ASSESSING THE IMPACT OF PHYLOGENETIC INCONGRUENCE ON TAXONOMY, FLORAL EVOLUTION, BIOGEOGRAPHICAL HISTORY, AND PHYLOGENETIC DIVERSITY[1]

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- *Premise of the study:* Phylogenetic incongruence between "gene trees" and "species trees" has been widely acknowledged in phylogenetic research. Conflicts may emerge from several processes including paralogy, hybridization, and incomplete lineage sorting. Although phylogenetic incongruence appears common, its impact on many phylogeny-based analyses remains poorly understood.
- *Methods:* We examined the occurrence of phylogenetic conflict between nuclear (ribosome ITS) and plastid (*rbcL*, *trnL-F*, *rpl20-rps12*, and *rps16* intron) loci in the ancient angiosperm family Chloranthaceae. Then we investigated how phylogenetic conflict bears on taxonomic classification within the family as well as on inferences on biogeographical history, floral evolution, and measures of phylogenetic diversity (PD).
- *Key results:* We found evidence for significant phylogenetic incongruence between plastid and nuclear data in the genus *Hedyosmum*. Within *Hedyosmum*, our results did not support previous subgeneric classification of the genus. Division of sections within subgenus *Tafalla* was supported by the ITS data but not by the plastid data set. As a consequence, we showed that inferring the evolution of key floral characters and geographical history within *Hedyosmum* depends on the phylogenetic data used. Both data sets yielded similar PD measures across genera, but we found contrasting PD measures in *Hedyosmum*, even after correcting for rate heterogeneity.
- *Conclusions:* Our study demonstrated that phylogenetic conflict not only affects the inference of organismal relationships but also impacts our understanding of biogeographical history, morphological evolution, and phylogenetic diversity.

Key words: biogeography; Chloranthaceae; *Hedyosmum*; phylogenetic conflict; phylogenetics; species trees.

In plant phylogenetic studies, plastid and nuclear ribosome DNA (18S-5.8S-28S), particularly the internal transcribed spacer (ITS), probably represent the most frequently used markers for phylogenetic inference (e.g., Qiu et al., 1999; Moore et al., 2007, 2010; Qiu et al., 2010). The advantages of plastid and ribosome DNA are clear and repeatedly demonstrated in previous studies. These include the presence of numerous duplications of homogenized copies that facilitate amplifications using polymerase chain reactions (PCR), easy design of universal primers on conserved regions at both ends, and inclusion of both conserved and rapidly evolving regions that are applicable across a broad range of taxonomic levels (e.g., land plants, seed plants, angiosperms, orders, families down to the genus, species, and populations levels; Chase et al., 1993; Soltis et al., 1999; Savolainen et al., 2000; Kong et al., 2002). Phylogenetic hypotheses arising from these markers have been used in diverse studies, encompassing phylogeography and historical biogeography, DNA barcoding and analyses of species diversification through time, as well as quantifying phylogenetic diversity (PD) to inform conservation and phylogenetic community ecology approaches (Soltis et al., 2006; Forest et al., 2007; Antonelli and Sanmartín, 2011; Li et al., 2011b; Liu et al., 2014).

In general, longer DNA sequences improve the resolution of phylogenetic relationships. Consequently, data from different loci are usually combined as total evidence in phylogenetic reconstruction. However, different loci, especially those that are genetically unlinked or from different genomes (e.g., plastid

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sequences vs. nuclear ribosome regions), could carry discordant phylogenetic information and yield conflicting phylogenetic trees (e.g., Pamilo and Nei, 1988; Page and Charleston, 1997; Sang et al., 1997; Calviño et al., 2008). Conflict arises from a diverse range of processes. These include paralogy, hybridization, incomplete lineage sorting of ancestral polymorphisms and horizontal gene transfer (HGT). Conflict may also result from systematic, methodological (e.g., misspecification of models) and stochastic errors (e.g., during sequencing and alignment), factors that are difficult to rule out (Pamilo and Nei, 1988; Page and Charleston, 1997; Keeling and Palmer, 2008; Smith, 2011; Knox, 2014). In the case of incomplete lineage sorting, considerable attention has been paid to understand the causes and consequences of phylogenetic incongruence in inferring the best "species tree" (Liu, 2008; Heled and Drummond, 2010).

In contrast, considerably less attention has been paid toward understanding the effects of phylogenetic incongruence on other phylogeny-based studies. For example, we know little about how phylogenetic incongruence bears on taxonomic classification (but see discussion in Baum, 2007), although molecular phylogeny has been widely used to evaluate traditional plant taxonomy (e.g., Angiosperm Phylogeny Group III [APG III, 2009]). Furthermore, phylogenetic conflict has been seldom considered in relation to possible implications for historical biogeography and estimations of phylogenetic diversity. These potential effects thus deserve further empirical examination.

Chloranthaceae are a small, early-diverging angiosperm family with ca. 77 species in four genera, *Sarcandra*, *Chloranthus*, *Ascarina*, and *Hedyosmum.* The family's extant distribution is large, spanning tropical Asia, tropical America, Australasia, and Madagascar (Smith, 1976; Todzia, 1988; Kong et al., 2002; Eklund et al., 2004). The intergeneric relationships are well resolved, being corroborated by several studies based on either morphological data alone (Doyle et al., 2003; Eklund et al., 2004), on plastid DNA (Zhang and Renner, 2003; Zhang et al., 2011), or combined DNA data from the plastid and nuclear genomes (Qiu et al., 1999; Antonelli and Sanmartín, 2011).

The intrageneric relationships in *Chloranthus* have been consistently supported by data sets from the nuclear ITS region and combined data from plastid and nuclear ITS sequences, respectively (Kong and Chen, 2000; Kong et al., 2002). In *Hedyosmum*, the most species rich and earliest-diverging genus in the family, however, largely incongruent intrageneric relationships have been found based on different morphological and molecular studies. Relationships recovered from combined data of the plastid gene *rbcL*, the *rps16* intron, and nuclear ITS sequences differ from those supported by the three plastid loci *rbcL*, *trnL-F*, and *rpl20-rps12* (Antonelli and Sanmartín, 2011; Zhang et al., 2011). A key difference, which largely impacts inferences of biogeographic history for the genus, concerns the placement of the sole Asian species, *Hedyosmum orientale*. *Hedyosmum orientale* was placed as the sister of all the other *Hedyosmum* taxa distributed in tropical America according to plastid data alone (*rbcL*, *trnL-F*, and *rpl20-rps12*; Zhang et al., 2011). In contrast, combined data of two plastid loci (*rbcL* and *rps16* intron) and nuclear ITS sequences, placed *H. orientale* within the neotropical (i.e., confined to the American tropics) *Hedyosmum*, with a lineage composed of several Central American and Caribbean species (all belonging to subgenus *Hedyosmum*) inferred as first diverging (Antonelli and Sanmartín, 2011). The extant amphi-Pacific disjunction in tropical Asia and America of *Hedyosmum* is a pattern also shared by some other taxa, e.g., *Persea*, *Gautheria*, and Symplocaceae (Bush et al., 2009; Li et al., 2011a; Fritsch et al., 2014). In *Hedyosmum*, it remains largely unexplored how the genus occupied its extant distribution in Asia and the neotropics and whether inferences of biogeographical history can be affected by phylogenetic conflict.

The different inferences for the phylogeny of *Hedyosmum* indicate phylogenetic conflict between the plastid data and the plastid and nuclear ITS combined data on one hand and between the molecular data and morphology on the other hand. The plastid and nuclear ITS combined data and morphology suggested that *H. orientale* is embedded within the neotropical congeners, although with some varied placements (Todzia, 1988; Eklund et al., 2004; Antonelli and Sanmartín, 2011; Zhang et al., 2011). Besides *H. orientale*, other species in *Hedyosmum*, some with key taxonomic and/or geographical significance, have been found as variably placed in the phylogenetic trees of the genus, shifting between different taxonomic units (e.g., subgenus and section) and/or between different assemblages consisting of taxa from different continental areas (Antonelli and Sanmartín, 2011; Zhang et al., 2011). These variable placements are very likely to affect the assessment of traditional taxonomy and inferences of morphological evolution and geographic history.

Phylogenetic diversity (PD), a measure of biological diversity in addition to traditional taxonomic diversity (TD; see Swenson, 2011), has been increasingly used for biological conservation decisions (Faith, 1992; Barker, 2002; Rolland et al., 2011). Taxonomic diversity is a measure of species richness quantified by morphological taxonomy and can be susceptible to vagaries of taxonomic circumscription. Phylogenetic diversity was originally defined as the sum of branch lengths connecting all the target taxa in a phylogenetic tree or as a derived proportion of the summed branch lengths of the target taxa to the total tree length (Faith, 1992). Phylogenetic diversity, especially when determined from multiple gene regions, may better represent evolutionary history because it is based on the divergence of DNA sequences, which can be more unambiguously and accurately quantified than morphological diversity. The advantage of PD is especially noteworthy in taxa with suspected cryptic morphological diversity and in those with particular evolutionary significance (e.g., the earliest-diverging extant angiosperm *Amborella*).

Although PD has been increasingly applied in evolutionary, ecological, and biological conservation studies, whether and how PD can be affected by the use of different DNA loci remain largely unexplored. PD can vary depending on differences in branch lengths estimated from phylogenetic trees based on DNA sequences from different loci. In addition, when taxa have complicated evolutionary histories, such as due to hybridization, different loci can reconstruct conflicting evolutionary patterns and lead to different sets of branch lengths for calculation of PD measures on the same set of taxa. In Chloranthaceae, such an effect should be mostly visible in the genus *Hedyosmum* because of the interspecific conflict of the taxa from different distributional areas demonstrated previously (Antonelli and Sanmartín, 2011; Zhang et al., 2011).

