

Developmental sequences of squamate reptiles are taxon specific

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SUMMARY Recent studies in comparative vertebrate embryology have focused on two related questions. One concerns the existence of a phylotypic period, or indeed any period, during development in which sequence variation among taxa is constrained. The second question concerns the degree to which developmental characters exhibit a phylogenetic signal. These questions are important because they underpin attempts to understand the evolution of developmental characters and their links to adult morphology. To address these questions, we compared the sequence of developmental events spanning the so-called phylotypic period of vertebrate development in squamate reptiles (lizards and snakes), from the formation of the primary optic placode to the first appearance of scale anlagen. We used Bayesian phylogenetic ancestral state reconstruction analyses and estimates of Bayesian posterior probabilities of the rank order of developmental events to determine the level of support for phylogenetically associated variation in development. We assessed the amount of variation in event sequences by

plotting the proportions of reconstructed ranks (excluding unlikely events, $PP < 0.05$) associated with each event. Sequence variability was the lowest towards the middle of the phylotypic period and involved three events (allantois contacts chorion, maximum number of pharyngeal slits, and appearance of the apical epidermal ridge [AER]); these events each had only two reconstructed ranks. Squamate clades also differed in the rank order of developmental events. Of the 20 events in our analyses, 12 had strongly supported ($PP \geq 0.95$) sequence ranks that differed at two or more internal nodes of the tree. For example, gekkotans are distinguished by the late appearance of the allantois bud compared to all other squamates (ranks 7 and 8 vs. rank 3, respectively) and Serpentes are distinguished by the earlier completion of torsion (rank 3) compared to acrodonts and pleurodonts (ranks 7 and 5, respectively). Clade specific sequences of developmental events mean that investigators should not extend observations on the development on particular squamate species to distantly related taxa for use in comparative studies.

INTRODUCTION

Recent studies on the evolutionary developmental biology of vertebrates have focused on two different, but ultimately related questions. One question concerns whether the magnitude of variability in the sequence of events among taxa changes during development. For example, von Baer's Law posits that variability should increase through time because early changes are more likely to destabilize development than later changes (Arthur 2004; Poe 2006). In contrast, the hourglass model posits that sequence variability is the highest early and late in development with an intermediate period of low variability associated with the so-called phylotypic period, the time in development when basic body plan characteristics such as the heart, pharyngeal arches, tail and limb buds appear (Raff 1996; Hall 1997; Bininda-Emonds et al. 2003). The argument for a conserved phylotypic period is that the developing embryo should be particularly resistant to changes in the sequence of developmental events when the most fundamental components of the body plan are formed. The existence of a phylotypic period, or indeed any period during development in which

sequence variation among taxa is constrained, has received mixed support (e.g., Richardson et al. 1997; Poe 2006; Prud'homme and Gompel 2010). Such mixed results are perhaps not surprising given that studies include a diversity of analytical approaches, use data from different segments of the embryonic period, and examine variation at taxonomic scales that range from species (e.g., zebrafish, mice) to vertebrates as a whole. Nonetheless, these studies provide evidence for both variability in the patterns of gene expression and in the sequence of morphological events during development at the species and higher taxonomic levels.

The second question concerns the degree to which developmental characters exhibit a phylogenetic signal. This question underpins attempts to understand the evolution of developmental characters and their links to adult morphology (Richardson et al. 2009; Koyabu et al. 2011; Sanger et al. 2012). It also is critical to comparative analyses that typically rely on developmental data of a limited subset of species in each included taxon. Thus, if the data are not representative of a taxon, comparative analyses are fundamentally flawed. For example, using one species to represent reptiles for comparative analyses

of vertebrate development (e.g., Richardson 1995) assumes conserved developmental patterns within the group. A general issue for comparative studies is the taxonomic level (genus, family, and so forth) for which developmental patterns described for one species are representative. To fully address these issues, analyses must be explicitly phylogeny. For example, mapping data on sequences of developmental events on a phylogeny provides information on a putative ancestral sequence for a designated group, on when changes occur throughout the phylogeny, and establishes the basis for hypothesis testing that can target particular groups or evolutionary phenomena.

Here, we integrate data on the sequence of developmental events and phylogeny to make the first assessment of the evolution of heterochronic changes in development (sensu Webster and Zelditch 2005) in a major group of vertebrates, the squamate reptiles (lizards and snakes). Squamates are the largest and most diverse group of non-avian reptiles (Pough et al. 2004), whose origin dates back 245 million years (Shedlock and Edwards 2009). Evolutionary relationships are well defined (see Materials and Methods Section). They are thus an appropriate model taxon to examine diversification in developmental sequences. We focused on development during the so-called phylotypic period for three reasons. First, early developmental events are known for relatively few species and later events (e.g., relative body, limb, and limb size, formation of scales and pigmentation) are likely to represent adaptation at relatively low taxonomic levels (e.g., species, genera). Second, the phylotypic period is at least putatively conserved, and should thus provide the most opportunities to detect taxon-specific differences in timing of developmental events. Third, the majority of experimental and comparative studies on squamates encompass the phylotypic period. Oviposition occurs when embryos are at phylotypic stages (Andrews and Mathies 2000), and experimental studies are typically initiated at oviposition. Comparative studies on the early development of organs such as limbs involve events of the phylotypic period (Cohn and Tickle 1999; Sanger et al. 2008; Noro et al. 2009). The phylotypic period therefore is an appropriate target for comparative studies on heterochronic change in developmental sequences.

Our objectives are to address fundamental questions about the amount of sequence variation during development and among taxa, provide preliminary insights into the evolution of developmental sequences in squamates and thereby assess the potential for more targeted analyses of character evolution. To meet these objectives, we detect shifts in developmental sequence rank across the squamate tree using Bayesian phylogenetic ancestral state reconstruction analyses. Our analytical method has the advantage of incorporating phylogenetic information, error phylogenetic reconstruction (branch length error, in this study), and most importantly, estimates Bayesian posterior probabilities of the reconstructed state—variables not completely accounted for in previous analyses of

developmental evolution. Therefore, if different sequence ranks of the same character are estimated in at least two clades with strong statistical support (Bayesian posterior probability ≥ 0.95), we can confidently infer that a shift in developmental sequence has occurred. Moreover, because we have estimated the posterior probability of every rank at every node of the tree, we can evaluate which developmental event ranks are the most variable across the sampled squamate taxa.

MATERIALS AND METHODS

Data sources and ranking of developmental events

Observations on squamate development were collected from the primary literature and from unpublished studies (Table 1). We selected developmental events that are commonly recorded in developmental studies on squamates. These events correspond to stages 23–36 in Dufaure and Hubert's (1961) normal table ("DH" hereafter) for the lizard *Zootoca (Lacerta) vivipara* and thus roughly the phylotypic period in amniote development. The 20 events selected ranged from the first appearance of the primary optic vesicle to the first appearance of scale anlagen (Table 2, Fig. 1). Events were ordered sequentially for each species and each event was assigned a sequence rank from 1 to 20 (or a parallel coded rank of "a" to "t" for phylogenetic analyses; Fig. 2). The rank of an event was based on its first appearance in development, not presence or absence. Events were treated as characters and ranks as character states (Poe and Wake 2004). If two or more events occurred at the same time, ranks were assigned multiple states (e.g., a/b). For species with partial sequences, ranks were assigned starting at 1 or "a" (if only the first part of the sequence was available) or at 20 or "t" (working backwards from the last event if only the latter part of the sequence was available). To account for absence of limb states for snakes, the mean ranks corresponding to limb events in the data set as a whole (j, l, n, and r) were skipped (i.e., coded as missing) in the snake sequences. *Anolis sagrei*, *Calotes versicolor*, *Calyptommatus sinebrachiatus*, *Nothobachia ablephara*, and *Pogona vitticeps* were missing data for one event each; the corresponding mean ranks were skipped in these cases as well.

