

Reconstructing the evolutionary and ecological history of the sea skaters *Halobates* spp. (Heteroptera: Gerridae)

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The genus *Halobates* includes the only five insect species that have been successful in a pelagic marine environment. Different scenarios of independent colonization events of that environment have been proposed, considering the appearance of pelagic distribution in several non-sister clades in the phylogeny of the genus. In this paper, we aim to update the phylogenetic hypothesis under the criterion of Bayesian inference, calibrate a molecular clock using the only fossil described in the genus and also analyze the diversity pattern of the lineage since its divergence. High support values were found in the phylogenetic reconstruction, which tend to decrease with an increase of the distances from the root. Low supports for the most derived clades or relatively recent divergences cast doubt on the delimitation of some species. Although the divergence time for *Halobates* was estimated at 42.01 mya (± 8.13) the emergence of the lineage probably happened a few million years before, so the estimated time of divergence probably also marked the start of diversification of the marine lineages of this genus. Since divergence, the richness of genus showed continuous linear growth for approximately 24.4 my, when the lineages began to diversify more quickly with a significantly lower extinction rate. The colonization of the pelagic environment which occurred nearly 42 mya, could also have been the starting point of the colonization of the marine pelagic environment when changes in their morphology, physiology and behaviour enabled them to exploit novel ecological niches. Ancestors of pelagic marine insects probably inhabited areas close to the seacoast more than 41 mya ago. The ecological history of *Halobates* was probably not limited to a pair of open water colonization events as indicated by earlier proposals. We hypothesize instead that at least three independent events of open water colonization by *Halobates* species have occurred. In this sense, the ecological character of coastal or pelagic distribution is considered to be a homoplastic character without direct implications on the net diversification of *Halobates*.

Reconstructing the evolutionary and ecological history of the sea skaters

Halobates spp. (Heteroptera: Gerridae)

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Abstract. The genus *Halobates* includes the only five insect species that have been successful in a pelagic marine environment. Different scenarios of independent colonization events of that environment have been proposed, considering the appearance of pelagic distribution in several non-sister clades in the phylogeny of the genus. In this paper, we aim to update the phylogenetic hypothesis under the criterion of Bayesian inference, calibrate a molecular clock using the only fossil described in the genus and also analyze the diversity pattern of the lineage since its divergence. High support values were found in the phylogenetic reconstruction, which tend to decrease with an increase of the distances from the root. Low supports for the most derived clades or relatively recent divergences cast doubt on the delimitation of some species. Although the divergence time for *Halobates* was estimated at 42.01 mya (\pm 8.13) the emergence of the lineage probably happened a few million years before, so the estimated time of divergence probably also marked the start of diversification of the marine lineages of this genus. Since divergence, the richness of genus showed continuous linear growth for approximately 24.4 my, when the lineages began to diversify more quickly with a significantly lower extinction rate. The colonization of the pelagic environment which occurred nearly 42 mya, could also have been the

starting point of the colonization of the marine pelagic environment when changes in their morphology, physiology and behaviour enabled them to exploit novel ecological niches. Ancestors of pelagic marine insects probably inhabited areas close to the seacoast more than 41 mya ago. The ecological history of *Halobates* was probably not limited to a pair of open water colonization events as indicated by earlier proposals. We hypothesize instead that at least three independent events of open water colonization by *Halobates* species have occurred. In this sense, the ecological character of coastal or pelagic distribution is considered to be a homoplastic character without direct implications on the net diversification of *Halobates*.

Keywords: Evolution, Phylogeny, Ecology, Pelagic *Halobates*, *Halobates micans*, Character reconstruction, Marine insects.

Background

One of the major challenges of biology is to explain why insects, the most abundant and diverse group on land, have not had the same success in the marine environment where only 1400 species are found [1] [48]. Among insects, Gerridae, with 700 species described in 70 genera [2] is the second highest family in number of species. Among these only 77 species are found in marine environments, 47 of which are in the genus *Halobates*, the largest number of species in this habitat of any genus [3]. Although many of the recognized marine species are only found in coastal areas exploiting resources from both terrestrial and marine ecosystems, only five species of *Halobates* have developed a complex life-history strategy that

has allowed them to survive permanently on the surface of open seawater [4] [5]. Among the remarkable morphological aspects of this group of marine species we find: (i) smaller size (1.5 - 6 mm) relative to other species of aquatic skaters (Gerridae) from coastal areas or inland waters, which allows a greater sustainability in the air-surface film (ii) preference for areas with low light intensity, (iii) scarce diurnal activity, (iv) preference for surface temperature between 24 - 28 °C and (v) the capacity to perform jumps near 0.1 m which together with the (vi) ability to soak for about 16 hours corresponds to an effective strategy to escape predation [6] [7] [8] [9] [10] [11] [12] [44].

