Phylogenomic and morphological evidence reveal a new species of spider lily, *Lycoris longifolia* (Amaryllidaceae) from China

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2 *Lycoris longifolia* (Amaryllidaceae) from China

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5 running title: A new species of spider lily, *Lycoris longifolia*

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20   **Abstract**: *Lycoris longifolia*, a new species from China, was described and illustrated here. Our

21   phylogenomic evidence based on whole plastomes strongly supported the separate phylogenetic

22   position of this new species, and it could also be easily distinguished by several morphological

23   characters, such as its long leaves with a distinct purplish-red midrib on the abaxial surface.

24   **Keywords:** *Lycoris*, morphological, phylogenomics, whole plastome

Introduction

26   The genus *Lycoris* Herb., including ca. 13-20 species of flowering plants in the family

27   Amaryllidaceae, subfamily Amaryllidoideae, is native to eastern and southern Asia. Herbert

28   described the first species in 1820, *L. aurea* (L'Hér.) Herb., which has important ornamental and

29   medicinal values (Hsu et al. 1994). In the mid-20th century, an American horticulturist, Hayward,

30   made much work on introducing and cultivating *Lycoris* species. Given the easily distinguished habit

31   of the populations of *L. aurea* distributed in northern Taiwan and southernmost Japan, i.e., the leaves

32   appear in autumn, about a month later than *L. aurea,* and no remains of leaf bases (Hsu et al. 1994).

33   Hayward described these populations as a new species, *L. traubii* (Hayward 1957; Hsu et al. 1994;

34   Kurita 1987). Having narrower perianth lobes and long-exserted stamens (Hsu et al. 1994; Ji and

35   Meerow, 2000), the populations from South Gansu (Kang Xian) and Northwest Hubei (Feng Xian)

36   were described as a variety of *L. aurea*, as *L. aurea* var. *angustitepala* (Hsu et al. 1994). In the last

37   decades, numerous new species or hybrids of *Lycoris* have been published in its diversity center, i.e.

38   mainland China, such as *L. hunanensis* (Quan et al. 2013), *L. × hubeiensis* (Meng et al. 2018), *L.*

39   *tsinlingensis* (Lu et al. 2020), and *L. wulingensis* (Zhang et al. 2021). Nowadays, more than 30

40   species and varieties have been recognized in the genus (Hsu et al. 1994; Ji and Meerow 2000; Kim

41   2004; Quan et al. 2013; Meng et al. 2018; Lu et al. 2020; Zhang et al. 2021), and nearly 20 of them

42   are from China.

43   During our recent field explorations in Sichuan Province, China, we collected a wild flowering

44   plant of *Lycoris*, which resembles *L. aurea* with yellow flowers. However, it could be easily

45   distinguished from *L. aurea* by markedly long leaves with a distinct purplish-red midrib on the

46   abaxial surface. Our morphological and molecular evidence strongly supported this population as a

47   new *Lycoris* species.

Materials and methods

49   Total genomic DNAs were extracted from 15mg of silica gel dried leaves using a modified

50   CTAB method (Li et al. 2013). The library was prepared at the Molecular Biology Experiment

51   Center, Germplasm Bank of Wild Species in Southwest China using a NEBNext® Ultra™ II DNA

52   Library Prep Kit (New England Biolabs, Ipswich, MA, USA). The paired-end (150 bp) reads have

53   been generated on the HiSeq 2500 (Illumina, Inc., San Diego, CA, USA) platform in Beijing

54   Genomics Institution (BGI) (Shenzhen, China), ca. 8 GB of raw data for this new species. The raw

55   reads have been deposited in the NCBI Sequence Read Archive in the BioProject (PRJNA857321)

56   with the Run number SRR20072320.

