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Review of the mudflat varunid crab genus *Metaplax* (Crustacea, Brachyura, Varunidae) from East Asia and northern Vietnam

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Abstract

Intertidal mudflat crabs of the genus *Metaplax* H. Milne Edwards, 1852 (Crustacea: Brachyura: Varunidae) from China, Taiwan, and northern Vietnam are taxonomically revised by morphological and molecular evidence. These crabs show sexual dimorphism and morphological variation of a considerable range in the infraorbital ridge, one of the primary features previously used for species identification. In this study, four species were identified from the region: *M. elegans* De Man, 1888; *M. longipes* Stimpson, 1858; *M. sheni* Gordon, 1930; and *M. tredecim* Tweedie, 1950. Based on the results of the morphological examination, and as confirmed by molecular evidence from mitochondrial cytochrome oxidase subunit I (COI), taxonomic confusion surrounding *M. longipes* was resolved, and *M. takahasii* Sakai, 1939, is considered a junior synonym of *M. longipes*. The geographical distribution of *Metaplax longipes* extends along the shores of China, north to Jiangsu, whereas the Southeast Asian *M. tredecim* was newly recorded from northern Vietnam and Hong Kong.

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* These authors contributed equally to this paper

Keywords
COI, *Metaplax elegans*, *M. longipes*, *M. sheni*, *M. takahasii*, *M. tredecim*, mitochondrial cytochrome oxidase subunit I, morphology

Introduction

Indo-West Pacific varunid crabs of the genus *Metaplax* H. Milne Edwards, 1852 commonly inhabit sheltered shores with silty muddy substrate often near or under shades of mangroves in tropical and subtropical regions. While some studies have reported on various biological aspects of selected species (e.g., Macnae 1963; Beinlich and Polivka 1989; Chakraborty and Choudhury 1994), the phylogenetic position of this group within the Thoracotremata remains obscure due to limited taxon sampling (see Kitaura et al. 2002; Chen et al. 2019; Liu et al. 2019).

Species of the genus *Metaplax* all share a broad, subquadrate, somewhat depressed carapace, which is shallowly marked, broad fronted (approximately 1/3 carapace width), and has lateral margins bearing at most five distinct teeth; slender and elongated ambulatory legs are also shared. One of the frequently used morphological features for species identification remains the number of lobes and tubercles along the infraorbital ridge (Tesch 1918; Tweedie 1950; Dai et al. 1986; Dai and Yang 1991), which are sexually dimorphic as in many varunid groups. The infraorbital tubercles are reported to serve a stridulatory function, which engage with the ridge along the anterior margin of the chelipedal merus (Macnae 1963; Beinlich and Polivka 1989). *Metaplax* contains around 12 species (Ng et al. 2008; but see Naderloo 2011 on the identity of *M. indicus occidentalis* Pretzmann, 1971), with an overall distribution extending from the shores of the Persian Gulf, the Arabian Sea, the Bay of Bengal to Southeast and East Asia, and easternmost to western Taiwan. Six species, namely *M. elegans* De Man, 1888; *M. gocongensis* Davie & Nguyen, 2003; *M. longipes* Stimpson, 1858; *M. sheni* Gordon, 1930; *M. takahasii* Sakai, 1939; and *M. tredecim* Tweedie, 1950, have been recorded from East and Southeast Asia, with only *M. elegans* recorded in the eastern Indian Ocean as well (De Man 1888; Ng and Davie 2002; Dev Roy and Bhadra 2011).

In East Asia, the northern limit of this genus appears to be around Zhejiang, China (Dai et al. 1986; Chen 1991; Dai and Yang 1991). *Metaplax* is absent from the east coast of Taiwan, the Ryukyus, the main islands of Japan, and Korea (Sakai 1939, 1940, 1976). Five species were previously reported from the region, including *M. elegans* De Man, 1888, *M. longipes* Stimpson, 1858, *M. sheni* Gordon, 1930, *M. takahasii* Sakai, 1939, and *M. tredecim* Tweedie, 1950. Among these, *M. takahasii* has been considered a junior synonym of *M. longipes* (see Davie and Nguyen 2003), whereas tropical *M. tredecim* had been first listed as part of the fauna of the East China and South China seas by Yang et al. (2008) without any illustration or elaboration.

In the present study, specimens of species of *Metaplax* were collected from various sites in East Asia (Fig. 1), their morphological features are examined and illustrated, and identifications are confirmed by molecular evidence from mitochondrial cytochrome oxidase subunit I (COI).

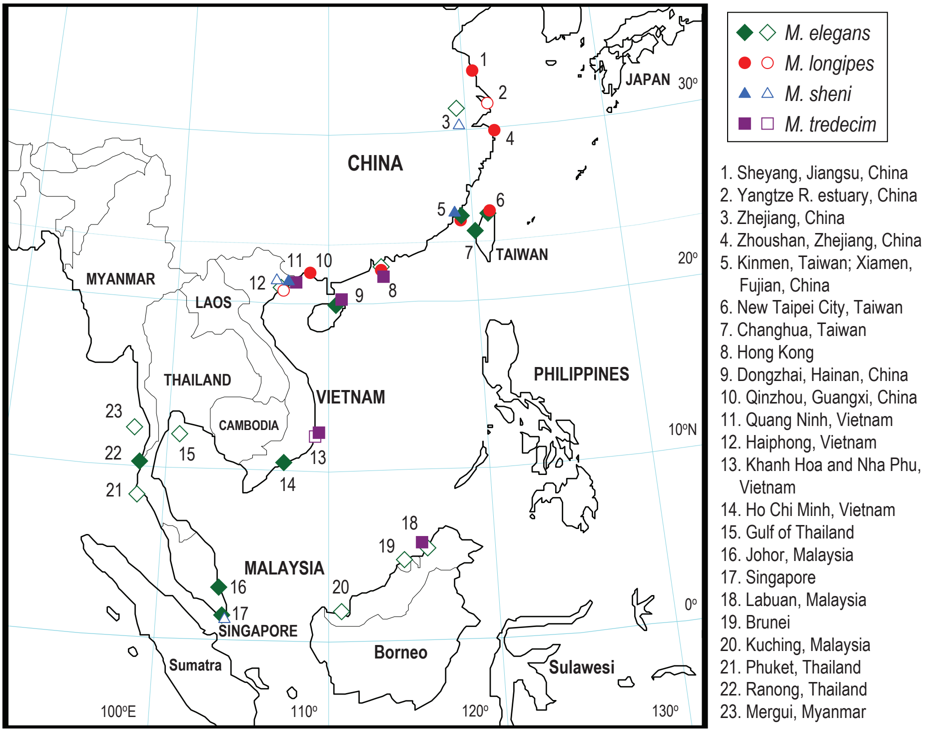

Figure 1. Collection sites (solid symbols) for species of the genus *Metaplax* used in this study: green rhombus for *M. elegans*; red circles for *M. longipes*; blue triangles for *M. sheni*; and purple squares for *M. tredecim*. Empty symbols mean the additional records from references (see synonym lists for each species).

Materials and methods

Specimens were collected from China, Taiwan, and Vietnam (Table 1, Fig. 1) and have been deposited in the Kanagawa Prefectural Museum of Natural History, Kanagawa, Japan (KPM-NH); the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOOL), and the Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore, Singapore (ZRC). The abbreviation G1 is used for male first gonopods. Measurement is of the maximum carapace width (CW) in millimeters.

To understand whether the number of infraorbital tubercles and lobes of each species is related to the sex and body size, the numbers on both sides for each specimen were calculated, averaged, and plotted against CWs. Specimens used were 21 males (CW 7.7–15.9 mm) and 19 females (CW 6.3–13.2 mm) for *M. elegans*, 69 males (CW 6.8–26.6 mm) and 29 females (CW 7.9– 23.6 mm) for *M. longipes*, 7 males (CW 8.6–12.8 mm) for *M. sheni* (no female specimen), and 23 males (CW 12.8–22.7 mm) and 13 females (CW 10.3–23.4 mm) for *M. tredecim*.

Genomic DNA was isolated from the muscle tissue using extraction kits following Shih et al. (2016). A portion of the *COI* gene was amplified with PCR using the primers

Table 1. The haplotypes and accession numbers (DNA Data Bank of Japan) of the COI gene of *Metaplax* specimens and outgroups from East Asia and northern Vietnam. For abbreviations of museums and universities, see Materials and methods.

