

Supplementary Material

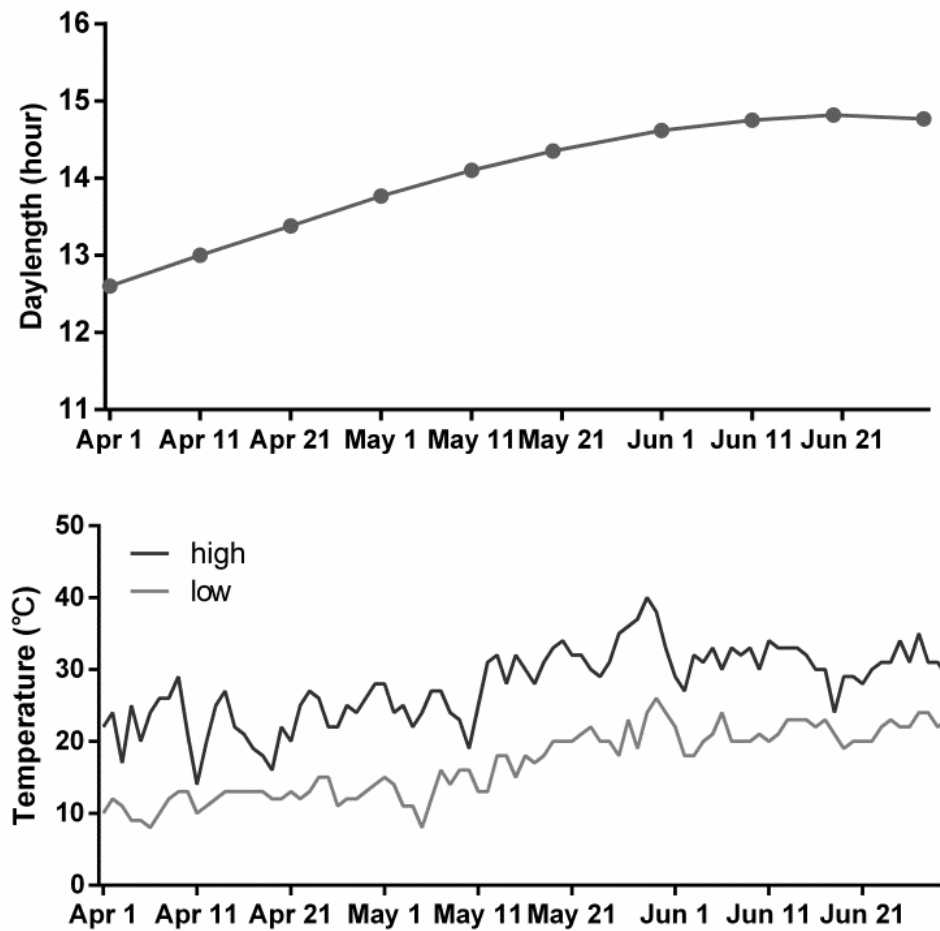


Fig.S1 The day length, minimum and maximum temperature of field environments (Shijiazhuang, Hebei Province, China) where the free-living Eurasian tree sparrow (*Passer montanus*) were sampled from between April 1 to June 30, 2014.

Table S1 The sense and antisense PCR primers used to clone *Dio2*, *Dio3*, *TRH*, *TSH*, *GnRH -I*, and *GnIH* cDNA (the coding regions of each gene were shown in Appendix A) for the Eurasian tree sparrow (*Passer montanus*) (The).

