

1 **A draft genome sequence of the miniature parasitoid wasp, *Megaphragma***
2 ***amalphitanum***

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26 **ABSTRACT**

27 Body size reduction, also known as miniaturization, is an important evolutionary
28 process that affects a number of physiological and phenotypic traits and helps animals
29 to conquer new ecological niches. However, this process is poorly understood at the
30 molecular level. Here, we report genomic and transcriptomic features of arguably the
31 smallest known insect – the parasitoid wasp, *Megaphragma amalphanum*
32 (Hymenoptera: Trichogrammatidae). In contrast to expectations, we find that the
33 genome and transcriptome sizes of this parasitoid wasp are comparable to other
34 members of the Chalcidoidea superfamily. Moreover, the gene content of *M.*
35 *amalphanum* compared to other chalcid wasps is remarkably conserved. Among the
36 very rare cases of apparent gene loss is *centrosomin*, which encodes an important
37 centrosome component; the absence of this protein might be related to the large number
38 of anucleate neurons in *M. amalphanum*. Intriguingly, we also observed significant
39 changes in *M. amalphanum* transposable element dynamics over time, whereby an
40 initial burst was followed by suppression of activity, possibly due to a recent
41 reinforcement of the genome defense machinery. Thus, while the *M. amalphanum*
42 genomic data reveal certain features that may be linked to the unusual biological
43 properties of this organism, miniaturization is not associated with a large decrease in
44 genome complexity.

45

46 **INTRODUCTION**

47 Miniaturization in animals is an evolutionary process that is frequently
48 accompanied by structural simplification and size reduction of organs, tissues and cells
49 [1, 2]. The parasitoid wasp *Megaphragma amalphitanum* (Hymenoptera:
50 Trichogrammatidae, subfamily Oligositinae) is one of the smallest known insects,
51 whose size (250 µm adult length) is comparable with unicellular eukaryotes and even
52 some bacteria (**Figure 1**). Parasitoids from the genus *Megaphragma* parasitize
53 greenhouse thrips *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae) developing
54 on the shrubs *Viburnum tinus* (Adoxaceae) and *Myrtus communis* (Myrtaceae) [3], and
55 possibly *Hercinothrips femoralis* (Thysanoptera: Thripidae) [4]. The wasp spends most
56 of its life cycle in host eggs, while the imago stage is very short and lasts only a few
57 days [3, 4]. *M. amalphitanum* belongs to chalcid wasps, which represent one of the
58 largest insect superfamilies (~23,000 described species)[5]. The higher-level taxonomic
59 relationships of Trichogrammatidae, Chalcidoidea and Hymenoptera have been
60 investigated in several recent studies [6-10] that helped to establish the placement of
61 this unique taxon that related to Mymaridae and Pteromalidae.

62

63 **Figure 1.** Size comparison of the parasitoid wasp *M. amalphitanum* and
64 bacterium *Thiomargarita namibiensis*. (A) An adult stage of the parasitoid wasp *M.*
65 *amalphitanum* (image adapted from [5]), (B) *T. namibiensis* – the largest known
66 bacterium (modified from Schulz et al. 1999) [11].

67

68 Amongst notable anatomical features of *M. amalphitanum*, this species has only
69 ~4600 neurons in its brain, which is substantially fewer than in the brains of other

70 wasps, e.g. the parasitoid chalcid wasp *Trichogramma pretiosum* (Trichogrammatidae:
71 Trichogrammatinae) (~18,000 neurons), *Hemiptarsenus sp.* (Chalcidoidea: Eulophidae)
72 (~35,000 neurons), and the honey bee *Apis mellifera* (Apidae) (~850,000-1,200,000
73 neurons). Moreover, by the final stage of *M. amalphitanum* development, up to 95
74 percent of the neurons of the central nervous system lose their nuclei [12, 13].
75 Nevertheless, adult wasps, which have an average lifespan of 5 days, still preserve the
76 basic functional traits of hymenopteran insects including flight, mating and oviposition
77 in hosts [14].

