Taxonomy of Freshwater Bdelloid Rotifers in the Genus *Rotaria* (Rotifera, Bdelloidea, Philodinidae) in Thailand

Rapeepan Jaturapruek

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Zoology
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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์ อนุกรมวิธานของเดลลอยด์โรติเฟอร์น้ำจืดสกุล *Rotaria* (Rotifera, Bdelloidea, Philodinidae) ในประเทศไทย

ผู้เขียน นางสาวรพีพรรณ จตุรพฤกษ์

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บทคัดย่อ

เดลลอยด์ เป็นโรติเฟอร์กลุ่มหนึ่งจัดอยู่ในไฟลัมโรติเฟอร์รา มีลักษณะเด่นคือ มีการสืบพันธุ์แบบไม่อาศัยเพศด้วยกระบวนการพาธีโนจีนีซิสตลอดชีวิต และสามารถทนต่อสภาพแวดล้อมที่ไม่เหมาะสม เช่น ภาวะแห้งของแหล่งอาศัย โดยมีกลไกการนำน้ำออกจากร่างกาย จากความสามารถดังกล่าวส่งผลให้พบการแพร่กระจายทั้งบนบกและในแหล่งน้ำ แม้ว่าเดลลอยด์โรติเฟอร์จะมีการศึกษาอย่างแพร่หลายในต่างประเทศโดยเฉพาะอย่างยิ่งในทวีปยุโรป แต่อย่างไรก็ตามในแถบเอเชียตะวันออกเฉียงใต้รวมทั้งในประเทศไทยยังมีการศึกษาน้อยมาก เนื่องจากมีข้อจำกัดหลายประการ เช่น ลักษณะสำคัญทางสัณฐานวิทยาสามารถสังเกตได้ขณะที่ตัวอย่างมีชีวิตเท่านั้น เดลลอยด์โรติเฟอร์สกุล *Rotaria* มีการแพร่กระจายกว้างขวาง ปัจจุบันมีจำนวนสมาชิกทั้งหมด 24 ชนิด ซึ่งในประเทศไทยเคยมีรายงานการพบเดลลอยด์โรติเฟอร์สกุลดังกล่าวเพียงหนึ่งชนิด คือ *Rotaria rotatoria* ดังนั้นงานวิจัยในครั้งนี้จึงสนใจศึกษาอนุกรมวิธานของเดลลอยด์โรติเฟอร์น้ำจืดสกุล *Rotaria* ในประเทศไทย โดยเก็บตัวอย่างในแหล่งน้ำจืดทั้งในฤดูร้อนและฝนจำนวนทั้งสิ้น 24 แหล่งน้ำทั่วประเทศไทย ศึกษาลักษณะสัณฐานภายนอก, โทรฟี และข้อมูลลำดับนิวคลีโอไทด์ จากการศึกษาพบเดลลอยด์โรติเฟอร์สกุล *Rotaria* ทั้งหมด 8 ชนิด ได้แก่ *R. mento*, *R. neptunia*, *R. neptunoida*, *R. ovata*, *R. rotatoria*, *R. tardigrada*, *Rotaria* sp. 1 และ *Rotaria* sp. 2 ซึ่งในจำนวนดังกล่าวมี 7 ชนิดที่มีรายงานการพบในประเทศไทยเป็นครั้งแรก นอกจากนี้ยังรายงานลักษณะทางสัณฐานวิทยาของ *R. ovata* เพิ่มเติมจากที่เคยมีรายงานก่อนหน้านี้ อีกทั้งพบว่า *Rotaria* sp. 1 และ *Rotaria* sp. 2 มีลักษณะทางสัณฐานวิทยาไม่สอดคล้องกับชนิดที่เคยมีรายงานมาก่อน ขนาดและจำนวนฟันของโทรฟีรวมถึงข้อมูลลำดับนิวคลีโอโทด์ สามารถใช้เป็นลักษณะร่วมในการระบุชนิด อีกทั้งยังแสดงถึงความสัมพันธ์เชิงวิวัฒนาการได้ จากการศึกษาครั้งนี้ทำให้พบความหลากหลายของเดลลอยด์โรติเฟอร์ในประเทศไทยเพิ่มขึ้นจาก 5 ชนิดเป็น 12 ชนิด แสดงให้เห็นว่าแหล่งน้ำในประเทศไทยมีความหลากหลายของสิ่งมีชีวิตกลุ่มนี้ หากมีการศึกษาเพิ่มเติมน่าจะพบความหลากชนิดเพิ่มขึ้น

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ABSTRACT

Bdelloids are a group of rotifers in Phylum Rotifera. The distinguished characteristics of this organism are obligated asexual reproduction by parthenogenesis and able to resist in unsuitable environments such as drying habitat in which they can remove water from their body. For above reasons, they can disperse in both moist terrestrial habitats and water bodies. Although bdelloids are well studied in other countries especially in Europe, but they have a few recorded in Southeast Asia including Thailand because the limitations in the studying bdelloids, for examples: the important features can be observed only when they are alive. In this research, genus *Rotaria* was chosen because they are expansive distribution. Twenty-four species are member in this genus, however, only one species *Rotaria rotatoria* has been recorded from Thailand. Therefore the present study focuses on the taxonomy of freshwater bdelloid rotifers in the genus *Rotaria* in Thailand. The specimens were collected in 24 freshwater habitats throughout Thailand in dry and rainy seasons. External morphology, trophi and nucleotide data were studied. A total of 8 species, namely *R. mento*, *R. neptunia*, *R. neptunoida*, *R. ovata*, *R. rotatoria*, *R. tardigrada*, *Rotaria* sp. 1 and *Rotaria* sp. 2 were found. Of which, seven species are new records for Thailand. Additional characters of *R. ovata* were included from the original description; the morphological features of *Rotaria* sp. 1 and *Rotaria* sp. 2 are different from other members in this genus. Size and teeth number of trophi including nucleotide data can be used to support the species identification and evolution. From this study, the species diversity of bdelloid rotifers in Thailand increased from 5 species to 12 species indicated that freshwater habitats in Thailand have high diversity of this fauna. More intensively study can be reveal species diversity of bdelloids rotifers.

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LIST OF ABBREVIATIONS

Cond.	=	conductivity
°C	=	degree Celsius
DO	=	dissolved oxygen
et al.	=	Et. Alii (Latin), and others
e.g.	=	exempli gratia (Latin)
fig.	=	figure
km^2	=	square kilometre
mgO_2/l	=	milligram oxygen per liter
O_2	=	oxygen
ppt	=	part per thousand
Temp.	=	temperature
µm	=	micrometer
µS	=	micro-Siemens
µS/cm	=	micro-Siemens per centimeter

Abbreviations in figures

a	=	antenna
AFR	=	Afrotropical
AL	=	antenna length
AUS	=	Australian
c	=	corona
cl	=	cloaca
CW	=	corona width
dt	=	distal minor teeth
e	=	eye
F	=	foot
FL	=	foot length
H	=	head
HL	=	head length

LIST OF ABBREVIATIONS (Continued)

m	=	mastax
ma	=	manubrium
mt	=	major teeth
NEA	=	Nearctic
NEO	=	Neotropical
o	=	oesophagus
ORI	=	Oriental
pt	=	proximal minor teeth
PAL	=	Palearctic
r	=	rostrum
ra	=	ramus
RL	=	rostrum length
RM	=	*Rotaria mento*
RN	=	*Rotaria neptunia*
RNu	=	*Rotaria neptunoida*
RR	=	*Rotaria rotatoria*
RT	=	*Rotaria tardigrada*
RX	=	*Rotaria* sp. 1
RY	=	*Rotaria* sp. 2
s	=	spur
SL	=	spur length
t	=	toe
T	=	trunk
TL	=	total length
u	=	uncus
wp	=	with aquatic plant
wop	=	without aquatic plant

CHAPTER 1

INTRODUCTION

1. Background

Phylum Rotifera consists of three classes; Seisonidea, Monogononta and Bdelloidea. Class Seisonidea comprises of two genera with three species and all of them are parasite of some marine crustaceans. The biggest class is the Monogononta, which comprises about 1,500 species and most researches focus on this class. However, Bdelloidea contains about 460 species, most of them are cosmopolitan species, but have a few taxonomic researches.

Bdelloid rotifers are truly asexual animals. They have solely reproduced by parthenogenesis and can withstand unusual environments through a form of dormancy, called anhydrobiosis (Ricci, 1987, 1998, 2001). They are common in moist terrestrial habitats such as mosses, lichens, damp soils, and freshwater habitats (Donner, 1965; Fontaneto *et al*., 2007a). Mostly, bdelloids are fortuitous found in plankton net and often ignored. They are well studied in Europe, but have a few records in Asia, especially Southeast Asia including Thailand owing to lack of specialist. Moreover, the difficulty of bdelloid rotifers studies can be divided into three reasons. Firstly, the important characters for identification such as corona, eyes, shape of foot, spurs and toes and number of toes can be observed in active animals only. Secondly, keys to species of bdelloid rotifers are quite tough to use, the most perfect book by Donner (1965) that has well-description and illustration was written in German. Bartoš (1951) is written in English, but the detail features is poor. Finally, most of aquatic animals are often preserved with chemical materials such as formalin and alcohol. These chemicals effect bdelloids identification because the key characters of them can be observed only in active specimens. Thus, almost all of bdelloid rotifers do not have type specimens and some original descriptions and drawings are often unreliable.

The genus *Rotaria* Scopoli, 1777 is one of members of family Philodinidae, composes of 24 described species. *Rotaria* are good-swimmers, most of them live in water bodies. They have wide distribution and have been recorded in many continents around the world e.g. Nearctic, Neotropical, Palearctic, Afrotropical and Australian regions (Segers, 2007). The morphological traits of most *Rotaria* are quite well identifiable. However, some species are species complex, for example, *R. rotatoria* and *R. sordida* (Ricci, 2001; Melone & Fontaneto, 2005; Fontaneto *et al*., 2007a, 2008a, b) with more than 30 cryptic taxa (Fontaneto *et al*., 2009). Trophi is also a choice of taxonomic character because the size of this morphological trail does not change with age (Fontaneto & Melone, 2005, 2006). Moreover, geometric morphometric was used for studying shape variation in genus *Rotaria* and the results showed that both size and shape of trophi depended on particular food that they consumed (Fontaneto *et al*., 2004; Fontaneto *et al*., 2007a, b; Melone & Fontaneto, 2005). Additionally, molecular studies are helpful for identifying different species or cryptic species and can be used to compare with other specimens which have been recorded in different places. Although *Rotaria* have wide spreading as mentioned above, only one species, *Rotaria rotatoria*, from all 24 members of this genus was reported from Koh Tan Island, Surat Thani, Thailand (Fontaneto *et al*., 2007c)**.** Thus, Thailand is interesting area and it is highly possible that bdelloids have more diversity than in the previous studies. The present study focuses on the taxonomy of freshwater bdelloid rotifers in the genus *Rotaria* of Thailand to contribute and fulfill the gap of knowledge in these microfauna in Thailand. Furthermore, phylogenetic analysis is applied as support information.

2. Literature review

2.1 Classification of the Bdelloid rotifers

Taxonomy is fundamental units in biology to identify, categorize and nominate organisms for compatible understand. The species is the major aim unit of zoological taxonomy based upon genetic, reproductive and ecological particularity (Mayr, 1963). However, in many other groups of creatures is not simply resolved task, bdelloid rotifer taxonomy is limited by the identification in only active animal as mention in the background including they have small size of body so that they always neglected.

The Bdelloidea is a class of phylum Rotifera consists of three orders; Adinetida, Philodinavida and Philodinida, four families, 19 genera and about 460 species are recognized on morphological features only (Donner, 1965; Melone & Ricci, 1995; Segers, 2007).

Phylum Rotifera Cuvier, 1817
- Class Eurotatoria De Ridder, 1957
  - Subclass Bdelloidea Hudson, 1884
    - Order Philodinavida
      - Family Philodinavidae Harring, 1913 (3 genera)
    - Order Philodinida
      - Family Philodinidae Ehrenberg, 1838 (11 genera)
      - Family Habrotrochidae Bryce, 1910 (3 genera)
    - Order Adinetida
      - Family Adinetidae Hudson and Gosses, 1889 (2 genera)

The word bdelloid is derived from the Greek mean leeches; it is according to their movements. The size of bdelloids ranges between 150-700 µm, however some species are long for example *Rotaria neptunia*, 1600 µm (Ricci & Melone, 2000). Most of bdelloid rotifers can swim, however all tend to creep on substrates. They have exclusive asexual reproduction by parthenogenesis, with two genital glands. Their life span about 30 to 40 days. The body of bdelloids can divide into three parts: head, trunk and foot (Fig. 1A).

The head have a retractable rostrum on forepart of head used for creeping and clearly visible when the rotifers creeping. The visual organs can be showed on the head or the rostrum. A dorsal antenna is present in all bdelloids. The remarkable feature of Bdelloidea is the rotatory apparatus, called the corona. The corona has different shape in the three orders (Melone & Ricci, 1995). The corona of the order Philodinida comprises of two ciliated discs, the trochi can be more or less wide. The trochi are able to contract into the head, and are used for creating currents in water to assemble food particles to the mouth (filter-feeding) and for swimming (Fig. 1B). The Adinetida have a ventral ciliated field, lined caudally by the hard feature look like the rake, for grazer-feeding. The corona of the Philodinavida has an aberrant corona, which reduced to a tiny ciliated field as in *Henoceros* and *Philodinavus*. Eyes are present in some genera and are located on brain or on the rostrum, *Rotaria*.

A muscular pharynx from the masticatory apparatus, called mastax. The locality of mastax occurs at two different levels in the body. The Adinetida and Philodinida have the mastax far from the mouth (deep); it is connected with oesophagus, therefore the mastax show in the anterior part of the trunk. The mastax of Philodinavida is close to the mouth opening (superficial) and can be partly protrude to take the food. The mastax is made from hard articulated pieces, called trophi and musculature concerned to them. Bdelloids present unique trophi morphology, called ramate (Melone *et al.*, 1998): a bilaterally symmetrical structure comprising of three paired parts; the manubrium, unci (with major and minor teeth) and rami (Fig. 1C). The shape of bdelloid trophi has very little variation and the number of teeth is not important character, but useful for identification when considered by other

morphological features. However, only one family of bdelloid group that can be identified from trophi structure is Philodinavidae (Ricci & Melone, 2000).

The trunk is the main part of bdelloid body, may be flattened or cylindrical. The trunk maintains the digestive, reproductive and excretory organs. In mature bdelloids, the paired of reproductive apparatus (germarium) and the huge gland (vitellaria) are present.

The foot is the posterior part of body to the cloaca and has the pedal gland inside. A pair of spurs mostly located at the ends of posterior foot. The toes are visible when they creeping. The variation of a number of toes between absent to four (Ricci & Balsamo, 2000).

Bdelloid rotifers dwell on water bodies and damp terrestrial habitats such as lichens, mosses, soils including temporary freshwater habitats, bird baths (Donner, 1965; Fontaneto, *et al*., 2007a). Furthermore, they can resist unsuitable environments by retract head and foot into their trunk and remove water from their body in dry period and have recovery process by themselves when condition become suitable again (Ricci, 1987, 1998, 2001).

2.2 Distribution of bdelloid rotifers

Bdelloidea are widely distributed, they can found in many continents around the world such as in America, Europe, Asia and Australia (Segers, 2007). These animals have been studied intensively in Europe; not only taxonomic study but also morphological changes during desiccation and anhydrobiosis process in bdelloid rotifers (Ricci & Caprioli, 2005; Marotta *et al*., 2010). However, there are few studies in Asia. They are reported from Korea and Taiwan; *Adineta gracilis* Jason, 1893, *Bradyscela hoonsooi* new species, *Macrotrachela sonorensis* Örstan, 1995, *M. timida timida* Milne, 1916, *M. timida inquires* Milne, 1916, *Habrotrocha acornis* Murray, 1911, *H. flaviformis* De Koning, 1947, *H. fuscochlaena* De Koning, 1947, *H. longula* Bryce, 1915, *H. visa* Donner, 1954, *H. pavida* Bryce, 1915, *H. scbropyga* Bartoš, 1958 *Philodina grandis* Milne, 1916, *Rotaria rotatoria* (Pallas, 1766) and *R. neptunia*

(Ehrenberg, 1830) have been reported from Korea (Kim & Park, 1969; Song, 1989, 2014; Song & Min, 2015) and *A. vaga* (Davis, 1873) were reported from Taiwan (Fontaneto *et al*., 2007a).

In Southeast Asia, few bdelloids were reported from Malaysia and Singapore, but rarely identified to species (Fernando & Zankai, 1981). In Thailand, there are few studies on bdelloids and most of records are fortuitous found in plankton net. According to A checklist of the freshwater rotifer of Thailand of Sa-adrit *et al.* (2013), the records of bdelloid rotifers in Thailand are *Habrotrocha ampulla* Murray, 1991, *H. angusticollis* (Murray, 1905), *Dissotrocha aculeata* (Ehrenberg, 1832), *D. macrostyla* (Ehrenberg, 1838) and *Rotaria rotatoria* (Pallas, 1766) (Koste, 1975; Chittapun & Pholpunthin, 2001; Chittapun *et al*., 2002, 2007; Fontaneto *et al*., 2007c, Sa-adrit *et al.*, 2013).

2.3 *Rotaria* Scopoli, 1777

Genus *Rotaria* have been named since 1777 by Scopoli. They are one of a member of family Philodinidae, now composes of 24 described species. *Rotifer* is the synonym of this genus that has been named by Cuvier in 1798. In 1965 Donner reported the most completed and well-illustrated key characters of *Rotaria*, however it was written in German. So it is limited to the German-speaking scientific community. According to Ricci & Melone in 2000, they proposed a simple and gainful about the key to identify the genera of bdelloid rotifers which was written in English. The key based on illustrations and descriptions it helps to understand easier.

The total length of species in genus *Rotaria* is 400-1600 µm and 200 µm in some species. All species are viviparous. Corona are well-developed. The rostrum, often with two eye spots sometimes with pigments and commonly visible between the trochi. Trophi, each uncus usually has two teeth, rarely three. The foot, long and slender with long pairs of spurs. Three toes, long and clearly visible sitting at the end of the foot (Fig. 1) (Ricci & Melone, 2000).

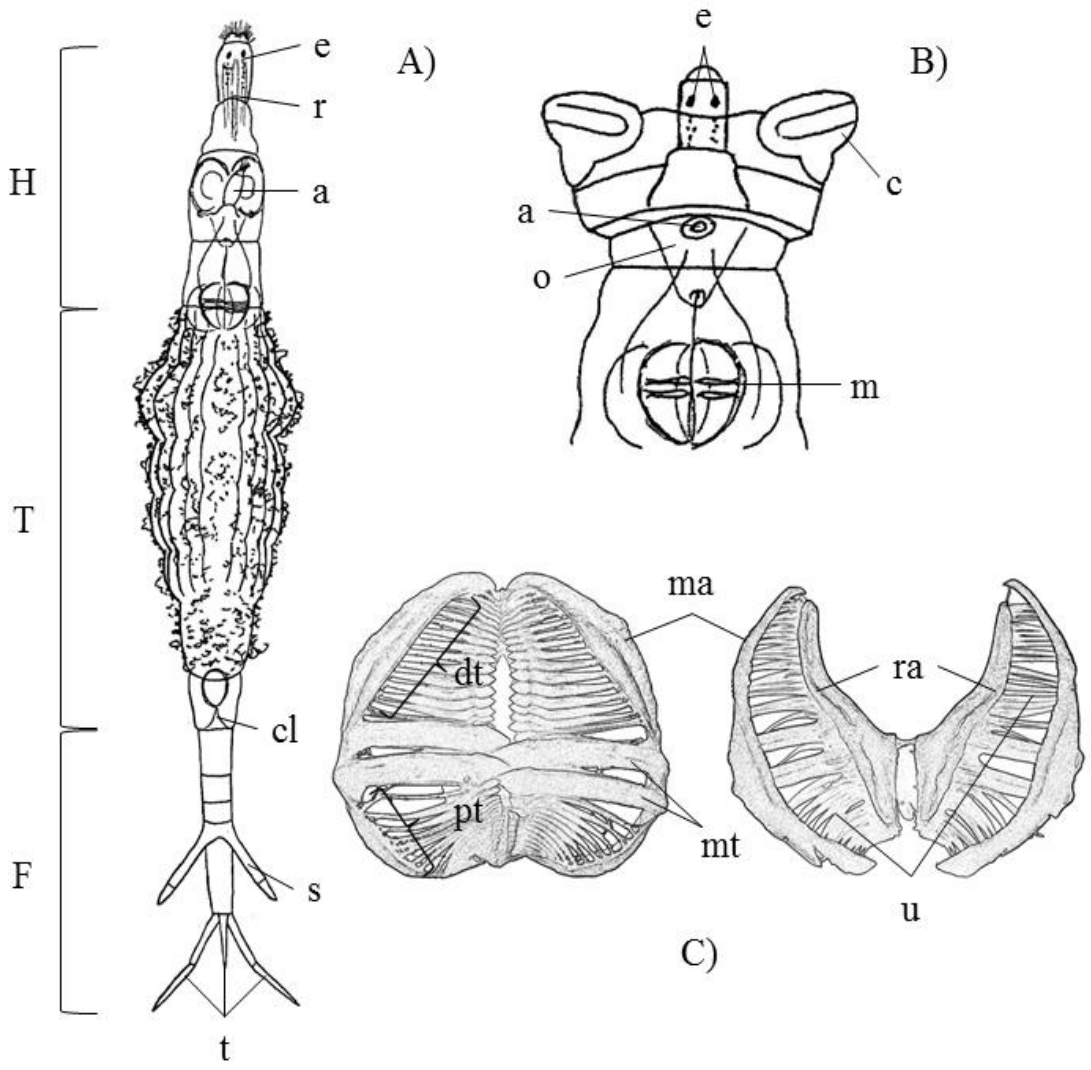


Figure 1. Morphology of bdelloid rotifers in the genus *Rotaria*; *R. tardigrada*. A, general morphology (dorsal view). B, head and anterior part of trunk. C, ramate trophi (Figure 1A and 2B modified from Donner, 1965).