Gene	Primer	Sequence (5' to 3')
TRH	Sense 1	TGCCASTGCTACTCCTYTG
	Antisense 1	CTGYCTCTTCTGAATGTCTCCA
	Sense 2	CACTGCTACTCCTTTGCTTGACCTC
	Antisense 2	GCTTTTCTTCTACCCTGTCTCTGCT
	Sense 3	CCAGCTGCCACTGCTACTCC
	Antisense 3	TGCCTCTTCTGAATGCCTCC
	Sense 4	CTCATTCTTCAGTCTGTCCT
	Antisense 4	TTCTCATTCCTCTGTTTCAC
	Sense 5	TCAAGGTCATCTTCCATCTCCC
	Antisense 5	ACAATGCCATCCATCCAGCT
TSH	Sense 1	ATGAGTCCCTTCTTTGTGAT
	Antisense 1	GTAGTTTGTCTCTGACCTCT
	Sense 2	CCATCCCCTACTACTCCTAC
	Antisense 2	TGAGCAGAGCAGTACATTTT
Dio2	Sense 1	GGAAGATGGGTTTGCTAAGTG
	Antisense1	TGCATCACCTTAAAGGGACA
Dio3	Sense1	AGCACAAGCCGCTRCGAT
	Antisense1	TTGCCAGCTCCTTTTAC
GnRH	Sense	GCAGTTACTCCACAACCTCTCTCAG
	Antisense	AACCTCACTCTGGCTCTGTGGCTCC
GnIH	Sense	ACAGATTCTGAGCTCTCTAAGTAAG
	Antisense	ACCAATAGGACATAAGTAGCACA
β-actin	Sense	TGCTATGTCGCCCTGGATTTT
	Antisense	GGATACCACAGGACTCCATACCC

Table S2 The sense and antisense primers of *TRH*, *TSH*, *Dio2*, *Dio3*, *GnRH*, *GnIH*, and β -actin for quantitative polymerase chain reactions (qPCR) in diencephalon of male Eurasian tree sparrow (*Passer montanus*).

Gene	Primer	Sequence (5' to 3')
<i>TRH</i>	Sense	CTGCTACAACGGCTTTCCAA
	Antisense	GCCTCTTCTGAATGCCTCCA
<i>TSH</i>	Sense	CTCACCACACCATCCCCCTAC
	Antisense	CTGACCCTCTCACGGACACA
<i>Dio2</i>	Sense	CGCCTGGAATCTCTCCCTCT
	Antisense	ACTCGCTCAAATGAAACCCC
<i>Dio3</i>	Sense	CAGAGGACCAGAGGGCTACA
	Antisense	CACACGGCTTTCAGCGACT
<i>GnRH</i>	Sense	GGGACCGTTCCAGGTGATTC
	Antisense	CTTCTGCCTTGTTCCCTCCGC
<i>GnIH</i>	Sense	AAGACTGGGGAACAAAAGACATC
	Antisense	CCTGTGTGATACCTTTGGAGCAT
β-actin	Sense	CACAGATCATGTTTGAGACCTTCA
	Antisense	GATGGGCACAGTGTGGGTAAC

Table S3. Regression results of plasma triiodothyronine (T₃), the latent variable (metabolism), mating season in the structural equation model explaining variation in body mass and testis size for Eurasian tree sparrows (*Passer montanus*).

Regression	Variable	Estimate	Se	z	p value
T₃	Mating season	0.877	0.097	9.055	<0.001
Metabolism	T ₃	0.558	0.262	2.130	0.033
	Mating season	-1.060	0.340	-3.119	0.002
Body mass	Metabolism	0.692	0.333	2.079	0.038
Testis size	Metabolism	-1.214	0.371	-3.276	0.001

Table S4. Statistical effects of latent variable (metabolism) including Glu, TG, UA in the structural equation model explaining variation in body mass and testis size for Eurasian tree sparrows (*Passer montanus*).

Latent variable	Variable	Estimate	Se	z	p value
Metabolism	Glu	1.000			
	TG	1.366	0.383	3.568	<0.001
	UA	-1.275	0.384	-3.316	0.001

Table S5. Covariances between body mass and testis size in the structural equation model explaining variation in body mass and testis size for Eurasian tree sparrows (*Passer montanus*).

