Hidden diversity and macroevolutionary mode of *Caulimoviridae* uncovered by euphyllophyte paleoviruses

Zhen Gong¹, & Guan-Zhu Han^{1*}

¹Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering
and Technology Research Center for Microbiology, College of Life Sciences, Nanjing
Normal University, Nanjing, Jiangsu 210046, China

*Correspondence and requests for materials should be addressed to G.-Z.H (email:
guanzhu@email.arizona.edu).

14 **Abstract**

15 Few viruses have been documented in plants outside angiosperms. Endogenous viral
 16 elements (paleoviruses) provide ‘molecular fossils’ for studying the deep history and
 17 macroevolution of viruses. Endogenous plant pararetroviruses (EPRVs) are
 18 widespread across angiosperms, but little is known about EPRVs in earlier branching
 19 plants. Here we use a large-scale phylogenomic approach to investigate the diversity
 20 and macroevolution of plant pararetroviruses (formally known as *Caulimoviridae*).
 21 We uncover an unprecedented and unappreciated diversity of EPRVs in the genomes
 22 of gymnosperms and ferns. The known angiosperm viruses only constitute a minor
 23 part of the *Caulimoviridae* diversity. By characterizing the distribution of EPRVs, we
 24 show that no major euphyllophyte lineages escape the activity of *Caulimoviridae*,
 25 raising the possibility that many exogenous *Caulimoviridae* remain to be discovered
 26 in euphyllophytes. We find that the copy numbers of EPRVs are generally high,
 27 suggesting that EPRVs define a unique group of repetitive elements and represent
 28 major components of euphyllophyte genomes. Phylogenetic analyses reveal an
 29 ancient monilophyte origin of *Caulimoviridae* and at least three independent origins
 30 of *Caulimoviridae* in angiosperms by cross-division transmissions. Our findings
 31 uncover the remarkable diversity of *Caulimoviridae* and have important implications
 32 in understanding the origin and macroevolution of plant pararetroviruses.

Endogenous viral elements (EVEs), the viral sequences integrated into their hosts' genomes, document past virus (paleovirus) infections and provide 'molecular fossils' for studying the deep history of viruses¹. EVEs lay the foundation of an emerging field, Paleovirology^{1,2}. The best characterized EVEs are endogenous retroviruses (ERVs)³. The replication of retroviruses requires integration into their hosts' genomes. On occasion, retroviruses infect germ lines of their hosts, and the integrated retroviruses, namely ERVs, become vertically inherited. ERVs are widespread and highly abundant in the genomes of vertebrates³; for example, ERVs make up 5%-8% of the human genome⁴. Recently, endogenous non-retroviral elements have been increasingly identified by comparative genomic analyses, which reveal the remarkable diversity, deep history, and macroevolution of related viruses^{5,6}. EVEs (especially ERVs) were pervasively co-opted for the hosts' biology, ranging from placentation, to inhibition of exogenous viral infection, to regulation of innate immunity⁷⁻⁹.

Like retroviruses, two families of viruses with double-stranded DNA genomes replicate through RNA intermediates, known as pararetroviruses or DNA reverse-transcribing viruses¹⁰. Unlike retroviruses, these pararetroviruses lack integrase, and thus integration into host genomes is not essential for their replication. Pararetroviruses infect vertebrates (*Hepadnaviridae*) and plants (*Caulimoviridae*). Evolutionary analyses suggest that *Hepadnaviridae* and *Caulimoviridae* originated independently from retrotransposons with long terminal repeats (LTRs)¹¹. Endogenous hepadnaviruses have been increasingly identified in the genomes of many species of Aves and reptiles¹²⁻¹⁴. The copy number of endogenous hepadnaviruses in the host genomes is very low (usually around ten copies)¹²⁻¹⁴. The identification of endogenous hepadnaviruses reveals the prevalent nature and deep history (more than 207 million years) of *Hepadnaviridae* in vertebrates¹²⁻¹⁵.

