

1 Running head: **Phylogenomics of *Triturus* newts**

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3 **Phylogenomics of the adaptive radiation of *Triturus* newts supports gradual ecological**
4 **niche expansion towards an incrementally aquatic lifestyle**

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18 **Abstract**

19 Newts of the genus *Triturus* (marbled and crested newts) exhibit substantial variation in the
20 number of trunk vertebrae (NTV) and a higher NTV corresponds to a longer annual aquatic
21 period. Because the *Triturus* phylogeny has thwarted resolution to date, the evolutionary
22 history of NTV, annual aquatic period, and their potential coevolution has remained unclear.
23 To resolve the phylogeny of *Triturus*, we generated a c. 6,000 transcriptome-derived marker
24 data set using a custom target enrichment probe set, and conducted phylogenetic analyses
25 using: 1) data concatenation with RAxML, 2) gene-tree summary with ASTRAL, and 3)
26 species-tree estimation with SNAPP. All analyses produce the same, highly supported
27 topology, despite cladogenesis having occurred over a short timeframe, resulting in short
28 internal branch lengths. Our new phylogenetic hypothesis is consistent with the minimal
29 number of inferred changes in NTV count necessary to explain the diversity in NTV observed
30 today. Although a causal relationship between NTV, body form, and aquatic ecology has yet
31 to be experimentally established, our phylogeny indicates that these features have evolved
32 together, and suggest that they may underlie the adaptive radiation that characterizes *Triturus*.

33

34 **Keywords:** morphology; phylogeny; sequence capture; systematics; target enrichment;
35 transcriptome

36 **1. Introduction**

37 Accurately retracing the evolution of phenotypic diversity in adaptive radiations requires
38 well-established phylogenies. However, inferring the true branching order in adaptive
39 radiations is hampered by the short time frame over which they typically unfold, which
40 provides little opportunity between splitting events for phylogenetically informative
41 substitutions to become established (resulting in low phylogenetic resolution; Philippe et al.,
42 2011; Whitfield and Lockhart, 2007) and fixed (resulting in incomplete lineage sorting and
43 discordance among gene-trees; Degnan and Rosenberg, 2006; Pamilo and Nei, 1988; Pollard
44 et al., 2006). Resolving the phylogeny of rapidly multiplying lineages becomes even more
45 complicated the further back in time the radiation occurred, because the accumulation of
46 parallel substitutions along terminal branches can lead to long-branch attraction (Felsenstein,
47 1978; Swofford et al., 2001). A final impediment is reticulation between closely related (and
48 not necessarily sister-) species through past or ongoing hybridization, resulting in additional
49 gene-tree/species-tree discordance (Kutschera et al., 2014; Leaché et al., 2014; Mallet et al.,
50 2016).

51 Phylogenomics, involving the consultation of a large number of markers spread
52 throughout the genome, has proven successful in resolving both recent (e.g. Giarla and
53 Esselstyn, 2015; Leaché et al., 2016; Léveillé-Bourret et al., 2018; Meiklejohn et al., 2016;
54 Nater et al., 2015; Scott et al., 2018; Shi and Yang, 2018) and more ancient (e.g. Crawford et
55 al., 2012; Irisarri and Meyer, 2016; Jarvis et al., 2014; McCormack et al., 2012; Song et al.,
56 2012) evolutionary radiations. The appeal of greatly increasing the amount of data available
57 for any given phylogenetic problem is that it often (but not always; see Philippe et al., 2011)
58 provides informative characters to resolve short branches in the tree of life. Advances in
59 laboratory and sequencing techniques, bioinformatics, and tree-building methods all facilitate
60 phylogenetic reconstruction based on thousands of homologous loci for a large number of

61 individuals, and promise to help provide the phylogenetic trees necessary to interpret the
62 evolution of eco-morphological characters involved in adaptive radiations (Alföldi et al.,
63 2011; Stroud and Losos, 2016). In this study, we conduct a phylogenomic analysis of an
64 adaptive radiation that moderately-sized multilocus nuclear DNA datasets (Arntzen et al.,
65 2007; Espregueira Themudo et al., 2009; Wielstra et al., 2014) have consistently failed to
66 resolve: the Eurasian newt genus *Triturus* (Amphibia: Urodela: Salamandridae), commonly
67 known as the marbled and crested newts.

68 One of the most intriguing features of *Triturus* evolution is the correlation between
69 certain aspects of their ecology and the number of trunk vertebrae (NTV; Fig. 1). Species
70 characterized by a higher modal NTV (which translates into a more elongate body build with
71 proportionally shorter limbs) are associated with a more aquatic lifestyle. Empirically, the
72 number of months a *Triturus* species spends in the water (defined at the population level as
73 the peak date of emigration, leaving a breeding pond, minus the peak in immigration,
74 entering it) roughly equals NTV minus 10 (Arntzen, 2003; Arntzen and Wallis, 1999;
75 Slijepčević et al., 2015). The intrageneric variation in NTV shown by *Triturus*, ranging from
76 12 to 17, is unparalleled in the family Salamandridae (Arntzen et al., 2015; Lanza et al., 2010)
77 and a causal relationship between NTV expansion and an increasingly aquatic lifestyle has
78 been presumed, but never adequately placed into a phylogenetic comparative analysis
79 (Arntzen, 2003; Arntzen et al., 2015; Arntzen and Wallis, 1999; Govedarica et al., 2017;
80 Slijepčević et al., 2015; Urošević et al., 2016; Vukov et al., 2011; Wielstra and Arntzen,
81 2011). A well-established *Triturus* species-tree is required to accurately retrace NTV
82 evolution and assess the concordance between aquatic lifestyle and NTV across the genus.