Covariances		Estimate	Se	z	p value
Body mass	Testis size	0.171	0.118	1.449	0.147

Appendix A Coding region of TRH, TSH, Dio2, Dio3, GnRH-I, GnIH of Eurasian tree sparrow

LOCUS Seq1 762 bp mRNA linear VRT 10-NOV-2019

DEFINITION Thyrotropin releasing hormone (TRH) mRNA, complete cds.

ACCESSION Seq1

VERSION

KEYWORDS .

SOURCE *Passer montanus* (Eurasian tree sparrow)

ORGANISM *Passer montanus*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda;
Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Passeridae; Passer.

REFERENCE 1 (bases 1 to 762)

AUTHORS hao,y.

TITLE Direct Submission

JOURNAL Submitted (10-NOV-2019) Key Laboratory of Animal Physiology,
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Life Sciences, Hebei Normal University, Shijiazhuang 050024,
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Hebei Province, shijiazhuang, hebei province 050024, China

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Bankit Comment: TOTAL # OF SEQS:1

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..762

/organism="Passer montanus"

/mol_type="mRNA"

/db_xref="taxon:9160"

/country="China: Shijiazhuang,Hebei,province"

/altitude="176 m."

/collected_by="Yinchao Hao"

gene 1..762

/gene="Thyrotropin releasing hormone (TRH)"

CDS 1..762

/gene="Thyrotropin releasing hormone (TRH)"

/note="The hormone is a kind of pituitary hormone secreted
by the hypothalamus, which promotes the secretion of
thyroid-stimulating hormone from the pituitary and further
controls the synthesis of thyroid hormone."

/codon_start=1

/product="Thyrotropin releasing hormone (TRH)"

/translation="MPSIQLPLLLLCLTSCGVCFNGGHLLPEESENMGKSPLDHILQR

SESLILQSVLKKAEEINKDSNAPVLQRLSKRQHPGKEYLSNLEKRQHPGKR DVEEETS

YGGIQKRQHPGKREMEDDL DVYLGLKRQQPSSRKSLLDQFAYSPRAQLTYMNELSKTE

HPGRRYP MYKHQRPSKRGWNYEVDVYDEKRQHPGKRHWNSDSSDDTGPCNFQDSL TCH

KGSLLLDLIDVSRDRVEEK RQHPGKRSAWESETEG"

BASE COUNT 249 a 164 c 189 g 160 t

ORIGIN

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121 atccttcaga gatctgaaag cctcattctt cagtctgtcc tcaagaaagc tgaagagatt
181 aataaagact caaatgcccc tgtgctacaa cggctttcca aaagacaaca ccctggggaa
241 aagtacctaa gtaacctgga aaagagacag catcctggaa aaagagatgt tgaggaagag
301 acatcttatg gaggcattca gaagaggcag catcccgaa aaagggagat ggaagatgac
361 cttgatgtct atctgggggt gaaaaggcaa cagccttcca gcagaaagtc actgttggat
421 cagtttgctt acagtcctag ggcacagcta acttacatga acgagttatc caaaacagaa
481 catccaggca gaagatatcc aatgtacaag caccagcgtc ctagcaaaag aggctggaat
541 tatgaggtag atgtatatga tgagaaacgc cagcatcctg ggaaaaggca ctggaattct
601 gacagctcag atgacacagg tccttgcaat ttcaggact cactcacttg tcacaaaggc
661 agcttggtgc ttgatttaat agatgtagc agagacaggg tagaagaaaa gcgtcagcac
721 ccaggaaaga gatcagcatg ggaaagtga acagagggat ga
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LOCUS Seq1 408 bp mRNA linear VRT 11-NOV-2019

DEFINITION thyroid stimulating hormone beta (TSHB) mRNA, complete cds.

ACCESSION Seq1

VERSION

KEYWORDS .

SOURCE *Passer montanus* (Eurasian tree sparrow)

ORGANISM *Passer montanus*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda;
Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;

Passeridae; Passer.

REFERENCE 1 (bases 1 to 408)

AUTHORS hao,y.

