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# Phylogeny of drepanosiphine aphids sensu lato (Hemiptera, Aphidoidea) inferred from molecular and morphological data

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## Abstract

As the second largest and most diverse group in the superfamily Aphidoidea, the phylogeny of drepanosiphine aphids sensu lato (s.l.) is critical for discussing the evolution of aphids. However, the taxa composition and phylogenetic relationships of drepanosiphine aphids s.l. have not been fully elucidated to date. In this study, based on total-evidence analyses combining 4 molecular genes (3 mitochondrial, *COI*, *tRNA-Leu/COII*, and *CytB*; 1 nuclear, *EF-1*α) and 64 morphological and biological characteristics, the phylogeny of this group was reconstructed for the first time at the subfamily level using different datasets, parsimonies and model-based methods. All of our phylogenetic inferences clearly indicated that the drepanosiphine aphids s.l. was not a monophyletic group and seemed to support the division of the drepanosiphine aphids s.l. into different groups classified at the subfamily level. Calaphidinae was also not a monophyletic group, and Saltusaphidinae was nested within this subfamily. Drepanosiphinae was not clustered with Chaitophorinae, which was inconsistent with the previous hypothesis of a close relationship between them, illustrating that their phylogeny remains controversial. Overall, some groups of drepanosiphine aphids s.l., including Phyllaphidinae, Macropodaphidinae, Pterastheniinae, Lizeriinae, Drepanosiphinae, Spicaphidinae, Saltusaphidinae, and Calaphidinae, clustered together and might constitute the actual drepanosiphine aphids s.l. To a certain extent, our results clarified the phylogenetic relationships among drepanosiphine aphids s.l. and confirmed their taxonomic status as subfamilies.

**Key words**: Calaphidinae, Drepanosiphidae, phylogenetics, Saltusaphidinae, total-evidence analyses

Drepanosiphine aphids sensu lato (s.l.), which is one of the largest and most diverse groups in the superfamily Aphidoidea, are characterized by developed dorsal processes of the body, typical knobbed cauda, and usually emarginate or bilobed anal plates (Qiao et al. 2005) or wishbone-shaped stiffening at the base rostrum in most species (Heie and Wegierek 2009a). These aphids comprise 13 subfamilies (Qiao et al. 2005), 96 genera and ~600 recognized species (Favret 2020, http://Aphid.SpeciesFile.org) (Figure 1). This group is distributed worldwide, in almost all zoogeographical regions except Antarctica, but the north temperate faunas are more diverse than those of other regions (Du et al. 2020). Their life history is monoecious holocyclic, with the sexual phase and parthenogenetic generations occurring on the same or closely related plants. Most species are solitarious, and they usually exhibit alate forms. They feed on a wide variety of host plants, mainly woody plants, although some species infest herbaceous plants of Fabaceae and Poaceae (Quednau

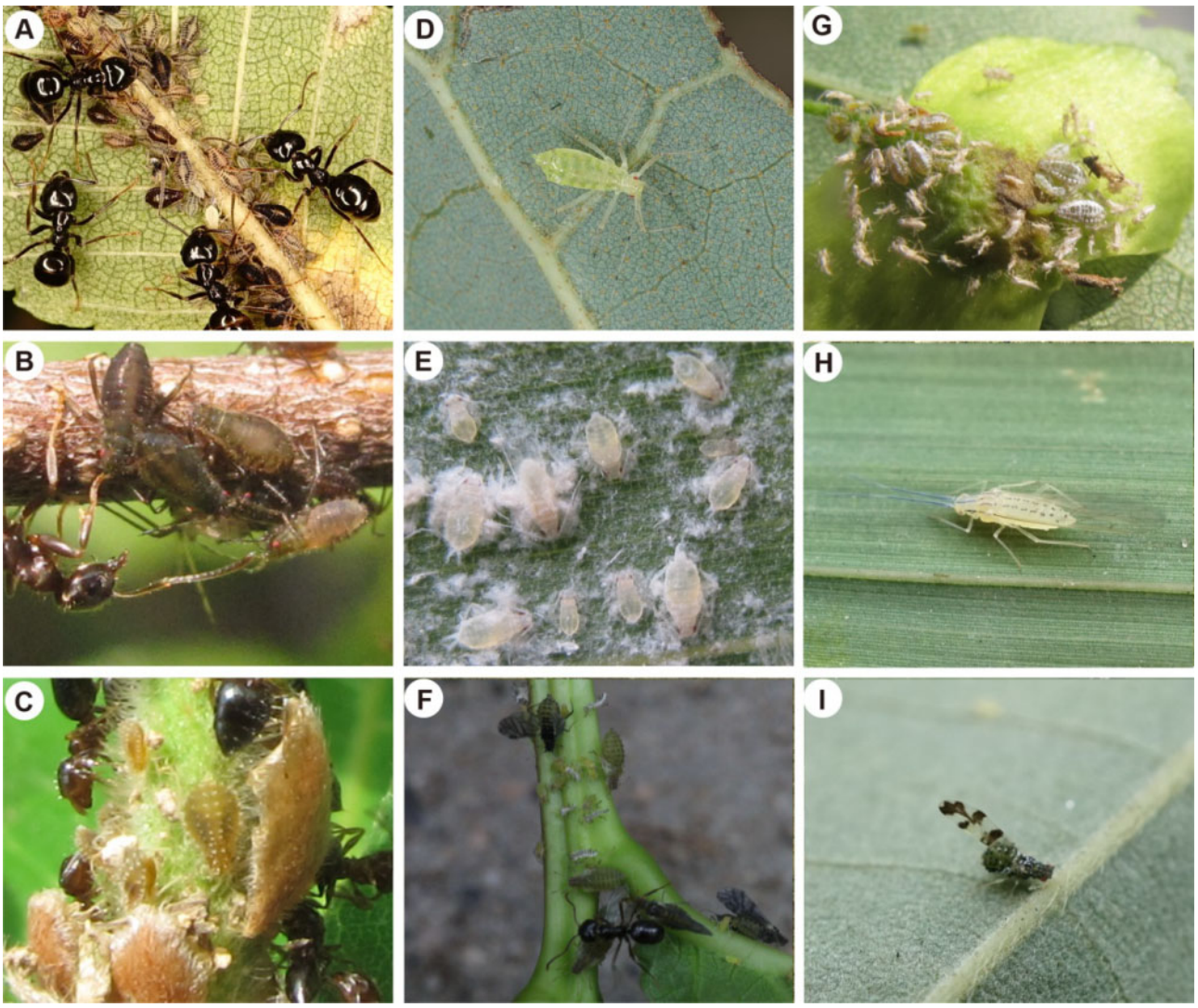


**Figure 1.** Pictures of representative species of Calaphidinae in the field. (A) *Sinochaitophorus maoi* (Takahashi). (B) *Symydobius carefasciatus* (Qiao and Zhang). (C) *Tuberculatus indicus* (L.K. Ghosh). (D) *Taoia chuansiensis* (Tao). (E) *Phyllaphoides bambusicola* Takahashi. (F) *Wanyucallis amblyopappos* (Zhang and Zhang). (G) *Shivaphis pteroceltis* Jiang, An, Li, and Qiao. (H) T*akecallis arundinariae* (Essig). (I) *Tuberculatus margituberculatus* (Zhang and Zhong). Photo Credit: Congcong Du and Rui Chen, Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology.

1999, 2003, 2010; Blackman and Eastop 2020). Many species of this group are economically important pests in agriculture, forestry, and horticulture, such as *Therioaphis trifolii* (Monell), *Melanocallis caryefoliae* (Davis), *Monellia caryella* (Fitch), *Sarucallis kahawaluokalani* (Kirkaldy), and *Shivaphis celti* Das (Stern et al. 1959; Wood et al. 1997; Halbert and Choate Pm 1998; Cottrell et al. 2010; Kondo and Cortes 2014). Due to their ecological and economic significance, there is an urgent need to address relevant issues regarding the evolutionary history of this group, such as the phylogenetic relationships within the drepanosiphine aphids s.l., which will be critical for further addressing their evolutionary biology and ecology.