Species	Locality	Sample size	Catalogue no. of NCHUZOOL (unless indicated)	Haplotype of COI	Access. no. of COI
M. elegans	Taiwan: Jhuwei, New Taipei City	1	15480	MXe1	LC498179
	Taiwan: Kinmen	1	15489	MXe2	LC498180
	Vietnam: Ho Chi Minh City	1	15499	MXe3	LC498181
	Singapore: Sungei Buloh	1	ZRC 1997.683	MXe4	LC498182
	Thailand: Ranong	1	15494	MXe4	LC498182
M. longipes	Taiwan: Danshuei, New Taipei City	1	NTOU	MXL2	LC498183
	Taiwan: Danshuei, New Taipei City	1	ZRC 1999.0708	MXL2	LC498183
	Taiwan: Kinmen	1	15460	MXL2	LC498183
	Taiwan: Kinmen	1	15462	MXL2	LC498183
	China: Zhoushan, Zhejiang	2	15466; 15465	MXL2	LC498183
	China: Xiamen, Fujian	1	15475	MXL2	LC498183
	China: Qinzhou, Guangxi	1	15449	MXL3	LC498185
	Hong Kong: Tung Chung	1	15450	MXL1	LC498184
M. sheni	Taiwan: Kinmen	1	15467	MXs1	LC498186
	China: Xiamen, Fujian	2	15465	MXs1, MXs2	LC498186, LC498187
	Vietnam: Dong Rui, Quang Ninh	1	15466	MXs1	LC498186
M. tredecim	Hong Kong: Nai Chung	1	15472	MXt1	LC498188
	Vietnam: Dong Rui, Quang Ninh	1	15477	MXt2	LC498189
	Vietnam: Nha Trang	1	15498	MXt3	LC498190
	Malaysia: Labuan	1	15475	MXt4	LC498191
Total		**22**			
Outgroups					
Gaetice depressus	Taiwan: Keelung		15544		LC498192
Helice formosensis	Taiwan: Shengang, Changhua		13083		AB334543
Hemigrapsus sanguineus	Taiwan: Yongsing, New Taipei City		15545		LC498193
Varuna litterata	Taiwan: Kenting, Pingtung		14816		LC498194

LCO1490 and HCO2198 (Folmer et al. 1994). PCR conditions for the above primers were denaturation for 50 s at 94 °C, annealing for 70 s at 45–47 °C, and extension for 60 s at 72 °C (40 cycles), followed by extension for 10 min at 72 °C. Sequences were obtained by automated sequencing (Applied Biosystems 3730), after verification with the complementary strand. Sequences of the different haplotypes have been deposited in the DNA Data Bank of Japan (DDBJ) (accession numbers are shown in Table 1). Outgroups were selected based on the phylogenetic tree of Kitaura et al. (2002: Fig. 2), as follows: *Gaetice depressus* (De Haan, 1835); *Helice formosensis* Rathbun, 1931; *Hemigrapsus sanguineus* (De Haan, 1835); and *Varuna litterata* (Fabricius, 1798).

The best-fitting model of sequence evolution was determined by PartitionFinder (ver. 2.1.1; Lanfear et al. 2017) and selected by the Bayesian information criterion (BIC). The obtained best model (GTR + I + G) was subsequently used for a Bayesian inference (BI) analysis. BI analysis was performed with MrBayes (ver. 3.2.3, Ronquist et al. 2012). Phylogenetic analysis was performed with four chains for 10 million generations and four independent runs, with trees sampled every 1000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended 0.01 (Ronquist et al. 2019), and the first 1000 trees were discarded as the burnin accordingly. Maximum likelihood (ML) analysis was conducted using RAxML (vers. 7.2.6, Stamatakis 2006). The model GTR + G (i.e. GTRGAMMA) was used with 100 runs and finding the best ML tree by comparing the likelihood scores. The robustness of the ML tree was evaluated by 1000 bootstrap pseudoreplicates under the model GTRGAMMA. Base pair (bp) differences and pairwise estimates of Kimura 2-parameter (K2P) distances (Kimura 1980) for genetic diversities between specimens were calculated with MEGA (ver. 10.0.5, Kumar et al. 2018).

Results

Systematics

Family Varunidae H. Milne Edwards, 1853
Genus *Metaplax* H. Milne Edwards, 1852

Metaplax elegans De Man, 1888

Figures 2A–C, 3, 7A–D

Metaplax elegans De Man, 1888: 164, pl. 11(4–6) (type locality: Mergui, Myanmar); Alcock 1900: 434 (East India: Godavari Delta; Myanmar: Mergui); Gordon 1931: 528 (Hong Kong); Rathbun 1931: 100 (China: Fujian; Guangdong); Tweedie 1936: 69 (Malaysia: Selangor; Singapore); Shen 1940a: 74, 95 (China: Zhejiang; Fujian); Shen 1940b: 236 (Hong Kong); Tweedie 1950: 353 (Malaysia: Labuan; Kuching); Macnae 1963: 104, 180 (list); Dai et al. 1986: 509, fig. 289 (1–2), pl. 72(5) (China: Guangdong); Fukui et al. 1989: 230, fig. 25 (W Taiwan); Dai and Yang 1991: 557, fig. 289 (1–2), pl. 72(5) (China: Guangdong); J.-T. Shih et al. 1991: 126 (Taiwan: New Taipei City); Davie 1992: 352, pl. 2B (Hong Kong); Choy and Booth 1994: 243 (Brunei); Huang 1994: 598 (list; China); Tan and Ng 1994: 82 (Singapore; Malaysia); Kuo 1995: 31, 82, 97, 191, 4 unnumbered figs (W Taiwan); Wang and Liu 1996a: 128, figs 171–172 (W Taiwan); Wang and Liu 1996b: 103–104, 2 unnumbered figs (W Taiwan); Fransen et al. 1997: 125 (syntype; Mergui, Myanmar); Ho and Hung 1997: 108–109, 1 unnumbered fig.; Kosuge et al. 1997: 182 (Vietnam: Haiphong); Jeng et al. 1998: 68, 3 unnumbered figs (Taiwan: Taichung); Wang and Liu 1998a: 128, figs 171–172 (W Taiwan); Wang and Liu 1998b: 142, 2 unnumbered figs (W Taiwan); Lee and Leung 1999: 68, pl. 11 (Hong Kong); Ng and Sivasothi 1999: 73, 2 unnumbered figs (Singapore); Jeng and Wang 2000: 38, 2 unnumbered figs (Taiwan: Taichung); Lee and Tung 2000: 70 (list); Dev Roy and Bhadra 2011: 36 (list; E India); Lee 2001:

114, 3 unnumbered figs (W Taiwan); Ng et al. 2001: 45 (list; Taiwan); Kitaura et al. 2002: 684 (Vietnam: Haiphong); Ng and Davie 2002: 379 (Thailand: Phuket); Wang and Liu 2003: 128, figs 171–172 (W Taiwan); Naiyanetr 2007: 112 (list: Gulf of Thailand); Dev Roy 2008: 135 (list; India); Huang 2008: 668 (list; China); Ng et al. 2008: 226 (list); Yang et al. 2008: 803 (list; East and South China seas); Rath and Dev Roy 2008: 72, pl. 4(5) (NE India); Liu and Wang 2010: 72, 3 unnumbered figs (W Taiwan); Lee et al. 2013: 108, 2 unnumbered figs (Taiwan: Tainan); Ng et al. 2017: 110 (list; Taiwan).

Materials examined. Taiwan: 8 ♂♂ (6.1–14.0 mm), 4 ♀♀ (8.0–13.2 mm) (NCHUZOOL 15479), Danshuei River mangroves, New Taipei City, coll. P.-Y. Hsu and J.-W. Hsu, 24 Mar. 2017; 4 ♂♂ (11.8–15.9 mm), 4 ♀♀ (14.1–15.3 mm) (NCHUZOOL 15480), Jhuwei, New Taipei City, 4 Oct. 1995; 1 ♂ (12.1 mm) (NCHUZOOL 15481), Sinfeng, Hsinchu, 15 Jan. 2014; 2 ♂♂ (8.4–10.5 mm), 5 ♀♀ (6.3–12.1 mm) (NCHUZOOL 15482), Siangshan, Hsinchu, 11 Aug. 2008; 1 ♂ (13.2 mm), 5 ♀♀ (8.7–9.7 mm) (NCHUZOOL 15483), Siangshan, Hsinchu, 13 Mar. 2008; 4 ♂♂ (10.1–11.3 mm), 1 ♀ (11.7 mm) (NCHUZOOL 15484), Haishangu, Hsinchu, 27 Aug. 2013; 1 ♂ (10.8 mm), 3 ♀♀ (8.8–11.6 mm) (NCHUZOOL 15485), Wufu Bridge, Miaoli, 2 Dec. 2015; 4 ♂♂ (12.0–15.1 mm) (NCHUZOOL 15486), Fangyuan, Changhua, 2014; 4 ♂♂ (10.0–12.2 mm), 6 ♀♀ (8.1–12.1 mm) (NCHUZOOL 15487), Yuliao R. estuary, Changhua, coll. J.-W. Hsu et al., 16 Jan. 2017; 4 ♂♂ (5.3–10.6 mm), 3 ♀♀ (10.2–12.9 mm) (NCHUZOOL 15488), area between Yunlin and Chiayi, coll. K.-C. Li and C.-T. Wang, 25 Aug. 2003; 1 ♂ (12.7 mm) (NCHUZOOL 15496), Dongshih, Chiayi County, coll. P.-Y. Hsu, 24 Jan. 2017; 4 ♂♂ (11.5–13.1 mm), 3 ♀♀ (6.7–10.7 mm) (NCHUZOOL 15489), Cihhu, Kinmen, coll. H.-T. Shih and P.-Y. Hsu, 29 June 2018. **China:** 3 ♀♀ (14.0–16.8 mm) (NCHUZOOL 15495), Dongzhai Harbor, Hainan, 23 June 2004; 3 ♀♀ (13.9–16.6 mm) (NCHUZOOL 15457), Dongzhai Harbor, Hainan, 23 June 2004. **Vietnam:** Ho Chi Minh: 8 ♂♂ (5.8–16.5 mm), 9 ♀♀ (6.5–13.7 mm) (NCHUZOOL 15490), Rung Sac, Long Hoa, 12 Oct. 2017; 1 ♂ (18.2 mm), 1 ♀ (12.7 mm) (NCHUZOOL 15491), TT. Can Thanh mangroves, Can Gio, 13 Oct. 2017; 5 ♂♂ (16.0–17.6 mm) (NCHUZOOL 15499), TT. Can Thanh mangroves, Can Gio, 13 Oct. 2017. **Malaysia:** 7 ♂♂ (7.7–12.6 mm), 14 ♀♀ (8.9–12.0 mm) (NCHUZOOL 15492), Mersing, Johor, 19 July 2010. **Singapore:** 1 specimen (not examined, only for DNA study; ZRC 1997.683), Sungei Buloh, 1996; 2 ♂♂ (9.4–11.0 mm), 1 ♀ (6.4 mm) (NCHUZOOL 15493), Lim Chu Kang, 4 Mar. 2012. **Thailand**: 1 ♀ (10.71 mm) (NCHUZOOL 15494), Ranong mangroves, 27 May 2012.

