

# Morphological and Molecular Evidence for a New Record of *Hesione cf. picta* (Polychaeta: Hesionida) from the Western Coast of the Gulf of Thailand

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## ABSTRACT

The bristle worm *Hesione picta* Müller, 1858 is a striped polychaete and a member of the family Hesionidae occurring throughout the North Atlantic and South Atlantic Oceans. This study reports the first record of *Hesione cf. picta* from the Western Gulf of Thailand. The morphological characteristics of the specimens were described including the prostomium, cirri, chaetigers, chaetae and aciculae. The relationships of the specimens was examined with others based on partial sequences of 28S, 18S and 16S ribosomal DNA (rDNA) genes. The results based on those rDNA sequences were highly consistent with the morphological taxonomy. The *Hesione cf. picta* collected in this study were not observed to form symbiotic associations with host species. The present study may contribute to the knowledge of the systematic biology and biodiversity of family Hesionidae.

**Keywords:** *Hesione cf. picta*, Gulf of Thailand, 28S rDNA, 18S rDNA, 16S rDNA

## INTRODUCTION

The hesionid polychaetes are bristle worms belonging to the family Hesionidae (suborder Phyllodocida) (Pleijel, 1998). The first known hesionid species was *Nereimyra punctata*, originally described by Müller (1776) from a Scandinavian habitat. The benthic hesionidae comprise more than 28 genera including approximately 170 species (Fauchald, 1977a; Pleijel and Rouse, 2005). These hesionids are active carnivores and are distributed primarily in marine environments worldwide in habitats ranging from shallow water to the deep sea in subtropical and tropical waters (Rouse and Pleijel, 2001). Representative species include *Glyphohesione nicoyensis* from the Gulf of Nicoya, Costa Rica (Dean, 1998); *Microphthalmus*

*hamosus* from Florida, USA (Westheide, 1982); *Parasyllidea humesi* from north of Pointe-Noire, West Africa (Martin *et al.*, 2012); and *Podarke latifrons* from the coast of Singapore (Tan and Chou, 1993). Pleijel *et al.* (2008) reported a new hesionid, *Vrijenhoekia balaenophila*, from a whale carcass in the waters off the California coast. Certain hesionids appear to live symbiotically in association with mollusc or echinoderm species (Martin and Britayev, 1998; Subida *et al.*, 2011).

*Hesione* spp. are relatively small hesionids belonging to the subfamily Hesioninae and ranging broadly from subtidal habitats to deep water (Pleijel, 1998). The genus includes common species such as *H. picta*, *H. intertexta*, *H. genetta* and *H. splendida* (Day, 1962; Wu *et al.*, 1980; Costa *et al.*, 2008). *Hesione picta* (Müller, 1858) is distinguished from other *Hesione* species by

transverse stripes of light yellow alternating with dark brown. It is widely distributed in the western Atlantic Ocean from Florida to Brazil (Fauchald, 1977b). However, no occurrence of *H. picta* has been reported from areas extending from Southeast Asia to the Malay Archipelago.

Nuclear genomic and mitochondrial sequences are frequently used in taxonomy to identify and define large metazoan taxa (Canales-Aguirre *et al.*, 2011; Machida and Knowlton, 2012). Ruta *et al.* (2007) evaluated the phylogenetic relationships among twenty bristle worms of the family Hesionidae using the 28S, 18S (nucleus) and 16S (mitochondria) ribosomal DNA (rDNA) sequences, but in that study they did not include sequences from *H. picta*.

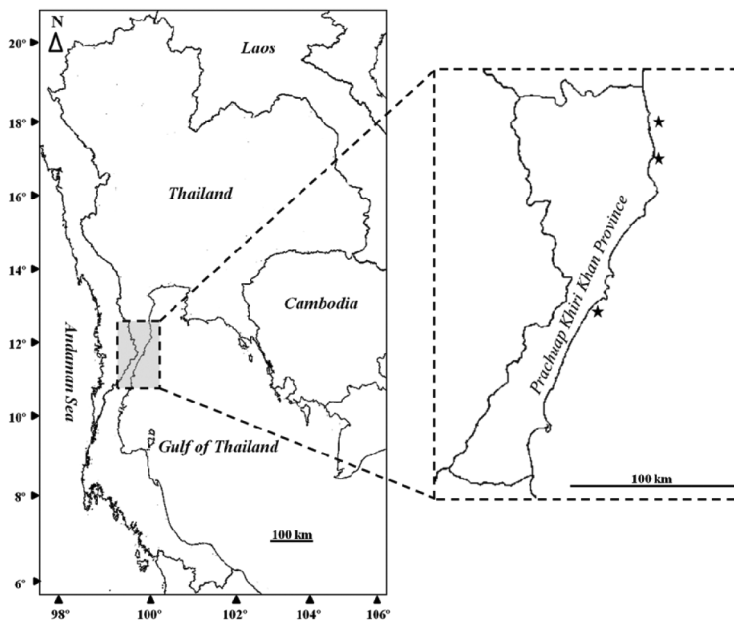
The aim of the current study was to report a new record of *Hesione cf. picta* from the western coast of Gulf of Thailand and to describe the morphological characteristics of the specimens. Moreover, the study assessed the relationships of *Hesione cf. picta* phylogenetically within the Hesionidae based on partial nucleotide sequences of 28S and 18S in nucleus and 16S rDNA in

mitochondria.