However, the classification of drepanosiphine aphids s.l. is still controversial and unsolved. Previously, taxonomists mainly performed intuitive classification on the basis of general morphology, which caused the classification of drepanosiphine aphids s.l. to vary according to different aphidologists. For example, based on the morphological features of the aphids' external body structure (the number of segments of the antennae, the presence or lack of siphunculata, and the wing vein and setae on body), early on, the drepanosiphine aphids s.l. group (genera: *Callipterus*, *Phyllaphis*, *Drepanosiphum*, and *Chaitophorus* sensu Mordvilko 1908; subtribe: Chaitophorina, Callipterina, and Drepanisiphina sensu Van der Goot 1913; tribe Callipterini sensu Baker 1920; 2 tribes: Chaitophorini and Callipterini sensu Börner 1930) was included in Aphidinae (Mordvilko 1908; Van der Goot 1913; Baker 1920; Börner 1930). Mordvilko (1928) distinguished the subfamily Callipterinae, including 2 tribes, Callipterea and Chaitophorina; Börner (1952), Börner and Heinze (1957), Shaposhnikov (1964) separated the Chaitophorini and Callipterini tribe sensu Börner 1930 into 2 independent families, Chaitophoridae and Callaphididae; however, Bodenheimer and Swirski (1957) once again joined these 2 groups into the family Callipteridae with Chaitophorinae and Callipterinae. Eastop (1966) suggested modifying Callipterinae sensu Mordvilko 1928 to the scientific name Drepanosiphinae, and then Eastop (1977) distinguished 1 family of Aphididae with 12 subfamilies, wherein Chaitophorinae and Drepanosiphinae constituted separate subfamilies. Heie (1980) divided Aphidoidea into 10 families, including Drepanosiphidae and Chaitophoridae, and then Heie (1982)

integrated them together to form the new Drepanosiphidae. Later, Heie (1987) further distinguished 3 subfamilies (Drepanosiphinae, Chaitophorinae, and Phyllaphidinae) within Drepanosiphidae on the basis of phylogenetic features. Zhang and Zhong (1983) also divided Drepanosiphidae and Chaitophoridae into 2 independent families. As knowledge and descriptions of new genera and species improved, more drepanosiphine aphids were found and included in different subfamilies, such as Tamaliinae and Parachaitophorinae (Remaudiere and Stroyan 1984), Pterastheniinae (Remaudiére and Quednau 1988), Neuquenaphidinae and Taiwanaphidinae (Quednau and Remaudière 1994). Finally, Remaudière and Remaudiere (1997) put forward a new classification system in which Aphididae was divided into 25 subfamilies, regarding various drepanosiphine groups as independent subfamilies (Supplementary Figure S9A). In this article, we refer to the Aphididae classification system proposed by Remaudière and Remaudiere (1997). However, under consideration of fossil taxa, Heie and Wegierek (2009a) presented a new classification of all aphids (Supplementary Figure S9B), in which he preferred to put the independent drepanosiphine subfamilies sensu Remaudière and Remaudiere (1997) together into 1 family, Drepanosiphidae, based on apomorphic characteristics, especially wishbone-shaped stiffening at the base rostrum (Supplementary Table S1).

In addition to the taxonomic status, there is also disagreement regarding the taxa composition of drepanosiphine aphids s.l., especially at the level of the subfamily or tribe. For example, in reference to the classification of aphids presented by Remaudière and Remaudiere (1997), different authors have divided drepanosiphine aphids s.l. into 3 (Heie 1987), 12 (Quednau 1999, 2003, 2010; Qiao et al. 2005), and 14 subfamilies (Quednau and Remaudière 1994), respectively (Supplementary Table S1). Quednau (2010) also mentioned that Chaitophorinae, Parachaitophorinae, and Tamalinae could perhaps be included among drepanosiphine aphids s.l. In this article, we refer to the taxa range of drepanosiphine aphids according to Qiao et al. (2005).

There have also been no previous comprehensive phylogenetic studies of the relationships of drepanosiphine aphids s.l., and only certain subfamily phylogenies have been proposed based on different datasets (Supplementary Figure S10). Some researchers have indicated that Saltusaphidinae is a sister group to Macropodaphidinae based on 6 morphological characteristics and host-plant characteristic (Zhang and Qiao 1998), whereas other studies have indicated that this group clusters together with Calaphidinae (von Dohlen and Moran 2000) or with Calaphidinae and Phyllaphidinae (Nováková et al. 2013; Chen et al. 2017) based on molecular data. For Drepanosiphinae, many lines of evidence have indicated that it is a sister group to Chaitophorinae (von Dohlen and Moran 2000; Ortiz-Rivas et al. 2004; Ortiz-Rivas and Martínez-Torres 2010), whereas Nováková et al. (2013) indicated that Chaitophorinae and Neophyllaphidinae clustered together. Quednau (2010) proposed a hypothetical phylogenetic tree for Aphidoidea that included nearly all drepanosiphine subfamilies based on his profound aphid knowledge; however, this tree remains to be verified. As seen above, the relationships among some drepanosiphine groups need to be further researched.

Although the phylogenetic analyses reported to date have revealed that drepanosiphine aphids s.l. are a polyphyletic taxon, almost all analyses have only included a few drepanosiphine subfamilies (Ortiz-Rivas et al. 2004; Ortiz-Rivas and Martínez-Torres 2010; Nováková et al. 2013; Chen et al. 2017). Based on the assessment of morphological characteristics, it appears justified to consider drepanosiphine aphids s.l., or at least some groups within them, as a monophyletic lineage (Zhang and Zhong 1983; Qiao et al. 2005; Heie and Wegierek 2009a; Quednau 2010), which lacks support from molecular systematics. Therefore, to comprehensively investigate the relationships of the drepanosiphine groups s.l. and their relatives, the subfamily-level phylogeny was reconstructed via total-evidence phylogenetic analysis including mitochondrial and nuclear DNA sequence markers and morphological characteristics. We primarily address the following 2 questions: (1) are drepanosiphine aphids s.l. formed as a monophyletic group, and (2) if not, which of them form a stable monophyletic group?

## Material and Methods

### Taxon sampling

In total, 60 species were sampled in this study, comprising 42 species representing all 13 subfamilies of Drepanosiphidae sensus Qiao et al. 2005 as ingroups and 18 species in 7 subfamilies of Aphididae, Adelgidae, and Phylloxeridae as outgroups (Supplementary Table S2). Aiceoninae, Greenideinae, Lachninae, and Thelaxinae were regarded as outgroups, as they shared some similar characteristics with drepanosiphine groups (Quednau 1974; Quednau and Martin 2006; Quednau 2010). Mindarinae was also included within the drepanosiphine aphids s.l. (Quednau and Remaudière 1994; Quednau 1999, 2003, 2010). Chaitophorinae might be a sister group to Drepanosiphinae (von Dohlen and Moran 2000; Ortiz-Rivas et al. 2004; Ortiz-Rivas and Martínez-Torres 2010), and Aphidinae is closely related to the drepanosiphine aphids s.l. according to some studies (Ortiz-Rivas et al. 2004; Ortiz-Rivas and Martínez-Torres 2010). Adelgidae and Phylloxeridae are closely related to Aphididae and were thus employed to root the obtained topologies. The samples used for slide mounting and molecular experiments were stored in 75% and 100% ethanol, respectively. The slide-mounted specimens were identified based on their external morphology by following the keys provided in authoritative monographs and the relevant literature (e.g., Quednau 1999, 2003, 2010; Qiao et al. 2005) and through comparison with identified specimens. All voucher specimens and samples were deposited in the National Zoological Museum of China (NZMC), Institute of Zoology, Chinese Academy of Sciences, Beijing, China. Voucher information is listed in Supplementary Table S2.