TITLE Direct Submission

JOURNAL Submitted (11-NOV-2019) Key Laboratory of Animal Physiology,

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Bankit Comment: TOTAL # OF SEQS:1

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..408

/organism="Passer montanus"

/mol_type="mRNA"

/db_xref="taxon:9160"

/country="China: Shijiazhuang,Hebei,province"

/altitude="176 m."

/collected_by="Yinchao Hao"

gene 1..408

/gene="TSHB"

/note="thyroid stimulating hormone subunit beta"

CDS 1..408

/gene="TSHB"

/note="Thyroid stimulating hormone is a hormone secreted by the pituitary gland to promote the growth and function of the thyroid gland."

/codon_start=1

/product="thyroid stimulating hormone beta (TSHB)"

/translation="MSPFFVMSLLFGLIFGQTASLCAPAEYTIHVEKRECAyclaint

TICAGFCMTRDSNGKKKLLLSALSQNVCTYKDMLYRTALIPGCPLHTIPYYSPVAL
SCKCGKCNTDYSDCVRERVRTNYCTKPQKLCNL"

BASE COUNT 95 a 120 c 99 g 94 t

ORIGIN

1 atgagtcctt tctttgtgat gtctctcttc ttggcctga ttttggaca aacagcatca
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121 gccatcaaca ccaccatctg cgctggattc tgcattgactc gggacagcaa tggcaagaag
181 aagctgctcc tgaagagtgc tctgtcccag aacgtgtgca cgtacaagga catgctgtac
241 cggacagcgc tgatcccgagg ctgccctctc cacaccatcc cctactactc ctaccccgctg
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361 gtcaggacaa actactgcac taagccacag aagctctgta acctgtaa

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LOCUS Seq1 840 bp mRNA linear VRT 11-NOV-2019

DEFINITION iodothyronine deiodinase 2 (DIO2) mRNA, complete cds.

ACCESSION Seq1

VERSION

KEYWORDS .

SOURCE *Passer montanus* (Eurasian tree sparrow)

ORGANISM *Passer montanus*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda;
Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Passeridae; Passer.

REFERENCE 1 (bases 1 to 840)

AUTHORS hao,y.

TITLE Direct Submission

JOURNAL Submitted (11-NOV-2019) Key Laboratory of Animal Physiology,
Biochemistry and Molecular Biology of Hebei Province, College of
Life Sciences, Hebei Normal University, Shijiazhuang 050024,
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Hebei Province, shijiazhuang, hebei province 050024, China

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Bankit Comment: TOTAL # OF SEQS:1

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..840

/organism="Passer montanus"

/mol_type="mRNA"

/db_xref="taxon:9160"

/country="China: Shijiazhuang,Hebei,province"

/altitude="176 m."

/collected_by="Yinchao Hao"

gene 1..840

/gene="DIO2"

/note="iodothyronine deiodinase 2 (DIO2)"

CDS 1..840

/gene="DIO2"

/note="It catalyzes the conversion of prohormone thyroxine

(3,5,3',5'-tetraiodothyronine, T4) to the bioactive

thyroid hormone (3,5,3'-triiodothyronine, T3) by outer

ring 5'-deiodination."

/codon_start=1

/product="iodothyronine deiodinase 2 (DIO2)"

/translation="MGLLSVDLLITVQVLPVFFSNCLFLALYDSVILLKHMVLFSLRS

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VGGKCGTKCHLLDFANSERPLVVNFGSATUPPFTSQLSAFSKLVEDFSGVADFLVYI

DEAHPSDGWAAPGISPSSFEVKKHKHSQEERCAAAHQLEHFSLPPQCQVADCMDNNA

NVAYGVVSFERVCIQVRQKIAYLGGKGPFYFYNLQEVRLWLEQNFSCRUNPFSTGVTSID

VSL"

BASE COUNT 191 a 209 c 227 g 213 t

ORIGIN

1 atgggtttgc taagtgtgga ttgttgatc acggttcagg tcttgccgt cttttctcc

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 181 cgctgcgtct ggaacagctt cctctggac gcctacaagc aggtcaaact gggaggagaa
 241 gcccctaaact ccagtgaat ccacatagcc aaggggagtg atggcagtag tggcagctgg
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 541 gttaagaaac acaaaagcca ggaagaaaga tgtgcagctg ctcaccagct ctagagcac
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LOCUS Seq1 825 bp mRNA linear VRT 11-NOV-2019

DEFINITION iodothyronine deiodinase (DIO3) mRNA, complete cds.

