The first example of extreme and independent host plant shifts mediated by parasitic plants in insects

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Abstract

Phytophagous insects have specialized on virtually every plant lineage. Parasitic plants, however, are uncommon hosts. Among insects, only a single lineage of weevils, the Smicronychini, has successfully radiated on both parasitic and non-parasitic plants in a large panel of distantly related Asterid families. This unusual pattern suggests that major host plant shifts have occurred over the course of their diversification. Through the analysis of a phylogenomic dataset, we reconstruct for the first time their evolutionary history and ancestral host plant associations. Our results show that independent host plant shifts occurred both from parasitic to non-parasitic hosts and between distinct parasitic lineages. These results suggest that host shift mechanisms can be driven by ecological opportunities provided by plant-plant interactions. This first evidence of extreme insect host plant shifts mediated by parasitic plant-plant interactions emphasizes the core importance of ecological interactions as driving forces behind insect host plant shifts.

Key words

Coevolution, Cuscuta, Curculionidae, host plant shift, parasitic plants, phylogenomics, phytophagous insects, plant-insect interactions, plant-plant interactions, target capture

Introduction

Plant-insect interactions are one of the main components shaping terrestrial ecosystems and have therefore been extensively studied throughout all major fields of Ecology and Evolution. Indeed, studying these interactions offer a promising opportunity for bridging micro- and macroevolutionary processes (Giron et al., 2018). To apprehend the fundamental processes associated with the evolution of plant-insect interactions, considering the notion of host repertoire (*sensu* Braga & Janz, 2021) is particularly relevant. Echoing the Hutchinsonian ecological niche concept (Hutchinson, 1957; see also Holt, 2009), phytophagous insects have a *fundamental* repertoire of suitable hosts and a *realized* repertoire of hosts actually used by the insect in its environment (Braga & Janz, 2021). The *realized* repertoire of hosts can either remain the same (i.e. niche conservatism; Peterson et al., 1999) or be subject to evolution via diet expansion (i.e. hosts acquisition without the loss of ancestral hosts; Janz et al., 2006), specialization (i.e. fewer hosts; Futuyma & Moreno, 1988; Jaenike, 1990), or host plant shifts (HPS) (i.e. loss of ancestral hosts followed by new hosts acquisition; Holloway & Hebert, 1979; see also Braga & Janz, 2021). With this point of view, HPS are the most extreme scenarios as they can be seen as a shift to a distinct biotic environmental space (Merrill et al., 2013; Singh et al., 2020). Adaptation to new host plants requires the adaptation to new structures, plant defenses, phenologies, micro-climates and so on (e.g. Berenbaum et al., 1996; Gatehouse, 2002; Connor, 2006; Yarnes et al., 2008; Mithöfer & Boland, 2012; Heidel-Fischer & Vogel, 2015, Brand et al., 2020). Altogether, this multidimensional nature of HPS is *de facto* much more challenging to study than other processes. As a result, underlying forces that enable such changes in the host repertoire of phytophagous insects remain poorly understood.

The distribution of host repertoire in phytophagous insects phylogenies follow these general patterns: *(i)* HPS are ubiquitous events at large taxonomic scales, *(ii)* phytophagous insect phylogenies do not match host plant phylogenies, and *(iii)* phylogenetic niche conservatism of host associations is more common at shallower taxonomic scales (see the reviews of Janz, 2011; Kergoat et al., 2017; Jousselin & Elias, 2019; Braga & Janz, 2021). The discovery of these patterns led to the formulation of several macroevolutionary scenarios such as the classical 'escape & radiate' arms race by Ehrlich and Raven (1964), the oscillation hypothesis (Janz & Nylin, 2008), and the musical chairs hypothesis (Hardy & Otto, 2014). All these scenarios posit that HPS are diversification drivers, a claim that has

often been demonstrated on empirical datasets (Hardy & Otto, 2014; Nylin et al., 2014; Edger et al., 2015; Wang et al., 2017; Allio et al., 2021; but see Nyman et al., 2010; Imada et al., 2011; Althoff et al., 2014; Jousselin & Elias, 2019). HPS have also been shown to be at the origin of genetic divergence between lineages, sometimes leading to reproductive isolation and even to ecological speciation (see the review of Forbes et al., 2017). Overall, the signature of HPS and adaptations to new hosts have been extensively studied, from complex genetic changes (Smadja et al., 2012; Edger et al., 2015; Allio et al., 2021; see also the reviews of Simon et al., 2015 and Vertacnik & Linnen, 2017) to transcriptomic signatures (Singh et al., 2020; Hou et al., 2021; Celorio-Mancera et al., 2023), and even morphological plasticity (Savković et al., 2023). However, although the importance of this phenomenon in the evolution of arthropods has been widely acknowledged, how HPS occur in the first place and what drives the directionality of these events has received very little attention.

