

# Coevolutionary Implications of Obligate Commensalism in Sea Turtles: the Case of the Genus *Hyachelia* Barnard, 1967 (Crustacea, Amphipoda)

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Obligate commensalism in the marine environment and its evolutionary role are still poorly understood. Although sea turtles may serve as ideal substrates for epibionts, within amphipods, only the genus *Hyachelia* evolved in obligate commensalism with turtles. Here, we report a new host record for *Hyachelia lowryi* on the hawksbill turtle and describe a larger distribution of the genus in the Atlantic Ocean on green and loggerhead turtles. *Hyachelia* spp. were sampled from nesting sites of *Caretta caretta* and feeding grounds of *Eretmochelys imbricata* and *Chelonia mydas* along the Brazilian coast. Insights regarding the coevolution of this remarkable genus with its hosts based on molecular analyses are inferred based on mitochondrial (*COI*) and nuclear (18S rRNA) genes using new and previously available sequences from the infraorder Talitrida. Divergence times for *Hyachelia* are around the Cretaceous (~127.66 Mya), corresponding to an ancient origin and in agreement with modern green turtle (Cheloniodea) radiation. Later, diversification of *Hyachelia* species is dated at about 26 Mya, suggesting a coevolutionary association between amphipods and Caretini/Chelonini sea turtles.

**Key words:** Distribution, Epibiosis, Invertebrate, Marine, Molecular evolution, New record, Talitrida, Taxonomy

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## BACKGROUND

Sea turtles can host a wide diversity of epibionts, from algae to macro- and microinvertebrates (Corrêa et al. 2014). According to Frick and Pfaller (2013), the variability of turtle epibiont communities depends on the geographic and ecological overlap of hosts and epibionts, with the likelihood of epibiosis resulting from a trade-off between the costs and benefits of the epibionts involved. The majority of these organisms are normally found in the surrounding marine environment (such as coral reefs, rocky shores and algal beds) (Frick and Pfaller 2013) and behave as facultative commensals (Wahl and Mark 1999). More rarely, some of the epibionts are found exclusively in association with sea turtles, and are thus referred to as obligate commensals (Frick and Pfaller 2013). The evolutionary roles of such strict associations are still poorly understood.

Among turtles and other large marine vertebrates, crustaceans represent one of the most diverse groups of epibionts, and amphipods show a great variety of ecological adaptations (Barnard 1967; Serejo and Sittrop 2009; Iwasa-Arai and Serejo 2018). Several species of amphipods are known as facultative commensals, such as *Caprella andreae* Mayer, 1890 and *Protohyale (Protohyale) grimaldii* (Chevreux, 1891), frequently found in association with sea turtles (Table 1). In contrast, only three species of amphipods are known as obligate commensals of sea turtles: *Podocerus chelonophilus* (Chevreux and Guerne, 1888), *Hyachelia tortugae* Barnard, 1967 and *H. lowryi* Serejo and Sittrop, 2009. *Podocerus chelonophilus* is a subcosmopolitan epibiont found on loggerhead [*Caretta caretta* (Linnaeus, 1758)], green [*Chelonia mydas* (Linnaeus, 1758)], olive ridley [*Lepidochelys olivacea* (Eschscholtz, 1829)] and hawksbill turtles [*Eretmochelys imbricata* (Linnaeus, 1766)] (Baldinger 2001; Lazo-Wasem et al. 2011; Iwasa-Arai et al. 2020). The genus *Podocerus* Leach, 1814 comprises over 60 species (Horton et al. 2021), but only three have particularly broad distributions (Hughes 2016), and the wide distribution of *P. chelonophilus* might be associated with its epibiont lifestyle.

The other two amphipod obligate commensal species, *H. tortugae* and *H. lowryi*, have a much more restricted distribution and host specificity. *H. tortugae* has been documented on green turtles while *H. lowryi* has been found on green and loggerhead turtles, and both amphipod species are found only in the Pacific Ocean (Serejo and Sittrop 2009; Yabut et al. 2014). In contrast with *Podocerus*, the genus *Hyachelia* Barnard, 1967 comprises only two species, and its evolution is likely correlated with sea turtle evolution. Due to its conspicuous morphological differentiation, *Hyachelia*

was transferred to its own subfamily Hyacheliinae Bousfield and Hendrycks 2002 within Hyalidae Bulyčeva, 1957 (Bousfield and Hendrycks 2002). In contrast, Hyalinae Bulyčeva, 1957 is composed of 148 species distributed among 11 genera (Horton et al. 2021). Free-living hyalids are predominantly found among algae and biofouling substrates in the intertidal and shallow infralittoral areas of tropical and subtropical zones (Serejo and Sittrop 2009).

While relationships within Hyalidae are yet to be elucidated, studies on the crown sea turtle evolutionary histories mostly agree on the species relationships and divergence times (Naro-Maciel et al. 2008; Duchene et al. 2012). Within sea turtles (Cheloniodea), the most speciose family is Cheloniidae Oppel, 1811, which comprises six of the seven extant species, and is divided into Chelonini and Carettini (*sensu* Naro-Maciel et al. 2008). Chelonini is composed by the green [*Chelonia mydas* (Linnaeus, 1758)] and the flatback sea turtles [*Natator depressus* (Garman, 1880)], which diverged around 34 Mya (Naro-Maciel et al. 2008). Carettini includes the hawksbill [*Eretmochelys imbricata* (Linnaeus, 1766)], separated from the remaining Carettini clade around 29 Mya (Naro-Maciel et al. 2008), the loggerhead [*Caretta caretta* (Linnaeus, 1758)], that diverged from *Lepidochelys* around 16 Ma (Bowen et al. 1991), the olive ridley [*Lepidochelys olivacea* (Eschscholtz, 1829)] and the Kemp's ridley [*Lepidochelys kempii* Garman, 1880], separated around 5 Mya (Bowen et al. 1991).

In the present study we report the first record of *H. lowryi* on a hawksbill turtle (*E. imbricata*), as well as the first records of both *H. lowryi* and *H. tortugae* for the Atlantic Ocean. In order to provide insights into the evolution of *Hyachelia*, we propose a phylogenetic hypothesis based on molecular analyses, including species within Talitrida (Brevitalitridae, Hyalidae, Hyalellidae and Talitridae). According to the new host association and the currently accepted sea turtle phylogeny, we hypothesize the emergence of *Hyachelia* ancestor species on the crown Cheloniidae, and further speciation into *H. tortugae* on Chelonini and *H. lowryi* on Carettini, with posterior dispersal to green turtles. Based on the data, we estimate the divergence times of *H. lowryi* and discuss its association with hosts and biogeographical aspects.

