The growth hormone gene has evolved independently in African Pygmy Mouse *Mus minutoides*

Sumito Matsuya¹, Hiroyuki Imai², Yasuo Kiso², Ken Takeshi Kusakabe² Kiyoshi Kano^{1*}

¹ *Laboratory of Developmental Biology, Joint-Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-8515, Japan*

² *Laboratory of Veterinar Anatomy, Joint-Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-8515, Japan*

Corresponding author.: E-mail: kanokiyo@yamaguchi-u.ac.jp

Running head: GROWTH HORMONE IN MUS MINUTOIDES

Abstract

Mus minutoides (the African pygmy mouse) is one of the smallest mammals. We determined the nucleotide sequence of the *growth hormone* (*Gh*) gene and the sequence of the putative coding region in *M. minutoides*, where is predicted to be distinct in the functional and transcriptional regulatory regions between *M. minutoides* and *Mus musculus* (the House mouse). To investigate the evolutionary characteristics of *Gh* in *M. minutoides*, we constructed a phylogenetic tree based on the putative amino acid sequences of *Gh* in *M. musculus* and mammals by neighbor-joining method, suggesting that *Gh* diverged relatively earlier than other *Mus* genus and may have evolved independently in *M. minutoides*. Furthermore, analysis of *Gh* gene expression levels showed a tendency to be higher in *M. minutoides* than in *M. musculus*. Our results suggest that *Gh* may have evolved independently in *M. minutoides* and may have different functions and signaling in *Mus* genus.

Keywords: body size, evolution, growth hormone, mouse, *Mus minutoides*

Mammals have various body sizes, ranging from large whales to small rodents. Each species also has an inherent size, but it is not clear how this is determined and maintained. *Mus minutoides* (African pygmy mouse) is one of the smallest mammals in the world, measuring about 30 mm in body length and about 3 grams in body weight. *M. minutoides* originally inhabit the south of Saharan in Africa and is now bred as a pet animal in many countries [6, 11]. The sexual maturity of *M. minutoides* is about 8 weeks old, the gestation period is about 20 days, and the lifespan is about 2 years [17], which is similar to that of the common laboratory mice, but the chromosomes of *M. minutoides* have unique characteristics that differ from those of *Mus musculus*. *M. minutoides* can be broadly divided into two major groups: the Southern Clade, which is widely distributed in South Africa, and the Eastern Clade, which is restricted to the eastern part of the South Africa region. Chromosome numbers are $2n=18, 32, 34$ in the Southern Clade, but not have been identified in the Eastern Clade [6].

Interestingly, in addition to the X and Y chromosomes, *M. minutoides* has a unique chromosome called X*, which is shorter than the original X chromosome and has a special sex-determination pattern [13]. X chromosomes inactivation and sex determination, male hormones, and characteristics of X*Y female have been well studied [9, 14, 16]; however *M. minutoides* is one of the smallest mammals, despite its obvious characteristics, few studies have focused on its size.

Many factors seem to be involved in determination of unique body size and growth rates in different animal species, and they are thought to interact in complex manner. One of the most representative factors involved in growth is growth hormone (Gh). Many studies have been conducted to identify the structure, functional and binding sites of Gh and Gh receptors, and to determine the regulatory mechanisms of growth and homeostasis [1, 4, 7, 10, 18]. In animals, Gh has been also well-studied, and the sequences of the Gh gene have been identified not only in rodents but also in dogs [2], cats [5], and pandas [12] and so on. In general, dwarfism is also due to impaired function of growth hormone regulators in the anterior pituitary gland and neuroendocrine and tissue transcription factors in the anterior pituitary in mice [3, 15].

In addition, understanding the regulatory mechanisms of Gh and other pituitary hormones is particularly important in body size determination mechanisms because many spontaneous dwarf mice may be deficient in pituitary hormones.

