

***Batillipes pennaki* Marcus, 1946, a New Addition to the Thai Tardigrade Fauna, with an Overview of Literature on the Species**

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Abstract. Tardigrades are a phylum of microscopic invertebrates (ca. 1100 species) with a worldwide distribution. Currently only ca. 17% of the total number of known species are considered entirely marine. Despite the fact that the first marine Tardigrada were described over a century ago, only two reports on marine tardigrades of Thailand have been published so far. In this paper we present the first record of *Batillipes pennaki* Marcus, 1946 from the Thai coast. We also summarise the current state of knowledge on the biology and ecology of the species. Additionally, a short summary of the Thai tardigrade fauna is given.

Key words: Arthrotardigrada, new record, marine Tardigrada, meiofauna, Thailand

INTRODUCTION

Tardigrada, known as water bears, are a phylum of micrometazoans that inhabit various ecosystems throughout the World, from the deepest seas to the highest parts of the Himalayas (Ramazzotti and Maucci, 1983). Terrestrial tardigrades are found in mosses, lichens, hepatics, leaf litter, soil and even on glaciers (in cryoconite holes and on glacier mice). Aquatic species inhabit both freshwater and marine habitats. In freshwater they live in sand, mud, detritus and on aquatic plants whereas in oceans they have been found from the intertidal zone to abyssal depths, in sediments (fine mud to coarse sand), on rocks, algae and on animals such as barnacles (Hansen, 2005). Currently, the majority of ca. 1100 known species (Degma *et al.*, 2012) are known from terrestrial ecosystems whereas only 183 species (and subspecies) are marine (Appeltans *et al.*, 2012).

Despite of a relatively high number of studies on the Asian terrestrial tardigrade fauna (*e.g.*, Mathews, 1936-1937; Iharos, 1968; Dastych, 1975;

Kaczmarek and Beasley, 2002; Dastych, 2004; Kaczmarek and Michalczyk, 2004, 2006; Li and Liu, 2005; Beasley and Miller, 2007; Li, 2007), and marine tardigrade fauna (Indian Ocean *e.g.* Rao, 1971; Renaud-Mornant, 1975; Grimaldi de Zio *et al.*, 1987; Gallo *et al.*, 2007 or the Pacific Ocean *e.g.* Tchesunov and Mokievsky, 1995; Rho *et al.*, 1999), the tardigrade fauna of Thailand is still very poorly known and until now only a few papers have been published. Thai terrestrial tardigrades have been studied only by Pilato *et al.* (2004) and Tumanov (2005, 2006). Pilato *et al.* (2004) reported three species including two new to science: *Pseudechiniscus* cf. *quadrilobatus* Iharos, 1969, *Calcarobiotus* (*Calcarobiotus*) *digeronimoi* Pilato, Binda and Lisi, 2004 and *Mixibius sutirae* Pilato, Binda and Lisi 2004. Tumanov (2005, 2006) reported ten taxa (also including two species new to science): *Paramacrobiotus alekseevi* (Tumanov, 2005), *Macrobiotus siamensis* Tumanov, 2006, *Macrobiotus* sp. (*harmsworthi* group), *Macrobiotus* sp., *Minibiotus* sp., *Calcarobiotus filmeri* Dastych, 1993, *Doryphoribius* sp., *Hypsibius convergens* (Urbanowicz, 1925), *Astatumen bartosi* (Węglarska, 1959), *Diphascon* sp. (*pingue* group). Marine tardigrades in Thailand have been studied only by Chang and Rho (1998), who described three new species: *Archechiniscus symbalanus* Chang and

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Rho, 1998, *Styraconyx craticuliformis* Chang and Rho, 1998 and *Echiniscoides andamanensis* Chang and Rho, 1998, and by Faurby *et al.* (2012) who reported three distinct molecular lineages of *Echiniscoides* sp. (interpreted as two or three different species). Thus, up to now the tardigrade fauna of Thailand counts eight terrestrial and three marine taxa identified to species level.