2.4 Distribution of *Rotaria* Scopoli, 1777

Most of *Rotaria* live in water bodies and some species can be found in soils and mosses (Ricci & Melone, 2000). Most of *Rotaria* were reported from Europe and Australia (Table 1) for example, *Rotaria elongata*, *R. exoculis*, *R. haptica*, *R. macroceros*, *R. macrura*, *R. montana*, *R. neptunia*, *R. neptunoida*, *R. rotatoria*, *R. sordida*, *R. tardigrada* and *R. tridens* were recorded from Australia in both freshwater and terrestrial habitats such as lake, billabong, creek, moss, *Sphagnum* and soil (Ricci *et al*., 2003). Some species are euryhaline such as *R. citrina* that were reported from the Mediterranean (Ricci & Fontaneto, 2003). Moreover, *R. rotatoria* and *R. tardigrada* were found in lakes above 1700 m in western Italian Alps (Fontaneto & Melone, 2003). In 1987 Bērziņš & Pejler study about rotifer occurrence in relation to pH the result show *R. neptunia* preferred habitats are highest pH (>7), highest conductivity (> 630 µS) (Bērziņš & Pejler, 1989a) and least O_2 (Bērziņš & Pejler, 1989b). In Thailand, *R. rotatoria* was reported in pond water from Koh Tan, Surat Thani province (Fontaneto *et al*., 2007c).

Table 1. Distribution of genus *Rotaria*.

	Species	Distribution	References
1	*Rotaria citrina* (Ehrenberg, 1838)	AFR, AUS, NEA, PAL; Mediterranean, Italy	Fontaneto *et al*., 2007c; Ricci & Fontaneto, 2003; Segers, 2007
2	*R. curtipes* (Murray, 1911)	AUS	Segers, 2007
3	*R. elongata* (Weber, 1888)	AFR, AUS; Australia, NEA, NEO, PAL	Ricci *et al*., 2003; Segers, 2007
4	*R. exoculis* (De Koning, 1947)	AUS; Australia, NEA, PAL	Ricci *et al*., 2003; Segers, 2007
5	*R. haptica* (Gosse, 1886)	AUS; Australia, PAL	Ricci *et al*., 2003; Segers, 2007
6	*R. laticeps* (Wulfert, 1942)	AUS, PAL	Segers, 2007
7	*R. macroceros* (Gosse, 1851)	AFR, AUS; Australia, NEA; USA, Neo, ORI, Pal; UK	Fontaneto *et al*., 2007c; Ricci *et al*., 2003; Segers, 2007
8	*R. macrura* (Ehrenberg, 1832)	AFR, AUS; Australia, NEA, NEO, PAL; Italy, UK	Fontaneto *et al*., 2007a, c; Ricci *et al*., 2003; Segers, 2007
9	*R. magnacalcarata* (Parsons, 1892)	PAL; Italy, Poland, UK	Fontaneto *et al*., 2007a, c; Segers, 2007
10	*R. mento* (Anderson, 1889)	NEA, ORI, PAL	Segers, 2007
11	*R. montana* (Murray, 1911)	AUS; Australia, ORI	Ricci *et al*., 2003; Segers, 2007
12	*R. murrayi* (Bartoš, 1951)	PAL	Segers, 2007
13	*R. neptunia* (Ehrenberg, 1832)	AFR, AUS; Australia, NEA, NEO, ORI; Myanmar, Pal; France, Korea, Italy, UK, China, Japan	Fontaneto *et al*., 2007a, c; Koste & Tobias, 1990; Ricci *et al*., 2003; Segers, 2007; Song, 1989
14	*R. neptunoida* Harring, 1913	AFR, AUS; Australia, NEA, ORI, PAL; Italy	Fontaneto *et al*., 2007a; Ricci *et al*., 2003; Segers, 2007
15	*R. ovata* (Anderson, 1889)	ORI	Segers, 2007
16	*R. quadrangularis* (Heinis, 1914)	NEA	Segers, 2007

Table 1. (Continued)

	Species	Distribution	References
17	*R. quadrioculata* (Murray, 1902)	AUS, PAL	Segers, 2007
18	*R. rotatoria* (Pallas, 1766)	AUS; Australia, NEA; Alaska, USA, NEO; Mexico, Pal; France, UK, Italy, Belgium, Russia, China, Japan, Korea, AFR; Tanzania, Iran ORI; Myanmar, Thailand, India, Sri Lanka	Bartoš, 1951; Fontaneto & Melone, 2003; Fontaneto *et al*., 2007a, b, c; Hakimzadeh Khoei *et al*., 2011, Kim & Park, 1969; Koste & Tobias, 1990; Koste & Zhuge, 1996; Mizuno & Takahashi, 1991; Murray, 1906; Ricci *et al*., 2003; Ricci & Fontaneto, 2009; Segers, 2007; Song & Kim, 2000; Thorpe, 1893; Wang, 1961
19	*R. socialis* (Kellicott, 1888)	NEA, NEO, PAL; Italy, UK	Fontaneto *et al*., 2007a, c; Segers, 2007
20	*R. sordida* (Western, 1893)	AUS; Australia, PAL; France, Spain, Sweden, Italy, UK, Japan, Korea, AFR; Tanzania, ORI; India, Taiwan, Indonesia	Bartoš, 1963; Fontaneto *et al*., 2007a, b, c, 2009, 2012; Mizuno & Takahashi, 1991; Murray, 1906; Ricci *et al*., 2003; Segers, 2007; Song & Kim, 2000
21	*R. spicata* (Murray, 1902)	NEO, PAL	Segers, 2007
22	*R. tardigrada* (Ehrenberg, 1830)	AFR, AUS; Australia, NEA; USA, Neo, Ori, PAL; Finland, Italy China, Japan, Korea	Fontaneto & Melone, 2003; Fontaneto *et al*., 2007a, c; Koste & Zhuge, 1996; Mizuno &Takahashi, 1991; Ricci *et al*., 2003; Segers, 2007; Song & Kim, 1996, 2000; Thorpe, 1893; Wang, 1961
23	*R. tridens* (Monter, 1915)	AUS; Australia, NEA, NEO, PAL	Ricci *et al*., 2003; Segers, 2007
24	*R. trisecata* (Weber, 1888)	NEA, NEO, PAL	Segers, 2007

The previous studies of *Rotaria* focused not only taxonomy and species diversity but also size and shape variation of trophi and phylogeny. According to Fontaneto & Melone in 2004 study shape diversity in trophi of nine species of *Rotaria* such as *R. macrura*, *R. magnacalcarata*, *R. neptunia*, *R. neptunoida*, *R. rotatoria*, *R. socialis*, *R. sordida*, *R. tardigrada* and *Rotaria* sp.1 by using geometric morphometric. The result showed that species-specific are presented in trophi of *Rotaria*. In 2005 they studied about trophi structure in bdelloid rotifers and mentioned that total number of unci teeth and trophi size resemble to each other, but the number of major teeth in bdelloid rotifers is not correlated with trophi size and total number of unci teeth. Actually, bigger trophi have the number of unci teeth more than smaller one (Melone & Fontaneto, 2005). Commonly, A few number of major teeth can be found in species that lived in water bodies for consume unicellular algae, whereas more major teeth are ordinary in species living in terrestrial habitats; mosses and lichens, where they probably consume bacteria. Moreover, the study from Fontaneto *et al*. (2007c) reported that shape and size of trophi in some species are different from other species and shape differentiation depended on adaptations to different food particles.

3. Research questions

1. How many species of freshwater bdelloid rotifers in the genus *Rotaria* distribute in Thailand?

2. What are the characteristics in each species of freshwater bdelloid rotifers in the genus *Rotaria*?

4. Objectives

1. To study the morphology, geometric morphometric of freshwater bdelloid rotifers in genus *Rotaria* in Thailand.

2. To describe and revise the status of freshwater bdelloid rotifers in genus *Rotaria* in Thailand.

3. To examine the diversity and distribution of freshwater bdelloid rotifers in genus *Rotaria* in Thailand.

CHAPTER 2

MATERIALS AND METHODS

1. Sampling sites

Thailand is located on the Indo-Chinese Peninsula. This tropical country situated between latitude 97°30'E to 105º45'E, and longitude 5º45'N to 20º30'N. Thailand divided into six regions: the northern, northeastern, eastern, western, central and southern regions, consists of 77 provinces. The weather in Thailand is hot and rather moist, particularly in the southern region. The southern has only two seasons, dry and rainy seasons, while the north and northeast regions have lower temperature in some months.

Thailand is one of the countries in Southeast Asia that has high biodiversity because of various appropriate factors. First, the location of Thailand is tropical area that the temperature rather stable and suitable for most biota. Second, the differences of geography: mountain, plateau, lowland, marine, freshwater, coastal and climate lead to species diversity in mainland and water habitats. Third, Thailand is a boundary of distribution (biogeography) of fauna and flora and concerned with evolution. Lastly, the rate of catastrophe is low. The freshwater habitat is a residence of numerous aquatic organisms. Although bdelloids are commonly found in moist terrestrial habitats, a preliminary survey in this study found a number of bdelloids in water bodies. Most of members in genus *Rotaria* were represented in freshwater localities. Thus freshwater habitats are interesting and were chosen for sampling sites of this study.

A total of 58 stations of 24 freshwater sampling sites (Table 2 and Fig. 2) such as freshwater swamps, peat swamps, marsh, lake and artificial pond (Fig. 3) were selected throughout Thailand covering five regions: North (N), Northeast (NE), East

(E), Central (C) and South (S). Samples were taken in dry (April-July 2015, March-April 2016) and rainy seasons (September-December 2015, January-February 2016).

Table 2. Sampling sites.

code	sampling sites	Provinces	Characters of each sampling site
N1	Phayao	Phayao	Lake, wop
N2	Chiang Saen	Chiang Rai	Lake, wp: *Nelumbo nucifera*, *Eichhornia crassipes*
NE1	Nong Han Kumphawapi	Udon Thani	Lake, wp: lotus, *Nelumbo nucifera*
NE2	Kud Thing	Bueng Kan	Lake, wp: *Utricularia aurea*, *Salvinia cucullata*
NE3	Khong Long	Bueng Kan	Swamp, wp: algae, lotus
NE4	Nong Han	Sakon Nakhon	Lake, wp: lotus, *Nelumbo nucifera*, *Eichhornia crassipes*, *Salvinia cucullata*
NE5	Nong wang	Chaiyaphum	Freshwater swamp, wp: lotus, *Hydrilla verticillata*
NE6	Huai Chorakhe Mak	Buri Ram	Freshwater swamp, wp: lotus, *Salvinia cucullata*
E1	Swamp in Rayong Botanic garden	Rayong	Freshwater swamp, peat swamp in some area, wp: lotus, *Utricularia aurea*
C1	Bueng Chawark	Suphan Buri	Freshwater swamp, wp: *Nelumbo nucifera*, *Eichhornia crassipes*
C2	Bueng Senat	Nakhon Sawan	Freshwater swamp, wp: lotus, *Nelumbo nucifera*
C3	Bueng Boraphet	Nakhon Sawan	Freshwater swamp, wp: lotus, *Eichhornia crassipes*
C4	Si Fai	Phichit	Freshwater swamp, wp: *Nelumbo nucifera*, *Salvinia cucullata*
S1	Pluckpraya	Satun	Freshwater swamp, wp: lotus, *Nelumbo nucifera*

Table 2. (Continued)

code	sampling sites	Provinces	Characters of each sampling site
S2	Pond behind department of biology building in PSU	Songkhla	Artificial pond, wp: *Salvinia cucullata*, *Pistia stratiotes*
S3	Pond connected to PSU reservoir	Songkhla	Pond, wp: algae
S4	Klong Lam Chan	Trang	Freshwater swamp, wp: algae
S5	Thale-Noi	Phatthalung	Marsh, wp: lotus, *Hydrilla verticillata*, *Utricularia aurea*
S6	Thungtong	Surat Thani	Marsh , wp: algae, *Nelumbo nucifera*
S7	Nong Han	Phuket	Artificial pond next to the sea, wop
S8	Maikhao	Phuket	Peat swamp, wop
S9	Jik	Phuket	Freshwater swamp, wp: lotus
S10	Jea Son	Phuket	Freshwater swamp, wop
S11	Yai	Chumpon	Freshwater swamp, wp: algae

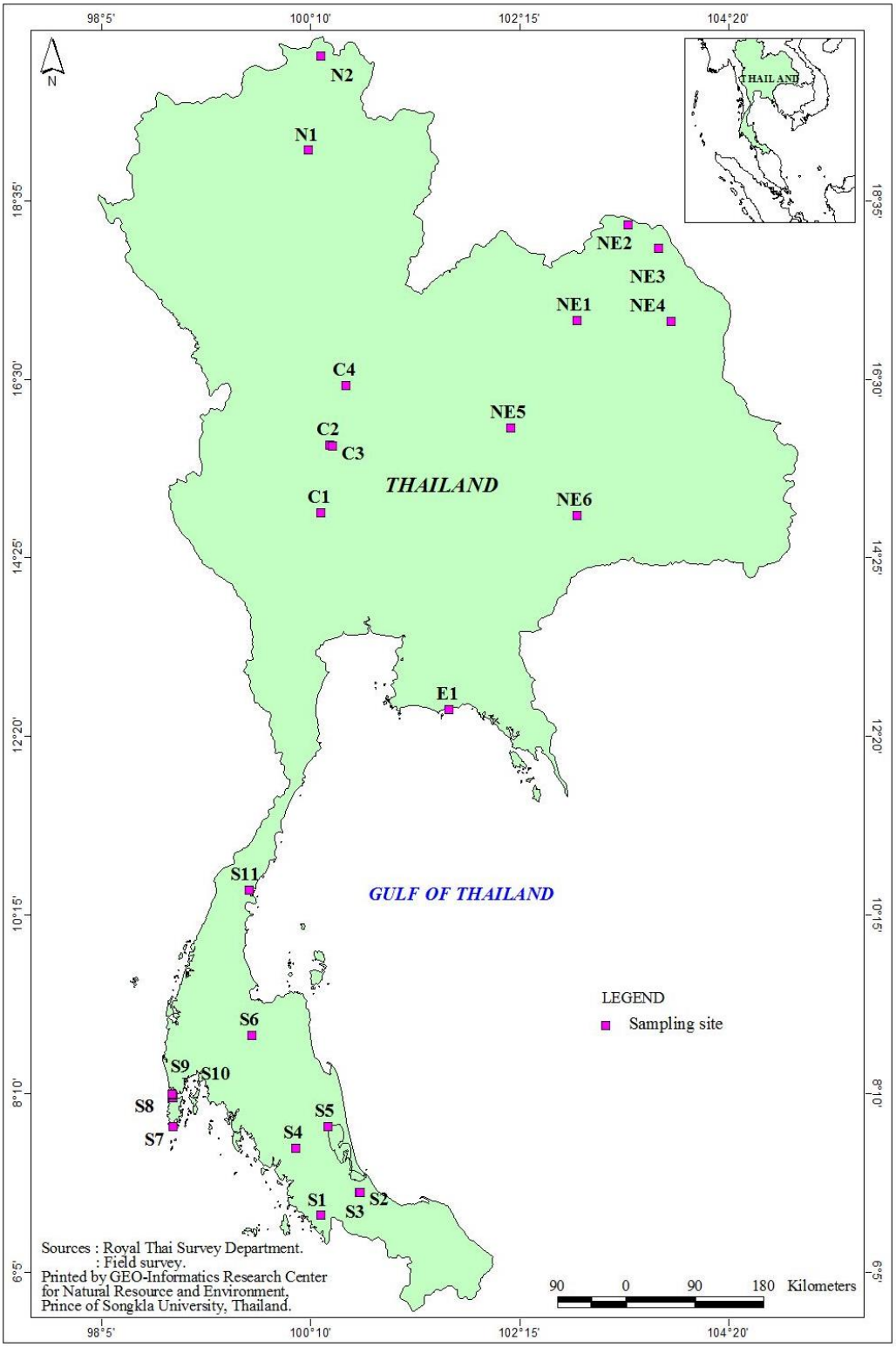


Figure 2. Geographic localization of sampling sites in Thailand (see sampling sites code in Table 2).

a b c d e f

Figure 3. Sampling sites of freshwater habitats in five parts in Thailand. a, N1; b, N2; c, NE1; d, NE2; e, NE3; f, NE4; g, NE5; h, NE6; i, E1; j, C1; k, C2; l, C3; m, C4; n, S1; o, S2; p, S4; q, S5; r, S6; s, S7; t, S8; u, S9; v, S10; w, S11 (see sampling sites code in Table 2). (N = North, NE = Northeast, C = Central, E = East, S = South).

g

h

i

j

k

l

Figure 3. (Continued)

m n

o p

q r

Figure 3. (Continued)

s

t

u

v

w

Figure 3. (Continued)

2. Sampling methods

2.1 Bdelloid rotifer sampling

Bdelloid rotifers in the genus *Rotaria* were sampled in both dry (April to July 2015 and March to April 2016) and rainy seasons (September to December 2015 and January to February 2016). Samples were collected by qualitative method by plankton net of mesh sizes 20 and 60 µm. In addition, submerged macrophytes were collected and maintained animal alive in plastic containers by providing oxygen and light. All samples were not preserved in chemical material.

All samples were brought to the laboratory as soon as possible for sorting and extracting the animals. Then, the morphological and molecular analyses were carried out.

2.2 Measurement of environmental factors

Five environmental factors were recorded in each sampling sites (Table 3). pH was measured by YSI 60 model 60/10 FT. Water temperature, conductivity and salinity were measured by YSI 30 model 30/10 FT. Dissolved oxygen was measured by dissolved oxygen test kit V-unique v-color 9780.

Table 3. Environmental factors at each sampling sites in Thailand.

Sampling sites	Stations	Seasons	Date	pH	Temp. (°C)	Cond. (µS/cm)	Salinity (ppt)	DO (mgO_2/L)
1. Phayao	1	Dry	11/7/2015	7.12	33.46	229	0.09	7.7
		Rainy	5/2/2016	7.28	27.61	532	0.24	8.15
	2	Dry	11/7/2015	7.34	33.73	226	0.09	6.72
		Rainy	5/2/2016	7.29	29.14	563	0.25	8.1
2. Chiang Saen	1	Dry	10/7/2015	6.45	30.21	32	0.01	0.72
		Rainy	5/2/2016	6.93	26.4	43	0.02	5.72
	2	Dry	10/7/2015	5.75	34.59	14	0	4.58
		Rainy	5/2/2016	6.46	24.72	38	0.02	6.64
3. Nong Han Kumphawapi	1	Dry	19/6/2015	6.62	34.97	468	0.18	2.85
		Rainy	8/1/2016	7.02	29.02	336	0.15	6.64
	2	Dry	19/6/2015	6.09	37.12	841	0.32	3.86
		Rainy	8/1/2016	6.33	29.69	482	0.21	3.55
4. Kud Thing	1	Dry	20/6/2015	6.49	32.83	550	0.23	4.04
		Rainy	9/1/2016	6.13	23.92	132	0.06	4.53
	2	Dry	20/6/2015	6.28	34.54	168	0.06	4.54
		Rainy	9/1/2016	6.54	23.2	72	0.03	4.89
	3	Rainy	9/1/2016	6.06	24.39	41	0.02	6.81
5. Khong Long		Dry	20/6/2015	5.1	37.08	16.7	0	5.21
		Rainy	9/1/2016	5.41	25.19	14	0.01	4.46
6. Nong Han	1	Dry	21/6/2015	6.38	30	269	0.11	3.78
		Rainy	9/1/2016	6.37	28.04	213	0.09	7.49
	2	Dry	21/6/2015	6.16	31.25	272	0.11	3.54
		Rainy	9/1/2016	6.34	25.93	234	0.11	7.46
	3	Dry	21/6/2015	6.82	33.16	175	0.07	7.05
		Rainy	9/1/2016	6.9	25.69	136	0.06	7.8
7. Nong wang		Dry	9/6/2015	8.32	34.63	3,621	1.57	6.79
		Rainy	21/12/2015	6.69	24.61	3,078	1.61	6.58
8. Huai Chorakhe Mak		Dry	21/6/2015	6.95	34.42	215	0.08	4.37
		Rainy	10/1/2016	6.87	26.07	143	0.06	4.2
9. Rayong Botanic garden	1	Dry	1/7/2015	5.47	31.32	201	0.08	4.61
		Rainy	4/1/2016	6.87	29.06	121	0.05	2.44
	2	Dry	1/7/2015	5.79	32.1	96	0.04	6.86
		Rainy	4/1/2016	6.32	28.03	78	0.03	3.63
	3	Dry	1/7/2015	5.82	30.84	78	0.03	3.06
		Rainy	4/1/2016	5.78	27.06	62	0.03	4.37
	4	Dry	1/7/2015	5.82	31.65	120	0.05	1.34
		Rainy	4/1/2016	6.45	27.16	127	0.06	2.78
	5	Dry	1/7/2015	5.6	31.72	203	0.08	3.44
		Rainy	4/1/2016	6.34	29.01	133	0.06	4.5
	6	Rainy	4/1/2016	6.41	29.04	133	0.06	4.4

Table 3. (Continued)

