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PHENETIC STUDY OF ENDEMIC AND WIDESPREAD *KNIPHOFIA* SPECIES IN ETHIOPIA USING *TRN*L (UAA) 3'-*TRN*F (GAA) INTERGENIC SPACER OF CHLOROPLAST DNA

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ABSTRACT: The phenetic relationship of four endemic and two widespread *Kniphofia* species in Ethiopia was studied using sequence data of *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer of chloroplast DNA. The species were *Kniphofia foliosa* Hochst., *K. hildebrandtii* Cufod., *K. insignis* Rendle, *K. isoetifolia* Hochst., *K. pumila* (Ait.) Kunth, and *K. schimperi* Baker. The length of *trn*L-*trn*F intergenic spacer sequences were from 355 base pairs in *K. foliosa* ("fol-10"), *K. isoetifolia*, and *K. hildebrandtii* to 360 base pairs in *K. foliosa* ("fol-13"), *K. insignis* and *K. schimperi*. The mean base frequency across the taxa was 0.33(A), 0.38(T), 0.14(G), and 0.15(C). The *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer sequences of the six species were remarkably similar except for five positions: one length mutation of 5 bp (indels) and four substitutions (transversions). The mutations were about 1% of the aligned sequences. The Tamura and Nei pair wise genetic distance calculated among the species was from 0.0 to 0.009 and with a mean of 0.005 $\pm$ 0.003. It indicated over 99% similarity and very low differentiation of the *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer of the chloroplast DNA among the species. The intergenic spacer appeared to be highly conserved in the endemic and widespread *Kniphofia* species in Ethiopia.

Key words/phrases: Chloroplast; Endemic; Ethiopia; *Kniphofia*; *trn*L- *trn*F

INTRODUCTION

The genus *Kniphofia* is useful in the field of horticulture. It is grown in home and botanical gardens and used as cut flower. The species of *Kniphofia* in cultivation are known by common names, such as red-hot pokers and torch-lights (Ramdhani, 2006). The secondary metabolites from *Kniphofia* species have medicinal use: knipholone and related natural phenylanthraquinones are considered to be a new group of potential antimalarial and anthraquinone aloe-emodin known to exhibit antileukemia properties (Ermias Dagne and Steglich, 1984; Esaiyas Berhanu *et al.*, 1986; Bringmann *et al.*, 1999). The roots of *Kniphofia foliosa* are used in traditional Ethiopian medicine for treatment of abdominal cramps. Furthermore, *Kniphofia* species naturally occurring in the wild are important

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honeybee-plants for pollen source and nectar (Fichtl and Admasu Adi, 1994).

The genus *Kniphofia* has five centers of endemism, one of which is Tropical East Africa and shows a strong affinity to Afromontane grassland (Ramdhani, 2006). Sebsebe Demissew and Nordal (1997) recognized seven species of the genus *Kniphofia* in the Flora of Ethiopia and Eritrea. They are *Kniphofia foliosa* Hochst., *K. hildebrandtii* Cufod., *K. insignis* Rendle, *K. isoetifolia* Hochst., *K. pumila* (Ait.) Kunth, *K. schimperi* Baker and *K. thomsonii* Baker. Of these, *K. foliosa*, *K. hildebrandtii*, *K. insignis* and *K. isoetifolia* are endemic to Ethiopia while *K. schimperi* occurs in land Eritrea. *Kniphofia pumila* and *K. thomsonii* are widely distributed from western Africa to eastern and central Africa (Marais, 1973).

The endemic *Kniphofia* species in Ethiopia are distributed between 6^0 00' N to 18^0 00' N and 33^0 00' E to 44^0 46' E that fall within the mountainous area of the country with a disjunct distribution. *K. foliosa* and *K. isoetifolia* are widespread whereas *K. insignis* and *K. hildebrandtii* are geographically restricted to the central highlands. Their habitat varies from montane grassland (characterized by *Olea europaea* subsp. *cuspidata*, *Juniperus procera*, *Celtis africana*, *Euphorbia ampliphylla*, *Carissa spinarum*, *Rosa abyssinica*, *Mimusops kummel* and *Ekebergia capensis*), to sub-alpine *Erica arborea* zone (characterized by *Erica arborea*, *Lobelia rhynchopetalum* and species of grass genera mainly of *Festuca*, *Poa* and *Agrostis*).

