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The genus *Chiropsoides* (Chirodropida: Chiropsalmidae) from the Andaman Sea, Thai waters

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Abstract

Box jellyfish *Chiropsoides buitendijki* from the coastal zone along the Andaman Sea, southwestern Thailand are characterized by a box-shaped body with unilateral branched tentacles and lack of interradial furrows. Tentacular banding was first reported in the present study with 1–3–2–3–2–3–2–3–1 patterns (1–major band, 2–thicker minor band and 3–thinner minor band). The DNA sequences of 18 S ribosomal RNA genes indicated that the specimen examined were genetically similar to *C. buitendijki* that was previously identified from the Nam Bor Bay, Phuket, Thailand, and distinct to the other known taxa in the order Chirodropida. In addition, a significant genetic divergence based on 16S mitochondrial gene was observed within the *C. buitendijki* samples. This indicates a population genetic differentiation but needs further confirmation.

Key words: *Chiropsoides*, Cubozoa, Andaman Sea, jellyfish, tentacular banding

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1 Introduction

The genus *Chiropsoides* Southcott (1956) replaced *Drepanochirus* Krumbach (1925) because this generic name was preoccupied (Southcott, 1956). The distinctive characters of *Chiropsoides* is having abaxially branched pedalia in a sequential linear form.

Gershwin (2006) revised the genus *Chiropsoides* and recognized 2 species of *Chiropsoides*, viz., *C. quadrigatus* (Haeckel, 1880) and *C. buitendijki* (Horst, 1907). The latter Indonesian species has a conspicuous, unilateral pedalia fork with knobs in a conspicuous unilateral manner and flat, ribbon-like tentacles. The main canal of *C. buitendijki* has a series of knobs between the forks, whereas *C. quadrigatus* has knobs on the forks themselves but not between.

The type of *C. quadrigatus* is immature, in a poor condition, and considered a nomendubium. However, Gershwin (2006) mentioned that it should be maintained as a separate species, but if *C. buitendijki* and *C. quadrigatus* are found to be identical, then *C. quadrigatus* would have priority.

C. buitendijki has been reported from India, Malay Archipelago and Indochina, whereas the distribution of *C. quadrigatus* is widely debated. Gershwin (2006) mentioned that all Chirodropida from Australia, Philippines, Japan, and throughout Southeast Asia should belong to other species than *C. quadrigatus*. Aungtonya and Chanachon (2012) found 4 cubozoan families with 5 species in coastal areas of Phuket Province, southwestern Thailand. *Chiropsoides buitendijki* was the only species of this genus. Sucharitakul et al. (2016) studied sequences of *C. buitendijki* from the Nam Bor Bay, Phuket, southwest Thailand (submitted to the National Center for Biotechnology Information (NCBI) with regard to KJ135023).

Chiropsoides buitendijki was reported to cause the rapid demise of two teenagers in Pulau Langkawi off the southwest coast of Malaysia bordering Thailand, the Andaman Sea (Fenner et al., 2010). The characteristic skin wound markings of the victims implied stung by Chirodropid. *Chiropsoides buitendijki* was blamed to be cause of these skin markings. However, Fenner et al. (2010) did not report the size of this box jellyfish.

2 Materials and methods

2.1 *Specimens collection and treatment*

Samples from the Andaman Sea in this study were collected during the surveys of distribution of venomous jellyfish project during 2009–2014. The surveys were conducted on the coast of Ranong, Phang-nga, Phuket, Krabi, Trang and Satun for 6, 12, 12, 8, 5 and 6 times during the northeast monsoon (November–April) and 3, 11, 9, 10, 4 and 4 times during the southwest monsoon (May–October), respectively. The trapped jellyfish were collected from shrimp trammel net. Additional specimens were occasionally received from fishermen. The specimens were examined, photographed, bell height measured and the amount of branching pedalia counted.

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Means and standard deviations of bell height and number of tentacles of *C. buitendijki* were calculated and compared by box plots.

2.2 *DNA extraction, PCR amplification and sequencing*

A small piece (about 0.5 cm) of tentacle or rhopalia or mesoglea was cut off from the selected samples, and preserved in 99% EtOH at a ratio of 10–acl: 1 tissue (v:v). The fixed tissues were stored at 4°C for DNA analysis.