## MATERIALS AND METHODS

Living sea turtles were sampled on nesting and feeding grounds from the hosts *C. caretta*, *C. mydas* and *E. imbricata*, in the municipalities of Feliz Deserto (10°17'59.0"S, 36°17'25.6"W), Arembepe

**Table 1.** Records of amphipods associated with sea turtles

Amphipod	Sea turtle host				References
	<i>C. caretta</i>	<i>C. mydas</i>	<i>L. olivacea</i>	<i>E. imbricata</i>	
<i>Ampithoe ramondi</i> Audouin, 1826	X				Caine 1986; Frick et al. 1998
<i>Ampithoe riedli</i> Krapp-Schickel, 1968		X			Zakhama-Sraieb et al. 2010
<i>Apocorophium acutum</i> (Chevreux, 1908)	X				Kitsos et al. 2005; Zakhama-Sraieb et al. 2010; Domènech et al. 2014
<i>Apohyale prevostii</i> (H. Milne Edwards, 1830) (as <i>Hyale nilssoni</i> )	X				Fuller et al. 2010
<i>Caprella andreae</i> Mayer, 1890	X				Caine 1986; Frick et al. 1998; Kitsos et al. 2005; Pfaller et al. 2008; Zakhama-Sraieb et al. 2010; Cabezas et al. 2013; Domènech et al. 2014; Iwasa-Arai et al. 2020
<i>Caprella equilibra</i> Say, 1818	X				Caine 1986; Frick et al. 1998; Pfaller et al. 2008; Domènech et al. 2014
<i>Caprella fretensis</i> Stebbing, 1878	X				Fuller et al. 2010
<i>Caprella penantis</i> Leach, 1814	X				Frick et al. 1998; Kitsos et al. 2005; Pfaller et al. 2008; Zakhama-Sraieb et al. 2010; Domènech et al. 2014
<i>Caprella scaura</i> Templeton, 1836	X				Pfaller et al. 2008
Caprellidae				X	Corrêa et al. 2014
<i>Cerapus</i> sp.	X				Frick et al. 2004
<i>Dulichella appendiculata</i> (Say, 1818)	X				Frick et al. 1998
<i>Elasmopus rapax</i> Costa, 1853	X				Caine 1986; Frick et al. 1998; Martín and Díaz 2003; Kitsos et al. 2005; Zakhama-Sraieb et al. 2010
<i>Erichthonius brasiliensis</i> (Dana, 1853)	X				Caine 1986; Frick et al. 1998
<i>Erichthonius punctatus</i> (Spence Bate, 1857)	X				Zakhama-Sraieb et al. 2010
Gammaridae				X	Corrêa et al. 2014
<i>Hyachelia lowryi</i> Serejo & Sittrop, 2009	X	X		X	Serejo and Sittrop 2009; Yabut et al. 2014; Present study
<i>Hyachelia tortugae</i> J.L. Barnard, 1967		X			Barnard 1967; Yabut et al. 2014; Robinson et al. 2016; Valencia et al. 2018
<i>Hyachelia</i> sp.				X	Loghmannia et al. 2021
<i>Hyale</i> sp.	X				Krapp-Schickel 1993; Zakhama-Sraieb et al. 2010
Hyalidae				X	Corrêa et al. 2014
<i>Jassa</i> sp.	X				Myers 1989; Zakhama-Sraieb et al. 2010
<i>Monocorophium acherusicum</i> (Costa, 1853)	X				Kitsos et al. 2005; Zakhama-Sraieb et al. 2010; Domènech et al. 2014
<i>Paracaprella tenuis</i> Mayer, 1903	X				Caine 1986; Frick et al. 1998; Domènech et al. 2014
Podoceridae	X				Fuller et al. 2010
<i>Podocerus brasiliensis</i> (Dana, 1853)	X				Caine 1986
<i>Podocerus cheloniae</i> (Stebbing, 1888)	X				Caine 1986; Frick et al. 1998
<i>Podocerus chelonophilus</i> (Chevreux & Guerne, 1888)	X	X	X		Baldinger 2000; Kitsos et al. 2005; Zakhama-Sraieb et al. 2010; Lazo-Wasem et al. 2011; Robinson et al. 2016; Iwasa-Arai et al. 2020
<i>Protohyale (Protohyale) grimaldii</i> (Chevreux, 1891)	X				Zakhama-Sraieb et al. 2010; Kitsos et al. 2005; Domènech et al. 2014
<i>Protohyale (Protohyale) schmidtii</i> (Heller, 1866)	X				Fuller et al. 2010
<i>Stenothoe minuta</i> Holmes, 1905	X				Caine 1986; Frick et al. 1998; Domènech et al. 2014
<i>Stenothoe</i> sp.	X				Zakhama-Sraieb et al. 2010

(12°45'54.8"S, 38°10'11.5"W) and Santa Cruz (19°57'40.3"S, 40°07'57.5"W), in the states of Alagoas, Bahia and Espírito Santo, Brazil, respectively. Epibionts were manually collected *in situ* with tweezers, fixed in a 70–99% alcohol solution, stored at room temperature and then later analysed under a stereomicroscope for species identification. Specimens were identified according to the original description from Barnard (1967) and Serejo and Sittrop (2009), and remarks from Yabut et al. (2014). One specimen of each species was dissected and mounted on permanent slides. All materials were deposited at the Zoology Museum at Universidade Estadual de Campinas (ZUEC).

Two specimens of *H. lowryi* and two of *H. tortugae*, and species of Hyalidae present in the southeast Brazilian coast [*Hyale macrodactyla* Stebbing, 1899, *Parhyale hawaiiensis* (Dana, 1853), *Ptilohyale littoralis* (Stimpson, 1853) and *Serejohyale youngi* (Serejo, 2001)] were subjected to molecular analyses. Total genomic DNA was obtained using a CTAB extraction protocol (Doyle and Doyle 1987). Fragments of the mitochondrial gene cytochrome oxidase I (*COI*, ~720 bp) and the nuclear gene 18S rRNA (~1500 bp) were amplified using the UCOIF (5' TAW ACT TCD GGR TGR CCR AAA AAY CA 3') and UCOIR (5' ACW AAY CAY AAA GAY ATY GG 3') primers for *COI* (Costa et al. 2009) and the 18SGF (5' GGATAACTGTGGTAATTCCAGAGCT 3') and 18SGR-2 (5' TAGTAGCGACGGGCGGTGTGTA 3') primers for 18S rRNA (Hou et al. 2007). Amplification reactions included approximately 50 ng of genomic DNA, 1 U of DNA polymerase (QIAGEN), 1.5 µL of QIAGEN DNA Polymerase Buffer (5×), 0.2 mM of dNTPs, 2.5 mM of MgCl<sub>2</sub> and 0.3 µM of each primer. PCR conditions were: one cycle of 3 min at 95°C followed by 35 cycles of 30 s at 95°C, 45 s at 48°C, and 1 min at 72°C. All PCR products were purified using the PEG purifying protocol (<http://labs.icb.ufmg.br/lbem/protocolos/peg.html>) and sequenced in both directions using ABI 3500 automated DNA Sanger sequencers (Applied Biosystems).

