RESEARCH ARTICLE



# Geosesarma mirum, a new species of semi-terrestrial sesarmid crab (Crustacea, Decapoda, Brachyura) from central Taiwan

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## Abstract

A new species of semi-terrestrial sesarmid crab of the genus *Geosesarma* De Man, 1892, is described from central Taiwan. *Geosesarma mirum* **sp. nov.** is distinct in possessing a strong transverse crest on the inner surface of the male chela and a diagnostic male first gonopod which is relatively long and stout, with the distal chitinous part broad and spatuliform. Like most *Geosesarma* species, *G. mirum* **sp. nov.** has large eggs and direct development, contrasting with the only other species known from Taiwan, *G. hednon* Ng, Liu and Schubart, 2004, which has small eggs and planktotrophic larvae.

## Keywords

Direct development, East Asia, freshwater, large eggs, Sesarmidae, taxonomy

# Introduction

*Geosesarma* De Man, 1892, is a large genus of semi-terrestrial and terrestrial crabs occurring in many freshwater habitats in Southeast Asia, Andamans, and western Pacific. At the moment, 64 species have been recorded, with the majority from Indonesia (Ng et al. 2008; Ng 2015, 2017; Ng et al. 2015; Manuel-Santos et al. 2016; Ng and Grinang 2018; Ng and Wowor 2019; Ng and Ng 2019). Thus far, the most northerly record of the genus is Taiwan and Luzon in the Philippines, with two known species: *G. vicentense* (Rathbun, 1914) and *G. hednon* Ng, Liu & Schubart, 2004 (Ng et al. 2004; Ng and Lemaitre 2017). Both species, however, are atypical members of *Geosesarma* as they have small eggs and planktotrophic larvae (see Ng et al. 2004; Ng and Lemaitre 2017). Most species of *Geosesarma* (when egg sizes are known) (over 90%) have large eggs and direct development.

In late 2018, the authors became aware of a semi-terrestrial freshwater sesarmid living in the lowlands of Chiayi County in central-western Taiwan with large eggs. Specimens were eventually obtained and studies showed that they belonged to a new species of *Geosesarma*. This is the first record of a large-egged *Geosesarma* from such a high latitude. The description of the new species and comparisons with congeners form the basis of the present paper.

#### Materials and methods

The terminology used here follows Ng (1988) and Davie et al. (2015). Measurements provided are the carapace width and length, respectively. The abbreviations G1 and G2 are used for the male first and second gonopods, respectively. Specimens examined are deposited in the zoology collections of the National Taiwan Ocean University (**NTOU**), Keelung, Taiwan; and the Zoological Reference Collection (**ZRC**) of the Lee Kong Chian Natural History Museum, National University of Singapore. Specimens with eggs or young crabs were not collected and released after study.

#### Systematic accounts

Family Sesarmidae Dana, 1851

### Genus Geosesarma De Man, 1892

**Type species.** *Sesarma* (*Geosesarma*) *nodulifera* De Man, 1892, subsequent designation by Serène and Soh (1970).

*Geosesarma mirum* sp. nov. http://zoobank.org/60FAAFB1-8BC8-416C-AF80-295A6EEA45D0 Figures 1–5

**Material examined.** Holotype: TAIWAN - male (11.9 x 10.8 mm); Chiayi County, Jhongpu, Lunziding Canal; 23.44914°N 120.48227°E; 28 Jan. 2019, leg. J.-Y. Shy; under rocks near stream; NTOU F10395. Paratypes: TAIWAN - 3 males (11.5 x 10.3 mm, 10.1 × 8.9 mm, 9.9 × 8.7 mm), 5 females (12.2 × 10.1 mm, 10.6 × 9.2 mm,



**Figure 1.** Geosesarma mirum sp. nov. **A**, **B** male  $(9.9 \times 8.6 \text{ mm})$  (NTOU F10396) **C** ovigerous female with eggs (specimen not collected) **D** brooding female with young crabs (specimen not collected). Photographs **A–C** J-Y Shy, **D** Peter Wang.

 $9.9 \times 8.9$  mm,  $9.1 \times 7.7$  mm,  $7.9 \times 7.2$  mm), 1 juvenile female (6.6 × 5.7 mm); same data as holotype; ZRC 2019.0513. 2 males ( $9.9 \times 8.6$  mm,  $8.4 \times 7.3$  mm); Chiayi County, Jhongpu, Chilan River;  $23.43744^{\circ}$ N 120.48917°E ; 21 Febr. 2019, leg. J.-Y. Shy; NTOU F10396. 1 male ( $11.5 \times 9.8$  mm); Chiayi County, Jhongpu, branch of Chilan River; ca.  $23.43744^{\circ}$ N 120.48917°E; 22 March 2019; leg. J.-Y. Shy; NTOU F10397. 7 males ( $7.8 \times 6.5$  mm –  $12.2 \times 11.2$  mm), 5 females ( $9.3 \times 7.8$  mm –  $11.4 \times 10.0$  mm), same locality as holotype; 23 March 2019; leg. J.-Y. Shy & H.-T. Lai; ZRC 2019.0514.

**Diagnosis.** Carapace quadrate, slightly wider than long or subequal, adult width to length ratio 1.10–1.21, lateral margins gently sinuous, gently diverging posteriorly (Fig. 2A, B); dorsal surface with regions visible, anterior regions with small rounded granules on gastric regions, branchial regions with numerous striae (Fig. 2A, B); front distinctly deflexed, frontal lobes broad, with subtruncate margins in dorsal view; postfrontal, postorbital cristae prominent, rugose (Fig. 2A–C); external orbital tooth triangular to subtruncate, directed obliquely laterally, outer margin convex, shorter than inner margin, tip reaching lateral margin; second lateral tooth low, rounded, separated from external orbital tooth by deep notch (Fig. 2A, B). Merus of third maxilliped subovate, subequal to ischium; exopod slender, reaching to just before edge of merus, with long flagellum (Fig. 4A). Merus of cheliped with low ventral lobe with serrated margin, upper lobe relatively lower. Outer surface of palm of adult



**Figure 2.** Geosesarma mirum sp. nov. holotype male (11.9 × 10.8 mm) (NTOU F10395), Taiwan **A** overall dorsal view **B** dorsal view of carapace **C** frontal view of cephalothorax **D** anterior thoracic sternum and pleon.

male covered with small rounded granules and striae; inner surface granulated, with distinct, high transverse granulated ridge; dorsal margin of dactylus with 11 or 12 low, non-chitinous tubercles on proximal two-thirds (Fig. 3). Ambulatory legs with relatively stout, short merus, with sharp subdistal spine on dorsal margin, surfaces rugose (Fig. 1A). Part of male thoracic sternite 8 exposed when pleon closed. Male pleonal locking mechanism formed by expanded posterior edge of thoracic sternite 4. Male pleon triangular; somite 6 wide, with convex lateral margins; telson triangular, not recessed into distal margin of somite 6, margins convex (Figs 2D, 4B). G1 relatively long, stout, gently curved outwards; outer margin of subdistal part of subterminal segment with subangular shelf-like structure (Fig. 4C, D), distal chitinous part broad, tip spatuliform, margin uneven (Fig. 4C–K). G2 short, ca. a third length of G1 (Fig. 4L).

**Etymology.** The name is derived from the Latin for "surprise", alluding to the unexpected discovery of this species in Taiwan.



**Figure 3.** *Geosesarma mirum* sp. nov. holotype male (11.9 × 10.8 mm) (NTOU F10395), Taiwan. Right chela **A** outer view **B** outer view of dactylus **C** dorsolateral view **D** frontolateral view showing crest on inner surface **E–G** different views of inner surface.

**Remarks.** Geosesarma mirum sp. nov. belongs to the group of species which have large eggs (ca. 1.0 mm or greater in diameter, measured in situ), the carapace is trapezoidal to subquadrate, the ambulatory meri are relatively short and stout, the exopod of the third maxilliped has a long flagellum, the inner surface of the male chela has a strong granulated transverse ridge and the G1 is relatively stout with the distal chitinised part spatuliform. The species in this group are: *G. amphinome* (De Man, 1899) [western Borneo], *G. peraccae* (Nobili, 1903) [Singapore and Peninsular Malaysia], *G. penangense* (Tweedie, 1940) [Penang, Peninsular Malaysia], *G. sarawakense* (Serène, 1968) [Sarawak, Borneo] and *G. pylaemenes* Ng, 2015 [western Borneo]. *Geosesarma mirum* can be distinguished from these species mainly by the distinctive form of its G1. Compared to *G. amphinome*, the distal chitinised part of the G1 of *G. mirum* is distinctly shorter and the tip is not bilobed (cf. Ng 2015: fig. 1A, B, 2A, G–K).



**Figure 4.** *Geosesarma mirum* sp. nov. holotype male (11.9 × 10.8 mm) (NTOU F10395), Taiwan **A** left third maxilliped **B** pleon **C**, **D** different views of ventral surface of left G1 **E** dorsal surface of left G1 **F–H** different views of ventral surface of distal part of eft G1 **I** mesial view of distal part of left G1 **J**, **K** different views of dorsal surface of distal part of left G1 **L** left G2. Scale bars: 0.5 mm (**A**, **C**, **D**, **L**); 1.0 mm (**B**); 0.2 mm (**F–K**).

Compared to *G. peraccae*, the G1 of *G. mirum* is stouter overall with the chitinous part proportionately much shorter (cf. Ng 1988: fig. 56A, D–F; Ng 2015: fig. 5A, B). The G1 of *G. mirum* differs from that of *G. penangense* in having the distal part gently curved rather than strongly bent, with the ambulatory leg merus proportionately stouter (cf. Ng 1988: fig. 58A, D, E). When compared to *G. sarawakense*, the carapace of *G. mirum* is distinctly more granulated and rugose, with the G1 proportionately stouter and shorter (Ng 2015: figs 6A, B, 7D–F). In contrast to *G. pylaemenes*, the external orbital tooth of *G. mirum* is more acute and the G1 is relatively stouter (cf. Ng 2015: fig. 3A, B, 4D–G).



**Figure 5.** *Geosesarma mirum* sp. nov. paratype female (10.6 x 9.2 mm) (ZRC 2019.0513), Taiwan **A** overall dorsal view **B** anterior thoracic sternum and pleon **C** sternopleonal cavity and vulvae.

In Taiwan and other parts of the Indo-West Pacific, *Geosesarma mirum* can be confused with species of *Scandarma* Schubart, Liu & Cuesta, 2003 (type species *Scandarma lintou* Schubart, Liu & Cuesta, 2003), and *Pseudosesarma* Serène & Soh, 1970 (type species *Sesarma edwardsii* De Man, 1887) because in these genera, the male chelipeds do not have pectinated ridges on their chelae and there are no stridulatory granules on the dorsal margin of the dactylus. *Geosesarma mirum* can be easily distinguished from species of *Scandarma* as the outer surface of the male chela does not have a distinct swelling and the G1 is very short and stout (see Schubart et al. 2003; Naruse and Ng 2007; Ng 2013; Naruse and Ng 2019). From *Pseudosesarma*, many members of which live in freshwaters, *G. mirum* can be distinguished by the G1 morphology, with those of *Pseudosesarma* species short, very stout with the median or distal parts prominently dilated and with sharp chitinised "beaks" (see Ng and Schubart 2017).

**Ecology.** *Geosesarma mirum* sp. nov. has a semi-terrestrial habit and has been found in small streams in lowlands, with the crabs digging burrows under stones near the edge of the water. The first author investigated 28 sites in and around the type locality of species, in the Ba-Jhang River region (total area of ca. 200 km<sup>2</sup>). Most of the sites examined were badly polluted, cemented, built over or no longer had permanent water. There were some sites with clean water but these were very close to the hills



**Figure 6.** Habitat of *Geosesarma mirum* sp. nov. **A** Chilan River, Jhongpu, Chiayi County, Taiwan, 23.43744°N, 120.48917°E **B** Lunziding Canal, Jhongpu, Chiayi County, Taiwan, 23.44914°N, 120.48227°E.

(altitude higher than ca. 100 m) but these sites only had the primary freshwater crab, *Geothelphusa olea* Shy, Ng & Yu, 1994 (Potamidae). Of the 28 sites surveyed, *Geosesarma mirum* was only found in six sites (in an area of ca. 4 km<sup>2</sup>). These six sites were from different branches of the river and the crabs were relatively abundant in each of these locations (sometimes more than 50 individuals/m<sup>2</sup>). From the surveys done, the species seems to have a relatively localised distribution, with some of the sites where they were found only a few dozen square metres in area, and the crabs absent from sites further upstream or downstream. This may not be the natural situation as the sites where the crabs were absent were invariably badly polluted or extensively concreted.

The development of *G. mirum* is direct (i.e., abbreviated), with the eggs measuring ca. 1.0 mm in diameter (specimen not preserved) (Fig. 1C). The egg sizes of *Geosesarma* species average between 1.2 and 1.8 mm (Ng 2017; Ng and Wowor 2019). Despite the slightly smaller egg size, we have observed females of *G. mirum* brooding young crabs under the pleon (Fig. 1D) and it is clear that the development is completely abbreviated (see Ng and Tan 1995). This contrasts with catadromous grapsoid species like *Eriocheir japonica* (De Haan, 1835) (Varunidae) which have much smaller eggs (ca. 0.34 mm; cf. Lai et al. 1986). Their eggs, however, are still smaller than those of primary freshwater species like *Nanhaipotamon formosanum* (Parisi, 1916) (Potamidae) which have egg diameters of up to 4.0 mm (cf. Lai et al. 2012).

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RESEARCH ARTICLE



# Resolution of the Portunus gladiator species complex: taxonomic status and identity of Monomia gladiator (Fabricius, 1798) and Monomia haanii (Stimpson, 1858) (Brachyura, Decapoda, Portunidae)

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## Abstract

The United States Food and Drug Administration (FDA) has recently adopted DNA barcoding for the purpose of determining the species identity of commercial seafood products. This effort has revealed instances of incongruence between current scientifically accepted taxon names and those utilized by the seafood industry in product labelling. One such case is that of "*Portunus haanii*", a name utilized by the seafood industry to label commercial products under the market name "red swimming crab." However, carcinologists currently regard *P. haanii* as synonym of *Portunus gladiator* Fabricius, 1798, which itself is the subject of debate over whether it is a secondary homonym of *Cancer gladiator* Fabricius, 1793. Further complicating matters, DNA barcode sequences from commercial products match GenBank sequences identified as *Portunus gladiator* complex is reviewed and a resolution proposed based on combined morphological descriptions and molecular phylogenetic analyses. It is demonstrated that, given the provisions of the International Code of Zoological Nomenclature and the current elevation of *Monomia* Gistel, 1848, to full genus rank, its type species, *Portunus gladiator* Fabricius, 1798, should be treated as a valid and available taxon name. It is also shown, upon examination and comparison of types and topotypic material that *Monomia haanii* (Stimpson, 1858) is a distinct taxon from *M. gladiator*, and *Portunus pseudoargentatus* 

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Stephenson, 1961, is a junior subjective synonym of *M. haanii* (Stimpson, 1858). Furthermore, it is shown that crab meat sold in the US currently labeled as "*Portunus haanii*" and/or "red swimming crab" is in fact *M. haanii* using comparative analysis of DNA barcode sequences between museum-vouchered reference specimens, whole crabs provided directly by a seafood importer, and processed commercial products purchased at retail.

#### **Keywords**

Commercial species, DNA barcoding, molecular phylogenetics, morphology, seafood, swimming crab, taxonomy

# Introduction

The United States Food and Drug Administration (FDA) has adopted DNA barcoding for the purpose of species identification to assure the accurate labelling of seafood products as well as to address issues with species substitution and fraud (Handy et al. 2011; Eischeid et al. 2016). This effort has identified instances of incongruence between currently accepted taxon names and the names utilized by industry in product labelling. At the same time that FDA was beginning to build its reference library of decapod crustacean DNA standards (Food and Drug Administration 2017), species substitution of brachyuran crabs was highlighted by a survey of crab cakes from restaurants in the Maryland / Washington, DC metropolitan area (Warner et al. 2015). DNA testing from that survey of products advertised by restaurants to contain crabmeat only from "local" Callinectes sapidus Rathbun, 1896, revealed at least one of six species of substituted portunoid crab in 38% of the crab cakes tested (Warner et al. 2015). According to the report, the most commonly detected substitute species was Portunus pelagicus (Linnaeus, 1758) followed by P. pseudoargentatus Stephenson, 1961, and P. sanguinolentus (Herbst, 1783). The reported DNA match to P. pseudoargentatus was of particular interest to Warner et al. (2015) who noted that this species was, at the time, not included in the FDA Guide to Acceptable Market Names for Seafood Sold in Interstate Commerce (The Seafood List) (Food and Drug Administration 2012) nor in the FAO fishery species list (Food and Agriculture Organization of the United Nations 2010–2018), raising concern that this species was unknown to regulators. As a result, in addition to the existing entry for P. haanii, P. pseudoargentatus was added to The Seafood List in 2015 (Food and Drug Administration 2015). Concerns over an unknown species that was apparently common in the US food supply led us to further investigate the identity of P. pseudoargentatus and its close relatives within the Portunus gladiator species complex to determine their relationship to what was currently being harvested and marketed as "P. haanii" and/or "red swimming crab."

At the time of the study by Warner et al. (2015), only three sequences from three loci belonging to one specimen identified as "*Portunus pseudoargentatus* (ZMMU Ma 3368)," which was collected from Nhatrang Bay, Vietnam, had been deposited

in GenBank (JX398121, JX398079, JX398094) (Spiridonov et al. 2014). Further complicating matters, there is a set of DNA barcode sequences (BOLD: AAO6694) identified as *"Portunus haanii"* from the Coral Sea in the Barcode of Life Database (BOLD), which led to more uncertainty about the true identity of crabs in the US seafood supply. The specimens in question, which are deposited in the Muséum national d'Histoire naturelle (MNHN) in Paris under the revised name of *P. gladia-tor* (MNHN-IU-2008-12570-77), have since been examined by one of the present authors (AMW) and confirmed to be neither *P. gladiator* nor *P. pseudoargentatus*, but morphologically and genetically closer to *Monomia lucida* Koch and Ďuriš, 2017. *Portunus haanii* was included on the FDA Seafood List prior to this study without knowledge that *P. haanii* is currently an unaccepted species name, having been synonymized under *P. gladiator* Fabricius, 1798 (Stephenson and Cook 1973, Ng et al. 2008).

Within the seafood industry, *Portunus haanii* and/or "red swimming crab" is the species/market name used for crabs harvested extensively from China and Vietnam (Monterey Bay Aquarium Seafood Watch 2013; Fishsource 2016). Photographs identified as *P. haanii* on websites advertising "*Portunus haanii*" (e.g., Fishsource 2016; Alibaba.com 2018) bear a striking resemblance to the color photograph of "*Portunus pseudoargentatus* (ZMMU Ma 3368)" (Chertoprud et al. 2012: pl. 51 fig. H) and Stephenson's (1961) original description of *P. pseudoargentatus*. The names *Portunus gladiator* and *Portunus pseudoargentatus* were not found associated with any specific crabmeat products in neither our on-line searches nor our discussions with industry representatives.

# **Taxonomic history**

The taxonomy of the *Portunus gladiator* complex is so convoluted that it makes a chronologically arranged taxonomic history difficult to compile. Here, we present the significant taxonomic actions for *P. gladiator*, *P. haanii*, and *P. pseudoargentatus*.

Fabricius (1798) described a swimming crab collected from "Oceano Asiatico Dom. Daldorff" (probably Tranquebar, India; see Ng et al. 2008), which he named *Portunus gladiator*, based on an unspecified quantity of specimens. Fabricius (1798: 368) gave a brief description in Latin, stating: "P. thorace tomentoso utrinque novemdentato: dente postico maiore, minibus sanguineo maculatis. Habitat in Oceano Asiatico Dom. Daldorff. Praecedentibus affinis at minor. Thorax holosericeus, parum inaequalis, hine inde scaber. Chelae sanguineo maculatae digitis apice dentibusque albis."

Previously, however, Fabricius (1793) had given the same specific epithet to a different species of swimming crab (from "Nova Hollandia" = Australia), *Cancer gladiator*. Fabricius (1793: 449) provided this description: "C. thorace laevi: lateribus octodentatis, postico maximo, minibus angulatis. Cancer hastatus. Mant. Ins. r. 319. 34. Habitat in Nova Hollandia Mus. Dom. Banks. Minutus pullo modo Cancer hastatus Linnaei. Palmae anticae bidentatae, chelae angulatae. Palmae posticae angulatae."

Latreille (1825) in his treatment of *Portunus*, considered *Cancer gladiator* Fabricius, 1793, a junior synonym of *Portunus sanguinolentus* (Herbst, 1783), and clearly also considered *Portunus gladiator* Fabricius, 1798, to be a distinct species. From this point on, it appears that the name "*Portunus gladiator*" has been almost exclusively associated with the species described in 1798. Stephenson and Cook (1973) would later accept Latreille's (1825) synonymization and selected a male specimen of *P. sanguinolentus* from Queensland, Australia (QM W3683) to be the neotype of *C. gladiator* Fabricius, 1793, in order to stabilize its taxonomy.

De Haan (1833) then established the subgenus *Portunus (Amphitrite)*, to which Miers (1886) subsequently designated *Neptunus (Amphitrite) gladiator* (Fabricius, 1798), as its type species. Prior to Miers' action, however, Gistel (1848) had proposed a replacement name, *Portunus (Monomia)*, for this subgenus as the name *Amphitrite* had been previously used for a polychaete annelid genus, viz. *Amphitrite* Müller, 1771. By virtue of these, *Portunus gladiator* Fabricius, 1798, is the type species of *Portunus (Monomia)* Gistel, 1848.

Stimpson (1858) gave the name *Amphitrite haanii* to a species from Tanegashima and Kagoshima, Japan and the 'China Seas, above 23°N latitude,' previously identified by De Haan (1833, 1835) as *Portunus (Amphitrite) gladiator* (Fabricius, 1798). Stimpson (1858: 38) wrote: "Amphitrite Haanii. *A. gladiator*, De Haan; loc. cit. p. 29, pl. i. f. 5. (v ix *L. gladiator*, M. Edwards.) In mari Sinensi, lat. bor. 23°; ad insulam 'Tanegasima'; et in sinu 'Kagosima'; in fundis arenosis prof. 12-20 org."

As Stimpson (1858) had material from Japan and also referred to De Haan's (1833) citations of the species as *P*. (*A*.) gladiator, all of Stimpson's and De Haan's specimens are effectively syntypes. Later, Stimpson (1907: 79) provided a slightly more detailed explanation, clearly opining that De Haan's (1833, 1835) Japanese *P. gladiator* was a distinct species from H. Milne Edwards' (1834) *Lupea gladiator* (= *P. gladiator* Fabricius, 1798) from the Indian Ocean. There are 53 extant syntypes of *Amphitrite haanii* in the collection of the Naturalis Biodiversity Center in Leiden as recorded by Yamaguchi and Baba (1993), and among these they selected a male specimen (RMNH 379, CW = 42 mm, CL = 24 mm, C. Fransen pers. comm.) to be the lectotype.

Stephenson (1961) described *Portunus pseudoargentatus* based on one male specimen from the Abrolhos Islands, off the western coast of Australia. He cited differences in the morphology of the male 6<sup>th</sup> pleomere and male gonopod 1 (G1) between *P. gladiator* and the new species. Crosnier (1962) in his treatment of the swimming crabs of Madagascar commented on the confusion in the identities of specimens from various localities labelled as "*P. gladiator*." He highlighted the differences in the morphology of the male 6<sup>th</sup> pleomere and G1, wherein *P. pseudoargentatus* tends to have the male 6<sup>th</sup> pleomere with less sinuous lateral borders, and the G1 more greatly bent in the middle (midlength) compared to *P. gladiator*. He also referred to Japanese material that he examined to *P. pseudoargentatus*, apparently not considering Stimpson's (1858, 1907) earlier reports on *P. haanii*. Stephenson and Cook (1973) did an extensive study on the *Portunus gladiator* complex, and they argued that, with both being then classified in the genus *Portunus, P. gladiator* Fabricius, 1798, effectively became a secondary homonym of *P. gladiator* (Fabricius, 1793) (= *P. sanguinolentus*), and a replacement name was needed. They went on to suggest *Amphitrite haanii* Stimpson, 1858, as the earliest available replacement name, eliminating an earlier name, *Cancer menestho* Herbst, 1803, on the basis of a difference in colouration and in the armature of the cheliped merus in the illustration of the latter (viz. Herbst, 1803:pl. 55 fig. 3). They also considered *Portunus pseudoargentatus* Stephenson, 1961, as a junior synonym of *Portunus haanii* (Stimpson, 1858). Additionally, Stephenson and Cook (1973) described aberrant "forms" within *P. haanii*, on the basis of the G1 morphology (e.g., "normal" vs. "unusual" specimens; viz. Stephenson and Cook 1973: figs 6, 7). These "unusual" specimens were two crabs from the Bay of Jeddo, Japan, and the holotype of *P. pseudoargentatus*. They did not, however, take steps to formally distinguish these forms as separate species, subscribing instead to the concept of "*Portunus haanii*" as a morphologically variable species.

Ng et al. (2008: 156, 157) provided a detailed account on the nomenclature of Fabricius' two species with the epithet *gladiator*, essentially stating that, contrary to Stephenson and Cook (1973), there is no secondary homonymy unless *Cancer gladiator* is considered a distinct species from *Portunus sanguinolentus*: "... as *Cancer gladiator* Fabricius, 1793, is regarded as a junior synonym of *Portunus sanguinolentus* (Herbst, 1783), the name '*Portunus gladiator* (Fabricius, 1793)' has not been recognized or used anywhere. This being the case, there is no homonymy with *Portunus gladiator* Fabricius, 1798, and this name should remain available for use under the Code. The issue of secondary homonymy will only arise if *Cancer gladiator* Fabricius, 1793, is regarded as a valid species of *Portunus* distinct from *Portunus sanguinolentus* (Herbst, 1783). If this were to happen (for example, if the widespread *P. sanguinolentus* was to prove to be a complex of several cryptic species), then the name *Portunus gladiator* Fabricius, 1798, would have to be replaced by the next available name...". Ng et al. (2008), however, maintained the synonymy of *Portunus gladiator* and *Amphitrite haanii*, while still considering *Portunus pseudoargentatus* a valid species.

Chertoprud et al. (2012) in their report on the commercially valuable brachyuran species of Vietnam, commented that Stephenson and Cook's (1973) mention of "*Portunus gladiator* (Fabricius, 1793)" was sufficient to activate Article 59 of the Code (ICZN 1999), which therefore necessitated the use of *Portunus haanii* (Stimpson, 1858) as a replacement name for *Portunus gladiator* Fabricius, 1798.

As things stand, the issue on the validity of the names "Portunus gladiator" and "Portunus haanii" has not been satisfactorily settled. Recent publications on the systematics of Portunidae have bolstered the concept of Monomia Gistel, 1848, as a valid genus-level taxon distinct from Portunus, but these have also shown that the problem with the taxonomy of the type species, M. gladiator (Fabricius, 1798), and its closely related congeners, M. haanii (Stimpson, 1859) and M. pseudoargentata (Stephenson, 1961), remains unresolved (Chertoprud et al. 2012; Spiridonov et al. 2014; Koch et al. 2017; Koch and Ďuriš 2018). This problem, unless addressed,

is expected to have wide-ranging consequences on the taxonomy of *Monomia*. As such, the identities of *M. gladiator*, *M. haanii*, and *M. pseudoargentata* need to be firmly established.

An integrative approach with morphological and molecular phylogenetic analyses was undertaken to resolve and stabilise the taxonomy of the *Portunus gladiator* (=*Monomia gladiator*) complex. The molecular results of the morphologically verified and vouchered reference specimens, which included whole specimens from Asian fish ports and a seafood importer, were then used as standards to identify the contents of cans of pasteurized lump crabmeat labeled as "*Portunus haanii*" and/or "red swimming crab" through comparative analysis of DNA barcode sequences.

# Materials and methods

# Taxonomic methods

Materials examined are deposited at the US National Museum of Natural History, Smithsonian Institution (USNM); Florida Museum of Natural History, University of Florida (UF); Western Australian Museum (WAM); and Lee Kong Chian Natural History Museum, National University of Singapore (ZRC). These included the holotype of *Portunus pseudoargentatus* (WAM-C7506), as well as crabs purchased at fish ports in India, Thailand, and Taiwan, and whole crabs identified by a US seafood importer as "*Portunus haanii*." Photographs of the type specimens of *Portunus gladiator* and *Amphitrite haanii*, housed at Zoological Museum at the University of Copenhagen (ZMUC) and Naturalis Biodiversity Center, Leiden (RMNH), respectively, were also examined. Details on all specimens utilized in morphological examinations are provided in the material examined subsection of the taxonomic account below. The morphological terminology largely follows Wee and Ng (1995) and Apel and Spiridonov (1998). The following abbreviations are used:

- **CL** carapace length, taken along the dorsal midline from the tips of the frontal teeth to the posterior margin of the carapace;
- **CW** carapace maximum width, taken at the level of its widest point;
- P1–P5 first to fifth percopods, respectively (P1, chelipeds; P2–P5, first to fourth ambulatory legs);
- G1, G2 first and second male pleopods, respectively.

The term, pleomere (first to sixth), here refers to the six somites of the pleon. When possible, DNA was extracted from the specimens utilized for the morphological studies. Details on all specimens utilized in the molecular phylogenetic component of this study are given in Table 1, Nomenclatural decisions are based on the provisions of the International Code of Zoological Nomenclature, here referred to as "the Code" (ICZN 1999).

Table 1. Material examined in molecular analyses with details on voucher identification numbers, sex, country in which the specimen was collected, the fish port
or body of water, and pertinent GenBank Accession Numbers. Voucher ID abbreviations: IOM= Institute of Oceanology and Museum, Nha Trang; MNHN=
Muséum National d'Histoire Naturelle, Paris; NHMUK= The Natural History Museum, London; UF= University of Florida Natural History Museum, Gainesville;
UO= University of Ostrava, Ostrava; USNM= United States National Museum, National Museum of Natural History, Washington, D.C.; WAM= Western Austral-
ian Museum, Perth; ZMMU= Zoological Museum of the Moscow University, Moscow; ZRC= Zoological Reference Collection, Lee Kong Chian Natural History
Museum, Singapore.

E		¢	c		9	enBank Accession N	umbers
laxon name	Voucher 1D	Sex	Country	Fort/Body of Water	12S	16S	COI
Monomia gladiator	ZRC 2016.0145	М	India	Pazhayar Fish Landing, Bay of Bengal	MK270964		MK281257
Monomia gladiator	ZRC 2016.0149	ц	India	Pondicherry, Bay of Bengal	MK270959	MK271060	MK281259
Monomia gladiator	WAM C61156	Ц	Australia	Pilbara Shelf, Indian Ocean	MK270957	MK271053	MK281253
Monomia gladiator	WAM C26459	М	Australia	Dampier Archipelago, Cape Brugieres	MK270956	MK271047	MK281247
Monomia gladiator	UF 36251	ц	Singapore	Singapore Strait	MK270963	MK271029	MK281229
Monomia gladiator	USNM 127068	н	Thailand	Andaman Sea	MK270962	MK271030	MK281230
Monomia gladiator	ZRC 2000.0842	М	Thailand	Pichai Fish Port, Phuket, Andaman Sea	MK270958		MK281230
Monomia gladiator	ZRC 2003.0114	М	Thailand	Pattani Fish Port, Gulf of Thailand	MK270960	MK271055	
Monomia gladiator	ZRC 2003.0197	М	Thailand	Saiburi Crab Landing, Gulf of Thailand	MK270961	MK271056	
Monomia gladiator	ZRC 2002.0297	Ч	Thailand	Pichai Fish Port, Phuket, Andaman Sea			
Monomia gladiator	MNHN-IU-2014-10087		Vietnam	Của Bé Fishing Port		KY524466 <sup>1</sup>	
Monomia gladiator	ZMMU Ma 3366		Vietnam	Của Bé Fishing Port		I	$JX398095^{2}$
Monomia haanii	ZRC 1999.0084	М	Japan	Pacific Ocean	MK270948	MK271054	MK281255
Monomia haanii	WAM C61155	ц	Australia	Pilbara Shelf	MK270946	MK271052	MK281252
Monomia haanii	WAM C34767	ц	Australia	Exmouth Gulf	MK270944	MK271048	MK281248
Monomia haanii	WAM C34900	М	Australia	Exmouth Gulf	MK270945	MK271049	MK281249
Monomia haanii	WAM C44737	ц	Australia	Ningaloo Marine Park	MK270954	MK271051	MK281251
Monomia haanii	WAM C34938	ц	Australia	Shark Bay	MK270949	MK271050	MK281250
Monomia haanii	WAM C55510	М	Australia	Shark Bay	MK270943	MK271045	MK281245
Monomia haanii	WAM C7506*	Σ	Australia	Abrolhos Islands	MK270953	MK271046	MK281246
Monomia haanii	USNM 1421161	М	China	South China Sea, FAO Fishing Area 61	MK270934	MK271033	MK281233
Monomia haanii	USNM 1421181	Σ	China	South China Sea, FAO Fishing Area 61	MK270935	MK271034	MK281234
Monomia haanii	USNM 1421182	Σ	China	South China Sea, FAO Fishing Area 61	MK270936	MK271035	MK281235
Monomia haanii	USNM 1421185	Σ	China	South China Sea, FAO Fishing Area 61	MK270937	MK271036	MK281236
Monomia haanii	USNM 1421187	Μ	China	South China Sea, FAO Fishing Area 61	MK270950	MK271037	MK281237

H		C	ļ		9	enBank Accession N	umbers
laxon name	Voucher 1D	Sex	Country	Port/Body of Water	12S	16S	COI
Monomia haanii	USNM 1421191	М	China	South China Sea, FAO Fishing Area 61	MK270938	MK271038	MK281238
Monomia haanii	USNM 1421194	Х	China	South China Sea, FAO Fishing Area 61	MK270951	MK271039	MK281239
Monomia haanii	USNM 1421195	Σ	China	South China Sea, FAO Fishing Area 61	MK270939	MK271040	MK281240
Monomia haanii	USNM 1421196	Σ	China	South China Sea, FAO Fishing Area 61	MK270940	MK271041	MK281241
Monomia haanii	USNM 1421202	М	China	South China Sea, FAO Fishing Area 61	MK270941	MK271042	MK281242
Monomia haanii	USNM 1421204	Σ	China	South China Sea, FAO Fishing Area 61	MK270952	MK271043	MK281243
Monomia haanii	USNM 1421206	М	China	South China Sea, FAO Fishing Area 61	MK270942	MK271044	MK281244
Monomia haanii	USNM 1420827	ц	Taiwan	Daxi Fishery Port	MK270933	MK271031	MK281231
Monomia haanii	USNM 1420828	Х	Taiwan	Daxi Fishery Port	MK270955	MK271032	MK281232
Monomia haanii	UF 29509	ц	Taiwan	Daxi Fishery Port	MK270930	MK271026	MK281227
Monomia haanii	UF 29511	ц	Taiwan	Daxi Fishery Port	MK270931	MK271027	MK281228
Monomia haanii	UF 29512	н	Taiwan	Daxi Fishery Port	MK270932	MK271028	
Monomia haanii	ZRC 1998.0186	Σ	Taiwan	Daxi Fishery Port	MK270947	MK271059	MK281254
Monomia haanii	MNHN-IU-2014-10086		Vietnam	Của Bé Fishing Port		KY524463 <sup>1</sup>	
Monomia haanii	UO 12J-Vn12		Vietnam	Của Bé Fishing Port		$KY524464^{1}$	
Monomia haanii	ZMMU Ma 3368		Vietnam	Của Bé Fishing Port			$JX398094^{2}$
Monomia argentata	MNHN-IU-2014-10076		Vietnam	Của Bé Fishing Port		$KY524480^{1}$	
Monomia argentata	MNHN-IU-2014-10075		Vietnam	Của Bé Fishing Port		KY524479 <sup>1</sup>	
Monomia argentata	IOM		Vietnam	Của Bé Fishing Port		KY524478 <sup>1</sup>	
Monomia lucida	ZRC 2016.0150	Σ	Vanuatu	South Pacific Ocean	MK270965	MK271061	
Monomia lucida	ZMMU Ma 3365		Vietnam	Của Bé Fishing Port			$JX398096^{2}$
Monomia lucida	NHMUK 2017.402		Vietnam	Của Bé Fishing Port		MG563792 <sup>3</sup>	
Monomia lucida	MNHN-IU-2014-10083		Vietnam	Của Bé Fishing Port		MG563793 <sup>3</sup>	
Monomia lucida	MNHN-IU-2014-10085		Vietnam	Của Bé Fishing Port		MG563794 <sup>3</sup>	
Monomia petrea	UF 188		Guam	Tepungan Channel		$\mathrm{KT365606^4}$	$\mathrm{KT365743^{4}}$
Outgroup Taxa							
Portunus sanguinolentus	ZRC 2016.0146	Σ	India	Pazhayar Fish Landing	MK270966	MK271057	
Portunus pelagicus	ZRC 2016.0147	Μ	India	Porto Novo	MK270967	MK271058	MK281258
Sequences mined fron	n GenBank are attributed	to <sup>1</sup> K	och and Ď	uriš (2018), <sup>2</sup> Spiridonov et al. (2014)	, <sup>3</sup> Koch et al. (2	(017), and <sup>4</sup> Evans	(2018). * <i>Holotype o</i>
	1071 1071						
Portunus pseudoargenue	1148 Stepnenson, 1901						

# **Commercial products**

Four cans (454 g each) of pasteurized lump crabmeat labeled as "Portunus haanii" were purchased from grocery stores in Maryland and Virginia in 2016 and 2017. Portions of 10 lumps (i.e., single piece of crabmeat reasonably expected to be from an individual crab), five from the top and five from the bottom, from each tub were sampled for DNA extraction (N=40). The DNA barcode region of the cytochrome oxidase subunit I (COI) was amplified and sequenced from samples following the methods described below.

