

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

Tammy Iwasa-Arai^{1,2,3,*}, Sônia C.S. Andrade³, Camila Miguel⁴, Silvana G.L. Siqueira², Max Rondon Werneck⁵, Fosca P.P. Leite², Lara Moraes³, Marcelo Renan D. Santos⁴, Luciana S. Medeiros^{6,7}, Uylia H. Lopes⁷, and Cristiana Serejo⁸

¹*Programa de Pós-Graduação em Biologia Animal, Universidade Estadual de Campinas (UNICAMP). Campinas, São Paulo, Brazil. *Correspondence: E-mail: araitammy@gmail.com (Iwasa-Arai)*

²*Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas 13083-970, SP, Brazil. E-mail: soniacsandrade@ib.usp.br (Andrade); silvsbio@gmail.com (Siqueira); fosca@unicamp.br (Leite)*

³*Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo 05508-090, SP, Brazil. E-mail: soniacsandrade@ib.usp.br (Andrade); larademoraess@gmail.com (Moraes)*

⁴*Projeto Chelonia mydas - Instituto Marcos Daniel, R. José Alexandre Buaiz, 190 - Sala 1713 - Enseada do Suá, Vitória, 29050-545, ES, Brazil. E-mail: camila.miguel@acad.pucrs.br (Miguel); mrenansantos@gmail.com (Santos)*

⁵*Instituto BW para Conservação e Medicina da Fauna Marinha. Araruama, Rio de Janeiro, Brazil. E-mail: maxrwerneck@gmail.com (Werneck)*

⁶*Programa de Pós-Graduação em Ciência Animal, Universidade Federal de Alagoas (UFAL). Viçosa, 57700-970, AL, Brazil. E-mail: luasmvet@hotmail.com (Medeiros)*

⁷*Instituto Biota de Conservação, Maceió, 57038-770, AL, Brazil. E-mail: uyllalopes@gmail.com (Lopes)*

⁸*Laboratório de Carcinologia, Departamento de Invertebrados, Museu Nacional/UFRJ, Rio de Janeiro, 20940-040, RJ, Brazil. E-mail: csserejo@acd.ufrj.br (Serejo)*

ORCID:

Tammy Iwasa-Arai: <https://orcid.org/0000-0002-3654-8671>

(Received 1 March 2023 / Accepted 23 October 2023 / Published -- 2023)

Communicated by Ryuji Machida

Obligate commensalism in the marine environment and its evolutionary role are still poorly investigated. Although sea turtles may serve as ideal substrates for epibionts, within amphipods,

only the genus *Hyachelia* evolved as obligate commensal. 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 (18SrRNA) genes using new and available sequences from the infraorder Talitrida. Divergence times for *Hyachelia* are inferred around the Cretaceous (~127.66 Ma), corresponding to an ancient origin, in agreement with modern green turtles (Chelonioida) radiation. Further on, diversification of *Hyachelia* species dated about 26 Ma, suggesting a coevolutionary association between amphipods and Carettini/Chelonini sea turtles.

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

Citation: Iwasa-Arai T, Andrade SCS, Miguel C, Siqueira SGL, Werneck MR, Leite FPP, Moraes L, Santos MRD, Medeiros LS, Lopes UH, Serejo C. 2023. Coevolutionary implications of obligate commensalism in sea turtles: the case of the genus *Hyachelia* Barnard, 1967 (Crustacea, Amphipoda). *Zool Stud* **62:54**.

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 host and epibionts, with the likelihood of epibiosis resulting from a trade-off between cost and benefits of the epibionts involved. The majority of these organisms is 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 thus referred to as obligate commensals (Frick and Pfaller 2013). The evolutionary roles of such strict association are still poorly understood.

Within turtles and other large marine vertebrates, crustaceans represent one of the most diverse group 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.

Table 1. Records of amphipods associated with sea turtles

Amphipod	Sea turtle host				Reference
	<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; Domenech 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; Domenech 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; Domenech 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; Domenech 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>Dulichchiella appendiculata</i> (Say, 1818)	X				Frick et al. 1998
<i>Elasmopus rapax</i> Costa, 1853	X				Caine 1986; Frick et al. 1998; Martín & 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 & 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; Domenech et al. 2014
<i>Paracaprella tenuis</i> Mayer, 1903	X				Caine 1986; Frick et al. 1998; Domenech 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; McGrath & Myers 1989; Domenech 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; Domenech et al. 2014
<i>Stenothoe</i> sp.	X				Zakhama-Sraieb et al. 2010

The other two amphipod obligate commensal species, *H. tortugae* and *H. lowryi* have a much more restricted distribution and host specificity. *Hyachelia tortugae* previously reported on green turtles and *H. lowryi* on green and loggerhead turtles, both species 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 related to 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 in 11 genera (Horton et al. 2021). Free living hyalids are predominantly found among algae and biofouling substrates of tropical and subtropical zones inhabiting the intertidal and shallow infralittoral areas of the world (Serejo and Sittrop 2009).

While relationships within Hyalidae are yet to be understood, 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 (Chelonioidea), the most speciose family is Cheloniidae Oppel, 1811, which comprises six of the seven extant species, and it 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 from each other around 34 Ma (Naro-Maciel et al. 2008). Carettini includes the hawksbill [*Eretmochelys imbricata* (Linnaeus, 1766)], separated from the remaining Carettini clade around 29 Ma (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 Ma (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

Sea turtles were sampled alive 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 (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 in permanent slides. All material is 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 gene (*COI*, ~720 bp) and the nuclear gene 18SrRNA (~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 18SGF (5' GGATAACTGTGGTAATTCCAGAGCT 3') and 18SGR-2 (5' TAGTAGCGACGGGCGGTGTGTA 3') primers for 18SrRNA (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 MgCl₂ 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 sequences obtained 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 (Kato 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 18SrRNA 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 18SrRNA.