The genus *Halobates* is distributed in the tropical and subtropical areas of the world, with a large number of endemic species in the vicinity of isolated or groups of islands (e.g. *H. robustus*, *H. kelleni*, *H. salotae*, *H. bryani*, *H. fijiensis* and *H. tethys*) [4]. Two ecological categories for species of the genus are recognized according to their spatial location in regions of the ocean: coastal species associated with estuarine and mangrove areas [11] [13] [14] and pelagic species distributed in the open sea. The geographical distribution of the species of *Halobates* is well established [15]. Among the five pelagic species described, *H. micans* is widely distributed in the Atlantic, Indian and Pacific Oceans. Moreover, *H. sobrinus* and *H. splendens* are located in the eastern Pacific Ocean, *H. germanus* is distributed in both the Pacific Ocean and the Indian Ocean. Finally, *H. sericeus* is found throughout the Pacific Ocean except between 15° north and south of where *H. micans* is found [16] [46].

Some interesting ecological aspects of *Halobates* have been described, that could be considered as unique for this genus. Sea skaters feed on other animals of the

pleustonic community and are themselves prey of marine and pelagic birds [16] [17] [18] [47]. The eggs are deposited on floating objects [5] [16] [19] and sometimes even on live heteropods [20]. The *Halobates* adults are always wingless, but can be dispersed by surface currents along the coast, island chains or even through stretches of open water. The consideration of their ability to evade predation has been crucial in interpreting the evolutionary success of *Halobates* in marine environments, since it is an important selective pressure on species which do not have temporary or permanent defense shelters, but also determines the respiratory physiology of these insects that can survive for long periods of immersion sometimes even at considerable depths [1].

The systematics of *Halobates* has been limited to few morphological and molecular phylogenetic analysis that have suggested, in most of them, the monophyly of the genus and the first invasion to the marine habitat 45 million years ago [4] [14]. Herring [21] based on morphological data questioned the monophyly of the genus, indicating that *H. sobrinus*, *H. micans* and *H. splendens* are more related to coastal species such as *H. flaviventris* and *H. hawaiiensis*, than the other pelagic species *H. germanus* and *H. sericeus*. In this sense, there has been much speculation about the origin and evolution of *Halobates*, but questions about how, when and where these species achieved this unique way of life among insects have been of particular interest [15] [22]. Evolutionary questions of this nature can only be answered through inference based on reliable hypotheses about the phylogenetic relationships among the species involved [24]. It has been proposed that during the course of the evolutionary process, pelagic species have colonized this environment at least twice, but in addition, *H. flaviventris* and *H. hawaiiensis* returned to the coasts, thus eliminating the independence of pelagic colonization

events. In this paper a new hypothesis of evolutionary relationships within the genus *Halobates*, based on molecular data, is proposed. Ecological characteristics of their distribution were also reconstructed using ocean zoning (littoral and pelagic) to quantify the number of times each species has colonized open water environments.

Material and Methods

Sequence obtention and alignment

Many of the sequences available in databases correspond to species with broad geographic distribution or are a consequence of particular interest by some researchers in biogeographic, population genetics [23] and evolutionary aspects [24], among others. The remaining species have very restricted distribution ranges and in most cases have not been included in previously published molecular analyzes. Therefore, only data for 20 species corresponding to 42.5% of genus richness are included in the analysis. All sequences of the 16s, 18s, 28s, EF1 and COI genes published in GenBank [25] for species of *Halobates* (Table 1) were included in the analysis. Protein coding genes were aligned with respect to the translation into amino acids according to a guided tree in ClustalW [26], specifying a Gap opening penalty of 10, Gap Extension penalty corresponding to 0.1, two alignment iterations and starting from the first codon. The alignments were translated in reverse (to DNA) and exported for concatenation with the ribosomal genes, which were previously aligned by the algorithm Muscle (10 iterations varying the distance measurement, the grouping method, and weights rooting alignment scheme) [27]. Finally, partitions of protein coding loci and

ribosomal genes were concatenated into a single matrix with a length corresponding to 5337 bp.

