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INCLUSION OF *KICKXIA ABHAICA* D.A. SUTTON IN THE GENUS *NANORRHINUM* (PLANTAGINACEAE): EVIDENCE FROM ITS NUCLEAR RIBOSOMAL DNA SEQUENCES

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Abstract

The nuclear ribosomal DNA (nrDNA) internal transcribed spacers (ITS) sequences is extensively used in the plant molecular phylogenetics for plant taxonomic identification and DNA barcoding purposes because the nrDNA ITS gene is easy to amplify by using the universal primers, its length is shorter and thus easy to sequence, and has strong discrimination power to distinguish the taxon at the species level. The present molecular phylogenetic analysis of ITS nrDNA sequences focuses to determine the taxonomic status of an unresolved endemic taxon *Kickxia abhaica* D.A. Sutton (Family Plantaginaceae, tribe Antirrhineae) reported from Saudi Arabia. The analysis supports the transfer of *K. abhaica* under the genus *Nanorrhinum.*

Introduction

The tribe Antirrhineae which comprises ca. 30 genera (Sutton, 1988) has undergone several taxonomic changes during last two decades. The genus *Kickxia* Dumort. (Family Plantaginaceae, tribe Antirrhineae) comprises ca. 25 accepted species (APG III, 2009). Based on the mode of dehiscence of capsule, the genus *Kickxia* has been divided into sections i.e. *Kickxia* sect. *Kickxia* and *Kickxia* sect. *Valvatae* (Sutton, 1988). The sections *Kickxia* sect. *Kickxia* and *Kickxia* sect. *Valvatae* were raised to the rank of subgenera (Smith, 1973). The species with valvate capsules were treated under *Pogonorrhinum* and *Nanorrhinum* (Betsche, 1984). Ghebrehiwet (2001) considered *Kickxia* and *Nanorrhinum* as two distinct genera on the basis of morphological analysis. The molecular phylogeny of mediterranean genera *Chaenorhinum*, *Kickxia* and *Nanorrhinum* based on nrDNA ITS and *rpl*32-*trn*L sequence data also supports the recognition of the clade comprising *Kickxia* sect. *Valvatae* as *Nanorrhinum*; as a result, new combinations i.e. *Nanorrhinum petranum* (Danin) Yousefi & Zarre, *Nanorrhinum judaicum* (Danin) Yousefi & Zarre and *Nanorrhinum scariosepalum* (Tackh. & Boulos) Yousefi & Zarre were established from *Kickxia petrana* Danin, *Kickxia judaica* Danin and *Kickxia scariosepala* Tackh. & Boulos, respectively (Yousefi *et al.*, 2016).

The genus *Kickxia* in Saudi Arabia is represented by nine species and one subspecies [*Kickxia abhaica* D.A. Sutton, *K. acerbiana* (Boiss.) Tackh. & Boulos, *K. aegyptiaca* (L.) Nab., *K. collenetteana* D.A. Sutton, *K. corallicola* D.A. Sutton, *K. elatine* subsp. *crinita* Greuter, *K. hastata* (R.Br. *ex* Benth.) Dandy, *K. petiolata* D.A. Sutton, *K. pseudoscoparia* V.W. Smith and *K. scalarum* D.A. Sutton] described under the family Scrophulariaceae (Chaudhary, 2001), out of which the taxonomic status of *K. abhaica* D.A. Sutton, *K. acerbiana* (Boiss.) Tackh. & Boulos and *K. hastata* (R.Br. *ex* Benth.) Dandy is still unresolved (http://www.theplantlist.org/), *K. abhaica* D.A. Sutton [Rev. Antirrhinea: 241 (1988). Plate Scroph. 17.] have been reported as

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endemic to Saudi Arabia (Chaudhary, 2001). The present study aims to resolve the taxonomic status of the *K. abhaica* based on the molecular phylogenetic analysis of ITS nrDNA sequences.