The genus *Kniphofia*, comprising 71 species, has a complex alpha taxonomy and species relationships are poorly understood (Ramdhani and Barker, 2009). The objective of this study was to investigate the phenetic relationship among *Kniphofia* species and between populations within each species using sequence data of *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer of chloroplast DNA. Previously, no such studies have been conducted although the basic taxonomy has been studied as part of the Ethiopian Flora Project. The results may assist in future molecular taxonomic study of the species.

MATERIALS AND METHODS

Biological material

The biological materials used in this study were fresh leaves from *Kniphofia foliosa* Hochst., *K. hildebrandtii* Cufod., *K. insignis* Rendle, *K. isoetifolia* Hochst., *K. pumila* (Ait.) Kunth. and *K. schimperi* Baker. The leaves were collected from plants grown in the greenhouse of the University of Oslo;

plants were grown from seeds of *Kniphofia* species collected from Ethiopia. The seeds were collected from each experimental site in Ethiopia from August to February in 1998 and 1999 (Table 1). The seeds of the individual mother plants were collected separately. Voucher specimens of each species are deposited in the National Herbarium (ETH) of Addis Ababa University and in the Biology Department of the University of Oslo.

Table 1. Experimental sites used for collection of seeds from the genus *Kniphofia* species in Ethiopia

Species	Site of seed collection		Population number
	Floristic Region	Locality	
K foliosa	Northern Shewa	Ali Doro	10
	Bale	Dinsho	13
K. hildebrandtii	Western Shewa	Gheddo	4
K. insignis	Northern Shewa	Torban Ashie	8
	North-Eastern Shewa	Bullo Workie	12
K. isoetifolia	Bale	Dinsho	1
	Arussi	Bekojji	2
K. schimperi	Western Shewa	Goro Wonchi	5
	Central Shewa	Entoto	14
K. pumila	Central Sidamo	Yirba Muda	6

Seed collection sites

A total of ten seed collection experimental sites were selected in Ethiopia based on the data obtained from the National Herbarium of Addis Ababa University (ETH), Royal Botanical Gardens; Kew (K), and the Natural History Museum of London (BM). This data set was augmented by observations made during time of seed collection. Two experimental sites were selected for each species, except for *K. hildebrandtii* and *K. pumila*, which was one each (Table 1).

Chloroplast DNA (cpDNA) sequencing

Total DNA was extracted from fresh leaves of six *Kniphofia* species using the modified CTAB (hexadecyltrimethlyammonium bromide) method of Dolye and Dolye (1990). Randomly selected five pot plants that were grown from seeds collected from ten experimental sites in Ethiopia were used for the extraction of the total DNA. Polymerase chain reaction (PCR) was conducted using standard PCR reaction mixture: 5 µl 20 mM $MgCl_2$, 2 µl 2 mM dNTPs (PH=7.0), 2.5 µl of the primers: "e"(5'-GGTTCAAGTCCCTCT

ATCCC-3') and "f"(5'-ATTGAACTGGTGACGCGAG-3') intergenic spacer (Taberlet *et al.*, 1991), 5 µl template DNA, 0.25 µl *Taq* DNA polymerase (5 U / µl) and 34.5 µl dH2O was added to make the final volume 50 µl into a 0.5 ml thin-walled microcentrifuge tube and covered with a hot lid. Then,

the sample was loaded to Genius Thermocycler (Techne Ltd, Cambridge). Five µl of PCR product was used to run 1% (0.5g/50 ml of TE) agarose gel electrophoresis with $EtBr_2$ and ϕX174/HaeIII molecular marker to check the PCR amplification products. The PCR products to be sequenced were purified with enzymes that are a combination of exonuclease I and shrimp alkaline phosphate (Amarsham, US 70995). The procedure followed was according to the manufacturer's manual (Amersham Life Science, Cleveland, Ohio). Then, about 200 ng of purified PCR product was mixed with 0.7 µl DMSO (reaction buffer), 1 µl 1pmol/µl of the fluorescent dye-labeled universal primer (Amersham Life Science, Cleveland, Ohio) and dH_2O was added to a final volume of 14 µl. The universal primer used for the sequencing was primer "f". The sequences were analyzed by loading 1.3 µl of the sequencing reaction into automatic sequencer, ABI PRISMTM310 Genetic Analyzer. The procedure followed was according to the manufacturer's manual (Perkin-Elimer, 1995).