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 1.000 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 10^8 generations, sampling every 1.000 generations. *Quadrimeaera inaequipes* (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+18SrRNA 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 10^8 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

TANONOMY

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)

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 found on 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 1.2 x 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 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* from 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.

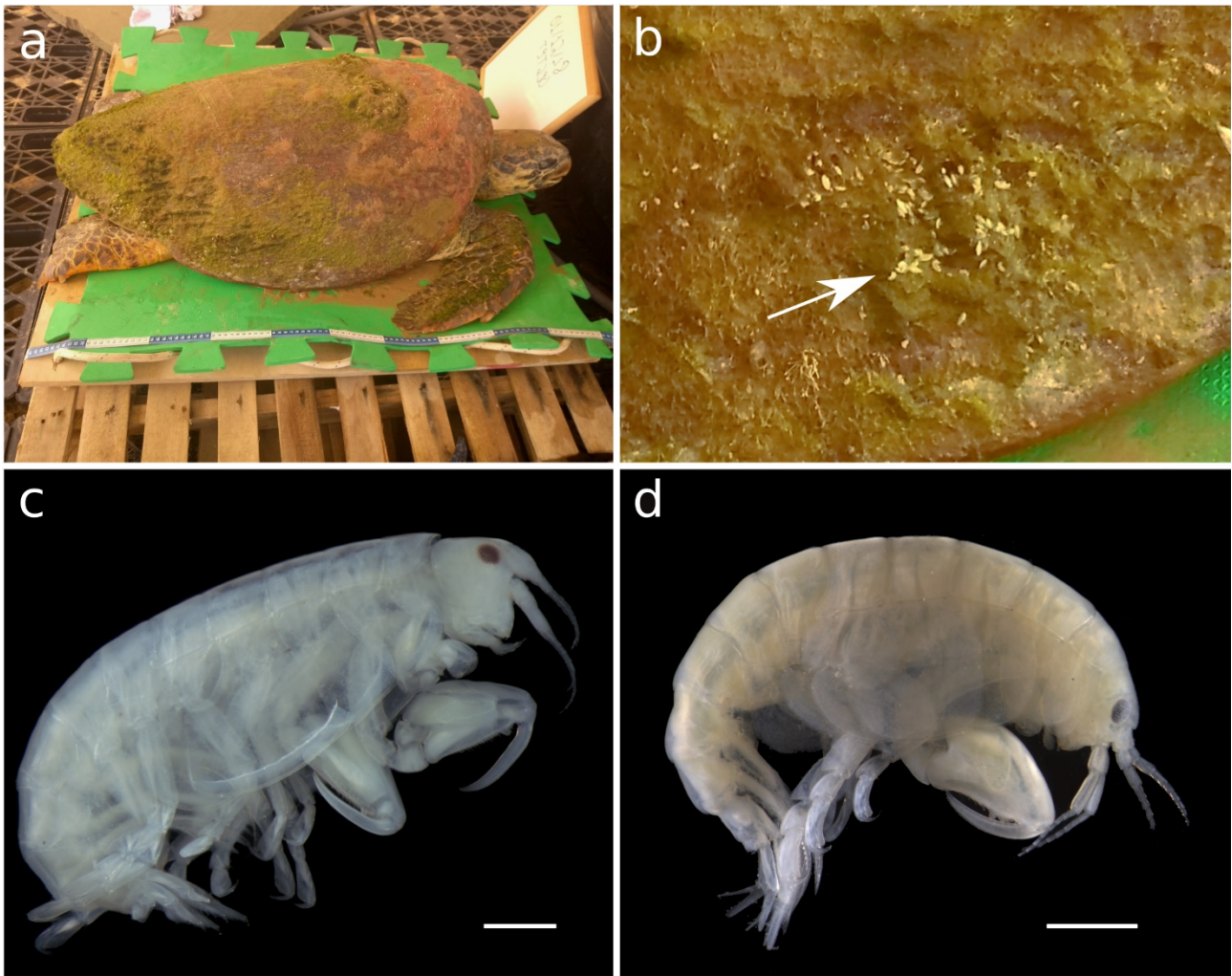


Fig. 1. a, Hawksbill turtle (*Eretmochelys imbricata*) stranded in Alagoas, northeast Brazil. b, Amphipods (*Hyachelia lowryi*) associated to *E. imbricata*, arrow indicates *H. lowryi* specimens. c, *Hyachelia lowryi* Serejo and Sittrop, 2009 (ZUEC CRU 4385). d, *Hyachelia tortugae* Barnard, 1967 (ZUEC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