Diagnosis. Carapace (Figs 2A, 3A, F) trapezoidal, 1.45 times as broad as long (N = 127, SD = 0.06), longitudinally convex, broadest between lateral teeth 2 (exorbital angle included), surface sparsely but regularly furnished with short, stiff setae; front broad, divided into two broad lobes, medially concave; lateral margins markedly converging posteriorly, interrupted by four notches, delineating five teeth (including exorbital angle), exorbital angle most distinct, posterior two indistinct; posterolateral facet faintly defined, decorated by two oblique granular ridges. Infraorbital ridge

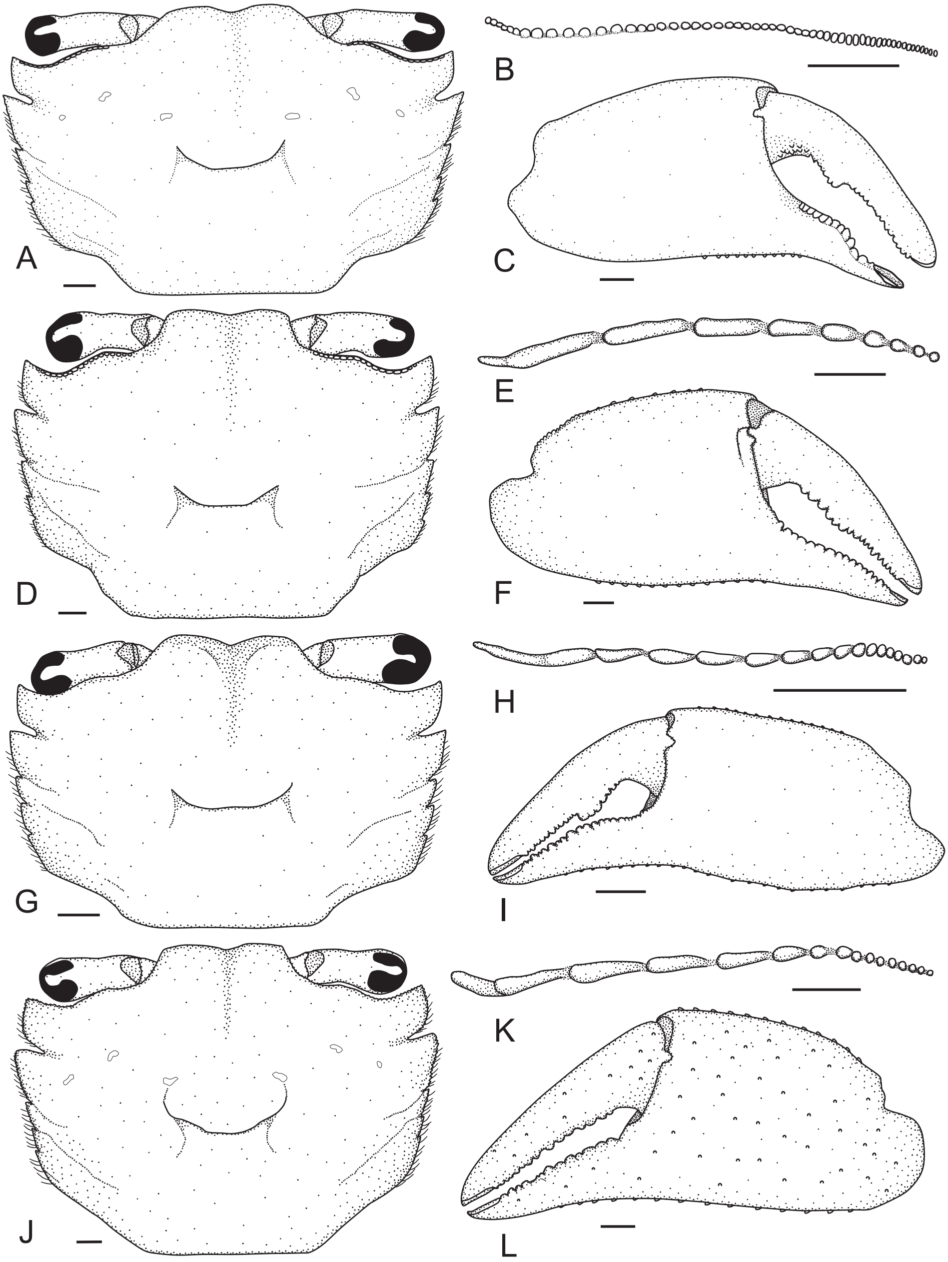

Figure 2. *Metaplax elegans* De Man, 1888 (**A–C** NCHUZOOL 15496, male, 12.7 mm), *M. longipes* Stimpson, 1858 (**D–F** ZRC 2019.0581, male, 14.9 mm), *M. sheni* Gordon, 1930 (**G–I** NCHUZOOL 15466, male, 9.9 mm), and *M. tredecim* Tweedie, 1950 (**J–L** paratype, ZRC 1964.7.14.4-18, 16.2 mm). **A, D, G, J, A** carapace **B, E, H, K** left infraorbital ridge **C, F, I, L** outer view of right cheliped. Scale bars: 1.0 mm.

(Figs 2B, 3C) distinctly sexual dimorphic, males with 47–61 tubercles, medial seven closely set, almost fusing, lateral ca. 20 tubercles slightly vertically elongated; females with 33–42 isomorphic tubercles. Chelipeds (Figs 2C, 3D) symmetrical, in males more