# Molecular methods

Genomic DNA was extracted from muscle tissue dissected from ethanol preserved or fresh specimens using the DNeasy Tissue Kit (Qiagen) according to the manufacturer's animal tissue protocol. Portions of three mitochondrial genes were amplified: a 658 bp barcode region of the cytochrome c oxidase I gene using the primers JgLCO1490 and JgHCO2189 (Geller et al. 2013), a 531 bp region of the 16S ribosomal gene using the primers 16S-ar and 16S-br (Palumbi 1996), and a 375 bp region of the 12S ribosomal gene using the primers 12Sf (Mokady et al. 1994) and 12S1R (Shull et al. 2005). PCR was carried out for 35 cycles with an annealing temperature of 48 °C for COI and 52 °C for 12S and 16S using Promega GoTaq G2 hot start master mix (Promega M7432). PCR products were visualized by agarose gel electrophoresis (1.5% agarose) and purified with ExoSAP-IT (Affymetrix) prior to sequencing. Sequencing reactions were performed using 1  $\mu$ L of purified PCR product in a 10  $\mu$ L reaction containing 0.5  $\mu$ L primer, 1.75  $\mu$ L Big Dye buffer and 0.5  $\mu$ L Big Dye (Life Technologies).

Geneious 9.1.7 (Biomatters) was used to visualize, trim, edit, and assemble contigs from forward and reverse sequences. All PCR, sequencing, and analytics were carried out at the Laboratories of Analytical Biology at USNM. Sequences have been deposited in GenBank (NCBI) with accession numbers listed in Table 1.

Partial sequences for each locus were also amplified from *Portunus pelagicus* (ZRC 2016.0147) and *P. sanguinolentus* (ZRC 2016.0146) to serve as outgroup taxa. Multiple sequence alignments were generated using the L-INS-i alignment strategy in MAFFT version 7 (Katoh and Standley 2013). The aligned sequences were then concatenated using Sequence Matrix (Vaidya et al. 2011). In the concatenated data set, positions 1–380 are 12S, 381–914 are 16S, and positions 915–1572 are COI.

A best-fit model of nucleotide sequence evolution compatible with MrBayes and partitioning arrangement for each locus was determined using Partition Finder 2 (Lanfear et al. 2016) with the greedy algorithm selected (Lanfear et al. 2012). The GTR+I+G model was chosen for all three loci. Phylogenetic analyses were performed on the concatenated dataset using maximum likelihood (ML) with RAxML (Stamatakis 2006) and Bayesian Inference (BI) performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) on the FDA's Raven2 high performance computing cluster. ML options for RAxML included the GTRCAT model of nucleotide evolution (-m), rapid bootstrap

analysis, and search for best-scoring ML tree (-f a), and 1000 bootstrap replicates. BI analysis was carried out for 10 million generations with two independent runs, each with four chains, and with trees sampled every 1000th generation. Model parameters (tratio, statefreq, shape, pinvar) were unlinked among partitions, and the rate prior (prset ratepr) was set to "variable." To calculate posterior probabilities, a "burn-in" of 25% of the total trees sampled per run adequately removed trees prior to convergence.

In addition to the concatenated dataset, a COI-only dataset which incorporated sequences from GenBank and BOLD was analysed to identify the species of crab found in four cans of pasteurized lump crabmeat labelled as "*Portunus haanii*". For visualization purposes, a neighbour joining tree of the 658bp alignment was built using the Jukes-Cantor model in the Geneious Tree Builder. Patristic and K2P distances were calculated for each alignment using MEGA7 (Kumar et al. 2016).

# Results

## Molecular phylogenetics

Molecular phylogenetic analyses of the concatenated dataset of three mitochondrial loci show that there is a well-supported (98/1) separation between *M. gladiator* and *M. haanii*. *Monomia petrea* (Alcock, 1899) (UF188; KT365743, KT365606) is a strongly supported sister to both (95/1) (Fig. 5). Analysis also confirms that voucher specimens purchased at the Daxi Fishery Port in northern Taiwan as well as those supplied by U.S. seafood importer, Newport International, both under the name "*P. haanii*," are the same species as the specimen of "*M. pseudoargentata*" (ZMMU 3368; JX398094) from Spiridonov et al. (2014). Furthermore, our specimens with color patterns similar to "*M. haanii*" in Chertoprud et al. (2012) (= *M. gladiator* ZMMU 3366 in Spiridonov et al. 2014; JX398095) are the same species: *M. gladiator* s. str. (Fig. 5). Included in the clade of *M. haanii* is the holotype of *Portunus pseudoargentatus* (WAM-C7056). The topology of the ML phylogram is congruent with the morphological findings.

DNA barcode sequence analyses for species identification of products confirm that crabmeat sold as "*Portunus haanii*" is indeed what we have identified herein as *Monomia haanii s. str.* (Fig. 6). The mean K2P distance between reference *M. haanii* and the 40 product samples is 0.72% compared to the 7.85% between *M. gladiator* and product samples. Similarly, COI sequences from *M. haanii* and *M. gladiator* reference samples have a mean K2P distance of 7.74% (Table 2) and is consistent with congeneric divergences observed in other decapods (Costa et al. 2007).

**Table 2.** Mean K2P distances between specimens genetically identified as *M. gladiator*, *M. haanii*, and commercial products calculated from a neighbor-joining distance tree built in Geneious.

	Monomia gladiator	Monomia haanii
Monomia haanii	7.74%	
Commercial Products	7.85%	0.72%

# **Taxonomic accounts**

Portunoidea Rafinesque, 1815 Portunidae Rafinesque, 1815 Portuninae Rafinesque, 1815

### Monomia Gistel, 1848

**Type species.** *Portunus gladiator* Fabricius, 1798, type species of *Amphitrite* De Haan, 1833, by subsequent designation (Miers, 1886); pre-occupied by *Amphitrite* Müller, 1771 [Polychaeta]; *Monomia* Gistel, 1848, replacement name for *Amphitrite* De Haan, 1833.

#### Monomia gladiator s. str. (Fabricius, 1798)

Figs 1A–D, 3A–C, 4A–D

Portunus gladiator Fabricius, 1798: 368; Latreille 1825: 189; Crosnier 1962 (in part): 51, figs 72, 76, 78, 82, 83, pl. 3 fig. 2; Stephenson and Rees 1967a: 14; 1967b: 25; 1968: 293 (in part); Stephenson 1972a: 16, 39 (in part); 1972b: 135 (in part); Bhadra 1998: 410; Dev Roy and Bhadra 2005: 425; 2011: 147.

Cancer menestho Herbst, 1803: 34, pl. 55 fig. 3.

- Lupea gladiator, H. Milne Edwards 1834: 456.
- *Neptunus gladiator*, A. Milne-Edwards 1861: 330; Richters 1880: 152; Müller 1887: 475; De Man 1888: 69; Henderson 1893: 367.
- *Neptunus (Amphitrite) gladiator*, Miers, 1886: 177; Alcock 1899: 35, 36; Laurie 1906: 412.
- *Callinectes gladiator*, Stebbing 1915: 58. Non *Callinectes gladiator* Benedict, 1893 (fide Stephenson and Cook 1973).
- *Monomia gladiator*, Barnard 1950: 156; Fourmanoir 1954: 9; Spiridonov et al. 2014: table 1; Trivedi et al. 2018: 66, table 1.
- *Portunus (Monomia) gladiator*, Jeyabaskaran et al. 2000: 51, pl. 36c; Biju Kumar et al. 2007: 286; Ng et al. 2008: 151, 156, 157 (list and discussion).

Portunus (Monomia) gladiator [sic], Krishnamoorthy 2009: 6 (list).

- *Portunus haanii*, Stephenson and Cook 1973: 429 (in part), figs 6A–E, 7A–E, 8A–E, 9A, 10A, C, D, G; Stephenson 1975: 178. Non *Amphitrite haanii* Stimpson, 1858.
- Monomia haanii, Chertoprud et al. 2012: 314, pl. 51 fig. G. Non Amphitrite haanii Stimpson, 1859.
- Non *Cancer gladiator* Fabricius, 1793: 449 (= *Portunus sanguinolentus* (Herbst, 1783), fide Latreille 1825).
- Non *Portunus (Amphitrite) gladiator*, De Haan 1833: 65; 1835: pl. 18 fig. 1 (= *Portunus orbitosinus* Rathbun, 1911).
- Non *Portunus gladiator*, Stephenson and Campbell 1959: 110, Figs 2J, 3J, pl. 3 fig. 2, pl. 4 fig. J, pl. 5 fig. J (= *Portunus australiensis* Stephenson and Cook, 1973).

**Material examined.** INDIA: ZRC.2016.0145, 2 males, 1 female, Pazhayar Fish Landing, Nagapattinam District, Tamil Nadu, coll. NK Ng et al., 17 Sep. 2011; ZRC.2016.0149, 1 female, sandy beach, Pondicherry, Union Territory of Puducherry; ZRC.2018.1189, 5 males, Jeppiar Fishing Port, Muttom, Tamil Nadu, coll. PKL Ng et al., 19 Sep. 2016; USNM127069, 1 male, SW of Mumbai, IIOE Anton Bruun, 14 Nov. 1963.

AUSTRALIA: WAM-C26459, 2 males, Dampier Archipelago, Cape Brugieres, Western Australia, coll. Slack-Smith and Hewitt, 17 Jul. 1999; WAM-C61155, 2 females, WAM-C61156, 1 female, Pilbara Shelf, Western Australia, coll. E Morello et al. (CSIRO Pilbara Survey), 13 Jun. 2013.

PENINSULAR MALAYSIA: ZRC.2000.1308, 4 males, Perhentian, coll. anon., 16 May 1976.

MYANMAR: ZRC.2016.0030, 1 female, Ayeyarwady Delta, coll. EAF-Nansen Project (Myanmar cruise), 19 May 2015; ZRC.2016.0034, 1 male, Tanintharyi Coast, coll. EAF-Nansen Project (Myanmar cruise), 26 May 2015.

SINGAPORE: ZRC.1965.10.22.1-2, 1 male, 1 female, Siglap, coll. M.W.F. Tweedie, Jul. 1933; ZRC.1984.338-348, 4 males, 7 females, Horsburgh Lighthouse, South China Sea near Singapore, coll. H Huat, 15 Dec. 1982; ZRC.1984.5451-5453, 3 males, Tuas fishery port, coll. WM Lee, 25 Sep. 1982.

THAILAND: USNM127068, 2 females, Andaman Sea, north of Phuket, IIOE Anton Bruun R/V, 31 July. 1963; ZRC.2000.0779, 3 males, 13 females, Phuket, Pichai Fish Port, coll. NK Ng et al., 17–20 Jan. 2000; ZRC.2000.0842, 2 males, Phuket, Pichai Fish Port (Andaman Sea), coll. PKL Ng et al., 3–6 May 2000; ZRC.2002.0297, 4 males, 1 female, Phuket, Pichai Fish Port, coll. JCY. Lai, 2–3 Sep. 2001; ZRC.2002.0298, 3 males, 1 female, Phuket, Pichai Fish Port, coll. JCY Lai, 2–3 Sep. 2001; ZRC.2003.0114, male, Pattani Fishing Port, Pattani Province; ZRC.2003.0197, 1 male, Saiburi Crab Landing, Pattani Province, coll. Z Jaafar et al., 8 Jun. 2003.

**Diagnosis.** Carapace (Fig. 1A–D) transversally hexagonal, CW/CL ratio 1.79– 1.83, with dorsal surface, except patches of granules, densely covered by short tomentum. Regions moderately defined; with discrete patches of granules on gastric, branchial, cardiac and intestinal regions. Front subdivided into four teeth with rounded apices, median pair distinctly smaller than lateral; median sulcus between teeth continuing ventrally to triangular projection appressed to median epistomial tooth. Epistome well defined, median tooth projecting beyond front. Supraorbital margin finely granulate, with two distinct notches; inner orbital angle tooth-like, with glabrous ventromesial ledge. Infraorbital margin with deep, V-shaped notch laterally; in antero-ventral view, mesial part of infraorbital margin granulate, ventrally a large triangular tooth projecting anteriorly, visible from dorsal view. Anterolateral margin armed with nine teeth, including external orbital angle, with granulate margins; first tooth larger than following teeth except 9<sup>th</sup>, with straight outer margin; 2<sup>nd</sup>–8<sup>th</sup> subequal in size, sharp, projecting outward, slightly curved anteriorly; 9<sup>th</sup> tooth largest, projecting laterally; just underneath anterolateral margin is thick coat of long soft setae which sometimes obscures



Figure I. Dorsal habitus of A lectotype of *Portunus gladiator* Fabricius, 1798, deposited in Copenhagen Museum (ZMUC-Cru 4705) B *Cancer menestho* Herbst, 1803 (= *Monomia gladiator*), probably from Indian Ocean (color print from Herbst, 1803: pl. 55 fig. 3 C *Monomia gladiator* (Fabricius, 1798), fresh colouration, Phuket, Thailand (not collected), photo by Rueangrit Promdam D *Monomia gladiator* (Fabricius, 1798), fresh coloration, Jeppiar, Tamil Nadu, India (ZRC), photograph by PKL Ng; E "*Neptunus (Amphitrite) gladiator*" [sic] (=*Monomia haanii*) from Sagami Bay, Japan (color print from Sakai, 1939: pl. 47 fig. 3) F *Monomia haanii* (Stimpson, 1858), fresh colouration, South China Sea (USNM 1421161) shipped frozen by US seafood importer.

teeth. Posterolateral margin concave, posterolateral angle rounded; posterior margin lined with small granules, straight to slightly convex; posterior margin with ventrally directed, smooth flange, lateral extremities of which coincide with posterolateral angle of carapace, each armed with small, lamelliform tooth.

Basal article of antennule completely filling antennular fossa, subsequent two articles slender. Basal article of antenna short, with broad, lateral projection entering but not obstructing orbital hiatus; flagellum long, exceeding well beyond orbit. Eyes with well-developed corneas, short, thick peduncles. Proepistome well developed, anterior tip with projecting conical tooth; epistome not extensively projecting posteriorly. Endostome with well-developed lateral ridges.

Third maxillipeds finely granulate on ischium, merus and exopod, setose on external surfaces, extensively pilose on mesial margins; ischium longer than wide, subrectangular, with deep, submesial sulcus; merus longer than wide, rhomboidal, anterolateral angle strongly projecting laterally; palp articles subcylindrical. Exopod stout, with subdistal triangular projection on inner medial border; flagellum well developed.

Male thoracic sternites covered with thick tomentum, thickest on exposed surfaces of sternites 5–8 (Fig. 3A–C); sternites 1–4 distinctly granulate, with granules becoming larger, coarser in large specimens; sternites 1, 2 fused, separated from sternite 3 by straight suture; sternites 3, 4 almost completely fused except for remnants of suture at lateral extremities, replaced mesially by smooth, setose groove; sternite 4 with narrow median groove on exposed surface, continuing posteriorly into sterno-pleonal cavity. Sutures 4/5, 5/6, and 6/7 present on exposed surface of thoracic sternum but interrupted within sterno-pleonal cavity; suture 7/8 present on most of exposed thoracic sternal surface, but disappearing just before sternopleonal cavity. Median longitudinal line evident at level of sternites 6, 7, 8, absent elsewhere. Press-button tubercle of sternopleonal locking mechanism located on posteromesial projection of sternite 5.

Chelipeds (P1), long, robust, surfaces tomentose; slightly heterochelous, major chela usually with modified cutting/crushing tooth proximally on cutting margin of dactylus. Merus long, with 4, sometimes 5, curved spines along flexor margin, and 2 distal spines on extensor margin; both margins densely setose. Carpus with sharp spine on inner angle, and flattened spine on external surface continuing as a strong carina, with additional, shorter carina above it. Dorsal surface of palm (propodus) with two straight, longitudinal granular crests, inner one distally ending distally in strong spine; small proximal spiniform tooth at articulation with carpus; two additional, curved granular crests on external surface of palm, first ending at level of articulation with dactylus, second, lower, ending near gape, creating cristate, proximoventral margin of palm; inner surface of palm with two wide, distinct rows of granules. Fingers generally straight except for curved, pointed tips; subequal in length to palm; with two granulate crests each on external and internal surfaces; lowest carina on fixed finger extending into palm; numerous teeth on cutting margins, arranged in groups so that each group has large central tooth flanked by smaller teeth of decreasing size, giving the cutting margins appearance of having three or more denticulate, triangular lobes.

First to third ambulatory legs (P2–P4), long, slender; decreasing in length and size, with P2 largest, P4 smallest; flexor margins of meri, carpi, propodi and dactyli heavily setose. Fifth ambulatory (natatory) leg (P5) with quadrate merus, pentagonal carpus, flat, subrectangular propodus, and flat, oval dactylus; margins of articles regularly setose; propodus with four raised glabrous longitudinal bands, including flexor and extensor margins, interspersed with tomentum; dactylus with five raised glabrous bands, including flexor and extensor margins, interspersed with tomentum, distal third with low median crest continuing proximally as narrow tomentose stripe; in fresh specimens, P5 propodus with white band on postero-distal margin, no purple spot, P5 dactylus with small white spot on distal end.

Male pleon (Fig. 3A–C) 'inverted T'-shaped, external surfaces mostly tomentose, 3<sup>rd</sup>–5<sup>th</sup> pleomeres fused. First pleomere very thin, less wide, mostly obscured by flange of posterior margin of carapace. Second pleomere much wider than first, lateral edges resting on P5 coxa, with prominent transverse keel running along entire width. Third pleomere widest, formed like a wedge, visible from both dorsal and ventral view; strong transverse crest somewhat forming posterior margin of cephalothorax, with shallow notch medially, posterolateral angles sharp, acute; sulcus between fused 3<sup>rd</sup> and 4<sup>th</sup> pleomere moderately deep, glabrous. Fourth pleomere subrectangular, wider than long, lateral margins convex, central region with low transverse crest. Fifth pleomere subrate sub-trapezoidal, basal margin wider than anterior. Sixth pleomere subrectangular; median length about 1.2 times maximum width; width at maximum lateral convexity greater than basal width; anterior margin concave, basal margin straight, lateral margins convex anteriorly, concave posteriorly. Telson subtriangular, apex rounded, lateral margins slightly concave, basal margin convex; median length 1.2 times basal width, with rounded tip, broadly rounded posterior margin.

G1 (Figs 4A–D) with proximal half relatively wide, somewhat flattened, strongly bent medially, by at least 45° but much less than 90°, distal half very slender, filiform; apically slightly recurved, rounded; distal tip much narrowed to small unarmed aperture. G2 about half length of G1, slender, distal tip minutely bilobed. Penis long, slender, uncalcified; emerging from sternocoxal condyle of P5.

**Remarks.** Following the recognition of *Monomia* Gistel, 1848, as a genus distinct from *Portunus* Weber, 1795 (see Mantelatto and Robles 2007; Mantelatto et al. 2009; Schubart and Reuschel 2009; Chertoprud et al. 2012; Spiridonov et al. 2014), the taxonomy of its type species, *Monomia gladiator* (Fabricius, 1798), needs to be assessed.

Firstly, there are five specimens identified as syntypes of *Portunus gladiator* Fabricius, 1798, in the Zoological Museum of the University of Copenhagen (ZMUC) (see http://www.zmuc.dk/inverweb/invertebrater/Crustacea%20databases/Fabricius%20collection.htm). All are dry specimens with the catalog numbers ZMUC-CRU 4704 through 4708 (see Ng et al. 2008). Examination of their photographs (available online) show that one of them is clearly not conspecific nor even congeneric (viz. ZMUC-CRU 4707). Accompanying this specimen in the photograph is a handwritten label identifying it as "*Achelous Whitei* A. M. Edw.," a name now considered a junior synonym of *Lupocycloporus gracilimanus* (Stimpson, 1858) (viz. Ng et al. 2008), a widespread species known from several localities in the Indo-West Pacific region; and a check with the available literature (e.g., Stimpson, 1907: pl. 10 fig. 3) confirms this identification. To stabilize the taxonomy of *Portunus gladiator* Fabricius, 1798, one of the other four syntypes, a male ZMUC-CRU 4705 (Fig 1A), is hereby designated as the lectotype, and the other three specimens (ZMUC-CRU 4704, 4706 and 4708) become paralectotypes.

Secondly, we agree with Ng et al. (2008) that there is no secondary homonymy between *Portunus gladiator* Fabricius, 1798, and *Cancer gladiator* Fabricius, 1793, because the two species were originally described in different genera, and the name "*Cancer gladiator* Fabricius, 1793" is no longer available by virtue of its synonymization under *Portunus sanguinolentus* (Herbst, 1783) (viz. Latreille 1825; Stephenson and Cook 1973). Although Chertoprud et al. (2012) cite the use of the name "*Portunus gladiator* (Fabricius, 1793)" by Stephenson and Cook (1973) as meeting the requirement of Article 59.1 of the Code, we view this as a misinterpretation of the Code's provisions for homonymy. Furthermore, with the recognition of *Monomia* Gistel, 1848, as a full genus, thus absolutely eliminating any reservations concerning secondary homonymy, the species name *Monomia gladiator* (Fabricius, 1798) is, therefore, valid.

Thirdly, there is the matter of the confusion between *M. gladiator* and *M. haanii*. Stephenson and Cook (1973) proposed *Amphitrite haanii* Stimpson, 1858, as a replacement name for *P. gladiator* Fabricius, 1798, and this has led to the current confusion between the two names. As the following discussion will show, it is evident that *Monomia gladiator* and *M. haanii* (Stimpson, 1858) are distinct and valid species, and, therefore, the latter cannot be used as a replacement name for the former.

Monomia gladiator differs from M. haanii primarily in these three morphological characters: (1) in the fresh specimens of *M. gladiator*, there is a white band on the postero-distal margin of the P5 propodus, but no purple spot, and a small white spot on the distal tip of the P5 dactylus (Fig. 1C, D; also Chertoprud et al. 2012: pl. 51 fig. G) (vs. in *M. haanii*, there is a large purple spot on the distal tip of the P5 propodus, and the distal one-third of the P5 dactylus is colored purple; Fig. 1E, F; also Sakai, 1939: pl. 47 fig. 3; 1965: pl. 57, fig. 1; Chertoprud et al. 2012: pl. 51 fig. H); (2) the anterolateral margins of the male  $6^{th}$  pleomere are more flared out and rounded in M. gladiator, and at their widest extent exceed the basal width of the 6th pleomere (Fig. 3A-C; also Stephenson and Cook, 1973: fig.10A) (vs. straight, convergent anteriorly, and separated from concave posterolateral margin by an angular convexity in M. haanii, widest at base, Figs 2B, 3D-F); and (3) the G1 is bent at an angle of about 45° at mid-length and the distal tip is slender and relatively narrower in *M. gladiator* (Fig. 4A-D) (vs. G1 bent at or almost at 90°, and distal tip is somewhat wider than the immediate subdistal region in M. haanii (Figs 2C, 3E-H). Aside from these are some minor differences; for instance, the mesial part of the infraorbital margin of M. gladiator is granulate and there is no tooth on the mesial end as it abuts the basal article of the antenna, there is, however, a large tooth immediately ventral to this margin, which projects outward and is visible from dorsal view as if it were part of the infraorbital margin. In *M. haanii*, the mesial end of the infraorbital margin has a large tooth which abuts against the basal article of the antenna. Also, the spines on the flexor margin of the P1 merus of *M. gladiator* tend to be more projecting and recurved than those of *M. haanii*, which are relatively smaller and less curved. Examination of available freshcolor photographs (viz. Chertoprud et al. 2012: pl. 51 Figs G, H) also show that these meral spines tend to be entirely white in *M. gladiator* while they are red at the base and white at the tips in *M. haanii*.

Furthermore, the molecular phylogenetic analysis corroborates the morphological evidence, clearly showing two distinct and well-supported clades corresponding to the two species. Specimens identified as *M. gladiator* based on the characters described above, including a topotypic specimen from India (ZRC.2016.0149), form a well-supported clade distinct from another clade containing specimens with the morphological attributes of *M. haanii*, which also includes a topotypic specimen from Japan (ZRC.2000.0084). Furthermore, the same molecular phylogenetic analysis shows that the specimen referred to by Chertoprud et al. (2012) as "*Monomia haanii*" (JX398095) falls within the *M. gladiator* clade, whereas the specimen referred to by Chertoprud et al. (2012) as "*Monomia pseudoargentata*" (JX398094) falls within the *M. haanii* clade. These specimens should now be identified as *M. gladiator* and *M. haanii*, respectively.

Finally, *Lupea gladiator* H. Milne Edwards, 1834 (Indian Ocean), is re-included in the synonymy of *Monomia gladiator* (Fabricius, 1898), and *Cancer menestho* Herbst, 1803 (probably from Indian Ocean) is hereby considered a junior subjective synonym of *Monomia gladiator* (Fabricius, 1798). We believe that Stephenson and Cook (1973) erred in removing these two names from the synonymy of *gladiator sensu* Fabricius, 1798. In the case of H. Milne Edwards' specimen, the error is due simply to their conflation of *haanii* with *gladiator*. In the case of Herbst's species, we disagree with them that the illustration of *Cancer menestho* does not show 2 spines on the posterior margin of the cheliped merus. What they call a "non-protruding" spine is an artifact of perspective. We have seen in our photographs of *M. gladiator*, that this second spine can appear non-protruding when the merus is viewed from directly above and if the marginal setae obscure its entire outline. Once this so-called difference is eliminated, there is no compelling reason why *C. menestho* should also not be treated as a synonym of *M. gladiator*.

# Monomia haanii s. str. (Stimpson, 1858)

Figs 1E, F, 2, 3D-F, 4E-H

- Portunus (Amphitrite) gladiator: De Haan 1833: 39; 1835: pl. 1 fig. 5, pl. A. Non Portunus gladiator Fabricius, 1798.
- *Amphitrite haanii* Stimpson, 1858: 38; 1907: 79; Ng et al. 2008: 151 (synonymy), 156 (discussion).



**Figure 2.** *Monomia haanii* (Stimpson, 1858), lectotype, male (RMNH 379), Japan. **A** dorsal habitus **B** thoracic sternum and pleon, ventral view **C** sterno-pleonal cavity and G1s, ventral view. Photographs by CHIJ Fransen (RMNH). Scale bars: A, B 10 mm; C 3 mm.

- Neptunus (Amphitrite) gladiator: Ortmann 1893: 73; Lanchester 1902: 544; Parisi 1916: 173; Balss 1922: 107; Sakai 1934: 303; 1936: 129, pl. 36 fig. 3; 1939: 390, fig. 5a, pl. 47 fig. 3; Shen 1937: 101, fig. 2; Lin 1949: 19. Non Portunus gladiator Fabricius, 1798.
- Portunus gladiator: Rathbun 1902: 26, Miyake 1961: 172; Sakai 1965: 118, pl. 57 fig. 1; 1976: 341, fig 180a, pl. 120 fig. 1; Stephenson and Rees 1967: 24; 1968: 293; Takeda and Miyake 1968: 551; Stephenson 1972a: 16, 39; 1972b: 135; Ko and Lee 2013: 40, pl.-fig. 34. Non Portunus gladiator Fabricius, 1798.
- Portunus (Achelous) gladiator; Rathbun 1910: 36 (from Gulf of Siam). Non Portunus gladiator Fabricius, 1798.
- *Portunus pseudoargentatus* Stephenson, 1961: 109, Figs 2A, 3F, pl. 2 fig. 4, pl. 4 fig. F, pl. 5 fig. D; Stephenson and Rees 1967b: 25; 1968: 294; Yang and Dai1994: 137, fig. 13.
- *Portunus haanii*: Stephenson and Cook 1973 (in part): 429, figs 6F–H, 7F–H, 8F–H, 9B; Dai and Yang 1991: 223, fig. 120(2), pl. 27(4); Moosa 1996: 521; Apel and Spiridonov 1998 (in part): 291; Ng et al. 2001: 16 (list).
- *Portunus (Monomia) gladiator*: Sakai 1976: 341, fig. 180, pl.120 fig. 1; Kim and Chang 1985: 52. Not *Portunus gladiator* Fabricius, 1798.
- Portunus haanii: Miyake 1983: 85, pl. 29 fig. 2; Takeda 1989: 152
- Portunus (Monomia) haanii Yamaguchi & Baba, 1993: 396, figs 137A-C.
- *Monomia pseudoargentata*: Chertoprud et al. 2012: 315, pl. 51 fig. H; Spiridonov et al. 2014: 412, 427, fig. 3I, tab. 1.
- Monomia haanii: Ng et al., 2017: 68 (list).
- Not Amphitrite media Stimpson, 1858: 39; 1907: 79, pl. 10 fig. 1.
- Not *Monomia haanii*, Chertoprud et al. 2012: 314, pl. 51 fig. G (=*Monomia gladiator* (Fabricius, 1798)).

**Material examined.** JAPAN: RMNH 379, lectotype, male, Japan, coll. P.F. von Siebold, 1823–1829 (photographs only); ZRC.1999.0084, 1 male, 1 female, off Hota, Uchibo, coast of Boso Peninsula, coll. T Komai, 22 Aug. 1997; USNM5255, 4 male, 1 female, coll. FC Dale and PL Jouy, Palos R/V; USNM26254, 1 male, 1 female, off Wakanoura, coll. DS Jordan and JO Snyder, 1900; USNM45882, 1 female, off Wakanoura, Kishu; USNM54519, 1 female, Yamagata Prefecture, coll. M Sasaki, Aug. 1917; USNM72540, 2 males, Enoshima, Bay of Jeddo, coll. ES Morse; USNM60250, 1 male, Toyama Bay, coll. M Sasaki, 1925; USNM112423, 1 male, Shimizu, Sugura, Albatross R/V, 14 Oct. 1906.

AUSTRALIA: WAM-C7506, 1 male (holotype of *Portunus pseudoargentatus* Stephenson, 1961), Abrolhos Islands, Western Australia, coll. RW George, 11 May 1960; WAM-C34767, 1 female, Exmouth Gulf, Western Australia , coll. S Morrison and P Unsworth, 5 Nov. 2004; WAMC34900, 1 female, Exmouth Gulf, Western Australia, coll. S Morrison and P Unsworth, 12 Mar. 2004; WAM-C34938, 1 gravid female, Shark Bay, Western Australia, coll. S Morrison et al., 4 Oct. 2002; WAM-C5510, 1 male, Shark Bay, Western Australia, coll. S Morrison et al., 5 Mar. 2003; WAM-C44737, 1 female, Ningaloo Marine Park, Western Australia, coll. MP Salotti and SM Slack-Smith, 1 Feb. 2008.

SOUTH CHINA SEA (FAO Area 61): USNM1421161, 1 male, USNM1421181, 1 male, USNM1421182, 1 male, USNM1421185, 1 male, USNM1421187, 1 male, USNM1421191,1 male, USNM1421194,1 male, USNM1421195,1 male, USNM1421186, 1 male, USNM121202, 1 male, USNM1421204, 1 male, USNM1421206, 1 male.

TAIWAN: UF29509, 1 female, Daxi Fishery Port; UF29511, 1 female, Daxi Fishery Port; UF29512, 1 female, Daxi Fishery Port; USNM1420827, 1 female, Daxi Fishery Port; USNM1420828, 1 male, Daxi Fishery Port; ZRC.1998.0186, 2 males, 1 female, Daxi Fishery Port, coll. PKL Ng, 3–4 Aug. 1996; ZRC.2016.0408, 4 males, 3 females, Daxi Fishery Port, coll. PKL Ng, 1 Jul. 2016.

**Diagnosis.** Similar to *Monomia gladiator* except in the following morphological characters. Infraorbital margin granulate, terminating mesially in small triangular tooth, in line with rest of margin. Sixth pleomere (Fig. 2B, 3D–F) longer than wide, maximum width at base; anterior half of lateral margins convergent anteriorly, posterior half concave to straight, anterior and posterior halves separated by angular convexity. G1 (Fig. 3C, 4E–H) bent at 90° at midlength, tapering distally toward a minutely spatulate tip, slightly broader than immediate subdistal area. In fresh specimens, P5 propodus with dark purple spot on distal tip, distal one-third of P5 dactylus colored dark purple as well (Fig. 1E, F).



Figure 3. Male thoracic sternum and pleon. A-C Monomia gladiator (Fabricius, 1798), A ZRC 2018.1189, Jeppiar, Tamil Nadu, India B ZRC 2016.0145, Pazhayar Tamil Nadu, India; C) ZRC 2000.0842, Phuket, Thailand. D-F Monomia haanii (Stimpson, 1858) D WAM-C7506, holotype of Portunus pseudoargentatus Stephenson, 1961, Abrolhos Is., Western Australia E ZRC 1999.0084, Boso Peninsula, Japan F ZRC 2016.0408, Daxi Fishery Port, Taiwan. Scale bar: 10 mm.

**Remarks.** The primary morphological differences between *Monomia haanii* (Stimpson, 1858) and *M. gladiator* (Fabricius, 1798) have already been discussed in the Remarks for the latter species. Yamaguchi and Baba (1993) had previously reported on the type material of *Amphitrite haanii* Stimpson, 1858, listing several syntypes col-



Figure 4. Left G1, sternal view (except D and E). A–D Monomia gladiator (Fabricius, 1798): A) ZRC 2018.1189, Jeppiar, Tamil Nadu, India B ZRC 2016.0034, Tanintharyi coast, Myanmar C ZRC 2016.0145, Pazhayar, Tamil Nadu, India D ZRC 2000.0842, Phuket, Thailand (flipped right G1). E–H Monomia haanii (Stimpson, 1858) E WAM-C7506, holotype of Portunus pseudoargentatus Stephenson, 1961, Abrolhos Is., Western Australia (flipped right G1) F ZRC 1999.0084, Boso Peninsula, Japan G ZRC 2016.0408, smaller male, Daxi Fishery Port, Taiwan H ZRC 2016.0408, larger male, Daxi Fishery Port, Taiwan. Scale bar: 3 mm.