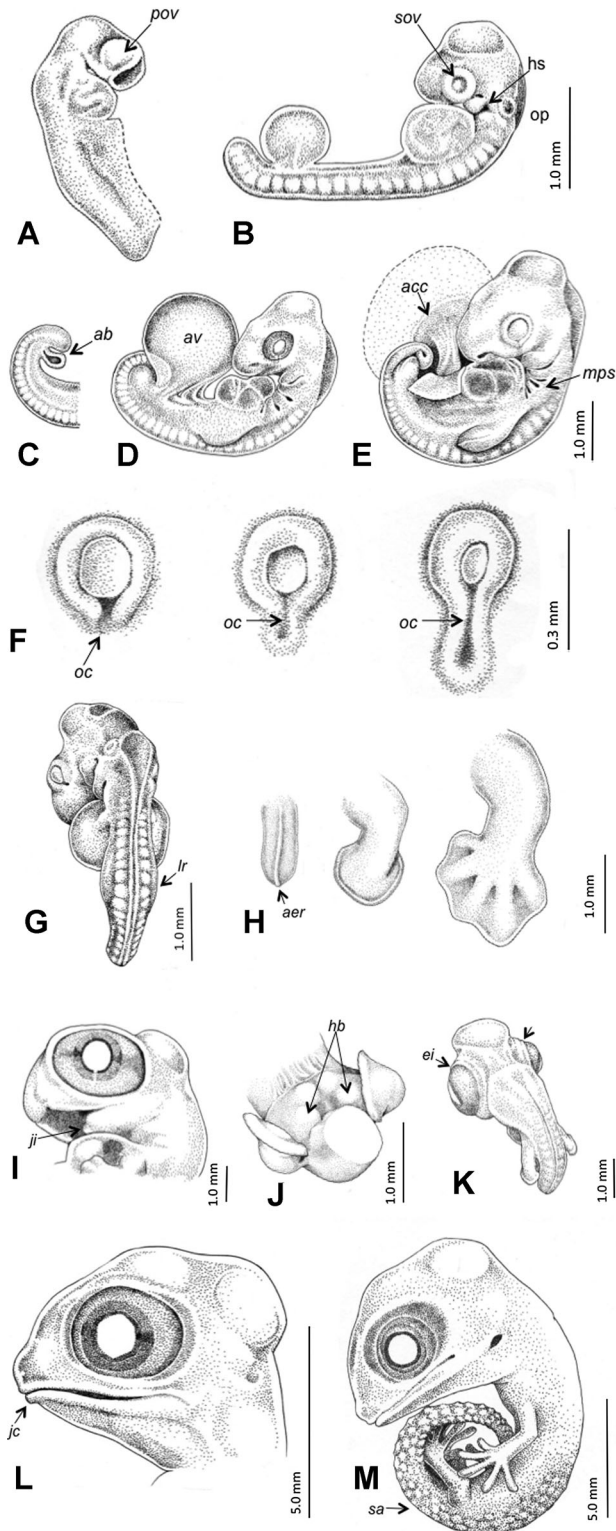
Our character scoring strategy may introduce two types of errors. In general, errors result from imposing absolute criterion for first appearance of a developmental event on what is a continuous process (Love 2010). As criteria for recognizing events are investigator specific, exact scoring may vary among studies. This is an unavoidable problem associated with any meta-analysis. Errors in scoring also concern sample size per se; when the embryos examined are too few to comprise a relatively continuous set of specimens, events may be scored as simultaneous when they not. Both these types of errors are confounded by intraspecific (i.e., individual) variation in the

Table 1. Species and sources of information on developmental sequences. Species used to construct phylogenies using molecular sequences and their GenBank numbers are shown in parentheses (see text for details)

Family	Species	Source
Agamidae	<i>Calotes versicolor</i> (<i>Calotes calotes</i> , AY662584)	Thapliyal et al. (1973) and Muthukkaruppan et al. (1973)
Agamidae	<i>Agama impalearis</i> (<i>Phrynocephalus raddei</i> , AY662586)	El Mouden et al. (2001)
Agamidae	<i>Pogona vitticeps</i> (<i>Physignathus cocincinus</i> , AY662582)	Andrews (unpubl. data)
Chameleonidae	<i>Furcifer lateralis</i> (<i>Furcifer lateralis</i> , AFA43856)	Blanc (1974)
Chameleonidae	<i>Chamaeleo calytratus</i> (<i>Chamaeleo rudis</i> , AY662578)	Andrews et al. (2008) and Andrews (unpubl. data)
Colubridae	<i>Thamnophis sirtalis</i> (<i>Dinodon</i> sp., AY662611)	Zehr (1962)
Diplodactylidae	<i>Oedura lesueurii</i> (<i>Pseudothecadactylus lindneri</i> , AY662626)	Andrews (unpubl. data)
Diplodactylidae	<i>Strophurus williamsi</i> (<i>Strophurus elderi</i> , JQ173763)	Andrews (unpubl. data)
Eublepharidae	<i>Eublepharis macularius</i> (<i>Eublepharis turcmenicus</i> , AY662622)	Andrews (unpubl. data)
Gekkonidae	<i>Chondrodactylus turneri</i> (<i>Gekko gekko</i> , AY662625)	Andrews (unpubl. data)
Gekkonidae	<i>Gehyra variegata</i> (<i>Gehyra variegata</i> , FJ855439)	Andrews (unpubl. data)
Gymnophthalmidae	<i>Nothobachia ablephara</i> (<i>Colobosaura modesta</i> , EU108527)	Roscito and Rodrigues (2012)
Gymnophthalmidae	<i>Calyptommatius sinebrachiatus</i> (<i>Gymnophthalmus underwoodi</i> , AY487364)	Roscito and Rodrigues (2012)
Lacertidae	<i>Zootoca (Lacerta) vivipara</i> (<i>Eremias</i> sp., AY662615)	Dufaure and Hubert (1961)
Liolaemidae	<i>Liolaemus gravenhorstii</i> (<i>Liolaemus pictus</i> , AY662595)	Lemus (1967)
Liolaemidae	<i>Liolaemus tenuis</i> (<i>Liolaemus lineomaculatus</i> , FJ356740)	Lemus and Duvauchelle (1966) and Lemus et al. (1981)
Phrynosomatidae	<i>Uta stansburiana</i> (<i>Phrynosoma mcallii</i> , AY662590)	Andrews and Greene (2011)
Polychrotidae	<i>Anolis sagrei</i> (<i>Anolis sagrei</i> , JN112657)	Sanger et al. (2008)
Scincidae	<i>Mabuya</i> sp. (<i>Trachylepis perrotetii</i> , HM161160)	Ramirez-Pinilla (unpubl. data)
Varanidae	<i>Varanus rosenbergi</i> (<i>Varanus rosenbergi</i> , AY662608)	Andrews (unpubl. data)
Viperidae	<i>Vipera aspis</i> (<i>Gloydus halys</i> , AY662614)	Hubert and Dufaure (1968)