The genus *Batillipes* was established by Richters (1909) and belongs to the monotypic marine family Batillipedidae Ramazzotti, 1962 (Kristensen and Mackness, 2000). Currently, *Batillipes* consists of twenty six valid species, which makes it the largest marine tardigrade genus (Degma *et al.*, 2012). *Batillipes* has a worldwide distribution, with many localities in seas and oceans surrounding Europe (*e.g.*, Richters, 1909; Kristensen, 1978; Pollock, 1971; Morone De Lucia *et al.*, 1988), the Americas (*e.g.*, Marcus, 1946; McGinty, 1969; McKirdy, 1975), Africa (*e.g.* Renaud-Mornant, 1979), Asia (*e.g.*, Renaud-Mornant, 1967; Rho *et al.*, 1999) and Australia (Kristensen and Mackness, 2000).

In the present study seven specimens of *B. pennaki* are reported from a single sample collected from a sandy beach on the coast of Thailand. We also provide a literature-based overview of the taxonomy, distribution and ecology of the species. Finally, this report summarises and discusses the current knowledge on the tardigrade fauna of Thailand.

MATERIALS AND METHODS

Tardigrades were collected from a sandy beach of the Kho Khao Island in February 2008. Material was taken using a 30 cm long perspex core with an inner diameter of 3.6 cm and immediately fixed with a 4% buffered formaldehyde water solution and stained with Bengal Rose. The upper 5 cm of sediment was taken for the analysis. A detailed description of the study area and the sampling methodology can be found in Grzelak *et al.* (2009).

In the laboratory, tardigrades were extracted and mounted on microscope slides in Hoyer's medium and examined using Phase Contrast Microscopy. Specimens were identified according

Marcus (1946) and with the key to the World Tardigrada fauna by Ramazzotti and Maucci (1983) and later species descriptions (McKirdy, 1975; Gallo D'Addabbo *et al.*, 2005). All measurements are given in micrometres (μm). Structures were measured only if their orientations were suitable. Body length was measured from the anterior to the posterior end, excluding the caudal appendages. Head width was measured as the distance between the bases of the lateral cirri (McKirdy 1975). Toes were numbered from the anterior to the posterior body parts (Kristensen and Mackness, 2000). Only toes on legs I and IV were measured. All specimens (slides P9/1-P9/7) are deposited in the Department of Animal Taxonomy and Ecology, A. Mickiewicz University, Poznań, Poland.

RESULTS

Phylum: Tardigrada (Spallanzani, 1777)
 Class: Heterotardigrada Marcus, 1927
 Order: Arthrotardigrada Marcus, 1927
 Family: Batillipedidae Ramazzotti, 1962
 Genus: *Batillipes* Richters, 1909

Batillipes pennaki Marcus, 1946
 (Fig. 1)

Terra typica

West Atlantic Ocean.

Material examined

Seven specimens were found in meiofaunal sample collected in February 2008 by Witold Szczuciński.

Location

Kho Khao beach, North-Western coast of the Kho Khao Island, Thailand: 09°00'N, 98°15'E, ca. 0 m asl.

Description (Fig. 1, Table I)

Eyes absent in mounted individuals. Cuticle with clearly visible lateral processes between legs III-IV (Fig. 1A). Minute granulation present on the body surface (Fig. 1B). The unpaired median cirrus, paired internal cirri and external cirri present. Internal and external cirrus longer than the median cirrus. Lateral cirri A present. Clava with a constriction between the thicker basal and the