### Molecular data

The molecular data came from 3 partial mitochondrial genes (cytochrome oxidase subunit I, *COI*, 658 bp; *tRNA-Leu* and cytochrome oxidase subunit II, *tRNA-Leu/COII*, 741 bp; cytochrome b, *CytB*, 745 bp) and 1 nuclear gene (elongation factor-1a, *EF-1α*, 777 bp) (Supplementary Table S2). Mitochondrial genes were selected to provide a sufficient resolution for lower taxonomic levels (generic and specific) (Zhang et al. 2011; Chen et al. 2014), and nuclear genes were used to provide an appropriate resolution deeper within the subfamilies (Ortiz-Rivas et al. 2004; von Dohlen et al. 2006; Zhang and Qiao 2008; Ortiz-Rivas and Martínez-Torres 2010; Chen et al. 2014). However, molecular data were not available for 11 ingroup species of 5 drepanosiphine subfamilies (Lizeriinae, Spicaphidinae, Israelaphidinae, Pterastheniinae, and Baltichaitophorinae) because these groups are very small with few species, and are distributed in limited regions, and they have therefore not been sampled.

The total genomic DNA was extracted from a single aphid individual selected from the ethanol-preserved candidates using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany)

according to the manufacturer's protocol. The DNA extracts were stored at −20°C. For the subsequent polymerase chain reaction (PCR) step, all primers used in this study are listed in Supplementary Table S3. Typical PCR mixtures were prepared in a 25 μL volume containing 25 μL of 10 × EasyTaq DNA Polymerase Buffer ($+Mg^{2+}$) (TransGen Biotech, Beijing, China), 1.5 U EasyTaq DNA Polymerase (TransGen Biotech), each dNTP at 2.5 mM (TransGen Biotech), 5 pmole of each primer, 2 μL DNA extract, and 18.2 μL double-distilled water. All PCR thermal regimes are provided in Supplementary Table S4. PCR products were detected by 1.5% agarose gel electrophoresis, and then purified using an EasyPure Quick Gel Extraction Kit (TransGen Biotech). The eligible products were then sequenced directly. Sequencing reactions were performed using the corresponding PCR primers from both directions with the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and run on an ABI 3730 automated sequencer (Applied Biosystems).

The returned sequence chromatograms were cleaned and assembled using SeqMan II (DNAStar, Madison, WI) with visual inspection and verification and manual editing. The positions of the introns in the nuclear gene were determined by following the GT–AG rule and aligning the sequences with cDNA sequences from *Mindarus keteleerifoliae* (GenBank accession no. JX489760), and the introns were removed before further analysis. The sequences were verified using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), confirming the high similarity of our submitted sequences to the available sequences of aphid species. Concurrently, the assembled sequences were translated into amino acid sequences at the online TranslatorX server (Abascal et al. 2010) to detect stop-codons that may indicate pseudogenes or misalignments. GenBank accession numbers are provided in Supplementary Table S2.

Alignments of individual gene regions were performed with MAFFT (Katoh and Standley 2013) using the Q-INS-i iterative refinement algorithm. All alignments were inspected by eye in MEGA version 7.0 (Kumar et al. 2016). Possible substitution saturation for each protein-coding gene and the corresponding nucleotide composition (codon positions 1, 2, and 3 were examined separately) were checked using DAMBE version 7 (Xia 2018). Multiple alignments of different genes were concatenated in a single matrix using SequenceMatrix version 1.7.8 (Vaidya et al. 2011). The basic alignment statistics for each gene and partition, including the number of sites, number of variable sites, and number of parsimony-informative sites, were calculated using AMAS (Borowiec 2016) and are presented in Supplementary Table S5. The final concatenated molecular dataset was composed of 2,921 bp and 49 terminals.

## Morphological and biological data

In total, 62 morphological and 2 biological characteristics were scored for all 60 valid species. The morphological characteristics were evaluated in apterous and alate viviparous females and embryos. The morphological characteristics were scored on the basis of the direct observation of specimens under a Leica DM2500 microscope. For 11 species without available slides, characteristic evaluations were conducted based on morphological descriptions from the literature and monographs (Quednau 1999, 2003, 2010). Unobserved states were scored with "?," and inapplicable states were denoted with "–." All specimens examined in this study were deposited in the NZMC, Institute of Zoology, and Chinese Academy of Sciences, Beijing, China. Descriptions of the characteristic states are provided in Supplementary Table S6, and the characteristic state matrix is shown in Supplementary Table S7.

## Phylogenetic analyses

Based on our data type, 3 datasets were used: the molecular dataset, the morphological dataset, and the total-evidence dataset combining all obtained gene fragment alignments and morphological and biological characteristics. Furthermore, total-evidence analyses were carried out for 2 other datasets, as molecular data were not available for 11 ingroup species (Supplementary Table S1). The first dataset included complete taxon sampling data ("all taxa"), whereas the second excluded the 12 species without molecular data ("reduced taxa"). All morphological and biological characteristics were treated as unordered. Therefore, 4 complementary datasets ("molecular dataset," "morphological dataset," "reduced taxa total-evidence dataset," and "all taxa total-evidence dataset") were used to conduct phylogenetic analyses.

For maximum-likelihood (ML) and Bayesian inference (BI) analyses, the best evolutionary model of the above 4 molecular datasets was estimated using PartitionFinder version 2 (Lanfear et al. 2016) with a heuristic search employing the "search=user" option and the corrected Akaike information criterion for model selection. Each partition was treated as a separate data block in PartitionFinder version 2, thus preventing the concatenation of data blocks. The whole matrix of morphological and biological characteristics was analyzed under the Mkv evolutionary model (Lewis 2001) combined with gamma-distributed rates (+G) with a shared shape parameter to account for variation in the substitution rates (Gillung and Winterton 2019) (Supplementary Table S5).

The ML analysis was implemented with IQ-TREE. Based on the resulting partitioning schemes and the corresponding best evolutionary models estimated by PartitionFinder version 2 (Lanfear et al. 2016), 1,000 ultrafast bootstrap (BS) replications (Minh et al. 2013) were performed to investigate nodal support across the topology.

For the BI analysis inferred with MRBAYES version 3.2.0 (Ronquist and Huelsenbeck 2003) via Cipres Science Gateway version 3.3 (Miller et al. 2010), based on the resulting partitioning schemes and their corresponding best evolutionary models estimated by PartitionFinder version 2 (Supplementary Table S5), 4 Markov chain Monte Carlo (MCMC) chains ($n$ runs = 2, $n$ chains = 4) were run simultaneously for 50 million generations, and MCMC performance assessed using tracer version 1.7 (Rambaut et al. 2018) and the output of MRBAYES. The trees were sampled every 1,000 generations, and the first 25% were discarded as burn-in. Then, the chains were combined—the combined effective sample size for each parameter was >200, and the average standard deviation of split frequencies was <1%.

For the maximum-parsimony (MP) analyses with TNT version 1.6 (Goloboff et al. 2008a), heuristic searches were performed using new technology algorithms (Goloboff 1993; Goloboff et al. 2008b). The total-evidence for the "all taxa" and "reduced taxa" sets was analyzed with the following settings: sectorial search in default mode, 200 iterations of ratcheting, 20 cycles of drift, and 10 rounds of tree fusing. Node support was evaluated by Bremer support (Bremer 1994) with the Bremer.run script, and symmetric resampling (standard bootstrapping) (Goloboff et al. 2003) was expressed as the difference in the CG (contradicted/present groups) frequency (1,000 replications). Additionally, to explore the effect of homoplasy on the results of equal weighting (EW), implied weighting (IW) was also performed (Goloboff 1993) for the "all taxa total-evidence dataset," with constants of concavity (k) set to different integer values of 3–35 separated by 1 digit. It has been demonstrated that properly downweighting characteristics according to their homoplasy produces more strongly supported groups and more

stable results in analyses of morphological datasets (Goloboff et al. 2008b). All resulting total-evidence trees for "all taxa" under different weighting regimes and equal weights were compared using SPR distances (Goloboff 2008). The tree with the highest mean similarity was chosen as the working hypothesis tree to optimize the characteristics. Only unambiguous changes were mapped on the tree using Winclada-ASADO version 1.61 (Nixon 2002).