Table 2. Developmental events scored listed in order of approximate DH stages and illustrated in Figure 1. Event numbers are arbitrary within DH stages and identical to those in Figures 2 and 4

Developmental event	DH stages	Figure panel	Event no.
Primary optic vesicle	23	A	1
Otic placode	25	B	2
Allantois bud (small thick-walled out-pouching)	25	C	3
Torsion complete	25	B	4
Secondary optic vesicle	25	B	5
Hyomandibular slit	25	B	6
Allantois vesicle (thin-walled bag)	–	B	7
Choroid fissure open (horse-shoe shaped)	27	F	8
Limb ridge	27	G	9
Allantois contacts chorion (allantois flattened above embryo like umbrella)	–	E	10
Maximum pharyngeal slits	30	E	11
Limb AER	30	H	12
Hemipenial buds form on cloacal lip	31	J	13
Three-segmented limb (autopodium, zeugopodium, and stylopodium)	32	H	14
Jaw initiated	33	I	15
Eyelid forms as a thin ribbon-like sheet of tissue overlapping the eyeball	33	K	16
Pharyngeal slits closed	–	I	17
Digits differentiated in limb paddle	34	H	18
Jaw complete; mandible meets tip of maxilla	35	L	19
Scale anlagen visible	36	M	20



relative time of appearance of developmental events (Andrews and Greene 2011). These sources of variation, however, should affect sequences largely with regard to the position of adjacent events. They thus introduce “random” errors that reduce the ability to infer ancestral ranks for events with high posterior probability rather than to systematically bias results.

Phylogenetic analyses

To interpret differences in developmental timing in a phylogenetic context, we conducted phylogenetic analyses of the nuclear recombination activation gene 1 (RAG1) and subsequently estimated reconstructions of ancestral character states (developmental sequence ranks). We chose RAG1 for phylogenetic analyses because the typically sequenced fragment is long (~2800 bp), and therefore provides many potential phylogenetically informative characters, and it has been used to infer strongly supported phylogenies across squamates (e.g., Townsend et al. 2004; Hugall et al. 2007; Brandley et al. 2011, 2012). We did not utilize the extensive mitochondrial DNA sequences available for squamates because of the difficulty of mtDNA to reconstruct “deep” lineages (Brandley et al. 2011). RAG1 data were downloaded from GenBank. Data were not available for most species used in our developmental analyses, and in these situations, we used RAG1 data for a closely related species or representative of the same lineage (Table 1). There exist comparatively few RAG1 sequences for gymnophthalmid lizards and none for the two species with developmental data, *N. ablephara* and *C. sinebrachiatus*. Instead, we used molecular data for *Gymnophthalmus underwoodi* and *Colobosaura modesta*. These two species share a common ancestor that is older than *N. ablephara* and *C. sinebrachiatus* (Castoe et al. 2004), although we expect this to have a trivial effect on

Fig. 1. Illustrations of developmental events used for sequence analyses. Event, species, and nominal DH stage of the embryo are shown. (A) Primary optic vesicle, *Cc*, stage 22. (B) Allantois vesicle, secondary optic vesicle, hyomandibular slit, otic placode, *Us*, stage 25. (C) Allantois bud, *Ct*, stage 24.5. (D) Allantois vesicle, *Ct*, stage 28.5. (E) Allantois contacts chorion, maximum (number of) pharyngeal slits, *Em*, stage 30. (F) Choroid fissure open, from left to right *Ct*, *Em*, *Us*, stages 27, 26, 28, respectively. (G) Limb ridge, *Em*, stage 27. (H) Limb states, from left to right, AER, three-segmented, five-digits differentiated, *Em*, stages 30, 33, 34. (I) Jaw initiated, pharyngeal slits closed, *Ct*, stage 32. (J) Hemipenial buds, *Ct*, stage 33. (K) Eyelid initiated, *Ct*, stage 32. (L) Jaw completed, *Ct*, stage 35, and (M) scale anlagen, *Ct*, stage 35.5. Species: *Chamaeleo calypttratus*, *Cc*; *Chondrodactylus turneri*, *Ct*; *Eublepharis macularius*, *Em*; *Uta stansburiana*, *Us*. Abbreviations: allantois bud, ab; allantois vesicle, av; apical epidermal ridge, aer; allantois contacts chorion, acc; eyelid initiated, ei; hemipenial bud, hb; hyomandibular slit, hs; jaw initiated, ji; jaw complete, jc; limb ridge, lr; open choroid fissure, oc; primary optic vesicle, pov; otic placode, op; maximum pharyngeal slits, mps; secondary optic vesicle, sov; scale anlagen, sa.

		Ranked sequence of events																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>Strophurus williamsi</i>												11	12	13	15	14	16	17	18	19	20
<i>Oedura lesueurii</i>										10	11	12	13	14	16	15	17	18	19	20	
<i>Eublepharis macularius</i>						3	8	7	9	10	11	12	13	14	16	17	15	18	19	20	
<i>Chondrodactylus turneri</i>						3	8	7	9	10	11	12	13	16	14	15	17	18	19	20	
<i>Gehyra variegata</i>	1	2	4	6	5	3	8	7	9	10	11	12	15	13	17	14	16	18	19	20	
<i>Mabuya</i> sp.	1	2	3	5	7	4	6	10	8	9	11	12	13	14	15	16	17	18	19	20	
<i>Zootoca (Lacerta) vivipara</i>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
<i>Calyptommatus sinebrachiatus</i>								8	9	md	11	12	15	17	13	14	16	18	19	20	
<i>Nothobachia ablephara</i>												12	11	15	13	14	17	md	18	19	20
<i>Thamnophis sirtalis</i>	1	3	4	2	7	6	5	8	10	D	11	D	13	17	D	15	16	D	19	20	
<i>Vipera aspis</i>	1	2	4	6	3	5	7	8	10	D	11	D	13	D	15	16	17	D	19	20	
<i>Varanus rosenbergi</i>											11	8	12	13	16	15	14	17	18	19	20
<i>Uta stansburiana</i>	1	2	3	7	6	4	5	8	10	9	11	12									
<i>Anolis sagrei</i>												11	15	12	md	17	14	16	18	19	20
<i>Liolaemus gravenhorstii</i>	1	3	5	2	8	4	6	7	11	10	9	12	13	17	14	15	16	18	19	20	
<i>Liolaemus tenuis</i>	1	3	5	2	4	8	7	6	10	9	11	12	17	13	14	15	16	18	19	20	
<i>Chamaeleo calytratus</i>	1	3	4	5	7	2	6	8	9	10	11	12	13	14	15	16	17	18	19	20	
<i>Furcifer lateralis</i>	1	2	3	5	6	4	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
<i>Pogona vitticeps</i>									9	md	11	12	13	14	15	16	17	18	19	20	
<i>Calotes versicolor</i>	1	md	3	7	5	6	4	8	10	9	11	12	13	14	15	17	16	18	19	20	
<i>Agama impalearis</i>	1	2	3	6	4	5	7	8	9	10	11	12	13	14	16	17	15	18	19	20	

Fig. 2. Ranked sequence of events for 21 species of squamate reptiles. Ranks from left (1) to right (20) represent the order of appearance of events in development for each species. Numbers in data cells correspond to event numbers in Table 2. Cells without numbers indicate the section of the development sequence that was not scored. Cells with two or more events represent ties. Rank placement of an event that is not expressed (i.e., limbs of snakes) and for which data are missing are indicated in cells by “D” and “md,” respectively.

the results as the posterior distribution of the length of branch joining these two species is large, effectively including very shallow and deep branch lengths (Fig. 3).