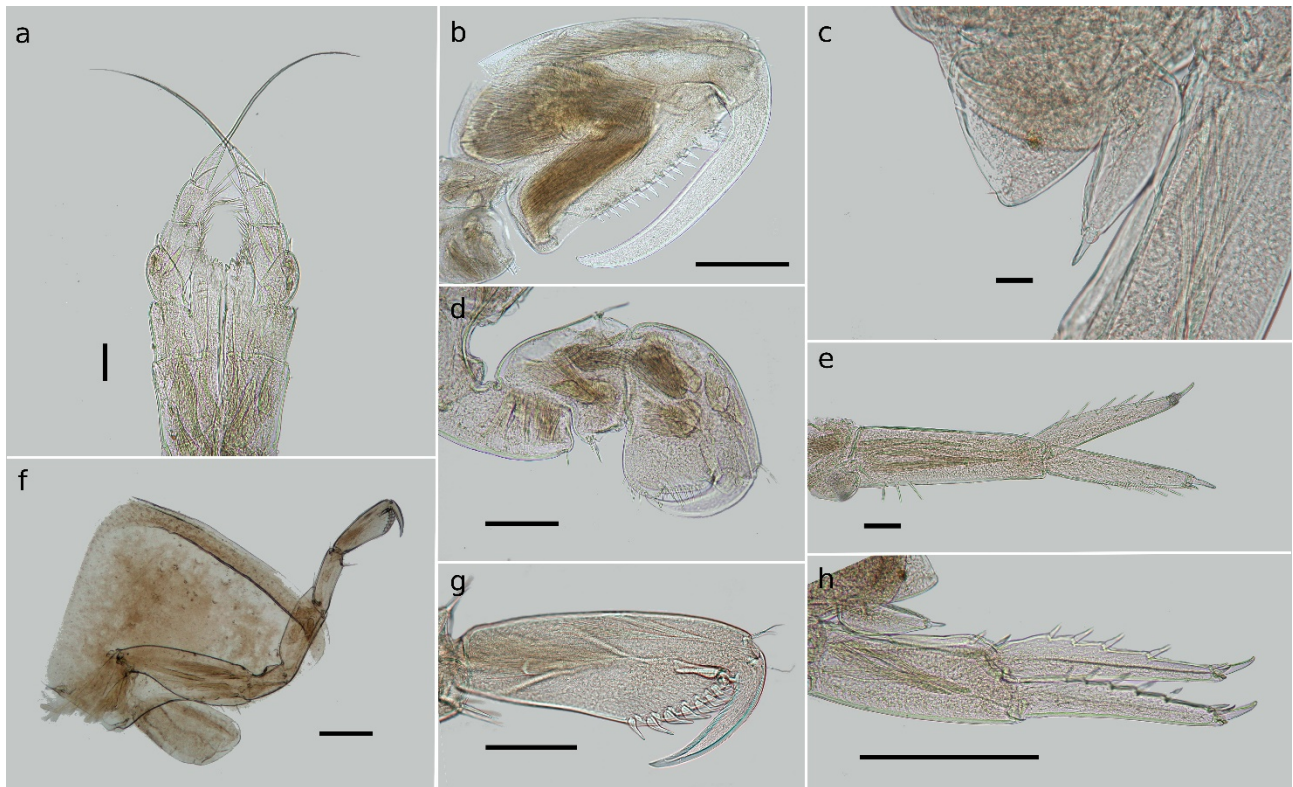


Fig. 2. *Hyachelia lowryi* Serejo and Sittrop, 2009 (ZUEC CRU 4385). a, Maxilliped, scale bar: 0.1 mm. b, Gnathopod 2, scale bar: 1.0 mm. c, Uropod 3, scale bar: 0.1 mm. d, Gnathopod 1, scale bar: 0.5mm; e) Uropod 1, scale bar: 0.5 mm. f, Pereopod 4, scale bar: 0.5 mm. g, Pereopod 7, scale bar: 0.5mm;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

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. 2017: 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 turtles hosts.