Sampling sites	Stations	Seasons	Date	pH	Temp. (°C)	Cond. (µS/cm)	Salinity (ppt)	DO (mgO_2/L)
10. Bueng Chawark	1	Dry	28/5/2015	6.83	32	220	0.09	5.9
		Rainy	19/12/2015	6.91	28.58	280	0.12	4.48
	2	Dry	28/5/2015	5.83	28.74	445	0.2	0.84
		Rainy	19/12/2015	6.91	28.75	261	0.11	6.67
	3	Dry	28/5/2015	7.32	31.72	255	0.1	5.72
		Rainy	19/12/2015	6.38	27.91	249	0.11	4.51
11. Bueng Senat	1	Dry	10/6/2015	7.08	33.66	405	0.1	3.11
		Rainy	22/12/2558	6.57	26.07	386	0.18	3.48
	2	Rainy	22/12/2015	6.31	25.85	411	0.19	2.37
12. Bueng Boraphet	1	Dry	10/6/2015	7.13	32.74	560	0.2	3.7
		Rainy	22/12/2015	6.74	27.57	571	0.26	5.58
	2	Dry	10/6/2015	7.42	37.27	593	0.23	5
		Rainy	22/12/2015	6.57	26.68	568	0.26	3.76
	3	Dry	10/6/2015	7.48	34.29	549	0.22	6.35
		Rainy	22/12/2015	6.53	27.06	571	0.26	5.38
13. Si Fai	1	Dry	9/6/2015	7.75	35.63	361	0.14	3.64
		Rainy	21/12/2015	7.57	29.6	189	0.08	7.12
	2	Dry	9/6/2015	7.94	35.41	354	0.14	6.56
		Rainy	21/12/2015	6.43	28.71	327	0.14	2.63
	3	Dry	9/6/2015	7.21	33.04	296	0.12	5.65
		Rainy	21/12/2015	6.47	27.98	273	0.12	4.34
14. Pluckpraya	1	Dry	15/3/2016	5.42	31.8	73.05	0	5.5
		Rainy	5/9/2015	6.3	30.3	79.65	0	7.75
	2	Dry	15/3/2016	6.78	30.3	83.2	0	6.5
		Rainy	5/9/2015	6.82	30.1	75.55	0	6.5
15. Pond behind department of biology building in PSU		Dry	16/4/2015	7.0	28.9	250	0.1	-
		Rainy	28/7/2014	7.4	29.2	252	0.1	-
16. Pond connected to PSU reservoir		Dry	18/4/2015	7.0	28	250	0.1	-
		Rainy	23/7/2014	7.0	27.3	245	0.1	-
17. Klong Lam Chan	1	Dry	15/3/2016	6.28	27	55.25	0	5.5
		Rainy	5/9/2015	6.96	26.9	40.3	0	7.75
	2	Dry	15/3/2016	5.85	28.3	56.05	0	6
		Rainy	5/9/2015	6.71	27	70.6	0	6
18. Thale-Noi	1	Dry	13/5/2015	5.91	28.9	837	0.4	10.65
		Rainy	26/7/2014	-	-	-	-	-
	2	Dry	13/5/2015	6.25	32	515.5	0.2	14.82
		Rainy	26/7/2014	-	-	-	-	-
	3	Dry	13/5/2015	8.95	32	1,555	0.7	9.08
		Rainy	26/7/2014	-	-	-	-	-
	4	Dry	13/5/2015	8.74	32	970.5	0.5	13.26
		Rainy	26/7/2014	-	-	-	-	-

Table 3. (Continued)

Sampling sites	Stations	Seasons	Date	pH	Temp. (°C)	Cond. (µS/cm)	Salinity (ppt)	DO (mgO_2/L)
19. Thungtong	1	Dry	20/3/2016	9.04	32.2	53.7	0	5
		Rainy	26/10/2015	7.3	31.2	48.85	0	6
	2	Dry	20/3/2016	8.74	32.1	57.8	0	5.5
		Rainy	26/10/2015	8.72	34.2	49.4	0	6.5
	3	Dry	20/3/2016	9.17	30.6	52.35	0	3
		Rainy	26/10/2015	6.26	34.9	43.4	0	4.5
20. Nong Han	1	Dry	9/4/2016	6.28	32.2	55,750	34.2	6
		Rainy	14/11/2015	8.14	36.3	52,100	31.4	8
	2	Dry	9/4/2016	5.14	31.6	55,050	33.9	5.5
		Rainy	14/11/2015	7.95	38.2	50,450	30.5	7
21. Maikhao	1	Dry	10/4/2016	3.48	34.6	49,900	23.9	7
		Rainy	15/11/2015	6.99	34.2	14,115	7.7	4.5
	2	Dry	10/4/2016	6.3	33.4	49,010	29.1	6
		Rainy	15/11/2015	6.38	34.5	13,960	7.5	2.5
	3	Dry	10/4/2016	7.84	34.4	8,195	4.1	4
		Rainy	15/11/2015	7.53	35.6	7,465	3.8	9.5
22. Jik	1	Dry	10/4/2016	9.04	29.7	134.05	0.1	1.5
		Rainy	15/11/2015	5.8	31.9	106.3	0.1	1
	2	Dry	10/4/2016	8.73	30	152.25	0.1	1.5
		Rainy	15/11/2015	6.21	34.1	245.8	0.1	1.5
	3	Dry	10/4/2016	7.93	28.7	135.45	0.1	1.5
		Rainy	15/11/2015	6.01	32.8	96.4	0	1
23. Jeh Son	1	Dry	10/4/2016	8.3	35.2	167.45	0.1	6
		Rainy	15/11/2015	7.02	37.8	132.7	0.1	7
	2	Dry	10/4/2016	-	-	-	-	-
		Rainy	15/11/2015	6.57	35	129.3	0.1	6
24. Yai	1	Dry	19/3/2016	9.05	31.7	120.15	0.1	9.5
		Rainy	25/10/2015	7.95	30.7	104.85	0.1	7.5
	2	Dry	19/3/2016	8.26	32.5	118.35	0.1	5
		Rainy	25/10/2015	6.93	30.6	109.6	0.1	7
	3	Dry	19/3/2016	7.31	32.5	123.45	0.1	6
		Rainy	25/10/2015	6.63	31.2	107.7	0.1	4.5

3. Specimens preparation for examination

3.1 Sorting

Live specimens were sorted under stereo microscope (Olympus SZ40) with stereo zoom lens (Olympus 110AL2X WD38). The bdelloid rotifers were taken from the samples for identification. The specimens were prepared in a small drop of water on glass slide and covered with round cover glass for morphological study. Their characteristics were investigated under compound microscope (Olympus CX21).

3.2 Identification and morphological study

The active bdelloid rotifers in the genus *Rotaria* were examined and identified to species level following a guidebook of Donner (1965) and recent publications such as Ricci & Melone (2000). The external morphological characteristics such as shape and length of whole body, rostrum, trunk, foot, spurs and toes and appearance of eyes were observed. Several videos of the animals in many positions were recorded by using camera Olympus Camedia C-7070 Wide Zoom with Olympus C5060-ADUS 5F16185 for morphological and geo-morphological study and drawing.

3.3 Measurement of the external morphology

The important characteristics such as total length, width of the corona, length of the rostrum, length of the foot, length of the spurs, and distance between spurs and toes were measured following the recent standardization of Iakovenko *et al*. (2013) (Fig. 4).

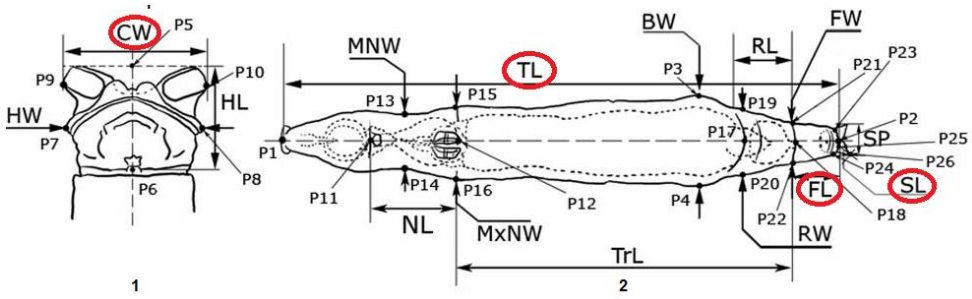


Figure 4. Measurements of external features of *Rotaria*. 1, head (dorsal view). 2, total body (dorsal view) (Iakovenko *et al*., 2013).

3.4 Preparation of trophi

The identified bdelloids were preserved in 95% alcohol before extracting the trophi for scanning electron microscopy (SEM). Trophi were prepared for SEM photograph with the standard method (De Smet, 1998; Segers *et al*., 1993), by adding a drop of 4% NaOCl to one single identified bdelloid to digest the body tissue. The only biological object that was left after the digestion was trophi. It was picked up and rinsed several times in drops of distilled water to remove the excess NaOCl. Lastly, the trophi were air dried on a cover slide. Dried trophi on cover slides were then coated with gold and their SEM photographs were taken with scanning electron microscope FEI Quanta 400.

3.5 Geometric morphometrics of the trophi

One of the important features of rotifers is the masticatory apparatus (mastax) with a hard jaw inside, called trophi. In bdelloid rotifers, trophi is ramate type (Melone *et al.*, 1998): ramate trophi have a rather uniform morphology and are found in class Bdelloidea only. Trophi shape has been shown to be species-specific in rotifers (Wallace *et al*., 2006), and trophi are currently used as a reliable and useful feature in species descriptions for monogonont rotifers (e.g. De Smet, 1998), whereas the ramate type of bdelloids, due to the uniform morphology, are less taxonomically relevant. Yet, in the genus *Rotaria*, interspecific differences in shape and size of

trophi have been found since the applications of geometric morphometrics are developed (Fontaneto *et al*., 2004).

Geometric morphometrics was chosen for studying shape variation of trophi in *Rotaria* and set the landmarks to compare forms (Bookstein, 1991; Marcus *et al*., 2000). Trophi are symmetric structures, so only one side of them was considered to reduce redundancy. However, when trophi are closed and their unci are fit together, the configuration of the major teeth is asymmetrical and the oblique of the first major tooth can be identified side of trophi. The figures with the first distal major tooth in the left half and in the right half were chosen and produced mirror image by electronically mirror-reflected.

To compare shape of the trophi of Thai specimens with trophi of the same species from other biogeograpgical areas, all the available SEM pictures of trophi from previous studied (Fontaneto *et al.*, 2004, 2007a) were included and re-digitised.

Tps series software was used in this study (Rohlf, 2005). In cephalic view, six landmarks were digitized on the pictures of trophi to describe uncus shape using the tpsDig program, accounting for proximal and distal point of the uncus plate, and for the bases and tips of the two major teeth (Fig. 5A) as already done in a previous study (Fontaneto *et al*., 2007a). A standard Generalized Procrustes Analysis (GPA) (Adams *et al*., 2013; Cardini, 2013) was used to analyze landmarks describing uncus plates in cephalic view.

In caudal view, twenty equally spaced of semi-landmarks were digitized on the trophi to describe the outline of the manubrium using the tpsDig2 program (Fig. 5B). The first and the last landmark were fixed at the distal and proximal ends of the manubrium, respectively. The other 18 semi-landmarks were permitted to slide between near landmarks to reduce the amount of shape changes between each specimen and the GPA average of all the samples (Fontaneto *et al*., 2007a).

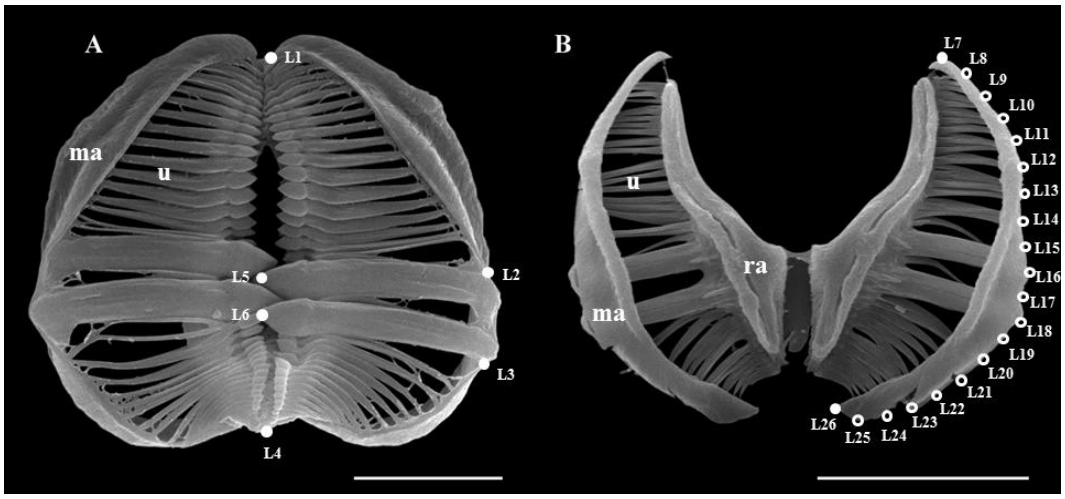


Figure 5. SEM picture of trophi *R. tardigrada* with the position of landmarks (closed circles) and sliding landmarks (open circles). A, cephalic view. B, caudal view. Scale bars: A = 10 µm; B = 20 µm.

3.6 Trophi meristic features

Size of the trophi does not change with age (Fontaneto & Melone, 2005, 2006). All SEM pictures of trophi from Thailand and the available SEM pictures in the different biogeographical areas (Fontaneto *et al.*, 2004, 2007a) were used in this study. The length of the right ramus of trophi in the caudal view was measured. Moreover the number of distal, proximal minor teeth, and major teeth of both side of trophi were counted.

3.7 DNA taxonomy

Fixed single specimen from various localities in Thailand were extracted with Hotshot lysis buffer 30 µl and Neutral solution 30 µl for genemic DNA. The partial mitochondrial cytochrome *c* oxidase subunit I (COI) gene were amplified by the polymerase chain reaction (PCR) with the universal primers of Folmer *et al*. (1994) (forward: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3', reverse; 5' TAA ACT TCA GGG TGA CCA AAA AAT CA-3') in a final volume of 25 µl.

The PCR-cycling conditions included initial denaturing at 94 °C for 5 min, 35 Cycles with denaturing at 94 °C for 1 min, annealing temperature 48 °C for 1 min and

elongation at 72 °C for 50 s; followed by a final elongation at 72 °C for 7 min and then dropped to 4 °C. Purified PCR products were sent to Macrogen Inc., Republic of Korea for DNA sequencing. The COI sequences from Thailand including the sequences from GenBank were aligned with Multiple Alignment Program for Nucleotide Sequences (MAFFT) (Katoh & Standley, 2013).

4. Data analysis

4.1 Taxonomy of *Rotaria* species in Thailand

The taxonomy of bdelloid rotifers in the genus *Rotaria* consist of scientific drawing with description of characters and measurements of external morphology in each species.

4.2 Geometric morphometric, size and teeth number of trophi

The trophi of the species that were found in this survey were digitized landmarks in both, caudal and cephalic view. Moreover the available pictures of the same species found elsewhere in the world (Fontaneto *et al.*, 2004, 2007a) were re-digitized and included for compare the shape of trophi. Analysis of Variance (ANOVA) with R program version 3.1.0 (R Core Team, 2015) was used to test differences of shape variation between species.

The length of right ramus and the teeth number of the trophi that found in this investigation were compared with the available SEM pictures of the same species that found in the different biogeographical areas (Fontaneto *et al.*, 2004, 2007a). Analysis of Variance was used to test the significant differences between species. After that, the differences of the trophi size and number of teeth of Thai animals and the same species that collected in other biogeographical areas were tested.

4.3 DNA taxonomy

All species of *Rotaria* from Thailand were compared with the DNA sequences of the same species that available from GenBank. The other sequences of each species of the genus that did not found in this study were included for the phylogenetic relationship. *Dissotrocha macrostyla* was added as outgroup. The previous studies recode exceedingly high level of cryptic diversity found in *R. rotatoria* (Fontaneto *et*

al., 2009) so that any sequence of them did not included to reduce misunderstanding of the pattern of diversity.

All sequences were aligned with Multiple Alignment Program for Nucleotide Sequences (MAFFT) (Katoh & Standley, 2013). The phylogenetic tree was reconstructed by Maximum likelihood analysis (ML) which performed in PhyML 3.1 (Guindon *et al*., 2010) with automatic model selection for taxa studied. GTR+I+G model was selected as the best model for the data set. Maximum likelihood support was estimated using 1000 bootstrap replicates. Moreover, the closest hit matched of the species identification from morphology was checked on BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

CHAPTER 3

RESULT

1. Morphological study

A total of eight *Rotaria* species were identified from the survey in permanent freshwater habitats throughout Thailand. Six species already reported from other countries: *Rotaria mento* (Anderson, 1889), *R. neptunia* (Ehrenberg, 1832), *R. neptunoida* Harring, 1913, *R. rotatoria* (Pallas, 1766) and *R. tardigrada* (Ehrenberg, 1830) and *R. ovata* (Anderson, 1889), while the characteristics of *Rotaria* sp. 1 are different from other species in this genus and *Rotaria* sp. 2 cannot be identified to species level. Here, all species were studied in detailed of the external morphological characters with their habitats and distributions in Thailand.

1. *Rotaria mento* (Anderson, 1889)

Synonym: *Rotifer mento* Anderson, 1889

Reference: Anderson, 1889: p.350-351

Type locality: India

Differential diagnosis: Body broad and covered with tube that built by itself. Antenna long and large. End of trunk protrude like notch. Foot short and stout. Spurs short and no jointed. Three toes equal and very short.

Description: Body broad, can created a tube covered body by themselves, body color depend on particles on water bodies, mostly light brown. Corona broad and neck rather short. A pair of eyes on rostrum. Antenna long and big. End of trunk bulge like notch. Foot short and chubby. Spurs short, rather stout and no jointed. Toes shorter than body length, equal and quite difficult to observe (Fig. 6A-E).

Trophi ramate, symmetric. Ramus length 18.21-24.21 µm (n=5). Several minor teeth: 20-23 of distal minor teeth and 14-18 of proximal minor teeth (n=2). The number of major teeth are equal; major teeth formula 2/2 (Fig. 13B, C and 14C, D).

Measurements: Total length (TL) 254-380 µm (n=6), Corona width (CW) 51-74 µm (n=11), Spurs length (SL) 8-12 µm (n=7), Foot length (FL) 68-143 µm (n=5), Rostrum length (RL) 33.33 µm and antenna length (AL) 21-40 µm (n=6).

Distributions: Phayao, Phayao (N1), Nong Han Kumphawapi, Udon Thani (NE1), Nong wang, Chaiyaphum (NE5), Rayong Botanic garden, Rayong (E1), Bueng Chawark, Suphan Buri (C1), Thale-Noi, Phatthalung (S5), Thungtong, Surat Thani (S6), Maikhao, Phuket (S8) and Yai, Chumpon (S11).

Habitats: Most of *R. mento* can be found in freshwater habitats that have tiny particles in water bodies. The environmental factors: pH 5.82-8.32; temperature 28.58-35.6 ºC; conductivity 43.4-7,465 µS/cm; salinity 0-3.8 ppt; dissolved oxygen 1.34-9.5 mgO_2/l.

Remarks: They use the particles in water bodies to build the tube cover their body and able to renovate more than once. From the observation, the tube construction can occur in short time. *R. mento* resemble *R. macroceros* (Gosse, 1851), but length of antenna are different in which antenna of *R. macroceros* is very long and apparently visible.

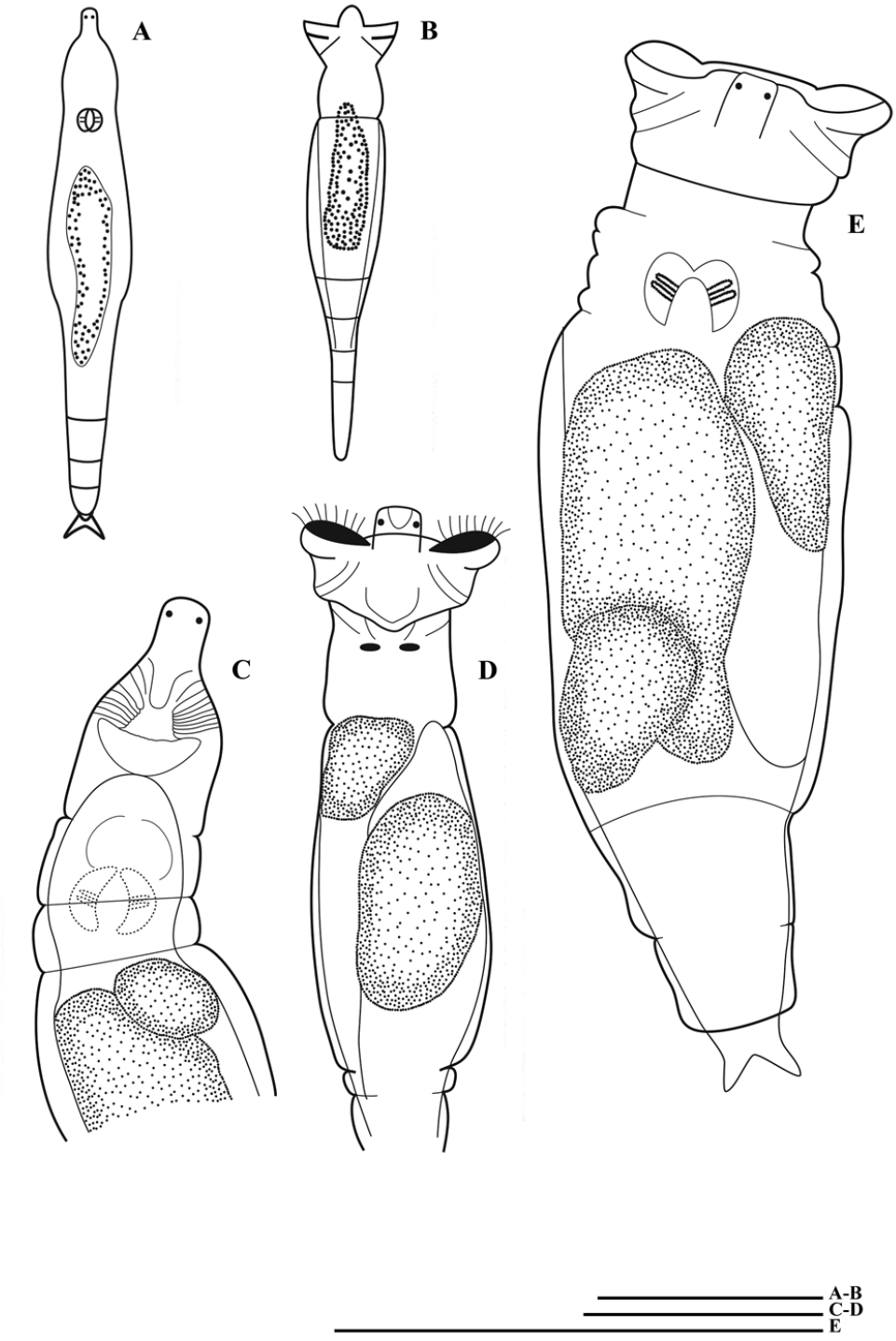


Figure 6. *Rotaria mento* (Anderson, 1889). A and C, creeping. B and C, feeding (dorsal view). D, feeding head (ventral view). Scale bars: C, D = 50 µm; A, B, E = 100 µm.

