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Research

Historical Biogeography of Holarctic *Cymbiodyta* Water Scavenger Beetles in the Times of Cenozoic Land Bridge Dispersal Routes

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Abstract

The genus *Cymbiodyta* Bedel, 1881 (Coleoptera: Hydrophilidae: Enochrinae) comprises 31 species distributed in both the Old and New World portions of the Holarctic realm. Although the species and taxonomy are relatively well known, the phylogenetic relationships among *Cymbiodyta* and the evolutionary history of the genus remain unexplored. To understand the systematics and evolution of this lineage, we sequenced five gene fragments for about half of the species in the genus, including most major morphological groups. We also estimated divergence times to test the hypothesis that *Cymbiodyta* beetles took advantage of the different land bridges connecting the Palearctic and Nearctic regions, that became subaerial in the Cretaceous and Paleocene. Our results recover the eastern Nearctic genus *Helocombus* Horn, 1890 nesting within *Cymbiodyta*. Therefore, we synonymize *Helocombus* syn. n. with *Cymbiodyta*, resulting in one new combination, *Cymbiodyta bifidus* (LeConte 1855) comb. n. Our dating analyses and ancestral range estimation support a Nearctic origin of *Cymbiodyta* in the late Cretaceous about 100 million year ago. The placement of the unique Palearctic species on a long branch as sister to the rest of the clade and the dating results cannot reject a role of the De Geer and/or Thulean routes in the colonization of the Palearctic region from the Nearctic; however, they do not support a role for Beringia in the more recent colonization of the Oriental region.

Key words: Bayesian relaxed clock, Bering route, De Geer route, Enochrinae, water beetle evolution

The role of Cenozoic terrestrial land bridges on the biogeographic history of lineages is of importance to understand how early Holarctic biotic elements originated and evolved (e.g., Sanmartin et al. 2001, Brikiatis 2014). These land bridges connecting major landmasses in Laurasia and later-on between the Nearctic, Oriental and Palearctic regions, existed during periods of sea-level lowstands fostered by climatic oscillations (Zachos et al. 2001). Among the different Holarctic land bridge hypotheses, three are most commonly invoked to explain Cenozoic patterns of dispersal between the Nearctic, Oriental, and Palearctic (Eastern and Western) regions. Beringia (or the Bering route) is a land bridge that existed within two windows of time, ca. 58 and ca. 65.5 million years ago (Ma). This land bridge facilitated dispersal between the Nearctic region and the Eastern Palearctic and Oriental regions. On the other hand, the De Geer (ca. 65.5 and 69 Ma) and the Thulean (ca. 56 and 57 Ma) routes connected the Nearctic and Western Palearctic regions via Greenland and Fennoscandia. Despite some controversy regarding the exact timing and extent of these land bridges, it is hypothesized that these geological corridors allowed the geodispersal of megafauna and other lineages throughout the Cenozoic (Sanmartin et al. 2001, Archibald et al. 2011, Brikiatis 2014). The recent developments into our understanding of periods when these land bridges were subaerial and allowed terrestrial dispersal is important to reconstruct the biogeographic history of lineages nowadays distributed across the Holarctic region.

The genus *Cymbiodyta* Bedel, 1881 (Hydrophilidae, Enochrinae) is a relatively common group of small- to medium-sized water scavenger beetles that occurs throughout North America and into southern Mexico, with one species occurring as far south as Guatemala (Smetana 1974, Hansen 1999). In addition to these New World taxa, one species, *Cymbiodyta marginella* (Fabricius 1792), is distributed throughout much of the western Palearctic region. Recently, two species have been found and described from the Eastern Palearctic and Oriental regions, considerably expanding the geographic range of the genus. *Cymbiodyta orientalis* Jia and Short 2010 was described from Guangdong Province in Southern China as the first representative of the genus in the Oriental region (Jia and Short 2010, Jia 2014). This species was originally only known from females found in small temporary pools with grass and fallen leaves until the males could be identified in 2014 from a wet cliff in the surroundings of the original pool, confirming the preference of this species for seepage habitats (Jia 2014). More recently still, *Cymbiodyta lishizheni* Jia and Lin 2015 was described from Jiangxi Province in southeastern China on wet rock seepages, similar to the habitat of *C. orientalis*. This lineage represents the second and only additional species described from this region to date (Jia and Lin 2015). We include in our phylogeny a third (and likely undescribed) Asian species from Malaysia. *Cymbiodyta* species are found in a variety of habitats, most often occurring in forested streams and pools with abundant detritus, although they may be found in river margins and a few are known to prefer springs or seepage habitats (Smetana 1974). Notably, *Cymbiodyta* is the only genus of water scavenger beetles with its center of diversity in North America. Other genera of Hydrophilidae that have comparatively large numbers (greater than five species) of Nearctic species (e.g., *Berosus* Leach, 1817, *Enochrus* Thomson, 1859, *Tropisternus* Solier, 1834, *Laccobius* Erichson, 1837) occur at even higher levels of diversity elsewhere.

