A new species of *Ampharete* Malmgren, 1866 (Annelida: Ampharetidae) from Washington and redescription of *A. cirrata* Webster & Benedict, 1887 and *A. labrops* Hartman, 1961

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Abstract

Ampharete acutifrons (Grube, 1860), originally described from Greenland, has long been considered a widely distributed arctic-boreal species. However, recent morphological re-assessment of the holotype indicates that most previous records of *A. acutifrons* were misidentifications, and molecular sequence data also suggest that *A. acutifrons* is a multispecies complex. This study focuses on specimens of the *A. acutifrons* species complex from Washington, USA with publicly available COI sequence data. Specimens from Washington belonging to the Invertebrate Zoology Collection of the Florida Museum of Natural History were examined. Additional specimens were examined for morphological comparison, including type material of *A. cirrata* Webster & Benedict, 1887 and *A. labrops* Hartman, 1961. Detailed morphological descriptions of specimens and photographs of the diagnostic characters were made. The molecular analysis includes 37 published COI sequences of *Ampharete* and *Anobothrus* species sourced from public databases. Redescriptions of type material of *A. cirrata* and *A. labrops* are provided. *Ampharete paulayi* **n. sp**. is described as a new species from Washington, USA based on morphological and COI sequences data. Photographs of living specimens are presented, a hypothesis on the development of buccal tentacles in *Ampharete* species is proposed, and the use of Methyl green stain is recommended as a standard practice in future descriptions of ampharetids.

Introduction

The family Ampharetidae Malmgren, 1866 comprises small, tubicolous benthic marine annelids commonly found in soft sediments from intertidal zones to deep sea environments (Rouse, Pleijel & Tilic, 2022). Within Ampharetidae, the genus *Ampharete* Malmgren, 1866 is the most species-rich, currently comprising about 50 nominal species and six synonymized genera (Kim et al., 2025; Read & Fauchald, 2025).

Ampharete acutifrons (Grube, 1860), the type species of *Ampharete*, was described based on a single specimen from Greenland. Historically, *A. acutifrons* was considered a broadly distributed arctic-boreal species, characterized by the presence of long dorsal cirri on abdominal neuropodia (*e.g.*, Hessle, 1917;, Annenkova, 1929; Jirkov, 2001). In addition, *A. grubei* Malmgren, 1866 and *A. cirrata* Webster & Benedict, 1887 have been considered as junior synonyms of *A. acutifrons* (Hessle, 1917).

Recent re-examination and redescription of the holotype of *A. acutifrons* revealed that the neuropodial cirri are absent in the type (Krüger et al., 2022). Unfortunately, the holotype was noted to be in poor condition, strongly contracted and without branchiae in the original description (Grube, 1860), and no other specimens from Greenland have been described for comparison. Despite this, re-examination of type material allows the reestablishment of *A. grubei* and *A. cirrata* as valid species, which differ from *A. acutifrons* in the shape of the paleae and the presence and size of the neuropodial cirri (Krüger et al., 2022). Based on this morphological information, many prior records of *A. acutifrons* are likely to be misidentifications. This idea is also supported by molecular data, which suggest that *A. acutifrons* is a species complex (Krüger et al., 2022), and includes some specimens from Washington, USA.

In light of these recent discoveries, previous records of *A. acutifrons* are in need of re-evaluation. This study focuses on *Ampharete* specimens from Washington with associated cytochrome c oxidase subunit I (COI) barcode sequences available in public databases. Detailed morphological examination of museum specimens and analysis of their COI sequences reveal three *Ampharete* species in Washington, including a new species formally described here: *A. paulayi* **n. sp.**

Materials & Methods

The studied material belongs to the following collections: Invertebrate Zoology Collection of the Florida Museum of Natural History (UF), University of Florida, USA; Natural History Museum (BMNH), London, UK; Polychaete Collection of the Natural History Museum (LACM-AHF), Los Angeles County, USA; Swedish Museum of Natural History (SMNH), Stockholm, Sweden.

Species descriptions are based solely on external characters observed in holotype specimens. Additional morphological information from paratypes or non-type specimens is included in a separate “Variation” section. The material examined citations follow the standardized format of Chester et al. (2019).

In *Ampharete*, the prostomium is fused ventro-laterally with the peristomium to form a structure commonly referred to as the lower lip. As the prostomium and peristomium (including lower lip) are pre-segmental regions (Rouse, Pleijel & Tilic, 2022), they are excluded from the

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body segment counts in this study. The arrangement and number of branchiae are commonly used to distinguish species (Reuscher, Fiege & Wehe, 2009); however, their origin and subsequent enumeration often vary among descriptions due to the lack of standardization. Therefore, this study considers that there are only one pair of branchiae per segment (Orrhage, 2001); the first thoracic segment is achaetous and has no branchiae; and the second segment bears the first pair of branchiae and is the first thoracic chaetiger, as it has paleae. To avoid confusion, segments are indicated by Roman numerals, while chaetigers are indicated by Arabic numerals (Reuscher, Fiege & Wehe, 2009). Following Chávez-López, Alvestad & Moore (2025), the intermediate uncinigers are included in the abdominal segment count.

Some specimens were temporarily stained with Shirlastain-A or Methyl green to improve the visibility of nephridial papillae, the branchial arrangement and neuropodial cirri (see 'Morphology' section). One thoracic and abdominal unciniger (generally TU4 and AU4) were dissected to describe uncini morphology. Permanent slides of tentacles, paleae and uncinigers were prepared using Euparal mounting medium and incorporated into the corresponding collection. Digital photographs of complete specimens and diagnostic characters were made using a Canon T8i digital camera equipped with a microscope adapter. Series of digital photographs were combined through focus stacking (Z-stacking) using Helicon Focus v.8.2.15 software (HeliconSoft Ltd., 2025). Figures were prepared using Adobe Photoshop v.25.12.3 software (Adobe Inc., 2024).

The following abbreviations are used in the text and figures: abdominal unciniger (AU), branchiae originating from segment II (Br1), branchiae from segment III (Br2), branchiae from segment IV (Br3), branchiae from segment V (Br4), lateral cirri??? (Lc), thoracic chaetiger (TC), thoracic unciniger (TU), peristomium (Pe).

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Molecular analysis

Thirty-seven COI sequences published in the NCBI GenBank and Barcode of Life Data System (BOLD) databases were used for molecular analysis (Table 1). All molecular analyses were performed in MEGA version 11 (Tamura, Stecher & Kumar, 2021). COI sequences were aligned using MUSCLE (Edgar, 2004). Model testing was performed using Maximum Likelihood; the best-fitting substitution model was selected according to the Bayesian Information Criterion (BIC). A Maximum Likelihood tree was reconstructed applying the General Time Reversible (GTR) model using a discrete Gamma distribution (+G) with five rate categories and by assuming invariable sites (+I). Model-corrected inter- and intraspecific genetic distances were estimated using Kimura's two-parameter (K2P; Kimura, 1980) distance model.

Nomenclatural acts

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it

contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:F771A2B4-4F5F-4168-BBAD-A519175C83AE. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS."