2. *Rotaria neptunia* (Ehrenberg, 1832)

Synonym: *Actinurus neptunius* Ehrenberg, 1832; *Rotifer actinurus* Jason, 1893

Reference: Ehrenberg, 1830: p.68

Type locality: Siberian

Differential diagnosis: Body long and cylinder, rod shape. Foot extraordinary long and thin. Toes long and thin.

Description: Body long, slender and cylinder. Integument smooth and without projections. Corona slightly wider than the head. Rostrum with two eyes. Trunk long, narrow and cylindrical. Foot extremely long about 3/5 of the total length of body, slender and skinny. A pairs of spurs equal, thin, two joints: first joint few longer than second joint and second joint pointed. Distance between spurs and toes longer than *R. ovata*. Three toes equal, slender and very long (Fig. 7A-F).

Trophi ramate, symmetric. Ramus length 20.36-22.93 µm (n=8). Several minor teeth: 22-25 of distal minor teeth and 12-16 of proximal minor teeth (n=3). The number of major teeth are equal; major teeth formula 2/2 (Fig. 13D, E and 14G-J).

Measurements: Total length (TL) 686-954 µm (n=7), Corona width (CW) 63-74 µm (n=5), Spurs length (SL) 25-30 µm (n=5), Foot length (FL) 314-571 µm (n=9), Rostrum length (RL) 29-34 µm (n=4) and distance between spurs and toes 126-286 µm (n=7).

Distributions: Phayao, Phayao (N1), Nong Han Kumphawapi, Udon Thani (NE1), Rayong Botanic garden, Rayong (E1), Bueng Boraphet, Nakhon Sawan (C3), Si Fai, Phichit (C4), Pond connected to PSU reservoir, Songkhla (S3).

Habitats: The environmental factors: pH 6.45-7.57; temperature 27.06-34.97 ºC; conductivity 127-571 µS/cm; salinity 0.06-0.26 ppt; dissolved oxygen 2.78-7.12 mgO_2/l.

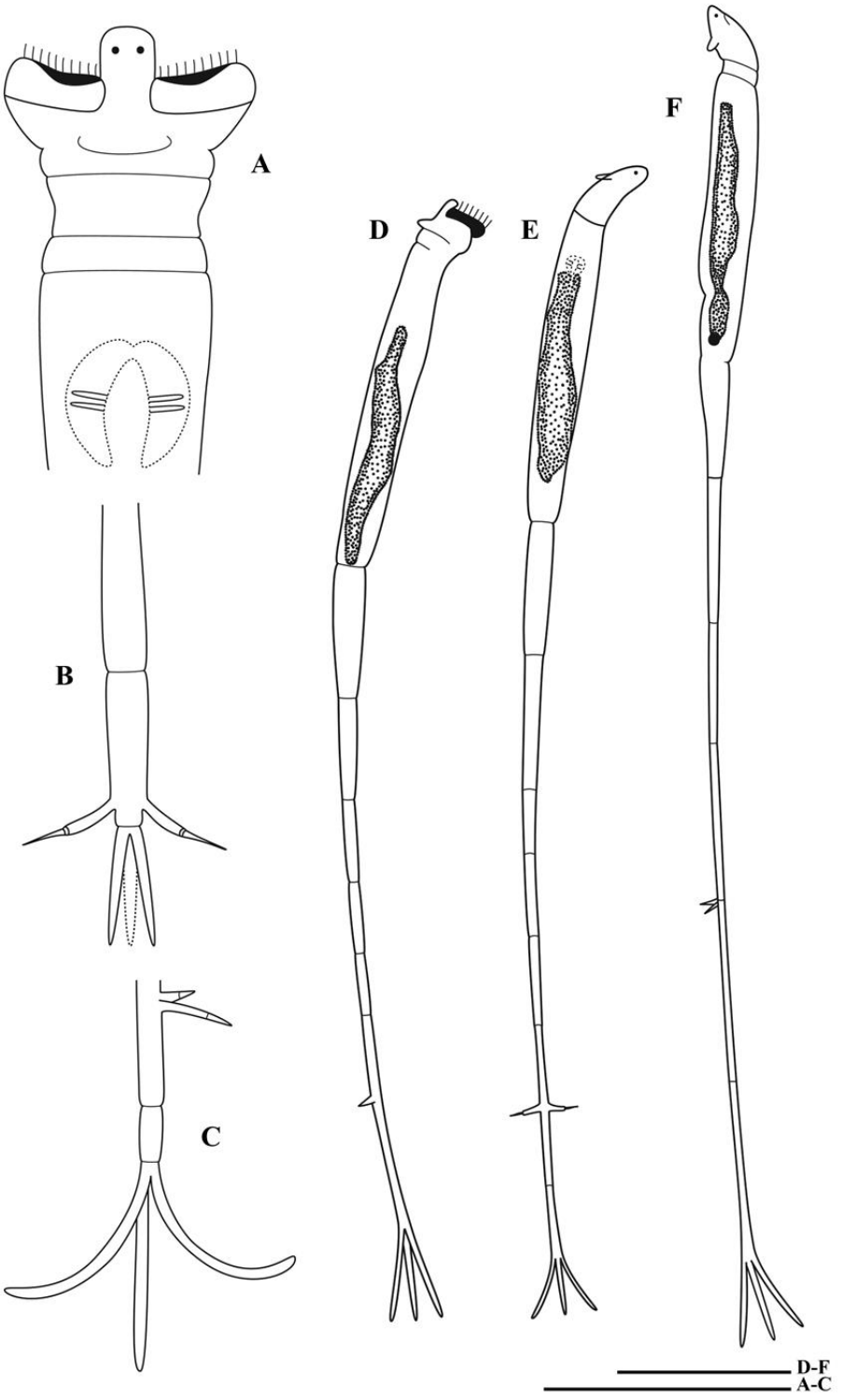


Figure 7. *Rotaria neptunia* (Ehrenberg, 1832). A, feeding head. B, foot, spurs, toes (dorsal view). C, spurs and toes (lateral view). D, feeding (lateral view). E and F, creeping. Scale bars: A-C = 50 µm; D-F = 100 µm.

3. *Rotaria neptunoida* Harring, 1913

Synonym: *Rotifer neptunius* Milne, 1886 (non *Rotifer neptunius* Ehrenberg, 1832)

Reference: Harring, 1913: p.92

Type locality: United States

Differential diagnosis: Rostrum long. Foot short. Spurs extremely long, slender and obviously visible.

Description: Body smooth and transparent. Corona little wider than the head. Rostrum very long, cylindrical with two eye-sports. Trunk covered with thin mucus layer, cylindrical, long and wide. Foot very short. Spurs very long, slender, clearly visible with two joints; the first joint long, the second joint short and sharply pointed. Three toes unequal; the ventral toes sharp and longer than the middle doesal toe (Fig. 8A-F).

Trophi ramate, symmetric. Ramus length 26.13 µm. Several minor teeth: 24-26 of distal minor teeth and 10-16 of proximal minor teeth (n=2). The number of major teeth are equal; major teeth formula 2/2 (Fig. 13G, H).

Measurements: Total length (TL) 346-571 µm (n=11), Corona width (CW) 63-106 µm (n=5), Spurs length (SL) 35-57 µm (n=11), Foot length (FL) 86-143 µm (n=8), Rostrum length (RL) 46-63 µm (n=6) distance between spurs and toes 40-57 µm (n=4).

Distributions: Cosmopolitan, pancontinental in Australia; between water plants, on insects and crustaceans. Phayao, Phayao (N1), Bueng Chawark, Suphan Buri (C1), Si Fai, Phichit (C4), Pond behind department of biology building in PSU, Songkhla (S2), Maikhao, Phuket (S8), Jik, Phuket (S9), Jea Son, Phuket (S10) and Yai, Chumpon (S11).

Habitats: The environmental factors: pH 5.8-7.57; temperature 28.58-35 ºC; conductivity 106.3-13,960 µS/cm; salinity 0.08-7.5 ppt; dissolved oxygen 1-7.7 mgO_2/l.

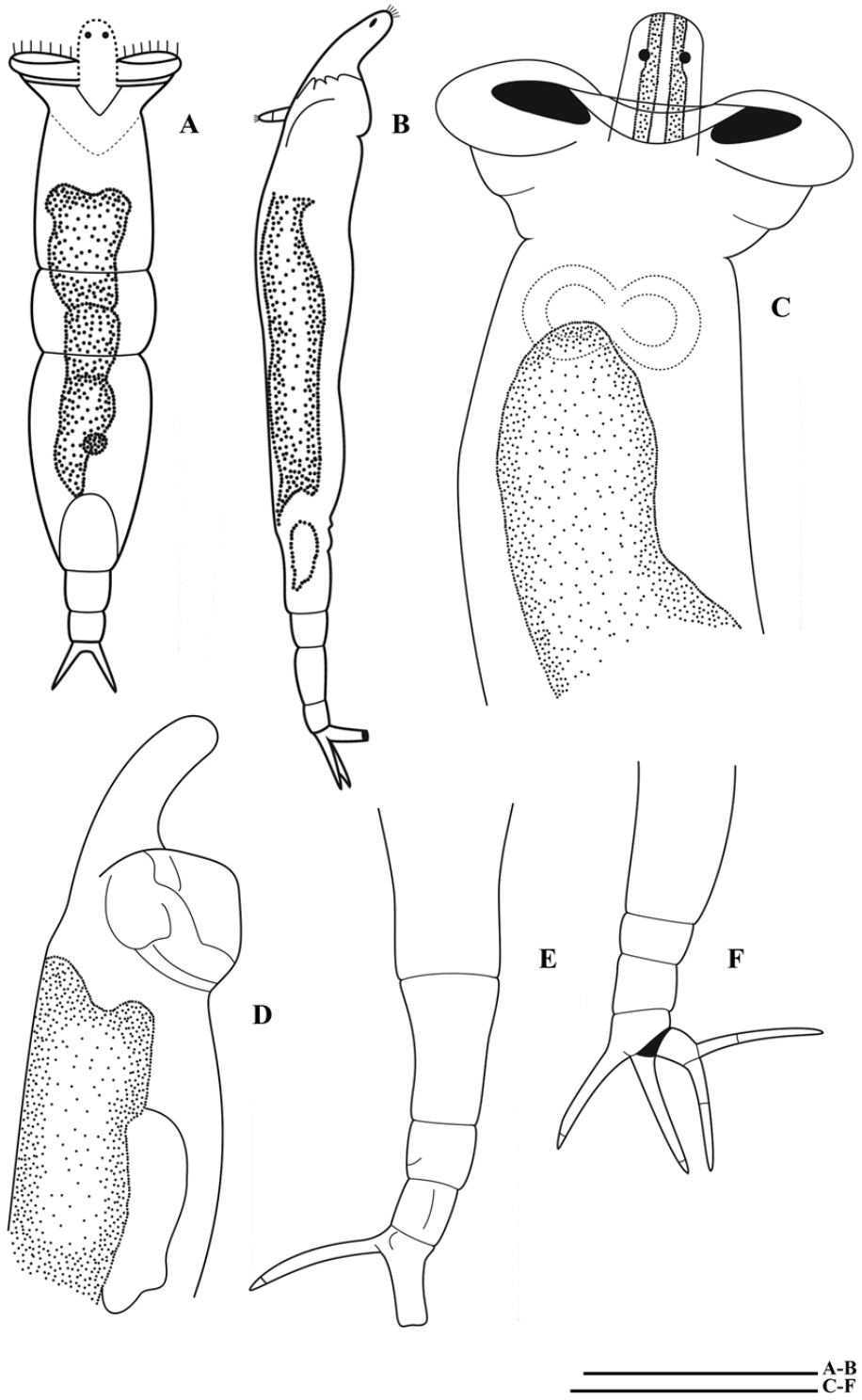


Figure 8. *Rotaria neptunoida* Harring, 1913. A, feeding (ventral view). B, creeping (lateral view). C, feeding head. D, creeping head (lateral view). E, foot (lateral view). F, spurs and toes (lateral view). Scale bars: C-F = 50 µm; A-B = 100 µm.

4. *Rotaria ovata* (Anderson, 1889)

Synonym: *Actinurus ovatus* Anderson, 1889

Reference: Anderson, 1889: p.351-352

Type locality: India

Differential diagnosis: oval shape of trunk, foot extraordinary long and thin, spurs short and slim, three toes equivalent, long and slender. The space between spurs and foot is shorter than *R. neptunia*.

Description: Total length 445-834 µm. Body smooth and transparent. Rostrum rather long and wide with two eyespots in the frontal area. The tipped of frontal rostrum with cilia. Corona small. Antenna short. Mastax near the rotatory apparatus. Oval shape of trunk; on lateral view, posterior of trunk bulges. Foot slender, extremely long; it is more than half of the total length of the body, 5 jointed fit into one another joints and can retract into their body. The spurs on the fifth jointed, no jointed, very short, thin, and pointed. The distance between spurs and toes shorter than *R. neptunia*. Three toes equal, also very long, slender, and sharp tip (Fig. 9A-C).

Trophi ramate, ramus length 18.11-19.79 µm (n=5). Several minor teeth: 19-23 of distal minor teeth and 13-18 of proximal minor teeth (n=6). The number of major teeth are unequal; has only one major tooth on one ramus, whereas have two major teeth on the other side (Fig. 13K-O and 14K).

Measurements: Total length (TL) 445-834 µm (n=11), Corona width (CW) 45-68 µm (n=20), Spurs length (SL) 7-14 µm (n=10), Foot length (FL) 246-548 µm (n=15), Rostrum length (RL) 29-46 µm (n=20) and distance between spurs and toes 51-86 µm (n=9).

Distributions: this species can be found in 10 habitats, 3 parts of Thailand, excepted North and Central. Consist of Nong Han Kumphawapi, Udon Thani (NE1), Nong Han, Sakon Nakhon (NE4), Nong wang, Chaiyaphum (NE5), Rayong Botanic garden, Rayong (E1), Pond behind department of biology building in PSU, Songkhla (S2),

Pond connected to PSU reservoir, Songkhla (S3), Thungtong, Surat Thani (S6), Jik, Phuket (S9), Jea Son, Phuket (S10) and Yai, Chumpon (S11).

Habitats: freshwater with aquatic plant such as algae, *Utricularia aurea*, *Salvinia cucullata*, *Pistia stratiotes* or detritus, swamp**.** The environmental factors: pH 5.8-7.4; temperature 29.2-35 ºC; conductivity 48.85-468 µS/cm; salinity 0-0.18 ppt; dissolved oxygen 1-7.05 mgO_2/l.

Remarks: *R. ovata* resemble *R. neptunia*, but their shape of trunk, length of foot and spurs are different. The trunk of *R. ovata* is oval, while *R. neptunia* is cylindrical. Spurs, foot and distance between spurs and toes are shorter than *R. neptunia*.

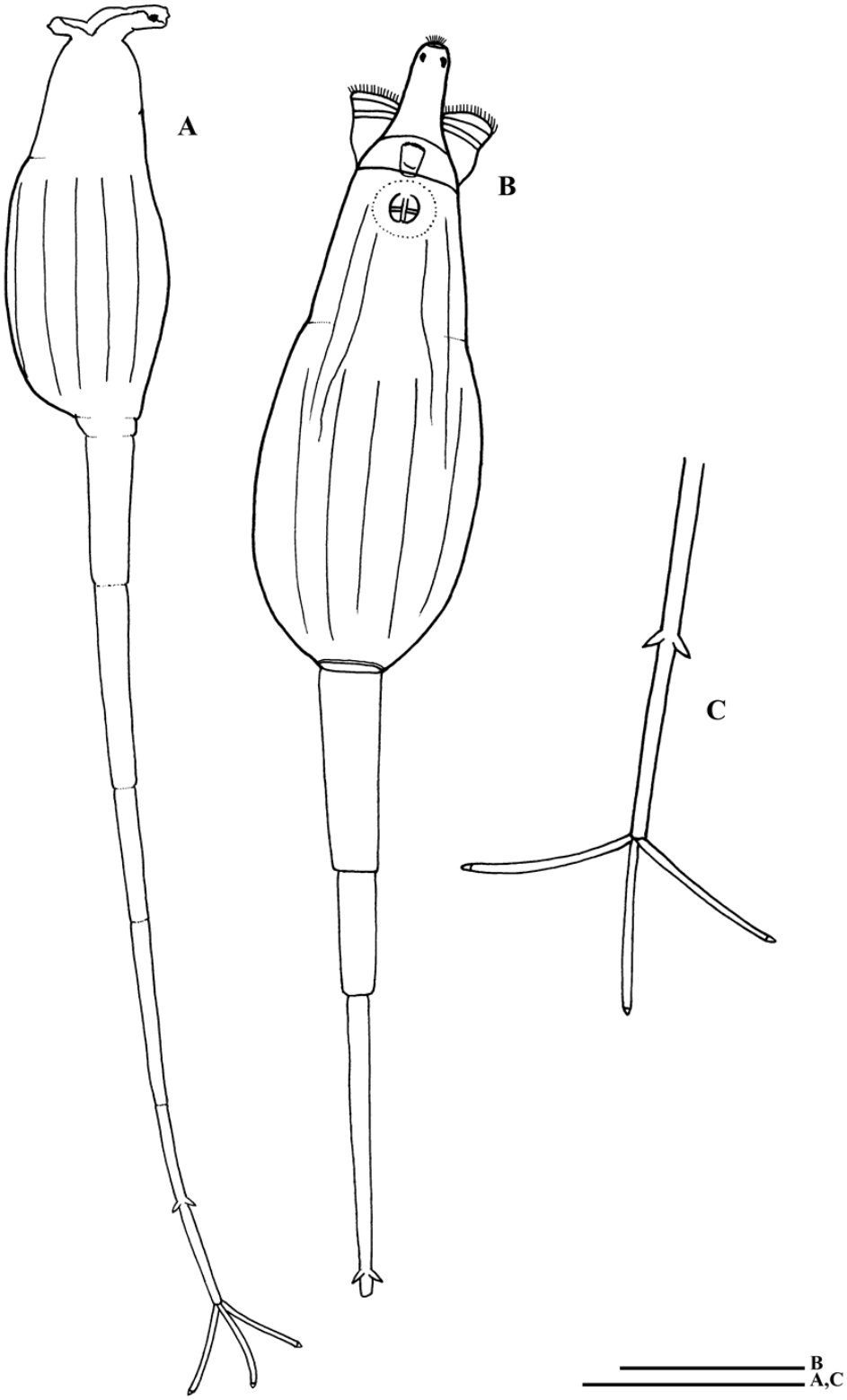


Figure 9. *Rotaria ovata* (Anderson, 1889). A, creeping (lateral view). B, feeding (dorsal view). C, spurs and toes. Scale bars: A-C = 100 µm.

5. *Rotaria rotatoria* (Pallas, 1766)

Synonym: *Brachionus rotatorius* Pallas, 1766; *Esechielina bakeri* Bory de St. Vincent, 1826; *Esechielina leuwenhoekii* Bory de St. Vincent, 1826; *Esechielina mulleri* Bory de St. Vincent, 1826; *Rotaria brachyurus* Ehrenberg, 1831; *Rotaria monteti* Bērziņš, 1955; *Rotaria saprobica* Bērziņš, 1955; *Rotaria redivivus* Cuvier, 1798; *Rotaria vulgaris* Schrank, 1801

References: Pallas, 1766: p.94

Type locality: no data

Differential diagnosis: Body long. Foot long. Spurs short and sharp tip. Three toes equal and short.

Description: Body long, slender. Two red eyes on short rostrum. Corona slightly wider than the head. Foot long. Spurs short and tapering to sharp point. Three toes equal and short (Fig. 10A-F).

Trophi ramate, ramus length 19.17 µm. symmetric several minor teeth: 16-18 of distal minor teeth and 13-15 of proximal minor teeth (n=2). The number of major teeth are equal; major teeth formula 2/2 (Fig. 13F and 14L).

Measurements: Total length (TL) 226-514 µm (n=20), Corona width (CW) 57-76 µm (n=7), Spurs length (SL) 14-29 µm (n=18), Foot length (FL) 90-206 µm (n=19), Rostrum length (RL) 14-46 µm (n=16), distance between spurs and toes 15-23 µm (n=23) and Antenna length 17-24 µm (n=2).

Distributions: Chiang Saen, Chiang Rai (N2), Nong wang, Chaiyaphum (NE5), Bueng Chawark, Suphan Buri (C1), Bueng Boraphet, Nakhon Sawan (C3), Si Fai, Phichit (C4), Pond behind department of biology building in PSU, Songkhla (S2), Pond connected to PSU reservoir, Songkhla (S3), Thale-Noi, Phatthalung (S5), Thungtong, Surat Thani (S6) and Maikhao, Phuket (S8).

Habitats: The environmental factors: pH 5.75-9.04; temperature 27.3-35.63 ºC; conductivity 14-13,960 µS/cm; salinity 0-7.5 ppt; dissolved oxygen 2.5-13.26 mgO_2/l.

Remark: *R. rotatoria* is rather difficult to observe because they creep very fast and previous studies reported they are species complexity.