Materials and Methods

Collection of the leaf material of Kickxia abhaica:

The leaf material of *K. abhaica* was collected from the specimen [Dharb-Abha Road, 5-4-1982, S. Chaudhary 3907 (KSUH)] deposited at the Herbarium (Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia). A total of 16 species of *Kickxia* was employed in this study (Table 1). The taxonomic identification of the herbarium specimens were reconfirmed with the taxonomic description mentioned in recent Flora of the Kingdom of Saudi Arabia (Chaudhary, 2001).

Table 1. The GenBank accessions of the ingroup and outgroup taxon included in the molecular phylogenetic analysis of *Kickxia abhaica.*

No.	Taxon	GenBank Acc. No.
Ingroup		
1.	*Nanorrhinum cabulicum* (Benth.) Podlech & Iranshahr	KT031916
2.	*Kickxia sagittata* (Poir.) Rothm.	KT031902
3.	*K. scoparia* (Brouss. ex Spreng.) G.Kunkel & Sunding	KT031903
4.	*K. urbanii* (Pit.) K.Larsen	KT031915
5.	*K. scariosepala* Täckh. & Boulos	KT031911
6.	*K. macilenta* (Decne.) Danin	KT031908
7.	*K. petrana* Danin	KT031909
8.	*K. judaica* Danin	KT031907
9.	*Kickxia lanigera* (Desf.) Hand.-Mazz.	KX061033
10.	*K. spuria* (L.) Dumort.	KT031914
11.	*K. sieberi* (Rchb.) Dörfl. & Allan	KT031912
12.	*K. cirrhosa* (L.) Fritsch	KT031896
13.	*K. aegyptiaca* (L.) Nab.	KT031905
14.	*K. elatine* (L.) Dumort.	KT031898
15.	*K. commutate* (Bernh. ex Rchb.) Fritsch	KT031897
16.	*K. abhaica* D.A. Sutton **[= *Nanorrhinum abhaicum* (D.A. Sutton) Ajmal Ali *comb. nov.*]**	MH628533
Outgroup		
17.	*Anarrhinum bellidifolium* (L.) Willd.	AY878116

Extraction of genomic DNA, amplification and sequencing of nrDNA ITS gene:

The leaf material was crushed with liquid nitrogen using 'Qiagen Tissue Lyser' (# 85300). The robotic workstation 'QIAcube' (# 9001292) using 'DNeasy Plant Mini Kit' (# 69104) was used for automated purification of the total genomic DNA. The nuclear ribosomal DNA ITS sequences (ITS1-5.8S and ITS2) were amplified in the thermal cycler (Applied Biosystems Veriti) via polymerase chain reaction using the primers (White *et al*., 1990) [forward primer ITS1 (5' GTCCACTGAACCTTATCATTTAG3') and the reverse primer ITS4 (5'TCCTCCGCTTATT GATATGC3')] and PCR Mix (# K-2011, Bioneer, Daejeon, Republic of Korea). The DNA sequencing of the amplified product was performed using kit (# 4337455, BigDye Terminator

cycle sequencing kit, Perkin-Elmer, Applied Biosystems) in DNA Analyzer (Perkin- Elmer, Applied Biosystems, # ABI PRISM 3730XL).

Molecular phylogenetic analysis of the nrDNA ITS gene sequences:

The nrDNA ITS sequences of a total number of 16 species of *Kickxia s.s.* and *s.l.* and outgroup sequence (Table 1) were retrieved from NCBI GenBank. The ITS sequences of nrDNA of *Anarrhinum bellidifolium* (GenBank accession No. AY878116) was used as outgroup in the molecular phylogenetic analysis because the genus *Anarrhinum* shows close relationships to the genus *Kickxia* (Yousefi *et al*., 2016). The alignment software 'CLUSTAL X v.1.81' (Thompson *et al*., 1997) was used to align the FASTA format DNA sequences. The parsimony (maximum parsimony, MP) (Nei and Kumar, 2000; Eck and Dayhoff, 1996) analysis using bootstrap method (Felsenstein, 1985) and maximum likelihood (ML) analysis using maximum composite likelihood method (Tamura *et al*., 2004) were used to conduct the molecular phylogenetic analyses using the molecular phylogenetic analysis software MEGA X (Kumar *et al.*, 2018).

