



Correction

Correction: An Aqueous Extract of *Fagonia cretica* Induces DNA Damage, Cell Cycle Arrest and Apoptosis in Breast Cancer Cells via FOXO3a and p53 Expression

Matt Lam, Kirsten Wolff, Helen Griffiths, Amtul Carmichael

In our article entitled “An Aqueous Extract of *Fagonia cretica* Induces DNA Damage, Cell Cycle Arrest and Apoptosis in Breast Cancer Cells via FOXO3a and p53 Expression” (1), we described the effects of plant material from Pakistan that we referred to as *Fagonia cretica*. Linnaeus in Species Plantarum (2) described three species, namely, *cretica*, *arabica* and *hispanica*, while we now have over 160 names published in the genus (3, 4). The circumscription of species in *Fagonia* is reported to be complex due to variability of morphological characters (3, 4).

After the publication of our article it was raised to our attention that in light of the widespread distribution of *F. indica* and the absence from Pakistan of the more narrowly distributed species *F. cretica* the plant material may originate from *F. indica* and not *F. cretica* as indicated in the article. Some reports suggest that in Pakistan that the name *F. cretica* is used as name for what actually is *F. indica* var. *schweinfurthii* Hadidi (5). Recent studies on *Fagonia* elucidated relationships within the genus using morphological and molecular data (3,4). In light of this, we completed a series of DNA sequencing analyses on different batches of plant material used in our studies in order to establish if the material originated from *F. indica* or *F. cretica*. These additional analyses have confirmed that the material used in the study originated from *F. cf. indica*.

The methodology for the analyses was as follows: DNA was extracted from batches of plant material using a Qiagen DNeasy Plant minikit, following manufacturer’s instructions. The chloroplast trnLeu intragenic spacer was amplified using primers trnL-c and trnL-d (6), and the the rDNA Internal Transcribed Spacer region was amplified using primers ITS4 and ITS5 (7). Amplification, purification and sequencing were performed as described by Houston and Wolff (8). Sequencing was carried out with the Big Dye Terminator cycle sequencing kit v. 3.1 (Applied Biosystems) according to manufacturer’s instructions. Sequencing products were purified using a Genetix column purification and the sequences of these samples were visualised using an ABI 3100 automated sequencer (Applied Biosystems).

The resulting sequences, both trnL (439 bases) and ITS (489 bases), were blasted to sequences available on GenBank and showed the highest similarity with those of *F. indica* and of *F. paulayana*, while being clearly different from those for *F. cretica* (Table 1). The alignment shows that for trnL the query sample differed from *F. indica* only for the number of repeats in two A-repeats, and one nucleotide substitution with *F. paulayana*. The trnL sequence differed from the *F. cretica* sequences for 12 substitutions and 6 insertions/deletions (Table 2). The ITS query sample differed from *F. indica* and *F. paulayana* for 1 and 3 substitutions, respectively, while the difference with *F. cretica* was 36 substitutions and 1 insertion/deletion (Table 3). The alignment of ITS showed ambiguities at certain positions; this is most likely due to a well-known phenomenon of incomplete sequence conversion between the rDNA paralogs, causing intra-individual

sequence diversity. Beier et al. (4) describe that *F. indica* and *F. paulayana* occur sympatrically and cannot be distinguished on the basis of sequence data, but that a single morphological character (persistence of sepals) discriminates the two species.

We conclude that *F. indica* or its sister species *F. paulayana* is the genetic identity of the plant material being commonly referred to as *F. cretica* in Pakistan and which has cytotoxic activity towards breast cancer cells. We conclude that it is more likely to be *F. indica* than *F. paulayana* for three reasons. Firstly, our ITS sequence was more similar to that of *F. indica*. Secondly, the trnL sequence only had repeat number differences with *F. indica*, which is a more likely evolutionary step than the substitution that was found in the comparison with *F. paulayana*. Thirdly, the distribution of *F. indica* encompasses the region of origin of our samples. The article title, abstract and main text should therefore refer to *F. cf. indica* and not *F. cretica*. The active compound has now been isolated and is undergoing molecular characterization.

We are grateful to Dr. Schori, an expert plant systematist, who alerted us to the likely misidentification of the species.