The genomic DNA was extracted using the commercial animal tissue extraction kit or a CTAB/chloroform method described by Dawson et al. (1998). The nuclear 18S and mitochondrial 16S gene fragments were amplified using the primer pairsL18S: 5′–CGGAAGGGCACCACCAGGAG–3′, 18Sb: 5′–GATCCTTCTGCAGGTTCACCTAC–3′ and BRDGP–1: 5′–TCGACTGTTTACCAAAAACATAGC–3′, BRDGP–2: 5′–ACGGAATGAACTCAAATCATGTAAG–3′, respectively (Bayha, 2005; Bayha et al., 2010). PCR reactions were performed on the thermal cycler following the optimized protocol (Liu et al., 2016). The amplicons were directly sequenced bi-directionally on the ABI3130XL genetic analyzer. In cases where direct sequencing was not applicable, amplicons were cloned using the TA cloning kits and then sequenced.

2.3 *Sequence analysis*

The resulting sequences were cleaned for vectors, primers and ambiguous nucleotides and subsequently aligned with the reference sequences downloaded from GenBank using Clustal W algorithm (Thompson et al., 1994). The phylogenetic relationship between unknown specimen and references was analyzed using maximum likelihood (ML) and neighbor joining (NJ) methods. Robustness of resulting phylogenetic trees was assessed by boostrapping for 1 000 replicates. The best assignment of the unknown specimen was hypothesized, and the results from different genes were compared to evaluate the consistency of the conclusions.

3 Results and discussion

3.1 *Sample measurements*

Average bell height sizes from Ranong (n=29): 38.37±8.92, Phang-nga (n=401): 40.84±7.09, Phuket (n=171): 42.40±9.05, Krabi (n=48): 44.31±8.22, Trang (n=66): 32.75±5.76 and Satun (n=99): 44.33±11.09. The values are not significantly different (Fig. 1 and Table 1). However, the number of tentacle of *C. buitendijki* in Trang is significant different from that of Phuket and Krabi (Fig. 2 and Table 1).

3.2 *Systematic account*

Order Chirodropida

Family Chiropsalmidae Thiel, 1936

Genus *Chiropsoides* Southcott, 1956

***Chiropsoides* Southcott, 1956**

Type species: *Chiropsalmus buitendijki* Horst, 1907 (by monotypy).

Diagnosis: Chirodropida with smooth, unbranched, finger-like gastic saccules, lacking filaments, unilaterally branching pedalia (Gershwin, 2006).

Remarks: *Chiropsoides quadrigatus* is a nomen dubium species, meaning a species commonly in doubt and debate. Although it best resides in Genus *Chiropsoides*, a new genus may

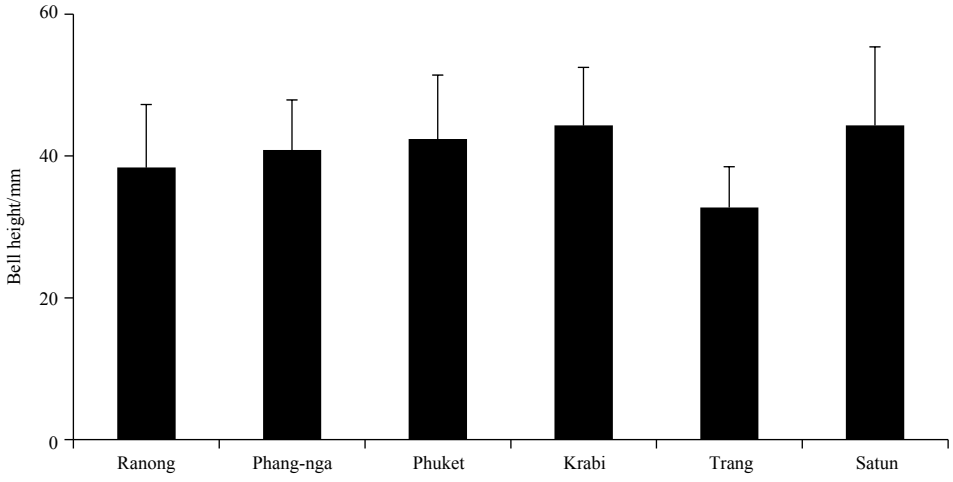

Fig. 1. Means and standard deviations of bell heights in provinces along the Andaman Sea: Ranong (n=29), Phang-nga (n=401), Phuket (n=171), Krabi (n=48), Trang (n=66) and Satun (n=99).