Phylogenetic reconstruction

The reconstruction of the evolutionary history of *Halobates* was performed using Bayesian algorithms in the software BEAST 1.7 [28] on the array of aligned and concatenated genes. The evolutionary model more consistent with the mutational characteristics of each gene was determined using the Akaike information criterion (AICc) in TOPALi 2.7 [29], which indicated GTR as the most suitable pattern on loci 18s, 28s and EF1; GTR + I + G for 16s and GTR for COI. In the analysis, two independent chains, each for 10,000,000 generations with a sampling frequency equal to 1,000 generations and burnin 10,000 were run. The determination of the consensus tree was performed in TreeAnnotator, retaining clades with support values (pp) higher than 0.3 [28].

Molecular dating and character state reconstruction

Molecular dating and reconstruction of characters. The absolute dating of the phylogenetic tree was made in BEAST 1.7, which uses Bayesian criteria for calibration of a molecular clock and allows the variation in evolutionary rates between the branches of the phylogenetic tree according to a log-normal distribution and modelling the distribution of the years in the nodes as a process Yule speciation [30]. The only fossil described for *Halobates* (*H. ruffoi*) [22] was used in the calibration of the MRCA for the ingroup, assuming a normal probability density function for modelling the ancestor-descendant uncertainty.

A mean value and standard deviation of 44.5 mya and 5.79, respectively, were used for the normal probability function. The evolutionary models were assigned according to the methods described for the phylogenetic reconstruction. Two independent MCMC were implemented in BEAST with 10.000.000 generations each, using a sampling frequency of 1.000 generations and burnin equivalent to 10.000.

Transitions between pelagic and littoral distribution of the *Halobates* species were assessed by performing the reconstruction of the ancestral characteristics in MESQUITE [31]. The binary consensus topology derived in BEAST was used as the input and the probability of occurrence of each character state per node and was approximated by maximum likelihood under the characteristics of stochastic mapping of categorical characters [32] according to the discrete evolutionary model Mk [33]. The correlation between diversification in *Halobates* and the appearance of the pelagic feature was analysed by the calculation of Sister Diversification index MESQUITE, which contrasts the null hypothesis which establishes that uniform clades with state 1 are smaller than clades with uniform status 0 among those having different values. Both speciation and extinction rates were calculated as the ratio between the two indexes from models under reduced maximum likelihood model BiSSE [31].

MCMC diagnostics

Inspection of the convergence of MCMC chains was assessed according to the length of the runs and the values of burnin was confirmed by Tracer 1.4 [34] from

graphical analysis of the likelihood of the tree and also considering as significant values of ESS above 100.

Results

Significant support values were found for most of the branches of the phylogenetic tree (Fig. 1), with posterior probability supports varying between 34.2 and 100. This analysis confirmed the molecular support for the monophyly of *Halobates*, in which two species diverged first: *H. mjobergi* and *H. micans*, and also two major lineages: (i) *H. alluaudi*, *H. salotae*, *H. mariannarum*, *H. bryani*, *H. nereis*, *H. robustus*, *H. sexualis* and *H. poseidon* in the first clade and in the second clade the species (ii) *H. flaviventris*, *H. sobrinus*, *H. splendens*, *H. hawaiiensis*, *H. whiteleggei*, *H. proavus*, *H. germanus*, *H. matsumurai*, *H. sericeus* and *H. hayanus*.

The low support for clades *H. alluaudi* + *H. salotae*, *H. brayani* + *H. nereis* and *H. matsumurai* + *H. sericeus* (34.2, 46.9 and 39.3 respectively) casts doubt on the validity of the interspecific separation of the morphological species and raises great uncertainty about the delimitation within the three clades, where the incipient differentiation may be result of gene flow between populations or even a consequence of the recent divergence time between the populations involved. The separation between *H. matsumurai* and *H. sericeus* occurred about 4.01 mya ago (1.64 - 11.18), a long time on an ecological scale, but probably early for an interspecific differentiation (microevolutionary scale) in accordance with the

evolutionary rate of the lineage. In the *H. alluaudi* + *H. salotae* and *H. brayani* + *H. nereis* clades the divergence times were also estimated as relatively recent.