In recent comprehensive molecular phylogenies of the family Hydrophilidae by Short and Fikáček (2013), Toussaint et al. (2016), and Toussaint and Short (2018), the two included species of *Cymbiodyta*, the Palearctic *C. marginella* and the Nearctic *C. semistriata*, were recovered as paraphyletic with respect to *Helocombus* Horn, 1890, a monotypic genus that occurs in woodland pools in eastern North America. The two genera formed a clade recovered as sister to the rest of the subfamily Enochrinae. In Toussaint et al. (2016) and Toussaint and Short (2018), this clade (i.e., *Cymbiodyta* + *Helocombus*) was estimated to have originated ca. 120 Ma in the Lower Cretaceous. However, the reduced taxon sampling prevented us from testing the mechanisms responsible for the widespread geographic range of this genus in the Holarctic region.

Here, we use substantially expanded taxon sampling of the genus *Cymbiodyta* from throughout its range to 1) test the monophyly of the genus with respect to *Helocombus*, and 2) reconstruct the biogeographic history of the genus to test hypotheses of dispersal and vicariance that may have shaped its distribution between the various regions in which it is found.

Materials and Methods

Taxon Sampling and Morphology

For the molecular data set, we included 12 species of the genus *Cymbiodyta* out of the 31 described. We include one species from Asia (Malaysia) that was only known to us by a single female specimen. It does not appear to be one of the two described Asian species as both those species have 10 complete rows of punctate elytral striae, while our voucher specimen only has serial striae on the lateral half of each elytron. We were not able to positively identify one voucher of *Cymbiodyta* from Oregon (United States), but do not believe it to be a new species. We also included representatives of other genera of the subfamily Enochrinae as outgroups (Table 1). Specimens for molecular analysis were preserved in 96% ethanol and kept frozen at −20°C, and were prepared for extraction by separating the thorax and abdomen between the pronotum and elytra using sterilized forceps. Total genomic DNA was extracted from entire beetles with blood and tissue kits (Qiagen Inc., Valencia, CA) according to the smanufacturer instructions and subsequently stored at −20°C. We used the PCR protocols listed in Table 2 to amplify and sequence the following five gene fragments: cytochrome oxidase subunit 1 (COI, 810 bp), carbamoyl-phosphate synthetase 2 (CAD, 711 bp), histone 3 (H3, 333 bp), 18S (~1,784 bp) and 28S (~1,003 bp). The DNA sequences were edited in Geneious R8.1.8 (Biomatters, http://www.geneious.com/), aligned using Muscle (Edgar 2004), and the reading frames checked in Mesquite 3.31 (build 859; http://mesquiteproject.org). New sequences were deposited in GenBank (see Table 1).

Molecular Phylogenetics

We used maximum likelihood (ML) and Bayesian inference (BI) to reconstruct phylogenetic relationships using the concatenated data set comprising the five gene fragments. For both the ML and BI analyses, the partitions and corresponding optimal models of substitution were searched under PartitionFinder 2.1.1 (Lanfear et al. 2017) using the '*greedy*' algorithm and the set of models corresponding to the different programs used (see below). The Akaike information criterion corrected (AICc) was used to compare the fit of the different models of substitution. The ML analyses were performed using IQ-TREE 1.5.5 (Nguyen et al. 2015). The data set was partitioned with the PartitionFinder 2.1.1 scheme and the best-fit models of substitution were searched using the '*Auto*' function on the IQ-TREE web server based on the AICc. We performed 1,000 ultrafast bootstrap replicates (Minh et al. 2013) and 1,000 SH-aLRT replicates (Guindon et al. 2010) to investigate nodal support across the topology.

