

1 **Title page**

2 *Title*

3 Recalibration of insect evolutionary timescale using Monte San Giorgio fossils suggests survival of
4 key lineages through the End-Permian Extinction

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23

24 *Running title*

25 A revised evolutionary timescale for insects

26 **Abstract**

27 Insects are a highly diverse group of organisms and constitute more than half of all known animal
28 species. They have evolved an extraordinary range of traits, from flight and complete metamorphosis
29 to complex polyphenisms and advanced eusociality. Although the rich insect fossil record has helped
30 to chart the appearance of many phenotypic innovations, data are scarce for a number of key periods.
31 One such period is that following the End-Permian Extinction, recognized as the most catastrophic
32 of all extinction events. We recently discovered several 240-million-year-old insect fossils in the
33 Monte San Giorgio Lagerstätte (Switzerland-Italy) that are remarkable for their state of preservation
34 (including internal organs and soft tissues), and because they extend the records of their respective
35 taxa by up to 200 million years. By using these fossils as calibrations in a phylogenomic dating
36 analysis, we present a revised timescale for insect evolution. Our date estimates for several major
37 lineages, including the hyperdiverse crown groups of Lepidoptera, Hemiptera Heteroptera, and
38 Diptera, are substantially older than their currently accepted post-Permian origins. We found that
39 major evolutionary innovations, including flight and metamorphosis, appeared considerably earlier
40 than previously thought. These results have numerous implications for understanding the evolution
41 of insects and their resilience in the face of extreme events such as the End-Permian Extinction.

42

43 **1. Introduction**

44 Insects have diversified over the past ~400 million years (Myr) into an estimated 5.5 million species
45 (ranging from 2.6 to 7.8 million species) [1] that occupy nearly all continental habitats. Insects were
46 among the first animals to colonize the land following the establishment of plants, evolving an array
47 of traits including flight, complete metamorphosis, and advanced sociality [2]. Among the hypotheses
48 put forward to explain the extreme diversity of insects, two that have received support are the great
49 antiquity of insects and a relatively low extinction rate [3,4].

50 Compared with other animal groups, insects appear to have been relatively unaffected by
51 major extinction events [2], with possible exceptions being the catastrophic End-Permian Extinction

52 (EPE; 251.9 Ma) [5–7] and the Cretaceous–Paleogene mass extinction (66 Ma) [8]. In the case of the
53 EPE, two distinct extinction events during an approximately 60,000-year period caused the
54 disappearance of 80–96% of marine species and 70% of terrestrial vertebrate species [9–11]. Extant
55 insect orders are generally represented in fossils post-Permian, whereas numerous extinct groups are
56 only found in deposits from the Permian or earlier [5]. For example, a number of major insect groups
57 (e.g., Palaeodictyoptera, Megasecoptera, and Archaeorthoptera) appear to have gone extinct at the
58 end of the Permian, or soon afterwards. A similar pattern emerged from a recent phylogenomic study
59 on insects [12], which estimated that a number of major groups, including Diptera and Lepidoptera,
60 emerged after the EPE.

61 Reconstructions of insect diversity through evolutionary time are dependent on the presence
62 of representative entomofauna across all stratigraphic stages since the origin of the group, estimated
63 to have occurred during the late Silurian [2,13]. One period that is depauperate of insect fossils is that
64 immediately following the EPE [2,14]. The relatively small number of fossils from this period
65 presents a hindrance to our understanding of how insects responded to this mass extinction event.

66 Here we investigate the impact of eight exceptionally well-preserved fossil insects from the
67 Monte San Giorgio (MSG) Lagerstätte (Italian-Swiss border; 240–239 Ma; Figure S1) on estimation
68 of the insect evolutionary timescale. Although the MSG has been recognized for its important
69 vertebrate assemblage [15], its entomofauna has received relatively little attention. Nonetheless, its
70 importance has recently been highlighted by the discovery of the oldest representatives of some extant
71 groups, including a jumping bristletail (Archaeognatha: Machilidae) [16,17] and a lace bug
72 (Hemiptera: Tingidae) [18].

73 We performed a phylogenomic dating analysis of a sequence alignment of 220,615 amino
74 acids generated by Misof et al. [12]. This data set has comprehensive coverage of insect ordinal
75 diversity. Although there have been further intra-ordinal phylogenomic studies of insects over the
76 past five years (e.g., [19]), we analysed the data set of Misof et al. [12] because their estimate of the
77 evolutionary timescale is the most widely recognized.

