



**Combined Molecular-Morphological Approach to Species Complexes
of Brachionid and Lecanid Rotifers (Eurotatoria: Monogononta:
Brachionidae and Lecanidae) in Thailand**

Phanee Sa-artrit

**A Thesis Submitted in Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Biology
Prince of Songkla University
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I hereby certify that this work has not been accepted in substance for any other degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์	ชีววิทยาเชิงโมเลกุลและสัณฐานวิทยาของชนิดทับซ้อนของโรติเฟอร์ในวงศ์ Brachionidae และวงศ์ Lecanidae (Eurotatoria: Monogononta: Brachionidae and Lecanidae) ในประเทศไทย
ผู้เขียน	นางสาวพรรณณี สอาดฤทธิ
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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อวิเคราะห์หาโรติเฟอร์ชนิดซ้อนเร้นที่อยู่ภายในโรติเฟอร์ชนิดทับซ้อนในวงศ์ Brachionidae และวงศ์ Lecanidae ที่พบในประเทศไทย โดยศึกษาความแตกต่างของนิวคลีโอไทด์ของยีน COI และ ITS1 ด้วยวิธีการทางอนุกรมวิธานชีวโมเลกุล 3 วิธี ได้แก่ ABGD, PTP และ GMYC ในการกำหนดขอบเขตของชนิดและประมาณจำนวนชนิดที่พบในโรติเฟอร์ชนิดทับซ้อน ความสอดคล้องของจำนวนชนิดที่ประมาณได้จากทุกวิธีการ จะใช้ยืนยันการมีอยู่ของชนิดซ้อนเร้นภายในโรติเฟอร์ชนิดทับซ้อน การประมาณจำนวนชนิดที่แท้จริงภายในชนิดทับซ้อน นอกจากจะสามารถนำไปใช้ประมาณความหลากหลายทางชีวภาพของโรติเฟอร์ที่แท้จริงในประเทศไทยแล้ว ยังนำไปสู่ความเข้าใจทางนิเวศวิทยา และวิวัฒนาการของโรติเฟอร์ในกลุ่มที่มีความสัมพันธ์ใกล้ชิดกันมากขึ้น นอกจากนี้การศึกษาค้นคว้าความแตกต่างทางพันธุกรรมกับการแพร่กระจายทางภูมิศาสตร์ของชนิดซ้อนเร้นในประเทศไทย ยังถูกศึกษาโดยวิเคราะห์ด้วยวิธีการทางสถิติ Mantel test

ผลการศึกษาโดยอาศัยวิธีการทางอนุกรมวิธานชีวโมเลกุล ชี้ให้เห็นถึงชนิดซ้อนเร้นภายในโรติเฟอร์ชนิดทับซ้อนทั้งสองวงศ์ที่พบในประเทศไทย โดยพบจำนวนชนิดซ้อนเร้นมากที่สุดในกลุ่ม *Lecane bulla* โดยพบชนิดซ้อนเร้นอย่างน้อย 20 ชนิด อยู่ภายในชนิดทับซ้อนของ *Lecane bulla* การศึกษานี้ได้นำลักษณะทางสัณฐานวิทยาของ *Lecane bulla* มาวิเคราะห์ร่วมด้วย พบว่าลักษณะทางสัณฐานวิทยาสามารถนำมาใช้อธิบายความแตกต่างของบางชนิดได้เพียงเล็กน้อย แต่ไม่สามารถใช้จำแนกชนิดซ้อนเร้นทั้งหมดที่อยู่ภายใน *Lecane bulla* นอกจากนี้ยังพบชนิดซ้อนเร้นภายในชนิดทับซ้อนอื่น ๆ ได้แก่ *Brachionus angularis*, *Brachionus calyciflorus*, *Brachionus caudatus*, *Brachionus quadridentatus*, *Keratella cochlearis*, *Keratella tropica*, *Lecane bulla*, *Lecane closterocerca*, *Lecane crenata*, *Lecane curvicornis*, *Lecane hamata*, *Lecane leontina*, *Lecane ludwigii*, *Lecane luna*, *Lecane lunaris*, *Lecane nitida*,

Lecane quadridentata, *Lecane signifera* และ *Lecane unguitata* และจากการวิเคราะห์ด้วย DNA taxonomy พบว่าผลที่ได้จากการวิเคราะห์ลำดับนิวคลีโอไทด์ของยีน COI มีความสอดคล้องกันของจำนวนชนิดที่ประมาณได้ในแต่ละวิธี ขณะที่การประมาณจากยีน ITS1 มีความแตกต่างกันในแต่ละวิธี และจากการวิเคราะห์เปรียบเทียบความแตกต่างทางพันธุกรรมภายในชนิดซ่อนเร้นในแต่ละพื้นที่ของประเทศไทย พบว่าไม่มีความแตกต่างทางพันธุกรรมระหว่างพื้นที่ทางภูมิศาสตร์ ยกเว้น *Lecane curvicornis* ซึ่งให้เห็นว่าโรติเฟอร์ชนิดซ่อนเร้นที่พบในประเทศไทยมีการกระจายทางภูมิศาสตร์ที่ซ้อนทับกันและกระจายตัวได้ในบริเวณกว้างในประเทศไทย

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Author	Miss Phannee Sa-artrit
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ABSTRACT

This study aims to analyze potential species complexes of rotifers within the families Brachionidae and Lecanidae, to test the hypothesis whether cryptic diversity is indeed as common as is being put forward. Moreover, this study analyzed the geographic distribution and genetic divergence within species complexes in Thailand. The tests for the presence of cryptic species were applied using three methods on both COI and ITS1 datasets: Automatic Barcode Gap Discovery (ABGD), a Poisson Tree Processes (PTP) model, and the Generalized Mixed Yule Coalescent (GMYC) model. Genetic divergence and geographic distance were analyzed using Mantel test. The results from three DNA taxonomy approaches, ABGD, PTP, and GMYC from COI and ITS1 markers clearly indicated the existence of cryptic species in both families. The highest estimate of species diversity in this study was *Lecane bulla* with at least 20 species existing in the complex. Morphological characters were examined. However, morphology cannot be used to separate some cryptic species in *Lecane bulla* complex. Moreover, this study reveals the existence of cryptic species in *Brachionus angularis*, *Brachionus calyciflorus*, *Brachionus caudatus*, *Brachionus quadridentatus*, *Keratella cochlearis*, *Keratella tropica*, *Lecane bulla*, *Lecane closterocerca*, *Lecane crenata*, *Lecane curvicornis*, *Lecane hamata*, *Lecane leontina*, *Lecane ludwigii*, *Lecane luna*, *Lecane lunaris*, *Lecane nitida*, *Lecane quadridentata*, *Lecane signifera*, and *Lecane unguitata*. The species estimated using COI marker is congruent among different DNA taxonomy methods, while there is incongruence among different methods based on ITS1 marker. Only *Lecane curvicornis* shows significant correlation between genetic and geographic distances. No correlation occurs between genetic and geographic distances in other species complexes. Thus, there is a geographic overlap in rotifer distribution in Thai waters.

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Rotifer is one of the four main groups of freshwater zooplankton. Rotifers are important in freshwater environments because their reproductive rate is the fastest among the metazoans and they often reach high population densities (Wallace *et al.*, 2006). Moreover, they form a critical link between the microbial loop and higher trophic levels in some freshwater food webs. They are permanently and obligatorily connected to aquatic habitats in all active stages, only their resting stages are drought-resistant (Segers, 2008). They are ubiquitous, occurring in almost all types of freshwater habitats, from large permanent lakes to small temporary puddles.

Phylum Rotifera comprises about 2,030 known species classified in three main groups, Seisonida (3 species), Monogononta (1,570 species), and Bdelloidea (461 species) (Segers, 2007). Although the diversity of monogonont rotifers currently turns out to be higher than previous estimates, many species are still insufficiently diagnosed and require a comprehensive description. One of the knowledge gaps concerns the taxonomy of species complexes and speciation in rotifers. Rotifers seem to meet conditions for an active speciation (Serra *et al.*, 1997). Sympatric speciation is more probable in rotifers than in bisexual animals with continuous reproduction, due to seasonal ecological specialization, which allows seasonal succession and partitioning of resources (Ortells *et al.*, 2003) and because of the role of the parthenogenetic phase in creating variation for mating systems (Serra *et al.*, 1997). Seasonal variation and timing of sex may allow allochronic divergence. Mutations affecting the mate recognition system may spread during the parthenogenetic phase. They are neutral, affecting a significant set of individuals before reproduction, and hence promoting their reproductive isolation (Serra *et al.*, 1998).

Mate recognition of rotifers involves contact chemoreception without visual cues (Snell, 1989). Consequently, speciation can be cryptic, with little or no morphological

divergence (Gómez and Snell, 1996). Such species are called “cryptic species” which are difficult or impossible to distinguish by phenotype (Birky *et al.*, 2005; Gómez, 2005).

Since the advent of molecular phylogenetics, there is increasing evidence that aquatic invertebrate species, which were previously believed to be single, cosmopolitan species, in fact might be cryptic species complexes (Suatoni *et al.*, 2006). Rotifer taxonomists have since long attempted to reveal cryptic diversity within a number of cosmopolitan species. Consequently, many rotifer species formerly seen as cosmopolitan are now recognized as cryptic species. The best documented example of such is *Brachionus plicatilis* (King and Zhao, 1987; Gómez *et al.*, 2002; Mills *et al.*, 2016), but it has also been shown in other species, as diverse as *Keratella cochlearis* (Derry *et al.*, 2003), *Lecane bulla* (Walsh *et al.*, 2009), and *Brachionus calyciflorus* (Xiang *et al.*, 2010). However, information on these species complexes is largely anecdotal.

Although morphology remains the predominant taxonomic tool for identifying rotifer species, it is ineffective to discriminate some taxa in groups of rotifer that have little or no morphological differences. Morphology is not always a reliable guide to the identification of species that may be well-defined on a molecular basis (Suatoni *et al.*, 2006). Identifying morphologically inseparable cryptic species requires a new set of taxonomic tools. Molecular tools are useful for understanding species complex (Gómez, 2005). This has led a number of rotifer researchers to apply molecular methods, such as allozyme analysis and DNA sequencing, to their work (Proudlove and Wood, 2003; Gómez, 2005).

Initial evidence from genetic variability and ecological specialization among different clonal groups indicated that *Brachionus plicatilis* is a species complex (Gómez *et al.*, 1995). The accumulation of diverse evidence over more than a decade, such as differences among clones regarding morphometric variation (Serra and Miracle, 1987), geometric morphometry (Fontaneto *et al.*, 2007), ecological specialization (Serra *et al.*, 1998), mating behavior (Gómez and Serra, 1995; Gómez and Snell, 1996), mate recognition pheromone (MRP) (Rico-Martinez and Snell, 1995; Kotani *et al.*, 2001), karyotype (Rumengan *et al.*, 1991 cited in Segers, 1995), and allozyme composition (Gómez *et al.*, 1995; Gómez and Snell, 1996; Kotani *et al.*, 2005), lead to the recognition within the *Brachionus plicatilis* complex of *Brachionus plicatilis sensu strictu*,

Brachionus rotundiformis, *Brachionus ibericus*, and *Brachionus manjavacas* (Segers, 1995; Círos-Pérez *et al.*, 2001).

Recently, evidence from nucleotide sequence variation from both mitochondrial (cytochrome *c* oxidase subunit I: COI) and nuclear (ribosomal internal transcribed spacer 1: ITS1) genes revealed nine genetically different lineages within the *B. plicatilis* complex (Gómez *et al.*, 2002). These lineages are *B. plicatilis s.s.*, *B.* ‘Nevada’, *B.* ‘Austria’, *B.* ‘Manjavacas’, *B. ibericus*, *B.* ‘Cayman’, *B.* ‘Tiscar’, *B.* ‘Almenara’, and *B. rotundiformis*. The study of Suatoni *et al.* (2006) revealed even more cryptic species, increasing the number of species within *B. plicatilis* complex. Molecular sequence and genetic patterns in COI and ITS1 indicate the existence of at least 15 species within *B. plicatilis* complex. This is the highest estimate of species-level diversity in a cryptic species complex in Rotifera thus far (Mills *et al.*, 2016).

Deoxyribonucleic acid (DNA) sequences or gene sequences of organisms are useful to assign individual organisms to species (Birky, 2007). Mitochondrial DNA sequences are better suited for identifying closely related animal species than nuclear sequences because mitochondrial DNA sequences are five- to ten-fold more different among closely related animal than nuclear sequences (Stoeckle *et al.*, 2005). Moreover, mitochondrial protein-coding genes contain more differences than ribosomal genes.

The mitochondrial protein-coding gene, cytochrome *c* oxidase subunit 1 (*cox1* or COI), is a widely accepted marker for molecular identification of taxa at the species level, across diverse animal groups (Buhay, 2009). For most eukaryotes, COI variation appears to be lower among individuals within a species than among individuals belonging to different species (Marshall, 2005). It allows effective distinguishing amongst closely related species (Stoeckle *et al.*, 2005). Moreover, the rate of evolution of COI seems to be ideal for differentiating species in rotifers (Birky, 2007) and universal invertebrate primers are available (Folmer *et al.*, 1994). Thus, it is suitable to be used for discriminating cryptic species and to study phylogeography (Birky *et al.*, 2005; Gómez, 2005; Pauls *et al.*, 2010).

Recently, DNA taxonomy methods were applied to delimit species (Tang *et al.*, 2014; Fontaneto *et al.*, 2015). Furthermore, phylogenetic delimitations were used to estimate the number of species. These tools offer taxonomists and ecologists to assess biodiversity. Thus, DNA taxonomy approaches are useful for the discovery of cryptic

species within small animals with high levels of cryptic diversity and frequent morphological stasis (Fontaneto *et al.*, 2009). DNA taxonomy employs genetic data, such as DNA sequences of selected loci or complete genome, to delimit species by analyzing variation to discover within this information (Tautz *et al.*, 2003). Moreover, multilocus approaches to species delimitation is an active field of research, and these will undoubtedly predominate in future studies. At present, there are 3 popular DNA taxonomy approaches for species delimitation: Automatic Barcode Gap Discovery (ABGD), Poisson Tree Processes (PTP) model, and the Generalized Mixed Yule Coalescent (GMYC) model. First, the ABGD was used to assign organisms into species based on the barcode gap. Gap detections are applied to observe the divergence among organisms. The barcode gap among organisms belonging to the same species is smaller than among organisms from different species (Puillandre *et al.*, 2012). Moreover, ABGD is a fast and simple method to split sequence alignment dataset into candidate species that should be complemented with other evidence in an integrative taxonomic approach (Puillandre *et al.*, 2012). Second, Poisson Tree Processes (PTP) is a model to delimit species boundaries on a given phylogenetic input tree (Zhang *et al.*, 2013). Third, the Generalized Mixed Yule Coalescent (GMYC) is a likelihood method to delimit species using single-locus, and fitting within- and between-species branching models to reconstruct gene trees (Fujisawa and Barraclough, 2013). Therefore, it is efficient to discover cryptic diversity of organisms. Recently, all of ABGD, PTP, and GMYC are well-known to delimit species and discover cryptic species within several organisms such as gastropods (Modica *et al.*, 2014), moths (Kekkonen *et al.*, 2015), beetles (Pentinsaari *et al.*, 2016), crayfishes (Larson *et al.*, 2016), coleopterans (Eberle *et al.*, 2016), nemertean (Leasi *et al.*, 2016), and parasitoid wasps (Schwarzfeld and Sperling, 2015). In rotifers, 15 species were discovered from *B. plicatilis* species complex through DNA taxonomy (Mills *et al.*, 2016).

Monogononta is the most diverse taxon among the three main groups of rotifers as they form the most abundant group of rotifers in Thailand. In this study, I aimed to analyze potential species complexes in selected morphotaxa of some monogonont rotifers, and to test the hypothesis whether cryptic diversity is indeed as common as is being put forward. I will further analyze the geographical distribution and genetic divergence within species complexes in Thailand. This study focused on species complexes within the

families Brachionidae and Lecanidae, using the cytochrome *c* oxidase subunit I (COI) gene, to discriminate sequence differences among taxa. In addition, the ribosomal internal transcribed spacer 1 (ITS1) gene was used for supporting species delimitation. Improved species recognition in monogonont rotifers might reveal cryptic species within a cosmopolitan species and lead to better understanding of the distribution of these organisms. Appropriate identification leads to more accurate predictions of the actual number of individual evolutionary units, or species, in rotifers. In fact, the diversity of rotifers may be much higher than presently believed.

1.2 Literature review

1.2.1 General background

1) The study of freshwater Rotifera in Thailand

The study of freshwater rotifers in Thailand started with the publication by Weber (1907). To date, 401 species-level taxa of rotifers have been recorded (Table 1) (Meksuwan *et al.*, 2013, 2014, 2015; Sa-ardrit *et al.*, 2013). A number of older records were recently re-evaluated by Segers and Savatnalinton (2010). Other dubious or otherwise problematic records are the following:

- *Brachionus dichotomus* Shephard, 1911 and *B. dichotomus reductus* Koste and Shiel, 1980: Both names can be found in literature dealing with Thai Rotifera, however, the “typical” *B. dichotomus* has not been recorded from the Oriental region and these reviews therefore indicate that the presence of *B. dichotomus* Shephard requires confirmation.

- *Brachionus plicatilis* Müller, 1786: Records under this name most likely do not refer to this species as presently understood (Ciros-Pérez *et al.*, 2001), but any of the warm-water representatives of this cryptic species group (see, for example, Suatoni *et al.*, 2006).

- *Keratella tropica* Apstein, 1907: This species was included under the name *Keratella valga* (Ehrenberg, 1834) by Boonsom (1984), considering that the distinction between the cold-water, acidophilic *K. valga* and the warm-water, euryoecious *K. tropica* has long remained problematic, and that it is unlikely that the former would occur in the habitats studied by Boonsom (1984).

- Koste’s (1975) record of the *nomen nudum* “*Lepadella monostyla* f. *caudata* (Koste)” is most likely a lapsus regarding the taxon *Lepadella monodactyla caudata* Koste, 1972, at present considered a junior subjective synonym of the nominal taxon.

- The presence in Thailand of *Filinia terminalis* (Plate, 1886) is in need of confirmation, considering that this cold-stenotherm is easily confused with the warm-water *F. novaezealandiae* Shiel and Sanoamuang, 1993 (see Segers *et al.*, 1996). However, while the two records of *F. terminalis* indeed precede the recognition of *F. novaezealandiae*, the presence of this species in the North of Thailand cannot *a priori* be excluded.

- The record of *Habrotrocha recumbens* Bartoš, 1963 by Koste (1975) is not included in the checklist. The name is unavailable in the sense of the International Code of Zoological Nomenclature (ICZN, 1999), as it is based solely on the case inhabited by the animal, and not the animal itself.

- Of the remaining species not included in Segers (2007), *Collotheca algicola* (Hudson, 1886) and *Stephanoceros millsii* (Kellicott, 1885) were recently treated as valid by Meksuwan *et al.* (2011), while *Trichocerca inermis* (Linder, 1904) has recently been recognized as a junior synonym of *Trichocerca dixonnutalli* (Jennings, 1903) (Jersabek *et al.*, 2011) and *Trichocerca tenuidens* (Hauer, 1931) remains insufficiently described *species inquirenda* (Segers, 2003)

The majority of Thai Rotifera belongs to family Lecanidae (1 genus with 97 species, 24.2%), Lepadellidae (4 genera with 45 species, 11.2%), Brachionidae (5 genera with 44 species, 11.0%), Trichocercidae (1 genus with 36 species, 9.0%), and Flosculariidae (9 genera with 36 species, 9.0%). The most diverse genus was *Lecane*, comprising 97 species, followed by *Trichocerca* (36 species), *Lepadella* (31 species), and *Brachionus* (30 species).

Regarding the regional distribution of rotifers in the six geographical regions of Thailand (northern, north-eastern, central, western, eastern, and southern) (Setapan, 1999), Thailand has been quite comprehensively investigated. However, studies have focused on four main parts: the northern, the north-eastern, the central, and the southern regions. The number of rotifers on record is highest in the north-eastern part (275 species) (Sanoamuang *et al.*, 1995; Sanoamuang and Savatnalinton, 1999, 2001; Segers *et al.*, 2004; Savatnalinton and Segers, 2005), followed by the southern part (261 species) (Pholpunthin, 1997; Segers and Pholpunthin, 1997; Pholpunthin and Chittapun, 1998; Chittapun *et al.*, 1999; Chittapun and Pholpunthin, 2001; Segers and Chittapun, 2001; Chittapun *et al.*, 2002, 2003, 2005, 2007; Sanoamuang, 2007; Meksuwan *et al.*, 2011), the central part (182 species) (De Ridder, 1971; Koste, 1975; Sanoamuang and Segers, 1997; Jithland and Wongrat, 2006; Teeramaethee *et al.*, 2006; Savatnalinton and Segers, 2008; Chittapun *et al.*, 2009; Chittapun, 2011), and the northern part (115 species) (De Ridder, 1971; Sanoamuang, 1998).

That the number of rotifer species recorded from north-eastern Thailand is the highest of all regions. This result should not automatically be interpreted as indicating that rotifer diversity is the highest there. Admittedly, this region is the largest of all Thai regions, but it is also the most intensively studied, as can be judged from the number and comprehensiveness of the relevant studies (e.g., Sanoamuang *et al.*, 1995). The southern region has the second largest rotifer record, which is attributed to the high diversity and abundance of its freshwater habitats, in addition to intensive study. The record from other Thai regions is much lower, mostly because the number of studies is lower. Nevertheless, when compared to other countries in Southeast Asia, the inventory confirms that Thailand is the best documented of all countries in the region (Segers, 2001).

The Thai rotifer fauna is composed largely of widespread, cosmopolitan or tropicopolitan species, but there is a sizeable fraction of Oriental (13 species, 3.3%) or even local, Thai endemics (13 species, 3.3%; Table 2). It can be expected that the latter number will decrease as research in the countries neighboring. Thailand may reveal the presence of these strict Thai endemics, as is the case with species like *Cephalodella songkhlaensis* Segers and Pholpunthin, 1997 and *Ptygura thalenoensis* Meksuwan *et al.*, 2011.

One of the knowledge gaps of Thai rotifer fauna concerns understudied regions in Thailand. Some taxonomic groups are insufficiently documented. This is the case for taxa such as Notommatidae and bdelloids (respectively 23 and 4 Thai records compared to 48 and 58 from the Oriental region: see Segers, 2008), the two groups, which are notoriously difficult to study because of methodological constraints. Furthermore, the studies in Thailand lack understanding of the ecology and evolution of rotifers, and of their role in ecosystem processes (Wallace *et al.*, 2006). Targeted efforts on understudied regions and taxonomic groups, preferably using modern molecular tools will undoubtedly raise the record of rotifer diversity in Thailand while experimental studies are needed to elucidate their contribution to ecosystem processes and functions.

Table 1. Species list of Rotifera in Thailand.

Legend: N-Northern; NE-Northeastern; C-Central; S-Southern; - no detail about region in publication; 1-De Ridder (1971); 2-Koste (1975); 3-Boonsom (1984); 4-Sanoamuang *et al.* (1995); 5-Pholpunthin (1997); 6-Sanoamuang and Segers (1997); 7-Segers and Pholpunthin (1997); 8-Pholpunthin and Chittapun (1998); 9-Sanoamuang (1998); 10-Chittapun *et al.* (1999); 11-Sanoamuang and Savatentalinton (1999); 12-Chittapun and Pholpunthin (2001); 13-Sanoamuang and Savatentalinton (2001); 14- Segers and Chittapun (2001); 15-Chittapun *et al.* (2002); 16- Chittapun *et al.* (2003); 17- Segers *et al.* (2004); 18-Athibai *et al.* (2005); 19-Chittapun *et al.* (2005); 20- Savatentalinton and Segers (2005); 21-Jithland and Wongrat (2006); 22-Teeramaethee *et al.* (2006); 23-Chittapun *et al.* (2007); 24-Sanoamuang (2007); 25-Savatentalinton and Segers (2008); 26-Chittapun *et al.* (2009); 27-Segers and Savatentalinton (2010); 28-Chittapun (2011); 29-Meksuwan *et al.* (2011) ; 30-Meksuwan *et al.* (2013); 31-Meksuwan *et al.* (2015)

Species	Distribution	References
Monogononta		
Family Asplanchnidae		
1 <i>Asplanchna brightwellii</i> Gosse, 1850	NE, C	1, 3, 4, 13, 17, 20, 21, 22
2 <i>Asplanchna priodonta</i> Gosse, 1850	N, NE, C	3, 4, 9, 13, 17, 20, 21, 22, 24
3 <i>Asplanchna sieboldii</i> (Leydig, 1854)	N, NE, C, S	4, 9, 17, 23, 24, 26, 28
4 <i>Asplanchna tropica</i> Koste & Tobias, 1989	NE, S	4, 5, 7
5 <i>Asplanchnopus hyalinus</i> Harring, 1913	NE	4, 17
6 <i>Asplanchnopus multiceps</i> (Schrank, 1793)	NE	4
7 <i>Harringia rousseleti</i> de Beauchamp, 1912	S	23
Family Atrochidae		
8 <i>Acyclus inquietus</i> Leidy, 1882	S	29
9 <i>Cupelopagis vorax</i> (Leidy, 1857)	C, S	2, 5, 7
Family Brachionidae		
10 <i>Anuraeopsis coelata</i> de Beauchamp, 1932	NE, C, S	4, 5, 7, 17, 18, 20, 21, 22, 23, 24
11 <i>Anuraeopsis fissa</i> Gosse, 1851 Syn.: <i>Anuraeopsis fissa fissa</i> Gosse, 1851: Koste, 1975	N, NE, C, S	1, 2, 3, 4, 5, 7, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26
12 <i>Anuraeopsis navicula</i> Rousselet, 1911	NE, C, S	4, 5, 7, 10, 21, 23, 24, 26
13 <i>Brachionus angularis</i> Gosse, 1851 Incl. <i>Brachionus angularis</i> Gosse f. <i>typica</i> , f. <i>chelonis</i> : Sanoamuang <i>et al.</i> , 1995	N, NE, C, S	1, 3, 4, 5, 7, 9, 10, 13, 17, 18, 20, 21, 22, 23, 24, 25, 26
14 <i>Brachionus angularis bidens</i> Plate, 1886	NE	4
15 <i>Brachionus bennini</i> Leissling, 1924	NE	4
16 <i>Brachionus bidentatus</i> Anderson, 1889	NE, C	4, 13, 17, 21, 24
17 <i>Brachionus budapestinensis</i> Daday, 1885	NE	4, 24
18 <i>Brachionus calyciflorus</i> Pallas, 1766 Syn.: <i>Brachionus calyciflorus calyciflorus</i> Pallas, 1766: Sanoamuang <i>et al.</i> , 1995 Incl. <i>Brachionus calyciflorus</i> f. <i>typica</i> , f. <i>monstruosus</i> , f. <i>amphiceros</i> : Sanoamuang <i>et al.</i> , 1995	N, NE, C, S	1, 3, 4, 5, 7, 9, 13, 17, 18, 20, 21, 22, 23, 24, 26
19 <i>Brachionus caudatus</i> Barrois and Daday, 1894 Incl. <i>Brachionus caudatus</i> f. <i>aculeatus</i> : Sanoamuang <i>et al.</i> , 1995; Teeramaethee <i>et al.</i> , 2006; <i>Brachionus caudatus</i> f. <i>apsteini</i> : Teeramaethee <i>et al.</i> , 2006 <i>Brachionus caudatus</i> f. <i>personatus</i> : Sanoamuang <i>et al.</i> , 1995	N, NE, C, S	1, 3, 4, 5, 7, 9, 17, 20, 21, 22, 24, 26

Table 1. (continued)

	Species	Distribution	References
20	<i>Brachionus dichotomus</i> Shephard, 1911	NE, C, S	5, 7, 20, 21, 23, 24
21	<i>Brachionus dichotomus reductus</i> Koste & Shiel, 1980 Syn.: <i>B. dichotomus</i> Shephard f. <i>reductus</i> Koste & Shiel, 1980: Sanoamuang <i>et al.</i> , 1995; Sanoamuang, 1998; Sanoamuang <i>et al.</i> , 2001; Segers <i>et al.</i> , 2004; Savatentalinton & Segers, 2005; Teeramaethee <i>et al.</i> , 2006; Sanoamuang, 2007	N, NE	4, 9, 13, 17, 18, 22
22	<i>Brachionus diversicornis</i> (Daday, 1883)	N, NE, C	3, 4, 9, 17, 20, 21, 22, 24, 26
23	<i>Brachionus donneri</i> Brehm, 1951	N, NE, C, S	3, 4, 5, 7, 9, 13, 17, 20, 22, 23, 24
24	<i>Brachionus durgae</i> Dhanapathi, 1974	NE	4, 24
25	<i>Brachionus falcatus</i> Zacharias, 1898	N, NE, C, S	1, 3, 4, 5, 7, 9, 10, 13, 17, 18, 20, 21, 22, 23, 24, 26, 28
26	<i>Brachionus forficula</i> Wierzejski, 1891 Incl. <i>Brachionus forficula</i> f. <i>typica</i> , f. <i>reductus</i> : Sanoamuang <i>et al.</i> , 1995	N, NE, C, S	1, 3, 4, 5, 7, 9, 13, 17, 18, 20, 21, 22, 23, 24, 25, 26, 28
27	<i>Brachionus kostei</i> Shiel, 1983	N, NE	4, 9, 17
28	<i>Brachionus leydigii</i> Cohn, 1862	-	3
29	<i>Brachionus lyratus</i> Shephard, 1911	NE, S	4, 17, 23, 24
30	<i>Brachionus murphyi</i> Sudzuki, 1989 Syn.: <i>Brachionus niwati</i> Sanoamuang, Segers & Dumont, 1995: Sanoamuang <i>et al.</i> , 1995; Pholpunthin, 1997; Segers & Pholpunthin, 1997	NE, S	4, 5, 7, 17, 23
31	<i>Brachionus nilsoni</i> Ahlstrom, 1940	C	22
32	<i>Brachionus plicatilis</i> Müller, 1786	NE	3, 4
33	<i>Brachionus quadridentatus</i> Hermann, 1783 Syn.: <i>Brachionus quadridentatus quadridentatus</i> Hermann, 1783: Sanoamuang <i>et al.</i> , 1995; Athibai <i>et al.</i> , 2005 <i>Brachionus quadridentatus</i> f. <i>typica</i> : Sanoamuang <i>et al.</i> , 1995; Incl. <i>Brachionus quadridentatus</i> f. <i>brevispinus</i> : Sanoamuang <i>et al.</i> , 1995; Athibai <i>et al.</i> , 2005; <i>Brachionus</i> <i>quadridentatus</i> f. <i>cluniorbicularis</i> : Athibai <i>et al.</i> , 2005	N, NE, C, S	1, 3, 4, 5, 7, 9, 10, 13, 15, 17, 18, 20, 21, 22, 23, 24, 26
34	<i>Brachionus quadridentatus melhemi</i> Barrois & Daday, 1894 Syn.: <i>B. quadridentatus</i> f. <i>melhemi</i> Barrois & Daday, 1894: Athibai <i>et al.</i> , 2005	-	18
35	<i>Brachionus quadridentatus mirabilis</i> Daday, 1897 Syn.: <i>B. quadridentatus</i> f. <i>mirabilis</i> Daday, 1897: Chittapun & Pholpunthin, 2001; Chittapun <i>et al.</i> , 2002; Athibai <i>et al.</i> , 2005	S	12, 18
36	<i>Brachionus rotundiformis</i> Tschugunoff, 1921	C, S	18, 19, 23, 26
37	<i>Brachionus rubens</i> Ehrenberg, 1838	NE, C, S	4, 5, 7, 10, 17, 18, 20, 24, 26
38	<i>Brachionus sericus</i> Rousselet, 1907	-	18
39	<i>Brachionus sessilis</i> Varga, 1951	NE	4
40	<i>Brachionus srisumona</i> Segers, Kothetip & Sanoamuang, 2004	NE	17
41	<i>Brachionus urceolaris</i> Müller, 1773	NE, C, S	3, 4, 14, 18, 19, 23, 26, 28
42	<i>Brachionus variabilis</i> Hempel, 1896	S	10
43	<i>Keratella cochlearis</i> (Gosse, 1851) Incl. <i>Keratella cochlearis</i> f. <i>typica</i> , f. <i>micracantha</i> : Sanoamuang <i>et al.</i> , 1995	N, NE, C, S	1, 3, 4, 5, 7, 9, 13, 17, 18, 20, 21, 22, 23, 24, 25
44	<i>Keratella edmondsoni</i> Ahlstrom, 1943	NE	4, 13, 17, 18
45	<i>Keratella javana</i> Hauer, 1973	S	12, 15
46	<i>Keratella lenzi</i> Hauer, 1953	N, NE, C, S	2, 4, 5, 7, 9, 13, 17, 18, 20, 21, 22, 24, 26
47	<i>Keratella mixta</i> (Oparina-Charitonova, 1924)	S	15
48	<i>Keratella procurva</i> (Thorpe, 1891)	NE, C	3, 4, 17, 18, 20, 22
49	<i>Keratella taksinensis</i> Chittapun, Pholpunthin & Segers, 2002	S	15

Table 1. (continued)

	Species	Distribution	References
50	<i>Keratella tecta</i> (Gosse, 1851)	NE, C	4, 17, 18, 20, 21, 22
51	<i>Keratella tropica</i> (Apstein, 1907) Syn.: <i>Keratella valga tropica</i> Apstein, 1907: Koste, 1975	N, NE, C, S	1, 2, 3, 4, 5, 7, 9, 10, 13, 17, 18, 20, 21, 22, 23, 24, 25, 26
52	<i>Platyonus patulus</i> (Müller, 1786) Syn.: <i>Brachionus patulus</i> Müller, 1786: Boonsom, 1984 <i>Platyonus patulus</i> (Müller) f. <i>typica</i> : Sanoamuang <i>et al.</i> , 1995	N, NE, C, S	3, 4, 5, 7, 9, 10, 12, 13, 15, 17, 18, 20, 21, 22, 23, 24, 26
53	<i>Platyias quadricornis</i> (Ehrenberg, 1832) Syn.: <i>Platyias quadricornis quadricornis</i> (Ehrenberg, 1832): Chittapun <i>et al.</i> , 2002 Incl. <i>Platyias quadricornis</i> f. <i>brevispinus</i> : Chittapun <i>et al.</i> , 2002	N, NE, C, S	3, 4, 5, 7, 9, 10, 13, 15, 17, 18, 21, 22, 23, 24, 26
Family Conochilidae			
54	<i>Conochilus (Conochiloides) coenobasis</i> (Skorikov, 1914)	NE	4, 13, 20, 24
55	<i>Conochilus (Conochiloides) dossuarius</i> Hudson, 1885	N, NE	4, 9
56	<i>Conochilus (Conochiloides) natans</i> (Seligo, 1900)	NE, S	4, 13, 20, 23, 24
57	<i>Conochilus (Conochilus) hippocrepis</i> (Schrank, 1803)	NE, S	4, 20, 24, 29
58	<i>Conochilus (Conochilus) unicornis</i> Rousselet, 1892	NE	3, 13
Family Collothecidae			
59	<i>Collotheca algicola</i> (Hudson, 1886)	S	29
60	<i>Collotheca ambigua</i> (Hudson, 1883)	S	29
61	<i>Collotheca campanulata</i> (Dobie, 1849) Incl. <i>Collotheca campanulata</i> var. <i>longicaudata</i> : Meksuwan <i>et al.</i> , 2011	NE, S	4, 29
62	<i>Collotheca edentata</i> (Collins, 1872)	C	2
63	<i>Collotheca ferox</i> (Penard, 1914)	S	30
64	<i>Collotheca heptabrachiata</i> (Schoch, 1869)	S	29
65	<i>Collotheca orchidacea</i> Meksuwan, Pholpunthin & Segers, 2013	S	30
66	<i>Collotheca ornata</i> (Ehrenberg, 1832)	S	29
67	<i>Collotheca stephanochaeta</i> Edmondson, 1936	S	29
68	<i>Collotheca tenuilobata</i> (Anderson, 1889)	NE, S	13, 29
69	<i>Collotheca trilobata</i> (Collins, 1872) <i>Collotheca</i> cf. <i>trilobata</i> (Collins, 1872): Teeramaethee <i>et al.</i> , 2006	S	29
70	<i>Stephanoceros fimbriatus</i> (Goldfusz, 1820)	S	29
71	<i>Stephanoceros millsii</i> (Kellicott, 1885)	S	29
Family Dicranophoridae			
72	<i>Aspelta circinator</i> (Gosse, 1886)	NE	13
73	<i>Dicranophoroides caudatus</i> (Ehrenberg, 1834) Syn.: <i>Dicranophorus caudatus</i> (Ehrenberg, 1834): Sanoamuang and Savatentalinton, 2001	N, NE	4, 9, 13, 17, 20
74	<i>Dicranophoroides claviger</i> (Hauer, 1965) Syn.: <i>Dicranophorus claviger</i> (Hauer): Pholpunthin, 1997	NE, S	4, 5, 7, 13, 17, 24
75	<i>Dicranophorus epicharis</i> Haring & Myers, 1928 <i>Dicranophorus</i> cf. <i>epicharis</i> Haring & Myers, 1928: Sanoamuang <i>et al.</i> , 1995	NE, C, S	2, 10, 12, 13, 17, 20, 23, 26
76	<i>Dicranophorus grandis</i> (Ehrenberg, 1832)	N, NE	4, 9, 13
77	<i>Dicranophorus prionacis</i> Haring & Myers, 1928	S	5, 7
78	<i>Dicranophorus robustus</i> Haring & Myers, 1928	-	3
79	<i>Encentrum longidens</i> Donner, 1943	S	14
80	<i>Encentrum pornsilpi</i> Segers & Chittapun, 2001	S	14, 19, 23
Family Epiphanidae			
81	<i>Cyrtonia tuba</i> (Ehrenberg, 1834)	S	5, 7
82	<i>Epiphanes clavulata</i> (Ehrenberg, 1832)	N, NE	4, 9, 17, 20, 24
83	<i>Epiphanes macroura</i> (Barrois & Daday, 1894)	NE	4
84	<i>Proalides subtilis</i> Rodewald, 1940	NE	4
85	<i>Proalides tentaculatus</i> de Beauchamp, 1907	NE	4