The BI analyses were performed using MrBayes 3.2.6 (Ronquist et al. 2012) as implemented in CIPRES (Miller et al. 2010). Two simultaneous and independent runs consisting of four MCMC chains (one cold and three incrementally heated) were run for 40 million generations, with a tree sampling every 5,000 generations to calculate posterior probabilities (PP). We assessed convergence of the runs by investigating the average standard deviation of split frequencies and effective sample size (ESS) of all parameters in Tracer 1.6 (http://BEAST.bio.ed.ac.uk/Tracer). A value of ESS > 200 was acknowledged as a good indicator of convergence. All posterior trees that predated the time needed to reach a log-likelihood plateau were discarded as burn-in, and the remaining samples were summarized to generate a 50% majority rule consensus tree.

Divergence Time Estimates

We used BEAST 1.8.4 (Drummond et al. 2012) to infer absolute divergence time estimates using the concatenated molecular matrix. We set up multiple analyses in BEAUti 1.8.4 (Drummond et al. 2012), to assess the importance of clock partitioning, and tree model choice on downstream inferences. The data set was partitioned in five partitions corresponding to the result of a PartitionFinder 2.1.1 analysis based on a priori division of the matrix per locus. The models of nucleotide substitution for each partition were selected in PartitionFinder 2.1.1 with the corresponding set of models. The number of clocks was set in two different ways: 1) one uncorrelated lognormal relaxed clock (Drummond et al. 2006) for the mitochondrial gene fragment CO1, and another for all nuclear gene fragments; 2) one uncorrelated lognormal relaxed clock for each partition. The continuous-time Markov chain (CTMC) rate reference prior (Ferreira and Suchard 2008) was specified for the ucld.mean rates of the different clocks. The tree model was set to birth–death (Gernhard 2008) or Yule (Yule 1925, Gernhard 2008) in distinct analyses. The age of the node corresponding to the split between *Guyanobius* Spangler, 1986 and the rest of the sampled species (Fig. 1) was constrained to the interval [108.6–178.4] corresponding to the 95% credibility interval inferred in Toussaint et al. (2016) for the origin of Enochrinae. The IQ-TREE topology was enforced as a fixed topology by manually editing the

Table 1. Taxon sampling, indicating the nuclear and mitochondrial loci analyzed for each taxon

Genus	Species	Code	CO1	18S	28S	CAD	H3	Locality (collecting event)
Anacaena	*solstitialis*	MSC1815	KC935223	KC934998	KC992528	—	—	Costa Rica: Alajuela Prov., Florencia (AS-04-049)
Anacaena	*suturalis*	MSC1816	KC935226	KC935001	KC992531	—	—	United States: Maryland: Allegany Co., Little Orleans (AS-04-068)
Cymbiodyta	sp.	SLE0463	MN170784	MN118006	—	—	MN170796	Malaysia: Negeri Selangor
Cymbiodyta	*beckeri*	SLE1111	MN170786	MN118003	MN118008	MN118015	MN170794	United States: Kansas: Douglas Co., Lawrence
Cymbiodyta	*chamberlaini*	SLE0986	MN170788	MN118002	MN118010	MN118017	MN170801	United States: Pennsylvania: Lebanon Co., Lebanon
Cymbiodyta	*fraterculus*	SLE0988	MN170783	MN118004	MN118007	—	MN170797	United States: Texas: Jeff Davis Co., Davis Mtns (AS-03-019)
Cymbiodyta	*imbellis*	SLE0985	MN170780	MN117997	MN118013	MN118019	MN170793	United States: California: Napa Co., Calistoga (AS-03-063)
Cymbiodyta	*marginella*	FUSE	AM287088	AM287126	AM287134	—	MN170790	Germany
Cymbiodyta	*punctatostriata*	SLE0987	MN170781	MN117998	—	MN118020	MN170798	United States: California: Napa Co., Calistoga (AS-03-063)
Cymbiodyta	*semistriata*	AES0013	KC935252	KC935029	KC992557	—	—	United States: Maryland: Allegany Co., Little Orleans (AS-04-068)
Cymbiodyta	*dorsalis*	SLE1223	MN170782	MN117999	MN118014	MN118021	MN170799	United States: Oregon: Josephine Co., Cave Junction (US16-0906-06A)
Cymbiodyta	sp.	SLE1224	MN170789	MN118000	MN118011	—	MN170803	United States: Oregon: Douglas Co., Glendale (US16-0906-04A)
Cymbiodyta	*toddi*	SLE1065	MN170785	MN118005	MN118009	MN118016	MN170795	United States: Kansas: Douglas Co., Lawrence
Cymbiodyta	*vindicata*	SLE0984	MN170787	MN118001	MN118012	MN118018	MN170802	United States: New York: Tompkins Co., Ithaca (AS-04-060)
Enochrus	*ochraceus*	SLE0248	KC935260	KC935036	KC992564	—	MN170792	United States: Delaware: New Castle Co., Newark
Enochrus	*testaceus*	GenBank	AM287089	AJ810719	AJ810754	—	—	Germany
Guyanobius	*lacuniventris*	SLE0024	KC935267	KC935043	KC992571	—	—	Venezuela: Merida States
Helocombus	*bifidus*	FUSE	KC935272	KC935048	KC992576	—	MN170791	United States: Delaware: New Castle Co., Newark
Notionotus	*liparus*	MSC1820	KC935293	KC935073	KC992598	—	MN170800	Venezuela: Aragua State: Henri Pittier National Park (AS-06-023)