78 We used eight key MSG fossils (Figure 1; Table S1; Text S1) plus the fossils used by Misof
79 et al. [12] to calibrate the molecular clock for our dating analysis (Table S2). Each of these fossils
80 was chosen according to strict criteria [20]. Briefly, the eight MSG fossils that we used for additional
81 calibrations were (Figure 1): *i*) the bristletail *Gigamachilis triassicus* [16], the oldest fossil of extant
82 Machilidae; *ii*) the mayfly *Tintorina meridensis* [21]; *iii*) the oldest lace bug fossil, *Archetingis*
83 *ladinica* [18]; *iv*) the adaphagan beetle *Praedodromeus sangiorgiensis* [22]; *v*) the stonefly
84 MCSN8462 [22]; *vi*) the webspinner MCSN8457 [22], ~130 Myr older than the second-oldest
85 confirmed webspinner, *Sorellembia estherae* [23]; *vii*) the polyphagan (not Staphyliniformia) beetle
86 MCSN8464 [22]; and *viii*) a reticulated beetle elytron of *Notocupes* sp. [21] (detailed information for
87 each fossil is provided in Text S1). In addition, due to their exceptional features, we report here an
88 unusually large holometabolous larva MCSN8531 (Figure 1H) and a specimen with a grasshopper-
89 like body plan MCSN8457 (Figures 1I and 1J); these fossils were not used for calibrations in our
90 dating analysis because of their uncertain phylogenetic positions.

91 The MSG insect fossils are remarkable because of their preservation of soft tissues and
92 internal organs, which are typically absent in invertebrate compression fossils. For example, in the
93 stonefly nymph MCSN8462 (Figure 1E), there is clear preservation of part of the cerebrum, the
94 suboesophageal ganglion, the meso- and metathoracic wing pads, and part of the alimentary canal
95 (possibly the midgut). In the winged male webspinner MCSN8457 (Figure 1F), phosphatized
96 enlarged basal fore-tarsomers (diagnostic of Embioptera) and leg muscular bundles are preserved. In
97 association with the impression of the folded wings, these features suggest that the insect died
98 suddenly in its burrow and was rapidly transported to an anoxic depositional basin, where
99 phosphatization of organic matter and fossilization occurred. Within the abdomen of the grasshopper-
100 like fossil MCSN8457 (currently unassigned; Figures 1I and 1J), Malpighian tubules are evident,
101 representing the first case, to our knowledge, of preservation of this organ in compression fossils.

102

103 **2. Materials and Methods**

104 *Selection of fossils used for calibrations*

105 To calibrate our molecular date estimates, we included 35 fossils previously used by Misof et al. [12]
106 and selected according to the criteria proposed by Parham et al. [20] (Table S2). In addition, we used
107 eight MSG fossils for calibration (Figure 1; Table S1; Text S1). Descriptions of diagnostic characters
108 and taxonomic assignments of the MSG fossils were provided in previous publications and a PhD
109 thesis [16–18,21,22,24]; in addition, a brief description is reported in electronic supplementary
110 material (Text S1). With the exception of *Notocupes* sp. [21] and the stonefly nymph [22], the MSG
111 fossils satisfy the criteria proposed by Parham et al. [20] for reliable calibrations. Museum identifiers,
112 taxonomic assignments, and references of the eight MSG fossils are provided in Table S1. The
113 specimens included as calibrations are or will be deposited at Museo Cantonale di Storia Naturale in
114 Lugano (CH).

115

116 *Genomic data set*

117 In order to evaluate the impacts of the MSG fossil entomofauna on phylogenomic estimation of the
118 insect evolutionary timescale, we chose to focus on the genomic data set recently published by Misof
119 et al. [12]. The transcriptomic data of 141 arthropod species consisting of 1,478 single-copy nuclear
120 genes, from which we obtained the data set analysed in this study (220,615 aligned amino acids), are
121 available from the Dryad Digital Repository (<https://doi.org/10.5061/dryad.3c0f1> [12,25]).