The *Caulimoviridae* family, pararetroviruses infecting plants, is classified into eight genera, namely *Caulimovirus*, *Soymovirus*, *Tungrovirus*, *Badnavirus*, *Solendovirus*, *Cavemovirus*, *Rosadnavirus*, and *Petuvirus*¹⁶. The genome size of

63 *Caulimoviridae* is usually between 6,000 and 8,000 base pairs (bps), encoding one to
 64 eight open reading frames (ORFs). The proteins (or domains) common to
 65 *Caulimoviridae* include movement protein (MP), coat protein (CP), aspartic protease
 66 (AP or PR), reverse transcriptase (RT), and RNase H1 (RH)¹⁶. While the replication of
 67 *Caulimoviridae* does not require integration into host genomes, endogenous plant
 68 pararetroviruses (EPRVs) were identified in many angiosperms at pre-genomic era¹⁷,
 69 for example banana¹⁸ and tobacco¹⁹. Genome-scale data provide important resource to
 70 explore the distribution and diversity of EPRVs within plant genomes, which would
 71 improve our understanding of the macroevolution of *Caulimoviridae* and the
 72 relationship between viruses and their hosts. By mining a variety of plant genomes,
 73 Geering et al.²⁰ identified a novel lineage of EPRVs in flowering plants (angiosperms),
 74 which was designated ‘Florendovirus’ and was thought to constitute a new genus
 75 within *Caulimoviridae*. However, EPRVs have not been detected in the genomes of
 76 plants outside angiosperms²⁰.

77

78 In this study, we use a large-scale phylogenomic approach to investigate whether
 79 EPRVs are present in the genomes of plants outside angiosperms. By mining ten
 80 gymnosperm and six fern genomes, we identified EPRVs in the genomes of nearly all
 81 these gymnosperms and ferns. Phylogenetic analyses using the newly identified
 82 EPRVs together with other angiosperm viruses reveal an unappreciated diversity of
 83 *Caulimoviridae* and show that the known angiosperm viruses only constitute a minor
 84 part of the *Caulimoviridae* diversity. The newly identified EPRVs in gymnosperms
 85 and ferns provide important and novel insights into the diversity, distribution, and
 86 macroevolution of *Caulimoviridae*.

87

88 **Results**

89 **Identification of EPRVs in gymnosperms and ferns**

90 We used a combined similarity search and phylogenetic analysis approach to
 91 screen the genomes of ten gymnosperms, six ferns, and four other earlier branching
 92 plant species (*Selaginella moellendorffii*, *Physcomitrella patens*, *Marchantia*

93 *polymorpha*, and *Klebsormidium flaccidum*) for the presence of EPRVs (Fig. 1 and
 94 Supplemental Table 1). Briefly, similarity search with the protein sequences of
 95 representative *Caulimoviridae* was performed against these plant genomes (Fig. 1 and
 96 Supplemental Table 1). Given RT and RH of *Caulimoviridae* share significant
 97 similarity with retrotransposons and other reverse-transcribing viruses, EPRVs were
 98 further identified and confirmed by phylogenetic analyses (see Methods). We found
 99 that EPRVs are present in the genomes of nearly all the gymnosperms and ferns
 100 investigated in this study (Fig. 1), suggesting that EPRVs are prevalent and
 101 widespread in gymnosperms and ferns. EPRVs were not identified in the genome of
 102 the fern *Ceratopteris richardii*, which does not necessarily indicate the absence of
 103 EPRVs but is more likely due to the low-density coverage (1.082×) of its genome
 104 sequencing (only 1% of its genome is covered by the genome assembly)²¹. No EPRV
 105 was detected in the genomes of the lycophyte *S. moellendorffii*, the moss *P. patens*,
 106 the liverwort *M. polymorpha*, and the charophyte *K. flaccidum* (Fig. 1). Together with
 107 previous reports of EPRVs in angiosperms¹⁷⁻²⁰, we conclude that EPRVs are
 108 widespread in the genomes of euphyllophytes (ferns and seed plants).

109
 110 The copy numbers of EPRVs within the genomes of gymnosperms and ferns
 111 appear to vary widely across plant species. We estimated that the genomes of the
 112 gymnosperms and ferns contain 112 – 20,579 copies of EPRVs (Table 1). However,
 113 the genomes of some gymnosperms and ferns are of low coverage, these estimated
 114 numbers should be taken with cautions. On the other hand, for the three genomes
 115 that >90% are covered by genome assembly (*Pinus taeda*, *Picea glauca*, and *Ginkgo*
 116 *biloba*), the EPRV copy numbers were estimated to be 6,733, 2,520, and 481,
 117 respectively. Our results suggest that EPRVs might represent major components of
 118 plant genomes.