83 Our goal is to obtain a genome-enabled phylogeny for *Triturus* and use it to reconstruct
84 the eco-morphological evolution of NTV and aquatic/terrestrial ecology across the genus. As
85 the large size of salamander genomes hampers whole-genome sequencing (but see Elewa et

86 al., 2017; Nowoshilow et al., 2018; Smith et al., 2018), we employ a genome-reduction
87 approach in which we capture and sequence a set of transcriptome-derived markers using
88 target enrichment, an efficient technique that affords extremely high resolution at multiple
89 taxonomic levels (Abdelkrim et al., 2018; Bi et al., 2012; Bragg et al., 2016; Gnirke et al.,
90 2009; McCartney-Melstad et al., 2016; McCartney-Melstad et al., 2018). Using data
91 concatenation (with RAxML), gene-tree summarization (with ASTRAL) and species-tree
92 estimation (with SNAPP), we fully resolve the *Triturus* phylogeny and place the extreme
93 body shape and ecological variation observed in this adaptive radiation into an evolutionary
94 context.

95

96 **2. Materials and Methods**

97

98 *2.1 Target capture array design*

99 Nine *Triturus* newts (seven crested and two marbled newt species) and one banded newt
100 (*Ommatotriton*) were subjected to transcriptome sequencing. Transcriptome assemblies for
101 each species were generated using Trinity v2.2.0 (Grabherr et al., 2011), clustered at 90%
102 using usearch v9.1.13 (Edgar, 2010), and subjected to reciprocal best blast hit analysis (Bork
103 et al., 1998; Camacho et al., 2009; Tatusov et al., 1997) to produce a set of *T. dobrogicus*
104 transcripts (the species with the highest quality transcriptome assembly) that had putative
105 orthologues present in the nine other transcriptome assemblies. These transcripts were then
106 annotated using blastx to *Xenopus tropicalis* proteins, retaining one annotated transcript per
107 protein. We attempted to discern splice sites in the transcripts, as probes spanning splice
108 boundaries may perform poorly (Neves et al., 2013), by mapping transcripts iteratively to the
109 genomes of *Chrysemys picta* (Shaffer et al., 2013), *X. tropicalis* (Hellsten et al., 2010),
110 *Nanorana parkerii* (Sun et al., 2015) and *Rana catesbeiana* (Hammond et al., 2017). A single

111 exon \geq 200bp and \leq 450bp was retained for each transcript target. To increase the ability of
112 the target set to capture markers across all *Triturus* species, orthologous sequences from
113 multiple species were included for targets with $>$ 5% sequence divergence from *T.*
114 *dobrogicus* (Bi et al., 2012). We generated a target set of 7,102 genomic regions for a total
115 target length of approximately 2.3 million bp. A total of 39,143 unique RNA probes were
116 synthesized as a MyBaits-II kit for this target set at approximately 2.6X tiling density by
117 Arbor Biosciences (Ann Arbor, MI, Ref# 170210-32). A detailed outline of the target capture
118 array design process is presented in Supplementary Text S1.

119

120 2.2 Sampling scheme

121 We sampled 23 individual *Triturus* newts (Fig. 2; Supplementary Table S1) for which tissues
122 were available from previous studies (Wielstra et al., 2017a; Wielstra et al., 2017b; Wielstra
123 et al., 2013). Because the sister-group relationship between the two marbled and seven
124 crested newts is well established (Fig. 1), while the relationships among the crested newt
125 species have defied resolution, we sampled the crested newt species more densely, including
126 three individuals per species to include intraspecific differentiation and to avoid misleading
127 phylogenies resulting from single exemplar sampling (Spinks et al., 2013). Because *Triturus*
128 species show introgressive hybridization at contact zones (Arntzen et al., 2014), we aimed to
129 reduce the impact of interspecific gene flow by only including individuals that originate away
130 from hybrid zones and have previously been interpreted as unaffected by interspecific genetic
131 admixture (Wielstra et al., 2017a; Wielstra et al., 2017b). The reality of phylogenetic
132 distortion by interspecific gene flow was underscored in a test for the phylogenetic utility of
133 the transcripts used for marker design which included a genetically admixed individual
134 (details in Supplementary Text S1).