It appears that, under the right conditions, phytophagous insects can easily make HPS, at least towards plants in their fundamental repertoire. For example, through a process called ecological fitting (*sensu* Agosta, 2006), numerous HPS have been documented following geographical opportunities, such as new potential hosts introduction or parasites invasion in a new geographical range (Graves & Shapiro, 2003; Singer et al., 2008 in Tilmon, 2008; Gougherty & Davies, 2021; Braga, 2023). For those species, it seems that the only barrier to host shifts was the host's geographical range. Other barriers associated with the plant as a niche, such as the plant's phenology (Wood et al., 1990; Whipple et al., 2009), physical structures (e.g. Center & Johnson, 1974; Jaime et al., 2010), detectability (Li et al., 2017), and physiology or chemistry (e.g. Berenbaum et al., 1996; Groen & Whiteman, 2021), can also be overcome and ultimately lead to HPS. These barriers therefore hold an important role in the respective diversification of plants and insects (Ehrlich & Raven, 1964; Farrell & Mitter, 1998; Becerra, 1997; Kergoat et al., 2005; Edger et al., 2015; van der Linden et al., 2021). The phylogenetic proximity between related plants that share most of these characteristics (on average) may in turn explain the ubiquitous 'minor' HPS observed at shallow taxonomic scales (Brooks & McLennan, 2002; Agosta, 2006; Celorio-Mancera, 2016). Nevertheless, several experiments have succeeded in forcing host shifts towards plants supposedly outside of the insect's host repertoire, showing that, under the right conditions (i.e. a consistent exposition to a new host opportunity), host preference is a relatively labile trait (e.g. Messina & Jones, 2009; Savković et al., 2016). The key factors

that explain 'major' insect HPS - between distantly related plant species - may therefore rely on rarer ecological opportunities that allow insects to cross those barriers. Interestingly, such opportunities can be found *in natura* in the form of interactions between parasitic plants and their hosted phytophagous insects.

Parasitic plants (*ca.* 4,500 species from 28 families; Yoshida et al., 2016) have a unique heterotrophic lifestyle associated with the development of a multicellular organ - the haustorium - which allows them to absorb water and nutrients but also proteins, nucleotides or pathogens (Yoshida et al., 2016). Phytophagous insects that live on parasitic plants are directly impacted by the nature of the compounds exchanged with the parasitized plants (Adler, 2002; Rowntree et al., 2014). As a result, parasitic plants are a unique model to study the evolution of phytophagous insects thanks to the physiological proximity provided by the parasitic interaction. Especially, the haustorium may give opportunities to insects feeding on the parasitic plant to shift towards the parasitic plant's host, breaking the 'physiological barrier' of distantly related plants. In this study, we explore this hypothesis by focusing on the only group of insects known to have successfully radiated on parasitic plants: weevils of the tribe Smicronychini (Coleoptera: Curculionoidea; Anderson, 1962, 1970, 1993; Caldara et al., 2014; Haran et al., 2017; Haran, 2018, 2021; Haran & Perrin, 2017). Smicronychini is a widespread weevil tribe known from every bioregion and consisting of *ca*. 200 species classified in six genera, the genus *Smicronyx* being by far the most diverse with 156 described species (Caldara et al., 2014). They are essentially monophagous or narrowly oligophagous on closely related plants and their endophagous larvae develop in stems, roots or fruits, sometimes inducing galls in their host (see Figure 1 and Table S1 for details). Although this tribe is known for its association with parasitic plants, it has a diverse repertoire of phylogenetically unrelated hosts plants, belonging to four Asterid families: some Smicronychini specifically develop in holoparasitic plants (obligatory parasites with no or little photosynthetic activity; Irving & Cameron, 2009), like dodders (*Cuscuta* - Convolvulaceae) or broomrapes (*Orobanche* - Orobanchaceae), others develop in hemiparasitic plants (obligatory or facultative parasites that still have some degree of photosynthetic activity, Irving & Cameron, 2009; such as *Euphrasia* or *Striga* - Orobanchaceae), while the rest solely develop in non-parasitic plants belonging to the Asteraceae, Convolvulaceae and Gentianaceae families (Figure 1; APG, 2016; Anderson, 1962, 1970; Haran, 2017, 2018, 2021). This remarkably diverse host repertoire contrasts with what is expected at this taxonomic scale in most weevils, especially in this subfamily:

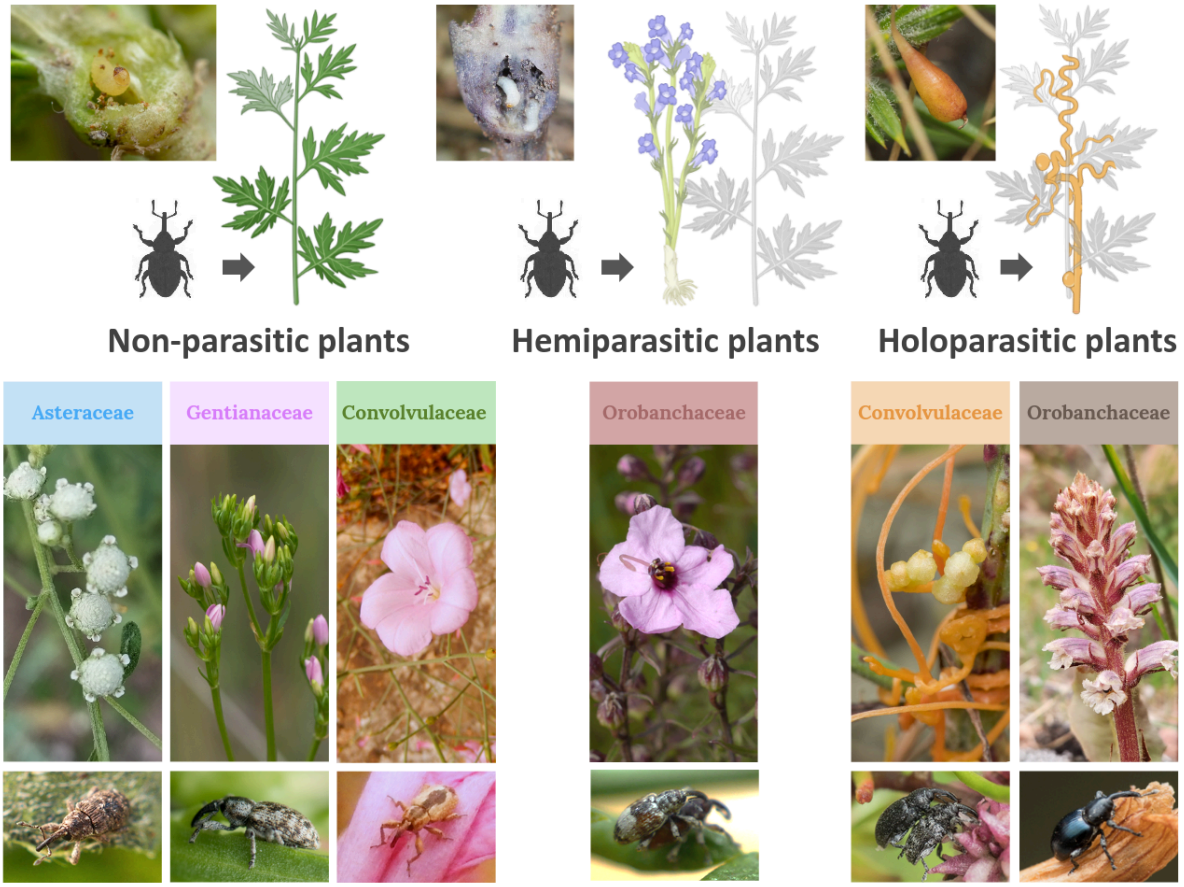

Figure 1: Host repertoire of Smicronychini weevils. Diagrams represent parasitic associations between the Smicronychini and their host plants, with pictures of larvae and galls. Host plant families that make up the host repertoire of Smicronychini are shown below each corresponding diagram, with examples of associated weevils. Credits: Zelvelder & Haran, except for Orobanchaceae (Clémence Massard) and Asteraceae (@sinaloasilvestre, iNaturalist) pictures.

the Curculioninae (e.g. Oberprieler et al., 2007; Hernández-Vera et al., 2019; Haran et al., 2023). Exploring the phylogenetic relationship within this group and their ancestral host plant associations is thus crucial to appreciate the role that parasitic plants may play in this unusual evolution pattern.

To test whether HPS in Smicronychini were mediated by parasitic plant-plant interactions, we produced the first robust and well-sampled molecular phylogeny of the tribe following a two-stage approach. Firstly, we gathered and assembled hundreds of genome-wide markers using Anchored Hybrid Enrichment (AHE) capture, thus taking advantage of specimens held in museum collections to sample a representative dataset of the tribe. Secondly, we expanded our taxon sampling by grafting additional Smicronychini

specimens/species for which mitochondrial markers (subunit I of the cytochrome *c* oxidase: COI) data was available. We then used the resulting phylogeny in combination with species-level ecological information to reconstruct the evolution of ancestral associations between Smicronychini and their host plants. Determining the ancestral host plant of this tribe allows us to assess whether the parasitic plants have indeed provided new ecological opportunities at the origin of extreme HPS in these weevils, or whether their broad host repertoire has been acquired independently of parasitic plant-plant interactions.

Materials and methods

Taxon sampling

The taxon sampling consists of specimens intended for target capture (hereafter *AHE dataset*) and specimens for which COI data were either generated or available (hereafter *COI dataset*).

AHE dataset - Smicronychini weevils were collected throughout ten years of field work carried out by J. Haran and by collecting specimens from Museum collections (Table S2). This sampling covers the Afrotropical, Palearctic and Oriental bioregions, representing all major genera encountered in this geographical range (i.e. *Afrosmicronyx*, *Sharpia*, *Smicronyx* and *Topelatus*), with the exception of the monospecific genus *Hedychrous*. Additional individuals were sampled in seven tribes (Anthonomini, Cionini, Curculionini, Derelomini, Erirhinini, Rhamphini and Styphlini) related to Smicronychini to serve as outgroups for phylogenetic inferences. These tribes were selected based on the most recent and comprehensive phylogenomic study of the Curculioninae subfamily (Haran et al., 2023).

COI dataset - COI sequences have been extracted from AHE sequencing data using MitoFinder v1.4.1 (Allio et al., 2020). When no COI sequence was found with MitoFinder (23 out of 35), COI sequences were recovered from additional individuals sequenced separately. Then, to expand our taxon sampling and include taxa native to the Nearctic bioregion, like the genus *Promecotarsus* and the *Smicronyx* subgenera *Desmoris*, *Pachyphanes* and *Pseudosmicronyx*, additional COI sequences of individuals were either generated by our research group using specimen in museum collections or downloaded from BOLD v4 and GenBank (Table S2).

The objective of this sampling was to cover the entire morphological diversity and host repertoire of the Smicronychini, considering both botanical families and the parasitic

ecology of some of their hosts, making it the most comprehensive sampling ever assembled for this tribe.

Target capture and DNA sequencing

Specimens from the *AHE dataset* were prepared following the target enrichment protocol of Faircloth et al. (2015). Tissues from ethanol- and dry-preserved specimens were extracted non-destructively using the EZ-10 Spin Column Animal Genomic DNA Miniprep Kit (Biobasic Inc., Canada) with an overnight lysis step. DNA concentration was normalized (except for low quality samples) and mechanically fragmented to 300-600 bp fragments using a Bioruptor Pico (Diagenode; 15s ON, 90s OFF, eight cycles). Low quality DNA were then reconcentred with AMPure XP beads and libraries were constructed according to the NEBNext® Ultra II DNA Library Prep for Illumina® kit recommendations (New England Biolabs, Ipswich, MA, USA). Each library was individually labeled and subjected to 12 PCR cycles for final enrichment and pooled in equal quantities, grouping 24 libraries. The myBaits Hyb Capture for AHE kit (Arbor Biosciences, Ann Arbor, MI, USA) was applied to these two pools following the instructions of the manufacturer and assayed by qPCR. The resulting AHE library was sequenced paired-end, on a 2x150 bp SP line on an Illumina NovaSeq sequencer (Illumina, San Diego, CA, USA) at the GenomiX platform in Montpellier, France (MGX). Resulting reads were then demultiplexed with cutadapt v2.8 (Martin, 2011) and cleaned with Trimmomatic v0.39 (Bolger et al., 2014).