Table 1. Bell height and tentacle number of *Chiropsoides buitendijki* in the Andaman Sea

Province	Number of specimens	Bell height/mm			Number of tentacle		
		Mean	SD	Range (Min–Max)	Mean	SD	Range (Min–Max)
Ranong	29	38.37	8.92	18.81–58.80	3.55	0.57	3–5
Phang-nga	401	40.84	7.09	19.40–63.40	3.63	0.54	3–5
Phuket	171	42.40	9.05	19.00–62.90	4.75	0.69	3–6
Krabi	48	44.31	8.22	21.90–62.00	4.27	0.64	3–5
Trang	66	32.75	5.76	24.00–47.00	3.06	0.24	3–4
Satun	99	44.33	11.09	21.80–73.30	3.99	0.68	3–6

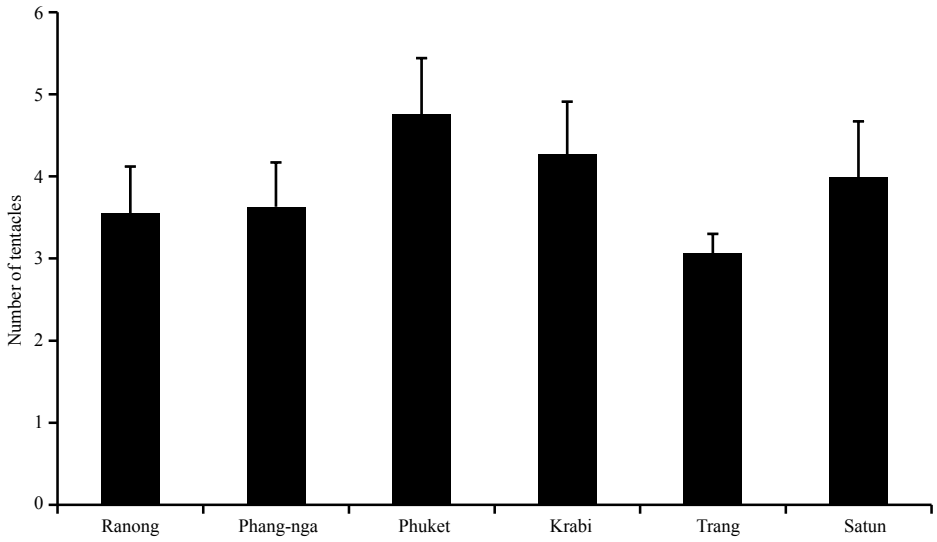

Fig. 2. Number of tentacles and standard deviations in provinces along the Andaman Sea: Ranong (n=29), Phang-nga (n=401), Phuket (n=171), Krabi (n=48), Trang (n=66) and Satun (n=99).

soon be necessary.

***Chiropsoides buitendijki* (Horst, 1907)**

(Figs 1, 2, 3a–h and Table 1)

Chiropsalmus buitendijki Horst, 1907: 101–106, Pl. 2, Figs 1–5.

Chiropsoides buitendijki – Gershwin, 2006: 18–22, Pls 3a–d, 5b, c, with synonymy; – Sucharitakul et al., 2016: 7–14.

3.2.1 *Morphological study*

Description: A total of 814 specimens were collected and examined. A set of specimens preserved in a good condition was selected for registration (Appendix Table A1). Cuboidal bell, with a rounded dome-shape apex; smooth exumbrellar surface without nematocyst warts. Thick mesoglea at bell apical and interradial pillars. Adradial body walls have thinner mesoglea layer; becoming thicker in upper-half of the bell. Adradial furrows shallow in lower-half bell and disappear in upper bell. Interradial furrows absent.

Eight gastric saccules attach to upper subumbrellar cavity; 2 pouches each pillar, unbranched, finger-like in shape, longer than 2/3 of the bell height. Single rooted phacellae arrange itself in a horseshoe-shape, attaching to each corner. Each filament is short and unbranched (Figs 3d, e).