ACCESSION Seq1

VERSION

KEYWORDS .

SOURCE *Passer montanus* (Eurasian tree sparrow)

ORGANISM *Passer montanus*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
 Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda;
 Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
 Passeridae; Passer.

REFERENCE 1 (bases 1 to 825)

AUTHORS hao,y.

TITLE Direct Submission

JOURNAL Submitted (11-NOV-2019) Key Laboratory of Animal Physiology,

Biochemistry and Molecular Biology of Hebei Province, College of
Life Sciences, Hebei Normal University, Shijiazhuang 050024,
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Bankit Comment: TOTAL # OF SEQS:1

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..825

/organism="Passer montanus"

/mol_type="mRNA"

/db_xref="taxon:9160"

/country="China: Shijiazhuang,Hebei,province"

/altitude="176 m."

/collected_by="Yinchao Hao"

gene 1..825

/gene="DIO3"

/note="iodothyronine deiodinase 3 (DIO3)"

CDS 1..825

/gene="DIO3"

/note="It catalyzes the inactivation of thyroid hormone by inner ring deiodination of the prohormone thyroxine (T4) and the bioactive hormone 3,3',5-triiodothyronine (T3) to inactive metabolites, 3,3',5'-triiodothyronine (RT3) and 3,3'-diiodothyronine (T2), respectively."

/codon_start=1

/product="iodothyronine deiodinase 3 (DIO3)"

/translation="MLHSVGVHTLQLLTQAAACILLFPRFLLTAVMLWLLDFLCIRKK

MLTMPTADEAASASEEPPPDDPPVCVSDSNRMFTLESLKAVWHGQKLDFFKSAHVGSL

APNPEVIQLDQGKRLRILDFARGKRPLILNFGSCTUPPFMARLRSFQRLAAHFVDIAD

FLLVYIEEAHPSDGWWSSDAAYNIPKHQCLQDRLRAAQLMREGAPDCPLAVDTMDNAS

SAAYGACFERLYIIQEEKVMYQGGRGPEGYKISELSWLDQYKTRLQSPSTVVIQV"

BASE COUNT 157 a 279 c 243 g 146 t

ORIGIN

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1 atgtccact ccgtcggcgt tcacacctg cagctgctca cccaggcggc cgcctgcatc
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121 attaggaaga agatgctgac gatgccacg gcggacgagg cggccagcgc cagcgaggag
181 ccgccccccg acgaccccc ggtctgcgtg tccgactcca accgcatgtt cacgctggag
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601 aggggaagggg cgcccattg cccctggcc gtggacacca tggacaatgc ttccagtgcc
661 gcctacgggtg cctgcttga gaggctctac atcattcagg aggagaaggt gatgtaccag
721 ggaggcagag gaccagaggg ctacaagatc tcggagctga ggagctggct agaccagtac
781 aaaacccggc tccagagccc cagcacgggtg gtcaccaag tgtaa

//

LOCUS Seq1 272 bp mRNA linear VRT 31-MAR-2020

DEFINITION gonadotropin-releasing hormone I (GnRH - I) mRNA, complete cds.

ACCESSION Seq1

VERSION

KEYWORDS .

SOURCE Passer montanus (Eurasian tree sparrow)

ORGANISM Passer montanus

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda;
Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Passeridae; Passer.

REFERENCE 1 (bases 1 to 272)

AUTHORS hao,c.