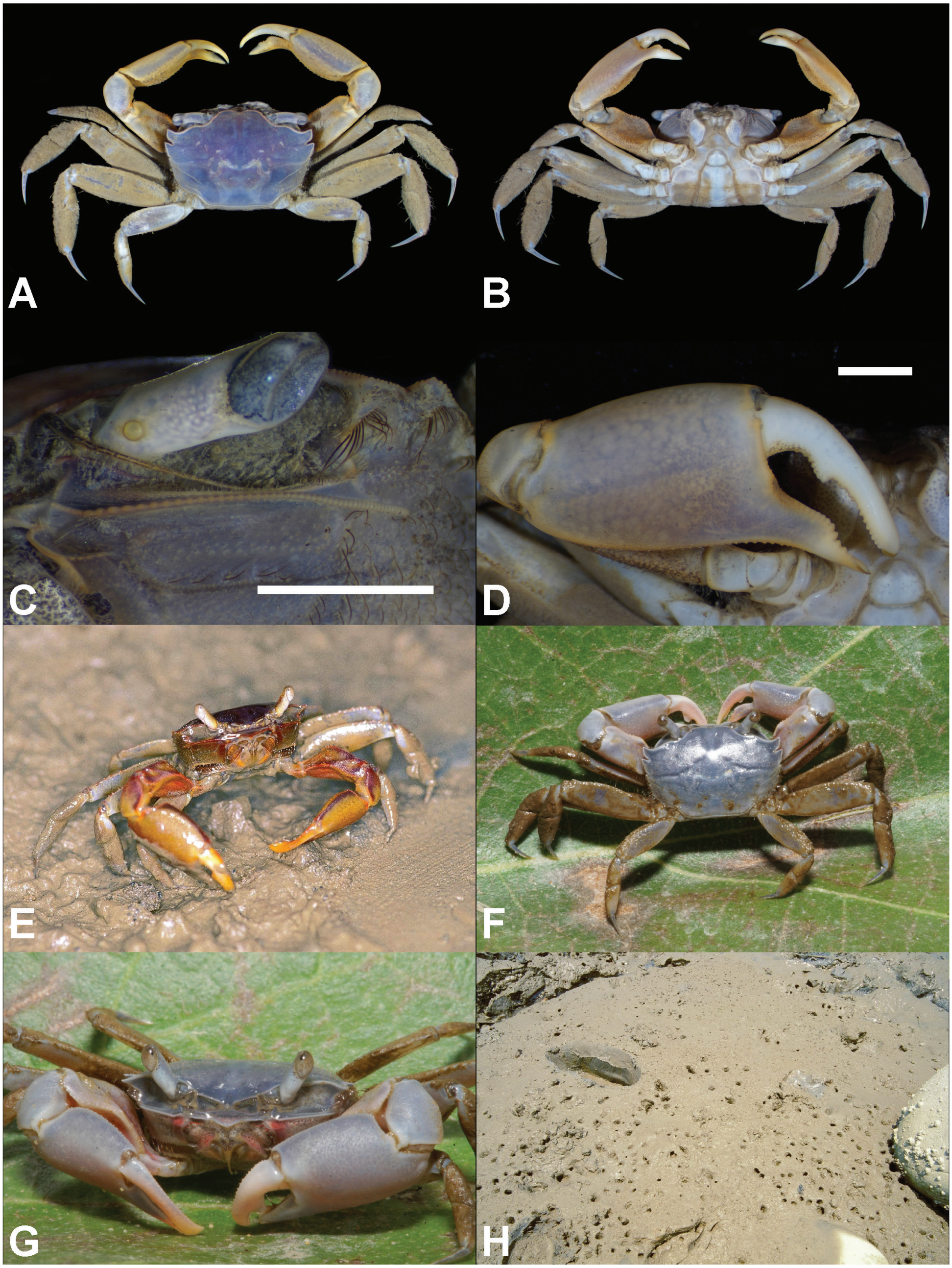

Figure 3. *Metaplax elegans* De Man, 1888. **A** Dorsal view **B** ventral view **C** left infraorbital ridge **D** right cheliped **A–D** male (CW 12.7 mm; NCHUZOOL 15496; Dongshih, Chiayi County, Taiwan) **E, F** live coloration **E** photo taken in Gaomei, Taichung, Taiwan (specimen not collected) **F–G** male (CW 13.1 mm; NCHUZOOL 15489; Kinmen, Taiwan) **G** the typical habitat (Gaomei, Taichung, Taiwan). Scale bars: 2.0 mm.

Table 2. Comparison of characters among four species of *Metaplax* from East Asia and northern Vietnam.

Characters	*M. elegans*	*M. longipes*	*M. sheni*	*M. tredecim*
lateral margin	five teeth	five teeth	five teeth	five teeth
infraorbital ridge	46–61 tubercles in males (lateral 20 vertically elongated); 33–42 isomorphic tubercles in females	7–13 tubercles in males (mesial ones broad, and gradually decreasing in size); 14–22 isomorphic tubercles in females	16–20 tubercles in males (mesial ones broad, decreasing in size laterally; mesial-most one more than twice the breadth of the next)	13–20 tubercles in males (mesial ones broad and decreasing in size, lateral 4–5 roughly same size); 20–27 isomorphic tubercles in females
cheliped	palm 2.2 times as long as broad, total length of palm nearly 1.8 times than length of dactyl, cutting edge of dactylus with distinct large teeth	palm 2.3 times as long as broad, length of palm nearly 1.3 times than length of dactyl, cutting edge of dactylus with low triangular molar, pollex unarmed	markedly elongated, palm 2.8 times as long as broad, length of palm nearly 2.0 times than length of dactylus, cutting edge of dactylus with triangular molar	palm 2.3 times as long as broad, length of palm nearly 1.6 times than length of dactyl, cutting edge of both fingers unarmed
ambulatory legs	short, broad	long, slender	long, slender	long, slender

elongated and robust, palm 2.2 times as long as broad, length of palm approximately 1.8 times longer than dactyl ($N = 20$), pollex of chela with low sinuous tooth along cutting margin, dactylus with distinct triangular molar. Ambulatory legs elongated, meri broad, merus of P4 distally armed with several short spines on anterior margin; anterior margins of all ambulatory legs fringed with setal patches. G1 (Fig. 7A–D) long, slender, distal process triangular, distinctly curved outward.

Distribution. The Bay of Bengal, Southeast and East Asia: China (Zhejiang; Fujian; Guangdong; Hainan), western Taiwan (including Kinmen), Vietnam, Malaysia (Selangor; Sarawak; Labuan), Singapore, Brunei, Thailand, Myanmar (Mergui), and eastern India (Tamil Nadu, Godavari Delta) (Fig. 1).

Habitat. At Wazihwei Wetland, estuaries of Danshuei River, northwestern Taiwan, this species is found along shores with substantial freshwater influence, on banks with substrates plastic-muddy, somewhat distant from mangrove stands.

Remarks. According to previous descriptions (De Man 1888, Dai et al. 1986, Dai and Yang 1991), the infraorbital ridges of *M. elegans* bear 50–60 tubercles in males and 35–40 tubercles in females. In the present study of 21 males and 19 females, CW ranging from 7.7 to 15.9 mm, and the range of variation is slightly wider than previously reported, being 47–61 in males and 33–42 isomorphic tubercles in females (Table 2, Fig. 8).

Metaplax longipes Stimpson, 1858

Figures 2D–F, 4, 7E–H

Metaplax longipes Stimpson, 1858: 97 (type locality: Hong Kong); Koelbel 1897: 711, pl. 1(5–6) (Hong Kong); Stimpson 1907: 99 (Hong Kong); Tesch 1918: 116 (key; no new specimens); Gee 1926: 164 (China: "Chin Bey"); Gordon 1931: 528 (Hong Kong; China: Amoy (= Xiamen), Fujian); Shen 1940a: 74, 95 (China: Zhejiang;

Fujian); Shen 1940b: 236 (Hong Kong); Shen and Dai 1964: 133, 1 unnumbered fig. (China: Zhejiang; Fujian; Guangdong); Dai et al. 1986: 508, fig. 288 (1–2), pl. 72(3) (China: Zhejiang; Fujian; Guangdong) (part); Chen 1991: 441, fig. 416; Dai and Yang 1991: 556, fig. 288 (1–2), pl. 72(3) (China: Zhejiang; Fujian; Guangdong) (part); Davie 1992: 352 (key); Huang 1994: 598 (list; China); Wang and Liu 1996c: 227 (list); Lee 2001: 115, 3 unnumbered figs (W Taiwan); Ng et al. 2001: 54 (list; Taiwan); Davie and Nguyen 2003: 384 (no specimen examined); Wang 2003: 111, 1 unnumbered fig. (Taiwan: Kinmen); Liu and He 2007: 167: 1 unnumbered fig. (China: Yangtze R. estuary); So and Lui 2007: 36, 3 unnumbered figs (Hong Kong); Huang 2008: 668 (list; China); Ng et al. 2008: 226 (list); Yang et al. 2008: 803 (list; East and South China seas); Ng et al. 2017: 110 (list; Taiwan).

Metaplax takahasii Sakai, 1939: 698, text-fig. 127 (type locality: Tansui (= Danshuei), Taiwan); Sakai 1940: 58 (list; Japan; Taiwan); Lin 1949: 31 (list; Taiwan); Fukui et al. 1989: 230, fig. 24 (Taiwan: New Taipei City); Dai and Yang 1991: 556, fig. 288 (3–4), pl. 72(4) (China: Fujian; Guangdong); J.-T. Shih et al. 1991: 126 (Taiwan: New Taipei City); Davie 1992: 352, pl. 2A (Hong Kong); Lee and Leung 1999: 69 (Hong Kong).

Metaplax takahashii: Horikawa 1940: 30 (list; Taiwan); Sakai 1976: 673, text-fig. 371 (Taiwan: Danshuei); Dai et al. 1986: 508, fig. 288 (3–4), pl. 72(4) (China: Fujian; Guangdong); Huang 1994: 598 (list; China); Kosuge et al. 1997: 182 (Vietnam: Haiphong); Muraoka 1998: 54 (Danshuei R., Taiwan); Ng et al. 2001: 46 (list; Taiwan); Kitaura et al. 2002: 684 (Vietnam: Haiphong); Davie and Nguyen 2003: 384 (no new specimens); Ng et al. 2017: 110 (list; Taiwan); Huang 2008: 668 (list; China); Ng et al. 2008: 226 (list; Taiwan).