**Figure 5.** Maximum likelihood phylogram of three mitochondrial loci showing genetic distinction between *M. gladiator* and *M. haanii* along with three other members of the genus *Monomia*. Abbreviations: IOM = Institute of Oceanology and Museum, Nha Trang; MNHN = Muséum National d'Histoire Naturelle, Paris; NHM = The Natural History Museum, London; UF = University of Florida Natural History Museum, Gainesville; UO = University of Ostrava, Ostrava; USNM = United States National Museum, National Museum of Natural History, Washington, D.C.; WAM = Western Australian Museum, Perth; ZMMU = Zoological Museum of the Moscow University, Moscow; ZRC = Zoological Reference Collection, Lee Kong Chian Natural History Museum, Singapore; CN = China; IN = India; JP = Japan; SG = Singapore; TH = Thailand; TW = Taiwan; VN = Vietnam.

lected by P.F. von Siebold from Japan in the years 1823 to 1829, deposited in the then Rijkmuseum van Natuurlijke Historie (RMNH) in Leiden, and preserved either dry or in alcohol. From these they selected a lectotype, a young male (42 by 24 mm; RMNH 379) preserved in alcohol. No further or detailed descriptions of the material were provided. Although they did provide photographs of several specimens, the lectotype was photographed still inside the bottle (viz. Yamaguchi and Baba, 1993: fig. 137A) and no definitive or diagnostic features of its morphology could be discerned. The collections of the RMNH are now housed in the Naturalis Biodiversity Center, Leiden, and photographs of the lectotype (Fig. 2) were kindly provided to the authors by Dr. Charles Fransen. From these photographs, the diagnostic morphology of the 6<sup>th</sup> pleomere (Fig. 2B) and the 90-degree bend of the G1 (Fig. 2C) can already be observed even in such



**Figure 6.** Neighbor joining phylogram of the barcode region of COI to visualize relationships between museum-vouchered reference specimens and 40 samples from four cans of pasteurized lump crabmeat (Product A–D) labeled "*Portunus haanii*" and/or "red swimming crab"

a young specimen. Furthermore, although the fresh coloration of this species was not recorded by either De Haan (1833) or Stimpson (1858), later observations of topotypic material show that the purple spots on the P5 propodus and dactylus, as well as the more profuse but scattered spotting on the dorsal carapace, and the dark colored spines on the anterior margin of the P1 merus are consistently observed (Fig. 1E, F; also Sakai, 1976: pl. 120 fig. 1; Miyake, 1983: pl. 29 fig. 1).

Stephenson and Cook (1973) had also previously synonymized *Portunus pseudoargentatus* Stephenson, 1961, under *P. haanii* on the basis of their similar morphology. We confirm that *P. pseudoargentatus* is a junior subjective synonym of *Monomia haanii* (Stimpson, 1858) *sensu stricto* on the basis of similarities in the dentition of the infraorbital margin, the shape of the male 6<sup>th</sup> pleomere (Fig. 3D), and the ~90° angle of the bend of the G1 at midlength (Fig. 4E; also Stephenson, 1961: fig. 2A). In a photograph of the presumably newly preserved holotype, the pigmented spots on the left P5 propodus and dactylus are still there (Stephenson, 1961: pl. 2 fig. 4), although these have since faded and can no longer be seen during the present examination of the holotype.

Specimens morphologically identifiable as *M. haanii* comprise a highly-supported clade that includes specimens from Japan, the type locality of *M. haanii*, as well as the holotype of *Portunus pseudoargentatus* Stephenson, 1961, and the Vietnamese specimens referred to by Chertoprud et al. (2012) (JX398094) and by Koch et al. (2017) and Koch and Duris (2018) (KY524463, KY524464) as "*Monomia pseudoargentata*". Both morphological and molecular phylogenetic analyses support the recognition of *Monomia haanii* as a full species. Molecular results also support a morphological basis for synonymy of *P. pseudoargentatus* Stephenson, 1961, under *M. haanii*.

# Discussion

The need to address and resolve the *Portunus gladiator* species complex was brought about largely because of the incongruence in taxon names utilized by the scientific community and the seafood industry. This incongruence was highlighted by Warner et al. (2015) who matched sequences from crab cakes to a single sequence of "*P. pseudoargentatus*" and raised concerns that this was a species in the US food supply unknown to regulators. Subsequently, "*P. pseudoargentatus*" was added to The Seafood List (Food and Drug Administration 2015), based on that report. Pasteurized, lump crab meat labeled as "Portunus haanii" or "red swimming crab" is routinely imported into the United States; however, the species name *P. haanii* has been synonymised under *P. gladiator* for some time (Ng et al., 2008). As it turned out, this complex had a very complicated taxonomic past, but here we have used traditional morphological methods in concert with molecular phylogenetic analyses to establish the morphological and molecular boundaries between the two species we recognize herein, *Monomia gladiator* and *Monomia haanii*.

Revising and describing the morphological differences between these two species was necessary to verify the identity of the individual specimens used to generate reference DNA sequences for identification of picked crab meat samples labelled as "*Portunus haanii*." The morphological findings of two distinct species were corroborated

in our multi-locus phylogenetic analysis that showed complete congruence between morphologically derived identity and genetic clade membership. This reciprocally informative approach has enabled us to confirm that the commercial products that we tested, that were labelled and sold as *Portunus haanii*, were in fact *Monomia haanii* and should be labelled as such. Based on our findings, the FDA's seafood labeling guidance to industry, The Seafood List, can be emended to reflect this current understanding of the species in question.

Commercially important species are often presumed to be well understood because they have tangible value, but in the case of decapod crustaceans molecular phylogenetic analyses are re-writing much of what carcinologists thought they knew about species-, genus-, and family-level relationships (Keenan et al. 1998; Ma et al. 2009; Lai et al. 2010; Bracken-Grissom et al. 2013; de Carvalho et al. 2013; Windsor and Felder 2014; Evans 2018; Tavares and Santana 2018). For example, Lai et al. (2010) determined that what was then known as *Portunus pelagicus* was actually a complex of four species; and the status of the genus *Penaeus* Fabricius, 1798, has been highly debated in the literature (Pérez Farfante and Kensley 1997; Lavery et al. 2004; Dall 2007; Flegel 2007; 2008; McLaughlin et al. 2008; Ma et al. 2009; Ma et al. 2011). These name changes are to be expected as new methods of DNA analysis are developed and applied to illuminate and clarify evolutionary relationships.

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# Appendix I

The types of *Monomia gladiator* (Fabricius, 1798) are currently deposited in the Natural History Museum of Denmark (NHMD) in Copenhagen. The lectotype (ZMUC-Cru 4705) has the corresponding catalog number, NHMD-82551. The paralectotypes (ZMUC-Cru 4704, 4706, and 4708) have the corresponding catalog numbers, NHMD-82550, -82552, and -82554, respectively (J. Olesen pers. comm.)

RESEARCH ARTICLE



# A new stonefly species (Plecoptera, Perlidae) from the Interior Highlands USA, with morphological and molecular comparison to other congeneric species

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# Abstract

Thirty-one species of Nearctic *Perlesta* Banks, 1906 (Plecoptera: Perlidae) are recognized. A new species is described from western Arkansas and eastern Oklahoma, USA, *Perlesta sublobata* South & DeWalt, **sp. nov.**, from the adult male, adult female, and egg. *Perlesta sublobata* males are differentiated from other congeners by a combination of a prominent ventral caecum and a distinct dorsal extension of the lateral sclerites of the aedeagus. A preliminary molecular phylogenetic hypothesis is proposed for *Perlesta* based on 17 congeners and three outgroup taxa using partial mitochondrial cytochrome c oxidase subunit I sequence data. Illustrations, stereomicroscope images, and scanning electron micrographs support the description and comparison to other *Perlesta*.

# Keywords

Arkansas, Nearctic, new species, Oklahoma, Perlesta sublobata South & DeWalt, stonefly

# Introduction

Needham and Claassen (1925) described *Perlesta* Banks, 1906 as a Nearctic genus of small, brown, triocellate stoneflies with yellow costal wing margins, long cerci, and highly variable coloration of the head and wing membrane. For over a century, the

name of the type species of the genus, *Perlesta placida* (Hagen, 1861), has been used for innumerable specimens that once critically reviewed, were revealed to encompass many cryptic species (Stark 1989, DeWalt et al. 2001). Stark (1989) revised the genus, removing several species from synonymy, describing seven new species, recognizing a total of 12 species, and providing the first useful key to the *P. placida* complex. Stark's revision prompted additional work, with eight new species described over the next 14 years (Poulton and Stewart 1991, Kirchner and Kondratieff 1997, Stark and Rhodes 1997, DeWalt et al. 1998, Kondratieff and Baumann 1999, Kondratieff and Kirchner 2002, 2003). Subsequently, a revised taxonomic key was necessary, in which was included 21 Nearctic species (Stark 2004). The genus has since expanded to 31 Nearctic species through the works of Kondratieff et al. (2006, 2008, 2011), Kondratieff and Myers (2011), and Grubbs and DeWalt (2011, 2012, 2018).

Two species are recognized from China (Murányi and Li 2016) and undetermined nymphs have been reported from Costa Rica (Gutiérrez-Fonseca and Springer 2011). Described species could easily surpass 40, given the amount of presumed new, undescribed *Perlesta* material currently present in North American collections (Grubbs and DeWalt 2018).

At least eight species of *Perlesta* co-occur in the United States Interior Highlands, a mountainous region defined by the United States Geological Survey as encompassing southern Missouri, western Arkansas, eastern Oklahoma, and extreme southeastern Kansas (Omernik 1987). This area of the central United States was extensively examined for stoneflies by Poulton and Stewart (1991). Surprisingly, a remarkably distinct and undescribed *Perlesta* species from the Interior Highlands was revealed through recent examination of undetermined Arkansas material donated to the Illinois Natural History Survey (INHS) Insect Collection by the late Kenneth W. Stewart (DeWalt et al. 2018) and from eastern Oklahoma material borrowed from the K. C. Emerson Entomological Museum, Oklahoma State University (**OKSU**) at Stillwater. Using freshly collected and properly prepared specimens, we describe this new species, *Perlesta sub-lobata* sp. nov., and compare it to similar regional congeners. Moreover, we provide the first comparative molecular study of the genus by exploring partial mitochondrial cytochrome c oxidase subunit I (COI) DNA sequence data to examine monophyly of the new species, delimit congeners, and construct a preliminary phylogeny.

The holotype male and all paratypes are deposited in the Illinois Natural History Survey (**INHS**) Insect Collection. Other material is deposited in the INHS Insect Collection with the exception of nine vials borrowed and returned to the OKSU Insect Collection.

### Materials and methods

### Collection and morphological analyses

Terminology of all stages follows Stark (1989). Fresh specimens of the new species (108 males and 40 females) were collected from six Arkansas stream systems, 13–19 June



**Figure 1.** Collection sites (1932–2016) for *Perlesta sublobata* sp. nov. in Oklahoma (OK) and Arkansas (AR), USA. Red circle represents type locality, 2016. Purple circles represent paratype localities, 2016.

2016 (Fig. 1). Methods included sweep netting during the day and ultraviolet light trapping at night. Live male specimens were anesthetized in a dry ice  $CO_2$  chamber and subsequently squeezed with forceps to evert the aedeagus, the source of the most informative morphological characteristics distinguishing species. All specimens were preserved in 95% EtOH. Select individuals of the fresh material and several related species were stack photographed and processed with a Zeiss AxioCam HRc Rev. 3 digital camera and Helicon Focus 6 software in the Sam W. Heads laboratory, INHS. The aedeagus and paraproct were sketched from the stereomicroscope images using Adobe Illustrator CC 2018. Scanning electron micrographs (SEM) of eggs and female terminalia were prepared at the Beckman Institute Microscopy Suite, University of Illinois by critical point drying, placing on an aluminum carbon disk, sputter coating with gold-palladium alloy, and imaged with a Thermo-Fisher FEI Quanta FEG 450 ESEM.

# **Molecular studies**

Genomic DNA from 20 *P. sublobata* specimens, 17 congeners, and three outgroup taxa was extracted using the Qiagen DNeasy Kit, amplified for a fragment of the

**Table 1. Haplotypes.** Description of COI haplotypes for 18 *Perlesta* species and three outgroup taxa. Number of specimens (N) and GenBank accession number are listed for each corresponding haplotype. Multiple specimens sharing the same haplotype are listed consecutively. All specimens collected from the USA except *P. nelsoni* Stark, 1989 (Canada). State or province is listed by standard postal abbreviation. Sequences obtained from GenBank are denoted with \*. INHS = Illinois Natural History Survey Insect Collection record number.

Species	Sex	GenBank	Ν	INHS	Lat./ Long.	Stream State/ C		Collector(s)
							Prov.	
Beloneuria georgiana	8	MH778486	1	909265	34.69804N,	tributary of Dukes	GA	E. J. South
(Banks, 1914)					-83.78149W	Creek		
Perlesta adena Stark, 1989	3	MH778426	1	793345	36.39021N,	Rocky Creek	TN	S. A. Grubbs
					-86.25096W			
Perlesta armitagei Grubbs &	3	MH778427	1	457510	39.0342N,	Little Salt Creek	IN	R. E. DeWalt
DeWalt, 2018					-86.16788W			
Perlesta bjostadi	3	MH778428	1	793346	36.84673N,	Nottoway River	VA	В. С.
Kondratieff & Lenat, 2006					-77.56095W			Kondratieff
Perlesta browni Stark, 1989	Ŷ	MH778429	1	658464	38.45202N,	South Moreau Creek	МО	E. J. South
					-92.48643W			
Perlesta cinctipes	3	MH778430	1	658465	38.45202N,	South Moreau Creek	MO	E. J. South
(Banks, 1905)					-92.48643W			
	3	MH778431	1	658466	38.45202N,	South Moreau Creek	MO	E. J. South
					-92.48643W			
	Ŷ	MH778432	1	658467	38.45202N,	South Moreau Creek	MO	E. J. South
					-92.48643W			
	3	MH778433	1	658468	38.45202N,	South Moreau Creek	MO	E. J. South
					-92.48643W			
Perlesta decipiens	3	MH778434	1	658778	41.3337N,	Kankakee River	IL	A. Yanahan
(Walsh, 1862)					-88.18761W			
	Ŷ	MH778435	1	658777	41.3337N,	Kankakee River	IL	A. Yanahan
					-88.18761W			
Perlesta ephelida	3	MH778436	1	658780	44.72652N,	Platte River	MI	R. E. DeWalt, S.
Grubbs & DeWalt, 2012					-86.14303W			K. Ferguson
	Ŷ	MH778437	1	658781	44.72652N,	Platte River	MI	R. E. DeWalt, S.
					-86.14303W			K. Ferguson
	3	MH778438	1	658469	37.49727N,	Osage Fork of	MO	E. J. South
					-92.63033W	Gasconade River		
	3	MH778439	1	658470	37.49727N,	Osage Fork of	MO	E. J. South
					-92.63033W	Gasconade River		
	Ŷ	MH778440	1	658477	37.49727N,	Osage Fork of	MO	E. J. South
					-92.63033W	Gasconade River		
Perlesta frisoni Banks, 1948	3	*HQ568861	2	NA	35.62276N,	West Prong Little	TN	R. E. DeWalt
					-83.44288W	Pigeon River		
	3	*JF884174	2	NA	35.4968N,	Twentymile Creek	NC	R. E. DeWalt
					-83.8337W			
Perlesta golconda	8	MH778441	1	550392	41.67397N,	Iowa River	IA	M. Kippenhon
DeWalt & Stark, 1998					-91.56452W			
Perlesta lagoi Stark, 1989	3	MH778442	9	658456	38.45202N,	South Moreau Creek	MO	E. J. South
~					-92.48643W			

Species	Sex	GenBank	N	INHS	Lat./ Long.	Stream	State/	Collector(s)
							Prov.	
Perlesta lagoi Stark, 1989	8	MH778443	9	658457	38.45202N,	South Moreau Creek	MO	E. J. South
					-92.48643W			
	8	MH778444	9	658460	38.45202N,	South Moreau Creek	МО	E. J. South
					-92.48643W			-
	3	MH778445	9	658461	38.45202N,	South Moreau Creek	МО	E. J. South
					-92.48643W			2
	3	MH778446	9	658462	38.45202N.	South Moreau Creek	МО	E. I. South
			-		-92.48643W			
	Ŷ	MH778447	9	658471	38 45202N	South Moreau Creek	МО	F. I. South
				0,01,1	-92.48643W			
	Ŷ	MH778448	9	658472	38 45202N	South Moreau Creek	МО	F. I. South
	Ŧ			0,01,2	-92.48643W	ooun moreue oreen		Liji ooddi
	Q	MH778449	9	658473	38 45202N	South Moreau Creek	МО	F. I. South
	Ŧ			0,01,5	-92.48643W	ooun moreue oreen		Liji ooddi
	3	MH778450	9	658474	38 45202N	South Moreau Creek	МО	F I South
	0	10111/ / 0190		0,01/1	-92 48643W	South Moreau Creek	1010	1. j. oouun
	3	MH778451	1	658459	38 45202N	South Moreau Creek	МО	F I South
	0	10111//04/1	1	0)01))	-92 48643W	South Moreau Creek	WIC	L. J. 500001
	2	MH778/52	1	658/175	38 /5202N	South Moreau Creek	MO	F I South
	0	10111//04/2	1	0)04/ )	_92 48643W	South Moreau Creek	WIO	L. J. 50000
	2	MH778/53	1	658/176	-92.40049 W	South Moreau Creek	MO	F I South
	0	IVII 1/ / 04)J	1	0)04/0	_92 48643W	South Moreau Creek	WIO	L. J. 50000
	2	MH778/5/	1	658776	/1.66065N	Bates Creek	ОН	E I South R
	0	10111//01/1	1	0)0//0	-81 11747W	Dates Creek	011	E. J. South, R. F. DeWalt
Parlacta milucorum	2	MH778/55	1	7033/7	42 4401N	Hannacroix Creek	NV	L. De wat
Kondratieff & Myers 2011	0	IVII 1/ / 04))	1	/////	-73 8137W	T faillactory Creek	191	L. Iviyers,
Devlacta malconi Stork 1090	2	*VD1///200	1	NIA	45.076N	St. John Divor	NR	K Heard et al
1 enesua neisona Stark, 1969	0	KK144270	1	1 1/1	-66 719W	St. John River	IND	K. I Italu et al.
Perlesta ouabache	2	MH778456	1	516699	42 45994N	Sugar River	П	R F DeWalt
Grubbs & DeWalt 2011	0	10111/ / 0490	1	)100))	-89 23985W	Sugai River	112	et al
Perlesta roblei Kondratieff &	3	MH778457	1	793348	36.4684N	Kirbys Creek	NC	BC
Kirchner 2003	0	10111//04//	1	/ / / / / / /	-77 1443W	Kilbys Cleck	ne	Kondratieff et al
Perlecta sublobata sp. pov	3	MH778458	8	793207	36 12052N	War Fagle Creek	AR	F I South
<i>1 trasai subiobia</i> sp. 110v.	0	10111/ / 0490	0	////	-93 69319W	wai Lagie Creek	7110	L. J. 500001
	3	MH778459	8	793212	34 03869N	Antoine River	AR	F. I. South
	0		Ū	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	-93.41752W			Liji ooddi
	3	MH778460	8	793218	34 17985N	Caddo River	AR	F I South
	0	10111/ / 0100	0	/ ) ) 210	-93 07021W	Caddo Faver	7110	1. j. oouur
	8	MH778461	8	793230	36.04161N	War Fagle Creek	AR	F. I. South
	0	10111/ / 0101	0	795250	-93 70482W	War Eagle Oreek	7110	1. j. oouur
	8	MH778462	8	793266	33 95608N	Little Missouri River	AR	F. I. South
	0		0	/ / / / / /	-93.44362W			Liji ooddi
	2	MH778463	8	793271	33.95608N	Little Missouri River	AR	E. I. South
	0		0	,,,,,,1	-93.44362W			2
	8	MH778464	8	793273	33.95608N	Little Missouri River	AR	E. I. South
			-		-93.44362W			

Species	Sex	GenBank	Ν	INHS	Lat./ Long.	Stream	State/	Collector(s)
							Prov.	
Perlesta sublobata sp. nov.	8	MH778465	8	793288	33.95608N,	Little Missouri River	AR	E. J. South
					-93.44362W			
	3	MH778466	1	793208	36.12052N,	War Eagle Creek	AR	E. J. South
					-93.69319W			
	3	MH778467	3	793209	35.66925N,	Mulberry River	AR	E. J. South
					-93.83033W			
	8	MH778468	3	793233	33.97121N,	Cossatot River	AR	E. J. South
					-94.22292W			2
	Ŷ	MH778469	3	793324	33.95608N,	Little Missouri River	AR	E. J. South
					-93.44362W			2
	3	MH778470	2	793211	35.66925N,	Mulberry River	AR	E. J. South
	-				-93.83033W	,		2
	3	MH778471	2	793224	33.95608N.	Little Missouri River	AR	E. I. South
					-93.44362W			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
	3	MH778472	1	793214	34.17985N,	Caddo River	AR	E. I. South
				,,,,	-93.07021W			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
	3	MH778473	1	793234	33.97121N.	Cossatot River	AR	E. I. South
	0			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	-94.22292W			
	3	MH778474	1	793299	33 95608N	Little Missouri River	AR	E. I. South
	0			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	-93 44362W	Little Millioutin ruver		2. j. ooddi
	3	MH778475	1	793312	33 95608N	Little Missouri River	AR	E. I. South
	0			,,,,,,,	-93 44362W	Little Millioutin ruver		2. j. ooddi
	Ŷ	MH778476	1	793328	33.95608N.	Little Missouri River	AR	E. I. South
			-	,,,,,,,	-93.44362W			
	Q	MH778477	1	793331	33 95608N	Little Missouri River	AR	E. I. South
	1		-	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	-93.44362W			
Perlesta teavsia Kirchner &	3	MH778478	1	515560	39.1355N.	tributary of Middle	IN	R. E. DeWalt
Kondratieff, 1997					-86.1601W	Fork Salt Creek		
	Ŷ	MH778479	1	457522	39.1355N,	tributary of Middle	IN	R. E. DeWalt
					-86.1601W	Fork Salt Creek		
Perlesta WI-1 (undescribed)	3	MH778480	1	516410	46.07721N,	St. Croix River	WI	R. E. DeWalt
	-				-92.24608W			et al.
	Ŷ	MH778481	1	552631	46.07721N.	St. Croix River	WI	R. E. DeWalt,
					-92.24608W			S. K. Ferguson
	3	MH778482	1	576963	45.57953N.	Menominee River	WI	R. E. DeWalt
					-87.78796W			et al.
	3	MH778483	1	658779	45.77348N,	St. Croix River	MN	R. E. DeWalt
					-92.78164W			
	Ŷ	MH778484	1	583370	45.82306N,	Snake River	MN	R. E. DeWalt
					-92.77001W			
Perlesta xube Stark &	3	MH778485	1	790543	39.40911N,	Mud Creek	IL	E. J. South,
Rhodes, 1997					-88.89952W			R. E. DeWalt
Perlinella drymo	3	MH778487	1	514716	40.2942N,	Wabash River	IN	R. E. DeWalt,
(Newman, 1839)					-87.2546W			M. Pessino
Perlinella ephyre	3	MH778488	1	548835	42.32815N,	Huron River	MI	R. E. DeWalt
(Newman, 1839)					-83.8595W			et al.

mitochondrial gene encoding for the COI subunit via polymerase chain reaction using either primers LCO1490 and HCO2198 (Folmer et al. 1994) or jgLCO1490 and jgHCO2198 (Geller et al. 2013), and sequenced with Sanger technology at the University of Illinois W. M. Keck Core Sequencing Facility. Thermocycling conditions consisted of one 94 °C for 5 min denaturation cycle, 40 cycles at 94 °C for 45 s, 50 or 53 °C for 1 min, 72 °C for 1.5 min, and one 72 °C for 5 min extension cycle. Amplification success was verified with gel electrophoresis. Forward and reverse sequences were aligned to create contigs, and all 63 aligned contigs were truncated to a uniform length of 606 nucleotides, visually edited with Sequencher 5.4, aligned in MUSCLE 3.8, and the sequences and supporting data deposited in GenBank (Table 1). Sequences were tested to determine the model of evolution in jModelTest2 (Darriba et al. 2012), and a gamma distribution with a proportion of invariable sites was used to model rate variation across sites (invgamma). Akaike Information Criterion (AIC) results indicated that the General Time Reversible nucleotide substitution model (GTR+I+G) was best for the Maximum Likelihood and Bayesian analyses. These models were applied in subsequent phylogenetic tree generation analyses.

We generated a maximum likelihood tree using MEGA 7.0 (Kumar et al. 2016) and calculated pairwise genetic distances for both sequences generated for this study, as well as additional sequences accessioned from GenBank, using the Kimura 2-parameter model (K2P) (Kimura 1980), the de facto standard for measuring mitochondrial pairwise distances (Collins et al. 2012). A Bayesian analysis was performed for all haplotypes using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) with a burn-in length of 500,000, subsampling frequency of 500, and a chain length of 5,100,000.

# Results

### Perlesta sublobata South & DeWalt, sp. nov.

http://zoobank.org/1FB6141B-3C6E-4B64-983C-406649DE6830 http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:505368 Figs 1–6

**Diagnosis.** Males are distinguished by a combination of a prominent ventral caecum with a broad ventral setal patch and a distinct dorsal extension of the lateral sclerites of the aedeagus. Females possess a subgenital plate with a deep V-shaped notch and truncate lobes. Eggs have a smooth chorion and a well-developed, distally flanged collar.

**Male**. Habitus moderately dark (Fig. 2A). **Wings:** Membrane brown with dark brown venation and pale intercostal margin (Fig. 2B, C). **Forewing:** Length 8–9 mm (mean =  $8.3 \pm 0.3$  SD, n = 95); membrane with two lightly pigmented longitudinal bands: one posterior to the posterior cubital vein and a second anterior to the median vein (Fig. 2B). **Head:** Pale with dark brown quadrangular patch covering intercoellar



**Figure 2.** *Perlesta sublobata* sp. nov., paratype males from Little Missouri River, Arkansas A habitus, dorsal view (INHS Insect Collection 793329) **B** dorsal view of right forewing showing intercostal margin (IM), posterior cubital vein (CuP), and median vein (M) **C** right hind wing, dorsal view (INHS Insect Collection 793270) **D** head and pronotum (INHS Insect Collection 793226) **E** head and pronotum (INHS Insect Collection 793229). Scale bars: 1 mm (**A**); 1.2 mm (**B**, **C**); 200 μm (**D**, **E**)



**Figure 3.** *Perlesta sublobata* sp. nov., terminalia of paratype males from Little Missouri River, Arkansas **A** tenth tergite and paraprocts, dorsal view (INHS Insect Collection 793335) **B** paraprocts, caudal view **C** paraprocts, oblique lateral view showing anteromedially directed carina (AC) and spine (AS) **D** right paraproct of extruded male, oblique lateral view (INHS Insect Collection 793226). Scale bars: 200  $\mu$ m (**A**); 100  $\mu$ m (**B**), 50  $\mu$ m (**C**, **D**).

region; brown subtriangular patches anterolateral and anteromedial to median ocellus (Fig. 2D, E); diffuse brown pigmentation posterior to ecdysial suture (Fig. 2D); ecdysial suture extends slightly to moderately beyond ocelli as a distinct dark line; antenna darkly pigmented on ca. distal 2/3 of flagellum and dorsomedian region of scape; proximal antennal segments pale with tan dorsal pigmentation. *Thorax:* Pronotum brown with vermiculated rugosities and faint, pale median stripe (Fig. 2D, E); mesothoracic



**Figure 4.** *Perlesta sublobata* sp. nov., aedeagus of paratype male from Little Missouri River, Arkansas (INHS Insect Collection 793295) **A** lateral view showing dorsal caecum (DC), ventral caecum (VC), envelope (E), tube (T), sac (S), sac extension (SE), dorsal patch (DP), ventral seta-like spines (VS), lateral sclerite (LS), and dorsal extension of the lateral sclerites (DLS) **B** lateral view showing partially extruded dorsal caecum **C** dorsal view showing dorsal patch (DP) and dorsal extension of the lateral sclerites (DLS) **D** dorsal view showing partially extruded dorsal caecum **E** ventral view showing fine seta-like spines covering sac venter and ventral caecum (VC) **F** ventral view. Scale bars: 200  $\mu$ m (**A**, **B**, **C**, **D**); 50  $\mu$ m (**E**); 500  $\mu$ m (**C**, **F**); 50  $\mu$ m (**F**).

and metathoracic nota brown; mesothoracic and metathoracic basisterna pale; femur and tibia pale, brown dorsally. *Abdomen*: Sterna pale; terga pale medially and light brown laterally, or uniformly brown. *Terminalia*: Tergum 10 with dark subquadrate pigment patch (Fig. 3A) and 10–20 small, sensilla basiconica (visible at 80× magnification); cercus long (holotype = 15 segments), pale proximally and dark brown distally; paraprocts broad basally and narrowed distally in caudal aspect (Fig. 3B); anteapi-



Figure 4. Continued.

cal paraproct spine and carina directed anteromedially—best visible in oblique lateral view of unextruded individuals (Fig. 3C, D). *Aedeagus:* Dorsal caecum moderately produced, ca. as long as wide and broad apically (Fig. 4A, B); dorsal patch broad over sac, moderately expanded proximally, constricted subapically, and broadly expanded over caecum; prominent lateral sclerites merge dorsally to form a distinct V-shaped pattern extending more than 1/2 tube length (Fig. 4C, D); prominent ventral caecum, narrowed apically, with a broad patch of fine seta-like spines covering venter and apex, length ca. 2/5 sac width; sac with fine seta-like spines covering venter (Fig. 4E, F).





Figure 4. Continued.

**Female**. Female habitus similar to male, but of larger size and wings of lighter pigmentation (Fig. 5A). Pronotum tan with brown vermiculated rugosities and pale median stripe (Fig. 5B). Wings with subhyaline membrane, tan venation, and pale intercostal margin (Fig. 5C, D). Forewing length 9–11 mm (mean =  $9.8 \pm 0.6$  SD, n = 40); often with two unpigmented longitudinal bands: one posterior to the posterior cubital vein and a second anterior to the median vein (Fig. 5C). Subgenital plate lobes truncate medially and truncate to slightly rounded laterally, slightly to moderately pigmented, covered with long bristle-like hairs, and separated by a deep V-shaped notch (Fig. 5E, F).



**Figure 5.** *Perlesta sublobata* sp. nov., paratype females from Little Missouri River, Arkansas **A** habitus, dorsal view (scale bar) **B** head and pronotum (INHS Insect Collection 793329) (scale bar  $\mu$ m) **C** dorsal view of right forewing showing intercostal margin (IM), posterior cubital vein (CuP), and median vein (M) **D** right hind wing, dorsal view (INHS Insect Collection 793322) **E** subgenital plate (INHS Insect Collection 793329) **F** subgenital plate, SEM (INHS Insect Collection 793328). Scale bars: 1 mm (**A**); 200  $\mu$ m (**B**, **E**); 500  $\mu$ m (**C**, **F**); 750  $\mu$ m (**D**).

**Egg.** Length ca. 360 µm, width ca. 280 µm. Chorion smooth with fine pitting (Fig. 6A). Collar well developed, ribbed, and flanged distally (Fig. 6B). Micropylar orifices distinct near anterior pole (opposite collar) (Fig. 6C).



**Figure 6.** *Perlesta sublobata* sp. nov., egg of paratype female from Little Missouri River, Arkansas (INHS Insect Collection 793316) **A** entire egg **B** posterior pole and collar **C** micropyle. Scale bars: 200 μm (**A**); 100 μm (**B**); 10 μm (**C**).

Molecular analyses. Perlesta sublobata formed a monophyletic group with strong support (ML bootstrap support = 97%, Bayesian posterior probability = 92%). The nearest neighbor species to P. sublobata was P. decipiens (Walsh, 1862) at 1.8% sequence divergence. Maximum intraspecific COI genetic distances were less than minimum interspecific distances within all tested *Perlesta* (Table 2). All intraspecific distances were less than the arbitrary threshold of 3.5%, suggesting that the new species was monophyletic without other cryptic species present within the new taxon (Hebert et al. 2003, Zhou et al. 2010). All haplotypes (total = 47) were confined to their respective genera and presumptive species in the ML and Bayesian analyses (Figs 7, 8, respectively). The three tested species within the P. frisoni group, consisting of five Nearctic species that lack an aedeagal dorsal caecum, formed a monophyletic grouping. Four of the five "dark" species studied in Grubbs and DeWalt (2018) also formed a monophyletic grouping. The placement of P. adena Stark, 1989 outside this group may be spurious, indicating additional genes or populations are needed for further refinement. The relatively distant placement of P. golconda DeWalt & Stark, 1998 from P. sublobata is congruent with the species' distinctly different morphologies, apart from the male genitalic similarities.

**Remarks.** The shape and armature of the aedeagus are the most distinct morphological features of *P. sublobata*. Stark (1989) illustrated a lateral view of an undetermined species from Arkansas (*P. sublobata*), demonstrating spinule patterns and shape of the aedeagal telescoping sections: envelope, tube, and sac. He noted that lateral sclerites of the tube joined dorsally. This dorsal extension of the lateral sclerites was not illustrated or specified in the literature for any other *Perlesta*. Furthermore,

Species	N	Maximum intraspecific distance (%)	Nearest neighbor	Nearest neighbor distance (%)
P. adena	1	0	P. nelsoni	19.3
P. armitagei	1	0	P. xube	7.2
P. bjostadi	1	0	P. decipiens	4.5
P. browni	1	0	P. armitagei	15.1
P. cinctipes	4	0.7	P. armitagei	10.4
P. decipiens	2	1.2	P. lagoi	1.5
P. ephelida	5	0.7	P. ouabache	4.8
P. frisoni	2	0	P. nelsoni	14.9
P. golconda	1	0	<i>P.</i> WI-1	17.8
P. lagoi	13	0.8	P. decipiens	1.5
P. mihucorum	1	0	P. sublobata	4.8
P. nelsoni	1	0	P. teaysia	9.4
P. ouabache	1	0	P. sublobata	2
P. roblei	1	0	P. ephelida	15.6
P. sublobata	20	0.8	P. decipiens	1.8
P. teaysia	2	0.2	P. frisoni	15.9
<i>P</i> . WI-1	5	2.4	P. ouabache	3.9
P. xube	1	0	P. armitagei	7.2

**Table 2.** Intra and interspecific distance. Maximum intraspecific and minimum interspecific (nearest neighbor) Kimura 2-parameter values for COI within *Perlesta*. Key: N = number of specimens, *P*. WI-1 is an undescribed species from Wisconsin.



**Figure 7.** Maximum Likelihood phylogenetic reconstruction of 44 unique *Perlesta* CO1 haplotypes using the GTR+I+G nucleotide substitution model. Haplotypes represented by more than one specimen are indicated in parentheses beside corresponding GenBank accession numbers. Outgroup taxa: *Beloneuria georgiana, Perlinella drymo*, and *Perlinella ephyre*. Bootstrap scores from 1,000 replicates are displayed at nodes. Scale bar represents the estimated number of nucleotide substitutions per site.

a ventral caecum is present in *P. sublobata* and only one other described congener, *P. golconda*. However, the ventral caecum of *P. golconda* is less prominent and without a distinct ventral patch of fine seta-like spines. Additionally, the dorsal caecum of *P. sublobata* is moderately developed, compared to the poorly developed dorsal caecum of *P. golconda* (Fig. 9).

The known distribution of *P. golconda*, originally limited to Illinois (DeWalt and Stark 1998), has expanded to include Iowa, Indiana, Michigan, and Nebraska (DeWalt et al. 2019), as well as Missouri (Stark 2004) and Louisiana (INHS Insect Collection 564765). Arkansas is bordered by Missouri to the north and Louisiana to the south. A sympatric distribution with *P. sublobata* is expected due to this geographic adjacency and overlap of the Interior Highlands' habitat. Consequently, re-examination of some museum specimens may be required. The male and female habitus easily distinguish *P. golconda* from *P. sublobata*. The ocelli of *P. golconda* are usually connected by a moderately dark V-shaped pattern on a pale background (Fig. 10A, B), whereas *P. sublobata* has a dark subquadrate interocellar region. The pronotum of *P. golconda* is primarily pale with light tan rugosities on the lateral margins, whereas *P. sublobata* has a dark pronotum with a pale narrow median stripe. Additionally, *P. golconda* females are dis-



**Figure 8.** Bayesian phylogenetic reconstruction of 44 unique *Perlesta* CO1 haplotypes using the GTR+I+G nucleotide substitution model. Haplotypes represented by more than one specimen are indicated in parentheses beside corresponding GenBank accession numbers. Outgroup taxa: *Beloneuria georgiana, Perlinella drymo*, and *Perlinella ephyre*. Posterior probabilities are indicated at nodes. Scale bar represents the estimated number of nucleotide substitutions per site.

tinguished by a very short egg collar (Grubbs and DeWalt 2008, their fig. 17) and rounded subgenital plate lobes (Fig. 11A, B).