135

136 *2.3 Laboratory methods*

137 DNA was extracted from samples using a salt extraction protocol (Sambrook and Russell,
138 2001), and 10,000ng per sample was sheared to approximately 200bp-500bp on a BioRuptor
139 NGS (Diagenode) and dual-end size selected (0.8X-1.0X) with SPRI beads. Dual-indexed
140 libraries were prepared from 375-2000ng of size selected DNA using KAPA LTP library
141 prep kits (Glenn et al., 2017). These libraries were pooled (with samples from other projects)
142 into batches of 16 samples at 250ng per sample (4,000ng total) and enriched in the presence
143 of 30,000ng of c0t-1 repetitive sequence blocker (McCartney-Melstad et al., 2016) derived
144 from *T. carnifex* (casualties from a removal action of an invasive population (Meilink et al.,
145 2015)) by hybridizing blockers with libraries for 30 minutes and probes with
146 libraries/blockers for 30 hours. Enriched libraries were subjected to 14 cycles of PCR with
147 KAPA HiFi HotStart ReadyMix and pooled at an equimolar ratio for 150bp paired-end
148 sequencing across multiple Illumina HiSeq 4000 lanes (receiving an aggregate of 18% of one
149 lane, for a multiplexing equivalent of 128 samples per lane).

150

151 *2.4 Processing of target capture data*

152 A total of 3,937,346 read pairs from the sample receiving the greatest number of reads were
153 used to *de novo* assemble target sequences for each target region using the assembly by
154 reduced complexity (ARC) pipeline (Hunter et al., 2015). A single assembled contig was
155 selected for each original target region by means of reciprocal best blast hit (RBBH) (Rivera
156 et al., 1998), and these were used as a reference assembly for all downstream analyses.
157 Adapter contamination was removed from sample reads using skewer v0.2.2 (Jiang et al.,
158 2014), and reads were then mapped to the reference assembly using BWA-MEM v0.7.15-
159 r1140 (Li, 2013). Picard tools v2.9.2 (<https://broadinstitute.github.io/picard/>) was used to add
160 read group information and to mark PCR duplicates, and HaplotypeCaller and

161 GenotypeGVCFs from GATK v3.8 (McKenna et al., 2010) were used jointly to genotype the
162 relevant groups of samples (either crested newts or crested newts + marbled newts depending
163 on the analysis; see below). SNPs that failed any of the following hard filters were removed:
164 $QD < 2$, $MQ < 40$, $FS > 60$, $MQRankSum < -12.5$, $ReadPosRankSum < -8$, and $QUAL < 30$
165 (Poplin et al., 2017). We next attempted to remove paralogous targets from our dataset with a
166 Hardy Weinberg Equilibrium (HWE) filter for heterozygote excess. Heterozygote excess p-
167 values were calculated for every SNP using vcftools 0.1.15 (Danecek et al., 2011), and any
168 target containing at least one SNP with a heterozygote excess p-value < 0.05 was removed
169 from downstream analysis. More detail on the processing of the target capture data can be
170 found in Supplementary Text S2.

171

172 *2.5 Phylogenetic analyses*

173 A concatenated maximum likelihood phylogeny was inferred with RAxML version 8.2.11
174 (Stamatakis, 2014) based on an alignment of 133,601 SNPs across 5,866 different targets. We
175 included all 23 *Triturus* individuals in this analysis. For gene-tree summary, ASTRAL v5.6.1
176 (Zhang et al., 2017) was used to estimate the crested newt species-tree from 5,610 gene-trees
177 generated in RAxML. The 21 crested newt samples were assigned species membership, and
178 no marbled newts were included because estimating terminal branch lengths is not possible
179 for species with a single representative. For species-tree estimation, SNAPP v1.3.0 (Bryant et
180 al., 2012) within the BEAST v2.4.8 (Bouckaert et al., 2014) environment was used to infer
181 the crested newt species-tree from single biallelic SNPs randomly selected from each of
182 5,581 post-filtering targets. All three individuals per crested newt species were treated as a
183 single terminal and marbled newts were again excluded given our single exemplar sampling
184 of both species. We also estimated divergence times in SNAPP for the crested newts. The
185 split between *T. carnifex* and *T. macedonicus*, assumed to correspond to the origin of the

186 Adriatic Sea at the end of the Messinian Salinity Crisis 5.33 million years ago, was used as a
187 single calibration point (Arntzen et al., 2007; Wielstra and Arntzen, 2011) to produce a rough
188 estimate of the timing of cladogenesis. A detailed description of our strategy for phylogenetic
189 analyses is available in Supplementary Text S3.

190

191 **3. Results**

192 Samples received a mean of 2,812,980 read pairs (s.d. = 585,815). Enrichment was highly
193 efficient, especially given the large genome size of *Triturus*, with an average of 44.5% of raw
194 reads mapping to the assembled target sequences (s.d. = 2.6%). After removing PCR
195 duplicates, which accounted for an average of 22.6% of mapped reads, the unique read on
196 target rate was 34.4% (s.d. = 1.9%). The 23 samples in the final RAxML alignment contained
197 an average of 10.1% missing data (min = 3.2%, max = 31.8%) after setting genotype calls
198 with GQ scores of less than 20 to missing.

199 The concatenated analysis with RAxML supports a basal bifurcation in *Triturus*
200 between the marbled and crested newts (Fig. 3), consistent with the prevailing view that they
201 are reciprocally monophyletic (Arntzen et al., 2007; Espregueira Themudo et al., 2009;
202 Wielstra et al., 2014). RAxML also recovers each of the crested newt species as
203 monophyletic, validating our decision to collapse the three individuals sampled per species in
204 a single terminal in ASTRAL and SNAPP. Furthermore, all five *Triturus* body builds are
205 recovered as monophyletic (cf. Arntzen et al., 2007; Espregueira Themudo et al., 2009;
206 Wielstra et al., 2014). The greatest intraspecific divergence is observed in *T. carnifex*
207 (Supplementary Text S1; Supplementary Fig. S1; Supplementary Table S2).