? *Metaplax longipes*: Naiyanetr 2007: 112 (list: Gulf of Thailand).

Not *Metaplax longipes*: Davie 1992: 352 (key) (= *Metaplax tredecim* Tweedie, 1950).

Not *Metaplax longipes*: Chertoprud et al. 2012: 276, pl. 47F (Nha Phu, southeastern Vietnam) (= *Metaplax tredecim* Tweedie, 1950).

Materials examined. China: 2 ♂♂ (20.7–22.7 mm) (NCHUZOOL 15443), Sheyang, Jiangsu, coll. W.-R. Lin, 24 Aug. 2015; 3 ♂♂ (11.3–22.3 mm), 1 ♀ (21.6 mm) (NCHUZOOL 15444), Mamu, Zhoushan, Zhejiang, 26 July 2018; 6 ♂♂ (15.9–26.6 mm), 3 ♀♀ (18.0–19.15 mm) (NCHUZOOL 15446), Mamu, Zhoushan, Zhejiang, Sep. 2018; 2 ♂♂ (13.9–15.6 mm), 5 ♀♀ (13.6–23.6) (NCHUZOOL 15447), Mamu, Zhoushan, Zhejiang, 26 July 2018; 1 ♀ (16.8 mm) (NCHUZOOL 15445), Buqiangwan, Zhoushan, Zhejiang, 26 July 2018; 2 ♂♂ (12.0–12.1 mm), 4 ♀♀ (9.8–18.6 mm) (NCHUZOOL 15448), Liuwudian, Xiamen, Fujian, 31 July 2018; 1 ♂ (19.2 mm) (NCHUZOOL 15449), Qinzhou, Guangxi, 10 May 2009. **Hong Kong:** 1 ♂ (18.3 mm) (NCHUZOOL 15451), Tung Chung, coll. K. J. H. Wong, 21 Mar. 2009; 1 ♂ (15.4 mm) (ZRC 2019.0581), 4 ♂♂ (11.2–16.7 mm) (NCHUZOOL 15450), Tung Chung, coll. K. J. H. Wong, 9 Apr. 2016; 1 ♂ (9.3 mm), 2 ♀♀ (8.6–9.8 mm) (NCHUZOOL 15452), Tung Chung, coll. K. J. H. Wong, 11 July 2015; 15 ♂♂ (6.6–12.7 mm), Tung Chung, coll. K. J. H. Wong, 17 July 2015; 4 ♂♂ (6.0–

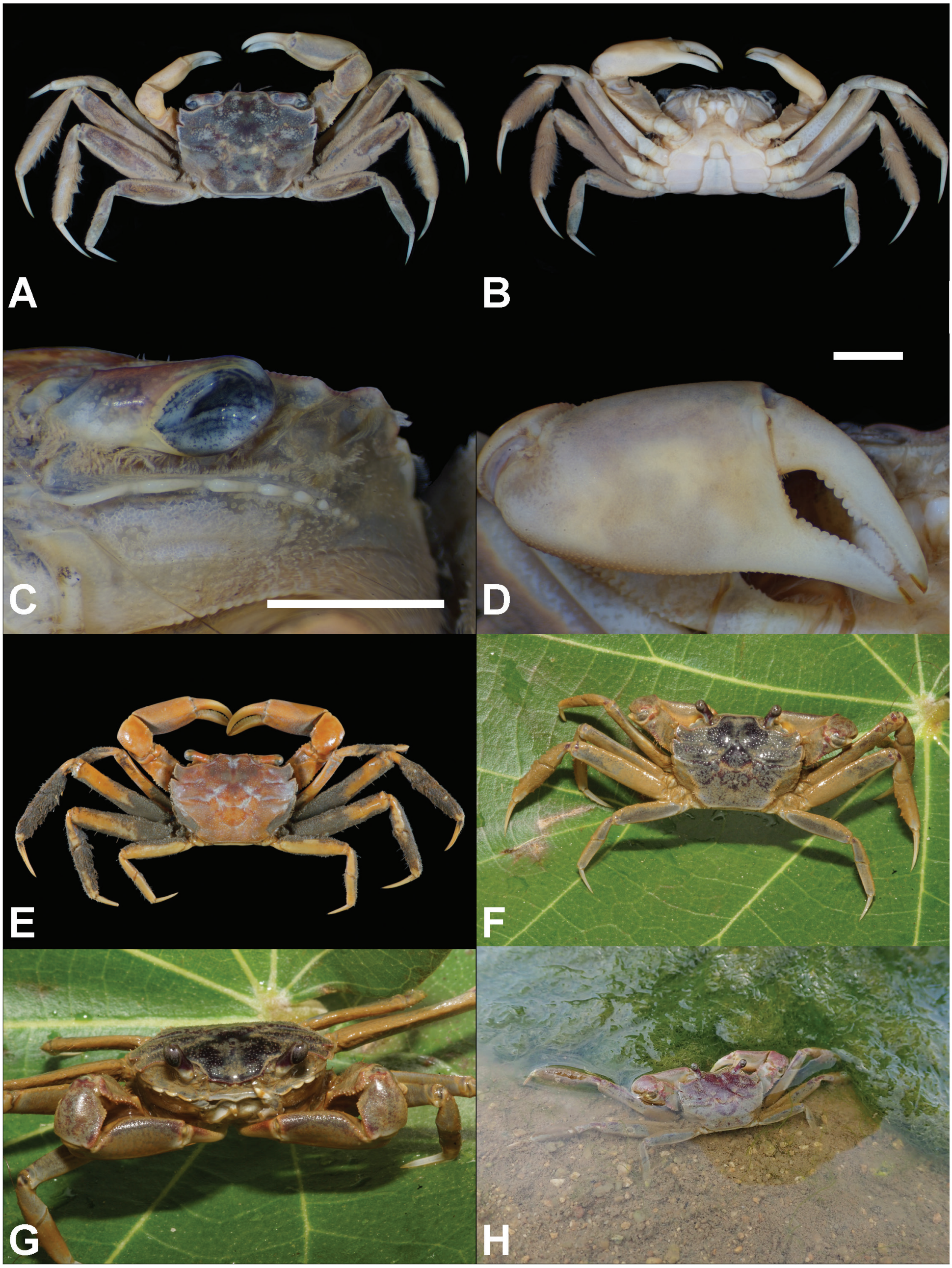

Figure 4. *Metaplax longipes* Stimpson, 1858. **A, E** Dorsal view **B** ventral view **C** left infraorbital ridge **D** right cheliped. **A–D** Male (CW 15.4 mm; ZRC 2019.0581; Hong Kong) **E** male (CW 24.1; ZRC 1999.0708; Danshuei, northwestern Taiwan) **F–H** live coloration **F–G** male (CW 20.6 mm; NCHUZOOL 15553; Lieyu, Kinmen, Taiwan) **H** male (CW 23.7 mm; NCHUZOOL 15501; Hong Kong). Scale bars: 2.0 mm.

8.8 mm) (NCHUZOOL 15455), Tung Chung, coll. K. J. H. Wong, 18 July 2011; 3 ♂♂ (17.7–23.7 mm), 1 ♀ (13.3 mm), Tung Chung, coll. K. J. H. Wong, 22 Apr. 2019; 3 ♂♂ (6.8–8.6 mm), 3 ♀♀ (10.0–14.7 mm) (NCHUZOOL 15503), Tung Chung, coll. H.-T. Shih and K. J. H. Wong, 2 June 2019; 1 ♂ (17.6 mm) (NCHUZOOL 15502), Lantau Island, 2 June 2019; 2 ♂♂ (9.9–22.3 mm); 1 ♂ (24.8 mm), 1 ovig. ♀ (19.0 mm) (ZRC 2019.0542), ca. 22.495486N, 114.029947E, mudflats at mangroves, Mai Po Nature Reserve, coll. K. J. H. Wong, 24 May 2019. **Macao:** 1 ♀ (17.5 mm) (NCHUZOOL 15454), Coloane, coll. K. J. H. Wong, 3 July 2015. **Taiwan**: 1 ♀ (17.7 mm) (KPM-NH 0107076), Danshuei, New Taipei City, coll. S. Takahashi (?), 1933 (?); 1 ♀ (14.7 mm) (NTOU), Danshuei, New Taipei City, 25 May 1984; 1 ♀ (14.4 mm) (NTOU), Danshuei, New Taipei City, coll. L.-H. Hsieh, 7 May 1992; 2 ♂♂ (10.6–20.0 mm) (ASIZ), Danshuei River mangroves, New Taipei City, 17 Mar. 1986; 1 ♂ (24.1 mm) (ZRC 1999.0708), Danshuei, New Taipei City, 8 July 1999; 1 ♂ (21.8 mm) (ZRC 1999.0708), Danshuei, New Taipei City, 8 July 1999; 3 ♂♂ (6.9–17.4 mm), 2 ♀♀ (7.9–10.3 mm) (NCHUZOOL 15458), Wujiang R. estuary, Kinmen, 6 Mar. 2008; 1 ♂ (19.1 mm), 1 ♀ (16.9 mm) (NCHUZOOL 15459), Wujiang R. estuary, Kinmen, 16 Aug. 2011; 7 ♂♂ (7.4–14.6 mm), 1 ♀ (9.3 mm) (NCHUZOOL 15460), Wujiang R. estuary, Kinmen, coll. H.-T. Shih and P.-Y. Hsu, 29 June 2018; 2 ♂♂ (7.0–7.1 mm) (NCHUZOOL 15461), Cihhu, Kinmen, coll. H.-T. Shih and P.-Y. Hsu, 29 June 2018; 1 ♀ (19.9 mm) (NCHUZOOL 15462), Mashan, Kinmen, 17 Aug. 2011; 1 ♀ (21.1 mm) (NCHUZOOL 1551), 1 ♀ (5.9 mm) (NCHUZOOL 15552), 1 ♂ (20.6 mm), 1 ♀ (17.5 mm) (NCHUZOOL 15553), 1 ♀ (20.1 mm) (NCHUZOOL 15554), Lieyu, Kinmen, coll. H.-T. Shih and P.-Y. Hsu, 28 June, 2018; 1 ♂ (18.1 mm) (NCHUZOOL 15463), Cingshuei, Matsu, coll. J.-H. Li, 9 July 2005; 1 ♂ (20.0 mm) (NCHUZOOL 15464), Cingshuei, Matsu, coll. J.-H. Li, 9 July 2005.

