

PHYLOGENETIC ANALYSIS OF CYTOCHROME OXIDASE SUBUNIT I (COI) GENE SEQUENCES OF MITOCHONDRIAL DNA OF *PARNASSIUS* SPP. (LEPIDOPTERA; PAPILIONIDAE; PARNASSIINAE)

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ABSTRACT

Cytochrome oxidase subunit I (COI) gene sequences of mitochondrial DNA of different species of the genus *Parnassius* downloaded from NCBI (www.ncbi.nlm.nih.gov) were aligned and analyzed to determine the diversity in respect to the percentage of Adenine, Thymine, Guanine and Cytosine, AT content, G + C content, basic melting temperature, relative melting temperature. Mean values of AT content, GC content and Relative melting temperature were 71.7713, 28.2564 and 0.363666 respectively showing a linear regression between GC content and the mean value of Relative melting temperature.

INTRODUCTION

The species under the genus *Parnassius* are basically white swallowtail butterflies (Lepidoptera, Papilionidae, Parnassiinae) having transparent wings, lightly scaled with black, red and blue spots, varying in colour and form depending on the altitude. They have been adapted to high altitudes showing darkened colouration at the wing base (altitudinal melanism). Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae) has been studied (Keyghobadi *et al.*, 1999). Although they are within swallowtail group of butterflies, they do not possess any tail like projections at their hind wings. Defensive organ, osmeterium is present in all larval forms as found in other papilionids (Katoh *et al.*, 2005). Preferred larval food plants belong to the families of stonecrop (Crassulaceae), Poppy (Papaveraceae), Saxifrage (Saxifragaceae) and Caltrop (Zygophyllaceae) (Kehimkar, 2008). The exact number of species within the genus is disputed and numbers range from 38 to 47 (Nazari, 2006). It has been reported that the genus may consist of about fifty species and upto ten species groups or subgenera have been proposed on the basis of morphological and behavioral studies (Weiss, 1992). The *Parnassius* species of butterflies are often hard to identify and the literature regarding phylogeny is scanty. Present work is an attempt to study the nucleotide diversity and molecular characters of *Parnassius* species of butterflies on the basis of cytochrome oxidase subunit I (COI) gene sequences of mitochondrial DNA of different species of *Parnassius* downloaded from NCBI (www.ncbi.nlm.nih.gov).

MATERIALS AND METHODS

Cytochrome oxidase subunit I (COI) gene sequences of mitochondrial DNA of different species of *Parnassius* were downloaded from NCBI (www.ncbi.nlm.nih.gov). The sequence data were aligned using the 'ClustalW Submission Form' (<http://www.ebi.ac.uk/clustalw/>) and analyzed by ClustalW (Thompson *et al.*, 1994). Sequences were analyzed to determine the diversity in respect to the percentage of Adenine, Thymine, Guanine and Cytosine, AT content, G + C content, basic melting temperature, relative melting temperature of Cytochrome oxidase subunit I (COI) gene sequences of mitochondrial DNA of different species of *Parnassius*. Evolutionary distances were calculated using the method of Jukes and Cantor (Jukes and Cantor, 1969) and a Phylogenetic tree was prepared by 'neighbor-joining' method (Saitou and Nei, 1987; Tamura *et al.*, 2007). Relative melting temperature based on nearest-neighbour thermodynamics (Santalucia, 1998) by $T_m = \text{SumDeltaH}/\text{SumDeltaS}$, where SumDeltaH and SumDeltaS are the summation of the dinucleotide enthalpy and entropy, respectively. T_m is relative because we do not have information on the concentration of the hybridizing strand.