Kirsten Wolff was responsible for the additional DNA analyses described in this Correction and she has been added as a co-author. The author list should therefore be revised to read as follows:

Matt Lam, Kirsten Wolff, Helen Griffiths, Amtul Carmichael.

Dr. Wolff is affiliated at School of Biology, Newcastle University and has no competing interests in relation to this work.

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Table 1. Sequence similarity of our query sample and most similar sequences available at Genbank for gene for trn-Leu and rDNA Internal Transcribed Spacer.

<i>trn Leu</i>			<i>Internal transcribed spacer rDNA</i>		
<i>Species</i>	<i>Similarity</i>	<i>Genbank nr</i>	<i>Species</i>	<i>Similarity</i>	<i>Genbank nr</i>
<i>F. indica</i>	99%	AY641593.1	<i>F. indica</i>	99%	AY641631.1
<i>F. indica</i>	99%	AY300769.1	<i>F. paulayana</i>	99%	AY641652.1
<i>F. indica</i>	99%	AY641592.1	<i>F. indica</i>	99%	AY641630.1
<i>F. paulayana</i>	99%	AY641607.1	<i>F. subinermis</i>	99%	AY641642.1
<i>F. paulayana</i>	98%	AY641606.1	<i>F. paulayana</i>	99%	AY641650.1
<i>F. subinermis</i>	98%	AY641610.1	<i>F. indica</i>	99%	AY641632.1
<i>F. mahrana</i>	96%	AY641600.1	<i>F. paulayana</i>	99%	AY641654.1
<i>F. lahovarii</i>	96%	AY641596.1	<i>F. mahrana</i>	98%	AY641639.1
<i>F. hadramautica</i>	95%	AY641590.1	<i>F. gypsophila</i>	98%	AY641627.1
<i>F. gypsophila</i>	95%	AY641589.1	<i>F. lahovarii</i>	97%	AY641635.1
<i>F. acerosa</i>	95%	AY641579.1	<i>F. latistipulata</i>	97%	AY641636.1
<i>F. indica</i>	95%	AY300770.1	<i>F. scabra</i>	97%	AY641645.1
<i>F. harpago</i>	95%	AY641591.1	<i>F. charoides</i>	97%	AY641621.1
<i>F. longispina</i>	94%	AY641599.1	<i>F. minutistipula</i>	96%	AY641641.1
<i>F. latistipulata</i>	94%	AY641598.1	<i>F. longispina</i>	96%	AY641637.1
<i>F. bruguieri</i>	94%	AY641582.1	<i>F. glutinosa</i>	96%	AY641626.1
<i>F. minutistipula</i>	93%	AY300771.1	<i>F. bruguieri</i>	96%	AY641619.1
<i>F. cretica</i>	92%	AJ387942.1	<i>F. olivieri</i>	96%	AY641646.1
<i>F. densa</i>	92%	AY641587.1	<i>F. mollis</i>	95%	AY641643.1
<i>F. laevis</i>	92%	AY641594.1	<i>F. bruguieri</i>	95%	AY641620.1
<i>F. villosa</i>	92%	AY641611.1	<i>F. rangei</i>	95%	AY641647.1
<i>F. mollis</i>	92%	AY641601.1	<i>F. harpago</i>	95%	AY641629.1
<i>F. laevis</i>	92%	AY641595.1	<i>F. acerosa</i>	95%	AY641617.1
<i>F. scabra</i>	92%	AY300768.1	<i>F. luntii</i>	95%	AY641638.1
<i>F. charoides</i>	91%	AY641583.1	<i>F. laevis</i>	95%	AY641634.1
<i>F. orientalis</i>	91%	AY641603.1	<i>F. laevis</i>	95%	AY641633.1
<i>F. rangei</i>	91%	AY641609.1	<i>F. palmeri</i>	95%	AY641653.1
<i>F. cretica</i>	91%	AY641585.1	<i>F. orientalis</i>	94%	AY641648.1
<i>F. arabica</i>	91%	AY641580.1	<i>F. boveana</i>	94%	KF850598.1
<i>sF. cretica</i>	91%	AY641586.1	<i>F. pachyacantha</i>	94%	AY641651.1
<i>F. pachyacantha</i>	90%	AY641604.1	<i>F. pachyacantha</i>	94%	AY641649.1
<i>F. pachyacantha</i>	90%	AY300772.1	<i>F. villosa</i>	94%	AY641640.1
<i>F. zilloides</i>	90%	AY641612.1	<i>F. hadramautica</i>	94%	AY641628.1
			<i>F. arabica</i>	93%	AY641618.1
			<i>F. chilensis</i>	93%	AY641622.1
			<i>F. densa</i>	93%	AY641625.1
			<i>F. cretica</i>	93%	AY641624.1
			<i>F. scoparia</i>	90%	AY641644.1
			<i>F. cretica</i>	90%	AY641623.1