The phylogenetic trees were visualized and edited using FIGTREE version 1.4.0 (Rambaut 2014) (http://tree.bio.ed.ac.uk/software/fgtree/).

## Topology tests

To evaluate the tree topologies resulting from our own and previously existing phylogenetic inferences using different data sets and approaches, the constrained trees were searched separately via likelihood and parsimony analyses. Here, we mainly focused on several specific hypotheses concerning the following phylogenetic questions: (1) are Drepanosiphidae sensus Qiao et al. 2005 formed as a monophyletic taxon; (2) are Saltusaphidinae and Phyllaphidinae sister groups; (3) is Calaphidinae, comprising 2 tribes (Calaphidini and Panaphidini), a monophyletic group; and (4) are Drepanosiphinae and Chaitophorinae sister groups?

For likelihood analysis, the total-evidence ML tree for "all taxa" (Figure 2) was considered an unconstrained tree, whereas constrained topologies were obtained based on the opposite condition. Shimodaira–Hasegawa (SH) and approximately unbiased (AU) tests (Shimodaira 2002) were performed between the unconstrained and constrained topologies. First, the site-wise log likelihoods for each topology were calculated using TREE-PUZZLE version 5.3 (Schmidt et al. 2002) and then combined for the comparison and calculation of the *P*-values of the SH and AU tests for every group of unconstrained and constrained trees with CONSEL version 0.1j (Shimodaira and Hasegawa 2001), which further helped assess the level of statistical support for the alternative topologies.

For parsimony analysis, the most parsimonious tree (k = 19) (Figure 3) was considered an unconstrained tree, whereas constrained topologies were obtained based on the opposite condition. The relative fit difference (RFD), which accounted for the amount of evidence favoring the unconstrained tree in relation to the evidence contradicting it (i.e., favoring the constrained tree) (Goloboff and Farris 2001), was performed to test the presented phylogenetic

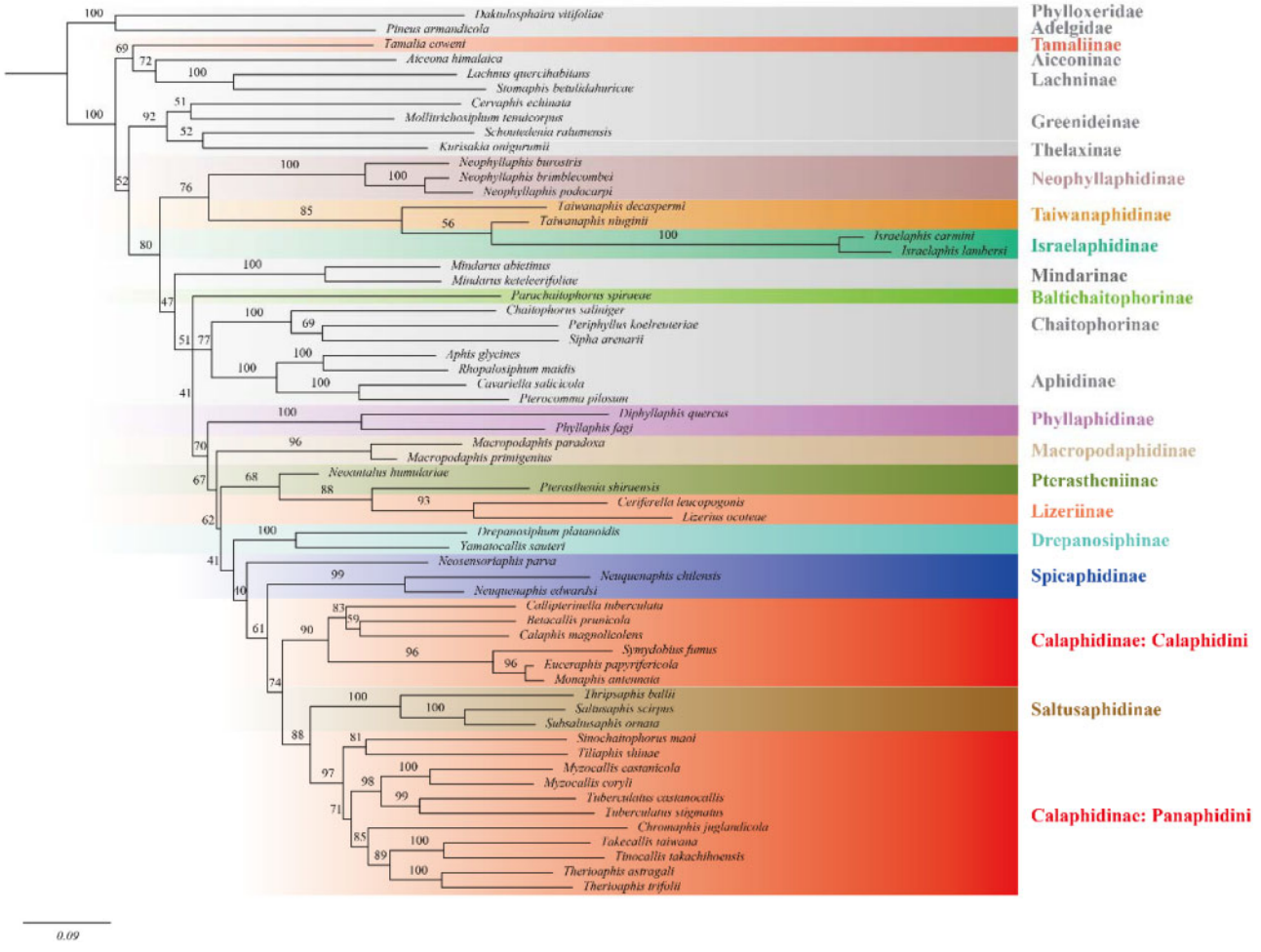


**Figure 2.** ML tree based on the combined analysis of DNA sequence data and morphological and biological characteristics for "all taxa" of the drepanosiphine aphids s.l. (colored taxa are subfamilies belonging to Drepanosiphidae sensus Qiao et al. 2005) and related subfamilies (gray taxa) in Aphididae. The numbers close to the nodes are ultrafast BS values.