Table 1. (continued)

	Species	Distribution	References
	Family Euchlanidae		
86	<i>Beauchampella eudactylota</i> (Gosse, 1886) Syn.: <i>Manfredium eudactylotum</i> (Gosse, 1886); Sanoamuang <i>et al.</i> , 1995; Sanoamuang, 1998; Chittapun <i>et al.</i> , 2002; Segers <i>et al.</i> , 2004	N, NE, S	4, 9, 15, 17
87	<i>Dipleuchlanis propatula</i> (Gosse, 1886) Incl. <i>Dipleuchlanis propatula</i> f. <i>macroactyla</i> : Chittapun <i>et al.</i> , 2002	N, NE, C, S	3, 4, 5, 7, 9, 12, 13, 15, 17, 20, 23, 24, 26
88	<i>Euchlanis dilatata</i> Ehrenberg, 1832	N, NE, C, S	1, 2, 3, 4, 5, 7, 9, 10, 12, 13, 17, 20, 22, 23, 24, 26
89	<i>Euchlanis incisa</i> Carlin, 1939 Incl. <i>Euchlanis incisa</i> f. <i>mucronata</i> : Chittapun <i>et al.</i> , 1999	N, NE, C, S	4, 9, 10, 12, 13, 15, 17, 20, 22, 24
90	<i>Euchlanis lyra</i> Hudson, 1886 Incl. <i>Euchlanis lyra</i> f. <i>myersi</i> : Chittapun <i>et al.</i> , 1999	S	10
91	<i>Euchlanis meneta</i> Myers, 1930	NE, C, S	2, 10, 13
92	<i>Euchlanis triquetra</i> Ehrenberg, 1838	C	2
93	<i>Tripleuchlanis plicata</i> (Levander, 1894)	N, NE, C	1, 3, 4, 9, 13, 17, 21, 22
	Family Flosculariidae		
94	<i>Beauchampia crucigera</i> (Dutrochet, 1812) Syn.: <i>Beauchampia crucigera crucigera</i> (Dutrochet, 1812): Koste, 1975	C, S	2, 29
95	<i>Floscularia armata</i> Segers, 1997	S	29
96	<i>Floscularia bifida</i> Segers, 1997	S	29
97	<i>Floscularia conifera</i> (Hudson, 1886)	NE, C, S	2, 12, 13, 17, 19, 23, 29
98	<i>Floscularia decora</i> Edmondson, 1940	C	2
99	<i>Floscularia melicerta</i> (Ehrenberg, 1832)	C	2
100	<i>Floscularia pedunculata</i> (Joliet, 1883)	S	29
101	<i>Floscularia ringens</i> (Linnaeus, 1758)	NE, C, S	2, 13, 29
102	<i>Floscularia wallacei</i> Segers & Shiel, 2008	S	29
103	<i>Lacinularia flosculosa</i> (Müller, 1773)	S	29
104	<i>Lacinularoides coloniensis</i> (Colledge, 1918)	S	29
105	<i>Limnias ceratophylli</i> Schrank, 1803	C, S	2, 29
106	<i>Limnias melicerta</i> Weisse, 1848	C, S	2, 14, 29
107	<i>Octotrocha speciosa</i> Thorpe, 1893	S	29
108	<i>Pentatrocha gigantea</i> Segers & Shiel, 2008	S	29
109	<i>Ptygura agassizi</i> Edmondson, 1948	S	29
110	<i>Ptygura barbata</i> Edmondson, 1939	S	29
111	<i>Ptygura beauchampi</i> Edmondson, 1940	S	29
112	<i>Ptygura brachiata</i> (Hudson, 1886)	C	2
113	<i>Ptygura crystallina</i> (Ehrenberg, 1834)	S	29
114	<i>Ptygura ctenoida</i> Koste & Tobias, 1990	S	29
115	<i>Ptygura elsteri</i> Koste, 1972 Syn.: <i>Ptygura elsteri elsteri</i> Koste, 1972: Koste, 1975	C, S	2, 29
116	<i>Ptygura furcillata</i> (Kellicott, 1889) Syn.: <i>Ptygura furcillata furcillata</i> (Kellicott, 1889): Koste, 1975 Incl.: <i>Ptygura furcillata</i> f. <i>variabilis</i> : Koste, 1975	NE, C, S	2, 13, 29
117	<i>Ptygura kostei</i> José de Paggi, 1996 Syn.: <i>Ptygura elsteri</i> f. <i>thailandis</i> Koste, 1975: Sanoamuang and Savatentalinton, 2001	NE	13
118	<i>Ptygura longicornis</i> (Davis, 1867)	S	29
119	<i>Ptygura melicerta</i> Ehrenberg, 1832	NE, C	2, 13
120	<i>Ptygura mucicola</i> (Kellicott, 1888)	C, S	2, 29
121	<i>Ptygura noodti</i> (Koste, 1972)	S	29
122	<i>Ptygura pedunculata</i> Edmondson, 1939	S	29
123	<i>Ptygura tacita</i> Edmondson, 1940	NE, S	13, 29
124	<i>Ptygura thalenoensis</i> Meksuwan, Pholpunthin & Segers, 2011	S	29
125	<i>Ptygura wilsonii</i> (Anderson & Shephard, 1892)	S	29

Table 1. (continued)

	Species	Distribution	References
126	<i>Sinantherina ariprepres</i> Edmondson, 1939	NE, C	13, 20, 22
127	<i>Sinantherina semibullata</i> (Thorpe, 1893)	N, NE, S	4, 9, 20, 29
128	<i>Sinantherina socialis</i> (Linnaeus, 1758)	C, S	2, 29
129	<i>Sinantherina spinosa</i> (Thorpe, 1893)	NE, C, S	4, 13, 17, 20, 22, 24, 26, 29
	Family Gastropodidae		
130	<i>Ascomorpha agilis</i> Zacharias, 1893	C	21
131	<i>Ascomorpha ecaudis</i> Perty, 1850	N, NE, C, S	4, 5, 7, 9, 20, 21
132	<i>Ascomorpha ovalis</i> (Bergendal, 1892)	NE, C, S	3, 4, 7, 13, 17, 20, 21, 22, 23, 24
133	<i>Ascomorpha saltans</i> Bartsch, 1870	NE, C, S	3, 4, 5, 7, 13, 17, 20, 21, 22
134	<i>Gastropus hyptopus</i> (Ehrenberg, 1838)	NE	4
	Family Hexarthridae		
135	<i>Hexarthra fennica</i> (Levander, 1892)	NE	4
136	<i>Hexarthra intermedia</i> (Wiszniewski, 1929)	N, NE, C, S	4, 5, 7, 9, 13, 17, 20, 21, 22, 24
137	<i>Hexarthra intermedia brasiliensis</i> (Hauer, 1953)	S	31
138	<i>Hexarthra mira</i> (Hudson, 1871)	N, NE, C, S	4, 5, 7, 9, 13, 17, 19, 20, 22, 23, 24
139	<i>Hexarthra oxyuris</i> (Sernov, 1903)	NE	4
	Family Ituridae		
140	<i>Itura aurita</i> (Ehrenberg, 1830)	NE	4
141	<i>Itura symmetrica</i> Segers, Mbogo & Dumont, 1994	NE	4
	Family Lecanidae		
142	<i>Lecane abanica</i> Segers, 1994	S	12, 23, 27
143	<i>Lecane acanthinula</i> (Hauer, 1938)	NE, S	4, 6, 14, 23, 27
144	<i>Lecane aculeata</i> (Jakubski, 1912)	N, NE, C, S	2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 27, 28
145	<i>Lecane acus</i> (Harring, 1913)	C	1, 3
146	<i>Lecane aeganea</i> Harring, 1914	N, NE, C	3, 4, 9, 21, 27
147	<i>Lecane agilis</i> (Bryce, 1892)	C	25, 27
148	<i>Lecane arcuata</i> (Bryce, 1891)	NE, C, S	1, 2, 3, 4, 10, 12, 25, 27
149	<i>Lecane arcua</i> Harring, 1914 Syn.: <i>Lecane strandi</i> Berzins, 1943: Jithlang & Wongrat, 2006	N, NE, C, S	4, 5, 7, 8, 9, 10, 13, 15, 17, 20, 22, 23, 24, 27
150	<i>Lecane armata</i> Thomasson, 1971	-	27
151	<i>Lecane aspasia</i> Myers, 1917	N, NE	4, 9, 17, 27
152	<i>Lecane baimaii</i> Sanoamuang & Savatentalinton, 1999	NE	11, 17, 27
153	<i>Lecane batillifer</i> (Murray, 1913)	NE, C, S	4, 5, 7, 17, 20, 22, 23, 27
154	<i>Lecane bifastigata</i> Hauer, 1938	NE, C	4, 6, 22, 27
155	<i>Lecane bifurca</i> (Bryce, 1892)	NE, S	5, 7, 10, 19, 20, 23, 24, 27
156	<i>Lecane blachei</i> Bērziņš, 1973	N, NE, C, S	4, 6, 8, 9, 13, 17, 20, 27
157	<i>Lecane braumi</i> Koste, 1988	NE, S	12, 13, 15, 27
158	<i>Lecane bulla</i> (Gosse, 1851) Syn.: <i>Lecane bulla bulla</i> (Gosse, 1851): Segers & Savatentalinton, 2010	N, NE, C, S	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 26, 27, 28
159	<i>Lecane bulla diabolica</i> (Hauer, 1936)	-	27
160	<i>Lecane calcaria</i> Harring & Myers, 1926	NE, S	7, 12, 17, 27
161	<i>Lecane chinesensis</i> Zhuge & Koste, 1996	-	27
162	<i>Lecane clara</i> (Bryce, 1892)	NE, S	5, 7, 12, 20, 27
163	<i>Lecane closteroerca</i> (Schmarda, 1859)	N, NE, C, S	1, 2, 3, 4, 5, 7, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26, 27
164	<i>Lecane cornuta</i> (Müller, 1786)	C	1, 2, 3
165	<i>Lecane crenata</i> (Harring, 1913)	C	2, 27
166	<i>Lecane crepida</i> Harring, 1914	N, NE, C, S	2, 4, 5, 7, 9, 10, 12, 13, 17, 20, 22, 23, 24, 27
167	<i>Lecane curvicornis</i> (Murray, 1913) Incl. <i>Lecane curvicornis</i> f. <i>typica</i> : Sanoamuang <i>et al.</i> , 1995	N, NE, C, S	1, 3, 4, 5, 7, 8, 9, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26, 27, 28
168	<i>Lecane decipiens</i> (Murray, 1913)	NE, S	10, 11, 12, 13, 17, 20, 24, 27
169	<i>Lecane donneri</i> Chengalath & Mulamoottil, 1974	NE	11, 17, 24, 27

Table 1. (continued)

	Species	Distribution	References
170	<i>Lecane doryssa</i> Harring, 1914 <i>Lecane cf. doryssa</i> Harring, 1914: Sanoamuang and Savatentalinton, 2001	N, NE, S	4, 9, 12, 13, 15, 17, 20, 23, 24, 27
171	<i>Lecane elegans</i> Harring, 1914	N, NE, C	2, 9, 13, 17, 20, 24, 26, 27, 28
172	<i>Lecane enowi</i> Segers and Mertens, 1997	S	15, 27
173	<i>Lecane eswari</i> Dhanapathi, 1976	NE	6, 17, 27
174	<i>Lecane flexilis</i> (Gosse, 1886)	NE, C, S	4, 10, 13, 17, 20, 22, 23, 27
175	<i>Lecane furcata</i> (Murray, 1913)	N, NE, C, S	2, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 27
176	<i>Lecane galeata</i> (Bryce, 1892) Syn.: <i>Lecane (Monostyla) pygmaea</i> Daday, 1897: Koste, 1975	C	2
177	<i>Lecane grandis</i> (Murray, 1913)	NE, S	10, 11, 23, 27
178	<i>Lecane haliclysta</i> Harring & Myers, 1926	N, NE, C, S	4, 9, 12, 13, 17, 20, 22, 23, 24, 27
179	<i>Lecane hamata</i> (Stokes, 1896)	N, NE, C, S	2, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 17, 20, 21, 22, 23, 24, 26, 27
180	<i>Lecane hastata</i> (Murray, 1913)	N, NE, C, S	3, 4, 9, 17, 20, 21, 22, 23, 24, 27
181	<i>Lecane hornemanni</i> (Ehrenberg, 1834)	N, NE, C, S	4, 5, 7, 8, 9, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26, 27
182	<i>Lecane inermis</i> (Bryce, 1892)	NE, C, S	2, 4, 5, 7, 10, 12, 15, 19, 20, 23, 26, 27
183	<i>Lecane inopinata</i> Harring & Myers, 1926	N, NE, C, S	3, 4, 9, 10, 12, 13, 17, 20, 21, 22, 24, 27, 28
184	<i>Lecane intrasinuata</i> (Olofsson, 1917)	C	2
185	<i>Lecane isanensis</i> Sanoamuang & Savatentalinton, 2001	NE	13, 27
186	<i>Lecane junki</i> Koste, 1975	C, S	2, 6, 16, 27
187	<i>Lecane kunthuleensis</i> Chittapun, Pholpunthin & Segers, 2003	S	16, 27
188	<i>Lecane lamellata</i> (Daday, 1893)	-	3
189	<i>Lecane lateralis</i> Sharma, 1978	N, NE, C, S	4, 5, 7, 8, 9, 10, 13, 15, 17, 20, 23, 24, 27, 26, 28
190	<i>Lecane latissima</i> Yamamoto, 1955 Syn.: <i>Lecane thailandensis</i> Segers & Sanoamuang, 1994; Sanoamuang <i>et al.</i> , 1995; Sanoamuang and Segers, 1997; Pholpunthin & Chittapun, 1998; Sanoamuang & Savatentalinton, 2001; Segers <i>et al.</i> , 2004; Savatentalinton & Segers, 2005	NE, S	4, 6, 8, 13, 17, 20, 27
191	<i>Lecane lauterborni</i> Hauer, 1924	-	3, 27
192	<i>Lecane leontina</i> (Turner, 1892)	N, NE, C, S	2, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26, 27
193	<i>Lecane ludwigii</i> (Eckstein, 1883)	N, NE, C, S	2, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 19, 20, 22, 23, 24, 27
194	<i>Lecane luna</i> (Müller, 1776)	N, NE, C, S	1, 3, 4, 5, 7, 8, 9, 10, 13, 17, 20, 21, 22, 23, 24, 26, 27, 28
195	<i>Lecane lunaris</i> (Ehrenberg, 1832)	N, NE, C, S	2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 25, 27
196	<i>Lecane lungae</i> Savatentalinton & Segers, 2005	NE	20, 27
197	<i>Lecane martensi</i> Savatentalinton & Segers, 2008	C	25, 27
198	<i>Lecane micrognatha</i> Segers & Savatentalinton, 2010	-	27
199	<i>Lecane minuta</i> Segers, 1994	S	5, 6, 7, 27
200	<i>Lecane mitis</i> Harring & Myers, 1926	S	12, 27
201	<i>Lecane monostyla</i> (Daday, 1897)	NE, C, S	3, 4, 10, 12, 15, 17, 22, 23, 24, 27
202	<i>Lecane nana</i> (Murray, 1913)	NE, C, S	1, 3, 4, 5, 7, 13, 17, 20, 27, 28
203	<i>Lecane nelsoni</i> Segers, 1994	NE	13, 27
204	<i>Lecane nitida</i> (Murray, 1913) Syn.: <i>Lecane curvicornis</i> (Murray) f. <i>nitida</i> (Murray): Sanoamuang <i>et al.</i> , 1995	NE	4, 27
205	<i>Lecane niwati</i> Segers, Kothetip & Sanoamuang, 2004	NE	17, 27

Table 1. (continued)

	Species	Distribution	References
206	<i>Lecane obtusa</i> (Murray, 1913)	N, NE, C, S	4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 17, 19, 20, 22, 23, 24, 27
207	<i>Lecane opias</i> (Harring & Myers, 1926)	NE	20, 27
208	<i>Lecane palinacis</i> Harring & Myers, 1926	C, S	12, 23, 26, 27, 28
209	<i>Lecane papuana</i> (Murray, 1913)	N, NE, C, S	1, 3, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 20, 21, 22, 24, 26, 27, 28
210	<i>Lecane paxiana</i> Hauer, 1940	NE, C	20, 25, 27
211	<i>Lecane pertica</i> Harring & Myers, 1926	N, NE, S	5, 7, 9, 12, 13, 15, 17, 27
212	<i>Lecane punctata</i> (Murray, 1913) Syn: <i>Lecane harringi</i> Ahlstrom, 1934: Boonsom, 1984; Jithlang and Wongrat, 2006	NE, C	3, 11, 20, 21, 22, 27
213	<i>Lecane pusilla</i> Harring, 1914	N, NE, S	4, 9, 12, 13, 17, 20, 27
214	<i>Lecane pyriformis</i> (Daday, 1905)	NE, C, S	2, 4, 10, 12, 13, 14, 15, 17, 20, 22, 23, 24, 26, 27
215	<i>Lecane quadridentata</i> (Ehrenberg, 1830)	N, NE, C, S	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 17, 22, 23, 24, 26, 27
216	<i>Lecane rhenana</i> Hauer, 1929	N, NE, C, S	4, 5, 7, 8, 9, 13, 17, 20, 22, 23, 24, 26, 27
217	<i>Lecane rhytida</i> Harring & Myers, 1926	N, NE, C, S	5, 7, 9, 10, 12, 14, 17, 22, 23, 27
218	<i>Lecane robertsonae</i> Segers, 1993	NE, S	17, 20, 23, 27
219	<i>Lecane rutneri</i> Hauer, 1938	NE	4, 13, 17, 20, 24, 27
220	<i>Lecane segersi</i> Sanoamuang, 1996	NE, C, S	6, 14, 17, 20, 23, 26, 27
221	<i>Lecane serrata</i> (Hauer, 1938)	NE	11, 17, 27
222	<i>Lecane shieli</i> Segers & Sanoamuang, 1994	NE, S	4, 6, 10, 13, 17, 20, 27
223	<i>Lecane signifera</i> (Jennings, 1896) f. <i>ploenensis</i> (Voigt, 1902)	N, NE, C, S	2, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26, 27
224	<i>Lecane simonneae</i> Segers, 1993	NE, S	12, 13, 15, 17, 27
225	<i>Lecane sola</i> Hauer, 1936	NE	3, 4, 17, 20, 24, 27
226	<i>Lecane stenroosi</i> (Meissner, 1908)	N, NE, C, S	2, 4, 8, 9, 10, 17, 20, 21, 22, 26, 27
227	<i>Lecane stichaea</i> Harring, 1913	C	2, 21, 27
228	<i>Lecane stichoclysta</i> Segers, 1993	NE	20, 27
229	<i>Lecane subtilis</i> Harring & Myers, 1926	NE, S	17, 23, 27
230	<i>Lecane superaculeata</i> Sanoamuang & Segers, 1997	N, NE, C, S	6, 9, 15, 17, 23, 27
231	<i>Lecane sympoda</i> Hauer, 1929	S	5, 7, 27
232	<i>Lecane syngenes</i> (Hauer, 1938)	S	12, 27
233	<i>Lecane tenuiseta</i> Harring, 1914	NE, C, S	2, 4, 5, 7, 8, 10, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 27, 28
234	<i>Lecane thalera</i> (Harring & Myers, 1926)	NE, C	4, 22, 27
235	<i>Lecane thienemanni</i> (Hauer, 1938)	NE, C, S	11, 12, 17, 20, 21, 22, 23, 24, 26, 27
236	<i>Lecane undulata</i> Hauer, 1938	N, NE, C, S	4, 5, 7, 9, 12, 13, 17, 20, 23, 24, 27, 28
237	<i>Lecane unguitata</i> (Fadeev, 1926)	N, NE, C, S	4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 19, 20, 22, 23, 24, 26, 27
238	<i>Lecane ungulata</i> (Gosse, 1887)	N, NE, C, S	2, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26, 27
	Family Lepadellidae		
239	<i>Colurella adriatica</i> Ehrenberg, 1831	NE, C, S	1, 3, 4, 12, 13, 20, 23, 24, 25
240	<i>Colurella colurus</i> (Ehrenberg, 1830)	N, NE, C, S	2, 4, 9, 10, 12, 15, 23, 26
241	<i>Colurella colurus compressa</i> (Lucks, 1912)	S	14
242	<i>Colurella hindenburgi</i> Steinecke, 1917	C	21
243	<i>Colurella obtusa</i> (Gosse, 1886)	NE, C, S	2, 4, 5, 7, 10, 12, 13, 14, 15, 17, 20, 21, 23, 24
244	<i>Colurella psammophila</i> Segers & Chittapun, 2001	S	14, 23
245	<i>Colurella sanoamuangae</i> Chittapun, Pholpunthin & Segers, 1999	C, S	10, 14, 23, 26
246	<i>Colurella sulcata</i> (Stenroos, 1898)	NE, S	12, 13, 20, 23
247	<i>Colurella tessellata</i> (Glascott, 1893)	S	12, 23
248	<i>Colurella uncinata</i> (Müller, 1773)	N, NE, C, S	5, 7, 9, 12, 13, 15, 17, 20, 22, 23, 24, 26

Table 1. (continued)

	Species	Distribution	References
249	<i>Colurella uncinata bicuspidata</i> (Ehrenberg, 1832) Syn.: <i>Colurella uncinata</i> (Müller) f. <i>bicuspidata</i> (Ehrenberg): Sanoamuang <i>et al.</i> , 1995; Chittapun <i>et al.</i> , 2002	NE	4
250	<i>Lepadella (Lepadella) acuminata</i> (Ehrenberg, 1834)	NE, C, S	1, 3, 4, 10, 13, 17, 20, 21, 23, 24, 26
251	<i>Lepadella (Lepadella) akrobeles</i> Myers, 1934	NE	11, 17
252	<i>Lepadella (Lepadella) amphitropis</i> Harring, 1916	NE	20
253	<i>Lepadella (Lepadella) apside</i> Harring, 1916	NE, S	4, 5, 7, 10, 13, 23
254	<i>Lepadella (Lepadella) benjamini</i> Harring, 1916	NE	13, 17
255	<i>Lepadella (Lepadella) biloba</i> Hauer, 1958	NE, S	4, 5, 7, 13, 17
256	<i>Lepadella (Lepadella) costatoides</i> Segers, 1992	N, NE, C, S	4, 9, 13, 17, 20, 22, 23, 24
257	<i>Lepadella (Lepadella) cristata</i> (Rousselet, 1893)	NE, S	12, 13
258	<i>Lepadella (Lepadella) dactyliseta</i> (Stenroos, 1898)	NE, S	4, 5, 7, 12, 24
259	<i>Lepadella (Lepadella) desmeti</i> Segers & Chittapun, 2001	S	14, 23
260	<i>Lepadella (Lepadella) discoidea</i> Segers, 1993	N, NE, C, S	4, 9, 10, 12, 13, 15, 17, 22, 24
261	<i>Lepadella (Lepadella) elliptica</i> Wulfert, 1939	NE	3, 20
262	<i>Lepadella (Lepadella) elongata</i> Koste, 1992 <i>Lepadella</i> cf. <i>elongata</i> Koste, 1992: Sanoamuang & Savatenalinton, 2001	NE	11, 17, 20
263	<i>Lepadella (Lepadella) eurysterna</i> Myers, 1942	NE, S	13, 17, 23
264	<i>Lepadella (Lepadella) latusinus</i> (Hilgendorf, 1899)	N, NE, C, S	2, 4, 5, 7, 9, 13, 23
265	<i>Lepadella (Lepadella) lindau</i> Koste, 1981	NE, C, S	5, 7, 13, 17, 20, 28
266	<i>Lepadella (Lepadella) minoruoides</i> Koste & Robertson, 1983	S	5, 7, 23
267	<i>Lepadella (Lepadella) minuta</i> (Weber & Montet, 1918)	C	25
268	<i>Lepadella (Lepadella) ovalis</i> (Müller, 1786)	N, NE, C, S	2, 3, 4, 5, 7, 9, 10, 12, 13, 17, 20, 22, 23, 24, 26
269	<i>Lepadella (Lepadella) patella</i> (Müller, 1773)	N, NE, C, S	2, 3, 4, 5, 7, 9, 10, 12, 13, 15, 17, 20, 22, 23, 24, 26
270	<i>Lepadella (Lepadella) punctata</i> Wulfert, 1939	S	16
271	<i>Lepadella (Lepadella) quadricarinata</i> (Stenroos, 1898)	N, NE, S	4, 5, 7, 9, 10, 12, 13, 17
272	<i>Lepadella (Lepadella) quinquecostata</i> (Lucks, 1912)	N, NE	9, 13, 17
273	<i>Lepadella (Lepadella) rhomboides</i> (Gosse, 1886)	N, NE, C, S	4, 5, 7, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26
274	<i>Lepadella (Lepadella) triba</i> Myers, 1934	NE, C, S	2, 5, 7, 10, 13, 17, 20, 23, 24
275	<i>Lepadella (Lepadella) triptera</i> (Ehrenberg, 1832) Incl. <i>Lepadella triptera</i> f. <i>alata</i> ; Sanoamuang and Savatenalinton, 2001; Segers, 2004 <i>Lepadella (Lepadella) vandenbrandei</i> Gillard, 1952	NE, S N, NE, S	4, 13, 17, 20, 23 4, 5, 7, 9, 12, 13, 15, 17, 20, 23, 24
276	<i>Lepadella (Heterolepadella) apsicora</i> Myers, 1834	NE, S	4, 5, 7, 13, 15, 17, 20, 23, 24
277	<i>Lepadella (Heterolepadella) ehrenbergii</i> (Perty, 1850)	N, NE, C, S	4, 9, 13, 15, 17, 20, 22, 23
278	<i>Lepadella (Heterolepadella) heterostyla</i> (Murray, 1913)	NE, S	5, 7, 17, 20
279	<i>Lepadella (Xenolepadella) monodactyla</i> Berzins, 1960 Syn.: <i>Lepadella monostyla</i> f. <i>caudata</i> (Koste, 1972)	NE, C, S	2, 13, 15, 23
280	<i>Paracolarella aemula</i> (Myers, 1934)	S	16
281	<i>Squatinella lamellaris</i> (Müller, 1786) Syn.: <i>Squatinella mutica</i> (Ehrenberg): Chittapun & Pholpunthin, 2001; Chittapun <i>et al.</i> , 2007 Syn.: <i>Squatinella lamellaris</i> (Müller) f. <i>mutica</i> (Ehrenberg): Sanoamuang <i>et al.</i> , 1995; Sanoamuang & Savatenalinton, 2001; Segers <i>et al.</i> , 2004	NE, S	4, 5, 7, 13, 17, 23
282	<i>Squatinella leydigii</i> (Zacharias, 1886) Incl. <i>Squatinella leydigii</i> f. <i>longiseta</i> : Chittapun <i>et al.</i> , 1999; Chittapun <i>et al.</i> , 2002	S	10, 15

Table 1. (continued)

Species	Distribution	References
Family Lindiidae		
283 <i>Lindia (Lindia) torulosa</i> Dujardin, 1841	S	19
Family Mytilinidae		
284 <i>Lophocharis salpina</i> (Ehrenberg, 1834)	N, NE, C	4, 9, 13, 17, 20, 24, 26
285 <i>Mytilina acanthophora</i> Hauer, 1938	N, NE	4, 9, 13, 17
286 <i>Mytilina bisulcata</i> (Lucks, 1912)	N, NE, C	3, 9, 13, 17, 22, 24, 26
287 <i>Mytilina compressa</i> (Gosse, 1851)	NE, S	4, 5, 7
288 <i>Mytilina crassipes</i> (Lucks, 1912)	C	21
289 <i>Mytilina michelangellii</i> Reid & Turner, 1988 Syn.: <i>Mytilina ventralis</i> f. <i>diversicantha</i> Wulfert, 1965; Jithlang & Wongrat, 2006	C	21
290 <i>Mytilina mucronata</i> (Müller, 1773)	C	21
291 <i>Mytilina unguipes</i> (Lucks, 1912)	N, NE, C	4, 9, 13, 17, 22, 26
292 <i>Mytilina ventralis</i> (Ehrenberg, 1830)	N, NE, C, S	3, 4, 9, 10, 12, 13, 17, 20, 21, 22, 23, 24, 26
293 <i>Mytilina ventralis brevispina</i> (Ehrenberg, 1830)	C	21
Family Notommatidae		
294 <i>Cephalodella forficula</i> (Ehrenberg, 1830) Syn.: <i>Cephalodella forficula forficula</i> (Ehrenberg, 1830): Koste, 1975	NE, C, S	2, 4, 13, 20, 23, 24
295 <i>Cephalodella gibba</i> (Ehrenberg, 1830)	NE, C, S	2, 4, 5, 7, 13, 15, 17, 19, 20, 23, 28
296 <i>Cephalodella</i> cf. <i>hyalina</i> Myers, 1924	S	10
297 <i>Cephalodella innesi</i> Myers, 1924	C, S	10, 14, 15, 19, 23, 28
298 <i>Cephalodella megalocephala</i> (Glascott, 1893)	S	14
299 <i>Cephalodella mucronata</i> Myers, 1924	NE, C, S	12, 13, 22
300 <i>Cephalodella</i> cf. <i>pachyodon</i> Wulfert, 1937	NE	4
301 <i>Cephalodella plicata</i> Myers, 1924	S	14
302 <i>Cephalodella songkhlaensis</i> Segers & Pholpunthin, 1997	NE, S	7, 20
303 <i>Cephalodella tenuior</i> (Gosse, 1886)	NE, S	13, 23
304 <i>Cephalodella tenuiseta</i> (Burn, 1890)	C	28
305 <i>Cephalodella ventripes</i> (Dixon-Nuttall, 1901)	NE	4
306 <i>Eosphora</i> cf. <i>thoides</i> Wulfert, 1935	NE	4
307 <i>Monommata actices</i> Myers, 1930	NE, S	5, 7, 17
308 <i>Monommata dentata</i> Wulfert, 1940	S	15, 23
309 <i>Monommata grandis</i> Tessin, 1890	S	10, 12, 15, 23
310 <i>Monommata longiseta</i> (Müller, 1786)	S	10, 12, 15
311 <i>Monommata maculata</i> Harring & Myers, 1930	S	12
312 <i>Notommata copeus</i> Ehrenberg, 1834	NE, S	4, 5, 7, 13, 23
313 <i>Notommata pachyura</i> (Gosse, 1886) Incl. <i>Notommata pachyura</i> f. <i>spinosa</i> : Chittapun & Pholpunthin, 2001	N, NE, S	4, 9, 12, 13, 17, 20
314 <i>Notommata pseudocerberus</i> de Beauchamp, 1908	S	5, 7
315 <i>Notommata pygmaea</i> Harring & Myers, 1922	S	10
316 <i>Notommata saccigera</i> Ehrenberg, 1830	S	12, 15, 23
317 <i>Taphrocampa annulosa</i> Gosse, 1851	S	10, 23
Family Scaridiidae		
318 <i>Scaridium bostjani</i> Daems & Dumont, 1974	N, NE, S	4, 5, 7, 9, 23
319 <i>Scaridium elegans</i> Segers & De Meester, 1994	NE, S	11, 12, 13
320 <i>Scaridium grande</i> Segers, 1995	N, NE, S	9, 12, 13, 15
321 <i>Scaridium longicaudum</i> (Müller, 1786)	N, NE, C, S	4, 5, 7, 9, 10, 12, 13, 15, 17, 20, 22, 23, 24
Family Synchaetidae		
322 <i>Ploesoma lenticulare</i> Herrick, 1885	NE, C	13, 22
323 <i>Ploesoma hudsoni</i> (Imhof, 1891)	NE, C	4, 13, 17, 20, 22
324 <i>Polyarthra euryptera</i> Wierzejski, 1891	-	3
325 <i>Polyarthra longiremis</i> Carlin, 1943	NE	4
326 <i>Polyarthra major</i> Burckhardt, 1900	N, NE	4, 9
327 <i>Polyarthra minor</i> Voigt, 1904	S	5, 7, 10, 15

Table 1. (continued)

	Species	Distribution	References
328	<i>Polyarthra remata</i> Skorikov, 1896	S	5, 7
329	<i>Polyarthra vulgaris</i> Carlin, 1943 <i>Polyarthra</i> cf. <i>vulgaris</i> Carlin, 1943: Sanoamuang, 2007	N, NE, C, S	1, 3, 4, 5, 7, 9, 10, 12, 13, 17, 20, 21, 22, 23, 26
330	<i>Synchaeta longipes</i> Gosse, 1887	NE	4
331	<i>Synchaeta pectinata</i> Ehrenberg, 1832	N, NE	4, 9, 17
332	<i>Synchaeta stylata</i> Wierzejski, 1893	NE, C	4, 13, 17, 20, 21
	Family Tetrasiphonidae		
333	<i>Tetrasiphon hydrocora</i> Ehrenberg, 1840	S	12
	Family Testudinellidae		
334	<i>Pompholyx complanata</i> Gosse, 1851	N, NE, C	1, 3, 4, 9, 17, 20, 21, 22, 24, 26
335	<i>Testudinella ahlstromi</i> Hauer, 1956 Syn.: <i>Testudinella incisa ahlstromi</i> (Hauer): Chittapun & Pholpunthin, 2001	N, NE, S	9, 12, 13, 17, 20, 24
336	<i>Testudinella amphora</i> Hauer, 1938	NE, S	12, 13, 17, 23
337	<i>Testudinella brevicaudata</i> Yamamoto, 1951	N, NE, S	4, 5, 7, 9, 13, 17, 24
338	<i>Testudinella dendradenade</i> Beauchamp, 1955	S	31
339	<i>Testudinella emarginula</i> (Stenroos, 1898)	NE, S	4, 17, 23
340	<i>Testudinella greeni</i> Koste, 1981	N, NE	9, 13, 17, 24
341	<i>Testudinella mucronata</i> (Gosse, 1886)	S	12
342	<i>Testudinella parva</i> (Ternetz, 1892) Syn.: <i>Testudinella insinuata</i> Hauer, 1938: Segers <i>et al.</i> , 2004; <i>T.</i> cf. <i>insinuata</i> Hauer, 1938: Sanoamuang and Savatenalinton, 2001	NE, C, S	4, 12, 13, 15, 17, 20, 21, 24
343	<i>Testudinella patina</i> (Hermann, 1783) Syn.: <i>Testudinella patina</i> (Hermann) f. <i>typica</i> : Sanoamuang <i>et al.</i> , 1995 Incl.: <i>Testudinella patina</i> f. <i>intermedia</i> : Sanoamuang <i>et al.</i> , 1995; Jithlang & Wongrat, 2006	N, NE, C, S	3, 4, 5, 7, 9, 10, 12, 13, 15, 17, 20, 21, 22, 23, 24, 26
344	<i>Testudinella tridentata</i> Smirnov, 1931	N, NE, C, S	4, 9, 12, 13, 15, 17, 20, 24, 26
345	<i>Testudinella walkeri</i> Koste & Shiel, 1980	NE, S	5, 7, 13, 17, 20
	Family Trichocercidae		
346	<i>Trichocerca abilioi</i> Segers & Sarma, 1993	NE	13, 17
347	<i>Trichocerca bicristata</i> (Gosse, 1887)	N, NE, C	3, 4, 9, 13, 17, 20, 22, 24
348	<i>Trichocerca bidens</i> (Lucks, 1912)	NE, C, S	4, 13, 17, 20, 22, 23, 24
349	<i>Trichocerca braziliensis</i> (Murray, 1913)	N, NE, C, S	4, 9, 10, 12, 13, 15, 17, 20, 22, 23, 24, 26
350	<i>Trichocerca capucina</i> (Wierzejski & Zacharias, 1893)	N, NE, C, S	4, 9, 13, 17, 20, 21, 22, 23, 24
351	<i>Trichocerca chattoni</i> (de Beauchamp, 1907)	NE, C, S	4, 17, 20, 21, 23, 24
352	<i>Trichocerca collaris</i> (Rousselet, 1896)	NE, S	12, 13
353	<i>Trichocerca cylindrica</i> (Imhof, 1891)	NE, C, S	3, 4, 5, 7, 13, 17, 20, 21, 22, 24
354	<i>Trichocerca dixonnutalli</i> (Jennings, 1903) Syn.: <i>Trichocerca inermis</i> (Linder, 1904): Sanoamuang and Savatenalinton, 2001; Sanoamuang, 2007	NE, S	7, 13, 24
355	<i>Trichocerca elongata</i> (Gosse, 1886)	NE, C	4, 13, 17, 21
356	<i>Trichocerca flagellata</i> Hauer, 1937	N, NE, C, S	4, 5, 7, 9, 12, 13, 17, 22, 23, 24
357	<i>Trichocerca</i> cf. <i>gracilis</i> (Tessin, 1890)	S	23
358	<i>Trichocerca hollaerti</i> De Smet, 1990	NE, S	4, 5, 7, 12, 13, 17, 23, 24
359	<i>Trichocerca insignis</i> (Herrick, 1885)	N, NE, S	4, 5, 7, 9, 12, 13, 17, 20, 24
360	<i>Trichocerca insulana</i> (Hauer, 1937) Syn.: <i>Trichocerca montana</i> Hauer, 1956: Sanoamuang & Savatenalinton, 2001	NE, C, S	4, 10, 13, 20, 23, 24, 26, 28