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Table 2. Aligned sequences of the trn-Leu gene intron for our query sample and a selection of the most similar sequences and most distant sequences within the genus *Fagonia* available at Genbank.

	10	20	30	40	50	60
trnL intron query	GTGATCACTT	TCAAATTCAG	AGAAACCTCG	GAATTAGAAA	TGGGCAATCC	TGAGCCAAAT
AJ387943 F.indica	GTGATCACTT	TCAAATTCAG	AGAAACCTCG	GAATTATAAA	TGGGCAATCC	TGAGCCAAAT
AY641592 F.indica	-----CTT	TCNAATTCAG	AGAANCCTCG	GAATTAGAAA	TGGGCAATCC	TGAGCCAAAT
AY641608 F.paulayana	GTGATCACTT	TCAAATTCAG	AGAAACCTCG	GAATTAGAAA	TGGGCAATCC	TGAGCCAAAT
AY641610 F.subinermi	GTGATCACTT	TCAAATTCAG	AGAA-CCCTG	GAATTASAAA	TGGGCAATCC	TGAGCCAAAT
AY641600 F.mahrana	GTGATCACTT	TCAAATTCAG	AGAAACCTCG	GAATTAGAAA	TGGGCAATCC	TGAGCCAAAT
AY641596 F.lahovarii	--GATCACTT	TCAAATTCAG	AGAAACCTCG	GAATTAGAAA	TGGGCAATCC	TGAGCCAAAT
AJ387942 F.cretica	GTGATCACTT	TCAAATTCAG	AGAAACCTCG	GAATTAGAAA	TGGGCAATCC	TGAGCCAAAT
	70	80	90	100	110	120
trnL intron query	CCTGTTTTCC	TAAAAAAAAA	AAAA--GAAG	TCAATTA---	---TAAAATC	AAAAAAGTAA
AJ387943 F.indica	CCTGTTTTCC	TAAAAAAAAA	AAAA--GAAG	TCAATT----	---TAAAATC	AAAAAAGTAA
AY641592 F.indica	CCTGTTTTCC	TAAAAAAAAA	AAA---GAAG	TCAATTA---	---TAAAATC	AAAAAAGTAA
AY641608 F.paulayana	CCTGTTTTCC	TAAAAAAAAA	AAAA--GAAG	TCAATTA---	---TAAAATC	AAAAAAGTAA
AY641610 F.subinermi	CCTGTTTTCC	TAAAAAAAAA	A-----GAAG	TCAATTA---	---TAAANTC	AAAAAAGTAA
AY641600 F.mahrana	CCTGTTTTCC	TAAAAAAAAA	AAAAAGAAG	TCAATTA---	---TAAAATC	AAAAAAGTAA
AY641596 F.lahovarii	CCTGTTTTCC	TAAAAAAAAA	AAAA--GAAG	TCAATTAATA	GAATAAATC	AAAAAAGTAA
AJ387942 F.cretica	CCTGTTTTCC	TAAAAAAAAA	AAA---GAAG	TAAATCAAAA	GAATCAAATC	AAAAAAGTAA
	130	140	150	160	170	180
trnL intron query	AA---AAAA	GGATAGGTGC	AGAGACTCAA	TGGAAGCTGT	TCTAAAAAAT	GGAGTTGACT
AJ387943 F.indica	AA---AAAA-	GGATAGGTGC	AGAGACTCAA	TGGAAGCTGT	TCTAAAAAAT	GGAGTTGACT
AY641592 F.indica	AA---AAAA-	GGATAGGTGC	AGAGACTCAA	TGGAAGCTGT	TCTAAAAAAT	GGAGTTGACT
AY641608 F.paulayana	AA---AAAA-	GGATAGGTGC	AGAGACTCAA	TGGAAGCTGT	TCTAAAAAAT	GGAGTTGACT
