RESEARCH ARTICLE

Plastome sequencing reveals phylogenetic relationships among *Comastoma* and related taxa (Gentianaceae) from the Qinghai-Tibetan Plateau

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Abstract

Genus *Comastoma* (subt. Swertiinae, Gentianaceae) contains species, such as "Zangyinchen," that are important herbs in Tibetan medicine. The phylogenetic relationship of this within Gentianaceae and the circumscriptions of its species have long been controversial with conflicting morphological and molecular data reported. Here, we used whole chloroplast genome sequences for *Comastoma* species and related taxa to reconstruct their phylogeny and clarify their taxonomic relationships. The results revealed that the length of all plastome sequenced varied from 149 to 151 kb and have high similarity in structure and gene content. Phylogenomic analysis showed that *Comastoma* is a monophyletic group, closely related to the genus *Lomatogonium*. The divergence time estimation showed that Gentianaceae diverged at about 21.81 Ma, while the split of *Comastoma* occurred at 7.70 Ma. However, the results suggested the crown age of species formation in this genus is after 4.19 Ma. Our results suggest that QTP uplift, the alternation of Quaternary glaciation and interglaciation, and monsoon changes might have acted as drivers of speciation in *Comastoma*.

KEYWORDS
chloroplast, *Comastoma*, Gentianaceae, phylogeny, plastome, Qinghai-Tibetan Plateau

Yu Zhang and Jingya Yu contributed equally to this work.

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1 | INTRODUCTION

As one of the most important global biodiversity hotspots, the Qinghai-Tibetan Plateau (QTP) and its adjacent regions harbor the world’s richest recently diverged flora with high endemcity (Khan et al., 2018; Wu, 1988). The QTP is an important center of origin for many alpine taxa (Liu et al., 2002; Ren et al., 2015; Zhang et al., 2012) where lineages exhibit accelerated evolution as a consequence of the region’s complex geological history (Muellner-Riehl, 2019; Spicer, 2017). Gentianaceae is mainly distributed in cold temperate regions and comprises ~80 genera and ~700 species worldwide (Ho & James, 1995). As the biogeographical source area for several large alpine lineages, the QTP mountains host 22 genera and about 419 species (Ebersbach et al., 2017; Favre et al., 2016; Ho & Liu, 2001). Of these, the genus Comastoma (Gentianaceae) has about 15 species distributed in Asia, Europe, and North America. However, 11 of their species are only confined to the southwest and northwest of China. For local inhabitants in the QTP, Comastoma is one of the original plants of “Zangyinchen,” which are important traditional Tibetan medicine, widely used to treat hepatitis, liver fibrosis, and cholecystitis (Tang Li et al., 2008, 2020). Comastoma is named for the hairy bases of its corolla lobes, which has adaptive significance for reproductive success in the harsh environment of alpine regions (Zhang et al., 2018). While there have been many taxonomic and systematic treatments of Gentianaceae, the origin and phylogenetic position of Comastoma remains controversial (Hagen & Kadereit, 2002; Kissling et al., 2009; Schonswetter et al., 2004; Yuan & Kupfer, 1995). For example, Yuan and Kupfer (1995) divided the subtribe Gentianinae into two independent evolutionary branches as Gentiana and Gentianella, placing Comastoma in the second branch and suggested that Comastoma is monophyletic (Yuan & Kupfer, 1995). Furthermore, recent molecular phylogenetic surveys revealed Comastoma, Lomatogonium, and Gentianella are not monophyletic and are located in one more derived clades (Xi et al., 2014).

Further, results from Xi et al. showed Lomatogonium and Gentianella are on the same evolutionary branch which is corroborated by evidence that these genera can cross with each other (2014). Xi et al.’s (2014) results were parallel to Liu and Ho (1996), who hypothesized that Comastoma has the closest relationship with Gentianella based on the embryological characteristics (Xi et al., 2014). Toyokuni, however, considered Comastoma as a genus most closely related to Lomatogonium and distant from Gentiana and Gentianella (1961). This hypothesized relationship between Comastoma and Lomatogonium has been supported by molecular phylogenetic investigations (Chassot et al., 2001). Similarly, based on flower morphological characteristics, Wu et al. (2003) treated Comastoma as a separate genus and suggested that it is more primitive than Gentianella in phylogenetic position and started a new debate about its evolutionary history and position.