BEAUti.xml files. All analyses were run twice to detect potential local optima and ensure convergence. The runs consisted of 50 million generations with a tree and parameter sampling every 5,000 generations, resulting in 10,000 posterior samples. To compare the different analyses, we calculated the marginal likelihood of each run using the path sampling/stepping-stone sampling (Baele et al. 2013) implemented in BEAST 1.8.4. These analyses were performed with default parameters. Specifically, we used 100 path steps, with chains running for 1 million generations, with a log-likelihood sampled every 1,000 generations, and with a beta distribution of path steps. All analyses were run on the CIPRES Science Gateway cluster (Miller et al. 2010). The convergence of the runs, mixing of the chains, and ESS were checked in Tracer 1.6 (http://BEAST.bio.ed.ac.uk/Tracer). The maximum clade credibility chronograms were generated in TreeAnnotator 1.8.4 (Drummond et al. 2012), with the posterior samples of each analysis after applying a conservative burn-in of 25%.

Table 2. Comparison of BEAST run marginal likelihoods

Analysis	Clock partitions	Tree model	BEAST MLE	SS MLE
A1	2	Yule	−17,438.37	−16,957.44
A2	2	Birth–death	−17,212.54	−16,645.09
A3	5	Yule	−17,747.10	−16,994.17
A4	5	Birth–death	−17,529.48	−16,951.88

Ancestral Range Estimation

To understand the directionality of colonization events in *Cymbiodyta*, we conducted ancestral range estimation and root ancestral state optimization in an ML framework. We reconstructed ancestral ranges under the Dispersal Extinction Cladogenesis (DEC) model as implemented in Lagrange v. 20130526 (Ree et al. 2005, Ree and Smith 2008), to run comparative root optimizations. The best maximum credibility clade chronogram obtained in BEAST (see Results) was used after removing all outgroups. We considered three biogeographic regions: Nearctic (N), Oriental (O), and Palearctic (P). The adjacency and dispersal rate scaler matrices were left unconstrained because the objective was to test the potential role of ancient Cenozoic land bridges as facilitator of dispersal in *Cymbiodyta*. A 2-log likelihood unit threshold was used to select the preferred root ancestral state (Ree et al. 2005, Ree and Smith 2008).