The female habitus of *P. sublobata* resembles two Interior Highlands congeners, *P. decipiens* and *P. ephelida* Grubbs and DeWalt, 2012. However, *P. sublobata* differs from *P. decipiens* and *P. ephelida* by subgenital plate morphology. *Perlesta decipiens* has a deep U-shaped notch bordered by truncate lobes, typically with darker pigmentation on the posterior margins (Fig. 12). *Perlesta ephelida* has a shallow V-shaped notch enclosed by truncate lobes, usually pale to lightly pigmented with posteromedially upturned margins (Fig. 13; Grubbs and DeWalt 2012, their fig. 7). These characters are contrasted to the deep V-shaped notch and moderately pigmented, truncate lobes of *P. sublobata*. Furthermore, *P. sublobata* has a shorter forewing length than *P. decipiens* (*P. sublobata*).



**Figure 9.** *Perlesta golconda*, aedeagi of males from Missouri River, Nebraska **A** lateral view showing dorsal caecum (DC) and ventral caecum (VC) (INHS Insect Collection 660209) **B** lateral view (INHS Insect Collection 660210) **C** dorsal view showing dorsal patch (DP) (INHS Insect Collection 660209). Scale bars: 200 μm.

= 9–11 mm; *P. decipiens* = 12–13 mm, Stark 2004). Egg chorion and collar are similar to *P. decipiens* (Stark 2004, his figs 7.397–7.399) and *P. ephelida* (Grubbs and DeWalt 2012, their figs 14–21).

**Habitat.** With the exception of one locality (OK, Washington Co., Caney River), all collection sites for *P. sublobata* are within or closely adjacent to the Interior Highlands, a region containing four contiguous U. S. Environmental Protection Agency (EPA) Level III Ecoregions: Ozark Highlands, Boston Mountains, Arkansas Valley, and Ouachita Mountains. Collection sites for *P. sublobata* within the Interior Highlands are partially canopied, hardwood forested, wadeable, low gradient streams (ca. 15–20 m wide) with substrata composed mostly of sand, gravel, and cobble. The type



**Figure 10.** *Perlesta golconda*, head and pronotum, Missouri River, Nebraska **A** male (INHS Insect Collection 660210) **B** female (INHS Insect Collection 658790). Scale bars: 200 μm.



**Figure 11.** *Perlesta golconda*, subgenital plates of females from Missouri River, Nebraska **A** notch indicated by arrow (INHS Insect Collection 658788) **B** (INHS Insect Collection 658789). Scale bars: 200 µm.



**Figure 12.** *Perlesta decipiens*, female subgenital plates **A** Kankakee River, Illinois (INHS Insect Collection 577949) **B** Caddo River, Arkansas, SEM (INHS Insect Collection 793908). Scale bars: 200 µm (**A**); 500 µm (**B**).



**Figure 13.** *Perlesta ephelida*, female subgenital plates **A** Platte River, Michigan, notch indicated by arrow (INHS Insect Collection 658781) **B** Sugar Creek, Indiana, SEM (INHS Insect Collection 658791). Scale bars: 200 μm (**A**); 500 μm (**B**).

locality is a low gradient run (ca. 25 m wide) of the Little Missouri River (Fig. 14), located 45 km downstream of Lake Greeson and 65 km upstream from its confluence with the Ouachita River in the extreme north EPA Level III Ecoregion 35 (South Central Plains). The substrate is primarily gravel and sand, with some large woody debris. Other stonefly species collected with the new species at the type locality included *Acroneuria frisoni* Stark & Brown, 1991, *Acroneuria* nr. *ozarkensis* Poulton & Stewart,



Figure 14. Little Missouri River, Pike County, Arkansas, USA. Type locality for Perlesta sublobata sp. nov.

1991, Agnetina flavescens (Walsh, 1862), Neoperla falayah Stark & Lentz, 1988, N. robisoni Poulton & Stewart, 1986, P. decipiens, and Perlinella ephyre (Newman, 1839).

**Etymology.** The specific epithet is derived from *sub*, Latin for under, and *lobata*, the feminine adjectival form of *lobus*, Latin for a rounded projection or protuberance (Brown 1956). The name references the ventral caecum of the aedeagus, a character shared by only one other described congener, *P. golconda*, though it is most prominent in *P. sublobata*.

### Material examined: Perlesta sublobata

**Holotype:**  $\eth$ , in 95% ethanol, **USA: Arkansas:** Pike Co., Little Missouri River, 10.0 km SSE Delight at AR-19, 33.95608, -93.44362, 15.vi.2016, E. J. South (INHS Insect Collection 793224).

**Paratypes. USA: Arkansas:** Clark Co., Caddo River, 6.7 km NNW Arkadelphia at Super 8 Motel at US-67, 34.17985, -93.07021, 14.vi.2016, E. J. South, 6♂ (INHS Insect Collection 793213–793218); Franklin Co., Mulberry River, 2.3 km SSW Cass at AR-23, 35.66984, -93.82962, 13.vi.2016, E. J. South, 3♂ (INHS Insect Collection 793209–793211); Madison Co., War Eagle Creek, 5.8 km NE Huntsville at AR-412, 36.12076,

-93.69354, 13.vi.2016, E. J. South, 3♂ (INHS Insect Collection 793206–793208), same but 16.vi.2016, E. J. South, ♂ (INHS Insect Collection 793228), same but 17.vi.2016, E. J. South, 4♂ (INHS Insect Collection 793229–792232); Pike Co., Antoine River, Antoine at AR-26, 34.03899, -93.41803, 14.vi.2016, E. J. South, ♂ (INHS Insect Collection 793212). Same data as holotype, E. J. South 10♂ (INHS Insect Collection 793219–793227), same but 18.vi.2016, E. J. South, 71♂, 20♀ (INHS Insect Collection 793261–793343); Sevier Co., Cossatot River, 5.1 km W Lockesburg at AR-24, 33.97145, -94.22274, 18.vi.2016, E. J. South, 9♂, 19♀ (INHS Insect Collection 793263–793260).

Other material examined. USA: Arkansas: Franklin Co., Mulberry River, Hwy 23 at Turner's Bend, 35.66984, -93.82962, 5.vii.1986, B. C. Poulton, 4d (INHS Insect Collection 795241); Howard Co., Cossatot River, 12.9 km W Umpire at Hwy 4, 34.29584, -94.17787, 26.vi.1981, H. W. Robison, 10 (INHS Insect Collection 794630), same but Saline River, 8 km S Umpire at Hwy 4, 34.21096, -94.05099, 9.vii.1982, H. W. Robison, D. Koym, 7∂, 5♀ (INHS Insect Collection 794640), same but 1.6 km W Athens at Hwy 84, 34.31498, -93.99048, 9.vii.1984, H. W. Robison, D. Koym, 3∂, 10♀ (INHS Insect Collection 794629, 794634); Johnson Co., Mulberry River, 4.8 km W Ozark at Wolf Pen, 35.67376, -93.63271, 16.vii.1983, H. W. Robison, D. Koym, 88 (INHS Insect Collection 794643); Madison Co., War Eagle Creek, 4.8 km NE Huntsville at Hwy 68, 36.12076, -93.69354, 27.v.1978, J. McGraw, 17& (INHS Insect Collection 794636); Nevada Co., Little Missouri River, 17.7 km N Prescott at AR-19, 33.95571, -93.44388, 3.vii.1982, D. Koym, 8∂, 11♀ (INHS Insect Collection 794641), Little Missouri River, Nubbin Hill Rd., 33.93804, -93.35393, 1.vi.1982, D. Kovm, 8♂ (INHS Insect Collection 794637); Pike Co., Antoine River, Antoine at AR-26, 34.03899, -93.41803, 18.vi.1982, D. Koym, 10∂, 8♀ (INHS Insect Collection 794638); Saline Co., Middle Fork Saline River, 1.6 km NW Owensville, 34.63066, -92.82711, 10.vii.1981, H. W. Robison, S. Harris, 4d (INHS Insect Collection 794631); Scott Co., Shadley Creek, 0.4 km S Bates, 34.90626, -94.38661, 12.vi.1983, H. W. Robison, D. Koym, 10∂ (INHS Insect Collection 794642); Sevier Co., Cossatot River, AR-24, 33.97145, -94.22274, 26.vi.1982, H. W. Robison, 10<sup>3</sup> (INHS Insect Collection 794632); Van Buren Co., South Fork Little Red River, 4 km NE Scotland at AR-95, 35.54868, -92.58541, 22.vi.1985, H. W. Robison, 15d (INHS Insect Collection 793774); Washington Co., Cove Creek, 24.1 km S Prairie Grove, 35.79531, -94.36519, 6.vi.1962, O. Hite, M. Hite,  $2\sqrt[3]{}$ ,  $2\heartsuit$  (INHS Insect Collection 794639). Oklahoma: Atoka Co., motel, Atoka, 34.38538, -96.12788, 4.vi.1969, D. C. Arnold, 2d (OKSU Midwest Plecoptera 19534); Le Flore Co., Big Creek, Page, 34.71595, -94.55016, 23.vi.1937, Standish, Kaiser, 8<sup>(1)</sup> (OKSU Midwest Plecoptera 19529); McCurtain Co., Broken Bow, 34.02983, -94.73871, 29.vii.1937, Standish, Kaiser, 34♂, 30♀ (OKSU Midwest Plecoptera 19517, 19518), Sherwood, 34.33121, -94.77833, 27.vi.1937, Standish, Kaiser, 5 $\stackrel{?}{\circ}$ , 24 $\stackrel{?}{\circ}$  (OKSU Midwest Plecoptera 19521, 19522), West Fork Glover River, Battiest, 34.39393, -94.94166, 14.vi.1972, D. C. Arnold, 2 (OKSU Midwest Plecoptera 19523), Mountain Fork, Beaver's Bend State Park, 34.13960, -94.70704,

11.vi.1985, D. C. Arnold,  $3\Diamond$  (OKSU Midwest Plecoptera 19526, 195277), same but 10.vi.1985, D. C. Arnold,  $\Diamond$ ,  $4\heartsuit$  (OKSU Midwest Plecoptera 19528); Pontotoc Co., Ada, 34.77447, -96.67892, 16.vii.1937, Standish, Kaiser,  $2\Diamond$ ,  $2\heartsuit$  (OKSU Midwest Plecoptera 19519, 19520); Washington Co., Caney River, Bartlesville, 36.75401, -95.97137, 31.v.1978, D. C. Arnold,  $\Diamond$  (OKSU Midwest Plecoptera 19525).

# Material examined: Perlesta golconda

**USA: Illinois:** Carroll Co., Mississippi River, Savanna, 42.09622, -90.16227, 19.vi.1999, R. E. DeWalt,  $\bigcirc$  (INHS Insect Collection 566462). **Indiana:** Ohio Co., Arnold Creek, 6.9 km WSW Rising Sun at IN-262 and White Rd., 38.93676, -84.93167, 14.v.2018, E. A. Newman,  $\bigcirc$  (INHS Insect Collection 660320). **Iowa:** Cedar Co., Cedar River, Cedar Bluff at Hwy F28, 41.78790, -91.31340, 2.viii.2000, D. Heimdal,  $\bigcirc$ , 2 $\bigcirc$  (INHS Insect Collection 36061). **Louisiana:** East Baton Rouge Co., Mississippi River, Baton Rouge at Centroplex Pier N I-10Br., 30.44532, -91.19184, 12.vi.1992, R. E. DeWalt,  $\bigcirc$ ,  $\bigcirc$  (INHS Insect Collection 564765). **Minnesota:** Winona Co., Mississippi River, 3.6 km N La Crescent, rest stop at I-90, 43.85981, -91.30351, 18.vi.2012, R. E. DeWalt,  $\bigcirc$  (INHS Insect Collection 577372). **Nebraska:** Nemaha Co., Missouri River, Brownville, 200 m downstream US-136, 40.39335, -95.64948, 17.vi.2018, R. E. DeWalt,  $\oslash$ ,  $\bigcirc$  (INHS Insect Collection 660254), same but 24.vi.2018, R. E. DeWalt,  $\bigotimes$ , 10 $\bigcirc$  (INHS Insect Collection 660209–660220).

# Modified key to first couplet in Stark (2004) for identification of males of *Perlesta* sublobata and *P. golconda*

1	Fully everted aedeagus with dorsal caecum (fig. 7.273) 1a
_	Fully everted aedeagus without dorsal caecum (fig. 7.361)
1a	Aedeagus with ventral caecum1b
_	Aedeagus without ventral caecum
1b	Ventral caecum prominent, length ca. 2/5 aedeagal sac width, fine ventral
	seta-like spines present; dorsal caecum moderately developed (Fig. 4A); dis-
	tinct dorsal extension of the aedeagal lateral sclerites with proximal V-shaped
	pattern (Fig. 4C); ocellar area with dark subquadrate patch P. sublobata
_	Ventral caecum less prominent, length ca. 1/3 aedeagal sac width, without dis-
	tinct fine ventral seta-like spines; dorsal caecum poorly developed; dorsal aedea-
	gal patch with lateral margins darker than mesal field, appearing as two tracks
	(fig.7.364); ocelli usually connected by V-shaped area with pale center

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RESEARCH ARTICLE



# A new species of Diaspididae, Megacanthaspis guiyangensis (Hemiptera, Coccomorpha) from China

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### Abstract

A new species of armoured scale insect, *Megacanthaspis guiyangensis* **sp. nov.** is described and illustrated. The new species is morphologically similar to *M. hangzhouensis. Megacanthaspis guiyangenis* infests leaves of *Oligostachyum lubricum* in China. A key to all eight species of *Megacanthaspis* now known is provided.

### **Keywords**

Armoured scale, diagnosis, illustration, key, taxonomy

# Introduction

Armoured scale insects (Hemiptera: Coccomorpha: Diaspididae), are the largest family of the Coccoidea, and have a worldwide distribution, including 426 genera and 2624 species currently identified (García Morales et al. 2016). The morphology of adult females is extremely reduced: no legs, antennae reduced to unsegmented tubercles, head, thorax and abdomen are fused, and these adults are wingless (Balachowsky 1948; Takagi 1993; Andersen et al. 2010; Henderson 2011).

The genus *Megacanthaspis* Takagi, 1961 belongs to the tribe Diaspidini. The genus was originally established by Takagi (1961) with *Megacanthaspis actinodaphnes* Takagi,

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1961 designated as the type species. The genus is currently composed of seven species (García Morales et al. 2016).

The genus *Megacanthaspis* is distributed in China, Japan, and Nepal (García Morales et al. 2016). Takagi (1961) collected the type species of this genus in Japan. Later he described another species, *M. litseae*, from Taiwan, China (Takagi, 1970), and added two new species (*M. langtangana* and *M. leucaspis*) from Japan (Takagi, 1981). Takagi (1981) also transferred the species *Nanmuaspis phoebia* Tang, collected in China (Tang, 1977), into *Megacanthaspis*. Most recently Wei (2012) recorded two new species of this genus (*M. hangzhouensis* and *M. hainanensis*) from China.

A new species of *Megacanthaspis* was discovered in China and is described and illustrated in this work. This discovery raises the number of species recorded in the genus to eight, five of which have been reported from China. A key to all species of the genus *Megacanthaspis* is provided.

### Materials and methods

Samples of plants infested by the new species described in this study were collected in Guiyang City (Guizhou Province, China). Permanent slide mounts of adult females from the samples collected were slide-mounted using the protocol described by Henderson (2011).

Illustrations of adult female of the new species were drawn from the slide-mounted specimens, showing an overview of the dorsum on the left side and the venter on the right; enlarged details of the significant features are illustrated but not drawn in direct proportion to each other.

All specimens of the new species, *Megacanthaspis guiyangensis*, were deposited in the Entomological Museum, Northwest A&F University, Yangling, Shaanxi, China (**NWAFU**).

#### Taxonomy

### Megacanthaspis Takagi, 1961

Megacanthaspis Takagi, 1961: 97.

Type species. Megacanthaspis actinodaphnes Takagi by monotype and original designation.

**Generic diagnosis. Adult female.** Body elongate and slender, with metathorax and free abdominal segments not strongly produced; derm membranous. Each antenna with a long seta. Anterior spiracles with disc pores, and posterior spiracles of some species also with disc pores. Gland tubercles present caudad of anterior spiracles, laterocaudad of posterior spiracles and submarginally on 1–3 anterior abdominal segments. Pygidium rounded along posterior margin, all species without lobes, some species with a marginal series of serrate processes or plates. Marginal gland spines pre-
sent on the abdomen, each with one or more microducts. Dorsal macroducts present on abdomen and arranged in segmental rows but not in a well-defined series. Ventral ducts are the same size or smaller than dorsal ducts. Anal opening situated in the centre of pygidium. Perivulvar pores with five groups or connected to form an arc.

**Remarks.** The genus *Megacanthaspis*, like other groups such as *Thysanaspis* and *Pygalataspis*, has non-glanduliferous plates that are well developed but does not have distinct lobes. *Thysanaspis* and *Pygalataspis* have no gland spines. Members of the genera *Megacanthaspis*, *Kuwanaspis*, and *Nikkoaspis* all have plates and gland spines.

### Megacanthaspis guiyangensis sp. nov.

http://zoobank.org/1B30EFDA-374A-4EC7-B01E-15E325415428

**Material studied.** Holotype female: CHINA, Guizhou Province, Guiyang city, 26°24'35"N; 106°40'13"E. Collected on *Oligostachyum lubricum* leaves by Niu & Wei, 21.vii.2015, fist specimen from the left end of a row of 5 adult females, clearly indicated on the slide label (NWAFU).

Paratypes: 59 specimens, same data as holotype (at, 1 slide with 1 adult female, 1 slide with 2 adult females, 1 slide with 3 adult females, 4 slides each with 4 adult females, 5 slides each with 5 adult females, 2 slides each with 6 adult females (NWAFU).

Description. Adult female. (Figs 1-8) Body outline oblong fusiform, with indistinct segmentation. Each antenna with a long seta and a tubercle. Anterior and posterior spiracles without disc pores. The pygidium with sharp marginal processes on abdominal segments VII-VIII: 2 on each side of abdominal segment VII, 2 on abdominal segment VIII between the marginal gland spines (Fig. 8). Marginal gland spines each associated with one microduct, present on abdominal segments V-VIII: one pair, widely separated, on abdominal segment VIII and two pairs on abdominal segments V-VII. Some individuals have one pair of marginal gland spines on abdominal segment IV. Gland tubercles absent. Dorsal macroducts arranged in irregular rows on abdominal segments II-VIII and numbering approximately 23-50 on each side; Marginal macroducts arranged one on each side of abdominal segment VII, and absent on abdominal segment VIII between the gland spines. Ventral microducts smaller than dorsal macroducts, scattered loosely on the cephalothorax and abdomen. Anus rounded, located near the centre of the pygidium. Five groups of perivulvar pores form arcs: 3-5 in the median group, 5-8 in each laterocephalic group, and 4-8 in each laterocaudal group.

**Diagnosis.** *Megacanthaspis guiyangensis* sp. nov. resembles *M. hangzhouensis* (Wei & Feng, 2012) in body outline, absence of gland tubercles and with 1 microduct on each of the marginal gland spines. The important differences between the pygidia of the two species are shown in Table 1.

Host. Oligostachyum lubricum (Poaceae).

Etymology. Named after Guiyang, the type locality.

Distribution. China (Guizhou).

Pygidium character state	M. guiyangensis sp. nov.	M. hangzhouensis
Marginal processes	present	absent
Pairs of gland spines on abdominal segment VII	2	1
Number of dorsal macroducts on each side of abdomen	23-50	about 17

Table 1. Morphological differences between the pygidia of *M. guiyangensis* sp. nov. and *M. hangzhouensis*.



Figures 1–8. *Megacanthaspis guiyangensis* sp. nov., adult female 1 body 2 antennae 3 anterior spiracle 4 detail of dorsal gland duct 5 ventral microducts 6 perivulvar pore 7 pygidium 8 margin of pygidium (sharp marginal processes).

## Key to adult female Megacanthaspis Takagi

1	Marginal gland spines each with a single microduct
_	Marginal gland spines each with 2 or more microducts
2	Marginal processes absent
_	Marginal processes present
3	Marginal sharp processes on abdominal segment VII–VIII.
_	Marginal serrate processes on abdominal segment V and VI as well as VII-
	VIII
4	Gland spines on abdominal segment VIII close together
_	Gland spines on abdominal segment VIII separated
5	Marginal gland spines present on segment II M. langtangana Takagi
_	Marginal gland spines absent from segment II
6	With a macroduct between median gland spines on abdominal segment
	VIII
_	Without macroduct between median gland spines on abdominal segment
	VIII
7	Marginal serrate processes present on abdominal segment VI as well as on
	segments VII–VIII
_	Marginal serrate processes present on abdominal segments VII–VIII

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RESEARCH ARTICLE



# A new species and new records of the genus Alexeter Förster (Hymenoptera, Ichneumonidae, Ctenopelmatinae) from Beijing with a key to Chinese species

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## Abstract

A new species, *Alexeter beijingensis* Sheng, **sp. nov.**, and two new records for China, *A. angularis* (Uchida, 1952) and *A. shakojiensis* Uchida, 1930, collected in Mentougou, Beijing, belonging to the tribe Mesoleiini of the subfamily Ctenopelmatinae (Hymenoptera, Ichneumonidae), are reported. A key to the six species of *Alexeter* known from China is given.

## Keywords

Mesoleiini, host, taxonomy

# Introduction

*Alexeter* Förster, 1869, belonging to the tribe Mesoleiini of the subfamily Ctenopelmatinae (Hymenoptera: Ichneumonidae), comprises 32 species (Yu et al. 2016), of which eleven are from the Eastern Palaearctic Region (seven of them also occur in the Western Palaearctic region) (Šedivý 1971, Uchida 1930, 1952, Yu et al. 2016), 18 from Western Palaearctic (Aubert 1998, Hinz 1996, Heinrich 1949, 1953, Meyer 1936), three from the Neotropical (Gauld et al. 1997), and seven from the Nearctic Region (Yu et al. 2016).

To date, three species are known from China (Chao 1976, Luo et al. 2019, Roman 1936). *Alexeter clavator* (Müller, 1776) was mentioned by Chao (1976) from Gansu, NW China, *A. multicolor* (Gravenhorst, 1829) from Jiangxi and Henan, S China, was reported by Luo et al. (2019), *A. segmentarius* (Fabricius, 1787) was reported from Gansu by Roman (1936) and Chao (1976).

The known hosts of *Alexeter* are sawflies, belonging to Diprionidae and Tenthredinidae (Aubert 1998, 2000, Constantineanu and Istrate 1973, Gauld et al.1997, Hinz 1961, 1996, Yu et al. 2016).

In this paper we deal with all *Alexeter* species from China, including the description of a new species and a key to all known species from the country.

## Materials and methods

Specimens were collected by interception traps (IT) (Li et al. 2012) in the forest of Mentougou, Beijing, P.R. China. The forest of Mentougou is composed of mixed deciduous angiosperms and evergreen conifers (Zong et al. 2013). Images were taken using a Leica M205A stereomicroscope with LAS Montage MultiFocus. Morphological terminology is mostly based on Gauld (1991).

The specimens of *A. clavator* (Müller, 1776), *A. coxalis* (Brischke, 1871), *A. multicolor* (Gravenhorst, 1829), *A. nebulator* (Thunberg, 1822), *A. niger* (Gravenhorst, 1829), *A. rapinator* (Gravenhorst, 1829), *A. segmentarius* (Fabricius, 1787) provided by Dr. Gavin Broad (The Department of Life Sciences, the Natural History Museum, London, UK) (**NHMUK**), were examined. The photos of the types, described by Uchida and deposited in Hokkaido University Museum, Hokkaido University, Japan, taken by Dr. Kyohei Watanabe (Kanagawa Prefectural Museum of Natural History, Odawara, Japan) (**KPMNH**), were examined and compared to the new species by the corresponding author.

Type specimens are deposited in the Insect Museum, General Station of Forest and Grassland Pest Management (**GSFGPM**), National Forestry and Grassland Administration, People's Republic of China.

#### Alexeter Förster, 1869

**Type species.** *Mesoleptus ruficornis* Gravenhorst, 1829 (= *segmentarius* Fabricius, 1787).

**Diagnosis.** (Förster 1869, Gauld et al. 1997, Townes 1970). Clypeus separated from face, apical margin blunt, weakly concave or centrally truncate. Occipital carina

dorsally complete, reaching to hypostomal carina distinctly above base of mandible. Epomia absent. Notaulus usually long and sharp. Mesopleuron with very fine to large punctures. Median longitudinal carina of propodeum usually distinct and complete, pleural carina complete. Fore wing vein 1cu-a opposite or distal of 1-M. Areolet present, or 3rs-m absent (Gauld et al. 1997). Hind wing vein 1-cu longer than cu-a. First tergite slender, median dorsal carina absent, or vestigial or indistinct. Glymma present. Second tergite with fine, weak, or indistinct punctures. Ovipositor short, with a distinct dorsal subapical notch.

## Key to species of Alexeter known from China

1	Body brown to reddish brown. Postocellar line 1.3 times as long as ocular-ocellar
	line. Malar space approximately 0.2 times as long as basal width of mandible.
	Median longitudinal carina of propodeum distinct and complete. Third tergite
	approximately 1.3 times as long as maximum width A. clavator (Müller)
_	Body, at least mesosoma, black. Other characters variable2
2	Median longitudinal carinae of propodeum complete, area superomedia dis-
	tinctly constricted (Fig. 7) or expanded medially. Metasomal tergites black, or
	almost entirely black
_	Median longitudinal carinae of propodeum almost parallel (Fig. 14), or di-
	vergent posteriorly, or incomplete (Fig. 13). Basal or median tergites, or all
	tergites brown to red-brown
3	Median longitudinal carinae of propodeum distinctly expanded medially
	(Fig. 12). Tegula, scutellum and postscutellum pale yellow.
	A. multicolor (Gravenhorst)
_	Median longitudinal carinae of propodeum distinctly constricted medially.
	Tegula, scutellum and postscutellum black4
4	Fore wing vein 2m-cu connecting to areolet basad of its posterior angle.
	Ovipositor sheath narrowed backwardly. Middle tarsus and hind leg entirely
	black
_	Fore wing vein 2m-cu connecting to 4-M slightly distal of posterior angle of
	areolet. Ovipositor sheath parallel-sided. Subbasal portion of hind tibia widely
	white. Middle tarsus with at least median portion white or yellowish brown.
	Hind tarsomeres 2-4 and basal half of 5 white
5	Median longitudinal carinae of propodeum almost parallel (Fig. 14). Third
	tergite distinctly longer than its apical width. Hind femur and second and
	subsequent tergites reddish brown. Ovipositor sheath whitish yellow
	A. shakojiensis Uchida
_	Median longitudinal carinae of propodeum divergent backwardly or incomplete
	(Fig. 13). Third tergite shorter than its apical width. Hind femur, fifth and subse-
	quent tergites black. Ovipositor sheath black A. segmentarius (Fabricius)

## Alexeter beijingensis Sheng, sp. nov.

http://zoobank.org/A36502D6-F805-4493-AFA6-4EAC2B14A464 Figs 1–11

**Etymology.** The name of the new species is derived from the type locality.

**Material examined.** Holotype female, Mentougou, Beijing, 20 August 2004, leg. Tao Wang and Shi-Xiang Zong (GSFGPM).

**Diagnosis.** Apical portion of clypeus shiny, apical margin weakly and evenly concave. Outer profiles of middle and hind tibiae with distinct spines. Propodeum (Fig. 7) shagreened, area between median longitudinal carinae shiny, almost smooth; posterolateral portion with long and dense grey setae. Head, mesosoma and metasoma almost entirely black. Subbasal portions of all tibiae and median bands of middle and hind tarsi white.

**Description.** Female. Body length approximately 11.5 mm. Fore wing length 10.0 mm.

**Head.** Inner margins of eyes weakly indented opposite antennal sockets. Face (Fig. 2) 1.3 times as wide as long, uppermedian portion slightly convex; shagreened, with dense fine indistinct punctures; upper margin with a small median smooth tubercle. Clypeus (Fig. 2) transversely convex medially; basal portion shagreened, with indistinct short transverse wrinkle; apical portion smooth, shiny; apical margin weakly and evenly concave. Mandible with dense dark grey setae, lower tooth slightly longer than upper tooth. Malar space 0.5 times as long as basal mandibular width. Gena almost evenly convergent backward, in dorsal view approximately 0.8 times as long as width of eye. Vertex (Fig. 3) and frons with sculpture as that of face. Postocellar line 0.5 times as long as ocular-ocellar line. Antenna with 50 flagellomeres; ratios of lengths from first to fifth flagellomeres: 4.8:2.2:2.0:1.9:1.7; ultimate flagellomere twice as long as penultimate flagellomere. Occipital carina complete.

Mesosoma. Pronotum shagreened, upper portion with dense fine transverse wrinkles; hind margin of lateral concavity with short transverse oblique wrinkles. Epomia indistinct. Mesoscutum (Fig. 4) finely shagreened, with relatively sparse shallow punctures, distance between punctures mostly subequal to one diameter of puncture. Notaulus weak, present on front half of mesoscutum. Scutoscutellar groove with dense longitudinal wrinkles. Scutellum and postscutellum finely shagreened. Mesopleuron (Fig. 5) with sculpture almost as that of mesoscutum, with indistinct fine punctures. Speculum very small, upper portion shagreened. Mesopleural fovea vestigial. Upper end of epicnemial carina almost reaching anterior margin of mesopleuron, at level of upper 0.6 of pronotum. Metapleuron almost flat, with sculpture as that of mesopleuron; posterior margin with short transverse wrinkles. Submetapleural carina distinct, complete. Wings (Fig. 6) slightly infuscate. Fore wing with vein 1cu-a distal to 1-M by 0.3 times length of 1cu-a. Areolet triangular, with long petiole, 0.4 times length of its height. 2m-cu slightly reclivous, connecting to posterior angle of areolet. Hind wing vein 1-cu 1.5 times as long as cu-a. Outer profiles of middle and hind tibiae with relative dense spines. Ratio of length of hind tarsomeres from first to fifth is



Figure 1. Alexeter beijingensis sp. nov. Holotype. Female. Habitus (without wings), lateral view.



Figure 2. Alexeter beijingensis sp. nov. Holotype. Female. Head, anterior view.



Figure 3. Alexeter beijingensis sp. nov. Holotype. Female. Head, dorsal view.



Figure 4. Alexeter beijingensis sp. nov. Holotype. Female. Mesoscutum and scutellum, dorsal view.



Figure 5. Alexeter beijingensis sp. nov. Holotype. Female. Mesosoma, lateral view.

4.0:2.0:1.5:0.8:1.0. Tarsal claws simple, hind claw strongly thick and curved (Fig. 8). Propodeum (Fig. 7) with distinct posterior transverse and strong complete median longitudinal carinae, latter strongly constricted medially. Area between median longitudinal carinae shiny, with indistinct, irregular transverse oblique fine striae. Area petiolaris



Figure 6. Alexeter beijingensis sp. nov. Holotype. Female 6 wings 6a areolet.



Figure 7. Alexeter beijingensis sp. nov. Holotype. Female. Propodeum, dorsal view.

with irregular longitudinal wrinkles. Remainder with sculpture as that of mesopleuron. Posterolateral portion with long dense grey hairs. Propodeal spiracle circular.

**Metasoma** (Figs 9–11). Tergites shagreened. First tergite (Fig. 9) 2.4 times as long as apical width; median dorsal carina absent; dorsolateral carina indistinct, almost absent; spiracle distinctly convex, located slightly before mid of the tergite. Second tergite (Fig. 10) 1.1 times as long as apical width. Lateral margins of tergites 3 and 5, in dorsal view, almost parallel. Third tergite 1.1 times as long as apical width. Fourth tergite 0.8 times as long as apical width. Ovipositor sheath 0.5 times apical depth of metasoma. Ovipositor (Fig. 11) tapered from base to apex, with a large, deep, almost quadrangular notch.

**Coloration** (Fig. 1). Black, except for the following. Apical half of clypeus, anterior side and apical portion of fore femur red brown. Dorsoposterior portion of pronotum, fore tibia, basal halves of middle and hind tibiae except bases, mid tarsomeres 3 and 4, and hind tarsomeres 2–5 white. Apicomedian portion of scutellum yellowish white. Pterostigma and wing veins brownish black.



Figure 8. Alexeter beijingensis sp. nov. Holotype. Female. Apex of hind tarsus with claw, lateral view.



Figures 9. Alexeter beijingensis sp. nov. Holotype. Female. First tergite, dorsal view.



Figures 10. Alexeter beijingensis sp. nov. Holotype. Female. Tergites 2-8, dorsal view.



Figures 11. Alexeter beijingensis sp. nov. Holotype. Female. Apex of metasoma with ovipositor, lateral view.

**Comparative diagnosis.** The new species is similar to *A. niger* (Gravenhorst, 1829) in coloration: mesosoma and metasoma black; basal and apical portions of hind tibiae black, median portions white or yellowish white, but can be distinguished from the latter by the following combinations of characters: median longitudinal carina of propodeum complete, strong (absent or indistinct in *A. niger*); fore wing vein 2m-cu connecting to posterior angle of areolet (basad in *A. niger*); antenna, face and tegula black (antenna yellow-brown, face and tegula yellow in *A. niger*).

## Alexeter angularis (Uchida, 1952)

**Material examined.** CHINA: 1 female, Mentougou, Beijing, 29 September 2009, leg. Tao Wang.

Distribution. China, Japan. New record for China.

# Alexeter clavator (Müller, 1776)

**Material examined.** CHINA: 105 females, 121 males, Mt. Liupanshan, Ningxia Hui Autonomous Region, 7 July to 19 September 2005, IT. 1 female, Qinling, Shaanxi province, 5 July 2017, leg. Tao Li.

**Distribution.** China: Gansu, Ningxia, Shaanxi; Finland; Germany; Netherlands; Sweden; Switzerland.

# Alexeter multicolor (Gravenhorst, 1829)

Fig. 12

Material examined. CHINA: 1 female, 2 males, Tianzhu, Guizhou province, April 1996, leg. Yi-Han Li. 1 female, Jiulianshan, Jiangxi province, 5 August 2012, IT. 2 males, Wugongshan, 580 m, Jiangxi province, 16 May 2016, leg. Yu Yao. 1 male, Baotianman National Natural Reserve, 1300–1500 m, Henan province, 12 July 1998, leg. Mao-Ling Sheng.

**Distribution.** China: Guizhou, Henan, Jiangxi; Europe (Yu et al. 2016).

# Alexeter segmentarius (Fabricius, 1787)

Fig. 13

Material examined. CHINA: 1 male, Zhenfengshan, Helong, Jilin province, 2 August 1982, leg. Bing-Zong Ren.

**Distribution.** China: Gansu, Jilin; Mongolia, Russia, Europe (Yu et al. 2016)

# Alexeter shakojiensis Uchida, 1930

Fig. 14

Material examined. CHINA: 6 females, Mentougou, Beijing, 29 August to 22 September 2008, leg. Tao Wang. 1 female, 1 male, Mentougou, Beijing, 4 August to 8 September 2009, leg. Tao Wang. 1 male, Yanqing, Beijing, 12 June 2012, leg. Shi-Xiang Zong.

Distribution. China, Korea. New record for China.



Figure 12. Alexeter multicolor (Gravenhorst, 1829). Propodeum, dorsal view.



Figure 13. Alexeter segmentarius (Fabricius, 1787). Propodeum, dorsal view.



Figure 14. Alexeter shakojiensis Uchida 1930. Propodeum, dorsal view.

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RESEARCH ARTICLE



# Two new European long-legged hoverfly species of the Eumerus binominatus species subgroup (Diptera, Syrphidae)

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## Abstract

*Eumerus* Meigen (Diptera, Syrphidae) is one of the most speciose hoverfly genera in Europe, with several species groups recognized within. As part of the *tricolor* group of species, a subgroup of long-legged representatives stands out. We name it *Eumerus binominatus* subgroup and provide descriptions for two new European species which belong to this subgroup: *E. grallator* **sp. nov.** from mainland Spain and *E. tenuitarsis* **sp. nov.** from Lesvos and Evros, Greece. A key for all five recognized species of the *binominatus* subgroup is provided.