57   The raw data generated from the Illumina platform was trimmed by Trimmomatic v.0.40

58   (Bolger et al. 2014) with the default parameters. The clean data has been checked by FastQC

59   (Andrews 2010) for quality control. We used the successive assembly approach (Zhang et al. 2015),

60   combining the reference-based and the de novo assembly methods to assemble the chloroplast

61   genome; this method has been performed well in various angiosperm lineages (e.g., Liu et al. 2019,

62   2020a, 2020b, 2021, 2022; Wang et al. 2020). We annotated the assembled chloroplast genome with

63   two reference genomes (MK353216 and MH118290) downloaded from NCBI, and checked the start

64   and stop codons carefully by translating the coding sequences of plastome into proteins in Geneious

65   Prime (Kearse et al. 2012). We also verified the boundary of two reverse complementary repeats in

66   the plastome using Find Repeats embedded in Geneious Prime (Kearse et al. 2012). The assembled

67   chloroplast genome has been submitted to GenBank with the accession number ON960856. The gene

68   map of the new species *Lycoris longifolia* chloroplast genome was drawn by

69   OrganellarGenomeDRAW (OGDRAW) version 1.3.1 (Greiner et al. 2019).

70   We downloaded 15 chloroplast genomes from GenBank as the ingroup and *Narcissus poeticus*

71   L. as the outgroup for phylogenomic analysis. Given the potential effect of the missing data for the

72   accurate phylogenetic inference, we used the whole plastome (WP) and 78 coding sequences (CDS)

73   to estimate the phylogeny, respectively. Because of the nearly identical sequence of two inverted

74   repeats (IR) in plastomes, We only included one repeat of IR region for downstream WP analyses.

75   Each CDS sequence extracted separately by Geneious Prime and the WP matrix were aligned with

76   MAFFT v. 7.480 (Nakamura et al. 2018) with default parameters. The whole plastome alignment

77   was trimmed using trimAL v1.2 (Capella-Gutiérrez et al. 2009) with a heuristic method to decide on

78   the best-automated method. All 78 CDS sequences of each plastome were concatenated by AMAS

79   (Borowiec 2016). The best-fit partitioning schemes and/or nucleotide substitution models for the 78

80   CDS sequences were estimated using PartitionFinder2 (Stamatakis 2006; Lanfear et al. 2016), under

81   the corrected Akaike information criterion (AICc) and linked branch lengths, as well as with rcluster

82   (Lanfear et al. 2014) algorithm options. The resulting optimal partitioning schemes and evolutionary

83   model for each CDS sequence were applied for the following tree inference. We used IQ-TREE2 v.

84   2.1.3 (Minh et al. 2020) with 1000 SH-aLRT and the ultrafast bootstrap replicates and RAxML

85   8.2.12 (Stamatakis 2014) with GTRGAMMA model for each partition and clade support assessed

86   with 200 rapid BS replicates for the Maximum Likelihood (ML) analysis. The BI was performed

87   with MrBayes 3.2.7 (Ronquist et al. 2012). The Markov Chain Monte Carlo (MCMC) analyses were

88   run for 10,000,000 generations. The stationarity was regarded to be reached when the average

89   standard deviation of split frequencies remained below 0.01. Trees were sampled at every 1,000

90   generations, and the first 25% of samples were discarded as burn-in. The remaining trees were used

91   to build a 50% majority-rule consensus tree. Considering the possible different evolutionary forces in

92   the chloroplast genome, we also used ASTRAL-III (Zhang et al. 2018) for estimating a coalescent-

93   based species tree based on the 78 CDS sequences.

94   **Results**

95   The four ML trees (Figure 1, S1, S3, S4), two Bayesian trees (Figure S2, S5), and the species

96   tree (Figure S6) resulted in a consistent phylogenetic position, and this new species, *Lycoris*

97   *longifolia*, formed a separate clade (Figure 1). This result showed that this new species has been

98   distant from other species in *Lycoris*. The examined morphological characters, long leaves and

99   purplish-red midrib abaxially, also supported its distinguished status.

Taxonomy

Lycoris longifolia L.H.Lou, **sp. nov.**

Chinese name: 长叶石蒜

Figure 2, 3

Diagnosis. Most similar to *L. aurea* but differs from it by markedly longer leaves, abaxially with a distinct purplish-red midrib.