Most of the phylogenetic hypotheses about Comastoma evolution have been based on a few chloroplast markers as well as the ITS region and some morphological characters (Toyokuni, 1961; Wu et al., 2003; Xi et al., 2014). These molecular phylogenies used noncoding plastid regions with uniparental inheritance to infer the true species tree for groups with complex evolutionary histories (Hung et al., 2009; Valcárcel et al., 2003). Unfortunately, the most variable regions of the chloroplast genome were not known and even the most informative plastid regions did not have the resolving power of low-copy nuclear markers (Shaw et al., 2007). To compensate for the comparably low variation in plastid genetic markers, whole plastome sequences are required to construct robust species trees (Hollingsworth et al., 2011; McCormack et al., 2013). Studies have proven that whole chloroplast genome sequencing can resolve phylogenetic relationships at various taxonomic levels and while also elucidating the molecular evolution of plastome structure and function (Jansen et al., 2007; Moore et al., 2007, 2010). Such valuable information has shown the effectiveness of full plastome data to resolve broader level questions at family, order, tribal, generic, and species levels (Barrett et al., 2016; Givnish et al., 2016).

Utilizing the high-throughput sequencing technology and advanced statistical tools, we report for the first time a robust phylogenetic and evolutionary history of genus Comastoma based on whole chloroplast sequences. The monophyly of Comastoma was tested in a phylogenetic context; if monophyletic, Comastoma species would form their cloud to the exclusion of species from all other genera. To this end, we sequenced the whole chloroplast genomes of five species of Comastoma and 11 other species representing four genera from Gentianaceae. Also, we included 19 complete chloroplast sequences from the NCBI: as Svertria (4 spp.), Helenia conrniculata, and Gentiana (14 spp.). The final aim is to (1) investigate the functional and structural differences in plastome of Comastoma and its allied taxa and (2) provide a robust phylogeny and evolutionary history of Comastoma.