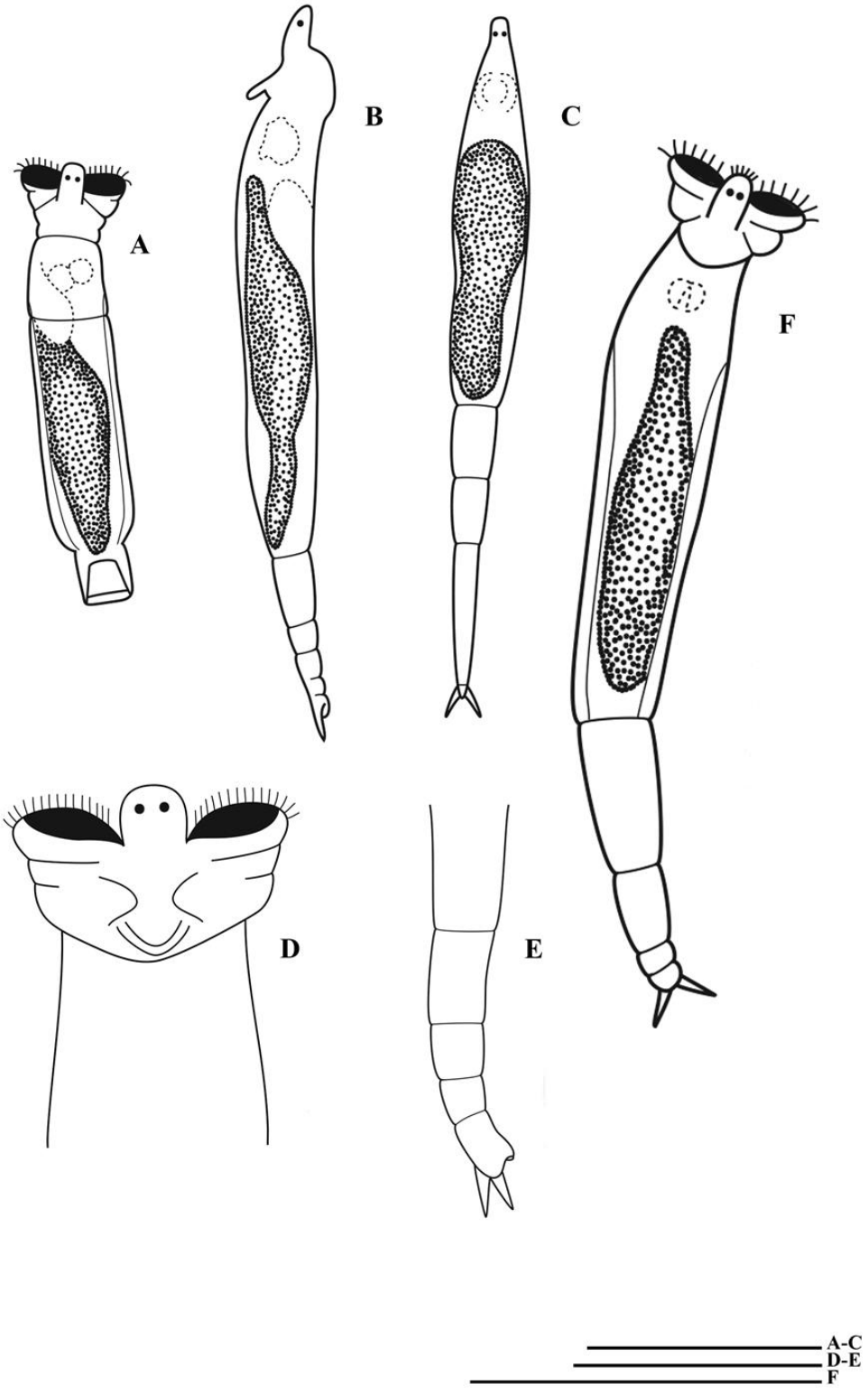


Figure 10. *Rotaria rotatoria* (Pallas, 1766). A and F, feeding (dorsal view). B and C, creeping. D, feeding head (ventral view). E, foot and spurs (lateral view). Scale bars: D-E = 50 µm; A, B, C, F = 100 µm.

6. *Rotaria tardigrada* (Ehrenberg, 1830)

Synonym: *Rotifer maximus* Bartsch, 1870; *Rotifer tardigradus* Ehrenberg, 1830; *Rotifer tardus* Ehrenberg, 1838.

Reference: Ehrenberg, 1830: p.68

Type locality: Siberian

Differential diagnosis: Trunk rough, covered with detritus, commonly brown. Foot short and colorless. Spurs long, thick, two joints. Toes unequal; ventral toes especially long with two joints, dorsal toe shorter.

Description: Body long, rod shape, a few broad in trunk. Head and foot colorless. Corona wider than the head. Trunk rough, covered with detritus, color depend on the suspensions in water bodies, mostly brown. Foot short and stout. Spurs long, thick, two joints: the first joint long, the second joint short and blunt. Three toes unequal, the middle dorsal toe shorter than ventral toes, very long, two segmented and bent. (Fig. 11A-F).

Trophi ramate, ramus length 28.06-28.57 µm. (n=2). Symmetric several minor teeth: 16-18 of distal minor teeth and 12-15 of proximal minor teeth (n=2). The number of major teeth are equal; major teeth formula 2/2 (Fig. 13I, J and 14E, F).

Measurements: Total length (TL) 300-503 µm (n=5), Corona width (CW) 55-86 µm (n=4), Spurs length (SL) 18-46 µm (n=9), Foot length (FL) 51-86 µm (n=4), Rostrum length (RL) 28-51 µm (n=4) and distance between spurs and toes 27-34 µm (n=3).

Distributions: Nong Han Kumphawapi, Udon Thani (NE1), Kud Thing, Bueng Kan (NE2), Nong Han, Sakon Nakhon (NE4), Nong wang, Chaiyaphum (NE5), Bueng Boraphet, Nakhon Sawan (C3), Si Fai, Phichit (C4), Pond behind department of biology building in PSU, Songkhla (S3), Pond connected to PSU reservoir, Songkhla (S3), Thale-Noi, Phatthalung (S5) and Thungtong, Surat Thani (S6).

Habitats: The environmental factors: pH 5.91-8.74; temperature 23.92-37.12 ºC; conductivity 43.4-3,621 µS/cm; salinity 0-1.57 ppt; dissolved oxygen 3.76-10.65 mgO_2/l.

Remarks: *R. tardigrada* creep slowly. For food particles feeding, they always attach to the substrate and use the corona for creates currents water to collect foods, while other species in this genus swimming.

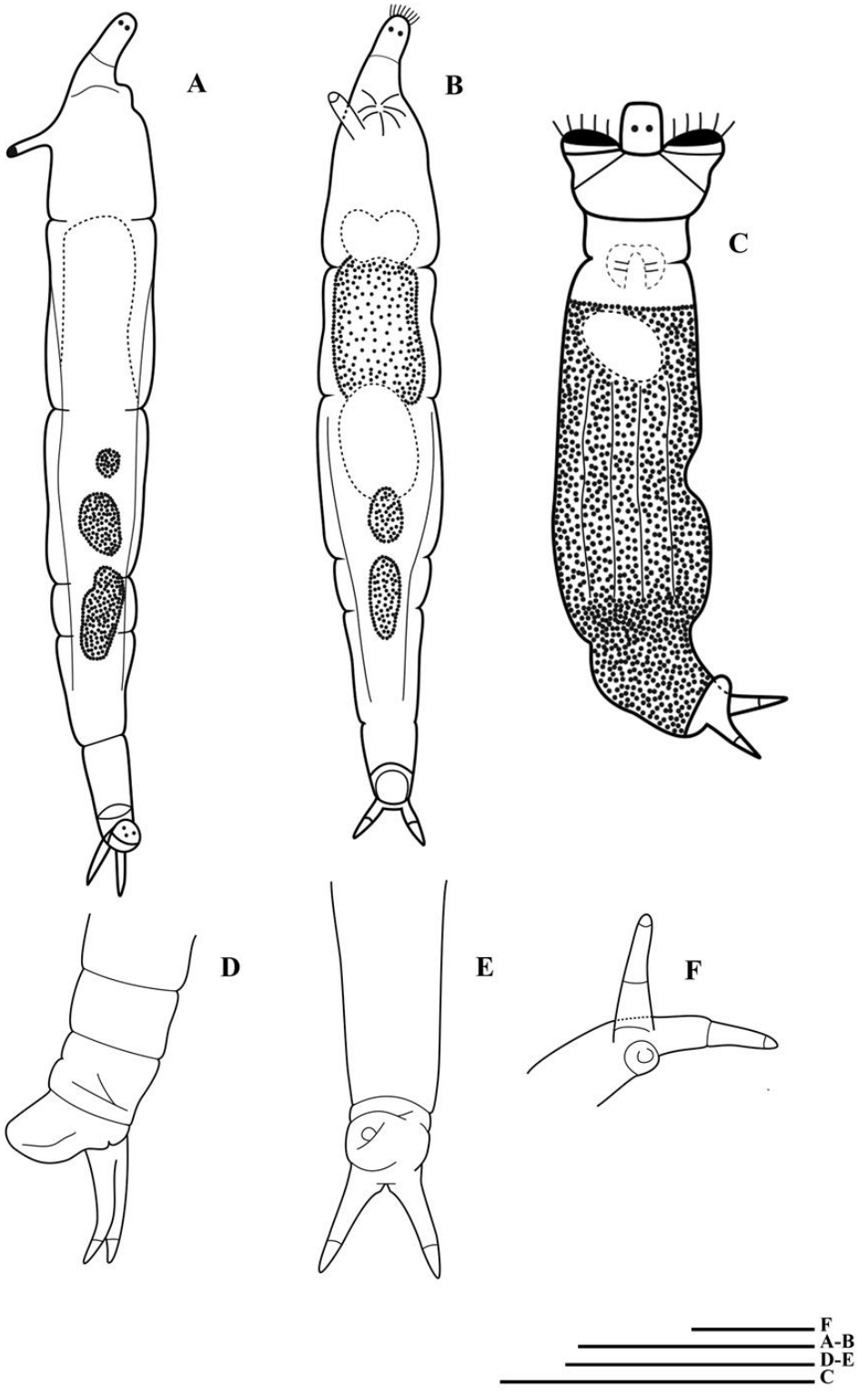


Figure 11. *Rotaria tardigrada* (Ehrenberg, 1830). A and B, creeping: A, lateral view; B, ventral view. C, feeding. D and E, foot and spurs: D, lateral view; E, ventral view. F, toes. Scale bars F = 25 µm; D, E = 50 µm; A, B, C = 100 µm.

7. *Rotaria* sp. 1

Differential diagnosis: two red eyespots on rostrum. Rostrum outstanding, huge and extreamly long. Corona rather wider than the head. Antenna short.

Description: Total length attains about 457-628 µm. Body smooth and covered with thin layer. Rostrum is outstanding, very long and wider than rostrum of other species in this genus. The tipped of rostrum with cilia. Large red eyespots. Corona slightly wider than the head, the length less than their rostrum, and similar to V-shaped. The cylindrical trunk longer than its foot. Foot rather short. Spurs long with two-jointed; the first joint very long and clearly visible, the second joint short and sharp. Three toes; middle dorsal toe very shorter than ventral toes, ventral toes very long and blunt tip (Fig. 12A-E).

Trophi ramate, symmetrical. Ramus length 27.47-29.67 µm (n=2). The shape of distal manubrium narrower than others, but wide in the middle. Each side of trophi has two major teeth and several minor teeth: 17-24 of distal minor teeth and 8-10 of proximal minor teeth (n=2) (Fig. 13A and 14A, B).

Measurements: Total length (TL) 491-628 µm (n=10), Corona width (CW) 77-91 µm (n=5), Spurs length (SL) 23-37 µm (n=11), Foot length (FL) 86-189 µm (n=11), Rostrum length (RL) 63-90 µm (n=10), distance between spurs and toes 29-35 µm (n=3) and Antenna length 40-46 µm (n=2).

Distributions: this species can be found in 10 habitats, 4 parts of Thailand, excepted Central. Consist of Chiang Saen, Chiang Rai (N2), Nong Han Kumphawapi, Udon Thani (NE1), Khong Long, Bueng Kan (NE3), Nong Han, Sakon Nakhon (NE4), Rayong Botanic garden, Rayong (E1), Pond connected to PSU reservoir, Songkhla (S3), Thale-Noi, Phatthalung (S5), Thungtong, Surat Thani (S6), Jik, Phuket (S9) and Jea Son, Phuket (S10).

Habitats: swamp, freshwater with hydrophytes such as algae, *Utricularia aurea*, *Salvinia cucullata*, *Pistia stratiotes* or litter. Rather shallow more than deep pond. The environmental factors: pH 5.75-8.73; temperature 25.93-37.08 ºC; conductivity 14-

13,960 µS/cm; salinity 0-7.5 ppt; dissolved oxygen 1-7.46 mgO_2/l. Moreover, in this study we found *Rotaria* sp. 1 with other species such as *R. mento*, *R. neptunia*, *R. neptunoida*, *R. ovata*, *R. rotatoria*, *R. tardigrada*, *Dissotrocha aculeata*, and *D. macrostyla*.

Remarks: the morphological characteristics of *Rotaria* sp. 1 are similar to *R. neptunoida* and *R. rotatoria*. *Rotaria* sp. 1 and *R. neptunoida* have long rostrum, however the length of spurs, rostrum and the width of corona are different; the spurs of *Rotaria* sp. 1 are shorter and the rostrum is longer than *R. neptunoida*. Its corona is narrower than *R. neptunoida*. While their body and length of spurs rather similar to *R. rotatoria*, but length of toes is dissimilar, toes of *Rotaria* sp. 1. are unequal; middle toe is shorter than ventral toes. Moreover some specimens have transparent mucus covered their body.

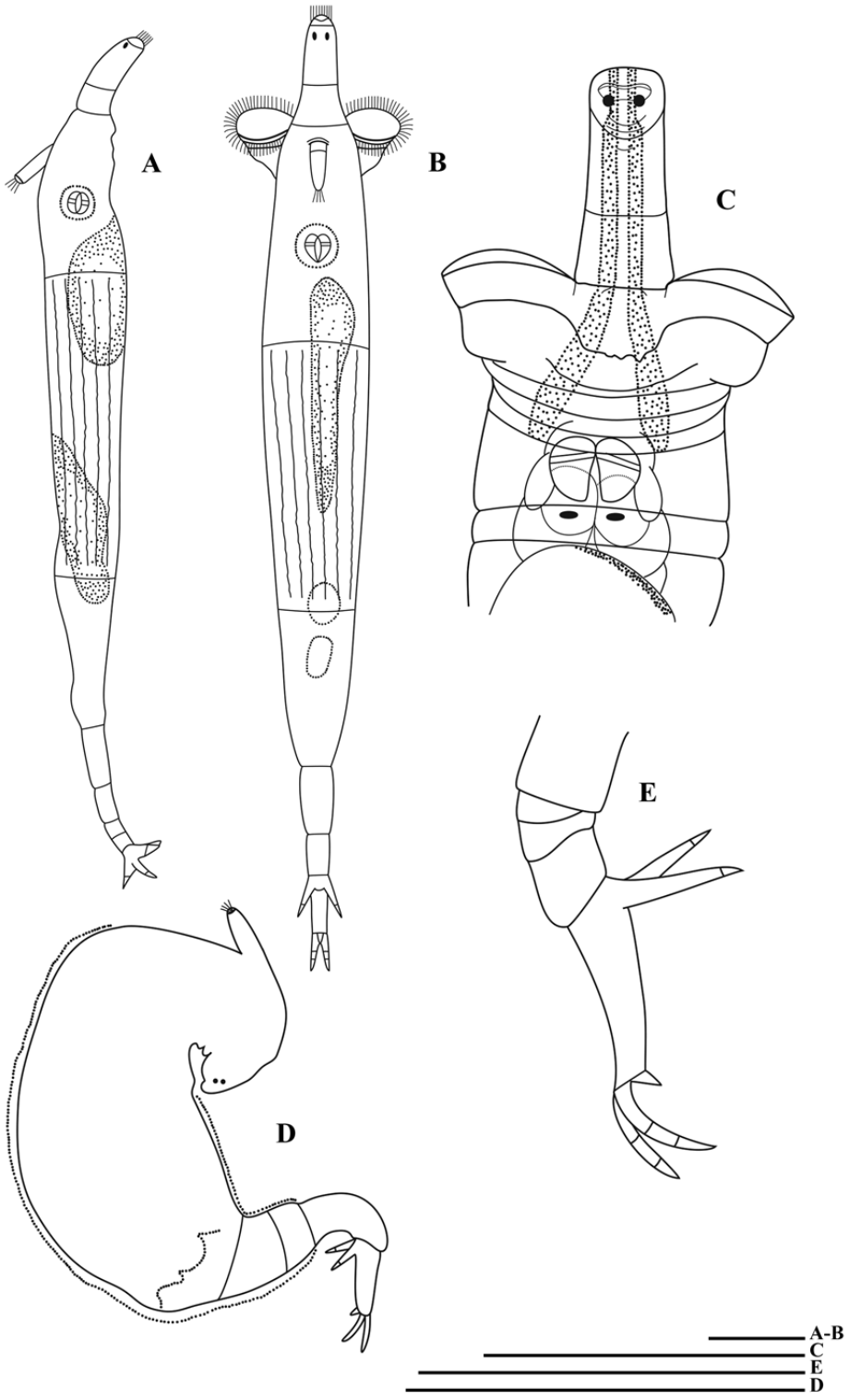


Figure 12. *Rotaria* sp. 1. A and D, creeping (lateral view). B, feeding (dorsal view). C, feeding head (ventral view). E, foot, spurs, toes (lateral view). Scale bars: A-E = 100 µm.

8. *Rotaria* sp. 2

Description: Body smooth and transparent. Small eye sports like dot. Trunk cylindrical and long. Foot long. Three toes unequal, middle dorsal toe longer than ventral toes.

Distribution: Pond connected to PSU reservoir, Songkhla (S3).

Habitat: The environmental factors: pH 7; temperature 27.3 ºC; conductivity 245 µS/cm; salinity 0.1 ppt.

Remarks: Body shape of *Rotaria* sp. 2 similar to *R. rotatoria*, but toes is different; three toes of *R. rotatoria* are equal, while middle dorsal toe of *Rotaria* sp. 2 shorter than ventral toes. Only one specimen was found and used for DNA taxonomic studied, howerver no VDO recorded and illustration for observed data.

Table 4. Morphological characteristics of *Rotaria* in Thailand.

Characters	***R. mento* (Anderson, 1889)**	***R. neptunia* (Ehrenberg, 1832)**	***R. neptunoida* Harring, 1913**	***R. ovata* (Anderson, 1889)**	***R. rotatoria* (Pallas, 1766)**	***R. tardigrada* (Ehrenberg, 1830)**	***Rotaria* sp. 1**	***Rotaria* sp. 2**
Rostrum	short	short	long	short	short	rather long	extraordinary long	no data
Shape of trunk/ integument	broad, end of trunk protrude like notch/ cover with tube	cylindrical, narrow/ transparent	cylindrical, rather broad/ transparent	oval/ transparent	cylindrical/ transparent	cylindrical/ mostly with detritus	cylindrical/ sometimes with mucus	cylindrical
Length of foot	short, stout	extraordinary long, thin	short	extraordinary long, thin	long	long	long	long
Distance between foot and toes	cannot observed	longer than length of spurs 5 times or more	rather long	shorter than *R. neptunia*	short	rather short	long	cannot observed
Spurs/ number of segment	short, stout/ no segment	short, thin/ 2	very long, slender, sharp tips/ 2	very short, slim, sharp tips/ no segment	short, sharp tip/ no segment	long, thick, blunt tips/ 2	long, sharp tips / 2	no data
Toes	3/ equal	3/ equal	3/ unequal	3/ equal	3/ equal	3/ unequal	3/ unequal	3/ unequal
Length of toes	very short	long	ventral toes longer than the middle dorsal toe	longer than spurs 4 times	short	ventral toes very long and bent, two segmented, the middle dorsal toe shorter	middle dorsal toe shorter than ventral toes 3 times	middle dorsal toe longer than ventral toes

Dichotomous key to species of *Rotaria*

1 Foot length longer than body length 2 times……..…….…….2

Foot length shorter than body length……..…..……...3

2(1) Trunk oval shape, spurs no joint……...…..…...*R. ovata* (Anderson, 1889)

Trunk cylindrical shape, spurs with two joints…....*R. neptunia* (Ehrenberg, 1832)

3(1) Foot length ½ of body length…….…….……...…….4

Foot length ¼ of body length…….……5

4(3) Rostrum short, three toes equal……..*R. rotatoria* (Pallas, 1766)

Rostrum extraordinary long, three toes unequal…….…….……*Rotaria* sp. 1

5(3) Spurs short, stout and no joint……...*R. mento* (Anderson, 1889)

Spurs long with two joints……..…….……..6

6(5) Spurs slender and sharp tips, trunk transparent…….....*R. neptunoida* Harring, 1913

Spurs thick and blunt tips, trunk with detritus….*R. tardigrada* (Ehrenberg, 1830)

2. Trophi study

2.1 Trophi morphometric

All 161 SEM pictures of *Rotaria* in cephalic view were obtained. Sixteen pictures were from Thai specimens (number of individual: *Rotaria* sp. 1 = 1, *R. mento* = 2, *R. neptunia* = 3, *R. neptunoida* = 2, *R. ovata* = 5, *R. rotatoria* = 1, *R. tardigrada* = 2), and 145 pictures of the same species available in the literature were from other parts of the world (*R. neptunia* = 19, *R. neptunoida* = 23, *R. tardigrada* = 103). The differences of uncus shape between species was significant ($df = 115$, $p = 0.001$).

All 277 SEM pictures in caudal view were obtained. Fifteen pictures were from Thail spcimens (number of individual: *Rotaria* sp. 1 = 2, *R. mento* = 5, *R. neptunia* = 4, *R. ovata* = 1, *R. rotatoria* = 1, *R. tardigrada* = 2), and 262 pictures of the same species available in the literature were from other parts of the world (*R. neptunia* = 17, *R. neptunoida* = 35, *R. tardigrada* = 210). The differences of manubrium shape between species was significant ($df = 6$, $p < 0.001$).

2.2 Trophi size and teeth number

All 452 SEM pictures were measured. Fourty-three pictures of SEM trophi were from Thai specimens (19 specimens of cephalic view and 24 specimens of caudal view), and 409 pictures of the same species available in the literature were from other parts of the world.