Table 1. (continued)

	Species	Distribution	References
361	<i>Trichocerca longiseta</i> (Schrank, 1802)	NE, C	13, 17, 20, 21, 24
362	<i>Trichocerca mus</i> Hauer, 1938	C, S	22, 23
363	<i>Trichocerca orca</i> (Murray, 1913)	NE	13
364	<i>Trichocerca porcellus</i> (Gosse, 1851)	NE	11, 13, 17
365	<i>Trichocerca pusilla</i> (Jennings, 1903)	N, NE, C, S	1, 3, 4, 9, 10, 13, 17, 19, 20, 21, 22, 23, 24, 25, 26
366	<i>Trichocerca obtusidens</i> (Olofsson, 1918) Syn.: <i>Trichocerca relicta</i> Donner, 1950; Sanoamuang <i>et al.</i> , 1995; Pholpunthin, 1997; Segers & Pholpunthin, 1997	NE, S	4, 5, 7
367	<i>Trichocerca rosea</i> (Stenroos, 1898)	NE	13
368	<i>Trichocerca rousseleti</i> (Voigt, 1902)	NE	4
369	<i>Trichocerca ruttneri</i> Donner, 1953	NE, S	4, 5, 7, 13, 23
370	<i>Trichocerca scipio</i> (Gosse, 1886) Syn.: <i>Trichocerca jenningsi</i> Voigt, 1957; Sanoamuang & Savatentalinton, 2001; Chittapun <i>et al.</i> , 2002	NE, S	13, 15, 17
371	<i>Trichocerca siamensis</i> Segers & Pholpunthin, 1997	NE, S	7, 13, 15, 20
372	<i>Trichocerca similis</i> (Wierzejski, 1893)	N, NE, C, S	3, 4, 5, 7, 9, 10, 13, 17, 20, 21, 22, 23, 24
373	<i>Trichocerca similis grandis</i> Hauer, 1965 Syn.: <i>Trichocerca similis</i> (Weirzejski) f. <i>grandis</i> Hauer: Chittapun & Pholpunthin, 2001; Chittapun <i>et al.</i> , 2002	C, S	12, 15, 26
374	<i>Trichocerca simoneae</i> De Smet 1990	NE	13, 17, 24
375	<i>Trichocerca stylata</i> (Gosse, 1851)	NE, C	4, 13, 17, 22, 24
376	<i>Trichocerca tenuidens</i> (Hauer, 1931)	NE	4
377	<i>Trichocerca tenuior</i> (Gosse, 1886)	N, NE, S	3, 9, 13, 14, 17, 19, 20, 23, 24
378	<i>Trichocerca tigris</i> (Müller, 1786)	NE, S	4, 13, 20, 23
379	<i>Trichocerca voluta</i> (Murray, 1913) Syn.: <i>Trichocerca tropis</i> Hauer, 1937; Sanoamuang <i>et al.</i> , 1995; Pholpunthin, 1997; Segers & Pholpunthin, 1997; Chittapun & Pholpunthin, 2001; Sanoamuang & Savatentalinton, 2001; Segers <i>et al.</i> , 2004	NE, S	4, 5, 7, 12, 13, 17
380	<i>Trichocerca vernalis</i> (Hauer, 1936)	NE	13
381	<i>Trichocerca weberi</i> (Jennings, 1903)	NE, S	13, 17, 20, 23
	Family Trichotriidae		
382	<i>Macrochaetus collinsii</i> (Gosse, 1867)	N, NE, C, S	2, 4, 9, 10, 12, 13, 15, 17, 20, 22, 23, 24
383	<i>Macrochaetus danneelae</i> Koste & Shiel, 1983	N, NE	9, 13, 17, 24
384	<i>Macrochaetus longipes</i> Myers, 1934	N, NE, C	4, 9, 13, 17, 22, 24
385	<i>Macrochaetus sericus</i> (Thorpe, 1893)	NE, S	4, 5, 7, 13, 17, 20, 24
386	<i>Macrochaetus subquadratus</i> (Perty, 1850)	NE, C, S	12, 13, 17, 22
387	<i>Trichotria tetractis</i> (Ehrenberg, 1830)	N, NE, C, S	4, 5, 7, 9, 12, 13, 15, 17, 20, 21, 22, 24
388	<i>Volga spinifera</i> (Western, 1894)	NE	11, 17, 24
	Family Trochosphaeridae		
389	<i>Filinia brachiata</i> (Rousselet, 1901)	C	3, 21
390	<i>Filinia camasecla</i> Myers, 1938	N, NE, C	4, 9, 13, 17, 20, 21, 22, 24, 26, 28
391	<i>Filinia longiseta</i> (Ehrenberg, 1834) Incl.: <i>Filinia longiseta</i> var. <i>limnetica</i> : Jithlang & Wongrat, 2006	N, NE, C, S	1, 3, 4, 9, 10, 17, 20, 21, 22, 23, 24, 26
392	<i>Filinia novaezealandiae</i> Shiel & Sanoamuang, 1993	NE, C, S	10, 13, 17, 20, 21, 22, 24, 26, 28
393	<i>Filinia opoliensis</i> (Zacharias, 1898) Syn.: <i>Tetramastix opoliensis</i> Zacharias, 1898: De Ridder, 1971	N, NE, C, S	1, 3, 4, 9, 10, 12, 13, 17, 20, 21, 22, 23, 24, 26
394	<i>Filinia pejleri</i> Hutchinson, 1964	N, NE	4, 9
395	<i>Filinia saltator</i> (Gosse, 1886)	N, NE	4, 9, 17

Table 1. (continued)

	Species	Distribution	References
396	<i>Filinia terminalis</i> (Plate, 1886)	NE	3, 4
397	<i>Trochosphaera aequatorialis</i> Semper, 1872	N, NE, C	3, 4, 9, 17, 26
	Bdelloidea		
	Family Habrotrichidae		
398	<i>Habrotricha ampulla</i> Murray, 1911	C	2
399	<i>Habrotricha angusticollis</i> (Murray, 1905)	C	2
	Family Philodiniidae		
400	<i>Dissotrocha aculeata</i> (Ehrenberg, 1832)	C, S	2, 12, 15, 23
401	<i>Dissotrocha macrostyla</i> (Ehrenberg, 1838)	C	2

Table 2. List of Oriental and Thai (*) taxa.

	Species
1	<i>Brachionus donneri</i> Brehm, 1951
2	<i>Brachionus murphyi</i> Sudzuki, 1989
3	<i>Brachionus srisumoniae</i> Segers, Kothetip & Sanoamuang, 2004*
4	<i>Cephalodella songkhlaensis</i> Segers & Pholpunthin, 1997 ¹
5	<i>Colurella psammophila</i> Segers & Chittapun, 2001*
6	<i>Colurella sanoamuangae</i> Chittapun, Pholpunthin & Segers, 1999
7	<i>Encentrum pornsilpi</i> Segers & Chittapun, 2001*
8	<i>Filinia camasecla</i> Myers, 1938
9	<i>Habrotricha recumbens</i> Bartoš, 1963
10	<i>Keratella edmondsoni</i> Ahlstrom, 1943
11	<i>Keratella taksinensis</i> Chittapun, Pholpunthin & Segers, 2002*
12	<i>Lecane acanthinula</i> (Hauer, 1938)
13	<i>Lecane blachei</i> Berzins, 1973
14	<i>Lecane bulla diabolica</i> (Hauer, 1936)
15	<i>Lecane isanensis</i> Sanoamuang & Savatnalinton, 2001*
16	<i>Lecane junki</i> Koste, 1975*
17	<i>Lecane kunthuleensis</i> Chittapun, Pholpunthin & Segers, 2003*
18	<i>Lecane lungae</i> Savatnalinton & Segers, 2005*
19	<i>Lecane martensi</i> Savatnalinton & Segers, 2008*
20	<i>Lecane micrognatha</i> Segers & Savatnalinton, 2010*
21	<i>Lecane minuta</i> Segers, 1994
22	<i>Lecane niwati</i> Segers, Kothetip & Sanoamuang, 2004*
23	<i>Lecane segersi</i> Sanoamuang, 1996*
24	<i>Lecane superaculeata</i> Sanoamuang & Segers, 1997 ^{1,2*}
25	<i>Octotrocha speciosa</i> Thorpe, 1893
26	<i>Ptygura thalenoensis</i> Meksuwan, Pholpunthin & Segers, 2011

¹ Reliably recorded from Cambodia by Sor (2011)² Recorded, without substantiation, from NE India by Sharma and Sharma (2005)

2) Review of species complexes in families Brachionidae and Lecanidae

The Thai rotifer fauna is composed largely of widespread, cosmopolitan or tropicopolitan species. Notwithstanding that the Thai rotifer fauna is the richest of all of Southeast Asian countries, this by no means implies that the fauna is adequately studied. One of the knowledge gaps concerns the taxonomy of species complexes, especially cryptic species. These are quite difficult to identify as they are morphological similar. Some studies in Thailand suggested that species complexes are common in monogonont rotifers, and cited as possible examples *Lecane bulla*, *Lecane leontina*, *Lecane ludwigii*, *L. lunaris*, and *L. quadridentata* species groups (Segers and Savatnalinton, 2010). Moreover, many species are known to belong to species complexes, such as *Brachionus angularis*, *Brachionus calyciflorus*, and *Brachionus quadridentatus* (Snell, 1989). In addition, there are no less than three different species within the taxon *Keratella cochlearis* (Hoffman, 1980 cited in Serra *et al.*, 1997). These cases illustrate the difficulty of estimating the actual species diversity of rotifers in Thailand.

Several Thai literature sources indicate that species complexes occur mainly in the families Brachionidae and Lecanidae. The biogeography and distribution of each known species complex in families Brachionidae and Lecanidae in Thailand are reviewed and listed in Tables 3 and 4, respectively. According to present knowledge, each species complex is cosmopolitan and can be found in all regions of Thailand. Because these families form the majority of rotifers in Thailand, erroneous identification of species complexes in these families may directly affect to estimate of the actual species number of rotifer in Thailand and may lead to misunderstand about biogeography and biodiversity. I expect that targeted efforts on understudied taxonomic groups, preferably using modern molecular tools will undoubtedly raise the record of rotifer diversity in Thailand.

Table 3. Species complex in Family Brachionidae in Thailand.

Species	Remarks	Biogeography	Records in Thailand (ref.)			
			N	NE	C	S
<i>Brachionus angularis</i> Gosse, 1851	Species complex (Reference: Athibai, 2008, Athibai <i>et al.</i> , 2013) (Thai recorded: <i>B. angularis</i> f. <i>typica</i> , <i>B. angularis</i> f. <i>bidens</i> , <i>B. angularis</i> f. <i>chelonis</i>)	AFR, AUS, NEA, NEO, ORI, PAL	5, 21	1, 8, 11, 13	14, 15, 18, 19	2, 3, 6, 16, 17
<i>Brachionus calyciflorus</i> Pallas, 1766	Species complex, probably concealing cryptic species. The taxonomy of <i>B. calyciflorus</i> is particularly confused. Validation of the numerous proposed subspecies and infrasubspecific variants awaits a thorough revision of the taxon. (References: Segers, 2007; Xiang <i>et al.</i> , 2010; Xiang <i>et al.</i> , 2011; Athibai <i>et al.</i> , 2013) (Thai recorded: <i>B. calyciflorus</i> f. <i>typical</i> , <i>B. calyciflorus</i> f. <i>amphiceros</i> , <i>B. calyciflorus</i> f. <i>anuraeiformis</i> , <i>B. calyciflorus</i> f. <i>monstruosus</i>)	AFR, ANT, AUS, NEA, NEO, ORI, PAL	5, 21	1, 8, 11, 13, 21	14, 15, 19, 21	2, 3, 16, 17, 21
<i>Brachionus caudatus</i> Barrois and Daday, 1894	Species complex. The taxonomy of <i>B. caudatus</i> is particularly confused. Validation of the numerous proposed subspecies and	AFR, AUS, NEA, NEO, ORI, PAL	5, 21	1, 11, 13, 21	14, 15, 19, 21	2, 17

Table 3. (continued)

Species	Remarks	Biogeography	Records in Thailand (ref.)			
			N	NE	C	S
	infrasubspecific variants awaits a thorough revision of the taxon (References: Giri and Jose de Paggi, 2006; Segers, 2007) (Thai recorded: <i>B. caudatus</i> f. <i>apsteini</i> , <i>B. caudatus</i> f. <i>aculeatus</i> , <i>B. caudatus</i> f. <i>personatus</i>)					
<i>Brachionus plicatilis</i> Müller, 1786	The <i>B. plicatilis</i> complex is an as yet incompletely resolved cryptic species complex (Reference: Segers, 2007)	AFR, AUS, NEA, NEO, ORI, PAC, PAL	-	1	-	-
<i>Brachionus quadridentatus</i> Hermann, 1783	Species complex (Reference: Athibai, 2008) (Thai recorded: <i>B. quadridentatus</i> f. <i>brevispinus</i> , <i>B. quadridentatus</i> f. <i>cluniorbicularis</i> , <i>B. quadridentatus</i> f. <i>melhemi</i> , <i>B. quadridentatus</i> f. <i>mirabilis</i> , <i>B. quadridentatus</i> f. <i>typica</i>)	AFR, ANT, AUS, NEA, NEO, ORI, PAC, PAL	5, 21	1, 8, 11, 13, 21	14, 15, 19, 21	2, 3, 6, 7, 10, 16, 17, 21
<i>Keratella cochlearis</i> (Gosse, 1851)	Species complex. The taxonomy of <i>K. cochlearis</i> is particularly confused. Validation of the	AFR, ANT, AUS, NEA, NEO, ORI,	5	1,8,11,13	14,15,18	2, 3, 16, 17

Table 3. (continued)

Species	Remarks	Biogeography	Records in Thailand (ref.)			
			N	NE	C	S
	numerous proposed subspecies and infrasubspecific variants awaits a thorough revision of the taxon (References: Derry <i>et al.</i> , 2003; Gómez, 2005; Segers, 2007) (Thai recorded: <i>K. cochlearis</i> f. <i>typica</i> , <i>K. cochlearis</i> f. <i>micracantha</i>)	PAL				
<i>Keratella tropica</i> (Apstein, 1907)	Species complex, variation in length of posterior spine (Reference: Green, 1980)	AFR, AUS, NEA, NEO, ORI, PAL	5	1, 8, 11, 13	14, 15, 18, 19	2, 3, 6, 16, 17

Biogeographical abbreviation: AFR-Afrotropical region; ANT-Antarctic region; AUS-Australian region; NEA-Nearctic region; NEO-Neotropical region; ORI-Oriental region; PAC-Pacific region; PAL-Palearctic region.

References:1-Sanoamuang *et al.*, 1995; 2-Pholpunthin, 1997; 3-Segers & Pholpunthin, 1997; 4-Pholpunthin & Chittapun, 1998; 5-Sanoamuang, 1998; 6-Chittapun *et al.*, 1999; 7-Chittapun & Pholpunthin, 2001; 8-Sanoamuang & Savatentalinton, 2001; 9-Segers & Chittapun, 2001; 10-Chittapun *et al.*, 2002; 11- Segers *et al.*, 2004; 12-Chittapun *et al.*, 2005; 13- Savatentalinton & Segers, 2005; 14-Jithland & Wongrat, 2006; 15-Teeramaethee *et al.*, 2006; 16-Chittapun *et al.*, 2007; 17-Sanoamuang, 2007; 18-Savatentalinton & Segers, 2008; 19-Chittapun *et al.*, 2009; 20-Chittapun, 2011; and 21-Athibai *et al.*, 2013.

Table 4. Species complex in Family Lecanidae in Thailand.

Species	Remarks	Biogeography	Records in Thailand (ref.)			
			N	NE	C	S
<i>Lecane bulla</i> (Gosse, 1851)	Species complex, morphologically variable taxon (References: Segers, 2007; Segers & Savatentalinton, 2010; Walsh <i>et al.</i> , 2009) (Thailand recorded: <i>L. bulla</i> , <i>L. bulla bulla</i> , <i>L. bulla diabolica</i>)	AFR, AUS, NEA, NEO, ORI, PAC, PAL	5	1, 8, 11, 13	14, 15, 19, 20	2, 3, 4, 6, 7, 9, 10, 12, 16, 17
<i>Lecane closterocerca</i> (Schmarda, 1859)	Species complex (Reference: Segers, 1996)	AFR, ANT, AUS, NEA, NEO, ORI, PAC, PAL	5	1, 8, 11, 13	14, 15, 19	2, 3, 6, 7, 10, 16, 17
<i>Lecane curvicornis</i> (Murray, 1913)	Species complex (References: Segers, 1996) (Thailand recorded: <i>L. curvicornis</i> f. <i>typica</i>)	AFR, AUS, NEA, NEO, ORI, PAL	5	1, 8, 11, 13	14, 15, 19, 20	2, 3, 4, 7, 10, 16, 17
<i>Lecane hamata</i> (Stokes, 1896)	Species complex (Reference: Segers, 1996)	AFR, AUS, NEA, NEO, ORI, PAC, PAL	5	1, 8, 11, 13	14, 15, 19	2, 3, 4, 6, 7, 9, 10, 16, 17
<i>Lecane hornemanni</i> (Ehrenberg, 1834)	Species complex (Reference: Segers, 1996)	AFR, AUS, NEA, NEO, ORI, PAC, PAL	5	1, 8, 11, 13	14, 15, 19	2, 3, 4, 7, 10, 16, 17

Table 4. (continued)

Species	Remarks	Biogeography	Records in Thailand (ref.)			
			N	NE	C	S
<i>Lecane leontina</i> (Turner, 1892)	Species complex, morphologically variable taxon, probably concealing cryptic species (References: Segers, 2007; Segers & Savatnalinton, 2010)	AFR, AUS, NEA, NEO, ORI, PAL	5	1, 8, 11, 13	14, 15, 19	2, 3, 4, 6, 7, 10, 16, 17
<i>Lecane ludwigii</i> (Eckstein, 1883)	Species complex (Reference: Segers & Savatnalinton, 2010; Segers & Shiel, 2003)	AFR, AUS, NEA, NEO, ORI, PAC, PAL	5	1, 8, 11, 13	15	2, 3, 4, 6, 7, 10, 12, 16, 17
<i>Lecane lunaris</i> (Ehrenberg, 1832)	Species complex, morphologically variable taxon, probably concealing cryptic species (Reference: Segers, 2007; Segers & Savatnalinton, 2010)	AFR, ANT, AUS, NEA, NEO, ORI, PAC, PAL	5	1, 8, 11, 13	14,15, 18	2, 3, 4, 6, 7, 10, 16, 17
<i>Lecane obtusa</i> (Murray, 1913)	Species complex (Reference: Segers, 1996)	AFR, AUS, NEA, NEO, ORI, PAL	5	1, 8, 11, 13	15	2, 3, 4, 6, 7, 9, 10, 12, 16, 17
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	Species complex (Segers & Savatnalinton, 2010)	AFR, AUS, NEA, NEO, ORI, PAC, PAL	5	1, 8, 11	15, 19	2, 3, 4, 6, 7, 16, 17

Table 4. (continued)

Species	Remarks	Biogeography	Records in Thailand (ref.)			
			N	NE	C	S
<i>Lecane signifera</i> (Jennings, 1896)	Species complex (Reference: Segers, 1996)	NEA	5	1, 8, 11, 13	14, 15, 19	2, 3, 4, 6, 7, 10, 16, 17
<i>Lecane unguitata</i> (Fadeev, 1926)	Species complex (Reference: Segers, 1996)	AFR, AUS, ORI, PAL	5	1, 8, 11, 13	15, 19	2, 3, 4, 6, 7, 10, 12, 16, 17
<i>Lecane ungulata</i> (Gosse, 1887)	Species complex (Reference: Segers, 1996)	AFR, AUS, NEA, NEO, ORI, PAL	5	1, 8, 11, 13	14, 15, 19	2, 3, 4, 6, 7, 10, 16, 17

Biogeographical abbreviation: AFR-Afrotropical region; ANT-Antarctic region; AUS-Australian region; NEA-Nearctic region; NEO-Neotropical region; ORI-Oriental region; PAC-Pacific region; PAL-Palearctic region.

References: 1-Sanoamuang *et al.*, 1995; 2-Pholpunthin, 1997; 3-Segers & Pholpunthin, 1997; 4-Pholpunthin & Chittapun, 1998; 5-Sanoamuang, 1998; 6-Chittapun *et al.*, 1999; 7-Chittapun & Pholpunthin, 2001; 8-Sanoamuang & Savatentalinton, 2001; 9-Segers & Chittapun, 2001; 10-Chittapun *et al.*, 2002; 11-Segers *et al.*, 2004; 12-Chittapun *et al.*, 2005; 13-Savatentalinton & Segers, 2005; 14-Jithland & Wongrat, 2006; 15-Teeramaethee *et al.*, 2006; 16-Chittapun *et al.*, 2007; 17-Sanoamuang, 2007; 18-Savatentalinton & Segers, 2008; 19-Chittapun *et al.*, 2009; and 20-Chittapun, 2011.

1.3 Research questions

- 1.3.1 Are there cryptic species complexes within the families Brachionidae and Lecanidae in Thailand?
- 1.3.2 Are there correlations between genetic divergence and geographical distribution of species complexes within the families Brachionidae and Lecanidae in Thailand?

1.4 Objectives

- 1.4.1 To investigate species complexes of rotifers in families Brachionidae and Lecanidae in Thailand
- 1.4.2 To investigate genetic divergence and geography of species complexes in Brachionidae and Lecanidae in Thailand

CHAPTER 2

RESEARCH METHODOLOGY

2.1 Study area

Thailand is a tropical country situated between latitude 5°37' N to 20°27' N and longitude 97°22' E to 105°37' E (Setapan, 1999). The geography of the country is quite heterogeneous, with large plains and relatively high mountains, and several watersheds generating abundance and diversity of freshwater habitats.

Samples were taken across all the country from several water bodies of different type to cover the largest possible diversity of rotifers. Samples were collected from several habitat types, such as lakes, swamps, peat-swamps, marshes, and ponds. Habitat types were classified by the following characteristics. A lake was considered as a large area (at least 80,000 m²) filled with water, apart from rivers that serves to drain the lake, regardless of whether the water body is of natural or artificial origin. Peat swamp was defined as a wetland where waterlogged soil prevents dead leaves and wood from fully decomposing; over time, this creates a thick layer of acidic peat. A swamp was defined as a wetland that is forested and occurs along large rivers where it is critically dependent upon natural water level fluctuations; some swamps could be covered by aquatic vegetation. A pond was defined as a body of standing water, either natural or artificial, that is smaller than a lake (less than 80,000 m²). A marsh was defined as a wetland dominated by herbaceous rather than by woody plant species.

A total of 133 samples from 90 sites were collected during November 2011 - April 2016 (Figure 1 and Table 5). Some samples were collected from, broadly speaking, the same site, for example the same lake, but in different places and/or on different occasions: limnological variables could be rather different among such samples even from the same lake; thus each sample was treated separately.

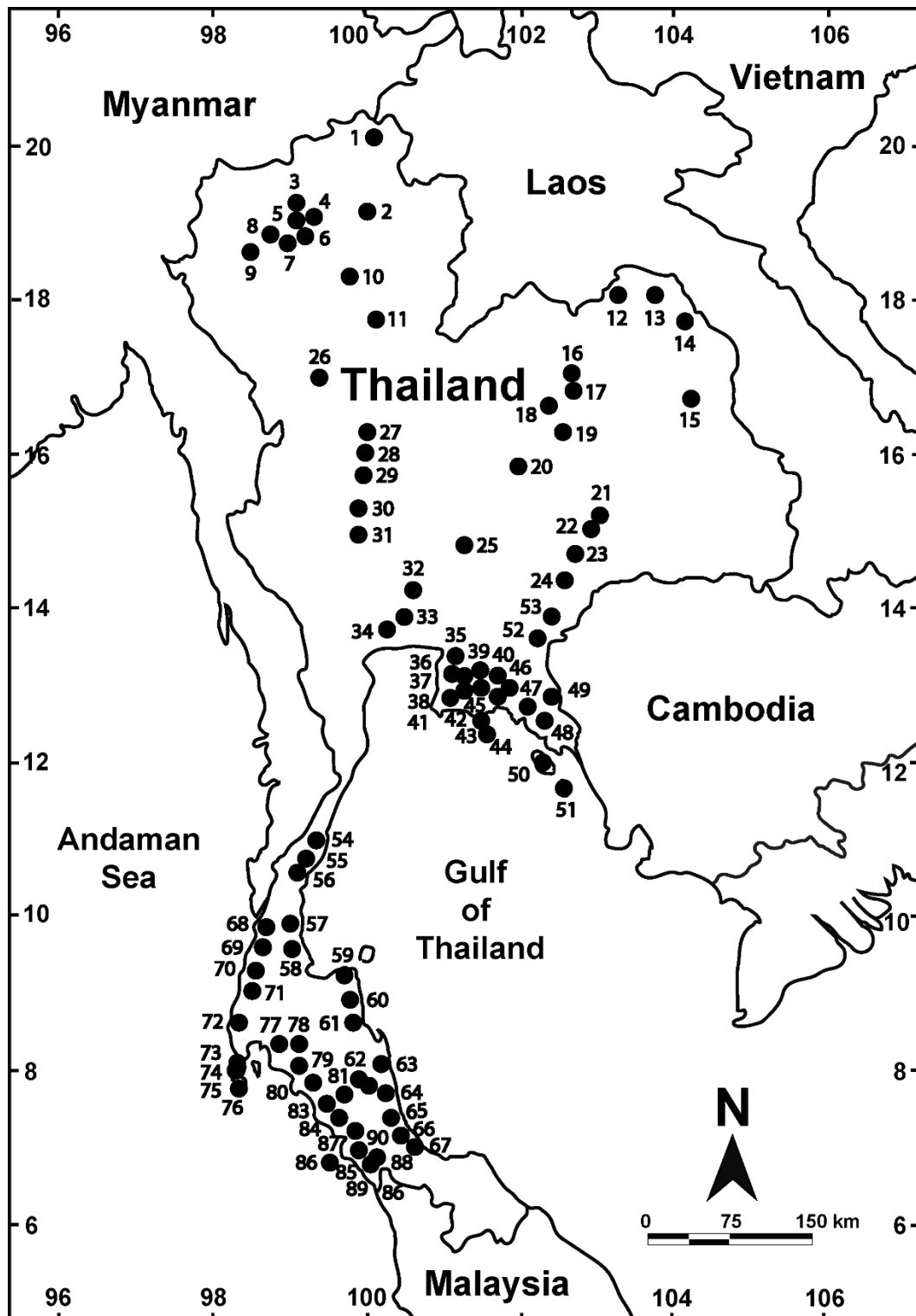


Figure 1. Map of sampling sites in this study (black circles). The number of sampling sites was shown in Table 5.

Table 5. Sampling sites in this study. Numbers given for each sampling location are corresponding to those in the map of Figure 1.

Sites	
1. Chiang Saen Lake	32. Sra Silapacheep
2. Kwan Phayao	33. Nong Phuttha HP
3. Huai Kang Reservoir	34. Bang Kruai
4. Mae Kuang Reservoir	35. Bang Phra Reservoir
5. Huai Jo Reservoir	36. Nong Kho Reservoir
6. Nong Han	37. Nong Klang Dong Reservoir
7. Huai Tung Tao Reservoir	38. Bangphi
8. Sra Pimtawan	39. Dok Krai Reservoir
9. Huaitong Reservoir	40. Nong Pla Lai Reservoir
10. Mae Kham Reservoir	41. Nong Thasipheth
11. Bueng Thungkalo	42. Khao Nguang Chang Reservoir
12. Nong Khon	43. Samet Reservoir
13. Nong Kut Thing	44. Sra Anodad
14. Bueng Khong Long	45. Botanical garden
15. Nong Han	46. Khao Chuk Reservoir
16. Thale Buadaeng	47. Pru Kung Krabaen
17. Srabua Khon Kaen	48. Nong Bua Chanthaburi
18. Srabua Ubonrat	49. Kirithan Reservoir
19. Nong Khot	50. Koh Chang Reservoir
20. Nong Wang	51. Aosalad Reservoir
21. Nong Tabeang	52. Sra Klong Had
22. Jorakhemak Reservoir	53. Nong Nonsa-at
23. Thung Laem Reservoir	54. Sra Klongbod
24. Nong Bon	55. Nong WatZaitong
25. Sra Fountain Tree	56. Pru Ching
27. Bueng Si Phi	57. Nong Bua Chumphon
28. Bueng Sanat	58. Sra Khanthulee
29. Bueng Borapet	59. Srabou Khanom
30. Bueng Thepo	60. Nong Sichon
31. Bueng Chawak	61. Nong Baansrabua

Table 5. (continued)

Sites	
62. Thale Noi	77. Nong Klong Muang
63. Nong Pakbung	78. Nong Baanlaempho
64. Sra Pangtree	79. Nong Banthamapraw
65. Bueng Baanwatmai	80. Srabua Rajamangala
66. Srabua PSU	81. Nong Klong Lamchan
67. Buengbua Natab	82. Nong Ban Chu Pa
68. Nong Klong Kapur	83. Nong Nachumhed
69. Srabua Kapur	84. Nong Thungsabo
70. Khaowong Reservoir	85. Sra Lalita Sabo
71. Bueng Kurod	86. Bulone
72. Nong Taymueng	87. Nong Pan Ya
73. Pru Jaeson	88. Nong Pan Ya
74. Pru Jik	89. Huai Yiao Reservoir
75. Pru Mai Khao	90. Nong Prakpraya
76. Nong Han	

2.2 Field study

2.2.1 Sample collections

1) Live and preserved samples

Rotifer samples were collected among vegetation and open water using a plankton net of 22 μm mesh size. The samples were divided into two parts: live and preserved samples. Live samples were kept in plastic bottles and carried from each site to the laboratory under slightly cooled conditions. For preserved samples, each sample was concentrated by filtering water through 22 μm sieve and immediately preserved in 95% ethanol and kept on ice in cool box until they were transferred to laboratory. Diagram of this study is shown in Figure 2.

In the field (At each sampling site)

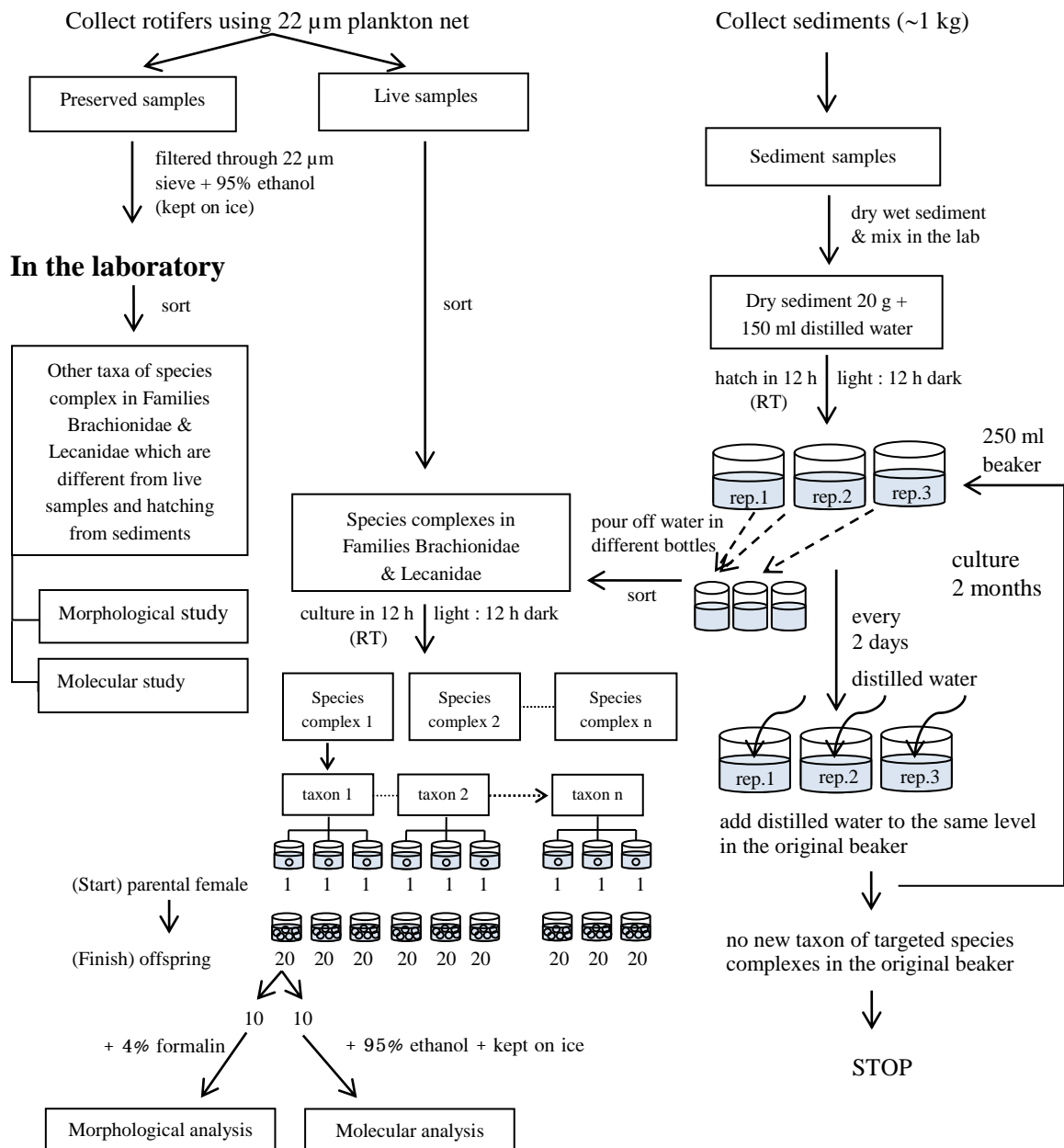


Figure 2. The diagram shows this study in the field and in the laboratory. RT = room temperature (27–33 °C). n = total number of species complex or taxon in each site.

2) Sediment samples

Sediments, including resting eggs of rotifers, were collected randomly from each sampling site. The top layer of sediment (approximately 1 cm) were taken from each site, approximately 1 kilogram. Sediments were transferred to laboratory and stored in dry, dark and cool conditions until they can be processed.

2.2.2 Environmental measurements

Physical and chemical factors, including pH, temperature ($^{\circ}\text{C}$), conductivity ($\mu\text{S cm}^{-1}$), salinity (ppt), and dissolved oxygen (mg L^{-1}), were measured at each site. pH was measured using Adwa AD12 pH meter. Temperature, conductivity, and salinity were determined using YSI Model 30 meter. Dissolved oxygen (DO) was measured by titration method using HANNA HI3810 Dissolved Oxygen Test Kit. Coordinates (latitude, longitude) and elevation (m) were obtained by Garmin eTrex H Handheld GPS Navigator meter.