The ancestor of *Halobates* occurred about 42.01 mya ago (± 8.13) and since then the richness of genus showed a continuous linear growth for approximately 24.4 my, when the families began a higher diversification rate than that recorded before and also a significantly lower extinction rate, that allowed since that time the increasing net diversification within the lineage and the current richness (Fig. 2). *Halobates* has an average evolutionary rate close to that of 0.05 % substitutions / site / million years ($\pm 0.01\%$) and the standard deviation was 0.71% substitutions / site / million years. Cladogenesis rate for the Yule speciation model was 0.06% (± 0.02).

The ancestral reconstruction of the ecological character of binary distribution in ocean zoning (littoral and pelagic) indicated the existence of three major events of colonization of the pelagic habitat by the five species that exhibit this habit. This is a homoplastic character and therefore does not support the monophyly of a clade that brings together all pelagic species (Fig. 3). The analysis also generates uncertainty about the more probable distribution for the ancestral of *Halobates*. The appearance of the pelagic feature in *Halobates* has not been determinant in the diversification of the lineages, being restricted to isolated species and absent in uniform sister clades (Sister Diversification $p > 0.05$). The lineage speciation

rate (λ) was 0.061, extinction (μ) equal to 1.28×10^{-5} and $-\log$ likelihood of speciation / extinction ratio was 72.36%.

Discussion

The number of colonizations of the marine environment by gerromorph insects has probably occurred independently at least 14 times [4]. Some previous estimates placed the divergence of *Halobates* 10 my before the only fossil described, suggesting (i) a sigmoidal increase of the ancestral population of *Halobates* and (ii) dating marine habitats to invasion by Gerridae prior to 45 mya [41]. The characteristic of living in the open sea or coastal areas in *Halobates* species has been widely discussed, and is considered by some classifications as a key character for the designation of the different monophyletic groups. In a strict sense, the previous proposals have a high degree of agreement, but the influence of plesiomorphic characters in the designation of monophyletic groups and the use of sensitive methodological approaches to establish phylogenetic relationships, distorts the understanding of the evolutionary characteristics of the genus.

Generally, similar ecological responses are the result of evolutionary convergences that are a consequence of environmental and biotic structure. These evolutionary trends suggest that the emergence and evolution of the pelagic distribution of *Halobates* is homoplastic, and supports the rejection of hypotheses that base their monophyly on this character, instead supporting a hypothesis of independent multiple colonizations of the open sea. In this sense, Andersen & Cheng [41] discussed in detail various aspects of the evolution of *Halobates* based on a phylogenetic reconstruction which presents some topological differences from that obtained in

the present work. In both topologies, the monophyly of pelagic species is unsupported and independent evolution of this feature is hypothesized for two different clades. Our hypothesis does not completely contradict the previous proposal on the historical biogeography of the genus, but creates uncertainty for new relationships established and suggests a different interpretation to gradualism in ecological change as proposed above. Moreover, our analysis indicates *H. germanus* is the sister species of the clade *H. sericeus* + *H. matsumurai*, which contradicts the progressive change in the geographical distribution from the coastal to pelagic transition, but does not reject this slow, continuous and gradual change from one pelagic to coastal environment.

Herring [21] recognized some groups of species within the genus by linking them together in an idealized "phylogenetic tree". Still, some of these groups were based exclusively on plesiomorphic characters and therefore, do not represent strictly monophyletic groups. Herring [21] also proposed a group of open water *Halobates* composed by *H. micans*, *H. sobrinus*, *H. splendens*, *H. germanus* and *H. sericeus* and suggested that it is monophyletic. Andersen [14] questioned the monophyly of the open sea group in the proposal of Herring [21] from a cladistic analysis and indicated that the oceanic species, *H. sobrinus*, *H. micans* and *H. splendens*, are more closely related to species that live near the coast (*H. flaviventris* and *H. hawaiiensis*) than to the other two oceanic species (*H. germanus* and *H. sericeus*). This implies for Andersen two possible evolutionary scenarios: one in which the path to ocean life happened only once and where *H. flaviventris* + *H. hawaiiensis*

reverted to nearshore habitats; but another scenario involving that the ocean habits were acquired independently twice [24] [35].