Trophi size was represented by ramus length which measured on the caudal view. The significant differences were showed in *R. neptunia*, *R. neptunoida* and *R. tardigrada* ($df = 2$, $p = 0.000$). The differences of three species of Thai animals were tested with the same species from other biogeological areas. Trophi size of *R. neptunia* from Thailand is slightly smaller ($df = 1$, $p = 0.02$) and only one specimen of *R. neptunoida* from Thailand slightly bigger ($df = 1$, $p = 0.001$) than other

biogeological areas, whereas no significantly different between *R. tardigrada* from Thailand and other biogeographical areas (*df* = 1, *p* = 0.741) (Table 5).

The number of distal minor teeth in both sides of all specimens was higher than the number of proximal minor teeth, ranging from 17 to 29 and from 8 to 18, respectively (Table 6). The number of distal minor teeth between species was significantly different in some species: the right side of *R. neptunia*, *R. neptunoida*, *R. ovata* and *R. tardigrada* (*df* = 1, *p* = 0.000); the left side of *R. neptunia*, *R. neptunoida* and *R. tardigrada* (*df* = 2, *p* = 0.000). For the other species, the number of specimens is not permitted to mesure the differences. The number of proximal minor teeth was significant differences only on left side in some species namely, *R. neptunia*, *R. neptunoida* and *R. tardigrada* (*df* = 2, *p* = 0.001). Three species from Thailand were tested with animals from other part of the world, *R. neptunia*, *R. neptunoida* and *R. tardigrada*.

The number of major teeth was not related to trophi size, except for *R. ovata*. Most species have two teeth and stable in both sides, whereas *R. ovata* has only one or two major teeth on the unci plates (Table 6). In addition, tip of major teeth of *R. neptunia* and *R. tardigrada* are quite blunt and bigger than that of other species.

Table 5. Trophi size of *Rotaria* from Thailand and other biogeological areas, presented as ramus length in micrometers with average and ± standard deviation. References are Fontaneto *et al.* (2004, 2007a) (Fontaneto *et al.*, 2004, 2007a). Significant codes *** = 0, ** = 0.001, * = 0.01, # = 0.05, ^ = 1, - = no data

Species	**ramus length (µm)**				***p*-values**
	Thailand	**n**	**Reference**	**n**	
R. mento	21.09±2.49	5	-	-	#
R. neptunia	21.24±0.70	8	22.04±0.78	19	***, #
R. neptunoida	26.13±0.00	1	21.1±0.84	36	***, **
R. ovata	18.82±0.64	5	-	-	#
R. rotatoria	19.17±0.00	1	-	-	-
R. tardigrada	28.32±0.36	2	27.24±4.57	217	***, ^
Rotaria sp. 1	28.57±1.56	2	-	-	-

Table 6. The range of the number of distal and proximal minor teeth including the number of major teeth from Thailand and other biogeological areas. The number in parentheses shows the sample size. (THAI = specimens from Thailand, REF = specimens from other parts of the world (Fontaneto *et al.*, 2004, 2007a)).

Species	**Distal minor teeth**		**Proximal minor teeth**		**Major teeth**	
	THAI (n)	**REF (n)**	**THAI (n)**	**REF (n)**	**THAI (n)**	**REF (n)**
R. mento	20-23 (2)	-	14-18 (2)	-	2	-
R. neptunia	22-25 (3)	21-29 (22)	12-16 (3)	9-16 (22)	2	2
R. neptunoida	24-26 (2)	20-25 (22)	10-16 (2)	11-18 (22)	2	2
R. ovata	19-23 (6)	-	13-18 (6)	-	1-2	-
R. rotatoria	16-18 (2)	-	13-15 (2)	-	2	-
R. tardigrada	16-18 (2)	11-25 (93)	12-15 (2)	8-18 (93)	2	2
Rotaria sp. 1	17-24 (2)	-	8-10 (2)	-	2	-

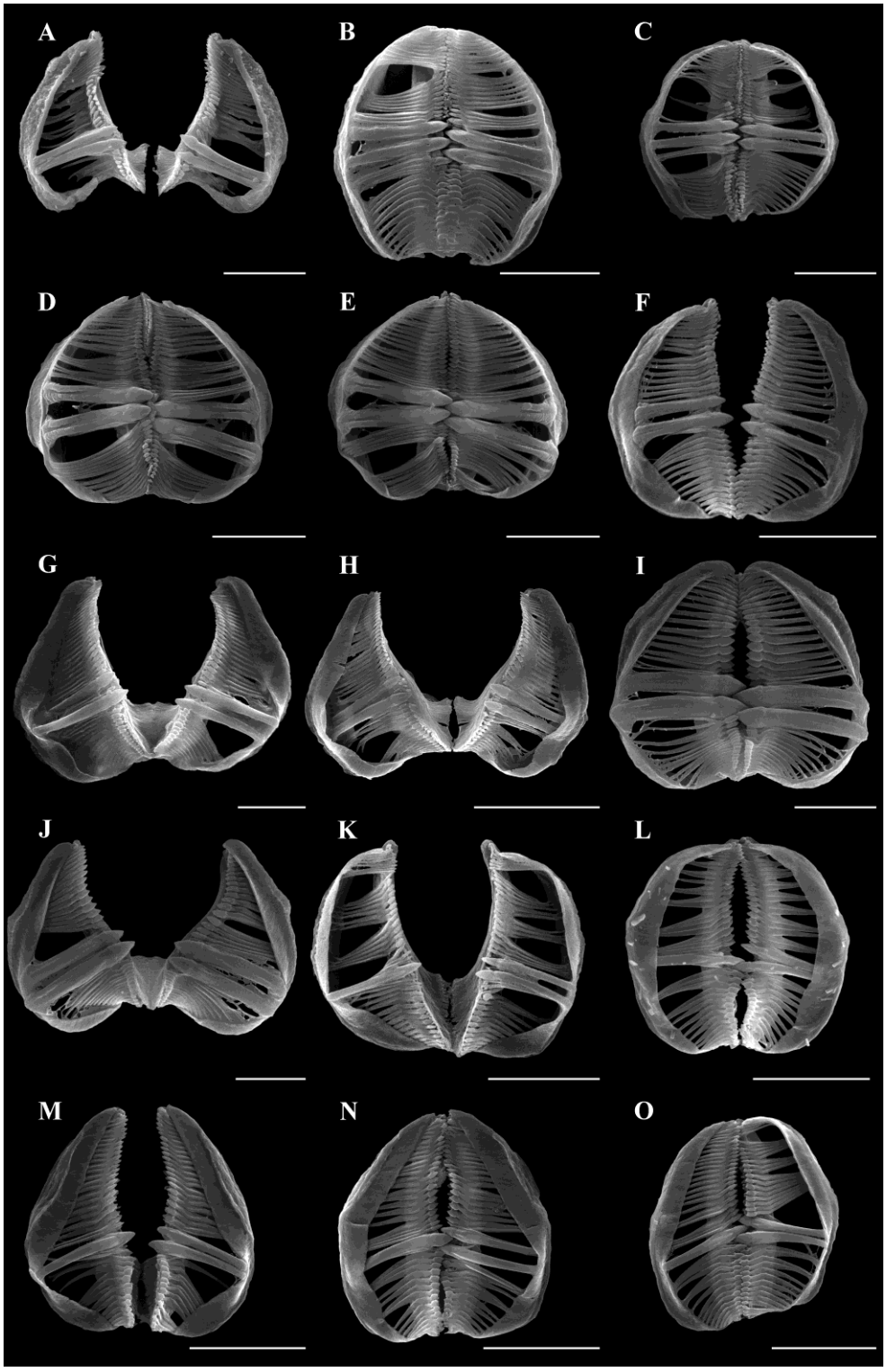


Figure 13. SEM trophi of *Rotaria* from Thailand in cephalic view. A, *Rotaria* sp. 1. B and C, *R. mento*. D and E, *R. neptunia*. F, *R. rotatoria*. G and H, *R. neptunoida*. I and J, *R. tardigrada*. K-O, *R. ovata*. Scale bars: A-G, I-O = 10 µm; H = 20 µm.

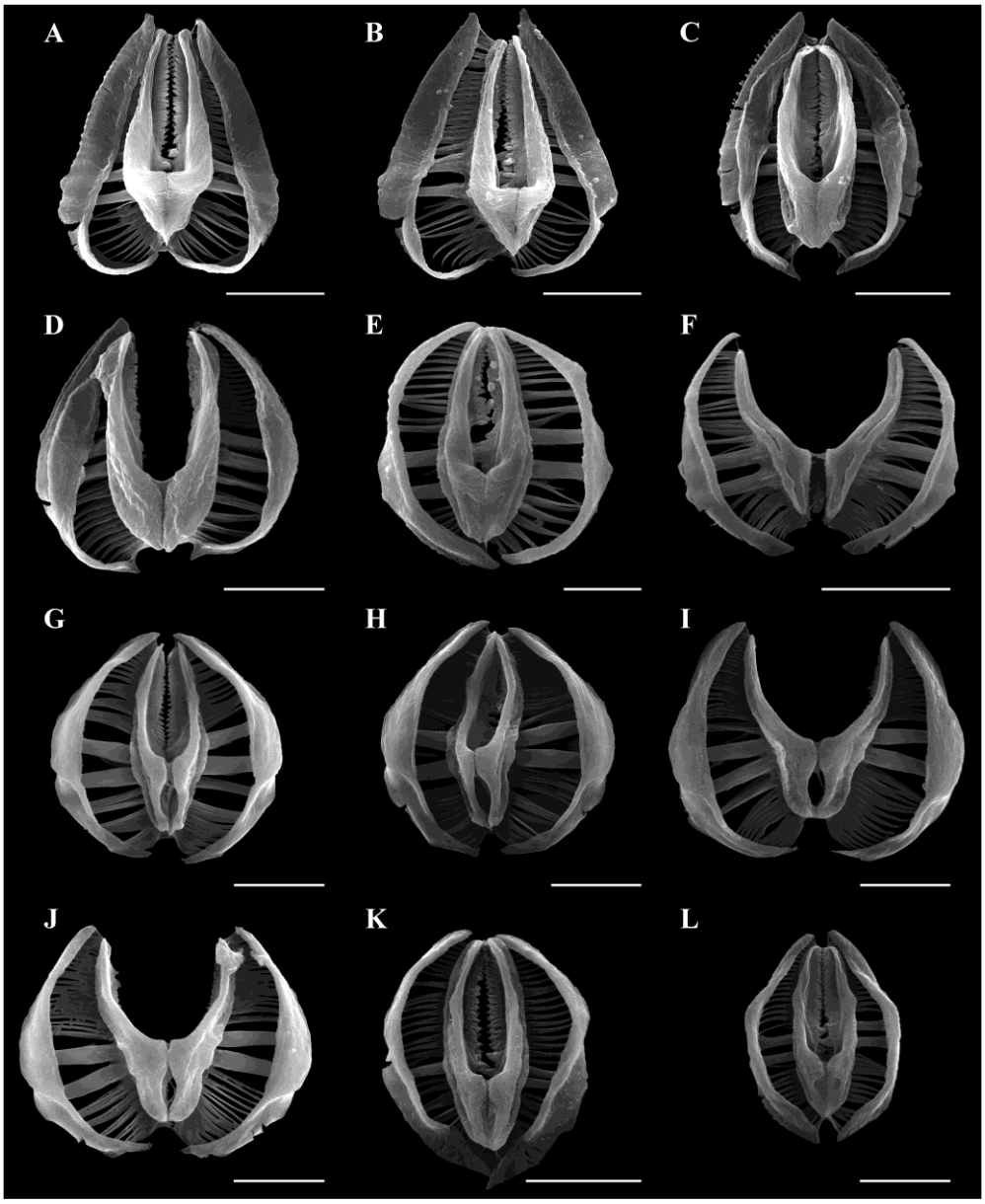


Figure 14. SEM trophi of *Rotaria* from Thailand in caudal view. A and B, *Rotaria* sp. 1. C and D, *R. mento*. E and F, *R. tardigrada*. G-J, *R. neptunia*. K, *R. ovata*. L, *R. rotatoria*. Scale bars: A-E, G-L = 10 µm; F = 20 µm.

3. DNA taxonomy

Nineteen COI sequences of eight species of *Rotaria* from Thailand with the other available sequences from GenBank were analysed. *Dissotrocha macrostyla* was added as outgroup.

Comparison on the COI sequences from Thai specimens to the available sequences on GenBank through BLAST revealed the closest hit of 2 species, *R. nepunia*, *R. rotatoria* with the identity value 0.85 and 0.87, respectively (Table 7) that support for most *R. neptunia* and *R. rotatoria* of the AY218094 and DQ656843 animals. However, the closest hit of other species did not support species identification from morphology. Moreover, misidentification was found in genus level in some cases (Table 7).

The sequences of *Rotaria* can divided into three groups, group A, B and C, and all of them separated from the outgroup, *Dissotrocha macrostyla*. Group A consists of *Rotaria* sp. 1 and *R. rotatoria*. Group B comprises of *R. citrina*, *R. mento*, *R. neptunoida*, *R. tardigrada* and *Rotaria* sp. Group C consists of *R. macroceros*, *R. macrura*, *R. magnacalcarata*, *R. neptunia*, *R. ovata*, *R. socialis* and *R. sordida* (Fig. 15 and Table 8).

Thai samples of *R. neptunia* formed a well-supported clade with three available sequences from GenBank (bootstrap = 84.5%) ever if one sequence (*R. neptunia*_THAI_1) separated from each other, but still clustered with the same species. *R. neptunoida* from Thailand grouped close to the other two available sequences of the same species (bootstrap <80%). *R. tardigrada* also clustered in a clade along with other sequences from the same species (bootstrap <80%). There is no available sequence of *R. mento*, *R. ovata*, *Rotaria* sp. 1, and *Rotaria* sp. 2 from GenBank (except for *R. rotatoria* which I did not added any sequence from GenBank) for analysis. However, the phylogenetic tree still supported their identity as each of them formed their own clade. *R. ovata* who have external morphological characters nearby *R. neptunia* and *R. macrura*, but their nucleotide sequences provided evidence that they likely to be distancly related. The morphological features of *Rotaria* sp. 1

resemble *R. neptunoida* and *R. rotatoria*, but the nucleotide sequences revealed they are not closely related. The unidentified species, *Rotaria* sp. 2 is close to *R. citrina* (Fig. 15 and Table 8).

Table 7. List of specimens from Thailand with species identification from external morphology including the accession number from GenBank, species name with the closest hit and percent identity.

Specimens	Species	GenBank Accession number	Identity (%)	Name of the closest hit with accession number
RX1	*Rotaria* sp. 1	KY006176	86	*Adineta vaga*, EF173252
RX2	*Rotaria* sp. 1	KY006177	86	*Adineta vaga*, EF173252
RX3	*Rotaria* sp. 1	KY006178	86	*Rotaria tardigrada*, KM043211
RX4	*Rotaria* sp. 1	KY006179	85	*Rotaria rotatoria*, JQ309445
RM1	*R. mento*	KY006180	88	*Rotaria rotatoria*, JQ309329
RM2	*R. mento*	KY006181	90	*Rotaria rotatoria*, JQ309329
RN1	*R. neptunia*	KY006182	88	*Rotaria rotatoria*, JQ309505
RN2	*R. neptunia*	KY006183	86	*Rotaria sordida*, EF173268
RN3	*R. neptunia*	KY006184	87	*Rotaria neptunia*, AY218094
RN4	*R. neptunia*	KY006185	87	*Rotaria neptunia*, AY218094
RN5	*R. neptunia*	KY006186	87	*Rotaria neptunia*, AY218094
RN6	*R. neptunia*	KY006187	87	*Rotaria neptunia*, AY218094
RNu1	*R. neptunoida*	KY006188	88	*Rotaria tardigrada*, KM043212
RO1	*R. ovata*	KY006189	85	*Rotaria rotatoria*, JQ309501
RO2	*R. ovata*	KY006190	85	*Rotaria rotatoria*, JQ309501
RR1	*R. rotatoria*	KY006191	85	*Rotaria rotatoria*, DQ656843
RR2	*R. rotatoria*	KY006192	85	*Rotaria rotatoria*, DQ656843
RT1	*R. tardigrada*	KY006193	86	*Habrotrocha* sp., DQ078528
RY1	*Rotaria* sp. 2	KY006194	90	*Rotaria rotatoria*, JQ309414

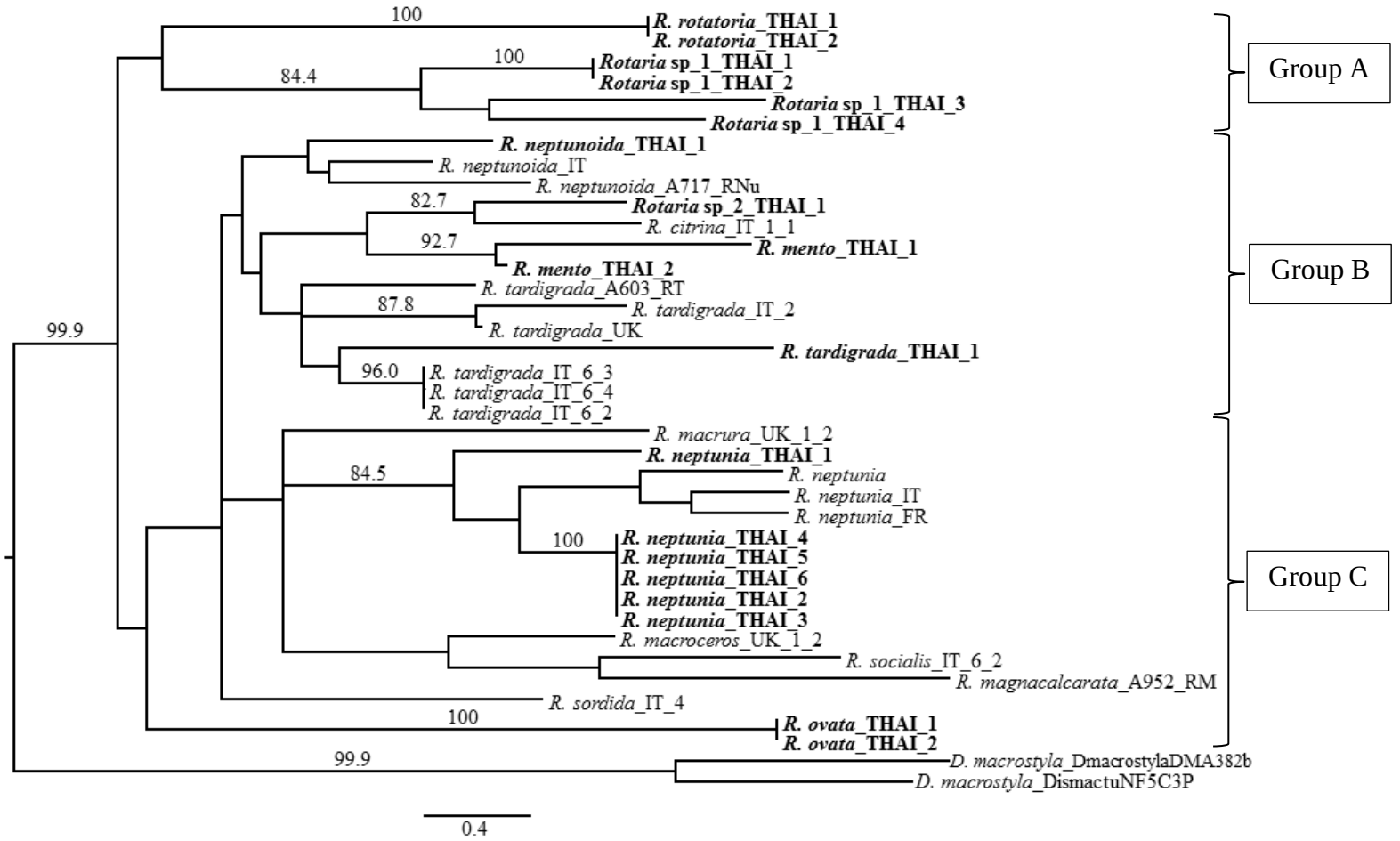


Figure 15. Phylogenetic relationship of *Rotaria*. The phylogenetic reconstruction of all 29 cytochrome *c* oxidase subunit I (COI) data from Thailand (bold type) and available sequences from GenBank (regular type) performed with maximum likelihood. Bootstrap support values above 80% are shown above each branch (see code in Table 8).