The purpose of this study is to elucidate the basic mechanisms that determine the size of *M. minutoides* by analyzing firstly the nucleotide sequences of Gh, which is involved in animal body size. The results indicated that growth hormone evolved independently within the *Mus* genus at the presumed amino acid sequence level, suggesting that it may be responsible for the characteristic small body size of *M. minutoides*.

All animal experiments were approved by the Experimental Animal Care and Use Committee of Yamaguchi University (protocol number: 291). *Mus minutoides* used in this study were prepared for experiments immediately after purchase (Increase, Inc., Himeji, Japan). *Mus musculus* (C57BL/6J) were purchased from Japan SLC (Hamamatsu, Japan) and housed in groups with *ad libitum* access to water and food. Primers were designed based on the nucleotide sequence of the Ensembl database (Mouse (GRCm38.p6)) of the *growth hormone (Gh)* gene, and the genomic DNA extracted from the tail of *Mus minutoides* was used for sequencing. The primers used for nucleotide sequencing were designed to divide the entire gene length into three regions based on the sequence of *M. musculus*, and the sequence of the putative genomic region of the *Gh* gene in *M. minutoides* was determined. The primer sequences are listed in Table 1. Genomic PCR was performed and sequenced to search for differences between *M. musculus* and genes that may have a significant effect on body size determination. The *Gh* gene was amplified by PCR using the genome extracted from *M. minutoides* and compared with *M. musculus*. The *Gh* gene of *M. musculus* consists of a 1695 bp sequence and contains five exons. Alignment of the nucleotide sequence in the Ensembl database of *M. musculus* showed substitutions, deletions, and insertions in all regions including exons in *M. minutoides* (Fig. 1A). We have already registered the DNA sequence of the *Gh* gene of *M. minutoides* to DNA Data Bank of Japan (accession number: LC5671320).

Furthermore, the sequence of *M. minutoides* of the *Gh* gene was translated into a putative amino acid sequence and compared with the amino acid sequence of *M. musculus* using BLAST. The length of the putative amino acid sequence was 216 amino acids in *M. minutoides*, and identical to that of *M. musculus*. At the DNA sequence level, the *Gh* gene in *M. minutoides* had many sequences that differed from those in *M. musculus*, but the homology at the putative amino acid level was very high except for two amino acids, suggesting that *M. minutoides* also may have a functional Gh. The results showed that only the 25th and 37th amino acid sequences of *M. musculus* are serine, respectively, while two sites are different in *M. minutoides*: glycine and alanine (Fig. 1B). These mutations were found in the N-terminal region responsible for signal transduction and in the region that promotes GH protein maturation, respectively [8]. The 25th amino acid in *M. musculus* is serine, while the 25th amino acid in *M. minutoides* is glycine, suggesting that the binding ability of GH protein to receptors may be altered. Serine, the 37th amino acid in *M. musculus*, is part of the site responsible for the maturation of the GH protein, suggesting that the maturation process or post-translational modification of the GH protein in *M. minutoides* may be different from that in *M. musculus*.

Next, to examine whether these amino acid differences affect Gh gene expression, Gh gene expression in the pituitary gland of adult male *M. minutoides* (n=3). Table 2 lists the primers used to detect *Gapdh* and *Gh*. *M. musculus* was examined using qRT-PCR and found that the expression of the *Gh* gene was higher in *M. minutoides* than in *M. musculus* (Fig. 2). It is unlikely that the sequence of the *Gh* gene is directly related to the secretion of Gh protein, but the relatively high expression of the *Gh* gene in *M. minutoides* may not be directly related to body size. In dogs, growth promotion has been correlated with the level of *Igf1*, a downstream factor of GH, rather than with the GH secretory [8]. In our preliminary experiments, we have found that the expression level of the *Igf1* gene in *M. minutoides* is much lower than that in *M. musculus*. Although the levels of Gh and *Igf1* in the serum need to be measured, the relatively high expression of the *Gh* gene in *M. minutoides* may be due to negative feedback of *Igf1* levels.