RESULTS AND DISCUSSION

Different species of genus *Parnassius* and their GenBank Entry numbers of the Cytochrome oxidase subunit I (COI) gene sequences of mtDNA have been shown in Table 1. Nucleotide base composition (Mole %) of the sequences of different

Table 1: Different species of *Parnassius* and their GenBank Entry

<i>Parnassius behrii</i>	gi 256010658 gb FJ756838.1
<i>Parnassius behrii</i>	gi 256010656 gb FJ756837.1
<i>Parnassius behrii</i>	gi 256010654 gb FJ756836.1
<i>Parnassius behrii</i>	gi 256010652 gb FJ756835.1
<i>Parnassius behrii</i>	gi 256010650 gb FJ756834.1
<i>Parnassius behrii</i>	gi 256010648 gb FJ756833.1
<i>Parnassius behrii</i>	gi 256010646 gb FJ756832.1
<i>Parnassius smintheus</i>	gi 256010644 gb FJ756831.1
<i>Parnassius smintheus</i>	gi 256010642 gb FJ756830.1
<i>Parnassius smintheus</i>	gi 256010640 gb FJ756829.1
<i>Parnassius smintheus</i>	gi 256010638 gb FJ756828.1
<i>Parnassius smintheus</i>	gi 256010636 gb FJ756827.1
<i>Parnassius smintheus</i>	gi 256010634 gb FJ756826.1
<i>Parnassius smintheus</i>	gi 256010632 gb FJ756825.1
<i>Parnassius smintheus</i>	gi 256010630 gb FJ756824.1
<i>Parnassius smintheus</i>	gi 256010628 gb FJ756823.1
<i>Parnassius smintheus</i>	gi 256010626 gb FJ756822.1
<i>Parnassius smintheus</i>	gi 256010624 gb FJ756821.1
<i>Parnassius smintheus</i>	gi 256010620 gb FJ756819.1
<i>Parnassius phoebus</i>	gi 256010618 gb FJ756818.1
<i>Parnassius phoebus</i>	gi 256010616 gb FJ756817.1
<i>Parnassius clodius</i>	gi 256010614 gb FJ756816.1
<i>Parnassius tianschanicus</i>	gi 227436763 gb FJ663915.1
<i>Parnassius stubbendorffii</i>	gi 227436761 gb FJ663914.1
<i>Parnassius stubbendorffii</i>	gi 227436759 gb FJ663913.1
<i>Parnassius stubbendorffii</i>	gi 227436757 gb FJ663912.1
<i>Parnassius staudingeri jacobsoni</i>	gi 227436755 gb FJ663911.1
<i>Parnassius staudingeri infernalis</i>	gi 227436753 gb FJ663910.1
<i>Parnassius staudingeri illustris</i>	gi 227436749 gb FJ663908.1

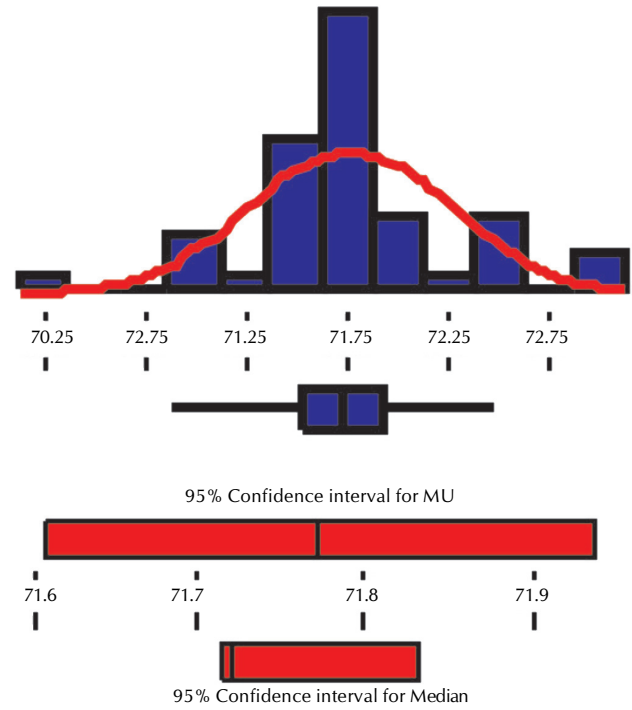


Figure 1: Graphical representation of histogram of AT content

Table 2: Nucleotide base composition (Mole %) of the Cytochrome oxidase subunit I (COI) gene sequences of mitochondrial DNA of different species of *Parnassius*