## Keywords

*Binominatus* subgroup, *Eumerus*, hoverflies, identification key, long-legged syrphids, *tricolor* group, Turano-Mediterranean distribution

# Introduction

The genus *Eumerus* Meigen (Merodontini) is one of the most species-rich hoverfly genera in the World. There are over 300 species known (Evenhuis and Pape 2019) and new ones are described on an almost yearly basis. The European fauna is fairly well known, although

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even here new species are regularly found, especially in the Mediterranean. Since 2010 no less than 17 species have been described from southern Europe and the adjacent Middle East (Ricarte et al. 2012, 2018; Grković et al. 2015, 2017, 2019; Markov et al. 2016; Smit et al. 2017; Steenis et al. 2017; Chroni et al. 2018) and many more await description.

The members of *Eumerus* recorded in Southeast Europe belong to the following groups, identified on the basis of molecular markers in Chroni et al. (2017) as well as on the basis of morphological similarity: *tricolor* (Chroni et al. 2017; Grković et al. 2017), *strigatus* with subgroup *bactrianus* (Speight et al. 2013; Grković et al. 2017, 2019), *clavatus* (Grković et al. 2017), *minotaurus* (Chroni et al. 2018), *barbarus* (van Steenis et al. 2017), *olivaceus, ornatus, obliquus* (Smit et al. 2017), *basalis* and *pulchellus*. The *Eumerus tricolor* species group, defined by Chroni et al. (2017) based on DNA sequencing and described by Grković et al. (2017) displays a wide spectrum of species, but is clearly separated from the other members of the genus by a set of apomorphic characters, including a radially wrinkled basoflagellomere and a fossette clearly expressed, most often with partially to completely red abdominal tergites, but also includes species without red markings. This group makes up about 30% of all *Eumerus* species in the Mediterranean Region.

In this paper we add yet another two European species to the list of *tricolor* group, one from the western Mediterranean and one from the eastern part of it. Both species belong to a species subgroup not previously recorded from the western Palearctic, characterized by slender elongated legs in the male, which is a unique feature within the family of Syrphidae, a long pilose thorax, pilose eyes and a stout abdomen. We included these species into the *binominatus* subgroup named after the Asian long-legged species *E. binominatus* Hervé-Bazin, 1923 (Fig. 1A, B), first described by Becker as *E. maculipennis*, a name preoccupied by Bezzi (1915) for an African species from the *ornatus* group.

## Material and methods

The characters used in the key, descriptions, and drawings follow the terminology established by Thompson (1999). Terminology referring to male genitalia follows Doczkal (1996) and Hurkmans (1993). Color characters are described from dry-mounted specimens. Male genitalia were stored in microvials containing glycerol after clearing in warm 10% potassium hydroxide (KOH) for a few minutes and neutralising in acetic acid for 5–10 seconds. Label information is given in quotes with the lines separated by a slash '/', additional information is provided in square brackets.

The drawings and part of the figures were created using photographs taken with a Leica DFC 320 (Wetzlar, Germany) camera attached to a Leica MZ16 binocular stereomicroscope and then processed in Adobe Photoshop CS3 v10.0 (Adobe Systems, San Jose, CA, USA). The figures of *Eumerus binominatus* and *E. tadzhikorum* were created using photographs taken with a Canon EOS D6 equipped with a Canon MP-E 65 macro zoom lens. Several photos for each figure were processed with Zerene Stacker and further edited with the Photoshop program GIMP 2.8.22.

The distribution map was created in Adobe Illustrator CS6 V 16.0.0 software (Adobe Systems, San Jose, CA, USA).

The following acronyms for museums and entomological collections are used in the text:

AEPC	A. van Eck private collection, Tilburg, The Netherlands
CEUA	Colección Entomológica de la Universidad de Alicante, CIBIO-Alicante
	University, Spain
CSCA	California State Collection of Arthropods, Sacramento, California, USA
DDPC	D. Doczkal private collection, Malsch, Germany
FSUNS	University of Novi Sad, Department of Biology and Ecology, Novi Sad, Serbia
NBC	Natural Biodiversity Center, Leiden, The Netherlands
SBPC	S. Bot private collection, Haren, The Netherlands
USNM	United States National Museum of Natural History, Smithsonian Institu-
	tion, Washington, United States
ZISP	Zoological Museum, Russian Academy of Sciences, St. Petersburg, Russia
ZMHU	Museum für Naturkunde der Humboldt Universität zu Berlin, Berlin, Germany

# Results

Taxonomy

Family Syrphidae Subfamily Eristalinae Tribe Merodontini Genus *Eumerus* Meigen, 1822 Species group *tricolor* 

# Species subgroup binominatus

**Diagnosis.** Eyes densely whitish, pilose. Basoflagellomere relatively small, only about twice size of pedicel, oval to squarish in shape, with only a few short radial wrinkles. Male eyes holoptic or narrowly dichoptic. Abdomen short and stout (Fig. 4C). Posterior lobe of surstylus simple, well developed.

**Remarks.** The *Eumerus binominatus* subgroup shares all characters of the *tricolor* group (Grković et al. 2017) but can easily be recognized within this group by the extremely long and slender legs in males, especially obvious in the metaleg, where the width of the widest part of metafemur is equal or less than one fifth of the length of the metafemur (Fig. 4D–F). *Eumerus niveitibia* is a Mediterranean species from the *tricolor* group which shares several characters with members of *binominatus* subgroup, e.g. long pilosity on eyes and thorax, similar heart-shaped abdomen with large white pollinose maculae on tergites, but it is clearly differentiated by the long eye-contiguity

in male, metafemur clearly thickened and by characteristic snow-white pilosity dorsally on metatibia. Furthermore, the posterior lobe of the surstylus in *E. niveitibia* is much smaller than in *E. binominatus* species subgroup. Females of *E. niveitibia* are similar in appearance with the females of *binominatus* subgroup, but can be differentiated by a slenderer metafemur and characteristic curvature on the metatibia in the *binominatus* subgroup females.

*E. selevini* Stackelberg, 1949 is a middle-Asian species similar to the *binominatus* subgroup, based on the slender metafemur. The head of this species is very similar to that in *E. binominatus* and *E. tadzhikorum* but with smaller, equilateral ocellar triangle, placed medially on vertex, which is in the other two species large, elongated and placed closer to the upper eye margins. It differs by the normal shaped metatarsus, not elongated as in *binominatus* subgroup; elongate abdomen in comparison to the length of head and thorax together and with a characteristic lateral notch in the second metatarsal segment; the pilosity on the thorax is very short in *E. selevini* in contrast to species in the *binominatus* subgroup which makes this species easily recognizable.

The following species belong to the *binominatus* subgroup:

*E. binominatus* Hervé-Bazin, 1923 (Fig. 1A, B) = *E. maculipennis* Becker, 1921 preocc. Bezzi, 1915 *E. grallator* sp. nov. (Fig. 3A, B) *E. longitarsis* Peck, 1979 *E. tenuitarsis* sp. nov. (Fig. 3C, D) *E. tadzhikorum* Stackelberg, 1949 (Fig. 1C, D)

# Eumerus binominatus Hervé-Bazin, 1923

Fig. 1A, B

**Notes.** This species was originally described by Becker (1921) as *E. maculipennis* from Transcaspia (south part of Kazakhstan). Hervé-Bazin (1923) revealed this name as a junior homonym of *Eumerus maculipennis* Bezzi, 1915 from Nigeria and named Becker's species *E. binominatus*. The holotype is held in ZMHU and has been examined.

Material examined. Holotype d' Eumerus maculipennis Becker, 1921: "Transkaspien / 57442", "maculipennis / Beck / det Becker", "Holotypus" [red label], "Zool. Mus. / Berlin", "Holotype d' / Eumerus maculipennis / Becker, 1921 / det. J. van Steenis, 2016, (ZMHU).

**Diagnosis.** Male eyes separated by width of ocellus. Face black, covered in whitish pilosity with few black pilosities above antennae (Fig. 2A). Antenna brown-red and slightly higher than long (Fig. 2B). Wing with a dark spot (Fig. 1B). Metafemur with row of about 7 rather long black setae, which are about 1/2 as long as width of metafemur. Abdomen partly red (Fig. 1A).

This species is similar to *E. tadzhikorum* but differentiated by the shape and color of the basoflagellomere.



**Figure 1.** *Eumerus binominatus*, male holotype of *E. maculipennis* **A** dorsal view **B** lateral view. *Eumerus tadzhikorum*, male, Kazachstan **C** dorsal view **D** lateral view. Scale bar: 1.0 mm.



**Figure 2.** *Eumerus binominatus*, Male holotype of *E. maculipennis* **A** head, fronto-lateral view **B** antenna, lateral view. *Eumerus tadzhikorum*, male, Kazachstan **C** head lateral view **D** antenna lateral view. Scale bar: 1.0 mm.

#### Eumerus grallator Smit, sp. nov.

http://zoobank.org/F77091EF-7681-4560-8370-447DDB792687 Figs 3A, B; 4E; 5A, B, D, G

**Type material. Holotype.** SPAIN *A*, Castilla la Mancha, Villahermosa. Original label: "España, Castilla / la Mancha, Villahermosa / [UTM] 30S WH19329-88405 / 23.vi.2003. 980 m / leg. J.T. Smit". The holotype is in good condition with no apparent signs of wear, except for wingtips, which are both damaged. The holotype is deposited in the NBC. **Paratypes**. SPAIN • 1  $\Diamond$  same data as for holotype (NBC); 1 $\bigcirc$ , Andalusia, Los Marines, 600 m, 37°17'05"N 06°22'08"W, 10.vi.2015, leg. J. and I. Smit (NBC); 1<sup>♀</sup>, Foia Ampla, 1060 m, Agres, Alicante, 3–17.vii.2001, leg. Pérez-Bañon, Marcos-García y Rojo (FSUNS); 1<sup>Q</sup>, Foia Ampla, 1060 m, Agres, Alicante, 2–16. vii.2002, leg. Pérez-Bañon, Marcos-García y Rojo (CEUA); 1♂, Mas del Parral, 900 m, Bocairent, Valencia, 5–19.vi.2001, leg. Pérez-Bañon, Marcos-García y Rojo (FSUNS); 13, "FO: 5335 Spanien / 36°58'29"N, 04°00'59"W / Bosque del Puerto Navazo, Alhama de / 1180 m NN / A; Thapsia villosa / leg. A. Ssymank, 12.06.2003" (DDPC); 13, "España, Madrid, Aranjuez / UTM 30T 4484430, 545 m a.s.l. / 17.vi.2015, leg. P.A. Fidalgo" (AEPC); 13, "España, Burgos, Peñahorada / UTM 30T 4474705, 910 m a.sl. / 13.vii.2016, leg. P.A. Fidalgo" (AEPC); 2 d "España, Soria, Herrera de Soria / UTM 30T 4984624 1095 m a.s.l. / 14.vii.2016, leg. P.A. Fidalgo" (AEPC).

**Diagnosis.** Male. Ocellar triangle isosceles. Basoflagellomere blackish, small, rounded, with one or two short radial wrinkles. Constriction of elongated metafemur located in posterior half (Fig. 4E). Greatest width of metafemur is approximately equal to one fifth of length of metafemur. Metatibia noticeably shorter than metafemur. Abdomen black, without red markings. Ventral margin of hypandrium with medial triangular protuberance (Fig. 5D: vp). Anterior lobe of surstylus with a single pilose row (Fig 5A).

Description. Male. Body length (excluding antenna): 11.5 mm; wing length: 7 mm. Head. Eyes separated by the width of an ocellus and covered in dense white pilosity. Eye margins in anterior view almost parallel, slightly broadening ventrally. Face completely black pilose, covered in silver pollinosity, most expressed in middle. Frons, vertical triangle and occiput black; silver pollinosity well expressed along eye margin on frons, on vertex anteriorly and dorsally on occiput behind eye margin, but most distinctive laterally. Ocellar triangle isosceles and predominantly black pilose, becoming intermixed with white pile in front of ocellar triangle and turning predominantly white behind it. Distance from anterior to posterior ocellus same as distance from latter one to upper eye corner. Lower facial margin in lateral view not protruding. Scape and pedicel brown to black. Basoflagellomere dark brown, rounded and slightly longer than broad with one or two short radial wrinkles. Ventral pile of pedicel black, not longer than its depth. Thorax. Scutum and scutellum densely punctate, shiny, with a bluish tinge; covered in long dense white pilosity. Two vittae of white pollinosity on scutum faint and thin, hardly reaching base of wings. Pleurae black. Anepisternum entirely white pilose, except for some black pile just behind the anterior spiracle. Anepimeron white pilose with some black pile posteriorly. Katepisternum and katepimeron black



**Figure 3.** *Eumerus grallator* sp. nov., male holotype **A** dorsal view **B** lateral view. *Eumerus tenuitarsis* sp. nov. male holotype **C** dorsal view **D** lateral view. Scale bar: 1 mm.

pilose. Wing. Hyaline with pterostigma about same color as the wing. Vein R<sub>4+5</sub> slightly curved. Wing covered in microtrichia except for basal cells mostly bare. Costal setae black. Halter blackish. Legs. Metaleg slender with all segments very elongated (Fig. 4E). Femora black, yellowish posteriorly, covered in black pilosity. Pro- and mesofemur black with yellowish tips; metafemur black, turning lighter in apical third, becoming orange at apex; slightly thickened in basal half; with a few scattered black setae in apical half. Tibiae white pilose. Pro- and mesotibia mostly black, yellowish in basal third and with yellowish apices; metatibia in basal half yellowish, apical half black turning lighter towards apex. Metatibia slightly thickened apically and slightly curved in apical half (curvature being species-specific in all three species with black abdomen). Tarsi brown to black; basitarsus of metaleg lighter ventrally; metatarsus longer than tibia (Fig. 4E). Abdomen. Black, punctate, pilose, tapered (Fig. 3A). Terga 2-3 with pairs of wide white pollinose maculae, slightly obscured towards medial part of terga; tergum 4 with pair of white pollinose maculae with apices upwards. Tergum 2 with long white pile laterally; pilosity adpressed, in area of pollinose maculae white, black on rest of terga. Punctation is visible through pollinose maculae. Genital capsule covered in erect black pilosity. Sterna entirely black pilose; sternum 4 flat with longer pilosity apico-laterally (Fig. 5G). Terminalia. (Fig. 5A, B, D). Posterior surstyle lobe simple, beak-like in lateral view, with long strong setae laterally on outer surface (Fig. 5A: ps); in ventral view, outer margin convex, pilose (Fig. 5B: om). Cerci oval, slightly pointed apico-dorsal, uniformly pilose (Fig. 5A: c). Interior accessory lobe of surstyle lobe densely pilose (5B:



**Figure 4.** *Eumerus tenuitarsis* sp. nov., male **A** head, dorsal view **B** head, lateral view **C** abdomen **D** metaleg, lateral view. *Eumerus grallator* sp. nov., male **E** metaleg, lateral view. *Eumerus longitarsis*, male **F** metaleg, lateral view. Scale bar: 1 mm.

in). Hypandrium curved, broad with folded thecal ridge near base; medially with triangular protuberance on ventral margin (Fig. 5D: vp), and wide notch dorsally near base. **Female.** Body length (excluding antennae): 11.5 mm; wing length: 7 mm. Similar to male except normal sexual dimorphism and for following differences: *Head*. Entirely white pilose. Basoflagellomere oval, with three to four radial wrinkles. Width of frons in narrower part is less than one fourth of width of head in anterior view. *Thorax*. White pollinose vittae obscured. Pleurae white pilose. Segments of metaleg only slightly elongated. *Abdomen*. Tergum 4 with longer white pile posteriorly. All sterna white pilose.

**Etymology.** The specific epithet is the Latin word *grallator* meaning "one who walks on stilts", which refers to the very slender and elongated legs of this species. It should be treated as a noun in apposition.



**Figure 5.** *Eumerus grallator* sp. nov., male **A** epandrium, lateral view **B** surstyle lobe, ventral view **D** medial part of hypandrium, lateral view **G** fourth sternum. *Eumerus tenuitarsis* sp. nov., male **C** hypandrium, lateral view **E** surstyle lobe, lateral view **F** epandrium, ventral view **H** fourth sternum. **Abbreviations: c** – cercus, **dn** – dorsal notch of hypandrium, **in** – interior accessory lobe of surstyle lobe, **om** – outer margin of posterior surstyle lobe in ventral view, **ps** – posterior lobe of surstylus, **th** – thecal ridge of hypandrium, **vp** – ventral protuberance of hypandrium. Scale bars: 0.2 mm (**A–F**); 0.5 mm (**G, H**).

## Distribution. Spain.

**Remarks.** The male holotype and one male paratype specimens were swept from a stand of some large yellow Apiaceae along a road, in an open park-like landscape of an oak dehesa. Accompanying hoverfly species were *Eristalinus taeniops* (Wiedemann, 1818), *Eristalis arbustorum* (Linnaeus, 1758), *Eumerus barbarus* (Coquebert, 1804), *E. nudus* Loew, 1848, *Spilomyia digitata* (Rondani, 1865) and *Xanthogramma marginale* (Loew, 1854).

## Eumerus longitarsis Peck, 1979

Fig. 4F

**Notes.** This species was described from Tajikistan and is known from Asia Minor and south-central Asia. This species is likely to consist of a complex of closely related species in this region (Doczkal pers. comm.).

**Material examined.** Holotype ♂ *Eumerus longitarsis* Peck, 1979: "Tajikistan, Hissar mountains / Takob ravine / Tian Shan h = 1700 m / leg. 23.vii.1976", "399", "Holotypus ♂ / *Eumerus / longitarsis* Peck" (ZISP).

Additional material: "[Russia] So.[uthern] Primor'e [Primorsky Krai] / Kamenushka / A.Shatalkin [leg.]", 1 (USNM).

**Diagnosis.** Male. Ocellar triangle equilateral. Face with black pile. Constriction of the elongated metafemur is located in posterior half. Metatarsus remarkably longer than metatibia (Fig. 2F). Anterior lobe of surstylus with multiple rows of long pilosity (see fig. 6 in Peck 1979). Abdomen black, without red markings.

## Eumerus tadzhikorum Stackelberg, 1949

Figs 1C, D; 2C, D; 6

**Notes.** Described from Tajikistan and known from southern Kazakhstan, Kyrgyz, Tajikistan, Turkmenistan and Uzbekistan (Peck 1988). The holotype is held in ZISP and has been examined by the last author. Additional material of *E. tadzhikorum* identified by Stackelberg was studied too.

Material examined. Holotype & Eumerus tadzhikorum Stackelberg, 1949: "16. VI.[19]44", "Eumerus typ. '46 / tadzhikorum sp. nov. / Stackelberg det.", "Holotypus '49 / Eumerus / tadzhikorum Stack." [red label, partly handwritten], "Lectotypus Eumerus / tadzhikorum Stack / design. V. Richter" [red label, partly handwritten], (ZISP).

Additional material. Kazakhstan: "KZ Oblast Almaty / Tamgaly 886 m / lat 43.802 lng 75.534 / 8 V 2015 leg. S. Bot", 1 (SBPC); "KAZAKHSTAN 29.V.2001 / SE Chilik 700m / 43°40'N 78°29'E / leg. M. Hauser", 1 (CSCA). Armenia: "Мегри на р. Аракс / Армения / В. Рихтер" with added handwritten "2.5 km В. m. g. / станции / 7.V.974", 1 (ZISP).

**Diagnosis.** Male. Eyes clearly separated by width of basoflagellomere. Wing with a dark spot (Fig. 1C, D). Antenna black and as high as long (Fig. 2D). Metafemur with row of about 7 rather long black setae, which are about 1/3 as long as width of metafemur. Abdomen partly red (Fig.1C, D). Posterior surstyle lobe anteriorly with fan-like protruding structure, separated by deep incision.

**Remarks.** This species is extremely similar to *E. binominatus* and it is possibly a subjective junior synonym of this species. The length of the setae on the apico-ventral side of the metafemur seems to vary in number and length. Due to lack of material no conclusion will be drawn here.

## Eumerus tenuitarsis Grković & Vujić sp. nov.

http://zoobank.org/4c2cf1d2-c5f5-4e5e-9c2e-562329fdf1f5 Figs 3C, D; 4A–D; 5C, E, F, H; 7

**Type material. Holotype**. GREECE •  $\Diamond$ , Lesvos, Agiassos. Original label: "Agiassos, 601 m / Lesbos, Greece / 39°4'16"N / 26°22'23"E / 23.vi.2003 / leg. M. Hull". **Paratype**. GREECE • 1 $\bigcirc$ , Evros, Dadia, 26–28.vii.2013, 40,9943N 26.0933E leg. M. Kourtidou (FSUNS).



**Figure 6.** *Eumerus tadzhikorum*, male terminalia **A** epandrium, lateral view **B** surstyle lobe, ventral view **C** hypandrium, lateral view. Scale bar: 0.2 mm.

**Diagnosis.** Male. Ocellar triangle isosceles (Fig. 4A). Abdomen black, without any red markings (Fig. 3C). Constriction of elongated metafemur is located in anterior half (Fig. 4D). Metatibia with characteristic curvature in posterior half, also well noticeable in female. Ventral margin of hypandrium with oval medial protuberance (Fig. 5C: vp). Anterior lobe of surstylus with a single row of pilosity (Fig 5E).

Description. Male. Body length (excluding antennae): 12 mm; wing length: 8 mm. Head. Eyes slightly dichoptic, separated by width of two ommatidia (Fig. 4A); covered in long dense white pilosity. Eye margins in anterior view almost parallel, slightly broadening ventrally. Face completely black pilose, gently pollinose, with distinctive thin line of silvery-white pollinosity in middle, slightly narrower in upper part. Frons, vertical triangle and occiput black; silvery-white pollinosity well expressed along eye margin on frons, on vertex anteriorly and dorsally on occiput behind eye margin, on the posterior margin as a patch, but most distinctive laterally. Ocellar triangle isosceles and predominantly black pilose, becoming intermixed with white pile in front of the ocellar triangle and turning predominantly white behind it. Vertical triangle and occiput with metallic blue reflection. Distance from anterior to posterior ocellus same as distance from latter one to upper eye corner. Lower facial margin in lateral view not protruding (Fig. 4B). In lateral view, white pilosity on eyes make contrast to black long pile on face, reaching one third to half of their length. Scape and pedicel dark colored, almost black. Basoflagellomere lacking in the holotype. Ventral pile of pedicel black, longer than its depth. Thorax. Scutum and scutellum densely punctate, covered in long dense white pilosity. Scutum with pair of white vittae of pollinosity, along almost two thirds of scutum length. Scutellum and lateral area of scutum with metallic blue tinge. Pleurae black. Anepisternum predominantly covered in long white pilosity, except behind anterior spiracle with patch of black pilosity. Katepisternum, anepimeron and katepimeron black pilose. Wing. Hyaline with pterostigma the same color as wing. Vein R4+5 slightly curved. Wing covered in microtrichia except for basal cells mostly bare. Costal setae black. Halter blackish. Legs. Metaleg slender with all segments very elongated (Fig. 4D). Femora black with yellowish tips covered in black



**Figure 7.** *Eumerus tenuitarsis* sp. nov., female **A** head, dorsal view **B** head, lateral view **C** metaleg, lateral view. Scale bar: 0.5 mm.

pilosity; metafemur very narrow in apical half with only few scattered inconspicuous setae. Tibiae white pilose. Pro- and mesotibia mostly black, yellowish in basal third and with yellowish apices; metatibia in the basal half yellowish, the apical half black turning lighter towards apex. Metatibia with characteristic curvature in posterior half (Fig. 4D). Tarsi brown to black; metatarsus longer than tibia. Abdomen. Black, punctate, pilose, tapered (Figure 3C; 4C). Terga 2–3 with pairs of wide white pollinose maculae, slightly obscured towards medial part of terga; tergum 4 with pair of white pollinose maculae with apices upwards. Tergum 2 with long white pile laterally; pilosity adpressed, in area of pollinose maculae white, black on rest of terga. Punctures are visible through pollinose maculae. Sterna entirely black pilose; sternum 4 flat with uniformly long pilosity (Fig. 5H). Terminalia. (Fig. 5C, E, F). Posterior surstyle lobe simple, beaklike in lateral view, with long strong setae laterally on outer surface (Fig. 5E: ps); in ventral view, outer margin slightly convex, pilose (Fig. 5F: om). Cerci oval (Fig. 5E: c), uniformly pilose. Interior accessory lobe of surstyle lobe densely pilose (Fig. 5F: in). Hypandrium curved, broad with folded thecal ridge near base (Fig. 5C: th); medially with oval protuberance on ventral margin (Fig. 5C: vp) and wide notch dorsally near base (Fig. 5C: dn). Female. Body length (excluding antennae): 11 mm; wing length: 7 mm. Similar to male except normal sexual dimorphism and for following characteristics: Head. White pilose except on ocellar triangle, with black pilosity. Basoflagellomere oval, dark, reddish anteriorly, with three radial wrinkles (Fig. 7B). Width of the frons in narrower part is narrower than one fourth of width of head in anterior view. (Fig. 7A). Thorax. Bluish sheen not noticeable. White pollinose vittae present along almost entire length of scutum. Pleurae white pilose. Segments of metaleg only slightly elongated. Metatibia with characteristic curvature (Fig. 7C). Abdomen. Tergum 4 with longer white pile posteriorly. Sterna black pilose except sternum 4 which is covered in white pile.



Figure 8. Current known distribution of the species of the *Eumerus binominatus* subgroup. Black dots stand for *Eumerus grallator* sp. nov., black triangle for *Eumerus longitarsis* Peck, 1979 Far East record only, black squares for *E. tenuitarsis* sp. nov. and grey area inferred distribution of Middle Asian species of the *Eumerus binominatus* subgroup, including *E. binominatus*, *E. longitarsis* and *E. tadzhikorum*.

**Etymology.** The species name is derived from the Latin words "tenui" and "tarsus" and refers to the extremely long and slender tarsi, especially obvious in the male metalegs.

**Distribution.** Only known from the holotype and female paratype taken on Lesvos and Evros (Greece) respectively.

# Identification key for the males of the Eumerus binominatus subgroup

Females of the subgroup are very difficult to distinguish morphologically. We have species with red abdomen from middle Asia which can be *E. binominatus* or *E. tadzhikorum* and black species which are in Spain *E. grallator*, in Greece *E. tenuitarsis* and in Middle Asia and Asia minor *E. longitarsis*. The distribution of *E. tenuitarsis* and *E. longitarsis* is not likely to be overlapping in Asia Minor and confusion between the female specimens of these species is not an issue.

2	Segments of metaleg extremely elongated, especially metatarsus as long as or
	longer than metafemur (Fig. 4D–F)binominatus subgroup, 3
_	Segments of metaleg not extremely elongated, metatarsus about 2/3-3/4 of
	length of metafemur
	other <i>Eumerus</i> species from <i>tricolor</i> group (not treated here)
3	Terga with red markings laterally (Fig. 1). Eyes separated for a large distance.
	Face white pilose. Wing with a dark spot4
_	Terga completely black (Fig. 3). Eyes touching in one point or very slightly
	separated by distance of few ommatidia. Face black pilose. Wing without a
	dark spot5
4	Basoflagellomere brown-red and slightly higher than long (Fig. 2B)
	<i>E. binominatus</i> Hervé-Bazin
_	Basoflagellomere black, as high as long (Fig. 2D) E. tadzhikorum Stackelberg
5	Greatest width of metafemur is approximately equal to one fifth of length of
	metafemur (Fig. 4E, F). Metatibia noticeably shorter than metafemur6
_	Greatest width of metafemur is approximately equal to one eighth of length of
	metafemur (Fig. 4D). Metatibia about same length or very slightly shorter than
	metafemur. Metatibia with characteristic curvature E. tenuitarsis sp. nov.
6	Metatarsus noticeably longer than metatibia and metafemur (Fig. 4F)
_	Metatarsus approximately the equally long as metatibia and metafemur (Fig.
	(F) E grallator sp. poy

# Discussion

The *Eumerus binominatus* subgroup is a group of long-legged species sharing all diagnostic characters with *E. tricolor* group (Chroni et al. 2017; Grković et al. 2017). The main characteristic for the species subgroup is the extremely thin and elongated legs which is an uncommon character for the family as a whole. The herein described species belonging to the *binominatus* subgroup share some characters with the two most similar species from the *tricolor* group – *Eumerus niveitibia* Becker, 1921 and *E. azabense* Ricarte & Marcos-García, 2018. Those characters are shape of abdomen and wide pollinose maculae on the terga, type of pilosity, shape of male epandrium and the presence of a folded thecal ridge on the hypandrium (Fig. 3C).

The remarkable long legs, especially conspicuous in males, could be behaviourally evolved or be part of a form of mimicry (Zimmer et al. 2003). In several hoverfly genera like *Platycheirus* and *Eumerus* (Dziock 2002; Reemer et al. 2009) the males use their legs in signalling for female attraction. In other genera the legs are used as part of the mimicry, like in *Spilomyia* which wave their prolegs in front of their head imitating the long antennae of Hymenoptera (van Steenis 2000; Penny et al. 2014) or in *Sphegina* which have their long metalegs hanging down in flight recalling sphecid wasps (Hippa et al. 2015). The remarkable long legs in the *binominatus* subgroup can have either of these functions and behavioural studies should clarify this in the future.

As shown here, the Turano-Mediterranean region represents a diversity center for the binominatus subgroup. The species of the binominatus subgroup are, however, not entirely restricted to this region given that we have one specimen of *E. longitaris* from the Russian Far East. This disjunct Turano-Mediterranean distribution is already recorded in several insect orders, in Acari and also in vipers (Ribera and Blasco-Zumeta 1998; Sanmartín 2003; Vujić et al. 2011; Ferchaud et al. 2012; García-Vázquez et al. 2016). It has also been discussed in the genus Eumerus within the bactrianus subgroup whose species exhibiting disjunct distribution between western and eastern Mediterranean regions extending to the Asiatic steppes to the east (Grković et al. 2019). Some authors discussed whether the Turano-Mediterranean distribution pattern in birds and beetles originate from dispersal or vicariant events (Voelker 1999; Sanmartín 2003). In the genus Eumerus it is unclear if the appearance of the geographic barrier occurred after the origin of the *binominatus* subgroup and thus would classify as vicariant event. The last ice age can be such an event separating the western and eastern Mediterranean and causing the speciation process forming E. grallator sp. nov. and E. tenuitarsis sp. nov. Further research including additional sampling in the Turanian area and DNA analysis could clarify this issue.

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RESEARCH ARTICLE



# Description of a new species of Music frogs (Anura, Ranidae, *Nidirana*) from Mt Dayao, southern China

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### Abstract

A new species of Music frogs, *Nidirana yaoica* **sp. nov.** is described based on a series of adult male specimens collected from Mt Dayao, Guangxi, southern China, providing valuable new information on the phylogeny, bioacoustics, and biogeography of related species within the genus *Nidirana*. The new species forms the sister taxon to *N. daunchina* from western China and together the sister taxon to *N. chapaensis* from northern Vietnam. *Nidirana yaoica* **sp. nov.** can be distinguished from all known congeners by a significant genetic divergence in the mitochondrial 16S and CO1 genes, the advertisement call containing 1–3 rapidly repeated regular notes, and the combination of morphological characteristics including a medium-sized body with SVL 40.4–45.9 mm in adult males; lateroventral grooves on every digit, not meeting at the tip of disk; tibio-tarsal articulation reaching the nostril; the presence of a pair of subgular vocal sacs in males; and one single developed nuptial pad on dorsal surface of first finger in males.

### Keywords

bioacoustic, Guangxi, mitochondrial DNA, morphology, Nidirana yaoica sp. nov.

# Introduction

The taxonomic treatment of the Music frog genus *Nidirana* Dubois, 1992 was controversial for a long time (Dubois 1987, 1992; Chen et al. 2005; Frost et al. 2006; Fei et al. 2009, 2010; Chuaynkern et al. 2010). The recent contribution to the phylogeny of genus *Nidirana* reconsidered it as a distinct genus, on the basis of comprehensive evidence of morphology, molecular phylogeny, bioacoustics, and biogeography (Lyu et al. 2017). Eight Music frog species were recognized from subtropical eastern and southeastern Asia (Lyu et al. 2017; Frost 2019): *N. okinavana* (Boettger, 1895) from Yaeyama of southern Ryukyu, and eastern Taiwan; *N. adenopleura* (Boulenger, 1909) from Taiwan and southeastern mainland China; *N. nankunensis* Lyu, Zeng, Wang, Lin, Liu & Wang, 2017 from Mt Nankun of Guangdong and *N. hainanensis* (Fei, Ye, & Jiang, 2007) from Mt Diaoluo of Hainan, both in southern China; *N. daunchina* (Chang, 1933) from western China; *N. pleuraden* (Boulenger, 1904) from southwestern China; and *N. chapaensis* (Bourret, 1937) and *N. lini* (Chou, 1999) from the northeastern Indochinese peninsula.

During our herpetological field surveys in Mt Dayao (MDY), Guangxi, south China, we collected a series of specimens of a small-sized frog that could be assigned to the genus *Nidirana* by possessing large suprabrachial gland in breeding males. Further detailed comprehensive analyses of molecules, bioacoustics, and morphology indicated that this frog was distinctive from all known congeners of *Nidirana*. Therefore, we propose it as a new species based on this study.

# Materials and methods

### Taxon sampling

Eight muscular samples of the unnamed species from MDY were used for molecular analysis. All samples were attained from euthanasia specimens and then preserved in 95% ethanol and stored at -40 °C. In addition, 36 sequences from all known *Nidirana* species and two sequences from the out-group *Babina* were obtained from GenBank and incorporated into our dataset. Detail information of these materials is shown in Table 1 and Fig. 1.