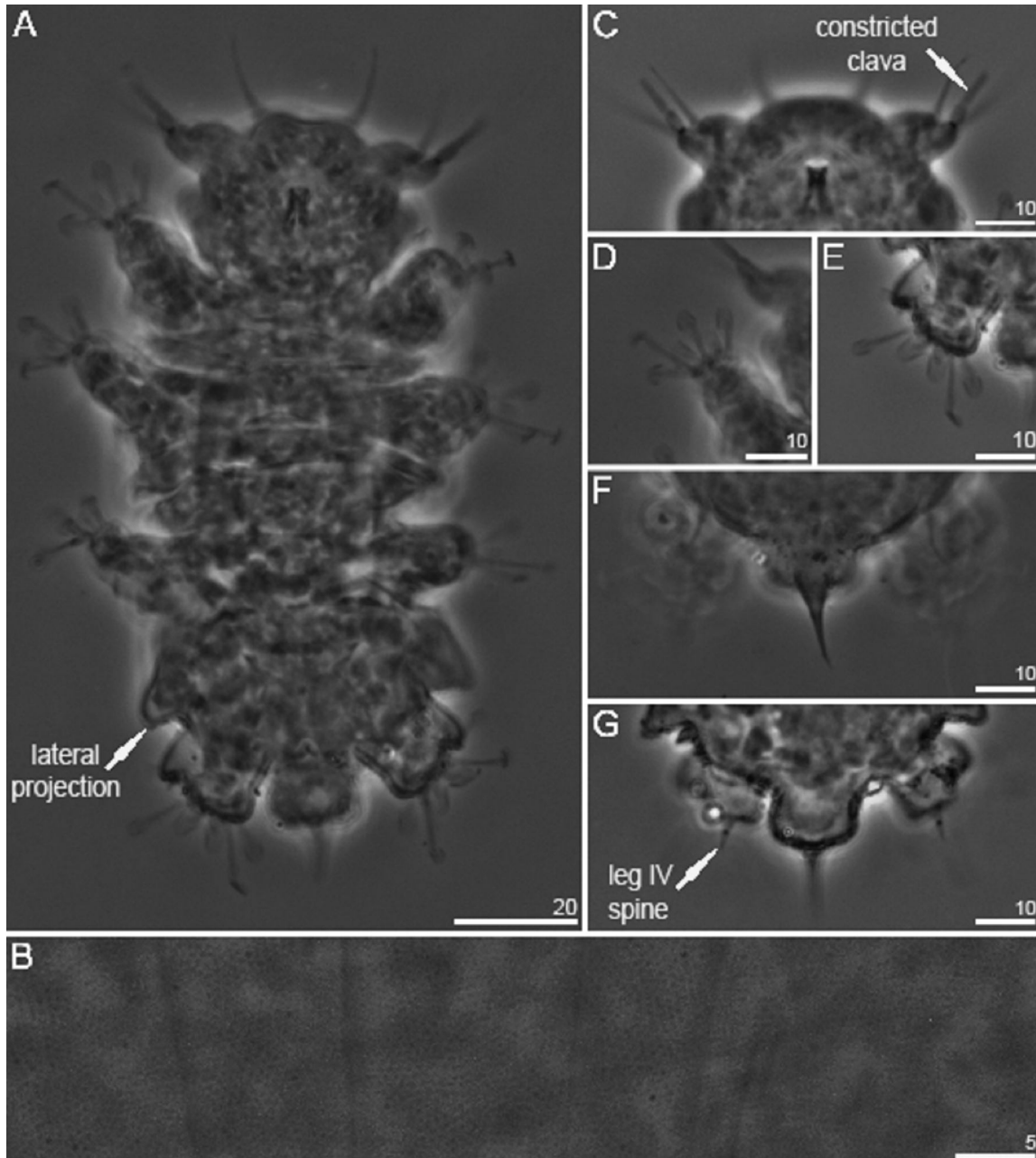


Fig. 1. *Batillipes pennaki*: A, habitus (ventral view); B, granulation on the cuticle; C, clava; D, toes of leg I; E, toes of leg IV; F, the caudal appendage; G, spines on legs IV.

thinner distal part (Fig. 1C). Cirrus A and clava have a common base (Fig. 1C). Dorsal cirrus E present. Caudal spine with a sharply pointed termination (Fig. 1F). Bucco-pharyngeal apparatus with a thin and long buccal tube and a short oval buccal pharynx with three pharyngeal bars. All legs with

six toes. Toe 2 on legs I-III without a peduncle (Fig. 1D). Toe 3 and 4 on legs IV almost equal in length (Fig. 1E). Short spines on all legs present (Fig. 1G), sometimes not visible in Hoyer's medium. Gonophores are not visible in Hoyer's medium.