Type. CHINA. Sichuan: Ya'an, Yucheng, Bifengxia, Houyancun, Yanjiashan, under the shrub along the stream, elevation ca. 950 m, 10 May 2021, *L.H. Lou & Y.L. Lou 8765* (holotype PE [barcode 02347459]!; isotypes KUN!, PE [barcode 02347457]!).

   Additional Specimens examined. CHINA. Sichuan: Ya'an, Yucheng, Bifengxia, Houyancun, Yanjiashan, under the shrub along the stream, elevation ca. 950 m, 30 July 2021, *L.H. Lou & Y.L. Lou 8766* (paratype PE [barcode 02347458]!).

   Description. Bulbous perennial. Bulbs subglobose, 3–6 cm diam., tunics membranous, dark brown. Leaves ligulate, acute at the apex, ca. 80–120 × 1.5–2 cm, absent at the flowering time and appearing in autumn, dark green, with a prominent midrib on the abaxial surface, abaxial midrib distinctly purplish-red. Inflorescence scapose, umbellate; scape solid, 70–75 cm long, ca. 2.0 cm diam. at base, light green with purplish-red base; involucral bracts 2, lanceolate, 5.0-9.0 cm long by 1.8 cm wide at base, membranous, light green; bracteoles membranous, lanceolate, 1.0–4.0 cm long. Flowers 5–7 per umbel; pedicels 2–2.5 cm long; perianth with 6 tepals; tube ca. 1.5 cm; lobes yellow, abaxially with white mid-vein, strongly recurved, narrowly oblanceolate, ca. 7 × 0.8–1.0 cm, margin strongly undulate. Stamen filaments 6, creamy-yellow, slightly longer than perianth; anther light purplish, dorsifixed, 8–10 mm long before anthesis. Style creamy-yellow but rose-red at apex, slightly exceeding filaments; stigma purplish-red; ovary green, ovoid, ca. 5 mm long.

   Phenology. Scape produced from July to August, and vegetative growth from September to May next year.

   Etymology. The specific epithet refers to the distinguished leaf blade of this new species.

   Distribution. This new species has been narrowly discovered in Ya'an, Sichuan, China. Some localities of Southwestern China have been poorly discovered, and a comprehensive floristic

128   investigation will help elucidate the germplasm resources.

129

130   Key to the species of *Lycoris* in China

131   1. Flowers actinomorphic…………………………………………………………….2

132   1. Flowers zygomorphic…………………………………………………………….6

133   2. Margin of perianth lobes not undulate……………………………………………3

134   2. Margin of perianth lobes basally minutely undulate…………………..………………4

135   3. Perianth pale purple but apically blue…………………………..……..…………*L. sprengeri*

136   3. Perianth white or yellow……………………………………………………*L. longituba*

137   4. Perianth purple………………………………………………..……………*L. squamigera*

138   4. Perianth not purple……………………………………………………….…5

139   5. Perianth yellow………………………………………………..…………*L. anhuiensis*

140   5. Perianth white, abaxially with purple midvein…………………………..…………*L. incarnata*

141   6. Leaves appearing in autumn…………………………………………………..7

142   6. Leaves appearing in spring…………………………………………………...………15

143   7. Perianth yellow or ocher-yellow…………………………………………………8

144   7. Perianth bright red, deep red, rose-red, or white……………………………………………11

145   8. Perianth yellow; leaves 1.5–5 cm wide……………………………………...………9

146   8. Perianth ocher-yellow; leaves 1.0–1.5 cm wide………………………………………10

147   9. Leaves ensiform, ca. 60 × 2–5 cm………………………..……………………*L. aurea*

148   9. Leaves ligulate, ca. 100 × 1.5–2 cm………………………………………….…*L. longifolia*

149   10. Leaves ensiform, apex acuminate…………………………………….…………*L. straminea*

150   10. Leaves ligulate, apex obtuse………………………………………………*L. hunanensis*

151   11. Perianth bright red, deep red, or rose-red…………………………………………12

152   11. Perianth white………………………………………………………*L. houdyshelii*

153   12. Perianth bright red or deep red, lobes strongly recurved…………….…………….…..13