In the present study, we explored phylogenetic conflict between the plastid DNA and nuclear ribosome ITS sequences and assessed its impact on phylogeny-based inferences in the plant family Chloranthaceae. First, we examined the extent of phylogenetic incongruence between the plastid and nuclear data by reconstructing and comparing phylogenetic trees based on each genomic source. Then we evaluated the influence of

our results on the systematics, morphological evolution, historical biogeography, and PD of the family. Our results showed that phylogenetic conflict can severely impact all aspects surveyed.

MATERIALS AND METHODS

Plant materials, DNA extractions, PCR amplifications, and sequencing— We sampled leaf fragments and performed DNA extractions from 59 accessions of 54 species that represented all the higher taxonomic units proposed to date above the species level in Chloranthaceae. Our samples also represented most of the family's geographical range (Appendix 1). The cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) and Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) were used to extract DNA from silica-dried leaf materials and herbarium specimens, respectively. In addition to the sequences from the plastid loci (*rbcL*, *trnL-F*, *rpl20-rps12*, and *rps16* intron) and nuclear ribosome ITS that were obtained and used in our previous studies (Antonelli and Sanmartín, 2011; Zhang et al., 2011), we amplified and sequenced several *rps16* and ITS sequences that were previously missing. The primers used for amplification and sequencing were the same as in our previous studies (Antonelli and Sanmartín, 2011; Zhang et al., 2011) except that primer pair rps16F (GTGGTAGAAAGCAACGTGCGACTT) and rps16R2 (TCGGGATCGAACATCAATTGCAAC) (Oxelman et al., 1997) and internal primer pairs rps16iF (TGTCTAAACCCAATGATTCAAAGC) and rps16iR (CGTACTCATAACTCAAGTTGGGT) were designed and used in the present study for the plastid *rps16* intron region. The PCR profiles began with initial denaturation for 3 min at 94°C; followed by 34 cycles of 30 s at 94°C, 40 s at 52°C, and 90 s at 72°C (60s for ITS); and a final extension step for 10 min at 72°C. For some extractions that failed in the first round of amplifications, a second PCR round was conducted with an internal pair of primers, using the products from the first round of PCR amplifications with external primer pairs as templates. The PCR products were purified using a TIANgel Midi Purification Kit (Tiangen Biotech) according to the manufacturer's instructions and then sequenced on an ABI 3730 DNA Sequencer (Applied Biosystems International, Foster City, California, USA) using Big Dye Terminator (Applied Biosystems, Shanghai, China). Only two ITS sequences from the two closely related *Hedyosmum nutans* and *H. brenesii* could not be directly sequenced due to unclear peaks, so we cloned these PCR products and then sequenced a single clone for each of them.

Sequence alignment and phylogenetic analysis— Forty-six *rps16* intron and ITS sequences were newly generated and added to 158 sequences obtained and used in our previous studies for Chloranthaceae (Antonelli and Sanmartín, 2011; Zhang et al., 2011). We also retrieved from GenBank 54 sequences of 24 Chloranthaceae species and 16 sequences of *Calycanthus floridus*, *Drimys granadensis*, *Magnolia tripetela*, and *Illicium oligandrum*, which were selected as outgroups according to phylogenomic studies (Jansen et al., 2007; Moore et al., 2007). The sample information can be found in Appendix 1. All sequences were aligned using the program CLUSTAL X v. 1.81 (Thompson et al., 1997) and adjusted manually with the program Bioedit v. 5.0.9 (Hall, 1999) according to the similarity criterion described by Simmons (2004). We did not include outgroups for aligning the ITS sequences because the ITS1 and ITS2 regions were too divergent to be aligned between the ingroup and outgroup. We also deleted the ITS sequences of *Ascarina* due to the fact that many different copies from the same individuals were retrieved, perhaps as a result of pseudogenization of those ITS sequences, because we observed abnormal higher substitution rate (more than 3-fold) and decreasing GC content (51% vs. 57%) in them. These sequences also led to spurious phylogenetic relationships in preliminary analyses. Taxon sampling of *Ascarina* was also scarce in our study; therefore, the relationships within the genus require future attention. The deletion of those sequences should have little effect, however, on analyses within *Hedyosmum*, the focus of this study (The matrices and trees are deposited in TreeBase, http://purl.org/phylo/treebase/phylows/study/TB2:S17095).

Because previous studies demonstrated possible phylogenetic conflict within the genus *Hedyosmum*, we tested for congruence among the different loci. First, the Akaike information criterion (AIC; Akaike, 1983) implemented in the program MODELTEST v. 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004) was used to select the best-fitting model of substitution for each locus. Phylogenetic trees based on data from each locus alone were then reconstructed using maximum likelihood (ML; Felsenstein, 1981) searches in the program RAxML v. 7.0.3 (Stamatakis et al., 2008), and the 70% majority-rule consensus trees from 1000 bootstrap searches (Felsenstein, 1985) were compared with each other. For these searches, we performed 1000 bootstrap searches and applied the GTR+G+I substitution model chosen by MODELTEST, leaving all other settings as default. Then, we computed the difference in likelihood values between the ML trees generated based on the ITS data with and without constraining the topology to fit the topology of the plastid combined tree. We further calculated the probability distribution of significance (P) of that difference and repeated the same procedure but based on the plastid combined data with and without topological constraints to fit the ITS tree. Finally, we tested for recombination of the ITS sequences of *Hedyosmum* that could mislead phylogenetic reconstruction, using all methods available in the recombination detection program RDP v. 3.44 (Martin et al., 2010).

Because strong conflicting phylogenetic signals were detected between the plastid and the ITS sequences in the above analyses, phylogenetic trees were reconstructed based on the plastid data and ITS data separately. We used both maximum parsimony (MP) as implemented in the program PAUP* v. 4.10 (Swofford, 2002) and the Bayesian method (Rannala and Yang, 1996) in the program MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001). For the MP analysis, 1000 replicates of random stepwise addition with tree-bisection-reconnection (TBR) branch swapping were performed using heuristic searches, with all most parsimonious trees saved at each replicate. Support for each branch was assessed using bootstrap analyses with 1000 bootstrap replicates, each with 10 random stepwise additions and TBR branch swapping. In the Bayesian analysis, two independent runs with four Markov chain Monte Carlo (MCMC) runs (three heated and one cold) were run simultaneously, sampling every 3000 generations for 30 million generations and starting with a random tree. The first 4.5 million generations (15% of the total) were excluded as burn-in after convergence of the chains, which was evaluated by the average standard deviation of splitting frequencies reaching below 0.01.

For displaying the extent of phylogenetic incongruence of the phylogenetic trees based on the plastid and ITS data, the Bayesian majority consensus trees from each locus with branches whose support values were lower than 50% were collapsed and then combined using the software SplitsTree4 ver. 4.10 (Huson and Bryant, 2006). This procedure allowed the construction of a tree showing different phylogenetic positions of taxa in the two tree sources.

Dating analyses— We did not use the plastid and ITS combined data in the dating analyses because of the conflicting phylogenetic signals detected between the two data sets (see below). Instead, we performed separate dating analyses and evaluated the results individually. First, we used the same matrix of 64 accessions with one to three missing plastid loci for a few species (named "plastid matrix" hereafter) used for phylogenetic reconstruction. To test the influence of long missing DNA regions (1–3 loci missing) of a few accessions on the dating analysis, we additionally compiled a matrix of only 52 accessions for which all four plastid loci were available ("full plastid matrix" hereafter). Second, we dated a data set consisting of ITS sequences for 44 Chloranthaceae accessions. Because of difficulties in confidently aligning ingroups and outgroups and the presence of heterogeneous substitutions particularly in the genus *Ascarina*, this data set did not include any *Ascarina* species. The results (chronograms) from the dating analyses were used for biogeographical reconstruction and the calculation of PD measures.

Initial likelihood ratio tests for rate consistency were conducted for each locus in PAUP* 4.0b10. The null hypothesis of strict clock was rejected for each of the four regions ($P < 0.01$). Time estimations were therefore performed under a Bayesian relaxed clock (BRC) in BEAST v. 1.5.3 (Drummond and Rambaut, 2007) and a relaxed molecular clock under the penalized likelihood (PL) method in the program r8s v. 1.71 (Sanderson, 2002), respectively. In the BRC analysis (in Beast v. 1.5.3), the relaxed clock method with uncorrelated lognormal distribution (UCLN) was used. The same best fitting substitution model previously selected in MODELTEST for the ML analysis on each plastid locus (GTR+I+G) was also used for the combined plastid data. Convergence of runs was evaluated in the program Tracer v. 1.6 (Rambaut et al., 2014). After 50 million generations of MCMC searches with one sample every 5000 generations, the effective sampling sizes (ESS) for all the relevant parameters were well above 200. The post burn-in samples from two independent searches were combined to calculate the mean times and 95% highest posterior densities (HPD), which could then be displayed in FigTree v. 1.0 (Rambaut, 2006). For the PL analysis, the optimal smoothing parameter (λ) was determined by cross-validation. The input trees required by the method were the phylograms generated from the former BRC analysis (in BEAST) with the outgroup *Illicium* subsequently pruned, as required by r8s. Confidence intervals for node ages were calculated with 1000 nonparametric bootstrap analyses.

To calibrate the plastid phylogenetic tree, we used two fossils and a deep secondary calibration to constrain the three corresponding ancestral nodes in the phylogeny and transform branch lengths into absolute times, following the morphological discussion in our previous study (Zhang et al., 2011). The earliest *Hedyosmum*-like fossil (*Asteropollis*; ca. 112 Ma) was used to constrain the ancestral stem node of *Hedyosmum* according to morphological cladistic analyses (Doyle et al., 2003; Eklund et al., 2004). In addition, the earliest *Chloranthus*-like fossil (*Chloranthistemon*; ca. 90 Ma) was used to constrain the stem lineage leading to both *Chloranthus* and *Sarcandra* according to the adjustment of cross-validations in a previous dating analysis (Zhang et al., 2011). Finally, the median age of the Chloranthaceae/Magnoliids divergence was set to 150 Ma (95% confidence interval: 140–160 Ma) according to the converging range of several angiosperm-scale dating analyses (Wikström et al., 2001; Moore et al., 2007; Zhang et al., 2012; Zeng et al., 2014). The details of calibration settings are listed in Appendix S1 (see Supplemental Data with the online version of this article). To calibrate the ITS phylogenetic tree, we used the earliest *Asteropollis* fossil as the only calibration to constrain the root height (crown Chloranthaceae or stem *Hedyosmum*) because the other two calibration nodes used in the plastid phylogeny were not included in the ITS tree.