Four pedalia present, interradial, with 3–6 tentacular branches abaxially, aligned and size reduced downward; terminal branch is the smallest. Main pedalia curve into oral direction below the bell. Pedalial canals with a long, narrow spike shape (Fig. 3b), point into each pillar mesoglea. Abaxial ridge of pedalial canals have a short spike between 2 tentacular canals while adaxial side is fine without any spike. Pedalial canals radially flat as ribbon-like tentacles but smaller in diameter. Tentacular banding comes with 1–3–2–3–2–3–2–3–1 patterns (1–major band, 2–thicker minor band and 3–thinner minor band; Fig. 3h).

Four rhopalial niches present, perradial, with a tiny upper scale; lower edge is slightly concave. Ostia oval shaped and shallow, without rhopaliar horns. Six eyes in each rhopalium, with 2 median lensed eyes and 4 lateral pigment spots (Fig. 3g).

Velarium wide; 2 velarial canals per octant then dendritically branched into countless canals in highly complex form. Perradial lappets pyramidal shape, about equal in each side, with lateral and distal branches, complex dendritic form (Fig. 3f). Transparent and colourless living body, with yellowish-white tentacles.

Remarks: The character of rhopalium agrees with that described by Gershwin (2006) and Sucharitakul et al. (2016, Figs 2A, B). Gershwin (2006) found that *C. buitendijki* has a phacellae arranged in V-shape as a typical Chirodropida being, but all specimens examined in this study show a conspicuous horseshoe-shaped phacellae. The tentacular banding pattern was revealed in this study because of the good specimen condition, as well as the staining technique. A preserved tentacle in formalin normally has a yellowish-white color.

Even though the number of specimens varys in each province, the range and mean of bell height from Ranong, Phang-nga, Phuket, Krabi and Satun show an increasing trend (Table 1). The specimens from Trang, however, were slightly lower in bell height.

Distribution: India, Thailand (Andaman Sea), Malay Archipelago, and Indochina.

3.2.2 *Phylogeny*

The partial sequences of 16S and 18S genes were generated from eight samples. The 18S sequences of the eight samples were 100% identical from each other, and showed two nucleotide differences from the deposited sequence of *C. buitendijki* (accession No. KJ135023) from Sucharitakul et al. (2016). Due to the length variation, KJ135023 was excluded from the following phylogenetic analysis.

These eight 18S gene sequences formed a monophyletic clade sister to *Meteorona* (LC033478, LC033479 and LC033480), a new genus observed in Japan (Toshino et al., 2015, Fig. 4).

The 16S gene sequences, on the other hand, showed significant divergence within the eight samples and formed two reciprocally monophyletic clades, one enclosed specimens of PMBC 25893 (accession No. KY980651–2) and PMBC 27976 (accession No. KT982728), the other consisted of the five specimens of PMBC 27985 (accession No. KT982726), PMBC 27972 (accession No. KT982727), PMBC 27986 (accession No. KT982729), PMBC 27989 (accession No. KT982730) and PMBC 27971 (accession No. KT982725) (Fig. 5).