Results and Discussion

The aligned nrDNA ITS data (ITS1, 5.8S, and ITS2 region) matrix was 622 bp (base pair) long. The most parsimonious tree out of nine parsimonious trees (length = 84) showed consistency index (CI) 0.781 and retention index (RI) 0.932. The ITS region (ITS1-5.8S-ITS2) of *K. abhaica* possessed 613 bp [ITS1: 228 bp, GC content 69%; 5.8S: 164 bp, GC content 54%; ITS2: 221 bp, GC content 71%].

The present molecular phylogenetic analysis of nrDNA ITS sequences revealed that *Kickxia s.l.* is monophyletic and sister to *Kickxia s.s.* The maximum parsimony phylogenetic tree (Fig. 1) showed two main clades i.e. *Kickxia s.s.* clade (BS 96%) and *Nonorrhinum* clade (BS 100%). *K. abhaica* nested within the *Kickxia s.l./Nonorrhinum* clade (BS 90%). The *Kickxia s.l.* (*K. scoparia* - *K. urbani* - *K. sagittata*) clade forms a distinct group (BS 90%). The ML tree with the highest log likelihood (-1294.69) recovered phylogenetic tree topology similar to MPT (Fig 1).

The tribe Antirrhineae (under Scrophulariaceae *s.l.*), with c. 300 species distributed in c. 30 genera constitutes a major clades of Plantaginaceae (Albach *et al*., 2005). The member of the tribe Antirrhineae are characterized by their herbaceous habit; two-lipped tubular corolla, 3-lobed lower lip and 2-lobed upper lip, gibbose, sometimes spurred at the base; 5 epipetalous stamens out of which 2 or 4 fertile, 2-carpelled fruits, operculate / valvate capsules (Sutton, 1988), and unique antirrhinosides / iridoid glycosides (Beninger *et al*., 2008). The systematic position of both the tribe and genera of the tribe Antirrhineae has been much debated (Ghebrehiwet *et al*., 2000), and the generic limits is still unresolved especially in the case of the genera *Chaenorhinum*, *Kickxia* and *Nanorrhinum* (Ghebrehiwet *et al*., 2000; Albach *et al*., 2005).

The morphological characteristics of taxon at lower level vary under different geographical and environmental condition; hence, requires sufficient taxonomic expertise for taxon identification based on morphology. In contrast, the DNA sequences have least or hardly influence by the geographical or environmental condition, and even remain unchanged during the developmental stages; therefore, the DNA barcode sequence such as ITS, ycf5, *rbc*L, *mat*K, *rpo*C1, *psb*A-*trn*H, *ndh*F, *trn*L-F, and *rps*16 based species identification together with morphological features gaining wide acceptance recently (Marcon *et al*., 2005; Liu *et al*., 2011; Rai *et al*., 2012; Ali *et al*., 2014). The ML tree showed two main clades i.e. *Kickxia s.s.* clade (BS 99%) and *Nonorrhinum* clade (BS 100%). *K. abhaica* nested within the *Kickxia s.l./Nonorrhinum* Clade (BS 71%), the *Kickxia s.l.* (*K. sagittata- K. scoparia-K. urbani*) clade forms a distinct group (BS 90%). Previously, *K. scoparia*, *K. urbani* and *K. sagittata* were recognized as *Nanorrhinum*