***Biogeographical reconstructions*—**The ancestral geographical distribution of nodes was reconstructed based on the plastid and ITS trees using Fitch parsimony in the program Mesquite v. 2.01 (Maddison and Maddison, 2007) and maximum likelihood in the program Lagrange v. 20110117 (Ree and Smith, 2008). The maximum clade credibility tree produced by the BRC dating analysis with all four Chloranthaceae genera included ("plastid familial tree") was used as the input tree. To test the effect of topological variations on the results, we also used the ITS chronogram with *Hedyosmum*, *Chloranthus*, and *Sarcandra* ("ITS familial tree") for biogeographical reconstruction under these two methods. In addition, the plastid and ITS trees with only *Hedyosmum* taxa included ("*Hedyosmum* plastid" and "*Hedyosmum* ITS" trees) were also used. We followed this approach to test the influence of long branches on geographical inferences, since previous studies showed long stem branches (with associated long time spans) for all four genera in Chloranthaceae (Antonelli and Sanmartín, 2011; Zhang et al., 2011).

We divided the distribution range of extant Chloranthaceae into seven areas: East and Southeast Asia (A), India-Sri Lanka (B), Central America (C), the Antilles (D), South America (E), the Pacific islands (F), and Madagascar (G). These areas are very different in geological history, being separated from each other today or once separated by oceans, within the timeframe of Chloranthaceae evolution as indicated by geological and paleontological evidence and molecular dating analysis (Friis et al., 1986, 1999; Eklund et al., 1997; Zhang et al., 2011).

The parameters in the Lagrange analysis were left as the default settings except that a maximum of two ancestral areas (except the combination of ABF which are occupied by the species *Chloranthus erectus* and cannot be excluded) were set as constrained for all ancestral nodes, on the assumption that no ancestral taxa occupied more than two large continental-scale units as defined here (only AB, AC, AD, BF, BG, AF, CD, CE, DE and EF permitted; the other potential combinations were excluded since the continents they represent were never close to one another during the period considered). We based this assumption on the observation that most extant taxa (ca. 71/77) are only restricted to one of the defined units, except three species that occupy the adjacent areas between Panama and northern South America (*Hedyosmum bonplandianum*, *H. goudotianum*, and *H. scaberrimum*) and three species in neighboring regions in the Asian/Indo-Pacific Islands (*Sarcandra glabra*, *Chloranthus erectus*, and *C. nervosus*).

***Phylogenetic diversity*—**The PD and mean nearest taxon distance (MNTD) of each genus and the geographical assemblages of South and Central American *Hedyosmum* taxa were calculated according to the two time-calibrated trees as well as the two ML phylograms based on either the plastid or the ITS, respectively, using the package Picante (Kembel et al., 2010) implemented in the platform R v. 2.11.1 (R Development Core Team, 2010).

***Floral evolution*—**To test the effect of phylogenetic incongruence on morphological changes, we reconstructed and compared the evolution of four morphological characters of taxonomic significance, based on the *Hedyosmum* plastid and ITS trees separately. These characters included: (1) sexual organization (dioecy; monoecy); (2) sexual structure (solitary pistillate flowers; pistillate flowers clustered into many cymules; pistillate flowers clustered into 1–3 cymules); (3) bract texture of pistillate flowers (chartaceous; fleshy); and (4) bract arrangement (bracts not enclosing or partially enclosing [enveloping the lower half or less of the pistillate flower]; completely enclosing the pistillate flowers [only the stigma uncovered]). These states follow the previous literature on the family and were coded numerically in Appendix S2 (see online Supplemental Data) (Todzia, 1988). The reconstruction was performed using Fitch parsimony in Mesquite.

RESULTS

***Data assemblage and congruence tests*—**After trimming the 5′ and 3′ ends with missing sites in most sequences, the *rps16* region of Chloranthaceae had a length variation from 794 to 850 bp with an average GC content of 33.7%. The alignment had a length of 969 characters, of which 171 were variable including 116 parsimony informative characters. The ITS region of Chloranthaceae ranged from 365 to 664 bp after pruning both flanks, with an average GC content of 58.5%. The final alignment had 766 bp, encompassing 330 variable characters with 258 parsimony informative characters. Information about the other loci used is described in our previous study (Zhang et al., 2011).

No obvious conflict was observed among the four plastid ML trees as shown in Appendices S3–S7 (see online Supplemental Data). However, moderately (here defined as bootstrap support [BS] ≥ 70% and/or Bayesian posterior probability [BPP] ≥ 0.80) to strongly supported (BS ≥ 90% and/or BPP ≥ 0.95) conflict was observed between some clades in the combined plastid tree and the ITS tree in *Hedyosmum* generated using all three phylogenetic methods (MP, ML and MrBayes) (Fig. 1). Conflict in *Hedyosmum* was further confirmed by significant log likelihood differences (X^2 = 382 or 299 based on the plastid or ITS data, respectively; df = 29; $P < 0.01$ according to χ^2 tests) between trees estimated without topological constraints vs. trees constrained to the topology obtained from the other data set (i.e., the ITS tree reflecting the plastid tree and vice versa; see Table 1). The conflict between the plastid tree on the one hand, and the ITS tree and morphological classification in *Hedyosmum* on the other hand, was also supported by common indels among species from several taxonomic levels (e.g., subgenus and section; see in Fig. 1 and more details below). These results suggest that phylogenetic conflict in *Hedyosmum* cannot be attributed to stochastic (e.g., sequencing) errors. Additionally, no recombination was detected for the ITS sequences of *Hedyosmum*.

***Phylogenetic relationships*—**The plastid data yielded 58 330 most parsimonious trees with a length of 1156 steps, consistency index (CI) = 0.74 and retention index (RI) = 0.93, while the ITS data produced only 21 most parsimonious trees with 416 steps, CI = 0.67 and RI = 0.9. Both maximum parsimony and Bayesian methods produced majority consensus trees with very similar topologies for the same data set. Figure 1 shows the 50% majority consensus Bayesian trees from each of the two data sources, with both Bayesian posterior probabilities and bootstrap support values mapped.

Both the plastid and ITS consensus trees revealed concordant generic relationships and intrageneric relationships within *Chloranthus*, which are also in line with previous phylogenetic analyses (Antonelli and Sanmartín, 2011; Zhang and Renner, 2003; Zhang et al., 2011). In the small genus *Sarcandra*, the plastid tree supports *S. glabra* is sister to *S. glabra* var. *hainanensis* (BPP = 0.98; BS = 57%), which are together sister to *S. chloranthoides*, a morphologically divergent and