Data analysis

The sequence data of the intergenic spacer of chloroplast DNA was used for the analysis of phenetic relationships among *Kniphofia* species. The multiple alignment of the six *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer sequences was carried out manually in SeqApp (Gilbert, 1992) and with CLUSTAL-W (Thompson *et al.*, 1994). The distance method of analysis was performed using PAUP 4.02 (Swofford, 1999) settings. All characters were treated as unordered characters with equal weight and gaps as missing data. The chloroplast DNA sequences were analyzed with DNA sequencing analysis software; Chromas vertion 1.43 (MaCarthy, 1997).

RESULTS

The length of *trn*L-*trn*F intergenic spacer sequences was from 355 base pairs in *K. foliosa* ("fol-10"), *K. isoetifolia* and *K. hildebrandtii* to 360 base pairs in *K. foliosa* ("fol-13"), *K. insignis* and *K. schimperi*. The mean base frequency across the taxa was 0.33(A), 0.38(T), 0.14(G), and 0.15(C) (Fig. 1).

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1          11         21         31         41        50
CATTTTTCAT AA-----GTA GTTTAAAGAA AATTCAATAT CTTTCTCATT   45   fol-10
CATTTTTCAT AACATAAGTA GTTTAAAGAA AATTCAATAT CTTTCTCATT   50   fol-13
CATTTTTCAT AA-----GTA GTTTAAAGAA AATTCAATAT CTTTCTCATT   45   hild-4
CATTTTTCAT AACATAAGTT GTTTAAAGAA AATTCAATTT CTTTCTCATT   50    ins-8
CATTTTTCAT AACATAAGTT GTTTAAAGAA AATTCAATTT CTTTCTCATT   50   ins-12
CATTTTTCAT AA-----GTA GTTTAAAGAA AATTCAATAT CTTTCTCATT   45    iso-1
CATTTTTCAT AA-----GTA GTTTAAAGAA AATTCAATAT CTTTCTCATT   45    iso-2
CATTTTTCAT AA-----GTA GTTTAAAGAA AATTCAATAT CTTTCTCATT   45 iso-1-16
CATTTTTCAT AACATAAGTA GTTTAAAGAA AATTCAATTT CTTTCTCATT   50    sch-5
CATTTTTCAT AACATAAGTA GTTTAAAGAA AATTCAATTT CTTTCTCATT   50   sch-14
CATTTTTCAT AA-----GGG GTTTAAAGAA AATTCAATAT CTTTCTCATT   45    pum-6

51         61         71         81         91       100
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT   95   fol-10
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT  100   fol-13
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT   95   hild-4
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT  100    ins-8
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT  100   ins-12
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT   95    iso-1
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT   95    iso-2
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT   95 iso-1-16
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT  100    sch-5
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT  100   sch-14
GATTCTACCC TTTCCCAAAC AAATAGGTCT GAACAGAAAT CTTTTTGTCT   95    pum-6

101        111        121        131        141       150
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  145   fol-10
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  150   fol-13
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  145   hild-4
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  150    ins-8
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  150   ins-12
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  145    iso-1
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  145    iso-2
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  145 iso-1-16
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  150    sch-5
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  150   sch-14
TATACCAAAT TTGGTTTGAA TAGATACGAT ACCTGTGCAT ATGAATATAT  145    pum-6

151        161        171        181        191       200
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  195   fol-10
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  200   fol-13
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  195   hild-4
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  200    ins-8
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  200   ins-12
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  195    iso-1
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  195    iso-2
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  195 iso-1-16
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  200    sch-5
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  200   sch-14
ATGGGCAAGG AATTTCCATT GTTGAATCAT TCACAGTCCA TATCATTCTT  195    pum-6

201        211        221        231        241       250
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  245   fol-10
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  250   fol-13
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  245   hild-4
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  250    ins-8
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  250   ins-12
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  245    iso-1
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  245    iso-2
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  245 iso-1-16
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TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  250    sch-5
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  250   sch-14
TTTCCGTTTA CAAATAAAAA GAAAGTCTTC TTTTTGAAGA TCTAAGAAAT  245    pum-6