Dataset assemblies and alignments

AHE dataset - This dataset was produced following the pipeline detailed in this section and illustrated in Figure 2. Before that, a first filtering was applied to each individual based on raw sequencing results and cross-contamination detection to avoid unreliable data and we generated a new set of AHE reference sequences using a close reference genome (see Supplementary Material & Methods). Then, reads were assembled with an iterative approach using IBA (Breinholt et al., 2018). The assembled loci obtained were then aligned to their corresponding AHE reference sequences using the *--add* option and *--adjustdirectionaccurately* implemented in MAFFT v7.453 and using the L-INS-I model (Katoh & Frith, 2012; Katoh & Standley, 2013), specially designed to be robust to divergent flanking regions around a core easier to align, corresponding to the AHE structure. To avoid any bias linked to missing data (Xi et al., 2016), we only retained loci represented by more

than 70% of the individuals in our dataset, a value established as a good ratio between the quantity of loci retained and total percentage of missing data. Although AHE probes target coding regions, assembled sequences can be surrounded by non-coding flanking regions. Alignments were therefore cleaned up differently depending on their coding or non-coding nature. Firstly, partitions were defined based on the region targeted by the probes (custom python script: *make_gapped_parts.py*). Secondly, using the annotation of the aforementioned genome of *A. grandis*, we were able to refine the boundaries of coding and non-coding regions more precisely (custom R script: *improve_parts.R*). This process led to the identification of potential overlapping sequences between loci that were consequently pruned with cutadapt. Thirdly, coding and non-coding partitions were split using AMAS (Borowiec, 2016) and processed with the OMM_MACSE pipeline (Ranwez et al., 2021) and HMMCleaner (Di Franco et al., 2019) followed by PaulBlocks (provided by Paul Simion; to remove positions with more than 50% of missing data), respectively. All of these alignments were then concatenated into one supermatrix and translated into usable formats for phylogenetic inference using *seqCat.pl* and *seqConverter.pl*.

Combined phylogenetic approach

Phylogenomic inferences based on AHE dataset - Phylogenetic inferences were conducted either locally or on the CIPRES Gateway cluster (Miller et al., 2010) within a Maximum Likelihood (ML) framework using IQTREE v1.6 or 2.2, depending on the cluster used (Nguyen et al., 2015; Minh et al., 2020). The supermatrix was partitioned so that each flanking region, each inserted region and each coding position (Figure 3B) was allowed to have its own evolution rate using the *-p* option (Chernomor et al., 2016). Model estimation was systematically conducted using ModelFinder (*-MFP+MERGE* option) with *-rclusterf 20* to improve partition merging performance (Kalyaanamoorthy et al., 2017; Lanfear et al., 2017). A perturbation level of heuristics of 0.2 (*-pers 0.2* option; recommended for a phylogenomic dataset according to IQTREE documentation) was implemented and symmetry tests were performed using the *--symtest-remove-bad* option (Naser-Khdour et al., 2019). The robustness of the nodes was estimated with ultra fast bootstraps (UFBS; Hoang et al., 2018), with the *-bnni* option to compensate for model violations and the Shimodaira-Hasegawa approximate likelihood-ratio test (SH-aLRT; Guindon et al., 2010), supposed to be more robust to the latter. A node was considered highly-supported for values of SH-aLRT ≥ 80% and UFBS ≥ 95% (following IQTREE authors' recommendations).