The obtained sequences were trimmed using GeneStudio 2.2.0.0. (GeneStudio Inc.). Multiple sequence alignment of all markers was performed with MAFFT v.7 using the strategy G-INS-i (Katoh et al. 2005), with the following parameters: gap penalty of 1.53 for *COI* and 3.0 for 18S rRNA; scoring matrix for nucleotide sequences of 200PAM/K2; offset value of 0.0. Sequences were deposited in GenBank (Table S1).

Sequences available for both *COI* and 18S rRNA of other Talitroidea families were also included in the analyses. The best partition schemes and models were determined in ModelFinder 1.5.4 (Kalyaanamoorthy et al. 2017) based on the modified Akaike Information

Criterion (AICc) available on IQ-TREE 2 web server (Nguyen et al. 2015). The optimal partitioning strategy and evolutionary models consisted of GTR+F+I+G4 for the three *COI* codon partitions and 18S rRNA. A maximum likelihood gene tree was inferred using IQTree web server (Nguyen et al. 2015; <http://iqtree.cibiv.univie.ac.at/>), and the support of the nodes was evaluated with 1000 ultrafast bootstrap replications. Bayesian Inference analyses were conducted in BEAST 1.10.4 (Drummond et al. 2012) on the CIPRES server (Miller et al. 2010) using 10<sup>8</sup> generations, sampling every 1000 generations. *Quadrinemaera inaequipis* (A. Costa in Hope 1851) (Hadziida: Maeridae) was used to root the phylogeny based on previous analyses by Copilaş-Ciocianu et al. (2020), whereas three species of Corophiida, Gammaridae and Crangonyctidae were also used as outgroups (Table S1).

Divergence times were calculated with BEAST 1.10.4 (Drummond et al. 2012) using the *COI*+18S rRNA dataset with an uncorrelated relaxed clock with a lognormal distribution (Drummond et al. 2006) and codon partitioning for *COI*. For the tree model, a random starting tree was used, and speciation was modelled using the Birth-Death Process. The MCMC chain was run for 10<sup>8</sup> iterations, with a thinning of 1000. Estimated divergence times were based on the fossil calibration scheme described in detail by Copilaş-Ciocianu et al. (2019 2020) with three calibration points on fossil Crangonyctidae (minimum age of 35 Ma), Gammaridae (minimum age of 9 Ma) and Talitridae (minimum age of 25 Ma). Effective sample sizes of parameters and convergence were checked with Tracer 1.7.1 (Rambaut et al. 2018) after discarding 20% of the trees as burn-in. Two independent runs were performed with concordant results. The resulting files were then combined using LogCombiner 1.8 (Drummond et al. 2012), and the maximum clade credibility tree was produced using TreeAnnotator 1.8 (Drummond et al. 2012). Sea turtle phylogenetic relationships and their estimated ages were used based on the molecular phylogeny proposed by Naro-Maciel et al. (2008).

## RESULTS

### TAXONOMY

**Order Amphipoda Latreille, 1816**  
**Family Hyalidae Bulyčeva, 1957**  
**Subfamily Hyacheliinae Bousfield and**  
**Hendrycks, 2002**  
**Genus *Hyachelia* J. L. Barnard, 1967**



***Hyachelia lowryi* Serejo and Sittrop, 2009**

(Figs. 1 and 2)

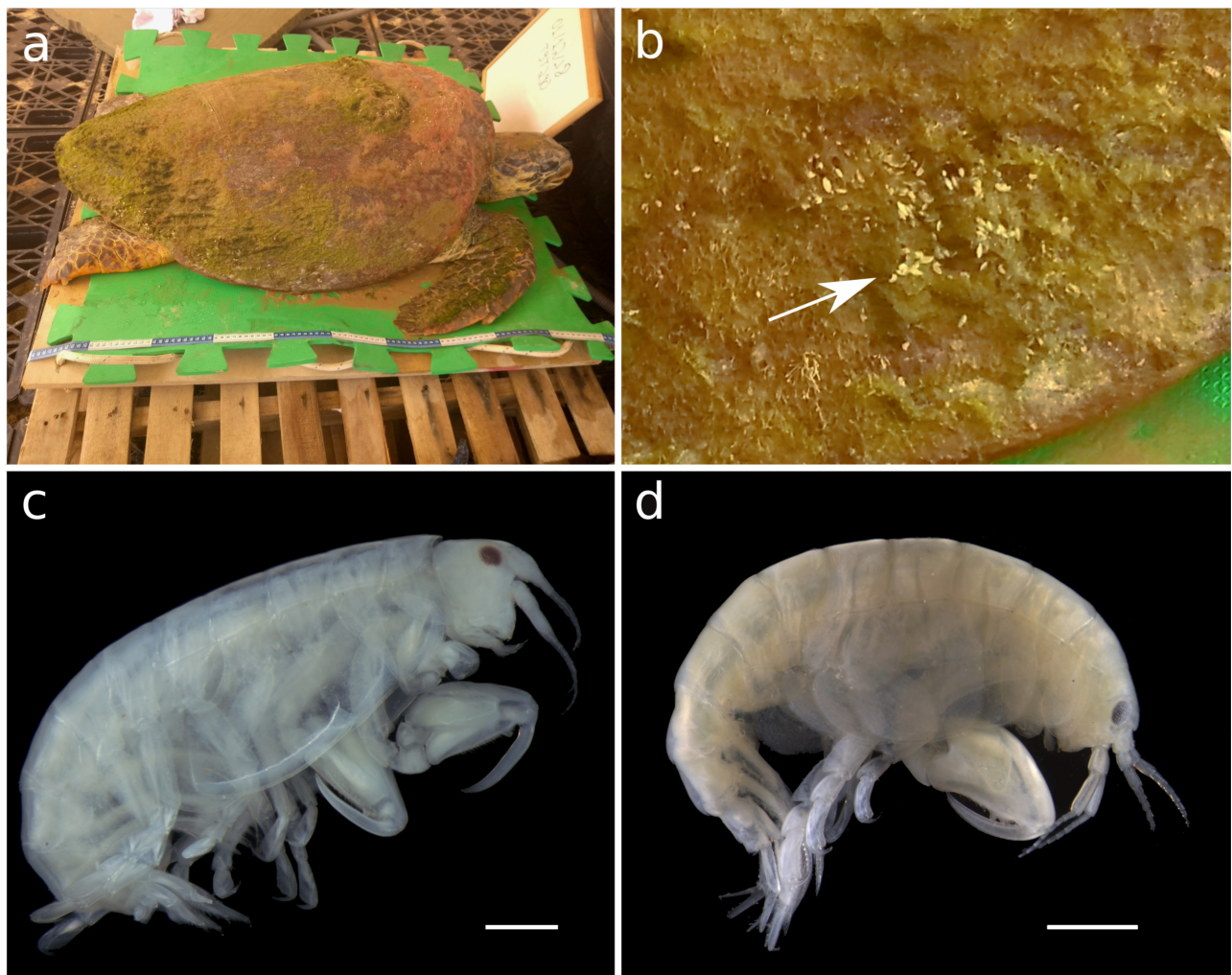
*Hyachelia lowryi* Serejo and Sittrop, 2009: 441–444, figs. 1–2; Yabut et al. 2014: 5–6, figs. 1B, 5B, 6.