AY641610 F.subinermi	AA---AAAA-	GGATAGGTGC	AGAGACTCAA	TGGAAGCTGT	TCTAAAAAAT	GGAGTTGACT
AY641600 F.mahrana	AA---AAAA-	GGATGGGTGC	AGAGACTCAG	TGGAAGCTGT	TCTAAAAAGT	GGAGTTGACT
AY641596 F.lahovarii	AA---AAAA-	GGATAGGTGC	AGAGACTCAA	TGGAAGCTGT	TCTAAAAAAT	GGAGTTGACT
AJ387942 F.cretica	AATAAAAAA	GGATAGGTGC	AGAGACTCAA	CGGAAGCTGT	TCTAAAAAAT	GGAGTTGACT
	190	200	210	220	230	240
trnL intron query	ACTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	A-----GG
AJ387943 F.indica	ACTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	AA-----GG
AY641592 F.indica	ACTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	A-----GG
AY641608 F.paulayana	ACTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	A-----GG
AY641610 F.subinermi	ACTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	A-----GG
AY641600 F.mahrana	ACTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	A-----GG
AY641596 F.lahovarii	ACTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	A-----GG
AJ387942 F.cretica	GCTGCATTAC	GTTAGTCAAG	TCAAGGAATC	CTTGCATCGA	AACTTTTTCA	AAGATAAAGG
	250	260	270	280	290	300
trnL intron query	ATAACCTTTC	TTTCTATCAA	ACTCTAAAAA	TAAAAAATAT	AAAGACTTTT	TAAATTAATT
AJ387943 F.indica	ATAACCTTTC	TTTCTATCAA	ACTCTAAAAA	TAAAAAATAT	AAAGACTTTT	TAAATTAATT
AY641592 F.indica	ATAACCTTTC	TTTCTATCAA	ACTCTAAAAA	TAAAAAATAT	AAAGACTTTT	TAAATTAATT
AY641608 F.paulayana	ATAACCTTTC	TTTCTATCAA	ACTCTAAAAA	TAAAAAATAT	AAAGACTTTT	TGAATTAATT
AY641610 F.subinermi	ATAACCTTTC	TTTCTATCAA	ACTCTAAAAA	TAAAAAATAT	AAAGACTTTT	TCAATTAATT
AY641600 F.mahrana	ATAACCTTTC	TTTCTATCAA	ACTATAAAAA	TAAAAAATAT	AAAGACTTTT	T-----
AY641596 F.lahovarii	ATAACCTTTC	TTTCTATCAA	ACTCTAAAAA	TAAAAAATAT	AAAGACTTTT	T-----
AJ387942 F.cretica	ATAACCTTTT	TTTCTATCAA	ACTCTAAAAA	TAAAAAAGAT	AAAGACTTTT	-----
	310	320	330	340	350	360
trnL intron query	GGAATTAATT	GGAAGTTGAA	GAAAGAATCA	AATATGATTT	TATCAAATCA	TTTACTCCAA
AJ387943 F.indica	GGAATTAATT	GGAAGTTGAA	GAAAGAATCA	AATATGATTT	TATCAAATCA	TTTACTCCAA