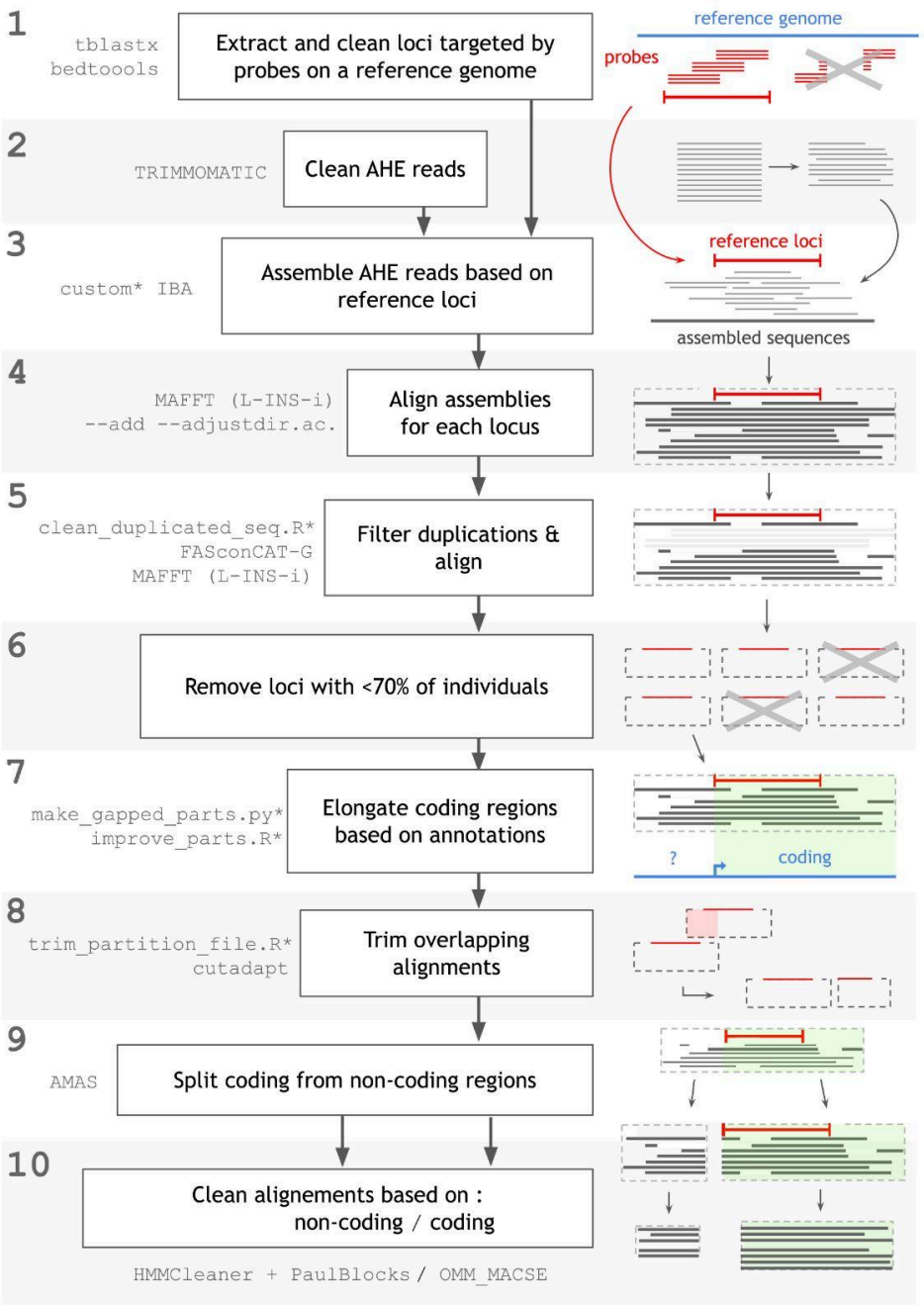

Figure 2: Flowchart of the phylogenomic pipeline developed to process AHE data. Each step forming our pipeline, numbered from 1 to 10 as referenced in the main text, are represented, from raw reads to the complete supermatrix. External tools and custom scripts (marked with an *) are given, along with a diagram representing each step of the pipeline. To get access to scripts and details from this pipeline, please refer to corresponding authors.

Extending the phylogeny with the COI dataset - The tree obtained from the *AHE dataset* was pruned to contain only ingroup individuals. This tree was then used to constrain a second ML phylogenetic inference (*-g* option) on the *extended dataset* (i.e. including both *AHE* and

COI datasets). This approach allows to densify the taxon sampling while retaining high confidence in the relationships at the species-level (Talavera et al., 2022; see Upham et al., 2019; Azevedo et al., 2022; Espeland et al., 2023 for empirical examples).

Ancestral character state estimations

Ancestral character state estimations (ASE) of host plants were carried out using the R package *Phytools* (Revell, 2012). The encoded characters correspond to known host plant families of Smicronychini (Asteraceae, Convolvulaceae, Gentianaceae and Orobanchaceae) associated with their degree of parasitism (non-parasitic, hemiparasitic and parasitic; Table S1). Six categories and one unknown state were thus defined as follows: non-parasitic Asteraceae, non-parasitic Convolvulaceae, parasitic Convolvulaceae, non-parasitic Gentianaceae, parasitic Orobanchaceae and hemiparasitic Orobanchaceae. The ASE were carried out by comparing the likelihood of three Markov models (Mk), based on three different character state transition matrices, one with equal rates (ER), one with symmetrical rates (SYM) and one with all rates different (ARD). These models were then applied to the ingroup of the extended phylogenetic tree (reduced to one representative per species in order to limit ancestral trait estimation biases) with the *fitMk* function, and AIC values were compared using the *aic.w* function. Based on the best fitting model, each internal node of the phylogeny was assigned with a posterior probability for each character state using the *make.simmap* function over 1,000 simulations by a continuous-time reversible Markov process.

Results

Phylogenomic pipeline

The filtering process allowed us to keep 37 out of 53 individuals sampled in the ingroup of the *AHE dataset* and 140 out of 228 individuals from the *COI dataset*, representing 46 different species of Smicronychini in total. Specimens selected for the *AHE dataset* then followed our pipeline. From 832 AHE reference sequences used for the iterative assembly, 819 loci were successfully recovered. At the end of our pipeline, 559 loci were remaining to make up the supermatrix. Most loci that were filtered out (238 out of 260) were represented by less than 70% of our samples (Figure 2, step 6). The supermatrix built from the *AHE dataset* comprises 189,932 nucleotides from 51 individuals including outgroups.