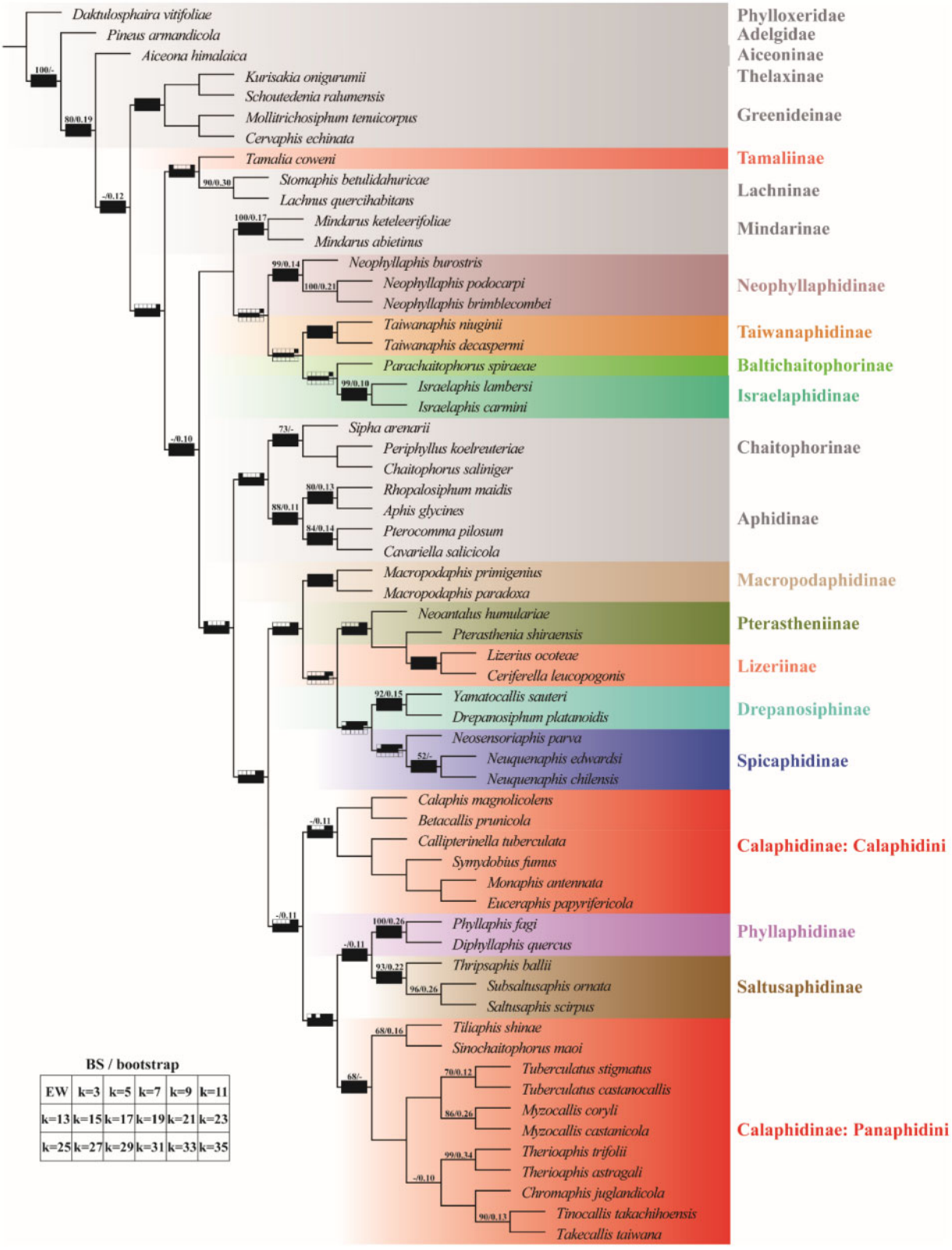


**Figure 3.** Best MP tree (k = 19) resulting from the analysis of the total-evidence dataset for "all taxa" of the drepanosiphine aphids s.l. (colored taxa are subfamilies belonging to Drepanosiphidae sensus Qiao et al. 2005) and related subfamilies (gray taxa) under different weighting regimes and equal weights compared using SPR distances. Navajo rugs indicate the results of stability analysis (black squares indicate clades that were recovered, white squares indicate clades that were not recovered). The numbers above or below each node represent the Bremer support (>0.10) or BS values (BS > 0.50), respectively.

hypotheses (Azevedo et al. 2018). RFD was calculated using formula 1 – (C/F), where C was the sum of the fits of characteristics showing an increased fit under the constrained hypothesis and F represented the characteristics showing decreased fits under the constrained hypothesis compared with the reference unconstrained topology. Therefore, the higher the RFD value is, the higher the support for the unconstrained tree.

## Results

### Molecular data analyses

The topologies of BI, ML, and MP trees constructed based on the combined molecular dataset (49 taxa, 2,921 bp) were nearly consistent (Supplementary Figures S11–13). Drepanosiphine aphids s.l. were recovered as a non-monophyletic group. Five subfamilies (Neophyllaphidinae, Phyllaphidinae, Macropodaphidinae, Drepanosiphinae, and Saltusaphidinae) were constructed as a monophyletic group with strong support (BI: PP > 0.95, ML: BS > 0.70, MP: BS > 0.50, and Bremer support >0.10), although the support for the monophyly of Macropodaphidinae and Drepanosiphinae was low in the MP analysis (BS < 0.50, Bremer support <0.10). Calaphidinae was recovered as a paraphyletic group, with Saltusaphidinae first clustering with Panaphidini of Calaphidinae, which together formed a sister group to the other tribe of Calaphidinae. The support for (Panaphidini + Saltusaphidinae) + Calaphidini was high in both BI and ML analyses (PP > 0.95, BS > 0.70) and low in the MP analysis (BS < 0.50, Bremer support <0.10) (Table 1).

### Morphological data analyses

The BI and MP trees analyzed from the morphological dataset (60 taxa, 64 characteristics) showed a pectinated pattern (Supplementary Figures S14 and S16). However, the monophyly of most subfamilies within the drepanosiphine aphids s.l. was still well supported, such as that of Neophyllaphidinae, Israelaphidinae, Phyllaphidinae, Macropodaphidinae, Lizeriinae, Drepanosiphinae, and Saltusaphidinae (Table 1), especially in the ML analysis (Supplementary Figure S15). In addition, all resulting topologies showed that Phyllaphidinae + Saltusaphidinae formed a sister group. In the ML tree, Calaphidinae was not recovered as a monophyletic group, and 2 constituent tribes within it were also not monophyletic. The sister groups of Phyllaphidinae and Saltusaphidinae, Taiwanaphidinae, Israelaphidinae, and Calaphidinae (except *Callipterinella tuberculata*) together formed a large monophyletic group, although it was weakly supported (Supplementary Figure S15). The drepanosiphine aphids s.l. was also not recovered as a monophyletic group.

### Total-evidence analyses

In the total-evidence ML tree of all taxa (60 taxa, 2,985 characteristics) (Figure 2), Aphididae was retrieved as monophyletic with strong support (BS = 1.00). Within Aphididae, the drepanosiphine aphids s.l. was not recovered as a monophyletic group. The monophyly of 7 subfamilies consistent with those identified in the combined molecular and morphological hypothesis was revealed, including Neophyllaphidinae, Israelaphidinae, Phyllaphidinae, Macropodaphidinae, Lizeriinae, Drepanosiphinae, and Saltusaphidinae. Moreover, Tamaliinae clustered together with Aiceoninae and Lachninae, which split off earliest from the other taxa. Greenideinae and Thelaxinae grouped together (BS = 0.92) and split from the remaining taxa, forming another well-supported clade (BS = 0.80). Neophyllaphidinae, Taiwanaphidinae, and Israelaphidinae were grouped together (BS = 0.76) and positioned as a sister group to the remaining groups. Phyllaphidinae, Macropodaphidinae, Pterastheniinae, Lizeriinae, Drepanosiphinae, Spicaphidinae, Saltusaphidinae, and Calaphidinae clustered together as a monophyletic group (BS = 0.70). In addition, Calaphidinae was recovered as a paraphyletic group, with Saltusaphidinae clustered within it, which was also consistent with the result of the combined molecular hypothesis.

In the total-evidence BI analysis of all taxa (Supplementary Figure S20), the resulting topology was almost consistent with the ML hypothesis (Figure 2), but it presented a decrease in resolution and recovered different placements of several taxa (Table 1).

The MP analysis of the total-evidence dataset for all taxa under equal weights resulted in 1 most parsimonious tree with 7,908 steps (consistency index [CI] = 0.241, retention index [RI] = 0.348) (Supplementary Figure S21). For the implied weight analyses, when the k value was equal to 3–7, the resulting MP tree was changeable, and some groups were no longer monophyletic, such as the tribe of Calaphidini. However, starting at k = 9, the topologies of the corresponding MP trees were identical. For all MP trees, as the IW tree with k = 19 presented the highest mean similarity to the rest of the EW and IW trees (Supplementary data, Table S8), it was used as the best MP tree for the optimization of characteristics (Figure 4). In contrast to the ML and BI trees, for drepanosiphine aphids s.l., the MP tree (IW, k = 19) was different to some extent (Figure 3). Tamaliinae was no longer clustered with Aiceoninae and Lachninae but was still the earliest group to split from the drepanosiphine aphids and their relatives; Baltichaitophorinae nested within the group of Neophyllaphidinae, Taiwanaphidinae, and Israelaphidinae, which together formed a monophyletic group; and Phyllaphidinae and Saltusaphidinae formed a sister group and then clustered within Calaphidinae.