2 | MATERIALS AND METHODS

2.1 | Species sampling

Fresh leaves from five Comastoma species, each represented with one individual, and 11 other species from the allied genera Gentianella (2 spp.), Gentianopsis (4 spp.), Lomatogonium (4 spp.), and Tripterospermum vulnerabile were sampled from the QTP (Table 1). Of these, species included are those previously showed paraphyly in Comastoma (Xi et al., 2014). The leaves were silica gel dried during the fieldwork and kept at ~20°C until total DNA extraction in the laboratory. Vouchers for all the samples are deposited into Qinghai-Tibetan Plateau Museum of Biology (HNWP), University of Chinese Academy of Sciences. Besides this, 19 species from Gentianaceae are included from the GenBank of NCBI: Svertria (4 spp.), H. conrniculata, and Gentiana (14 spp. Table 1). Species from Apocynaceae, Asclepiadaceae, and Rubiaceae were also downloaded from GenBank as outgroups.
| Species                     | Voucher No.       | Location         | Longitude | Latitude | GenBank Accession No. |
|-----------------------------|-------------------|------------------|-----------|----------|----------------------|
| *Comastoma pedunculatum*    | chensl0546        | Nangqian, QH     | 97°39′   | 34°07′   | MN627282             |
| *Comastoma pulmonarium*     | chensl0683        | Nangqian, QH     | 96°36′   | 32°18′   | MN627286             |
| *Comastoma polycladum*      | chensl0726        | Zaduo, QH        | 95°52′   | 32°56′   | MN627288             |
| *Comastoma jigzhiense*      | chensl0430        | Jiuzhi, QH       | 101°14′  | 33°24′   | MN627283             |
| *Comastoma falcatum*        | chensl0120        | Banma, QH        | 99°02′   | 34°49′   | MK331815             |
| *Gentianella azurea*        | zhang2016495      | Nangqian, QH     | 96°36′   | 32°13′   | MN627289             |
| *Gentianella arenaria*      | zhang2018070      | Zeku, QH         | 101°33′  | 35°04′   | MN627277             |
| *Tripterospermum volubile*  | zhang20120123     | Bomi, XZ         | 95°43′   | 29°37′   | MN627287             |
| *Gentianopsis barbata*      | chensl0629        | Shiqu, SC        | 97°21′   | 32°53′   | MN627280             |
| *Gentianopsis barbata* var. stenocalyx* | chensl0691 | Nangqian, QH     | 96°34′   | 31°52′   | MN627276             |
| *Gentianopsis paludosa*     | chensl0762        | Zaduo, QH        | 95°27′   | 32°51′   | MN627278             |
| *Gentianopsis paludosa* var. aipina* | chensl0448 | Aba, SC          | 102°05′  | 32°44′   | MN627279             |
| *Lomatogonium carinthiacum* | chensl0722        | Zaduo, QH        | 96°01′   | 32°57′   | MN627284             |
| *Lomatogonium gamosepalum*  | chensl0810        | Qumalai, QH      | 95°50′   | 34°01′   | MN627285             |
| *Lomatogonium perenne*      | chensl0383        | Banma, QH        | 100°35′  | 32°49′   | MN627290             |
| *Lomatogonium macranthum*   | chen2014554       | Yushu, QH        | 97°12′   | 32°34′   | MN627281             |
| *Swertia verticillifolia*   | —                 | —                | —         | —        | MF795137             |
| *Swertia mussotii*          | —                 | —                | —         | —        | NC031155             |
| *Swertia hispidalcyx*       | —                 | —                | —         | —        | NC044474             |
| *Swertia bimaculata*        | —                 | —                | —         | —        | MH394372             |
| *Halenia corniculata*       | —                 | —                | —         | —        | NC042674             |
| *Gentiana veitchiorum*      | —                 | —                | —         | —        | NC037985             |
| *Gentiana tibetica*         | —                 | —                | —         | —        | NC030319             |
| *Gentiana straminea*        | —                 | —                | —         | —        | NC027441             |
| *Gentiana stipitata*        | —                 | —                | —         | —        | NC037984             |
| *Gentiana siphonantha*      | —                 | —                | —         | —        | NC039573             |
| *Gentiana robusta*          | —                 | —                | —         | —        | KT159969             |
| *Gentiana ornate*           | —                 | —                | —         | —        | NC037983             |
| *Gentiana oreodoxa*         | —                 | —                | —         | —        | NC037982             |
| *Gentiana officinalis*      | —                 | —                | —         | —        | NC039574             |
| *Gentiana obconica*         | —                 | —                | —         | —        | NC037981             |
| *Gentiana hexaphylla*       | —                 | —                | —         | —        | NC037980             |
| *Gentiana dahurica*         | —                 | —                | —         | —        | MH261259             |
| *Gentiana caelestis*        | —                 | —                | —         | —        | MG192304             |
| *Gentiana crassicaulis*     | —                 | —                | —         | —        | KY595457             |
| *Calotropis procera*        | —                 | —                | —         | —        | NC041440             |
| *Carissa macrocarpa*        | —                 | —                | —         | —        | NC033354             |
| *Dunnia sinensis*           | —                 | —                | —         | —        | NC039965             |
| *Mitragyna speciosa*        | —                 | —                | —         | —        | NC034698             |
| *Emmenopterys henryi*       | —                 | —                | —         | —        | NC036300             |

Note: QH, Qinghai Province, P.R. China; XZ, Xizang Autonomous Regions, P.R. China; SC, Sichuan Province, P.R. China.
2.2 | DNA extraction, sequencing, and bioinformatics

Total genomic DNA was extracted following a modified CTAB protocol (Englen & Kelley, 2000) and was used to prepare Illumina sequencing libraries as described Thomson et al. (2018) and processed on an Illumina NovaSeq 6000 platform (Novogene) with 150 PE chemistry.

About 10 Gb raw data were obtained for each sample and processed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to quality control; ~9 Gb clean reads were retained by Fastp (Chen et al., 2018). The reads were extracted by BWA v0.7.17 (Li & Durbin, 2009) and BLASTed against a reference plastome using BLAST v2.2.25 (Kent, 2002) and assembled by SPAdes v3.15.1 (Bankevich et al., 2012).

The final plastome was annotated by Geseq modular from CHLOROBOX (https://chlorobox.mpimp-golm.mpg.de/geseq.html) and draw support from GENEIOUS (Matthew et al., 2012). We used the online module OGDRAW (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) to draw the circular diagram of plastome. Nucleotide variation and important characteristics (number of genes, genes content, gene loss, and IR border regions) were analyzed using DNASP v5.0 (Librado & Rozas, 2009). All chloroplast genome sequences obtained in this study were submitted to NCBI and assigned GenBank accession numbers (Table 1).