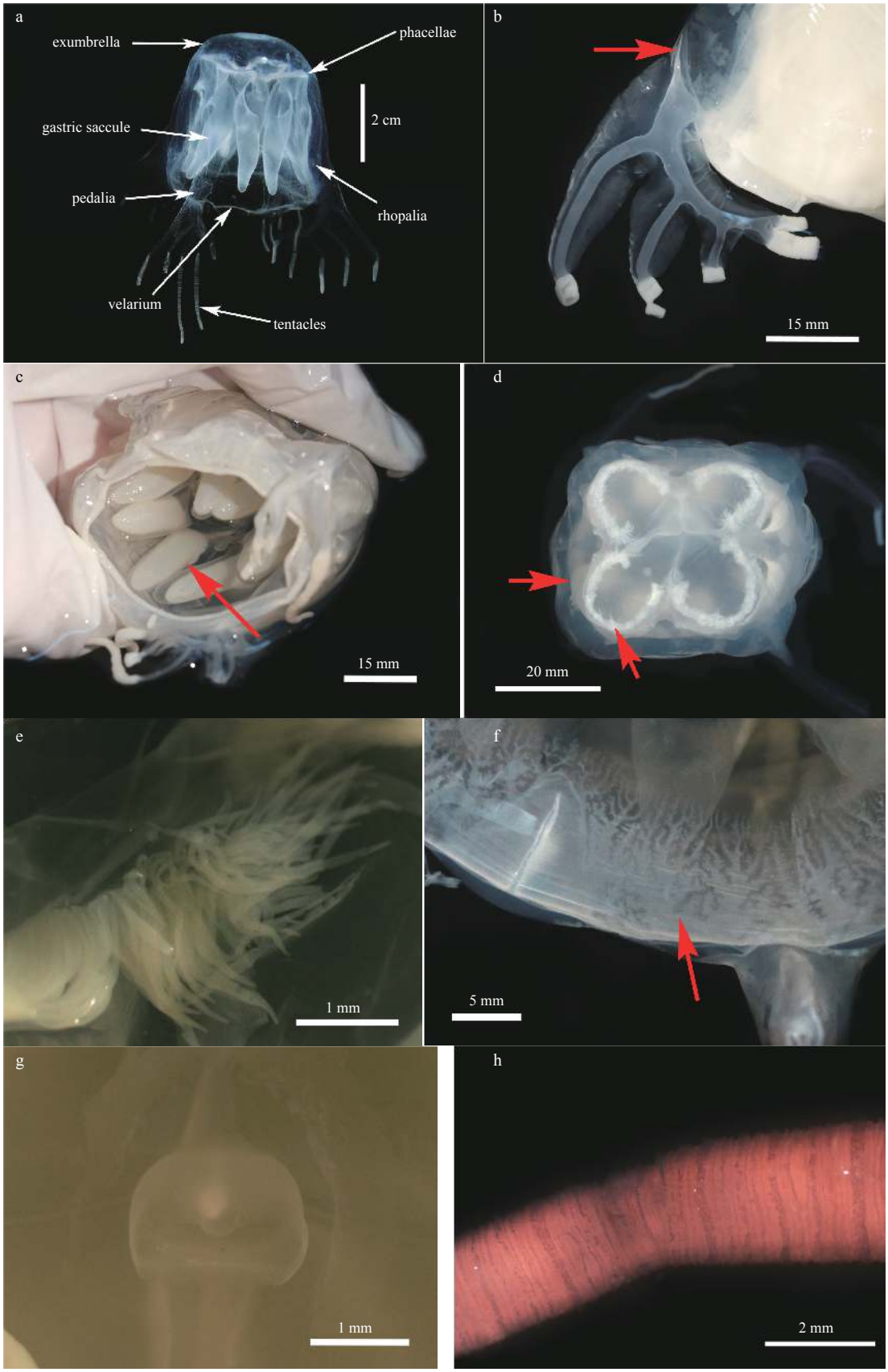

Fig. 3. Morphological characters of *Chiropsoides buitendijki*. a. Overall morphology of *C. buitendijki* (modified after Aungtonya and Chanachon, 2012); b. pedalium; c. gastric saccule; d. apical view shows phacella arrangement (arrow); e. phacellae; f. velarial canals (arrow); g. rhopalial niche ostium; and h. color stained tentacular banding.