Table 2. Cont.

	310	320	330	340	350	360
AY641592 F.indica	GGAATTAATT	GGAAGTTGAA	GAAAGAATCA	AATATGATT	TATCAAATCA	TTTACTCCAA
AY641608 F.paulayana	GGAATTAATN	GGAAGTTGAA	GAAAGAATCA	AATATGATT	TATCAAATCA	TTTACTCCAA
AY641610 F.subinermi	GGAATTAATT	GGAAGTTGAA	GAAAGAATCA	AATATGATT	TATCAAATCA	TTTACTCCAA
AY641600 F.mahrana	- GAATTAATT	GGAAGTTGAA	GAAAGAATCA	AATATGATT	TATCAAATCA	TTTACTCCAA
AY641596 F.lahovarii	- GAATTAATT	GGAAGTTGAA	GAAAGAATCA	AATATGATT	TATCAAATCA	TTTACTCCAA
AJ387942 F.cretica	GGAATCAATT	GGAAGTTTAA	GAAAGAATCA	AATATGATT	TATCAAATCA	TTTACTCCAA
	370	380	390	400	410	420
trnL intron query	AGTCTTATAT	ATCTTTTTCA	AAAAA-GATT	AGTCAGACGA	GAATAAAGAT	AGAGTCCCAT
AJ387943 F.indica	AGTCTTATAT	ATCTTTTTCA	AAAAA-GATT	AGTCAGACGA	GAATAAAGAT	AGAGTCCCAT
AY641592 F.indica	AGTCTTATAT	ATCTTTTTCA	AAAAA-GATT	AGTCAGACGA	GAATAAAGAT	AGAGTCCCAT
AY641608 F.paulayana	AGTCTTATAT	ATCTTTTTCA	AAAAA-GATT	AGTCAGACGA	GAATAAAGAT	AGAGTCCCAT
AY641610 F.subinermi	AGTCTTATAT	ATCTTTTTCA	AAAAA-GATT	AGTCAGACGA	GAATAAAGAT	AGAGTCCCAT
AY641600 F.mahrana	AGTCTTATAT	ATCTTTTTCA	AAAAA-GATT	AGTCAGACGA	GAATAAAGAT	AGAGTCCCAT
AY641596 F.lahovarii	AGTCTTATAT	ATCTTTTTCA	AAAAA-GATT	AGTCAGACGA	GAATAAAGAT	AGAGTCCCAT
AJ387942 F.cretica	AGTCTGATAT	ATCTTTTTCA	AAAAAAGATT	AGTCAGGCGA	GAATAAAGAT	AGAGTCCCAT
	430	440	450	460	470	480
trnL intron query	TCTATATGTC	AATATTGACA	ACAATGAAAT	TTATAGTGAG	AGGAAAATCC	GTCGATTTTA
AJ387943 F.indica	TCTATATGTC	AATATTGACA	ACAATGAAAT	TTATAGTGAG	AGGAAAATCC	GTCGATTTTA
AY641592 F.indica	TCTATATGTC	AATATTGAC-	-----	-----	-----	-----
AY641608 F.paulayana	TCTATATGTC	AATATTGAC-	-----	-----	-----	-----
AY641610 F.subinermi	TCTATATGTC	AATATTGAC-	-----	-----	-----	-----
AY641600 F.mahrana	TCTATATGTC	AATATTGAC-	-----	-----	-----	-----
AY641596 F.lahovarii	TCTATATGTC	AATATTGAC-	-----	-----	-----	-----
AJ387942 F.cretica	TCTATATGTC	AATATTGACA	ACAATGAAAT	TTATAGTGAG	AGGAAAATCC	GTCGATTTTA
	490					
trnL intronquery	TCAATCGTGA	GGGT				
AJ387943 F.indica	TCAATCGTGA	GGG-				
AY641592 F.indica	-----	----				
AY641608 F.paulayana	-----	----				
AY641610 F.subinermi	-----	----				
AY641600 F.mahrana	-----	----				
AY641596 F.lahovarii	-----	----				
AJ387942 F.cretica	TCAATGTGGA	GGGT				

doi:10.1371/journal.pone.0040152.t002

Table 3. Aligned sequences of the rDNA Internal Transcribed Spacer for our query sample and a selection of the most similar sequences and most distant sequences within the genus *Fagonia* available at Genbank.