**Material examined:** 5 males, Feliz Deserto, Alagoas, Brazil (10°17'59.0"S, 36°17'25.6"W), ZUEC CRU 4385; 13 males, 9 females and 4 juveniles, Arembepe, Bahia, Brazil (12°45'54.8"S, 38°10'11.5"W).

**Distribution:** Type locality: Mon Repos, Queensland, Australia (~24°48'S, 152°26'E), on loggerhead turtle *Caretta caretta* (Linnaeus, 1758) and green turtle *Chelonia mydas* (Linnaeus, 1758) (Serejo and Sittrop 2009). Palmyra Atoll National Wildlife Refuge (5°53'N, 162°5'W), on *C. mydas* (Yabut et al. 2014). Atlantic Ocean: Feliz Deserto, Alagoas (10°17'59.0"S, 36°17'25.6"W), Brazil, on hawksbill

turtle *Eretmochelys imbricata* (Linnaeus, 1766) (present study) (Fig. 2). Arembepe, Bahia (12°45'54.8"S, 38°10'11.5"W), Brazil, on *C. caretta* (present study).

**Remarks:** The genus *Hyachelia* presents two species, *Hyachelia lowryi* and *H. tortugae* that are obligate commensals of marine turtles. *Hyachelia lowryi* is very distinct from *H. tortugae* and the Brazilian material displayed the overall morphology of the original description from Queensland, Australia provided by Serejo and Sittrop (2009). Differences between *H. lowryi* and *H. tortugae* (in parentheses) are: palp of maxilla 1 reaching the base of outer lobe setal-teeth (vs vestigial); presence of a long whip-like seta on the male palp of maxilliped (vs short seta); coxa 4 wider, about 1.2x wider than long (vs as long as wide); propodus of pereopods 3–7 with 7 robust setae (vs 4 robust setae); and the inner ramus of uropods 1–2 with



**Fig. 1.** a, Hawksbill turtle (*Eretmochelys imbricata*) stranded in Alagoas, northeast Brazil. b, Amphipods (*Hyachelia lowryi*) associated to *E. imbricata*, arrow indicates *H. lowryi* specimens. c, *Hyachelia lowryi* Serejo and Sittrop, 2009 (ZUEC CRU 4385). d, *Hyachelia tortugae* Barnard, 1967 (ZUEC CRU 4386).

4–5 setae (vs lacking setae) (Serejo and Sittrop 2009). Previous records from the Palmyra Atoll National Wildlife Refuge reported both *Hyachelia* species, *H. lowryi* and *H. tortugae* on green turtles (*Chelonia mydas*), where they can co-occur in the same turtle host (Yabut et al. 2014). In our study, *H. lowryi* were absent on the sampled green turtles. This is the first record of *H. lowryi* on a hawksbill turtle [*Eretmochelys imbricata* (Linnaeus, 1766)], as well as the first observation in the Atlantic Ocean for both hawksbill and loggerhead turtles.

### *Hyachelia tortugae* Barnard, 1967

(Figs. 1 and 3)

*Hyachelia tortugae* Barnard, 1967: 120–125, figs. 1–4; Aguirre et al. 1998: 93; Yabut et al. 2014: 5, figs. 1A, 3, 4, 5A; Robinson et al. 2016: 1235–1237; Valencia et al. 2018: 86–88, figs. 1, 2.

**Material examined:** 8 males, 5 females, 2 juveniles, Santa Cruz, Aracruz, Espírito Santo, Brazil (19°57'40.3"S, 40°07'57.5"W), ZUEC CRU 4386.