Results

Molecular comparison

The COI barcode sequences for some specimens examined here were previously deposited in BOLD and identified as *Ampharete acutifrons*. The phylogenetic analysis of COI recovered three distinct genetic lineages, herein identified as: *A. cirrata* Webster & Benedict, 1887, *A. labrops* Hartman, 1961, and *A. paulayi* **n. sp.** (Fig. 1). *Ampharete labrops* from Washington were closely related to specimens identified as *A. labrops* from Vancouver Island, with a K2P-corrected genetic distance of 1.3–1.7%.

The intraspecific K2P genetic distance variation was low within the *A. cirrata* population from Washington (0.2–0.5%). The K2P-corrected genetic distance between the Baltic Sea and Washington populations is 4.4–4.9% (Table 2), yet few morphological differences distinguish these populations and the syntype of *A. cirrata* (see "*A. cirrata* Variation"). This suggests a recent history of genetic isolation in these populations and may reflect and ongoing, incomplete speciation process.

All included *Ampharete* species had K2P-corrected COI distances between 15.4–22% for *A. paulayi* **n. sp.** (Table 2, Table S1), a result consistent with the morphological analysis. The sequences within *A. paulayi* **n. sp.** were identical. This result supports the hypothesis that paratypes of *A. paulayi* are juveniles with an incomplete development of some structures, such as the buccal tentacles or abdominal pinnulae (see "*A. paulayi* **n. sp.** Remarks"). At the same time, it confirms earlier accounts of ontogenetic changes in some *Ampharete* species.

Morphology

The shape of thoracic and abdominal neuropodia has long been considered an important character for distinguishing ampharetid species. In *Ampharete*, neuropodia may bear dorsal marginal cirri (*e.g.*, Figs. 3I, 4E) or papillae (*e.g.*, Fig. 12E–F). In other annelids, the term 'cirri' generally refers to dorsal or ventral appendages of the prostomium or parapodia (*e.g.*, Rouse, Pleijel & Tilic, 2022), rather than to the overall shape of the parapodial ramus; in the latter context, the term 'cirriform' is more appropriate. In contrast, 'papillae' traditionally refers to epidermal rugosities containing glandular cells and pores, which may have secretory, sensory or combined functions (*e.g.*, Fauchald & Rouse, 1997; Vodopyanov, Tzetlin & Zhadan, 2014). In ampharetids, however, there is no evidence that the 'neuropodial papillae' are secretory. Instead, the distinction between neuropodial papillae and cirri lies only in their shape: cirri are elongated and pointed, whereas papillae are small and rounded. Therefore, referring to the same structure

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by different names depending on its shape is problematic and confusing. Moreover, in some species (*e.g.*, *A. cirrata*), the so-called 'neuropodial papillae' of anterior segments gradually transform into 'neuropodial cirri' in posterior segments. Based on these observations, the terms '**neuropodial cirrus**' (singular) and '**neuropodial cirri**' (plural) were chosen in this study as the most appropriate to refer to the dorsal projection of the neuropodia, which can be rounded or elongated.

Systematics

Family Ampharetidae Malmgren, 1866

Subfamily Ampharetinae Malmgren, 1866

***Ampharete* Malmgren, 1866**

Type species. *Amphicteis acutifrons* Grube, 1860, by subsequent designation (Uschakov, 1955: 366).

Diagnosis. Ampharetins with 17 thoracic segments: 15 chaetigers (including paleal segment), 12 uncinigers from segment VI (TC4). Prostomium subdivided into two lobes, without glandular ridges. Segment II with paleae. Four pairs of branchiae; three pairs of branchiae arranged in a transverse row with an interbranchial gap, fourth pair immediately behind them. Two dorsal nephridial papillae next to fourth pair of branchiae, well separated from each other. Buccal tentacles pinnate. Pygidium with two lateral cirri and some with several surrounding anal cirri.

Remarks. Several genera have historically been treated as junior synonyms of *Ampharete* Malmgren, 1866: *Asabellides* Annenkova, 1929, *Branchiosabella* Claparède, 1863, *Heterobranchus* Wagner, 1885, *Pseudosabellides* Berkeley & Berkeley, 1943, *Pterampharete* Augener, 1918 and *Sabellides* Milne-Edwards in Deshayes & Milne-Edwards, 1838 (Fauvel, 1897; Day, 1964; Jirkov, 1994; Jirkov, 1997; Jirkov, 2018). However, I do not follow the proposed synonymies of *Asabellides*, *Pseudosabellides*, *Pterampharete* and *Sabellides* with *Ampharete*. The diagnostic characters of these genera are inconsistent with Malmgren's (1866) definition of *Ampharete*, particularly regarding the presence of paleae (i.e., chaetae often longer and wider than typical capillary notochaetae) on segment II, and the presence of 12 thoracic uncinigers. Accordingly, the diagnosis of *Ampharete* provided in this study is restricted to adult specimens, and excludes the characters attributed to species of *Asabellides*, *Pseudosabellides*, *Pterampharete* and *Sabellides*.

***Ampharete paulayi* n. sp.**

Figures 2–5

Diagnosis. *Ampharete* with 26–28 paleal chaetae per side on segment II. All thoracic neuropodia entire, without cirri. Abdomen with 12 segments. Each abdominal pinnules (AU3–12) with an elongate neuropodial cirrus, progressively increasing in length toward posterior segments. Pygidium with two lateral cirri and 14 long cirri surrounding the anus.

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Etymology. The species is name in honor of Dr. Gustav Paulay, curator of the Invertebrate Zoology collection at UF, who collected most of the specimens reviewed in this work and who supported the examination of material in the collection under his care. The species name is a noun in the genitive case (ICZN, 1999, Art. 31.1.2).

Material examined

Holotype

WASHINGTON • 1 complete spec.; Kitsap County, North Hood Canal, south of the bridge; 47.83758°, -122.62895°; depth 21 m; 17 Apr. 2019; Gustav Paulay leg.; soft bottom; BOLD COI: BBPS043-19; **UF 7977**.

Paratypes

WASHINGTON • 1 complete spec.; Pierce County, west of Devils Head, eastern end of Nisqually Reach; 47.17060°, -122.78051°; 10 Apr. 2019; depth 109.25 m; Gustav Paulay leg.; soft bottom; BOLD COI: BBPS1016-19; **UF 7637** • 1 incomplete spec.; same data as previous; BOLD COI: BBPS1017-19; **UF 7692**.

Description. Holotype complete (**UF 7977**), body pale-yellow, 33 mm long, 5 mm wide (Fig. 2A). Prostomium subdivided into two lobes (Figs. 2B, 3B), middle lobe anteriorly rounded, one pair of nuchal organs as ciliated pits (Fig. 3B), eyespots not observed. Buccal tentacles partially everted (Fig. 2C–D), bipinnate (Fig. 4A). Lower lip entire (Figs. 2C, 3C).

Thorax with 17 segments: 15 chaetigers, 12 uncinigers. Segment I achaetous, clearly distinguishable in ventral view, not fused with peristomium (Figs. 2E, 3D). Segment II with paleae arranged in spiral (Fig. 3B, E), 28 paleae on the right side, 26 paleae on the left. Paleae yellowish, thick and flat with caudate tips (Fig. 4B). Paleal tip very fragile, most paleae with broken tips. Paleae 1–1.8 mm long; longest paleae protruding beyond prostomium (Fig. 2B–D), four times longer than TC2 notochaetae (0.4 mm), and thrice as long as the longest notochaetae (TC8, 0.6 mm). Some ciliate epibionts attached to paleae (Fig. 4C).