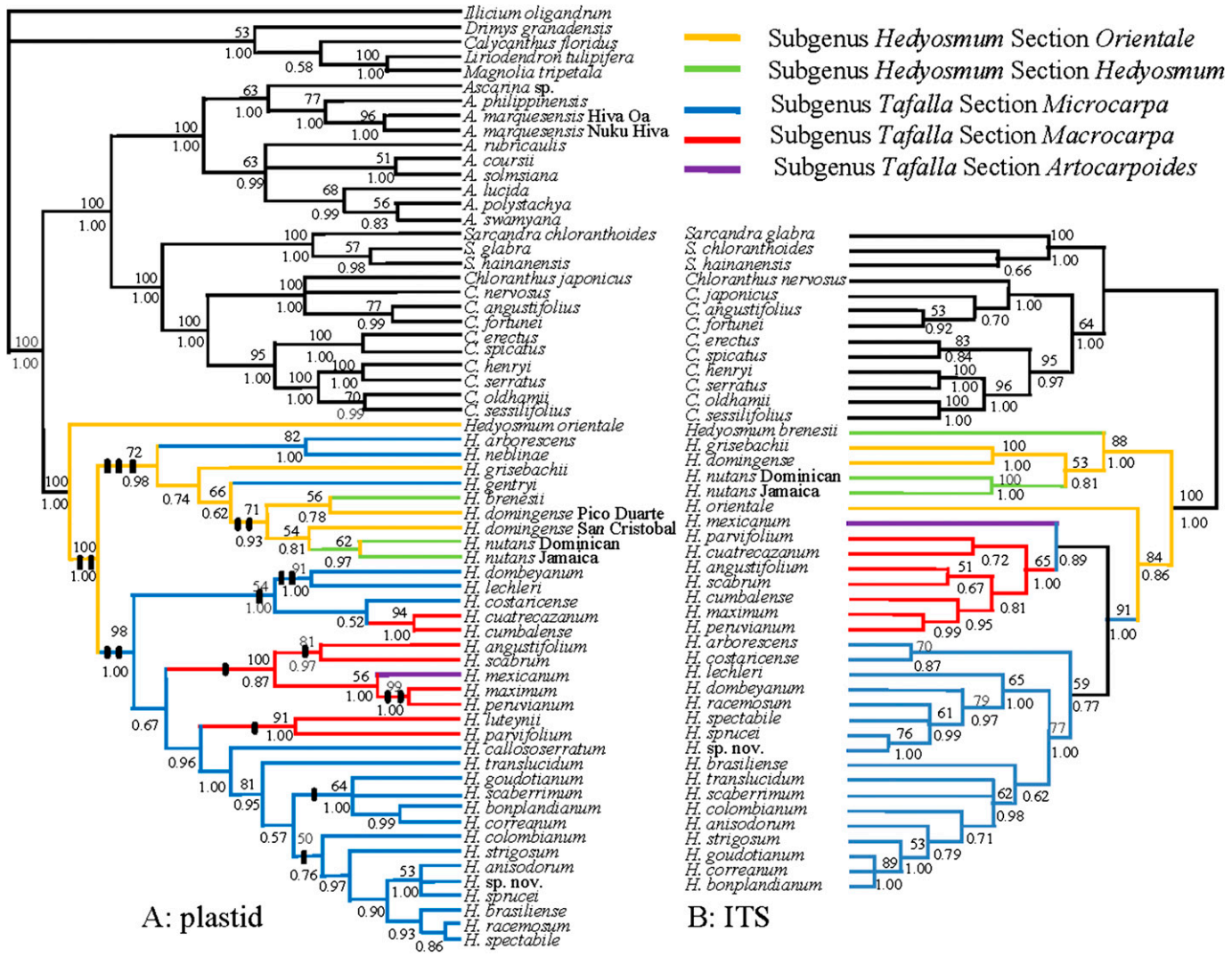

Fig. 1. (A) plastid and (B) ITS phylogenies of Chloranthaceae and the traditional taxonomic classifications in the genus *Hedyosmum*. Bayesian consensus cladograms were generated based on data of the four plastid loci (*rbcL*, *trnL-F*, *rpl20-rps12*, and *rps16*; A) and the ITS (B), with posterior probabilities and maximum parsimony bootstrap supports indicated below and above branches, respectively. The classification follows Todzia (1988). Vertical bars on the branches within *Hedyosmum* in the plastid tree represent common indel(s) of the taxa subtended by each of the branches. *A.*, *Ascarina*; *C.*, *Chloranthus*; *H.*, *Hedyosmum*; *S.*, *Sarcandra.*

geographically isolated species. The ITS MP tree also supports the same relationships (BS = 76%), but the ITS Bayesian tree instead suggests a close relationship of *S. glabra* var. *hainanensis* and *S. chloranthoides* with weak support (BPP = 0.66). In *Ascarina*, the plastid tree topology was identical to our previous plastid tree without the *rps16* intron added (Zhang et al., 2011).

In *Hedyosmum*, many phylogenetic conflicts were uncovered by comparing the plastid and ITS majority consensus trees. Terminals with variable phylogenetic positions shift position over multiple nodes and/or among higher taxonomic groups (subgenera and sections), i.e., units reflecting rather distinct morphologies. For instance, *H. orientale* from subgenus *Hedyosmum* represents the first-diverging lineage in the plastid tree (BS = 100%; BPP = 1.00). This position is further supported by two common indels: one deletion of seven nucleotides and one insertion of eight nucleotides in all other *Hedyosmum* except *H. orientale* for the *rpl20-rps12* region (Fig. 1). However, *H. orientale* is nested within the tropical American taxa in the ITS tree, being sister to a large clade consisting of taxa previously placed in subgenus *Tafalla* (BS = 84%; BPP = 0.86).

Other major shifts between subgenera include *H. arborescens* and *H. gentryi* (subgenus *Tafalla*) placed within that subgenus in the ITS tree, but nested within subgenus *Hedyosmum* in the plastid tree. This shift happened despite moderately to strongly

Table 1. The best fitting substitution model, differences and the significance (*P*) of likelihood values of maximum likelihood (ML) trees with and without topological constraints for the plastid and ITS data of *Hedyosmum*.

Data	Optimal model	−ln L	−ln L topological constraint	No. taxa (df)	*P*
Plastid	K81uf+I+G	7289.40969	7671.76928	31 (29)	<0.01
ITS	TrNef+G	2434.64011	2733.82926	31 (29)	<0.01

supported nodes in each phylogeny, as well as several common indels (one deletion of five nucleotides and one insertion [except in *H. arborescens*] of six nucleotides for the *rpl20-rps12* region; and one deletion of two nucleotides for the *trnL-F* region; see Fig. 1).

Within subgenus *Tafalla*, the ITS tree supports the taxonomic division of the subgenus into three morphologically distinctive sections. In the plastid tree, *H. cuatrecazanum* and *H. cumbalense* from section *Macrocarpa* form a strongly supported clade with *H. dombeyanum*, *H. lechleri*, and *H. costaricense* from section *Microcarpa* (BPP = 1.0; BS = 54%; and one common indel: one insertion of five nucleotides for the *trnL-F* region). A lineage including *H. luteynii* and *H. parvilifolium* from section *Macrocarpa* is sister to another larger lineage consisting of most taxa from section *Microcarpa* with high BPP = 0.96 (but BS < 50%).

Besides the striking phylogenetic conflicts listed above, many additional conflicts were identified within lower taxonomic units (i.e., sections and groups), whose taxa are more morphologically homogeneous, and some species also shift position over several moderately to strongly supported nodes (Fig. 1). The general scope and extent of phylogenetic conflict within *Hedyosmum* is illustrated by the combination of the plastid and ITS Bayesian majority consensus trees into a single network (Fig. 2).

***Divergence times and ancestral distributions*—**The dating results from different data sets and methods, in comparison with ages obtained in previous studies, are listed in Appendix S1. According to the combined plastid data and the BRC dating method, the four genera successively diverged from each other from the Early Cretaceous to the early Cenozoic (Fig. 3A). The most recent common ancestor (MRCA) in all genera, however, appeared much later, which either represents a delay in their species diversification or (more likely) the confounding effect of extinction on phylogenetic trees (Antonelli and Sanmartín, 2011).

The ancestral area reconstruction depended on methodology, sampling (familial vs. generic levels), and tree topology from different sources of data (plastid vs. ITS). Overall, we found quite different results for some nodes, particularly in *Hedyosmum*. Our biogeographical analyses suggest that the ancestral lineages of all genera were restricted to, or at least include, Asia. *Ascarina*, with a current distribution on South Pacific islands and Madagascar, had its stem lineage in Asia (Mesquite) or was widely distributed in both Asia and the Pacific islands (Lagrange). Similarly, the stem lineages of both *Chloranthus* and *Sarcandra* and the crown *Chloranthus* originated and diversified in East and Southeast Asia, from where some lineages in both genera independently expanded into India/Sri Lanka at least twice. The crown ancestor of *Sarcandra* could either be restricted to East and Southeast Asia (Mesquite) or occupied both East and Southeast Asia and India–Sri Lanka (Lagrange) (Fig. 3A, B).

Both the plastid and ITS familial trees suggest that the crown ancestor of *Hedyosmum* was in Asia (Mesquite) or occupying a wider distribution in both Asia and the Antilles (Lagrange) followed by dispersals into South America and Central America (Fig. 3A, B). Multiple shifts among the Antilles, Central America, and South America are suggested. The directionality of most continental dispersals could not be determined with confidence, except for three cases that could be determined regardless of the trees and methods used. These included one dispersal into

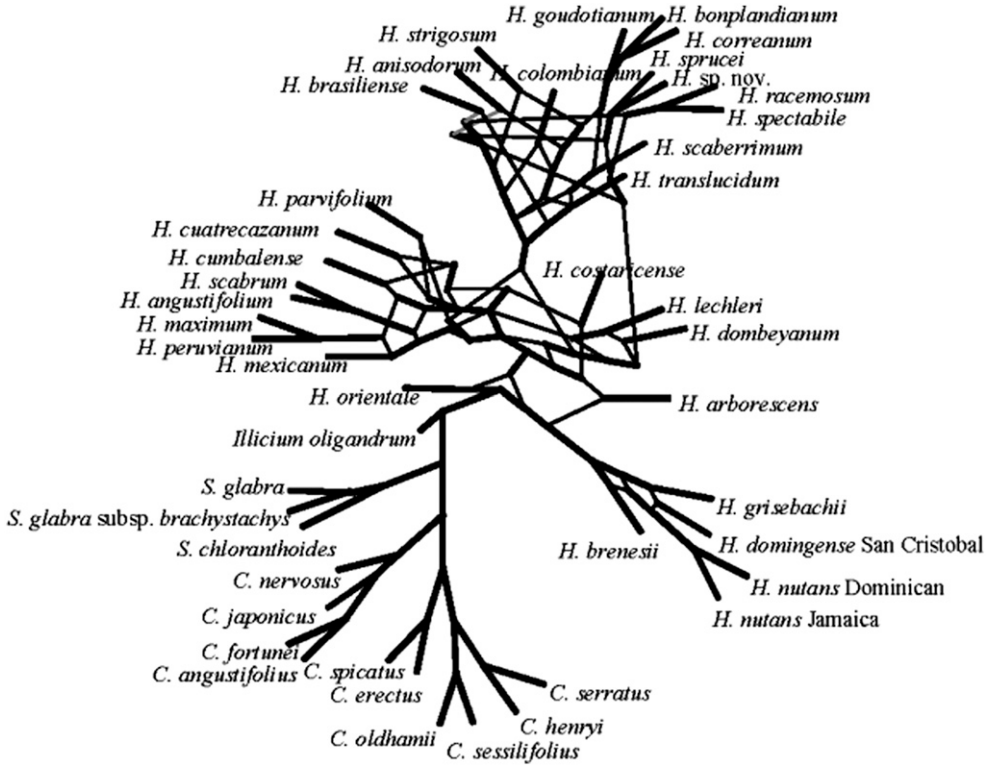

Fig. 2. Phylogenetic incongruence between the plastid and ITS trees in *Hedyosmum*. The network was combined from the plastid (*rbcL*, *trnL-F*, *rpl20-rps12* and *rps16*) and ITS Bayesian majority trees using the program SplitsTree. See Fig. 1 for abbreviations of the generic names.