154   12. Perianth rose-red, lobes slightly recurved…………………………………………14

155   13. Perianth bright red……………………………….…………………………*L. radiata*

156   13. Perianth deep red with white but faintly pale red filaments…………………….…*L. hubeiensis*

157   14. Leaves ligulate, ca. 0.8 cm wide………………………………………..…………*L. rosea*

158   14. Leaves narrowly ligulate, ca. 0.5 cm wide……………………………….………*L. wulingensis*

159   15. Perianth white…………………………………………………………………………16

160   16. Perianth white without pink stripes……………………………………………*L. caldwellii*

161   16. Perianth white with pink stripes…………………………………………………*L. shaanxiensis*

162   15. Perianth yellow or orange-red……………………………………………………………..17

163   17. Perianth yellow in bud, becoming orange-red as buds develop……………….……*L. tsinlingensis*

164   17. Perianth yellow…………………………………………………………………...……18

165   18. Perianth lobes without red stripes…………………………………………..……*L. chinensis*

166   18. Perianth lobes abaxially with red stripes……………………………………..…*L. guangxiensis*

167   ## Acknowledgements

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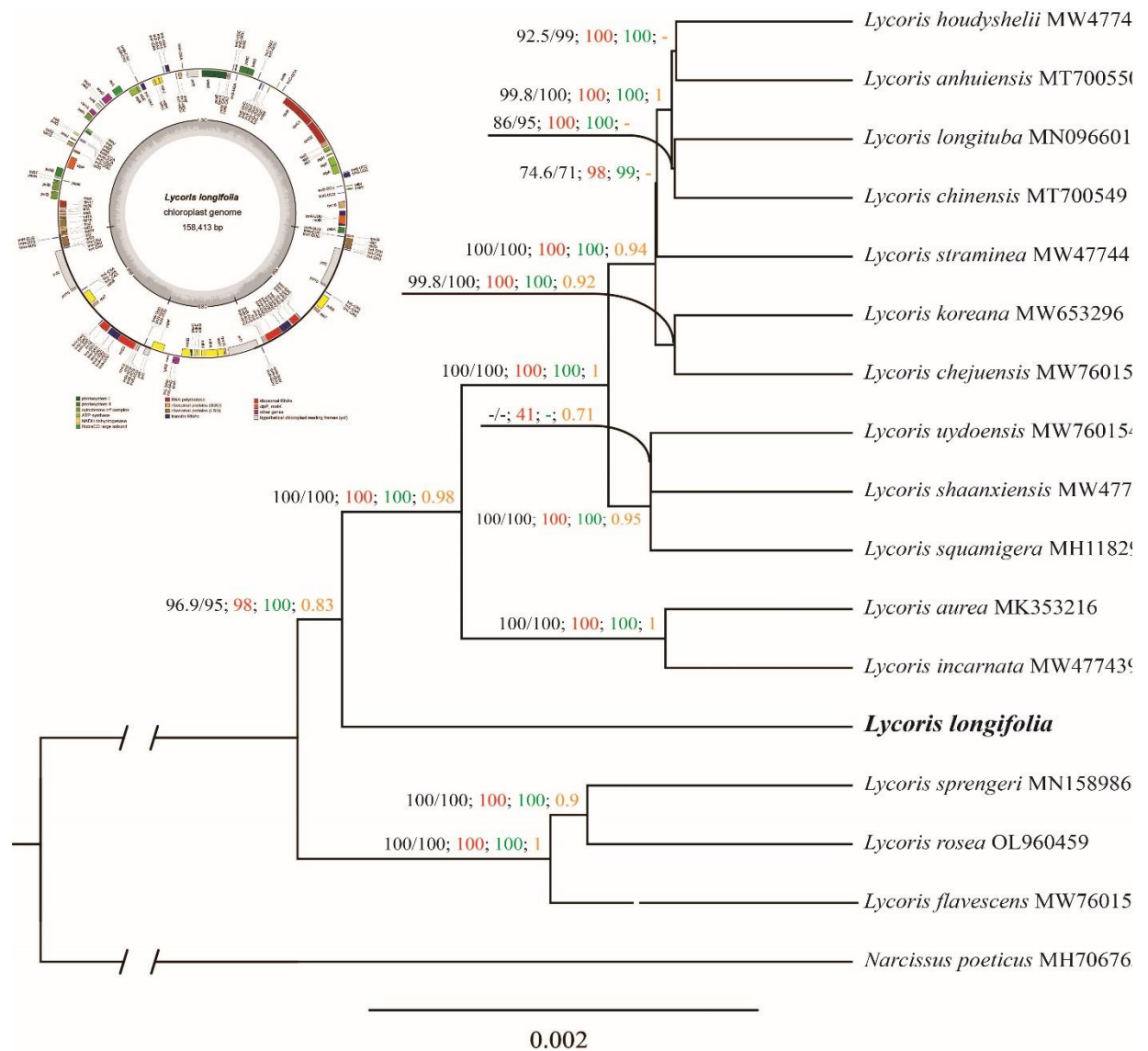258   new species of Amaryllidaceae from Hunan, China. PhytoKeys 177: 1-9.