119 120 **Diversity and classification of *Caulimoviridae***

121 To explore the relationship between the newly identified gymnosperm and fern
 122 EPRVs and the known angiosperm *Caulimoviridae*, we inferred a phylogeny of

representative exogenous and endogenous viruses of *Caulimoviridae* using the highly conserved RT-RH proteins with the retrotransposon *Ty3* as the outgroup. Our phylogenetic analysis reveals an extraordinarily large diversity of the *Caulimoviridae* family, which has never been appreciated previously (Fig. 2). The known eight viral genera and florendoviruses fell well within the diversity of EPRVs of gymnosperms and ferns. It follows that the previously known angiosperm *Caulimoviridae* constitute only a minor part of its diversity.

Our phylogenetic analysis identified at least seven monophyletic groups of EPRVs with high supports (Bayesian posterior probability > 0.95) in gymnosperms and ferns. These clades were designated gymnosperm endogenous florendovirus-like virus 1-3 (GEFLV 1-3), fern endogenous florendovirus-like virus (FEFLV), gymnosperm endogenous caulimovirus-like virus 1-2 (GECLV 1-2), and fern endogenous caulimovirus-like virus (FECLV), respectively (Fig. 2). The host of each clade is restricted to one plant division (except GECLV 1) (Fig. 2). The divergence within one of these clades is comparable to and even greater than that of one known *Caulimoviridae* genus. Some gymnosperm and fern EPRVs are not readily classified, either because one virus formed one branch or because the branches are not strongly supported.

Macroevolutionary mode of *Caulimoviridae*

To estimate the relative importance of co-speciation and host switching in the macroevolution of *Caulimoviridae*, we performed a global assessment of the correspondence between *Caulimoviridae* and host phylogenetic trees using the event-based approach. The analyses found no significant signal for co-speciation (p values > 0.05; Table 2), suggesting co-speciation might not play a predominant role in the diversification of *Caulimoviridae*.

Our phylogenetic analysis shows that some newly described fern EPRVs occupy important phylogenetic positions -- basal to all the other known *Caulimoviridae*.

153 Ancestral state reconstruction reveals that the *Caulimoviridae* family originated in
154 ferns (Supplemental Fig. 1). Our phylogenetic analysis also shows that the
155 angiosperm viruses form three independent monophyletic groups: two consist of
156 known eight genera of exogenous viruses and one consists of florendoviruses (Fig. 2).
157 The three angiosperm viral groups are only distantly related to each other. The
158 phylogenetic relationship among euphyllophyte viruses indicate that the angiosperm
159 viruses originated multiple times probably through cross-division transmission from
160 gymnosperms.

161

162 **Genome structure evolution of *Caulimoviridae***

163 To explore the genome structure evolution within the *Caulimoviridae* family, we
164 reconstructed the consensus genome sequences of EPRVs (Supplemental Data 1-5).
165 Given the fern genomes are of low coverage, we only reconstructed the genomes of
166 gymnosperm EPRVs, and one representative for each of the five gymnosperm EPRV
167 clades were inferred. These gymnosperm EPRV genomes vary wildly in size (from
168 6,061 to 8,109 bps) and ORF organization (Fig. 3 and Supplementary Data).
169 Conserved Domain (CD) searches show that protein domains common to all the
170 EPRVs include MP, AP, RT, and RH, suggesting that the gymnosperm EPRVs exhibit
171 a similar protein architecture as the angiosperm *Caulimoviridae* (Fig. 3). No CP
172 homologs were identified in the consensus genome sequences, possibly due to the
173 rapid nature of its evolution. However, we identified the zinc-finger CCHC motif, a
174 hallmark of the CP protein, in PtaeV_2 (GECLV1) and GbiIV (GECLV2) (Fig. 3). CD
175 searches did not find any integrase-like domain, a pattern similar to the angiosperm
176 *Caulimoviridae* and indicates integration might not be necessary for the replication of
177 gymnosperm EPRVs either.

178

179 **Age estimate of EPRV bursts**

180 Because the genomes of loblolly pine (*P. taeda*) and ginkgo (*G. biloba*) were of
181 relatively high quality and contain rather distinct numbers of EPRVs (Table 1), they
182 were used to infer the evolutionary dynamics of EPRVs within the host genomes.