## MATERIALS AND METHODS

### Polychaete collection

Samples of the striped *Hesione cf. picta* were found at a depth of 10–15 m using an Agassiz trawl during a survey of all polychaete worms from the intertidal zone of three sites in Prachuap Khiri Khan province, Gulf of Thailand (12°33'36.41" N, 99°59'44.46" E; 12°28'42.47" N, 99°59'10.48" E; and 11°52'25.72" N, 99°55'45.55" E) (Figure 1). The samples were collected from marine mud and from the shells of dead molluscs, *Pinna* sp. (Pterioidea: Pinnidae). Sample sizes were 120 cm<sup>2</sup> in the muddy area based on the small-scale structure of communities of polychaete fauna from the report of Martin *et al.* (1993). In the field, the living worms of *Hesione cf. picta* were placed in small aquaria (35 × 21 × 23 cm) containing natural seawater and equipped with air pumps. The aquaria were then transported to the laboratory. Seven individuals of *Hesione cf. picta* were found in this field survey. To obtain further descriptions of the



**Figure 1** Study area of *Hesione cf. picta* collections from the western coast of the Gulf of Thailand. Asterisks (★) indicate the localities where bristle worm samples were collected.

specimens, four living worms were anaesthetised with 7% MgCl<sub>2</sub> in cool seawater, and their general morphology was photographed and drawn under an SMZ1000 stereomicroscope (Nikon Instech Co. Ltd; Tokyo, Japan). The study was conducted from June 2012 to April 2013. The specimens were fixed in 10% formaldehyde and stored in 70% ethanol.

The *Hesion* classification used in this study was based on the criteria in a key to the genera of the Hesionidae by Salazar-Vallejo and Orensanz (2006), and *H. picta* was identified according to Fauchald (1977b). The nomenclature used to describe polychaete morphology followed that of Rouse and Pleijel (2001).

#### DNA extraction and polymerase chain reaction analysis

Three *Hesion* cf. *picta* samples were separated for the molecular procedure each from a different representative site. The total genomic DNA of fresh *Hesion* cf. *picta* was extracted from the visceral organs using a DNeasy tissue kit (Qiagen; Hilden, Germany) according to the manufacturer's protocol. For 28S and 18S rDNA sequencing, two-pair primers were used for amplifying rDNA target sequences in genomic DNA; 5'-ACCCGCTGAATTTAAGCAT-3' and 5'-TCCGTGTTTCAAGACGG-3' for 28S rDNA amplification (approximately 800 bp) (Lê *et al.*, 1993) and 5'-TACCTGGTTGATCCTGCCAGTAG-3' and 5'-GATCCT TCCGCAGGT TCACCTAC-3' primer pairs with a length of 1665 bp for 18S rDNA (Giribet *et al.* 1996). The analysis was based on the 5'-CGCCTGTTTATCAAAAACAT-3' with 5'-CCGGTCTGAACTCAGATCACGT-3' for 16S rDNA (450 bp) (Palumbi *et al.*, 1996). The polymerase chain reaction (PCR) conditions used were as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. The PCR products were electrophoresed on a 1.5% agarose

gel, stained with ethidium bromide and viewed on an ultraviolet transilluminator. DNA bands were purified using the QIAquick gel extraction kit (Qiagen; Hilden, Germany). DNA sequencing was performed by Macrogen DNA Sequencing Service, Seoul, Korea. The sequencing results were searched for local regions of similarity using the online program Basic Local Alignment Search Tool (Benson *et al.*, 2005; Zhang and Madden, 1997). The partial sequences of the striped bristle worm 28S, 18S and 16S rDNA genes have been deposited in GenBank under accession numbers KF006977, KJ748004 and KF006978, respectively.