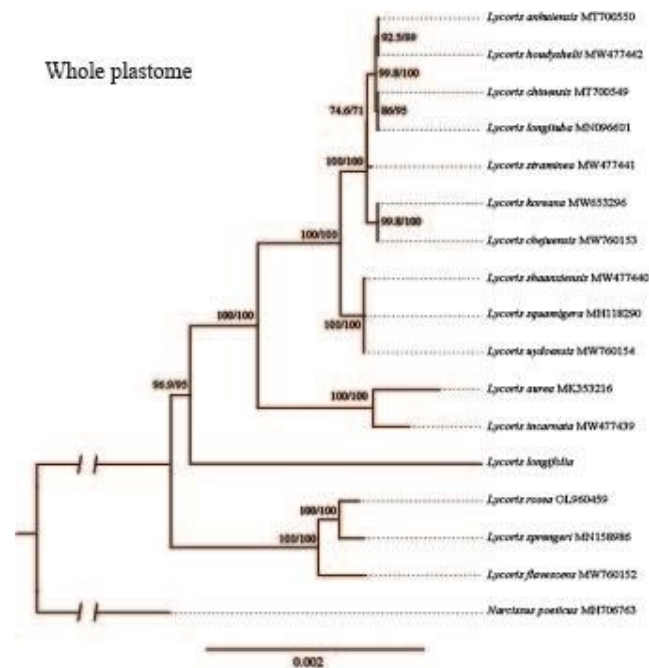259   https://dx.doi.org/10.3897/phytokeys.177.62741

260

Figure 1. Maximum likelihood phylogeny of *Lycoris* inferred from RAxML analysis of the whole plastome data. Numbers above the branches 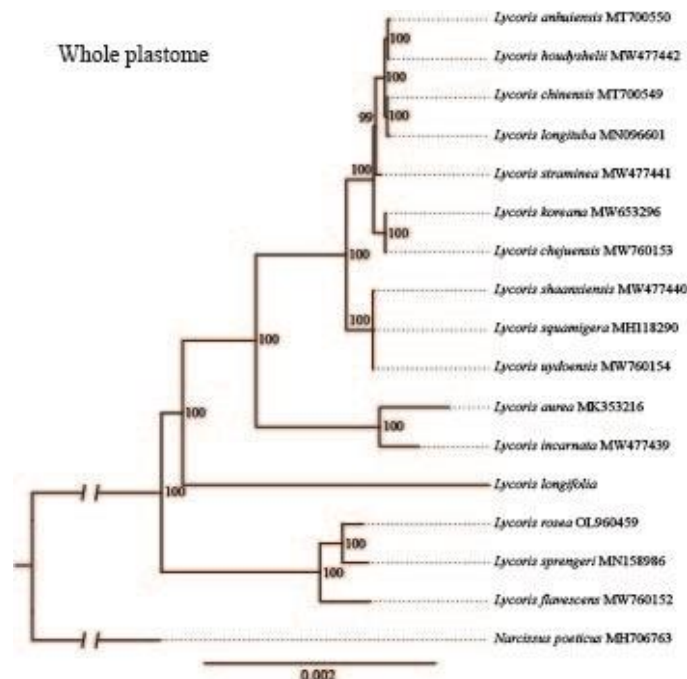indicate the SH-aLRT support and Ultrafast Bootstrap support (black) by IQ-TREE2, the bootstrap support (red) by RAxML, the posterior probabilities (green) by MrBayes 3, and the local posterior possibility (orange) by ASTRAL-III. The upper-left inset was a gene map of the new species *Lycoris longifolia* chloroplast genome.