Four pairs of branchiae originating from segments II–V (Figs. 2B, 3B). Branchiae arranged in two transverse rows. Three pairs of branchiae arranged in a transverse row on segments II–III, separated by a median gap as long as branchial width; 4th pair shifted behind innermost (Br3) and middle (Br1) branchiae of transverse row with bases extended to segment V (TC3) (Fig. 3B). Most branchiae lost, only visible as scars, except outermost branchiae (Br2), left side (Figs. 2A–D, 3C–D). Branchia filiform, smooth, 3.5 mm long, reaching back to thoracic chaetiger 5 (TU2); branchia detached by handling, but present in vial. Two dorsal nephridial papillae between 4th pair of branchiae (Figs. 2B, 3B). Nephridial papillae well separated by a wide gap of 1 mm, ~7 times as long as nephridial papillae width (Fig. 3B).

Notopodia with capillary chaetae on 14 segments from segment IV (Fig. 2E). Segment III achaetous, fused dorsally with segment II, easily distinguishable on ventral and lateral views (Figs. 2E, 3D), with rudimentary notopodia (Fig. 3E). First two thoracic notopodia slightly displaced dorsally (TC2–3) (Fig. 3D). All notopodia lobes of similar size, as long as wide.

Notochaetae arranged in two rows, each with 8–10 capillary bilimbate chaetae. Nephridial papillae below notopodial lobes of TC4 (TU1).

Neuropodia tori present on 12 segments from chaetiger 4 (segment VI) (Fig. 3C). Thoracic neuropodia smooth, without cirri (Fig. 4D). Thoracic uncini with two rows of denticles in front view, 7–8 denticles in lateral view (Fig. 4F). Ventral shields along chaetigers 1–13 (segments II–XV) (Fig. 3C). Continuous median ventral groove visible from chaetiger 14 (segment XVI, TU11) to posterior end.

Abdomen with 12 chaetigers (Fig. 2A). Small and rounded dorsal tubercle (rudimentary notopodia?) along abdominal segments 1–9 (Fig. 3F–G). First two segments with intermediate uncinigers (Figs. 2G). Third and following abdominal segments with pinnules. Abdominal pinnules with elongate neuropodial cirri (Fig. 3H–I). Anterior pinnulae with shorter cirri, as long as pinnulae length (Figs. 3I, 4E). Neuropodial cirri progressively longer towards posterior segments, slightly exceeding ~~the~~ pinnula length (Figs. 2F, 4I). Abdominal uncini with two rows of denticles in frontal view, 6–7 denticles in lateral view (Figs. 4G–H).

Pygidium with two long broad lateral cirri, one bifurcated, and about 14 long cirri, surrounding anus in one row (Figs. 2F, 4I). Pygidial cirri 0.3–0.9 mm long, longest cirri ~11 times longer than wide, reaching forward to last abdominal segment.

Methyl green staining pattern. Prostomium intensely stained, except for margins of middle lobe and nuchal organs (Fig. 3A–B). Peristomium and segment I completely stained, except for intersegmental areas (Figs. 3C–D). Buccal tentacles completely stained, except basally lacking lateral filaments; base of tentacular filaments strongly stained (Fig. 3A). Thorax dorsally spotted (Fig. 3A). Thoracic notopodia intensely stained, except laterally (Fig. 3D–E). Thoracic neuropodia intensely stained around uncini row (Figs. 3D, 4D). Ventral shields strongly stained from TC1–13 (Fig. 3C). Abdomen spotted (Fig. 3F–H). Abdominal pinnula with neuropodial cirri intensely stained (Figs. 3I, 4E). All anal cirri intensely stained (Fig. 4I).

Body coloration in life. Holotype with body pink, dorsally iridescent (Fig. 5A). Prostomium pink, with anterior margin whitish (Fig. 5B–D). Branchiae two-toned: innermost and middle pairs greenish-orange, outermost pair and 4th pair pale; all branchiae with dark blood vessels visible by transparency (Fig. 5A–D). Paleae translucent yellowish. Notochaetae translucent white. Pygidial cirri pale (Fig. 5A).

Paratypes with translucent orange to melon-colored body (Fig. 5E–G). Paratype **UF 7637** with a pygidial eyespot (Fig. 5E–F). Paratype **UF 7692** with branchiae translucent green to pale (Fig. 5G).

Variations. The type series of *A. paulayi* **n. sp.** consisted of three specimens: complete holotype and two paratypes, one complete (**UF 7637**) and another one incomplete (**UF 7692**). Complete specimens 15–33 mm long, 1.7–5 mm wide, 14–28 paleae per side (0.8–1.8 mm long), 12 abdominal segments, and 10–17 long anal cirri. The complete paratype (15 mm long, 1.7 mm wide) has short smooth tentacles, and abdominal pinnula with rounded cirri instead of bipinnate tentacles and abdominal neuropodia with elongate cirri. The incomplete paratype (5 mm long,

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1.2 mm wide), with only one abdominal segment, also has short smooth tentacles, but there is a single larger bipinnate tentacle.

Remarks. *Ampharete paulayi* **n. sp.** has 15 thoracic chaetigers, 12 thoracic uncinigers, paleae on segment II, rudimentary notopodia without chaetae on segment III, 12 abdominal segments, abdominal pinnulae with elongate neuropodial cirri (as long as the pinnula length), and 10–17 long cirri surrounding the anus.

Paratypes of *A. paulayi* have short, smooth tentacles and abdominal pinnules with rounded neuropodial cirri. The presence of smooth tentacles differs with the holotype and is even inconsistent with the diagnosis of *Ampharete*. However, considering the size of the specimens, it is likely that the holotype (33 mm long) is an adult specimen, whereas the paratypes (<15 mm long) are juveniles of *A. paulayi* **n. sp.** In the larval development of *A. acutifrons* (*e.g.*, Clavier, 1984), *A. grubei* (*e.g.*, Fauvel, 1897) and *A. labrops* (Blake, 2017), the only three *Ampharete* species studied to date, the buccal tentacles are smooth and ciliated in larval stages but become bipinnate in adults. The presence of one larger bipinnate tentacle in the paratype **UF 7692** of *A. paulayi* **n. sp.** reinforces the hypothesis that pinnate tentacles develop in later juvenile stages. The same may be true for the development of neuropodial cirri in the abdominal segments.

There are no differences in other diagnostic characters nor in the COI sequences of the *A. paulayi* **n. sp**. specimens studied here (Fig. 1). Therefore, I consider those paratypes with smooth tentacles to be juveniles of *A. paulayi* **n. sp.** with underdeveloped buccal tentacles and abdominal neuropodial cirri.