2.3 | Chloroplast phylogenomics and diversification analyses

All sequence alignments were performed with MAFFT v7.471 (Katoh et al., 2019). Two different datasets were used to re-construct Comastoma phylogeny: The first one included the complete genome of chloroplast while the second dataset included only protein-coding sequences. Both the datasets included 35 ingroup and five outgroup species (details below) and were analyzed with maximum likelihood (ML) with IQ-TREE v1.6.10 (Nguyen et al., 2014) and Bayesian inferences (BI) statistics MRBAYES v.3.2.3 (Ronquist & Huelsenbeck, 2003). Optimal substitution models were assessed with jMODELTEST using Akaike information criterion (Nguyen et al., 2014) and Bayesian inferences (BI) statistics MRBAYES v.3.2.3 (Ronquist & Huelsenbeck, 2003). Optimal substitution models were assessed with jMODELTEST using Akaike information criterion (Nguyen et al., 2014; Posada & Buckley, 2004). The ML analysis was performed using the GTR+F+R3 model and 1000 bootstrap replicates. The BI was conducted with two parallel runs of one million MCMCs, sampling after 1000 generations. A consensus tree was estimated after the first 25% of trees were discarded as burn-in. In both ML/BI approaches, the trees were rooted with the outgroups including Calotropis procera (Asclepiadaceae), Carissa macrocarpa (Apocynaceae), Mitragyna speciose, Dunnia sinensis, and Emmenopterys henryi (Rubiaceae).

To assess the evolutionary history, we calibrated the divergence time of Comastoma and its related groups as implemented in BEAST (Drummond & Rambaut, 2007). For this analysis, we only used the whole chloroplast dataset in a concatenated fashion setting GTR as a substitution model with lognormal relaxed clock (Thomas et al., 2007).

We used 45-Ma-old infructescence and fruit fossils recovered for genus Emmenoptery (Rubiaceae) and seed fossil of 5 Ma age of Gentiana. Divergence times have been calibrated with sect. Cruciata (Gentianaceae) as this is the most recent common ancestor of genus Comastoma (Favre et al., 2016; Pirie et al., 2015).

The analysis was followed for 50 million generations with three independent MCMC runs, taking samples after every 5000 generations. We used the program Tracer v1.7.1 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that the ESS (effective sampling size) of each parameter was more than 200. After all parameters converged to exceed 200 ESS, we used Logcombiner v2.5.2 (https://www.onlin.edown.net/soft/84600.htm) to combine all three runs. Finally, we used the program Tree Annotator v2.5.2 (Luo & Zhao, 2011) to generate the tree after burning the first 20% MCMC. The trees were displayed, annotated, and saved with the help of Figtree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

3 | RESULTS

3.1 | Plastome comparison

All 16 sequenced chloroplast genomes displayed a typical quadripartite structure, including a large single-copy region (LSC), a small single-copy region (SSC), and two reverse repeat regions (IRa and IRb; Figure 1; Figure 2). Plastome size varied from 149,001 to 151,699 bp. The species belongs to the genus Comastoma possess 134 genes, including 89 protein-coding genes (PCGs), 37 tRNA, and 8 rRNA genes. The details of protein-coding genes were available in Appendix S1. The number of PCGs in other species ranged from 131 to 133, where excluding 89 protein-coding genes (PCGs), 37 tRNA, and 8 rRNA genes. The number of rRNAs is the same in all species; however, the tRNAs are slightly different, for example, trnS has been lost in nine species (except Comastoma) and rps16 has been lost in nine species (except Comastoma) and rpl33 in three species (Gentianella aurea, Gentianella arenaria, and T. volubile). Similarly, the number of rRNAs is the same in all species; however, the tRNAs are slightly different, for example, trnSCU was lost in Gentianopsis paludosum and Gentianopsis barbata; and trnAUC was lost in T. volubile. Our results found that the variation of plastome length is mainly due to the expansion and extraction of LSC and SSC regions. The IR regions were relatively conserved (Figure 3).