Diagnosis. Carapace (Figs 2D, 4A, E, F) subquadrate, 1.36 times broader than long (N = 98, SD = 0.05), mildly convex longitudinally and laterally, region faintly defined; front medially slightly concave; lateral margin nearly parallel, interrupted by four notches (cutting into five teeth), anterior two lateral teeth pronounced, posterior two very indistinct; posterior facet depressed, decorated by two oblique granular ridges, anterior one extended from second notch. Infraorbital ridge (Figs 2E, 4C) marked sexually dimorphic: males with 7–13 lobes and tubercles, medial 2 broad, decreasing in breadth laterally, innermost four or five decreasing in size, lateral ones small, isomorphic; females with 16–22 small isomorphic tubercles. Chelipeds (Figs 2F, 4D) symmetrical, robust, palm 2.3 times as long as broad, length of palm approximately 1.3 times length of dactyl (N = 16), merus denticulate along anterior and posterior margins; chelae surface smooth, pollex and dactylus unarmed of pronounced molars along cutting edge. Ambulatory legs slender, elongated, meri unarmed along anterior margin, proximal half of meri, and propodi of all furnished with setal mats. G1 (Fig. 7E–H) elongated, relatively stout, almost straight.

Distribution. Western Taiwan (including Matsu and Kinmen), China (Jiangsu; Zhejiang; Fujian; Guangdong; Guangxi), and northern Vietnam (Haiphong) (Fig. 1). The record in the Gulf of Thailand (Naiyanetr 2007: 112) requires further verification.

Habitat. At Tung Chung Wetland, Hong Kong, where numerous specimens were collected, the habitat of this species is composed of muddy substrates and substantial freshwater influences. Considerable numbers flourish under fringes of mangrove stands, as well as the adjacent more open mudflats.

Remarks. The identity of *Metaplax longipes* had long remained unclear since the publication of *M. takahasii* Sakai, 1939. The confusion between the two nominal species was mainly caused by two crucial morphological features used for species identification: the number of tubercles and lobes along the male infraorbital ridge, and the number of teeth on the lateral margin of the carapace.

Originally described from Hong Kong by Stimpson (1858, 1907), type material(s) of *M. longipes* was destroyed in the Great Chicago Fire in 1871 (Evans 1967). Illustrations based on material from Hong Kong were eventually presented by Koelbel (1897: pl. 1(5–6)), and further records from Hong Kong and elsewhere in South China include those by Gee (1926), Gordon (1931), Shen (1940a, b) and Shen and Dai (1964). Morphology of the infraorbital ridge in males serves as a good taxonomic character (Tesch 1918); delimitations provided in various work under the name *M. longipes* range from 7 to 10: "seven-lobed" (Stimpson 1907), "fogak száma összesen tehát kilencz" (= total number of nine teeth; Koelbel 1897), "number of lobules or teeth … 7–9" (Tesch 1918, Gordon 1931), and "9 to 10 tubercles" (Shen and Dai 1964).

Interpretations of *M. longipes* by Dai et al. (1986) and Dai and Yang (1991) brought much confusion. These authors illustrated two forms of infraorbital ridges based on specimens from South China (Guangdong to Zhejiang), one bearing 17 lobes and tubercles, and the other bearing nine (fig. 288(1) in Dai et al. 1986 and Dai and Yang 1991). It appeared very likely that their material was composite (also see Remarks under *M. tredecim*). Anyhow, this "shift" in the number of tubercles was subsequently followed by various authors: "with 15–17 lobules and teeth" (Davie 1992) and "about 9–17 tubercles" (Lee and Leung 1999). Reflecting this confused situation, the dichotomous key to the *Metaplax* species provided by Davie (1992: 352), which differentiated "*M. longipes*" (15 to 17 lobules and teeth) from "*M. takahasii*" (9 teeth), was problematic. Following diagnoses given by Stimpson (1858, 1907), and authors such as Gordon (1931) and Shen and Dai (1964), only those with around 9 lobes or tubercles, should be considered as the true *M. longipes*.

Without accessing any material of *M. longipes* from South China, Sakai (1939) described a similar form named *M. takahasii* based on one male specimen from Tansui (= Danshuei), northwestern Taiwan, after the naturalist and collector, Sadae Takahashi (or Sadae Takahasi in another translation). The species was subsequently reported elsewhere in China, including Guangdong and Fujian (Dai et al. 1986; Dai and Yang 1991) and Hong Kong (Davie 1992). Regarding the correct spelling of the species epithet, "*takahasii*" (original as in Sakai 1939), instead of "*takahashii*" as in Sakai (1976), should be maintained (ICZN 1999: Article 32.2).

Nevertheless, *M. takahasii* was described with an infraorbital ridge composed of 8 tubercles and the lateral margin of the carapace cut into five teeth. Considering the original descriptions of *M. longipes* and *M. takahasii* (Stimpson 1907 and Sakai 1939, respectively), holotypes of the two (CW 15.5 mm and 14.2 mm, respectively) differ by the numbers of lateral carapace teeth (four vs. five) of the carapace and the infraorbital lobes and tubercles (seven vs. eight). The number of infraorbital tubercles of both forms overlap might be explained by variation between intraspecific individuals (see "Note on the number of infraorbital tubercles and lobes"; Fig. 8), whereas the posterior-most notch along the lateral margin, however, can be very indistinct and often obscured by a layer of sediment-laden setae and easily omitted (Davie and Nguyen 2003; see Remarks under *M. tredecim* below). This led Davie and Nguyen (2003: 384) to the view that *M. longipes* is "almost certain(ly) … a senior synonym of *M. takahashii*". In enumerating Chinese species of *Metaplax*, Yang et al. (2008: 803), probably following Davie and Nguyen's (2003) suggestion, listed *M. takahasii* as a junior synonym of *M. longipes* without further elaboration. In our material referred to *M. longipes*, the number of infraorbital tubercles and lobes varies from 7–13 for males and 14–22 in females (Table 2; Fig. 8)

In the present study, we compared specimens from Hong Kong (identified as *M. longipes*) and various lots from Taiwan main island (originally labeled as *M. takahasii*: see Materials examined above) with morphological and molecular approaches. As noted by Davie and Nguyen (2003; also see above), the number of notches (hence teeth) on the lateral margin of the carapace is easily underestimated unless the surface is carefully denuded. This aspect is well-illustrated in the case of *M. tredecim* (as discussed below), and also between specimens of *M. longipes* from Hong Kong (Fig. 4A) and "*M. takahasii*" from Danshuei, Taiwan (Fig. 4E), the two being identical. Molecular analyses also support only one clade of specimens from various localities of China and Taiwan (Table 1; Fig. 9).

***Metaplax sheni* Gordon, 1930**

Figures 2G–I, 5, 7I–L

Metaplax sheni Gordon, 1930: 525 (type locality: Amoy (= Xiamen), Fujian, China); Gordon 1931: 553, figs 31–32 (China: Xiamen, Fujian); Tweedie 1936: 69, fig. 15(5) (Singapore); Shen 1940a: 74, 95 (China: Zhejiang; Fujian); Shen and Dai 1964: 133, 1 unnumbered fig. (China: Fujian); Dai et al. 1986: 509, fig. 289 (3–4), pl. 72 (6) (China: Fujian); Chen 1991: 441, fig. 415 (China: Zhejiang); Dai and Yang 1991: 558, fig. 289 (3–4), pl. 72(6) (China: Fujian); Huang 1994: 598 (list; China); Tan and Ng 1994: 82 (Singapore); Kosuge et al. 1997: 182 (Vietnam: Haiphong); Kitaura et al. 2002: 684 (Vietnam: Haiphong); Davie and Nguyen 2003: 383 (Malaysia: Johor; Singapore); Huang 2008: 668 (list; China); Ng et al. 2008: 226 (list); Yang et al. 2008: 803 (list; China: Fujian).