78 In this study, we present a draft *M. amalphitanum* genome, and adult
79 transcriptome, and compare these with several parasitoid wasp species of different body
80 sizes from the Chalcidoidea and Ichneumonoidea hymenopteran superfamilies. We
81 performed a comprehensive analysis of its coding potential, including both general gene
82 ontology and pathway analyses as well as specific gene categories of interest, such as
83 chemosensory receptors, venom components etc. Additionally, we investigated
84 transposable element (TE) content and dynamics across several parasitoid wasp species
85 and analyzed the major components of the genome defense machinery. As body size
86 reduction and loss of physiological or phenotypic traits is often correlated with genome
87 size diminution [15, 16] and/or gene networks reduction [17], including chromatin
88 diminution from the somatic tissues during embryogenesis[18, 19], we initially
89 anticipated that the *M. amalphitanum* genome would be greatly simplified during
90 miniaturization.

91

92 MATERIAL AND METHODS

93 Detailed information is presented in *Supplementary Information*

94 *Nucleic acid extraction and library construction.* *M. amalphitanum* individuals were
95 reared in the laboratory conditions from eggs of *Heliothrips haemorrhoidalis*
96 (Thysanoptera: Thripidae) collected in Santa Margherita, Northern Italy. DNA was
97 extracted from ten individuals (males and females) using NucleoSpin Tissue XS kit
98 (Macherey-Nagel, Germany) for each DNA-library. Three DNA libraries (DNA-
99 library1 – whole insects; DNA-library2 – thorax and abdomen; DNA-library3 – head)
100 were constructed using Ovation Ultralow Systems V2 kit (NuGEN, USA). Limited
101 amount of biological material and low quantity of starting material (1 – 3 ng) did not
102 permit construction of mate-paired libraries. Genome was sequenced used Illumina
103 HiSeq 1500 (Illumina, USA) with 150 bp paired-end reads. RNA was extracted from
104 ten *M. amalphitanum* individuals (males and females) using the Trizol reagent (Thermo
105 Fisher Scientific, USA) by a standard protocol, and cDNA libraries were constructed
106 using Ovation RNA-Seq System V2 kit (NuGEN, USA) with poly(A) enrichment.
107 *Genome de novo assembly.* The output from Illumina sequencing of the genomic DNA
108 library (source format *.fastq) was used for *de novo* genome assembly. To assemble the
109 complete genome of *M. amalphitanum*, we used 102,188,833 paired-end reads. Genome
110 assemblies have been constructed using different assembly algorithms, and their
111 performance was compared to each other (**Figure S1**). Additionally, genomic DNA-
112 libraries from thorax and abdomen (DNA-library2) of *M. amalphitanum* (SRR5982987)
113 and from head (DNA-library3) of *M. amalphitanum* (SRR5982986) were prepared.
114 Totally, 79,317,970 (paired-end sequencing: 2×100 bp) and 85,409,775 (single--end

115 sequencing: 50 bp) DNA reads were sequenced and were used for *M. amalphantanum*
116 coverage increase and as additional evidence during the search for missing genes (**Table**
117 **S1**).

118 *Transcriptome de novo assembly*. Illumina RNA sequencing generated a total of
119 59,790,973 paired-end reads. Transcriptome *de novo* assembly was conducted using the
120 default k-mer size in the Trinity software package (v. 2.4.0) [20], which combines three
121 assembly algorithms: Inchworm, Chrysalis and Butterfly. Annotation of the *M.*
122 *amalphantanum* transcriptome assembly was performed using the Trinotate pipeline
123 (<https://trinotate.github.io/>).