2.3 Sample processes in the laboratory

2.3.1 Live sample process

Living specimens belonging to selected species complexes of monogonont rotifers were isolated and put into chambers. Parental females of each taxon of selected species complexes were cloned from each site. In case of parental females which were insufficient for studying, they were isolated from hatching experiment from the resting egg in the sediment. Each parental female was cloned with the method of Athibai (2008). One parental female was cultured in a chamber with two milliliters of water from the same locality and fed on *Chlorella vulgaris* at density of 1×10^6 cells ml^{-1} . The cultured containers were incubated at room temperature ($27\text{--}33^{\circ}\text{C}$) with a twelve-hour light and a twelve-hour dark light regime to let parental female produce her offsprings by parthenogenesis. All offsprings of the same parents were cultured together in their parental containers. Twenty offsprings are enough for studying. After culturing, ten specimens were fixed in 4% formalin for morphological analysis, and ten specimens were preserved in 95% ethanol and immediately kept on ice.

2.3.2 Sediment sample process

Sediments were dried, mixed and stored in dark and cool conditions until they were processed. Dry sediments were mixed and cultured to allow rotifer resting eggs to hatch after applying the method of Chittapun *et al.* (2005). Three replicates of sediments from each site were cultured. For each replicate, 20 grams of sediments were placed into 250 ml beaker and 150 milliliters of distilled water were added. Importantly, the beakers were incubated at room temperature (27–33°C) with a twelve-hour light and twelve-hour dark light regime. Every two days during two month period, the water in these beakers were poured off into a different bottle, and refilled back to the same level in the original beaker. Sediments were incubated until no new taxon of targeted species complexes appeared in the original beaker. The targeted species complexes were isolated from the water which were poured off from beaker every two days. The targeted species complexes were cloned at the same procedure as that for the live samples via the approach described by Athibai (2008). After incubated, ten specimens were fixed in 4% formalin for morphological study and ten specimens were fixed in 95% ethanol and immediately kept on ice for molecular analysis.

2.3.3 Preserved sample process

Preserved samples were searched for other targeted species complexes which are different from the live samples and the ones hatching from sediments. Specimens of each taxon of other targeted species complexes were selected from each site. The specimens were photographed before molecular analysis. Geometric morphometric analysis was investigated from specimens whose sequences can be obtained

2.4 Morphological analysis

Animals were sorted in the lab under a dissecting microscope and targeted species complexes of the families Brachionidae and Lecanidae were identified, according to the recent literature reviews of Koste (1978); Koste and Shiel (1987); Sanoamuang *et al.* (1995); Segers (1995); Segers and Wang (1997); Segers and Rong (1998); Segers and Savatentalinton (2010); and Athibai *et al.* (2013). Nomenclature follows the most recent updates of the candidate List of Available Names for rotifer species (Segers *et al.*, 2012, 2015). For each taxon, specimens at each site in targeted species complexes in Brachionidae and Lecanidae were identified and photographed using Leica DM1000 LED microscope before DNA extraction. The representatives of each taxon derived from culture were kept as vouchers in the reference collection at Princess Maha Chakri Sirindhorn Natural History Museum, Faculty of Science, Prince of Songkla University.

In addition, photographs of rotifers were taken before DNA extraction. Next, geometric morphometric analysis was investigated from specimens whose sequences can be obtained. Also, lorica morphology was employed to describe variations among species and population.

2.4.1 Geometric morphometric analysis

Morphological characters of ventral lorica were obtained from the photographs with the same magnification (40x). Because ventral lorica is stable, ventral lorica was used to examine the morphological characters of lorica in order to avoid distortion. In this process, three methods were performed: a measurement, a landmark, and a semi-landmark sliding. The lorica measurements were performed using ImageJ v.1.50i program as shown in Figure 3. Furthermore, the coordinates of a set of the topographically corresponding landmark were utilized in geometric morphometrics (Marcus *et al.*, 2000; Zelditch *et al.*, 2004) in order to compare the form of organisms. The landmark and semi-landmark slidings were digitized on photograph (Figure 4) through Tps program series: version 1.68 of tpsUtil, version 2.22 of tpsDig2, and version 1.61 of tpsrelw program.

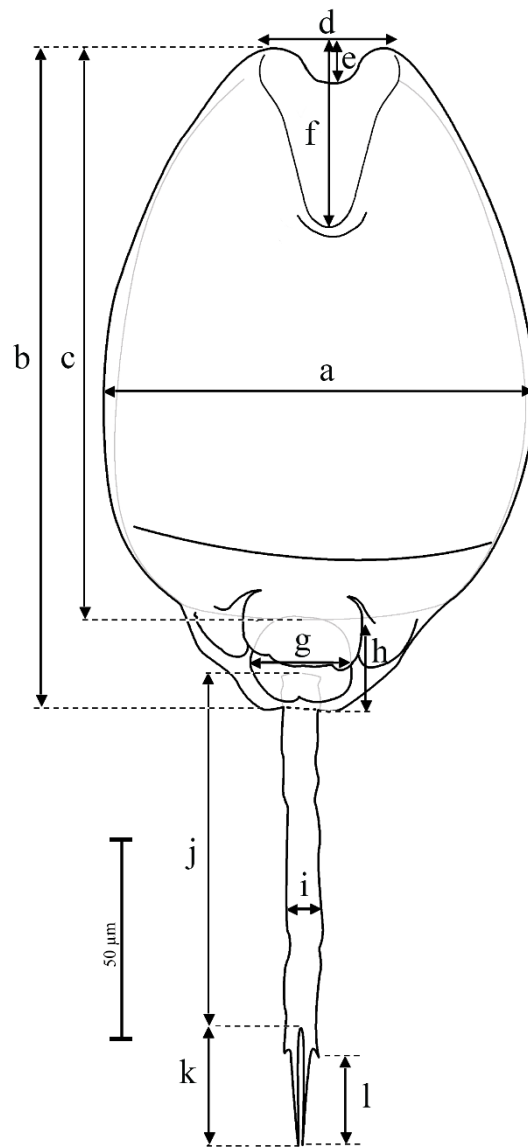


Figure 3. Morphological characters measured in this study.

- | | |
|--------------------------------|------------------------------|
| a. ventral lorica width | g. foot pseudosegment width |
| b. ventral lorica length | h. foot pseudosegment length |
| c. dorsal lorica length | i. maximum toe width |
| d. head aperture width | j. toe length |
| e. head aperture dorsal depth | k. terminal fissure length |
| f. head aperture ventral depth | l. pseudoclaw length |

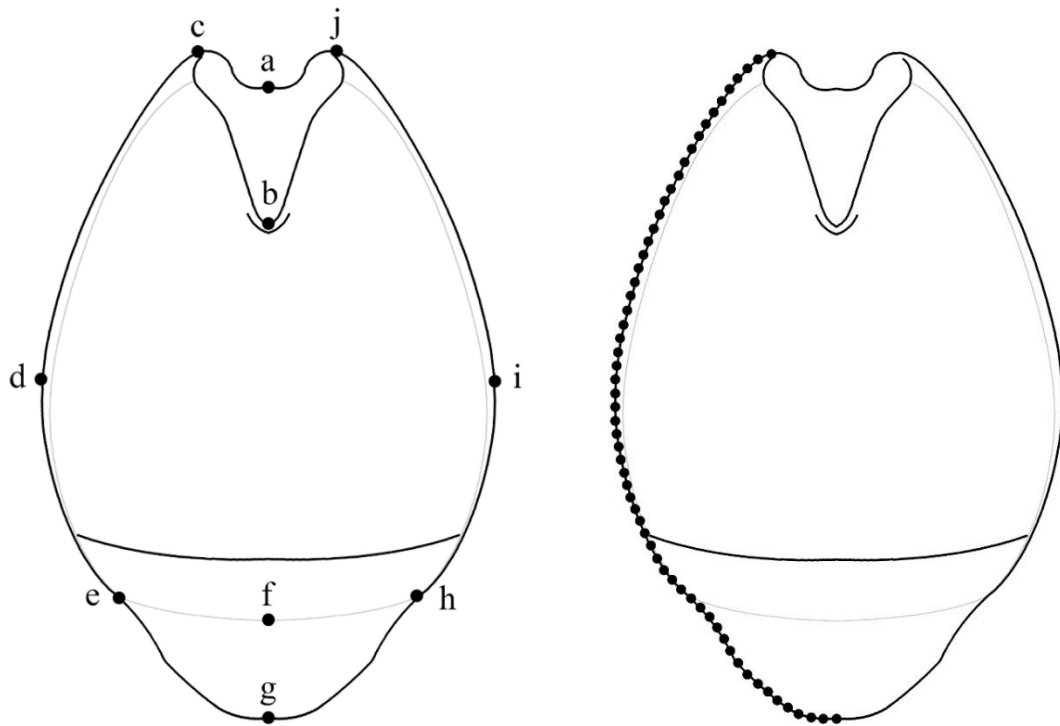


Figure 4. Lorica landmark (left) and semi-landmark slidings (right).

- a. the median of dorsal head aperture margin
- b. the median of ventral head aperture margin
- c. left antero-lateral corner (lateralmost point of head aperture margin)
- d. the maximum width of ventral lorica (left)
- e. point where the postero-lateral margins of ventral and dorsal lorica plates cross (left)
- f. dorsal point of dorsal lorica plate
- g. median point of distal margin of ventral lorica
- h. point where the postero-lateral margins of ventral and dorsal lorica plates cross (right)
- i. the maximum width of ventral lorica (right)
- j. right antero-lateral corner (lateralmost point of head aperture margin)

2.4.2 Data Analysis

Morphological analyses were conducted among species complex using R version 3.3.1 (R Core Team, 2016) and RStudio Version 0.99.903 (2009–2016 RStudio, Inc.). Significant differences of morphological characters were examined using ANOVA and Turkey test.

2.5 Molecular analysis

2.5.1 DNA extraction

Each specimen preserved in 95% ethanol was transferred into a PCR (0.2 mL) tube and dried. The whole body of a single specimen was used for DNA extraction according to García-Morales and Elías-Gutiérrez (2013) with a modified HotSHOT protocol (Montero-Pau *et al.*, 2008) or Chelex100 (Instagene Matrix BioRad) with a modified protocol (Walsh *et al.*, 2009). For the HotSHOT protocol, a single specimen was digested in 30 µl of alkaline lysis buffer (NaOH 25 mM, disodium EDTA 0.2 mM, pH 8.0) in 0.2 mL tube and crushed against the side at the bottom of the tube under a compound microscope. Each sample was incubated at 95 °C for 30 min and then at 4 °C for 4 min. A further 30 µl of neutralizing buffer (Tris-HCL 40 mM, pH 5.0) was added to each tube and spinned down. The supernatant was used as DNA template for polymerase chain reactions (PCRs). For the Chelex protocol, a single specimen was digested using 30 µl of Chelex matrix in a 0.2 mL tube and vortexed for 20 seconds. Samples were then incubated at 99 °C in ThermoMixer machine (Eppendorf) with continuous shaking at 1000 rpm for 10 min. Extracted DNA was stored at -20 °C for future amplification.

2.5.2 DNA amplification, purification and sequencing

Target fragments of DNA, the mitochondrial cytochrome *c* oxidase subunit I (COI or *cox1*) and the nuclear ribosomal internal transcribed spacer 1 (ITS1), were amplified using polymerase chain reactions (PCRs) with universal primers. Amplification of the COI region was performed using primers LCO1490 (5'-GGTCAACAAATCA TAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAT CA-3') (Folmer *et al.*, 1994). Amplification of the ITS1 region was done using the

primer III (5'-CACACCGCCCGTCGCTACTACCGATTG-3') and VIII (5'-GTGCG TTCGAAGTGTGCGATGATCAA-3') (Palumbi, 1996).

The PCR reactions were performed in 20 µl final volume containing 10 µl of EmeraldAmp GT PCR Master Mix (Takara), 0.5 µl of each primer (10 µM), 2–6 µl of DNA template (depending on DNA concentration), and topped up with PCR grade water to the 20 µl final volume. The reactions were amplified under the following thermal cycler conditions:

Initial denaturation	1 cycle	3 minutes at 95 °C
Amplification	38 cycles	15 seconds at 94 °C
		60 seconds at 50 °C
		90 seconds at 72 °C
Final extension	1 cycle	10 minutes at 72 °C

PCR products were electrophoresed on 1.2% agarose gels with SYBR Safe DNA Gel Stain (10 ml agarose gel per 1 µl SYBR Safe) and observed under UV light using Gel-Doc.

PCR products of expected sizes were purified using FavorPrep™ Gel/PCR purification kit (Favorgen) according to manufacturer's protocol. The purified products were sequenced in both directions using the amplification primers at Macrogen Inc., Seoul, Korea.

2.5.3 Data analysis

1) Sequence preparation

1.1) Mitochondrial cytochrome *c* oxidase subunit I (COI or *cox1*)

Chromatograms for all sequences were checked and manually edited in case of problems using Chromas Lite version 2.1.1 (Technelysium Pty Ltd., 2012). Overlapping sequences, both forward and reverse directions, were assembled into a consensus using program GENDOC version 2.6.002 (Nicholas *et al.*, 1997). Haplotypes were defined from genetic distance using pairwise-distance (<0.001). Haplotypes were used for alignment using MAFFT version 7 web server (Kato and Standley, 2013; <http://mafft.cbrc.jp/alignment/server/>).

All alignments were double-checked by eye using BioEdit version 7.1.3.0 (Hall, 1999) and MESQUITE version 3.11 (Maddison and Maddison, 2016) to ensure that no small errors and no stop codons were present. Haplotypes were used to investigate phylogenetic reconstruction and species delimitation.

1.2) Nuclear ribosomal internal transcribed space 1 (ITS1)

Chromatograms for all sequences were checked and manually edited using Chromas Lite version 2.1.1. The double peaks read from forward and reverse chromatograms were manually coded following the IUPAC ambiguity codes. Heterozygous sequences were resolved manually in case of single ambiguities, whereas we used Champuru v1.0 (<http://seqphase.mpg.de/champuru/>) to resolve length variant heterozygotes and SeqPHASE (<http://stephenslab.uchicago.edu/phase/download.html>) for all other heterozygotes. All alleles were aligned using MUSCLE web server (<http://www.ebi.ac.uk/Tools/msa/muscle/>), and the alignments were double-checked by eye using BioEdit version 7.1.3.0. Only one copy was used for each allele in order to avoid redundancy, similarly to what was done for haplotypes of COI.

2) Phylogenetic reconstructions

Phylogenetic trees were reconstructed using haplotypes of COI and of ITS1. In the case of COI, each individual had only one haplotype, whereas, in case of ITS1, each individual could have one or two different copies of the amplified fragment. Previously published sequences of the same COI and ITS1 region in species complexes in families Brachionidae and Lecanidae were included in the analyses. The species in the complex were identified and estimated the number of species within the complex using a combination of ABGD, PTP, and GMYC methods. Although COI marker was mainly used for identifying species, ITS1 marker (137 haplotypes from 103 individuals: 1 copy from 67 individuals and 2 copies from each of 36 individuals) was used for supporting species delimitation.

Phylogenetic trees were reconstructed using CIPRES web server (<https://www.phylo.org/>) with two approaches: Maximum Likelihood (ML) using RAxMLv.8.2.9 (Stamatakis, 2014) and Bayesian Inference (BI) using BEAST v.1.8.3 (Drummond and Rambaut, 2007) to examine the phylogenetic relationships among haplotypes.

RAxML (Randomized Axelerated Maximum Likelihood) is a program for sequential and parallel maximum likelihood based inference of large phylogenetic trees. BEAST (Bayesian Evolutionary Analysis Sampling Trees) is a cross-platform program for Bayesian analysis of molecular sequences using Markov Chain Monte Carlo (MCMC) method.

For ML, this method generated phylogenetic tree using maximum likelihood and rapid bootstrapping. The settings were performed using default mode.

For BI, 2 xml input files were created using BEAUti v.1.8.3 (Drummond *et al.*, 2012). First, the tree prior was set according to the Coalescent process speciation prior with the following settings: the general time-reversible model of evolution, including estimation of invariable sites and assuming a discrete gamma distribution (GTR+I+G) as substitution model calculated by jModelTest v.2.1.7 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012), uncorrelated lognormal relaxed clock (mean molecular clock rate set as normal). MCMC chains were run, from random trees for 100 million generations and sampling every 10,000th generation. For all other prior, the default settings were used. Second, tree prior was reconstructed using the Yule process speciation prior (rate of linear birth in the Yule model of speciation set as lognormal). Other settings were performed in the same way as Coalescent model. Tracer v1.6 (Drummond and Rambaut, 2007) was used for investigating effective sample size for parameters and determining the burn-in. TreeAnnotator v.1.8.3 (Drummond and Rambaut, 2007) was used for summarizing trees, with 10 millions discarded as burn-in.

Brachionus calyciflorus and *Lecane bulla* were used as the outgroup for phylogenetic reconstructions of families Lecanidae and Brachionidae, respectively. Trees were generated using COI and ITS1. Moreover, phylogenetic tree inferred from mitochondrial DNA haplotypes were used to explain processes, such as population subdivision, speciation, and geographical distribution patterns within the species complex.

3) Species delimitation and DNA taxonomy

The tests for the presence of cryptic species were performed using several methods on both COI and ITS1 datasets: Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.*, 2012), a Poisson Tree Processes (PTP) model (Zhang *et al.*, 2013; <http://species.h-its.org/>), and the Generalized Mixed Yule Coalescent (GMYC) model (Fujisawa and Barraclough, 2013). Moreover, haploweb was also used for the ITS1 dataset. ABGD was performed using web server. DNA barcoding and ABGD require a matrix of pairwise genetic distances, while PTP and GMYC require only a phylogenetic tree for analysis. All haplotypes were analyzed in ABGD web server (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>) with the following settings: 1.0 X (relative gap width), Jukes-Cantor (JC69). For others, the default settings were used. Phylogenetic trees obtained from RAxML and BEAST were used as input for PTP and GMYC models. PTP was performed using a web server (<http://species.h-its.org/>) and GMYC was performed on the ultrametric tree with R version 3.3.1 (R Development Core Team, 2016) and RStudio version 0.99.903 (2009–2016 RStudio, Inc.). The GMYC model uses a maximum likelihood approach to optimize the threshold identifying the shift in the branching patterns of the gene tree from interspecific branches (Yule model) to intraspecific branches (coalescent). Thus, independent entities from GMYC model can be assumed to be cryptic species.

All alleles identified from ITS1 were used to reconstruct haplotype networks PopART (Population Analysis with Reticulate Trees) (Bandelt *et al.*, 1999; Clement *et al.*, 2002; French *et al.*, 2013; <http://popart.otago.ac.nz>). On the network, different alleles were connected if they were found co-occurring in the same individual, demarcating fields of recombination with evidence of gene flow according to the haploweb method (Flot *et al.*, 2010).

4) The correlation between genetic distance and geography within species complex in Thailand.

The pairwise genetic distance and geographic distance of each species complex in Thailand were examined using Mantel test in R program.

CHAPTER 3

RESULTS

3.1 Species list of rotifers in Families Brachionidae and Lecanidae in Thailand

A total of 60 species 3 subspecies in 5 genera from families Brachionidae and Lecanidae were recorded in Thailand (Tables 6 and 7). Of these, 20 taxa at the species and subspecies levels from 4 genera belonged to the family Brachionidae. Brachionidae was found in 96 samples; the highest number of species for the family was recorded from Nong Han (2012.03.15), with 8 species. The most common species in family Brachionidae was *Platyonus patulus* (found in 28 of the 133 samples), followed by *Brachionus angularis* (25 samples), *Brachionus caudatus*, *Brachionus falcatus*, *Brachionus forficula*, and *Keratella tropica* (22 samples). *Platyonus leloupi* was a new record for Thailand, whereas all other species were already known from the country. Completeness of the survey for this family is demonstrated by the cumulative curve reaching saturation (Figure 5), and by estimates of expected richness matching the observed richness (S) of 20 (Table 8). Yet, the species list for the family, even if representative for the samples I collected, cannot be considered representative for Thailand given that the currently known species for the country is more than twice what I recorded.

In the monogeneric family Lecanidae, 43 taxa at the species and subspecies level were found. Lecanidae was found in 118 samples: the highest number of species for the family was recorded from Bueng Kurod (2011.11.19), with 17 species. The most common species in family Lecanidae was *Lecane bulla* (94 samples), followed by *Lecane leontina* (43 samples), *Lecane curvicornis*, and *Lecane hamata* (42 samples). Eight species, *Lecane abanica*, *Lecane braumi*, *Lecane decipiens*, *Lecane grandis*, *Lecane haliclysta*, *Lecane monostyla*, *Lecane nana*, and *Lecane undulata*, were found only once. All Lecanidae found in this survey were already known from Thailand. In contrast to Brachionidae, the cumulative curve (Figure 5) did not reach saturation. Moreover, the expected number of species, which on average ranged from 45 to 50 depending on the metric (Table 8), was higher than the observed one (S = 43). Yet,

these estimates do not reach the total number of species actually known from the country ($S = 97$).

A total of 26 species complexes were recorded from families Brachionidae and Lecanidae (Table 9). Of these, 9 species 2 subspecies and 3 forms (*Brachionus calyciflorus* f. *amphiceros*, *B. calyciflorus* f. *anuraeiformis*, and *Brachionus quadridentatus* f. *brevispinus*) belonged to family Brachionidae. Seventeen species and 1 subspecies belonged to family Lecanidae. The distribution of each species complex was shown in Table 9.

3.2 Environmental correlates with species richness (S)

Species richness (S) of Brachionidae, but apparently not of Lecanidae, was significantly influenced by some of predictors I tested in the statistical models (Table 10). Gaussian models had better fit than Poisson models, therefore I only showed the results obtained from Gaussian models. Latitude had a positive effect on richness of Brachionidae; temperature had a marginally significant negative effect on richness of Brachionidae; none of the variables seemed to affect species richness (S) of Lecanidae, except for habitat type, albeit not significantly. The effect of none of the other predictor could be supported by the models (Table 10).

3.3 Environmental correlates with species composition

Regarding proportion of explained variance in species composition of Brachionidae, expressed as Jaccard distances, habitat type was the most important variable, explaining 5.5% of the variance in species composition, followed by conductivity with 3.9% (Table 11; Figure 6). All other variables were not significant and explained less than 2% (Table 11). For Lecanidae, habitat type (7.3% of the variance in species composition), conductivity (1.7%), and latitude (1.5%) had an effect on explaining differences in species composition (Table 11; Figure 6).

Table 6. List of rotifers in families Brachionidae and Lecanidae from this study. The sample number were given in corresponding to those in Table 7. (* = new record for Thailand)

Species	Sample numbers
Family Brachionidae	
<i>Brachionus angularis</i> Gosse, 1851	6, 12, 25, 27, 29, 33, 41, 48, 51, 53, 55, 57, 68, 73, 74, 88, 90, 91, 92, 93, 105, 109, 110, 125, 127
<i>Brachionus angularis bidens</i> Plate, 1886	8, 9, 26, 46, 89
<i>Brachionus calyciflorus</i> Pallas, 1766	8, 27, 35, 38, 44, 47, 51, 52
<i>Brachionus caudatus</i> Barrois & Daday, 1894	5, 6, 12, 34, 36, 37, 38, 41, 44, 46, 47, 51, 52, 53, 57, 67, 92, 107, 119, 120, 125, 131
<i>Brachionus dichotomus reductus</i> Koste & Shiel, 1980	6, 51, 67, 119
<i>Brachionus diversicornis</i> (Daday, 1883)	9, 38, 44, 49, 52, 53
<i>Brachionus donneri</i> Brehm, 1951	7, 9, 11, 92, 93, 112
<i>Brachionus falcatus</i> Zacharias, 1898	6, 7, 8, 11, 33, 34, 51, 53, 55, 57, 61, 73, 74, 83, 92, 93, 109, 112, 120, 125, 129, 130
<i>Brachionus forficula</i> Wierzejski, 1891	6, 9, 12, 26, 27, 29, 35, 42, 43, 49, 50, 52, 53, 55, 69, 70, 106, 107, 112, 120, 125, 130
<i>Brachionus kostei</i> Shiel, 1983	71, 72, 109
<i>Brachionus quadridentatus</i> Hermann, 1783	8, 11, 17, 44, 62, 79, 80, 87, 90, 91, 112, 117, 122, 123, 125
<i>Brachionus quadridentatus melheni</i> Barrois & Daday, 1894	10, 15, 18, 22, 23, 28, 36, 47, 54, 55, 73, 74
<i>Brachionus rotundiformis</i> Tschugunoff, 1921	57, 83, 84, 110, 123, 130
<i>Keratella cochlearis</i> (Gosse, 1851)	6, 7, 9, 11, 14, 22, 25, 33, 60, 61, 62, 64, 67, 68, 107, 112, 116, 118, 131
<i>Keratella lenzi</i> Hauer, 1953	16, 109
<i>Keratella tecta</i> (Gosse, 1851)	9, 68
<i>Keratella tropica</i> (Apstein, 1907)	9, 11, 12, 25, 33, 34, 35, 43, 46, 49, 52, 53, 56, 73, 74, 76, 77, 78, 88, 89, 112, 125
<i>Plationus patulus</i> (Müller, 1786)	1, 3, 8, 11, 14, 15, 16, 17, 22, 26, 30, 68, 77, 78, 79, 81, 83, 84, 92, 93, 94, 95, 96, 111, 112, 116, 125, 126
<i>Platyias leloupi</i> Gillard, 1967*	10, 24, 94
<i>Platyias quadricornis</i> (Ehrenberg, 1832)	15, 43, 57, 73, 74, 94, 95, 96, 111, 112, 116
Family Lecanidae	
<i>Lecane abanica</i> Segers, 1994	102
<i>Lecane aculeata</i> (Jakubski, 1912)	69, 88, 102, 106, 113, 131
<i>Lecane acus</i> (Harring, 1913)	43, 133
<i>Lecane arcula</i> Harring, 1914	69, 106
<i>Lecane batillifer</i> (Murray, 1913)	79, 107, 129
<i>Lecane braumi</i> Koste, 1988	60
<i>Lecane bulla</i> (Gosse, 1851)	1, 2, 3, 7, 8, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 36, 37, 38, 39, 40, 43, 44, 54, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 68, 69, 70, 73, 74, 75, 78, 79, 80, 81, 82, 83, 86, 87, 88, 89, 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 106, 108, 109, 110, 111, 112, 113, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 129, 131, 132, 133
<i>Lecane bulla diabolica</i> (Hauer, 1936)	83
<i>Lecane clara</i> (Bryce, 1892)	102, 113, 115, 117, 122
<i>Lecane clostercerca</i> (Schmarda, 1859)	70, 98, 108, 109
<i>Lecane crenata</i> (Harring, 1913)	14, 17, 19, 30, 31, 58, 59, 64, 81, 97, 98
<i>Lecane crepida</i> Harring, 1914	73, 74, 78, 79, 80, 102, 106

Table 6. (continued)

Species	Sample numbers
<i>Lecane curvicornis</i> (Murray, 1913)	3, 8, 10, 11, 15, 19, 22, 23, 24, 26, 27, 28, 32, 38, 43, 57, 60, 61, 63, 69, 70, 73, 74, 75, 83, 86, 87, 88, 89, 94, 95, 96, 101, 102, 106, 108, 109, 112, 115, 125, 129, 133
<i>Lecane decipiens</i> (Murray, 1913)	128
<i>Lecane grandis</i> (Murray, 1913)	110
<i>Lecane haliclysta</i> Harring & Myers, 1926	76
<i>Lecane hamata</i> (Stokes, 1896)	3, 10, 13, 19, 25, 26, 27, 28, 29, 30, 69, 70, 71, 72, 73, 74, 75, 76, 79, 80, 81, 82, 86, 88, 89, 94, 95, 96, 102, 108, 109, 111, 113, 114, 115, 117, 118, 119, 122, 123, 126, 129
<i>Lecane hastata</i> (Murray, 1913)	7, 22, 25, 52, 56, 69, 70, 112
<i>Lecane hornemanni</i> (Ehrenberg, 1834)	75, 102, 129
<i>Lecane lateralis</i> Sharma, 1978	3, 11, 20, 25, 29, 37, 40, 55, 56, 68, 69, 70, 79, 80, 87, 93, 94, 102, 109, 119, 124
<i>Lecane latissima</i> Yamamoto, 1955	17, 98, 102
<i>Lecane leontina</i> (Turner, 1892)	3, 4, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 31, 32, 36, 37, 52, 54, 58, 64, 69, 79, 80, 81, 82, 85, 86, 87, 94, 95, 96, 100, 101, 102, 111, 112, 115, 117, 118, 121, 122, 123, 131
<i>Lecane ludwigii</i> (Eckstein, 1883)	3, 15, 17, 19, 25, 36, 63, 68, 81, 86, 94, 104, 109, 112, 129, 130, 131
<i>Lecane luna</i> (Müller, 1776)	11, 19, 24, 25, 26, 27, 30, 33, 39, 43, 67, 75, 86, 88, 89, 94, 120, 129
<i>Lecane lunaris</i> (Ehrenberg, 1832)	11, 14, 15, 19, 25, 30, 66, 71, 72, 75, 79, 80, 81, 82, 87, 88, 89, 97, 98, 102, 103, 106, 107, 112, 114, 118, 121, 129, 131, 132
<i>Lecane minuta</i> Segers, 1994	115, 117, 122, 126
<i>Lecane monostyla</i> (Daday, 1897)	106
<i>Lecane nana</i> (Murray, 1913)	75
<i>Lecane nitida</i> (Murray, 1913)	3, 14, 16, 17, 18, 22, 25, 30, 60, 64, 94, 102, 133
<i>Lecane obtusa</i> (Murray, 1913)	109, 113, 114, 115, 123
<i>Lecane papuana</i> (Murray, 1913)	4, 6, 11, 15, 21, 22, 25, 29, 45, 47, 49, 50, 52, 55, 71, 72, 79, 80, 83, 84, 86, 87, 95, 96, 101, 110, 113, 116, 124, 125, 126
<i>Lecane punctata</i> (Murray, 1913)	69, 70, 88, 89
<i>Lecane pyriformis</i> (Daday, 1905)	11, 88, 89, 95, 96, 106, 115
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	1, 14, 15, 18, 19, 22, 23, 24, 31, 36, 37, 69, 70, 86, 102, 109, 111, 112, 118, 119, 120, 121, 129, 132, 133
<i>Lecane rhenana</i> Hauer, 1929	30, 102
<i>Lecane rhytida</i> Harring & Myers, 1926	62, 122
<i>Lecane signifera</i> (Jennings, 1896)	3, 14, 15, 17, 18, 19, 23, 25, 30, 32, 36, 58, 70, 71, 72, 73, 81, 82, 86, 87, 94, 95, 96, 98, 101, 102, 106, 117, 118, 121, 130
<i>Lecane stenroosi</i> (Meissner, 1908)	22, 25, 28
<i>Lecane superaculeata</i> Sanoamuang & Segers, 1997	88, 89, 130
<i>Lecane thalera</i> (Harring & Myers, 1926)	28, 88
<i>Lecane undulata</i> Hauer, 1938	108
<i>Lecane unguitata</i> (Fadeev, 1925)	1, 3, 14, 15, 16, 17, 19, 20, 22, 26, 30, 36, 39, 68, 86, 97, 101, 102, 104, 109, 111, 112, 120, 121, 126, 129, 131, 133
<i>Lecane ungulata</i> (Gosse, 1887)	1, 3, 10, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27, 28, 30, 36, 39, 52, 54, 87, 88, 94, 95, 96, 111, 112, 115, 119, 120

Table 7. Physical and chemical factors in each sampling site.