#### Phylogenetic analysis of partial 28S, 18S and 16S rDNA sequences

For the aligned sequences of 28S, 18S and 16S rDNA sequences, representing fifteen species of Hesionidae and two outgroup species, the source of nucleotide data was searched from GenBank (Benson *et al.*, 2005) as accession numbers DQ442598, DQ442584 DQ442569, JN631346, JN631335, JN631323, JN631346, JN631337, JN631323, DQ442603, DQ442587, DQ442574, DQ442604, JN631329, JN631319, JN631343, JN631330, JN631320, DQ442605, DQ442589, DQ442575, JN631349, JN631338, JN631325, DQ442606, DQ779661, DQ442577, DQ442607, EU555039, DQ442578, DQ442609, DQ442594, DQ442580, DQ442611, JN631332, DQ442582, DQ442612, DQ442596, DQ442583, DQ513309, JN631333, DQ513303, EU100079, AF116011, AF315058, HQ691218, HQ691211 and HQ691206 (Table 1). The multiple sequence alignments were performed with the ClustalW2 program (Larkin *et al.*, 2007) and 28S rDNA sequences were linked to 18S and 16S rDNA sequences using BioEdit Sequence Alignment Editor Version 7.0.7 (Abbott Laboratories; IL, USA.).

The phylogenetic analyses based on 28S, 18S and 16S rDNA sequences were performed separately. The sequencing was analyzed from

**Table 1** Lists of hesionid and other polychaete, with accession numbers for 28S, 18S and 16S rDNA sequences.

Species	rDNA	Accession number	Reference
<b>Ingroup</b>			
<i>Amphiduros fuscescens</i>	28S	DQ442598	Ruta <i>et al.</i> , 2007
	18S	DQ442584	Ruta <i>et al.</i> , 2007
	16S	DQ442569	Ruta <i>et al.</i> , 2007
<i>Gyptis brunnea</i>	28S	JN631346	Pleijel <i>et al.</i> , 2012
	18S	JN631335	Pleijel <i>et al.</i> , 2012
	16S	JN631323	Pleijel <i>et al.</i> , 2012
<i>G. pacifica</i>	28S	JN631346	Pleijel <i>et al.</i> , 2012
	18S	JN631337	Pleijel <i>et al.</i> , 2012
	16S	JN631323	Pleijel <i>et al.</i> , 2012
<i>G. rosea</i>	28S	DQ442603	Ruta <i>et al.</i> , 2007
	18S	DQ442587	Ruta <i>et al.</i> , 2007
	16S	DQ442574	Ruta <i>et al.</i> , 2007
<i>Hesione cf. picta</i>	28S	KF006977	This study
	18S	KJ748004	This study
	16S	KF006978	This study
<i>Hesiospina aurantiaca</i>	28S	DQ442604	Ruta <i>et al.</i> , 2007
	18S	JN631329	Pleijel <i>et al.</i> , 2012
	16S	JN631319	Pleijel <i>et al.</i> , 2012
<i>H. vestimentifera</i>	28S	JN631343	Pleijel <i>et al.</i> , 2012
	18S	JN631330	Pleijel <i>et al.</i> , 2012
	16S	JN631320	Pleijel <i>et al.</i> , 2012
<i>Leocrates chinensis</i>	28S	DQ442605	Ruta <i>et al.</i> , 2007
	18S	DQ442589	Ruta <i>et al.</i> , 2007
	16S	DQ442575	Ruta <i>et al.</i> , 2007
<i>Neogyptis carriebowcayi</i>	28S	JN631349	Pleijel <i>et al.</i> , 2012
	18S	JN631338	Pleijel <i>et al.</i> , 2012
	16S	JN631325	Pleijel <i>et al.</i> , 2012
<i>Nereimyra punctata</i>	28S	DQ442606	Ruta <i>et al.</i> , 2007
	18S	DQ779661	Rousset <i>et al.</i> , 2007
	16S	DQ442577	Ruta <i>et al.</i> , 2007
<i>Ophiodromus flexuosus</i>	28S	DQ442607	Ruta <i>et al.</i> , 2007
	18S	EU555039	Wiklund <i>et al.</i> , 2009
	16S	DQ442578	Ruta <i>et al.</i> , 2007
<i>Podarkeopsis arenicolus</i>	28S	DQ442609	Ruta <i>et al.</i> , 2007
	18S	DQ442594	Ruta <i>et al.</i> , 2007
	16S	DQ442580	Ruta <i>et al.</i> , 2007
<i>Sirsoe methanicola</i>	28S	DQ442611	Ruta <i>et al.</i> , 2007
	18S	JN631332	Pleijel <i>et al.</i> , 2012
	16S	DQ442582	Ruta <i>et al.</i> , 2007
<i>Syllidia armata</i>	28S	DQ442612	Ruta <i>et al.</i> , 2007
	18S	DQ442596	Ruta <i>et al.</i> , 2007
	16S	DQ442583	Ruta <i>et al.</i> , 2007
<i>Vrijenhoekia balaenophila</i>	28S	DQ513309	Pleijel <i>et al.</i> , 2008
	18S	JN631333	Pleijel <i>et al.</i> , 2012
	16S	DQ513303	Pleijel <i>et al.</i> , 2008
<b>Outgroup</b>			
<i>Hirudo medicinalis</i>	28S	EU100079	Borda <i>et al.</i> , 2008
	18S	AF116011	Apakupakul <i>et al.</i> , 1999
	16S	AF315058	Halanych <i>et al.</i> , 2001
<i>Lumbricus terrestris</i>	28S	HQ691218	Novo <i>et al.</i> , 2011
	18S	HQ691211	Novo <i>et al.</i> , 2011
	16S	HQ691206	Novo <i>et al.</i> , 2011