208 Phylogenetic inference based on data concatenation with RAxML (Fig. 3), gene-tree
209 summary with ASTRAL (Fig. 4a) and species-tree estimation with SNAPP (Fig. 4b) all
210 recover the same crested newt topology, with a basal bifurcation between the *T. karelinii*-

211 group (NTV = 13; *T. ivanbureschi* as the sister taxon to *T. anatolicus* + *T. karelinii*) and the
212 remaining taxa, which themselves are resolved into the species pairs *T. carnifex* + *T.*
213 *macedonicus* (NTV=14; the *T. carnifex*-group), and *T. cristatus* (NTV=15) + *T. dobrogicus*
214 (NTV=16/17). Despite the rapidity of cladogenesis, we obtain strong branch support for
215 every internal node. Even with the uncertainty in dating given a single biogeographically-
216 derived calibration date, the bifurcation giving rise to the four crested newt species groups (cf.
217 Fig. 1) must have occurred over a relatively short time frame (Fig. 5), reflected by two
218 particularly short, but resolvable internal branches (Fig. 3; Fig. 4).

219 The phylogenomic analyses suggest considerable gene-tree/species-tree discordance in
220 *Triturus*. The normalized quartet score of the ASTRAL tree (Fig. 4a), which reflects the
221 proportion of input gene-tree quartets consistent with the species-tree, is 0.63, indicating a
222 high degree of gene-tree discordance. Furthermore, the only node in the SNAPP tree with a
223 posterior probability below 1.0 (i.e. 0.99) is subtended by a very short branch (Fig. 4b).
224 Consistent with the high level of gene-tree/species-tree discordance, we also found that the
225 full mtDNA-based phylogeny of *Triturus* produced a highly supported, but topologically
226 different, phylogeny (Supplementary Text S3; Supplementary Fig. S2; Wielstra and Arntzen,
227 2011).

228 Considering an NTV count of 12, as observed in the marbled newts as well as the most
229 closely related newt genera, as the ancestral state for *Triturus* (Arntzen et al., 2015; Veith et
230 al., 2018), three sequential single-vertebral additions to NTV along internal branches, and one
231 or two additions along the terminal branch leading to *T. dobrogicus* (in which NTV = 16 and
232 NTV = 17 occur at approximately equal frequency; Arntzen et al., 2015; Wielstra et al., 2016)
233 are required under a parsimony criterion (with either ACCTRAN or DELTRAN optimization)
234 to explain the present-day variation in NTV observed in *Triturus* (Fig. 3). This is the
235 minimum possible number of inferred changes in NTV count required to explain the NTV

236 radiation observed today (Supplementary Fig. S3; Supplementary Text S5). No NTV
237 deletions or reversals are required, implying a linear, stepwise, single-addition scenario for
238 NTV expansion in *Triturus*.

239

240 **4. Discussion**

241 We use a large, transcriptome-derived phylogenomic dataset to construct a phylogenetic
242 hypothesis and study the evolution of ecological and phenotypic diversity within the adaptive
243 radiation of *Triturus* newts. In contrast to previous attempts to recover a multilocus species-
244 tree (Arntzen et al., 2007; Espregueira Themudo et al., 2009; Wielstra et al., 2014), we
245 recover full phylogenetic resolution with strong support across the tree. Despite cladogenesis
246 having occurred in a relatively brief time window (Fig. 5), resulting in a high degree of gene-
247 tree/species-tree discordance, independent phylogenetic approaches based on data
248 concatenation (RAxML), gene-tree summarization (ASTRAL) and species-tree estimation
249 (SNAPP), all recover the same, highly supported topology for *Triturus* (Fig. 3; Fig. 4). Our
250 *Triturus* case study underscores that sequence capture by target enrichment is a promising
251 approach to resolve the phylogenetic challenges associated with adaptive radiations,
252 particularly for taxa with large and complicated genomes where other genomic approaches
253 are impractical, including salamanders (McCartney-Melstad et al., 2016).

254 Our new phylogenetic hypothesis allows us to place the eco-morphological
255 differentiation shown by *Triturus* into a coherent evolutionary context. Over time, *Triturus*
256 expanded its range of NTV to encompass higher counts (Fig. 3). The *Triturus* tree is
257 consistent with a maximally parsimonious scenario, under which four to five character state
258 changes are required to explain the radiation in NTV observed today. Any other possible
259 phylogenetic relationship among *Triturus* body builds would require a higher number of
260 inferred NTV changes (Supplementary Fig. S3). Three of these inferred changes are

261 positioned along internal branches, of which two are particularly short, suggesting that
262 changes in NTV count can evolve over a relatively short time. The fourth and fifth inferred
263 change are situated on the external branch leading to *T. dobrogicus*, the only *Triturus* species
264 with substantial intraspecific variation in NTV count (Arntzen et al., 2015; Wielstra et al.,
265 2016).