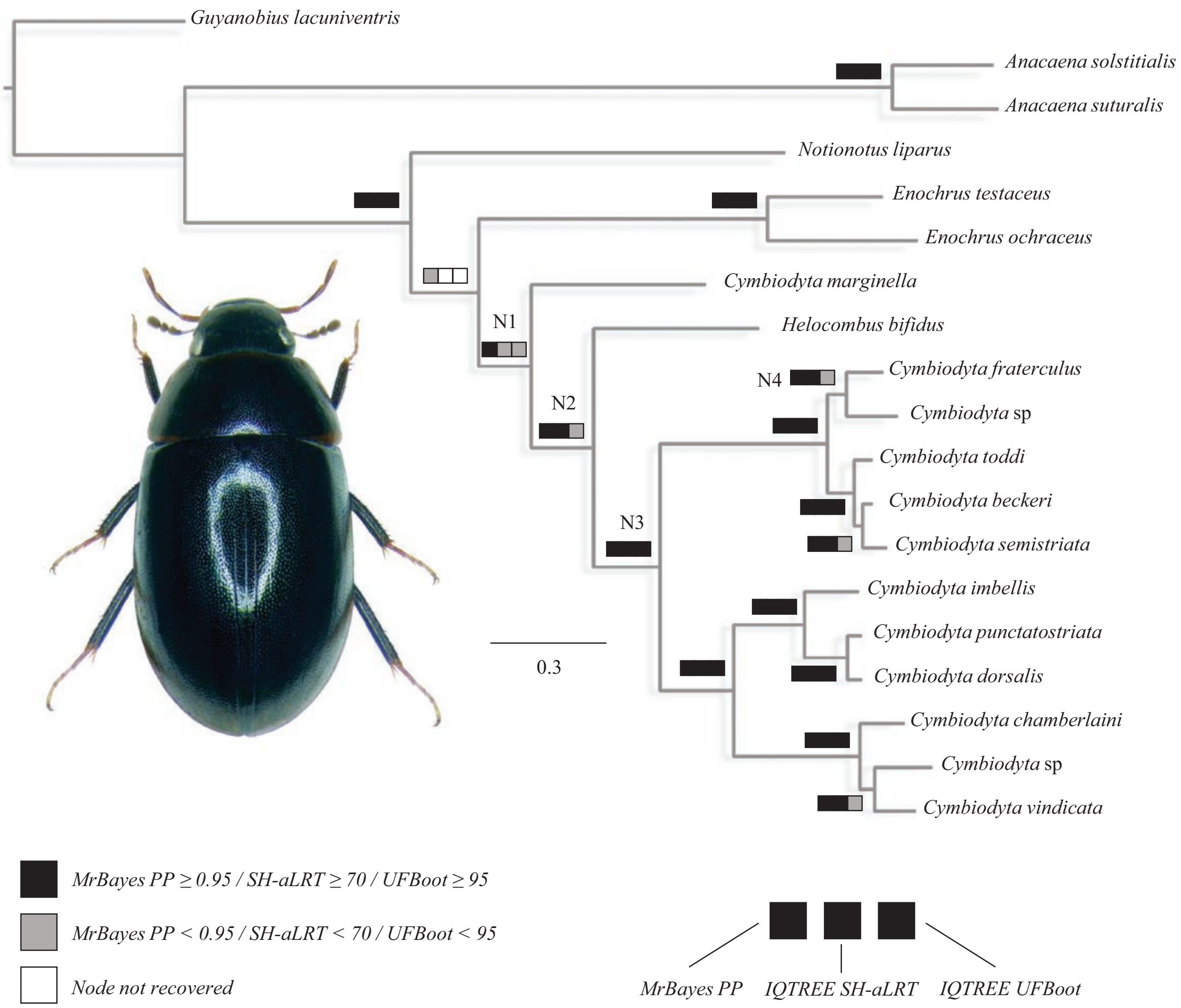

Fig. 1. Molecular phylogeny of the genus *Cymbiodyta* and phylogenetically closely related genera. MrBayes topology inferred based on the concatenated data set of five gene fragments. Nodal support for each node resulting from the MrBayes and IQ-TREE analyses is given following the inserted caption. A picture of *Cymbiodyta marginella* (Fabricius, 1792) is presented (Credit: Udo Shmidt).