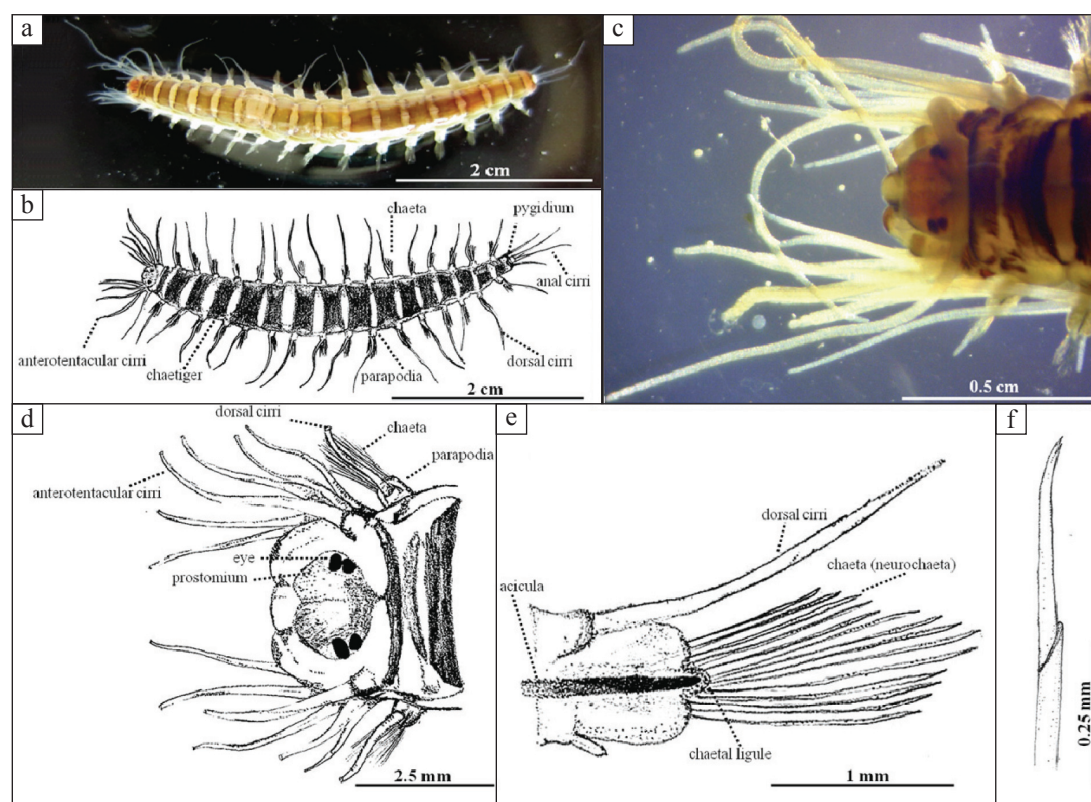
each individual in triplicate. A neighbour-joining analysis with 1,000 bootstrap replications was performed using the Molecular Evolutionary Genetics Analysis (MEGA) version 5.10 software package (Tamura *et al.* 2011) for Windows.

## RESULTS

The specimen of *Hesione cf. picta* sampled from the tropical seacoast of the Gulf of Thailand were almost 40 mm in length, dorsoventrally flattened, with a dark brownish dorsum and irregularly alternating transverse light stripes (Figures 2a and b) and 16 chaetigers, distinctly rectangular in shape, bearing pairs of 16 parapodia laterally. Four enlarged anal cirri were present on the end of the pygidium, with middle pairs longer than lateral pairs. The head was obtuse with four eyes on an orange-red prostomium,

eight pairs of anterotentacular cirri and no palps (Figures 2c and d). The parapodia were mucronate; all neuropodia were uniramous, the notopodia reduced, no less than 17 neurochaetae, and long slender dorsal cirri (Figure 2e). Aciculae embedded within the parapodia appeared from proximal to distal. The neurochaetae appeared as unidentate falcigers, with the blades serrate, similar to other chaetae on the chaetal ligule (Figure 2f).