Ampharete paulayi **n. sp.** (type locality: Washington) resembles *A. grubei* Malmgren, 1865 (TL: Svalbard, Arctic Ocean), *A. cirrata* Webster & Benedict, 1887 (TL: Maine, NE Atlantic), and *A. brevibranchiata* Treadwell, 1926 in the presence of 12 abdominal segments and pygidial cirri surrounding the anus. *Ampharete brevibranchiata*, described from the Bering Strait, differs in having a rounded neuropodial cirri, rather than elongate cirri as in the other three species. The presence of elongate cirri on the last thoracic neuropodia separates *A. cirrata* from *A. paulayi* **n. sp.** and *A. grubei*. *Ampharete paulayi* **n. sp.** additionally differs from *A. grubei* in the body size of specimens, the width of the interbranchial gap, and the size of pygidial cirri. Complete specimens of *A. paulayi* **n. sp.** (15–33 mm long, 1.7–5 mm wide) are smaller than those of *A. grubei* (27–50 mm long, 4.5–9 mm wide), have a shorter interbranchial gap (almost as wide as the branchial width, instead of 1.5–2 times wider than the branchiae as in *A. grubei*), and have longer and slender pygidial cirri (Krüger et al., 2022, fig. 11D).

***Ampharete cirrata* Webster & Benedict, 1887**

Figures 6–10

Ampharete cirrata Webster & Benedict, 1887: 747, pl. 8, figs. 110–112. Type locality. Gulf of Maine.

Diagnosis. *Ampharete* with 9–14 paleal chaetae per side on segment II. Neuropodial cirri from TU5–6. Anterior thoracic neuropodia with rounded cirri. Last 4–5 thoracic segments (from TU9–TU10) with elongated neuropodial cirri, protruding beyond ~~the~~ tori length. Abdomen with 12

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segments. All abdominal neuropodia with elongated cirri protruding beyond ~~the~~ pinnulae length. Pygidium with two lateral cirri and 8–18 long cirri surrounding the anus.

Material examined

Syntypes

MAINE • 3 spec., 4 slides; Eastport, North Atlantic; depth 11–18 m; Webster H. leg.; **USNM 457**.

Material additional

MAINE • 60 spec.; Muscongus Bay, near Hog Island, Audu~~o~~bon Camp; depth 5.4 m; mud; 29 Aug. 1955; M.H. Pettibone leg.; **USNM 44343**.

WASHINGTON • 1 incomplete spec.; San Juan County, Reads Bay; 48.49626°, -122.82139°; depth 8.5 m; soft bottom; 24 Apr. 2019; Gustav Paulay leg.; BOLD COI: BBPS506-19; **UF 8068** • 1 incomplete spec.; same data as for preceding; BOLD COI: BBPS507-19; **UF 8069** • 1 incomplete spec.; same data as for preceding; BOLD COI: BBPS508-19; **UF 8071** • 1 incomplete spec.; same data as for preceding; BOLD COI: BBPS509-19; **UF 8081** • 2 complete spec.; same data as for preceding; **UF 8082**• 3 complete spec.; same data as for preceding; **UF 8098**.

BALTIC SEA • 1 incomplete spec.; S Baltic Sea; 55°90'N, 14°40'E; depth 46 m; Örbeg leg.; **SMNH-120823**.

DENMARK • 3 spec.; South of Helsingor, Oresund; depth 18 m; 24 May. 1961; Eliason leg.; **USNM 43292**.

NORWAY • 2 spec.; Leirbugt, Posangerfjord; depth 10 m; 11 July 1939; G.I. Crausford leg.; **BMNH 1950.2.15.30**.

WESTERN RUSSIA • 8 spec.; Novaya Zemlya, Matotschkin scharr, western opening, Arctic Ocean; Jenissej exped. 1875, sta. 80; 72°30'N, 54°40'E; depth 4–9 m; mud less sand; **SMNH-120734**.

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Redescription. Syntype complete (**USNM 457**), body pale yellow, 9.5 mm long, 1.5 mm wide (Fig. 6C). Prostomium subdivided into two lobes, middle lobe anteriorly rounded with a pair of eyespots below the ciliated pits of the nuchal organs (Figs. 6D, 7A). Buccal tentacle inside the mouth, not visible. Lower lip entire (Fig. 6F).

Thorax with 17 segments: 15 chaetigers, 12 uncinigers (Fig. 6G). Segment I achaetous, clearly distinguishable in ventral view, not fused with peristomium (Fig. 7B). Segment II with paleae arranged in a semicircle, 12 paleae on the right side, 11 paleae on the left. Paleae bright golden, slender, gradually tapering with caudate tips. Paleal tips very fragile, most paleae with

broken tips. Paleae 0.9 mm long, as long as prostomium, about three times longer than first thoracic notopodium (notochaetae broken).

Four pairs of branchiae originating from segments II–V. Branchiae arranged in two transverse rows. Three pairs of branchiae in ~~the~~ first row on segments II–III, with a median gap shorter than branchial scars width, 4th pair of branchiae shifted behind ~~the~~ middle branchiae (Br1) of anterior row with bases extended to segment V (TC3). All branchiae lost, only scars visible. Two nephridial papillae between 4th pair of branchiae (Fig. 6D). Nephridial papillae well separated by a gap of 0.3 mm, ~8 times ~~the~~as long as width of ~~the~~ nephridial papillae.

Notopodia with capillary chaetae on 14 segments from segment IV. Segment III achaetous, fused dorsally with segment II, easily distinguishable in ventral and lateral view, no rudimentary notopodium visible. Notopodia lobes of similar size, twice longer than wide. Notochaetae arranged in two rows, each with 5–6 bilimbate chaetae. Several notochaetae broken in ~~the~~ first four notopodia. Nephridial papillae below notopodial lobes not observed.

Neuropodia tori present on 12 segments from chaetiger 4 (segment VI; Fig. 7B). Thoracic neuropodia with neuropodial cirri from TU8. Neuropodial cirri short and rounded on TU8–10. Posterior thoracic neuropodia (TU11–12) with an abruptly elongated cirri, ~9 times longer than wide, as long as ~~the~~ neuropodia width. Ventral shields along chaetigers 1–13 (segment XV; Fig. 6F–G). Continuous median ventral groove visible from chaetiger 14 (segment XVI, TU11) to posterior end.

Abdomen with 12 segments (Fig. 7C). First two segments with intermediate uncinigers. Pinnules from ~~the~~ third abdominal segment. All abdominal neuropodia with elongated cirri. Neuropodial cirri progressively longer along posterior segments until AU6–AU7, which have the longest cirri (~11 times longer than wide), then progressively shorter until be ~6 times longer than wide (Fig. 7D).

Pygidium with two lateral cirri and about eight long cirri surrounding anus in one row (Fig. 6E, H). Pygidial cirri ~0.2 mm long, 7–9 times longer than wide (Fig. 7E).

Methyl green staining pattern. Prostomium completely stained, except for margins of middle lobe and a transverse band including ~~the~~ eyespots and nuchal organs (Fig. 7A). Peristomium and segment I completely stained, except for junctional regions dividing each ring (Fig. 7A~~,~~ B). All neuropodial tori slightly stained, surrounding uncini row. Ventral shields strongly stained along TC1–13 (TU10; Figs. 6F–G, 7A). Neuropodial cirri not stained (Figs. 6G, 7C). Lateral cirri on pygidium slightly stained, surrounding cirri non-stained (Fig. 6H).