122 The genomic data set includes representatives of every major insect order, as well as
123 springtails and proturans. Ten taxa were used as outgroups: seven crustaceans, two myriapods, and
124 the tick *Ixodes*. Here we briefly repeat the methods used by Misof et al. [12] to prepare the data for
125 analysis. A detailed description is provided on electronic supplementary material (Text S1).

126

127 *Estimation of divergence dates*

128 We described our calibration priors as uniform priors with soft minimum bounds, reflecting the
129 uncertainty in fossil evidence in molecular dating [26]. The minimum age of each MSG fossil

130 corresponds to the age of the fossil deposit (i.e., 239.51 ± 0.15 Ma [27]). The maximum age of each
131 calibration was set to either 580 Ma, which is the approximate estimated age of the origin of
132 arthropods based on the oldest Ediacaran fossils; or to 450 Ma, when there was sufficient terrestrial
133 food to support Hexapoda [28]. The former maximum age limit was applied to each of the oldest
134 calibrated nodes, representing the split between two outgroup lineages and the split between the
135 outgroup and ingroup lineages. The latter, younger age limit was used for every other calibration
136 within the insect tree. Information on the nodes at which minimum age constraints were informed by
137 the MSG fossils are reported in electronic supplementary material (Text S1; Table S2; Figure S3).

138 We ran five sets of phylogenomic dating analyses to compare the effects of the MSG fossils
139 on estimation of the insect evolutionary timescale (details provided on electronic supplementary
140 material; Text S1). The tree topology was fixed for all of these analyses, having been estimated using
141 maximum likelihood by Misof et al. [12]. To infer the evolutionary divergence times in the tree, we
142 used the Bayesian dating approach in MCMCTREE [29], which is able to use an approximate
143 likelihood calculation to reduce computational burden [30,31]. For all of our phylogenomic dating
144 analyses, we used a Dirichlet-gamma prior for the mean substitution rate, with $\alpha=2$ and $\beta=20$, which
145 represents a diffuse prior distribution. We used a uniform prior for the relative node times. The
146 posterior distribution of node times was estimated using Markov chain Monte Carlo (MCMC)
147 sampling. The first 100,000 MCMC steps were discarded as burn-in before we drew samples every
148 50 steps over 2 million steps. For each of the five analyses, we combined all samples from the
149 respective 85 meta-partitions before calculating the combined 95% credibility intervals for each node
150 in the tree to produce a combined estimate of the evolutionary timescale.

151

152 **3. Results and Discussion**

153 *Impact of MSG fossils and uniform calibration priors on estimated divergence times*

154 We performed five separate phylogenomic dating analyses in MCMCTREE to examine the effects of
155 adding eight MSG fossils to a set of 37 fossils previously used in a phylogenomic analysis of insect

156 evolution [12] (Table S3). These analyses were performed using the same data set and fixed tree
157 topology (Fig. 1 of Misof et al. [12]), but with five different sets of fossil calibration priors. A
158 comparison of date estimates from three of these five analyses at 13 key nodes is shown in Fig. 2,
159 with a full comparison for all 140 node times provided in Tables S3-S4.

160 We first replicated the analyses of Misof et al. [12], who used a total of 37 fossil calibrations.
161 Lognormal priors were used for 20 of these calibrations, and uniform priors for the remaining 17
162 calibrations (Analysis 1). The results of these analyses were similar to those previously reported; in
163 terms of median posterior estimates, there was a mean difference of 8.16 Myr from the values across
164 the 140 nodes reported in Table S25 of Misof et al. [12] (see Tables S3-S4 and grey bars in Fig. 2).
165 Next, we added the eight MSG fossils with uniform calibration priors, retaining the original
166 lognormal and uniform priors for the fossil calibrations used by Misof et al. [12] (Analysis 2; 43
167 fossils used in total; note that two of the MSG fossil calibrations replaced those used by Misof et al.
168 [12]). This set of calibrations led to notable increases in the median posterior ages of some nodes
169 (Table S4), with an average increase of 13.4 Myr across the 140 internal nodes in the tree compared
170 with Analysis 1.