Metaplax indica: Rathbun 1931: 100 (China: Fujian); Shen 1940a: 74, 95 (list; South China). (not *M. indica* H. Milne Edwards, 1852)

Materials examined. China: 5 ♂♂ (8.6–12.8 mm) (NCHUZOOL 15465), Wuyuanwan, Xiamen, Fujian, coll. H.-T. Shih et al., 1 Aug. 2018. **Taiwan**: 1 ♂ (9.9 mm)

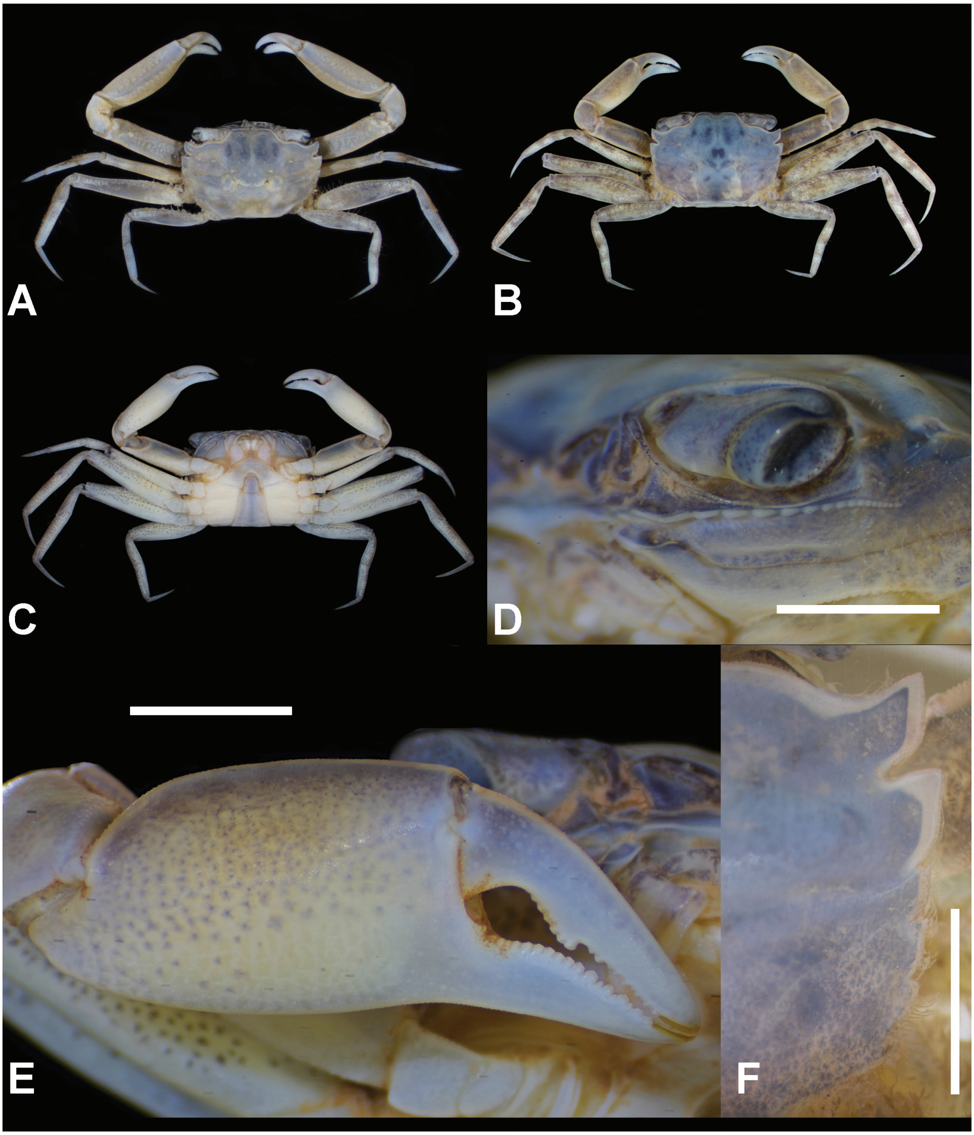

Figure 5. *Metaplax sheni* Gordon, 1930. **A, B** dorsal view **C** ventral view **D** left infraorbital ridge **E** right cheliped **F** right side of carapace showing the five teeth of lateral margin. **A, C** Male (CW 12.8 mm; NCHUZOOL 15465; Xiamen, Fujian, China) **B, D–F** male (CW 9.9 mm; NCHUZOOL 15466; Dong Rui, Quang Ninh, Vietnam). Scale bars: 2.0 mm.

(NCHUZOOL 15467), Kinmen. **Vietnam**: 1 ♂ (9.9 mm) (NCHUZOOL 15466), Dong Rui, Quang Ninh, coll. H.-T. Shih et al., 29 May 2016.

Diagnosis. Carapace (Figs 2G, 5A, B) subquadrate, 1.45 times broader than long (*N* = 7, SD = 0.04), longitudinally convex, regions faintly defined; frontal margin sinuous, medially noticeably concave; lateral margin markedly converging posteriorly, furnished with a row of soft setae, interrupted by four notches, cutting into five teeth, anterior two marked, triangular, last two weak, indistinct. Infraorbital ridge (Figs 2H, 5D) of males with 16–20 lobes and tubercles, innermost tubercle more than twice as broad as the adjacent, followed by six broad tubercles, decreasing in size. Chelipeds (Figs 2I, 5E) of males subequal, markedly elongated, palm 2.8 times as long as broad, length of palm approximately 2 times longer than length of dactyl (*N* = 6), merus and palm subequal in length; both fingers about half-length of palm, deflexed; cutting edges of pollex with low, serrated lobe, dactylus bearing distinct triangular molar. Ambulatory legs slender, elongated, anterior margins of meri finely serrated; anterior margins of carpi and propodi line with thick tomentum. G1 (Fig. 7I–L) elongated, relatively stout almost straight.

Distribution. China (Zhejiang, Fujian), Taiwan (Kinmen), Vietnam (Khanh Hoa; Haiphong; Quang Ninh), and Malay Peninsula (including Singapore) (Fig. 1).

Remarks. One curious record of *M. indica* was reported by Rathbun (1931) from Tsimei, Amoy (= Jimei, Xiamen), along the coasts of Fujian. Other than this record, there have been no definite records of *M. indica* from China, despite that of Shen (1940a) citing that of Rathbun's (1931). Given the considerable resemblance between *M. indica* (see Naderloo 2011: figs 15, 18c–d; Naderloo 2017: figs 31.11e, 32.2, 32.3) and *M. sheni*, and the latter described from the region (Amoy) shortly before, it is reasonable to consider Rathbun's (1931) record represented *M. sheni*.

Specimens collected from Kinmen, opposite Xiamen (Fujian, China), are confirmed as *M. sheni* based on molecular analyses (see below), being a new record to Taiwan.

***Metaplax tredecim* Tweedie, 1950**

Figures 2J–L, 6, 7M–P

Metaplax tredecim Tweedie, 1950: 354, fig. 6 (type locality: Labuan, Malaysia); Choy and Booth 1994: 243 (Brunei); Davie and Nguyen 2003: 383, fig. 1d–e (Malaysia: Labuan; Brunei); Ng et al. 2008: 226 (list); Yang et al. 2008: 803 (list; East China and South China seas).

Metaplax longipes: Dai et al. 1986: 508, fig. 288 (1–2), pl. 72(3) (China: Zhejiang, Fujian, Guangdong) (part); Dai and Yang 1991: 556, fig. 288 (1–2), pl. 72(3) (China: Zhejiang, Fujian, Guangdong) (part); Chertoprud et al. 2012: 276, pl. 47F (Vietnam: Nha Phu, Nha Trang, Khanh Hoa) (not *M. longipes* Stimpson, 1858).