Our analysis indicate that the first event of establishment in open water was by the species *H. micans* which diverged 24 mya and successfully colonized the open sea during the Oligocene-Miocene transition. This period was characterized by the occurrence of several events that probably shaped the life history of this aquatic species, but mainly the drying out of the Mediterranean Sea and the increase in temperature significantly influenced the proliferation and establishment of the first populations of this species in the open waters of the Indian, Atlantic and Pacific oceans. The second independent colonization event occurs for the species *H. germanus* and *H. sericeus* previously proposed as a monophyletic group [4] [35] [49]. The present phylogenetic reconstruction indicates that *H. sericeus* is closely related to *H. matsumurai* but could be due to its geographical proximity. *H. matsumurai* which is associated with coastal areas that connect the Pacific Ocean to the Sea of Japan [45], diverged 5 mya with respect to *H. germanus*, which also has a wider distribution that covers both Pacific and Indian oceans [35]. This event is conceived as a recolonization of *H. matsumurai* to the coastal area during the Pliocene period [36], where the temperature of the Pacific Ocean decreased with respect to the present day. This condition facilitated the movement of species in the aquatic environment, but a further increase in temperature influenced the geographic specialization of *H. matsumurai*. Despite this, it is important to note that according to the phylogenetic reconstruction, the divergence of these three species has low support so it can in fact be a single species with wide distribution

primarily a result of the ecological characteristics of *H. sericeus* and *H. matsumurai*.

Previous proposals make a significant recount of evolutionary features of *Halobates*, contextualizing the genus more broadly with respect to sister groups. Andersen & Cheng [41] indicates: “Since all of the c. 90 species belonging to the tribe Metrocorini live in lotic freshwater habitats, it is hypothesised that ancestral sea-skaters from such limnic habitats invaded nearshore marine habitats, probably through estuaries and coastal mangroves, where the three known species of *Asclepios* now live [42]. The existence of limnic species such as *A. rivularis*, *Halobates (Hilliella) robinsoni* [43], and *H. (s. str.) acherontis* [44] suggests that either the marine environment has been invaded more than once or, more likely, marine lineages have recolonized freshwater habitats independently several times. The oceanic way of life in some *Halobates* species probably evolved at least twice. This hypothesis, well supported by molecular data, is more parsimonious than the one that includes only one original transition to the open ocean, but three independent reversals to nearshore habitats in *H. hayanus*, *H. flaviventris*, and *H. hawaiiensis* [24].”

A big question remains with respect to the absence of *Halobates* populations (especially *H. micans*) from the Mediterranean Sea, because although these might have disappeared during the drying process that occurred about 5.6 Mya [38], *Halobates* had the spatial potential to reoccupy the sea after its refilling approximately 5.33 Mya. The time of divergence of *H. micans* does not explain the absence of the species from the Mediterranean Sea but indicates the potential Mediterranean recolonization constrained by the potential suitability of environment in the

continuous distribution. In this sense, both life history characteristics and thermal preferences [16] along with the physical barriers and the direction of currents that characterizes the Strait of Gibraltar [39] have been defined as improbable explanations of the absence of *H. micans* in the Mediterranean Sea. On the other hand, influx by other species of *Halobates* (*H. germanus*, *H. melleus* and *H. hayanus*) to the Mediterranean Sea has also been considered possible via the Suez Canal [3].

It is clear that many of the biological peculiarities of *Halobates* are not restricted only to the appearance of one feature, but have been the consequence of sequence variations within the lineage. Some biogeographical and ecological aspects have been questioned (e.g. absence of populations in the Mediterranean, exclusive distributions between species among others), but obtaining better answers at both the evolutionary and ecological levels are still a motive for further research and discussion. Multiple evidence supports the strong dependence of some species of *Halobates* upon the availability of certain resources (e.g. oviposition sites) or ecological preferences (e.g. thermal), offering a possibly more accurate way to predict the ecological influence on the distribution of *Halobates* species. The genus has varied markedly as a result of geological and environmental changes that have occurred since its appearance, and has been altered by changes in the thermal characteristics of the areas where it occurs as well as by the anthropogenic introduction of novel compounds to the marine environment. In this sense, the temporal continuity of diversity as regards at least to populations of species such as *H. sericeus* generates uncertainty about the magnitude of the interspecific interactions that can occur with the overlap in distributions with other species of

Halobates as a consequence for example of the increased availability of oviposition sites [40].

Finally, the colonization of the aquatic environment which occurred near 42 mya, could have also been the starting point of the colonization of insect species into the marine environment by exploiting new niches and finding broader ecological opportunities. This also supports the proposal for the occurrence of ancestors inhabiting areas close to the coast more than 41 mya ago. Probably, the ecological history of *Halobates* was not limited to a pair of events of colonization to open water as indicated by earlier proposals but at least three events of colonization of open waters by species of the genus have occurred. In this sense, the ecological characteristic of coastal or pelagic distribution is indicated as a homoplastic character without direct implications on the net diversification of *Halobates* until the present.