Sample number	Latitude	Longitude	Elevation (m)	pH	Temperature (°C)	Conductivity ($\mu\text{S cm}^{-1}$)	Salinity (ppt)	DO (mg L^{-1})
North								
1. Chiang Saen Lake (2012.05.11), [L]	20.2534	100.0477	378	8.00	30.6	21.3	0.00	6.8
2. Chiang Saen Lake (2015.07.10), [L]	20.2606	100.0470	411	6.45	30.2	32.0	0.01	7.2
3. Kwan Phayao (2012.05.10), [S]	19.1916	99.8583	408	7.28	32.8	154.7	0.10	4.5
4. Kwan Phayao (2015.07.11), [S]	19.1659	99.8960	401	7.12	33.5	229.0	0.09	7.7
5. Huai Kang Reservoir (2015.07.27), [L]	19.1283	99.0636	394	7.63	28.3	146.7	0.10	4.5
6. Mae Kuang Reservoir (2015.07.27), [L]	18.9425	99.1380	364	7.56	28.8	97.3	0.10	7.0
7. Huai Jo Reservoir (2015.07.27), [L]	18.9381	99.0611	397	7.84	28.8	104.0	0.10	7.8
8. Nong Han (2015.07.27), [S]	18.8742	99.0106	310	7.74	29.0	298.3	0.10	4.5
9. Huai Tung Tao Reservoir (2014.12.30), [L]	18.8656	98.9392	352	8.40	24.1	88.8	0.00	7.0
10. Sra Pimtawan (2014.12.30), [P]	18.9008	98.8317	780	7.91	23.4	337.6	0.20	14.5
11. Huaitong Reservoir (2015.07.28), [L]	18.7025	98.5489	997	7.27	23.5	68.9	0.00	5.0
12. Mae Kham Reservoir (2012.05.11), [L]	18.3853	99.7577	361	7.15	33.1	316.9	0.20	6.8
13. Bueng Thungkalo (2012.05.11), [M]	17.5960	100.1623	68	7.02	32.8	215.7	0.10	7.6
Northeast								
14. Nong Khon (2012.05.07), [S]	18.2455	103.1948	163	8.12	29.4	34.8	0.00	5.0
15. Nong Kut Thing (2012.05.07), [S]	18.3171	103.6784	147	7.36	32.9	69.7	0.00	6.5
16. Nong Kut Thing (2015.06.20), [S]	18.3171	103.6788	149	6.28	34.5	168.0	0.06	4.5
17. Bueng Khong Long (2012.05.07), [S]	18.0237	104.0136	162	7.97	30.9	16.6	0.00	7.9
18. Bueng Khong Long (2015.06.20), [S]	18.0237	104.0136	157	5.10	37.1	16.7	0.00	5.2
19. Nong Han ST.1 (2015.06.21), [S]	17.1666	104.1600	154	6.16	31.6	272.0	0.11	3.5
20. Nong Han ST.2 (2015.06.21), [S]	17.2512	104.1720	161	6.82	33.2	175.0	0.07	7.0
21. Thale Buadaeng ST.1 (2012.05.05), [S]	17.2137	103.0371	173	7.20	35.5	522.0	0.20	6.0
22. Thale Buadaeng ST.2 (2012.05.05), [S]	17.1784	103.0573	185	7.63	33.9	430.3	0.20	9.0
23. Thale Buadaeng ST.1 (2015.06.19), [S]	17.2136	103.0370	168	6.09	37.1	841.0	0.32	3.9
24. Thale Buadaeng ST.2 (2015.06.19), [S]	17.1782	103.0572	216	6.62	34.9	468.0	0.18	2.9
25. Srabua Khon Kaen (2012.05.04), [S]	17.1049	102.9361	182	7.88	32.9	291.1	0.10	5.0
26. Srabua Ubonrat (2012.05.04), [M]	16.7714	102.6268	189	7.40	32.4	432.6	0.20	5.3
27. Nong Khot (2012.05.04), [P]	16.4310	102.7947	156	8.01	35.7	466.0	0.20	11.0
28. Nong Wang (2015.06.09), [S]	15.9306	102.2802	229	8.32	34.6	3621.0	1.57	6.8
29. Nong Tabeang (2013.05.15), [S]	14.9386	103.0452	161	7.42	33.0	156.7	0.10	6.2

Table 7. (continued)

Sample number	Latitude	Longitude	Elevation (m)	pH	Temperature (°C)	Conductivity ($\mu\text{S cm}^{-1}$)	Salinity (ppt)	DO (mg L^{-1})
30. Jorakhemak Reservoir (2013.05.15), [L]	14.9113	103.0496	166	7.36	34.4	141.6	0.10	5.0
31. Jorakhemak Reservoir (2015.06.21), [L]	14.9081	103.0536	177	6.95	34.4	215.0	0.08	4.4
32. Thung Laem Reservoir (2013.03.15), [L]	14.6325	102.8310	191	7.78	35.8	151.5	0.10	8.8
33. Nong Bon (2013.05.16), [S]	14.2927	102.7388	249	7.70	35.4	184.1	0.10	7.3
34. Sra Fountain Tree (2013.01.01), [P]	14.6571	101.4542	329	6.70	26.0	688.0	0.30	4.6
West								
35. Nong Luang (2015.07.25), [S]	16.8983	99.1219	108	9.52	29.2	495.0	0.20	7.8
Central								
36. Bueng Si Phi (2012.05.12), [S]	16.4276	100.3333	46	7.23	33.0	201.3	0.10	5.2
37. Bueng Si Phi (2015.06.09), [S]	16.4260	100.3438	95	7.75	35.6	361.0	0.14	3.6
38. Bueng Sanat (2015.06.10), [S]	15.7290	100.1497	72	7.08	33.7	405.0	0.10	3.1
39. Bueng Borapet (2012.05.12), [S]	15.7146	100.1778	35	7.36	35.0	480.0	0.20	7.2
40. Bueng Borapet (2013.05.19), [S]	15.7128	100.1770	37	7.60	35.5	491.0	0.20	5.0
41. Bueng Thepo (2013.05.19), [S]	15.3769	100.0631	23	7.70	38.1	234.4	0.10	7.2
42. Bueng Thepo (2015.07.25), [S]	15.3769	100.0631	16	9.65	32.9	243.3	0.10	7.2
43. Bueng Chawak (2015.05.28), [S]	14.9333	100.0437	24	6.83	32.0	220.0	0.09	5.9
44. Sra Silapacheep (2015.01.02), [P]	14.1528	100.5200	7	7.99	27.2	597.0	0.30	12.0
45. Nong Phuttha HP (2013.01.04), [P]	13.7989	100.2883	4	7.21	30.9	2210.0	1.10	4.2
46. Nong Phuttha (2013.01.05), [P]	13.7705	100.3157	8	7.54	28.7	444.1	0.20	7.5
47. Bang Kruai (2013.01.05), [M]	13.8034	100.3490	11	7.52	28.2	667.0	0.30	4.9
East								
48. Bang Phra Reservoir (2012.07.29), [L]	13.2058	100.9885	30	7.07	31.2	182.4	0.10	7.0
49. Nong Kho Reservoir (2012.07.29), [L]	13.1441	101.0313	66	6.95	31.7	155.4	0.10	10.0
50. Nong Klang Dong Reservoir (2012.07.29), [L]	13.0378	101.0168	60	6.78	32.7	162.4	0.10	6.5
51. Bangphi (2012.07.29), [M]	12.7782	101.0205	59	6.91	31.2	136.6	0.10	8.5
52. Dok Krai Reservoir (2012.07.30), [L]	12.8875	101.2108	60	7.02	29.1	215.9	0.10	8.0
53. Nong Pla Lai Reservoir (2012.07.30), [L]	12.9420	101.2466	48	6.77	28.5	237.2	0.10	5.0
54. Nong Thasipheth (2012.07.30), [S]	12.6914	101.3234	12	6.96	28.4	111.8	0.10	3.3
55. Khao Nguang Chang Reservoir (2012.07.30), [L]	12.7957	101.3814	112	6.90	28.5	117.3	0.10	5.5

Table 7. (continued)

Sample number	Latitude	Longitude	Elevation (m)	pH	Temperature (°C)	Conductivity ($\mu\text{S cm}^{-1}$)	Salinity (ppt)	DO (mg L^{-1})
56. Samet Reservoir (2012.08.01), [L]	12.5657	101.4526	20	7.46	29.1	91.3	0.00	7.0
57. Sra Anodad (2012.08.01), [P]	12.5693	101.4648	3	6.89	29.0	713.0	0.30	0.5
58. Botanical garden ST.1 (2015.07.01), [PS]	12.6466	101.5511	23	5.47	31.3	201.0	0.08	4.6
59. Botanical garden ST.2 (2015.07.01), [PS]	12.6410	101.5516	11	5.79	32.1	96.0	0.04	6.9
60. Botanical garden ST.3 (2015.07.01), [PS]	12.6520	101.5362	8	5.82	31.7	120.0	0.05	1.3
61. Khao Chuk Reservoir (2012.08.01), [L]	12.8592	101.7650	44	6.99	30.2	65.2	0.00	7.0
62. Pru Kung Krabaen (2012.08.01), [PS]	12.6102	101.9203	14	6.40	28.7	433.7	0.20	1.5
63. Nong Bua Chanthaburi (2012.08.02), [P]	12.5467	102.1221	13	7.35	27.8	67.3	0.00	5.0
64. Kirithan Reservoir (2012.08.04), [L]	12.7379	102.3596	211	7.85	27.6	31.5	0.00	9.5
65. Koh Chang Reservoir (2012.08.03), [L]	12.0556	102.2992	10	7.45	29.7	25.0	0.00	6.0
66. Aosalad Reservoir (2014.07.12), [L]	11.6911	102.5781	92	7.06	29.2	28.2	0.00	8.0
67. Sra Klong Had (2012.08.04), [P]	13.4015	102.3063	185	7.00	31.6	191.6	0.10	10.0
68. Nong Nonsa-at (2012.08.05), [M]	13.7354	102.4561	49	7.07	29.1	170.8	0.10	3.5
South								
69. Sra Klongbod (2012.04.23), [P]	10.8982	99.4310	19	6.64	32.4	325.1	0.20	8.0
70. Sra Klongbod (2012.10.29), [P]	10.8982	99.4310	19	7.41	32.6	160.9	0.10	13.0
71. Nong WatZaitong (2012.04.24), [M]	10.5597	99.2717	5	6.84	29.0	187.9	0.10	2.0
72. Nong WatZaitong (2012.10.30), [M]	10.5597	99.2717	5	7.75	27.5	129.4	0.10	1.5
73. Pru Ching (2012.04.24), [P]	10.5677	99.2637	13	6.75	29.2	88.9	0.00	2.0
74. Pru Ching (2012.10.30), [P]	10.5677	99.2637	13	7.50	27.1	90.4	0.00	3.8
75. Nong Bua Chumphon (2012.04.24), [P]	9.8998	99.1511	2	6.94	34.6	284.3	0.10	6.0
76. Nong Bua Chumphon (2012.10.30), [P]	9.8998	99.1511	2	7.67	32.8	283.0	0.10	9.0
77. Sra Khanthulee (2012.04.24), [P]	9.6714	99.1562	10	7.01	34.2	65.7	0.00	5.0
78. Sra Khanthulee (2012.10.30), [P]	9.6714	99.1562	10	7.42	33.4	98.2	0.00	8.0
79. Srabou Khanom (2012.04.25), [P]	9.2768	99.8318	10	7.03	30.6	471.0	0.20	5.0
80. Srabou Khanom (2012.10.31), [P]	9.2768	99.8318	10	7.40	27.2	359.9	0.20	2.2
81. Nong Sichon (2012.04.25), [S]	8.9950	99.9120	9	7.60	33.6	237.4	0.10	6.0
82. Nong Sichon (2012.10.31), [S]	8.9950	99.9120	9	7.78	32.0	441.9	0.20	10.2
83. Nong Baansrabua (2012.04.25), [PS]	8.6256	99.9392	13	7.90	36.7	96.8	0.10	8.0
84. Nong Baansrabua (2012.10.31), [PS]	8.6256	99.9392	13	7.49	39.1	253.2	0.10	5.2
85. Thale Noi ST.1 (2013.06.18), [L]	7.7699	100.1336	6	7.66	29.6	177.9	0.10	2.0

Table 7. (continued)

Sample number	Latitude	Longitude	Elevation (m)	pH	Temperature (°C)	Conductivity ($\mu\text{S cm}^{-1}$)	Salinity (ppt)	DO (mg L^{-1})
86. Thale Noi ST.2 (2014.07.26), [L]	7.7890	100.1293	1	6.98	26.3	380.5	0.20	6.0
87. Thale Noi ST.3 (2014.11.02), [L]	7.7853	100.1729	11	7.05	28.7	1525.0	0.80	5.8
88. Nong Pakbung (2012.04.26), [P]	7.8836	100.3452	10	7.56	30.9	1008.0	0.50	4.0
89. Nong Pakbung (2012.11.01), [P]	7.8836	100.3452	10	7.80	30.5	2412.0	1.20	6.5
90. Sra Pangtree (2012.04.26), [P]	7.7060	100.3864	13	7.48	33.0	4630.0	2.40	6.0
91. Sra Pangtree (2012.11.01), [P]	7.7060	100.3864	13	8.12	32.0	5120.0	2.70	13.0
92. Bueng Baanwatmai (2012.04.26), [P]	7.4232	100.4618	19	7.49	33.4	451.1	0.20	5.0
93. Bueng Baanwatmai (2012.11.01), [P]	7.4232	100.4618	19	8.27	33.3	312.6	0.10	6.8
94. Srabua PSU (2016.04.27), [P]	7.0094	100.5061	30	5.84	32.6	131.0	0.10	3.4
95. Buengbua Natab (2012.04.26), [M]	7.0920	100.6787	15	6.67	31.2	320.7	0.10	2.0
96. Buengbua Natab (2012.11.01), [M]	7.0920	100.6787	15	7.54	31.5	507.0	0.30	3.5
97. Nong Klong Kapur (2011.11.19), [S]	9.7941	98.5930	22	8.12	29.1	22.6	0.00	6.0
98. Nong Klong Kapur (2012.03.13), [S]	9.7941	98.5930	22	8.65	32.8	69.2	0.00	6.0
99. Srabua Kapur (2011.11.18), [P]	9.5741	98.5886	10	8.05	30.5	4149.0	2.20	8.0
100. Khaowong Reservoir (2011.11.19), [L]	9.3096	98.3923	3	9.06	28.6	41.5	0.00	10.0
101. Khaowong Reservoir (2012.03.14), [L]	9.3096	98.3923	3	8.68	29.7	39.5	0.00	8.0
102. Bueng Kurod (2011.11.19), [P]	9.0891	98.4431	28	8.61	32.9	28.9	0.00	6.5
103. Bueng Kurod (2012.03.14), [P]	9.0891	98.4431	28	8.28	34.5	25.1	0.00	6.0
104. Nong Taymueng (2011.11.20), [S]	8.3961	98.2505	7	7.11	30.4	1568.0	0.70	7.0
105. Nong Taymueng (2012.03.15), [S]	8.3961	98.2505	7	6.99	30.6	3663.0	1.70	6.2
106. Pru Jaeson (2011.11.21), [PS]	8.1595	98.3008	22	7.01	31.8	93.5	0.00	7.6
107. Pru Jaeson (2012.03.15), [PS]	8.1595	98.3008	22	8.07	33.5	89.2	0.00	7.0
108. Pru Jik (2011.11.21), [PS]	8.1447	98.3009	17	6.88	29.6	101.3	0.10	3.0
109. Pru Mai Khao (2011.11.21), [PS]	8.1298	98.3012	12	6.93	30.5	978.0	0.50	3.0
110. Pru Mai Khao (2012.03.15), [PS]	8.1298	98.3012	12	7.22	36.6	5640.0	2.40	6.0
111. Nong Han (2011.11.20), [P]	7.7820	98.3107	10	8.24	31.5	246.0	0.10	7.5
112. Nong Han (2012.03.15), [P]	7.7820	98.3107	10	8.14	32.0	319.8	0.20	12.5
113. Nong Klong Muang (2011.11.22), [P]	8.0457	98.7635	3	6.91	26.7	2737.0	1.40	7.8
114. Nong Klong Muang (2012.03.16), [P]	8.0457	98.7635	3	7.52	28.0	2123.0	1.00	2.0
115. Nong Baanlaempho (2011.11.22), [P]	8.0269	98.8747	9	7.00	26.3	192.3	0.10	9.3
116. Nong Baanlaempho (2012.03.16), [P]	8.0269	98.8747	9	7.26	27.7	163.2	0.10	1.7

Table 7. (continued)

Sample number	Latitude	Longitude	Elevation (m)	pH	Temperature (°C)	Conductivity ($\mu\text{S cm}^{-1}$)	Salinity (ppt)	DO (mg L^{-1})
117. Nong Banthamapraw (2011.11.22), [P]	7.7351	99.1772	6	5.43	28.9	493.0	0.20	7.0
118. Nong Banthamapraw (2012.03.16), [P]	7.7351	99.1772	6	7.14	31.2	455.2	0.20	6.0
119. Srabua Rajamangala (2011.11.23), [M]	7.5288	99.3091	9	7.28	27.5	404.0	0.20	6.2
120. Srabua Rajamangala (2012.03.16), [M]	7.5288	99.3091	9	7.41	32.9	503.0	0.20	3.0
121. Nong Klong Lamchan (2014.07.26), [S]	7.5300	99.7546	41	8.66	28.9	45.8	0.00	9.0
122. Nong Ban Chu Pa (2011.11.22), [M]	7.3624	99.5171	15	3.98	30.6	308.6	0.10	7.5
123. Nong Ban Chu Pa (2012.03.16), [M]	7.3624	99.5171	15	6.29	34.7	250.0	0.10	5.0
124. Nong Nachumhed (2011.11.23), [M]	7.2045	99.5621	7	7.14	28.9	408.0	0.20	15.6
125. Nong Nachumhed (2012.03.17), [M]	7.2045	99.5621	7	7.44	31.8	386.2	0.20	3.5
126. Nong Thungsabo (2011.12.20), [M]	7.0284	99.6753	10	7.11	26.9	277.8	0.10	3.5
127. Sra Lalita Sabo (2015.01.17), [P]	7.0211	99.6775	6	7.48	27.9	3604.0	1.90	5.1
128. Bulone (2015.02.19), [P]	6.8278	99.5367	38	7.13	41.5	108.8	0.10	7.0
129. Nong Pan Ya (2011.12.20), [P]	6.8360	99.7865	16	7.11	29.8	225.8	0.10	5.0
130. Nong Pan Ya (2012.03.17), [P]	6.8360	99.7865	16	7.55	34.1	236.1	0.10	4.0
131. Huai Yiao Reservoir (2011.12.20), [L]	6.7688	99.8855	18	7.68	30.5	44.1	0.10	4.0
132. Huai Yiao Reservoir (2012.03.17), [L]	6.7688	99.8855	18	7.97	33.4	68.2	0.00	6.0
133. Nong Prakpraya (2015.01.17), [S]	6.7417	100.0425	18	5.81	26.4	74.6	0.00	1.3

The letters in [] show types of habitats: L-lake; M-marsh; P-pond; PS- peat swamp; and S-swamp

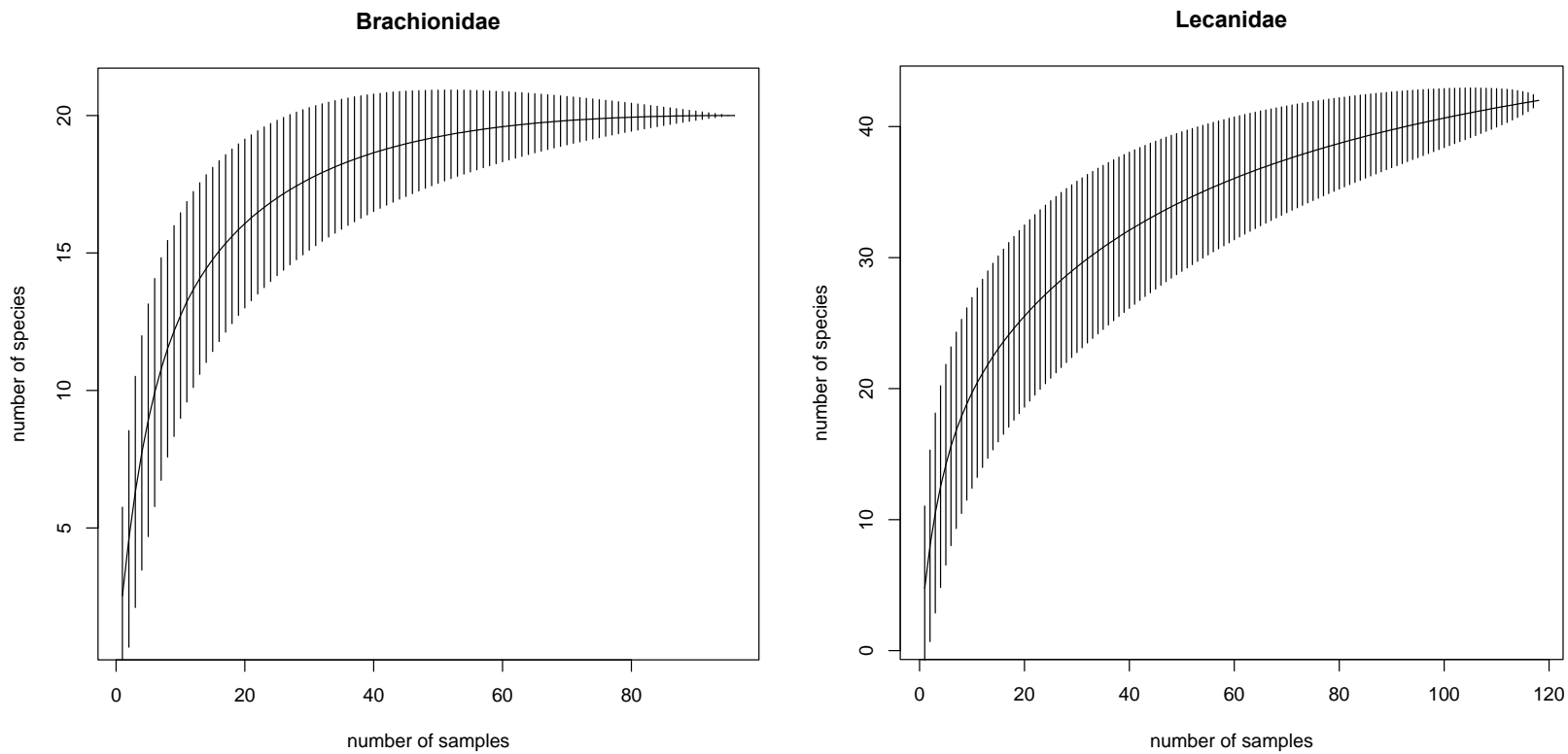


Figure 5. Curves of the cumulative number of rotifer species in relation to the number of samples for Brachionidae and Lecanidae.

Table 8. Estimates of the expected number of species according to three different metrics: Chao, first order jackknife, and bootstrap (Colwell and Coddington, 1994). Average values \pm standard errors are provided. The numbers known in Thailand are from Sa-ardrit *et al.* (2013).

Metric	Brachionidae	Lecanidae
Chao	20.0 \pm 0.0	48.3 \pm 5.8
jackknife	20.0 \pm 0.0	49.9 \pm 2.8
bootstrap	20.4 \pm 0.6	45.9 \pm 1.8
Observed (S)	20	43
known in Thailand	44	97

Table 9. Species complexes of families Brachionidae and Lecanidae and their distribution in Thailand from this study. (N-North, NE-Northeast, W-West, C-Central, E-East, and S-South)

Species complex	Region					
	N	NE	W	C	E	S
Family Brachionidae						
1 <i>Brachionus angularis</i> Gosse, 1851	+	+		+	+	+
<i>Brachionus angularis bidens</i> Plate, 1886	+	+		+		+
2 <i>Brachionus calyciflorus</i> Pallas, 1766 (included <i>B. calyciflorus</i> f. <i>amphicerus</i> ; <i>B. calyciflorus</i> f. <i>anuraeiformis</i>)	+	+	+	+	+	
3 <i>Brachionus caudatus</i> Barrois & Daday, 1894	+	+		+	+	+
4 <i>Brachionus quadridentatus</i> Hermann, 1783 (included <i>B. quadridentatus</i> f. <i>brevispinus</i>)	+	+		+	+	+
<i>Brachionus quadridentatus melheni</i> Barrois & Daday, 1894	+	+		+	+	+
5 <i>Brachionus rotundiformis</i> Tschugunoff, 1921					+	+
6 <i>Keratella cochlearis</i> (Gosse, 1851)	+	+			+	+
7 <i>Keratella lenzi</i> Hauer, 1953		+				+
8 <i>Keratella tecta</i> (Gosse, 1851)	+				+	
9 <i>Keratella tropica</i> (Apstein, 1907)	+	+	+	+	+	+
Family Lecanidae						
10 <i>Lecane acus</i> (Harring, 1913)				+		+
11 <i>Lecane bulla</i> (Gosse, 1851) <i>Lecane bulla diabolica</i> (Hauer, 1936)	+	+		+	+	+
12 <i>Lecane closterocerca</i> (Schmarda, 1859)						+
13 <i>Lecane crenata</i> (Harring, 1913)		+			+	+
14 <i>Lecane curvicornis</i> (Murray, 1913)	+	+		+	+	+
15 <i>Lecane hamata</i> (Stokes, 1896)	+	+				+
16 <i>Lecane hornemanni</i> (Ehrenberg, 1834)						+
17 <i>Lecane leontina</i> (Turner, 1892)	+	+		+	+	+
18 <i>Lecane ludwigii</i> (Eckstein, 1883)	+	+		+	+	+
19 <i>Lecane luna</i> (Müller, 1776)	+	+		+	+	+
20 <i>Lecane lunaris</i> (Ehrenberg, 1832)	+	+			+	+
21 <i>Lecane nitida</i> (Murray, 1913)	+	+			+	+
22 <i>Lecane obtusa</i> (Murray, 1913)						+
23 <i>Lecane quadridentata</i> (Ehrenberg, 1830)	+	+		+		+
24 <i>Lecane signifera</i> (Jennings, 1896)	+	+		+	+	+
25 <i>Lecane unguitata</i> (Fadeev, 1925)	+	+		+	+	+
26 <i>Lecane ungulata</i> (Gosse, 1887)	+	+		+	+	+

Table 10. Results of the statistical tests (generalized liner models with Gaussian error) with estimates, standard errors and p values, to test the significance of the effect of the predictor variables on species richness of Brachionidae and of Lecanidae in Thailand, together with the relative-importance values (RI) on the same models from multimodel averaging. Significant values are reported in bold.

Variable	Brachionidae			Lecanidae		
	F	p	RI	F	p	RI
Habitat type	1.24	0.292	0.16	2.07	0.086	0.42
Latitude	4.59	0.034	0.65	0.31	0.576	0.34
pH	0.89	0.346	0.36	0.42	0.517	0.31
Temperature	3.77	0.054	0.64	0.28	0.593	0.26
Conductivity	0.20	0.652	0.31	1.54	0.216	0.41
Dissolved Oxygen	0.27	0.601	0.26	0.07	0.786	0.28

Table 11. Results of the permutational multivariate analysis of variance using distance matrices (*adonis*), with a matrix of Jaccard distances in species composition between samples as response variable and the scaled transformed (when needed) limnological measurements and explanatory variables. F, R^2 and p values are reported; significant values are reported in bold.

Variable	Brachionidae			Lecanidae		
	F	R^2	p	F	R^2	p
Habitat type	1.40	0.055	0.032	1.81	0.073	0.001
Latitude	1.15	0.011	0.296	1.89	0.015	0.024
pH	1.52	0.015	0.092	1.30	0.011	0.196
Temperature	1.69	0.017	0.056	1.19	0.010	0.266
Conductivity	3.98	0.039	0.001	2.10	0.017	0.012
Dissolved Oxygen	1.59	0.016	0.082	0.53	0.004	0.925
Residuals		0.847			0.870	

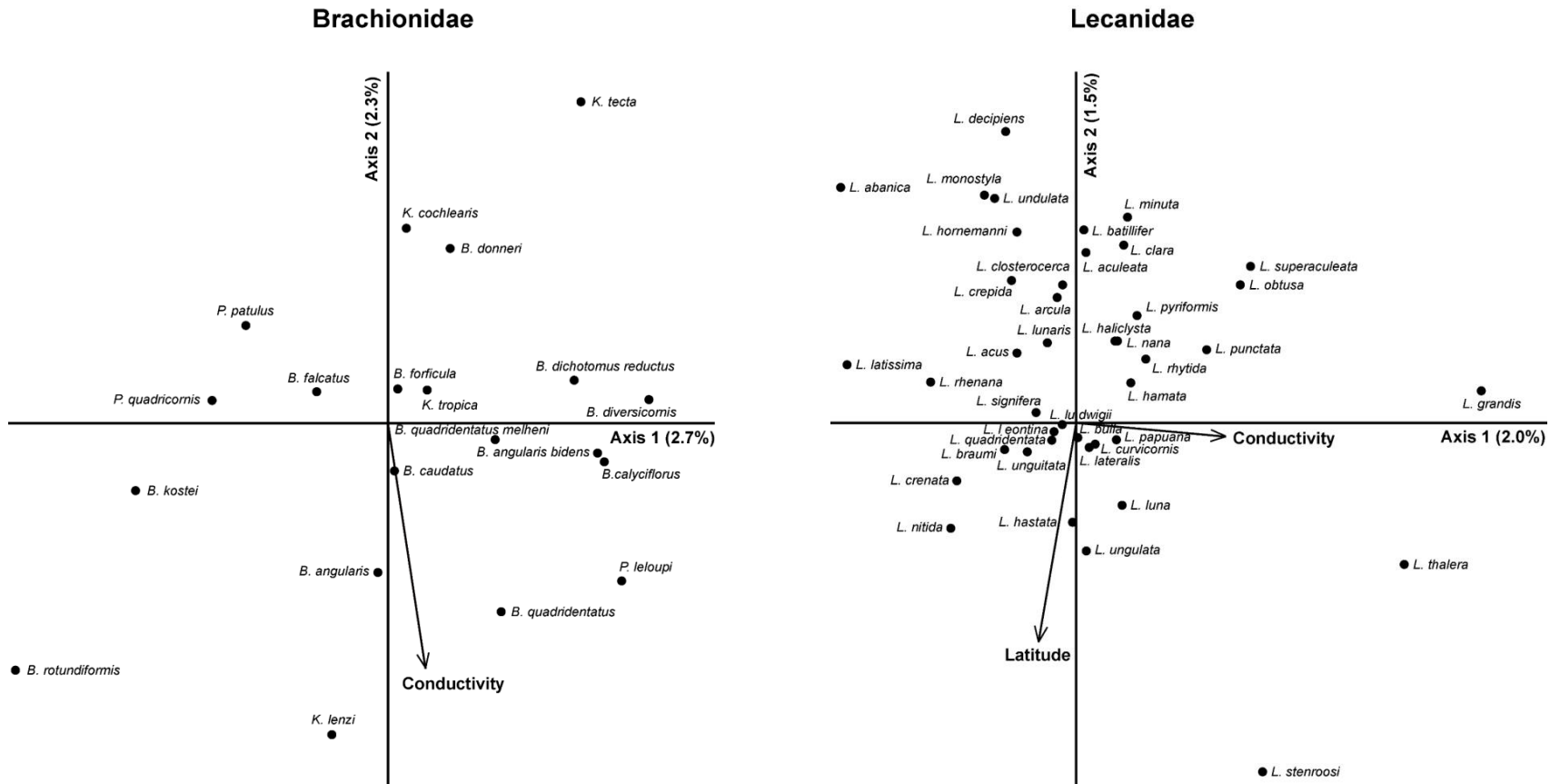


Figure 6. Plot of the first two axes of the Canonical Correspondence Analysis (CCA) between rotifer species and environmental factors for Brachionidae and Lecanidae. The first and second axes of the CCA include the explained percentage of the variability in species occurrence. Black circles represent species and arrow lines indicate environmental factors. The length of each line reflects the strength of its effect. Habitat type was not included because it is a categorical and not a continuous variable.

3.4 Phylogenetic tree of Family Brachionidae

Sixty-seven sequences were obtained from COI (659 bp unambiguous alignment) and 46 sequences from ITS1 (553 bp aligned, including gaps) for the family Brachionidae (Table 12). COI sequences were obtained from *Brachionus angularis*, *Brachionus calyciflorus*, *Brachionus caudatus*, *Brachionus diversicornis*, *Brachionus falcatus*, *Brachionus forficula*, *Brachionus quadridentatus*, *Keratella cochlearis*, *Keratella tropica*, *Plationus patulus*, and *Platyias leloupi*, while ITS1 ones were obtained from *B. angularis*, *B. calyciflorus*, *B. caudatus*, *B. quadridentatus*, *K. cochlearis*, and *K. tropica*. Sixty-seven haplotypes of COI and 33 haplotypes of ITS1 were analyzed. *Lecane bulla* was outgroup for phylogenetic tree reconstruction. For ITS1, 33 haplotypes were obtained from 26 individuals. Because animals could be heterozygous in their ITS1 sequences, all the copies were included in the analyses. Thus, of the 26 individuals, 19 had only 1 copy of ITS1 and 7 had 2 copies. Species clade support values within phylogenetic tree topologies from Bayesian analyses (BI) of COI and ITS1 markers were higher than maximum likelihood analyses (ML) (Figures 7–10). Species complexes, *B. angularis*, *B. calyciflorus*, *B. caudatus*, *B. quadridentatus*, *K. cochlearis*, and *K. tropica*, based on BI have high species clade support value with ≥ 0.93 and ≥ 0.77 posterior probabilities for COI and ITS1, respectively (Figures 8 and 10). *Brachionus angularis* and *B. caudatus* were sister group. These clades were connected with high support values (77% bootstrap and 0.99 posterior probability for COI; 100% bootstrap and 1 posterior probability for ITS1) (Figures 7–10). The bar at the bottom of the figure is the unit of branch length (nucleotide substitutions per site).

Table 12. Sequences of COI and ITS1 markers of family Brachionidae obtained from this study. Numbers of haplotypes are indicated in parentheses.

Species	Number of sequences			
	COI		ITS1	
	Thai	GenBank	Thai	GenBank
<i>Brachionus angularis</i>	2 (2)		2 (2)	
<i>Brachionus calyciflorus</i>	2 (2)	9 (9)	6 (5)	3 (3)
<i>Brachionus caudatus</i>	2 (2)		7 (4)	
<i>Brachionus diversicornis</i>	1 (1)	5 (5)		
<i>Brachionus falcatus</i>	1 (1)	1 (1)		
<i>Brachionus forficula</i>	2 (2)			
<i>Brachionus quadridentatus</i>	7 (7)	9 (9)	12 (8)	
<i>Keratella cochlearis</i>	3 (3)	9 (9)	10 (6)	
<i>Keratella tropica</i>	3 (3)	3 (3)	4 (3)	2 (2)
<i>Plationus patulus</i>	2 (2)	5 (5)		
<i>Platyias leloupi</i>	1 (1)			

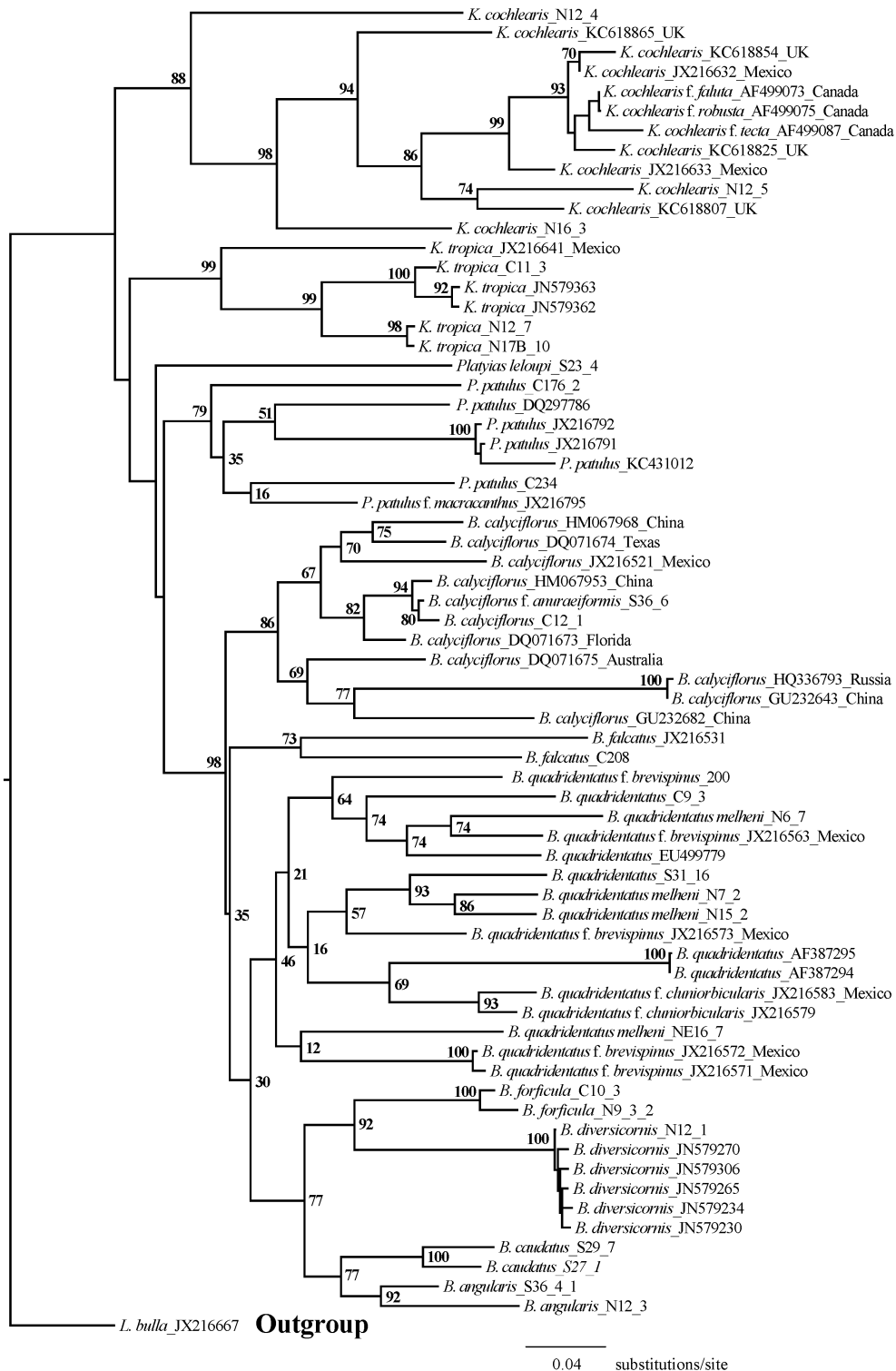


Figure 7. Phylogenetic tree inferred from COI haplotypes of family Brachionidae based on maximum likelihood analysis with bootstrap support values at the nodes.

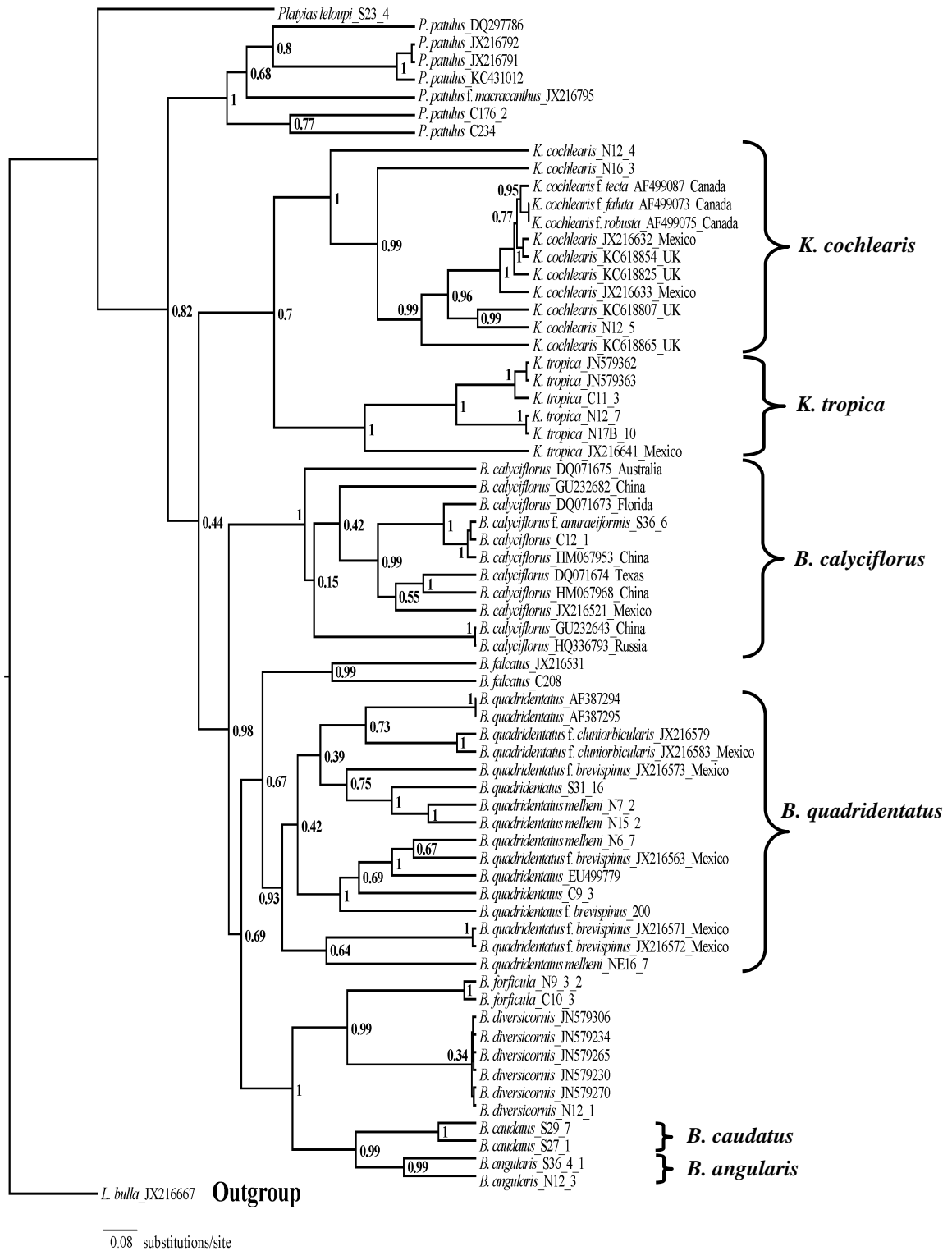


Figure 8. Phylogenetic tree inferred from COI haplotypes of family Brachionidae based on Bayesian analysis with posterior probabilities at the nodes.