For the phylogenetic inference for the reduced taxa (Supplementary Figures S17–19), the resulting topology was nearly consistent with the phylogenetic hypothesis analyzed from all taxa. The monophyly of Neophyllaphidinae, Phyllaphidinae, Macropodaphidinae, Drepanosiphinae, and Saltusaphidinae was highly supported. The clade of (Panaphidini + Saltusaphidinae) + Calaphidini was also retrieved, although its support in MP analysis was low (Table 1).

### Topology tests

The results of the parsimony analysis showed that topology Tests I, III, and IV presented relatively moderate RFD values (Table 2) , suggesting only weak signals favoring the constrained topology and supporting the unconstrained tree (the IW tree with k = 19). The rest of the phylogenetic hypotheses showed very low RFD values, which seemed to indicate that the corresponding constrained topology might be almost as reliable as the unconstrained tree. All SH and AU tests indicated that the unconstrained tree was the best tree ($P = 1.000$), with all constrained tree topologies presenting $P$-values < 0.01 (Table 3). Therefore, the results of the topology test supported that the drepanosiphine aphids s.l. group was not a monophyletic taxon; Calaphidinae was a paraphyletic group, with Saltusaphidinae first clustering with Panaphidini, together forming a sister group to the other tribe of Calaphidini; and Saltusaphidinae and Phyllaphidinae, Drepanosiphinae, and Chaitophorinae were not clustered as sister groups.

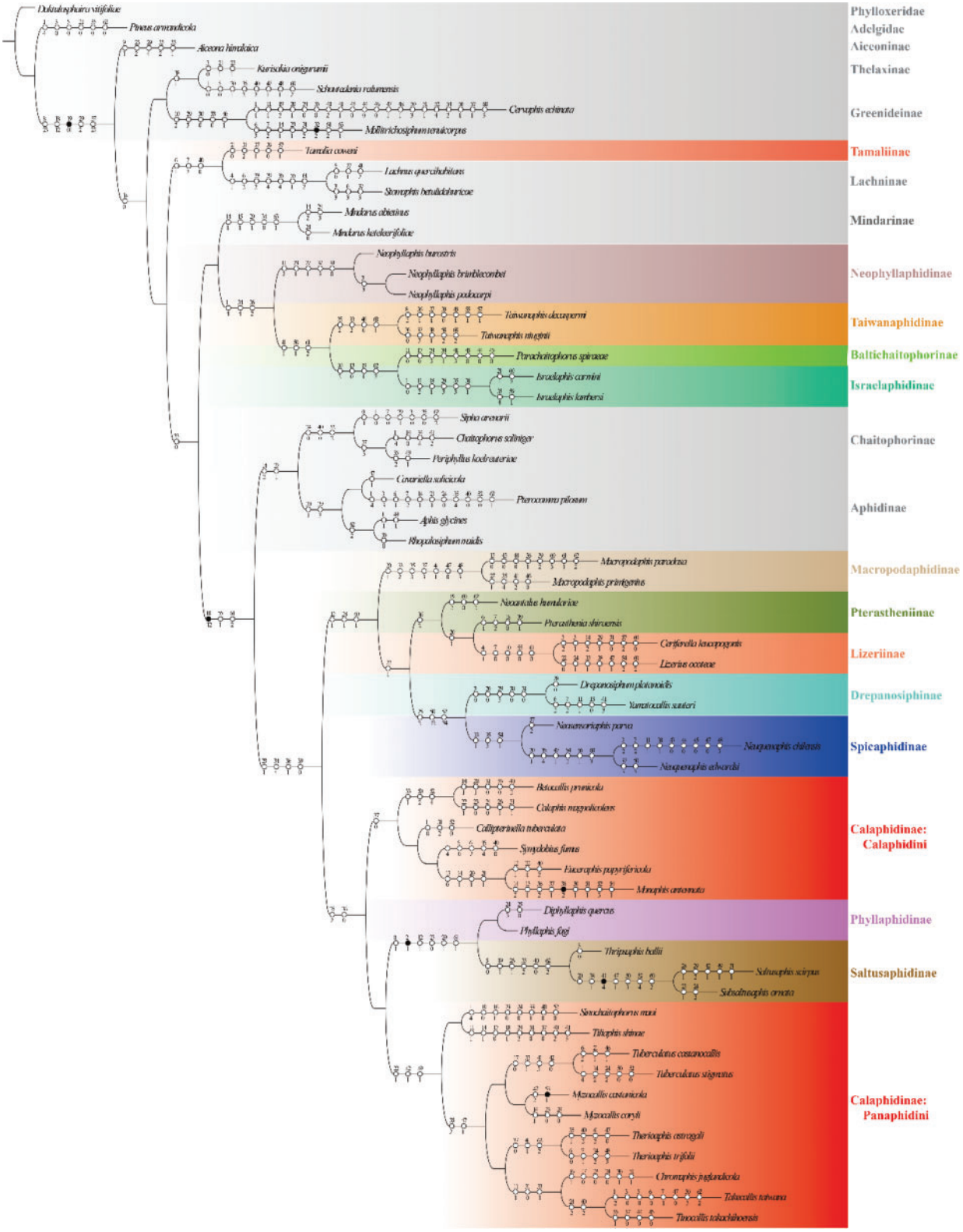


**Figure 4**. Characteristic optimization of the best total-evidence MP tree for "all taxa" of traditional drepanosiphine aphids s.l. (colored taxa are subfamilies belonging to Drepanosiphidae sensus Qiao et al. 2005) and related subfamilies (gray taxa). The numbers above and below the circles on the branches indicate characteristic numbers and states, respectively. White and black circles represent homoplasious and non-homoplasious states, respectively.

## Discussion

The phylogenetic hypotheses put forth for drepanosiphine aphids s.l. were analyzed for the first time based on the total-evidence analyses combining molecular data and morphological and biological characteristics. Based on 4 different datasets, parsimony and model-based phylogenetic inference methods, a total of 29 trees were constructed (Figures 2–3; Supplementary Figures S11–21). The support values for the particular phylogenetic relationships in each tree are summarized in Table 1. All resulting topologies recovered drepanosiphine aphids s.l. and Calaphidinae as a non-monophyletic group, respectively, and indicated a non-sister group between Drepanosiphinae and Chaitophorinae. The topology test further verified the above hypothesis. For the total-evidence analyses, the trees resulting from BI and ML analyses under the same dataset were nearly consistent, and both revealed a close relationship between Saltusaphidinae and Calaphidinae. Therefore, the total-evidence ML and MP trees (IW, k = 19) were presented as our working topologies (Figures 2–4).

Previously, there were no phylogenetic studies in terms of morphology, bionomy, or molecular data to discuss the phylogenetic links within all drepanosiphine taxa, especially at the level of a subfamily or a tribe. Over the past dozen years, some studies devoted to unraveling the high-level phylogenetic relationships of Aphidoidea based on different molecular datasets implied the non-monophyly of drepanosiphine aphids s.l. to some extent (von Dohlen and Moran 2000; Ortiz-Rivas et al. 2004; Ortiz-Rivas and Martínez-Torres 2010; Nováková et al. 2013; Chen et al. 2017), but they only involved small groups within the drepanosiphine aphids. Here, the polyphyly of drepanosiphine aphids s.l. was further confirmed by all resulting tree topologies (Figures 2–3; Supplementary Figures S11–21) and topology Test I (RFD = 0.17, SH test: $P < 0.01$, AU test: $P < 0.01$) (Tables 2–3). However, drepanosiphine aphids s.l. were classified as the family Drepanosiphidae by Zhang and Zhong (1983) according to the following morphological characteristics: the siphunculus was not reticulated, the anal plate was slightly incised or bilobate, the cauda was knobbed, a dorsal and marginal processes were developed, the antennae was 6-segmented, the empodial setae were mostly lobate and spinules on the tarsus were present or absent (Supplementary Table S1). Nevertheless, morphological specialization and characteristic convergence are commonly observed in drepanosiphine aphids s.l. and may prevent an objective understanding of their systematics and evolution, leading to a certain degree of subjectivity in the selection of key characteristics for phylogenetic inference. The >7 key characteristics all seemed to be non-synapomorphic (Supplementary Table S7) and were not shared by all groups. However, the total-evidence approach applied herein has been proven to overcome this common problem in some cases (Abrams et al. 2012), and our results showed that Drepanosiphidae sensus Qiao et al. (2005) was undoubtedly a polyphyletic group.