171 The use of restrictive lognormal priors can lead to underestimation of node ages [26,32], so
172 we performed two further analyses with uniform calibration priors only. The first analysis employed
173 only the fossils used by Misof et al. [12] (Analysis 3; 37 fossil calibrations), and is equivalent to the
174 analysis reported in Fig. 1B of Tong et al. [32] (see yellow bars in Fig. 2). The second analysis
175 involved the addition of the MSG fossils (Analysis 4; 43 fossil calibrations; see red bars in Fig. 2).
176 Median posterior node ages across the tree increased by an average of 42.9 Myr (Analysis 3) and 50.5
177 Myr (Analysis 4) compared with Analysis 1 (in which 20 lognormal calibration priors were used;
178 Table S3; Fig. 2). We discuss the results from Analysis 4 in detail in the following section. Finally,
179 we performed an analysis using both sets of fossil calibrations (43 taxa) and an autocorrelated relaxed-
180 clock model (Analysis 5). We did this analysis to allow comparison with the results of Analysis 4,
181 which employed an uncorrelated relaxed-clock model. Analyses 4 and 5 produced similar estimates

182 of node ages (average difference of 4.3 Myr in median posterior node ages; Tables S3-S4). Each of
183 the eight MSG fossils used for calibration was found to have a strong influence on the date estimate
184 for its respective node, with the dates being pushed back in time past the EPE in Analyses 2, 4, and
185 5 compared with Analyses 1 and 3 (Fig. S2).

186

187 *The evolution of early-branching insect lineages and key insect innovations*

188 The timescale of evolution estimated in our analysis that used uniform priors for 43 fossil calibrations
189 (Analysis 4) is shown in Figs. 3, S3. The median posterior ages of a total of 63 out of 140 internal
190 nodes, 32 of which represent the last common ancestors of major hexapod crown groups, increased
191 by more than 50 Myr (maximum 147 Myr) compared with those estimated in Analysis 1 (which were
192 similar to those reported in Misof et al. [12]). The median posterior ages of 96 internal nodes
193 (including 49 major lineages) increased by more than 25 Myr compared with the node-age estimates
194 from Analysis 1 (Table S4).

195 The increases in median ages between Analyses 1 and 4 were generally matched by similar
196 increases in the lower and upper bounds of the 95% CIs of each analysis (average increases of 34.6
197 and 62.6 Myr for the lower and upper bounds, respectively; Table S4). In the sections below, we
198 focus on differences in median posterior node ages between the two analyses and those of other
199 relevant studies. However, the 95% CIs for node ages across the tree were generally very wide (means
200 of 101.6 and 129.6 Myr across all nodes for Analyses 1 and 4 respectively; Table S3), such that there
201 was typically some overlap between the date estimates from the two analyses.

202 Our analysis supports an origin of crown group hexapods in the Cambrian to Ordovician
203 (~504 Ma, 95% CI 469–540 Ma). This is earlier than previous estimates in the Silurian [33] but agrees
204 with the results of other studies [12,32,39,40] (Fig. 2). We estimated that the last common ancestor
205 of extant insects (Ectognatha) appeared in the Ordovician 465 Ma (95% CI 439–493 Ma),
206 contemporary with the origin of land plants [41–44] (Fig. 3; Table S3). This result extends the median

207 estimate of the age of crown Ectognatha by ~20 Myr compared with some previous estimates [12,33]
208 (Fig. 2; Table S3), but is consistent with the results of other studies [39,45].

209 Crown Collembola was estimated to have originated in the Carboniferous to Triassic (285
210 Ma, 95% CI 209–365 Ma), with the median posterior age being ~30 Myr earlier than a previous
211 estimate ([12]; Table S3). This raises the possibility of an origin of the group before the EPE. The
212 last common ancestor of extant lineages of monocondylous insects (Archaeognatha) was estimated
213 to have arisen during the Devonian to Triassic (290 Ma, 95% CI 227–388 Ma; Fig. 2–3), with a
214 median posterior age ~140 Myr earlier than previously reported [12] (Table S3; Fig. 2–3). We
215 estimated an origin of crown Palaeoptera, including damselflies, dragonflies, and mayflies in the
216 Silurian to Carboniferous ~396 Ma (95% CI 327–439 Ma), with a median posterior age in the Early
217 Devonian rather than in the Late Devonian to Carboniferous [12,33] (Table S3). This median
218 posterior estimate is in agreement with those from other studies [32,39].