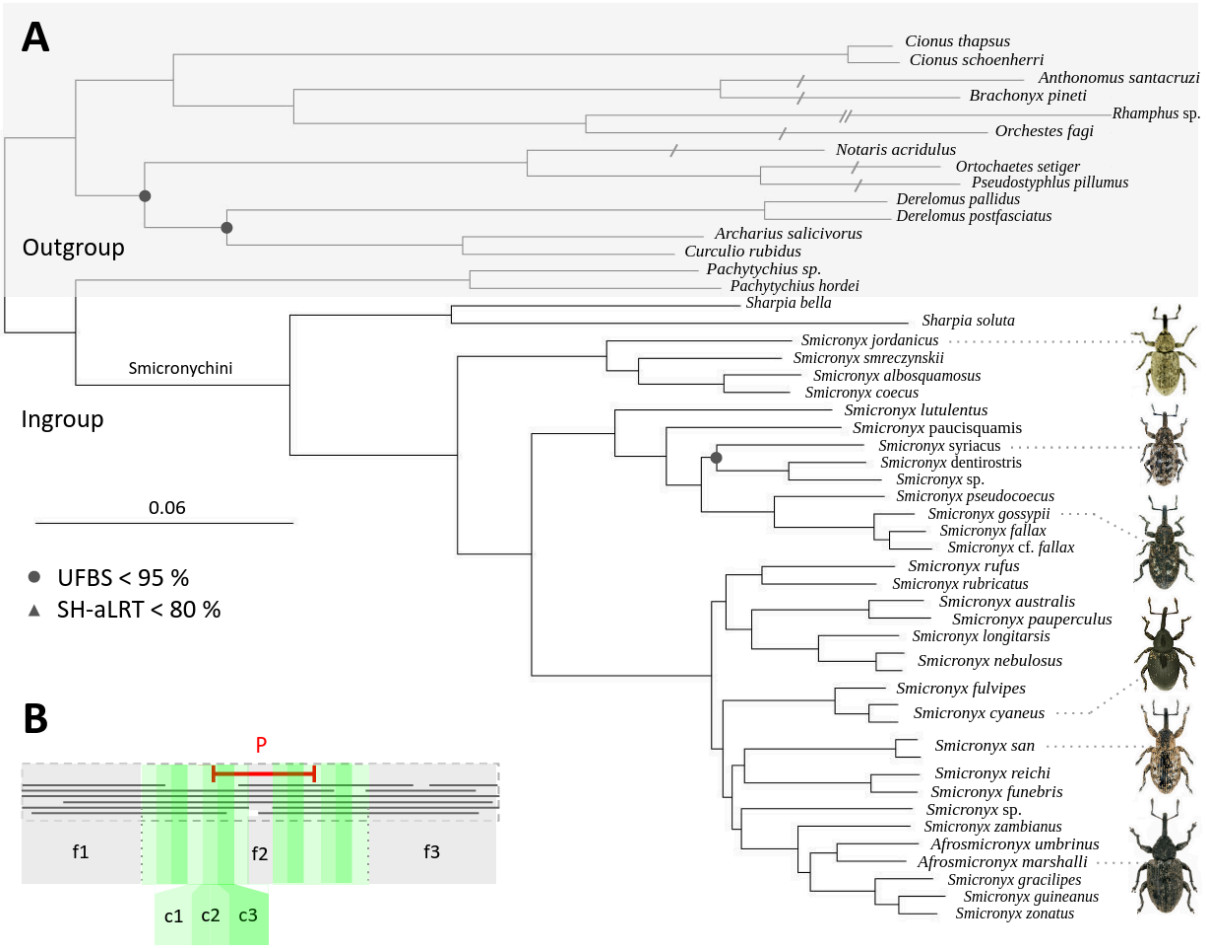

Figure 3: Phylogenomic tree based on the AHE dataset. A. Maximum likelihood tree of the AHE dataset. Nodes poorly supported [Ultrafast Bootstrap (UFBS) values < 95% and SH-approximate likelihood ratio tests (SH-aLRT) values < 80%] are respectively highlighted with gray circles or triangles. For graphical purposes, some branches were shortened to half of their length (//) or by a length of 0.06 (/). Pictures of weevils are linked to their corresponding species. B. Partitioning scheme of a theoretical AHE loci. Flanking and inserted partitions (f1-3) are highlighted in grey and coding partitions (c1-3) are highlighted in green. The region targeted by the probes (P) is shown in red but does not appear in the final supermatrix. Credits for weevil pictures: CBGP.

Interestingly, the elongation of the coding regions and excision of overlapping alignments (Figure 2, steps 7-8) reduces the size of the supermatrix by 17% but increase the coding part of the supermatrix from 51.2% to 60.5%, reducing the total number of partitions from 2,832 to 2,329 (see Figure S1 for implications of these steps on tree inferences). Lastly, the concatenation of partitions carried out by ModelFinder reduced the number of partitions to only 49, corroborating the high degree of homogeneity in the sequences obtained with AHE among this tribe.

Tree inferences

Following the phylogenomic pipeline on the *AHE dataset*, we obtain the phylogenetic tree shown in Figure 3A. This phylogeny recovers the Smicronychini as monophyletic with maximum support, and their most closely related outgroup is inferred to be from the genus *Pachytychius* (Curculioninae *uncert. sed.*; sometimes included in Smichronichini, e.g. Caldara et al., 2018). Every node of the ingroup is inferred with maximum support, except for one apical node that did not pass the UFBS threshold (UFBS = 86%). The genus *Sharpia* is recovered as sister to all remaining Smicronychini. The genus *Smicronyx* is paraphyletic with the genus *Afrosmicronyx* embedded among *Smicronyx* species. In this clade, we recover three major subclades (Figure 3A). The first subclade regrouping (*Smicronyx jordanicus* to *S. coecus*), being inferred as sister to the second (*S. lutulentus* to *S.* cf. *fallax*) and third subclades (*S. rufus* to *S. zonatus*). Finally, despite a low support for interspecific relationships, the addition of individuals issued from the *COI dataset* improves our taxon sampling by *ca*. 10 species and reveals a well supported clade of Nearctic species living on Asteraceae as a sister group of the *Smicronyx paucisquamis* clade living on dodders (Figure 4, Figure S2).