To perform a phylogenetic analysis within the Hesionidae, the 15 species considered were divided into two large clusters and one small cluster based on the partial 28S, 18S and 16S rDNA sequences. The large clusters consisted of seven and six worm species, respectively, with common cladistic properties. The seven polychaete species in the first large cluster were: *Vrijenhoekia balaenophila*, *Sirsoe methanicola*, *Hesiospina aurantiaca*, *H. vestimentifera*, *Syllidia armata*,



**Figure 2** General morphology of *Hesione cf. picta*: a–b = Dorsal views of whole body; c–d = Dorsal views of anterior part; e = Neuropodium; f = Unidentate falcigers.

*Nereimyra punctata* and *Amphiduros fuscescens*. The six species of the other large cluster were: *Gyptis brunnea*, *G. pacifica*, *G. rosea*, *Neogyptis carriebowcayi*, *Ophiodromus flexuosus* and *Podarkeopsis arenicolus*. *Hesionone cf. picta* was pooled in a small cluster with *Leocrates chinensis* as a monophyletic taxon. All hesionids were also highly distant from the outgroup (*Hirudo medicinalis* and *Lumbricus terrestris*) as shown in Figure 3.

### DISCUSSION

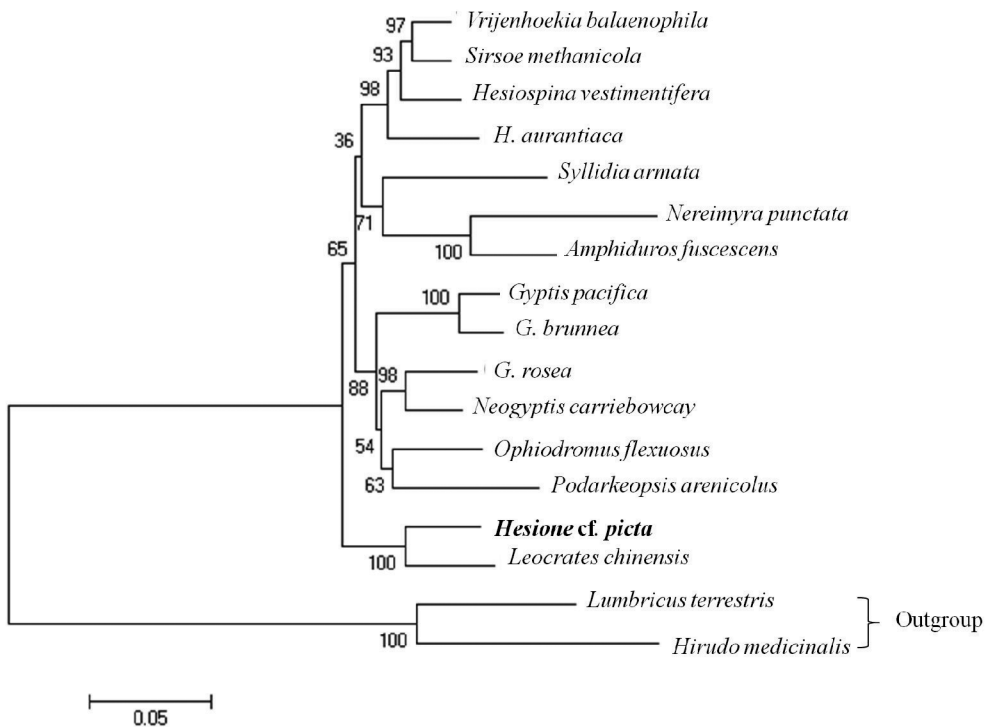
In terms of general morphology, these results corresponded to the characteristics specified in the guide to polychaetes in the report of Uebelacker (1984), in which *H. picta* was described from the Northern Gulf of Mexico.

Four closely related *Hesionone* species have been found in the South China Sea: *H. genetta*, *H.*

*splendida* from China, *H. reticulata* from Japan and *H. intertexta* from the Philippines (Paxton and Chou, 2000; Uchida, 2004). In Thailand, an unidentified *Hesionone* sp. was listed by Aungtunya *et al.* (2002) from Phuket Island, Andaman Sea. The specimens of *Hesionone cf. picta* collected in the current study are the first to be reported from the Gulf of Thailand and an area including the South China Sea and extending to the Malay Archipelago.

De Assis *et al.* (2012) reported a symbiotic association in the Southwestern Atlantic region between *H. picta* and reticulated brittle stars (*Ophionereis reticulata*) from the State of Paraiba, Brazil. In the present study, however, no symbiotic associations involving *Hesionone cf. picta* were observed.