108

109 Finally, to study the evolutionary characteristics of the amino acid sequence of Gh in *Mus minutoides*, we
110 constructed a phylogenetic tree of Gh in other *Mus* genus and mammals (Fig. 3) by neighbor-joining
111 method using the Poisson model by MEGAX with 1,000 bootstraps (The Biodesign Institute, Tanpe, AZ,
112 USA), suggesting that the Gh diverged relatively earlier than in the other *Mus* genus and may have
113 evolved independently in *M. minutoides*. This indicates that *M. minutoides* might have a unique growth
114 system of determining body size through mediated Gh hormone.

115

116 The present study suggests that *M. minutoides* evolved independently in Gh and may have different
117 functions and signaling from *M. musculus*. In order to further elucidate the characteristic growth-
118 related characteristics of *M. minutoides*, particularly Gh, it will be necessary to analyze related
119 factors such as growth hormone receptors and Igf1.

120

121 **POTENTIAL CONFLICTS OF INTEREST**

122 The authors have nothing to disclose.

123

124 **Acknowledgments**

125 The authors thank the ARCLAS animal facility and the technical expertise of The DNA Core facility of the
126 Center for Gene Research, Yamaguchi University. We also thank W. Fujii for his comments on the research.
127 We are grateful to anonymous reviewers for their insightful comments.

128

129 **Funding**

130 This work was supported by JSPS KAKENHI (Grant-in-Aid for JSPS Research Fellow; JP17J07902 and
131 Young Scientists; 20K15664 to H. I.) and Grants-in-Aid from the Foundation for Growth Science for KK.

Figure legends

Fig. 1 Alignment of a nucleic acid sequence of the genomic region and a putative amino acid sequence of *Gh* gene. (A) Alignment of a nucleic acid sequence of the genomic region of *Gh* gene between *M. minutoides* and *M. musculus*. Arrows indicate primers for the nucleotide sequence. The upper row is the sequence of *M. musculus* and the lower row is that of *M. minutoides*. * indicates matching nucleic acids between *M. minutoides* and *M. musculus*. Deletions and insertions across the entire region, including exons, was observed in the genomic DNA of *M. minutoides*. The shaded indicates five exons of *M. musculus*. (B) Alignment of a putative amino acid sequence of *Gh* between *M. musculus* and *M. minutoides*. The amino acid sequence of growth hormone was presumed by a nucleic acid sequence of *M. minutoides*. The 25th and 37th amino acid sequences of *M. musculus* are serine, respectively, while two sites are different in *M. minutoides*: glycine and alanine.

Fig. 2 Transcript levels of *Gh* in the pituitary gland of *M. minutoides* and *M. musculus*. Left: The relative expression level for *Gh* was normalized against the *Gapdh* expression level. The expression of the *Gh* gene in *M. minutoides* was higher than those in *M. musculus*. Data represent the mean $\pm$ SD. Right: RT-PCR analysis was performed for confirmation of the specificity of the primer set.

Fig. 3 Phylogenetic tree of the vertebrate growth hormone (Gh). The tree was constructed with the maximum likelihood method using MEGAX from *M. minutoides* and 16 other vertebrate Gh amino acid sequences. Gh amino acid sequences were obtained from the Ensembl database.

References

1. Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N. and Carter-Su, C. 1993. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell* **74**: 237-244.

158

159 2. Ascacio-Martinez, J. A. and Barrera-Saldana, H. A. 1994. A dog growth hormone cDNA codes
160 for a mature protein identical to pig growth hormone. *Gene* **143**: 277-280.

161

162 3. Bartke, A. 1964. Histology of the Anterior Hypophysis, Thyroid and Gonads of Two Types of
163 Dwarf Mice. *Anat. Rec.* **149**: 225-235.

164

165 4. Baumbach, W. R., Horner, D. L. and Logan, J. S. 1989. The growth hormone-binding protein in
166 rat serum is an alternatively spliced form of the rat growth hormone receptor. *Gene Dev.* **3**: 1199-1205.