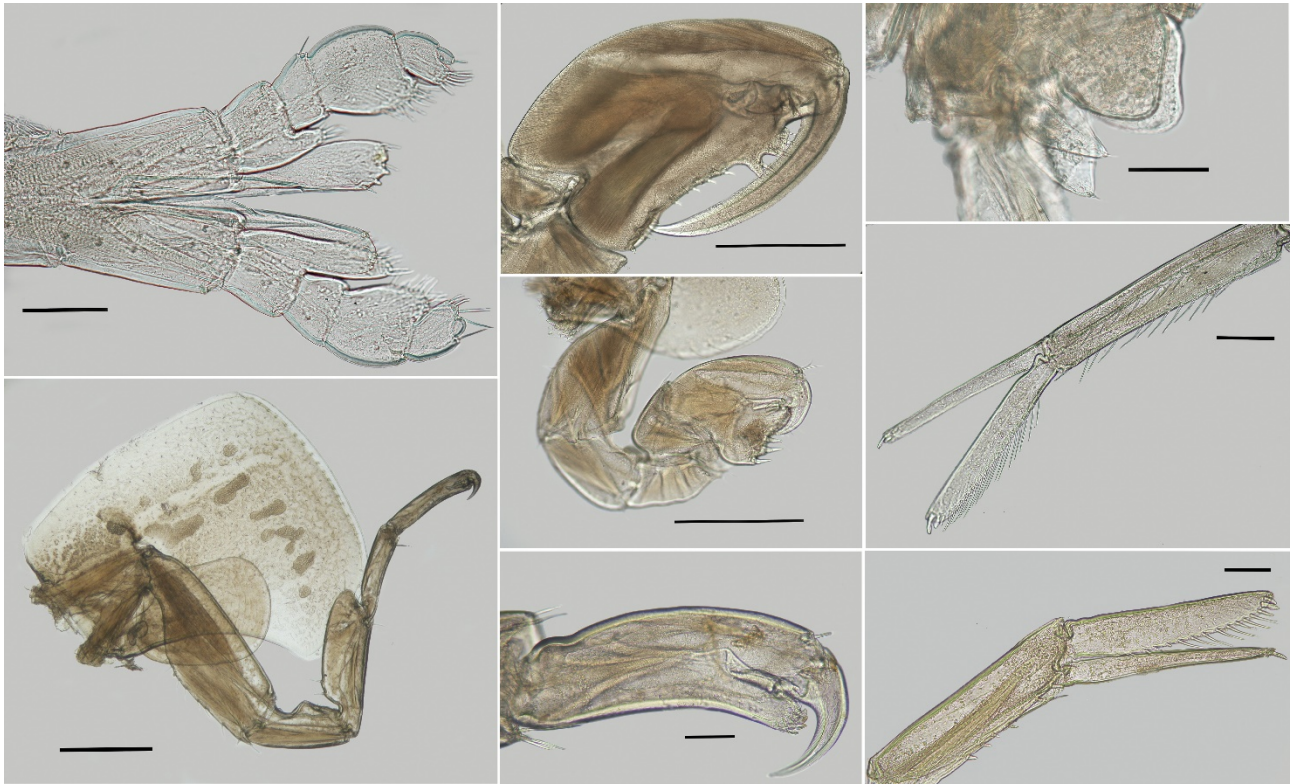


Fig. 3. *Hyachelia tortugae* Barnard, 1967 (ZUEC CRU 4386). a, Maxilliped, scale bar: 0.1 mm. b, Gnathopod 2, scale bar: 1.0 mm. c, Uropod 3, scale bar: 0.1 mm. d, Gnathopod 1, scale bar: 0.5 mm. e, Uropod 1, scale bar: 0.5 mm. f, Pereopod 4, scale bar: 0.5 mm. g, Pereopod 7, scale bar: 0.5 mm. h, Uropod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

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 to the relationships among *Hyachelia* spp. Within the infraorder Talitrida, there are four families represented in the present analysis: Brevitalitridae (terrestrial), Hyalidae (marine, shallow infralitoral and commensal), Hyalellidae (freshwater) and Talitridae (terrestrial or marine supralitoral). Hyalidae includes two subfamilies: Hyalinae is suggested as polyphyletic (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 other molecular markers and inclusion of more sibling taxa may help in understanding of 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 Ma [95% highest posterior density intervals (HPD): 87.00-174.64] and possibly represent 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 Ma (95% HPD: 9.36-46.43, Fig. 4).