Results and Discussion

Phylogenetic Relationships

Both BI and ML analyses recovered identical in-group tree topologies, with almost all nodes strongly supported (Fig. 1). *Cymbiodyta* was recovered as paraphyletic with respect to *Helocombus bifidus* in both BI and ML analyses with strong support. Specifically, the single known Palearctic species was resolved as sister to *Helocombus* and all remaining *Cymbiodyta* taxa, in line with previous molecular phylogenies (Short and Fikáček 2013, Toussaint et al. 2016, Toussaint and Short 2018). *Helocombus* was then found to be sister to the remaining *Cymbiodyta* species. The single Asian taxa included in our analysis *Cymbiodyta* sp. SLE0463 are found to be deeply nested with the otherwise Nearctic clade of *Cymbiodyta*.

Taxonomic Implications

Cymbiodyta Bedel, 1881
Hydrocombus Sharp, 1882 (syn: Horn, 1890)
Helocombus Horn, 1890 new synonymy

Cymbiodyta and *Helocombus* share a synapomorphy unique to the entire Hydrophiloidea in a reduction in the number of meso- and metatarsomeres from five to four, giving them a 5-4-4 tarsal formula (virtually all other hydrophilids are 5-5-5, with males of *Berosus* being 4-5-5). Combined with sharing a similar aedeagal form (simple, trilobed with an elongated basal piece, broad median lobe, and similarly tapering parameters) and habitat, their close relationship was expected. Indeed, when erecting the genus *Helocombus* for *H. bifidus*, Horn (1890) remarked that another recourse would be to 'continue it with *Cymbiodyta*'. He chose not to do so because of 1) the strongly elevated mesosternum (he presumed laminate in existing *Cymbiodyta*), 2) its long maxillary palps (he presumed shorter in *Cymbiodyta*), and 3) the slightly toothed claws in the males (he presumed not sexually dimorphic in *Cymbiodyta*). Over the next 129 yr, *Cymbiodyta* and *Helocombus* have been maintained as valid and their circumscriptions unchanged. However, in that intervening time, no new *Helocombus* have been found, whereas the number of *Cymbiodyta* has grown from 10 to 31, revealing an ever-increasing variation in the form of the mesosternum, palps, and claws among other characters. Indeed, none of these three characters can now be used to separate the two genera as originally propositioned by Horn (1890). Instead, *Helocombus* has been largely maintained as a valid genus due to its larger size (larger than any known *Cymbiodyta*) and its deeply grooved elytra (not known in any co-occurring *Cymbiodyta*). However, some species of *Cymbiodyta* from the western United States [e.g., *C. punctatostriata* (Horn, 1873)] also have deeply sulcate elytra. Indeed, when body size alone is excluded, there are no known adult morphological characters that would be candidate synapomorphies to separate *Helocombus* from *Cymbiodyta* as currently defined. Although the larvae of the *Cymbiodyta*–*Helocombus* clade are very poorly known (4 of 32 described), there may be one putative character unique to the Palearctic *C. marginella*: although the larval account of *C. marginella* is not published, the mandibles are described in a Masters thesis (Fikáček 2006) as being asymmetrical with regard to the number of interior teeth (two and one), whereas *H. bifidus* and two Nearctic species of *Cymbiodyta* have two interior teeth on each mandible (Archangelsky 1997). The larvae of the remaining 27 *Cymbiodyta* are unknown. Given that the closely related genus *Enochrus* includes lineages with varying numbers of mandibular teeth and no larger phylogeny exists to put this character into context, it is not possible to establish which state is derived or simply pleisiomorphic at this time.

To resolve the issue of a paraphyletic *Cymbiodyta*, we have chosen the simplest and most morphologically supported option of synonymizing *Helocombus* syn. n. with *Cymbiodyta* to maintain the monophyly of the latter. This creates a single new combination, *Cymbiodyta bifidus* (LeConte, 1855) comb. n., and establishes the tarsal formula 5-4-4 as a unique synapomorphy for *Cymbiodyta* sensu n. Other options for changing the classification require far more nomenclatural changes and would create genera without known diagnosable synapomorphies: to maintain *Helocombus* as a valid genus, *Cymbiodyta* would need to be split into two genera with *C. marginella* (as the type species of *Cymbiodyta*) placed in its own monotypic genus and the name *Hydrocombus* (currently a synonym of *Cymbiodyta*) would need to be resurrected for the remaining 30 species, creating 27 new combinations. However, there are no known adult characters that would serve as synapomorphies for the three respective genera. Another solution would have been to narrow *Cymbiodyta* to consist of only *C. marginella*, with the remaining taxa (*Helocombus* and the Nearctic and Asian *Cymbiodyta*) moved to the resurrected name *Hydrocombus* (*Hydrocombus* is an older name than *Helocombus*). This would also create a few dozen new combinations and create genera without clear synapomorphies. Therefore, we rejected these options.