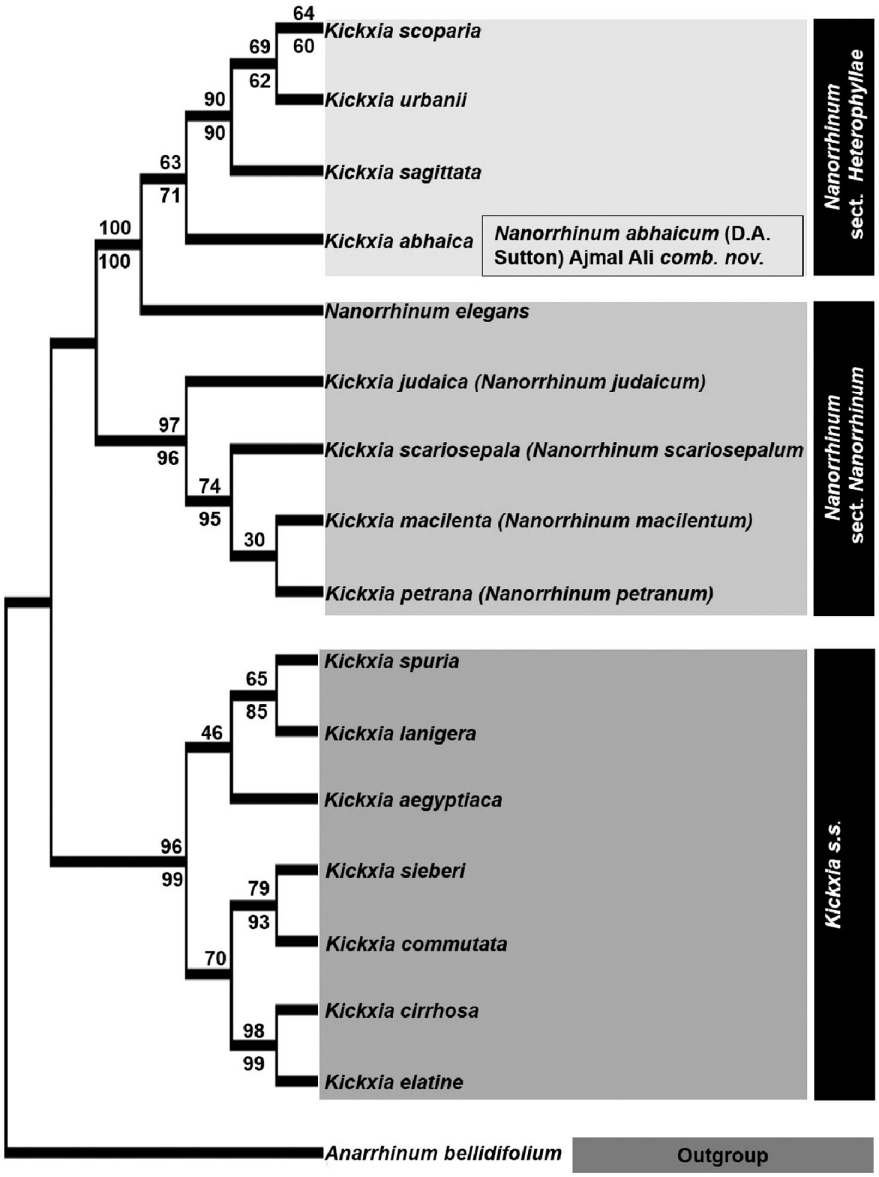

Fig. 1. The phylogenetic tree showing the systematic position of *Kickxia abhaica* [= *Nanorrhinum abhaicum* (D.A. Sutton) Ajmal Ali *comb. nov.*]. The phylogenetic analysis (1000 bootstrap replicates) was inferred using the Maximum Parsimony method. The numbers at the nodes are the bootstrap supports in MP (above) and ML (below) analysis.

(Smith, 1973) under the sect. *Heterophyllae* (Yousefi *et al*., 2016) or as *Pogonorrhinum* (Betsche, 1984). The taxonomic status of *K. collenetteana* (branches prostrate spreading, rigid, leafy; leaves all elliptic to oblong), *K. corallicola* (branches flexuous, tangled; petiole long, capillary, twining), *K. hastata* (annual delicate herb*), K. petiolata* (leaves homomorphic, without any basal lobe; petioles becoming thickened woody; spur coming out from corolla base) and *K. scalarum* (petioles

prominent capillary, often twining; corolla drying dark) reported from Saudi Arabia are unresolved, and its DNA sequence for any gene are not available in the GenBank. Therefore, the DNA sequencing of these taxon are required to know its taxonomic status within the tribe Antirrhineae. Moreover, the molecular phylogenetic analysis of nrDNA ITS sequence of *K. abhaica* [which was described as endemic to Saudi Arabia (Chaudhary, 2000)] supports its transfer to the genus *Nanorrhinum*, and thus the proposed new combinations in *Nanorrhinum* (new generic record for Saudi Arabia) is as follows.