Because the phylogeny of squamates has been inferred previously using much more extensive molecular data sets (Townsend et al. 2004; Vidal and Hedges 2005; Hugall et al. 2007; Wiens et al. 2012), our intention was not to re-estimate the branching relationships of the species used in the current study, but rather to estimate branch lengths for the tree used for our subsequent ancestral state reconstruction analyses. We therefore constructed a rooted cladogram of our sampled species (Fig. 3) that is congruent with the combined data maximum likelihood tree of Townsend et al. (2004, their Fig. 7B) and the analysis of iguanians of Townsend et al. (2011), and used this rooted cladogram as a constraint tree in our phylogenetic analyses (i.e., we conducted no tree swapping). Because the interrelationships of varanids, iguanians, and snakes are not strongly supported in any recent molecular analysis (Townsend et al. 2004; Vidal and Hedges 2005; Hugall et al. 2007; Wiens et al. 2010, 2012), we conducted additional analyses using modified constraint trees that place varanids as sister to snakes

and snakes sister to iguanians, for a total of three tree topologies. Furthermore, raw molecular branch lengths do not necessarily represent accurate estimates of the age of a group or rate of evolution (Dornburg et al. 2012); because our subsequent inferences of ancestral states are dependent on the branch lengths as well as the phylogenetic relationships, we instead used an uncorrelated relaxed clock model to accommodate rate heterogeneity amongst lineages (Drummond et al. 2006). Although these branch lengths do not represent absolute molecular ages (we could not estimate these given the taxon sampling relative to the squamate fossil record), they nonetheless provide estimates of relative branching times.

Phylogenetic analyses were conducted using BEAST v1.7.5 (Drummond and Rambaut 2007) holding one of three trees constant (see above), and employing an uncorrelated lognormal relaxed molecular clock, separate GTR + G models evolution for each of the three RAG1 codon positions, the program’s default prior distributions of model parameters, and with the age of the root of the phylogeny set to 1.0. Four analyses were run for 10⁷ generations for each of the three constraint trees. We determined convergence of the four analyses when the log-

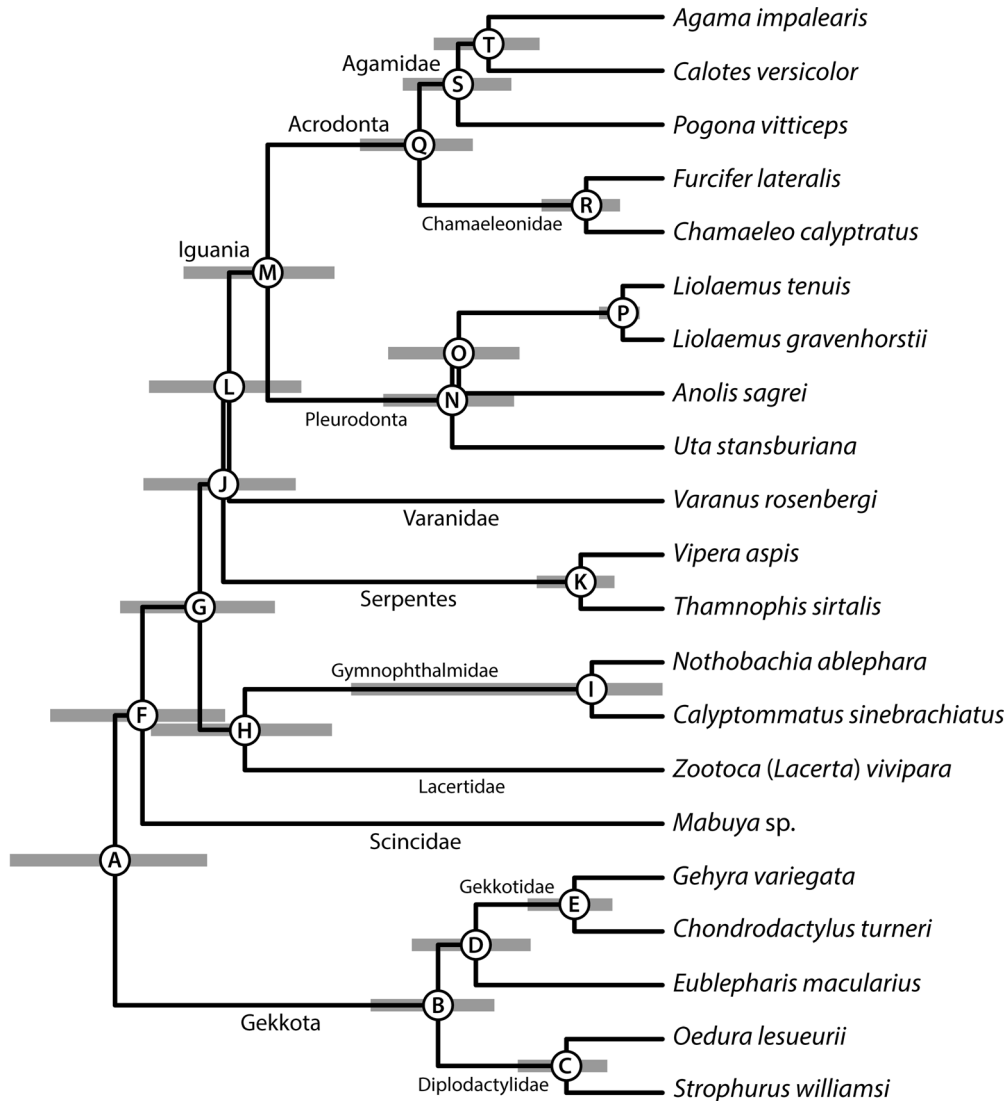


Fig. 3. The consensus phylogeny of the 21 species of squamates in this study inferred by Bayesian phylogenetic analyses of the nuclear RAG1 gene. The branching relationships fixed during the analysis conform to multiple recent phylogenetic analyses of squamate reptiles, but branch lengths and other model parameters were free to vary. Branch lengths represent relative branching times calculated using a lognormal uncorrelated relaxed clock model with the root age set to 1.0, and 95% credible intervals of branch lengths are represented by gray bars. Nodes labeled with capital letters refer to clades referenced in Table 3. Taxa shown are those from which we obtained developmental data, however species used in the phylogenetic analysis may differ (see Table 1).

likelihood values stabilized over time. The end result of these analyses produces a probability distribution of branch lengths given the tree and models of evolution. Our ancestral state reconstruction analyses (below) therefore incorporate variation in branch lengths as opposed to using a single, point estimate of phylogeny and branch lengths (e.g., the maximum likelihood tree).

Ancestral state reconstruction

To identify shifts in developmental sequence of each character, we estimated the probability of each character state at every node

of 1000 phylogenies sampled from the posterior distribution of phylogenies using the maximum likelihood function and Brownian motion model in BayesTraits v1.0 (Pagel et al. 2004). This distribution of 1000 reconstructed character states represents an estimate of the posterior probability distribution (PP) of each ancestral state. To evaluate whether shifts in developmental sequence have occurred, we assessed whether different states were reconstructed for two or more nodes with a PP > 0.95 (our criterion for “strong” Bayesian support). In some cases, we combine posterior probabilities of adjacent states if they sum to ≥ 0.95 and differ from any other strongly supported state (e.g., state 4 vs. states 2 and 3).