3.2 | Phylogenomics and diversification time

Both the ML and BI statistics recovered trees with the same topologies based on the whole chloroplast genome. Similarly, we recovered congruent trees based on only the protein-coding sequence data. In all results, Comastoma species formed a clade sister to a couple of Lomatogonium species; together these taxa were sister to a clade containing other Lomatogonium species with Gentianella (Figure 4). The
FIGURE 1  Showing comparison of junctions between the quadripartite structure in the LSC, SSC, and IR regions among 16 species. Distance in the figure is not to scale.
basal node of this clade was highly supported in both ML and BI reconstructions, suggesting Comastoma shares a common ancestor with genus Lomatogonium. Depending on the subset of data used, there were subtle inconsistencies in support for some branches and conflicting relationships among Comastoma species. In the ML/BI trees based on the whole chloroplast genome sequence, Comastoma pedunculatum has the closest relationship with C. polycladum, and then, these two species clustered together with Comastoma falcatum, while C. pedunculatum first aggregated with C. falcatum and then C. polycladum in the ML/BI trees using the protein-coding sequence. The robustness of the phylogenies of each branch is relatively high above 90%.

The genus Gentianopsis is the most basal lineage followed by Halenia corniculata and then Swertia. The relationship among the four species in Swertia is relatively complex, for example, Swertia bimaculata has the closest relationship with H. corniculata rather than with another three species in Swertia. All the 14 species of Gentiana clustered in the same lineage with the species T. volubile (Figure 4). Time to the most recent common ancestor for all the 35 species of Gentianaceae included coalesced at 21.81 Ma (Figure 5). The most basal genus Gentianopsis diverged at about 17.89 Ma, followed by Swertia, Halenia, Gentianella, Lomatogonium, and Comastoma. The genus Comastoma diverged from its allied genus Lomatogonium at 7.70 Ma and is the most recently evolved group. Also,
Comasatoma had similar plastome structures, there were distinctions among Comastoma and its close relatives, such as gene number and the rps16 pseudogene. According to different studies (Oxelman et al., 1996; Roy et al., 2010; Wallander & Albert, 2000; Wanntorp & Källersjö, 2002), the rps16 is in an intergenic region or intron non-coding region, where the evolution rate is much faster than most of the genes in the chloroplast. Therefore, we suggest that this region can be used in the systematic evolution and the kinship investigation of Gentianaceae. Similarly, rpl33 was lost from G. azurea, G. arenaria, and T. volubile. This phenomenon might be based on two reasons: (1) rpl33 transferred from chloroplast genome to nuclear genome and (2) this gene is functionally nonessential. According to relevant research on rpl33, this gene could maintain enough plastid translation ability in cold environments (Rogalski et al., 2008). Most of the species in Gentianaceae grow in alpine areas with an average altitude of 4000 m, where the climate is relatively cold throughout the year. In the light of our results and previous studies, rpl33 exists in most of Gentianaceae members with wide distribution and large numbers of species. In contrast, rpl33 is not as common in less species, like Gentianella and Tripterospermum. Therefore, the gene rpl33 may confer greater tolerance of cold climates; this explains the relatively smaller number of Gentianella and Tripterospermum on QTP. Similarly, in a study of the Tibetan herbs Swertia hispidiclyx, Gentiana lhassica, and Halenia elliptica, trnS^GCU sequencing was suggested to be the best strategy for genetic diversity analysis and molecular identification (Ni et al., 2015). In our study, we found the loss of trnS^GCU in G. paludosa and G. barbata plastomes, but not G. paludosa var. aipina and G. barbata var. stenocalyx. We can explore more
about the importance of trnS

4.2 | Phylogenomics of Comastoma

We found phylogenies based on whole chloroplast sequence data was more consistent and had higher bootstrap support values than using only CDS data. However, all the results showed Comastoma as one monophyletic group. Our result supports previous studies based on ITS sequences and embryological characteristics (Liu & Ho, 1996; Yuan & Kupfer, 1995). Our results are in contrast with Xi et al.'s results (Xi et al., 2014), where they found Comastoma as polyphyletic. In (Xi et al., 2014), ITS-based phylogeny showed C. pedunculatum in one cluster but matK phylogeny recovered C. pedunculatum and C. polycladum with other groups as polyphyletic.