Mixture model analyses of the genetic divergence between EPRV copies and their consensus nucleotide sequence show that there are four and three peaks in *P. taeda* and *G. biloba* (Supplemental Table 2), suggesting at least four and three independent EPRV integration events occurring along the lineages leading to *P. taeda* and *G. biloba*, respectively. Based on the mixture analyses and phylogenetic analyses (Supplemental Fig. 2), the EPRVs within the genomes of *P. taeda* and *G. biloba* were classified into four and three families.

We failed to find any orthologous integration of EPRVs in different species and cannot directly estimate the time of viral integration. The median pairwise genetic distance within each family was calculated to examine the age of burst for each EPRV family^{22, 23} (Supplemental Table 3). Our results show that the EPRV proliferation dynamics were of difference between *P. taeda* and *G. biloba* (Supplemental Table 3). But we found all of the EPRV families investigated here experienced proliferation peaks tens or hundreds of million years ago. Consistently, the EPRV copies contain many frame-shift mutations and premature stop codons (Supplemental Fig. 4). However, those analyses come with two caveats: i) it is uncertain whether the EPRV proliferation activity within the host genome follows the Gaussian distribution; ii) the evolutionary rate of EPRVs remains unclear. Nevertheless, our results suggest that EPRVs evolved within their host genomes for hundreds of millions of years and indicate an ancient origin of *Caulimoviridae*.

Discussion

In this study, we report the identification of EPRVs within the genomes of gymnosperms and ferns. Together with the previous reports of exogenous and endogenous *Caulimoviridae* in angiosperms, our results demonstrate that all the major lineages of euphyllophytes (ferns and seed plants) are/were infected by the *Caulimoviridae* family. No EPRVs detected in the genome of *C. richardii* does not necessarily mean the absence of EPRVs, which is probably due to the low coverage

212 nature of its genome assembly. Few viruses have been documented in plant species
 213 outside angiosperm²⁴. The identification of EPRVs in gymnosperms and ferns makes
 214 *Caulimoviridae* the only known viral family that infects all major lineages of
 215 euphyllophytes.

216
 217 Our findings show that the newly identified EPRVs exhibit an unprecedented
 218 diversity, and the known angiosperm viral diversity only accounts for a minority of
 219 the *Caulimoviridae* diversity. The current *Caulimoviridae* classification system¹⁶
 220 cannot readily account for the diversity of EPRVs in gymnosperms and ferns. Indeed,
 221 the divergence within one clade of gymnosperm or fern EPRVs is comparable to the
 222 divergence of one exogenous viral genus or florendoviruses. Therefore, an updated
 223 classification incorporating gymnosperm and fern EPRVs should be developed. Most
 224 of the EPRV clades lack the exogenous counterparts, either because the ancient viral
 225 lineages completely died out, or because many exogenous viruses remain to be
 226 discovered.

227
 228 Two possible macroevolutionary modes of *Caulimoviridae* could be conceived: i)
 229 Co-speciation model: the viruses have co-evolved with their euphyllophyte hosts
 230 for >400 million years and undergone sporadic cross-species transmission (Fig. 5a); ii)
 231 Cross-species transmission model: frequent cross-species transmissions predominated
 232 in the evolution of *Caulimoviridae* (Fig. 5b). In this study, we failed to find
 233 co-speciation signal between *Caulimoviridae* and its hosts, suggesting co-speciation
 234 might not be predominant in the macroevolution of *Caulimoviridae* (Table 2 and
 235 Supplemental Fig. 3). Indeed, it appears that the angiosperm viruses originated
 236 multiple times via independent cross-division transmission events (gymnosperms to
 237 angiosperms). For plant viruses, cross-division transmission events were rarely
 238 documented, partially because much remains unknown about the virosphere in plants
 239 outside angiosperms. Given multiple cross-division transmission events took place in
 240 *Caulimoviridae*, we think that cross-division transmission might not be a rare event
 241 for other plant viruses.

