and *C. parviflorum*. Among these taxon, *S. scleranthoides* (generic synopsis: stipules conspicuous, sepals hooded, ovules many per locule) shows proximity [98% BS (MPT), 100% BS (MLT)] with *C. parviflorum* (generic synopsis: sepals fused) and *P. lanuginosum* (generic synopsis: sometimes stipules, locules 3-5, ovules many per locule). *C. parviflorum* shows close relationship with *P. lanuginosum* [86% BS (MPT), 89% BS (MLT)]. While *P. capensis* (generic synopsis: sepals white, fringed; locules 2, ovule 1 per locule) shows close proximity [77% BS (MPT), 85% BS (MLT)] with *A. teretifolia* (generic synopsis: petals white, locule 1, ovule 1) and *P. quadrangularis* (generic synopsis: Perennials, stipules conspicuous, locules 3-5, ovule 1 per locule), *A. teretifolia* and *P. quadrangularis* clade together [98% BS (MPT), 100% BS (MLT)].

The genus *Hypertelis* was found to be polyphyletic in a previous study (Christin et al., 2011). *H. spergulacea* was found to be placed inside Molluginaceae s.s., and was nested within *Mollugo*, from which it was segregated based on its fleshy leaves with large stipules adnate to the leaf base and clasping the stem. This relationship was later supported based on single-copy nuclear genes and multiple plastid markers (Christin et al., 2011). The genus *Mollugo* is also polyphyletic within the family Molluginaceae (Arakaki et al., 2011; Christin et al., 2011).

In conclusion, the present molecular phylogenetic analyses revealed the polyphyly of the family Molluginaceae. *Hypertelis* was found at the base of the Molluginaceae clade. The genus *Glinus* is close to *Glischrothamnus* and *Mollugo*, *Suessenguthiella* showed proximity with *Coelanthum* and *Pharnaceum*, and *Polpoda* grouped with *Adenogramma* and *Psammotropha*.

### Conflicts of interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

Research supported by research fund of Chungnam National University, Daejeon, Republic of Korea. Research supported by the King Saud University, Deanship of Scientific Research, College of Science, Research Center.

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