	10	20	30	40	50	60
query ITS	AGAGCATACC	CCTTCTCGA	GTGTCGGGAG	GGAGACTTCC	TGACATTATA	ACGAACCCCG
AY641631 F.indica	AGAGCATACC	CCTTCTCGA	GTGTCGGGAG	GGAGACTTCC	TGACATTATA	ACGAACCCCG
AY641630 F.indica	AGAGCATACC	CCTTCTNGA	GTGTCGGGAG	GGAGACTTCC	NGACATTATA	ACGAACCCCG
AY641632 F.indica	AGAGCATACC	CCTTCTCGA	GTGTCGGGAG	GGAGACTTCN	TGACATTATA	ACGAACCCCG
AY641652 F.paulayana	AGAGCATACC	CCTTCTCGA	GTGTCGGGAG	GGAGACTTCC	TGACATTATA	ACGAACCCCG
AY641650 F.paulayana	AGAGCATACC	CCTTCTCGA	GTGTCGGGAG	GGAGACTTCC	TGACATTATA	ACGAACCCCG
AY641654 F.paulayana	AGAGCATACC	CCTTCTCGA	GTGTCNGGAG	GGAGACTTCN	TGACATTATA	ACGAACCCCG
AY641642 F.subinermi	AGAGCATACC	CCTTCTCGA	GTGTCAGGAG	GGAAACTTCC	TGACATTATA	ACGAACCCCG
AY641639 F.mahrana	AGAGCATGCC	CCTTCTCGA	GTGTCGGGAG	GGAGACTTCN	TGACATCATA	ACGAACCCCG
AY641627 F.gypsophil	AGAGCATACC	CCTTCTCGA	GTGTCAGGAG	GGAGACTTCC	TGACATTATA	ACGAACCCCG
AY641635 F.lahovarii	--AGCATGCC	CCTTCTCGA	GTGTTGGGAG	GGAGACTTCC	CGACATCATA	ACGAACCCCG
AY641636 F.latistipu	AGAGCATGCC	CCTTCTCGA	GTGTTGGGAG	GGAGACTTCC	CGACATCATA	ACGAACCCCG
AY641641 F.minutisti	AGAGCATGCC	CCTTCTCGA	GTGTCGGGAG	GGAGACTTCC	CGACATCATA	ACGAACCCCG
AY641624 F.cretica	AGAGCATGCC	CCTTCATCGA	GTGTTGAGAG	GGAGACTTCT	CGACATCATA	ACGAACCCCG
AY641623 F.cretica	AGAGCATGCC	CCTTCATCGA	GTGTTGAGAG	GGAGACTTCT	CGACATCATA	ACGAACCCCG
	70	80	90	100	110	120
query ITS	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	AAA-GGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641631 F.indica	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	AAA-GGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641630 F.indica	GCNTAAAAA	CGCCANGGAA	ANCAACAAA	AAA-GGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641632 F.indica	GCGTAAAAAM	CGCCAAGGAA	AMCAAMCAAA	AAA-GGARAC	TGCGTTCGCG	TGGCCTCCTT
AY641652 F.paulayana	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	AANAGGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641650 F.paulayana	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	TAA-GGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641654 F.paulayana	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	TAN-GGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641642 F.subinermi	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	AAA-GGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641639 F.mahrana	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	CAA-GGAGAT	TGCGTTCGCG	TRGCCTCCTT
AY641627 F.gypsophil	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	CAA-GGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641635 F.lahovarii	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	CAA-GGAGAT	TGCGTTCGCG	TGGCCTCCTT
AY641636 F.latistipu	GCGTAAAAA	CGCCAAGGAA	AACAACAAA	TAA-GGAGAT	TGCGTTCGCG	TGACCTCCTT
AY641641 F.minutisti	GCGTTAAAAG	CGCCAAGGAA	AACAACAAA	ACAAGGAGAC	TGCGTTCGCG	TGGCCTCCTT
AY641624 F.cretica	GCGTAAAAAG	CGCCAAGGAA	AACAACAAA	ACAAGGAGAT	TGCATTGCGT	TGGCCTCCTT
AY641623 F.cretica	GCGTAAAAAG	CGCCAAGGAA	AACAACAAA	ACAAGGAGAT	TACATTGCGT	TGGCCTCCTT
	130	140	150	160	170	180
query ITS	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641631 F.indica	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641630 F.indica	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641632 F.indica	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641652 F.paulayana	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641650 F.paulayana	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641654 F.paulayana	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641642 F.subinermi	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641639 F.mahrana	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641627 F.gypsophil	TGTATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641635 F.lahovarii	TGCATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641636 F.latistipu	TGCATTGAAT	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641641 F.minutisti	TGCATTGAGT	CAAAACGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641624 F.cretica	TGCATTGAAT	CAAAACGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATGAAGAAC
AY641623 F.cretica	TGCATTGAAT	CAAAACGACT	CTCGGCAACG	GATATCTCGG	CTCTGCATC	GATG-----

Table 3. Cont.