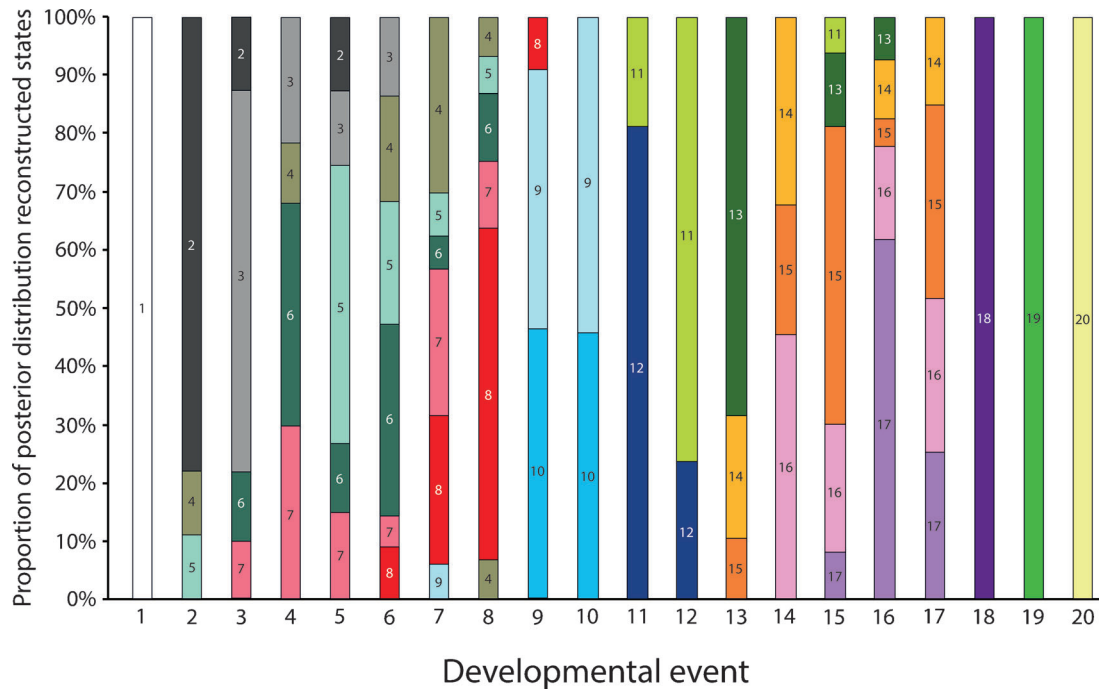


Fig. 4. The relative proportion of ranks present in the posterior distribution of phylogenetically reconstructed event ranks across the squamate phylogeny. Colors and numbers in columns represent event ranks. To mitigate the effects of missing data, only reconstructed ranks with a posterior probability ≥ 0.05 are shown (see text).

To assess the variation of rank (state) of each event (character), we developed a method that has the advantage of using phylogenetic information to resolve ambiguities of multistate events in extant taxa. This method calculates the relative proportion that each event rank was present in the posterior distribution of reconstructed ancestral event ranks for every node of the tree. Our justification for this analysis is that events with high variance among taxa should be represented by more reconstructed ranks across the squamate phylogeny than events with low variance (Fig. 4). This methodology has a drawback in that missing data introduces variation of reconstructed ranks. To account for this, we only included reconstructed ranks per node that were present in more than 5% of sampled trees (i.e., we ignored reconstructed event ranks with a posterior probability ≤ 0.05).

RESULTS

Developmental sequences were obtained for 21 species representing 14 families of squamates (Table 1). The mean number of events scored for each species was 15.5 with a range of 9–20 (Fig. 2). One event was invariable, the primary optic placode preceded all other events, and another event, scale anlagen, was the last (20th) event for all but two species (*Eublepharis macularius* and *Liolaemus tenuis*) for which jaw completion and scale anlagen occurred simultaneously. Apart

from these two events, the sequence in which events occurred varied substantially among species as illustrated by the wide range in ranks scored for most events. For example, the earliest (tied) rank for torsion completion was 2.5 (*Thamnophis sirtalis*) and the latest (tied) rank was 7.5 (*Furcifer lateralis*) among the 12 species scored for this event. Formation of the apical epidermal ridge (AER) was among the least variable event; the earliest (tied) rank was 11.5 (several species) and the latest rank was 13 (*A. sagrei*) among the 15 species scored.

All Bayesian phylogenetic analyses using tree constraints achieved stationarity within 2 million generations. The remaining post “burn-in” trees represent the estimated posterior probability distribution of branch lengths. There were no strongly supported differences amongst event rank reconstructions between the three constraint trees. This is unsurprising because the estimated internal branch lengths leading to these different clades in the three constraint trees are extremely short (functionally, a “hard” polytomy); a pattern seen in other phylogenetic analyses of these lineages (e.g., Townsend et al. 2004; Vidal and Hedges 2005). For the remainder of the paper, we restrict discussion to the tree shown in Figure 3.

We assessed amount of variation in event sequences by plotting the proportions of reconstructed ranks (excluding unlikely events, $PP < 0.05$) associated with each event (Fig. 4). For example, an event with six reconstructed ranks would be highly variable in its rank occurrence across taxa, and an event with only one reconstructed event would occur at the

same rank for all taxa. We found that the magnitude of variation fluctuated through development/time (apart from the low variance of the first and last few events which is, at least in part, an artifact of not having preceding or following events, respectively). The eight events that followed the primary optic vesicle were particularly variable in sequence, as judged by a high number (3–6) of reconstructed ranks associated with each event. In contrast, sequence variability of the following set of three events (allantois contacts chorion, maximum pharyngeal slits, and AER) was low; these events had only two reconstructed ranks. The next five events (hemipenial buds, three segmented limb, jaw initiated, eyelid forms, and pharyngeal slits closed) exhibited increased variability with 3–5 reconstructed ranks per event. Finally, the last three events (five digits in limb paddle,

jaw complete, and scale anlagen) were invariable in sequence with one reconstructed rank each.