Ancestral character states estimation

ASE analyses of host-plant associations are presented in Figure 4 and were conducted with an equal transition rate (ER) model based on the AICw comparisons. Results indicate that the most recent common ancestor for the *Sharpia* genus and the other genera of the tribe has a marginal posterior probability of 0.50 in favor of parasitic Convolvulaceae, 0.35 in favor of non-parasitic Convolvulaceae and less than 0.05 for every other character state. The parasitic Convolvulaceae character state is, however, strongly supported as the ancestral state of the rest of the tribe (marginal posterior probability of 0.97). Consequently, we infer several shifts from parasitic Convolvulaceae to other plant lineages such as non-parasitic Asteraceae (marginal posterior probability of 0.95) and parasitic Orobanchaceae (marginal posterior probability of 0.61). However, the ancestral host plant of weevils feeding on non-parasitic Gentianaceae and those feeding on hemiparasitic Orobanchaceae remains uncertain.

Discussion

This study provides the first robust phylogenetic hypothesis for the Smicronychini and reveals a remarkable pattern in the evolution of their host plant use. Phylogenomic inferences recover the monophyly of the tribe, but the paraphyly of the most speciose genus *Smicronyx* indicates that the current genus-level classification based on morphology is not satisfactory and requires a detailed re-investigation. That being said, the inferred *Smicronyx* clades consist of species with similar morphology and host plant associations.

The evolution of host plant preferences inferred herein reveals an unequivocal ancestral association with Convolvulaceae for Smicronychini and indicates that the lineage associated with parasitic Convolvulaceae (dodders) has experienced extreme secondary shifts toward phylogenetically distant botanical families encompassing both parasitic (Orobanchaceae) and non-parasitic Asterids (Asteraceae, Gentianaceae). To our knowledge this pattern is unique in phytophagous insects and also deviates from the expectation of niche conservatism generally observed at a shallow taxonomic level in weevils (see the review of Oberprieler et al., 2007). This scenario implies that an ecological context specific to this case has promoted such shifts and our results suggest that interactions between parasitic plants and their hosts has been a key driver of such events. The latter hypothesis is substantiated by the fact that dodders are known parasites of the families for which secondary shifts are inferred (Asteraceae, Gentianaceae). The case of *Smicronyx quadrifer* stands as a particularly telling example, as this species starts its larval development in dodders and systematically completes it in one of the few Asteraceae hosts parasitized by the dodder (Anderson, 1970). Host-parasite interactions are associated with an intricate spatial proximity of tissues and exchanges of primary and secondary compounds (Smith et al., 2016; Kokla & Melnyk, 2018). As such, the Smicronychini developing as larvae in the stems of parasitic plants are directly exposed to a physicochemical environment close to those of dodder's hosts that can promote the shift. However, ancestral state estimation analyses show that it is unclear whether Gentianaceae feeding weevils shifted from parasitic Convolvulaceae or hemiparasitic Orobanchaceae.