Table I. Morphometry of *Batillipes pennaki*. N is the number of specimens/structures measured; Min and Max are the smallest and the largest structure among all measured specimens, respectively; SD is standard deviation. Measurements are given in μm .

Character	N	Min	Max	Mean	SD
Body length	5	115	148	135	15
Body width	5	48.7	61.2	54.6	5.3
Head width	6	24.1	36.9	30.1	4.1
Median cirrus	3	6.4	9.5	8.0	1.6
Internal cirrus	7	8.8	13.2	11.8	1.4
External cirrus	5	8.7	14.5	11.9	2.1
Cirrus A	7	19.9	26.5	24.0	2.6
Clava	7	7.3	12.5	10.4	1.6
Cirrus E	1	15.3	15.3	15.3	–
Leg I spine	0	?	?	–	–
Leg II spine	4	6.3	12.2	8.3	2.7
Leg III spine	1	7.9	7.9	7.9	–
Leg IV spine	1	5.8	5.8	5.8	–
Caudal apparatus	6	11	16.1	13	1.9
Leg I					
toe 1	1	8.8	8.8	8.8	–
toe 2	1	5.3	5.3	5.3	–
toe 3	1	9.6	9.6	9.6	–
toe 4	1	6.4	6.4	6.4	–
toe 5	1	13	13	13	–
toe 6	1	8.2	8.2	8.2	–
Leg IV					
toe 1	2	6.8	9.6	8.2	2.0
toe 2	2	12.6	13.7	13.2	0.8
toe 3	2	5	6.2	5.6	0.9
toe 4	2	6	6.4	6.2	0.3
toe 5	2	13	13.1	13.1	0.1
toe 6	2	9.9	10.9	10.4	0.7

Differential diagnosis

B. pennaki is most similar to *B. gilmartini* McGinty, 1969 and *B. spinicauda* Gallo D'Addabbo, Sandulli and de Zio Grimaldi, 2005 by having a constricted clava and toe length configuration on legs IV, but differs from (i) *B. gilmartini* by a different basal support of the caudal spine (a wide basal support in *B. pennaki* vs. lacking that kind of base in *B. gilmartini*) and by a different tip shape of the caudal spine (sharp in *B. pennaki* vs. blunt in *B. gilmartini*); (ii) *B. spinicauda* by a different clava construction (two identical parts separated by a constriction in *B. pennaki* vs. a stiff and slightly wrinkled clava in *B. gilmartini*) and by some morphometric characters (shorter median, external and internal cirrus and clava in *B. pennaki*).

A chronological overview of knowledge on *Batillipes pennaki*

B. pennaki is a marine heterotardigrade, originally described from the Atlantic coasts of both American continents by Marcus (1946). During subsequent studies the species was also found in many other locations on both hemispheres (Kristensen and Mackness, 2000). The most important studies on *B. pennaki* along with their key conclusions are listed in Table II.

Table II. A chronological summary of the literature on *Batillipes pennaki*.