Syntype variation. The type series of *A. cirrata* consists of three syntypes (**USNM 457**): two complete and one incomplete specimen (Fig. 6A–C). The longest, complete syntype was used to redescribe the species (Fig. 6C–F). Smallest, complete syntype 5 mm long, 0.7 mm wide, with 12 abdominal segments (Fig. 6B); neuropodial cirri distinguishable along TU7–AU12, cirri small and rounded on TU7–9, cirri slightly elongated along TU10–AU1, and long cirri protruding the pinnulae length from AU2 to the last abdominal segment; pygidium and pygidial cirri not distinguishable. Incomplete syntype consisting of only posterior region of 10 mm long, 1.5 mm wide, with 11 abdominal segments (Fig. 6A); pygidium with a pair of long lateral cirri

and ~12 long surrounding cirri (4–5 times longer than wide), about three surrounding cirri lost (Fig. 7F).

There are four slides labeled as types of *A. cirrata*. **Slide 1032** includes anterior capillary chaetae. **Slide 1034** has thoracic parapodia. **Slides 1033** and **1035** have anterior and posterior uncini, respectively; therefore, no additional dissection of uncini was made of the complete syntype used in the redescription. Anterior and posterior uncini only visible in lateral view, with 5–6 denticles (Fig. 7G–H).

Variation non-type specimens. Sixty specimens from Maine (**USNM 44343**). Complete specimens of 8.5–19 mm long, 1.1–1.9 mm wide, with bipinnate tentacles, longest paleae 0.9–1.6 mm long, 12 abdominal segments, and about 10–12 long pygidial cirri. One specimen with posterior region bifurcated, probably the result of a mutation: each posterior abdominal regions with 8–9 AU and pygidium with lateral and surrounding cirri (Fig. 7I–J).

Nine specimens from Washington: five complete specimens and four incomplete with body split into two regions (probably due to tissue sampling for DNA extraction). Complete specimens 9–11 mm long, 1.1–1.4 mm wide (Fig. 8–9); with bipinnate tentacles (Fig. 9A), 12–14 paleae per side (Figs. 8B–C, 9B); thoracic neuropodia with elongate cirri from TU8–TU10 (Fig. 8A–B, E); 12 abdominal segments, all of them with long neuropodial cirri (Figs. 8E, 9D). All specimens with lateral and surrounding pygidial cirri, 8–9 surrounding long cirri interspersed with 8–9 short cirri (Figs. 8F, 9K). Neuropodial and pygidial cirri not stained by Methyl green (Figs. 8F, 9F).

Fifteen specimens from northeastern Atlantic Ocean with neuropodial and pygidial cirri stained with Methyl green. One incomplete specimen from the Baltic Sea, body 13 mm long, 2.1 mm wide with complete thorax and only 5 AU (**SMNH-120823**). Three specimens from Denmark (**USNM 43292**): two complete juvenile (5–7 mm long, 0.6–0.8 mm wide, with 12 AU, smooth tentacles, and 9–11 paleae per side, longest paleae of 0.5–0.7 mm long) and one incomplete specimen (9.5 mm long, 1.6 mm wide, with complete thorax and only 8 AU, bipinnate tentacles, 13–15 paleae per side, longest paleae of 1.2 mm long). Two complete, mature female specimens from Norway of 11–17 mm long, 1.3–2 mm wide with 12 AU, longest paleae 0.8–1.5 mm long (**BMNH 1950.2.15.30**). Eight specimens from Novaya Zemlya (**SMNH-120734**): seven complete specimens (9–12.5 mm long, 1.6–1.7 mm wide with 12AU, ~12 paleae per side, longest paleae of 1.2 mm long) and one incomplete (9 mm long, 1.8 mm wide with complete thorax and only 3 AU).

Body coloration in life. Specimens from Washington (**UF 8068**, **UF 8069**, **UF 8071**, **UF 8081**) with translucent, light melon body color. Branchiae green or orange with white horizontal lines (Fig. 9G–H). Paleae yellowish. Some internal organs visible through transparent body wall: dorsal blood vessel dark reddish, anterior lobe of stomach pinkish (Fig. 9I–J), stomach pale yellowish, intestine colorless (Fig. 5I). Abdomen colorless, translucent (Fig. 9G–J).

Remarks. *Ampharete cirrata* Webster & Benedict, 1887 was originally described based on specimens from Maine, USA collected from sediments at 11–18 m depth. Although the original description of *A. cirrata* is brief (Webster & Benedict, 1887: 747), it includes the relative size of branchiae (referred to as long as body width or slightly longer), the presence of neuropodial cirri

from 9th chaetiger segment (TU6), 14 pygidial cirri, body coloration (body light green and branchiae light green with white transverse bands on ventral surface), and the size of the body (24 mm long, 4 mm wide) and branchiae (4 mm long) of the largest specimen. The two complete syntypes of *A. cirrata* (**USNM 457**) match Webster & Benedict's description. The illustrations of *A. cirrata* by Webster & Benedict (1887: pl. 8, figs. 110–112) appears to be based on the largest and incomplete specimen, which is the only one with neuropodia dissected. Therefore, it is likely that the four slides were made from this incomplete syntype, which lacks most abdominal neuropodia. However, since it is an incomplete specimen with only 11 abdominal segments and pygidium, the best-preserved specimen of *A. cirrata* is redescribed here. Krüger et al. (2021) provided two micrographs of the same syntype specimen redescribed here.

The syntypes of *A. cirrata* and non-type specimens examined from the NE Pacific, NE Atlantic and the Artic Ocean have notable morphological similarities, including 12 abdominal segments, neuropodial cirri on thoracic and abdominal segments, and long pygidial cirri surrounding the anus. However, specimens from Washington (*e.g.*, **UF 8068**, **UF 8069**) differ from *A. cirrata sensu stricto* in the number of pygidial cirri and in the body coloration. Syntypes of *A. cirrata* have a pygidium with 8–12 long surrounding cirri of similar size (Fig. 3E, F) and a light green live body coloration, rather than 16 surrounding pygidial cirri (eight long cirri and eight short cirri interspersed; Fig. 5K), and a light melon-colored, translucent body (Fig. 5G–J) as in the Washington specimens. However, there are no differences in other characteristics, including the branchiae coloration pattern of the living specimens.

Specimens from Russia (**SMNH-120734**; Fig. 10), Norway (**BMNH 1950.2.15.30**), Denmark (**USNM 43292**) and the Baltic Sea (**SMNH-120823**) differ from *A. cirrata* syntypes and Washington specimens in the intense staining of the neuropodial and pygidial cirri with Methyl green (Fig. 10F). The Methyl green staining pattern of the northwest Atlantic specimens matches the records of Krüger et al. (2022) of *A. cirrata* in the Baltic Sea with specimens collected at 44–48 m depth.

There are no COI sequences of *A. cirrata* from Maine or the northwestern Atlantic, but molecular analysis revealed K2P-corrected distances of 4.4–4.9% between the Washington and the Baltic Sea populations, in contrast to the interspecific distance of 14.4–22.9% observed among morphological distinct *Ampharete* specimens (Table 2, Table S1). The subtle morphological differences observed among the specimens of *A. cirrata* examined here, and the relatively low genetic divergence between Washington and Baltic Sea specimens suggest recent population isolation. However, separating the NE Pacific and NE Atlantic populations into different species could lead to confusion and identification difficulties, given the strong morphological resemblance to *A. cirrata*. Obtaining COI sequences for specimens of *A. cirrata* from Maine or the northeast Atlantic and reviewing more material from the Arctic Ocean and surrounding areas could clarify the distribution range of *A. cirrata sensu stricto*.