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Competing interests

The authors declares that they have no competing interests

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Figures

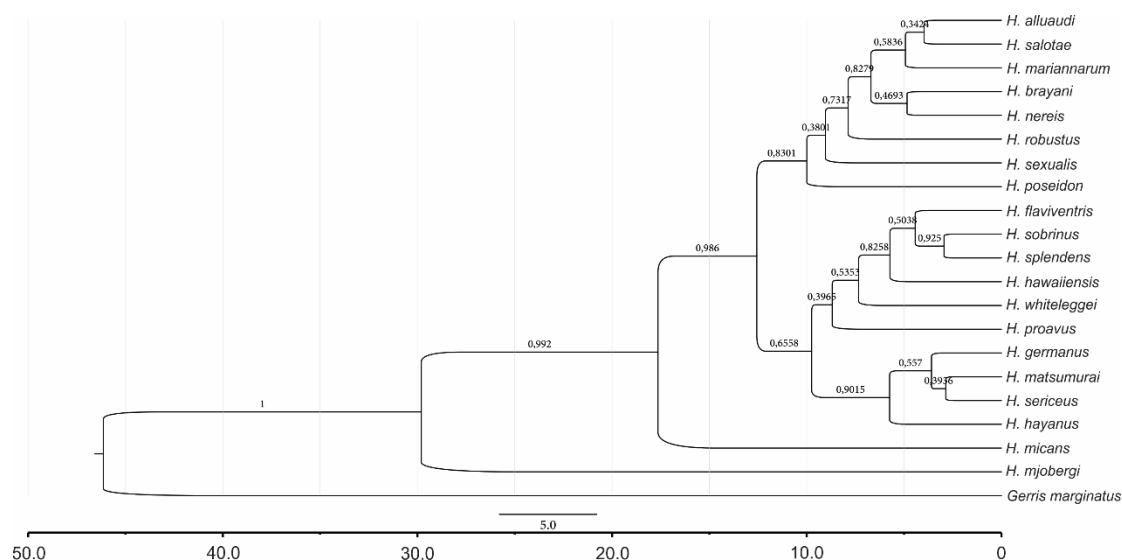


Figure 1. Timetree of *Halobates*. The support value (pp) is on each branch. Scale in millions of years (mya). *Halobates* is supported as a monophyletic group separated by two species which diverged first: *H. mjobergi* and *H. micans*. The remaining species are divided in two groups with a high value of support (>99) which diverged *ca.* 23 mya: (i) *H. alluaudi*, *H. salotae*, *H. mariannarum*, *H. bryani*, *H. nereis*, *H. robustus*, *H. sexualis* and *H. poseidon* in the first set and in the second group the species (ii) *H. flaviventris*, *H. sobrinus*, *H. splendens*, *H. hawaiiensis*, *H. whiteleggei*, *H. proavus*, *H. germanus*, *H. matsumurai*, *H. sericeus* and *H. hayanus*. The clades *H. matsumurai* + *H. sericeus* and *H. alluaudi* + *H. salotae* exhibited low support (39.6 and 34.24 respectively), indicating there might not be a clear genetic differentiation between these species. The ancestor of the genus appeared about 42 mya and the first significantly diversification occurred 30 mya. *Halobates* species with pelagic distribution appeared three times independently during the evolution of the genus. The first event occurred *ca.* 23 mya in the species *H. micans*. The second evolutionary event was about 5 mya

with the species *H. sobrinus* and *H. splendens*. The third event happened 4 mya in the species *H. germanus*, *H. sericeus* and *H. matsumurai*.