242
243 Phylogenetic analysis and ancestral state reconstruction show that the
244 *Caulimoviridae* family might have a monilophyte origin (Supplemental Fig. 1), which
245 is compatible with the fact that we did not find any EPRV in earlier branching plants
246 (lycophytes and nonvascular plants). One might argue that the absence of EPRVs in
247 earlier branching plants is due to no viral integration occurring. However, this
248 possibility seems to be unlikely, given viral integration is so widespread in
249 euphyllophytes. The paleoviruses provide ‘molecular fossils’ for estimating the age of
250 related viruses. Previously, the integration of banana streak virus into the *Musa*
251 *balbisiana* genome was estimated to occur 0.63 million years ago^{25, 26}. The
252 endogenization of florendoviruses in *Oryza* speices was estimated to take place at
253 least 1.8 million years ago²⁰. Although we cannot directly date when the EPRV
254 endogenization events occurred by finding orthologous integration of EPRVs in
255 closely related species, the proliferation dynamics analyses of EPRVs within two
256 representative genomes indicate they might have activated within the host genomes
257 for hundreds of millions of years. Taken together, our findings pinpoint a possible
258 ancient monilophyte origin of *Caulimoviridae*.

259
260 Unlike EVEs of non-retroviral source, the copy numbers of EPRVs are generally
261 high (>1,000 copies for 10 out of 16 gymnosperm and fern species), suggesting that
262 EPRVs contribute significantly to the complexity of host genomes. *Caulimoviridae*
263 are closely related to LTR retrotransposons¹¹. On the other hand, EPRVs lack LTRs,
264 which makes it inappropriate to be classified as an LTR retrotransposon. It seems to
265 be more appropriate to define EPRVs as a unique group of transposable elements¹⁹.

266
267 Our findings suggest that *Caulimoviridae* integrated into and amplified within
268 host genomes multiple times. However, the integration and amplification mechanisms
269 of EPRVs remain unclear, as the *Caulimoviridae* genomes lack integrase-like proteins
270 and integration is not essential for their replication. Several potential mechanisms
271 might be involved: i) Unlike the highly sequestered nature of animal germlines, plant

germline tissues might allow more frequent infections of viruses. However, the copy numbers of EVEs within animals seem to be generally similar to EVEs within plants⁵.⁶. It appears that the high copy numbers of EPRVs are due to its own biology. ii) EPRVs encode a ‘cryptic’ integrase without significant similarity with the known proteins that function in integration. No integrase domain was found in the *Petunia vein clearing virus* (PVCV) genome. But one of its proteins encodes two distinctive motifs [HHCC and DD(35)E] that are shared by the integrase domain of retroviruses and LTR retrotransposons²⁷. However, it remains unknown whether that protein performs function similar to integrase. iii) Microhomology-mediated recombination between EPRV sequences and host sequences during host gap repair process could result in the integration of viral sequences into the host genomes¹⁷. This mechanism requires the free ends of open circular viral sequences produced during virus replication^{19,28}. iv) Like short interspersed elements (SINE), EPRVs might integrate and amplify themselves within the host genomes via hijacking the integrase of other retrotransposons^{29,30}.

Method

Identification of EPRVs in plant genomes

The genome sequences of twenty plant species were used to screen the presence of EPRVs, including ten gymnosperms (*P. taeda*, *Pinus lambertiana*, *Pinus sylvestris*, *Picea abies*, *Picea glauca*, *G. biloba*, *Gnetum gnemon*, *Juniperus communis*, *Taxus baccata*, and *Abies sibirica*), six ferns (*C. richardii*, *Dipteris conjugata*, *Plagiogyria formosana*, *Pteridium aquilinum*, *Polypodium glycyrrhiza*, and *Cystopteris protrusa*), one moss (*P. patens*), one liverwort (*M. polymorpha*), one lycophyte (*S. moellendorffii*), and one charophyte (*K. flaccidum*)^{21,31,32} (Table S1). To identify putative EPRVs within these genomes, we employed a two-step phylogenomic approach. First, the tBLASTn algorithm was employed to search against the plant genomes using the RT-RH domain sequences of *Rice tungro bacilliform virus* (RTBV) and PVCV as queries with an *e* cutoff value of 10⁻¹⁰. Next, all the significant hits

301 obtained were aligned with RT-RH sequences of representative LTR retrotransposons,
 302 retroviruses, *Hepadnaviridae*, and *Caulimoviridae*³³ using MAFFT with default
 303 parameters³⁴. Putative EPRVs, which form a monophyletic group with other
 304 *Caulimoviridae* with high support values, were identified based on phylogenetic
 305 analyses. EPRVs were confirmed by further rounds of phylogenetic analyses with
 306 putative EPRVs and representative LTR retrotransposons, retroviruses, *Hepadnaviridae*,
 307 and *Caulimoviridae*. Phylogenetic analyses were performed using an approximate
 308 maximum likelihood method implemented in FastTree 2.1.9 with default parameters³⁵.
 309 The copy number of EPRVs within each species was then counted. If length between
 310 hits was less than 5,000 bps and the hits were in the same order as the query, the hits
 311 were treated as a single copy.