Species (Gen Bank Entry)	Adenine(Mole %)	Thymine(Mole %)	Guanine(Mole %)	Cytosine(Mole %)
gi 256010676 gb FJ756847.1	276	391	131	132
gi 227436749 gb FJ663908.1	277	390	130	133
gi 256010674 gb FJ756846.1	276	389	131	134
gi 256010670 gb FJ756844.1	278	391	131	132
gi 256010668 gb FJ756843.1	276	389	130	135
gi 256010666 gb FJ756842.1	277	390	131	132
gi 256010664 gb FJ756841.1	276	391	131	132
gi 256010662 gb FJ756840.1	277	391	130	132
gi 256010660 gb FJ756839.1	275	390	131	134
gi 256010658 gb FJ756838.1	276	390	131	133
gi 256010656 gb FJ756837.1	275	390	132	133
gi 256010654 gb FJ756836.1	275	391	132	132
gi 256010652 gb FJ756835.1	276	390	131	133
gi 256010650 gb FJ756834.1	275	390	132	133
gi 256010648 gb FJ756833.1	275	391	132	132
gi 256010646 gb FJ756832.1	275	389	131	135
gi 256010644 gb FJ756831.1	278	390	128	134
gi 256010642 gb FJ756830.1	278	392	128	132
gi 256010640 gb FJ756829.1	277	392	129	132
gi 256010638 gb FJ756828.1	277	392	129	132
gi 256010636 gb FJ756827.1	277	391	129	133
gi 256010634 gb FJ756826.1	280	390	128	132
gi 256010632 gb FJ756825.1	277	392	129	132
gi 256010630 gb FJ756824.1	278	390	128	134
gi 256010628 gb FJ756823.1	278	391	128	133
gi 256010626 gb FJ756822.1	278	390	128	134
gi 256010624 gb FJ756821.1	278	391	128	133
gi 256010622 gb FJ756820.1	279	393	127	131
gi 256010620 gb FJ756819.1	279	391	127	131
gi 256010618 gb FJ756818.1	278	396	133	123
gi 256010616 gb FJ756817.1	282	397	127	124
gi 256010614 gb FJ756816.1	286	392	126	125

Cont.....Table 2: Nucleotide base composition (Mole %) of the Cytochrome oxidase subunit I (COI) gene sequences of mitochondrial DNA of different species of *Parnassius*

Species (Gen Bank Entry)	Adenine(Mole %)	Thymine(Mole %)	Guanine(Mole %)	Cytosine(Mole %)
gi 227436763 gb FJ663915.1	197	271	94	96
gi 227436761 gb FJ663914.1	202	266	93	95
gi 227436759 gb FJ663913.1	204	266	96	95
gi 227436757 gb FJ663912.1	203	266	94	95
gi 227436755 gb FJ663911.1	204	259	90	105
gi 227436753 gb FJ663910.1	204	263	91	100
gi 227436749 gb FJ663908.1	202	263	91	100

species has been depicted in Table 2. Lengths of DNA, Molecular weight, AT and GC content have been shown in Table 3. Mean values of AT and GC content were 71.7713 and 28.2564 respectively. Histogram of AT and GC content are shown in Figs. 1 and 2 respectively. The variance values for AT and GC were 0.259411 and 0.4828 respectively having 95% confidence interval for mean and sigma. The mean value of Relative melting temperature was 0.363666 with the variance 0.000209. A linear regression has been established between GC content and Relative melting temperature (Fig. 3).