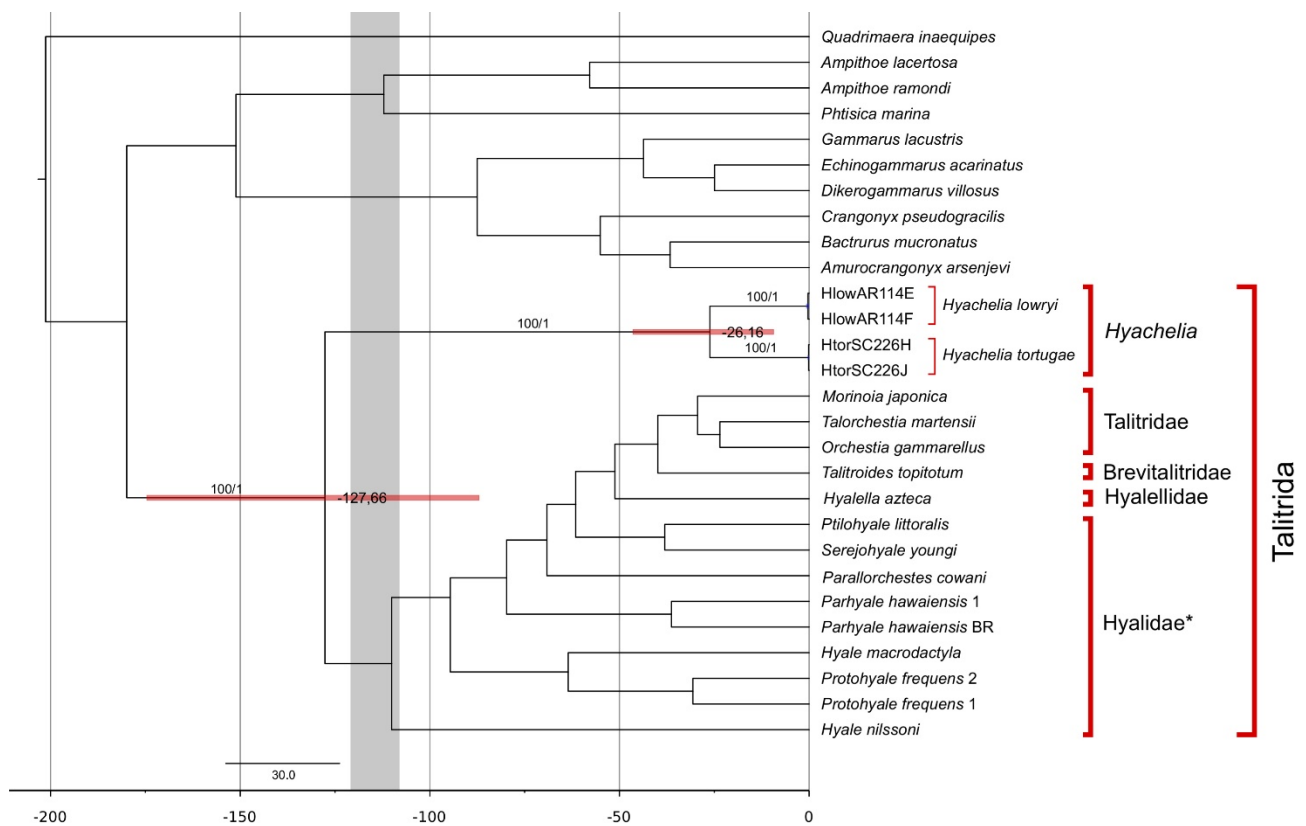


Fig. 4. COI+18SrRNA tree of Talitrida based on available sequences. Node values correspond to estimated divergence time of *Hyachelia*. Red bar shows the 95% highest posterior density. Bootstraps and posterior probability values are represented above branches for the Talitrida, *Hyachelia*, *H. lowryi*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

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 reported as facultative commensals on sea turtles all over the world (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; propodus 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 the widespread distribution of sea turtles hosts, while facultative commensal species are more commonly found (Pfaller et al. 2008; Zakhama-Sraieb et al. 2010; Domenech et al. 2014; Iwasa-Arai et al. 2020). Cleaning behaviour performed by fishes on sea turtles might change the epibiont fauna, as observed by Sazima et al. (2004a) 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 preamplexing notch in mature females of *H. tortugae*, as well as morphological similarities to *Hyale*, particularly in uropod 3 and 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 families Hyalidae, Hyalellidae and Talitridae, historically known as sister groups (Bulycheva 1957; Serejo 2004; Lowry and Myers 2013). In the present phylogenetic analysis, Hyalidae turned out to be paraphyletic, as species of Brevitalitridae, Talitridae and Hyalellidae were grouped together. As a large and complex group, the infraorder Talitrida includes 768 species within 4 superfamilies (Caspicoloidea Bisrtein, 1945; Hyaloidea Bulycheva, 1957; Kurioidea Barnard, 1964; Talitroidea Rafinesque, 1817) (Horton et al. 2021), and we do need a much more inclusive analysis to discuss it. Moreover, *Hyachelia* is recovered as a monophyletic group in both ML and BI trees with an early

divergence from the sister clade that includes the remaining Talitrida (Hyalidae, Hyalellidae and Talitridae) (Figs. 1 and S1). Species relationships were mainly recovered in both ML and BI analyses, as for *P. frequens* and *H. macrodactyla*, *S. youngi* and *P. littoralis*, and *T. martensii*, *O. gammarellus*, *M. japonica* 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, 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 Chelonioidea 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). According to the divergence times estimated by Naro-Maciel et al. (2008), Chelonini separated from Caretteni about 63 Ma (95% HPD: 35.59 Ma–91.38 Ma), whereas *Eretmochelys* split from *Caretta* and *Lepidochelys* about 29 Ma, and *Chelonia* populations from Pacific and Atlantic split around 7 Ma. Therefore, a coevolutionary pattern between the split of Chelonini/Caretteni and *H. lowryi*/*H. tortugae* is herein evidenced (Fig. 5). As the analysis inferred an 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 Caretteni, and later dispersion to *C. mydas*, is therefore plausible.