Comentado [JM15]: Agree

***Ampharete labrops* Hartman, 1961**

Figures 11–13

Ampharete labrops Hartman, 1961: 127–128, pl. 34, figs. 1–4. Type locality: Santa Barbara Point, California, 9.14 m, from a bottom of fine sand with kelp.

Diagnosis. *Ampharete* with the anterior margin of prostomium with rows of tiny eyespots. Segment II with paleae, 9–11 paleal chaetae per side. All thoracic neuropodia entire, without cirri. Abdomen with 13 segments. Abdominal pinnulae (AU3–13) with neuropodial cirri, as long as ~~the~~ pinnula length; cirri progressively shorter towards ~~the~~ posterior segments. Pygidium with one pair of long lateral cirri, surrounding cirri absent.

Material examined

Holotype

CALIFORNIA • 1 incomplete spec.; 2 miles from Santa Barbara Point light; 34.4041, -119.7611; depth 9.14 m; R/V Velero IV, Sta. AHF 6694–59; 3 Dec. 1959; Allan Hancock Foundation leg.; dark gray to black fine sand with worm tubes, kelp, and much woody debris; **LACM-AHF Poly 267**.

Additional material

WASHINGTON • 1 incomplete spec.; Jefferson County, Port Townsend, mouth of Kilisut Harbor; 47.09354, -122.73316; 1 Apr. 2019; depth 4 m; Gustav Paulay leg.; soft bottom; BoldSystems COI gene: BBPS810-19; **UF 8202**.

CALIFORNIA • 2 spec.; Orange County, off Oceanside; 33.2130, -117.4188; 18 Jul 1994; depth 13 m; soft sediment; Van Veen grab; **LACM-AHF Poly 12845** • 33 spec.; Los Angeles County, Palos Verdes Peninsula, off San Pedro; 33.704444, -118.310278; depth 21.95 m; R/V Velero IV, Sta. AHF 5028-57; 24 Apr. 1957; Allan Hancock Foundation leg.; coarse gray and black sand; Hayward Orange Peel grab; **LACM-AHF Poly 13546**.

Description. Holotype incomplete (**LACM-AHF Poly 267**), mature female, body stained blue, 11 mm long, 2 mm wide (Fig. 11A–B). Prostomium subdivided into two lobes, middle lobe anteriorly straight, with one pair of eyespots below nuchal organs (as ciliated pits; Fig. 11C). Anterior margin of prostomium with two or more rows of tiny brown eyespots, except in central slightly pointed region (Fig.11D). Buccal tentacles inside ~~the~~ mouth, partially visible, bipinnate. Lower lip entire (Fig.11D).

Thorax with 17 segments: 15 chaetigers, 12 uncinigers. Segment I clearly distinguishable in ventral and lateral view, not fused with peristomium (Fig. 11D). Segment II with paleae arranged in semicircle, 12 paleae per side (Fig. 11C). Paleae bright golden, thick, flat, gradually tapering with caudate tips (Fig. 12B). Paleae 0.3–0.7 mm long, longest paleae protruding beyond prostomium, twice longer than TC2 notochaetae (0.3 mm), and almost as long as longest notochaetae (TC6, 0.6 mm).

Four pairs of branchiae originating from segments II–V (Fig. 11E–F). Branchiae arranged in two transverse rows. Three pairs of branchiae arranged in a transverse row on segment II–III,

without median gap (Fig. 12A), 4th pair shifted behind innermost (Br3) and middle (Br1) branchiae of transverse row with bases extended to segment V (TC3). Branchiae filiform, 1.7–4.5 mm long (Fig. 11E–G); outermost branchia (Br2) longest, reaching back to thoracic chaetiger 11 (TU8). Three branchiae lost, only scar remains: Br1 and Br3 left side, Br2 right side. Two dorsal nephridial papillae between 4th pair of branchiae (Fig. 12A). Nephridial papillae well separated by a wide gap of 0.7 mm, ~6 times as long as nephridial papillae width (Fig. 12A).

Notopodia with capillary chaetae on 14 segments from segment IV (Fig. 11A). Segment III fused dorsally with segment II, easily distinguishable on ventral and lateral view, with an inconspicuous rudimentary notopodia without chaetae. Notopodia 2–3 times longer than wide, lobe progressively longer towards posterior region. Notochaetae arranged in two rows, each with 7–8 bilimbate chaetae. Nephridial papillae below notopodial lobes not observed.

Neuropodia tori present on 12 segments from chaetiger 4 (segment VI) (Fig. 11F–G). Thoracic neuropodia smooth, without cirri. Ventral shields along chaetiger 1–13 (segments II–XV) (Fig. 11B). Continuous median ventral groove visible from chaetiger 14.

Abdomen incomplete, only 4 chaetigers (Fig. 12C). Small and rounded tubercle (rudimentary notopodia?) along abdominal segments 1–4. First two segments with intermediate uncinigers. Third and four abdominal segments with pinnules. Abdominal pinnules with slightly elongated neuropodial cirri, smaller than ~~the~~ pinnula length.

Methyl green staining pattern of holotype. Prostomium, peristomium and segment I completely stained (Fig. 11E–G). Anterior margin of prostomium intensely stained. Few spots in dorsal region of thorax, near nephridial papillae (Fig. 11E, 12A). Thoracic notopodia and neuropodia with surrounding spots (Fig. 11F–G). Notopodia with a small, rounded lateral spot stained (Fig. 12C). Ventral shields strongly stained from TC1–13.

Variation. Complete specimens (**LACM-AHF Poly 126952**, **LACM-AHF Poly 13546**) 7–25 mm long, 1.2–2.7 mm wide, body white to pale brown, 8–13 paleae per side (0.5–1.1 mm long), 13 abdominal segments (rarely 14 AU), two long lateral anal cirri, reaching forward to the last 2–4 abdominal segments. Non-type~~s~~ specimens stained with Methyl green (Fig. 12D–F): ventral shields strongly stained from TC1–13 (Fig. 12D), abdominal pinnula with the base of neuropodial cirri stained, tip non-stained (Fig. 12E), pygidial lateral cirri strongly stained (Fig. 12F). Some specimens inside their tubes (Fig. 12G). Mature females with oocytes of ~70 µm diameter (Fig. 12H).

Incomplete specimen from Washington (**UF 8202**; Fig. 13) with prostomial middle lobe anteriorly pointed (Fig. 13A, E, H–I), with two pairs of eyespots (Fig. 13E). Buccal tentacles everted (Fig. 13A–C), bipinnate, with only two rows of short filaments (Fig. 13D); tentacles barely stained with methyl green, except for filaments and their bases which are strongly stained. Dorsal nephridial papillae separated by a gap of 0.3 mm, 5 times the width of the nephridial papillae (Fig. 13F). Ventral side of branchial surface with transverse rows of cilia, except in the outermost pair (Br2) of branchiae. Thoracic uncini with two rows of denticles in front view, 4–5 denticles over rostral tooth in lateral view, with subrostral process (Fig. 13G).

Comentado [JM16]: Check use of index, not used in descriptions of other species

Body coloration in life. Incomplete specimen (**UF 8202**) with body pinkish orange, abdomen with a lighter shade (Fig. 13H). Prostomium translucent with dark marginal eyespots, except in center region of middle lobe (Fig. 13I). Peristomium and first two segments pinkish orange. Ventral shields slightly white. Branchiae orange. Stomach dark reddish, visible through transparency. Thoracic notochaetae colorless.