	190	200	210	220	230	240
query ITS	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641631 <i>F.indica</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641630 <i>F.indica</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641632 <i>F.indica</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641652 <i>F.paulayana</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641650 <i>F.paulayana</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAN	NGAACCATCG	AGTCTTTGAA
AY641654 <i>F.paulayana</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641642 <i>F.subinermi</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641639 <i>F.mahrana</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	YGAACCATCG	AGTCTTTGAA
AY641627 <i>F.gypsophil</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	CGAACCATCG	AGTCTTTGAA
AY641635 <i>F.lahovarii</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641636 <i>F.latistipu</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	TGAACCATCG	AGTCTTTGAA
AY641641 <i>F.minutisti</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	CGAACCATCG	AGTCTTTGAA
AY641624 <i>F.cretica</i>	GTAGCGAAAT	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	CGAACCATCG	AGTCTTTGAA
AY641623 <i>F.cretica</i>	-----T	GCGATACTTG	GTGTGAATTG	CAGAATCCAG	CGAACCATCG	AGTCTTTGAA
	250	260	270	280	290	300
query ITS	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641631 <i>F.indica</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641630 <i>F.indica</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641632 <i>F.indica</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641652 <i>F.paulayana</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641650 <i>F.paulayana</i>	CGCAAGTTGC	GCCCCAAGCN	TTCGGGCTAA	GGGCACGTCT	GCNTGGGTGT	CACGCAATGT
AY641654 <i>F.paulayana</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641642 <i>F.subinermi</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641639 <i>F.mahrana</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641627 <i>F.gypsophil</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641635 <i>F.lahovarii</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641636 <i>F.latistipu</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641641 <i>F.minutisti</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGCTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAACGT
AY641624 <i>F.cretica</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGTTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
AY641623 <i>F.cretica</i>	CGCAAGTTGC	GCCCCAAGCC	TTCGGGTTAA	GGGCACGTCT	GCCTGGGTGT	CACGCAATGT
	310	320	330	340	350	360
query ITS	CGCTCTCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641631 <i>F.indica</i>	CGCTCTCCAC	TNCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641630 <i>F.indica</i>	CGCTCTCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641632 <i>F.indica</i>	CGCTCTCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641652 <i>F.paulayana</i>	CGCTCTCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641650 <i>F.paulayana</i>	CGCTCTCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641654 <i>F.paulayana</i>	CGCTCTCCAC	TNCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641642 <i>F.subinermi</i>	CGCTCTCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTTCTTTGTC
AY641639 <i>F.mahrana</i>	CGCTCTCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTATCTTTGC
AY641627 <i>F.gypsophil</i>	CGCTCCCCAC	TCCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTATCTTTGC
AY641635 <i>F.lahovarii</i>	CGCTCCCCAC	TCTATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTATCTNTGC
AY641636 <i>F.latistipu</i>	CGCTCCCCAC	TCTATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGGAG	CAATCTTTGC
AY641641 <i>F.minutisti</i>	CGCTCCCCAT	TTCATATTGA	TTGGATGAGC	GTATGATGGT	CTCCCGTGAG	CTATATTTGC
AY641624 <i>F.cretica</i>	CGCTCCCCAC	TCCAAATTGA	TTGGGTGAGT	GTATGATGGT	CTCCCGTGAG	CTATATTTGC

Table 3. Cont.