167

168 5. Castro-Peralta, F. and Barrera-Saldana, H. A. 1995. Cloning and sequencing of cDNA
169 encoding the cat growth hormone. *Gene* **160**: 311-312.

170

171 6. Chevret, P., Robinson, T. J., Perez, J., Veyrunes, F. and Britton-Davidian, J. 2014. A
172 phylogeographic survey of the pygmy mouse *Mus minutoides* in South Africa: taxonomic and karyotypic
173 inference from cytochrome b sequences of museum specimens. *PLoS One* **9**: e98499.

174

175 7. Cunningham, B. C., Ultsch, M., De Vos, A. M., Mulkerrin, M. G., Clauser, K. R. and Wells, J. A.
176 1991. Dimerization of the extracellular domain of the human growth hormone receptor by a single
177 hormone molecule. *Science* **254**: 821-825.

178

179 8. Ergun-Longmire, B. and Wajnrajch, M. 2000. Growth and Growth Disorders, MDText.com, Inc.,
180 South Dartmouth, MA.

181

182 9. Ginot, S., Claude, J., Perez, J. and Veyrunes, F. 2017. Sex reversal induces size and
183 performance differences among females of the African pygmy mouse, *Mus minutoides*. *J. Exp. Biol.* **220**:

184 1947-1951.

185

186 10. Godowski, P. J., Leung, D. W., Meacham, L. R., Galgani, J. P., Hellmiss, R., Keret, R., Rotwein, P.

187 S., Parks, J. S., Laron, Z. and Wood, W. I. 1989. Characterization of the human growth hormone receptor

188 gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism. *Proc. Natl.*

189 *Acad. Sci. U. S. A.* **86**: 8083-8087.

190

191 11. J.Britton-Davidiana, T.J.Robinsonb and F.Veyrunesa 2012. Systematics and evolution of the

192 African pygmy mice, subgenus *Nannomys*: A review. *Acta Oecologica* **42**: 41-49.

193

194 12. Liao, M. J., Zhu, M. Y., Zheng, X., Zhang, Z. H. and Zhang, A. J. 2003. cDNA cloning of growth

195 hormone from giant panda (*Ailuropoda melanoleuca*) and its expression in *Escherichia coli*. *Comp. Biochem.*

196 *Physiol. B. Biochem. Mol. Biol.* **135**: 109-116.

197

198 13. Rahmoun, M., Perez, J., Saunders, P. A., Boizet-Bonhoure, B., Wilhelm, D., Poulat, F. and

199 Veyrunes, F. 2014. Anatomical and molecular analyses of XY ovaries from the African pygmy mouse *Mus*

200 *minutoides*. *Sex Dev.* **8**: 356-363.

201

202 14. Saunders, P. A., Perez, J., Rahmoun, M., Ronce, O., Crochet, P. A. and Veyrunes, F. 2014. XY

203 females do better than the XX in the African pygmy mouse, *Mus minutoides*. *Evolution* **68**: 2119-2127.

204

205 15. Sornson, M. W., Wu, W., Dasen, J. S., Flynn, S. E., Norman, D. J., O'Connell, S. M., Gukovsky, I.,

206 Carriere, C., Ryan, A. K., Miller, A. P., Zuo, L., Gleiberman, A. S., Andersen, B., Beamer, W. G. and

207 Rosenfeld, M. G. 1996. Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor

208 defective in Ames dwarfism. *Nature* **384**: 327-333.

209

- 210 16. Veyrunes, F. and Perez, J. 2018. X inactivation in a mammal species with three sex
211 chromosomes. *Chromosoma* **127**: 261-267.
212
- 213 17. Willan, K. and Meester, J. 1978. Breeding biology and postnatal development of the African
214 dwarf mouse. *Acta Theriologica* **23**: 55-73.
215
- 216 18. Zhou, Y., Xu, B. C., Maheshwari, H. G., He, L., Reed, M., Lozykowski, M., Okada, S., Cataldo, L.,
217 Coschigamo, K., Wagner, T. E., Baumann, G. and Kopchick, J. J. 1997. A mammalian model for Laron
218 syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene
219 (the Laron mouse). *Proc. Natl. Acad. Sci. U. S. A.* **94**: 13215-13220.