# DNA Extraction, PCR amplification, and sequencing

Genomic DNA were extracted from muscle tissue samples, using DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Two mitochondrion genes, namely partial 16S ribosomal RNA gene (16S) and partial cytochrome C oxidase 1 gene (CO1), were amplified. Primers used for 16S were L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3'), and L2A (5'-CCAAACGAGC-

ID	Species	Localities (* = type localities)	Voucher	165	CO1
1	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007009	MK882271	MK895036
2	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007011	MK882272	MK895037
3	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007012	MK882273	MK895038
4	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007013	MK882274	MK895039
5	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007014/CIB 110013	MK882275	MK895040
6	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007020	MK882276	MK895041
7	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007021	MK882277	MK895042
8	<i>Nidirana yaoica</i> sp. nov.	China: Guangxi: Mt Dayao *	SYS a007022	MK882278	MK895043
9	Nidirana adenopleura	China: Zhejiang: Jingning	SYS a002725	MF807827	MF807866
10	Nidirana adenopleura	China: Fujian: Nanping	SYS a005911	MF807844	MF807883
11	Nidirana adenopleura	China: Fujian: Mt Wuyi	SYS a005940	MF807851	MF807890
12	Nidirana adenopleura	China: Fujian: Mt Wuyi	SYS a005941	MF807852	MF807891
13	Nidirana adenopleura	China: Fujian: Mt Wuyi	XM2827	KF771281	/
14	Nidirana adenopleura	China: Taiwan: New Taipei	UMMZ 189963	DQ283117	/
15	Nidirana adenopleura	Not given	NMNS 2384	AF458118	/
16	Nidirana adenopleura	Not given	A-A-WZ001	NC018771	NC018771
17	Nidirana chapaensis	Vietnam: Lao Cai: Sapa *	ROM 28070	AF206460	/
18	Nidirana chapaensis	Vietnam: Lao Cai: Sapa *	1999.5871	KR827710	/
19	Nidirana chapaensis	Vietnam: Lao Cai: Sapa *	T2483/2000.4850	KR827711	KR087625
20	Nidirana chapaensis	Vietnam: Gia Lai	AMSR176027	KU840598	/
21	Nidirana daunchina	China: Sichuan: Mt Emei *	0609	KU840597	/
22	Nidirana daunchina	China: Sichuan: Mt Emei *	CIB-WU37990	DQ359988	/
23	Nidirana daunchina	China: Sichuan: Mt Emei *	HNNU 20060103	KF185065	/
<b>2</b> 4	Nidirana daunchina	China: Sichuan: Mt Emei *	SYS a004594	MF807822	MF807861
25	Nidirana daunchina	China: Sichuan: Mt Emei *	SYS a004595	MF807823	MF807862
<b>2</b> 6	Nidirana daunchina	China: Sichuan: Hejiang	SYS a004930	MF807824	MF807863
27	Nidirana daunchina	China: Sichuan: Hejiang	SYS a004931	MF807825	MF807864
28	Nidirana daunchina	China: Sichuan: Hejiang	SYS a004932	MF807826	MF807865
29	Nidirana daunchina	Not given	Not given	/	HQ395353
30	Nidirana hainanensis	China: Hainan: Mt Diaoluo *	SYS a003741	MF807821	MF807860
31	Nidirana hainanensis	China: Hainan	Not given	KU840596	/
32	Nidirana lini	China: Yunnan: Jiangcheng *	SYS a003967	MF807818	MF807857
33	Nidirana lini	China: Yunnan: Jiangcheng *	SYS a003968	MF807819	MF807858
34	Nidirana lini	China: Yunnan: Jiangcheng *	SYS a003969	MF807820	MF807859
35	Nidirana lini	China: Yunnan: Lyuchun	HNNULC001	KF185066	/
36	Nidirana lini	Laos: Xieng Khouang	FMNH256531	KR264073	/
37	Nidirana lini	Laos: Xieng Khouang	FMNH256532	KR264074	/
38	Nidirana lini	Not given	Not given	/	HQ395352
39	Nidirana nankunensis	China: Guangdong: Mt Nankun *	SYS a005717	MF807838	MF807877
40	Nidirana nankunensis	China: Guangdong: Mt Nankun *	SYS a005718	MF807839	MF807878
41	Nidirana nankunensis	China: Guangdong: Mt Nankun *	SYS a005719	MF807840	MF807879
42	Nidirana okinavana	Japan: Okinawa: Iriomote Island *	Not given	NC022872	NC022872
43	Nidirana pleuraden	China: Yunnan: Mt Gaoligong	SYS a003775	MF807816	MF807855
44	Nıdirana pleuraden	China: Yunnan: Mt Gaoligong	SYS a003776	MF807817	MF807856
45	Babina holsti	Japan: Okinawa *	Not given	NC022870	NC022870
46	Babina subaspera	Japan: Kagoshima: Amami Island *	Not given	NC022871	NC022871

Table 1. Localities, voucher information, and GenBank numbers for all samples used in this study.



Figure 1. Localities of the samples used in this study. The numbers correspond to the ID numbers in Table 1.

CTAGTGATAGCTGGTT-3') and H10 (5'-TGATTACGCTACCTTTGCACG-GT-3'), and for CO1 were Chmf4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATCA-3'), and dgLCO (5'-GGT-CAACAAATCATAAAGAYATYGG-3') and dgHCO (5'-AAACTTCAGGGTGAC-CAAARAAYCA-3'), following Lyu et al. (2019). PCR amplifications were processed with the cycling conditions that initial denaturing step at 95 °C for 4 min, 35 cycles of denaturing at 94 °C for 40 s, annealing at 53 °C (for 16S) / 48 °C (for CO1) for 40 s and extending at 72 °C for 60 s, and a final extending step at 72 °C for 10 min. PCR products were purified with spin columns and then sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit per the guidelines, on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co, Ltd. All sequences were deposited in GenBank (Table 1).

## **Phylogenetic analyses**

DNA sequences were aligned by the Clustal W algorithm with default parameters (Thompson et al. 1997) and trimmed with the gaps partially deleted in MEGA 6 (Tamura et al. 2013). Two gene segments, 1041 base pairs (bp) of 16S and 573 bp of CO1, were concatenated seriatim into a 1614-bp sequence, and further divided into four partitions by codons. The partitions were tested in jmodeltest v2.1.2 with Akaike and Bayesian information criteria, all resulting the best-fitting nucleotide substitution models of GTR+I+G. Sequenced data was analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012), and maximum likelihood (ML) in RaxmlGUI 1.3 (Silvestro and Michalak 2012). Two independent runs were conducted in a BI analysis, each of which was performed for 10,000,000 generations and sampled every 1000 generations with the first 25% samples were discarded as burn-in, resulting a

potential scale reduction factor (PSRF) of < 0.005. In ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed.

### **Bioacoustic analysis**

Advertisement calls of the specimen SYS a007009 from MDY were recorded in the field at the air temperature of 18 °C using a SONY PCM D100 digital sound recorder. The sound files in wave format were sampled at 44.1 kHz with 24 bits in depth. Praat 6.0.27 (Boersma 2001) was used to obtain the oscillogram, sonogram, and power spectrum (window length = 0.005 s). Raven pro 1.5 (Cornell Lab of Ornithology, 2003–2014) was used to quantify the acoustic properties (window size = 256 points, fast Fourier transform, Hanning window with no overlap). The following measurements were taken for each call: call duration (the time between onset of the first note and offset of the last note in a call) and call PF (peak frequency; the frequency at which max power occurs within the call); the following measurements were taken for each and offset of a note), note rise time (the time between onset and max amplitude of a note), note interval (the time between adjacent notes in a call), note PF and note IQR-BW (inter-quartile range bandwidth; the difference between the first and third quartile frequencies within a note). Mean and standard deviation (SD) were calculated in R 3.3.2 (R Core Team 2016).

# Morphology

Comparison characters of all known congeners were obtained from the literature (Boettger 1895; Boulenger 1904, 1909; Schmidt 1925; Chang and Hsu 1932; Bourret 1937; Kuramoto 1985; Chou 1999; Fei et al. 2007, 2009; Matsui 2007; Chuaynkern et al. 2010; Lyu et al. 2017) and 55 examined museum specimens of six species which are listed in the Appendix 1. All specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol, and deposited in the Museum of Biology, Sun Yatsen University (**SYS**), Natural History Museum of Guangxi (**NHMG**), and Chengdu Institute of Biology, Chinese Academy of Sciences (**CIB**), China.

Morphological descriptions follow the consistent definition by Fei et al. (2009), Chuaynkern et al. (2010) and Lyu et al. (2017). External measurements were made for the unnamed specimens with digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm. Mean and standard deviation (SD) were calculated in R 3.3.2 (R Core Team 2016). These measurements were as follows:

**SVL** snout-vent length (from tip of snout to posterior margin of vent);

**HDL** head length (from tip of snout to the articulation of the jaw);

HDW head width (head width at the commissure of the jaws);

- **SNT** snout length (from tip of snout to the anterior corner of the eye);
- **IND** internasal distance (distance between nares);
- **IOD** interorbital distance (minimum distance between upper eyelids);
- **ED** eye diameter (from the anterior corner of the eye to posterior corner of the eye);
- TD tympanum diameter (horizontal diameter of tympanum);
- **TED** tympanum-eye distance (from anterior edge of tympanum to posterior corner of the eye);
- **HND** hand length (from the proximal border of the outer palmar tubercle to the tip of digit III);
- **RAD** radio-ulna length (from the flexed elbow to the proximal border of the outer palmar tubercle);
- FTL foot length (from distal end of shank to the tip of digit IV);
- **TIB** tibial length (from the outer surface of the flexed knee to the heel).

Sex and age were determined by secondary sexual characters, i.e., the presence of suprabrachial glands in males. Webbing formula was written according to Savage (1975).

## Results

The ML and BI analyses resulted in essentially identical topologies and were integrated in Fig. 2, in which the major nodes were sufficiently supported with the Bayesian posterior probabilities (BPP) > 0.95 and the bootstrap supports (BS) for maximum likelihood analysis > 70. This mitochondrial result is consistent with the phylogenic relationship in Lyu et al. (2017). The *Nidirana* specimens from MDY, southern China, grouped in a clade with strong supported values and small divergences, forming a sister taxon to *N. daunchina* from western China, then together forming the sister taxon to *N. chapaensis* from northern Vietnam.

Morphologically, the specimens from MDY significantly differ from the recognized congeners by the following characteristics: (1) medium-sized body, SVL 40.4– 45.9 mm in adult males vs. 33.3–37.1 mm in *N. nankunensis*; (2) finger IV longer than finger I vs. equal in *N. chapaensis*; (3) presence of lateroventral groove on every digit vs. absent on fingers and toes in *N. pleuraden*; absent or barely visible on fingers in *N. daunchina*; absent on finger I in *N. chapaensis*, *N. lini*, *N. nankunensis*, *N. adenopleura*, and *N. okinavana*; (4) tibio-tarsal articulation reaches the nostril vs. beyond the snout tip in *N. lini*; (5) the presence of a single nuptial pad vs. absent in *N. hainanensis*; divided into two parts in *N. chapaensis*; (6) the presence of a pair of subgular vocal sacs vs. absent in *N. okinavana*; (7) the absence of spinules on dorsal skin vs. present in *N. adenopleura*, *N. lini* and *N. pleuraden*. Detail comparison between the specimens from MDY and its congeners is listed in Table 2 with the characteristics item by item.

Further, the advertisement call from the frogs from MDY is different from the congeners by: (1) containing 1–3 fast-repeated identical regular notes (vs. containing



**Figure 2.** Bayesian inference and maximum-likelihood phylogenies. Number in parenthesis corresponds to the ID number in Table 1.

2–4 fast-repeated double-notes in *N. hainanensis*; containing a significantly different first note in *N. daunchina* and *N. nankunensis*); (2) the call notes last 30-54 ms vs. call notes last 115-252 ms in *N. adenopleura*; the first notes last 108-135 ms in *N. nankunensis*; the first notes last 162-197 ms and the others last 131-150 ms in *N. daunchina*; (3) the intervals between notes last 212-372 ms vs. last 98-213 ms in *N. adenopleura*; last 12-166 ms in *N. nankunensis*.

Therefore, based on the molecular, morphological, and bioacoustic differences, the specimens from MDY, southern China, represent an unnamed species which is described as a new species of genus *Nidirana*.

Characteristics	N. yaoica	N. daunchina	N. chapaensis	N. hainanensis	N. adenopleura	N. nankunensis	N. okinavana	N. lini	N. pleuraden
SVL of males	40.4-45.9	40.6-51.0	35.5-42.5	32.8-44.4	43.1–57.6	33.3-37.1	35.5-42.8	44.1 - 63.1	45.4-58.7
SVL of females	/	44.0-53.0	41.0-51.8	۸.	47.6-60.7	37.8–39.5	44.6-48.8	57.7-68.6	45.5-62.5
Body habitus	Stocky	Stocky	Stocky	Stocky	Elongated	Stocky	Stocky	Elongated	Elongated
Fingers tips	Dilated	Dilated	Dilated	Dilated	Dilated	Dilated	Dilated	Dilated	Not dilated
Lateroventral	Present	Absent or rarely	Present except	Present	Present except	Present except	Present except	Present except	Absent
groove on fingers		present	finger I		finger I	finger I	finger I	finger I	
Relative length of fingers	II < I < IV < III	II < I < IV < III	II < I = IV < III	II < I < IV < III	II < I < IV < III	II < I < IV < III	II < I < IV < III	II < I < IV < III	II < I < IV < III
Toes tips	Dilated	Dilated	Dilated	Dilated	Dilated	Dilated	Dilated	Dilated	Not dilated
Lateroventral	Present	Present	Present	Present	Present	Present	Present	Present	Absent
groove on toes									
Relative length of toes	I < II < V < III < V	I < II < V < III < IV	I < II < V < III < IV	I < II < V < III < V	I < II < V < III < IV	I < II < V < III < V	I < II < V < III < V	I < II < V < III < V	I < II < V < III < IV
Tibio-tarsal articulation	Nostril	Nostril	Nostril	Nostril	Snout tip or eye- snout	Nostril	Eye center-near nostril	Beyond snout	Eye-snout
Subgular vocal sacs	Present	Present	Present	Present	Present	Present	Absent	Present	Present
Nuptial pad	One	One	Two	Absent	One	One	Poorly one	One	One
Spinules on dorsal skin	Absent	Absent	Absent or few above vent	Absent	Entire or posterior dorsal skin	Absent or few above vent	Absent	Posterior dorsal skin	Posterior dorsal skin
Nest construction	<b>~</b> .	Present	Present	Present	Absent	Present	Present	Absent	Absent
Tadpole labial tooth row formula	<u>~</u> .	1:1+1/1+1:2 or 1:1+1/2+2:1	1:1+2/1+1:2	<u>~-</u>	1:1+1/1+1:2 or 1:0+0/1+1:1	1:1+1/1+1:2	1:1+1/1+1:2	1:1+1/1+1:2	1:1+1/1+1:2 or 1:1+1/2+2:1
Calling	1–3 fast-repeated notes	2–5 notes containing a specific first note	3 notes	2–4 fast-repeated double-notes	2-4 notes	13–15 fast-repeated notes containing a specific first note	10–25 fast-repeated notes	5-7 notes	4–7 notes
Cites	This study	Liu (1950); Fei et al. (2009); Lyu et al. (2017)	Chuaynkern et al. (2010)	Fei er al. (2009, 2012); Lyu er al. (2017)	Pope (1931); Chuaynkern et al. (2010); Lyu et al. (2017)	Lyu et al. (2017)	Matsui and Utsunomiya (1983); Chuaynkem et al. (2010); Lyu et al.	Chou (1999); Fei et al. (2009); Lyu et al. (2017)	Fei et al. (2009); Lyu et al. (2017)

Table 2. Diagnostic characters separating Nidinana yaoica sp. nov. from congeners.

### Nidirana yaoica sp. nov.

http://zoobank.org/D05423B2-1812-4AF4-890C-A0A1915BD8A6

**Chresonymy.** *Nidirana adenopleura*: Fei et al. 2009 (Mt. Dayao, Jinxiu, Guangxi); Mo et al. 2014 (Jinxiu, Guangxi)

Holotype. SYS a007022 (Fig. 3), adult male, collected by Zhi-Tong Lyu on 1 June 2018 from Mt Dayao (24.1602N, 110.2304E; ca 1190 m a.s.l.), Jinxiu Yao Autonomous County, Guangxi Zhuang Autonomous Region, China.

**Paratypes.** SYS a007009, 7011–7013, 7020–7021, SYS a007014/CIB 110013, seven adult males collected by Zhi-Tong Lyu, Yu-Long Li and Cheng-Yu Yang on 30 May–1 June 2018 from the same locality as the holotype. NHMG 1503043–47, five adult males collected by Yun-Ming Mo and Wei-Cai Chen on 19 March 2015 from the neighboring locality as the holotype (24.1035N,110.2294E; ca 1350 m a.s.l.).

**Etymology.** The specific name *yaoica* is an adjective derived from Yao, referring to the type locality of the new species, Mt Dayao in Jinxiu Yao Autonomous County, where the settlement of the Yao people is located. We suggest its English common name to be Mt Dayao music frog and its Chinese name Yao Qin Wa (瑶琴蛙).

**Differential diagnosis.** *Nidirana yaoica* sp. nov. is distinguished from its congeners by the following combination of the morphological characteristics: (1) body mediumsize and stocky, with SVL  $43.8 \pm 1.7$  (40.4-45.9, n = 13) mm in adult males; (2) disks of digits dilated, pointed; (3) lateroventral grooves present on every digit; (4) heels overlapping; (5) tibio-tarsal articulation reaching at the nostril; (6) mid-dorsal stripe present; (7) posterior of dorsal skin rough with dense tubercles but without spinules; (8) week supernumerary tubercles below the base of fingers III and IV, palmar tubercles prominent and distinct; (9) a pair of subgular vocal sacs present; (10) one single nuptial pad present on the first finger, nuptial spinules invisible; (11) suprabrachial gland large; (12) calling: 1–3 fast-repeated regular notes.

**Description of holotype.** Adult male. Body stocky, SVL 44.6 mm; head longer than wide (HDW/HDL 0.92), flat above; snout rounded in dorsal and lateral views, slightly protruding beyond lower jaw, longer than horizontal diameter of eye (SNT/ ED 1.26); canthus rostralis distinct, loreal region concave; nostril round, directed laterally, closer to the snout than to the eye; a longitudinal swollen mandibular ridge extending from below nostril through lower edges, eye and tympanum to above insertion of arm, where the ridge is intermittent, forming a maxillary gland and shoulder gland; supratympanic fold absent; interorbital space flat, narrower than internasal distance (IND/IOD 1.37); pupil elliptical, horizontal; tympanum distinct, round, TD/ED 0.72, and close to eye, TED/TD 0.38; pineal ocellus present; vomerine ridge present, bearing small teeth; tongue large, cordiform, notched behind.

Forelimbs moderately robust, lower arm 19% of SVL and hand 27% of SVL; fingers thin, relative finger lengths II < I < IV < III; tip of each finger slightly dilated and remarkable elongated, forming long pointed disks; well-developed lateroventral



**Figure 3.** Morphological features of the adult male holotype SYS a007022 of *Nidirana yaoica* sp. nov. in life. **A** dorsolateral view **B** ventral view **C** left hand **D** poorly developed nuptial pad **E** left foot **F** surface of posterior dorsum and hind limbs.

grooves on all fingers, not meeting at the tip of disks; fingers free of webbing; presence of weak lateral fringes on inner and outer sides of fingers II, III and IV, and on outer side of finger I; subarticular tubercles prominent and rounded; week supernumerary tubercles below the base of fingers III and IV; three elliptic, large, prominent and very distinct palmar tubercles.

Hindlimbs relatively robust, tibia 53% of SVL and foot 78% of SVL; heels overlapping when hindlimbs flexed at right angles to axis of body; tibio-tarsal articulation reaching the nostril when hindlimb is stretched along the side of the body; toes relatively long and thin, relative lengths I < II < V < III < IV; tip of each toe slightly dilated with remarkable elongated ventral callous pad, forming long and pointed disk; well-developed lateroventral grooves on toes, not meeting at the tip of disks; webbing moderate, webbing formula: I 2 - 2½ II 1⅔ - 3 III 2⅓ - 3½ IV 3½ - 2 V; presence of lateral fringes on inner and outer sides of each toes, forming distinct dermal flap on the lateral edges of toes I and V; subarticular tubercles rounded, prominent; inner metatarsal tubercle elliptic, twice as long its width; outer metatarsal tubercle indistinct, small and rounded; tarsal folds and tarsal tubercle absent.

Dorsal skin of head and anterior body smooth, posterior dorsum of body rough with dense tubercles but not bearing horny spinules; developed dorsolateral fold from posterior margin of upper eyelid to above groin but intermittent posteriorly; flank relatively smooth with dense tubercles on region nearly the dorsolateral fold; a large and smooth suprabrachial gland behind base of forelimb, slightly prominent; dorsal surface of upper arm with two longitudinal ridges and slightly extending to lower arm; the dorsal surfaces of thigh and tibia with several longitudinal ridges and tubercles bearing spinules. Ventral surface of head, body, and limbs smooth; large flattened tubercles densely arranged on the rear of thigh and around vent.

**Color in life of holotype.** Dorsal surface of head and body reddish brown; pineal ocellus yellowish; a longitudinal reddish brown mid-dorsal stripe edged with broad dark brown, beginning from snout, across pineal ocellus, posteriorly extending to vent; several black spots on upper eyelids and posterior dorsum of body; dorsolateral fold bicolor, upper part reddish brown and lower part black; upper flank yellowish brown with irregular black spots; lower flank yellowish white; suprabrachial gland yellowish brown. Dorsal forelimbs reddish brown; a longitudinal black stripe on the anterior surface of the forelimb; irregular black marks on dorsal surface of the forelimb; dorsal hindlimbs nonuniform dark brown, four black crossbars on the thigh, three on the tibia and three on the tarsus; irregular black marks on dorsal toes. Loreal and temporal regions black, tympanum dark brown; upper <sup>1</sup>/<sub>3</sub> iris bright brownish white and lower <sup>2</sup>/<sub>3</sub> iris reddish brown; maxillary gland and shoulder gland yellowish white. Lips and throat grey white, but two subgular vocal sacs slightly dark colored; ventral surface of body and limbs creamy white; rear thigh tinged with pink; ventral hand and foot pale white with large black patches.

**Color in preservative of holotype.** Dorsal surface faded, but dark brown edges of the mid-dorsal stripe more distinct; black spots on dorsum more distinct; upper flank black; limbs faded, the crossbars clearer; ventral surface faded, throat and posterior of chest with smoky gray markings.

**Variations.** Measurements of type series are given in Table 3. All specimens were similar in morphology. Dorsal surface light brown in SYS a007009 (Fig. 4A), 7011, 7013 and 7020; mid-dorsal stripe begins from pineal ocellus in SYS a007011, 7013, 7014, 7020 and 7021 (Fig. 4B), unclear in SYS a007009; pineal ocellus invisible in SYS a007009.

**Male secondary sexual characteristics.** A pair of subgular vocal sacs, a pair of slitlike openings at posterior of jaw; a single light brown nuptial pad on the dorsal surface of first finger, nuptial spinules invisible; suprabrachial gland present.

**Distribution and ecology.** Currently, *Nidirana yaoica* sp. nov. is known only from the type locality, Mt Dayao, Jinxiu, Guangxi, in southern China. This frog inhabits in

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Snecimens	SAS	SAS	SAS	SAS	SVS	SAS	SAS	SAS	NHMG	NHMG	NHMG	NHMG	NHMG	summarizing of
No.	a007022	a007009	a007011	a007012	a007013	a007014/	a007020	a007021	1503043	1503044	1503045	1503046	1503047	measurement (minimum-
	(holotype)					CIB 110013								maximum, mean ± SD)
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Males
SVL	44.6	42.1	44.1	44.9	43.2	45.5	45.6	44.6	42.6	40.4	43.9	42.5	45.9	$40.4-45.9, 43.8 \pm 1.7$
HDL	17.6	16.4	18.2	16.8	16.3	18.6	17.5	16.9	16.2	15.7	16.2	16.3	17.7	$15.7 - 18.6, 16.9 \pm 0.9$
MDW	16.2	15.3	16.4	16.2	15.0	16.7	16.3	16.1	16.0	15.6	15.7	15.7	17.2	$15.0-17.2, 16.0 \pm 0.6$
SNT	6.8	6.2	6.8	6.7	6.9	7.2	6.2	7.0	7.5	7.7	7.7	7.7	8.7	$6.2-8.7, 7.2 \pm 0.7$
IND	5.6	5.9	5.8	6.0	5.8	5.4	5.5	5.6	6.0	6.2	6.3	6.0	6.6	$5.4-6.6, 5.9 \pm 0.3$
IOD	4.1	4.3	4.9	4.7	5.1	4.8	4.4	4.5	4.2	3.8	3.5	3.5	4.2	$3.5-5.1, 4.3 \pm 0.5$
ED	5.4	5.1	5.2	4.7	4.6	5.4	5.4	5.0	5.3	5.1	5.2	5.1	5.0	$4.6-5.4, 5.1 \pm 0.2$
QT.	3.9	3.2	3.4	3.7	3.9	3.9	3.6	3.9	4.1	4.1	4.2	4.1	4.5	$3.2-4.5, 3.9 \pm 0.4$
TED	1.5	1.3	1.0	1.3	1.2	1.2	1.6	1.2	1.1	1.1	1.1	1.2	1.6	$1.0{-}1.6, 1.2 \pm 0.2$
<b>UNH</b>	12.0	10.3	11.1	10.4	10.3	11.5	10.9	12.4	10.4	10.9	12.0	10.2	12.8	$10.2-12.8, 11.1 \pm 0.9$
RAD	8.4	8.8	9.4	8.6	8.4	8.6	8.6	8.7	8.2	8.0	7.8	8.3	8.4	$7.8-9.4, 8.5 \pm 0.4$
FTL	34.9	33.1	34.3	34.6	34.3	35.0	33.7	35.7	32.3	3.1	32.9	32.3	35.6	$3.1-35.7, 31.4 \pm 9.0$
TIB	23.6	23.5	23.0	22.6	23.2	23.9	23.5	23.4	22.0	21.6	22.9	22.4	25.6	$21.6-25.6, 23.1 \pm 1.0$
HDL/SVL	0.39	0.39	0.41	0.37	0.38	0.41	0.38	0.38	0.38	0.39	0.37	0.38	0.39	$0.37 - 0.41, 0.39 \pm 0.01$
HDW/SVL	0.36	0.36	0.37	0.36	0.35	0.37	0.36	0.36	0.38	0.39	0.36	0.37	0.37	$0.35-0.39, 0.37 \pm 0.01$
HDW/HDL	0.92	0.94	06.0	0.96	0.92	0.89	0.93	0.95	0.99	66.0	0.97	0.96	0.97	$0.89 - 0.99, 0.95 \pm 0.03$
<b>SNT/HDL</b>	0.39	0.38	0.38	0.40	0.42	0.39	0.35	0.41	0.46	0.49	0.48	0.47	0.49	$0.35-0.49, 0.43 \pm 0.05$
<b>SNT/SVL</b>	0.15	0.15	0.15	0.15	0.16	0.16	0.14	0.16	0.18	0.19	0.18	0.18	0.19	$0.14-0.19, 0.16 \pm 0.02$
WDH/DNI	0.35	0.38	0.36	0.37	0.38	0.32	0.34	0.35	0.38	0.40	0.40	0.38	0.38	$0.32-0.40, 0.37 \pm 0.02$
IOD/HDW	0.25	0.28	0.30	0.29	0.34	0.29	0.27	0.28	0.26	0.24	0.22	0.22	0.24	$0.22 - 0.34, 0.27 \pm 0.03$
ED/HDL	0.31	0.31	0.28	0.28	0.28	0.29	0.31	0.30	0.33	0.32	0.32	0.31	0.28	$0.28-0.33, 0.30 \pm 0.02$
ED/SVL	0.12	0.12	0.12	0.10	0.11	0.12	0.12	0.11	0.12	0.13	0.12	0.12	0.11	$0.10-0.13, 0.12 \pm 0.01$
TD/ED	0.72	0.62	0.66	0.79	0.85	0.73	0.67	0.78	0.77	0.80	0.81	0.80	06.0	$0.62-0.90, 0.77 \pm 0.08$
TED/TD	0.38	0.42	0.29	0.35	0.31	0.29	0.43	0.31	0.27	0.27	0.26	0.29	0.36	$0.26-0.43, 0.32 \pm 0.06$
HND/SVL	0.27	0.24	0.25	0.23	0.24	0.25	0.24	0.28	0.24	0.27	0.27	0.24	0.28	$0.23-0.28, 0.25 \pm 0.02$
RAD/SVL	0.19	0.21	0.21	0.19	0.19	0.19	0.19	0.20	0.19	0.20	0.18	0.20	0.18	$0.18-0.21, 0.19 \pm 0.01$
FTL/SVL	0.78	0.79	0.78	0.77	0.79	0.77	0.74	0.80	0.76	0.08	0.75	0.76	0.78	$0.08-0.80, 0.71 \pm 0.20$
TIB/SVL	0.53	0.56	0.52	0.50	0.54	0.52	0.52	0.52	0.52	0.53	0.52	0.53	0.56	$0.50-0.56, 0.53 \pm 0.02$



**Figure 4. A, B** paratypes SYS a007009 and SYS a007021 of *Nidirana yaoica* sp. nov. **C** habitat of *Nidirana yaoica* sp. nov. in the type locality in Mt Dayao **D** the holotype SYS a007022 in wild.

the swamps and ponds surrounded by moist subtropical secondary evergreen broadleaved forests (Fig. 4C, D). The adult male calls in the brushwood at the bank, from mid-March to late May. Nevertheless, the females, tadpoles, and much of the ecology and behavior of this species remain unknown.

**Vocalization.** The call spectrograms are shown in Fig. 5 and the measurement parameters are listed in Table 4. The advertisement call (n = 87) of *Nidirana yaoica* sp. nov. contains 1–3 rapidly repeated, identical, regular notes with the PF of 516.8 Hz and note IQR-BW of 172.3 Hz or 0 generally. The one-note call (n = 25) has a duration of  $43.3 \pm 2.7$  ms with the rise time of  $10.1 \pm 4.5$  ms. The two-note call (n = 59)



**Figure 5.** Advertisement call spectrograms of *Nidirana yaoica* sp. nov. **A** one-note call **B** two-note call; **C** three-note call.

has a duration of 355.9  $\pm$  31.1 ms; the first note lasts 43.5  $\pm$  2.8 ms with the rise time of 8.5  $\pm$  4.6 ms, and the second lasts 39.6  $\pm$  3.3 ms with the rise time of 11.6  $\pm$  4.4 ms; the note interval last 272.8  $\pm$  31.7 ms.

	one-note call (n = 25)	two-note call (n = 59)	three-note call (n = 3)
Call duration (ms)	37–51, 43.3 ± 2.7	307-454, 355.9 ± 31.1	565-678, 628.0 ± 57.6
Note duration (ms)	$37-51, 43.3 \pm 2.7$	$1^{\text{st}}$ note: 36–51, 43.5 ± 2.8;	$1^{st}$ note: 42–54, 46.7 ± 6.4;
		$2^{nd}$ note: 30–49, 39.6 ± 3.3	$2^{nd}$ note: 37–40, 38.7 ± 1.5;
			$3^{rd}$ note: 35–52, 42.3 ± 8.7
Note rise time (ms)	$1.6-15.5, 10.1 \pm 4.5$	$1^{st}$ note: 2.0–16.0, 8.5 ± 4.6;	$1^{\text{st}}$ note: 3.7–13.7, 7.4 ± 5.5;
		$2^{nd}$ note: 1.7–17.9, 11.6 ± 4.4	$2^{nd}$ note: 13.1–15.8, 14.8 ± 1.5;
			$3^{rd}$ note: 14.0–16.1, 15.3 ± 1.1
Note interval (ms)	/	215–372, 272.8 ± 31.7	$1^{st}$ interval: 212–250, 234.0 ± 19.7;
			$2^{nd}$ interval: 222–302, 266.3 $\pm$ 40.7
Call PF (Hz)	516.8	516.8	516.8
Note PF (Hz)	516.8	1st note: 516.8 (98.3%) or 2584	1 <sup>st</sup> note: 516.8;
		(1.7%);	
		2 <sup>nd</sup> note: 516.8	2 <sup>nd</sup> note: 516.8;
			3 <sup>rd</sup> note: 516.8
Note IQR-BW (Hz)	172.3 (48.0%) or 0 (52.0%)	1st note: 344.5 (8.4%), 172.3	1 <sup>st</sup> note: 172.3 (33.3%) or 0 (66.6%);
		(45.8%) or 0 (45.8%);	
		2 <sup>nd</sup> note: 172.3 (54.2%) or 0	2 <sup>nd</sup> note: 172.3 (33.3%) or 0 (66.6%);
		(45.8%)	
			3 <sup>rd</sup> note: 172.3 (33.3%) or 0 (66.6%)

Table 4. Vocalization parameters of paratype SYS a007009 of Nidirana yaoica sp. nov.

### Discussion

The taxonomic status for the *Nidirana* population in MDY was suspected and suggested a further study by Fei et al. (2009), despite their work reported it as *N. adenopleura* tentatively which was followed by Mo et al. (2014). Currently this population is revealed as *N. yaoica* sp. nov. in present work. In morphology, this frog is similar to *N. hainanensis* by the presence of lateroventral groove on all digits, and further to *N. daunchina*, *N. chapaensis*, and *N. okinavana* by the absence of spinules on dorsal skin. Bioacoustically, *N. yaoica* sp. nov. has the same calling pattern as *N. adenopleura*, which contains several fast-repeated identical regular notes, but different from the pattern in *N. daunchina* and *N. hainanensis*. The phylogenetic tree showed that the new species is closer to *N. daunchina* with moderate supports (BPP 0.98 and BS 88), and then to *N. chapaensis* and *N. hainanensis*.

The genus *Nidirana* was recognized as a distinct genus recently based on comprehensive evidence by Lyu et al. (2017). For the interspecific relationship within the genus, Dubois (1992) constructed two morphological species groups: *N. pleuraden* group for *N. pleuraden* and *N. adenopleura* group for the other known species; Fei et al. (2009) proposed *N. daunchina* group for *N. daunchina* and *N. psaltes* Kuramoto, 1985 (= *N. okinavana*), and *N. adenopleura* group for *N. adenopleura*, *N. lini*, and *N. hainanensis*, but excluding *N. chapaensis*, and placing *N. pleuraden* in another genus *Pelophylax* Fitzinger, 1843; Chuaynkern et al. (2010) suggested three species groups for Music frogs based on the morphological characters and ecological behavior of nest construction: *N. pleuraden* group for *N. pleuraden*, *N. adenopleura* group for *N. adenopleura* and *N. lini*, and *N. okinavana* group for *N. daunchina*, *N. okinavana*, and *N. chapaensis*. From the current mitochondrial results (Lyu et al. 2017; this study), the *N. pleuraden* consistently formed the basal lineage of this genus, while the monophyly of the three species groups *N. adenopleura* group (Fei et al. 2009; Chuaynkern et al. 2010), *N. okinavana* group, and *N. daunchina* group, was challenged. The main conflicts are: (1) *N. okinavana* was suggested morphologically more similar to *N. daunchina* and *N. chapaensis* (Fei et al. 2009; Chuaynkern et al. 2010) while clustered with *N. adenopleura* in the phylogeny; (2) *N. hainanensis* was suggested morphologically more similar to *N. adenopleura* and *N. lini* (Fei et al. 2009) while clustered with *N. adenopleura* in the phylogeny; (3) *N. lini* was suggested morphologically more similar to *N. adenopleura* (Fei et al. 2009; Chuaynkern et al. 2010) while formed the basal lineage of the congeners except *N. pleuraden* in the phylogeny.

Thus we propose to follow Dubois's (1992) suggestion, regarding two species groups within the genus *Nidirana*: (1) *N. pleuraden* group, the lateroventral groove absent on fingers and toes: one species, *N. pleuraden*; (2) *N. adenopleura* group, the lateroventral groove present on toes, absent or present on fingers: eight species, *N. adenopleura*, *N. okinavana*, *N. nankunensis*, *N. hainanensis*, *N. chapaensis*, *N. daunchina*, *N. yaoica* sp. nov., and *N. lini*.

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# Appendix I

### Specimens examined

- Nidirana adenopleura (29): China: Fujian: Yanping District (type locality): SYS a005911–5916; Mt Wuyi: SYS a005939–5943; Jiangshi Nature Reserve: SYS a004112, 4132; Mt Yashu: SYS a005890–5891, 5901–5902; Jiangxi: Tongboshan Nature Reserve: SYS a001663–1665, 1667, 1698; Yangjifeng Nature Reserve: SYS a0000317, 0334; Jinggangshan Nature Reserve: SYS a004025–4027; Zhejiang: Jingning County: Dongkeng Town: SYS a002725–2726.
- *Nidirana daunchina* (5): **China: Sichuan:** Mt Emei (type locality): SYS a004594–4595; Hejiang County: Zihuai Town: SYS a004930–4932.
- Nidirana hainanensis (1): China: Hainan: Mt Diaoluo (type locality): SYS a003741.
- *Nidirana lini* (4): **China: Yunnan:** Jiangcheng County: Hongjiang Town (type locality): SYS a003967–3970.
- *Nidirana nankunensis* (12): **China: Guangdong:** Mt Nankun (type locality): SYS a003615, 3617–3620, 4019, 4905–4907, 5717–5719 (type series).
- Nidirana pleuraden (4): China: Yunnan: Mt Gaoligong: SYS a003775–3778.

RESEARCH ARTICLE



# Morphological and molecular analyses reveal two new insular species of *Cnemospis* Strauch, 1887 (Squamata, Gekkonidae) from Satun Province, southern Thailand

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### Abstract

We describe two new insular gecko species of the genus Cnemaspis from Tarutao, Adang, and Rawi islands in Satun Province, southern Thailand. The new species are distinguished from their congeners in having a unique combination of morphological, scalation, and color pattern characters, and by genetic divergence in the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene. Cnemaspis tarutaoensis sp. nov. was found to be a member of the C. kumpoli group, but is distinguished from all other species in that group by having 8-9 supralabials and 8 infralabials; 4-5 pore-bearing precloacal scales, pores rounded; 17-19 paravertebral tubercles randomly arranged; 27-29 subdigital lamellae under the fourth toe; subcaudal region yellowish, with smooth scales and a single enlarged median row; black gular markings in males and females; and 17.24-22.36% uncorrected pairwise sequence divergences. Cnemaspis adangrawi sp. nov. was found to be a member of the C. siamensis group, but is distinguished from all other species in that group by having 10 supralabials and 9 infralabials; 6-8 pore-bearing precloacal scales, pores rounded and arranged in a chevron shape; 23–25 randomly arranged, separated paravertebral tubercle rows; 26–28 subdigital lamellae under the fourth toe; subcaudal scales keeled, without enlarged median row; gular region, abdomen, limbs and subcaudal region yellowish in males only; gular marking absent in males and females; and 8.30-26.38 % uncorrected pairwise sequence divergences. Cnemaspis tarutaoensis sp. nov. occurs in karst formations on Tarutao Island, while Cnemaspis adangrawi sp. nov. is found near granitic, rocky streams on Adang and Rawi islands.