Figure 2. Field photos of *Lycoris longifolia*. **A-C** flowers **D** bulb **E** the distinct purplish-red midrib abaxially **F** vegetative growth period, showing the long leaves.

Figure 3. Illustration of *Lycoris longifolia*, drawn by Ai-Li Li (PE).
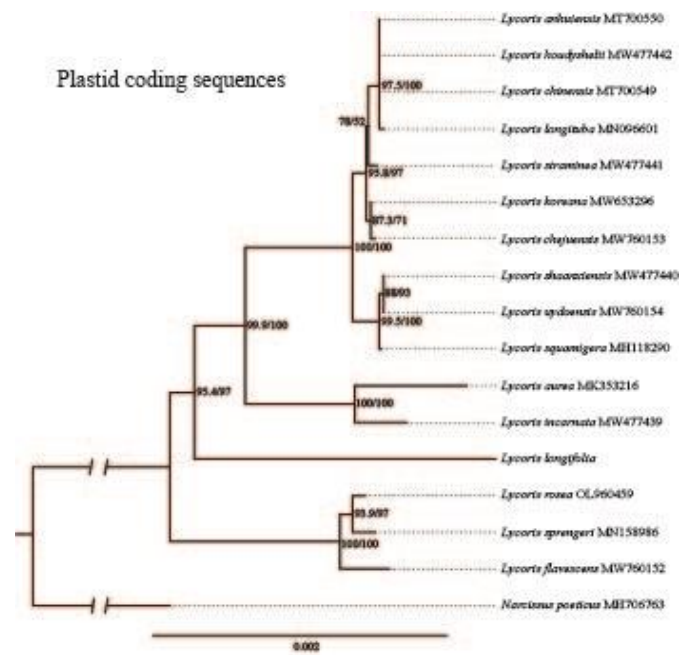
261

262   Supplementary material

Figure S1. Maximum likelihood phylogeny of *Lycoris* inferred from IQ-TREE2 analysis of the whole plastome data.

Numbers above the branches indicate the SH-aLRT support and Ultrafast Bootstrap support.

Figure S2. Bayesian inference phylogeny of *Lycoris* inferred from MrBayes 3 analysis of the complete chloroplast genome data.

Numbers above the branches indicate the posterior probabilities (PP).

Figure S3. Maximum likelihood phylogeny of *Lycoris* inferred from IQ-TREE2 analysis of

the concatenated 78 plastid coding genes.

Numbers above the branches indicate the SH-aLRT support and Ultrafast Bootstrap support.
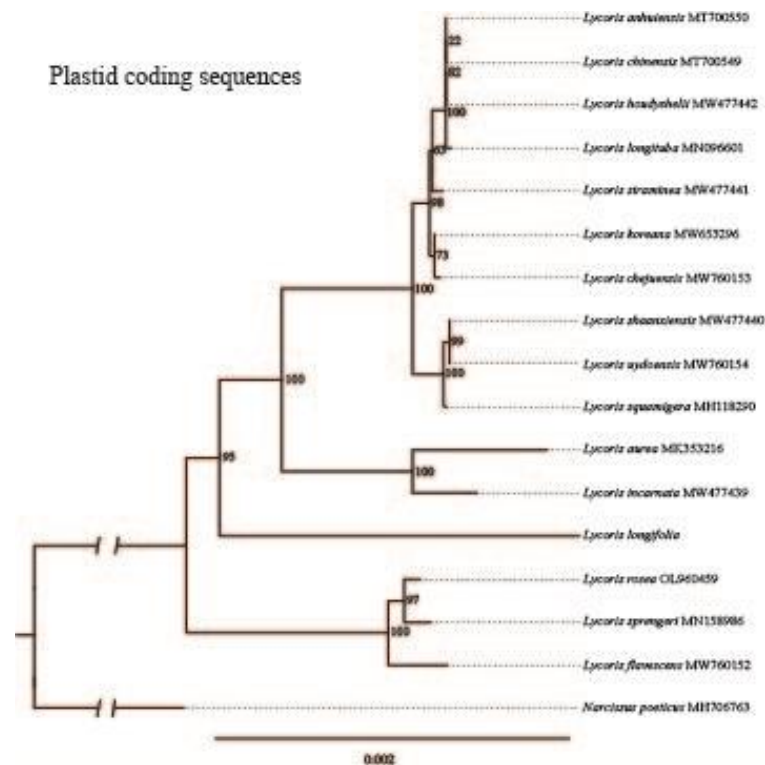