mus_musculus -----TGTCAGTGGCCCCAGCCATGAATAAATGTATAGGGGAAAGGCAGGAGCCTTGGGGTCGAGGAAACAGGTAGGGTATAAAAGGGCAGCGAAGGGACCAAGTCCAGCATC
mus_minutoides TTTTATTTTGTCCAGTGGCCCCAGCCATGAATAAATGTATAGGGGAAAGGCAGGAGCCTTGGGGTCGAGGAAACAGGTAGGGTATAAAAGGGCAGCGAAGGGACCAAGTCCAGCATC
***** * * *****

Exon 1
mus_musculus CTAGAGTCCAGATTCCAAACTGCTCAGAGTCTGTGGACAGATCACTGCTGGCAATGGCTACAGTAAGCATGCGCAATCCCGTGGGTGTGGTTTGGGACCCAGGGCCCTGAAGAT
mus_minutoides CTAGAGTCCAGATTCCAAACTGCTCAGGCTCTGTGGACAGATGCTGCGTGGCAATGGCTACAGTAAGCATG-----GGTGTGGTTTGGGACCAAGGGGCCCTGAAGAT
***** * * *****

mus_musculus GGATCTGAGGCTTCTAATGTGAGTGCCTTCAACTTCTGCATGTTGGGAATACTCTGGGTCCCTATGGGATTGGGAGAGATCGGCATTGCTCCAGGTTTCTCTGCCCTCTGTCT
mus_minutoides GGAGCTGAGGCTTCTAATGTGAGTGTGCTCAACTTCCACCATGTCGGGAACACTCTGGGTCCCCAGGGGATTGGGAGAGATTGGTCATTGCTCCCTGGTTTCTCTGCCCTCTGTCT
***** * * *****

Exon 2
mus_musculus CTCTCT-----AGACTCTCGGCACCTCTGGTCTCTGACCTGACCTGCTCTGCTCTGTCCTCAGGAGCTAGTGCTTTTCCGCCATGCCCTTGCTCAGTCTGTTTTCTAATGCTG
mus_minutoides CTCTCTCTAGACTCTCGACCTCTGGCTCTGCTGACCTGACCTGCTCTGCTCTGTCCTCAGGAGCTGGTGCTTTTCCCGCATGCCCTGTGCTCAGTCTGTTTGGCCAATGCTG
***** * * *****

Exon 2 F2
mus_musculus TGCTCCGAGCCAGCACCTGCACAGCTGGCTGCTGACACCTACAAGAGTTGCGTAAGTTCCTCC--AGAGATGGGTGCCGTTTGTGGAAGCAGGAAGGGGAGGTCTACCCCATACTCC
mus_minutoides TGCTCCGAGCCAGCACCTGCACAGCTGGCTGCTGACACCTACAAGAGTTTGTAAAGTTCCTCCAGAGATGGGTGCCGCTGTGTGGAAGCAGGAAGGGGAGGTCCACCCCATACTCC
***** * * *****

R1 Exon 3
mus_musculus TGCCCCAGGAAGTCAATTGAGGAAACTATGGGGTAGGGGAATCTTAGCCAATGCTGTACCATAGTAATGATGGTGACGAGACACAAGCTGGTCCCTCAGTGACCACCTCTCTCCAGGA
mus_minutoides TGCCCCAAGAAGTCAAAGAGGAAACTATGGGGTAGGGGAATCTTAGCCAATGCCATAACATAGTAATGACGGTGGCGAGGCCACAAGCTGGTACCTCAGTGACCACCTCTCTCCAGGA
***** * * *****