124 *Transposable element (TE) de novo identification and analysis*. For *de novo* TE library
125 construction, we used the REPET package [21] which combines three mutually
126 complementing repeat identification tools (RECON, GROUPER and PILER), yielding a
127 combined repeat library with the average consensus sequence length of 1.66 kb (ranging
128 from 157 to 14,640 bp). The outputs were subject to additional classification with the
129 RepeatClassifier tool from the RepeatMasker package (www.repeatmasker.org), which
130 was also used to build the corresponding TE landscape divergence plots.
131

132 **RESULTS AND DISCUSSION**

133 **Whole genome and transcriptome sequencing and assembly of *M. amalphantum***

134 To gain insight into the genomic signatures of miniaturization that would
135 distinguish *M. amalphantum* from other Hymenoptera, we performed whole-genome
136 shotgun sequencing of DNA (DNA-library1) isolated from ten adult individuals (males
137 and females), using the Illumina platform (**Table S1**). The resulting genome assembly
138 (PRJNA344956) has a cumulative length of 346 megabases (Mb), with a scaffold
139 N50 of 10,296 bp. The total genome coverage is 88.6-fold. Thus, the genome of *M.*
140 *amalphantum* is comparable in size with other Chalcidoidea wasps, such as
141 *Copidosoma floridanum*, *T. pretiosum* or *Nasonia vitripennis* [22, 23]. The best-
142 performing combination of assembly software yielded contig N50 of 4,285 bp and
143 allowed us to assemble 94,687 scaffolds from the quite low amounts of starting DNA
144 material (**Table S2-S3; Figure S1**).

145 The *M. amalphantum* genome assemblies were evaluated with the BUSCO
146 (benchmarking universal single-copy orthologs) arthropod gene set [24], which uses
147 2,675 near-universal single-copy orthologs to assess the relative completeness of
148 genome assemblies. Through this analysis, 7.55% of the conserved genes were initially
149 identified in the *M. amalphantum* assembly as missing (**Table S4**). More detailed
150 information on our extensive search for the missing genes in *M. amalphantum* genome
151 is presented below.

152 We also performed whole-body transcriptome analysis using RNA extracted
153 from ten *M. amalphantum* individuals (males and females). Transcriptome *de novo*
154 assembly (PRJNA344956) was performed using the Trinity software [20]. A total of

155 46,841 contigs were assembled with a mean length of 586 bp and an N50 of 633 bp
156 from the quite low amounts of starting RNA material (**Table S5**). The Illumina paired-
157 end RNA-Seq data from *M. amalphantanum* were mapped to the previously assembled
158 genome using bowtie2 [25]. Inspection of the alignments revealed that 79.95% of reads
159 could be mapped to the genome. The BUSCO statistics for the transcriptome assembly
160 is also presented in **Table S4**.

161 **Gene ontology analysis**

162 We used Gene Ontology (GO) analysis terms to describe characteristics of *M.*
163 *amalphantanum* gene products in three independent categories: biological processes
164 (**Figure S2**), molecular function (**Figure S3**), and cellular components (**Figure S4**).
165 BLASTX outputs were used to retrieve the associated gene names and GO terms in all
166 three categories (**Table 1**).

167

168

169 **Table 1.** Basic Gene Ontology (GO) analysis terms for *M. amalphantanum* gene
 170 products

GO assignments of the transcripts	Transcript counts and percentage from total		
Biological processes	8,812 counts, 49.72%		
	Transcription	Regulation of transcription	DNA integration
	15 %	10 %	8 %
Cellular components	4,802 counts, 27.10%		
	Nucleus and cytoplasm components	Integral membrane components	Plasma membrane components
	18 %	9 %	7%
Molecular functions	4,108 counts, 23.18%		
	ATP binding	Metal ion binding	Zinc ion binding
	17 %	12 %	10 %

171

172 All *M. amalphantanum* transcripts were matched to the Clusters of Orthologous
 173 Groups (COG) database to predict and classify their functions. In total, 8,810 genes
 174 were assigned to 25 COG functional categories. One of the largest groups is represented
 175 by the cluster for post-translational modification, protein turnover, and chaperones (988
 176 counts; 10.7%), followed by intracellular trafficking, secretion, and vesicular transport
 177 (659 counts; 7.2%), DNA replication, recombination and repair (606 counts; 6.6%),

178 signal transduction mechanisms (599 counts, 6.5%) and transcription (587; 6.4%)
179 **(Figure S5).**

180 To better understand incorporation of genes into diverse pathways, all annotated
181 transcripts were mapped against the KEGG database for pathway-based analysis. As a
182 result, 6,130 transcripts out of a total of 46,841 were assigned to a KEGG pathway, and
183 were present in 328 different KEGG pathways. The KEGG pathway distribution is
184 summarized in **Figure S6**. The top five pathways are metabolism (479 counts; 7.8%),
185 biosynthesis of secondary metabolites (150 counts; 2.4%), RNA transport (100 counts;
186 1.6%), biosynthesis of antibiotics (95 counts; 1.5%), spliceosome (94 counts; 1.5%).