219 Our analyses show that several innovations critical to the success of insects, including the
220 appearance of wings and complete metamorphosis, evolved earlier than previously proposed. We
221 estimated that crown winged lineages (Pterygota) emerged during the Silurian (434 Ma, 95% CI 413–
222 449). This median posterior estimate is at least ~30 Myr earlier than some estimates (e.g., [12,46];
223 Table S3) but in agreement with others (e.g., [39,45,47]). Our results are consistent with the
224 thermoregulatory hypothesis of insect winglet evolution and compatible with the evolution of insect
225 flight during a period of high atmospheric pO₂ [48], which is estimated to have occurred in the Early
226 to Middle Silurian [38]. The appearance in the ectothermic protopterygote of small winglets is
227 believed to have led to an increase in body temperature [49], which might have conferred an adaptive
228 advantage under the cool climate conditions prevalent during the Late Ordovician to Early Silurian
229 (Fig. 3). On the basis of our estimates, the ability to fold wings (crown group Neoptera) evolved
230 within ~10 Myr after their appearance (421 Ma, 95% CI 399–441).

231 We estimated the origin of insects exhibiting complete metamorphosis (crown Holometabola)
232 to have occurred during the Devonian (~389 Ma, 95% CI 359–419), with a median posterior age more

233 than 40 Myr earlier than proposed in the majority of previous estimates (e.g., [12,33,50]; Table S3),
234 but in agreement with a recent timescale inferred using transcriptome data [39]. The broader spectrum
235 of available ecological niches provided by communities of vascular and macrophyllous plants from
236 the Late Silurian to Early Devonian [43], as well as a global transition from hyperoxic to hypoxic
237 conditions [38], might have been associated with the evolution of complete metamorphosis and the
238 development of semaphoronts able to utilize different habitats and food resources.

239 The ancestor of crown polyneopterans, a group that includes, amongst others, ground lice,
240 stoneflies, crickets, leaf insects, cockroaches, and termites, was estimated here to have appeared
241 during the Devonian (389 Ma, 95% CI 347–428 Ma), with a median posterior age approximately 90
242 Myr earlier than previous estimates [12,33] (Table S3). Similarly, we found that the ancestor of crown
243 Zoraptera+Dermaptera, representing the sister lineage to the rest of the polyneopterans, occurred 345
244 Ma (95% CI 245–414 Ma), ~170 Myr earlier than previous estimates ([12]; Table S3, Figs 2, 3). The
245 last two node ages are in agreement with the results of Tong et al. [32], who included “roachoid”
246 fossils from the late Carboniferous in one of their analyses. During the Ordovician to Late Devonian,
247 the establishment of complex ecosystems dominated by woodland-like vegetation, fungi, and
248 arthropods increased the availability of food resources and opened new ecological niches [6]. This
249 might have promoted the appearance and diversification of crown polyneopterans, in agreement with
250 the hypothesis of an early origin of these lineages [51].

251 Parasitism is a key trait among insects. We estimated that the last common ancestor of
252 mosquitoes (based on representatives of *Anopheles* and *Aedes*) occurred 131 Ma (95% CI 62–229).
253 This raises the possibility of co-evolution of these insects with live-bearing mammals, whose last
254 common ancestor is thought to have originated in the Jurassic [52], or with a non-mammalian host.
255 Our results indicate that crown parasitic lice (order Phthiraptera) appeared in the Mesozoic (~129 Ma,
256 95% CI 54.5–213). This is inconsistent with the hypothesis that they diversified after the emergence
257 of avian and mammalian hosts [12,53], but supports the alternative hypothesis that they evolved on
258 feathered theropod dinosaurs [54].

259

260 *Multiple major insect groups may have survived the End-Permian Extinction*

261 Our finding that a large number of crown lineages potentially arose before the EPE (Figs 2, 3)
262 suggests that the most catastrophic extinction event in Earth's history might not have reduced insect
263 diversity to the same degree that it did in other animal groups. A similar finding was recently reported
264 for plants [55], another major group of organisms inhabiting continental ecosystems, suggesting that
265 these ecosystems were not as dramatically affected by the EPE as previously thought. The extensive
266 species diversity of phytophagous insect groups, including Orthoptera, Lepidoptera, Coleoptera,
267 Heteroptera, Hymenoptera, and Diptera, was previously thought to have been associated with the
268 diversification of angiosperms [56–58]. However, our results and those of others [19,59–62] raise the
269 possibility that these groups appeared prior to the EPE, and that their initial diversification occurred
270 in association with gymnosperms [63] rather than with flowering plants, which are thought to have
271 diversified in the Mesozoic [64,65].