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#### **Keywords**

Island, rock geckos, species diversity, systematics

# Introduction

Southeast Asia is a global biodiversity hotspot with extraordinary levels of species endemism (Myers et al. 2000). Southern Thailand serves as an important biogeographic transition zone between the Indochinese and Sundaic biotas, especially at the Isthmus of Kra and the Kangar-pattani line (Hughes et al. 2003; Woodruff and Turner 2009; Woodruff 2010; Parnell 2013). Southern Thailand has high levels of species diversity and endemism of reptiles (Sodhi et al. 2004; Grismer et al. 2010; Das and van Dijk 2013; Wood et al. 2017).

The rock gecko genus Cnemaspis Strauch, 1887 currently contains 57 recognized species distributed throughout Southeast Asia (Grismer et al. 2014; Riyanto et al. 2017; Wood et al. 2017; Uetz et al. 2018). The number of recognized Cnemaspis species has increased rapidly during the past two decades (e.g. Bauer and Das 1998; Das 2005; Bauer et al. 2007; Grismer et al. 2009, 2014, 2015b; Grismer and Chan 2010; Wood et al. 2013, 2017; Rivanto et al. 2017). Thailand currently contains 16 recognized species of Cnemaspis (Grismer et al. 2010, 2014; Wood et al. 2017; Uetz et al. 2018) ranging from Chanthaburi in the east (Bauer and Das 1998), Sai Yok to the west (Grismer et al. 2010), and south through the Thai peninsula to the Malaysian border and its offshore islands (Grismer et al. 2014; Wood et al. 2017). Species delimitation of Cnemaspis in Thailand has been hindered by their conserved morphology and microhabitat specialization (Bauer and Das 1998; Grismer et al. 2010, 2014; Wood et al. 2017). Earlier taxonomic studies on *Cnemaspis* relied on morphology (e.g. Smith 1925; Taylor 1963; Bauer and Das 1998; Das and Leong 2004) but recent studies have incorporated molecular data to aid clarifying species boundaries in Thailand (e.g. Grismer et al. 2010; 2014; Wood et al. 2017). Grismer et al. (2014) recognized four groups of *Cnemaspis* in Thailand on the basis of morphological and molecular data: the *siamensis* group, the chanthaburiensis group, the kumpoli group (= Pattani clade of Grismer et al. 2014), and the *affinis* group. The *siamensis* group contains species that occur throughout western Thailand, southward to southern Thailand, and include C. chanardi Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya, C. huaseesom Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya, C. kamolnoranathi Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya, C. omari Grismer, Wood, Anuar, Riyanto, Ahmad, Muin, Sumontha, Grismer, Onn, Quah & Pauwels, C. phangngaensis Wood, Grismer, Aowphol, Aguilar, Cota, Grismer, Murdoch & Sites, C. punctatonuchalis Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya, C. roticanai Grismer & Onn, C. siamensis Smith, C. thachanaensis Wood, Grismer, Aowphol, Aguilar, Cota, Grismer, Murdoch & Sites, and C. vandeventeri Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya. The *chanthaburiensis* group contains species that occur from the northern margin of the Gulf of Thailand, eastward to Cambodia and southern Vietnam, and include C. aurantiacopes Grismer & Ngo, C. caudanivea Grismer & Ngo,

C. chanthaburiensis Bauer & Das, C. lineogularis Wood, Grismer, Aowphol, Aguilar, Cota, Grismer, Murdoch & Sites, C. neangthyi Grismer, Grismer & Chav, C. nuicamensis Grismer & Ngo, and C. tucdupensis Grismer & Ngo. The kumpoli group is composed of four species, C. biocellata Grismer, Chan, Nasir & Sumontha, C. kumpoli Taylor, C. monachorum Grismer, Ahmad, Chan, Belabut, Muin, Wood & Grismer, and C. niyomwanae Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya, that occur from southern Thailand to northern Malaysia. The affinis group contains species that occur from southern Thailand to central Peninsular Malaysia, including C. affinis Stoliczka, C. harimau Chan, Grismer, Anuar, Quah, Muin, Savage, Grismer, Ahmad, Remigio & Greer, C. pseudomcguirei Grismer, Ahmad, Chan, Belabut, Muin, Wood, Grismer, C. shahruli Grismer, Chan, Quah, Muin Savage, Grismer, Ahmad, Greer & Remegio, C. mcguirei Grismer, Grismer, Wood & Chan, C. grismeri Wood, Quah, Anuar & Muin, C. flavolineata Nicholls, C. temiah Grismer, Wood, Anuar, Riyanto, Ahmad, Muin, Sumontha, Grismer, Onn, Quah & Pauwels, C. narathiwatensis Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya, C. hangus Grismer, Wood, Anuar, Riyanto, Ahmad, Muin, Sumontha, Grismer, Onn, Quah & Pauwels, C. selamatkanmerapoh Grismer, Wood, Mohamed, Chan, Heinz, Sumarli, Chan & Loredo, C. bayuensis Grismer, Grismer, Wood & Chan, and C. stongensis Grismer, Wood, Anuar, Riyanto, Ahmad, Muin, Sumontha, Grismer, Onn, Quah & Pauwels. Although a large number of *Cnemaspis* species have been reported from Thailand, only two species are known to occur on islands in Thailand: C. chanardi from Samui, Phangan and Ko Tao islands, and C. siamensis from Phuket Island (Grismer et al. 2010, 2014). It is evident that the diversity of *Cnemaspis* on the islands of southern Thailand, especially those containing isolated karst formations and granitic rocky streams, remains poorly studied.

During recent fieldwork in 2017–2018 on Tarutao, Adang, and Rawi islands, Satun Province, southern Thailand, specimens of *Cnemaspis* were collected that differed from all other named species. Herein, we evaluate the morphological and molecular distinctiveness of these specimens.

# Materials and methods

# Sampling

Specimens of *Cnemaspis* were collected from Tarutao, Adang, and Rawi islands in Tarutao National Park, Mueang Satun District, Satun Province, Thailand (Fig. 1) between November 2017–April 2018. Specimens were collected by hand during the day (1000–1800 h) and at night (1900–2200 h). Liver or muscle samples for genetic analysis were collected and preserved in 95% ethanol after euthanasia. Specimens were fixed in 10% formalin and later transferred to 70% ethanol for permanent storage. Specimens and tissue samples were deposited in the herpetological collection at the Zoological Museum of Kasetsart University, Bangkok, Thailand (**ZMKU**) and the Thailand Natural History Museum, Pathum Thani, Thailand (**THNHM**).



**Figure 1.** Map illustrating the holotype locality (red star) and paratype localities (red circles) of *Cnemaspis tarutaoensis* sp. nov. at Tarutao Island, Satun Province, Thailand; the holotype locality (green star) and paratype localities (green circles) of *Cnemaspis adangrawi* sp. nov. at Adang and Rawi islands, Mueang Satun District, Satun Province, Thailand.

# Morphology

Only adult individuals were used in the morphological analysis, as determined by the presence of hemipenes or precloacal pores in males, and the presence of calcium glands or eggs in females. Measurements were taken by the first author on the left side of preserved specimens to the nearest 0.1 mm using digital calipers under a Nikon SMZ 445 dissecting microscope. Sixteen measurements were taken following Grismer et al. (2014) and Wood et al. (2017): snout-vent length (SVL), taken from tip of snout to the anterior margin of vent; tail width (TW) at the base of the tail immediately posterior to the postcloacal swelling; tail length (TL), as distance from the vent to the tip of the tail, whether original or regenerated; forearm length (FL), taken on the dorsal surface from the posterior margin of the elbow while flexed 90° to the inflection of the flexed wrist; tibia length (TBL), taken on the ventral surface from the posterior surface of the knee while flexed  $90^{\circ}$  to the base of the heel; head length (**HL**), as distance from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout; head width (HW) at the angle of the jaws; head depth (HD), as the maximum height of head from the occiput to the throat; axilla-groin length (AG), taken from the posterior margin of the forelimb at its insertion point on the body to the anterior

margin of the hind limb at its insertion point on the body; eye diameter (**ED**), as the maximum horizontal diameter of the eyeball; eye-snout distance (**ES**), measured from the anterior margin of the eyeball to the tip of snout; eye-ear distance (**EE**), measured from the anterior edge of the ear opening to the posterior edge of the eyeball; eye-nostril distance (**EN**), measured from the anterior most margin of the eyeball to the posterior margin of the external nares; inner orbital distance (**IO**), as the width of the frontal bone at the level of the anterior edges of the orbit; internarial distance (**IN**), measured between the medial margins of the nares across the rostrum; and ear length (**EL**), taken from the greatest vertical distance of the ear opening.

Meristic characters of scale counts and external observations of morphology were taken following Grismer et al. (2014) and Wood et al. (2017): number of supralabial and infralabial scales, counted from below the middle of the orbit to the rostral and mental scales, respectively; texture of scales on the anterior margin of the forearm; number of paravertebral tubercles between limb insertions, counted in a straight line immediately left of the vertebral column; presence or absence of a row of enlarged, widely spaced, tubercles along the ventrolateral edge of the body flank between limb insertions; number of subdigital lamellae beneath the fourth toe (=4<sup>th</sup> toe lamellae), counted from the base of the first phalanx to the claw; general size (i.e., strong, moderate, weak) and arrangement (i.e., random or linear) of dorsal body tubercles; number, orientation and shape of precloacal pores; relative size of subcaudal and subtibial scales; and number of postcloacal tubercles on each side of tail base.

Comparative material was examined in the holdings of **THNHM** (Appendix 1), and comparative data were obtained from the original descriptions of other Thai species of *Cnemaspis* (Grismer et al. 2009; Grismer and Chan 2010; Grismer et al. 2010; Wood et al. 2017).

### Molecules

Genomic DNA was extracted from liver tissue of eight individuals of *Cnemaspis* (Table 1) using the Qiagen DNAeasy tissue kit (Valencia, CA, USA). An 1,296 bp fragment of mitochondrial (mt) DNA consisting of the NADH dehydrogenase subunit 2 (ND2) gene and the flanking tRNAs Trp, Ala, Asn and Cys was amplified by the polymerase chain reaction (PCR; 95 °C for 2 min, 95 °C for 35 s, 52 °C for 35s, 72 °C for 35 s) for 33 cycles using the primers L4437b (5'-AAGCAGTTGGGGCCCATACC-3'; Macey et al. 1997) and H5934 (5' AGRGTGCCAATGTCTTTGTGRTT-3'; Macey et al. 1997). PCR products were purified using the AccuPrep\* PCR Purification Kit (Bioneer, Daejeon, Korea), and were sequenced using the amplifying primers and the internal sequencing primer CyrtintF1 (5'-TAGCCYTCTCYTCYATYGCCC-3'; Siler et al. 2010) on an ABI 3730 automatic sequencer (Applied Biosystems, CA, USA). Sequences were edited and aligned using Geneious v.5.6.3 (Biomatters, Auckland, New Zealand). All new sequences were deposited in GenBank under accession numbers MK862112 to MK862119 (Table 1).

**Table 1.** Samples used in this study, including catalogue numbers, Genbank accession numbers and localities of voucher specimens. Voucher abbreviations are as follows: Monte L. Bean Life Science Museum at Brigham Young University (**BYU**), California Academy of Sciences (**CAS**), the Field Museum of Natural History, Chicago, Illinois, USA (**FMNH**), La Sierra University Herpetological Collection (**LSUHC**), Universiti Sains Malaysia Herpetological Collection at the Universiti Sains Malaysia, Penang, Malaysia (**USMHC**), and Zoological Museum of Kasetsart University (**ZMKU**).

Species	Locality	Collection no.	Genbank accession no.	Reference
Cyrtodactylus intermedius	Cambodia, Kampot	FMNH 263228	KT13107	Grismer et al. 2015a
Hemidactylus garnotii	Myanmar, Mon State, Kyaihto Township, Kyait Hti Yo Wildlife Sanctuary.	CAS 222276	EU68364	Bauer et al. 2008
Cnemaspis adangrawi sp. nov.	Thailand, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00767	MK862112	This study
	Thailand, Satun Province, Mueang Satun District, Adang Island	THNHM 28207	MK862113	This study
	Thailand, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00770	MK862114	This study
	Thailand, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00775	MK862115	This study
	Thailand, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00776	MK862116	This study
Cnemaspis affinis	Malaysia, Penang, Pulau Pinang	LSUHC 6787	KM024682	Grismer et al. 2014
Cnemaspis argus	Malaysia, Terengganu, Gunung Lawit	LSUHC 8304	KM024687	Grismer et al. 2014
	Malaysia, Terengganu, Gunung Lawit	LSUHC 10834	KM024688	Grismer et al. 2014
Cnemaspis aurantiacopes	Vietnam, Kien Giang Province, Hon Dat Hill	LSUHC 8610	KM024692	Grismer et al. 2014
	Vietnam, Kien Giang Province, Hon Dat Hill	LSUHC 8611	KM024693	Grismer et al. 2014
Cnemaspis biocellata	Malaysia, Perlis, Kuala Perlis	LSUHC 8817	KM024707	Grismer et al. 2014
	Malaysia, Perlis, Kuala Perlis	LSUHC 8817	KM024708	Grismer et al. 2014
	Malaysia, Perlis, Gua Kelam	LSUHC 8789	KM024709	Grismer et al. 2014
Cnemaspis boulengerii	Vietnam, Ca Mau Province, Con Dao Archipelago	LSUHC9278	KM024710	Grismer et al. 2014
	Vietnam, Ca Mau Province, Con Dao Archipelago	LSUHC9279	KM024711	Grismer et al. 2014
Cnemaspis caudanivea	Vietnam, Kien Giang Province, Hon Tre Island	LSUHC 8582	KM024714	Grismer et al. 2014
Cnemaspis chanardi	Thailand, Nakhon Si Thammarat Province, Thum Thong Panra	LSUHC 9567	KM024715	Grismer et al. 2014
Cnemaspis chanthaburiensis	Cambodia, Pursat Province, Phnom Dalai	LSUHC 9338	KM024716	Grismer et al. 2014
Cnemaspis grismeri	Malaysia, Perak, Lenggong	LSUHC 9969	KM024722	Grismer et al. 2014
Cnemaspis hangus	Malaysia, Pahang, Bukit Hangus	LSUHC 9358b	KM024728	Grismer et al. 2014
Cnemaspis harimau	Malaysia, Kedah, Gunung Jeri	LSUHC 9665	KM024730	Grismer et al. 2014
Cnemaspis huaseesom	Thailand, Kanchanaburi Province, Sai Yok National Park	LSUHC 9455	KM024733	Grismer et al. 2014
	Thailand, Kanchanaburi Province, Sai Yok National Park	LSUHC 9457	KM024734	Grismer et al. 2014
	Thailand, Kanchanaburi Province, Sai Yok National Park	LSUHC 9458	KM024735	Grismer et al. 2014
Cnemaspis karsticola	Malaysia, Kelantan, Gunung Reng	LSUHC 9054	KM024736	Grismer et al. 2014
	Malaysia, Kelantan, Gunung Reng	LSUHC 9055	KM024737	Grismer et al. 2014
Cnemaspis kumpoli	Malaysia, Perlis, Perlis State Park	LSUHC 8847	KM024745	Grismer et al. 2014
	Malaysia, Perlis, Perlis State Park	LSUHC 8848	KM024746	Grismer et al. 2014
Cnemaspis lineogularis	Thailand, Prachuap Khiri Khan Province, Kui Buri District, Wat Khao Daeng	BYU 62535	KY091231	Wood et al. 2017
	Thailand, Prachuap Khiri Khan Province, Kui Buri District, Wat Khao Daeng	ZMKU R 00728	KY091233	Wood et al. 2017

Species	Locality	Collection no.	Genbank accession no.	Reference
Cnemaspis mahsuriae	Malaysia, Kedah, Pulau Langkawi, Gunung Raya	LSUHC 11829	KT250634	Grismer et al. 2015b
Cnemaspis mcguirei	Malaysia, Perak, Bukit Larut	LSUHC 8853	KM024751	Grismer et al. 2014
Cnemaspis monachorum	Malaysia, Kedah, Langkawi Archipelago, Pulau Langkawi	LSUHC 9114	KM024754	Grismer et al. 2014
	Malaysia, Kedah, Langkawi Archipelago, Pulau Langkawi	LSUHC 10807	KM024755	Grismer et al. 2014
Cnemaspis narathiwatensis	Malaysia, Perak, Belum-Temengor, Sungai Enam	USMHC 1347	KM024762	Grismer et al. 2014
	Malaysia, Perak, Belum-Temengor, Sungai Enam	USMHC 1348	KM024763	Grismer et al. 2014
Cnemaspis neangthyi	Cambodia, Pursat Province, O'Lakmeas	LSUHC 8515	KM024767	Grismer et al. 2014
	Cambodia, Pursat Province, O'Lakmeas	LSUHC 8516	KM024768	Grismer et al. 2014
Cnemaspis niyomwanae	Thailand, Trang Province, Thum Khao Ting	LSUHC 9568	KM024773	Grismer et al. 2014
	Thailand, Trang Province, Thum Khao Ting	LSUHC 9571	KM024774	Grismer et al. 2014
Cnemaspis nuicamensis	Vietnam, An Giang Province, Nui Cam Hill	LSUHC 8646	KM024775	Grismer et al. 2014
	Vietnam, An Giang Province, Nui Cam Hill	LSUHC 8647	KM024776	Grismer et al. 2014
	Vietnam, An Giang Province, Nui Cam Hill	LSUHC 8648	KM024777	Grismer et al. 2014
Cnemaspis omari	Thailand, Satun Province, Phuphaphet Cave	LSUHC 9565	KM024780	Grismer et al. 2014
	Malaysia, Perlis, Perlis State Park	LSUHC 9978	KM024779	Grismer et al. 2014
Cnemaspis perhentianensis	Malaysia, Terengganu, Pulau Perhentian Besar	LSUHC 8699	KM024820	Grismer et al. 2014
Cnemaspis phangngaensis	Thailand, Phangnga Province, Mueang Phangnga District, Khao Chang, Phung Chang Cave	BYU 62537	KY091234	Wood et al. 2017
	Thailand, Phangnga Province, Mueang Phangnga District, Khao Chang, Phung Chang Cave	BYU 62538	KY091235	Wood et al. 2017
Cnemaspis punctatonuchalis	Thailand, Prachaup Khiri Khan Province, Thap Sakae	BYU 62539	KY091236	Wood et al. 2017
	Thailand, Prachaup Khiri Khan Province, Thap Sakae	BYU 62540	KY091237	Wood et al. 2017
Cnemaspis roticanai	Malaysia, Kedah, Pulau Langkawi, Gunung Raya	LSUHC 9430	KM024829	Grismer et al. 2014
	Malaysia, Kedah, Pulau Langkawi, Gunung Raya	LSUHC 9431	KM024830	Grismer et al. 2014
	Malaysia, Kedah, Pulau Langkawi, Gunung Raya	LSUHC 9439	KM024831	Grismer et al. 2014
Cnemaspis siamensis	Thailand, Chumpon Province, Pathio District	LSUHC 9474	KM024838	Grismer et al. 2014
	Thailand, Chumpon Province, Pathio District	LSUHC 9485	KM024839	Grismer et al. 2014
Cnemaspis tarutaoensis sp. nov.	Thailand, Satun Province, Mueang Satun District, Tarutao Island	ZMKU R 00761	MK862117	This study
	Thailand, Satun Province, Mueang Satun District, Tarutao Island	ZMKUR 00763	MK862118	This study
	Thailand, Satun Province, Mueang Satun District, Tarutao Island	ZMKU R 00764	MK862119	This study
Cnemaspis thachanaensis	Thailand, Surat Thani Province, Tha Chana District, Tham Khao Sonk Hill	BYU 62542	KY091239	Wood et al. 2017
	Thailand, Surat Thani Province, Tha Chana District, Tham Khao Sonk Hill	BYU 62543	KY091243	Wood et al. 2017
	Thailand, Surat Thani Province, Tha Chana District, Tham Khao Sonk Hill	BYU 62544	KY091244	Wood et al. 2017
Cnemaspis tucdupensis	Vietnam, An Giang Province, Tuc Dup Hill	LSUHC 8631	KM024852	Grismer et al. 2014
	Vietnam, An Giang Province, Tuc Dup Hill	LSUHC 8632	KM024853	Grismer et al. 2014
Cnemaspis vandeventeri	Thailand, Ranong Province, Suk Saran District, Naka	BYU 62541	KY091238	Wood et al. 2017

### Phylogenetic analyses

Homologous sequences of 56 *Cnemaspis* and the outgroups *Cyrtodactylus intermedius* and *Hemidactylus garnotii* (following Bauer et al. 2008; Grismer et al. 2015b) were downloaded from GenBank and aligned to the eight newly generated *Cnemaspis* sequences using Geneious v. 5.6.3 (Biomatters, Auckland, New Zealand). The aligned dataset was partitioned into four partitions consisting of ND2 first, second and third codon positions, and tRNAs.

Phylogenies were reconstructed with the maximum likelihood (ML) criterion using IQ-TREE v. 1.6.7 (Nguyen et al. 2014) on the IQ-TREE web server (Trifinopoulos et al. 2016). The best-fit model of substitution for each partition was estimated using IQ-TREE's ModelFinder function (Kalyaanamoorthy et al. 2017) under the Akaike Information Criterion (AIC). The selected models were TIM+F+R4 for each ND2 codon position partition, and HKY+F+R4 for the tRNA partition. Bootstrap analysis was performed using the ultrafast bootstrap approximation (Minh et al. 2013) with 1,000 replicates and 0.95 minimum correlation coefficient.

Phylogenies were also reconstructed with Bayesian Inference (BI) using MrBayes v. 3.2 on XSEDE on the Cyberinfrastructure for Phylogenetic Research (CIPRES; Miller et al. 2010) computer cluster. The best-fit model of substitution was estimated for each partition using jModelTest 2.1.10 (Posada 2008) under AIC. The selected models were GTR+ I+ $\Gamma$  for each ND2 codon position partition, and HKY+ I+ $\Gamma$  for the tRNA partition. Two simultaneous runs, each with three heated and one cold chain, were performed using the default priors for 10 × 10<sup>6</sup> generations, with trees sampled every 1,000 generations from the Markov Chain Monte Carlo (MCMC). Runs were halted after the average standard deviation of split frequencies was below 0.01 and convergence was assumed. The first 25% of the trees were discarded as burn-in using the sumt command. The convergence of the two simultaneous runs, and stationary state of each parameter, were evaluated using Tracer v. 1.6 (Rambaut et al. 2014). Runs were terminated when the effective sample sizes (ESS) of all parameters was greater than or equal to 200.

The most likely tree in the ML analysis, and the 50% majority-rule consensus of the sampled trees from the BI analysis, were visualized using FigTree v. 1.4.3 (Rambaut 2009). Nodes having bootstrap support (BS) of  $\geq$ 70 and posterior probabilities (PP) of  $\geq$ 0.95 were considered to be well-supported (Huelsenbeck and Ronquist 2001; Wilcox et al. 2002). Uncorrected pairwise sequence divergences were calculated using MEGA v. 7.0.26 (Kumar et al. 2016).

# Results

### Molecular analyses

The aligned dataset contained 1,296 characters of 64 individuals of *Cnemaspis* and two individuals of the outgroup species. The standard deviation of split frequencies among

the two simultaneous BI runs was 0.001478. The ESS values were greater than or equal to 3,630 for all parameters. A single most likely tree resulted from the ML analysis.

The most likely ML tree and the 50% majority rule consensus tree from the BI analysis had similar topologies (Fig. 2). *Cnemaspis* samples from Tarutao Island represented a well-supported lineage (100 BS, 1.0 PP) within the *kumpoli* group, and was recovered as the sister species to *C. monachorum* from Pulau Langkawi, Malaysia (100 BS, 1.0 PP). The Tarutao samples differed from one another by uncorrected *p*-distances of 0.00–0.31%, but from other members of the *kumpoli* group by uncorrected *p*-distances of 17.24–22.36 % (Table 2).

*Cnemaspis* samples from Adang and Rawi islands represented a well-supported lineage (100 BS, 1.0 PP) within the *siamensis* group, and was recovered as being closely related to a clade containing *C. chanardi*, *C. phangngaensis*, *C. omari*, and *C. roticanai* (Fig. 2). However, the exact sister taxon relationship of the Adang and Rawi islands was not resolved with strong support (Fig. 2). The Adang-Rawi samples differed from one another by uncorrected *p*-distances of 0.00–4.68 %, but from other members of the *siamensis* group by uncorrected *p*-distances of 8.30–26.38 % (Table 3).

### **Taxonomic hypotheses**

The Tarutao and Adang-Rawi samples of *Cnemaspis* differed from each other and all other congeners by being diagnosable in morphology and mitochondrial DNA. Owing to these independent lines of evidence, we hypothesize that the Tarutao and Adang-Rawi samples represent two new species to science, and are described below.

### **Systematics**

### Cnemaspis tarutaoensis sp. nov.

http://zoobank.org/91BAE519-9241-447C-8EB6-BED473B99529 Figures 3–6 Tarutao Rock Gecko Thai common name: Jing Jok Niew Yaow Ko Tarutao

**Holotype** (Figs 3A, 4, 5). ZMKU R 00763, adult male from Thailand, Satun Province, Mueang Satun District, Tarutao National Park, Tarutao Island, Pha Toe Boo (6°42.1854'N, 99°38.8956'E; 2 m a.s.l.; Fig. 7A), collected on 5 November 2017 by Natee Ampai, Attapol Rujirawan, Siriporn Yodthong, and Korkwan Termprayoon.

**Paratypes** (Figs 3b, 6). Twelve paratypes (adult males = 6, adult females = 6). ZMKU R 00761–00762, ZMKU R 00764 (3 adult males), THNHM 28201–28202, ZMKU R 00758–00760 (5 adult females), bear the same collection data as holotype. THNHM 28203 (1 adult male), same data as holotype except collected 5 April 2018. ZMKU R 00765 (1 adult male), same data as holotype except collected at Tham Chora-

No.	Species	n	1	2	3	4	5
1	<i>C. tarutaoensis</i> sp. n.	3	0.16 (0.00–0.31)				
2	C. monachorum	2	17.70 (17.24–18.17)	0.54 (0.00–1.09)			
3	C. biocellata	3	20.34 (20.19–20.50)	19.10 (19.79–19.41)	3.57 (0.00–7.14)		
4	C. kumpoli	2	21.84 (21.74–21.89)	22.28 (22.95–22.52)	13.51 (13.51–13.51)	0.16 (0.00–0.31)	
5	C. niyomwanae	2	21.35 (20.34–22.36)	21.20 (19.88–22.36)	14.44 (13.20–15.68)	12.89 (11.49–14.29)	1.79 (0.00–3.57)

**Table 2.** Mean (minimum–maximum) uncorrected *p*-distances (%) within the *Cnemaspis kumpoli* group based on 1,296 bp of the mitochondrial ND2 gene and flanking tRNAs. Numbers in bold are within species divergence. n = number of individuals.

khae (6°41.7966'N, 99°39.0426'E; 37 m a.s.l.; Fig. 7B), collected 7 November 2017. ZMKU R 00766 (1 adult female) and THNHM 28205 (1 adult male), same data as holotype except collected at karst forest near stream (6°39.759'N, 99°39.1596'E; 53 m a.s.l.; Fig. 7C), collected 5 April 2018.

**Referred specimens.** THNHM 28204 (one juvenile), same data as holotype except collected 5 April 2018.

**Diagnosis.** Cnemaspis tarutaoensis sp. nov. can be distinguished from all other Cnemaspis by having the following combination of characters: (1) adult males with maximum snout-vent length (SVL) 36.4 mm (mean  $34.7 \pm \text{SD } 1.5$ , n = 7) and females with maximum SVL 34.8 mm (mean  $33.7 \pm \text{SD } 0.6$ , n = 6); (2) 8–9 supralabials and 8 infralabials; (3) 4–5 pore-bearing precloacal scales, pores rounded; (4) 17–19 paravertebral tubercles, small in size, randomly arranged; (5) 27–29 subdigital lamellae under the 4<sup>th</sup> toe; (6) subcaudal region yellowish, scales smooth with a single enlarged median subcaudal row; (7) one postcloacal tubercles on each side; (8) no sexual dimorphism in dorsal and ventral patterns; and (9) black gular markings present in males and females. These differences are summarized for geographically close congeners in the *kumpoli* group (Table 4).

**Description of holotype.** Adult male; SVL 36.3 mm; head moderate in size (HL/SVL 0.28), elongate, narrow (HW/SVL 0.15), flattened (HD/HL 0.33), distinct from neck; snout moderate (ES/HL 0.42), in lateral view slightly concave; postnasal region constricted medially; scales of rostrum, raised, smooth, larger than conical scales on occiput; faint supraorbital ridges; gular and throat scales raised, smooth and round; shallow frontorostral sulcus; canthus rostralis nearly absent, smoothly rounded; eye large (ED/HL 0.18); pupil round; ear opening oval, taller than wide; rostral slightly concave, dorsal 80% divided by longitudinal median groove; rostral bordered posteriorly by supranasal and laterally by first supralabial; 9, 9 (right, left) slightly raised supralabials decreasing in size posteriorly; 8, 8 (right, left) infralabials decreasing in size posteriorly; 8, 8 (right, left) infralabials decreasing in size posteriorly; supranasal scales; mental large, triangular, bordered posteriorly by three large postmentals.

Body slender, elongate (AG/SVL 0.39); small, raised and equal in sized, dorsal scales throughout body intermixed with several large, multicarinate tubercles random-



**Figure 2.** The single best maximum likelihood tree of the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene and flanking tRNAs from geckos of the genera *Cnemaspis, Cyrtodactylus* and *Hemidactylus*, shown in full view (above) and close-up view of relevant clades (below). Support values at nodes are bootstrap values from a maximum likelihood analysis of the same dataset followed by posterior probabilities of the Bayesian analysis.

1,296 bp of the mitochondrial ND2 gene and	
<i>Cnemaspsis siamensis</i> group based on 1	individuals.
b-distances (%) within the	divergence. $n =$ number of
uncorrected	vithin species
(minimum-maximum)	. Numbers in bold are v
le 3. Mean	cing tRNAs.
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<b>Tab</b> flank	<b>le 3.</b> Mean (mini king tRNAs. Num	imuı iber:	n-maximum) s in bold are v	) uncorrected $\mu$ within species $\epsilon$	o-distances (% divergence. <i>n</i> :	) within the ( = number of i	<i>Cnemaspsis sian</i> individuals.	<i>nensis</i> group b	ased on 1,296	bp of the mi	tochondrial N	D2 gene and
No.	Species	n	1	2	3	4	5	6	7	8	6	10
	C. adangrawi	Ś	2.81									
	sp. nov.		(0.00 - 4.68)									
7	C. chanardi	-	11.40	0.00								
			(10.85–11.91)									
3	C. omari	7	9.36	11.81	2.13							
			(8.30-10.21)	(11.49–12.13)	(0.00 - 4.26)							
4	C. phangngaensis	7	10.19	11.38	11.17	0.11						
			(9.57–10.85)	(11.27–11.49)	(10.85–11.49)	(0.00-0.21)						
2	C. siamensis	7	25.83	24.40	27.77	25.00	0.00					
			(25.74-25.96)	(24.26–24.68)	(27.66–27.87)	(24.89–25.11)						
9	C. roticanai	3	8.92	11.77	9.01	8.90	28.16	0.11				
			(8.51–9.57)	(11.70–11.91)	(8.72–9.36)	(8.72–9.15)	(28.09 - 28.30)	(0.00-0.21)				
$\sim$	C. vandeventeri	1	24.26	24.04	26.60	25.21	12.34	26.88	0.00			
			(24.04-24.47)	(24.04–24.04)	(25.96–27.23)	(25.11–25.32)	(12.34–12.34)	(26.81–27.02)				
8	C. thachanaensis	3	25.50	24.40	28.30	26.13	13.35	27.66	14.47	0.53		
			(25.10-25.96)	(24.26–24.68)	(27.23–28.94)	(25.74–26.81)	(13.19 - 14.26)	(27.45–28.09)	(14.26–14.89)	(0.00-1.06)		
6	C. punctatonuchalis	7	25.23	25.53	26.38	25.00	19.36	25.60	21.06	21.13	0.00	
			(24.04-26.17)	(25.53–25.53)	(26.38–26.38)	(25.00-25.00)	(19.36 - 19.36)	(25.53-25.74)	(21.06–21.06)	(21.06-21.28)		
10	C. huaseesom	3	26.00	26.17	28.19	23.72	19.36	27.52	20.64	20.99	16.95	0.43
			(25.74-26.38)	(26.17–26.17)	(27.87–28.51)	(23.62-23.83)	(19.36–19.36)	(27.45–27.66)	(20.64–20.64)	(20.64-21.70)	(16.81–17.02)	(0.00-0.64)

**Table 4.** Meristic character states and color patterns of species in the *Cnemaspis kumpoli* group. Measurements are taken in millimeters and measurement abbreviations are defined in the text. var = character variable; - = data unavailable, ant = anterior.

Characters/Species	<i>C. tarutaoensis</i> sp. nov.	C. biocellata	C. kumpoli	C. monachorum	C. niyomwanae
Sample size	13	25	13	12	5
Maximum SVL	36.4	40.2	63.0	32.9	56.8
Supralabial scales	8–9	6–10	7–9	7–8	8-11
Infralabial scales	8	5–9	6–8	5–7	6–8
Ventral scales keeled (1) or smooth (0)	0	0	0	0	0
No. of precloacal pores	4–5	6–12	1-8	3	3
Precloacal pore continuous (1) or separated (0)	0.1	1	0	1	0.1
No. of paravertebral tubercles	17–19	21-27	28-35	11-20	26-31
Tubercles present (1) or absent (0) on lower flanks	0	1	1	0	0
No. of 4 <sup>th</sup> toe lamellae	27–29	29–37	34-41	24-30	31–34
Lateral caudal furrows present (1) or absent (0)	1	1	1	1	1
Lateral caudal tubercle row present (1) or absent (0)	0	ant	0	ant	0
Subcaudal scales keeled (1) or smooth (0)	0	0	0	0	0
Enlarge submetatarsal scales on $1^{st}$ toe (1) or not (0)	0	0	0	0	0
Enlarge median subcaudal scales row (1) or not (0)	1	1	1	1	1
No. of postcloacal tubercles in males	1	1	2.3	1–2	1.2
Subcaudal region yellow present (1) or not (0)	1	var	0	0	0
Ventral pattern sexually dimorphic present (1) or not (0)	0	1	0	1	-
Dorsal color pattern sexually dimorphic (1) or not (0)	0	1	1	0	1
Wide black and yellow bands on tail present (1) or not (0)	1	0	0	0	0
Gular marking (1) or not (0)	1	0	0	1	0

ly arranged; 19 paravertebral tubercles; tubercles absent on lower flanks; tubercles extend from occiput to base of tail; dorsal scales slightly raised and keeled; pectoral and abdominal scales smooth and round, flat to concave, slightly larger than dorsal scales and not larger posteriorly; ventral scales of brachia raised, smooth and juxtaposed; four pores-bearing precloacal scales arranged in a chevron, separated; precloacal pore rounded; precloacal depression absent; femoral pores absent.