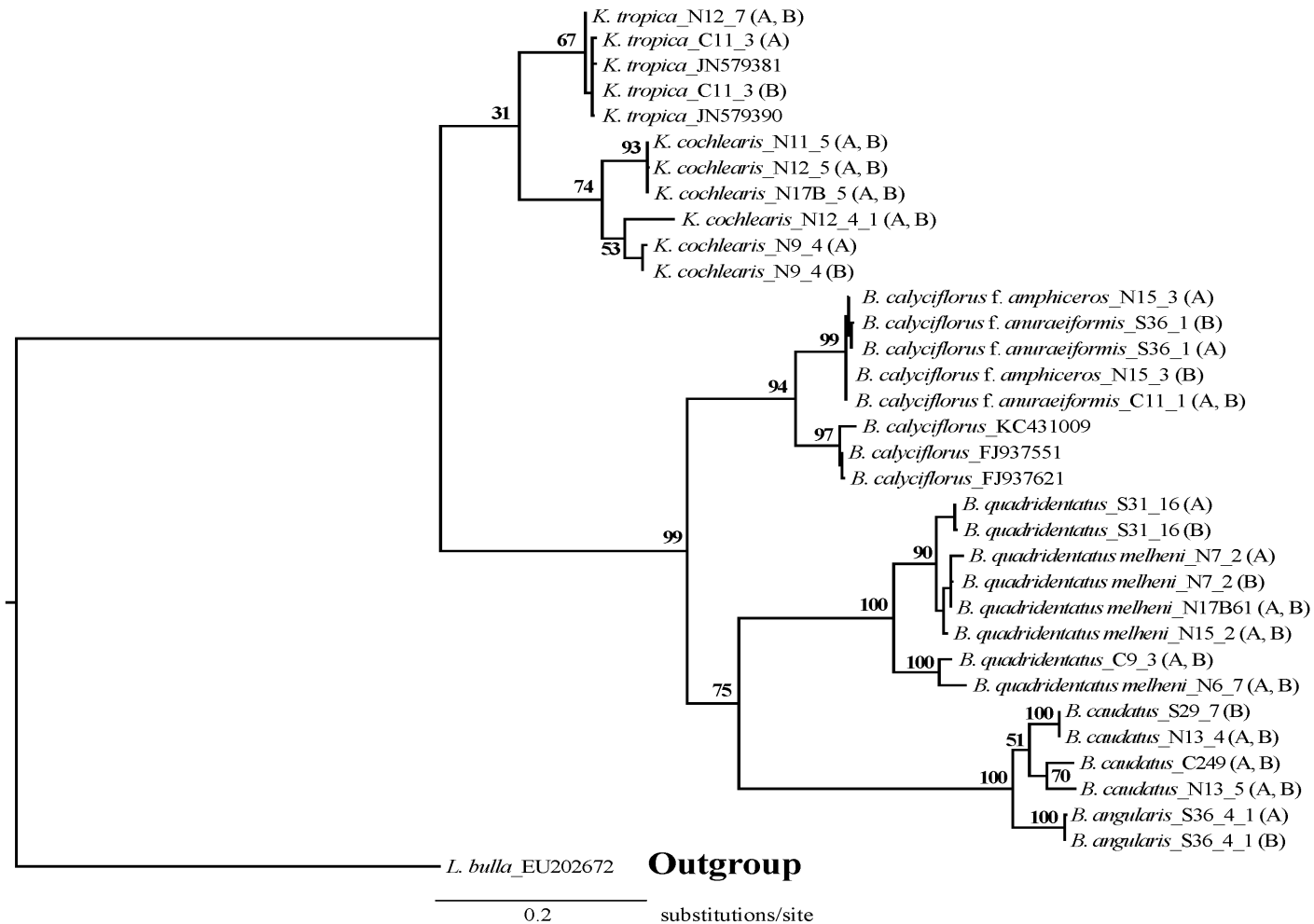


Figure 9. Phylogenetic tree inferred from ITS1 haplotypes of family Brachionidae based on maximum likelihood analysis with bootstrap support value at the nodes.

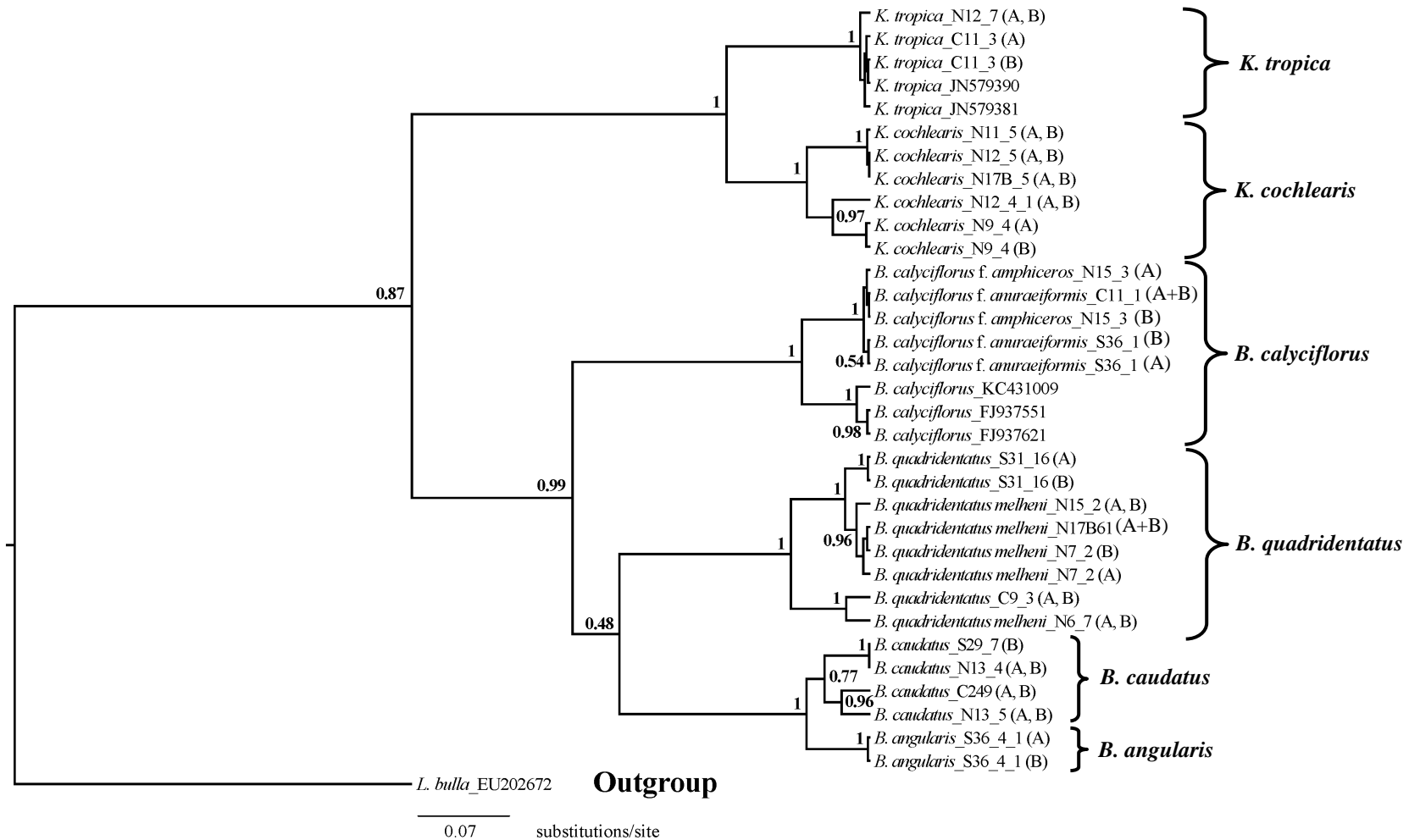


Figure 10. Phylogenetic tree inferred from ITS1 haplotypes of family Brachionidae based on Bayesian analysis with posterior probabilities at the nodes.

3.5 Phylogenetic analyses of Family Lecanidae

Two hundred and thirty-eight sequences were obtained from COI (661 bp unambiguous alignment) and 135 sequences from ITS1 (602 bp aligned, including gaps) for the family Lecanidae (Table 13). COI sequences were obtained from *Lecane acus*, *Lecane bulla*, *Lecane closterocerca*, *Lecane crenata*, *Lecane crepida*, *Lecane curvicornis*, *Lecane halsei*, *Lecane halyclista*, *Lecane hamata*, *Lecane lateralis*, *Lecane leontina*, *Lecane ludwigii*, *Lecane luna*, *Lecane lunaris*, *Lecane nitida*, *Lecane papuana*, *Lecane quadridentata*, *Lecane signifera*, *Lecane unguitata*, and *Lecane* sp., while ITS1 sequences were obtained from *L. bulla*, *L. curvicornis*, *L. halsei*, *L. leontina*, *L. ludwigii*, *L. luna*, *L. signifera*, and *L. unguitata*. One hundred and forty-eight haplotypes of COI and 104 haplotypes of ITS1 were analyzed. For ITS, 104 haplotypes were obtained from 77 individuals. Because animals could be heterozygous in their ITS1 sequences, all the copies were included in the analyses. Thus, of the 77 individuals, 48 had only 1 copy of ITS1 and 29 had 2 copies. Phylogenetic tree topologies from maximum likelihood (ML) and Bayesian analyses (BI) of COI markers were similar. *Lecane closterocerca*, *L. curvicornis*, *L. ludwigii*, *L. lunaris*, *L. nitida*, and *L. signifera*, have high species clades supports with $\geq 91\%$ bootstrap support and 1 posterior probability (Figures 11–12). *Lecane quadridentata* was grouped within *L. bulla* clade. These species clades were connected with 70% bootstrap support and 0.99 posterior probability (Figures 11–12). Phylogenetic tree topologies showed non-monophyly for the *Lecane leontina* complex, which had representatives in more than one clade. Species clade supports from ML (100% bootstrap supports) were higher than BI (≥ 0.64 posterior probabilities). *Lecane acus* and *L. crenata* were grouped within *L. lunaris* clade with 100% bootstrap support and 1 posterior probability. For ITS1 marker, phylogenetic tree topologies from maximum likelihood and Bayesian analyses were congruent (Figures 13–14). *Lecane curvicornis*, *L. leontina*, *L. ludwigii*, *L. luna*, and *L. signifera* clades have strong support values with 100% bootstrap support and 1 posterior probability. As for *Lecane bulla* clade, tree topology from Bayesian analysis (BI) shows higher support value than maximum likelihood (ML) analysis (0.94 posterior probability from BI and 62% bootstrap support from ML) (Figures 13–14). *Lecane*

halsei was a sister group of *L. ludwigii* and clades were connected with 76% bootstrap support and 0.97 posterior probability.

Table 13. Sequences of COI and ITS1 markers of family Lecanidae obtained from this study. Numbers of haplotypes were indicated in parentheses. *Australian specimens were obtained from Australian sediments which were cultured in this study.

Species	Number of sequences					
	COI			ITS1		
	Thai	*Australia	GenBank	Thai	*Australia	GenBank
<i>Lecane acus</i>	1 (1)					
<i>Lecane bulla</i>	79 (54)	2 (2)	12 (8)	94 (70)	4 (3)	13 (13)
<i>Lecane closterocerca</i>	1 (1)		4 (2)			
<i>Lecane crenata</i>	2 (2)					
<i>Lecane crepida</i>	1 (1)		7 (4)			
<i>Lecane curvicornis</i>	16 (10)		4 (2)	2 (2)		
<i>Lecane halsei</i> *		1 (1)			2 (1)	
<i>Lecane halyclista</i>	4 (2)					
<i>Lecane hamata</i>	4 (3)		2 (2)			
<i>Lecane lateralis</i>	5 (4)			2 (2)		
<i>Lecane leontina</i>	10 (5)		7 (2)	4 (3)		
<i>Lecane ludwigii</i>	2 (2)			4 (3)		
<i>Lecane luna</i>	6 (3)		12 (6)	4 (2)		
<i>Lecane lunaris</i>	9 (4)		11 (2)			
<i>Lecane nitida</i>	7 (4)					
<i>Lecane papuana</i>	2 (2)		6 (3)			
<i>Lecane quadridentata</i>	4 (3)		4 (2)			
<i>Lecane signifera</i>	6 (6)			4 (4)		
<i>Lecane unguitata</i>	6 (4)			2 (1)		
<i>Lecane</i> sp.	1 (1)					

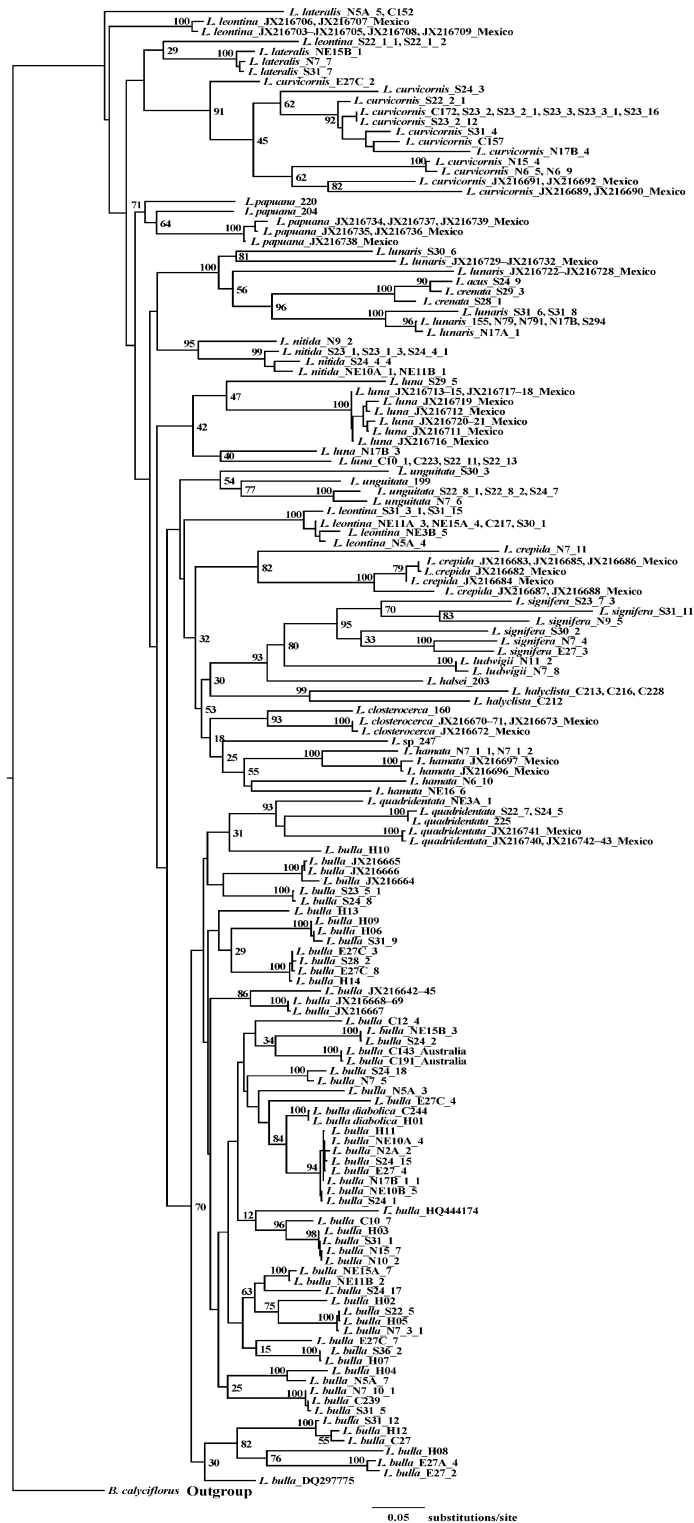


Figure 11. Phylogenetic tree inferred from COI haplotypes of family Lecanidae based on maximum likelihood analysis with bootstrap support values at the nodes.

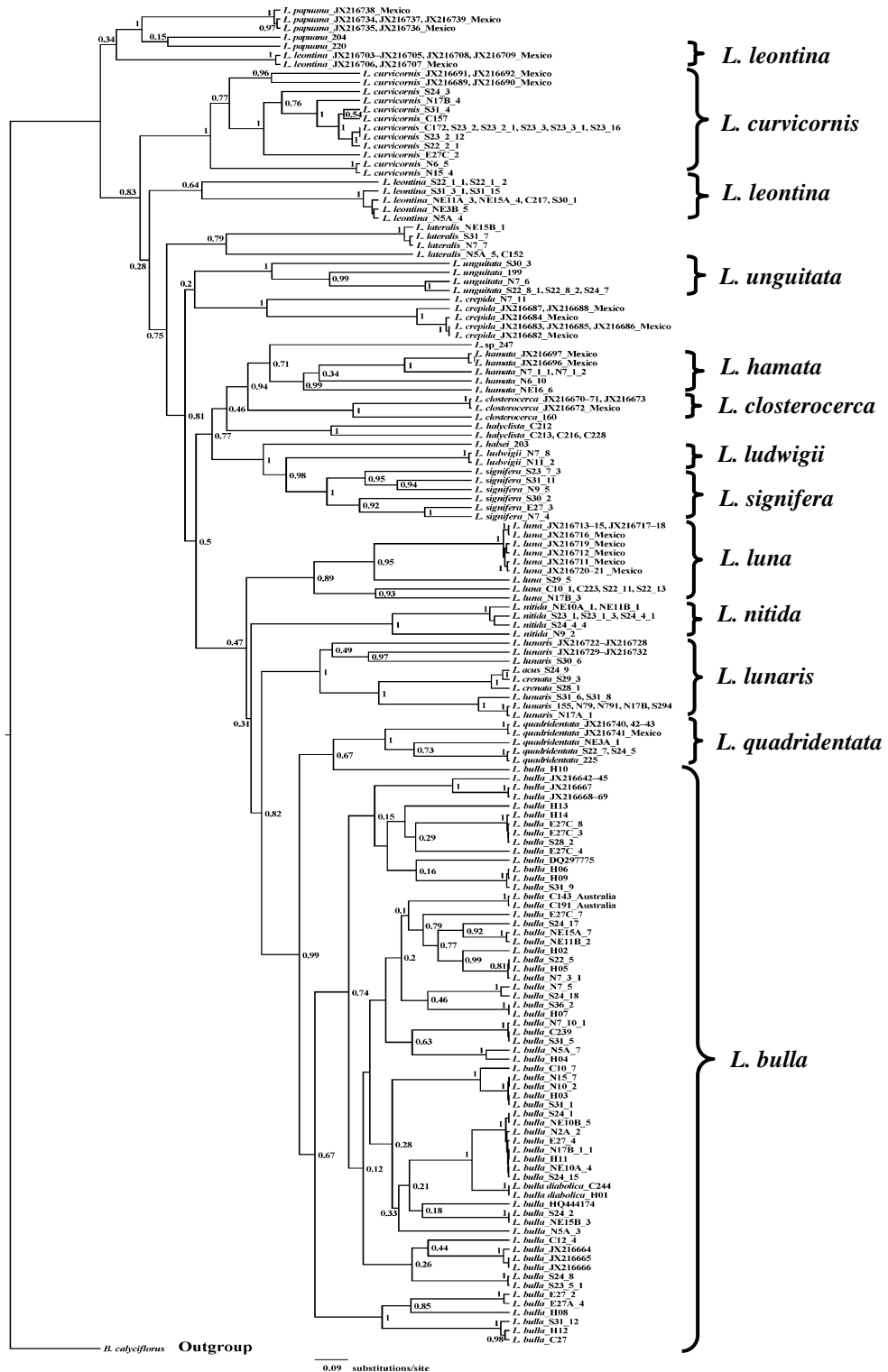


Figure 12. Phylogenetic tree inferred from COI haplotypes of family Lecanidae based on Bayesian analysis with posterior probabilities at the nodes.

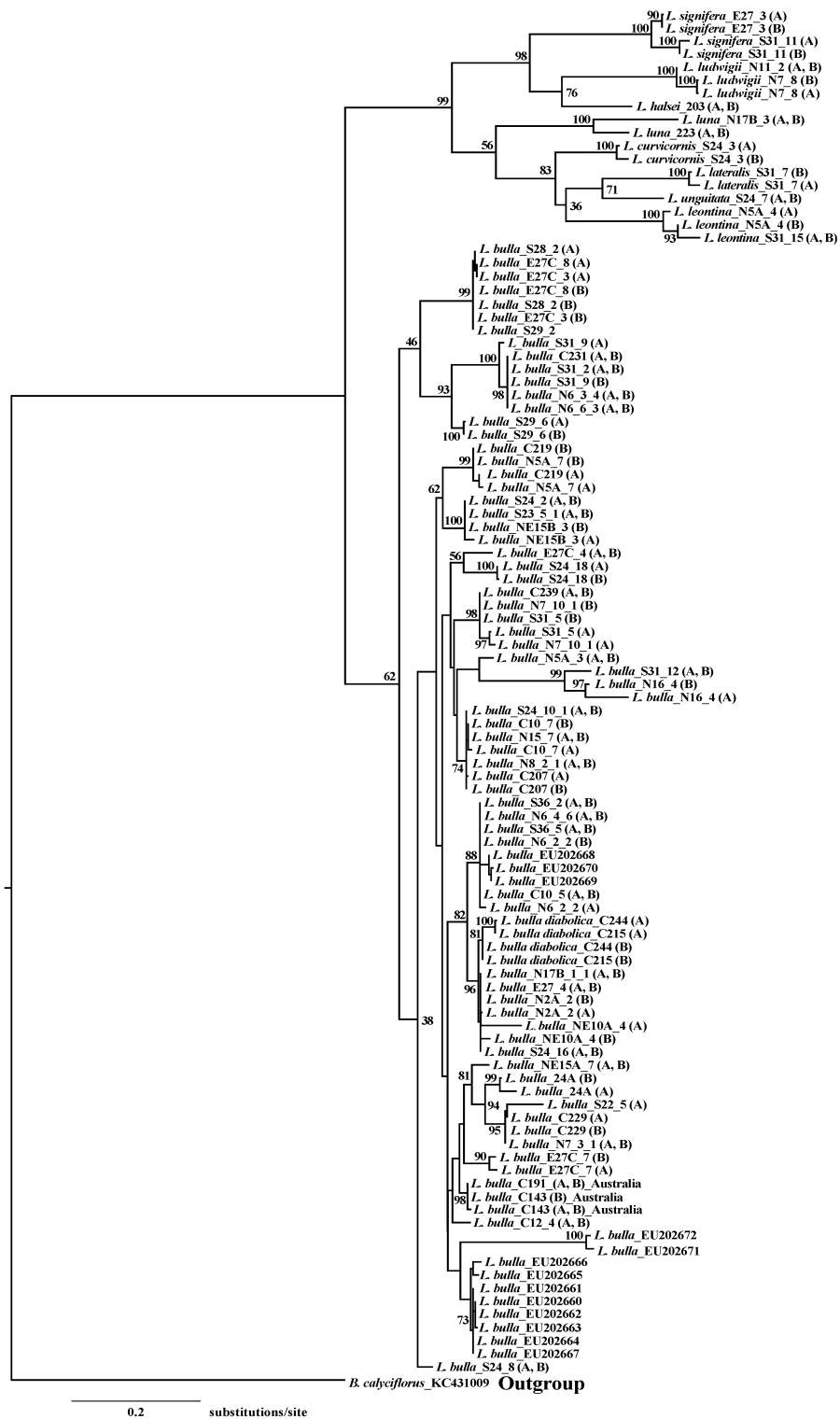


Figure 13. Phylogenetic tree inferred from ITS1 haplotypes of family Lecanidae based on maximum likelihood analysis with bootstrap support values at the nodes.

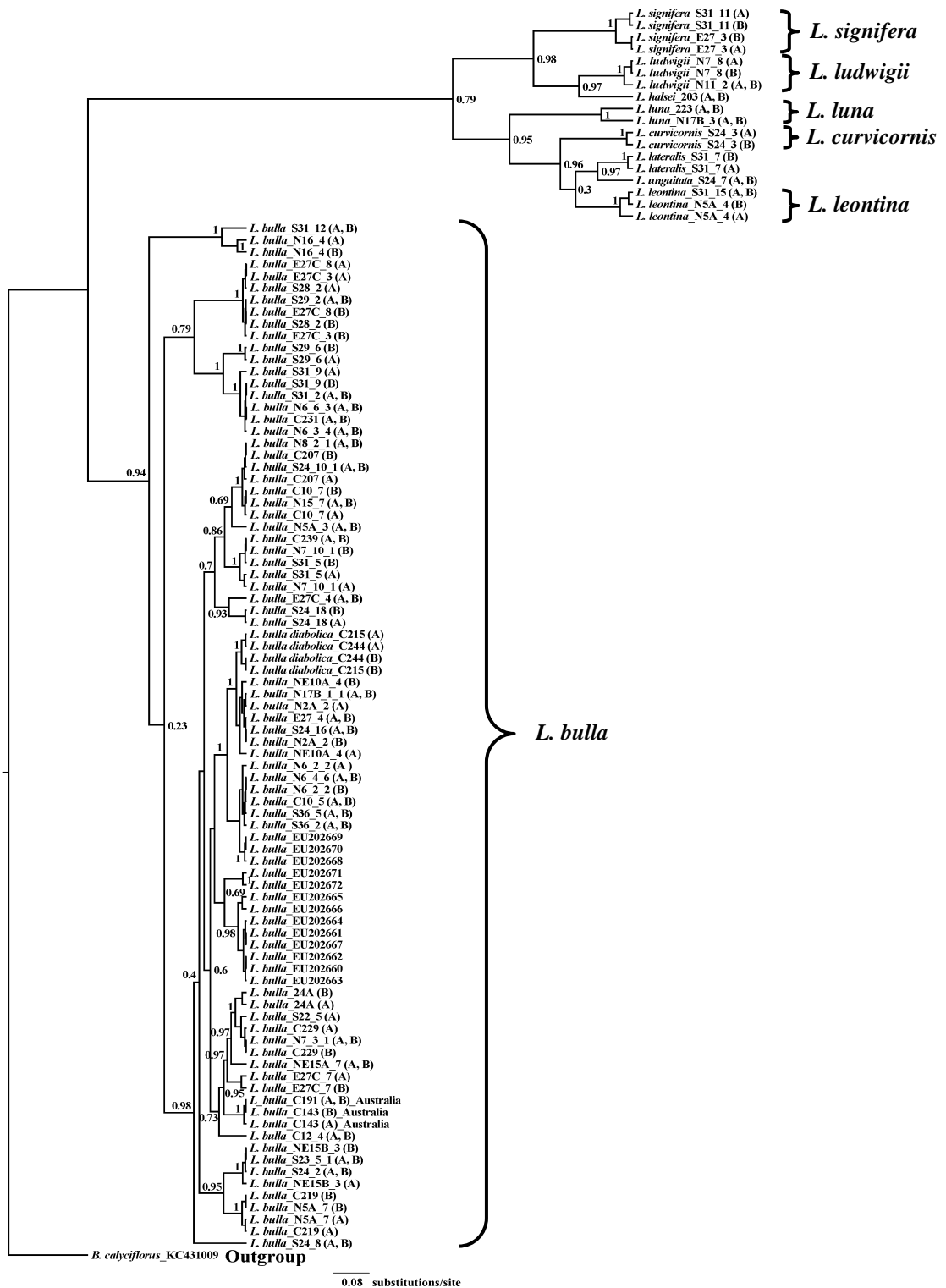


Figure 14. Phylogenetic tree inferred from ITS1 haplotypes of family Lecanidae based on Bayesian analysis with posterior probabilities at the nodes.

3.6 DNA taxonomy and species delimitation

Pairwise genetic distances calculated from COI marker ranged from 1.06% to 20.49% in family Brachionidae and 0.15% to 20.58% in family Lecanidae (Table 14). As for ITS1, genetic distances ranged from 0.21% to 27.73% in family Brachionidae and highly varied 0.21% to 33.73% in family Lecanidae. Species delimitation was estimated using DNA taxonomy approaches, ABGD, PTP, and GMYC (Tables 15 and 16). The species delimitation within complexes was mainly performed on COI haplotypes. The ITS1 marker was used for supporting existence of cryptic species within the species complex. In family Brachionidae, the estimate number of cryptic species from COI marker was congruent among three methods (Table 15). *Brachionus quadridentatus* is the complex that shows the highest level of species diversity with at least 13 species. The estimated numbers within Thailand are shown in parentheses in Table 15. The results from COI and ITS1 showed that the highest species diversity in Thailand is within *Brachionus quadridentatus* (7 species), followed by *Keratella cochlearis* (3 species), *Brachionus angularis*, *Brachionus caudatus*, and *Keratella tropica* (2 species), respectively (Table 15). For ITS1, the estimated numbers of species showed conflicts in some species complexes. The estimated numbers of species in the complexes of *Brachionus calyciflorus*, *B. caudatus*, *B. quadridentatus*, *K. cochlearis*, and *K. tropica* were in the ranges of 1–3 species, 2–3 species, 2–6 species, 3–5 species, and 1–3 species, respectively. In *Brachionus quadridentatus* complex, *B. quadridentatus*, *B. quadridentatus melheni*, and *B. quadridentatus* f. *brevispinus* were found in Thailand. Genetic distances within the complex are 9.26%–18.21% for COI and 0.22%–8.52% for ITS1. In *B. calyciflorus* complex, *B. calyciflorus*, *B. calyciflorus* f. *amphiceros* and *B. calyciflorus* f. *anuraeiformis* were found from this study. Genetic distances within the complex are 1.86% for COI and 0.23%–7.23% for ITS1.

In monogeneric family Lecanidae, the highest estimated number of species from COI in Thailand was *Lecane bulla* (26–29 species), followed by *Lecane curvicornis* (7–9 species), and *Lecane signifera* (6 species), respectively (Table 16). For ITS1, the estimated numbers of species showed conflicts in some species complexes. The estimated numbers of species in the complexes of *L. bulla*, *L. curvicornis*, and *L. signifera* were in the ranges of 20–40 species, 1–2 species, and 2–4 species, respectively. In *Lecane bulla* complex, *L. bulla* and *L. bulla diabolica* were found from this study. Genetic

distances within the complex are 0.15%–18.45% for COI and 0.23%–33.73% for ITS1. *Lecane nitida* was already known splitting from *L. curvicornis* and the result indicated 2–4 species for COI within *L. nitida*. Genetic distances within *L. curvicornis* ranged from 1.66% to 17.40% for COI and were 1.21% for ITS1. Within *Lecane nitida*, genetic distance varied from 4.08% to 11.95% for COI. As for *Lecane lunaris* complex, *L. acus*, *L. crenata*, and *L. lunaris* were shown in same clade. Genetic distance varied from 1.06% to 20.12% within the lineage of *L. lunaris* for COI (Table 14). No sequence of *L. lunaris* was obtained from ITS1.

Table 14. Percentage of genetic distances (pairwise distance) calculated from two genetic markers within the examined species complexes. (– no sequence was obtained in this study)

Species complex	COI	ITS1
Family Brachionidae		
<i>Brachionus angularis</i>	9.86	0.21 – 27.73
<i>Brachionus calyciflorus</i>	1.86	0.23 – 7.23
<i>Brachionus caudatus</i>	6.98	0.65 – 4.75
<i>Brachionus quadridentatus</i>	9.26 – 18.21	0.22 – 8.52
<i>Keratella cochlearis</i>	17.75 – 20.49	0.21 – 7.33
<i>Keratella tropica</i>	1.06 – 11.08	0.41 – 1.02
Family Lecanidae		
<i>Lecane bulla</i>	0.15 – 18.45	0.23 – 33.73
<i>Lecane crenata</i>	6.18	–
<i>Lecane curvicornis</i>	1.66 – 17.40	1.21
<i>Lecane hamata</i>	17.55 – 18.31	–
<i>Lecane leontina</i>	1.21 – 18.18	0.21 – 28.30
<i>Lecane ludwigii</i>	1.36	0.21 – 0.42
<i>Lecane luna</i>	14.67 – 18.46	0.93 – 25.91
<i>Lecane lunaris</i>	1.06 – 20.12	–
<i>Lecane nitida</i>	4.08 – 11.95	–
<i>Lecane quadridentata</i>	0.76 – 17.85	–
<i>Lecane signifera</i>	10.29 – 20.58	0.22 – 16.93
<i>Lecane unguitata</i>	5.60 – 18.00	–

Table 15. Summary of numbers of species estimated from this study using 3 DNA taxonomy methods for each species complex within family Brachionidae based on COI and ITS1 markers. DNA sequences from this study and GenBank were included in the analysis. Only Thai specimens were indicated in parentheses. (ML–Maximum likelihood; BI–Bayesian analysis; coal–coalescent)

Species	COI											ITS1										
	Haplo type	A B G D	PTP						GMYC			Haplo type	A B G D	PTP						GMYC		
			RAxML		BEAST				RAx ML	BEAST				RAxML		BEAST				RAx ML	BEAST	
			ML	BI	coal		yule			coal	yule			ML	BI	coal		yule			coal	yule
					ML	BI	ML	BI								ML	BI	ML	BI			
<i>Brachionus angularis</i> Gosse, 1851	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
<i>Brachionus calyciflorus</i> Pallas, 1766	11 (2)	8 (1)	9 (1)	9 (1)	8 (1)	8 (1)	8 (1)	8 (1)	9 (1)	9 (1)	9 (1)	8 (5)	2 (1)	2 (1)	2 (1)	3 (1)	3 (1)	3 (1)	3 (1)	6 (3)	3 (1)	3 (1)
<i>Brachionus caudatus</i> Barrois and Daday, 1894	2 (2)	1 (1)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	4 (4)	2 (2)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
<i>Brachionus quadridentatus</i> Hermann, 1783	16 (7)	13 (7)	14 (7)	14 (7)	14 (7)	14 (7)	14 (7)	14 (7)	14 (7)	14 (7)	14 (7)	8 (8)	2 (2)	3 (3)	3 (3)	4 (4)	4 (4)	5 (5)	5 (5)	6 (6)	5 (5)	5 (5)
<i>Keratella cochlearis</i> (Gosse, 1851)	12 (3)	6 (3)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)	6 (6)	3 (3)	3 (3)	4 (4)	3 (3)	3 (3)	3 (3)	3 (3)	5 (5)	3 (3)	3 (3)
<i>Keratella tropica</i> (Apstein, 1907)	6 (3)	3 (2)	3 (2)	3 (2)	4 (2)	4 (2)	4 (2)	4 (2)	3 (2)	3 (2)	3 (2)	5 (3)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	5 (3)	2 (2)	2 (2)

Table 16. Summary of numbers of species estimated from this study using 3 DNA taxonomy methods for each species complex within family Lecanidae based on COI and ITS1 markers. DNA sequences from this study and GenBank were included in the analysis. Only Thai specimens were indicated in parentheses. (ML–Maximum likelihood; BI–Bayesian analysis; coal–coalescent; – no sequence was obtained in this study)

Species	COI											ITS1										
	Haplo type	A B G D	PTP						GMYC			Haplo type	A B G D	PTP						GMYC		
			RAxML		BEAST				RAx ML	BEAST				RAxML		BEAST				RAx ML	BEAST	
			ML	BI	coal		yule			coal	yule			ML	BI	coal		yule			coal	yule
					ML	BI	ML	BI								ML	BI	ML	BI			
<i>Lecane bulla</i> (Gosse, 1851)	64 (56)	32 (26)	32 (27)	32 (27)	33 (29)	33 (29)	33 (29)	33 (29)	37 (26)	32 (26)	32 (26)	87 (72)	27 (22)	37 (33)	35 (31)	26 (23)	20 (20)	30 (24)	32 (25)	76 (40)	25 (22)	25 (22)
<i>Lecane closterocerca</i> (Schmarda, 1859)	3 (1)	2 (1)	2 (1)	2 (1)	2 (1)	2 (1)	2 (1)	2 (1)	3 (1)	2 (1)	2 (1)	–	–	–	–	–	–	–	–	–	–	–
<i>Lecane crenata</i> (Harring, 1913)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	–	–	–	–	–	–	–	–	–	–	–
<i>Lecane curvicornis</i> (Murray, 1913)	12 (10)	9 (7)	10 (8)	11 (9)	9 (7)	9 (7)	9 (7)	9 (7)	10 (7)	9 (7)	9 (7)	2 (2)	1 (1)	1 (1)	1 (1)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	1 (1)	1 (1)
<i>Lecane hamata</i> (Stokes, 1896)	5 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	5 (3)	4 (3)	4 (3)	–	–	–	–	–	–	–	–	–	–	–
<i>Lecane leontina</i> (Turner, 1892)	7 (5)	4 (3)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	8 (4)	5 (4)	5 (4)	3 (3)	3 (3)	3 (3)	3 (3)	2 (2)	2 (2)	3 (3)	3 (3)	3 (3)	2 (2)	2 (2)
<i>Lecane ludwigii</i> (Eckstein, 1883)	2 (2)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	1 (1)	1 (1)	3 (3)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	3 (3)	1 (1)	1 (1)
<i>Lecane luna</i> (Müller, 1776)	9 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (4)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
<i>Lecane lunaris</i> (Ehrenberg, 1832)	3 (3)	5 (3)	5 (3)	5 (3)	5 (3)	5 (3)	5 (3)	5 (3)	5 (3)	5 (3)	5 (3)	–	–	–	–	–	–	–	–	–	–	–
<i>Lecane nitida</i> (Murray, 1913)	4 (4)	2 (2)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	–	–	–	–	–	–	–	–	–	–	–
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	5 (4)	–	–	–	–	–	–	–	–	–	–	–
<i>Lecane signifera</i> (Jennings, 1896)	6 (6)	6 (6)	6 (6)	6 (6)	6 (6)	6 (6)	6 (6)	6 (6)	6 (6)	6 (6)	6 (6)	4 (4)	2 (2)	2 (2)	3 (3)	2 (2)	2 (2)	2 (2)	2 (2)	4 (4)	2 (2)	2 (2)
<i>Lecane unguitata</i> (Fadeev, 1926)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)

3.7 Molecular and morphological analyses to discover cryptic species of *Lecane bulla*

Because the largest number of sequences, both COI and ITS1 markers, were obtained from *Lecane bulla* complex, this species was further analyzed to estimate the number of species within the complex, and to confront results using molecular versus morphological analyses.