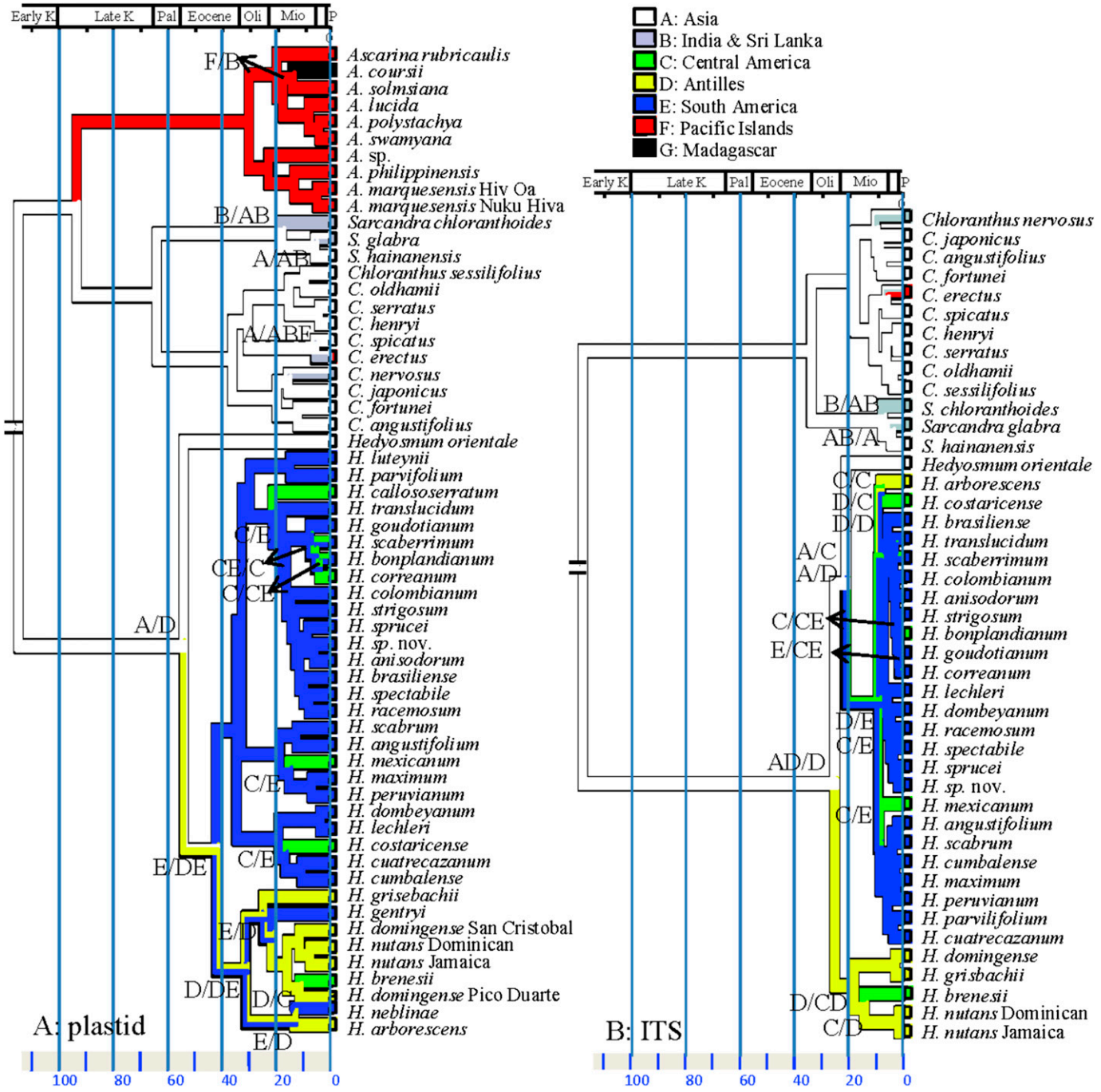

Fig. 3. Multiple shifts of geographical ranges and divergence times of Chloranthaceae based on the familial (A) plastid and (B) ITS trees. Ancestral reconstruction was generated by the Fitch MP method in Mesquite. Ancestral distributions inferred from a DEC likelihood model in Lagrange are also provided for nodes where there was a discrepancy between the results of these two methods. Early K, early Cretaceous; Late K, late Cretaceous; Pal, Paleocene; Oli, Oligocene; Mio, Miocene; P, Pleistocene.

Central America from the Antilles (leading to *H. brenesii*) and two dispersals from South America into Central America (leading to *H. scaberrimum-H. bonplandianum-H. correanum* and *H. callososerratum*; *H. callososerratum* is not included in the ITS tree due to a failure in obtaining its ITS sequence). As one example of the impact of phylogenetic conflict on the biogeographical results, the plastid familial tree suggests a possible first dispersal into South America (at the second deepest node in the tree) from the Antilles, but the ITS tree indicates the first dispersal into South America was equally possible at the second, third, or fourth deepest node (Mesquite) or at the third deepest node (Lagrange) and that it came either from Asia, Central America, or the Antilles. Similarly, it was not possible to determine whether the South American species *H. gentryi* and *H. neblinae* (only sampled in the plastid tree) originated in South America or whether it came instead from an ancestral Antillean lineage (Mesquite). In addition, a total of four dispersals into Central America from South America are clearly suggested by the plastid

familial tree, while only two of them are corroborated by the ITS tree regardless of optimization methods (Fig. 3).

When only *Hedyosmum* species are included, the plastid tree suggests a possible distribution either in Asia, the Antilles, or South America for the crown ancestor of the genus (Mesquite) or a wider distribution in both Asia and the Antilles (Lagrange), followed by several independent forward and reverse dispersals among the Antilles, Central America, and South America. These include one dispersal from the Antilles to Central America and four dispersals from South America into Central America, which could be identified by both biogeographical methods as shown in online Appendix S8. The *Hedyosmum* ITS tree also indicates an equivocal distribution of the crown ancestor, in any one of the four geographic units of Asia, the Antilles, Central America, or South America alone (Mesquite), but an almost equally possible wider distribution either in Asia and Central America or in Central America and the Antilles (instead of in Asia and the Antilles as suggested by the plastid tree and Lagrange). The *Hedyosmum* ITS tree also suggests a later possible first dispersal into South America than that suggested by the *Hedyosmum* plastid tree (no younger than at the fourth vs. the second deepest node in the ITS and plastid trees, respectively). Moreover, the *Hedyosmum* ITS tree only supports two of the four subsequent dispersals into Central America from South America inferred from the *Hedyosmum* plastid tree. The other two shifts (leading to *H. costaricense* and *H. mexicanum*) are not from South America into Central America but in the reverse direction according to the *Hedyosmum* ITS tree (Lagrange) (Appendix S8).

In summary, we detected considerable qualitative and quantitative differences between the plastid and nuclear trees inferred and also between the biogeographical methods used.

***Phylogenetic diversity*—**PD is correlated with TD among the three genera *Sarcandra*, *Chloranthus*, and *Hedyosmum*, regardless of being based on the plastid ($t = 14.15$, $r = 1.00$, df = 1, $P = 0.04$ for the phylogram; and $t = 45.27$, $r = 1.00$, df = 1, $P = 0.01$ for the chronogram) or the ITS data ($t = 5.05$, $r = 0.99$, df = 1, $P = 0.11$ for the phylogram and $t = 5.15$, $r = 0.99$, df = 1, $P = 0.11$ for the chronogram). However, the plastid and the ITS data generated contrasting PD measures within *Hedyosmum*. The plastid data resulted in a PD = 0.61, 1.02, and 2.15 total substitutions/sites (phylogram) and a PD = 112.33, 176.92, and 378.12 total million years of divergence (chronogram) for *Hedyosmum* in the Antilles, Central America and South America, respectively. In contrast, the ITS data resulted in a PD = 0.32, 0.26 and 0.24 total substitutions/sites (phylogram) and PD = 79.94, 84.90 and 71.32 total million years of divergence (chronogram) for the *Hedyosmum* from the same three areas, respectively (Table 2).

***Floral evolution*—**All four coded morphological characters showed different evolutionary pathways as inferred according to the plastid and ITS trees, respectively. In the plastid tree, the reconstruction clearly showed that the MRCA of *Hedyosmum* was dioecious, whereas it was equivocal (either dioecious or monoecious) according to the ITS tree as shown in online Appendix S9. The plastid tree further indicated that three shifts have taken place between the state of solitary pistillate flowers on the axis and flowers clustering into many (>3) cymules, with an additional three shifts between the state of pistillate flowers clustering into many cymules and clustering into only 1–3 cymules. In contrast, in the ITS tree, a single shift was reconstructed from solitary flowers into many cymules, and from many cymules into 1–3 cymules (Fig. 4). The plastid reconstruction also showed at least three to four independent shifts between fleshy and chartaceous bracts and from bracts not enclosing or partially enclosing, to completely enclosing bracts in the pistillate flowers. These results were in contrast to those from the ITS tree, in which these two characters evolved only once as shown in Appendix S10 and S11 (see Supplemental Data with the online version of this article).

DISCUSSION

Underlying causes for the phylogenetic conflict in* Hedyosmum*—Phylogenetic conflict between different sources (different loci or genomes) of DNA sequences is being increasingly acknowledged (Degnan and Rosenberg, 2006). Conflict can be divided into two broad categories according to whether the gene trees naturally reflect the evolutionary relationships of taxa (their species tree). In the first category, the gene tree does not reflect natural relationships of taxa due to incomplete lineage sorting of ancestral polymorphisms, paralogy, saturated substitutions, nonneutral evolution, and other stochastic, methodological, or systematic errors (Wendel and Doyle, 1998; Slowinski and Page, 1999; Degnan and Rosenberg, 2006). In the second category, different gene trees reflect different inheritance lines (i.e., maternal or paternal) of relationships when taxa experienced reticulate evolution (through hybridization or gene

TABLE 2. Diversity measures (phylogenetic diversity, PD; and taxonomic diversity, TD) for each defined unit in Chloranthaceae based on the plastid and ITS data, respectively, and related Pearson's correlation coefficients (r) and probabilities of significance (P). The three genera (*Chloranthus*, *Sarcandra* and *Hedyosmum*) with dense sampling and the three distributional areas with different tectonic histories and more than one *Hedyosmum* species were defined and compared among each other for their PD and TD diversities.