187 The annotation of *M. amalphitanum* and the available transcriptome assemblies
188 of other parasitoid wasps from the families Trichogrammatidae (*T. pretiosum*, a
189 lepidopteran egg parasitoid) and Braconidae including *Cotesia vestalis* (a diamondback
190 moth parasitoid), *Diachasma alloeum* (an apple maggot parasitoid) and *Fopius arisanus*
191 (tephritid fruit fly parasitoid) were used for comparative analysis of the most
192 represented gene functions in parasitoids. We also used transcriptome assemblies from
193 the Agaonidae fig wasp, *Ceratosolen solmsi*. We found significant similarities between
194 *M. amalphitanum*, *T. pretiosum* and *C. vestalis* major GO enrichment categories
195 **(Figure S7-S9)**. At the same time, a significant number of transcripts related to DNA
196 integration relative to other parasitoid wasps was found in *D. alloeum* and *M.*
197 *amalphitanum* **(Figure S7)** (see below). Complete information about reference datasets
198 used for *M. amalphitanum* genome and transcriptome data analysis is shown in **Table**
199 **S6**. The Trinotate statistics for annotation of *M. amalphitanum*, *C. solmsi*, *D. alloeum*,
200 *F. arisanus*, *C. vestalis* and *T. pretiosum* transcriptome assemblies is presented in **Table**
201 **S7**.

202 **Missing genes and missing or rapidly evolving gene clusters in the *M.***

203 ***amalphitanum* genome**

204 We clustered gene orthologs and identified gene clusters for each hymenopteran taxa
205 (Chalcidoidea: *M. amalphitanum*, *T. pretiosum*, *C. solmsi*, *C. floridanum*, and *N.*
206 *vitripennis*; Ichneumonoidea: *D. alloenum* and *F. arisanus*; Apoidea: *A. mellifera*) using
207 OrthoMCL [26]. The core gene set of all the hymenopteran species was composed
208 of 6278 gene clusters, 122 gene clusters were unique to the chalcid clade. 262 gene
209 clusters were not detected in any of the chalcids analysed (**Supplementary Dataset 3**;
210 NCBI BioProject: PRJNA344956), but found in all the other hymenoptera, consistent
211 with a similar recent analysis [27]. Our findings suggest that that these gene losses
212 occurred in the last common ancestor of chalcids or point to the possibility of parallel
213 genome evolution across these species. Interestingly, the missing/rapidly evolving genes
214 include homologs of genes that have important roles in embryonic patterning and
215 development in other insects (e.g., *krueppel-1*, *knirps* or *short gastrulation* [27]).

216 To determine whether miniaturization in *M. amalphitanum* is associated with
217 gene loss, genomic data of six larger hymenopteran species (*T. pretiosum*, *C. vestalis*,
218 *C. floridanum*, *F. arisanus*, *N. vitripennis*, and *N. giraulti*) – as well as the well-
219 annotated genome of the honeybee (*A. mellifera*) as reference – were used (body sizes
220 are presented in **Table S6**). We mapped the *M. amalphitanum* (DNA-library1), *T.*
221 *pretiosum*, *C. vestalis*, *C. floridanum*, *F. arisanus*, *N. vitripennis*, *N. giraulti* DNA reads
222 on the *A. mellifera* genome sequence (PRJNA13343, PRJNA10625) (**Figure S10-S11**),
223 and detected 115 genes that were not represented by *M. amalphitanum* sequencing reads
224 but were present in other parasitoid wasps. We next increased the coverage of the *M.*
225 *amalphitanum* genome to 146.8-fold by adding the reads from additional libraries