266 Newts annually alternate between an aquatic and a terrestrial habitat, and the
267 functional trade-off between adaptation to life in water or on land likely poses contrasting
268 demands on body build (Fish and Baudinette, 1999; Gillis and Blob, 2001; Gvoždík and van
269 Damme, 2006; Shine and Shetty, 2001). Considering the observed relationship between one
270 additional trunk vertebra and an extra month annually spent in the water (Fig. 1), the
271 extraordinary NTV variation observed in *Triturus* may reflect the morphological mechanism
272 by which more efficient exploitation of a wider range in hydroperiod (i.e. the annual
273 availability of standing water) evolved. Despite the evolvability of NTV count (Arntzen et al.,
274 2015), NTV evolution has been phylogenetically constrained in *Triturus*. Apparently the
275 change in NTV was directional and involved the addition of a single trunk vertebra at a time
276 (Fig. 3; Supplementary Fig. S3). Species with a more derived body build, reflected in a
277 higher NTV, have a relatively prolonged aquatic period and, because species with transitional
278 NTV counts remain extant, the end result is an eco-morphological radiation.

279 *Triturus* newts show a slight degree of intraspecific variation in NTV today. Such
280 variation is partially explained by interspecific hybridization (emphasizing the genetic basis
281 of NTV count; Arntzen et al., 2014), but there is standing variation in NTV count within all
282 *Triturus* species (Slijepčević et al., 2015). This suggests that, during *Triturus* evolution, there
283 has always been intraspecific NTV count polymorphism that could be subjected to natural
284 selection. Whether there is a causal relationship between the directional, parsimonious
285 evolution of higher NTV and the equally parsimonious evolutionary increase in aquatic

286 lifestyle, and, if so, which of these two may be the actual target of selection, remain important
287 open questions. A proper understanding of the functional relationship between NTV, body
288 build and fitness in aquatic/terrestrial environments in *Triturus* is still lacking (Gvođík and
289 van Damme, 2006), and functional studies exploring this fitness landscape across intra and
290 interspecific variation in NTV is an important next step in establishing a firm causal
291 relationship between variation, performance and fitness. The recent availability of the first
292 salamander genomes (Elewa et al., 2017; Nowoshilow et al., 2018; Smith et al., 2018) finally
293 offers the prospect of sequencing the genome of each *Triturus* species and exploring the
294 developmental basis for NTV and its functional consequences in the diversification of the
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296

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315

316 **Data availability**

317 Raw sequence read data for the sequence capture libraries of the 23 *Triturus* samples and the
318 12 transcriptome libraries are available at SRA (PRJNA498336). Transcriptome assemblies,
319 genotype calls (VCF) for the 21- and 23-sample datasets, input files for the RAxML,
320 ASTRAL and SNAPP analyses, and synthesized target sequences are available at Zenodo
321 (<https://doi.org/10.5281/zenodo.1470914>). Supplementary data associated with this article
322 can be found, in the online version, at [\[xxx\]](#)