We used rooted phylogenies for our ancestral state reconstruction analyses although we could not include the closest related non-squamate outgroup (*Sphenodon*) due to lack of developmental data. As a result of this, and variation or ambiguity of ranks scored for the tip taxa, many of the reconstructed ranks at the root and “backbone” nodes are not supported by a $PP \geq 0.95$ (Appendix). This limits our ability to track stepwise evolution of event ranks across the tree. Nonetheless, of the 20 developmental events in our analyses, twelve had strongly supported ($PP \geq 0.95$) sequence ranks that differed at two or more internal nodes of the tree (Table 3, Fig. 3). Gekkotans were distinguished by the rank order of four events:

Table 3. Reconstructed events that occur at different times in development across the squamate phylogeny (Fig. 3) as assessed by ancestral state reconstruction analyses. Ranks represent the numerical position of the event in a sequence of 20 events. Posterior probabilities represent the probability of the developmental event rank in the most recent common ancestor of the clade. Clades are judged to have strongly different ranks if the differing reconstructed ranks are both supported with a posterior probability ≥ 0.95

Developmental event (event number)	Node name	Node label	Rank	Posterior probability
Otic Placode (2)	Root	A	2	1.00
	<i>Liolaemus</i>	P	4, 5 (not 2)	1.00
Allantoic bud (3)	Gekkota	B	7, 8 (not 3)	0.96
	Non-gekkotans	F	3	0.98
Torsion complete (4)	Serpentes	K	3	0.98
	Acrodonta	Q	7	0.95
	Pleurodonta	N	5	0.97
Secondary optic vesicle (5)	Serpentes	K	7	0.99
	Acrodonta	Q	5	0.97
	<i>Liolaemus</i>	P	2, 3	1.00
Choroid fissure open (8)	Non-gekkotans, non-skinksnon-skinks	G	8	1.00
	<i>Liolaemus</i>	P	4, 5	1.00
Limb ridge (9)	Root	A	9	1.00
	Iguania	M	10	1.00
	Chamaeleonidae	R	9	0.99
Allantois contacts chorion (10)	Gekkota	B	10	1.00
	Varanidae + Iguania + Serpentes	L	9	1.00
	Chamaeleonidae	O	10	1.00
Maximum slits (11)	Root	A	11	0.97
	Gymnophthalmidae	I	12, 13	1.00
Hemipenial buds (13)	Gekkota	B	14	0.99
	Non-gekkotans	F	13	1.00
	Gymnophthalmidae	I	15	0.99
Three-segmented limb (14)	Gekkota	B	15	0.99
	Acrodonta	Q	14	0.95
	Pleurodonta	N	16	0.95
Jaw initiated (15)	Non-gekkotans	F	15	0.99
	Serpentes	K	16	0.99
Pharyngeal slits closed (17)	Root	A	17	0.95
	Serpentes	K	15	0.99
	Gymnophthalmidae	I	14	0.99
	<i>Liolaemus</i>	P	12–14 (not 17)	0.99
	<i>Agama</i> + <i>Calotes</i>	T	15, 16 (not 17)	1.00

the allantois bud (rank 7 or 8) and hemipenial buds (rank 14), developed later compared to non-gekkotans (ranks 3 and 13, respectively), but earlier than gymnophthalmids (rank 15); the allantois contacts the chorion (rank 10) later than most squamates (rank 9) with the exception of chamaeleonids in which it develops (independently) at the same rank (10); and the three-segmented limb (rank 15) develops later than acrodonts (rank 14), but earlier than pleurodonts (rank 16). Serpentes were distinguished by the rank order of four events; torsion (rank 3) is completed earlier compared to acrodonts and pleurodonts (ranks 7 and 5, respectively); the secondary optic vesicle (rank 7) appears later compared to acrodonts (rank 5) and *Liolaemus* (rank 2 or 3); initiation of the jaw (rank 16) occurs later than the root of gekkotans (rank 15); and the pharyngeal slits (rank 15) close early compared to the root of Squamata (rank 17), but later than gymnophthalmids (rank 14) and *Liolaemus* (ranks 12–14). In addition to the taxa and events listed above, the limb ridge develops earlier in chamaeleonids (rank 9) than other iguanian clades (rank 10) and pharyngeal slits are closed earlier in gymnophthalmid (rank 14), *Liolaemus* (ranks 12–14), and the *Agama + Calotes* clade (ranks 15 and 16) than most other squamate taxa (rank 17). In addition, gymnophthalmids were distinguished by the later appearance of the maximum number of open pharyngeal slits (ranks 12 or 13) than the root of Squamata (rank 11) and *Liolaemus* was distinguished by the earlier appearance of the open choroid fissure (ranks 4 and 5) compared to non-gekkotans and non-skinks (rank 8).

DISCUSSION

Our observations document substantial phylogenetically related variation in the sequence of development events among squamates; of the 20 developmental events in our analyses, twelve had strongly supported ($PP \geq 0.95$) sequence ranks that differed at two or more internal nodes of the tree (Table 3, Fig. 4). These events are distributed throughout the temporal span of events in our study and include a diversity of functional units (i.e., eyes, limbs, allantois, pharynx, hemipenes, etc.). Thus, no particular time in development or type of event provided more resolution than others. Resolution was, however, related to the number of species representing each clade. For example, family-level clades represented by one species each (varanids, lacertids, and scincids), were not associated with any resolved event rank. In contrast, clades represented by two or more species had at least one resolved event rank. Acrodonts, gekkotans, and snakes, for example, represented by five, five, and two species, respectively, had the greatest number (3–4) resolved developmental events (Table 3). These data suggest that our analyses underestimate the full diversity of developmental sequences, a situation that would be remedied by including more squamate representatives in future analyses of developmental patterns.

Our results are pertinent to two important issues of evolutionary developmental biology. The first concerns the question of whether sequence variability is constrained during the phylotypic period of vertebrate development. The second concerns the evolution of developmental sequences and hence the potential to test hypotheses of adaptive change. These issues will be discussed in the following sections. In general, however, our results indicate that comparative developmental biology is more nuanced than previously anticipated.

Evidence for a constrained period in vertebrate development

In contrast to studies using morphological character states to assess sequence variability among taxa (Richardson 1995; Richardson et al. 1997; Poe and Wake 2004; Poe 2006), we observed a conserved developmental sequence within the phylotypic period. For squamates, the lowest variation in the numbers of reconstructed ranks was centered in the middle of the squamate phylotypic period (Fig. 4). These conserved events do not appear to be an artifact of the particular events per se. For example, if these events were developmentally linked, they would have to follow a fixed sequence and hence would appear conserved. This is not the case; these events, contact of the allantois with the chorion, maximum number of open pharyngeal slits, and formation of the AER, are components of independent developmental units. Similarly, if these events were spaced more widely in time than earlier and later events, their rank order

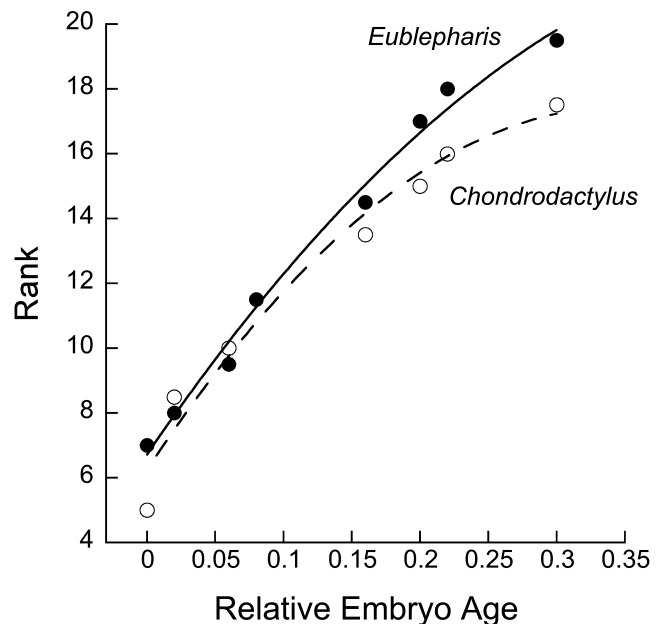


Fig. 5. Rank as a function of relative age (days from oviposition/incubation length) for *Chondrodactylus turneri* (open circles and dashed line) and *Eublepharis macularius* (closed circles and solid line).

would be assigned more accurately than events spaced more closely (see remarks in the Materials and Methods Section). To test this possibility, we plotted the rank assigned to events as a function of the mean age of embryos scored for that event for two gekkotan species (Table 1, Fig. 5). If sequence rank is unrelated to the time interval between events, observed curves should increase monotonically (i.e., fit a linear or quadratic function). If, however, sequence rank is affected by the interval between events, curves should exhibit changes in slope (i.e., cubic function). The former model was supported; the relationship between sequence rank and time interval was best fit by a quadratic function (*Chondrodactylus*: $F_{2,9} = 117.9$, $P < 0.0001$, $R^2 = 0.97$; *Eublepharis*: $F_{2,5} = 258.8$, $P < 0.0001$, $R^2 = 0.99$); coefficients for all terms were significant at $P \leq 0.05$. In contrast, coefficients for cubic terms were not significant (*Chondrodactylus*: $P = 0.28$; *Eublepharis*: $P = 0.18$) for fits to third-order polynomials.