226 (DNA-library2 and DNA-library3) (**Table S1**) and observed the apparent absence of
227 114 of the 115 genes. An additional TBLASTX search identified 36 of these genes as
228 present, yielding a total of 78 missing genes (**Table S8**). However, querying the *M.*
229 *amalphitanum* genome with the corresponding amino acid sequences from the closest
230 wasp ortholog (*N. vitripennis* or *T. pretiosum*) in TBLASTN searches reduced the
231 number of missing genes to just five: centrosomin, phosphoglycerate mutase 5,
232 phosphoglycerate mutase 5-2, 26S proteasome complex subunit DSS1, and mucin-
233 1/nucleoporin NSP1-like. We detected short *M. alphitanum* genome sequences
234 encoding protein fragments (~8-23 amino acid residues) with some similarity to four of
235 them, suggesting that they may be in the process of degeneration in this species. Despite
236 a careful search, we were unable to find any homologous sequence related to
237 *centrosomin* (*cnn*) gene either in the assembled genome or our cDNA libraries (**Figure**
238 **S15**). Although *cnn* is regarded as rapidly evolving [28], sequence homology can be
239 readily discerned and orthologs are present in every other insect, including the
240 parasitoid *T. pretiosum*, suggesting that this is a *bona fide* absent gene. Globally,
241 however, these analyses indicate that there has been little gene loss in *M.*
242 *amalphitanum*.

243 **Chemosensory genes in the *M. alphitanum* genome**

244 Chemosensory receptors are encoded by some of the largest gene families in
245 insect genomes, reflecting their important and wide-ranging roles in detection of
246 environmental odors and tastants. We asked how these gene families have evolved in *M.*
247 *amalphitanum*, whose central and peripheral nervous systems are highly reduced [2,
248 14]. The highly divergent sequences of chemosensory receptors and relatively short
249 genomic contig lengths available for *M. alphitanum* precluded accurate annotation of

250 full-length sequences in this species for the majority of loci. Nevertheless, comparison
251 with chemosensory receptor repertoires of other insects allowed us to define probable
252 orthologous relationships with receptors of known function in other species and obtain
253 initial estimates of the size of each family.

254 The most deeply conserved family of chemosensory receptors in insects are the
255 Ionotropic Receptors (IRs), which are distantly related to ionotropic glutamate receptors
256 [29, 30]. IRs function in heteromeric protein complexes comprising more broadly-
257 expressed co-receptors with selectively expressed “tuning” IRs that determines sensory
258 specificity. We identified orthologs of each of the co-receptors (*Ir8a*, *Ir25a* (two
259 paralogs), *Ir93a* and *Ir76b*), as well as four genes encoding tuning IRs related to acid-
260 sensing receptors in other species. We also identified orthologs of IR68a, which
261 functions in hygrosensation [31] and IR21a, which functions in cool temperature-
262 sensing [32, 33]. Overall, the repertoire of IRs in *M. amalphantanum* is therefore very
263 similar in size and content to that of *N. vitripennis* [30].

264 Insects possess a second superfamily of chemosensory ion channels –
265 distinguished by a heptahelical protein structure – comprising Odorant Receptor (OR)
266 and Gustatory Receptor (GR) subfamilies, which generally function in detection of
267 volatile and non-volatile stimuli, respectively [34-37]. Similar to IRs, ORs function in
268 heteromeric complexes of a conserved co-receptor (ORCO) and a tuning OR. We
269 identified an *M. amalphantanum* ortholog of *Orco* and 83 additional *Or*-related
270 sequences. We caution that many of these *Or* sequences are small fragments (often
271 located near the end of the assembled contigs), so it is currently difficult to determine
272 whether these are intact genes or pseudogenes. Within the GR repertoire, we identified
273 genes encoding proteins related to GR43a, a sensor of both external and internal

274 fructose [38], two others similar to other insect sugar-sensing GRs [39], and 25
275 additional *Gr* gene fragments. The sizes of these repertoires are smaller than in *N.*
276 *vitripennis* (300 *Ors* (including 76 pseudogenes) and 58 *Grs* (including 11 pseudogenes)
277 [40]), but similar to non-miniaturized parasitoid wasps *Meteorus pulchricornis* and
278 *Macrocentrus cingulum* [41, 42]. However, precise comparison with the latter two
279 species is difficult, as receptors in these wasps were identified from antennal
280 transcriptomes, thereby representing only one of these insects' chemosensory organs.