Evolution of Cymbiodyta

Our BEAST dating analyses converged well and the one with two clocks and a birth–death tree model (A2, see Table 2) was preferred based on comparison of marginal likelihoods (Table 2). The chronogram resulting from this analysis is presented in Fig. 2. This dating analysis indicates that the divergence between *C. marginella* and the remaining species of *Cymbiodyta* (including *Helocombus*) occurred in the Upper Cretaceous ca. 96 Ma (95% confidence interval: 69.59–130.14 Ma; Table 3). This estimate is slightly younger than the ones from Toussaint et al. (2016) and Toussaint and Short (2018), likely due to the use of secondary calibrations in this study and of multiple fossil calibrations in the two other ones. However, these differences in divergence time estimates are unlikely to change the biogeographic implications within *Cymbiodyta* (see below). The DEC analyses significantly supported a Nearctic origin of *Cymbiodyta* (log-likelihood [LnL] = −12.56) when compared with an Oriental (LnL = −17.83) or Palearctic (LnL = −15.39) origin. The biogeographic pattern within the phylogeny is extremely conserved with all nodes within the phylogeny having an ancestral range in the Nearctic region (Fig. 2).

Table 3. Comparison of BEAST run divergence time estimates

Analysis	Root	N1	N2	N3	N4
A1	143.60 (111.41–185.82)	96.02 (71.15–130.26)	85.58 (61.86–116.49)	70.01 (50.94–96.59)	26.60 (15.48–40.71)
A2	144.89 (110.17–186.97)	96.07 (69.59–130.14)	85.33 (61.55–117.51)	69.61 (48.78–96.72)	26.40 (14.94–40.95)
A3	144.30 (111.55–190.32)	80.05 (54.47–113.85)	71.71 (46.17–101.52)	59.99 (38.63–86.77)	12.17 (5.20–21.42)
A4	146.29 (111.19–190.26)	79.50 (53.45–112.20)	71.05 (45.03–100.06)	59.46 (37.46–85.79)	11.75 (4.61–20.70)

Median age estimates with 95% credibility interval in millions of years.