Table 8. List of species code from Thai animal and other available sequences from other coutries for phylogenetic tree with GenBank accession number.

code	GenBank Accession number	Species	countries
Rotaria sp_1_THAI_1	KY006176	*Rotaria* sp. 1	Thailand
Rotaria sp_1_THAI_2	KY006177	*Rotaria* sp. 1	Thailand
Rotaria sp_1_THAI_3	KY006178	*Rotaria* sp. 1	Thailand
Rotaria sp_1_THAI_4	KY006179	*Rotaria* sp. 1	Thailand
*R. mento*_THAI_1	KY006180	*Rotaria mento*	Thailand
*R. mento*_THAI_2	KY006181	*Rotaria mento*	Thailand
*R. neptunia*_THAI_1	KY006182	*Rotaria neptunia*	Thailand
*R. neptunia*_THAI_2	KY006183	*Rotaria neptunia*	Thailand
*R. neptunia*_THAI_3	KY006184	*Rotaria neptunia*	Thailand
*R. neptunia*_THAI_4	KY006185	*Rotaria neptunia*	Thailand
*R. neptunia*_THAI_5	KY006186	*Rotaria neptunia*	Thailand
*R. neptunia*_THAI_6	KY006187	*Rotaria neptunia*	Thailand
*R. neptunoida*_THAI_1	KY006188	*Rotaria neptunoida*	Thailand
*R. ovata*_THAI_1	KY006189	*Rotaria ovata*	Thailand
*R. ovata*_THAI_2	KY006190	*Rotaria ovata*	Thailand
*R. rotatoria*_THAI_1	KY006191	*Rotaria rotatoria*	Thailand
*R. rotatoria*_THAI_2	KY006192	*Rotaria rotatoria*	Thailand
*R. tardigrada*_THAI_1	KY006193	*Rotaria tardigrada*	Thailand
Rotaria sp_2_THAI_1	KY006194	*Rotaria* sp. 2	Thailand
*R. neptunoida*_IT	DQ656856	*Rotaria neptunoida*	other countries
*R. neptunoida*_A717_RNu	KM043212	*Rotaria neptunoida*	other countries
*R. citrina*_IT_1_1	DQ656871	*Rotaria citrina*	other countries
*R. tardigrada*_A603_RT	KM043211	*Rotaria tardigrada*	other countries
*R. tardigrada*_IT_2	DQ656858	*Rotaria tardigrada*	other countries
*R. tardigrada*_UK	DQ656859	*Rotaria tardigrada*	other countries
*R. tardigrada*_IT_6_3	EU076829	*Rotaria tardigrada*	other countries
*R. tardigrada*_IT_6_4	EU076830	*Rotaria tardigrada*	other countries
*R. tardigrada*_IT_6_2	EU076828	*Rotaria tardigrada*	other countries
*R. macrura*_UK_1_2	DQ656821	*Rotaria macrura*	other countries
R. neptunia	AY218094	*Rotaria neptunia*	other countries
*R. neptunia*_IT	DQ656830	*Rotaria neptunia*	other countries
*R. neptunia*_FR	DQ656831	*Rotaria neptunia*	other countries
*R. macroceros*_UK_1_2	EU076868	*Rotaria macroceros*	other countries
R. socialis_IT_6_2	EU076822	*Rotaria socialis*	other countries
*R. magnacalcarata*_A952_RM	KM043207	*Rotaria magnacalcarata*	other countries
*R. sordida*_IT_4	EF173269	*Rotaria sordida*	other countries
*D. macrostyla*_DmacrostylaDMA382b	KF582507	*Dissotrocha macrostyla*	other countries
*D. macrostyla*_DismactuNF5C3P	KF582506	*Dissotrocha macrostyla*	other countries

4. Species composition and distribution

In this study, a total of eight species of genus *Rotaria* were found, namely, *R. mento*, *R. neptunia*, *R. neptunoida*, *R. ovata*, *R. rotatoria*, *R. tardigrada*, *Rotaria* sp. 1 and *Rotaria* sp. 2. Although most species already recorded from other parts of the world including oriental region, seven species are new records for Thailand and two species occurred only in Thailand (Table 9). Almost all species are not restrict to a single habitat, except for *Rotaria* sp. 2 which is restricted in a pond connected to PSU reservoir, Songkhla (S3). *R. mento* and *R. neptunia* have highest distribution occured in five parts of Thailand. *R. rotatoria* and *Rotaria* sp. 1 distribute in four parts, while *R. neptunoida*, *R. ovata* and *R. tardigrada* presented in three parts (Table 9 and Fig. 16).

The sampling sites where had highest species diversity, five species, were a pond connected to PSU reservoir, Songkhla (S3) and Nong Han Kumphawapi, Udon Thani (NE1) (5 species). The sampling sites, where four species of genus *Rotaria* were found, were Rayong Botanic garden (E1), Si Fai (C4), Thale-Noi (S5), Thungtong (S6), Maikhao (S8), Pond behind department of biology building in PSU (S2) and Pond connected to PSU reservoir (S3). On the other hand, any species was not observed in Huai Chorakhe Mak (NE6), Bueng Senat (C2), Pluckpraya (S1), Klong Lam Chan (S4) and Nong Han (S7). Nong Han (S7) has highest water salinity among other sites (30.5-34.2 ppt). In this study, *Rotaria* were found in pH 5.57-9.04 and water salinity 0-7.5 ppt. *R. tardigrada* was present in broadest range of water temperature, 23.92-37.12 °C, while *R. rotatoria* was found at pH 5.57-9.04.

Rotaria presented in both seasons, except for *Rotaria* sp. 2 which is present in rainy season. Dominant species in dry and rainy seasons of the same part of Thailand are similar. In dry season: mostly, dominant species in Northeast is *R. ovata*, in South is *R. tardigrada* (Fig. 17), in rainy season: mostly, dominant species in Northeast is *R. ovata*, in South is *R. tardigrada* (Fig. 18).

Table 9. Distribution of *Rotaria* in freshwater habitats in Thailand and the present distribution in other biogeological areas according to Segers (2007). * = New record for Thailand.

species	Distribution	Thailand
Rotaria mento (Anderson, 1889)*	NEA, ORI, PAL	N1, NE1, NE5, E1, C1, S5, S6, S8, S11
Rotaria neptunia (Ehrenberg, 1830)*	AFR, AUS, NEA, NEO, ORI, PAL	N1, NE1, E1, C3, C4, S2, S3
Rotaria neptunoida Harring, 1913*	AFR, AUS, NEA, ORI, PAL	N1, C1, C4, S2, S8, S9, S10, S11
Rotaria ovata (Anderson, 1889)*	ORI	NE1, NE4, E1, S2, S3, S6, S9, S10, S11
Rotaria rotatoria (Pallas, 1766)	AFR, AUS, NEA, NEO, ORI, PAL	N2, NE5, C1, C3, C4, S2, S3, S5, S6, S8
Rotaria tardigrada (Ehrenberg, 1830)*	AFR, AUS, NEA, NEO, ORI, PAL	NE1, NE2, NE4, NE5, C3, C4, S2, S3, S5, S6
Rotaria sp. 1*	-	N2, NE1, NE3, NE4, E1, S3, S5, S8, S9, S10
Rotaria sp. 2*	-	S3

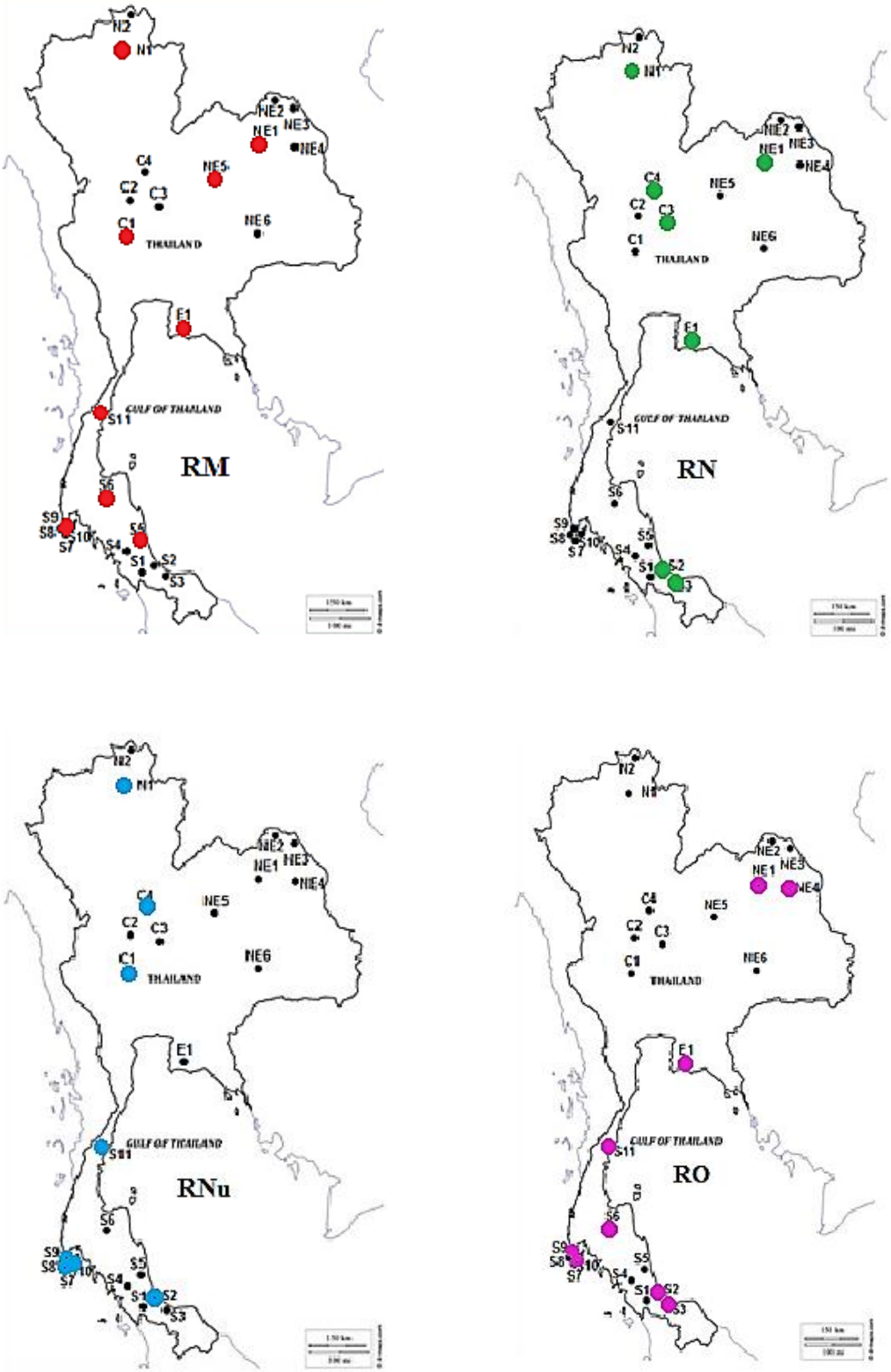
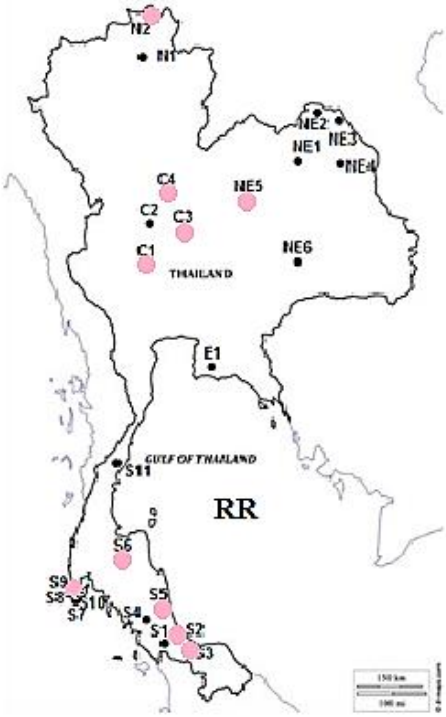
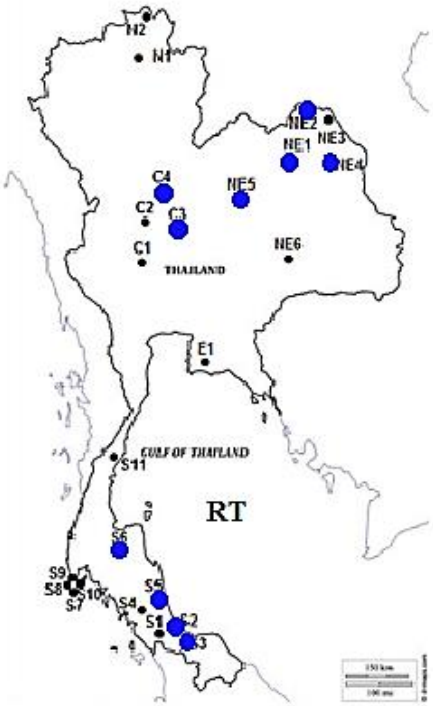


Figure 16. Distribution of *Rotaria* in freshwater habitats in Thailand.





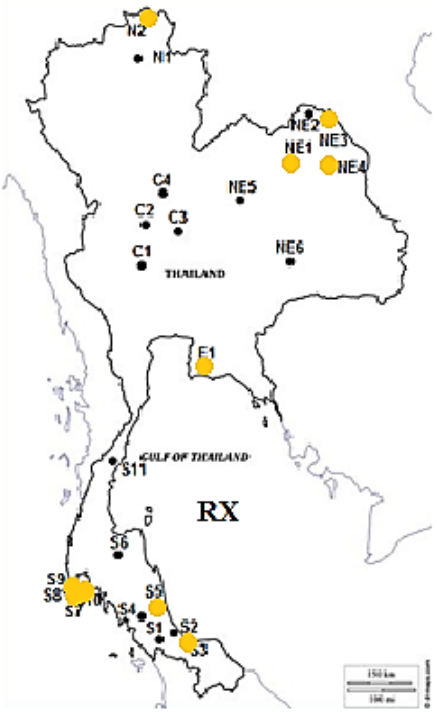


Figure 16. (Continued)

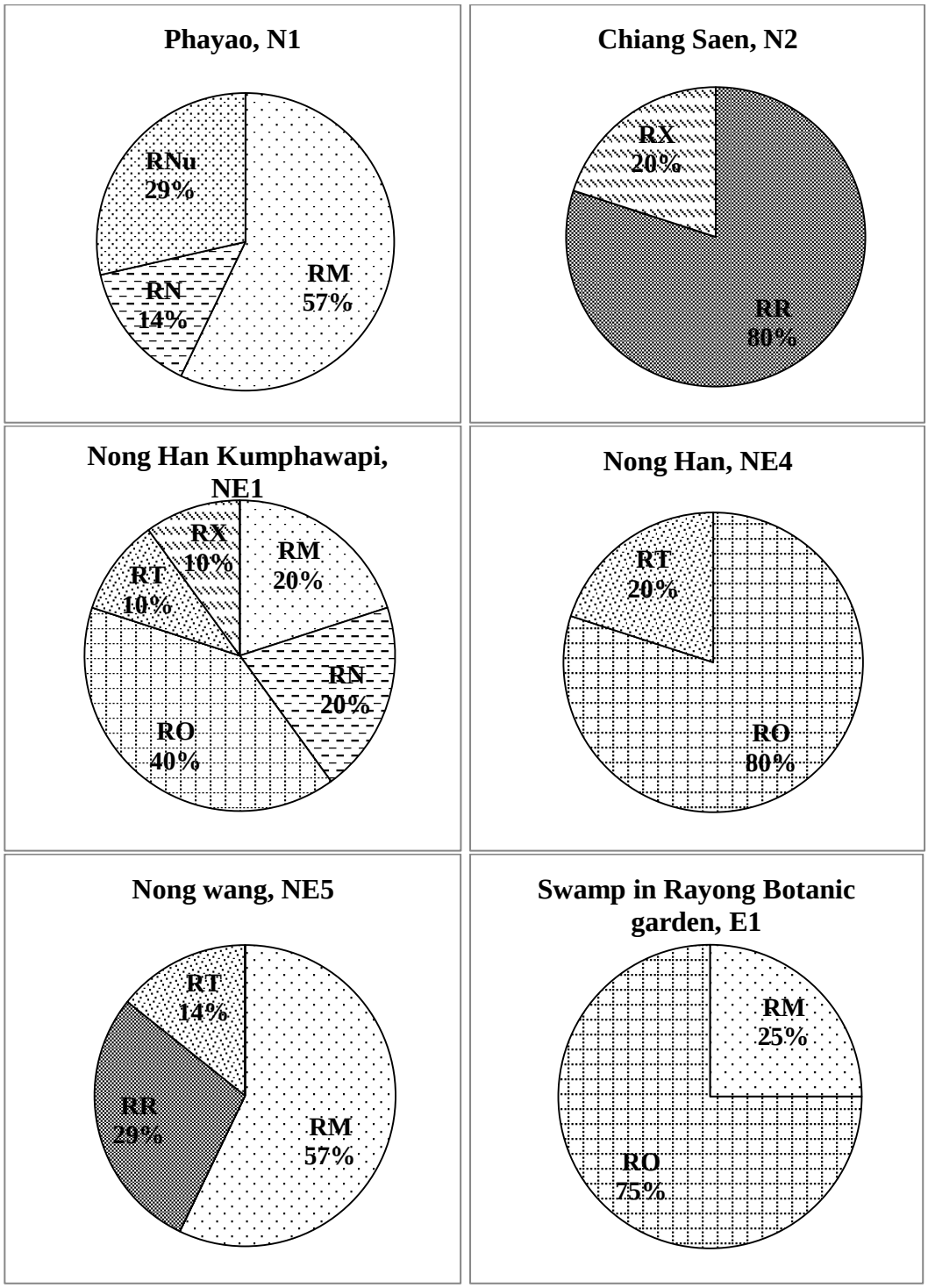


Figure 17. Percentage of individual of *Rotaria* from each habitat in dry season. (Represented only habitats where *Rotaria* were found more than one species).

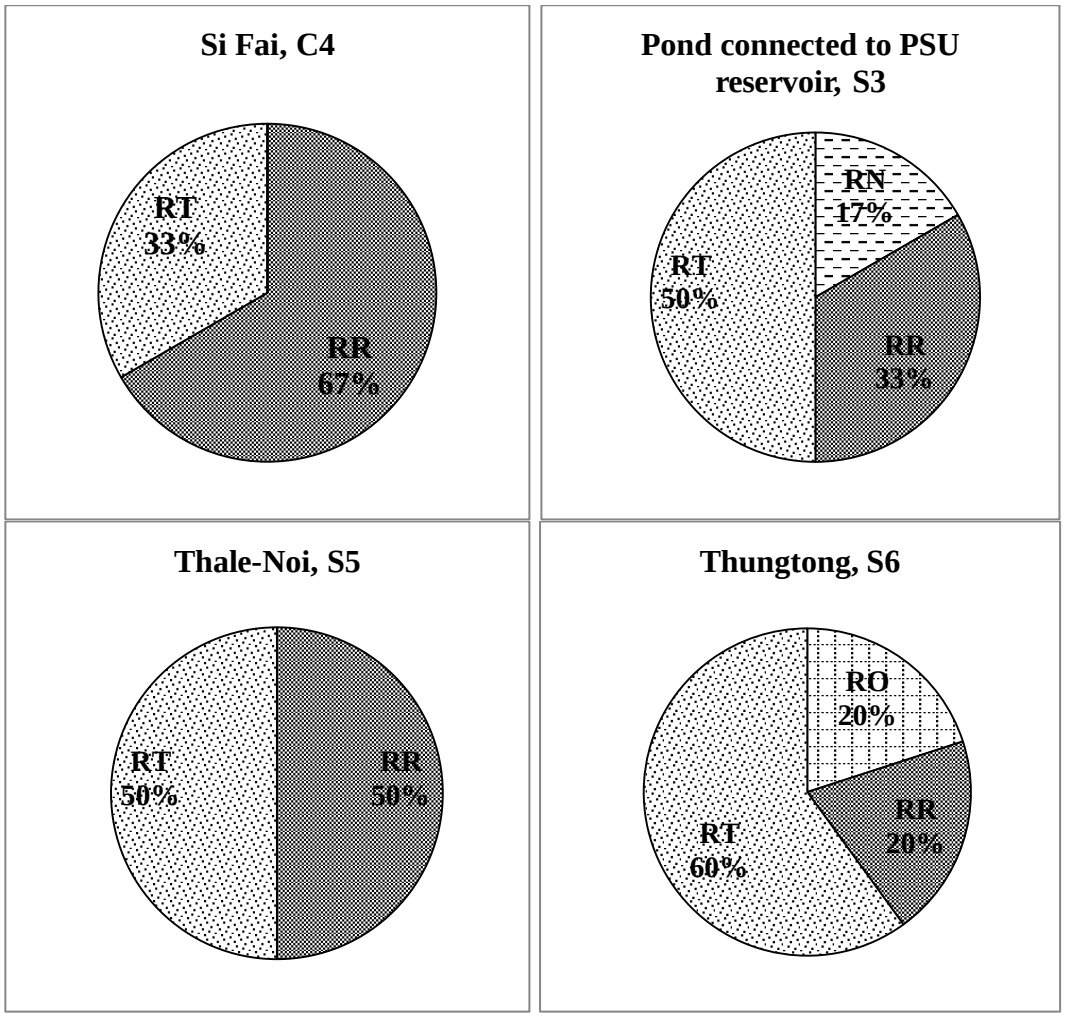


Figure 17. (Continued)

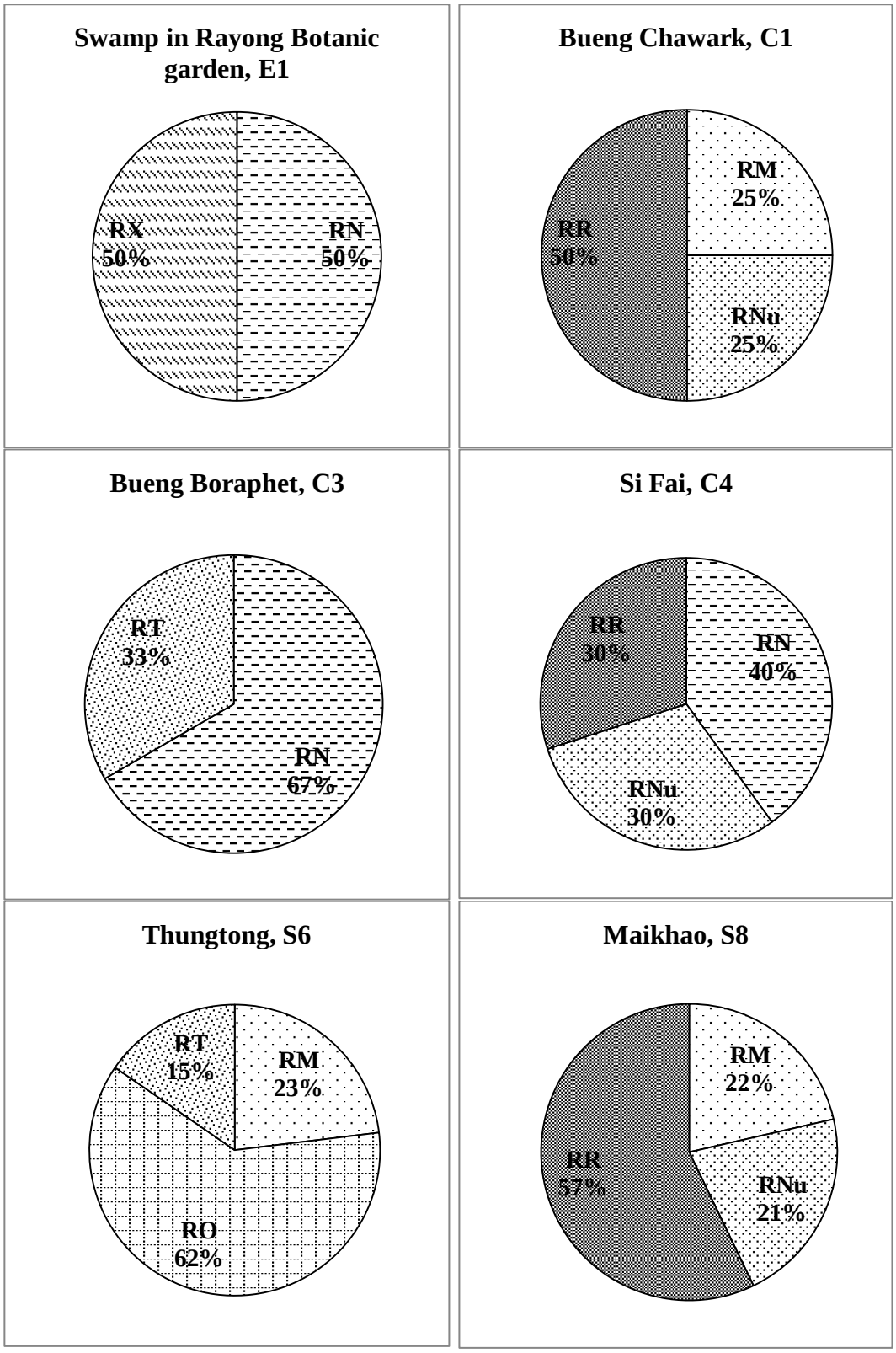


Figure 18. Percentage of individual of *Rotaria* from each habitat in rainy season. (Represented only habitats where *Rotaria* were found more than one species).