Pleijel (1998) reported that *H. splendida* was more closely related to *L. chinensis* than to *O. flexuosus* in an analysis of hesionid interrelationships based on morphological



**Figure 3** Neighbour-joining tree for selected polychaetes, showing the interrelationships of *Hesionone cf. picta* with 15 hesionids and 2 outgroup species using the partial nucleotide sequences of 28S, 18S and 16S rDNA.

characters. Ruta *et al.* (2007) also reported that *Hesion* sp. was associated with *L. chinensis* in a hesionid consensus tree inferred from DNA sequences and morphological data. In view of these phylogenetic data, it appears that the partial nucleotide sequences of 28S, 18S and 16S rDNA support the morphological data on which the cladistic relationships of the Hesionidae were based and were congruent with the morphological hypotheses about hesionid relationships.

In recent decades, the evident diversities of cryptic species have been reported on a large scale for many marine metazoans including sponges, crustaceans, cnidarians, mollusks polychaetes, fishes and mammals (Colborn *et al.*, 2001; Miller *et al.*, 2001; Goetze, 2003; Wada *et al.*, 2003; Kirkendale and Meyer, 2004; Dawson *et al.*, 2005; Nygren, 2013). Cryptic species appear as two or more distinct species which are similar in morphology as a single species but are quite distinct genetically (Jörger and Schröd 2013). In marine polychaetes, Bastrop *et al.* (1998) described cryptic communities of *Marenzelleria viridis* from North America to Europe based on mitochondrial 16S rDNA sequences. Kesaniemi *et al.* (2012) reported the cryptic evidence of *Pygospio elegans* from European samples using a partial mitochondrial cytochrome c oxidase subunit I sequences. Recently, Glasby *et al.* (2013) also reported that *Nereis denhamensis*, *Perinereis suluana* and *Pseudonereis anomala* collected from Australian coral reefs were cryptic nereidid polychaetes in the molecular phylogeny based on mitochondrial cytochrome c oxidase subunit I and nuclear histone H3. Taking all data into consideration, it may be impossible that the *H. picta* have a cosmopolitan distribution from the place of origin to the Gulf of Thailand. The current study's finding that *Hesion* sp. was *Hesion* cf. *picta* based on the morphology and molecular data of partial mitochondrial 28S, 18S and 16S rDNA sequences although additional data remain to be required for precise clarification of whether this is a cryptic species or not for this worm.

## CONCLUSION

To the best of the authors' knowledge, the current report on *Hesion* cf. *picta* represents a new record of the species from the western Gulf of Thailand. Based on the general morphology and molecular phylogeny of the specimens collected that were investigated using the nucleotide sequences of the 28S, 18S and 16S rDNA genes, the *Hesion* cf. *picta* DNA sequences obtained appear to represent a primary genetic source for further study of the systematic biology of polychaetes.

## LITERATURE CITED

- Apakupakul, K., M.E. Siddall and E.M. Bureson. 1999. Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences **Mol. Phylogenet. Evol.** 12: 350–359.
- Aungtunya, C., S. Thaipal and S. Bussarawit. 2002. A list of polychaetes (annelida) in the reference collection database of the Phuket Marine Biological Center, Thailand. **Phuket Mar. Biol. Cent. Spec. Publ.** 24: 21–32.
- Bastrop, R., K. Jürss and C. Sturmbauer. 1998. Cryptic species in marine polychaete and their independent introduction from North America to Europe. **Mol. Biol. Evol.** 15(2): 97–103.
- Benson D.A., I. Karsch-Mizrachi, D.J. Lipman, J. Ostell and D.L. Wheeler. 2005. GenBank. **Nucleic Acids Res.** 33: 34–38.
- Borda, E., A. Ocegüera-Figueroa and M.E. Siddall. 2008. On the classification, evolution and biogeography of terrestrial haemadipsoid leeches (Hirudinida: Arhynchobdellida: Hirudiniformes). **Mol. Phylogenet. Evol.** 46: 142–154.
- Canales-Aguirre, B., N. Rozbaczylo and C.E. Hernández. 2011. Genetic identification of benthic polychaetes in a biodiversity hotspot in the southeast Pacific. **Rev. Biol. Mar. Oceanogr.** 46: 89–94.