Reference	Key conclusions and remarks
Marcus (1946)	The original description of <i>B. pennaki</i> .
De Zio (1962)	First record of <i>B. pennaki</i> from the Mediterranean Sea with notes on a new differential characters.
De Zio and Grimaldi (1964)	1. Temperature is the key factor determining the distribution of <i>B. pennaki</i> . 2. Within the first meters off shore <i>B. pennaki</i> is usually found near the sand surface, whereas towards the beach it is found at greater depths in the sand. 3. Tardigrada and Nematoda are likely to compete for resources if present in the same spot.
De Zio and Grimaldi (1966)	Sediment characteristics do not influence <i>B. pennaki</i> distribution. The most important factor influencing <i>B. pennaki</i> distribution is the distance from the shore line.
Pollock (1970a)	<i>B. pennaki</i> is the most abundant tardigrade species on the beaches of Massachusetts (USA).
Pollock (1970b)	The male gonophore is oval-shaped whereas the female gonophore rosette-shaped.
Grimaldi de Zio and D'Addabbo (1975a)	Description of two larval stages of <i>B. pennaki</i> and an alternate type of the adult moult (not confirmed since).
Grimaldi de Zio and D'Addabbo (1975b)	1. A significant increase in reproduction in spring and autumn and reduction in summer (Mediterranean area). 2. Details of the morphology of the female gonophore.
Martinez (1975)	<i>B. pennaki</i> was dominant during September and November whereas <i>B. mirus</i> Richters, 1909 in June, August and January on the New York city beach.
McKirdy (1975)	1. Comparison of six species of the genus <i>Batillipes</i> . 2. Notes on the morphology of the species. Abundant in mid tidal heights. 3. <i>B. pennaki</i> recorded as a sympatric species of <i>B. bullacaudatus</i> McGinty&

- Higgins, 1968.
- Grimaldi de Zio *et al.* (1983) 1. *B. pennaki* shows preference towards medium grain size sand.
2. *B. pennaki* is a typical intertidal species.
- Victor Castro *et al.* (1999) Species distribution is directly affected via sedimentologic process, especially the erosive and depositional processes (Restinga do Paiva, Pernambuco, Brasil).
- Kristensen and Mackness (2000) Description of the world distribution of the species.
- D'Addabbo *et al.* (2000) Revision of the genus *Batillipes* with some remarks on the morphology of *B. pennaki*.
- Rocha *et al.* (2000) Spatio-temporal distribution of *B. pennaki* (the species was most numerous in January in the upper mesolittoral and almost absent in the infralittoral) (Itamaraca Island, Brasil).
- Grimaldi de Zio and D'Addabbo (2001) *B. pennaki* as subtidal and intertidal species. A summary of *B. pennaki* distribution (depths and substratum) in the Mediterranean sea.
- D'Addabbo *et al.* (2007) Sandy sediments (specifically medium fine sand) in areas of shallow waters are the most preferred habitat of the species.
- Ryu *et al.* (2007) First molecular data (mtDNA sequences) of the species, confirming its position within the Ecdysozoa.
- Jørgensen *et al.* (2010) First molecular phylogeny of the Arthrotardigrada (based on 28S rRNA sequences) with the position of *B. pennaki* on the phylogenetic tree of Arthrotardigrada.
- Schulze and Schmidt-Rhaesa (2011) Description of musculature based on staining of the f-actin with TRITC conjugated phalloid and observed under the confocal laser scanning microscopy. Somatic musculature of *B. pennaki* is grouped into dorsal, ventral, lateral, dorso-ventral and the leg musculature.

DISCUSSION

Studies on marine Thai tardigrades have been conducted sporadically, therefore it is not surprising that an analysis of a single hydrosammon sample resulted in a new record for the country. Given that there are nearly 200 known marine tardigrade species and the total number is thought to be close to 1500 species (Appeltans *et al.*, 2012), the four marine tardigrade species recorded so far from Thailand are probably a small fraction of the real biodiversity of this area. Terrestrial tardigrades are ubiquitous and in well studied areas/countries of a size comparable to that of Thailand, such as Poland

(Dastych, 1988), Costa Rica (Kaczmarek *et al.*, 2010) or New Zealand (Horning *et al.*, 1978), species numbers oscillate around a hundred. Thus, we should probably expect the terrestrial tardigrade fauna of Thailand to be at least several times larger than the current census of eight taxa.

Species of the genus *Batillipes* have been recorded from beaches throughout the world. *Batillipes* communities are found to be composed of either a single or several species (Kristensen and Mackness, 2000). Studies by Rho *et al.* (1999) carried out in Korea suggest, that more than one species of the genus is likely to inhabit Thai coasts. All records of *B. pennaki* are based solely on morphological traits, but if molecular data reveal great and discontinuous genetic variance between different populations of this taxon, the current opinion that the species is cosmopolitan may be refuted in favour of a species complex hypothesis.

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