	310	320	330	340	350	360
AY641623 F.cretica	CGCTCCCAC	TCCAATTGA	TTGGGTGAGT	GTATGATGGT	CTCCCGTGAG	CTATATTTGC
	370	380	390	400	410	420
query ITS	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGY	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641631 F.indica	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGN	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641630 F.indica	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGT	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641632 F.indica	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGT	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641652 F.paulayana	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGT	GTGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641650 F.paulayana	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGN	GCGAATTGTG	CCACGGTCTT	AGGTGGTNGA
AY641654 F.paulayana	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGN	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641642 F.subinermi	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGT	GCAAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641639 F.mahrana	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGC	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641627 F.gypsophil	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGC	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641635 F.lahovarii	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGT	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641636 F.latistipu	TTGTGGTTGG	CCTAAACATG	AGTCCTCGGT	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641641 F.minutisti	TTGTGGTTGG	CCTAAACACG	AGTCCTCGGC	GCGAATTGTG	CCACGGTCTT	AGGTGGTCGA
AY641624 F.cretica	TTGTGGTTGG	CCTAAACACG	AGTCCTCGGC	ACGGATTGTG	TCACGGTCTT	AGGTGGTCGA
AY641623 F.cretica	TTGTGGTTGG	CCTAAACACG	AGTCCTCGGC	ACGGATTGTG	TCACGGTCTT	AGGTGGTCGA
	430	440	450	460	470	480
query ITS	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641631 F.indica	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641630 F.indica	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641632 F.indica	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641652 F.paulayana	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641650 F.paulayana	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641654 F.paulayana	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAAAG	GAACCTGCAA	CCCTAGAGCA
AY641642 F.subinermi	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	AAACTTGCAA	CCCTAGAGCA
AY641639 F.mahrana	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641627 F.gypsophil	AAGCCTGAAG	GATCGTGTGC	CCATTTCA-G	CCATAGAGAG	GAACCTGCAA	CCCTAGAGCA
AY641635 F.lahovarii	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	TCATATAGAG	GAACCTGCAA	CCCTAGAGCA
AY641636 F.latistipu	AACCCTGAAG	GATCGTGTGC	CCATTTTA-G	TCATAGAGAG	GAACCTGTAA	CCCTAGAGCA
AY641641 F.minutisti	AACCCTGAAG	GATCGTGTGT	CCATTTTA-G	CCATAGAGAG	GAATTTGCAA	CCCTAGAGCA
AY641624 F.cretica	AACCCTGAAA	GATCGTGTGC	TCATTTCTTG	CCATAGTTAG	GAACCTGCAA	CCCTAAAGCA
AY641623 F.cretica	AACCCTGAAA	GATCGTGTGC	TCATTTCT-G	CCATAGCTAG	GAACCTGCAA	CCCTAAAGCA
query ITS	TGTCGATCT					
AY641631 F.indica	TGTCGATCT					
AY641630 F.indica	TGTCGATCT					
AY641632 F.indica	TGTCGATCT					
AY641652 F.paulayana	TGTNGATCT					
AY641650 F.paulayana	TGTCGATCT					
AY641654 F.paulayana	TGTCGATCT					
AY641642 F.subinermi	TGTCGATCT					
AY641639 F.mahrana	TGTCGATCT					
AY641627 F.gypsophil	TGTCAATCT					
AY641635 F.lahovarii	TGTCGATCT					
AY641636 F.latistipu	TGTCGATCT					
AY641641 F.minutisti	TGTCGAT--					
AY641624 F.cretica	TGTCGATCT					
AY641623 F.cretica	TGTCGATCT					

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References

1. Lam M, Carmichael AR, Griffiths HR (2012) An Aqueous Extract of *Fagonia cretica* Induces DNA Damage, Cell Cycle Arrest and Apoptosis in Breast Cancer Cells via FOXO3a and p53 Expression. PLoS ONE 7(6): e40152. doi:10.1371/journal.pone.0040152
2. Muller-Wille S, Reeds K (2007) A translation of Carl Linnaeus's introduction to *Genera plantarum* (1737). *Studies in history and philosophy of biological and biomedical sciences* 38:563-72
3. Beier BA (2005) A revision of the desert shrub *Fagonia* (Zygophyllaceae). *Systematics and Biodiversity* 3:221-63
4. Beier BA, Nylander JA, Chase MW, Thulin M (2004) Phylogenetic relationships and biogeography of the desert plant genus *Fagonia* (Zygophyllaceae), inferred by parsimony and Bayesian model averaging. *Molecular phylogenetics and evolution* 33:91-108
5. Nasit E, Ali SI. Eds. *Flora of West Pakistan* <http://www.tropicos.org/Name/34600187?projectid=32>
6. Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17:1105-1109
7. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T., (Eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego.
8. Houston K, Wolff K (2012) *Rhinanthus* minor population genetic structure and subspecies: Potential seed sources of a keystone species in grassland restoration projects. *Perspectives in Plant Ecology, Evolution and Systematics* 14:423-433