New combination in *Nanorrhinum*

Nanorrhinum abhaicum (D.A. Sutton) Ajmal Ali, **comb. nov.**

Basionym: *Kickxia abhaica* D.A. Sutton [Rev. Antirrhinea: 241 (1988). Plate Scroph. 17.]

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References

Albach, D.C., Meudt, H.M. and Oxelman, B. 2005. Piecing together the 'new' Plantaginaceae. Am. J. Bot. **92**: 297–315.

Ali, M.A., Gábor, G., Norbert, H., Balázs, K., Al-Hemaid, F.M.A., Pandey, A.K. and Lee, J. 2014. The changing epitome of species identification - DNA barcoding. Saudi J. Biol. Sci. **21**(3): 204–231.

APG III (Angiosperm Phylogeny Group). 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. **161**: 105–121.

Beninger, C.W., Cloutier, R.R. and Grodzinski, B. 2008. The iridoid glucoside, antirrhinoside from *Antirrhinum majus* L. has differential effects on two generalist insect herbivores. J. Chem. Ecol. **34**: 591–600.

Betsche, L. 1984. Taxonomische Untersuchungen an *Kickxia* Dumortier (*s.l.*). Die neuen Gattungen *Pogonorrhinum* n. gen. und *Nanorrhinum* n. gen. Cour. Forsch. Inst. **71**: 125–142.

Chaudhary, S. (Ed.) 2001. Flora of the Kingdom of Saudi Arabia. Ministry of Agriculture and Water, National Herbarium, National Agriculture and Water Research Center, Riyadh, Saudi Arabia, Vol. **II**(2), pp. 435–439.

Eck, R.V. and Dayhoff, M.O. 1966. Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Silver Springs, Maryland, USA.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**: 783–791.

Ghebrehiwet, M. 2001. Taxonomy, phylogeny and biogeography of *Kickxia* and *Nanorrhinum* (Scrophulariaceae). Nordic J. Bot. **20**: 655–690.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. **35**: 1547–1549.

Liu, C., Liang, D., Gao, T., Pang, X., Song, J., Yao, H., Han, J., Liu, Z., Guan, X., Jiang, K., Li, H. and Chen, S. 2011. PTIGS-IdIt, a system for species identification by DNA sequences of the *psb*A-*trn*H intergenic spacer region. BMC Bioinformatics **12**: S4.

Marcon, A.B., Barros, I.C. and Guerra, M. 2005. Variation in chromosome numbers, CMA bands and 45S rDNA sites in species of *Selaginella* (Pteridophyta). Ann. Bot. **95**: 271–276.

Nei, M. and Kumar, S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Rai, P.S., Bellampalli, R., Dobriyal, R.M., Agarwal, A., Satyamoorthy, K. and Narayana, D.A. 2012. DNA barcoding of authentic and substitute samples of herb of the family Asparagaceae and Asclepiadaceae based on the ITS2 region. J. Ayurveda Integr. Med. **3**: 136–140.

Smith, V.A. 1973. A revision of the genus *Kickxia* with particular reference to the section *Heterophyllae* in the Canary Islands. M. Sc. thesis, University of Reading, UK.

Sutton, D.A. 1988. A revision of the tribe Antirrhineae. Oxford Univ. Press.

Tamura, K., Nei, M. and Kumar, S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Nat. Acad. Sci. (USA) **101**: 11030–11035.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24**: 4876–4882.

Yousefi, N., Zarre, S. and Heubl, G. 2016. Molecular phylogeny of the mainly Mediterranean genera *Chaenorhinum, Kickxia* and *Nanorrhinum* (Plantaginaceae, tribe Antirrhineae), with focus on taxa in the Flora Iranica region. Nordic J. Bot. **34**: 455–463.

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