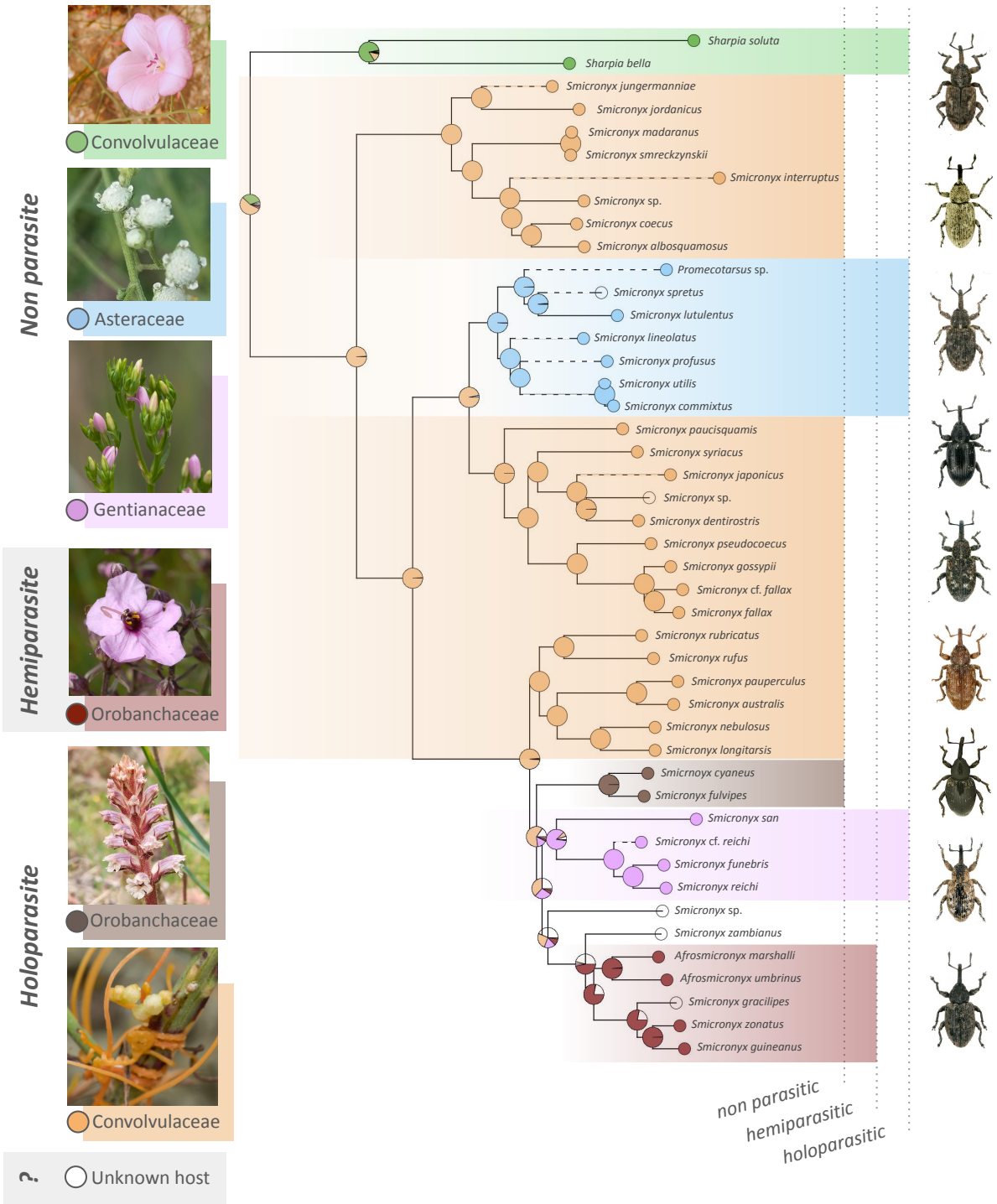

Figure 4 - Ancestral character state reconstruction analyses (ASE) of Smicronychini weevils and their host plant associations. Host plants of each species of weevil is characterized by both its botanical family (Asteraceae, Convolvulaceae, Gentianaceae, Orobanchaceae) and its parasitic type (non parasitic, hemiparasitic, holoparasitic), as illustrated on the left of the figure and on the phylogeny as background colors with variable length. Pie charts represented on each node correspond to marginal posterior probabilities of each character state computed by *Phytools*. The topology corresponds to that of the ML tree obtained from the extended dataset pruned to one branch per species, with grafted COI species represented at the tip of dashed branches. Credits: Zelvelder & Haran, except for Orobanchaceae (Clémence Massard) and Asteraceae (@sinaloasilvestre, iNaturalist) pictures, and CBGP for weevil pictures.

Another intriguing host plant shift pattern in Smicronychini is the shift inferred between phylogenetically distant parasitic plant lineages, namely from Convolvulaceae to Orobanchaceae. This scenario suggests that a convergent parasitic lifestyle between phylogenetically distant lineages of plants could have promoted the shifts. One feature of parasitic plants that could explain this pattern is the existence of similar chemical compounds between dodders and broomrapes (Rehker et al., 2012), like those involved in haustorium formation (e.g. Sun et al., 2018; Qiu et al., 2022; Westwood, 2022) that potentially facilitated the detection of these distant plants by one or a few Smicronychini lineages towards parasitic Orobanchaceae (although their detectability by Smicronychini have never been put to the test). Additionally, a second feature of parasitic plants is that they may share similar host plants and therefore show some degree of chemical similarity according to the proximity of their host plants. However, the host plant repertoire of Orobanchaceae feeding weevils' are not known with sufficient precision to corroborate this second hypothesis yet. Overall, with two independent shifts from parasites to non-parasites and between parasitic lineages, our results highlight for the first time the role of parasitic plants in insect host shifts.

Phytophagous insect diversity has been widely associated with major shifts between distant plant lineages leading to bursts of diversification (Ehrlich & Raven, 1964; Janz, 2011). Yet, few papers have questioned how these major shifts occur in the first place. Here, we showed that parasitic interactions have facilitated insect host shifts, both towards non parasitic plants and other parasitic plants. Although the patterns inferred in this paper are specific to parasitic interactions, the underlying mechanisms revealed here provide valuable insights on the processes leading to major host plant shifts in insects. From the insect perspective, plants with overlapping niches, for example with similar physiology, structure or chemistry, provide ecological opportunities at the basis of host shifting events. In other words, overlapping plant niches make up “facilitating bridges” that can be crossed by insects toward new potential hosts. Insects that shift from parasitic plants to their hosts make these opportunities obvious in all of its multifaceted parts. It is likely that a large share of historical shifts have been driven by these kinds of opportunities, but the scarcity of ancestral data, intermediate states and our limited ability to link ecological niches in their multifaceted aspects to host plant shifts make it difficult to conceptualize. In this context, “minor” shifts that typically occur within a plant lineage - generally described as niche conservatism - may also derive from such ecological opportunities provided by the general

relatedness (chemical, physiological, etc) between species in a given lineage. The decorrelation between phylogeny and niche relatedness inferred in the Smicronychini emphasizes the preeminence of ecological factors as prior components driving host shifts in phytophagous insects (Brooks & McLennan, 2002; Nyman, 2010). Similarly, several independent major host shifts were also proposed as a result of ecological niche similarities. For example, the structural and physiological similarities in flower structures were inferred to be at the origin of major host shifts from monocots to dicots in derelomine flower weevils (Haran et al., 2022). Altogether, these examples are consistent with a unified view of host shift mechanisms based on ecological opportunities arising from overlapping plant niches.

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Author contributions

RA, JH, GJK and BZ designed the study; JH collected data; LB and BZ conducted the experiments; BZ wrote the pipeline and performed all molecular analyses supervised by RA and GJK; BZ wrote the first draft; all authors contributed to the writing of the final draft.

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Supplementary material

Please refer to corresponding authors for accessing supplementary material.

- Supplementary Material & Methods
- Table S1 - Species represented in the extended dataset and their host plant associations
- Table S2 - Individual data and accession numbers
- Figure S1 - ML trees inferred from the AHE dataset on flanking and coding partitions only
- Figure S2 - ML tree inferred from the extended dataset