Remarks. *Ampharete labrops* Hartman, 1961 was originally described based on an incomplete specimen collected from California at 9.14 m depth. The original description includes 15 thoracic chaetigers, 12 thoracic uncinigers, four pairs of branchiae, pinnate tentacles, a subquadrate prostomium, 11 paleae per side on segment II, and the presence of rows of eyespots along anterior margin of the prostomium (Hartman, 1961: 127–128). Records of *A. labrops* from California by Hilbig (2000) expanded the species diagnosis by noting the presence of 13 abdominal segments and a pygidium with two long lateral cirri, which agrees with the additional specimens of *A. labrops* (**LACM-AHF Poly 12845, LACM-AHF Poly 13546**) from California examined in this study.

Holotype of *A. labrops* is permanently stained blue, probably due to the previous use of Methyl blue, which, unlike Methyl green, is not temporary. The notopodium and neuropodium of chaetiger 13 (segment XV), right side in dorsal view, were previously dissected, but the corresponding slides were not found. No dissections were made from the holotype, to preserve the condition of the specimen.

The presence of marginal eyespots on the prostomium has been the main diagnostic character to distinguish *A. labrops* from other *Ampharete* species (*e.g.*, Hilbig, 2000). However, loss of marginal eyespot pigmentation was observed in specimens from lot **LACM-AHF Poly 12845**. I examined these specimens in 2022, and photographs of the prostomial eyespots were taken; however, on a more recent examination (July 2025), there was no trace of the presence of marginal eyespots on either specimen. Some of the possible causes of loss of pigmentation of marginal eyespots could be exposure to sunlight or the preserving solution (*i.e.*, ethanol 70%). Marginal eyespots can be a useful attribute to quickly identify specimens of *A. labrops*, however, since eyespots pigmentation can be lost, the comparation of other structures is necessary.

The presence of 13 abdominal uncinigers in *A. labrops* is shared with *A. arctica* Malmgren, 1866, *A. eupalea* Chamberlin, 1920, *A. finmarchica* (M. Sars, 1865), *A. kudenovi* Jirkov, 1994, and *A. seribranchiata* Treadwell, 1926. However, *A. labrops* differs from *A. eupalea* and *A. seribranchiata* by the branchial arrangement into two transverse rows rather than in a single row. The mucronate tips on the paleae of *A. arctica* and *A. finmarchica* separate them from *A. labrops* with filiform paleal tips. The absence of abdominal neuropodial cirri in *A. kudenovi* differs from *A. labrops*, with small and rounded neuropodial cirri.

Although the Washington specimen (**UF 8082**) is incomplete, it exhibits all the diagnostic features of the holotype of *A. labrops*, except for the prostomial shape and the number of eyespots. In the holotype, the anterior margin of the prostomium is straight and slightly pointed in the center (Fig. 11C), whereas in the Washington specimen it is distinctly pointed (Fig. 13E, H–I). Notably, even when the tentacles are everted, the prostomium of the **UF 8082** specimen retains its pointed

shape (Fig. 11E). The Washington specimen has two pairs of eyespots (Fig. 11C, E) instead of the single pair described for *A. labrops*. Because these morphological differences are subtle and may be size-related, the specimen is here identified as *A. labrops*.

COI sequences of *A. labrops* from California are not currently available for comparison. However, molecular analysis showed that the incomplete specimen of *A. labrops* examined here (previously identified as *A. acutifrons*) is most closely related to Vancouver Island specimens also identified as *A. labrops* (Fig. 1).

Discussion

The detailed morphological analysis performed in this study enabled the identification of three *Ampharete* species from Washington: *A. cirrata* Webster & Benedict, 1887, *A. labrops* Hartman, 1961, and *A. paulayi* **n. sp**. COI sequences from most Washington specimens examined here were previously deposited in the BOLD database under the name *A. acutifrons*. These sequences were included in the phylogenetic analysis of Krüger et al. (2022), who suggested that there is more than one species included in *A. acutifrons*. The present study clarifies the taxonomic identity of the COI sequences and provides detailed morphological descriptions of the corresponding specimens. Because more than 80% of nominal *Ampharete* species remain unrepresented in genetic databases, the integration of molecular data with thorough morphological characterization is essential to generate a reliable molecular reference framework for Ampharetidae, and to enhance the accuracy of species identification.

The availability and good preservation of the Washington specimens, from which molecular sequences were obtained, enabled the detailed morphological comparisons and taxonomic reassessment presented in this study. In addition, the available photographs of living specimens allowed the description of pigmentation patterns, which can facilitate the identification of newly collected specimens. The above underscores the importance of applying standardized protocols for the collection, handling and preservation of newly obtained material to maximize data preservation and facilitate future comparisons of living and preserved specimens as well as genetic information.

The examination of *A. cirrata* specimens from arctic-boreal localities revealed subtle morphological and molecular differences. These findings raise new questions about the genetic connectivity and reproductive isolation populations of *A. cirrata* and presents opportunities for future research.

The results of this study show that the identification of *Ampharete* can be challenging, especially when dealing with juvenile specimens. As described in the Variation and Remarks sections of *A. paulayi* **n. sp.**, paratypes smaller than 15 mm in length have smooth tentacles and rounded cirri on abdominal neuropodia. Since no differences were observed in other diagnostic characters or in COI sequences, small paratypes were considered to have reached full segmental development of all 29 body segments as in adult specimens (*e.g.*, holotype, 33 mm in length), but had not completed the development of the buccal tentacles and abdominal pinnules. Therefore, I

hypothesize that the development of the tentacular surface and neuropodial cirri in *A. paulayi* **n. sp.**, and probably in other *Ampharete* species, occurs in late juvenile stages.

The use of Methyl green allowed the recognition of a distinctive staining pattern in each of the *Ampharete* species included in this study. Methyl green dye use has revealed interspecific differences in ampharetids (e.g., Jirkov, 2011; Kim et al., 2025) and other tubicolous annelids (e.g., Winsnes, 1985; Tovar-Hernández, de León-González & Bybee, 2017), due to its affinity to glandular areas. Because it is a temporary stain and disappears in alcohol after few hours (Winsnes, 1985), Methyl green can be used on type material without damaging it. Therefore, the description of Methyl green staining patterns should become standard practice in future descriptions of *Ampharete* and other ampharetid species.

Conclusions

The detailed morphological study of specimens previously recorded as *A. acutifrons* from Washington allow the identification three species, including the new species *A. paulayi* **n. sp**. Based on morphological and COI sequences data, a hypothesis on the development of buccal tentacles and abdominal neuropodia in *Ampharete* species is proposed. The findings of this study raise new questions about the morphological variation and geographic distribution of *A. cirrata* for future research.

Given the complexity in the taxonomic identification of *Ampharete* species, largely due to the morphological similarities among species, this study highlights the value of Methyl green staining as a useful tool for distinguishing closely related species. The use of Methyl green dye is recommended as a standard practice in future descriptions of ampharetids.