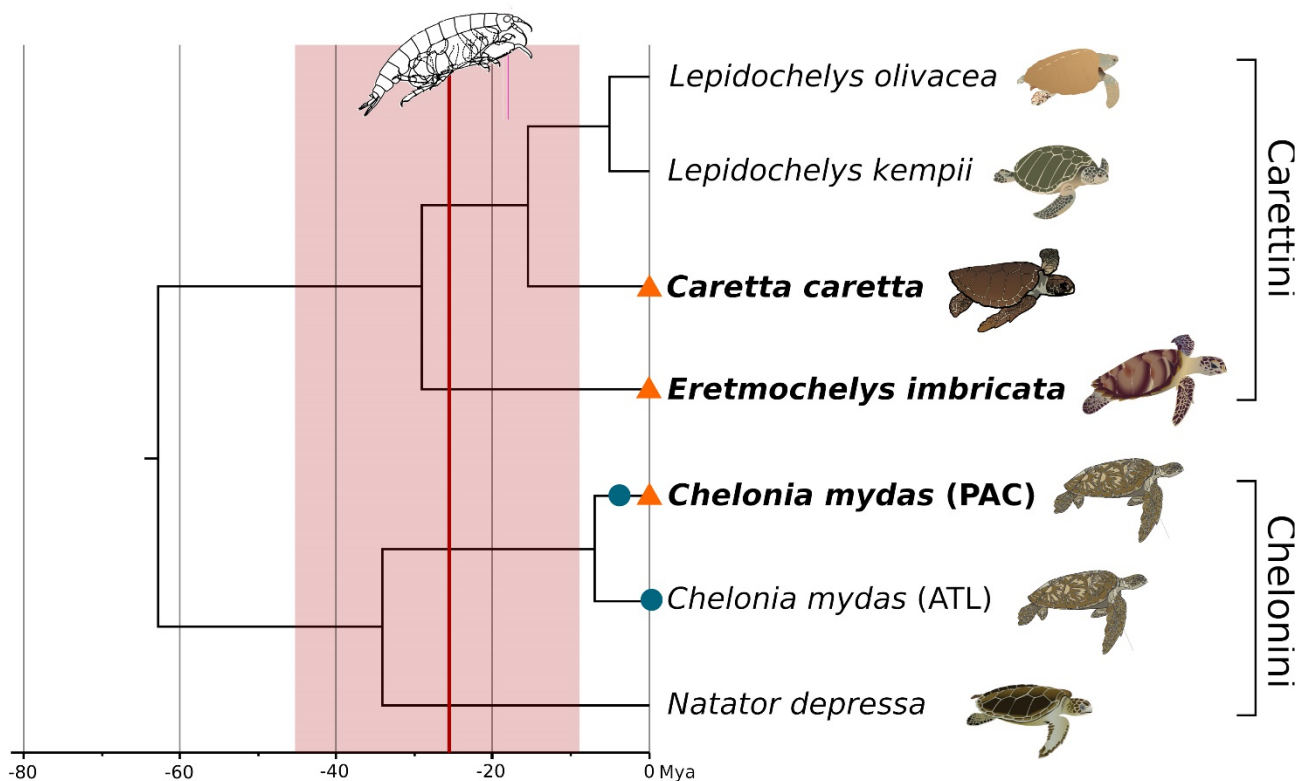


Fig. 5. Currently accepted Chelonidae relationships and divergence times. Red bar correspond to *Hyachelia lowryi* and *H. tortugae* divergence times of 26.16 Ma. (95% HPD: 9.36–46.43). Species

in bold represent *Hyachelia* occurrences. Turtle drawings adapted from Vecta.io and Biorender. Orange triangles represent *H. lowryi* known hosts and blue dots represent *H. tortugae* known hosts.

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. 2010; Reis et al. 2010; 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 unpuzzle 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, Hyalellidae and Talitridae. Further investigation on the genomics, ecology and systematics of Talitrida may help to elucidate the processes that drove the evolution of *Hyachelia* into an obligatory commensal lifestyle, essential since they are useful indicators of sea turtle health and migration.

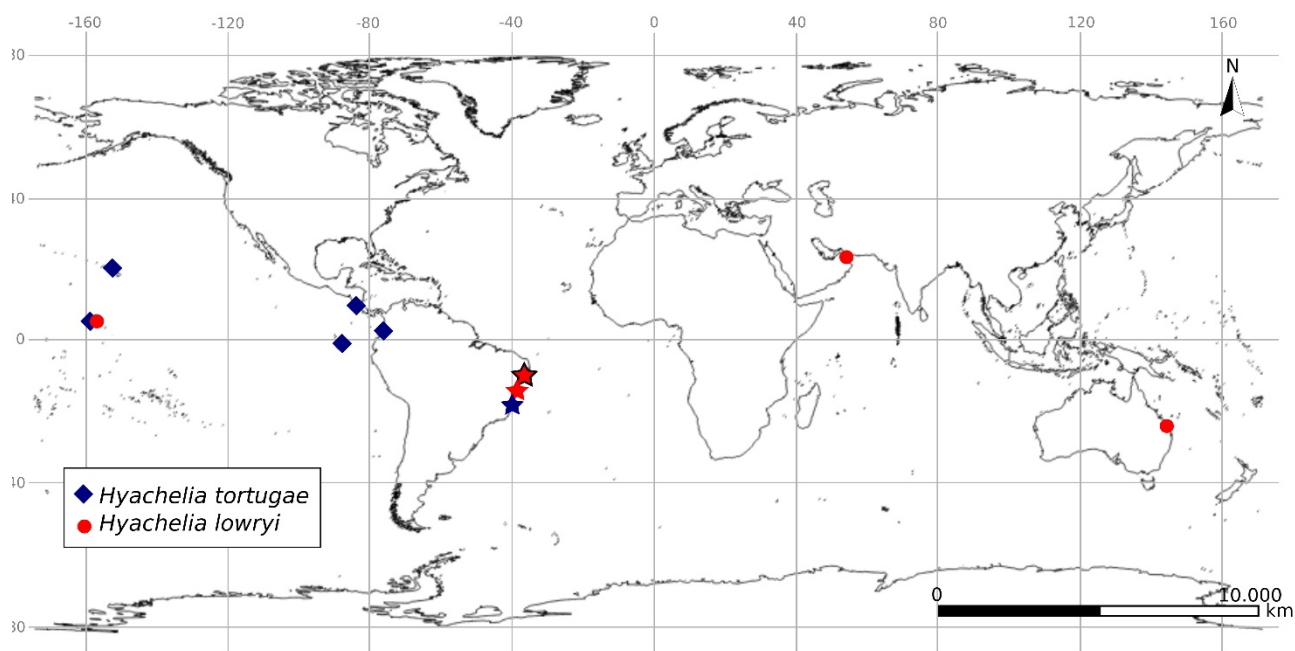


Fig. 6. Current known distribution of *Hyachelia*. Stars correspond to new records, star with black stroke correspond to new host record.

CONCLUSIONS

The genus *Hyachelia*, previously known only for the Pacific Ocean, was firstly reported for the Atlantic Ocean herein, 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 green turtles ancestors, while *H. lowryi* and *H. tortugae* diverged from each other about 26 Ma, suggesting a coevolutionary association between amphipods and Carettini/Chelonini sea turtles.

Acknowledgments: We are extremely grateful to the Instituto Biota and Projeto *Chelonia mydas* (Instituto Marcos Daniel) team that collected the samples. We thank Pedro Longo (Unicamp) for Hyalidae sequences. We are thankful to R. B. Bressan for the English review. We thank FAPESP for TI-A's scholarship (2018/00488-7) and TI-A, SS and FL grant (2018/10313-0). This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grants no. 2018/00488-7 and 2018/10313-0.