Despite our results substantiated the initial hypothesis that the genus is monophyletic by clustering all their five species, the interspecific relationship of species within the genus Comastoma showed inconsistencies based on different datasets. For example, C. pedunculatum, C. falcatum, and C. polycladum from a clade, but it is not clear whether C. falcatum or C. polycladum clustered with C. pedunculatum to the exclusion of the other. This distinction can be explained for two reasons. Firstly, evolutionary rates of coding and noncoding sequences vary and may contribute to discordant topologies (Tian & Li, 2002). Although some species Lomatogonium and Swertia for a clade in this study, more complete sampling of these genera are needed to fully understand the polyphyly of these genera. Secondly, we have included only one individual per species, which might lead to the inconsistency of results. This study supports that Comastoma is monophyletic, but there are not enough Comastoma species in this study, the clear phylogeny of Comastoma and Gentiana needs more in-depth study in the future.
The overall phylogeny of different groups of Gentianaceae revealed Comastoma clustering with two species in Lomatogonium (L. perenne and L. macranthum) in a more recently evolved branch. Four species in Gentianopsis diverged earlier, so they have a more distant relationship with Comastoma than Lomatogonium. Interestingly, L. gamosepalum and L. carinthiacum clustered together with two species of Gentianella in the ML/BI tree (Figure 4a,b). Similarly, four species of Swertia not clustered in one group, for example, S. bimaculata has a closer sister relationship with H. corniculata rather than with the other three Swertia species. Although some species in Lomatogonium and Swertia for a clade in this study, more complete sampling of these genera is needed to fully understand the polyphyly of these genera. The 14 species of Gentiana formed a well-supported clade. The overall topology of sect. Kudoa and sect. Cruciata is almost consistent with Sun’s (2018) and Zhou’s (2018) results. Support values for our topology were greater than those previously published (Sun et al., 2018; Xi et al., 2014; Zhou et al., 2018) because our whole plastome dataset includes vastly more phylogenetically informative characters.

4.3 | Calibrated divergence time of Comastoma

The origin, distribution, and differentiation of many species have a close association with the change in the geology of the QTP (Ebersbach et al., 2017; Ren et al., 2015; Wang et al., 2009). Comastoma and its related groups are mainly distributed on the QTP. The uplift of the QTP is mainly divided into three stages. Based on the molecular clock hypothesis, the differentiation time of Gentianaceae has been estimated to be about 21.87 Ma and the
The whole chloroplast genomes of Comastoma and its related taxa were sequenced for the first time to establish a robust hypothesis about the monophyly of this genus and its evolutionary relationships with other Gentianaceae. Analyzing our plastomes with 19 others from NCBI revealed the similarity of structure and content for our 16 species, underscoring the recent diversification of this lineage. Our results suggested that Comastoma is monophyletic and has the closest relationship with Lomatogonium through both ML and BI tree reconstruction. We further concluded that combined with the relevant geological and historical events, the uplift of the QTP, the alternation of Quaternary glaciation and interglaciation, and the change of monsoon may have created conditions inductive for speciation in the common ancestors of extant Comastoma. Additionally, we found that the whole chloroplast genome sequencing is an excellent strategy to better resolve the phylogeny of historically enigmatic groups at deep and recent divergences. We have substantiated the hypothesis of monophyly in Comastoma and suggest that such types of studies will provide helpful insight into solving similar problems in other groups.

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**AUTHOR CONTRIBUTIONS**

Yu Zhang: Data curation (equal); Formal analysis (equal); Investigation (equal); Visualization (equal); Writing-original draft (lead). Jingya Yu: Data curation (equal); Investigation (equal); Visualization (equal); Writing-review & editing (lead). Mingze Xia: Formal analysis (equal); Investigation (equal); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal). Xiaofeng Chi: Data curation (equal); Investigation (equal). Gulzar Khan: Formal analysis (equal); Investigation (equal); Writing-review & editing (supporting). Shilong Chen: Conceptualization (lead); Data curation (equal); Investigation (equal); Resources (equal); Supervision (equal); Writing-original draft (supporting). Faqi Zhang: Conceptualization (lead); Investigation (lead); Resources (supporting); Supervision (lead); Writing-original draft (supporting); Writing-review & editing (supporting).

**DATA AVAILABILITY STATEMENT**

DNA sequences: GenBank accessions MN627276–MN627290, MK331815.

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**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

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