312

313 **Phylogenetic analysis**

314 To further analyze the relationship among *Caulimoviridae*, phylogenetic analysis
 315 was performed using the RT-RH protein sequences from representative EPRV
 316 sequences of each gymnosperm and fern species, exogenous viruses, and
 317 florendoviruses. The *Ty3* retrotransposon sequence was used as the outgroup. These
 318 protein sequences were aligned using MAFFT algorithm with an accurate method
 319 with the L-INS-i strategy³⁴. Ambiguous regions within the alignment were removed
 320 using Gblocks 0.91b³⁶. The phylogenetic analysis was performed using a Bayesian
 321 method implemented in MrBayes 3.2.6 (ref. 37). The RtRev substitution model was
 322 used. A total of 17,000,000 generations in four chains were run, sampling posterior
 323 trees every 100 generations. The first 25% of the posterior trees were discarded for
 324 further analysis.

325

326 **Reconstruction of consensus genome sequences**

327 Relatively complete EPRV sequences with *MP-AP-RT-RH* domains with
 328 extended flanking regions (~5,000 bps for each end) were extracted. These sequences
 329 were then used as queries to search sequences with high similarity within their own
 330 host genome using the BLASTn algorithm with an *e* cutoff value of 10^{-25} . The

331 significant hits were aligned using MAFFT³⁴ and consensus sequences were generated
 332 using Geneious 10 (ref. 38) and manually edited. ORFs with the minimum size of 500
 333 were found using Geneious 10 (ref. 38). Protein domains within these reconstructed
 334 genomes were detected using the CD search³⁹.

335

336 **Analysis of EPRV activity within host genomes**

337 Because the genomes of *P. taeda* and *G. biloba* were of relatively high quality, we
 338 used their genomes to infer the evolutionary dynamics of EPRVs within the host
 339 genomes. The *AP-RT-RH* nucleotide sequences with length >500 bps of all the EPRVs
 340 within *P. taeda* and *G. biloba* genomes were extracted independently. These
 341 sequences were aligned using MAFFT and consensus sequences were inferred using
 342 Geneious 10 (ref. 38). The genetic distance between the consensus sequences and
 343 EPRVs was calculated based on the Kimura two-parameter model. To identify
 344 significant peaks in the genetic distance data sets, Gaussian mixture models were
 345 fitted using the R package mclust. The number of components (each component is
 346 modeled by the Gaussian distribution) was estimated by fitting models. The Bayesian
 347 Information Criterion (BIC) was used as the model selection criterion.

348

349 The phylogenetic trees of the *AP-RT-RH* nucleotide sequences extracted above
 350 within each host genome were reconstructed using FastTree 2.1.9 with a GTR+CAT
 351 model. The different EPRV families within the phylogenetic trees were allocated
 352 based on the results by mixture model analyses. The nucleotide sequences of each
 353 EPRV family were extracted and aligned using MAFFT³⁴. Pairwise genetic distances
 354 were calculated based on the Kimura two-parameter model. The age of burst (T) for
 355 each EPRV family was estimated through $T = D/2\mu$, where D represents the median
 356 pairwise distance and μ represents the evolutionary rate of host ($\sim 1.43 \times 10^{-9}$ – 2.2×10^{-9}
 357 substitutions per site per year³¹).

358

359 **Co-speciation analysis**

360 We explore the host-virus co-speciation signal at the level of class, for the

complex evolutionary history of EPRVs after integration might complicate co-speciation analysis. The relationships between virus and host phylogenetic trees were assessed using an event-based method implemented in Jane 4 (ref. 40). Briefly, five events (cospeciation, duplication, duplication & host switch, loss and failure to diverge) were assigned to a cost. The number of each event was estimated by finding the solution with the minimum total cost. The event-cost schemes (cospeciation-duplication-duplication & host switch-loss-failure to diverge) were set as follows, -1-0-0-0-0 (refs. 41, 42) and 0-1-1-2-0 (ref. 40). Host-virus phylogeny congruence was assessed by statistical tests with both two randomization methods, random tip mapping and random parasite tree, with the sample size of 500.