Phylogenetic tree (Fig. 4) was done on the basis of cytochrome oxidase subunit I (COI) gene sequences of mt DNA of different species of the genus *Parnassius*. Phylogenetic relationships were derived from a neighbor-joining analysis. The cluster containing the isolates of *Parnassius smintheus* and *Parnassius behrii* branched with isolates of *Parnassius phoebus*. Maximum identity (100%) was observed among the three isolates of *Parnassius stubbendorfi* (FJ663914.1, FJ663913.1 and FJ663912.1). The branch closest to this cluster contains the isolates of *Parnassius staudingeri* (FJ663911.1,

Table 3: Lengths of DNA, Molecular weight, AT and GC content of the Cytochrome oxidase subunit I (COI) gene sequences of mitochondrial DNA of different species of *Parnassius*

Gen Bank Entry	Length of DNA(bp)	Molecular Weight(Da)	ATcontent (%)	GC content (%)	BasicTm (°C)	Relative Tm(%)
gi 256010676 gb FJ756847.1	930	561949.00	71.72	28.28	76	0.36371
gi 256010674 gb FJ756846.1	930	561949.00	71.72	28.28	76	0.36369
gi 256010672 gb FJ756845.1	930	561983.00	71.51	28.49	76	0.36380
gi 256010670 gb FJ756844.1	930	561949.00	71.72	28.28	76	0.36370
gi 256010668 gb FJ756843.1	930	561932.00	71.83	28.17	76	0.36365
gi 256010666 gb FJ756842.1	930	561949.00	71.72	28.28	76	0.36370
gi 256010664 gb FJ756841.1	930	561949.00	71.72	28.28	76	0.36368
gi 256010662 gb FJ756840.1	930	561983.00	71.51	28.49	76	0.36382
gi 256010660 gb FJ756839.1	930	561949.00	71.72	28.28	76	0.36369
gi 256010658 gb FJ756838.1	930	561949.00	71.72	28.28	76	0.36370
gi 256010656 gb FJ756837.1	930	561932.00	71.83	28.17	76	0.36364
gi 256010654 gb FJ756836.1	930	561983.00	71.51	28.49	76	0.36382
gi 256010652 gb FJ756835.1	930	561966.00	71.61	28.39	76	0.36375
gi 256010650 gb FJ756834.1	930	561983.00	71.51	28.49	76	0.36383
gi 256010648 gb FJ756833.1	930	561966.00	71.61	28.39	76	0.36379
gi 256010646 gb FJ756832.1	930	562000.00	71.40	28.60	76	0.36387
gi 256010644 gb FJ756831.1	930	561932.00	71.83	28.17	76	0.36362
gi 256010642 gb FJ756830.1	930	561898.00	72.04	27.96	76	0.36353
gi 256010640 gb FJ756829.1	930	561915.00	71.94	28.06	76	0.36360
gi 256010638 gb FJ756828.1	930	561915.00	71.94	28.06	76	0.36358
gi 256010636 gb FJ756827.1	930	561932.00	71.83	28.17	76	0.36362
gi 256010634 gb FJ756826.1	930	00561898.	72.04	27.96	76	0.36358
gi 256010632 gb FJ756825.1	930	561898.00	72.04	27.96	76	0.36358
gi 256010630 gb FJ756824.1	930	561932.00	71.83	28.17	76	0.36362
gi 256010628 gb FJ756823.1	930	561915.00	71.94	28.06	76	0.36355
gi 256010626 gb FJ756822.1	930	561932.00	71.83	28.17	76	0.36361
gi 256010624 gb FJ756821.1	930	561932.00	71.83	28.17	76	0.36357
gi 256010622 gb FJ756820.1	930	561864.00	72.26	27.74	76	0.36344
gi 256010620 gb FJ756819.1	930	561898.00	72.04	27.96	76	0.36352
gi 256010618 gb FJ756818.1	930	561830.00	72.47	27.53	76	0.36341
gi 256010616 gb FJ756817.1	930	561745.00	73.01	26.99	76	0.36311
gi 256010614 gb FJ756816.1	930	561745.00	73.01	26.99	76	0.36307
gi 227436763 gb FJ663915.1	658	397672.00	71.12	28.88	76	0.36389
gi 227436761 gb FJ663914.1	930	561949.00	71.72	28.28	76	0.36361
gi 227436759 gb FJ663913.1	658	397638.00	71.43	28.57	76	0.36364
gi 227436757 gb FJ663912.1	658	397655.00	71.28	28.72	76	0.36373
gi 227436755 gb FJ663911.1	658	397757.00	70.36	29.64	76	0.36422
gi 227436753 gb FJ663910.1	658	397689.00	70.97	29.03	76	0.36405
gi 227436749 gb FJ663908.1	656	396489.00	70.88	29.12	76	0.36403