Under consideration of fossil taxa, Heie and Wegierek (2009a) also prefer to regard drepanosiphine aphids s.l. as the family Drepanosiphidae based on some apomorphic characteristics, especially wishbone-shaped stiffening at the base rostrum (Supplementary Table S1). They also pointed out that the feature of wishbone-shaped stiffening at the base rostrum was only known among Drepanosiphidae but did not appear in all of them. They explained that this was because subfamilies without this characteristic once acquired it, and then the characteristic was lost in their evolution. However, it was unusual that some rather primitive subfamilies (Mindarinae, Neophyllaphidinae, and Parachaitophorinae) never acquired this characteristic, as they stated were also divided into Drepanosiphidae. According to our results, the Drepanosiphidae sensus Heie and Wegierek (2009a) was also a polyphyletic group (Figures 2–3; Supplementary Figures S11–21). Furthermore, the total-evidence ML tree and the best MP tree (k = 19) of "all taxa" both showed that Phyllaphidinae, Macropodaphidinae, Pterastheniinae, Lizeriinae, Drepanosiphinae, Spicaphidinae, Saltusaphidinae, and Calaphidinae clustered together as a relatively robust monophyletic group (ML: BS = 0.70) (Figures 2–3). The >8 subfamilies were also stably recovered as monophyletic in the MP analyses, as supported by 14 of the 18 total EW and IW trees (Figure 3). More interestingly, the remaining subfamilies within Drepanosiphidae sensus Heie and Wegierek (2009a)

**Table 1.** Sensitivity of particular phylogenetic hypotheses to different datasets and phylogenetic analyses

| Phylogenetic hypothesis | Molecular dataset | | | Morphological dataset | | | Total-evidence dataset | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Reduce taxa | | | All taxa | | |
| | BI | ML | MP | BI | ML | MP | BI | ML | MP | BI | ML | MP (IW, k = 19) |
| Monophyly of Neophyllaphidinae | 1.00 | 100 | 100/0.43 | 0.99 | 99 | 88/- | 1.00 | 100 | 100/0.54 | 1.00 | 1.00 | 99/0.14 |
| Monophyly of Israelaphidinae | a | a | a | 1.00 | 100 | 98/- | a | a | a | 1.00 | 1.00 | 99/0.10 |
| Monophyly of Phyllaphidinae | 1.00 | 100 | 94/0.16 | – | 90 | -/- | 1.00 | 100 | 97/0.25 | 1.00 | 1.00 | 100/0.26 |
| Monophyly of Macropodaphidinae | 1.00 | 100 | -/- | 0.97 | 85 | *n* | 1.00 | 100 | 99/0.17 | 0.99 | 0.96 | -/- |
| Monophyly of Lizeriinae | a | a | a | 0.85 | 96 | *n* | a | a | a | 0.90 | 0.93 | 99/0.10 |
| Monophyly of Drepanosiphinae | 1.00 | 97 | -/- | 1.00 | 100 | -/- | 1.00 | 100 | 81/0.16 | 1.00 | 1.00 | 92/0.15 |
| Monophyly of Saltusaphidinae | 1.00 | 100 | 76/0.18 | 0.70 | 87 | -/- | 1.00 | 100 | 87/0.19 | 1.00 | 1.00 | 93/0.22 |
| (Panaphidini, Saltusaphidinae) | 1.00 | 100 | -/- | *n* | *n* | *n* | 1.00 | 98 | -/- | 0.95 | 0.88 | *n* |
| (Panaphidini, Saltusaphidinae), Calaphidini) | 1.00 | 100 | -/- | *n* | *n* | *n* | 1.00 | 99 | -/- | *n* | 0.74 | *n* |
| (Phyllaphidinae, Macropodaphidinae, Pterastheniinae, Lizeriinae, Drepanosiphinae, Spicaphidinae, Calaphidini, Saltusaphidinae, Panaphidini) | *n* | *n* | *n* | *n* | *n* | *n* | *n* | *n* | *n* | *n* | 0.70 | – |

The left and right BS values in the MP tree represent the Bremer support and standard BS values calculated by resampling, respectively. "n/a" indicates that a given phylogenetic hypothesis was not recovered in the corresponding tree or not included in its dataset. "-" indicates that a given phylogenetic hypothesis was recovered but that its node was not strongly supported (ML BS value <50%; Bayesian posterior probability was <70%; for the MP tree, BS value <50% or Bremer support <0.10).

all presented no wishbone-shaped stiffening at the base rostrum, and these 3 primitive subfamilies (Mindarinae, Neophyllaphidinae, and Parachaitophorinae) split relatively earlier. Thus, it was reasonable to speculate that these 8 subfamilies might constitute the actual drepanosiphine aphids s.l. This inference is supported by the unambiguous morphological synapomorphies of these aphids, such as 2–5 ventral setae on tarsal segment I, knobbed cauda, incised or bilobed anal plate, absent spinules on the tarsus, and other characteristics such as a dorsal body with developed processes or mostly tubercles, mostly lobate empodial setae, a generally small population, and alate viviparous females constituting the majority of individuals.

**Table 2.** RFDs of alternative phylogenetic hypotheses

| Test | Phylogenetic hypothesis | Fit | C | F | RFD |
|---|---|---|---|---|---|
| I | Non-monophyly of the drepanosiphine aphids s.l. | 221.11051 | – | – | – |
| | monophyly of Drepanosiphidae sensus Qiao et al. 2005 | 221.44739 | 1.66878 | 2.01586 | 0.17217 |
| II | Sister group of Saltusaphidinae and Phyllaphidinae | 221.11051 | – | – | – |
| | Non-sister group of Saltusaphidinae and Phyllaphidinae | 221.21631 | 3.83574 | 3.94149 | 0.02683 |
| | Sister group of Saltusaphidinae and Spicaphidinae | 221.25318 | 3.73535 | 3.87798 | 0.03678 |
| III | (Panaphidini, Saltusaphidinae), Calaphidini | 221.11051 | – | – | – |
| | Monophyly of Calaphidinae: (Calaphidini, Panaphidini) | 221.16754 | 1.06355 | 1.12055 | 0.05087 |
| | Monophyly of Calaphidinae: (Calaphidini, Panaphidini), Saltusaphidinae | 221.32574 | 1.28124 | 1.49644 | 0.14381 |
| IV | Non-sister group of Drepanosiphinae and Chaitophorinae | 221.11051 | – | – | – |
| | Sister group of Drepanosiphinae and Chaitophorinae | 221.35368 | 0.93944 | 1.18262 | 0.20563 |

C = sum of fits of characteristics increasing their fit in the constrained hypotheses; F = sum of the fits of characteristics showing a decreased fit under the constrained hypotheses. An unconstrained tree (k = 19) was used as a reference tree for the comparison of its fitness with each constrained hypothesis.