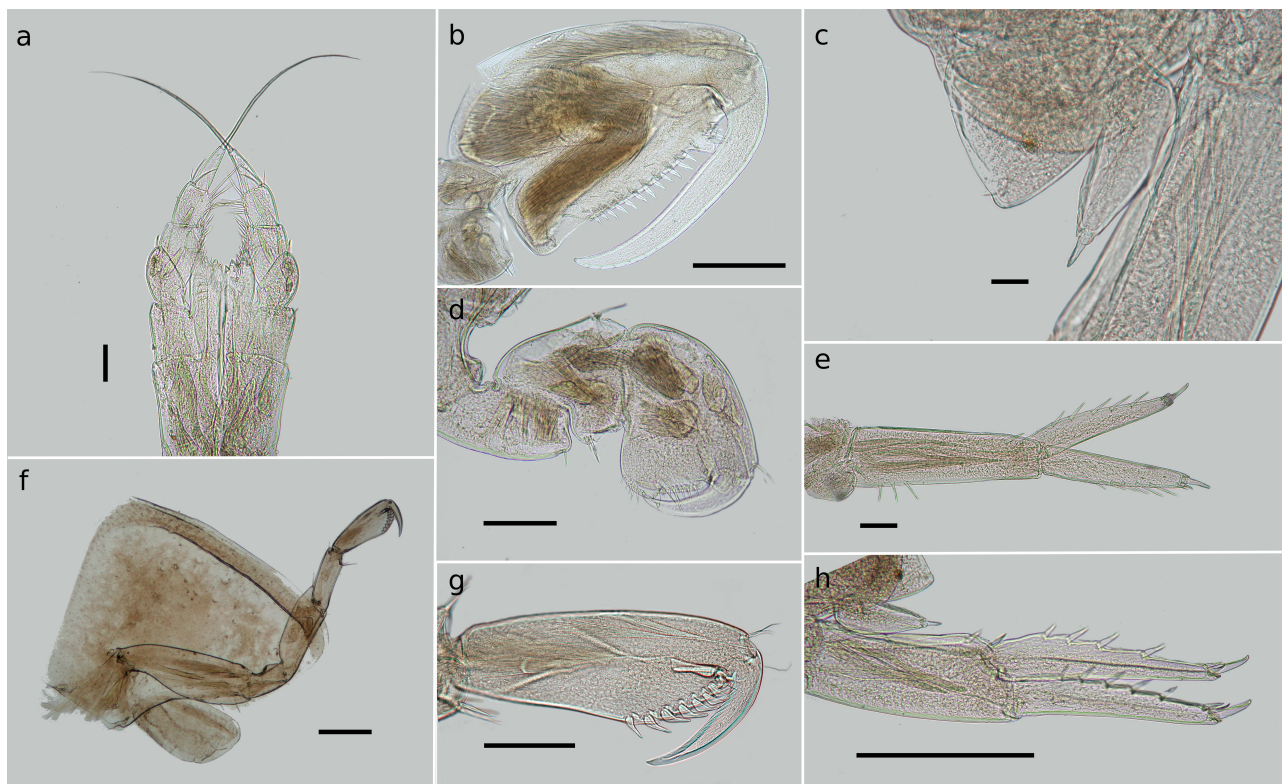
**Distribution:** Type locality: Porto Nuñez, Santa Cruz Island, Galapagos (~0°45'S, 90°20'W), on green turtle *Chelonia mydas* (Barnard, 1967). Palmyra

Atoll National Wildlife Refuge (5°53'N, 162°5'W), on *C. mydas* (Yabut et al. 2014). Parque Nacional Marino Las Baulas, Guanacaste, Costa Rica (10°20'N, 85°51'W), on *C. mydas* (Robinson et al. 2017). Gorgona Island, Colombia (2°58'00"N, 78°11'24"W), on *C. mydas* (Valencia et al. 2018). Atlantic Ocean: Santa Cruz, Aracruz, Espírito Santo, Brazil (10°17'59.0"S, 36°17'25.6"W), on *C. mydas* (present study).

**Remarks:** *Hyachelia tortugae* from the Brazilian green turtles displayed the overall morphology found in the original description provided by Barnard (1967) and remarks from Yabut et al. (2014). This species was observed only on green turtle hosts.

### Phylogenetic analysis

Sequences of species found on the Brazilian southeastern coast (*H. macrodactyla*, *P. hawaiiensis*, *P. littoralis* and *S. youngi*) as well as *H. lowryi* and *H. tortugae* are newly available. Due to the low sampling of Talitrida, which comprises more than 700 spp (Horton et al. 2023), we resume our discussion of the relationships among *Hyachelia* spp. Within the infraorder Talitrida, there are four families represented in the present analysis: Brevitalitridae (terrestrial),



**Fig. 2.** *Hyachelia lowryi* Serejo and Sittrop, 2009 (ZUEC CRU 4385). a, Maxilliped, scale bar: 0.1 mm. b, Gnathopod 2, scale bar: 1.0 mm. c, Uropod 3, scale bar: 0.1 mm. d, Gnathopod 1, scale bar: 0.5 mm; e) Uropod 1, scale bar: 0.5 mm. f, Pereopod 4, scale bar: 0.5 mm. g, Pereopod 7, scale bar: 0.5 mm; h) Uropod 2, scale bar: 1.0 mm.



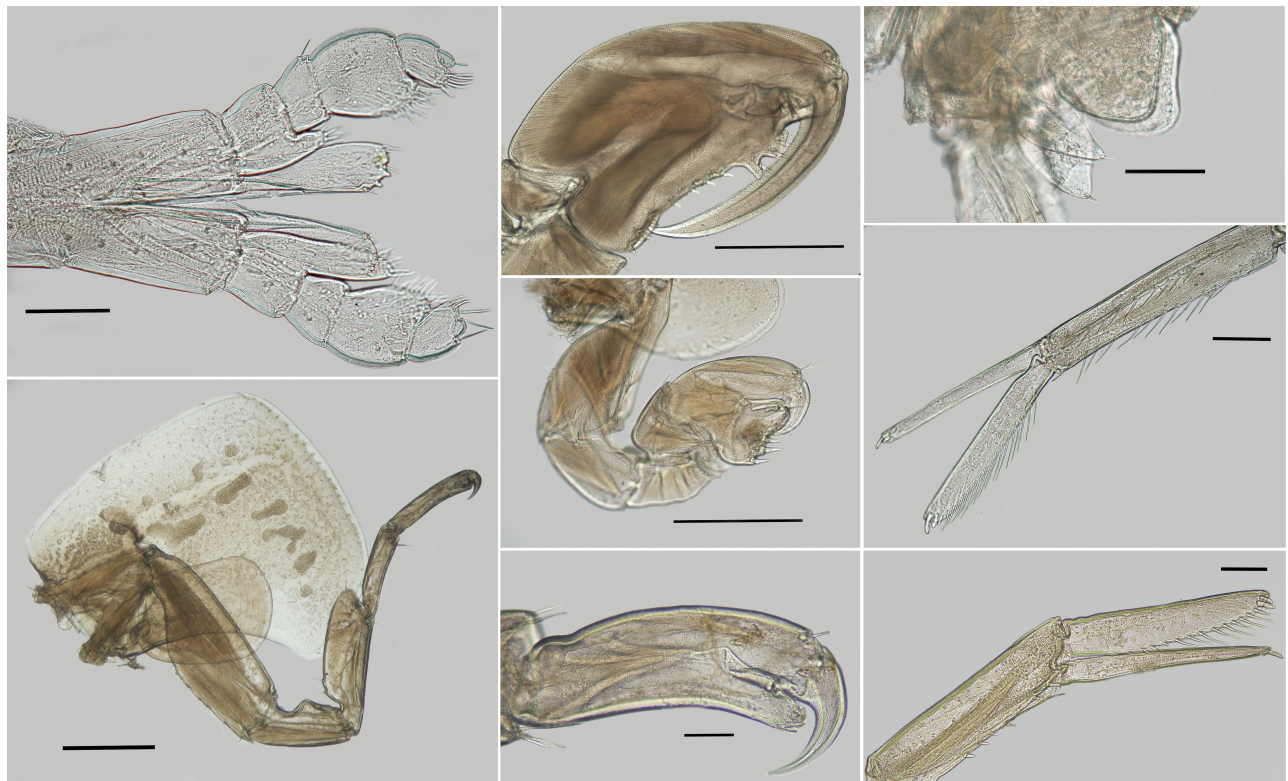
Hyalidae (marine, shallow infralittoral and commensal), Hyalellidae (freshwater) and Talitridae (terrestrial or marine supralittoral). Hyalidae includes two subfamilies: The possibly polyphyletic Hyalinae (Fig. 4); and Hyachelinae. Hyachelinae, comprised by *H. lowryi* and *H. tortugae*, was recovered both in ML and BI trees (Figs. 4 and S1). The *Hyachelia* clade showed long branch attraction, suggesting phylogenetic uncertainty, especially for 18SrRNA (Fig. S1), complicating attempts to map the evolution of the gene (Lindgren and Daly 2007). Therefore, a more comprehensive view of the evolutionary history requires knowledge of other molecular markers and inclusion of more sibling taxa to help understand the relationships.