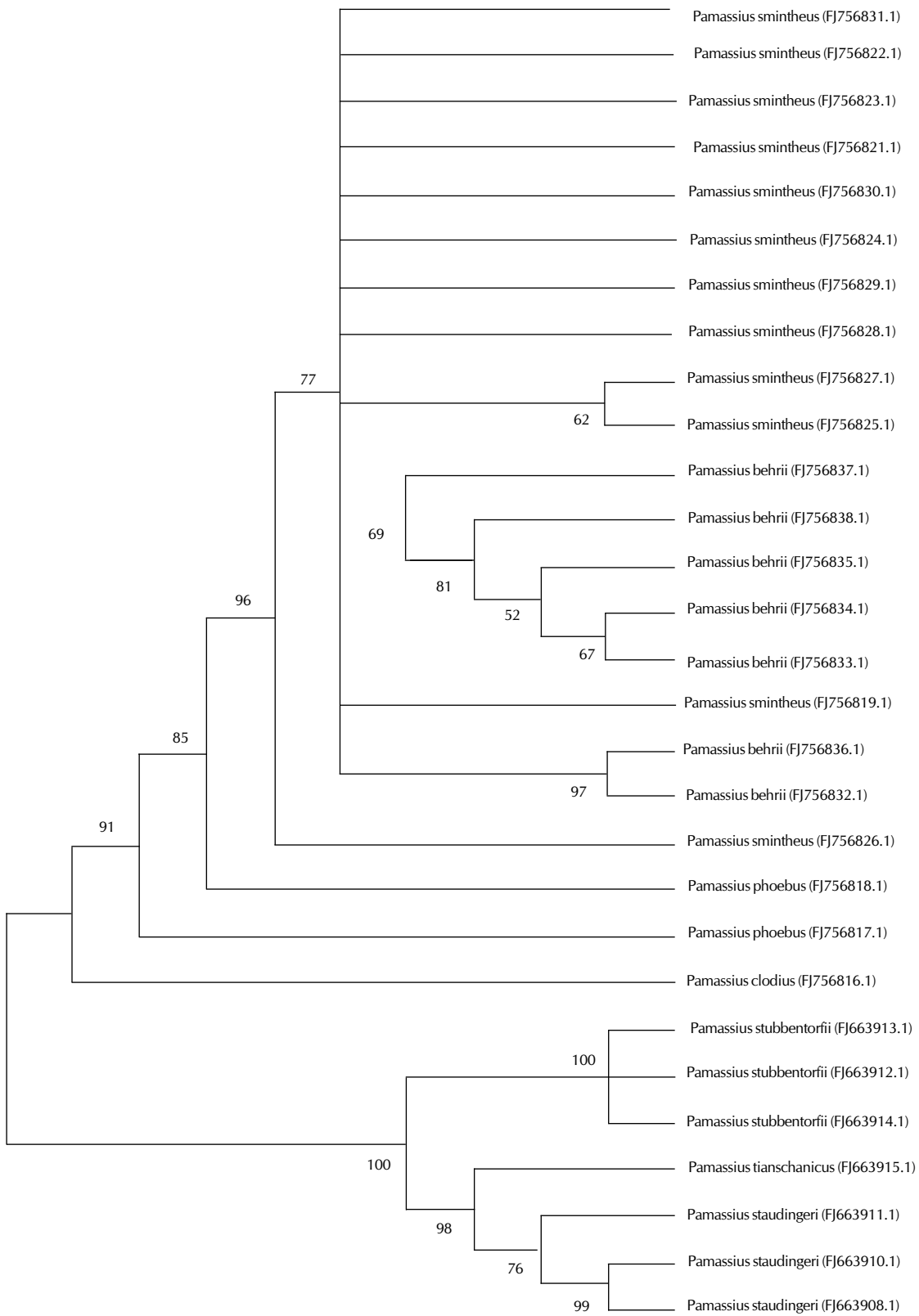


Figure 4: A Phylogenetic tree of different species of the genus *Parnassius*

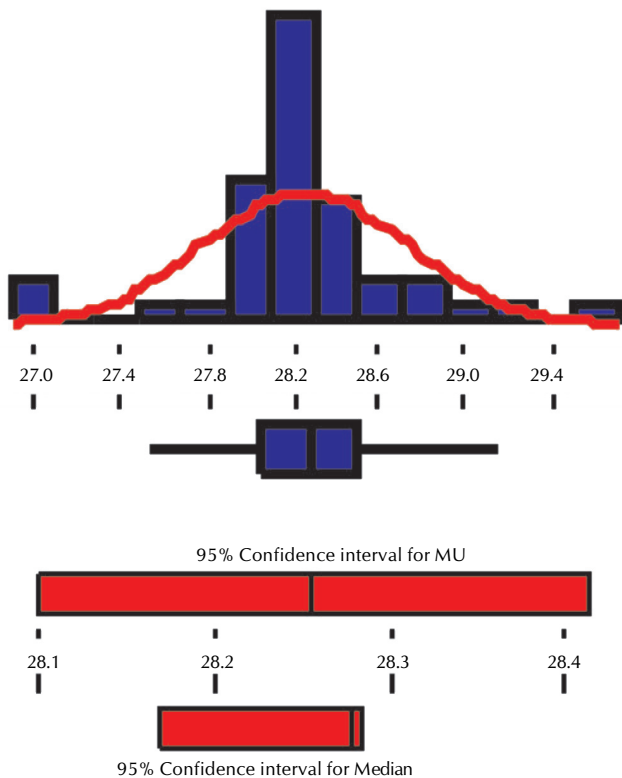


Figure 2: Graphical representation of histogram of GC content

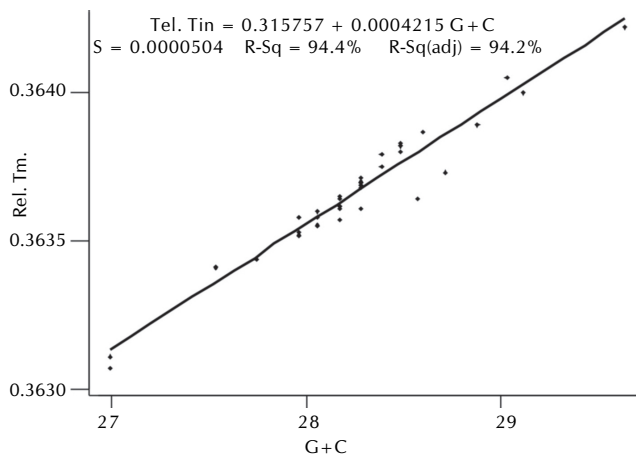


Figure 3: Regression Plot between GC content and Relative Tm

FJ663910.1, and FJ663908.1) and *Parnassius tianschanicus* (FJ663915.1) with 100% bootstrap support.

Statistical analysis

Coefficients:

Estimate	Std. Error	t value	Pr(> t)
(Intercept)	3.448e-01	8.642e-03	39.897 < 2e-16 ***
AT content	6.826e-05	8.539e-05	0.799 0.429
GC content	4.951e-04	9.008e-05	5.497 3.27e-06 ***

Relative Melting Temperature is highly co-related with GC content (%) and $Corr. (GC \text{ content}, Relative \ Tm.) = 0.9721334$. The regression equation is $Relative \ Tm. = 0.352 + 0.000424 \ GC \ \text{content}$. The study on but-terflies is of great interest in the field of biodiversity (Dennis *et al.*, 2000; Grill and Cleary, 2003; Schmitt *et al.*, 2005). So the present study bears relevant information in respect to the molecular diversity of different species of *Parnassius*.

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