Reconstruction of ancestral states

To detect the macroevolutionary pattern among *Caulimoviridae*, we performed ancestral state reconstruction with Mesquite 3.10 (ref. 43). We assigned the 96 viral taxa (Fig. 2) using their hosts (gymnosperm, angiosperm, and fern) as characters. For the outgroup Ty3, a ‘?’ character was assigned. The parsimony model was used to trace character evolution over the posterior trees sampled in the aforementioned Bayesian analysis.

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Author contributions

G.-Z.H. designed the study. G.Z. and G.-Z.H performed the experiments. G.-Z.H and G.Z. analyzed the data and wrote the manuscript.

389 **Competing Financial Interests**

390 The authors declare no competing financial interests.

391

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505 **Table 1. Copy number of EPRVs identified and estimated in plant genomes**

506

Species	Division	Proportion of genome covered by assembly (%)	No. of EPRVs identified	No. of EPRVs estimated ²
<i>Pinus lambertiana</i>	Pinophyta	89.04	4556	5117
<i>Pinus taeda</i>	Pinophyta	92.00	6194	6733
<i>Picea glauca</i>	Pinophyta	100	2520	2520
<i>Juniperus communis</i>	Pinophyta	16.04	18	112
<i>Taxus baccata</i>	Pinophyta	27.79	112	403
<i>Abies sibirica</i>	Pinophyta	14.08	130	923
<i>Pinus sylvestris</i>	Pinophyta	30.20	314	1040
<i>Picea abies</i>	Pinophyta	61.22	1238	2022
<i>Ginkgo biloba</i>	Ginkgophyta	100	481	481
<i>Gnetum gnemon</i>	Gnetophyta	55.66	1328	2386
<i>Dipteris conjugata</i>	Pteridophyta	9.51	144	1515
<i>Plagiogyria formosana</i>	Pteridophyta	0.31	64	20579
<i>Ceratopteris richardii</i>	Pteridophyta	3.11	0	0
<i>Pteridium aquilinum</i>	Pteridophyta	6.34	57	898
<i>Polypodium glycyrrhiza</i>	Pteridophyta	0.53	21	3947
<i>Cystopteris protrusa</i>	Pteridophyta	1.00	12	1199
<i>Physcomitrella patens</i>	Bryophyta	ND ¹	0	0
<i>Selaginella moellendorffii</i>	Lycopodiophyta	ND ¹	0	0
<i>Marchantia polymorpha</i>	Marchantiophyta	ND ¹	0	0
<i>Klebsormidium flaccidum</i>	Charophyta	ND ¹	0	0

507

508 ¹ Not determined.

509 ² No. of EPRVs estimated = No. of EPRVs identified/Proportion of genome covered by assembly.

510 **Table 2. Number of events experienced by virus lineages**
511

Event costs ¹	Total cost	Cospeciation ²	Duplication ²	Duplication & host switching ²	Loss ²	Failure to diverge ²	P-value ³	P-value ⁴
-1, 0, 0, 0, 0	-8	8-8	2-3	13-14	7-20	0-0	>0.05	>0.05
0, 1, 1, 2, 0	20	4-4	3-5	15-17	0-0	0-0	>0.05	>0.05

512
513 ¹Event costs are for cospeciation, duplication, duplication & host switching, loss, and failure to
514 diverge, respectively.
515 ²Number of events is expressed as ranges that result in the same cost.
516 ³Random tip mapping method with sample size of 500.
517 ⁴Random parasite tree method with sample size of 500.

518 **Figure Legends**

519 **Figure 1. Distribution of EPRVs within plant genomes.** The phylogenetic
520 relationship of plant species is based on Refs. 21, 31, 32, 44-46. Different plant
521 divisions were labelled in different colors, and angiosperms, gymnosperms, and ferns
522 were labelled in pink, green, and blue, respectively. The presence and absence of
523 EPRVs were marked with solid and open circles around the related species. The
524 half-filled circle indicates that EPRVs have been identified in some but not all the
525 angiosperms.