TITLE Direct Submission

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Bankit Comment: TOTAL # OF SEQS:1

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..272

/organism="Passer montanus"

/mol_type="mRNA"

/db_xref="taxon:9160"

/country="China: Shijiazhuang,Hebei,province"

/altitude="176 m."

/collected_by="Chenyang Hao"

gene <1..>272

/gene="GnRH1"

CDS <1..>272

/gene="GnRH1"

/note="It is a member of the gonadotropin-releasing

hormone (GnRH) family of peptides"

/codon_start=1

/product="gonadotropin-releasing hormone I (GnRH-I)"

/translation="MEKPRRMVATVLLCVLAVGLCLAQHWSFGLQPGGKRSAQVLLGP

FQVIPNEMEKLKEVQQSECPGSQQNPRIRDLKEAMERLAEEQGRRK"

BASE COUNT 67 a 64 c 100 g 41 t

ORIGIN

1 atggagaagc cccggaggat ggtggccact gtcctgctgt gtgtgctggc cgtggggctg
61 tgcctggccc agcactggtc cttcgggctc cagccagggg gcaagaggag cgcccaggtc
121 ctgctgggac cgttccaggt gattccaaat gaaatggaaa aattaaagga ggtgcagcag
181 agtgagtgcc caggctcgca gcagaacccc aggatcaggg atctgaagga agccatggag
241 aggctggcgg aggaacaagg cagaagaaag aa

//

LOCUS Seq1 522 bp mRNA linear VRT 31-MAR-2020

DEFINITION gonadotropin-inhibitory hormone pound (GnIH) mRNA, complete cds.

ACCESSION Seq1

VERSION

KEYWORDS .

SOURCE *Passer montanus* (Eurasian tree sparrow)

ORGANISM *Passer montanus*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda;
Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Passeridae; Passer.

REFERENCE 1 (bases 1 to 522)

AUTHORS Li,Y.

TITLE Direct Submission

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China., universities, No. 20, South Second Ring Road, Shijiazhuang,
Hebei Province, shijiazhuang, hebei province 050024, China

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Bankit Comment: TOTAL # OF SEQS:1

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..522

/organism="Passer montanus"

/mol_type="mRNA"

/db_xref="taxon:9160"

/country="China: shijiazhuang,Hebei,province"

/altitude="176 m."

/collected_by="Yaqing Li"

gene <1..>522

/gene="GnIH"

CDS <1..>522

/gene="GnIH"

/note="GnIH acts on hypothalamus GnRH neurons and
pituitary gonadotropins to inhibit gonadotropin synthesis
and release, thereby regulating bird reproduction"

/codon_start=1

/product="gonadotropin-inhibitory hormone (GnIH)"

/translation="MKVILSKKFILFALATVFFLTSSMCLNEPMKSRLQSREDNDDK

YYEIKDNILEEKQRSLNFEDMEDWGTKDIIKMNHFTASKMPNSVANLPLRFGRNYPEE

RSIKPFSNLPLRFGRAFGENILNHAPKVSHRLGRSPLVKGSSQSLNLNPQRFKSLAV

NLPRDIEESEPGI"

BASE COUNT 182 a 91 c 105 g 144 t

ORIGIN

1 atgaaagtca tttatccaa gaagtttatt ctgtttgctt tagctacagt tttctttctc
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121 aatgatgata aatattatga gattaaagat aatattttgg aagaaaagca gaggagcctc
181 aattttgaag acatggaaga ctggggaaca aaagacatca taaaatgaa ccattttaca
241 gcaagcaaga tgccaaattc agttgctaatt tacctctta gatttgaag aaattatcca
301 gaagaaagaa gcattaaacc attttctaatt ttgccctga gatttgaag agcttttga
361 gagaacatac ttaatcatgc tccaaaggta tcacacaggc ttgggagatc tccactgtt
421 aaaggttcca gtcaatcact tctaaatttg ccacagagat ttgggaagtc actggctgtc
481 aatctgcctc gagacattga ggaatctgaa ccagggatat ga

//