323

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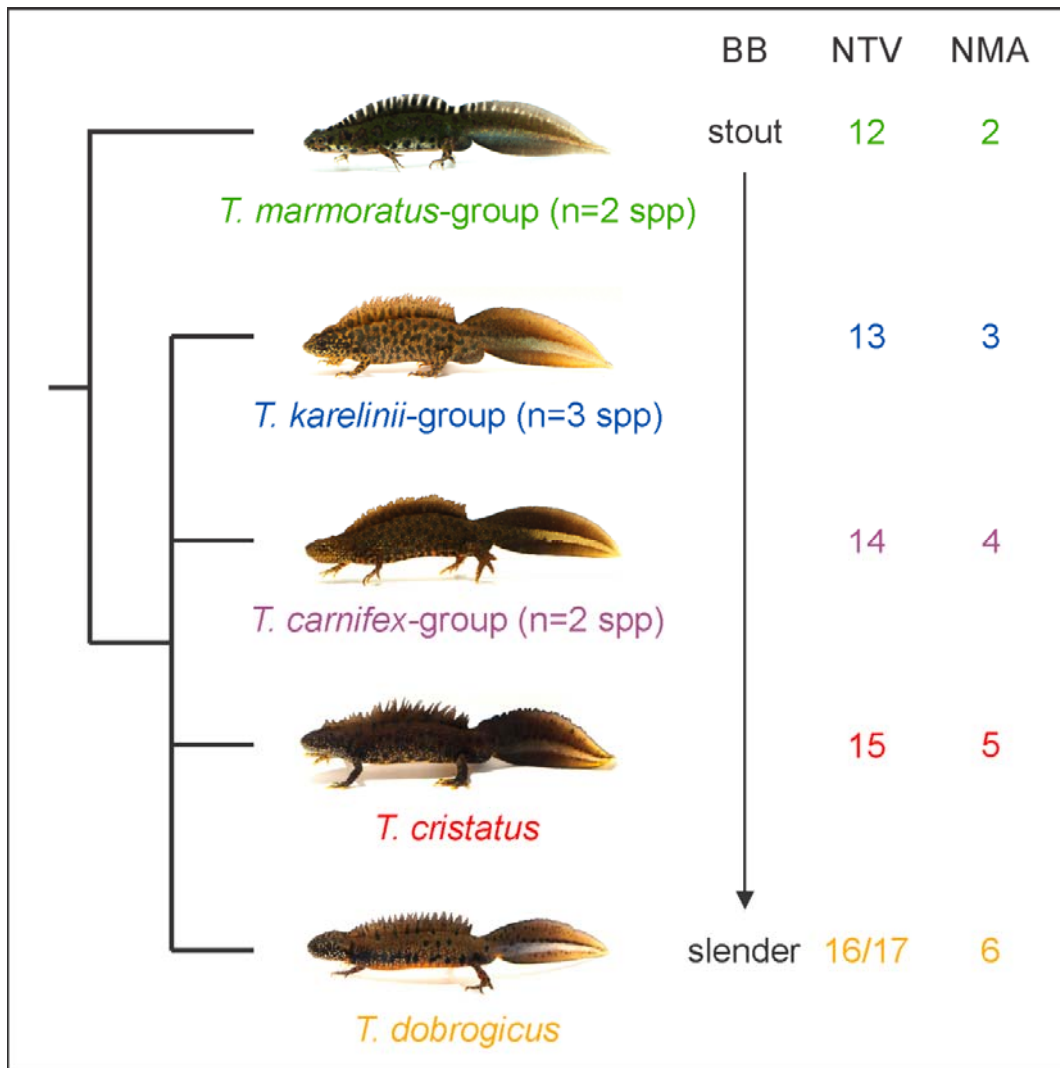
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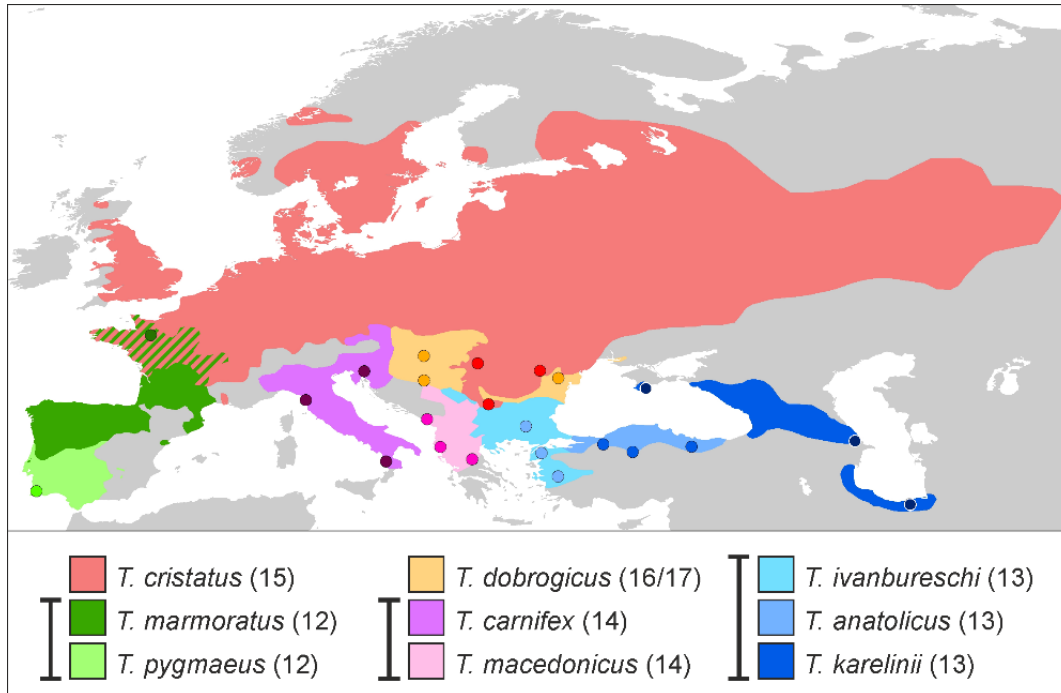
590 **Figures**