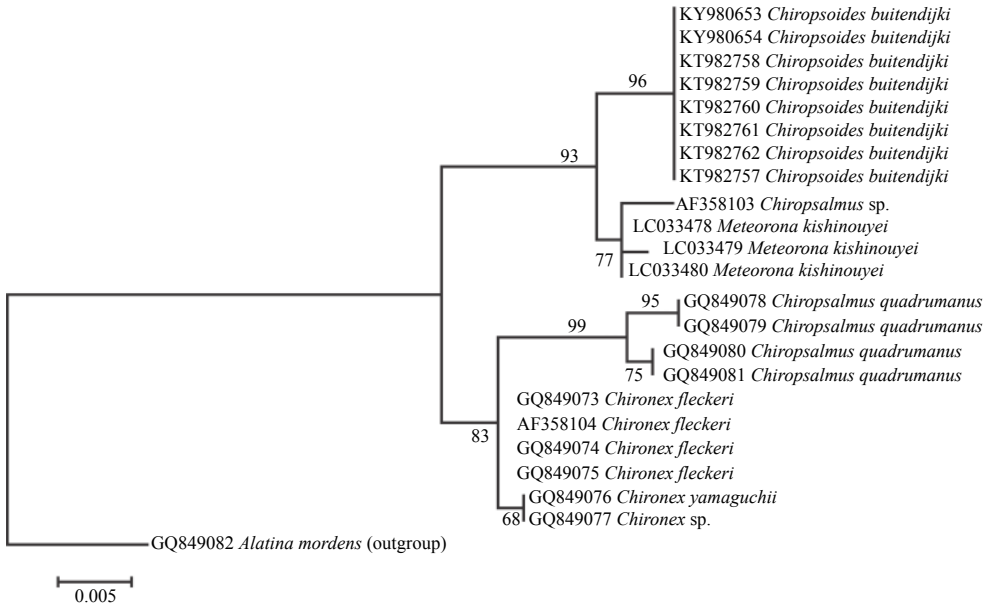

Fig. 4. The Maximum-Likelihood (ML) tree based on the partial 18S gene sequences. Numbers on the node are boos trapping support (>50%) after 1 000 replicates. Bold fonts are those from this study.

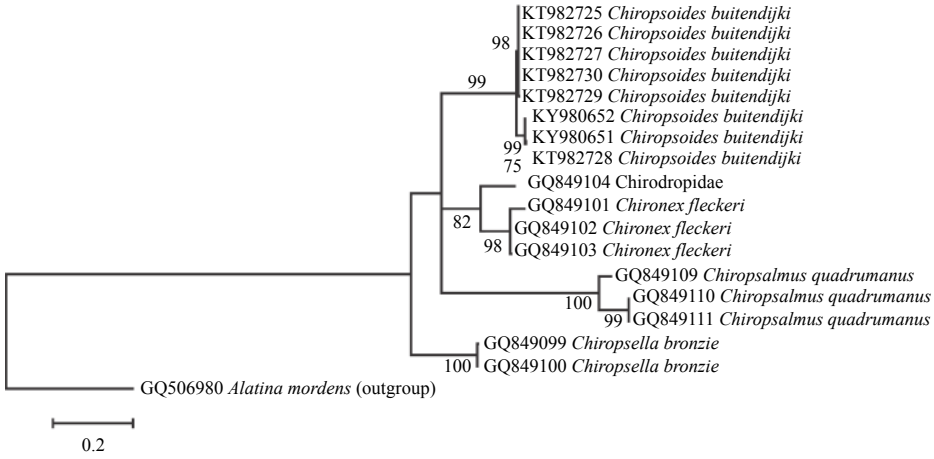

Fig. 5. The Maximum-Likelihood (ML) tree based on the partial 16S gene sequences.

Above all, these eight samples were genetically similar to *C. buitendijki* identified by Sucharitakul et al. (2016), and distinct to the other known taxa in Order Chirodropidae, including *Chironex*, *Chiropsella*, *Chiropsalmus* and *Meteorona*.

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Appendix:

Table A1. *Chiropsoides buitendijki*: selected materials for deposited in the PMBC Reference Collection