Divergence time estimates are in agreement with the ages of the infraorder Amphipoda inferred by Copilaş-Ciocianu et al. (2020). According to our analyses, the ancestor of the genus *Hyachelia* originated in the Cretaceous around 127.66 Mya [95% highest posterior density intervals (HPD): 87.00–174.64] and possibly represents the very first split of the infraorder Talitrida (Fig. 1). Within the genus, *Hyachelia tortugae* and *H. lowryi* split in the Miocene, around 26.16 Mya (95% HPD: 9.36–46.43 Mya, Fig. 4).

## DISCUSSION

This is the first approach on the phylogeny of Talitrida with molecular data, including new data on Brazilian species. Thirty-two species of amphipods are facultative commensals of sea turtles worldwide (Table 1). The unique morphology of *Hyachelia lowryi* and *H. tortugae* includes the presence of several synapomorphies, including: coxae 1–4 longer than wide, without posterior processes; propodi of pereopods 3–7 prehensile and with distal robust setae; and uropod 3 lacking rami, which are believed to result from its obligate commensal habit (Serejo and Sittrop 2009).

The first observation of the association between the genus *Hyachelia*, and sea turtles dates back to its description, based on the conspicuous morphological differences from the extant Hyalidae and Talitridae amphipods. Despite its morphological adaptations to the commensal lifestyle, there are only a few records of *Hyachelia* worldwide, regardless of the widespread distribution of sea turtle hosts, while facultative commensal species are more commonly found (Pfäller et al. 2008; Zakhama-Sraieb et al. 2010; Domènech et al. 2014; Iwasa-Arai et al. 2020). Cleaning behaviours



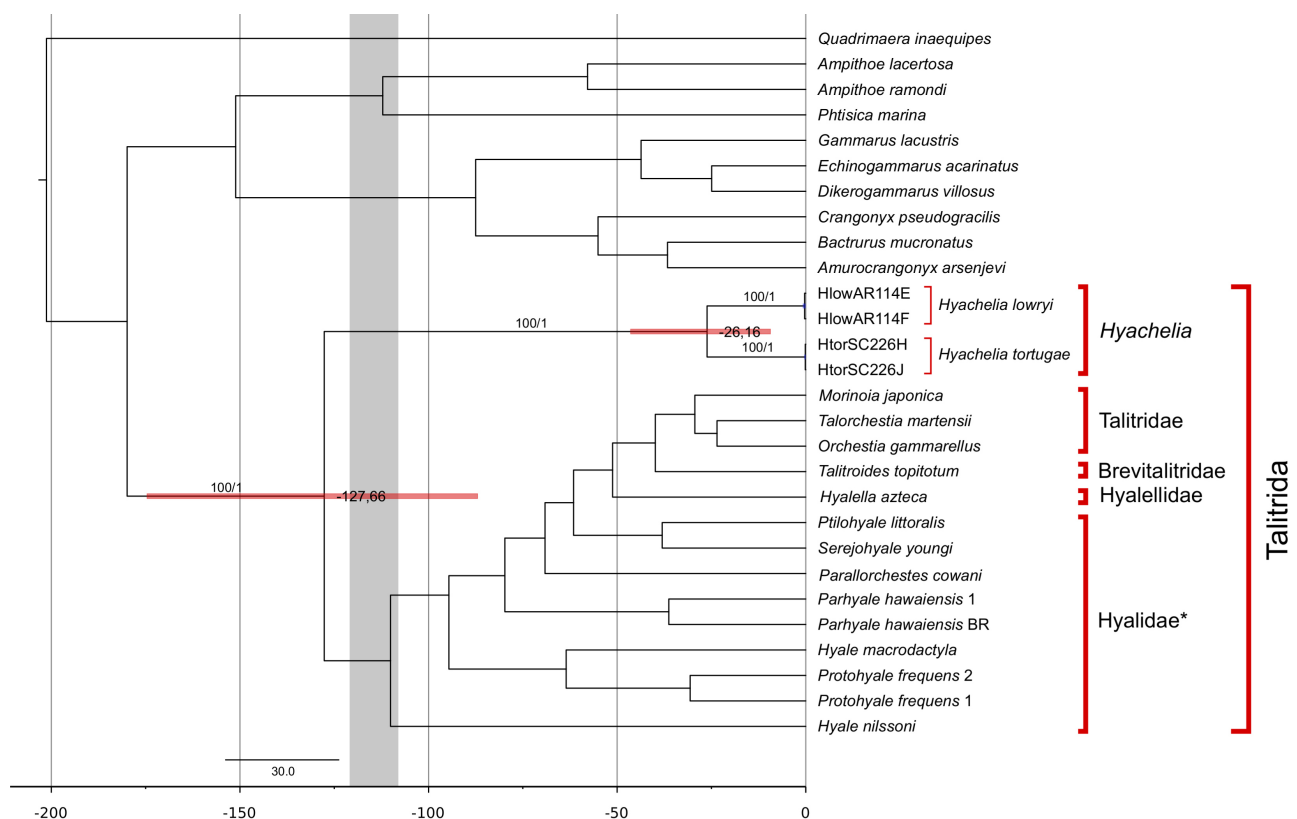
**Fig. 3.** *Hyachelia tortugae* Barnard, 1967 (ZUEC CRU 4386). a, Maxilliped, scale bar: 0.1 mm. b, Gnathopod 2, scale bar: 1.0 mm. c, Uropod 3, scale bar: 0.1 mm. d, Gnathopod 1, scale bar: 0.5 mm. e, Uropod 1, scale bar: 0.5 mm. f, Pereopod 4, scale bar: 0.5 mm. g, Pereopod 7, scale bar: 0.5 mm. h, Uropod 2, scale bar: 1.0 mm.

performed by fishes on sea turtles might change the epibiont fauna, as observed by Sazima et al. (2004) and Grossman et al. (2006) for *C. mydas* and *E. imbricata* in the oceanic island of Fernando de Noronha, northeastern Brazil.