Fore and hind limbs moderately long, slender; scales beneath forearm slightly raised, smooth and subimbricate; subtibial scales keeled; palmar scales smooth and juxtaposed; digits elongate, slender, inflected joint and bearing slightly recurved claws; subdigital lamellae unnotched; lamellae beneath first phalanges wide; lamellae beneath phalanx immediately following inflection granular; lamellae of distal phalanges wide; lamellae beneath inflection large; interdigital webbing absent; enlarge submetatarsal scales on 1<sup>st</sup> toe absent; fingers increase in length from first to fourth with fourth and fifth nearly equal in length; relative length of fingers IV>V>III>II>I; toes increase in length from first to fifth with fourth and fifth nearly equal in length; relative length of toes IV>V>III>II>I; total number of subdigital lamellae on 4<sup>th</sup> toe 28, 28 (right, left).



**Figure 3.** *Cnemaspis tarutaoensis* sp. nov. from Tarutao Island, Mueang Satun District, Satun Province, Thailand. **A** male holotype ZMKU R 00763 **B** female paratype ZMKU R 00758.

Caudal and subcaudal scales smooth, similar to dorsal scale size; lateral caudal furrow present; lateral caudal tubercle row absent; enlarge caudal tubercles at the base of tail not encircling tail; enlarged median subcaudal scales row present; tail length (TL) 34.3 mm with broken at tail tip; enlarged postcloacal tubercle 1, 1 (right, left) on lateral surface of hemipenial swellings at the base of tail.

**Coloration in life** (Figs 3, 4). Dorsal ground color of head light brown; top of the head bearing small black, sage and yellowish marking; snout yellowish; dorsal ground color of body, limbs and tail light brown with dark brown to black irregular blotches; ground color of ventral surfaces grayish white intermixed with light yellowish blotches; gular and throat regions are beige and light yellow; anterior gular region yellowish; midgular region with faint, dark lineate marking; thin, faint black postorbital stripe; light sage vertebral blotches extending from the nape to tail; flanks with irregular incomplete sage to yellowish blotches becoming smaller posteriorly; limbs yellowish brown with dark brown incomplete irregular spots subcaudal region yellowish; wide dark brown to black and yellow bands on tail.



**Figure 4.** Male holotype of *Cnemaspis tarutaoensis* sp. nov. from Tarutao Island, Mueang Satun District, Satun Province, Thailand (ZMKU R 00763) in life. **A** ventral view **B** dorsal view **C** precloacal region showing distribution of pore-bearing scales (black arrows).



**Figure 5.** Male holotype of *Cnemaspis tarutaoensis* sp. nov. from Tarutao Island, Mueang Satun District, Satun Province, Thailand (ZMKU R 00763) in preservative. **A** ventral **B** dorsal views.

**Coloration in preservative** (Fig. 5). Color pattern similar to that in life with some fading. Dorsal ground color of head, body, limbs and tail brown with vertebral blotches indistinct; irregular pale marking; top of head with indistinct darker marking; all yellow markings faded to whitish gray; dorsal surfaces of limbs with irregular light and dark blotches; entire ventral surface whitish gray; gular region with faint dark lineate marking.

**Variation.** Most paratypes approximate the holotype in general aspects of color pattern (Fig. 6), with most differences found in the degree of vertebral blotches. ZMKU R 00761 (adult male) has dark spots in gular region. ZMKU R 00762 and ZMKU R 00765 (two adult males) have lighter gular markings than the holotype. THNHM 28201 and ZMKU R 00760 (two adult females) have lighter dorsal markings than the holotype. ZMKU R 00762 and THNHM 28205 (two adult males) have a pattern that resembles transverse bands rather than paravertebral blotches. ZMKU R 00762 and ZMKU R 00765 (two adult males) have regenerated tails of uniform



**Figure 6.** Paratypes of *Cnemaspis tarutaoensis* sp. nov. in preservative. **A** dorsal view **B** ventral view; from left to right, top panel (females): ZMKU R 00758, ZMKU R 00759, ZMKU R 00760, ZMKU R 00766, THNHM 28201, and THNHM 28202; bottom panel (males): ZMKU R 00761, ZMKU R 00762, ZMKU R 00765, ZMKU R 00764, THNHM 28203, and THNHM 28205.

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	R 00763	R 00761	R 00762	R 00764	R 00765	28203	28205	R 00766	R 00758	28201	R 00759	R 00760	28202
Type series	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
Sex	М	M	Μ	Μ	Μ	М	Μ	н	ц	н	н	н	н
TAS	36.3	33.3	35.2	32.6	35.2	33.8	36.4	33.3	34.8	33.4	33.5	33.8	33.6
TL	34.3b	8.7b	42.3r	þ	40.8r	þ	17.3b	47.7	45.3	50.3	52.4	44.1r	13.6b
TW	3.5	3.4	3.5	3.2	3.5	3.2	3.6	3.5	3.8	3.5	3.4	3.4	3.3
FL	5.3	5.1	5.2	5.1	5.3	5.1	5.3	5.2	5.2	5.0	5.1	5.1	5.1
TBL	6.3	6.2	6.3	6.0	6.3	6.2	6.3	6.0	6.3	6.0	6.1	6.2	6.1
AG	14.3	14.1	14.3	14.1	14.3	14.1	14.3	14.3	14.2	14.0	14.1	14.0	14.0
HL	10.0	9.8	10.1	9.7	10.0	9.6	10.1	9.5	9.9	9.7	9.7	9.7	9.6
WH	5.6	5.4	5.5	5.4	5.6	5.3	5.6	5.5	5.6	5.2	5.3	5.4	5.3
HD	3.3	3.1	3.2	3.2	3.3	3.2	3.3	3.2	3.2	3.0	3.1	3.2	3.0
ED	1.8	1.9	1.9	1.8	1.8	1.8	1.9	1.8	1.8	1.8	1.9	1.9	1.9
EE	2.9	2.9	3.0	2.9	3.0	2.9	3.0	2.8	2.8	2.7	2.8	2.8	2.8
ES	4.2	4.1	4.1	4.0	4.2	4.0	4.2	4.1	4.2	4.0	4.1	4.0	4.0
EN	3.5	3.2	3.4	3.2	3.5	3.2	3.6	3.4	3.4	3.2	3.2	3.3	3.2
IO	2.2	2.1	2.2	2.0	2.1	2.0	2.2	1.9	2.2	2.1	2.1	2.1	2.0
EL	0.6	0.7	0.7	0.6	0.7	0.6	0.7	0.6	0.7	0.7	0.7	0.6	0.6
NI	0.8	0.7	0.8	0.7	0.8	0.8	0.8	0.7	0.7	0.7	0.8	0.8	0.7
Supralabials	9	9	8	9	6	9	6	6	8	8	8	8	6
Infralabials	8	8	8	8	8	8	8	8	8	8	8	8	8
No. of precloacal pores	4	4	4	4	4	4	5	I	I	I	I	I	I
Precloacal pore continuous (1) or separated (0)	0	0	0	0	0	0	1	I	I	I	I	I	I
No. of paravertebral tubercles	19	19	18	18	19	18	19	17	18	19	18	19	18
No. of 4th toe lamellae	28	29	29	29	29	29	28	29	27	27	27	27	28
Gular marking (1) or absent (0)	1	1	1	1	1	1	1	1	1	1	1	1	1
tan coloration. THNHM 28202 (adult female) and THNHM 28203 and THNHM 28205 (two adult males) have broken tails. THNHM 28205 (adult male) is an adult male with five continuous precloacal pores. Meristic and mensural variation within the type series are presented in Table 5.

**Distribution and natural history.** *Cnemaspis tarutaoensis* sp. nov. is known only from the type locality on Tarutao Island, approximately 40 km off the coast of Thailand. All specimens were found in karst forest near mangroves and karst outcrops near a stream (Fig. 7). Nine specimens (ZMKU R 00759–00760, ZMKU R 00762–00763, ZMKU R 00765–00766, and THNHM 28202–28204) were collected during the day (1100–1805 h) and five specimens (ZMKU R 00758, ZMKU R 00761, ZMKU R 00764, THNHM 28201 and THNHM 28205) were collected during the night (1920–2106 h). The male holotype was found during the day (1724 h) upside down on the interior surface of the karst formation.

Paratypes found during the day (ZMKU R 00759 and 00760, ZMKU R 00762 and 00763, ZMKU R 00765–00766, and THNHM 28202–28204) were in shaded areas, cracks, and crevices of rock boulders. When disturbed, some individuals would retreat into cracks and crevices, or hide in shaded areas of the rock boulder. Paratypes found at night (ZMKU R 00758, ZMKU R 00761, ZMKU R 00764, THNHM 28201 and THNHM 28205) were in deep crevices, within cracks on the shaded (by day) surfaces of boulders, or perched on vegetation near karst. Three gravid females (ZMKU R 00758, ZMKU R 00760, and THNHM 28202) contained two eggs during November 2017. THNHM 28204 (juvenile) was observed on vegetation near a rock boulder on 5 April 2018. At night, *Cyrtodactylus* cf. *astrum* was found in syntopy on rock boulders and karst formations with *C. tarutaoensis* sp. nov.

Etymology. The specific epithet refers to the type locality of the new species.

**Comparisons.** Cnemaspis tarutaoensis sp. nov. can be distinguished from all other members of the *kumpoli* group (*C. biocellata*, *C. kumpoli*, *C. monachorum*, and *C. ni-yomwanae*) by having a maximum SVL of 36.4 mm (vs 32.9 mm in *C. monachorum*, 40.2 mm in *C. biocellata*, 63.0 mm in *C. kumpoli*, and 56.8 mm in *C. niyomwanae*).

*Cnemaspis tarutaoensis* sp. nov. is further distinguished from *C. monachorum* by having eight infralabial scales (vs 5–7 in *C. monachorum*). The new species is further distinguished from *C. biocellata, C. monachorum* and *C. niyomwanae* by having 4–5 precloacal pores (vs 6–12 in *C. biocellata* and three in *C. monachorum* and *C. niyomwanae*). The new species is further distinguished from *C. biocellata, C. kumpoli*, and *C. niyomwanae* by having 17–19 paravertebral tubercles (vs 21–27 in *C. biocellata,* 28–35 in *C. kumpoli* and 26–31 in *C. niyomwanae*). The new species is further distinguished from *C. biocellata* and *C. kumpoli* by lacking tubercles on lower flanks (vs present in *C. biocellata* and *C. niyomwanae* by having 26–29 lamellae under the 4<sup>th</sup> toe (vs 29–37 in *C. biocellata,* 34–41 in *C. kumpoli*, and 31–34 in *C. niyomwanae*).

Cnemaspis tarutaoensis sp. nov. is further distinguished from C. kumpoli, C. monachorum and C. niyomwanae by having yellow coloration in the subcaudal region and wide black



**Figure 7.** Habitats of *Cnemaspis tarutaoensis* sp. nov. **A** Pha Toe Boo karst formation at type locality **B** habitat of paratypes in the exterior surface of karst cave at Tham Chorakae **C** habitat of paratypes in karst outcropped at Tarutao Island, Mueang Satun district, Satun Province, Thailand.

and yellow bands on tail (vs lacking in *C. kumpoli*, *C. monachorum*, and *C. niyomwanae*). The new species is further distinguished from *C. biocellata*, *C. kumpoli*, and *C. niyomwanae* by lacking a sexually dimorphic dorsal color pattern (vs present in *C. biocellata*, *C. kumpoli*, and *C. niyomwanae*). The new species is further distinguished from *C. monachorum* and *C. biocellata* by lacking lateral caudal tubercle row (vs present in *C. monachorum* and *C. biocellata*). The new species is distinguished from *C. biocellata*, *C. kumpoli*, and *C. niyomwanae* by having gular marking (vs lacking in *C. biocellata*, *C. kumpoli*, and *C. niyomwanae*).

## Cnemaspis adangrawi sp. nov.

http://zoobank.org/E783766E-6BA0-4F3D-A1BD-968C130AB52B Figures 8–10 Adang-Rawi Rock Gecko Thai name: Jing Jok Niew Yaow Ko Adang-Rawi

**Holotype** (Figs 8a, 9, 10). ZMKU R 00767, adult male from Thailand, Satun Province, Mueang Satun District, Tarutao National Park, Adang Island, Jonsalad Waterfall (6°30.7806'N, 99°18.0072'E; 84 m a.s.l.; Fig. 13A), collected on 9 November 2017 by Natee Ampai, Attapol Rujirawan, Siriporn Yodthong, and Korkwan Termprayoon.



**Figure 8.** *Cnemaspis adangrawi* sp. nov. from Adang Island, Mueang Satun District, Satun Province, Thailand **A** male holotype ZMKU R 00767 **B** female paratype ZMKU R 00768.

**Paratypes** (Figs 8b, 11, 12). Fourteen paratypes (adult males = 10, adult females = 4). ZMKU R 00768 (1 adult female), same locality and collectors as holotype. ZMKU R 00771 (1 adult female), and ZMKU R 00769–00770, THNHM 28206–28209 (6 adult males), same data as holotype except collected 6 April 2018. ZMKU R 00773, ZMKU R 00775, THNHM 28210 (3 adult males) and ZMKU R 00774 (1 adult female), same collectors as holotype except from Rawi Island (6°33.9084'N, 99°15.5088'E; 7 m a.s.l.; Fig. 13B), collected on 7 April 2018. ZMKU R 00776 (1 adult male) and THNHM 28211 (1 adult female), same collectors as holotype except from Rawi Island (6°33.3474'N, 99°15.0018'E; 7 m a.s.l.; Fig. 13C), collected on 8 April 2018.

**Referred specimens.** ZMKU R 00772 and THNHM 28212–28215 (five juveniles), same data as holotype except collected 6 April 2018.

**Diagnosis.** *Cnemaspis adangrawi* sp. nov. can be distinguished from all other *Cnemaspis* by having the following combination of characters: (1) adult males with maxi-

**Table 6.** Meristic character state and color pattern of species in the *Cnemapsis siamensis* group. Measurements are taken in millimeters and measurement abbreviations are defined in the text. – = data unavailable, w = weak.

						,				
Characters/Species	C. adangrawi sp. nov.	C. chanardi	C. huaseesom	C. omari	C. phangngaensis	C. punctatomuchalis	C. roticanai	C. siamensis	C. thachanaensis	C. vandeventeri
Sample size	15	25	5	8	2	5	8	12	6	3
Maximum SVL	44.9	40.9	43.5	41.3	42.0	49.6	47.0	39.7	39.0	44.7
Supralabial scales	10	8-10	7–10	8–9	10	8	8–9	8–9	10-11	8,9
Infralabial scales	9	8	6–9	7–8	10	7–8	7–8	6–8	9–11	7–9
Ventral scales keeled (1) or smooth (0)	1	1	0	1	1	0	1	1	1	1
No. of precloacal pores	6–8	6–8	5–8	3–6	4	0	3–6	0	0	4
Precloacal pore continuous (1) or separated (0)	0	0	1	0	1	-	0	-	_	0
Precloacal pores elongate (1) or round (0)	0	0	0	0	0	-	0	-	-	0
No. of paravertebral tubercles	23–25	22–25	18-24	22–29	22	24–27	25–27	19–25	15–19	25–29
Paravertebral tubercles linearly arranged (1) or more random (0)	0	0	w,0	w,0	1	w	0	0	1	0
Tubercles present (1) or absent (0) on lower flanks	0	1	1	w,1	0	1	1	1	1	1
No. of 4 <sup>th</sup> toe lamellae	26–28	26–29	21-31	25–28	29	29-31	26–29	24–26	24	24–28
Lateral caudal furrows present (1) or absent (0)	1	1	1	1	1	1	1	1	1	0
Subcaudal keeled (1) or smooth (0)	1	1	0	1	1	0	1	1	1	1
Enlarge median subcaudal scales row (1) or not (0)	0	1	0	0	0	1	w	1	0	1
No. of postcloacal tubercles in males	1	1	1,2	1	2	1–3	1-2	1-2	0	1–3
Subtibial scales keeled (1) or smooth (0)	1	1	0	1	1	1	1	1	1	1
Subcaudal region yellow present (1) or not (0)	1	1	1	1	1	0	1	0	0	0
Ventral pattern sexually dimorphic present (1) or not (0)	1	1	1	-	0	1	1	1	1	1
Dorsal color pattern sexually dimorphic (1) or not (0)	0	0	1	0	0	1	1	0	0	0
Lineate gular marking (1) or not (0)	0	0	0	0	0	-	0	1	1	0

mum SVL length 44.9 mm (mean 41.8  $\pm$  SD 2.5, n = 11) and females with maximum SVL 43.8 mm (mean 42.5  $\pm$  SD 1.5, n = 4); (2) 10 supralabials and 9 infralabials; (3) 6–8 pore-bearing precloacal scales with rounded pores arranged in chevron shape and separated; (4) 23–25 paravertebral tubercles randomly arranged; (5) 26–28 subdigital lamellae under the 4<sup>th</sup> toe; (6) subcaudal scales keeled and lacking enlarge median row; (7) one postcloacal tubercle each side; (8) gular region, abdomen, limbs and subcaudal region yellowish in males only; (9) mid-gular marking absent in males and females. These differences are summarized for geographically close congeners in the *siamensis* group (Table 6).

**Description of holotype.** Adult male; SVL 44.6 mm; head moderately sized (HL/ SVL 0.26), narrow (HW/SVL 0.15), flattened (HD/HL 0.38), and head distinct from



**Figure 9.** Male holotype of *Cnemaspis adangrawi* sp. nov. from Adang Island, Mueang Satun District, Satun Province, Thailand (ZMKU R 00767) in life. **A** ventral view **B** dorsal view **C** precloacal region showing distribution of pore-bearing scales (black arrows).

neck; snout moderate (ES/HL 0.47), snout slightly concave in lateral view; postnasal region constricted medially; scales of rostrum smooth, larger than conical scales on occiput; weak supraorbital ridges; lineate gular marking absent; gular and throat scales raised, keeled and round; shallow frontorostral sulcus; canthus rostralis nearly absent, smoothly rounded; eye large (ED/HL 0.20); pupil round; ear opening oval, taller than wide; rostral slightly concave; rostral bordered posteriorly by supranasals; 10, 10 (right, left) supralabials decreasing in size posteriorly; 9, 9 (right, left) infralabials decreasing in

size posteriorly; nostril elliptical, oriented posterodorsally, bordered by small postnasal scales; mental large, triangular, concave bordered posteriorly by three large postmentals.

Body slender, elongate (AG/SVL 0.42); small, keeled, dorsal scales equal in size throughout body intermixed with several large, keeled, multicarinate tubercles randomly arranged; 24 paravertebral tubercles; tubercles absent on lower flanks; tubercles extend from occiput to base of tail; dorsal scales raised and keeled; pectoral and abdominal scales keeled, round, flat to concave, slightly larger than dorsal and not larger posteriorly; ventral scales of brachia smooth, raised and juxtaposed; eight separated pore-bearing precloacal scales with rounded pores; precloacal depression absent; femoral pores absent.

Fore and hind limbs moderately long, slender; scales beneath forearm slightly raised, smooth and subimbricate; subtibial scales keeled; palmar scales smooth and juxtaposed; digits elongate, slender, inflected joint and bearing slightly recurved claws; subdigital lamellae unnotched; lamellae beneath first phalanges wide; lamellae beneath phalanx immediately following inflection granular; lamellae of distal phalanges wide; lamellae beneath inflection large; interdigital webbing absent; enlarged submetatarsal scales on 1<sup>st</sup> toe absent; fingers increase in length from first to fourth with fourth and fifth nearly equal in length; relative length of fingers IV>V>III>II>I; toes increase in length from first to fifth with fourth and fifth nearly equal in length; relative length of toes IV>V>III>III>I; total subdigital lamellae on 4<sup>th</sup> toe 28, 28 (right, left).

Caudal and subcaudal scales keeled, similar to dorsal scale size; lateral caudal furrow present; enlarge caudal tubercles arranged in segmented whorls, not encircling tail; enlarge median subcaudal scales row absent; caudal tubercles present on lateral furrow; tail length (TL) 58.3 mm with regenerated tail; enlarge, flat, postcloacal tubercle 1, 1 (right, left) on lateral surface of hemipenial swellings at the base of tail.

**Coloration in life** (Figs 8, 9). Dorsal ground color of head light brown, top of head bearing small, faint black and yellowish markings; thin, black postorbital stripes extending to nape; light-colored prescapular cresent; dorsal ground color of body, limbs and tail light brown with black irregular blotches; ground color of ventral surfaces grayish-white intermixed with yellowish blotches; ventral pattern sexually dimorphic, anterior gular region, abdominal region, and caudal region yellowish in males; two dark blotches on nape form a bipartite pattern; light sage vertebral blotches extending from the nape to tail; flanks with irregular incomplete brown to yellowish blotches becoming smaller posteriorly; tubercles on anterior and posterior of the body were white or yellow; widely separated, white or yellow tubercles occur on flanks; limbs beige with dark brown mottling; tail faintly marked with dark brown.

**Coloration in preservative** (Fig. 10). Color pattern similar to that in life with some fading of markings. Dorsal ground color of head, body, limbs and tail brown, darker with indistinct, irregular markings. All yellow coloration in gular region, ventral surfaces, flanks and tail faded to creamy white.

**Variation.** Most paratypes approximate the holotype in general aspects of morphology (Figs 11, 12), with most differences found in the degree of vertebral blotches. All adult female paratypes lack yellowish coloration in the gular, abdominal, and caudal regions. ZMKU R 00767, THNHM 28208, THNHM 28210, and ZMKU R



**Figure 10.** Holotype (adult male) of *Cnemaspis adangrawi* sp. nov. from Adang Island, Mueang Satun District, Satun Province, Thailand (ZMKU R 00767) in preservative. **A** ventral **B** dorsal views.

00776 (four adult males) have regenerated tails of uniform tan coloration. THNHM 28207–28209, ZMKU R 00773, and ZMKU R 00775 (five adult males) have lighter dorsal markings that appear more as transverse bands than as paravertebral blotches. THNHM 28211 (one adult female) has a broken tail. Differences in meristic and morphometrics within the type series are presented in Table 7.

**Distribution and natural history.** *Cnemaspis adangrawi* sp. nov. is known only from Adang and Rawi islands, 60 and 61 km off the coast of Thailand, respectively (Fig. 1). All Adang specimens were found in a granitic rocky stream (Fig. 13A). Rawi Island specimens were found in rock outcrops along a stream (Fig. 13B) and along a forest stream near mangroves (Fig. 13C). Sixteen specimens (ZMKU R 00767–00768, ZMKU R 00770–00772, ZMKU R 00775–00776, THNHM 28206–28209, and THNHM 28211–28215) were collected during the day (1047–1823 h) and four specimens (ZMKU R 00769, ZMKU R 00773–00774, and THNHM 28210) were collected at night (1927–2024 h). The male holotype was found during the day (1047–

	TERME		TTINITY.		THINHA	TUNE		ILZIMZ,	TERME	TUNUM.	TUTU	TUTINE	TATAT	TART	TUNUM.
	R 00767	R 00769	28206	28207	28208	R 00770	28209	R 00773	R 00775	28210	R 00776	R 00768	R 00771	R 0074	28211
Type series	Holotype	Paratype													
Sex	M	M	Μ	Μ	M	Μ	Μ	Μ	Μ	Μ	Μ	щ	ц	ц	ц
SVL	44.6	44.9	37.9	39.7	43.7	42.2	41.5	42.8	42.3	37.6	43.1	40.6	43.8	43.7	41.9
TT	58.3r	55.7	47.7	53.1	39.7r	56.5	49.8	56.2	51.0	45.6r	42.3r	50.5	50.3	42.6	40.5b
ML	4.3	4.3	3.9	3.8	4.3	4.1	4.0	4.1	4.0	3.8	4.2	4.0	4.1	4.3	4.1
FL	6.6	6.6	5.9	6.0	6.6	6.4	6.3	6.4	6.4	5.9	6.5	6.2	6.5	6.5	6.4
TBL	8.5	8.5	7.7	8.1	8.5	8.4	8.3	8.3	8.4	7.6	8.4	8.1	8.4	8.4	8.3
AG	18.8	18.7	15.2	17.2	18.5	18.3	18.3	18.4	18.3	15.2	18.5	18.3	18.6	18.6	18.4
HL	11.6	11.6	10.9	10.9	11.6	11.2	11.0	11.2	11.2	10.6	11.4	11.0	11.1	11.3	11.1
MM	7.0	7.0	6.4	6.5	7.0	6.8	6.7	6.8	6.8	6.4	6.9	6.6	6.7	6.9	6.8
HD	4.5	4.2	3.9	3.9	4.5	4.3	4.0	4.5	4.5	2.8	4.5	4.2	4.4	4.5	4.4
ED	2.4	2.4	2.1	1.9	2.4	2.2	2.1	2.3	2.3	2.0	2.4	2.2	2.4	2.4	2.2
EE	3.7	3.8	3.2	3.3	3.6	3.8	3.6	3.8	3.7	3.3	3.7	3.5	3.8	3.8	3.7
ES	5.5	5.4	4.5	4.6	5.2	5.2	5.0	5.1	5.1	4.4	5.1	5.0	5.2	5.2	5.0
EN	4.4	4.3	3.3	3.4	4.1	4.0	4.0	4.2	4.1	3.2	4.2	4.2	4.1	4.2	4.1
IO	2.9	2.9	2.6	2.6	2.9	2.8	2.7	2.8	2.8	2.7	2.8	2.7	2.9	2.8	2.7
EL	6.0	0.9	0.8	0.8	0.9	0.9	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.9	0.8
NI	0.9	0.9	0.9	0.0	0.9	0.9	0.9	0.9	0.8	0.8	0.9	0.8	0.8	0.0	0.9
Supralabials	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Infralabials	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
No. of precloacal pores	8	9	9	6	6	9	6	8	9	8	9	I	I	I	I
Precloacal pore continuous (1) or	0	0	0	0	0	0	0	0	0	0	0	I	I	I	1
separated (0)															
No. of paravertebral tubercles	24	25	25	24	25	24	25	23	24	23	25	25	25	23	23
No. of 4th toe lamellae	28	28	27	28	28	27	28	27	27	27	28	26	28	26	28

**Table 7.** Descriptive measurements in millimeters and characters of the type series of *Cnemaspis adangrawi* sp. nov. M = male; F = female; - = data unavailable or absent; b = broken; r = regenerated. h) on the base of a rock boulder with holes formed by the expansive soil between the ground and rock interface of a nearby stream.

Paratypes found during the day (ZMKU R 00767–00768, ZMKU R 00770– 00772, ZMKU R 00775–00776, THNHM 28206–28209, and THNHM 28211– 28215) were in crevices of boulders, shaded areas with holes in the soil at the base of a rock wall near a stream, and on boulder outcrops near streams. When disturbed, some individuals would retreat into rock crevices or into holes in the soil at the base of a rock wall. Paratypes found at night (ZMKU R 00769, ZMKU R 00773–00774 and THNHM 28210) were in shaded areas (by day), deep at the base of boulders, or perched on vegetation near a rocky stream. Two gravid females (ZMKU R 00771 and THNHM 28211) contained one or two eggs during November 2017. Some juveniles (not collected) were found in holes in the soil and perched on vegetation near a stream at Rawi Island on 8 April 2018. At night, *Cyrtodactylus macrotuberculatus* was found in syntopy on the rock wall and vegetation near a stream at Jonsalad Waterfall, Adang Island, with *Cnemaspis adangrawi* sp. nov.

**Etymology.** The specific epithet refers to Adang and Rawi islands where the new species is found, and is a noun in apposition.

**Comparisons.** Cnemaspis adangrawi sp. nov. can be distinguished from other members of the siamensis group (C. chanardi, C. huaseesom, C. omari, C. phangngaensis, C. punctatonuchalis, C. roticanai, C. siamensis, C. thachanaensis, and C. vandeventeri) by having a smaller maximum SVL of 44.9 mm (vs 47.0 mm in C. roticanai, 49.6 mm in C. punctatonuchalis) and by having a larger maximum SVL 44.9 mm (vs 40.9 mm in C. chanardi, 43.5 mm in C. huaseesom, 41.3 mm in C. omari, 42.0 mm in C. phangngaensis, 39.7 mm in C. siamensis, 39.0 mm in C. thachanaensis, and 44.7 mm in C. vandeventeri).

Cnemaspis adangrawi sp. nov. is distinguished from C. omari, C. punctatonuchalis, C. roticanai, C. siamensis, and C. vandeventeri by having 10 supralabial scales (vs eight in C. punctatonuchalis and 8–9 in C. omari, C. roticanai, C. siamensis, and C. vandeventeri). This species is distinguished from C. chanardi, C. omari, C. phangngaensis, C. punctatonuchalis, C. roticanai, and C. siamensis by having 9 infralabial scales (vs 8 in C. chanardi, 7–8 in C. omari, C. punctatonuchalis, C. roticanai, 10 in C. phangngaensis, and 6–8 in C. siamensis). This species is distinguished from C. huaseesom and C. punctatonuchalis by having keeled ventral and subcaudal scales (vs smooth ventral and subcaudal scales in C. huaseesom and C. punctatonuchalis).

*Cnemaspis adangrawi* sp. nov. is distinguished from *C. phangngaensis* and *C. vandeventeri* by having 6–8 precloacal pores (vs 4 in *C. phangngaensis* and *C. vandeventeri*). This species is distinguished from *C. punctatonuchalis*, *C. siamensis*, and *C. thachanaensis* by presence of precloacal pores (vs precloacal pores absent in *C. punctatonuchalis*, *C. siamensis*, and *C. thachanaensis*). This species is distinguished from *C. huaseesom* and *C. phangngaensis* by having a separated row of precloacal pores (vs continuous in *C. huaseesom* and *C. phangngaensis*).

*Cnemaspis adangrawi* sp. nov. is distinguished from *C. phangngaensis* and *C. thachanaensis* by having 23–25 paravertebral tubercles (vs 22 in *C. phangngaensis* and 15–19 in *C. thachanaensis*). This species is distinguished from *C. huaseesom*, *C. omari*, *C. punc*-



**Figure 11.** Male paratypes of *Cnemaspis adangrawi* sp. nov. in preservative. **A** dorsal view **B** ventral view; from left to right, top panel: ZMKU R 00769, ZMKU R 00770, ZMKU R 00773, ZMKU R 00775, and ZMKU R 00776; bottom panel: THNHM 28206, THNHM 28207, THNHM 28208, THNHM 28209, and THNHM 28210.



**Figure 12.** Female paratypes of *Cnemaspis adangrawi* sp. nov. in preservative. **A** dorsal view **B** ventral view; from left to right: ZMKU R 00774, THNHM 28211, ZMKU R 00768, and ZMKU R 00771.

*tatonuchalis, C. roticanai, C. siamensis, C. thachanaensis,* and *C. vandeventeri* by lacking tubercles on lower flanks (vs present in *C. huaseesom, C. omari, C. punctatonuchalis, C. roticanai, C. siamensis, C. thachanaensis,* and *C. vandeventeri*). This species is distinguished from *C. phangngaensis, C. punctatonuchalis,* and *C. thachanaensis* by having 26–28 lamellae under 4<sup>th</sup> toe (vs 29 in *C. phangngaensis,* 29–31 in *C. punctatonuchalis,* and 24 in *C. thachanaensis*). This species is distinguished from *C. chanardi* and *C. vandeventeri* by having lateral caudal furrows (vs lacking in *C. chanardi* and *C. vandeventeri*).



**Figure 13.** Habitats of *Cnemaspis adangrawi* sp. nov. **A** Jonsalad Waterfall at type locality of Adang Island **B** habitat of paratypes in outcropped near stream at Rawi Island **C** habitat of paratypes in forest stream near mangrove at Rawi Island, Mueang Satun district, Satun Province, Thailand.

Cnemaspis adangrawi sp. nov. can be further distinguished from C. chanardi, C. punctatonuchalis, C. siamensis, and C. vandeventeri by lacking enlarged median subcaudal scales (vs present in C. chanardi, C. punctatonuchalis, C. siamensis, and C. vandeventeri). This species is distinguished from C. huaseesom by having keeled subtibial scales (vs smooth subtibial scales in C. huaseesom). This species is distinguished from C. siamensis and C. thachanaensis by lacking lineate gular marking (vs present in C. siamensis and C. thachanaensis).

## Discussion

Studies on the taxonomy and systematics of *Cnemaspis* in Southeast Asia have increased in the past two decades (Bauer and Das 1998; Das 2005; Bauer et al. 2007; Grismer and Chan 2010; Wood et al. 2013; Grismer et al. 2014; Iskandar et al. 2017; Wood et al. 2017). Integrative taxonomic approaches that incorporated both morphological and molecular data have been especially useful in uncovering cryptic diversity of Thai *Cnemaspis* (Grismer et al. 2014; Wood et al. 2017). Our descriptions of *C. tarutaoensis* sp. nov. and *C. adangrawi* sp. nov. bring the total number of *Cnemaspis* to 59 species, of which 18 occur in Thailand.

Previously, the reported geographic distribution of *Cnemaspis* in Thailand was mostly restricted to the mainland (Smith 1925; Taylor 1963; Bauer and Das 1998; Grismer et al. 2010; Wood et al. 2017), with insular populations of Cnemaspis known only from four localities in Thailand, including Samui, Phangan and Ko Tao islands, Surat Thani Province (Gulf of Thailand; approximately 85 km offshore the mainland of Mueang Chumphon District, Chumphon Province; Grismer et al. 2010, 2014) and Phuket Island, Phuket Province (Andaman Sea; approximately 30 km offshore the mainland of Takua Thung District, Phangnga Province; Das and Leong 2004). The descriptions of C. tarutaoensis sp. nov. and C. adangrawi sp. nov. double the number of Cnemaspis species known from islands in Thailand from two (C. chanardi and C. siamensis) to four. These two new species occur on Tarutao, Adang and Rawi islands in the Andaman Sea, offshore of the Thai mainland of Mueang Satun District, Satun Province (at approximately 40 km, 60 km, and 61 km, respectively). These islands were connected to the mainland during the last glacial maximum as recently as 21,000 years before present (Voris 2000; Sathiamurthy and Voris 2006), but the timing of their divergence from other *Cnemaspis* species remains untested.

The complex geological history in Thailand created a large number of limestone and granitic formations in southern Thailand (Day and Urich 2000; Morley et al. 2011). The karst regions and granitic rocky streams of southern Thailand are proving to harbor a high diversity of range-restricted species of geckos (Smith 1925; Taylor 1963; Grismer et al. 2010, 2014; Wood et al. 2017). Further research and additional field surveys in unexplored karst regions on islands and the mainland are needed to better understand the taxonomy, ecology, distribution, biogeography, and conservation of *Cnemaspis* in southern Thailand.

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# Appendix I

List of comparative specimens examined.

- *Cnemaspis chanardi*: Thailand, Trang Province, Nayong District, Ban Chong: THN-HM 06983 (male holotype); Krabi Province, Klong Thom District: THNHM 012439–012440 (males); Mueang Krabi District: THNHM 012436 and 012437 (males), THNHM 012438 (female); Nakhon Si Thammarat Province, Tha Sala District: THNHM 020992 (male); Lansaka district: THNHM 014111 (immature male); Noppitam district: THNHM 013838 (male), THNHM 010705 (male); Surat Thani Province, Ang Thong Island, Mueang Surat Thani District: THNHM 016074 (female).
- *Cnemaspis huaseesom*: Thailand, Kanchanaburi Province, Sai Yok District, Sai Yok National Park: THNHM 15909 (male holotype).
- *Cnemaspis niyomwanae*: Thailand, Trang Province, Palean District, Thum Khao Ting: THNHM 15909 (female holotype).
- *Cnemaspis punctatonuchalis*: Thailand, Prachuap Khiri Khan Province, Thap Sakae District, Huay Yang National Park: THNHM 02001 (male holotype)
- Cnemaspis siamensis: Thailand, Nakhon Si Thammarat Province, Lansaka District: THNHM 013828 (male); Tha Sala District: THNHM 018265 (male); Phetchabun Province, Nam Nao District: THNHM 01336 (female), THNHM 01337 (male); Phetchaburi Province, Cha-am District: THNHM 01448 (male), THN-HM 01449 (immature male); Chumpon Province, Mueang Chumpon District: THNHM 0372 (male); Phato District: THNHM 01086 (male); Surat Thani Province, Vibhawadee District: THNHM 01084 (female); Ang Thong Island, Mueang Surat Thani District: THNHM 015624 (female).
- *Cnemaspis vandeventeri*: Thailand, Ranong Province, Kapur District, Klong Naka: THNHM 08261 (male holotype), THNHM 08260 (female).