Exon 3
mus_musculus GCGTGCTACATTCCCGAGGGACAGCGCTATTCATTCCAGTAATGCCAGGCTGCTTTCTGCTCTCAGAGACCATCCCGGCCCAACAGGCAGGAGGAGGGCCAGCAGAGAACCGTGAG
mus_minutoides GCGTGCTACATTCCCGAGGGACAGCGCTATTCATTCCAGTAATGCCAGGCTGCTTTCTGCTCTCAGAGACCATCCCGGCCCAACAGGCAGGAGGAGGGCCAGCAGAGAACCGTGAG
***** * * *****

mus_musculus TAGTCCCAGCTTTGTCTGCACAAATCTCTGTTTCCCTCCAGCAGCCCTAACCTGCACTCCAGGCAGGGACCACTCTCTCCCTGAAGCTGGGGTAACTGGGAGTCCAGGCAGAGGTC
mus_minutoides TAATCCAGGCCTTGTGTGCACAAATCTCTTTTCCCTCCAGCAGCCCTAACCTGCACTCCAGGCAGGGACCACTCTCTCCCTGAAGCTGGGGTAACTGGGAGTCCAGGCAGAGGTA
***** * * *****

Exon 4 F3
mus_musculus ACTAGGCAATACATAACCCAGCCCTTTTTCCTCCCTCAGGACATGGAATTGCTTGCTCTCTGCTCTCAGTCTATGCTGAGGCTGGGGCCCTGTCAGTCTCTCAGCAGGATTTT
mus_minutoides ACTAGGCAATACACTGACCCAGCCCTTTTTC--CCCTCCCTCAGGACATGGAATTGCTTGCTCTCTCTGCTGCTATCAGTCTGAGTCTGGGGCCCTGTCAGTCTCTCAGCAGGATTTT
***** * * *****

Exon 4 R2
mus_musculus CACCAACAGCCTGATGTTGGCACCTCGGACCGTGTCTATGAGAACTGAAGGACCTGGAAGAGGGCATCAGGCTCTGATGCAGGTGAGGATGGAC-----TAGCCTGGGGTTAT
mus_minutoides CACCAACAGCCTGATGTTTGGCACCTCAGACCGTGTCTATGAGAACTGAAGGACCTGGAAGAGGGCATCAGGCTCTGATGCAGGTGAGGATGGACCGGGTCTTAACCTGGGGTTAT
***** * * *****

mus_musculus GCTCTGAGGCTAGGTGGG--GCTCACTG--TCTCTGT--TTTACCGTCAAGCCTTAGACCTTGAAGAGGCTCTTCTTCTTCACTTTTCTTATAGGCTCTCAGGCTTTTCTTGGTCT
mus_minutoides ACCTGGAGGCTAGGTGGATGCTTACTGCTTCTCTGTTTATCGGTCAAGCCTTAGACCTTGAAGAGGCTCTTCT-----ATTCTCCCTTATGAGGCTCAGGCTTTTCTTAGGTC
***** * * *****

Exon 5
mus_musculus CTGGGGTGGAGGGAGGCACAGCTCCGAGTCTCTGCCCTTTTCTCCACGACAGGAGCTGGAAGATGGCAGCCCGGTTGGGCGATCTCTCAAGCAAACTATGACAAGTTTGACGC
mus_minutoides CTGGGGTGGAGGGAGGCACAGCTCCGAGTCTCTGCCCTTTTCTCCATGACAGGAGCTGGAAGAGGGCCCGGTTGGGCGATCTCTCAAGCAAACTATGACAAGTTTGATGC
***** * * *****

Exon 5
mus_musculus CAACATGCGCAGCGACGACCGCTGCTCAAAAACTATGGGCTGCTCTCTGCTTCAAGAGGACCTGCACAAGGCGAGACCTACTCGGGTTCATGAAGTGTCCGCGCTTGTGGAAG
mus_minutoides CAACATGCGCAGCGACGACCGCTGCTCAAAAACTATGGGCTGCTCTCTGCTTCAAGAGGACCTGCACAAGGCGAGACCTATCTCGGGTTCATGAAGTGTCCGCGCTTGTGGAAG
***** * * *****