526
527 **Figure 2. Phylogenetic relationship of representative exogenous and endogenous**
528 ***Caulimoviridae*.** The phylogenetic tree was inferred based on the RT-RH protein
529 sequences using a Bayesian method. The tree was rooted using the *Ty3* LTR
530 retrotransposon as the outgroup. Bayesian posterior probabilities were shown on the
531 selected nodes. *Caulimoviridae* of angiosperms, gymnosperms, and ferns were
532 highlighted in orange, green, and blue, respectively. Virus abbreviations: CVMV,
533 Cassava vein mosaic virus; SPCV, Sweet potato caulimo-like virus; SPVCV, Sweet
534 potato vein clearing virus; TVCV, Tobacco vein clearing virus; BSOLV, Banana streak
535 OL virus; CoYMV, Commelina yellow mottle virus; RTBV, Rice tungro bacilliform
536 virus; CaMV, Cauliflower mosaic virus; FMV, Figwort mosaic virus; RYV, Rose
537 yellow vein virus; PCSV, Peanut chlorotic streak virus; SoyCMV, Soybean chlorotic
538 mottle virus; CitPRV, Citrus endogenous pararetrovirus; PVCV, Petunia vein clearing
539 virus; VvinCV_sc1, *Vitis vinifera* C virus sequence cluster 1; CsatAV, *Cucumis*
540 *sativus* A virus; MescV, *Manihot esculenta* virus; FvesV_sc1, *Fragaria vesca* virus
541 sequence cluster 1; AtrichAV, *Amborella trichopoda* A virus; AtrichCV, *Amborella*
542 *trichopoda* C virus; EgranV_sc1, *Eucalyptus grandis* virus sequence cluster 1;
543 OsatBV, *Oryza sativa* B virus; SbicV, *Sorghum bicolor* virus. Species abbreviations:
544 Pabi, *Picea abies*; Pgla, *Picea glauca*; Ptae, *Pinus taeda*; Plam, *Pinus lambertiana*;
545 Psyl, *Pinus sylvestris*; Asib, *Abies sibirica*; Gbil, *Ginkgo biloba*; Jcom, *Juniperus*
546 *communis*; Ggne, *Gnetum gnemon*; Tbac, *Taxus baccata*; Pfor, *Plagiogyria formosana*;

547 Pgly, *Polypodium glycyrrhiza*; Cpro, *Cystopteris protrusa*; Paqu, *Pteridium aquilinum*;
548 Pgly, *Polypodium glycyrrhiza*.

549

550 **Figure 3. Genome structures of representative gymnosperm EPRVs and**
551 **exogenous *Caulimoviridae*.** Virus name abbreviation: RTBV, Rice tungro bacilliform
552 virus; PVCV, Petunia vein clearing virus; PglaV_1, *Picea glauca* virus 1; PglaV_2,
553 *Picea glauca* virus 2; PtaeV_1, *Pinus taeda* virus 1; PtaeV_2, *Pinus taeda* virus 2;
554 GbilV, *Ginkgo biloba* virus. The tRNA^{Met} which represents the beginning of the viral
555 replication, was indicated by an asterisk. The grey lines represent the genomes and the
556 rectangles represent the putative open reading frames (ORFs). The conserved protein
557 domains were labelled in different colors. Abbreviations: MP, movement protein;
558 PR/AP: protease/pepsin-like aspartate protease; RT, reverse transcriptase; RH,
559 ribonuclease H1; zf_CCHC, zinc-finger CCHC motif.

560

561 **Figure 4. Proliferation dynamics of EPRVs within the *P. taeda* (a and c) and *G.***
562 ***biloba* (b and d) genomes.** Yellow rectangles in **a** and **c** represent the distribution of
563 the genetic distances between EPRV copies and their consensus sequences. **b** and **d**
564 indicate Gaussian mixture models fitted.

565

566 **Figure 5. The models of the *Caulimoviridae* macroevolution.** The evolution of plant
567 hosts and viruses were indicated by blue lines and yellow dash lines, respectively. **(a)**
568 Co-speciation model: the viruses have co-evolved with their euphyllophyte hosts and
569 undergone sporadic cross-species transmission. **(b)** Cross-species transmission model:
570 frequent cross-species transmission predominated in the evolution of *Caulimoviridae*.

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PVCV (7,206 bps)

RTBV (8,002 bps)

GEFLV 1: PglaV_1 (7,421 bps)

GEFLV 2: PtaeV_1 (8,109 bps)

GEFLV 3: PglaV_2 (7,219 bps)

GECLV 1: PtaeV_2 (6,061 bps)

GECLV 2: GbiIV (6,081 bps)

a

Pinus taeda

b

Ginkgo biloba

c

Pinus taeda

d

Ginkgo biloba

a

b