251        261        271        281        291       300
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  295   fol-10
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  300   fol-13
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  295   hild-4
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  300    ins-8
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  300   ins-12
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  295    iso-1
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  295    iso-2
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  295 iso-1-16
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  300    sch-5
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  300   sch-14
TCATGGACTA GGTCAATTTT TTGAATACTT TAAATTAATA TTGAATTTCT  295    pum-6

301        311        321        331        341       350
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  345   fol-10
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  350   fol-13
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  345   hild-4
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  350    ins-8
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  350   ins-12
ATTAAATTTA TTGGTCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  345    iso-1
ATTAAATTTA TTGGTCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  345    iso-2
ATTAAATTTA TTGGTCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  345 iso-1-16
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  350    sch-5
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  350   sch-14
ATTAAATTTA TTGGGCTATT TAATTTACCA AGCACTCTAC TAGGATGGTG  345    pum-6

351
CGCGGGAAAT                                             355   fol-10
CGCGGGAAAT                                             360   fol-13
CGCGGGAAAT                                             355   hild-4
CGCGGGAAAT                                             360    ins-8
CGCGGGAAAT                                             360   ins-12
CGCGGGAAAT                                             355    iso-1
CGCGGGAAAT                                             355    iso-2
CGCGGGAAAT                                             355 iso-1-16
CGCGGGAAAT                                             360    sch-5
CGCGGGAAAT                                             360   sch-14
CGCGGGAAAT                                             355    pum-6
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Fig. 1. Sequence of the *trn*L (UAA)3'-*trn*F (GAA) intergenic spacer of the chloroplast DNA. The length mutation is underlined and substitutions (transversion) are bold-faced.

The *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer sequences of the six species were remarkably similar except for five positions: one length mutation of 5 bp (indels) and four substitutions (transversions). In the phenetic analysis, 355 of the 360 characters in the sequence data (aligned base sequences) were constant. The identified differentiation among the intergenic spacer was not sufficient to draw firm conclusion regarding the relationships of the *Kniphofia* species in Ethiopia. The mutations were about 1% of the aligned sequences (Table 2). Fig. 2 indicates the relation among the species.

Table 2. The rate and percentage of informative variation per aligned length (360 bp) of the *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer of chloroplast DNA.

*trn*L(UAA)-*trn*F(GAA) region	Informative variation	Rate	Percentage
Substitution	4	0.01	1 %
Length mutation (indels)	1	0.002	0.2 %

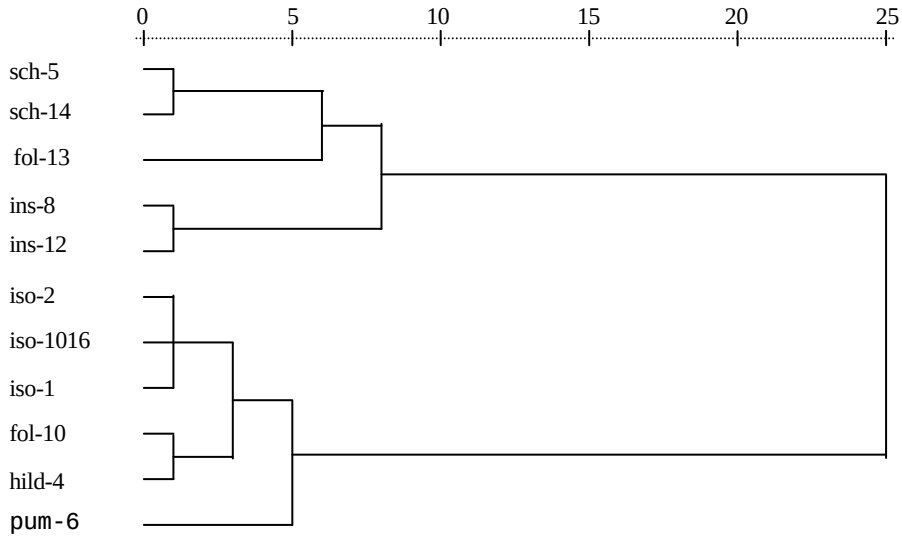

Fig. 2. Phenogram of the *Kniphofia* species using Average Linkage (Between Groups) based on the *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer sequences of chloroplast DNA.