1) Molecular analysis using DNA taxonomy

Species estimates based on COI showed congruence among all methods with 23 species in total (Table 17). Of these, 22 species were from Thai specimens, while 1 species was from Australia. For ITS1, species estimates varied between 21–52 species. The number estimates from RAxML with BI in PTP and GMYC were overestimated compared with other methods, while ABGD method of ITS1 showed lower estimate than other methods. However, the minimum number of estimate of *Lecane bulla* is 21 species with 20 of them from Thailand and 1 from Australia. Since ITS1 sequences of *L. bulla* are heterozygous, the haplotype networks of this gene were created to show allele sharing and evidence of gene flow in *L. bulla* (Figure 15). *Lecane bulla diabolica* was included in this complex. The groups are those connected by the dashed lines and shown in circles (Figure 15).

Table 17. Results of DNA taxonomy method for species delimitation of *Lecane bulla*.

Thai specimens were shown in parentheses.

DNA taxonomy method	Input data/ tree		Number of species		
			COI	ITS1	
ABGD	Alignment		23 (22)	21 (20)	
PTP	RAxML	ML	23 (22)	22 (21)	
		BI	23 (22)	27 (26)	
	BEAST	Coal.	ML	23 (22)	22 (21)
			BI	23 (22)	22 (21)
		Yule	ML	23 (22)	22 (21)
			BI	23 (22)	22 (21)
GMYC	RAxML		23 (22)	52 (51)	
	BEAST	Coal.	23 (22)	22 (21)	
		Yule	23 (22)	22 (21)	

2) Morphological analysis

The group of species within *Lecane bulla* was defined based on molecular results, COI and ITS1 markers. The analysis showed the significant characters among species, such as ventral lorica width, ventral lorica length, dorsal lorica length, foot pseudosegment width, foot pseudosegment length, maximum toe width, toe length, terminal fissure length, pseudoclaw length ($p < 0.001$), head aperture ventral depth ($p = 0.001$), and head aperture width ($p = 0.01$) (Table 18; Figure 16). The correlation among each character was shown in Figure 17. The ratio of some characters showed significant difference between species of *L. bulla*, such as ventral lorica width/ventral lorica length, toe length/ terminal fissure length, ventral lorica length/toe length ($p < 0.001$), and foot pseudosegment width/foot pseudosegment length ($p = 0.002$). The results of the landmark and landmark sliding were shown in Figures 18 and 19.

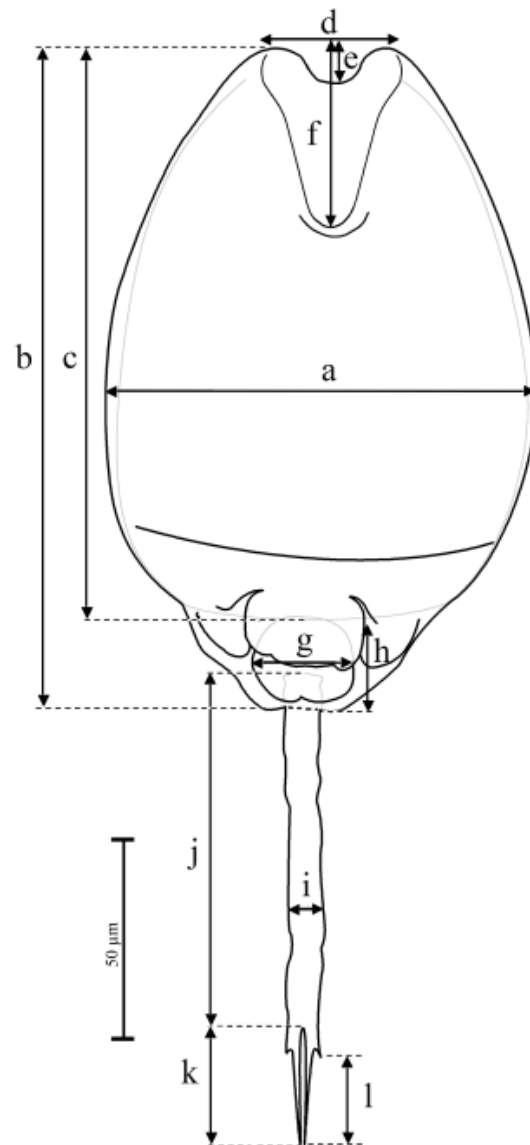


Figure 16. Morphological character for measurements of *Lecane bulla*.

- | | |
|--------------------------------|------------------------------|
| a. ventral lorica width | g. foot pseudosegment width |
| b. ventral lorica length | h. foot pseudosegment length |
| c. dorsal lorica length | i. maximum toe width |
| d. head aperture width | j. toe length |
| e. head aperture dorsal depth | k. terminal fissure length |
| f. head aperture ventral depth | l. pseudoclaw length |

Table 18. Analysis of Variance among morphological measurements of *Lecane bulla*. (significant codes: 0.001 ‘***’, 0.01 ‘**’, and 0.05 ‘*’)

Characters	Df	Sum Sq	Mean Sq	F value	Pr (>F)
ventral lorica width	23	8724.6	379.33	27.88	3.519e-15 ***
ventral lorica length	23	23135.3	1005.88	37.802	< 2.2e-16 ***
dorsal lorica length	23	15279.2	664.31	20.332	3.499e-13 ***
head aperture width	23	1894.6	82.375	2.2626	0.0165 *
head aperture dorsal depth	23	356.83	15.514	1.4276	0.1736
head aperture ventral depth	23	1317.36	57.276	3.1928	0.00131 **
foot pseudosegment width	23	543.12	23.6138	21.166	1.963e-13 ***
foot pseudosegment length	23	702.86	30.5591	21.001	2.196e-13 ***
maximum toe width	23	57.578	2.50339	17.721	2.476e-12 ***
toe length	23	14507.2	630.75	55.721	< 2.2e-16 ***
terminal fissure length	23	686.35	29.8414	6.6915	7.833e-07 ***
pseudoclaw length	23	585.08	25.4381	4.7636	3.202e-05 ***
ventral lorica width/ventral lorica length	23	0.0706	0.00307	3.668	0.000395 ***
head aperture width/head aperture ventral depth	23	1.293	0.05621	1.042	0.449
foot pseudosegment width/foot pseudosegment length	23	0.7221	0.0314	2.881	0.00298 **
toe length/terminal fissure length	23	13.670	0.5943	16.11	9.42e-12 ***
terminal fissure length/pseudoclaw length	23	0.3186	0.01385	1.17	0.336
ventral lorica length/toe length	23	9.094	0.3954	19.91	4.72e-13 ***

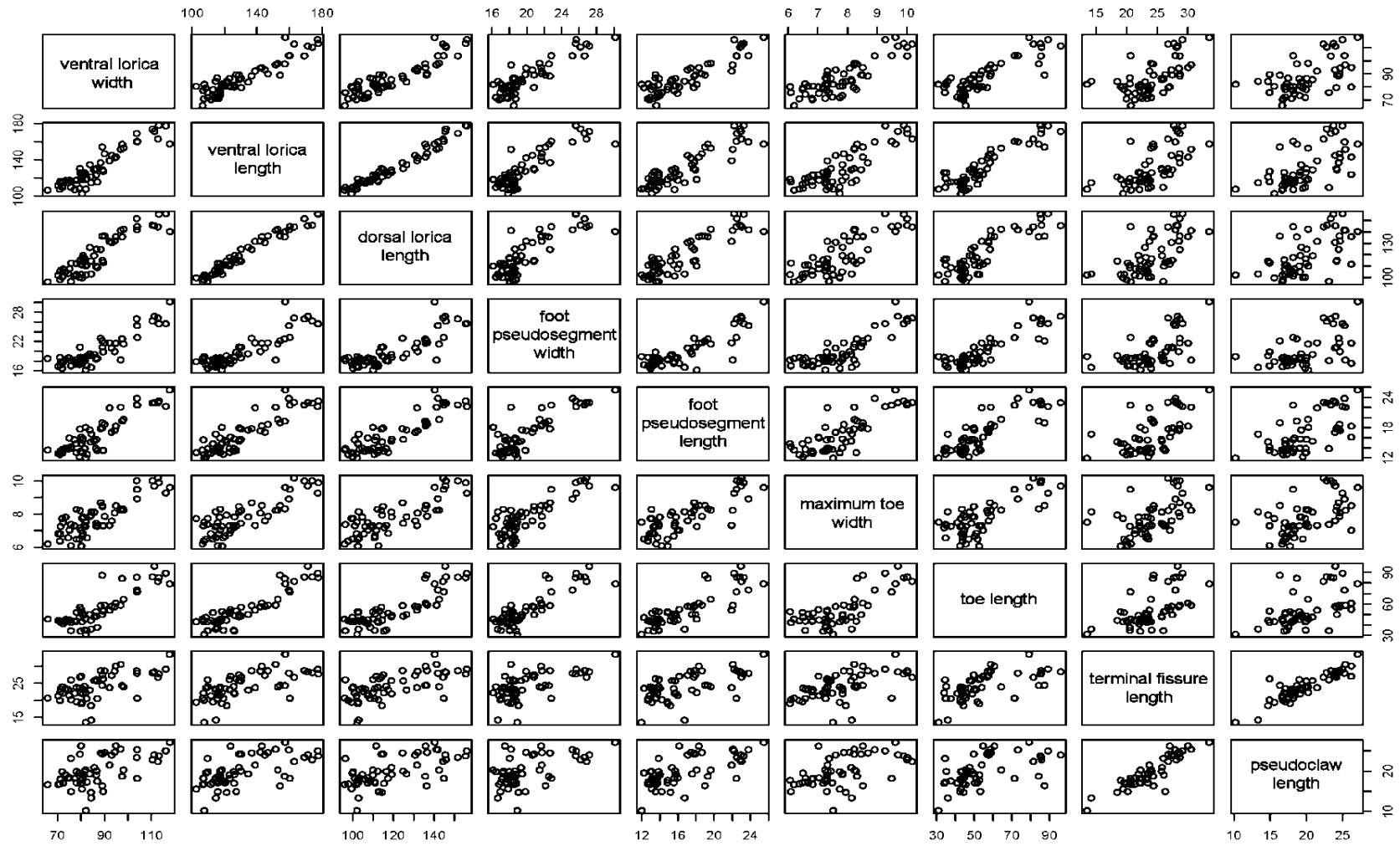


Figure 17. Graphs show correlation among each morphological character ($r^2 > 0.8$).

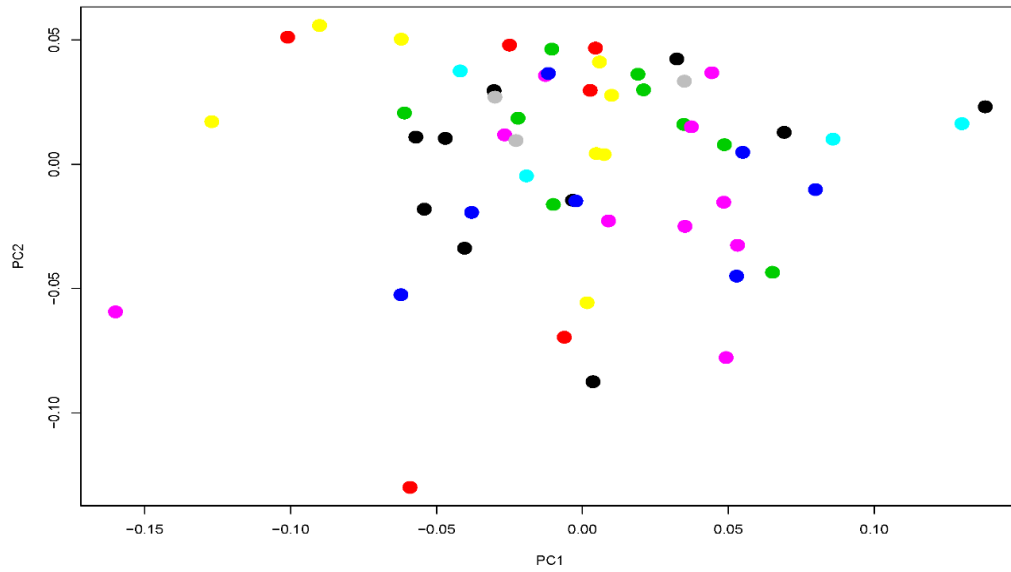


Figure 18. Graph shows correlation between landmark in each individual of *L. bulla* and PCA 1 and 2. Same species within *L. bulla* was represented in the same color.

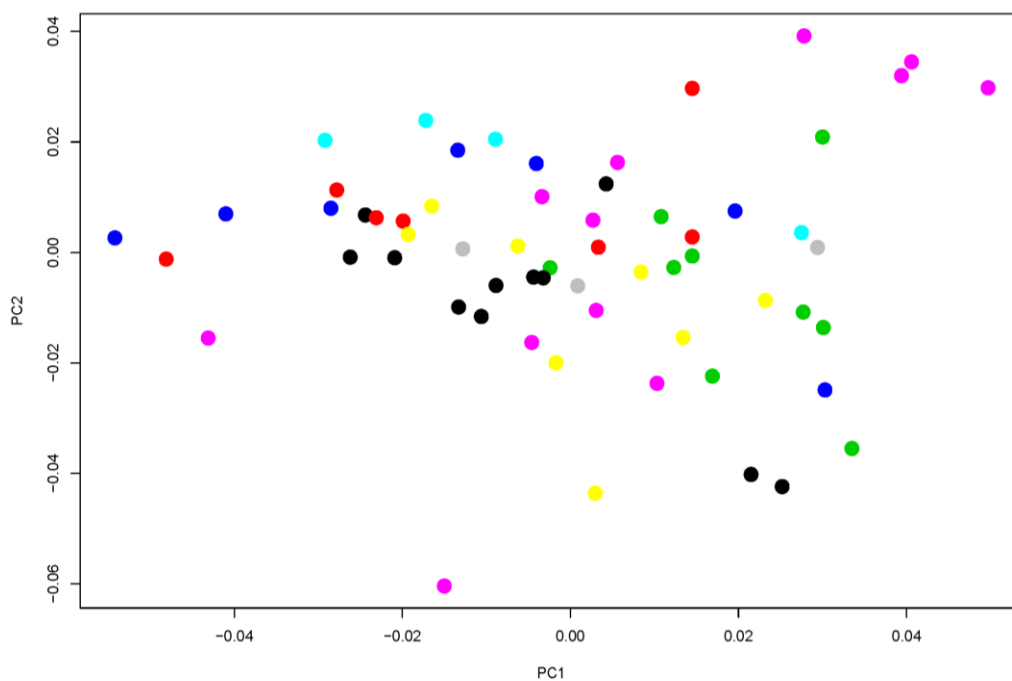


Figure 19. Graph shows correlation between semi-landmark sliding in each individual of *L. bulla* and PCA 1 and 2. Same species within of *L. bulla* was represented in the same color.

3) Molecular and morphological analyses

The consensus trees deduced from COI and ITS1 of *Lecane bulla* were congruent. Phylogenetic tree topologies show estimating number of species within the complex (23 species from COI and 22 species from ITS1) (Figures 20 and 21). Tree deduced from morphological characters shows 6 groups within the complex (Figure 22). The number of species estimates deduced from molecular and morphological analyses were shown in Figure 22. The result shows that least 4 groups within the complex can be separated using morphology (groups 2, 3, 4, and 6), COI (groups 20–23), and ITS1 genes (groups 19–22) (Figure 22). In contrast, group 1 from morphological analysis is the main group within the complex. Morphological characters cannot separate the difference among organism, while COI gene and ITS1 were different within this group and varied from 18 to 19 species. *Lecane bulla diabolica* (C244 in Figure 22) was included in this group.

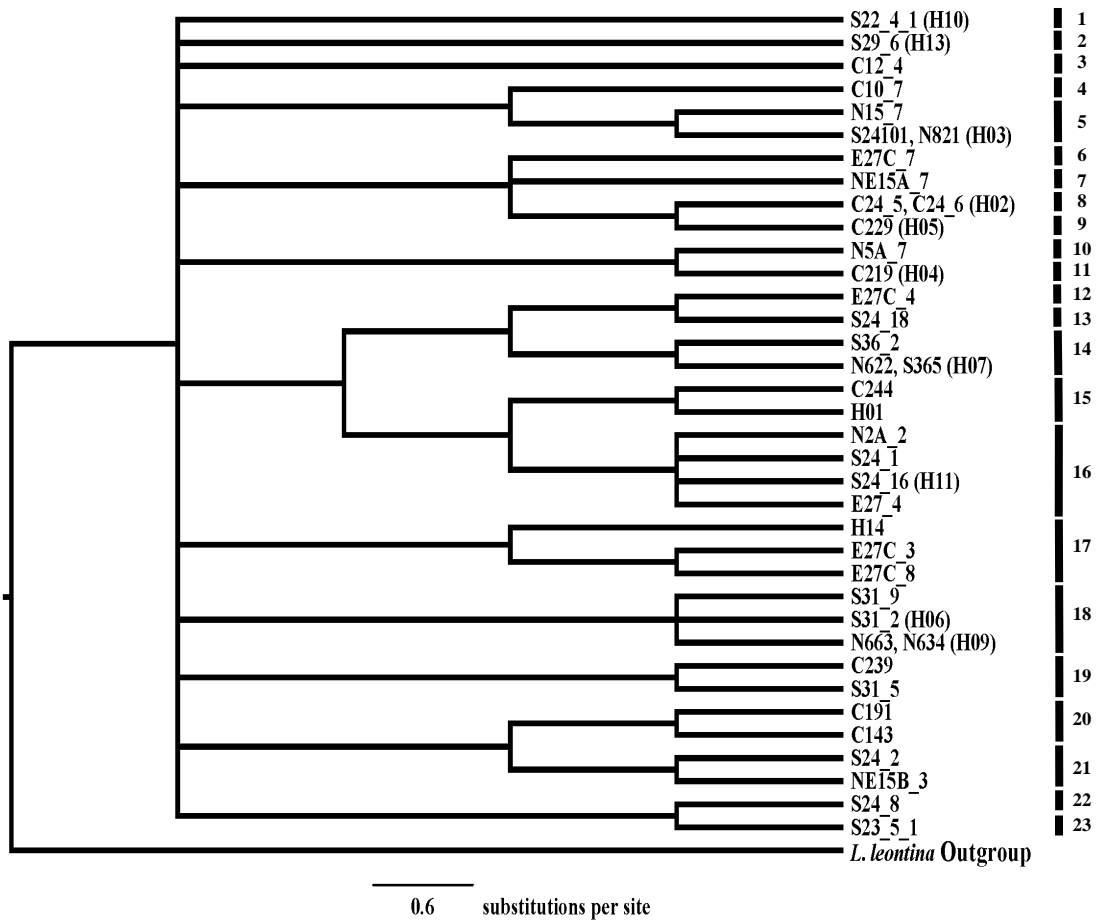


Figure 20. Consensus tree inferred from COI marker of *Lecane bulla*. The bar at the bottom of the figure is the unit of branch length (nucleotide substitutions per site).

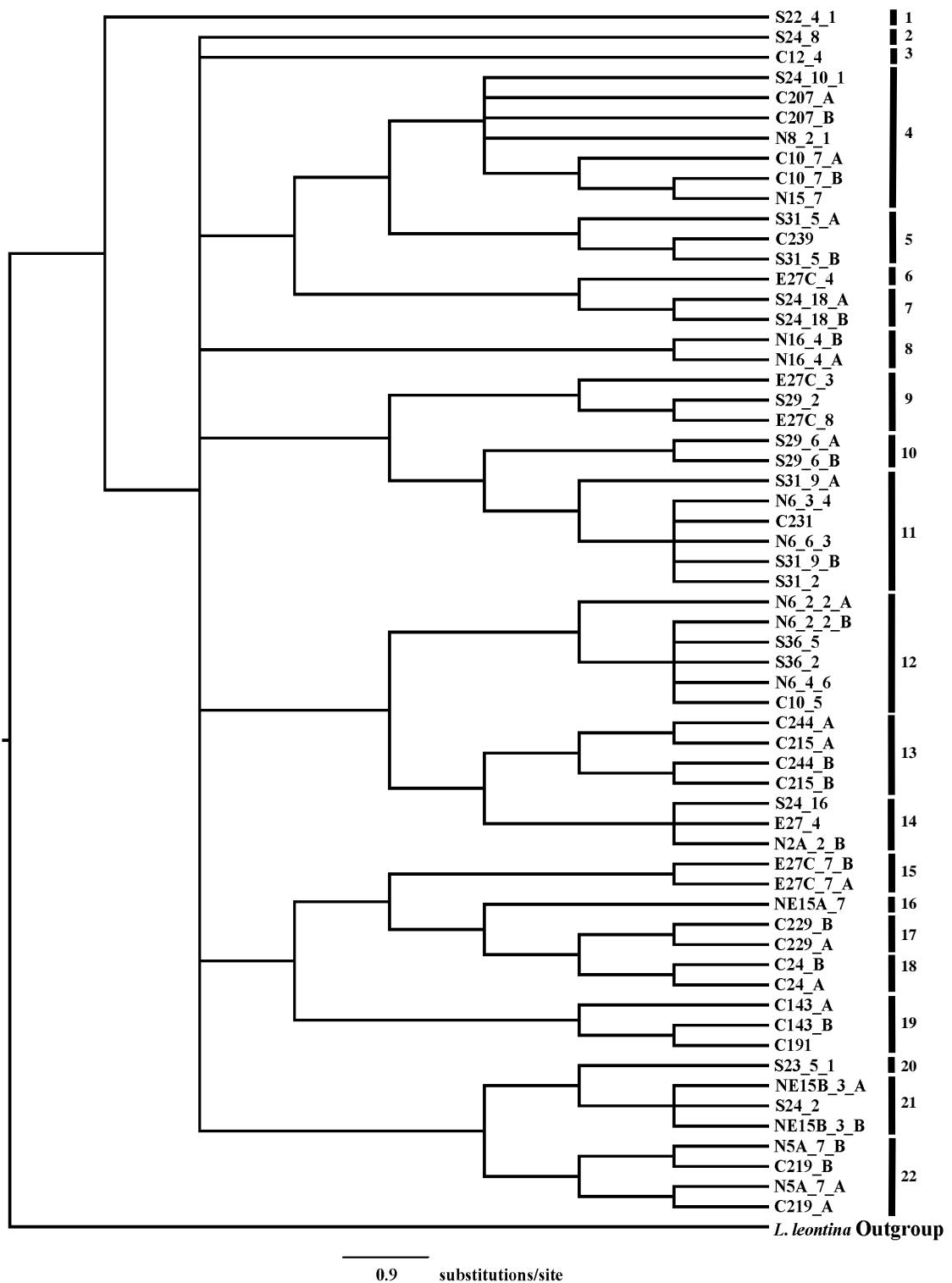


Figure 21. Consensus tree inferred from ITS1 marker of *Lecane bulla*. The bar at the bottom of the figure is the unit of branch length (nucleotide substitutions per site).

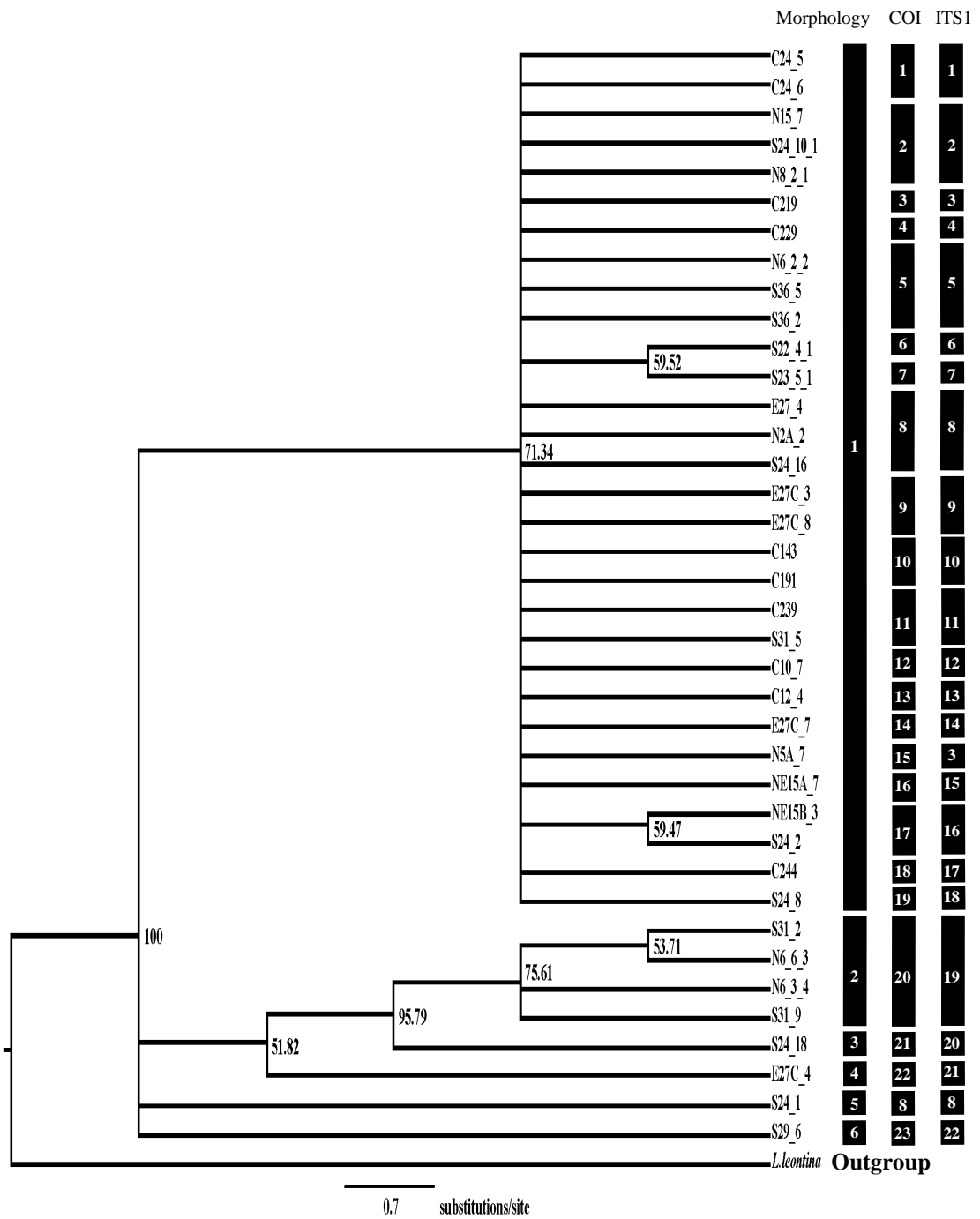


Figure 22. Tree deduced from morphological characters of *Lecane bulla*. The black bands show the number of species estimates among morphological and molecular analyses using COI and ITS1 markers. The bar at the bottom of the figure is the unit of branch length (nucleotide substitutions per site).

3.8 The correlation between genetic distance and geography

Pairwise genetic distance within COI marker of species complex ranged from 1.06% to 20.49% among Brachionidae. As for Lecanidae, genetic distance ranged from 0.15% to 20.58% (Table 14). The correlation between genetic distance and geography of COI in each species complex was analyzed in each species complex with at least three specimens (Table 19). Only *Lecane curvicornis* showed significant correlation between genetic distance and geographic distance ($r = 0.44$, $p = 0.019$) (Table 19). The genetic distance within this species ranged from 1.66 to 17.40 (Table 14). For other species complexes, there was no correlation between genetic distance and geographic distance (Figures 23 and 24). This result indicated evidence of gene flow within the *Lecane bulla* complex among geographic regions in Thailand.

Table 19. Mantel statistic for correlation between pairwise genetic distance and geography of species complexes in Thailand. (significant codes: * $p < 0.05$)

Species complex	Mantel statistic r	Significance
<i>Brachionus angularis</i>	0.160	0.186
<i>Brachionus quadridentatus</i>	-0.050	0.516
<i>Keratella tropica</i>	0.998	0.333
<i>Lecane bulla</i>	-0.029	0.828
<i>Lecane luna</i>	-0.773	0.833
<i>Lecane lunaris</i>	-0.425	0.833
<i>Lecane unguitata</i>	-0.235	0.5
<i>Lecane hamata</i>	-0.999	1
<i>Lecane curvicornis</i>	0.444	0.019*
<i>Lecane leontina</i>	0.057	0.187
<i>Lecane nitida</i>	0.117	0.375
<i>Lecane quadridentatus</i>	0.146	0.219
<i>Lecane signifera</i>	-0.149	0.665

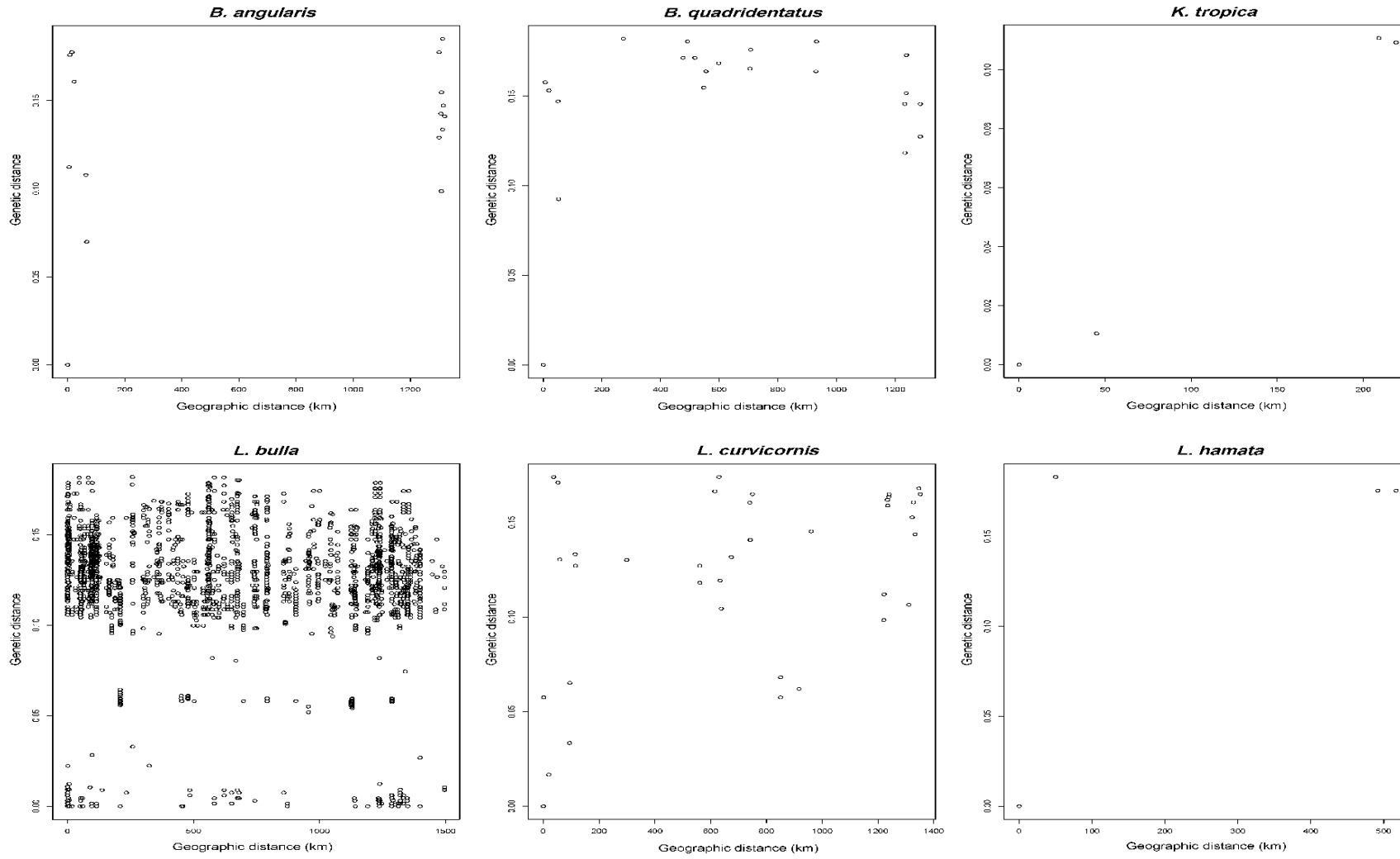


Figure 23. The correlation between genetic and geographic distances of some species complexes using Mantel test.