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.

REFERENCES

- Barnard JL. 1967. New genus of Galapagan amphipod inhabiting the buccal cavity of the sea-turtle, *Chelonia mydas*. In: Proceedings of Symposium on Crustacea Part 1:119–125. Mandapam Camp: Marine Biological Station of India.
- Barnard JL, Karaman GS. 1991. The families and genera of marine Amphipoda (except marine Gammaroids). *Rec Austr Mus* **13**:1–866. doi:10.3853/j.08127387.13.1991.91.
- Bousfield EL, Hendrycks EA. 2002. The talitroidean amphipod family Hyalidae revised, with emphasis on the North Pacific fauna: systematics and distributional ecology. *Amphipacifica* **3**:17–134.
- Bowen BW, Meylan AB, Avise JC. 1991. Evolutionary distinctiveness of the endangered Kemp's ridley sea turtle. *Nature* **352**:709–711. doi:10.1038/352709A0.
- Bowen BW, Karl SA. 2007. Population genetics and phylogeography of sea turtles. *Mol Ecol* **16**:4886–4907. doi:10.1111/j.1365-294X.2007.03542.x.
- Bulycheva AI. 1957. Beach-fleas of the seas of the USSR and adjacent waters (Amphipoda — Talitroidea). *Akademiia Nauk SSSR, Opredeliteli po Faune SSSR* **65**:1–185.
- Callahan CM. 2008. Molecular systematics and population genetics of whale lice (Amphipoda: Cyamidae) living on gray whale islands. Dissertation, Humboldt State University, USA.
- Casale P, Tucker AD. 2017. *Caretta caretta* (amended version of 2015 assessment). The IUCN Red List of Threatened Species 2017, e.T3897A119333622. doi:10.2305/IUCN.UK.20172.RLTS.T3897A119333622.en. Accessed 02 June 2021.

- Copilaş-Ciocianu D, Sidorov D, Gontcharov A. 2019. Adrift across tectonic plates: molecular phylogenetics supports the ancient Laurasian origin of old limnic crangonyctid amphipods. *Org Divers Evol* **19**:191–207. doi:10.1007/s13127-019-00401-7.
- Copilaş-Ciocianu D, Borko Š, Fišer C. 2020. The late blooming amphipods: global change promoted post-Jurassic ecological radiation despite Palaeozoic origin. *Mol Phylogenet Evol* **143**:106664. doi:10.1101/675140.
- Corrêa GVV, Ingels J, Valdes YV, Fonsêca-Genevois VG, Farrapeira CMR, Santos GAP. 2014. Diversity and composition of macro-and meiofaunal carapace epibionts of the hawksbill sea turtle (*Eretmochelys imbricata* Linnaeus, 1822) in Atlantic waters. *Mar Biodivers* **44**:391–401. doi:10.1007/s12526-013-0189-9.
- Costa FO, Henzler CM, Lunt DH, Whiteley NM, Rock J. 2009. Probing marine Gammarus (Amphipoda) taxonomy with DNA barcodes. *Syst Biodivers* **7**:365–379. doi:10.1017/S1477200009990120.
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* **19**:11–15. doi:10.1016/0031-9422(80)85004-7.
- Drummond AJ, Ho SY, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol* **4**:88. doi:10.1371/journal.pbio.0040088.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* **29**:1969–1973. doi:10.1093/molbev/mss075.
- Duchene S, Frey A, Alfaro-Núñez A, Dutton PH, Gilbert MT, Morin PA. 2012. Marine turtle mitogenome phylogenetics and evolution. *Mol Phylogenet Evol* **65**(1):241–250. doi:10.1016/j.ympev.2012.06.010.
- Frick MG, Pfaller JB. 2013. *Sea Turtle Epibiosis*. In: Wyneken J, Lohmann KJ, Musick JA (eds) *The Biology of Sea Turtles, Volume III*. CRC, Boca Raton, pp. 399–426.
- Grossman A, Sazima C, Bellini C, Sazima I. 2006. Cleaning symbiosis between hawksbill turtles and reef fishes at Fernando de Noronha Archipelago, off Northeast Brazil. *Chelonian Conserv Biol* **5**:284–288. doi:10.2744/1071-8443(2006)5[284:CSBHTA]2.0.CO;2.
- Horton T, Lowry J, De Broyer C, Bellan-Santini D, Coleman CO, Corbari L, ..., Zeidler W 2022. World Amphipoda Database. Hyalidae Bulyceva, 1957. World Register of Marine Species. Available at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=490602>. Accessed 2 June 2022
- Horton T, Lowry J, De Broyer C, Bellan-Santini D, Coleman CO, Corbari L, ..., Zeidler W. 2022. World Amphipoda Database. Talitrida. World Register of Marine Species. Available at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=490602>. Accessed 2 Feb. 2023.

Zoological Studies **62**:54 (2023)

Hou Z, Fu J, Li S. 2007. A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. *Mol Phylogenet Evol* **45**:596–611. doi:10.1016/J.YMPEV.2007.06.006.

Hughes LE. 2016. Designation of neotypes for *Cyrtophium orientale* Dana, 1853, *Podocerus brasiliensis* (Dana, 1853) and *P. cristatus* (Thomson, 1879) and the description of a new species *Podocerus cyrenensis* (Crustacea: Amphipoda: Podoceridae). *Raffles Bull Zool* **34**:312–330.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat Methods* **14**:587–589. doi:10.1038/nmeth.4285.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* **30**:772–780. doi:10.1093/molbev/mst010.