Figure S4. Maximum likelihood phylogeny of *Lycoris* inferred from RAxML analysis of the

concatenated 78 plastid coding genes.

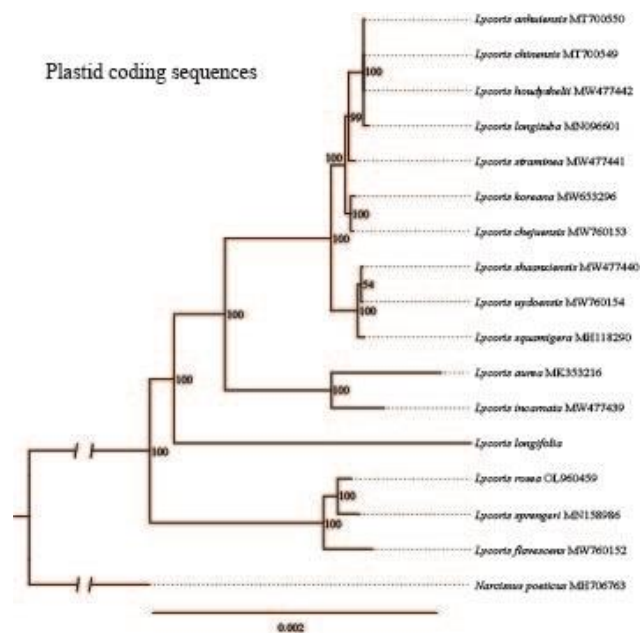Numbers above the branches indicate the bootstrap support.

Figure S5. Bayesian inference phylogeny of *Lycoris* inferred from MrBayes 3 analysis of the complete chloroplast genome data.

Numbers above the branches indicate the posterior probabilities (PP).
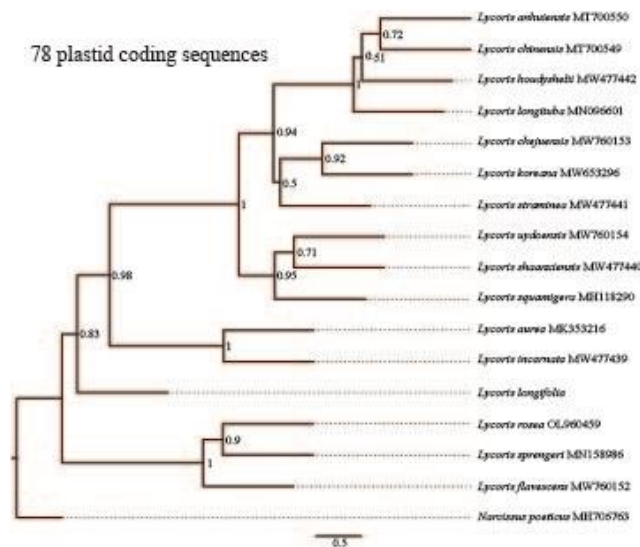

Figure S6. Species tree of *Lycoris* inferred from ASTRAL-III of the concatenated 78 plastid coding genes.

Numbers above the branches indicate the branch support values measuring the support for a local posterior possibility.

263