281 In sum, these analyses reveal that despite drastic nervous system reduction, *M.*
282 *amalphitanum* has retained the conserved chemosensory receptors of larger wasps (and
283 other insects), and appears to have numerous additional order- or species-specific
284 receptors to allow detection of environmental chemical cues.

285 **Venom components in the *M. amphitanum* transcriptomic data**

286 Parasitoid wasps often use venom to modify the metabolism of their hosts;
287 toxins and their known or presumed biological functions are described in various
288 species [43]. We investigated the presence of homologs of *N. vitripennis* toxin
289 constituents in *M. amphitanum* and other parasitoid wasps (*Megastigmus*
290 *spermatrophus*, *N. vitripennis*, *C. solmsi*, *T. pretiosum*), using previously published
291 venom data [44, 45] and the transcriptomes of chalcid wasps (**Table S6**). We identified
292 28 transcripts encoding putative venom proteins (**Figure 2; Table S9**); homologs of
293 these are found in all investigated Chalcidoidea species (**Table 2**). Assuming that most
294 of these candidates are truly conserved venom proteins among Chalcidoids, *M.*
295 *amalphitanum* venom's diversity does not seem to have been significantly affected by
296 size reduction.

297

298 **Figure 2.** A Venn diagram showing *Nasonia vitripennis* venom components in other
299 Chalcidoidea species: *M. spermotrophus*, *C. solmsi*, *T. pretiosum*, and *M.*
300 *amalphitanum*.

301 **Table 2.** Number of homologs of *N. vitripennis* venom (*N. vitripennis* toxin
302 constituents) in *M. amphitanum* and other Chalcidoidea species based on Universal
303 Chalcidoidea Database (<http://www.nhm.ac.uk/our-science/data/chalcidoids/database/>)
304

Parasitoid wasp species	Families of Chalcidoidea	Number of <i>N. vitripennis</i> venom constituents	Body size, mm	Approximate number of hosts
<i>M. amphitanum</i>	Trichogrammatidae	37	0.25	2 insect species from one order
<i>C. solmsi</i>	Agaonidae	38	2.7	2 plant species from one family
<i>M. spermotrophus</i>	Torymidae	41	2.8	13 plant species from one family
<i>T. pretiosum</i>	Trichogrammatidae	45	0.5	>140 insect species from 4 orders
<i>N. vitripennis</i>	Pteromalidae	64	2.2	>110 insect species from 8 orders

305 ***M. amalphanum* transposable elements and genome defense**

306 Transposable elements (TEs) constitute a measurable fraction of virtually all
307 eukaryotic genomes, and can play important roles in their function and evolution. In
308 insects, TE activity has been implicated in evolution of eusociality, based on
309 comparison of ten bee genomes with increasing degrees of social complexity [46]. We
310 performed *de novo* TE identification and comparative analysis of TE dynamics in *M.*
311 *amalphitanum* and in a representative set of larger wasp genomes for which TE content
312 has previously been reported: the parasitoid *N. vitripennis* and two primitively eusocial
313 aculeate wasps *Polistes canadensis* and *Polistes dominula* [12, 23, 47]. Additionally, we
314 analyzed TEs in the genomes of parasitoid wasps *T. pretiosum* from the family
315 Trichogrammatidae and *D. alloeum* from the family Braconidae, in which TE content
316 has not been studied.