*Hyachelia* is an intriguing genus that was originally described within Hyalidae by Barnard (1967), and later transferred to Ceinidae J.L. Barnard, 1972 by Barnard and Karaman (1991), based on the absence of a ramus on uropod 3 (Serejo 2004). Afterwards, Bousfield and Hendrycks (2002) returned *Hyachelia* to Hyalidae due to the presence of a preamplifying notch in mature females of *H. tortugae*, as well as morphological similarities to *Hyale*, particularly in uropod 3 and the telson, and proposed the subfamily Hyacheliinae. Later on, Serejo (2004) studied the superfamily Talitroidea based on cladistic analyses and recovered *Hyachelia* as part of the subfamily Hyacheliinae, family Hyalidae, suggesting that *Hyachelia* evolved from free-living hyalid-like ancestors. In a more recent revision that dealt with the phylogeny and establishment of the suborder Senticaudata, Hyacheliinae (*Hyachelia*) was again recovered as part of the Hyalidae family (Lowry

and Myers 2013).

In order to provide insights into the evolution of *Hyachelia* (Hyalidae), we propose a phylogenetic hypothesis based on molecular analyses, including species within the families Hyalidae, Hyaellidae and Talitridae, historically known as sister groups (Bulycheva 1957; Serejo 2004; Lowry and Myers 2013). In the present phylogenetic analysis, Hyalidae is paraphyletic, as species of Brevitalitridae, Talitridae and Hyaellidae are grouped together. As a large and complex group, the infraorder Talitrida includes 768 species within 4 superfamilies (Caspicoidea Birstein, 1945; Hyaloidea Bulycheva, 1957; Kurioidea Barnard, 1964; Talitroidea Rafinesque, 1817) (Horton et al. 2021), and we need a much more inclusive analysis to discuss it. Moreover, *Hyachelia* is recovered as a monophyletic group in both the ML and BI trees with an early divergence from the sister clade that includes the remaining Talitrida (Hyalidae, Hyaellidae and Talitridae) (Figs. 1 and S1). Species relationships were mainly recovered in both the ML and BI analyses for *P. frequens* and *H. macrodactyla*, *S. youngi* and *P. littoralis*, and *T. martensii*, *O. gammarellus*, *M. japonica*

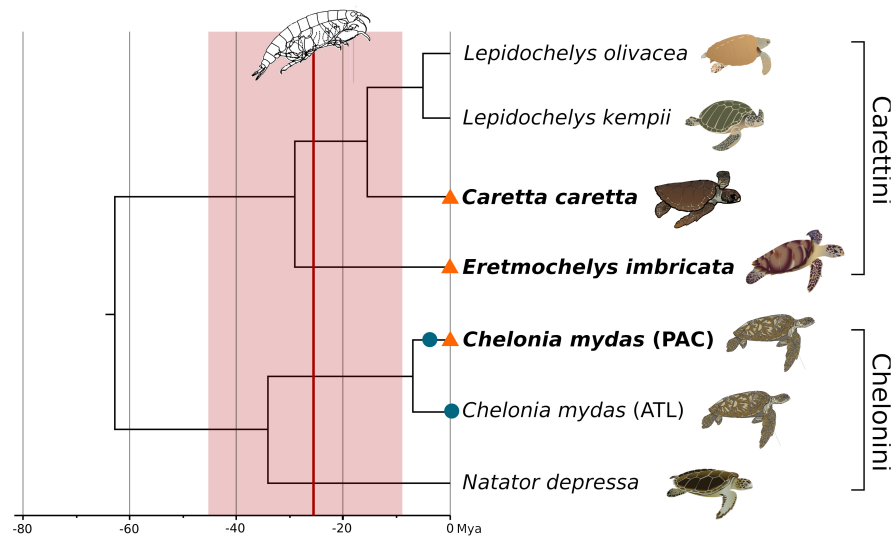


**Fig. 4.** COI+18S rRNA tree of Talitrida based on available sequences. Node values correspond to the estimated divergence time of *Hyachelia*. Red bars show the 95% highest posterior density. Bootstraps and posterior probability values are represented above branches for the Talitrida, *Hyachelia*, *H. lowryi* and *H. tortugae*. Red horizontal bars correspond to the 95% highest posterior density intervals (HPD). The gray vertical bar corresponds to Chelonioidea radiation according to Kear and Lee (2006).