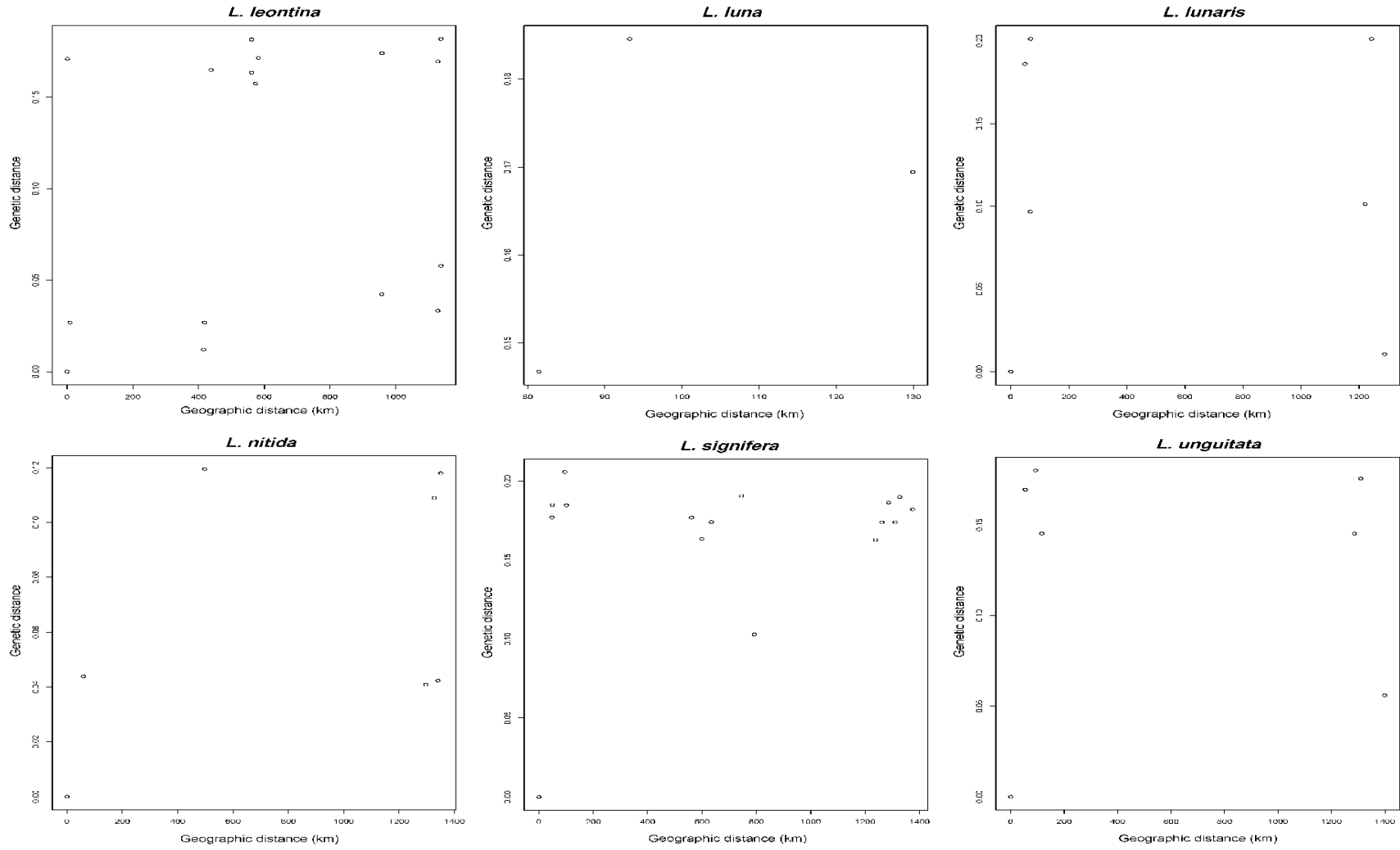


Figure 24. The correlation between genetic and geographic distances of species complexes using Mantel test.

CHAPTER 4

DISCUSSION

The species from the two families of rotifers found in this study were already known in Thailand, except for only one species, *Platyias leloupi*, which is a new record for the country and thus increases the total number of family Brachionidae found in Thailand from 44 (Sa-ardrit *et al.*, 2013) to 45 species. This study included almost half of the species of the family, and sampling design covered all possible habitat types in order to cover all the expected species. No new record was found for the family Lecanidae. The number of known species of Lecanidae from the country is 97 (Sa-ardrit *et al.*, 2013); however, only about half of that total number was found in this study. The discrepancies between the species in this study dataset and the ones known from the country could be due to the fact that this study did not sample all the types of habitats, nor all the geographical areas in Thailand, nor all the seasons.

The total number of rotifers in the two families in Thailand is higher than the one recorded from other southeast Asian countries (Segers, 2001), such as Cambodia with 27 species of Brachionidae and 58 species of Lecanidae (Meas and Sanoamuang, 2010; Meas and Sor, 2014), Laos (9 and 30 species, respectively) (Segers and Sanoamuang 2007), Philippines (12 and 3 species) (Papa and Zafaralla, 2011), and Vietnam (21 and 27 species) (Zhdanova, 2011; Trinh Dang *et al.*, 2013). Overall, the distribution of monogonont rotifers is generally poorly known, and there is a strong effect of sampling bias on the faunistic species lists from all over the world (Dumont, 1983; Segers and De Smet, 2008; Fontaneto *et al.*, 2012). Nevertheless, this extensive survey demonstrated that at least for Thailand and for the two families of Brachionidae and Lecanidae, this knowledge is rather good. Thus, this study dataset could be considered reliable for the inference that this study attempted on the limnological correlates of diversity. The fact that one more species was found in this survey is not contrary to the assumption of good faunistic knowledge, given that new species and new records of rotifers are rather common even in well-studied areas at temperate latitudes (e.g., De Smet, 2015).

Environmental correlates of species richness (S)

The most important correlate of species richness (S) for Brachionidae was clearly latitude. In fact, latitude itself cannot affect biological diversity, but it operates through indirect ways of environmental variables that change with latitude (Hawkins and Diniz-Filho, 2004). In this study case, latitude was highly correlated with elevation: samples at higher latitudes were also from sites at higher elevations, thus this study cannot disentangle which of the two variables actually correlated most with species richness (S) in Brachionidae. Interesting, species richness (S) of Brachionidae seems to be positively related with latitude, which means that more species are expected at higher latitudes. This is the opposite of the general trends seen in latitudinal and altitudinal diversity gradients, where species richness (S) increases with lower latitudes and/or lower elevation (Green, 1987, 1994; Hillebrand, 2004; Obertegger *et al.*, 2010). Yet, my study is at a low spatial scale, covering only from 6 to 20° North, and the significance of the effect is not high ($p = 0.034$). Thus, the apparent positive effect may be spurious.

The other variable with a marginally significant effect on species richness (S) of Brachionidae was temperature. Brachionidae is known to have higher species richness of some genera (e.g., *Brachionus*) in warm waters, and higher species richness of other genera (e.g., *Notholca*) in cold waters (Green, 1972, 1994). The effect that I observed in Thailand was negative: a lower number of species of the family were found in samples from warmer waters. Temperatures did not strongly correlate with latitude and ranged from 23 to 41 °C. These temperatures are much higher than the ones usually observed in temperate water bodies from which there is more information available on rotifer species richness. It is possible that indeed temperatures around 40 °C could have a limiting effect on species richness of Brachionidae. Yet, this is only speculation and there is no data to support it, except the overall negative effect of temperature observed in this dataset. However, a previous study in Cambodia confirmed that water temperature was the most important positive correlate of rotifer species richness (Meas and Sor, 2014). Furthermore, according to another study in Asia (China), the peak of maximum number of species of rotifers occurred in the seasons with the highest water temperature, even if water temperature was only between 16 and 21 °C (An *et al.*, 2012).

Contrary to Brachionidae, no effect of environmental variables was seen to influence species richness of Lecanidae. A lack of effect could be the actual pattern that is perhaps linked to different species replacing each other under different environmental conditions (Fischer *et al.*, 2001), and thus confounding this analysis. Another explanation could be that other environmental variables that I did not measure are more important for Lecanidae, but I cannot speculate on any of them from this empirical observations from the field.

Environmental correlates of species composition

Regarding differences in species composition, the situation is rather different: in this case, habitat type and conductivity were variables significantly affecting differences in species composition for both Brachionidae and Lecanidae. The effect of habitat type, which was stronger and more significant for Lecanidae than for Brachionidae, means that, at least for Lecanidae, there was a species-specific relationship between species and the habitat, even if this was not due to differences in temperature, pH, or dissolved oxygen between the habitat types. Thus, the situation, although significant, is not clear and the proportion of variance actually explained by habitat type in the statistical models is indeed low, about 5.5% for Brachionidae and 7.3% for Lecanidae.

Differences in conductivity was the other general driver of differences in species composition among samples. It is already known that conductivity and salinity (highly correlated in this dataset) affect occurrence of rotifers, and even slightly haline water bodies host species assemblages that are very different from those in freshwater habitats (Green and Mengestou, 1991; Kaya *et al.*, 2010). Different species of rotifers are adapted to different salinities, even within the same genus (Fontaneto *et al.*, 2006; De Smet *et al.*, 2015). Supported by previous studies in Thailand, conductivity was correlated to Brachionidae and their abundance (Athibai *et al.*, 2013). Thus, the significance of conductivity (and indirectly salinity) as an important correlate of differences in species composition in rotifers was expected.

Latitude affected differences in species composition for Lecanidae, but not for Brachionidae. As for differences in species richness (S), the explanation for the effect of latitude is that some other variables correlating with latitude could be the driver of

the differences (Hawkins and Diniz-Filho, 2004). In this case, elevation gradients in environmental variables, correlating with latitude, could be the cause for the differences. Although I cannot speculate which variable could be the important one as neither temperature nor dissolved oxygen had any effect in these analyses, an effect of latitude on species composition of Brachionidae and Lecanidae was previously described, but at larger spatial scales (Green, 1972; Segers, 1996).

Species delimitation

The results from three DNA taxonomy approaches, ABGD, PTP, and GMYC, based on COI and ITS1 markers clearly indicated the existence of cryptic species in both families. The highest estimate of species diversity in this study was *Lecane bulla* with at least 20 species in the complex. *Lecane bulla* is a cosmopolitan species which occurs in every region (Segers, 2007; Walsh *et al.*, 2009; Segers and Savatnalinton, 2010). A previous study showed cryptic speciation in this species complex in Chihuahuan Desert waters (Walsh *et al.*, 2009). Similar to this study, they occur sympatrically in Thailand regions and unrestricted of distribution among Thai geographic regions as indicated by Mantel test. The result from hapowebs showed evidence of gene sharing among *L. bulla*. The high genetic variation was found in mitochondrial cytochrome c oxidase subunit I of *L. bulla*. The variation of genetic distance based on COI within the *L. bulla* complex between 0.15% and 18.45% was higher than within other rotifers, which show approximately 12%–15% (Gomez *et al.*, 2002; Derry *et al.*, 2003; Gilbert & Walsh, 2005).

At present, DNA taxonomy approach becomes a reasonable method for delimiting species boundary within several organisms such as gastropods (Modica *et al.*, 2014), moths (Kekkonen *et al.*, 2015), beetles (Pentinsaari *et al.*, 2016), crayfishes (Larson *et al.*, 2016), coleopterans (Eberle *et al.*, 2016), nemerteans (Leasi *et al.*, 2016), and parasitoid wasps (Schwarzfeld and Sperling, 2015). Recently, 15 species were discovered in one of monogonont rotifers, *B. plicatilis* species complex, through DNA taxonomy (Mills *et al.*, 2016). This is among the highest estimates of species-level diversity in a cryptic species complex in Rotifera thus far. *Brachionus plicatilis* is a cosmopolitan species complex, which lives in coastal ponds and salt lakes

(Suatoni *et al.*, 2006). Only one of the species of the *B. plicatilis* complex, *B. rotundiformis*, was found from some coastal ponds in this study.

As for other popular species complexes, *Brachionus calyciflorus* has been previously recorded about the existence of three cryptic species by Xiang *et al.* (2010) and six cryptic species by Xiang *et al.* (2011) within the complex. The genetic divergences of ITS sequences among three clades ranged from 4.2% to 25.3% (Xiang *et al.*, 2010) and among six cryptic species ranged from 4.2% to 26.7%. Among DNA sequence of Thai and other countries data from GenBank, DNA taxonomy approaches indicated the existence of cryptic species ranging from 8 to 9 species and 2 to 6 species for COI and ITS1, respectively. Within the complex, *Brachionus calyciflorus*, *B. calyciflorus* f. *amphiceros* and *B. calyciflorus* f. *anuraejformis* were found from this study. Genetic distance within species complex is 1.86% for COI and 0.23%–7.23% for ITS1. This result showed genetic distance variation was lower than a previous study (Xiang *et al.*, 2010, 2011).

There are a few studies in other Brachionidae and Lecanidae. In a previous studies, the result from DNA barcoding of freshwater Rotifera in Mexico revealed cryptic species in *Brachionus calyciflorus*, *Keratella cochlearis*, *Lecane bulla*, *L. cornuta*, *L. crepida*, *L. curvicornis*, *L. hastata*, and *L. lunaris* (García-Morales and Elías-Gutiérrez, 2013). This result confirmed the existence of cryptic species within these species, including *Brachionus angularis*, *B. caudatus*, *B. quadridentatus*, *K. tropica*, *L. leontina*, *L. ludwigii*, *L. luna*, *L. signifera*, and *L. unguitata*. In this result, *Brachionus quadridentatus* is the one complex that showed a high number of species diversity within the complex with at least 13 species from COI marker. *Brachionus quadridentatus*, *B. quadridentatus melheni* and *B. quadridentatus* f. *brevispinus* were found in this study. Previously, a study discovered *B. quadridentatus* f. *brevispinus*, *B. quadridentatus* f. *cluniorbicularis*, *B. quadridentatus* f. *melhemi*, *B. quadridentatus* f. *mirabilis* and *B. quadridentatus* f. *typica* (Athibai, 2008).

For Lecanidae, the estimated number of species diversity of *Lecane curvicornis* based on COI marker ranged from 9 to 10 species. The intraspecific variance within this species is high. *Lecane curvicornis* f. *nitida* was included in the complex (Segers, 1995). However, *Lecane nitida* was already known to split from *L. curvicornis* based on important differences in morphology, ecology, and distribution

(Segers and Sanoamuang, 2007). In addition, the molecular studies lead to the defining of these taxa at different species-level rather than morphological variants (Gómez *et al.*, 2002). The mitochondrial DNA sequences from this result confirmed a clear difference clade between the two species. The analyses estimated the number of species based on COI marker within *L. nitida* was 2 to 4 species. For other closely related species, the analyses showed that *L. acus* and *L. crenata* were included within the same clade of *L. lunaris*. *Lecane halsei* was closely related to *L. ludwigii*, while *Brachionus angularis* was closely related with *B. caudatus*. *Brachionus caudatus* can be easily confused with morphological variation inducing with temperature (Athibai and Sanoamuang, 2008). Phylogenetic tree topologies based on COI marker showed a non-monophyly among *Lecane leontina* population.

The geometric morphometric analyses from this study cannot separate some *Lecane bulla* from one another, while DNA sequences based on COI and ITS1 are different. This study offers a basis for further analyses on the species complex, providing a phylogenetic framework for comparative studies. DNA taxonomy uses DNA for delimiting species within species complex. This approach uses DNA sequence variation to discover cryptic animals (Fontaneto *et al.*, 2015). Since COI is more variable than ITS1, this marker is still the best maker to explore the population genetic structure within species and for phylogeographic analysis (Gómez *et al.*, 2000; Mills *et al.*, 2016).

Moreover, there is a geographic overlap in rotifer distribution in Thai waters. No correlation occurs between genetic and geographic distances in all species complexes, except in *L. curvicornis*. Although *L. curvicornis* demonstrates a significant difference, only a few specimens were used for the analysis. More samples of the species complex are needed for further analysis.

CHAPTER 5

CONCLUSION

A total of 60 species 3 subspecies in 5 genera were recorded from the families Brachionidae and Lecanidae in Thailand. Of these, 18 species and 2 subspecies 4 genera belong to family Brachionidae and 42 species 1 subspecies 1 genus belong to family Lecanidae. The habitat type was the most important variable to species composition of Brachionidae. For Lecanidae, habitat type, conductivity, and latitude had an effect on differences in species composition.

This study showed the existence of cryptic species within the complexes, *Brachionus angularis*, *Brachionus calyciflorus*, *Brachionus caudatus*, *Brachionus quadridentatus*, *Keratella cochlearis*, *Keratella tropica*, *Lecane bulla*, *Lecane closterocerca*, *Lecane crenata*, *Lecane curvicornis*, *Lecane hamata*, *Lecane leontina*, *Lecane ludwigii*, *Lecane luna*, *Lecane lunaris*, *Lecane nitida*, *Lecane quadridentata*, *Lecane signifera*, and *Lecane unguitata*. The highest estimated number of species within the complex was found in *Lecane bulla* with at least 20 species supporting both COI and ITS1 markers. In contrast, morphological analyses can separate only some individuals. Only *Lecane curvicornis* shows significant correlation between genetic and geographic distances. For other species complexes, there are gene flows among Thai geographic regions. There is no significant difference between other target species complexes within this study.

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APPENDIX

Appendix 1. Haplotypes of COI of *Lecane bulla* (Gosse, 1851).

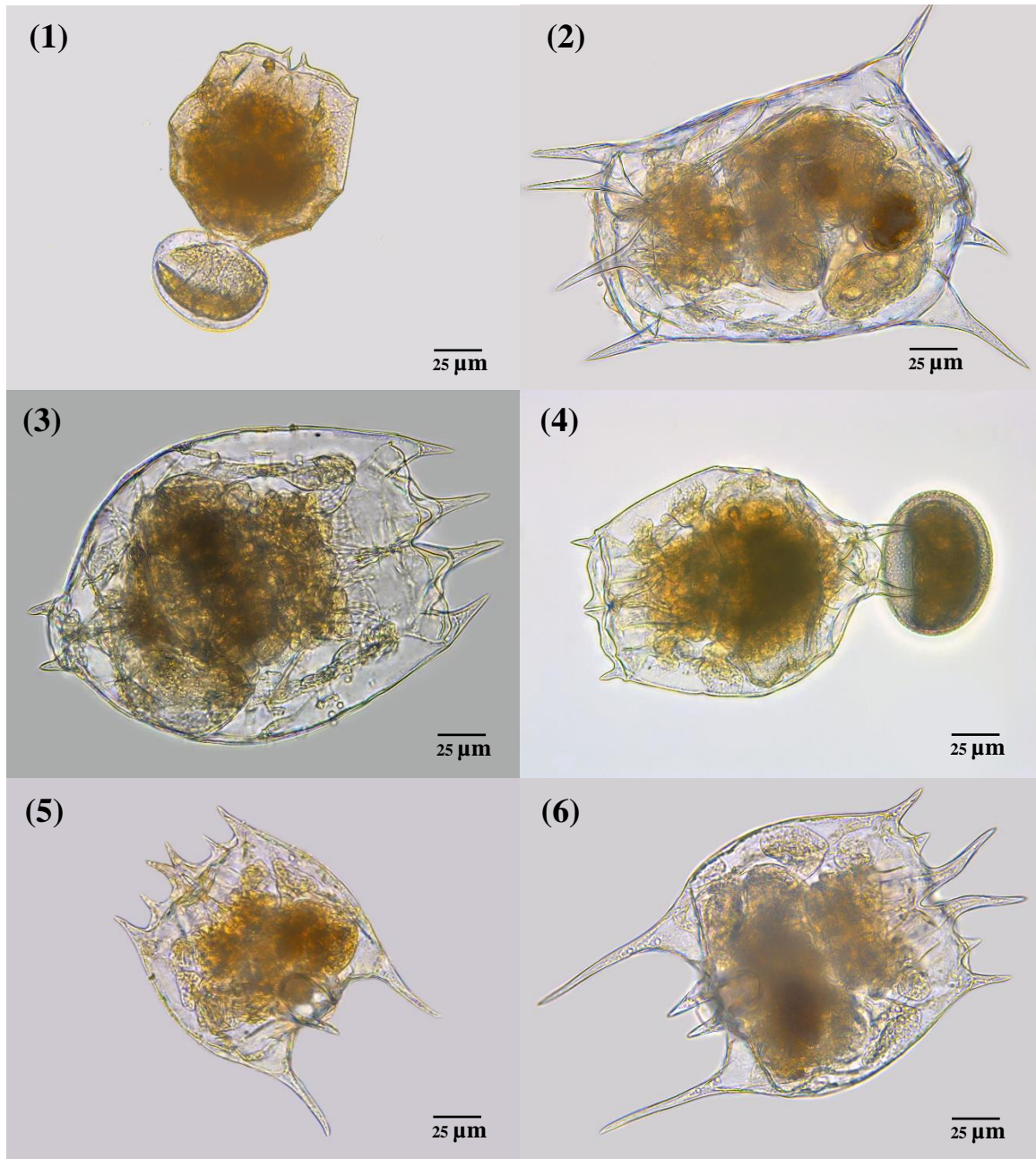
Haplotype	Specimen code
Haplotype 1 (H01)	C18, C129, C167, C215
Haplotype 2 (H02)	C24, C44
Haplotype 3 (H03)	C207, N7-10, N8-2-1, S24-10-1, S31-1-1
Haplotype 4 (H04)	C218, C219
Haplotype 5 (H05)	C229, N7-3
Haplotype 6 (H06)	C231, C236, S31-2
Haplotype 7 (H07)	C10-5, N6-2-2, N6-4-4, N6-4-6, N8-2, S36-5
Haplotype 8 (H08)	N16-4, N17B-9
Haplotype 9 (H09)	N6-3-3, N6-3-4, N6-6-3
Haplotype 10 (H10)	S22-4-1, S22-6
Haplotype 11 (H11)	S24-6, S24-16
Haplotype 12 (H12)	S27-2a, S27-2-1
Haplotype 13 (H13)	S29-1, S29-6
Haplotype 14 (H14)	S29-2, S29-8
Haplotype 15 (H15)	C27
Haplotype 16 (H16)	C143
Haplotype 17 (H17)	C191
Haplotype 18 (H18)	C239
Haplotype 19 (H19)	C244
Haplotype 20 (H20)	C10-7
Haplotype 21 (H21)	C12-4
Haplotype 22 (H22)	E27A-4
Haplotype 23 (H23)	E27C-3
Haplotype 24 (H24)	E27C-4
Haplotype 25 (H25)	E27C-7
Haplotype 26 (H26)	E27C-8

Appendix 1. (continued)

Haplotype	Specimen code	Haplotype	Specimen code
Haplotype 27 (H27)	E27-2	Haplotype 42 (H42)	N15-7
Haplotype 28 (H28)	N7-10-1	Haplotype 43 (H43)	N7-3-1
Haplotype 29 (H29)	NE10A-4	Haplotype 44 (H44)	S24-1
Haplotype 30 (H30)	NE10B-5	Haplotype 45 (H45)	S24-2a
Haplotype 31 (H31)	NE11B-2	Haplotype 46 (H46)	S24-8
Haplotype 32 (H32)	NE15A-7	Haplotype 47 (H47)	S28-2
Haplotype 33 (H33)	NE15B-3	Haplotype 48 (H48)	S31-1
Haplotype 34 (H34)	S22-5	Haplotype 49 (H49)	S31-5
Haplotype 35 (H35)	E27-4	Haplotype 50 (H50)	S31-9
Haplotype 36 (H36)	N2A-2	Haplotype 51 (H51)	S36-2
Haplotype 37 (H37)	N5A-3	Haplotype 52 (H52)	S23-5-1
Haplotype 38 (H38)	N5A-7	Haplotype 53 (H53)	S24-15
Haplotype 39 (H39)	N17B-1-1	Haplotype 54 (H54)	S24-17
Haplotype 40 (H40)	N7-5	Haplotype 55 (H55)	S24-18
Haplotype 41 (H41)	N10-2	Haplotype 56 (H56)	S31-12

Appendix 2. Haplotypes of ITS1 of *Lecane bulla* (Gosse, 1851).

Haplotype	Specimen code
Haplotype 1 (H01)	E27C_3_B, E27C_8_B, S28_2_B, S29_2_A
Haplotype 2 (H02)	E27C_3_A, E27C_8_A
Haplotype 3 (H03)	C239_A, N7_10_1_B, S31_5_B,
Haplotype 4 (H04)	E27_4_A, N2A_2_B, N17B_1_1_A, S24_16_A
Haplotype 5 (H05)	C244_B, D215_B
Haplotype 6 (H06)	NE15B_3_B, S23_5_1_A, S24_2_A
Haplotype 7 (H07)	C219_B, N5A_7_B
Haplotype 8 (H08)	C229_B, N7_3_1_B
Haplotype 9 (H09)	C10_5_A, N6_2_2_B, N6_4_6_A, S36_2_A, S36_5_A
Haplotype 10 (H10)	C143_B, D191_A
Haplotype 11 (H11)	C231_A, N6_6_3_A, N6_3_4_A, S31_2_A, S31_9_B
Haplotype 12 (H12)	S24_18_A, S24_18_B
Haplotype 13 (H13)	C207_B, N8_2_1_B, S24_10_1_A
Haplotype 14 (H14)	C10_7_B, N15_7_A



Appendix 3. Species complexes of Family Brachionidae from this study.

(1) *Brachionus angularis*

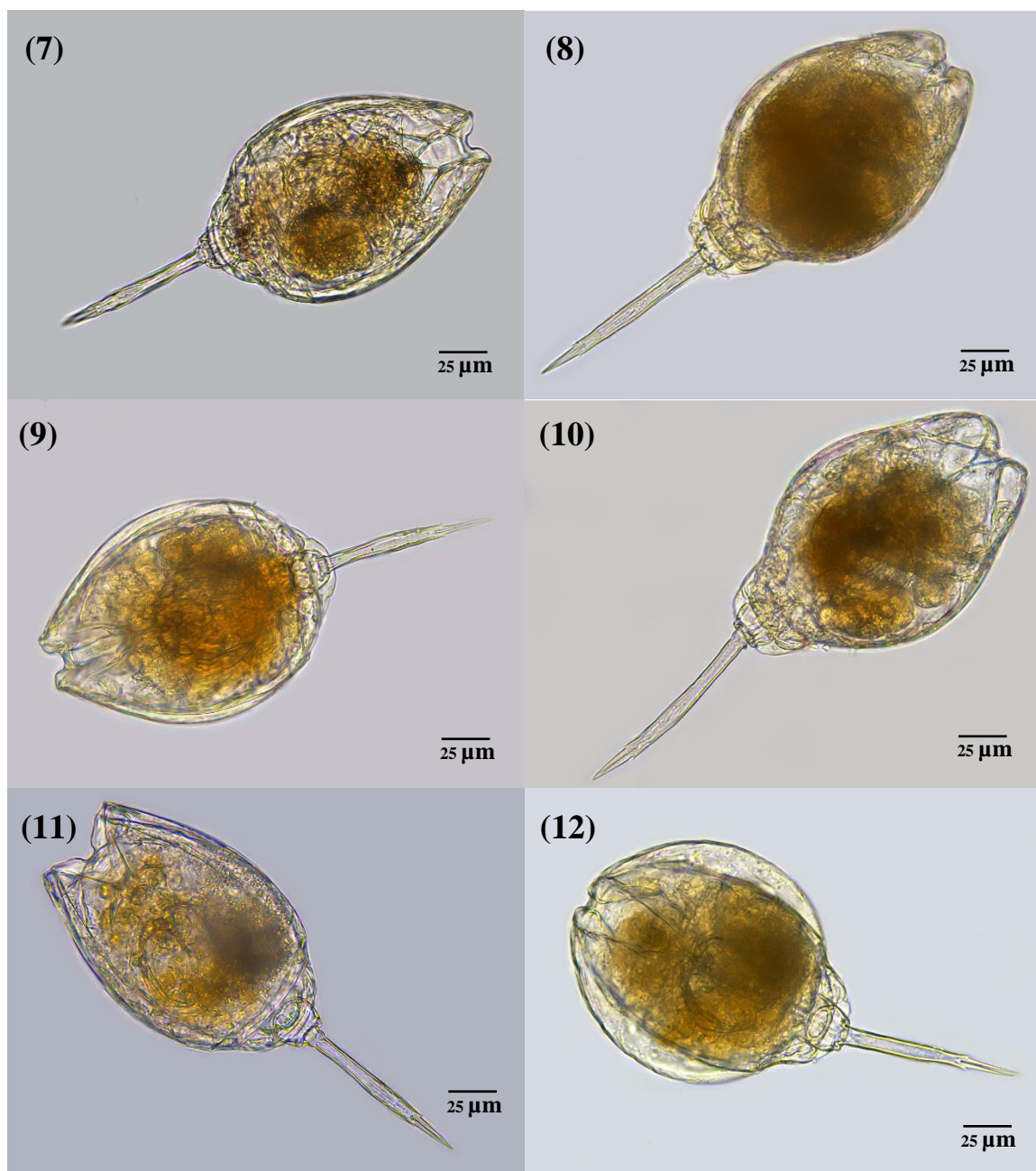
(2) *Brachionus calyciflorus* f. *amphiceros*

(3) *Brachionus calyciflorus* f. *anuraeiformis*

(4) *Brachionus caudatus*

(5) *Brachionus quadridentatus*

(6) *Brachionus quadridentatus* *melheni*



Appendix 4. *Lecane bulla* complex from this study.

(7) *Lecane bulla*

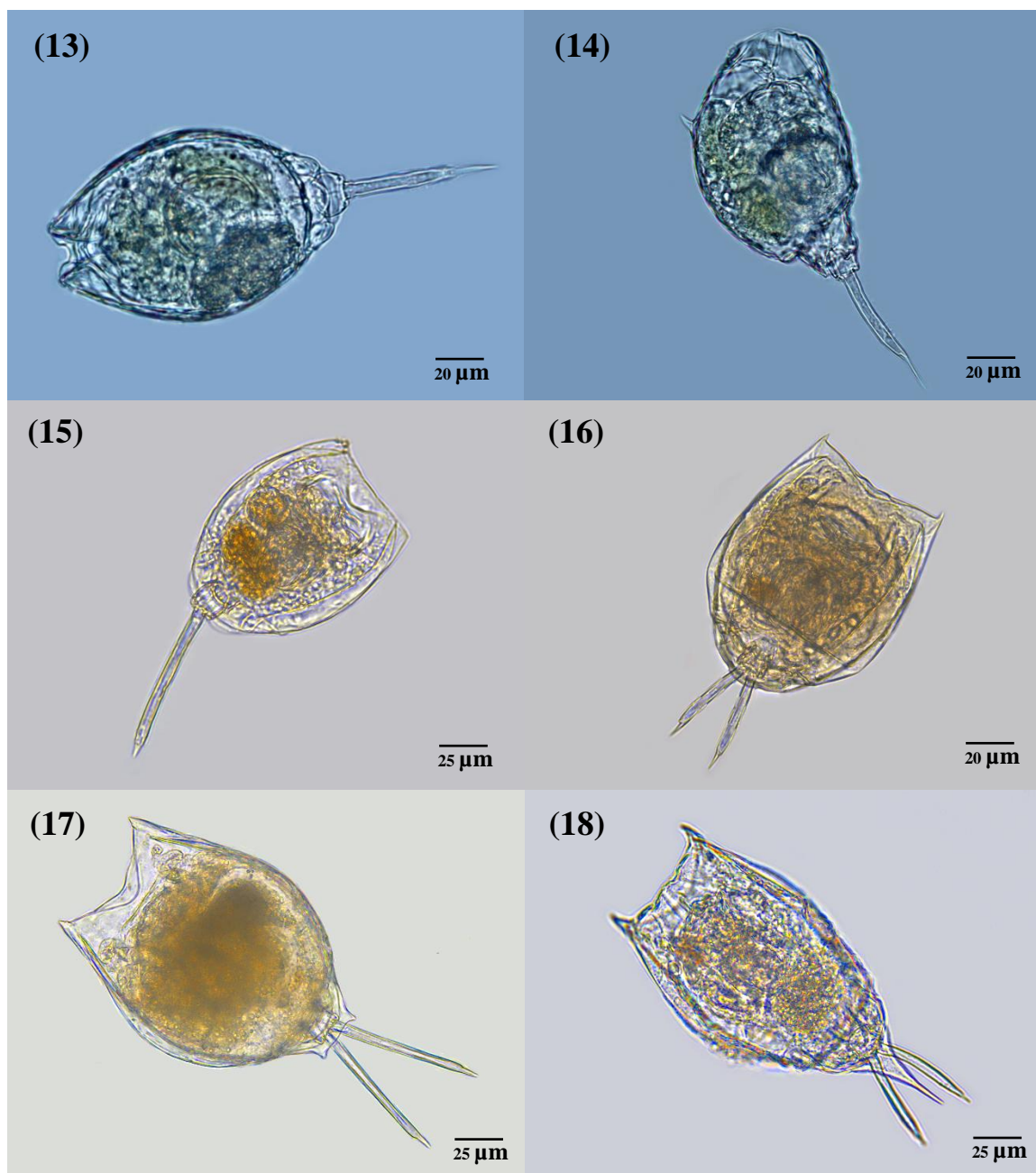
(8) *Lecane bulla*

(9) *Lecane bulla*

(10) *Lecane bulla*

(11) *Lecane bulla*

(12) *Lecane bulla*



Appendix 5. Species complexes of Family Lecanidae from this study.

(13) *Lecane bulla diabolica*

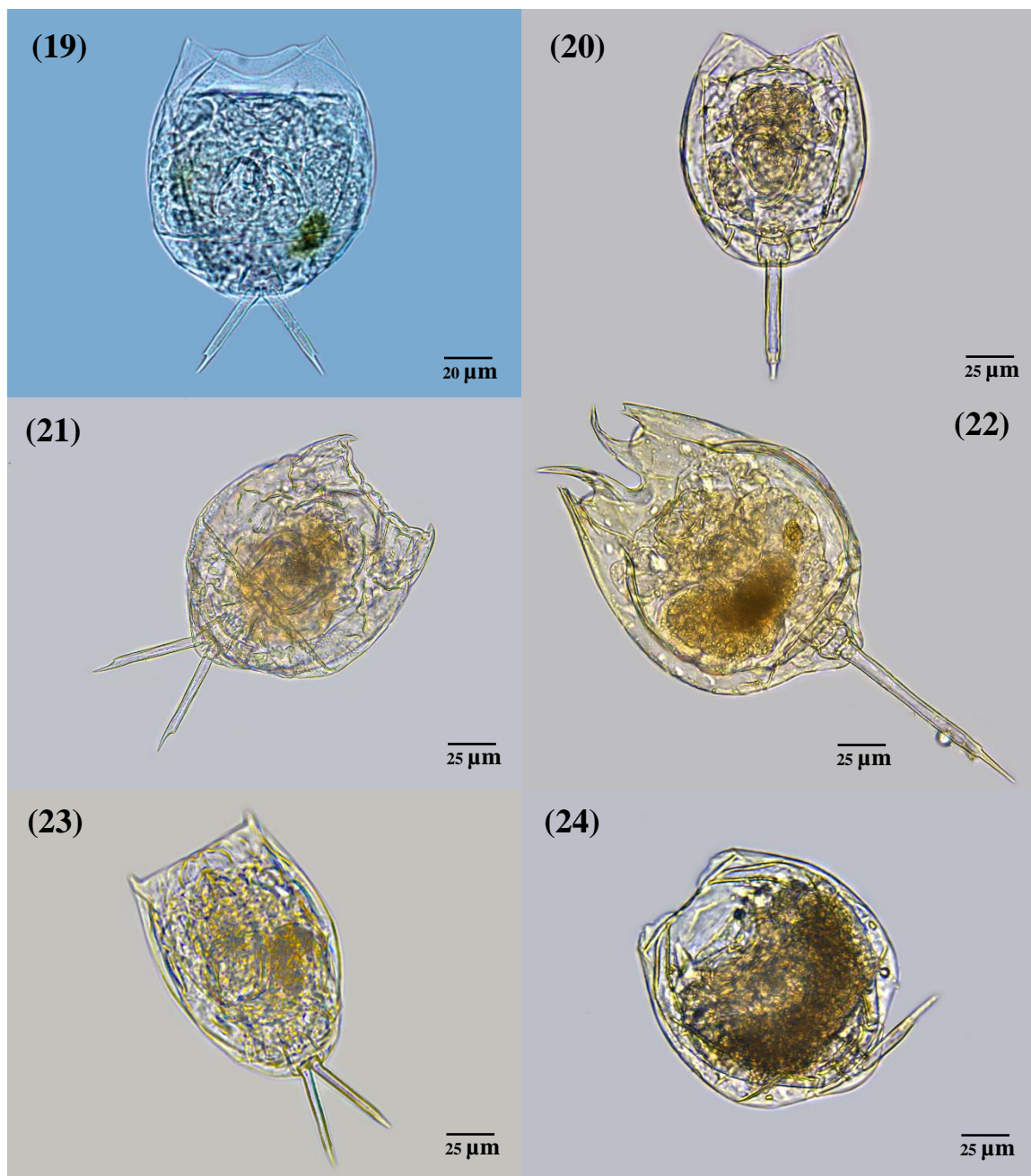
(14) *Lecane bulla diabolica* (lateral view)

(15) *Lecane crenata*

(16) *Lecane curvicornis*

(17) *Lecane leontina*

(18) *Lecane ludwigii*



Appendix 6. Species complexes of Family Lecanidae from this study.

(19) *Lecane luna*

(20) *Lecane lunaris*

(21) *Lecane nitida*

(22) *Lecane quadridentata*

(23) *Lecane signifera*

(24) *Lecane unguitata*

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Educational Attainment

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B.Sc. (Biology)	Prince of Songkla University	1998
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Scholarship Awards during Enrolment

- 1) Royal Golden Jubilee (RGJ) from Thailand Research Fund (TRF) (Grant no. PHD/0261/2552; 4.B.PS/52/D.2)
- 2) PhD Thesis Research Scholarship, Graduated School, Prince of Songkla University.

List of Publications and Proceedings

- Paper I: Sa-ardrit, P., Pholpunthin, P. and Segers, H. 2013. A checklist of the freshwater rotifer fauna of Thailand (Rotifera, Monogononta, Bdelloidea). *Journal of Limnology*. 72(s2): 361–375.
- Paper II: Sa-ardrit, P., Pholpunthin, P., Segers, H. and Fontaneto, D. 2016. The influence of environmental variables on freshwater rotifers of the family Brachionidae and Lecanidae in Thailand. *Tropical Zoology* (Accepted).