- Colborn, J., R.E. Crabtree, J.B. Shaklee, E. Pfeiler and B.W. Bowen. 2001. The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. **Evolution** 55: 807–820.
- Costa, D.A., J.E. De Assis and M.L. Christoffersen. 2008. New synonym of *Hesione splendida* (Hesionidae, Polychaeta, Annelida). **Biociencias** 16: 131–133.
- Day, J.H. 1962. Polychaeta from several localities in the western Indian Ocean. **Proc. Zool. Soc. Lond.** 139: 627–656.
- Dean, H.K. 1998. A new species Of Hesionidae, *Glyphohesione Nicoyensis* (Annelida : Polychaeta), from the Gulf of Nicoya, Costa Rica. **Proc. Biol. Soc. Wash.** 111: 257–262.
- De Assis, J.E., A.S. Bezerra, R.J. De Brito, A.I. Gondim and M.L. Christoffersen. 2012. An association between *Hesione picta* (Polychaeta: Hesionidae) and *Ophionereis reticulata* (Ophiuroidea: Ophionereididae) from the Brazilian Coast. **Zool. Stud.** 51: 762–767.
- Dawson, M.N., A. Sen Gupta and M.H. England. 2005. Coupled biophysical global ocean model and molecular genetic analyses identify multiple introductions of cryptogenic species. **Proc. Natl. Acad. Sci. USA** 102: 11968–11973.
- Fauchald, K. 1977a. The polychaete worms, definitions and keys to the orders, families and genera. **Nat. Hist. Mus. Los Angeles County, Sci. Ser.** 28: 1–188.
- Fauchald, K. 1977b. Polychaetes from intertidal areas in Panama, with a review of previous shallow-water records. **Smithson. Contr. Zool.** 221: 1–81.
- Giribet, G., S. Carranza, J. Baguna, M. Riutort and C. Ribera. 1996. First molecular evidence for the existence of a Tardigrada Arthropoda clade. **Mol. Biol. Evol.** 13: 76–84.
- Glasby, C.J., V. Wei and K.S. Gibb. 2013. Cryptic species of Nereididae (Annelida : Polychaeta) on Australian coral reefs. **Invertebr. Syst.** 27(3): 245–264.
- Goetze, E. 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. **Proc. Roy. Soc. Lond. B. Biol. Sci.** 270: 2321–2331.
- Halanych, K.M., R.A. Feldman and R.C. Vrijenhoek. 2001. Molecular evidence that *Sclerolinum brattstromi* is closely related to vestimentiferans, not to frenulate pogonophorans (Siboglinidae, Annelida). **Biol. Bull.** 201: 65–75.
- Jörger, K.M. and M. Schröd. 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. **Front. Zool.** 10(1): 59.
- Kesaniemi, J.E., P.D. Rawson, S.M. Lindsay and K.E. Knott. 2012. Phylogenetic analysis of cryptic speciation in the polychaete *Pygospio elegans*. **Ecol. Evol.** 2(5): 994–1007.
- Kirkendale, L.A. and C.P. Meyer. 2004. Phylogeography of the *Patelloida profunda* group (Gastropoda: Lottidae): diversification in a dispersal-driven marine system. **Mol. Ecol.** 13: 2749–2762.
- Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J. D Thompson, T.J. Gibson and D.G. Higgins. 2007. ClustalW and ClustalX version 2. **Bioinformatics** 23: 2947–2948.
- Lê, H.L. V., G. Lecointre and R. Perasso. 1993. A 28S rRNA based phylogeny of the gnathostomes: First steps in the analysis of conflict and congruence with morphologically based cladograms. **Mol. Phylogenet. Evol.** 14: 1–10.
- Machida, R.J. and N. Knowlton. 2012. PCR Primers for metazoan nuclear 18S and 28S ribosomal DNA sequences. **PLoS ONE** 7: 1–11.
- Martin, D., E. Ballesteros, J.M. Gili and C. Palacín. 1993. Small-scale structure of infaunal polychaete communities in an estuarine