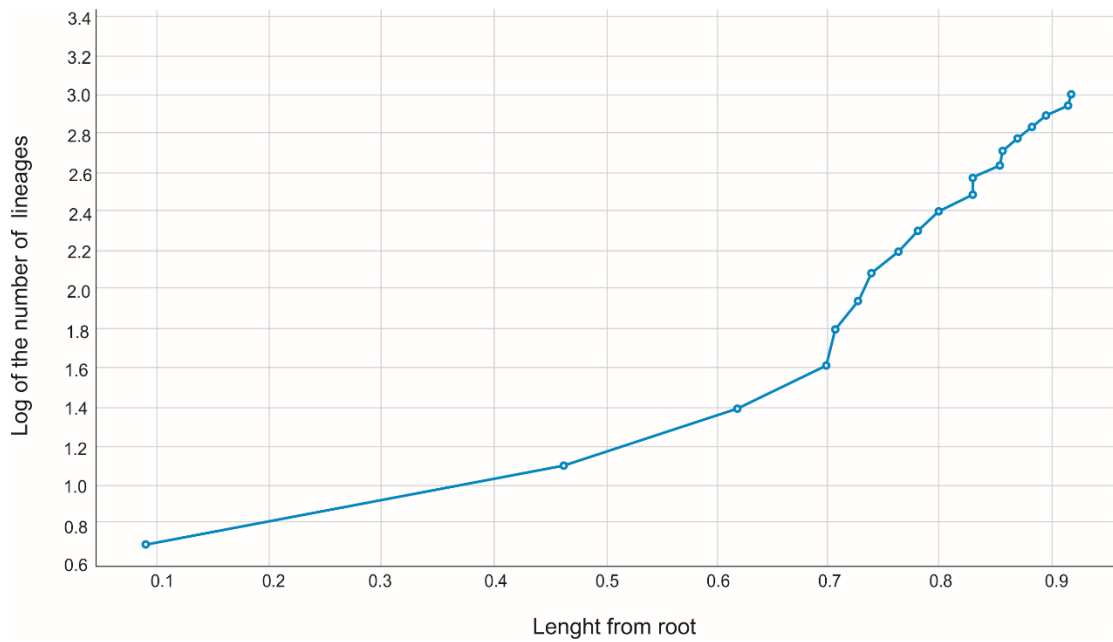


Figure 2. Lineages through time of the genus *Halobates*

The ancestor of *Halobates* occurred about 42.01 mya ago (± 8.13) and since then the richness of genus showed a continuous linear growth for approximately 24.4 my, when the families began a higher diversification rate than that recorded before and also a significantly lower extinction rate, that allowed since that time the increasing net diversification within the lineage and the current richness.