317 For uniformity of measurements, we applied the same workflow to all genomes,
318 without relying on pre-existing repeat libraries. We employed the REPET package for
319 *de novo* TE identification (also used in [46]), and RepeatMasker for repeat classification
320 and construction of TE landscape divergence plots. Comparison of the overall repeat
321 content across six wasp species did not reveal substantial differences between four
322 species (18.5% in *M. amalphanum* vs. 18.1%, 17.7% and 14.2% in *P. canadensis*, *P.*
323 *dominula* and *T. pretiosum*, respectively), while the *N. vitripennis* genome was found to
324 be 32.5% repetitive, in close agreement with the published estimate [23], and *D.*
325 *alloeum* was highly repetitive at 52.8% (pie charts in **Figure 3; Figure S12**).

326 Surprisingly, TE dynamics over time, which is shown on the corresponding TE
327 landscape divergence plots, was found to differ substantially for *M. amalphanum*,

328 which displayed a pronounced decline in recent TE activity after an initial increase, a
329 pattern that was not observed in any other wasp (**Figure 3**).

330

331 **Figure 3.** Comparison of TE landscape divergence plots and TE genome fraction pie
332 charts in four parasitoid wasp species: *M. amalphitanum*, *T. pretiosum*, *N. vitripennis*,
333 and *D. alloeum*.

334

335 While TE dynamics may be affected by different factors, the observed drop in
336 active TE content in *M. amalphitanum* may be relevant to the unique biology of this
337 highly miniaturized insect. Its closest relative, *T. pretiosum*, is about 2-fold larger in
338 body length. The spike in recent TE activity in *T. pretiosum* may have been caused by
339 *Wolbachia* infection, which typically results in abandonment of sexual reproduction
340 [48] and the concomitant TE proliferation in a non-recombining genome [49]. Other
341 wasps do not display notable drops or spikes in current TE activity; TE inactivation was
342 reported in two asexual mites [50], however it appears to be ancient and may have
343 occurred prior to the abandonment of sex. Overall, the continued decline in *M.*
344 *amalphitanum* TE activity over the span of several million years – not observed in *T.*
345 *pretiosum* which shares the most recent common ancestor with *M. amalphitanum* –
346 represents a highly unusual genomic feature in every hymenopteran we examined,
347 including ants (not shown). Interestingly, no traces of *Wolbachia* infection or other
348 representatives of the Rickettsiaceae family were found in *M. amalphitanum* individuals
349 [51], while the sequenced *T. pretiosum* carries the *Wolbachia* symbiont [52]; the
350 sequenced *Nasonia* strain was maintained on antibiotics to cure it of infection.

351 To gain insights into possible reasons for reduction in TE activity after the initial
352 burst, we investigated the major components of the genome defense machinery in *M.*
353 *amalphitanum*, including Dicer (Dcr)-like and Argonaute (Ago)/Piwi-like protein-
354 coding genes. In insects, *Ago-1* and *Dcr-1* homologs represent the key components of
355 the miRNA pathway; *Ago-2* and *Dcr-2* mediate antiviral RNA interference; and *Piwi*
356 and *Ago-3/Aub* suppress TE activity in the germline [53]. Both *M. amalphitanum* and *T.*
357 *pretiosum* possess equal numbers of *Dcr-1* and *Dcr-2* homologs, as well as *Ago-2* and
358 *Ago-3* homologs (**Figure S13**). However, in *M. amalphitanum*, the *Ago-1* and the
359 *Piwi/Aub* homologs underwent a relatively recent duplication in comparison to *T.*
360 *pretiosum* (**Figure 4**). This may indicate additional layers of enforcement in the miRNA
361 and piRNA pathways of *M. amalphitanum*, both of which should result in suppression
362 of TE activity.

363

364 **Figure 4.** Maximum likelihood analysis of phylogenetic relationships between
365 Piwi/Argonaute coding sequences. Percent identity is indicated for the duplicated Ago-1
366 and Piwi/Aub homologs, both at the DNA and protein (cds) level. Phylogeny analysis
367 and notations are as in Supplementary Figure S13.