Data						*Hedyosmum*				
Statistic	*Sarcandra*	*Chloranthus*	*Hedyosmum*	*r*	*P*	CA	Antillean	SA	*r*	*P*
Plastid										
PD	0.19 (36.50)	1.03 (168.62)	3.07 (572.03)	1.00 (1.00)	0.04 (0.01)	1.02 (176.92)	0.61 (112.33)	2.15 (378.12)	0.99 (1.00)	0.07 (0.06)
mntd	0.09 (16.36)	0.10 (15.52)	0.11 (22.00)			0.18 (34.68)	0.16 (29.64)	0.10 (19.24)	−0.92 (−0.89)	0.23 (0.30)
SR	3	10	34			8	5	24		
ITS										
PD	0.08 (22.70)	0.33 (92.13)	0.70 (196.27)	0.99 (0.99)	0.11 (0.11)	0.26 (84.90)	0.32 (79.94)	0.24 (71.32)	−0.80 (−0.86)	0.41 (0.34)
mntd	0.04 (11.78)	0.04 (11.87)	0.02 (7.27)			0.05 (15.66)	0.05 (13.92)	0.01 (3.60)	−0.99 (−0.96)	0.11 (0.20)
SR	3	10	31			7	4	21		

Notes: CA, Central American; chrono, chronogram; mntd, mean nearest taxon distance; *P*, probability; PD, phylogenetic diversity; phylo, phylogram; *r*, correlation coefficient; SA, South American; SR, species richness. See more details in Materials and methods.

Fig. 4. Contrasting morphological evolution of inflorescence structure in *Hedyosmum* based on (A) the plastid and (B) the ITS trees. The pistillate flowers are shown to undergo multiple shifts among the three coded states on the plastid tree (left). In contrast, the ITS tree (right) shows a single shift from solitary flowers to be clustered into many cymules, and one shift from flowers clustered into many cymules to clustered into 1–3 cymules. Bayesian posterior probabilities of clades are also mapped.

introgression; Soltis and Kuzoff, 1995; McKinnon et al., 2001; Okuyama et al., 2005; Frajman et al., 2009; Spinks and Shaffer, 2009).

The major conflict we found between the plastid and ITS trees in *Hedyosmum* seems robust, at least for those accessions that shift phylogenetic position over several clades with moderate to strong support in both the plastid and ITS trees. One example is *H. arborescens* and *H. gentryi* from subgenus *Tafalla,* which according to the plastid tree are instead embedded within subgenus *Hedyosmum.* A second example is species in section *Macrocarpa* (*H. cuatrecazanum–H. cumbalense* as well as *H. luteynii–H. parvilifolium*), which are nested with species from section *Microcarpa* in the plastid tree. Interestingly, the classification of these taxa with phylogenetically variable positions are consistently well supported by the ITS tree.

For the ITS sequences, no different paralog sequences were detected in most *Hedyosmum* taxa, as they were directly sequenced using the PCR products as templates (except for the closely related *H. brenesii* and *H. nutans*). Homoplasious substitutions and long branch attraction may be ruled out as the causes for the conflict, since the phylogenies obtained were largely insensitive to the different methods (under parsimony and maximum likelihood) and no high homoplasy index was obtained (CI = 0.67; RI = 0.90; HI = 0.33 for the ITS data; Bergsten, 2005). In addition, no long branches were observed to affect the phylogeny, except for three of the four sampled Mesoamerican taxa that showed consistently much longer branches. However, these taxa had no effect on the rest of the phylogeny, as assessed by excluding them in a separate phylogenetic analysis (not shown). Additionally, no recombination signals were detected using RDP, indicating that recombination can also be excluded as potential bias that would have misled the ITS phylogenetic reconstruction and account for the phylogenetic incongruence detected.

More importantly, the ITS phylogeny agrees considerably better with the traditional taxonomic classifications suggested by cladistic analyses of over 100 morphological characters in subgenus *Tafalla* (Todzia, 1988; Doyle et al., 2003; Eklund

et al., 2004). Therefore, despite the common and generally valid criticisms against the use of ITS in molecular phylogenetics (Álvarez and Wendel, 2003), we interpret the striking congruence between the ITS phylogeny and the morphology-based classification as an indication that the ITS tree is more reliable than the plastid tree to represent species relationships in *Hedyosmum*.

The placements of phylogenetically variable species are equally supported in the plastid tree with strong support, not only by nucleotide substitutions but also by shared indel(s). However, their placements largely contradict the morphology-based taxonomy and the ITS phylogeny. To force the phylogenetic placements of these taxa to fit with those suggested by the ITS tree (or the traditional taxonomy) would require collapses of several moderately to strongly supported nodes, which are supported by both nucleotide substitutions and common indels. Consequently, these distant shifts of phylogenetic positions seem unlikely to be attributable to deep incomplete lineage sorting, unidentified paralogy, saturated substitution, heterogeneous or nonneutral evolution, and other stochastic or systematic errors of the sequences obtained.

Hybridization has been widely reported in plant lineages and constitutes an important speciation mechanism, as more than 20% of extant taxa could have recent hybridization origins (Mallet, 2005, 2007). In hybrid species, sequences from different genomes usually reflect different lines of inheritance (e.g., the plastid from the maternal line, the mitochondrion from the paternal line and nuclear DNA from both parental lines), which could lead to phylogenetic conflict between these different data sources. In *Hedyosmum*, hybridization seems to provide the best explanation for some of the major phylogenetic conflicts detected, such as species shifting phylogenetic position over multiple well-supported clades, e.g., *H. gentryi* from subgenus *Tafalla*. This species also possesses multiple characters (e.g., chartaceous bract and cymule composed of only one flower) that are almost exclusive to subgenus *Hedyosmum*.

Our interpretation of a hybrid origin for certain lineages in Chloranthaceae precludes the use of methods that assume phylogenetic conflict to derive from a single evolutionary process, such as incomplete lineage sorting. One example is the multispecies, multilocus coalescent approach implemented in *BEAST (Heled and Drummond, 2010), which to date cannot deal with taxa of hybrid origin and could therefore not be used for this study due to a violation of its main assumption.

Within each of the morphologically homogeneous lineages (i.e., within a section or group), no lineage with more than four species shows internally consistent phylogenetic relationships between the plastid and ITS trees (Fig. 2). However, some of the conflicting nodes only received low to moderate support. At this taxonomic depth, it is difficult to locate distinctive characters that can be used to evaluate the validity of conflicting relationships as suggested by the plastid and nuclear ITS phylogenies. In addition, it is difficult to distinguish among different potential processes, e.g., hybridization from incomplete lineage sorting, which could both account for phylogenetic conflict. Moreover, plastid capture via androgenesis (cases where the nuclear genome is completely inherited from the paternal line) is uncommon, but cannot be completely excluded (Hedtke and Hillis, 2011). Assessing how much of the present conflicting phylogeny in *Hedyosmum* was caused by hybridization therefore requires further investigation. More data, especially sequences from many single-copy nuclear loci, will be required to discern between competing hypotheses of phylogenetic conflict in the genus.

Implications of phylogenetic conflict for taxonomy and morphological evolution—Hypotheses of evolutionary relationships unveiled by molecular phylogenetic trees have been widely used to test the validity of traditional taxonomy (e.g., APG III, 2009). However, taxonomic definition faces a dilemma when different sources of molecular data yield different relationships, with one supporting and the other rejecting the monophyly of taxa. We found clear evidence for such a situation within *Hedyosmum*. If the incongruent molecular phylogenies result from hybridization, any classification placing the hybrid species in either of the parent taxa becomes problematic. Although phylogenetic conflict and its taxonomic implications were detected and examined here for *Hedyosmum*, other studies based on DNA data from a single genomic source may fail and lead to confounding taxonomic conclusions in similar situations. A possible solution to compromise conflicting topologies is to place the suspected hybrid species in their own taxonomic unit, as is the case of the orchid genus *Cattleya* (van den Berg, 2014).

Discordant phylogenies may also have a major effect on the inferences of morphological evolution, as shown here. For reproductive functional evolution, both the plastid and ITS trees suggested independent origins of monoecy from dioecy in several species of *Hedyosmum* as shown in Appendix S9. In the ITS tree, our analyses showed an equivocal state for the crown ancestor of the genus, differing from that according to the plastid tree, which clearly suggested dioecy. Shifts between dioecy and monoecy overall were frequent with a general trend from bisexual into unisexual flowers, and from monoecy into dioecy in the whole family (a result echoed when both living and fossil taxa are coanalyzed; see Doyle and Endress, 2014).

For the remaining three of the four selected reproductive characters for our analysis, the ITS tree showed a single shift of states, whereas the plastid tree indicated multiple morphological shifts (Fig. 4; Appendices S10, S11). Three character states (solitary pistillate flowers in combination with chartaceous bracts not or partially enclosing the pistillate flowers; pistillate flowers clustering into many cymules with fleshy bracts partially enclosing the pistillate flowers; and pistillate flowers clustering into 1–3 cymules with flesh bracts completely enclosing the pistillate flowers) have been used as key taxonomic characters. These characters, which played a key role in taxonomic delimitation in the group, are unlikely to have undergone so many shifts. We find it more plausible that these character states originated only once, as suggested by the ITS tree.

Influence of phylogenetic conflict on historical biogeography—Our biogeographical reconstructions showed a major methodological effect on the results. Lagrange usually inferred a wider distributional range compared to Fitch optimization in Mesquite, but such a contrast is probably mainly derived from methodological differences because Fitch optimization precludes the inference of widespread ancestors (i.e., in more than one area). Due to the arguably low possibility for Chloranthaceae species to occupy more than one continental-level area (see Materials and Methods), we interpret the results of widespread ancestors with caution.