591



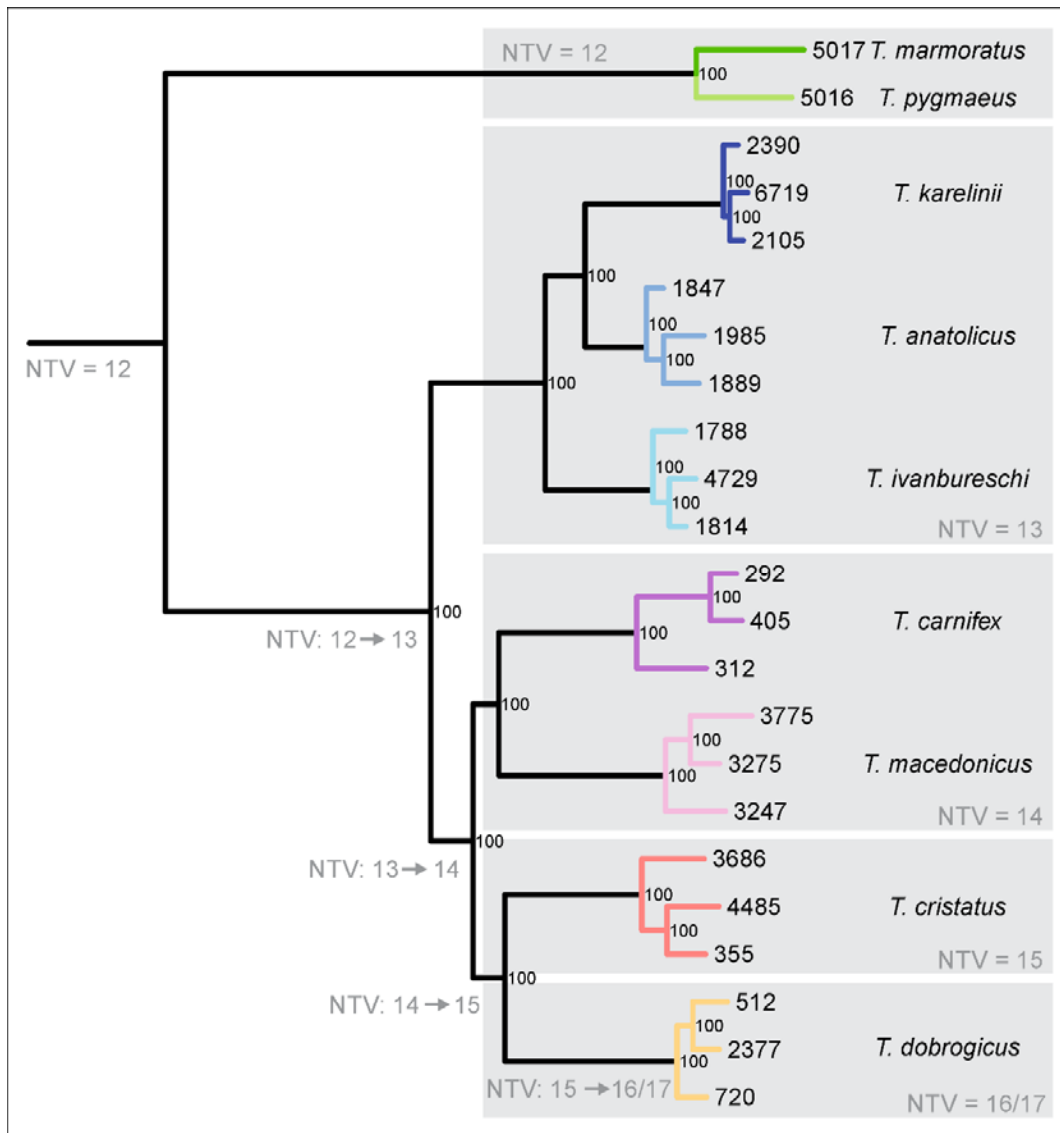
592

593 **Fig. 1. The adaptive radiation of *Triturus* newts.** Five body builds (BB) from stout to
 594 slender are observed in *Triturus* that are also characterized by an increasing number of trunk
 595 vertebrae (NTV) and number of annual aquatic months (NMA). The marbled newts (*T.*
 596 *marmoratus*-group) and crested newts (remaining four BBs) are sister clades. Relationships
 597 among the crested newts are not yet resolved and are the main focus of the present study.