272 Our median posterior estimate for the age of crown Hemiptera was in the Devonian to
273 Carboniferous (358 Ma, 95% CI 317–401), rather than in the middle Carboniferous to Permian as
274 previously estimated [12,32]. This is in accordance with a recent analysis of a comprehensive data
275 set comprising 2,395 protein-coding genes from 193 hemipteroid taxa [62]. Our date estimate for
276 crown Heteroptera (277 Ma, 95% CI 241–318) is consistent with the attribution of *Paraknightia*
277 *magnifica* Evans 1943, a highly debated fossil from the Late Permian, to this group [66]. Although
278 predation has been postulated as a plesiomorphic state of heteropterans [67,68], anatomical features
279 suggest that *Archetingis ladinica* was phytophagous, possibly feeding on gymnosperms or horsetails.
280 Therefore, it is possible that the ancestor of true bugs was phytophagous rather than predatory [67,68].
281 Similar results were also obtained for crown Coleoptera, which we dated at 283 Ma (95% CI 257–
282 305) in the Permian, with the last common ancestor of Coleoptera and Strepsiptera occurring in the
283 late Carboniferous to Early Permian (301 Ma, 95% CI 279–313). These findings are consistent with
284 other studies that placed the base of crown Coleoptera in the Permian [2,12,32,50,69–71].

285 Our analysis placed the crown age of true flies (Diptera) in the late Carboniferous to early
286 Triassic (~282 Ma, 95% CI 233–333), in agreement with the results of certain previous studies [32,72]
287 but not of others [12,50]. On the basis of our estimates, the last common ancestor of extant
288 Lepidoptera occurred ~271 Ma (95% CI 208–331). This is slightly earlier than the first glossatan
289 fossilized wing scales, recently discovered in an Upper Triassic deposit [73], but well before the first
290 fossils reliably identified as members of the Lepidoptera dated to the Early Jurassic [74–77].
291 Therefore, suctorial mouthparts for feeding on gymnosperm pollination drops might have evolved
292 earlier than generally thought. Molecular estimates of the age of crown Lepidoptera range from the
293 Early Cretaceous [12,50] to the Late Triassic [35] and the Middle Permian (this study; 32), suggesting
294 the potential benefits of further analyses of a more comprehensive data set, both in terms of taxa and
295 molecular data. The crown group of butterflies (here included as members of the genera *Polyommatus*
296 and *Parides*) was estimated to have appeared in the Cretaceous to Paleogene (82.8 Ma, 95% CI 36.1–
297 134 Ma), with a median posterior age approximately 45 Myr after the earliest appearance of eudicots
298 [35,64,78] and almost in agreement with a recent study based on ~6000 nucleotides [79].

299

300 **4. Conclusions**

301 Our phylogenomic dating analysis, calibrated using new MSG fossils from the Middle Triassic,
302 provides a revised timescale for the appearance of key insect innovations, shifting the median
303 estimates and 95% CIs for several nodes to substantially earlier than reported by previous studies.
304 Our results raise the possibility that the EPE had a lower impact on insect evolution than previously
305 thought. Our results indicate that terrestrial or amphibiotic protopterygotes evolved ~435 Ma, and
306 that the appearance of complete insect metamorphosis followed the origin of vascular plants (~390
307 Ma). A number of hyperdiverse crown groups such as Lepidoptera, Hymenoptera, Diptera,
308 Sternorrhyncha, and Heteroptera may have evolved prior to the EPE and were not strictly associated
309 with the evolutionary diversification of angiosperms. We tentatively propose that the EPE had only
310 a limited impact on insect evolution and on the faunal turnover postulated to have occurred following

311 this tremendous upheaval of Earth's biodiversity. Our results provide an updated timescale for
312 comparative analyses of insect evolution.