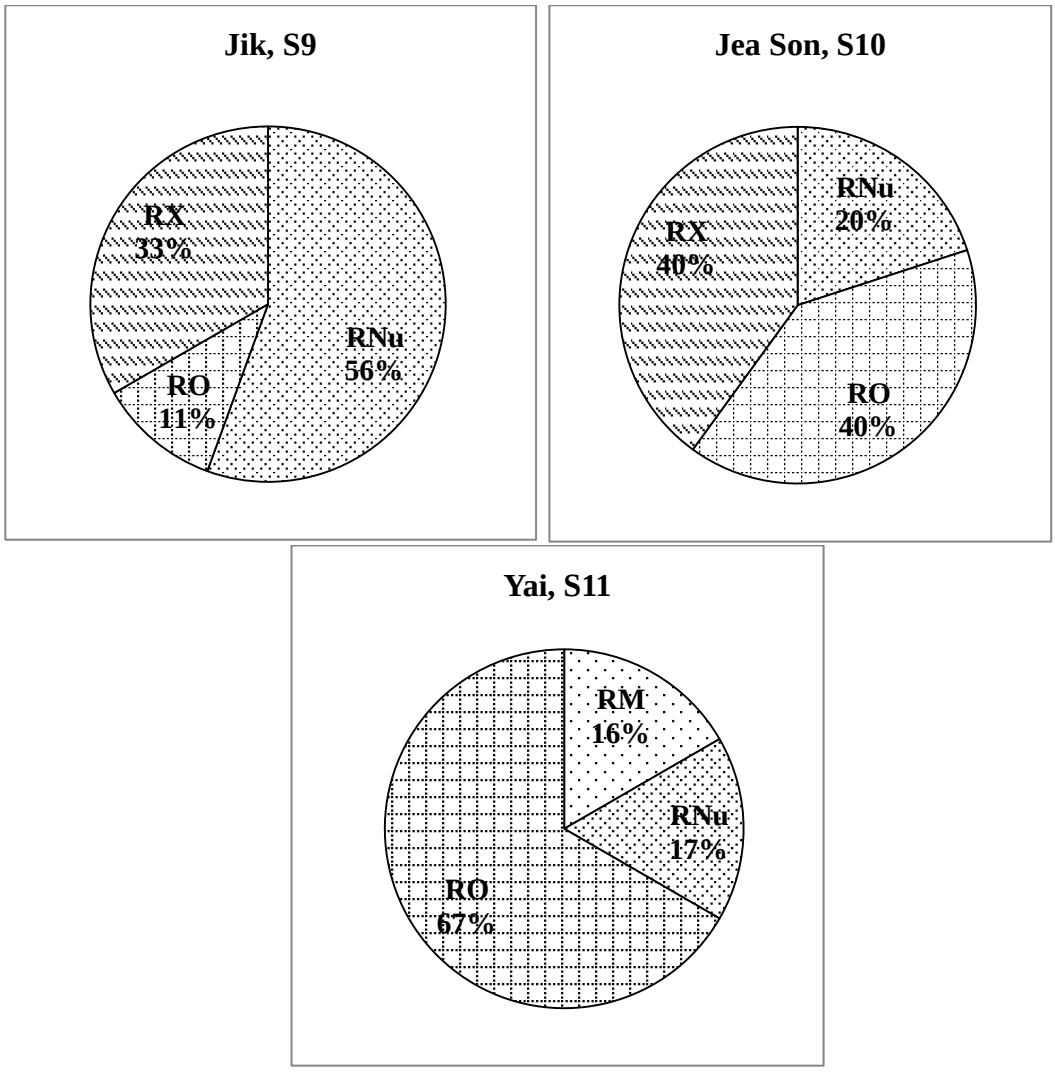


Figure 18. (Continued)

CHAPTER 4

DISCUSSION

1. Morphological taxonomy

Bdelloids are ignored organisms when compare with other groups of rotifers, monogononts. Records of bdelloid rotifers from Asia are rare, particularly Southeast Asia along with Thailand. Although many bdelloid's studies in Asia were recorded from Korea, but a few of *Rotaria* were reported. The recent study of Song & Min (2015) published a new species, new records including checklist of bdelloid rotifers from Korea, 68 taxa, of which 49 species and 19 subspecies. Nevertheless, only five taxa of *Rotaria*; four species and one subspecies were recorded (Song & Min, 2015). In Thailand, five species of bdelloids were recorded (Koste, 1975; Chittapun & Pholpunthin, 2001; Chittapun *et al*., 2002, 2007; Fontaneto *et al*., 2007c). However, those studies did not report the morphological details of them and some species such as *Dissotrocha aculeate* just have only SEM picture (Chittapun & Pholpunthin, 2001).

The morphological study of *Rotaria* is not easy because they have creep movement like leech and important characteristics can be observed when they are alive. Each species has different movement, some species move very fast, (e.g., *R. rotatoria*), while some are truly slow, (e.g., *R. tardigrada*). This behavior effects measurement of the body. Even if the best positions of *Rotaria* was chosen through the picture for measurement, but it is difficult to measure their real size. So, the good characters for identification should have less variation such as the appearance of eyes, shape of corona, body, foot, spurs and toes, and jointed number of spurs and toes. Although the length of their organ is not rather good feature, but sometimes it is useful to distinguish some species. For example, foot length of *R. neptunia* and *R. ovata*, and length and size of rostrum of *Rotaria* sp. 1 can distinguish them from other species. Shape variation of *Rotaria* in Thailand is not different. It might possible

that because the number of samples in this study is low. Furthermore bdelloids are obligated parthenogenetic reproduction, descendent are clone from their mother and most of them have a very similar basic features (Ricci, 1987). As mentioned above, misidentification is occurring frequently. Consequently, molecular study is useful tool for identify dissimilar species or cryptic species along with compare genetic relationship between domestic species and species form other country.

2. Shape, size and teeth number of trophi

Although bdelloid rotifers have only one trophi type, the ramus, its size is rather stable and do not change with age (Fontaneto & Melone, 2005, 2006). However, this study revealed significant differences of trophi morphology between some species. The intraspecific variations have also been observed in the same species from other biogeographical regions.

Investigation of shape variation of trophi by using geometric morphometric is helpful, but rather problematic in identification purpose compare to measurement trophi size and number of minor and major teeth (Donner, 1965**;** Ricci & Melone, 2000). In addition the sample preparation for geometric morphometric study is quite difficult. Thus, big sample size and perfect position of trophi in SEM picture are necessary.

Number of distal minor teeth of *Rotaria* is usually more than the number of proximal teeth. Previous studies reported that most of *Rotaria* have certain number of major teeth, mostly two, while three are rare (Ricci & Melone, 2000; Melone & Fontaneto, 2005). Nevertheless, this study found that *R. ovata* has only one major tooth which is support to the original description (Anderson, 1889). Moreover shape of major teeth of *R. ovata* rather slender and sharp tip when compared with other species. Shape and teeth number can used to approximate the habitats of bdelloid rotifers **(**Melone & Fontaneto, 2005). Commonly a few numbers of major teeth and several minor teeth appear in aquatic species in contrast with the terrestrial species because it is related to a food type (Melone & Fontaneto, 2005).

3. DNA taxonomy

Regarding the species identification by using DNA sequences, several morphological identifications were not supported by BLAST that compared raw genetic sequences without incorporating evolutionary models. However, the morphospecies were clearly grouped as monophyly in molecular phylogenetic analysis.

DNA taxonomy supported the species identification from morphology and estimated the closest relationship of unidentified species. Even if some species do not have available sequence from GenBank for comparison, but they still formed their monophyletic clade. *Rotaria* sp. 1, which has different morphology from each member in this genus, has its own distinct clade. Furthermore, DNA taxonomy is widespread tool to reveal cryptic species in rotifers (Fontaneto *et al.*, 2009; Kaya *et al.*, 2009). This scenario could be expected in bdelloid rotifers which have exclusively parthenogenetic reproduction (Gilbert, 1983). Therefore, the traditional taxonomy could be underestimated a large diversity of bdelloid rotifers that is hidden by morphological similarity (e.g. Fontaneto *et al.*, 2009; Kaya *et al.*, 2009, Mills *et al.*, 2016).

From the phylogenetic tree, foot shape and length, spurs joint and length and toes equilibrium and length are differentiate characters that can be separate each clade from each other. Group A have foot length a half of body length. Group B, foot length of the members is ¼ of body length, spurs jointed and long, mostly three toes unequal and ventral toes longer than middle dorsal toe. Group C, most of members have foot length longer than 2 times of body length, especially *R. neptunia* and *R. ovata*, however foot of *R. sordida* and *R. macroceros* shorter than their body length, three toes are equal and rather long, moreover both eyes/without eyes on rostrum found in this group.

4. Species composition and distribution

According to this study, eight species of *Rotaria* were found. This account for 33% of all members in genus *Rotaria*, and it account for 89% of bdelloids species recorded in Oriental region (Segers, 2007). Six species are new to Southeast Asia including *R. mento*, *R. neptunoida*, *R. ovata*, *R. tardigrada*, *Rotaria* sp. 1 and *Rotaria* sp. 2. The morphological characters of *Rotaria* sp. 1 and *Rotaria* sp. 2 are different from other species, while *R. ovata* are the first discovered outside type locality in India since 1889 by Anderson (1889).

Previous studies reported that *R. neptunia*, *R. rotatoria* and *R. tardigrada* are cosmopolitan taxa. They were found in many continents around the world; Afrotropical, Australian, Nearctic, Neotropical, Oriental and Palearctic. *R. neptunoida* had been found in many regions, except for Pacific and Neotropical. *R. mento* were recorded at Nearctic, Palearctic and Oriental (Segers, 2007) (Table 9). This study also found that *R. mento* and *R. neptunia* have wide distribution present in five parts of Thailand. The two species seem to have high ability to tolerate a broad range of environmal conditions. The other species such as *R. neptunia* can be found in a both water bodies and decomposition areas of shallow waters, moreover can survive in a broad range of environment factors for example temperature 12-26 ºC, pH 7.2-8.6, dissolved oxygen 7.5-10.4 mgO_2/l^{-1} (Thorpe, 1887; Whitlegge, 1889; Donner, 1965; Koste, 1976; Shiel *et al.*, 1982; Shiel, 1981; Koste & Shiel, 1986).

On the other hand, *R. ovata*, *Rotaria* sp. 1 and *Rotaria* sp. 2 are narrow distributions, being restricted to oriental region. *R. ovata* have never reported since the original description in India (Anderson, 1889; Segers, 2007). This study identified *R. ovata* in three parts of Thailand, extending its distribution record. *Rotaria* sp. 1 found only in Thailand, but they showed wide distribution presenting in four parts, except Central. *Rotaria* sp. 2, however, has been observed only in S3 in South of Thailand (Table 9).

One of the important factors that can affect bdelloid life is water salinity. Most bdelloids inhabit freshwater and moist terrestrial habitats. Some species are euryhaline, while some live in brackish water. Notwithstanding, in the saline water such as Nong Han, Phuket (30-34 ppt), no rotifers were found in this place because the high salinity may affect their normal physiology.

According to Ricci (1987) she mentioned that the depth of freshwater habitats and the distribution of bdelloid rotifers are inverse correlation (Ricci, 1987). Moreover Klimowicz (1972) proposed that increasing depth can decrease the number of species and population densities. The results of the present investigation agreed with the previous in which studies, most bdelloids occupy littoral zone that have more litters than in the depth. A number of factors promote the preference of bdelloids living in the literal zone. First, *Rotaria* species are able to swim, and they also creep on the surface of aquatic plants as semi-planktonic animals (Pontin, 1978; Koste & Shiel, 1986; Ricci & Melone, 2000). Second, bdelloids survival relies on food and nutrient along with dissolved oxygen in water surface in which much abandant in the litteral than in the deep zone. For present observations, most of *R. mento* were found in freshwater habitats where sediment is abandant. The species may need the particles to create the tube that cover their body. Although, bdelloids live in habitats that mentioned above, but their real behavior and habitat preference are doubtful because in preliminary observation, most bdelloid rotifers were presented in freshwater habitats with aquatic macrophytes, however in this study some places where possibly found, but did not appear.

This research demonstrates that *Rotaria* is a diverse genus in Thailand, since eight species were found from only freshwater habitat collection. Of which, seven species are new records to Thailand; one is different fron each other, and one seem to be a different species, but a single specimen was found and was not to comfirm its identity. Consequently, the present study increase the species diversity of bdelloid rotifers in Thailand from five to twelve species: two of *Dissotrocha*, two of *Habrotrocha* and eight of *Rotaria*. In addition, any study and sample collection in other habitats would increase other new records of this group of rotifer. This may reduce the “rotiferologist” effect (Fontaneto *et al*., 2012) in our biogeological

knowledge because many more bdelloid rotifers are yet to be discovered from Southeast Asia including Thailand, as well as understand the spatial patterns of these microscopic animals.

CHAPTER 5

CONCLUSION

1. Morphological taxonomy

The morphological identification of bdelloid rotifers in the genus *Rotaria* is difficult due to its movement. A good taxonomic character should be qualitative, but the length of rostrum, foot, spurs and toes is still needed in some cause to support the species identity. Although these features are less variable in the present study, it may due to a few sample size together with they are asexual animals lead to morphological resemblance and high hidden diversity.

2. Shape, size and teeth number of trophi

Variation in shape of trophi is observed. However, size and teeth number are useful for identification more than shape variation and related to their food and habitats. Furthermore two major teeth are usually found in bdelloid rotifers including *Rotaria*.

3. DNA taxonomy

DNA taxonomy is important tool to reveal the hidden species and used as strong evidences to support the morphological identification.

4. Species composition and distribution

Rotaria is a diverse genus in Thailand. This study added species diversity of the genus from one to eight species and the number of bdelloid rotifers in Thailand

rise to twelve species. Both widespread and narrow distributed species are observed. It is forced by environmental factors and their adapted ability. The real habitat preferences are still doubtful, but most of *Rotaria* were found in shallow water. Thus, studying in any habitats and increasing frequently of sample collections can uncover the diversity of bdelloid rotifers in Thailand.

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APPENDIX

Appendix 1. Size and teeth number of trophi of *Rotaria* in Thailand.

species	individual	view	ramus length (µm)	number of					
				right			left		
				distal minor	proximal minor	major	distal minor	proximal minor	major
RM	R_mento_S3b_t1	cephalic	-	21	17	2	23	18	2
RM	R_mento_S3b_t2	caudal	18.21	-	-	-	-	-	-
RM	R_mento_N1a_1_t1	cephalic	-	21	14	2	20	17	2
RM	R_mento_N1a_1_t2	caudal	21.59	-	-	-	-	-	-
RM	R_mento_N1a_1_t3	caudal	22.47	-	-	-	-	-	-
RM	R_mento_S3b_t3	caudal	24.21	-	-	-	-	-	-
RM	R_mento_S3b_t4	caudal	18.96	-	-	-	-	-	-
RX	R_megarostris_S3b_t2	cephalic	-	19	10	2	17	10	2
RX	R_megarostris_S3b_t3	cephalic	-	24	8	2	21	9	2
RX	R. megarostris_S10a_2_t1	caudal	27.47	-	-	-	-	-	-
RX	R. megarostris_S10a_2_t2	caudal	29.67	-	-	-	-	-	-
RN	R_neptunia_S3_t1	caudal	21.93	-	-	-	-	-	-
RN	R_neptunia_S3_t2	caudal	20.6	-	-	-	-	-	-
RN	R_neptunia_S3_t3	caudal	20.36	-	-	-	-	-	-
RN	R_neptunia_S3_t4	caudal	20.48	-	-	-	-	-	-
RN	R_neptunia_S3_t5	caudal	21.67	-	-	-	-	-	-
RN	R_neptunia_S3_t6	caudal	21.11	-	-	-	-	-	-
RN	R_neptunia_S3_t7	cephalic	-	23	13	2	22	13	2
RN	R_neptunia_S3_t8	caudal	21.67	-	-	-	-	-	-
RN	R_neptunia_S3_t9	cephalic	-	23	15	2	25	16	2
RN	R_neptunia_S3_t10	cephalic	-	24	16	2	23	12	2
RN	R_neptunia_S3_t11	caudal	22.14	-	-	-	-	-	-
RNu	R_neptunoida_S9a_1_t1	caudal	26.13	-	-	-	-	-	-
RNu	R_neptunoida_S9a_1_t2	cephalic	-	26	15	2	25	15	2
RNu	R_neptunoida_S9a_1_t3	cephalic	-	25	10	2	24	16	2
RO	R_ovata_S3b_t1	caudal	18.69	-	-	-	-	-	-
RO	R_ovata_S3b_t2	cephalic	-	20	13	2	22	15	1
RO	R_ovata_S3b_t3	cephalic	-	21	14	2	22	14	1
RO	R_ovata_E1b_4_t1	cephalic	-	23	14	1	21	15	2
RO	R_ovata_E1b_4_t2	caudal	19.04	-	-	-	-	-	-
RO	R_ovata_NE4b_3_t1	caudal	18.45	-	-	-	-	-	-
RO	R_ovata_S3b_t4	caudal	18.11	-	-	-	-	-	-
RO	R_ovata_S3b_t5	caudal	19.79	-	-	-	-	-	-
RO	R_ovata_S3b_t6	cephalic	-	23	18	1	22	14	2

Appendix 1. (Continued)

species	individual	view	ramus length (µm)	number of					
				right			left		
				distal minor	proximal minor	major	distal minor	proximal minor	major
RO	R_ovata_S3b_t7	cephalic	-	21	15	1	20	13	2
RO	R_ovata_S3b_t8	cephalic	-	22	15	1	19	14	2
RR	R_rotatoria_S3_t1	cephalic	-	18	15	2	16	15	2
RR	R_rotatoria_S3_t2	caudal	19.17	-	-	-	-	-	-
RR	R_rotatoria_S3_t3	cephalic	-	18	13	2	16	15	2
RT	R_tardigrada_S2b_t1	cephalic	-	16	13	2	17	12	2
RT	R_tardigrada_S2b_t2	caudal	28.06	-	-	-	-	-	-
RT	R_tardigrada_S2b_t3	caudal	28.57	-	-	-	-	-	-
RT	R_tardigrada_S2b_t4	cephalic	-	17	15	2	18	12	2

Appendix 2. Appearance of *Rotaria* in each parts of Thailand.

sampling sites	code	RM	RN	RNu	RO	RR	RT	RX
Phayao, Phayao	N1	+	+	+	-	-	-	-
Chiang Saen, Chiang Rai	N2	-	-	-	-	+	-	+
Nong Han Kumphawapi, Udon Thani	NE1	+	+	-	+	-	+	+
Kud Thing, Bueng Kan	NE2	-	-	-	-	-	+	-
Khong Long, Bueng Kan	NE3	-	-	-	-	-	-	+
Nong Han, Sakon Nakhon	NE4	-	-	-	+	-	+	+
Nong wang, Chaiyaphum	NE5	+	-	-	-	+	+	-
Huai Chorakhe Mak, Buri Ram	NE6	-	-	-	-	-	-	-
Rayong Botanic garden, Rayong	E1	+	+	-	+	-	-	+
Bueng Chawark, Suphan Buri	C1	+	-	+	-	+	-	-
Bueng Senat, Nakhon Sawan	C2	-	-	-	-	-	-	-
Bueng Boraphet, Nakhon Sawan	C3	-	+	-	-	+	+	-
Si Fai, Phichit	C4	-	+	+	-	+	+	-
Pluckpraya, Satun	S1	-	-	-	-	-	-	-
Pond behind department of biology building in PSU, Songkhla	S2	-	+	+	+	+	+	-
Pond connected to PSU reservoir, Songkhla	S3	-	+	-	-	+	+	+
Klong Lam Chan, Trang	S4	-	-	-	-	-	-	-
Thale-Noi, Phatthalung	S5	+	-	-	-	+	+	+
Thungtong, Surat Thani	S6	+	-	-	+	+	+	-
Nong Han, Phuket	S7	-	-	-	-	-	-	-
Maikhao, Phuket	S8	+	-	+	-	+	-	+
Jik swamp, Phuket	S9	-	-	+	+	-	-	+
Jea Son, Phuket	S10	-	-	+	+	-	-	+
Yai, Chumpon	S11	+	-	+	+	-	-	-

VITAE

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