Only analyses of gene expression have supported the hypothesis that the least variability of developmental sequence among vertebrate embryos occurs during the phylotypic period. For example, developmental genes whose origin is associated with ancestral bilaterians are most highly expressed during early development of mice while developmental genes whose origin is associated with ancestral vertebrates, ancestral tetrapods, and ancestral amniotes are the most highly expressed during the phylotypic period (Irie and Sehara-Fujisawa 2007). Similarly, the evolutionarily oldest (most conserved) transcriptome set is associated with the phylotypic period of zebrafish (Domazet-Lošo and Tautz 2010). Both studies indicate that the regulation of development during the middle of the embryonic period is associated with conserved sets of genes. In contrast, patterns of protein–protein interactions and molecular signaling, although variable during development, do not correspond to the phylotypic period of zebrafish and the molecular commonalities between zebrafish and mice were most similar late in development but not specifically during the phylogenetic period (Comte et al. 2010). In the context of the limited current understanding of the linkage between gene regulation and the attendant phenotype of embryos, our results are the first to suggest that conserved periods of development documented at the molecular level may be paralleled by conserved morphologies of developing embryos.

Evolution of developmental sequences

Sequence heterochrony, changes in the relative timing of developmental events, is a pervasive phenomenon among vertebrate taxa (Richardson et al. 1997, 2009, 1997, 2009; Smith 2002; Koyabu et al. 2011). Our study is the first to use both contemporary developmental data and a well-supported phylogeny to assess if, and the extent to which, the relative timing of developmental events is associated with phylogeny. We found that many clades were distinguished by unique developmental

sequences. For example, gekkotans, the sister taxon to all other squamates, snakes, which possess the most derived morphology of all squamates, acrodonts, pleurodonts (including *Liolaemus*, and gymnophthalmids, had a least two events whose rank order differs significantly from that of other clades. Clearly, one or even a few squamate species do not represent the diversity of developmental sequences of this group. This result has an important practical implication—normal tables developed for one species are not applicable to distantly related species.

Normal tables (descriptions of developmental events and their chronological sequence) are core research tools for embryological studies (Hopwood 2007). Normal tables provide reference points (stages) that can be used to initiate experiments with embryos of the same age, to statistically control for stage in analyses, and to facilitate comparisons among species (Blanco and Alberch 1992; Andrews 2004; Radder et al. 2008; Andrews et al. 2008). While other normal tables are available (Table 1), Dufaure and Hubert's (1961) table for *Zootoca* (*Lacerta*) *vivipara* is commonly referenced because it is the most comprehensive; stages span fertilization to hatching, and stage descriptions include multiple events. Use of the DH normal table for non-lacertid embryos assumes that inter-specific variation in developmental sequences is not related to phylogeny. Our results indicate that this assumption is not valid; events that occur at the same stage for *Lacerta*, may be separated chronologically in other taxa. For example, use of the DH normal table would suggest a later than actual stage for a snake embryo that had just completed torsion which occurs earlier in snakes than lizards. This means that investigators should use staging tables based on close phylogenetic relatives even to sort embryos of a single species into chronological sequence. Currently, however, normal tables of development are quite limited relative to squamate diversity. A researcher thus might have to choose between a comprehensive table and a poor or incomplete one, or have to construct a normal table appropriate for a specific research agenda for example, Sanger et al. (2008, 2012).

The association between phylogeny and sequences of developmental event also has a very positive spin—the evolution of developmental patterns is amenable to study. A pertinent question in this regard concerns whether sequence heterochonies in development represent adaptive variation. On the one hand, if the order in which neighboring events occur is selectively neutral, developmental sequences will drift over time. On the other hand, variation in sequences of developmental events may reflect episodes of directional selection for embryonic function and/or adult phenotype. For example, the extreme elongation of snakes post-hatching is paralleled by corkscrew coiling of embryos starting at about DH stage 28 (Zehr 1962). The early torsion exhibited by snake embryos (Table 3) may thus reflect the distortion of the ancestral squamate body shape by selection for the elongate body form. Body elongation, in association with limb reduction and loss, has occurred multiple times within squamates (Wiens et al. 2006; Brandley et al. 2008). If

elongation is functionally related to the relative timing of developmental events such as torsion, then we would expect to see parallel shifts in developmental sequences in other clades of elongate squamates. Unfortunately, ranks of early events for the snake-like gymnophthalmids were not described in the study we had available. Multiple shifts from oviparity to viviparity in squamates (Shine 1985) provide another model system for assessing adaptive shifts in developmental sequences. Similarly, parallel shifts in adult limb length associated with shifts in microhabitats have been used to assess the link between selection on adult morphology and modifications of embryonic limb development in several clades of *Anolis* lizards (Sanger et al. 2012). In conclusion, our results confirm that developmental sequences are taxon specific, and that adaptive hypotheses are testable with comparative developmental data.

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APPENDIX

Clade	State	Events																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	b	0.00	0.96	0.01	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	c	0.00	0.01	0.89	0.04	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	d	0.00	0.01	0.00	0.00	0.06	0.01	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	e	0.00	0.00	0.00	0.00	0.16	0.02	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	f	0.00	0.00	0.00	0.81	0.05	0.94	0.00	0.06	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	g	0.00	0.00	0.08	0.11	0.08	0.01	0.36	0.07	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	h	0.00	0.00	0.00	0.01	0.00	0.01	0.37	0.73	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	i	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.99	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	j	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	k	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	n	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.80	0.00	0.12	0.00	0.00	0.00	0.00
A	o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.85	0.01	0.00	0.00	0.00	0.00
A	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.07	0.71	0.00	0.00	0.00	0.00
A	q	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.13	0.99	0.00	0.00	0.00
A	r	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
A	s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
A	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
B	a	1.00	0.05	0.00	0.05	0.05	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	b	0.00	0.05	0.00	0.05	0.05	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	c	0.00	0.05	0.00	0.05	0.05	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	d	0.00	0.05	0.00	0.05	0.05	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Continued

APPENDIX. (Continued)