368

369 The drop in TE activity is also evident from the transcriptome analysis. The GO
370 radar plot (**Figure S7**) shows a substantial number of short contigs related to DNA
371 integration, most of which upon inspection were found to represent separate fragments
372 of gypsy-like and copia-like LTR retrotransposons, and a few belong to Polinton, P and
373 Ginger DNA TEs. Transcriptionally active copies fall into two groups: first, those
374 which apparently proliferated during the burst of TE activity and have since

375 accumulated debilitating mutations making them incapable of transposition, but still
376 retain a certain level of transcriptional activity; second, those that originate from recent
377 infections by retrovirus-like TEs and contain uninterrupted ORFs, but are not actively
378 proliferating and are present at very few genomic loci. Box plot of per cent identity
379 between BLASTN hits for *M. amalphitanum* integrase-related TE transcripts showed
380 that high-copy hits represent MITEs (**Figure S14**). We hypothesize that actively
381 proliferating TE copies can represent recent arrivals, possibly brought about by viruses
382 or host-parasite interactions [54].

383 Our study provides a first view of the genomic content of one of the smallest
384 insects currently known, the parasitoid wasp *M. amalphitanum*. In contrast to the
385 expectation that the small body size, in combination with the parasitic lifestyle, should
386 lead to significant reduction in the amount of genomic DNA and in gene content, we do
387 not observe a drastic reduction in the overall genome size or in the number of expressed
388 genes in comparison with larger parasitic wasps.

389 Of the rare cases of genes that were apparently lost from – or highly degenerated
390 in – the *M. amalphitanum* genome, the most intriguing is *centrosomin* (*cnn*), which is
391 universally present in insects, including *T. pretiosum*. In *Drosophila melanogaster*, Cnn
392 has important roles at the centrosome in mitotic spindle formation, cytoskeleton
393 organization and neuronal morphogenesis [55, 56], although these functions may not be
394 indispensable because this species (and possibly other insects) possesses centrosome-
395 independent mechanisms for spindle nucleation [57]. A fungal homolog of Cnn, called
396 *anucleate primary sterigmata*, is involved in nuclear migration [58-60]. We speculate
397 that the loss of this gene is linked to the unique biological feature of widespread neuron
398 denucleation in this tiny parasitoid wasp.

399 Surprisingly, transposable element dynamics over time was found to differ
400 greatly between the analyzed species, with *M. amalphantum* displaying a relatively
401 recent dramatic decline in TE activity preceded by a burst, a pattern not observed in
402 other parasitoid wasps. The decline in TE activity may have been associated with
403 evolution of additional *Ago* and *Piwi* copies, not present in *T. pretiosum*, which could
404 have reinforced the genome defense machinery to prevent uncontrolled TE expansion.

405 The relationship between body size and genome size has been discussed for a
406 long time. Significant correlations of these values have been described for flatworms
407 and copepods [16]; by contrast, such correlations were not found in ants [61]. Our
408 results show that body size reduction in hymenopterans, while being associated with
409 certain genomic adaptations, is not accompanied by greatly decreased transcriptomic
410 and genomic complexity. This observation begs the question of how miniaturization is
411 encoded genetically. We hypothesize that changes in regulatory sequences, rather than
412 gene content, were important in the process of body size reduction, similar to
413 mechanisms of morphological evolution that have driven adaptive diversification in all
414 animals, great or small [62].

415

416 **Acknowledgements**

417 This work has been carried out using computing resources of the federal collective
418 usage center Complex for Simulation and Data Processing for Mega-science Facilities
419 at NRC “Kurchatov Institute” (ministry subvention under agreement
420 RFMEFI62117X0016), <http://ckp.nrcki.ru/>. Support of this project was provided by the
421 Russian Scientific Foundation (RSF) grant #14-24-00175 and RSF grant #14-50-00060.
422 B.L. was supported by the Rosenthal Brown-MBL internship and the REU supplement
423 to NSF MCB-1121334 to I.A. R.B. was supported by a European Research Council
424 Consolidator Grant (615094).

425

426 **Data availability**

427 All genome and transcriptome assemblies and all SRA sequence data are publicly
428 available at the NCBI BioProject: PRJNA344956.

429

430 **Competing interests**

431 The authors declare that they have no competing interests.

432

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