Except for several MP trees, most of our trees recovered Calaphidinae as non-monophyletic (Figures 2–3; Supplementary Figures S11–21). The topology test also rejected the monophyletic hypothesis for Calaphidinae (SH test: $P < 0.01$, AU test: $P < 0.01$) (Tables 2–3). The non-monophyly of Calaphidinae has already been implied by some previous studies to some extent. For instance, using DNA of the obligate symbiotic bacteria *Buchnera aphidicola*, Nováková et al. (2013) reconstructed Calaphidinae as a non-monophyletic group in most single-gene and concatenated dataset analyses based on the hypothesis of parallelism (Supplementary Figure S10E). In the phylogenetic inference based on 4 combined molecular datasets (Ortiz-Rivas and Martínez-Torres 2010) and mitochondrial genome sequences (Chen et al. 2017), Calaphidinae, which were represented only by samples from the tribe of Panaphidini, were retrieved as a monophyletic group and showed a close relationship with Saltusaphidinae (Chen et al. 2017). Furthermore, Nováková et al. (2013) and Chen et al. (2017) also revealed the potential relationship among Calaphidinae, Saltusaphidinae, and Phyllaphidinae. In our results, the topology of (Saltusaphidinae + Panaphidini) + Calaphidini was strongly recovered in the BI and ML analyses of the molecular and total-evidence dataset (ML: BS > 0.70, BI: PP > 0.95) (Table 1; Figure 4; Supplementary Figures S8–9 and S14–15), although the support of this clade was low in the corresponding MP analysis. In addition, many total-evidence MP trees retrieved Phyllaphidinae clustered with Saltusaphidinae as a sister group and nested in the same position of Calaphidinae (Figure 3). The phylogenetic inference of the morphological dataset also revealed the sister group between Phyllaphidinae and Saltusaphidinae, and the potential relationship with Calaphidinae (Supplementary Figures S14–16). However, the final topology test indicated that the monophyly of (Saltusaphidinae + Panaphidini) + Calaphidini was better accepted (Tables 2–3). Anatomically, a peculiarity of Saltusaphidinae is the double-filter chamber of the midgut (Ponsen 1983), which is also known to occur in Panaphidini of Calaphidinae (Quednau 2010). The fore and middle legs or all legs of Saltusaphidini of Saltusaphidinae and Panaphidini are more or less enlarged and saltatorial (Heie and

**Table 3.** Statistical testing of particular phylogenetic hypotheses

| Test | Rank | Phylogenetic hypothesis | Obs | SH test (*P*-value) | AU *test (P*-value*)* |
|---|---|---|---|---|---|
| I | 1 | Non-monophyly of the drepanosiphine aphids s.l. | Best | 1.000 | 1.000 |
| | 2 | monophyly of Drepanosiphidae sensus Qiao et al. (2005) | 57,104.4 | $0^*$ | $1 \times 10^{-7*}$ |
| II | 1 | (Saltusaphidinae, Panaphidini), Calaphidini | Best | 1.000 | 1.000 |
| | 2 | Sister group of Saltusaphidinae and Phyllaphidinae | 2,386.2 | $0^*$ | $4 \times 10^{-51*}$ |
| | 3 | Sister group of Saltusaphidinae and Spicaphidinae | 3,105.1 | $0^*$ | $1 \times 10^{-50*}$ |
| III | 1 | (Saltusaphidinae, Panaphidini), Calaphidini | Best | 1.000 | 1.000 |
| | 2 | Monophyly of Calaphidinae: (Calaphidini, Panaphidini), Saltusaphidinae | 2,401.2 | $0^*$ | $3 \times 10^{-7*}$ |
| | 3 | Monophyly of Calaphidinae: (Calaphidini, Panaphidini) | 2,413.8 | $0^*$ | $2 \times 10^{-8*}$ |
| IV | 1 | Non-sister group of Drepanosiphinae and Chaitophorinae | Best | 1.000 | 1.000 |
| | 2 | Sister group of Drepanosiphinae and Chaitophorinae | 3,104.7 | $0^*$ | $4 \times 10^{-65*}$ |

Obs, observed log-likelihood difference from the best topology.
*indicates that the hypothesis receives a *P*-value < 0.01 and can be rejected.

Wegierek 2009b). Saltusaphidinae mainly feeds on herbs, so do some genera of Panaphidini. Therefore, Calaphidinae should be a paraphyletic group, with Saltusaphidinae nested within this subfamily.

All of our phylogenetic inferences recovered Chaitophorinae and Drepanosiphinae as 2 stable monophyletic groups, and they were not clustered together as sister groups (Figures 2–3; Supplementary Figures S11–21). The constrained tree test also rejected the phylogenetic hypothesis of Drepanosiphinae + Chaitophorinae (RFD = 0.21, SH test: $P < 0.01$, AU test: $P < 0.01$) (Tables 2–3). This challenged the well-known phylogenetic hypothesis that Drepanosiphinae is closely related to Chaitophorinae based on evidence from aphid parasites (Mackauer 1965) and fossils (Heie 1967), along with similarities of these groups in terms of morphology (e.g., the absence of sclerotization of rostral segment II and absence of wax glands) (Shaposhnikov 1981; Quednau 2010), anatomy (e.g., a gastrointestinal tract without a filter chamber) (Ponsen 1983), the internal male reproductive system (Wojciechowski and Wieczorek 2004), male genitalia (Wieczorek et al. 2011) or bionomy (e.g., associations with host plants and similar summer diapause morphs). A molecular phylogeny of Aphididae based on limited gene sequences also indicated that Drepanosiphinae and Chaitophorinae may be sister groups (von Dohlen and Moran 2000; Ortiz-Rivas et al. 2004; Ortiz-Rivas and Martínez-Torres 2010) and could even be combined in a single unit (von Dohlen 2009). Wieczorek et al. (2017) further discussed the relationship between these 2 subfamilies inferred from a molecular-based phylogeny and comprehensive morphological data and supported the separation of Chaitophorinae from Drepanosiphinae. Unfortunately, Wieczorek et al. (2017) only sampled these 2 species groups and did not combine molecular and morphological data to discuss their relationships in the evolutionary framework of Aphididae. However, the non-sister groups Chaitophorinae and Drepanosiphinae were also revealed by other molecular phylogenetic inferences (Nováková et al. 2013), even based on mitochondrial genome sequences (Chen et al. 2017). Thus, it could be seen that the similar morphological characteristics between Drepanosiphinae and Chaitophorinae might be the result of homoplasy. Interestingly, Quednau (2010) pointed out that Drepanosiphinae could have separated from the common trunk of drepanosiphine aphids s.l. prior to the existence of highly evolved groups, such as Calaphidinae, and evolved as a sister group of Spicaphidinae. This was validated by our results, especially those of the MP analyses. Thus, it can be seen that the hypothesis of a sister group between Chaitophorinae and Drepanosiphinae is doubtful, and they might be 2 independent monophyletic groups.

In summary, our total-evidence analyses clearly confirmed that the drepanosiphine aphids s.l. are not a monophyletic taxon, which seemed to support the classification system in which the drepanosiphine aphids were divided into different groups classified at the subfamily level. Calaphidinae is also not monophyletic, with Saltusaphidinae first clustering with Panaphidini in Calaphidinae, which together form a sister group to the other tribe of Calaphidinae. Drepanosiphinae was not clustered with Chaitophorinae, which was inconsistent with the phylogenetic hypothesis of a close relationship between them, illustrating that their phylogeny was still controversial. Overall, some groups within the drepanosiphine aphids s.l., including Phyllaphidinae, Macropodaphidinae, Pterastheniinae, Lizeriinae, Drepanosiphinae, Spicaphidinae, Saltusaphidinae, and Calaphidinae, clustered together and might constitute the actual drepanosiphine aphids s.l. Nevertheless, the relationships between some subfamilies of drepanosiphine aphids s.l. and other subfamilies in Aphididae were still uncertain in our analyses. Therefore, additional data from an increasing number of multisourced sequences from a broader range of taxa are needed to produce a more detailed and robust phylogeny to test the current hypothesis and obtain more useful information.

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## Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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