Clade	State	Events																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
D	r	0.00	0.05	0.00	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	1.00	0.00	0.00	
D	s	0.00	0.05	0.00	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
D	t	0.00	0.05	0.00	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
E	a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	b	0.00	0.98	0.01	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	c	0.00	0.00	0.99	0.04	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	d	0.00	0.01	0.00	0.00	0.06	0.01	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	e	0.00	0.00	0.00	0.00	0.16	0.02	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	f	0.00	0.00	0.00	0.84	0.05	0.97	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	g	0.00	0.00	0.00	0.11	0.08	0.00	0.58	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	h	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.86	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	i	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.06	0.73	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	j	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.26	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	k	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	n	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.02	0.00	0.00	0.00	0.00	0.00
E	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.83	0.01	0.00	0.00	0.00	0.00	0.00
E	q	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.97	0.00	0.00	0.00	0.00	0.00	0.00
E	r	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
E	s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
E	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
F	a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	b	0.00	0.99	0.00	0.01	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	c	0.00	0.00	0.99	0.30	0.07	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	d	0.00	0.00	0.00	0.01	0.01	0.06	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	e	0.00	0.00	0.00	0.01	0.46	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	f	0.00	0.00	0.00	0.58	0.13	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	g	0.00	0.00	0.00	0.08	0.24	0.00	0.73	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	h	0.00	0.00	0.00	0.00	0.00	0.00	0.01	1.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	i	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.71	0.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	j	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	k	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	n	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.97	0.02	0.31	0.00	0.00	0.00	0.00	0.00
F	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.03	0.12	0.13	0.00	0.00	0.00	0.00	0.00
F	q	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.84	0.50	0.00	0.00	0.00	0.00	0.00
F	r	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	1.00	0.00	0.00	0.00	0.00
F	s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
F	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
G	a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	b	0.00	0.99	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	c	0.00	0.00	0.99	0.18	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	d	0.00	0.00	0.00	0.00	0.00	0.06	0.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	e	0.00	0.00	0.00	0.00	0.16	0.15	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	f	0.00	0.00	0.00	0.35	0.04	0.77	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	g	0.00	0.00	0.00	0.42	0.72	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	h	0.00	0.00	0.00	0.00	0.01	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	j	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Continued

APPENDIX. (Continued)

Clade	State	Events																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
G	k	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	n	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00
G	o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.67	0.00	0.00	0.00
G	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.03	0.01	0.27	0.00	0.00	0.00
G	q	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.99	0.05	0.00	0.00	0.00
G	r	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.99	0.00	0.00
G	s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
G	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
H	a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00
H	b	0.00	0.48	0.24	0.01	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00
H	c	0.00	0.00	0.24	0.98	0.00	0.24	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00
H	d	0.00	0.24	0.00	0.01	0.00	0.24	0.24	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00
H	e	0.00	0.24	0.48	0.00	0.00	0.00	0.24	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00
H	f	0.00	0.00	0.00	0.00	0.00	0.48	0.24	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00
H	g	0.00	0.00	0.00	0.00	1.00	0.00	0.24	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00
H	h	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00
H	i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	1.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00
H	j	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00
H	k	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	1.00	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00
H	l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00	0.00
H	m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	1.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00	0.00
H	n	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.05	0.00	0.00	0.00
H	o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	1.00	0.05	0.00	0.00
H	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	1.00	0.00	0.00	0.05	0.00	0.00	0.00
H	q	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	1.00	0.00	0.05	0.00	0.00	0.00
H	r	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00
H	s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.05	1.00	0.00
H	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	1.00
I	a	1.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	b	0.00	0.96	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	c	0.00	0.00	0.97	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	d	0.00	0.01	0.00	0.00	0.01	0.02	0.78	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	e	0.00	0.01	0.00	0.01	0.55	0.76	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	f	0.00	0.00	0.00	0.42	0.13	0.16	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	g	0.00	0.00	0.00	0.51	0.05	0.01	0.16	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	h	0.00	0.00	0.00	0.01	0.04	0.02	0.01	0.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	i	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.17	0.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	j	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.81	0.03	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	k	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.05	0.00	0.00	0.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	l	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.05	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	m	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.05	0.00	0.00	0.00	0.00
I	n	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.07	0.01	0.00	0.00	0.00
I	o	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.03	0.00	0.00	0.00
I	p	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.00	0.18	0.78	0.00	0.00	0.00
I	q	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.67	0.15	0.00	0.00	0.00
I	r	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.99	0.00	0.00
I	s	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
I	t	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
J	a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	b	0.00	0.99	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	c	0.00	0.00	1.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Continued

APPENDIX. (Continued)

Clade	State	Events																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
J	d	0.00	0.00	0.00	0.00	0.01	0.02	0.80	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	e	0.00	0.00	0.00	0.00	0.56	0.78	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	f	0.00	0.00	0.00	0.44	0.13	0.16	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	g	0.00	0.00	0.00	0.53	0.04	0.01	0.16	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	h	0.00	0.00	0.00	0.00	0.04	0.02	0.01	0.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	j	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	k	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
J	l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.01	0.00	0.03	0.00	0.00	0.00
J	m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.03	0.00	0.00	0.00
J	n	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.00	0.00	0.07	0.00	0.00	0.00
J	o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.97	0.00	0.30	0.00	0.00	0.00
J	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.01	0.21	0.43	0.00	0.00	0.00
J	q	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.78	0.08	0.00	0.00	0.00
J	r	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	1.00	0.00	0.00
J	s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
J	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
K	a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	b	0.00	0.41	0.01	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	c	0.00	0.00	0.99	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	d	0.00	0.26	0.00	0.00	0.00	0.00	0.43	0.25	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	e	0.00	0.26	0.00	0.00	0.00	0.43	0.00	0.25	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	f	0.00	0.01	0.00	0.97	0.14	0.12	0.00	0.17	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	g	0.00	0.00	0.00	0.01	0.14	0.12	0.25	0.13	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	h	0.00	0.00	0.00	0.01	0.14	0.25	0.25	0.13	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	j	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
K	k	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.01	0.00	0.20	0.00	0.00	0.00	0.00
K	l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.01	0.00	0.20	0.00	0.12	0.00	0.00
K	m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.43	0.00	0.00	0.00	0.12	0.00	0.00
K	n	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.01	0.00	0.25	0.00	0.00
K	o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.02	0.26	0.00	0.43	0.00	0.00
K	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.95	0.26	0.01	0.00	0.00	0.00
K	q	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.98	0.00	0.00	0.00
K	r	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.99	0.00	0.00
K	s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.99	0.01
K	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.98
L	a	1.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	b	0.00	0.01	0.44	0.01	0.44	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	c	0.00	0.01	0.44	0.01	0.44	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	d	0.00	0.44	0.01	0.01	0.01	0.01	0.01	0.44	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	e	0.00	0.44	0.01	0.01	0.01	0.01	0.01	0.44	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	f	0.00	0.01	0.01	0.86	0.01	0.22	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	g	0.00	0.01	0.01	0.01	0.01	0.22	0.43	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	h	0.00	0.01	0.01	0.01	0.01	0.43	0.43	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	i	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.43	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	j	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.99	0.43	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L	k	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.96	0.00	0.01	0.00	0.20	0.00	0.00	0.00	0.00
L	l	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.02	0.47	0.01	0.00	0.20	0.00	0.13	0.00	0.00
L	m	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.46	0.43	0.00	0.00	0.00	0.13	0.00	0.00
L	n	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.15	0.00	0.01	0.00	0.25	0.00	0.00
L	o	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.15	0.02	0.26	0.00	0.43	0.00	0.00
L	p	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.15	0.96	0.26	0.01	0.00	0.00	0.00

Continued