Kear BP, Lee MSY. 2006. A primitive protostegid from Australia and early sea turtle evolution. *Biol Lett* **2**:116–119. doi:10.1098/rsbl.2005.0406.

Kelez S, Velez-Zuazo X, Pacheco AS. 2016. First record of hybridization between green *Chelonia mydas* and hawksbill *Eretmochelys imbricata* sea turtles in the Southeast Pacific. *PeerJ* **4**:1712. doi:10.7717/peerj.1712

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol Biol Evol* **34**(3):772–773. doi:10.1093/molbev/msw260.

Latreille PA. 1816. Amphipodes. *In*: Nouveau Dictionnaire d'histoire naturelle, appliquée aux arts, à l'Agriculture, à l'Economie rurale et domestique, à la Médecine, etc. Vol. 1. Par une Société de Naturalistes et d'Agriculteurs. Nouvelle Édition. Paris, Deterville, pp. 467–469.

Lindgren AR, Daly M. 2007. The impact of length-variable data and alignment criterion on the phylogeny of Decapodiformes (Mollusca: Cephalopoda). *Cladistics* **23**(5):464–476. doi:10.1111/j.1096-0031.2007.00160.x.

Loghmannia J, Nasrolahi A, Rezaie-Atagholipour M, Kiabi BH. 2021. Epibiont Assemblages on Nesting Hawksbill Turtles Show Site-Specificity in the Persian Gulf. *Front Ecol Evol* **9**: 690022. doi:10.3389/fevo.2021.69002.

Lowry JK, Myers AA. 2013. A phylogeny and classification of the Senticaudata subord. nov. (Crustacea: Amphipoda). *Zootaxa* **3610**(1):1–80. doi:10.11646/zootaxa.3610.1.1.

Mortimer JA, Donnelly M (IUCN SSC Marine Turtle Specialist Group). 2008. *Eretmochelys imbricata*. The IUCN Red List of Threatened Species 2008: e.T8005A12881238. Accessed 02 June 2021. doi:10.2305/IUCN.UK.2008.RLTS.T8005A12881238.en.

- Naro-Maciel E, Le M, FitzSimmons NN, Amato G. 2008. Evolutionary relationships of marine turtles: a molecular phylogeny based on nuclear and mitochondrial genes. *Mol Phylogenet Evol* **49**:659–662. doi:10.1016/j.ympev.2008.08.004.
- Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* **32**:268–274. doi:10.1093/molbev/msu300.
- Reis EC, Soares LS, Vargas SM, Santos FR, Young RJ, Bjorndal KA, ... Lobo-Hajdu G. 2010. Genetic composition, population structure and phylogeography of the loggerhead sea turtle: colonization hypothesis for the Brazilian rookeries. *Conserv Genet* **11**:1467–1477. doi:10.1007/s10592-009-9975-0.
- Reis EC, Soares LS, Lôbo-Hajdu G. 2010. Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. *Conserv Genet* **11**:1587–1591. doi:10.1007/s10592-009-9973-2.
- Sazima C, Grossman A, Bellini C, Sazima I. 2004. The moving gardens: reef fishes grazing, cleaning, and following green turtles. *Cybiurn* **28**:47–53.
- Schärer MT. 2003. A survey of the epibiota of *Eretmochelys imbricata* (Testudines: Cheloniidae) of Mona Island, Puerto Rico. *Rev Biol Trop* **51**:87–90.
- Seminoff JA. 2004. *Chelonia mydas*. The IUCN Red List of Threatened Species 2004: e.T4615A11037468. Accessed 02 June 2021. doi:10.2305/IUCN.UK.2004.RLTS.T4615A11037468.en.
- Serejo CS. 2004. Cladistic revision of talitroidean amphipods (Crustacea, Gammaridea), with a proposal of a new classification. *Zool Scr* **33**:551–586. doi:10.1111/j.0300-3256.2004.00163.x.
- Serejo CS, Sittrop DJ. 2009. Hyalidae. *Zootaxa* **2260**:440–452.
- Valencia B, Sampson L, Giraldo A. 2018. New record of *Hyachelia tortugae* Barnard, 1967, an amphipod epibiont on green turtles *Chelonia mydas* (Linnaeus, 1758) from Gorgona island (Colombian Pacific). *Boletín Científico del Museo de Historia Natural* **22(2)**:84–89.
- Vilaça ST, Vargas SM, Lara-Ruiz P, Molfetti E, Reis EC, Lobo-Hadju G, ... Santos FR. 2012. Nuclear markers reveal a complex introgression pattern among marine turtle species on the Brazilian coast. *Mol Ecol* **21**: 4300–4312. doi:10.1111/j.1365-294X.2012.05685.x.
- Wahl M, Mark O. 1999. The predominately facultative nature of epibiosis: Experimental and observational evidence. *Mar Ecol Progr Ser* **187**:59–66.
- Yabut MG, Lazo-Wasem EA, Sterling EJ, Gómez A. 2014. New Records of *Hyachelia tortugae* Barnard, 1967, and *H. lowryi* Serejo and Sittrop, 2009 (Amphipoda: Gammaridea: Hyalidae),

Zoological Studies **62**:54 (2023)

from Palmyra Atoll National Wildlife Refuge: Cooccurrence on Pacific Green Turtles
(*Chelonia mydas*). *Am Mus Novit* **3809**:1–12. doi:10.1206/3809.1.

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)