- environment: Methodological approach. **Est. Coast. Shelf Sci.** 36: 47–58.
- Martin, D. and T. Britayev. 1998. Symbiotic polychaetes: review of known species. **Oceanogr. Mar. Biol.** 36: 217–340.
- Martin, D., A. Cuesta, P. Drake, J. Gil, A. Nygren and F. Pleijel. 2012. The symbiotic hesionid *Parasyllidea humesi* Pettibone, 1961 (Annelida: Polychaeta) hosted by *Scrobicularia plana* (da Costa, 1778) (Mollusca: Bivalvia: Semelidae) in European waters. **Org. Divers. Evol.** 12: 145–153.
- Miller, K., B. Alvarez, C. Battershill, P. Northcote and H. Parthasarathy. 2001. Genetic, morphological, and chemical divergence in the sponge genus *Latrunculia* (Porifera: Demospongiae) from New Zealand. **Mar. Biol.** 139: 235–250.
- Müller, O.F. 1776. **Zoologiae Danicae Prodromus, seu Animalium Daniae et Norvegiae Indigenarum Characteres, Nomina, et Synonyma Imprimis Popularium.** Havniae, Hallager. 274 pp.
- Müller, O.F. 1858. Einiges über die Anneliden Fauna der Insel St. Catharina an der Brazilianischen Kuste. **Archiv. für Natur. (Berlin)** 24.1: 211–220.
- Novo, M., A. Almodovar, R. Fernandez, G. Giribet and D.J. Diaz Cosin. 2011. Understanding the biogeography of a group of earthworms in the Mediterranean basin-The phylogenetic puzzle of Hormogastridae (Clitellata: Oligochaeta) **Mol. Phylogenet. Evol.** 61(1): 125–135.
- Nygren, A. 2013. Cryptic polychaete diversity: a review. **Zool. Scr.** 43(2): 172–183.
- Pleijel, F. 1998. Phylogeny and classification of Hesionidae (Polychaeta). **Zool. Scr.** 27: 89–163.
- Pleijel, F. and G.W. Rouse. 2005. Reproductive biology of a new hesionid polychaete from the Great Barrier Reef. **Biol. Bull.** 208: 69–76.
- Pleijel, F., G.W. Rouse, C. Ruta, H. Wiklund and A. Nygren. 2008. *Vrijenhoekia balaenophila*, a new hesionid polychaete from a whale fall off California. **Zool. J. Linnean Soc.** 152: 625–634.
- Pleijel, F., G.W. Rouse, T. Sundkvist and A. Nygren. 2012. A partial revision of *Gyptis* (Gyptini, Ophiodrominae, Hesionidae, Aciculata, Annelida), with descriptions of a new tribe, a new genus and five new species. **Zool. J. Linnean Soc.** 165: 471–494.
- Palumbi, S.R., A. Martin, S. Romano and W.O. Mcmillan. 1996. **The Simple Fool's Guide to PCR.** University of Hawaii, Honolulu, HI, USA. 94 pp.
- Paxton, H. and L.M. Chou. 2000. Polychaetous annelids from the South China Sea. **Raffles B. Zool.** 8: 209–232.
- Rouse, G.W. and F. Pleijel. 2001. **Polychaetes.** Oxford University Press. Oxford, UK. 354 pp.
- Rousset, V., F. Pleijel, G.W. Rouse, C. Erseus and M.E. Siddall. 2007. A molecular phylogeny of annelids. **Cladistics** 23: 41–63.
- Ruta, C., A. Nygren, V. Rousset, P. Sundberg, A. Tillier and F. Pleijel. 2007. Phylogeny of Hesionidae (Aciculata, Polychaeta), assessed from morphology, 18S rDNA, 28S rDNA, 16S rDNA and COI. **Zool. Scr.** 36: 99–107.
- Salazar-Vallejo, S.I. and J.M. Orensanz. 2006. *Pleijelius longae* n. gen., n. sp., a remarkable deep water polychaete from the northwestern Atlantic (Polychaeta: Hesionida. **Sci. Mar.** 70 (3): 157–166.
- Subida, M.D., A.M. Arias, P. Drake, R. García, A. Rodríguez and J.A. Cuesta. 2011. On the occurrence of *Afropinnotheres monodi* Manning, 1993 (Decapoda: Pinnotheridae) in European waters. **J. Crust. Biol.** 31: 367–369.
- Tan, L. and L. Chou. 1993. Checklist of polychaete species from Singapore waters (Annelida). **Raffles B. Zool.** 41: 279–295.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary

- distance, and maximum parsimony methods. **Mol. Phylogenet. Evol.** 28: 2731–2739.
- Uchida, H. 2004. Hesionidae (Annelida, Polychaeta) from Japan. **Kuroshio Biosph.** 1: 27–92.
- Uebelacker, J.M. 1984. Family Hesionidae Sars, 1862, pp. 28–36. In J.M. Uebelacker and P. G. Johnson, (eds.). **Taxonomic Guide to the Polychaetes of the Northern Gulf of Mexico Vol. 4 Chapter 28.** Final Report to the Minerals Management Service. A.V. Barry and Associates Inc. Mobile, AL, USA.
- Wada, S., M. Oishi and T.K. Yamada. 2003. A newly discovered species of living baleen whale. **Nature** 426: 278–281.
- Westheide, W. 1982. *Microphthalmus hamosus* sp.n. (Polychaeta, Hesionidae) –an example of evolution leading from the interstitial fauna to a macrofaunal Interspecific relationship. **Zool. Scr.** 11: 189–193.
- Wiklund, H., A.G. Glover, P.J. Johannessen, and T.G. Dahlgren. 2009. Cryptic speciation at organic-rich marine habitats: a new bacteriovore annelid from whale-fall and fish farms in the North-East Atlantic. **Zool. J. Linn. Soc.** 155: 774–785.
- Wu, B., R. Sun and M. Chen. 1980. Zoogeographical studies on Polychaeta from the Xissha Islands and its adjacent water. **Acta Oceanol. Sin.** 2:111–130.
- Zhang, J. and T.L. Madden. 1997. "PowerBLAST: A new network BLAST application for interactive or automated sequence analysis and annotation. **Genome Res.** 7: 649–656.