The relation among the species was grouped into two clusters based on the absence and presence of the length mutation. *K. insignis* and *K. schimperi* made one cluster and, *K. hildebrandtii*, *K. isoetifolia* and *K. pumila* the other. The populations within a species were similar unlike the relation between *K. foliosa* from Ali Doro ("fol-13") and from Dinsho ("fol-10") that showed differentiation compared with the rest. The length mutation determined the clustering pattern (Fig. 2).

The Tamura and Nei (1993) pairwise genetic distance calculated among the species ranged from 0.0 to 0.009 with a mean of 0.005 $\pm$ 0.003 (Table 3) that indicated over 99% similarity and very low differentiation of the *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer of the chloroplast DNA among the species.

Table 3. Tamura and Nei (1993) pairwise comparison of the bases of the *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer of the chloroplast DNA of the *Kniphofia* species.

	fol-10	fol-13	hild-4	iso-1	iso-2	iso-1-16	ins-8	ins-12	sch-5	sch-14
fol-10	----									
fol-13	0.000	----								
hild-4	0.000	0.000	----							
iso-1	0.003	0.003	0.003	-----						
iso-2	0.003	0.003	0.003	0.000	----					
iso-1-16	0.003	0.003	0.003	0.000	0.000	----				
ins-8	0.006	0.006	0.006	0.008	0.008	0.008	----			
ins-12	0.006	0.006	0.006	0.008	0.008	0.008	0.000	----		
sch-5	0.003	0.003	0.003	0.006	0.006	0.006	0.003	0.003	----	
sch-14	0.003	0.003	0.003	0.006	0.006	0.006	0.003	0.003	0.000	----
pum-6	0.006	0.006	0.006	0.009	0.009	0.009	0.008	0.008	0.009	0.009

DISCUSSION

The *trn*L (UAA) 3'-*trn*F (GAA) intergenic spacer has been shown to evolve faster and possesses more length mutations than the *trn*L (UAA) 3' intron region of the chloroplast DNA (Taberlet *et al*., 1991; Gielly and Taberlet, 1994; Widmer and Baltisberger, 1999). However, in some studies, the conservative nature of the region in a closely related species or at the lower taxa level is an impediment to resolve phylogenetic relationships (Baker *et al*., 1999; McDade and Moody, 1999). In the endemic and indigenous *Kniphofia* species in Ethiopia, the intergenic spacer is highly conserved and the low distance value indicated the low differentiation found among the species (Ramdhani, 2006). The available sequence divergence among the taxa is less than what is described in Olmstead and Palmer (1994), which is between 5% and 10% and indicates an appropriate rate of divergence that can be used for relation analysis among species.

Despite the low sequence divergence rate of the *trn*L (UAA) 3'-*trn*F (GAA) of chloroplast DNA in the studied species, it signifies the conclusions drawn from the isoenzyme analysis (Tilahun Teklehaymanot *et al*., 2004). For example, *K. insignis* and *K. schimperi* come out more related than the rest in the isoenzyme cluster analysis. The Ethiopian *Kniphofia* species again are closely related at the *trn*L (UAA) 3'-*trn*F (GAA) of chloroplast DNA. This result showed the strange phenomenon of different species, in genetic respect, more behaving as conspecific populations indicating rather recent speciation (Mcclenaghan and Beauchmap, 1986; Ramdhani and Barker, 2009). This hypothesis needs, however, further testing. Fangan and Nordal (1993) experienced the same relation within the genus *Crinum*, i.e. distinct difference in flower morphology and very little genetic differentiation, again

explained by rapid evolution due to differential pollinator preferences (Hughes and Queller, 1993). However, the *Kniphofia* species are pollinated by the same pollinator, Tecezze sunbird (*Nectarina tecezze*) (Tilahun Teklehaymanot, 2001).

CONCLUSION

The results showed that all the studied Ethiopian *Kniphofia* species share a fairly recent common ancestor and radiation in Ethiopia, but have differentiated in floral and inflorescence character through rapid evolution. This hypothesis needs, however, further testing. Therefore, the phylogenetic relation of the species will be better explained by considering molecular markers that could provide a sufficient number of parsimony-informative characters with highly polymorphic nature, codominant inheritance, frequent occurrence in genome and high reproducibility, and also including more number of *Kniphofia* species from other parts of the country that are not considered in this study to draw a strong conclusion on the evolutionary history of the species and their species rank definitions.

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