Multiple large-scale range expansions or shifts have been documented in each of the crown genera. In *Chloranthus* and *Sarcandra*, at least two invasions into India/Sri Lanka from East and Southeast Asia since the Miocene were inferred, long after the Eocene collision of India with Eurasia (Zhu et al., 2005; Favre et al., 2015). *Hedyosmum* was also inferred to shift its range multiple times, including a shift between tropical Asia

and tropical America and multiple shifts among the Antilles, Central America, and South America since the Eocene onward (Fig. 3). We consider the majority of shifts between currently isolated continents to result from long-distance or stepping-stone dispersals, including the intrageneric continental-scale expansions, and some more obvious long-distance dispersal events (e.g., of *Ascarina* on the Pacific Islands and Madagascar). We base this interpretation on the younger timing of lineage splits as compared with the geological separations of the regions occupied. High dispersal ability in the family is probably facilitated by the small berry or drupe fruits for most species, which are likely mediated by avian vectors (Todzia, 1988).

The different topologies shown by the plastid and ITS trees also affected the ancestral distribution inferences, as shown in *Hedyosmum* (Fig. 3; Appendix S8). The most striking distribution, the amphi-Pacific disjunction in Asia and America of *Hedyosmum*, seems to have different explanations according to different tree topologies, as suggested by the plastid and ITS data. The plastid tree topology, together with generally old divergence time estimates for the genus (ranging from ca. 69 Ma–ca. 25 Ma according to different methods and matrices; see Appendix S1) and an extensive record of *Hedyosmum*-like fossils extending to high latitudes of the northern hemisphere (Walker and Walker, 1984; Friis et al., 1999), suggest that this disjunction could have been caused by vicariance over more or less continuous land, possibly across the boreotropical flora (Tiffney, 1985; Wolfe, 1997; Manchester, 1999; Azuma et al., 2001; Davis et al., 2002; Cuenca et al., 2008; Antonelli and Sanmartín, 2011).

In contrast, on the basis of the ITS tree, we tend to interpret that *Hedyosmum* originated in the Antilles with a subsequent dispersal into Asia (formation of the Asian endemic *H. orientale*), Central America, and South America. However, our results suggest that it was equally possible for the ancestor of crown *Hedyosmum* to have been confined to Asia, Central America, the Antilles, or South America or some of these areas combined—all depending on the tree and biogeographical method used. On the basis of the ITS tree, the divergence of *H. orientale* from its American congeners was estimated to be in general considerably younger, with a mean age around 18.0 (8.3–29.4) Ma, which would more likely be the result of long-distance dispersal over larger water gaps across the northern hemisphere.

Both the plastid and ITS phylogenies suggest that *Hedyosmum* underwent multiple dispersals among the Antilles and Central and South America. According to the plastid tree, *Hedyosmum* was inferred to colonize South America from Central America at the second- (Lagrange, but equivocal for Fitch optimization) or third-deepest node, earlier than a dispersal inferred at the fourth-deepest node based on the ITS tree. Furthermore, the plastid tree suggested a single dispersal into South America from Central America and at least four dispersals back into Central America for *Hedyosmum*. In contrast, the ITS tree suggested two independent earlier dispersals into South America from Central America and two reversals back into Central America (not taking into consideration *H. gentryi* and *H. neblinae* due to a failure in obtaining their ITS sequences). We lack objective means of confidently discerning between these alternative scenarios.

The different biogeographical reconstructions described above derive mainly from the differential relationship between two taxa, *H. mexicanum* and *H. costaricense* from Central America, in relation to South American *Hedyosmum*. These taxa are sister to the two South American lineages in the ITS tree, but embedded in the South American *Hedyosmum* clade in the plastid tree. The ITS tree indicates that these two taxa originated in Central America, rather than having originated from ancestors from South America as revealed by the plastid tree.

If the long generic branches in Chloranthaceae reflect the effect of extinction (either gradual or punctuated, e.g., at the Cretaceous/Paleogene mass extinction event), as previously suggested (Antonelli and Sanmartín, 2011), it would mean that many lineages are missing from the inferred tree. Such a pattern could lead to spurious geographical inferences when only extant taxa are used. For example, all the generic stem-lineage ancestors were inferred to be distributed in Asia (Fitch optimization) or cover Asia (Lagrange). However, both these results could be artifacts because fossil evidence indicates that the earliest unequivocal *Hedyosmum*-like fossils were found in the Albian of Europe (Portugal), and the earliest unambiguous *Chloranthus*-like fossils were found in the Turonian and Santonian-Campanian of Europe and North America (Friis et al., 1986, 1999; Herendeen et al., 1993; Eklund et al., 1997). This scenario implies ancestral distributions beyond the inferred geographical ranges inferred here from molecular data. Adding fossil taxa to the biogeographical analyses of Chloranthaceae could be a helpful way of further investigating this issue (e.g., Wood et al., 2013), but will require scoring a large number of morphological characters for both extinct and extant species to enable their inclusion in a combined phylogenetic analysis (e.g., Ronquist et al., 2012).

Our results showcase the large influence of phylogenetic conflict on biogeographical reconstructions and exemplify the potential biases in tracing and interpreting the biogeographical history of a group only based on a single genomic source.

Effect of different sources of data on PD measures—The PD measures based on the plastid and the ITS data show a congruent pattern among genera, indicating that PD is positively correlated with TD (i.e., species richness) for *Sarcandra*, *Chloranthus*, and *Hedyosmum* regardless of data set (Table 2). However, in *Hedyosmum*, different sources of data yielded very different PD measures. The plastid data suggested much higher diversity in South America than in Central America and the Antilles, which is consistent with the TD diversity. In contrast, the ITS data suggested higher diversity in the Antilles and Central America than in South America (Table 2). Results from different sources of data seem unlikely to be much altered by increased species sampling because the samples in the current study are very taxonomically and geographically representative (see Materials and Methods). The addition of missing species, which are likely close relatives to the sampled ones, should thus produce a minor effect on PD measures.

Two alternative but not exclusive underlying causes could account for the conflicting PD measures within *Hedyosmum*. First, nucleotide substitution rates in each DNA locus could differ among species and clades, resulting in true differences in PD between the ITS and plastid data sets (e.g., with high ITS rates in Antillean and Central American *Hedyosmum*). However, these differences should be leveled out in the calculation of PD from rate-smoothed chronograms, which reconcile the variations of substitution rates among lineages. Since the contrasting PD results between the plastid and ITS still exist (for Central American and Antillean *Hedyosmum* vs. South American *Hedyosmum*) regardless of being based on phylograms or chronograms (Table 2), we therefore consider the substitution rate variation across different lineages to have less effect. This lends support for a second potential explanation, which is that the contrasting PD measures mirror the different evolutionary

histories of the plastid and ITS sequences in *Hedyosmum*. Our study thus shows a potential caveat for the calculation and interpretation of PD, which has become an increasingly popular metric in the fields of evolution, ecology, and biological conservation as compared with raw species counts.

***Conclusions*—**We have shown that phylogenetic incongruence derived from the analyses of different genomes (nuclear vs. plastid) not only leads to disagreements in the evolutionary relationships of taxa; it also greatly affects other phylogeny-based inferences and interpretations, including taxonomy, morphological evolution, historical biogeography, and phylogenetic diversity measures. This study therefore calls for caution in phylogenetic reconstruction and related inferences based on single sources of data (regardless of size, since even a fully sequenced plastid genome still represents a single maternal inheritance and data source), or concatenation of different data with incongruent phylogenetic signals. Further studies based on denser taxonomic and genetic sampling, particularly sequences from unlinked nuclear loci and multiple intraspecific samples, may be required to produce robust and reliable species trees as proposed also by previous studies (e.g., Sang et al., 1997; Baum, 2007).

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APPENDIX 1. Sources of plant material and GenBank accession numbers of the DNA sequences used in this study.