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322

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502

503 **Figure legends**

504 **Figure 1.** Macro photographs of the MSG fossil insects. **a**, *Gigamachilis triassicus* (Archaeognatha:
505 Machilidae). **b**, *Tintorina meridensis* (Ephemeroptera: Tintorinidae). **c**, *Archetingis ladinica*
506 (Hemiptera: Tingidae). **d**, *Praedodromeus sangiorgiensis* (Coleoptera: Trachipachidae). **e**,
507 Completely phosphatized stonefly nymph (Plecoptera), with a total length of 9.38 mm. **f**,
508 Phosphatized webspinner (Embioptera) of 18.3 mm in length, preserved in ventral view; fore basitarsi
509 swollen, three segmented tarsi, hindlegs with enlarged femora; muscles and tendons are visible within
510 the legs. **g**, Coleoptera Polyphaga (not Staphyliniformia) of 2.9 mm in length preserved in
511 dorsal/lateral view. **h**, Phosphatized e holometabolous larva (MCSN8531) of ~4 cm in length (not
512 complete), not assigned to an order. **i**, Phosphatized specimens with grasshopper-like body plan;
513 within the abdomen, at its end, a group of tubules is visible, possibly identifiable as the Malpighian
514 tubules; in **j**, a magnification of these structures, scale bar=200 µm. Details of image acquisition are
515 reported in electronic supplementary material (Text S1).

516

517 **Figure 2.** Comparison of age estimates for 13 key nodes obtained from three analyses employing
518 different fossil calibration priors. For each lineage, vertical bars show the 95% credibility intervals
519 of the age estimates; circles denote median values, and the red triangle corresponds to the age of MSG
520 fossils. The purple dashed line corresponds to the Permo-Triassic boundary. The main comparisons
521 are between the results of Analysis 1 (grey bars, replicating the calibration scheme used by Misof et
522 al [12]), Analysis 3 (yellow bars, replicating the calibration scheme used to produce Fig. 1B in Tong
523 et al. [32]), and Analysis 4 (red bars, based on the addition of the MSG fossil calibrations). We also
524 include estimates for some nodes obtained by Wheat and Wahlberg [33] (purple bars), Ronquist et
525 al. [34] (green bars), and Wahlberg et al. [35] (blue bars); for some of these, squares denote estimated
526 mean values. The periods and epochs from the International Chronostratigraphic Chart v 2019/05
527 [36] are shown on the left. Abbreviations: Hexa=Hexapoda; Ecto=Ectognatha; Pter=Pterygota;
528 Holo=Holometabola; Arch=Archaeognatha; Poly=Polyneoptera incl. Zoraptera; Phth=Phthiraptera;
529 Orth=Orthoptera; Hemi: Hete=Hemiptera: Heteroptera; Hymenopt=Hymenoptera;
530 Lepidopt=Lepidoptera; Dipt=Diptera; Mississip.=Mississippian; N=Neogene;
531 Pennsylv.=Pennsylvanian; Q=Quaternary; S=Silurian.

532
533 **Figure 3.** Time-calibrated phylogenetic tree of insect evolutionary relationships. Tips show the names
534 of taxa at ordinal and sub-ordinal levels. Bars on nodes correspond to the 95% credibility intervals of
535 node-age estimates, with red indicating the lineages for which the origin is pushed back by more than
536 50 Myr. Green stars indicate the nodes calibrated with MSG fossils; yellow circles indicate nodes
537 calibrated with other fossils. Two key evolutionary innovations in insects (wings and complete
538 metamorphosis) are shown at the relevant nodes. Red triangles indicate Hexapoda and Insecta. Below
539 the tree the following are shown: i) bars with periods and epochs from the International
540 Chronostratigraphic Chart v 2019/05 [36]; ii) scale axis of the tree expressed in Myr; iii) origin of
541 plant groups; iv) bar with warmer (red) and cooler (light-blue/dark-blue) periods [37]; and, v) plot of
542 O₂ concentration vs time according to [38]. Abbreviations as in Fig. 2.

544 **Electronic supplementary material**

545

546 **Supplementary information**

547 **Text S1.** This file includes supplementary information on i. *Monte San Giorgio fossil collection*; ii.
548 *The eight MSG fossils used for calibration*; and iii. *Image acquisition and figure preparation*

549

550 **Supplementary Tables**

551 **Table S1.** Monte San Giorgio fossils used in the analyses for estimating divergence dates and
552 calibration point prior settings.

553

554 **Table S2.** Age constraints used for fossil calibrations. Minimum ages, maximum ages, and priors for
555 each calibration are listed.

556

557 **Table S3.** Estimated divergence dates of the five performed simulations.

558

559 **Table S4.** Comparisons of five phylogenomic dating analyses using different calibration schemes
560 and clock models with the original Misof et al. [12] estimates.