Exon 5
mus_musculus CAGCTGTGCTCTTACGCACTCAACAGTGTCTCTGCTCACTCTCTGCTCTCTGCTTGGCCCTCGGCAACTGCCACCCTTGGCTTTGTCTTAATAAAATTAAGATGCATCATACCC
mus_minutoides CAGCTGTGCTCTTACCACTCAACAGGCTCTCTGCTCACTCTCTGCTCTCTGCTTGGCCCTCGGCAACTGCCACCCTTGGCTTTGTCTTAATAAAATTAAGATGCATC-----
***** * * *****

R3
mus_musculus GGC TAGAGGTCTTTCTGTTATGGGATGGAGCAGTTGTGTCA
mus_minutoides

Figure 1 Matsuya *et al.*

B

Mus_musculus	MATDSRTSWLLTVSLLCLLWPQEASAFPAMPLSSLFSSNAVLRAQHLHQLAADTYKEFERA
Mus_minutoides	MATDSRTSWLLTVSLLCLLWPQEAGAFPAMPLSSLFANAVLRAQHLHQLAADTYKEFERA

Mus_musculus	YIPEGQRYSIQNAQA AFCFSETIPAPTGKEEAQQRDMELLRFSLLLIQSWLGPVQFLSR
Mus_minutoides	YIPEGQRYSIQNAQA AFCFSETIPAPTGKEEAQQRDMELLRFSLLLIQSWLGPVQFLSR

Mus_musculus	IFTNSLMFGTSDRVYEKLDLEEGIQALMQELEDGSPRVGQILKQTYDKFDANMRSDDAL
Mus_minutoides	IFTNSLMFGTSDRVYEKLDLEEGIQALMQELEDGSPRVGQILKQTYDKFDANMRSDDAL

Mus_musculus	LKNYGLLS CFKKDLHKAETYL RVMKCRRFVESSCAF
Mus_minutoides	LKNYGLLS CFKKDLHKAETYL RVMKCRRFVESSCAF

Fig. 1. Alignment of a putative amino acid sequence of Gh between *M. minutoides* and *M. musculus*. The amino acid sequence of growth hormone was presumed by a nucleic acid sequence of *M. minutoides*. The 25th and 37th amino acid sequences of *M. musculus* are serine, respectively, while two sites are different in *M. minutoides*: glycine and alanine.

Figure 2 Matsuya *et al.*

Fig. 2. Transcript levels of *Gh* in the pituitary gland of *M. minutoides* and *M. musculus*. Left: The relative expression level for *Gh* was normalized against the *GAPDH* expression level. The expression of the *Gh* gene in *M. minutoides* was higher than those in *M. musculus*. Data represent the mean + SD. Right: RT-PCR analysis was performed for confirmation of the specificity of the primer set.

Figure 3 Matsuya *et al.*

Fig. 3. Phylogenetic tree of the vertebrate growth hormone (Gh). The tree was constructed with the maximum likelihood method using MEGAX from *M. minutoides* and 16 other vertebrate Gh amino acid sequences. Gh amino acid sequences were obtained from the Ensembl database.

Table S1. Primer sequences for the genome sequence of *G.*

	Primer sequences (5'→3')
F1	TGTCAGTGGCCCCAGCCATG
R1	CCAGCTTGTGTCTCGTCACCATC
F2	GAGTTCGTAAGTTCCCCAGAGATGG
R2	CTGGATGCCCTCTTCCAGGTCC
F3	GCTCATCCAGTCATGGCTGGG
R3	TGACACAACCTGCTCCATCCCATAAC

Table S2 Primer sequences for RT-PCR

genes		Primer sequences (5'→3')
<i>Gh</i>	F	CGAGGGACAGCGCTATTCCATTC
	R	GCTTGAGGATCTGCCCAACACG
<i>Gapdh</i>	F	GTGCTGAGTATGTCGTGGAGTC
	R	CATACTTGGCAGGTTTCTCCAG