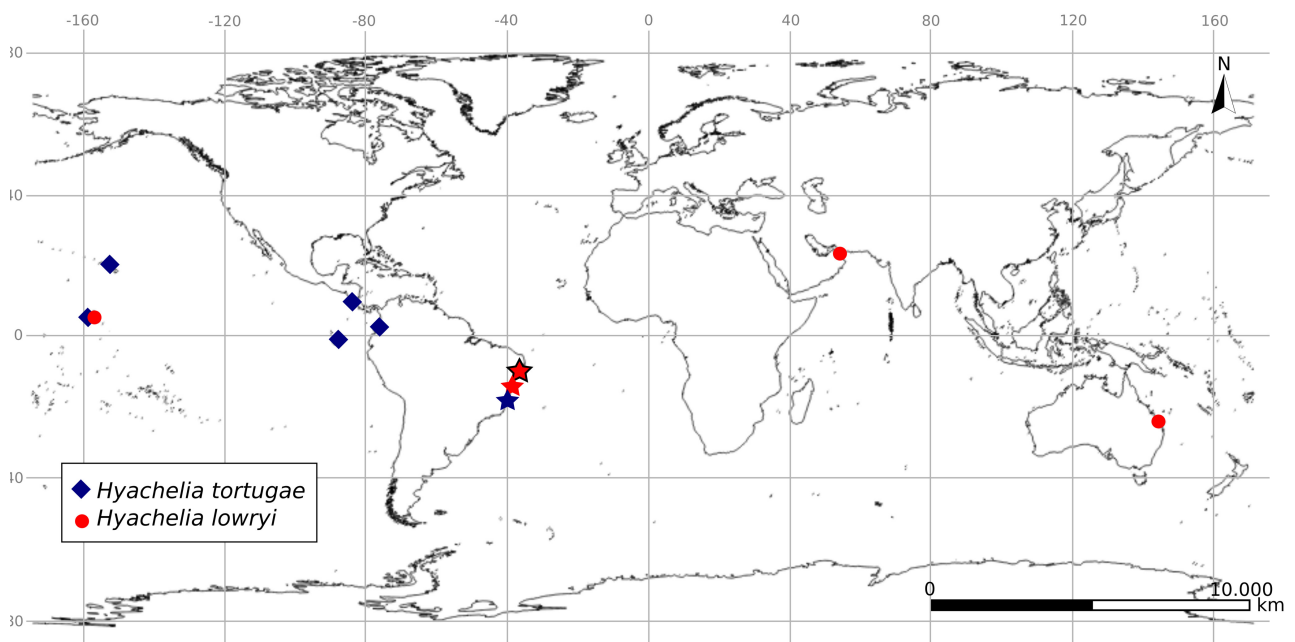
and *T. topitotum* (Figs. 1 and S1).

Divergence times of *Hyachelia* were estimated in the Cretaceous, around 127.66 Ma (95% HPD: 87.00–174.64 Ma, Fig. 1). Five distinct sea turtle lineages existed around 100 Ma (Kear and Lee 2006), which included the two lineages correspondent to the modern cheloniids and dermocheliids (leatherback turtles). Thus, *Hyachelia* ancestors could be associated with older Chelonioidae ancestors, or as free-living amphipods. In contrast, *H. lowryi* and *H. tortugae* showed a more

recent split in the Paleogene, around 26.16 Ma (95% HPD: 9.36–46.43 Ma). According to the divergence times estimated by Naro-Maciel et al. (2008), Chelonini separated from Caretteni about 63 Ma (95% HPD: 35.59–91.38 Ma), whereas *Eretmochelys* split from *Caretta* and *Lepidochelys* about 29 Ma, and Pacific and Atlantic *Chelonia* populations split around 7 Ma. Therefore, a coevolutionary pattern between the split of Chelonini/Caretteni and *H. lowryi*/*H. tortugae* is evidenced (Fig. 5). As the analysis inferred an



**Fig. 5.** Currently accepted Cheloniidae relationships and divergence times. The red bar corresponds to the *Hyachelia lowryi* and *H. tortugae* divergence time of 26.16 Ma. (95% HPD: 9.36–46.43). Species in bold represent *Hyachelia* occurrences. Turtle drawings are adapted from Vecta.io and Biorender. Orange triangles represent known *H. lowryi* hosts and blue dots represent known *H. tortugae* hosts.



**Fig. 6.** Current known distribution of *Hyachelia*. Stars correspond to new records, and stars with black outlines correspond to new host records.

ancient colonisation of sea turtles by these specialised amphipods, our initial hypothesis of emergence of the *Hyachelia* ancestor species followed by speciation into *H. tortugae* in Chelonini and *H. lowryi* in Carettini, and later dispersion to *C. mydas*, is therefore plausible.

The presence of *H. lowryi* in the three sea turtle species (*Chelonia mydas*, *Caretta caretta* and *Eretmochelys imbricata*) is corroborated by the known interspecific interactions and even hybridization between them (Bowen and Karl 2007; Reis et al. 2010a b; Vilaça et al. 2012; Kelez et al. 2016). During these interactions, both *Hyachelia* species could be transmitted from one host to another; however, possible competition between commensal species might favour the prevalence of *H. lowryi* in green, loggerhead and hawksbill turtles, while *H. tortugae* remains restricted to green turtles.

The new record of *H. lowryi* in the Atlantic Ocean reveals an important step to uncover the geographic distribution of this singular genus. With the cosmopolitan distribution of the marine turtles, it is expected that *Hyachelia* is a much more widespread genus, but more sampling is needed to understand this pattern (Fig. 6). For now, partnerships between carcinologists and local sea turtle monitoring organisations are the best option for obtaining more information regarding biogeographic patterns and other aspects of this unusual genus. Our study also broadens the knowledge of the association between the obligate commensal genus *Hyachelia* and its host the sea turtles. We also shed light on the molecular evolution of *Hyachelia* and related species within the families Hyalidae, Hyaellidae and Talitridae. Further investigation on the genomics, ecology and systematics of Talitrida may help to elucidate the evolutionary processes that drove *Hyachelia* into an obligatory commensal lifestyle, essential knowledge since they are useful indicators of sea turtle health and migration.

## CONCLUSIONS

The genus *Hyachelia*, previously agreed only in the Pacific Ocean, was herein documented for the first time in the Atlantic Ocean, with the first record of *Hyachelia lowryi* on the hawksbill turtle, showing broad distribution worldwide. The first molecular data of both *Hyachelia lowryi* and *H. tortugae* suggest the origin of the genus around the Cretaceous, possibly in ancestors of green turtles, while *H. lowryi* and *H. tortugae* diverged about 26 Ma, suggesting a coevolutionary association between amphipods and Carettini/Chelonini sea turtles.

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**Authors' contributions:** TI-A conceived this study, performed morphological identification, molecular analyses, and drafted the manuscript. SS performed the morphological dissection and provided microscopy figures. CM, MS, MW, UL and LM provided the specimens. LM performed molecular analyses. FL, CS and SA supervised and acquired funding. All authors read and approved the final manuscript.

**Competing interests:** The authors declare that they have no competing interests.

**Availability of data and materials:** The data that support this study are available in the article and accompanying online supplementary material.

**Consent for publication:** Not applicable.

**Ethics approval consent to participate:** Not applicable.

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## Supplementary materials

**Fig. S1.** Maximum likelihood tree of *COI*+18SrRNA of Hyalidae and outgroups Ampithoidae, Caprellidae, Crangonyctidae, Gammaridae, Hyaellidae and Talitridae. Numbers above branches correspond to bootstrap values above 80%. (download)

**Table S1.** Species used in the phylogenetic analyses and accession numbers. (download)