PMBC No.	Individual number	Locality	Province in Tailand	Collector	Date (year-month-day)
26260	1	LantaYai Island	Krabi	Wanida Onkaew	2008–11–29
26252	3	Nam Bor Bay	Phuket	Krittaya Chanachon	2009–09–02
26245	4	Nam Bor Bay	Phuket	Wanida Onkaew	2010–06–11
26284	10	Nam Bor Bay	Phuket	Krittaya Chanachon	2010–07–21
26285	6	Nam Bor Bay	Phuket	Krittaya Chanachon	2010–08–24
26297	4	Rang Yai Island	Phuket	Krittaya Chanachon	2010–09–22
26330	25	Nam Bor Bay	Phuket	Krittaya Chanachon	2010–10–19
26314	2	Chebilang Village	Satul	Krittaya Chanachon	2010–10–22
26338	2	Rang Yai Island	Phuket	Krittaya Chanachon	2010–12–22
26265	3	Sapum Bay	Phuket	Krittaya Chanachon	2010–12–22
26259	13	Nam Bor Bay	Phuket	Krittaya Chanachon	2011–04–25
26831	7	Sarai Island	Satul	Varintha Vasinamekhin	2011–07–12
25894	10	Song Phi Nong Island	Phang-nga	KrittayaChanachon	2012–06–12
26280	6	Sarai Island	Satul	Krittaya Chanachon	2012–06–18
26286	1	Tanyong Po Cape	Satul	Krittaya Chanachon	2012–06–18
26258	4	Siboya Island	Krabi	Krittaya Chanachon	2012–06–25
26247	5	MakNoi Island	Phang-nga	Krittaya Chanachon	2012–09–05
26248	18	Song Phi Nong Island	Phang-nga	Krittaya Chanachon	2012–09–05
25892	6	Panak Island	Phang-nga	Krittaya Chanachon	2013–01–16
26246	17	Khai Island	Phang-nga	Krittaya Chanachon	2013–02–14
26266	29	Sukorn Island	Trang	Krittaya Chanachon	2013–04–23
20499	5	Phang-nga Bay	Phang-nga	Krittaya Chanachon	2013–04–25
25893	19	Phang-nga Bay	Phang-nga	Piyawut Sukthong	2013–05–14
26253	9	LantaYai Island	Krabi	Piyawut Sukthong	2013–09–24
26254	2	Klong Dao Beach	Krabi	Charatsee Aungtonya	2013–10–30
27984	1	AoKhoei	Phang-nga	Armas Toema	2014–06–24
27994	5	Ban Chaiphattana	Phang-nga	Piyawut Sukthong	2014–06–30
27943	3	Ban LaemTukkae	Phuket	Charatsee Aungtonya	2014–10–22
27982	5	Mapraw Island	Phuket	Charatsee Aungtonya	2014–10–22
27971	15	Ban NaiRai	Phang-nga	Piyawut Sukthong	2014–11–28
27985	20	AoKhoei	Phang-nga	Piyawut Sukthong	2014–12–24
27992	1	Ban NaiRai	Phang-nga	Piyawut Sukthong	2014–12–26
27988	4	Ban Nam Khem	Phang-nga	Piyawut Sukthong	2015–01–08
27972	1	Ban Nam Khem	Phang-nga	Piyawut Sukthong	2015–02–05
27976	9	Koh Had Sai Dam Village	Ranong	Piyawut Sukthong	2015–04–21
27986	30	AoKhoei	Phang-nga	PiyawutSukthong	2015–04–22
27989	28	Ban Nam Khem	Phang-nga	Piyawut Sukthong	2015–04–23
27977	1	Koh Had Sai Dam Village	Ranong	Piyawut Sukthong	2015–05–19
27987	21	AoKhoei	Phang-nga	Piyawut Sukthong	2015–05–20
27980	1	Sin Hai Island	Ranong	Piyawut Sukthong	2015–06–23
27993	23	Ban NaiRai	Phang-nga	Piyawut Sukthong	2015–07–14
27978	31	Koh Had Sai Dam Village	Ranong	Piyawut Sukthong	2015–07–21
27990	25	Ban Nam Khem	Phang-nga	Piyawut Sukthong	2015–07–23
27983	9	AoKhoei	Phang-nga	Piyawut Sukthong	2015–08–04
27979	4	Koh Had Sai Dam Village	Ranong	Piyawut Sukthong	2015–08–04
27995	3	LantaYai Island	Krabi	Charatsee Aungtonya	2015–10–14
27996	14	LantaYai Island	Krabi	Charatsee Aungtonya	2015–11–11
27981	5	Ban LaemTukkae	Phuket	Piyawut Sukthong	2015–11–19
27991	31	Ban NaiRai	Phang-nga	Piyawut Sukthong	2015–11–28
27973	5	LantaYai Island	Krabi	Charatsee Aungtonya	2016–01–28
27974	1	LantaYai Island	Krabi	Piyawut Sukthong	2016–04–28
27997	2	LantaYai Island	Krabi	Piyawut Sukthong	2016–05–11
27944	6	Ao Nang	Krabi	Usawadee Detsri	2016–07–07
27975	1	Nai Thon Beach	Phuket	Charatsee Aungtonya	2016–09–22