Taxon	*rbcL*	*trnL-F*	*rpl20-rps12*	*rps16*	ITS	Collector (voucher)/Location
Ingroups						
Ascarina J.R. Forster & G. Forster						
A. coursii (Humbert & Capuron) J.F. Leroy & Jérémie	AY236844[c]	AY236747[c]	AY236721[c]			Ravelonarivo 1139 (MO)/Madagascar
A. lucida Hook. f.	AF238050[c]	AY236745[c]	AY236722[c]			Lange 3594 (AK)/New Zealand
A. marquesensis A.C. Smith	HQ336515[e]		HQ336557[e]			Feild/Hiva Oa, Marquesas, French Polynesia
A. marquesensis A.C. Smith	HQ336516[e]	HQ336598[e]	HQ336558[e]	KJ137957		Feild/Nuku Hiva, Marquesas, French Polynesia
A. philippinensis C.B. Rob.	HQ336518[e]	HQ336599[e]	HQ336559[e]	KJ137958		Feild/Mt. Gumi, Morobe Province, Papua New Guinea
A. polystachya Foster	AY236842[c]	AY237816[b]	AY236732[c]	KJ137960		Feild/Tahiti
A. rubricaulis Solms-Laub.	HQ336519[e]	AY236746[c]	HQ336560[e]	KJ137959		Feild/Mt. Dzumac, New Caledonia
A. solmsiana Schlechter. var. *grandifolia* Jérémie	HQ336520[e]	HQ336600[e]	HQ336561[e]			Feild/Mt. Dzumac, New Caledonia
Ascarina sp.[f]	HQ336517[e]	HQ336597[e]	HQ336556[e]	KJ137962		Feild/Mt. Shungol, Morobe Province, Papua New Guinea
A. swamyana A.C. Smith	AY236843[c]	AY236748[c]	HQ336562[e]	KJ137961		Feild/Des Vouex Peak, Taveuni, Fiji
Chloranthus **Swartz**						
C. angustifolius Oliver	AY236839[c]	AF364600[b]	AY236724[c]	KJ137964	AF280416[b]	Kong 97701 (PE)/China
C. erectus (Buch.-Ham.) Verdc.	AY236834[c]	AF329949[b]	AY236738[c]	KJ137966	AF280410[b]	Kong 97602 (PE)/China
C. fortunei (A. Gray) Solms-Laub.	AY236840[c]	AF364601[b]	HQ336563[e]	KJ137965	AF280419[b]	Kong/Mt.Wuzhishan, Ruyuan, Quangdong, China
C. henryi Hemsl.	AY236837[c]	AF364599[b]	AY236735[c]	KJ137968	AF280415[b]	Kong 97124 (PE)/China
C. japonicus Sieb.	L12640.2[a]	AF364603[b]	AY236723[c]	KJ137963	AF280418[b]	Chase 204 (NCU)/Japan
C. nervosus Coll. & Hemsl.	AY236841[c]	AF364602[b]	AY236733[c]		AF280417[b]	Kong 97603 (PE)/China
C. oldhamii Solms-Laub.	AY236838[c]	AF364598[b]	AY236734[c]	KJ137970	AF280414[b]	Wang 99001 (PE)/Taiwan, China
C. serratus (Thunb.) Roem. & Schult.	AY236836[c]	AF364596[b]	AY236736[c]	KJ137969	AF280412[b]	Kong 97402 (PE)/China
C. sessilifolius K.F. Wu	HQ336521[e]	AF364597[b]	HQ336564[e]	KJ137971	KJ137946	Kong/Mt. Jinfoshan, Nanchuan, Chongqing, China
C. spicatus (Thunb.) Makino	AY236835[c]	AF329950[b]	AY236737[c]	KJ137967	AF280411[b]	Kong 97101 (PE)/China
Hedyosmum **Swartz**						
H. angustifolium (Ruiz & Pavón) Solms-Laub.	HQ336524[e]	HQ336602[e]	HQ336568[e]	EU302175	EU302153	Persson 715 (GB)/Pasco, Peru
H. anisodorum Todzia	HQ336525[e]	HQ336603[e]	HQ336569[e]	KJ137972	KJ137947	Feild/Trocha Union, Peru
H. arborescens Swartz	L12649.2[a]	HQ336604[e]	AY236720[c]	EU302168	EU302146	Feild & Luke/St. Catherines, Jamaica

Appendix 1. Continued.

Taxon	*rbcL*	*trnL-F*	*rpl*20-*rps12*	*rps16*	ITS	Collector (voucher)/Location
H. brasiliense Miquel	HQ336526[e]	HQ336605[e]	HQ336570[e]	EU302183	EU302161	Antonelli & Andersson 297 (GB)/Rio de Janeiro, Brazil
H. brenesii Standl.	HQ336527[e]	HQ336606[e]	HQ336571[e]	KJ137973	KJ137948	Feild/Costa Rica
H. bonplandianum Humboldt	HQ336528[e]	AY236751[c]	AY236729[c]	EU302181	EU302159	Feild 1025/Estacion Cacao, Guanacaste, Costa Rica
H. bonplandianum var. *callososerratum* Oerst.			HQ336572[e]	KJ137974		Dwyer & Kirkbride 7839/Panama
H. columbianum Cuatrec.	HQ336529[e]	HQ336607[e]	HQ336573[e]	KJ137975	KJ137949	Todzia 2431/Colombia
H. correanum D'Arcy & Liesner	HQ336530[e]	HQ336608[e]	HQ336574[e]	EU302182	EU302160	Hammel 3064 (AAU)/Chiriquí, Panama
H. costaricense Burger	HQ336531[e]	HQ336609[e]	AY236718[c]	EU302169	EU302147	Feild 1023/Tapanti, Costa Rica
H. cuatrecazanum Occhioni	HQ336532[e]	HQ336610[e]	HQ336575[e]	EU302171	EU302149	Gavilanes & Tivira 653-A (AAU)/Napo, Ecuador
H. cumbalense H. Karsten	HQ336533[e]	HQ336611[e]	HQ336576[e]	EU302172	EU302150	Harling 25488 (GB)/Azuay, Ecuador
H. dombeyanum Solms-Laub.	HQ336534[e]	HQ336612[e]	HQ336577[e]	EU302176	EU302154	Steinbach 543 (S)/Cochabamba, Bolivia
H. domingense Urban	HQ336535[e]	HQ336613[e]	HQ336578[e]	EU302167	EU302145	Abbott 20902 (FLAS)/San Cristobal, Dominican Republic
H. domingense Urban	HQ336536[e]		HQ336579[e]	KJ137976		Feild/Pico Duarte, Dominican Republic
H. gentryi D'Arcy & Liesner		HQ336614[e]	HQ336580[e]	KJ137977		Dorr 8138/Venezuela
H. goudotianum Solms-Laub.	HQ336537[e]	AY236754[c]	HQ336581[e]	EU302180	EU302158	Pherson 15898 (MO)/Chiriqui, Panama
H. grisebachii Solms-Laub.	HQ336538[e]	HQ336615[e]	HQ336582[e]	EU302166	EU302144	Abbott 19948 (FLAS)/Holguin, Cuba
H. lechleri Solms-Laub.	HQ336539[e]	HQ336616[e]	HQ336583[e]	KJ137978	KJ137950	Solomon 13735/Bolivia
H. luteynii Todzia	HQ336540[e]	HQ336617[e]	HQ336584[e]	KJ137979		Sodiro (P)/Pichincha, Ecuador
H. maximum (Kuntze) K. Schum	HQ336541[e]	HQ336618[e]	HQ336585[e]	KM111252	KJ137951	Feild/Wayquechas Reserve, Peru
H. mexicanum Cordemoy	HQ336542[e]	HQ336619[e]	HQ336586[e]	EU302173	EU302151	Feild/Ojo de Agua, Talamancas, Costa Rica
H. neblinae Todzia			HQ336587[e]	KJ137980		Maquire/Brazil
H. nutans Swartz	HQ336543[e]		HQ336588[e]	EU302165	EU302143	Veloz et al., 2903 (JBSD)/Pico Duarte, Dominican Republic
H. nutans Swartz	HQ336544[e]	HQ336620[e]		KJ137981	KM111253	Feild & Luke/Vinegar Hill Trail, Jamaica
H. orientale Merr. & Chun	HQ336545[e]	AY236749[c]	AY236730[c]	EU302165	EU302142	Kong/Mt. Diaoluoshan, Lingshui, Hainan, China
H. parvifolium Cordemoy	HQ336546[e]	HQ336621[e]	HQ336589[e]	EU302170	EU302148	Todzia et al., 2432 (AAU)/ Cundinamarca, Colombia
H. peruvianum Todzia	HQ336547[e]	HQ336622[e]	HQ336590[e]	KJ137982	KJ137952	Feild/Trocha Union, Peru
H. racemosum (Ruiz & Pavón) G. Don	HQ336548[e]	HQ336623[e]	HQ336591[e]	EU302185	EU302163	Asplund 13151 (S)/Kosnipata Road, Peru
H. scaberrimum Standl.	HQ336549[e]	HQ336624[e]	HQ336592[e]	EU302178	EU302156	Santamaría S-1029 (GB)/Alajuela, Costa Rica
H. scabrum (Ruiz & Pavón) Solms-Laub.	HQ336550[e]	HQ336625[e]	HQ336593[e]	EU302174	EU302152	Andersson & Nilsson 2539 (GB)/Loja, Ecuador
H. spectabile Todzia	HQ336551[e]	HQ336626[e]	HQ336594[e]	EU302186	EU302164	Qllgaard & Madsen 90562 (GB)/ Zamora-Chinchipe, Ecuador
Hedyosmum sp.[f]	HQ336555[e]	HQ336628[e]	HQ336596[e]	KJ137983	KJ137953	Feild/Tono Alto, Peru
H. sprucei Solms-Laub.	HQ336552[e]	AY236752[c]	AY236719[c]	EU302184	EU302162	Harling & Andersson 24138 (GB)/ Zamora-Chinchipe, Ecuador
H. strigosum Todzia	HQ336553[e]	HQ336627[e]	HQ336595[e]	EU302179	EU302157	Andersson & Nilsson 2412 (GB)/ Sucumbíos, Ecuador
H. translucidum Cuatrec.	HQ336554[e]	AY236753[c]	AY236728[c]	EU302177	EU302155	Harling & Andersson 21980 (GB)/ Kosnipata Road, Peru
***Sarcandra* Gardner**						
S. chloranthoides Gardner	AY236833[c]	AY236740[c]	HQ336565[e]	KJ137984	KJ137954	Endress/Zurich (cultivated)
S. glabra (Thunb.) Nakai	HQ336522[e]	AF329948[b]	HQ336566[e]	KJ137985	KJ137955	Kong/China
S. glabra subsp. *brachystachys* (Blume) Verdc.	HQ336523[e]	HQ336601[e]	HQ336567[e]	KJ137986	KJ137956	Kong/China
Outgroups						
Calycanthus floridus L.	AJ428413	AJ428413	AJ428413			
Drimys granadensis (L.f.) J. F. Gmel.	DQ887676	EU669556	DQ887676			
Liriodendron tulipifera L.	DQ899947	DQ899947	DQ899947			
Magnolia tripetala (L.) L.	AF206791	AY009073	AY727308			
Illicium oligandrum Merr. & Chun	EF380354	EF380354	EF380354			

Notes: Some specimen numbers and vouchers were unavailable (or possibly lost) due to a change of departments. Sources: [a] Qiu et al. (1993); [b] Kong et al. (2002); [c] Zhang and Renner (2003); [d] Antonelli & Sanmartín (2011); [e] Zhang et al. (2011); [f] new taxa that have not been formally described.