Acknowledgements

I thank Dr. Gustav Paulay (UF) for the loan of materials and permission to use his photographs of live specimens. Thanks to Dr. Emma Sherlock (BMNH), Dr. Lena Gustavsson (SMNH), Leslie Harris (LACM-AHF), and Dr. Karen Osborn (USNM) for allow me to work with material from the collections in their care and for assisting me with sending material. Thanks are extended to Dr. Sergio I. Salazar-Vallejo (ECOSUR) and Dr. Jenna Moore (ZMH) for receiving material on my behalf and for their valuable comments to improve the manuscript. This research is part of the PhD Thesis of Y. Chávez-López at ECOSUR.

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Código de campo cambiado

Table 1. Specimens included in the molecular analysis, with museum voucher number, accession number, locality and reference to published sequences.

	Species	Voucher	Accession number		Locality	Coordinates / Depth	Reference
			BOLD	NCBI			
1	*A. californica*	SIO-BIC A9433	–	MT166986	Unknown	–	Stiller et al. (2020)
2	*A. cirrata*	–	–	OM470635	Baltic Sea	–	Krüger et al. (2022)
3	*A. cirrata*	–	–	OM470636	Baltic Sea	–	Krüger et al. (2022)
4	*A. cirrata*	UF 8068	BBPS506-19	–	Reads Bay, Washington	48.49626, -122.82139; 8.5 m	This study
5	*A. cirrata*	UF 8069	BBPS507-19	–	Reads Bay, Washington	48.49626, -122.82139; 8.5 m	This study
6	*A. cirrata*	UF 8071	BBPS508-19	–	Reads Bay, Washington	48.49626, -122.82139; 8.5 m	This study
7	*A. cirrata*	UF 8081	BBPS509-19	–	Reads Bay, Washington	48.49626, -122.82139; 8.5 m	This study
8	*A. falcata*	ZMBN 89835	POLNB011-13	OR891674	Norway	60.2691, 5.1157; 98 m	Parapar et al. (2017)
9	*A. falcata*	ZMBN 89842	–	OR891679	Norway	58.3466, 8.9283; 250 m	Parapar et al. (2017)
10	*A. falcata*	ZMBN 106563	–	MG270098	Norway	60.2817, 5.20067; 81–68 m	Eilersten et al. (2017)
11	*A. falcata*	ZMBN 95443	–	MG270099	Norway	58.3466, 8.9283; 250 m	Eilersten et al. (2017)
12	*A. finmarchica*	SIO-BIC A1100	–	JX423738	Norway, Svalbard, Hornsunddjupet	76.76, 14.95; 291 m	Stiller et al. (2013)
13	*A. finmarchica*	NTNU-VM 68245	PONOR080-13	OR891684	Norway, Porsangerfjorden	70.476, 25.397; 107 m	Parapar et al. (2017)
14	*A. finmarchica*	ZMBN 94828	POLYNB836-14	OR891672	Norway, Barents Sea	72.30983, 32.34133; 312 m	Parapar et al. (2017)
15	*A. labrops*	UF 8202	BBPS810-19	–	Mouth of Kilisut, Port Townsend, Washington	47.09354, -122.73316; 4 m	This study
16	*A. labrops*	–	BCPOL392-08	HM473291	Canada, Vancouver Island, Bamfield	48.831, -125.129; intertidal	Carr et al. (2011)
17	*A. labrops*	–	BCPOL396-08	HM473292	Canada, Vancouver Island, Bamfield	48.831, -125.129; intertidal	Carr et al. (2011)
18	*A. labrops*	–	BCPOL391-08	HM473290	Canada, Vancouver Island, Bamfield	48.831, -125.129; intertidal	Carr et al. (2011)
19	*A. lindstroemi*	ZMBN 89905	POLNB081-13	OR891673	Norway, Skagerrak	57.9282, 9.2809; 247–290 m	Parapar et al. (2017)
20	*A. lindstroemi*	ZMBN 89844	POLNB020-13	OR891681	Norway, Bergen area	60.269, 5.116; 98 m	Parapar et al. (2017)

Comentado [JM1]: Provide species names in full (author(s), year) in the first mention in Table

Comentado [JM2]: Not included in Reference list

Comentado [JM3]: Not included in Reference list

Comentado [JM4]: Not included in Reference list

Comentado [JM5]: Not included in Reference list

Table 1. Specimens included in the molecular analysis, with museum voucher number, accession number, locality and reference to published sequences.

	Species	Voucher	Accession number		Locality	Coordinates / Depth	Reference
			BOLD	NCBI			
21	*A. lindstroemi*	ZMBN 95899	–	OR891685	Norway, SW coast	59.03, 5.449; 58–60 m	Parapar et al. (2017)
22	*A. paulayi* **n. sp.**	UF 7977	BBPS043-19	–	North Hood Canal, Washington	47.83758, -122.62895; 21 m	This study
23	*A. paulayi* **n. sp.**	UF 7637	BBPS1016-19	–	Eastern end of Nisqually Reach, Washington	47.1706, -122.78051; 109.25 m	This study
24	*A. paulayi* **n. sp.**	UF 7692	BBPS1017-19	–	Eastern end of Nisqually Reach, Washington	47.1706, -122.78051; 109.25 m	This study
25	*A. santillani*	ZMBN 98157	MIWAP520-15	OR891683	Morocco, Atlantic	33.688, -7.614; 55 m	Parapar et al. (2017)
26	*A. santillani*	ZMBN 98164	MIWAP527-15	OR891670	Morocco, Atlantic	32.473, -9.274; 40 m	Parapar et al. (2017)
27	*A. santillani*	ZMBN 115540	GBAN17713-19	MG230531	Spain, Ría de Ferrol	43.47, -8.23; shallow subtidal	Parapar et al. (2017)
28	*A. santillani*	ZMBN 115542	GBAN17714-19	MG230532	Spain, Ría de Ferrol	43.47, -8.23; shallow subtidal	Parapar et al. (2017)
29	*A. undecima*	ZMBN 104774	–	OR891678	Norwegian Sea	63.0372, 4.689; 760–766 m	Parapar et al. (2017)
30	*A. undecima*	–	–	OR891676	Norwegian Sea	62.27, -0.02; 846 m	Parapar et al. (2017)
31	*A. undecima*	–	–	OR891675	Iceland, Denmark Strait	67.868, -23.696; 1,281 m	Parapar et al. (2017)
32	*Anobothrus gracilis*	ZMBN 95440	–	MG270106	Norway, Skagerrak	57.9282, 9.2809; 247–290 m	Eilersten et al. (2017)
33	*Anobothrus gracilis*	SIO-BIC A1106	–	JX423739	Norway	63.48, 10.37; –	Stiller et al. (2013)
34	*Sabellides manriquei*	–	CMBIA290-11	–	USA, California	33.573, -117.985; 57 m	Not published
35	*S. octocirrata*	SIO-BIC A1109	–	JX423770	Norway, Trondhejmsfjord	63.52, 10.42; 90 m	Stiller et al. (2013)
36	*S. octocirrata*	ZMBN 89909	POLNB085-13	OR891680	Norway, Skagerrak	57.928, 9.280; 193 m	Parapar et al. (2017)
37	*S. octocirrata*	ZMBN 91772	POLNB459-13	OR891671	Norway, W coast	62.369, 4.463; 192 m	Parapar et al. (2017)

Comentado [JM1]: Provide species names in full (author(s), year) in the first mention in Table