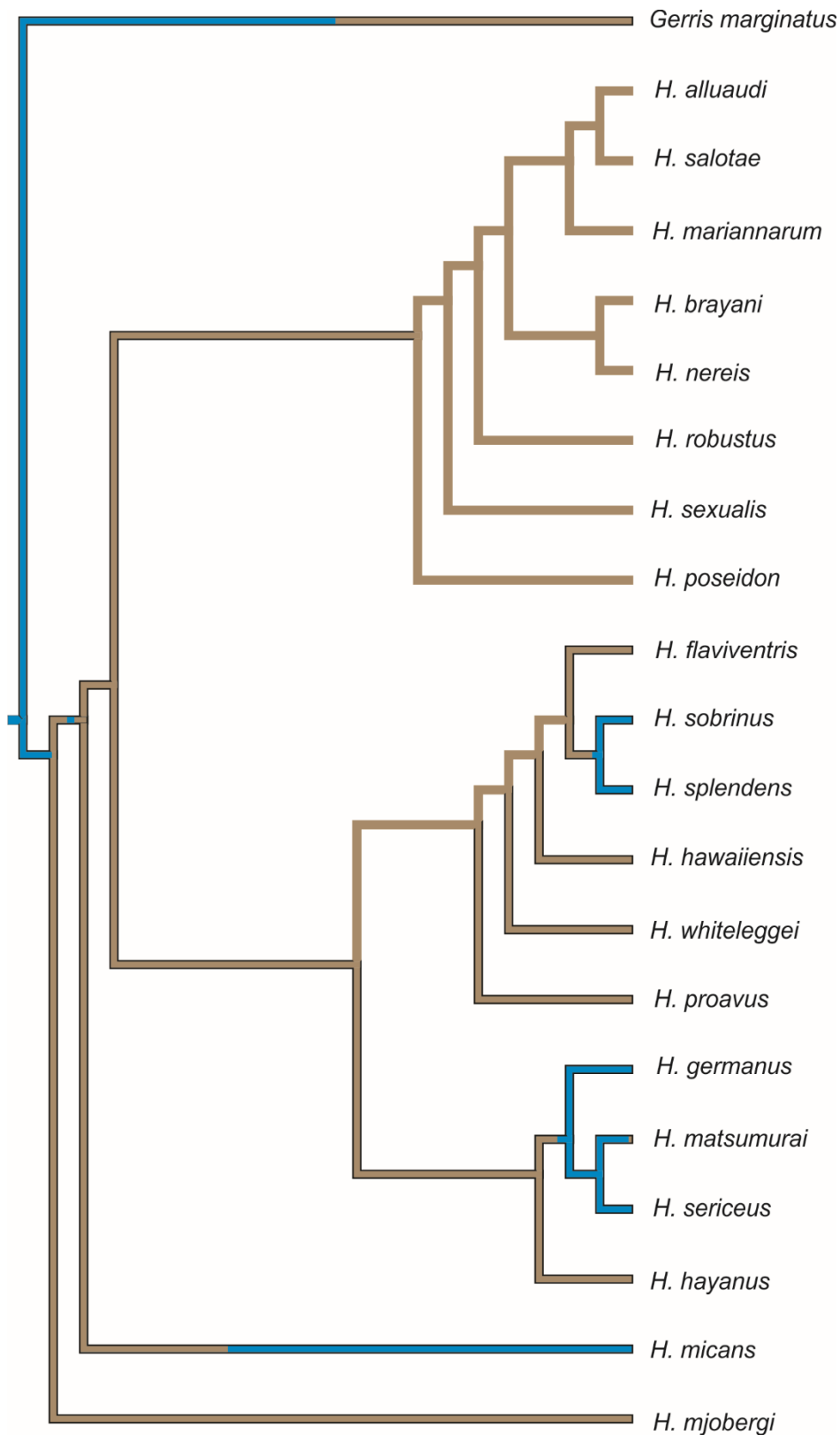


Figure 3. Reconstruction of the ecological binary character (pelagic/ littoral) in *Halobates*. Where, species with coastal distribution are indicated in brown and pelagic with blue. The reconstruction supports the existence of an ancestor with nearshore

distribution, *H. micans* present the first change from of the ancestral state, to a pelagic distribution. The second colonization event occurred by the species *H. sericeus* and *H. germanus* mostly related to *H. matsumurai*, a coastal species, and the third event corresponds to the species apomorphy that supports ecologically *H. splendens* and *H. sobrinus*.

Table 1. Accession numbers of the sequences analysed. Accession number of sequences analyzed from the GenBank [25] for 16s, 18s, 28s, COI and EF1 genes for the outgroup *Gerris marginatus* and of the ingroup *Halobates*: *H. alluaudi*, *H. bryani*, *H. flaviventris*, *H. germanus*, *H. hayanus*, *H. hawaiiensis*, *H. mariannarum*, *H. matsumurai*, *H. mjobergi*, *H. nereis*, *H. poseidon*, *H. proavus*, *H. robustus*, *H. salotae*, *H. sericeus*, *H. micans*, *H. splendens*, *H. sobrinus* and *H. sexualis*.

Species	Gene	Accession	Species	Gene	Accession
<i>Gerris marginatus</i>	16s	AY252646.1	<i>H. mjobergi</i>	16s	AY648134.1
	18s	AY252122.1		28s	AY648150.1
	COI	AY252904.1		COI	AF200291.2
	EF1a	AY425272.1	<i>H. nereis</i>	COI	AF200292.1
<i>H. alluaudi</i>	COI	AF200284.1	<i>H. poseidon</i>	COI	AF200299.1
<i>H. bryani</i>	COI	AF200285.1	<i>H. proavus</i>	COI	AF200300.1
<i>H. flaviventris</i>	COI	AF200286.1	<i>H. robustus</i>	COI	AF200301.1
<i>H. germanus</i>	COI	JN565119.1	<i>H. salotae</i>	COI	AF200302.1
	EF1a	JN565144.1	<i>H. sericeus</i>	16s	AB026608.1
<i>H. hayanus</i>	COI	AF200289.1		18s	JN565129.1
<i>H. hawaiiensis</i>	COI	AF200288.1		28s	AB034776.1
<i>H. mariannarum</i>	COI	AF200298.1	COI	AF200293.1	
<i>H. matsumurai</i>	16s	AB026607.1	EF1a	JN565131.1	
	28s	AB034778.1	<i>H. sexualis</i>	COI	AF200294.1
<i>H. micans</i>	16s	EU871204.1	<i>H. sobrinus</i>	COI	AF200295.1
	28s	EU871265.1	<i>H. splendens</i>	COI	AF200296.1
	18s	JN565130.1			
	COI	JN565118.1			
	EF1a	JN565143.1			