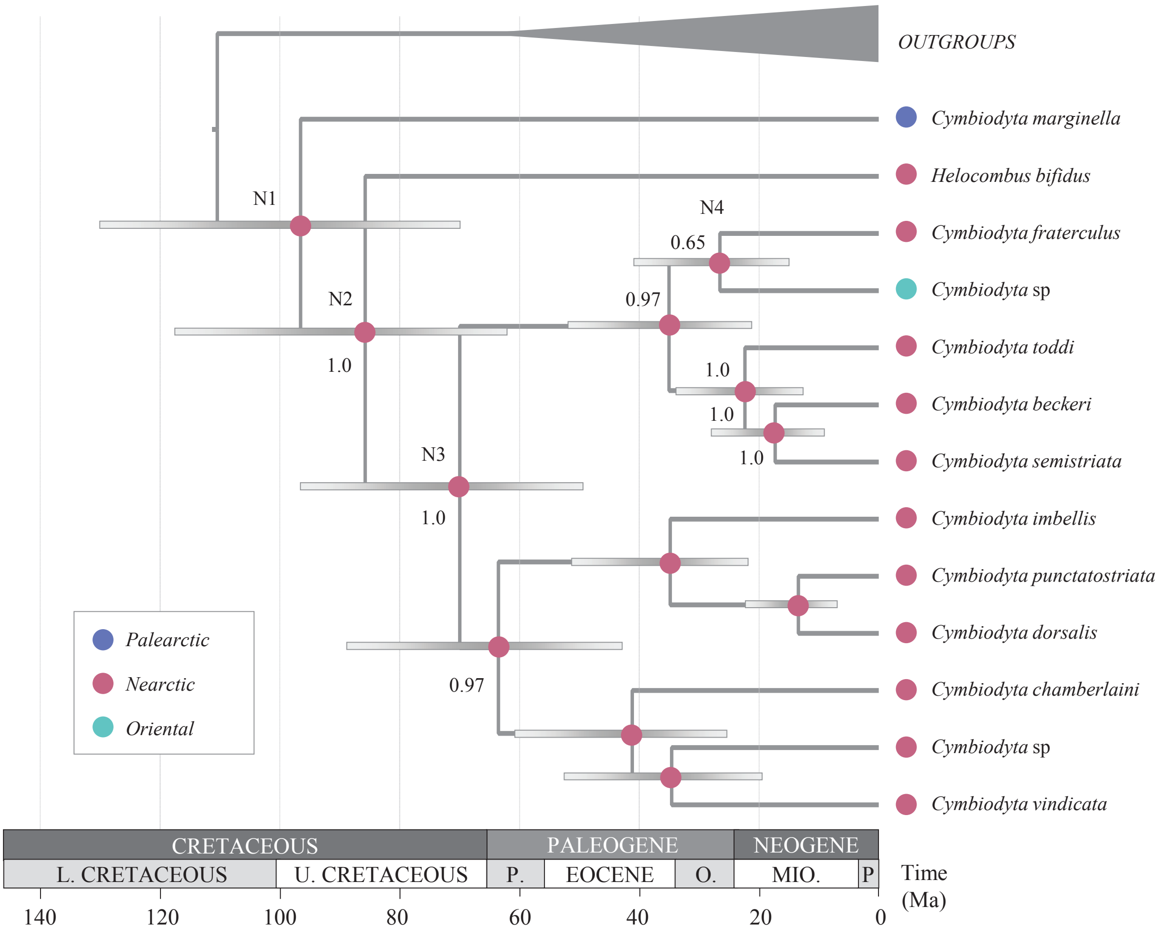

Fig. 2. Bayesian median divergence time estimates of *Cymbiodyta.* Maximum clade credibility chronogram resulting from the best BEAST analysis using secondary calibrations with outgroups pruned. The credibility intervals are indicated with gray horizontal boxes. The best ancestral range estimation scheme from the DEC analyses is presented with the most likely ancestral range for each node along the phylogeny of the genus. Color codes follow the inserted caption.

Our dating and biogeographic analyses suggest an origin of *Cymbiodyta* in the Nearctic region about 100 Ma, when the Nearctic and western Palearctic regions were connected by land within Laurasia (Seton et al. 2012). Although our data recover the Nearctic region as the most likely ancestral range with significant likelihood difference, additional sampling in particular among Enochrinae will be needed to test this hypothesis in a more comprehensive framework. An origin in the Palearctic region would also be plausible but is not preferred by our data. The range evolution of the genus is fully conserved throughout geological times (Fig. 2), indicating a geographic stasis in the Nearctic region with 'in situ' diversification most likely caused by fine-scale allopatric speciation events that cannot be investigated without a denser sampling across Nearctic *Cymbiodyta*. We infer a comparatively recent dispersal toward the Oriental region in the past 30 Ma. This dating is compatible with landmass configuration at that time because the Oriental and Nearctic regions were geographically close albeit separated by a water corridor (Seton et al. 2012). However, our divergence time estimates are incompatible with a Beringian route and associated geodispersal colonization that would require a split between the Nearctic and Oriental lineages ca. 58 or 65.5 Ma (Brikiatis 2014). The credibility interval we estimate for this particular split does not overlap with the existence of a Beringian land bridge in the Eocene, and therefore oversea dispersal is the most likely scenario based on our data (Fig. 2). The presence of *C. marginella* in the Palearctic is probably the result of dispersal from the Nearctic region; however, considering that this lineage is placed on a long branch and as sister to the rest of the genus, it is not possible to discuss in more detail the possible timing of this event. It is possible that the mechanisms of lineage dispersal from Nearctic to Palearctic are related to land bridges throughout the Cenozoic, albeit it is not possible to disentangle which between the De Geer and Thulean routes would be the most likely explanation for the colonization of this new geographic range.

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