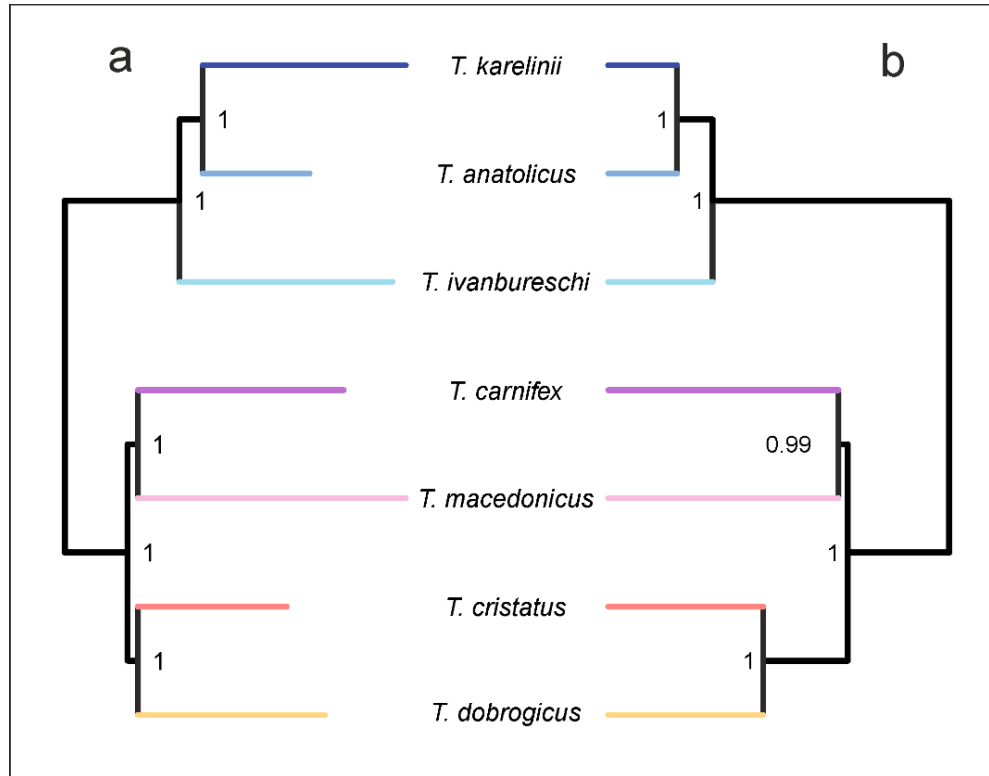
598

599 **Fig. 2. Distribution and sampling scheme for *Triturus*.** Dots represent sample localities
600 (details in Supplementary Table S1). For the marbled newts (in green) a single individual is
601 sampled for each of the two species and for the crested newts (other colours) three
602 individuals are sampled for all seven species. The number in parentheses reflects each species'
603 characteristic number of trunk vertebrae and whiskers link species that possess the same body
604 build (see Fig. 1).



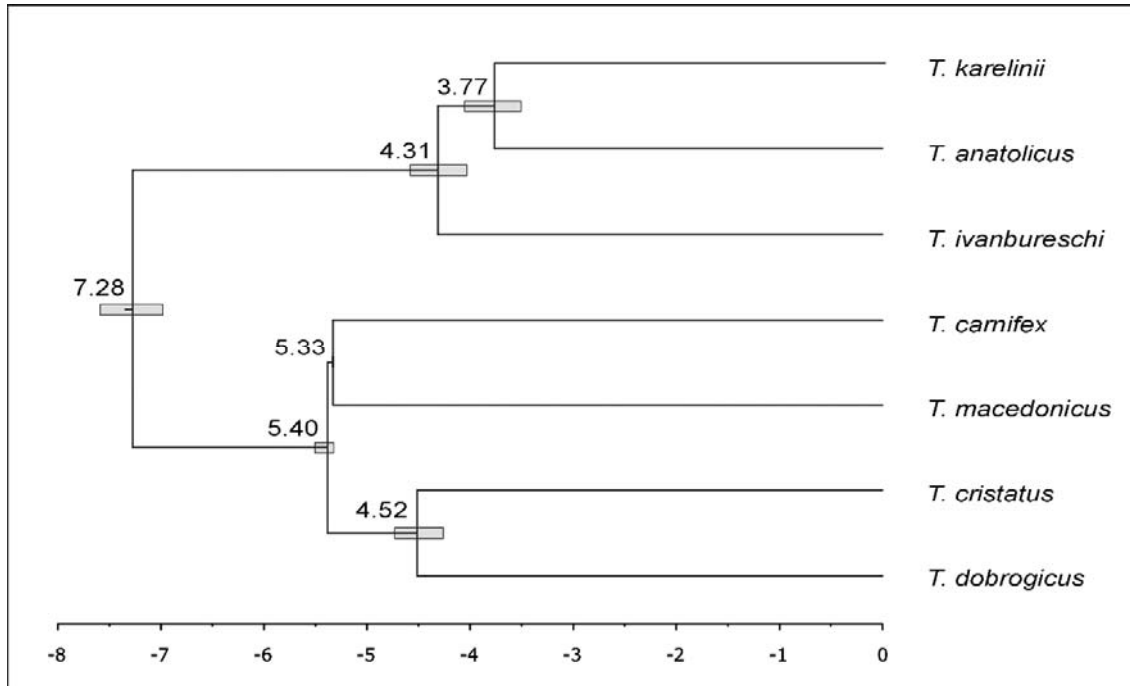
605

606 **Fig. 3. *Triturus* newt phylogeny based on data concatenation with RAxML.** This
 607 maximum likelihood phylogeny is based on 133,601 SNPs derived from 5,866 nuclear
 608 markers. Numbers at nodes indicate bootstrap support from 100 rapid bootstrap replicates.
 609 The five *Triturus* body builds (see Fig. 1) are delineated by grey boxes, with their
 610 characteristic number of trunk vertebrae (NTV) noted. Inferred changes in NTV under the
 611 parsimony criterion are noted along branches. Colours reflect species and correspond to Fig.
 612 2. Tip labels correspond to Supplementary Table S1.



613

614 **Fig. 4. Crested newt phylogeny based on gene-tree summary with ASTRAL and species-**
615 **tree estimation with SNAPP.** The ASTRAL tree (a) is based on 5,610 gene-trees. Numbers
616 at nodes indicate local quartet support posterior probabilities. The SNAPP tree (b) is based on
617 single biallelic SNPs taken from 5,581 nuclear markers. Numbers at nodes indicate posterior
618 probabilities. Colours reflect species and correspond to Fig. 2. Note that both topologies are
619 identical to the phylogeny based on data concatenation (Fig. 3).



620

621 **Fig. 5. Dated species-tree for the crested newts.** Divergence times were determined with

622 SNAPP, using a single *T. carnifex*–*T. macedonicus* inferred split date of 5.33 million years

623 ago as a calibration point. Numbers at nodes reflect median divergence times in millions of

624 years ago and bars the 95% credibility interval around the median.