Materials examined. Paratypes: 2 ♂♂ (15.6–16.2 mm), 1 ♀ (15.7 mm) (ZRC 1964.7.14.4-18), Labuan, Malaysia, coll. G. Nunong, Aug. 1938. **Others. Hong Kong**: 1 ♂ (14.2 mm) (NCHUZOOL 15468), Starfish Bay, coll. P.-C. Tsai and H. Y. Cheung, 19 July 2015; 3 ♂♂ (16.4–19.4 mm) (NCHUZOOL 15546), Starfish Bay, coll. K. J. H. Wong, 4 June 2019; 1 ♂ (16.1 mm) (NCHUZOOL 15469), Tai Tan, coll. K. J. H. Wong, 15 July 2015; 1 ♂ (18.1 mm) (NCHUZOOL 15470), Ting Kok, coll. K. J. H. Wong, 22 Aug. 2017; 2 ♂♂ (12.8–16.2 mm), 1 ♀ (15.6 mm) (NCHUZOOL 15471), Kei Ling Ha, coll. K. J. H. Wong, 31 Aug. 2011; 3 ♂♂

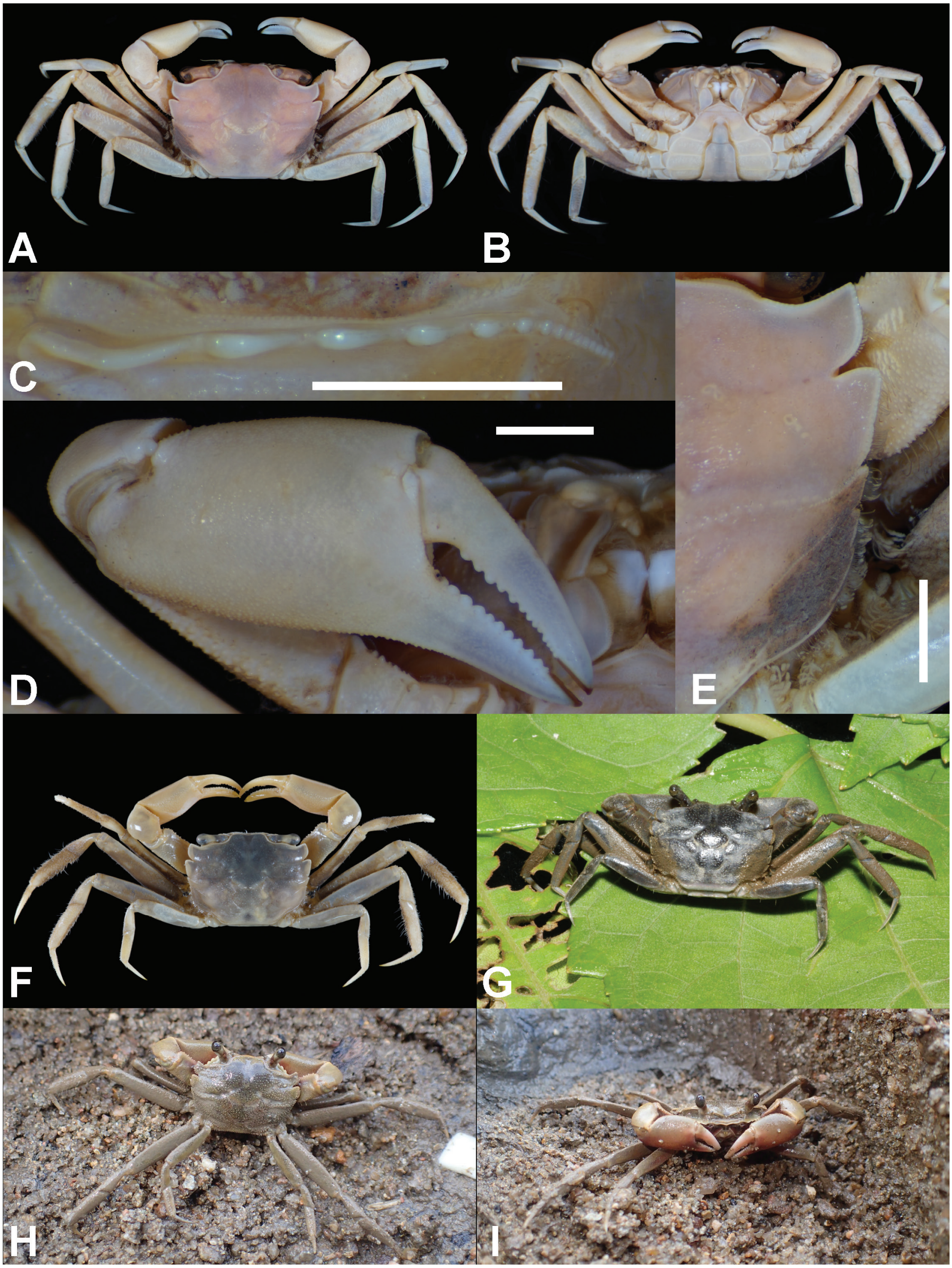

Figure 6. *Metaplax tredecim* Tweedie, 1950. **A, F** Dorsal view **B** ventral view **C** left infraorbital ridge **D** right cheliped **E** right side of carapace showing the five teeth of lateral margin. **A–E** paratype male (CW 16.2 mm; ZRC 1964.7.14.4-18; Labuan) **F** male (CW 16.5 mm; NCHUZOOL 15473; Hong Kong) **G** male (CW 15.4 mm; NCHUZOOL 15475; Labuan, Malaysia) **H, I** male (18.2 mm; NCHUZOOL 15705; Hong Kong) **G–I** color in life. Scale bars: 2.0 mm.

(16.9–21.0 mm) (NCHUZOOL 15472), Nai Chung, coll. K. J. H. Wong, 23 June 2015; 2 ♂♂ (16.5–21.5 mm) (NCHUZOOL 15473), Luk Keng, coll. C. W. Lau, 22 May 2016; 1 ♂ (18.2 mm) (NCHUZOOL 15705), Mak Pin, Sai Kung, coll. K. J. H. Wong, 7 July 2019. **China:** 4 ♂♂ (18.0–22.7 mm), 1 ♀ (10.7 mm) (NCHUZOOL 15474), Dongzhai Harbor, Hainan, 23 June 2004. **Vietnam:** Quang Ninh: 1 ♂ (21.8 mm), 2 ♀♀ (15.7–23.4 mm) (NCHUZOOL 15476), Dong Rui, 29 May 2016; 1 ♂ (22.4 mm) (NCHUZOOL 15477), Dong Rui, coll. H.-T. Shih and P.-Y. Hsu, 9 Oct. 2017; 1 ♀ (10.3 mm) (NCHUZOOL 15478), Dong Rui, coll. H.-T. Shih and P.-Y. Hsu, 9 Oct. 2017; Khanh Hoa: 4 ♂♂ (14.4–18.7 mm), 5 ♀♀ (12.6–16.1 mm) (NCHUZOOL 15498), Nha Trang, coll. I.-H. Chen and K. J. H. Wong, 24 Nov. 2010. **Malaysia**: Labuan, 1 ♂ (15.4 mm), 1 ♀ (16.2 mm) (NCHUZOOL 15475), coll. H.-T. Shih, 23 July 2010; 1 ♂ (17.1 mm) (NCHUZOOL 15497), coll. H.-T. Shih, 27 July 2010.

Diagnosis. Carapace (Figs 2J, 6A, F, G) subquadrate, 1.35 times broader than long (*N* = 39, SD = 0.03), regions defined by shallow grooves, slightly inflated, surface pitted; front nearly straight, medially slightly concave; lateral margin mildly convex, posteriorly converging, cut into five teeth, anterior two pronounced, quadrate, posterior two inconspicuous; posterolateral facet slightly depressed, behind second notch decorated with two short oblique granular ridges. Infraorbital ridge (Figs 2K, 6C) markedly sexually dimorphic: in males medial four or five roughly same size, decreasing in breadth laterally, laterally of a row of seven or eight small, rounded, isomorphic, tubercles; females with 21–27 small isomorphic tubercles. Chelipeds (Figs 2L, 6D) stout, symmetrical, palm 2.3 times longer than broad, length of palm approximately 1.6 times of length of dactyl (*N* = 12), meri slightly dilated anteriorly, lined with minute denticles along the margin; chela surface finely granulated, along cutting edges both fingers unarmed. Ambulatory legs slender, elongated, meri of P3 and P4 tomentum-covered on distal half, and propodi of P2 to P4 with thick mat of setae. G1 (Fig. 7M–P) elongated, slender, almost straight.

Distribution. Southeast and East Asia: northern Borneo (Labuan, Malaysia; Brunei), Vietnam (Quang Ninh; Khanh Hoa), and South China (Hong Kong) (Fig. 1).

Habitat. In Hong Kong, in comparison to *M. longipes*, *M. tredecim* tends to occur in habitats of coarser, grittier substrates, with less freshwater input, and frequently on open sandflats rather unsheltered by mangroves.

Remarks. Identification of the *Metaplax tredecim* had been confusing, particularly based on the number of teeth along the lateral margin of the carapace. Tweedie (1950: fig. 6) showed merely three conspicuous lobes, the posterior one occupying more than half of carapace length. However, as noted by Davie and Nguyen (2003), members of the genus often have the structures around the posterolateral facet obscured by setae-trapped sediments, and not visible unless carefully denuded. Reexamination of a paratype male (16.2 mm; ZRC 1964.7.14.4-18), after denudation, showed the lateral margin to be interrupted by 4 notches (hence 5 teeth) (Figs 2J, 6A, E), the posterior two being inconspicuously defined by the last notch.

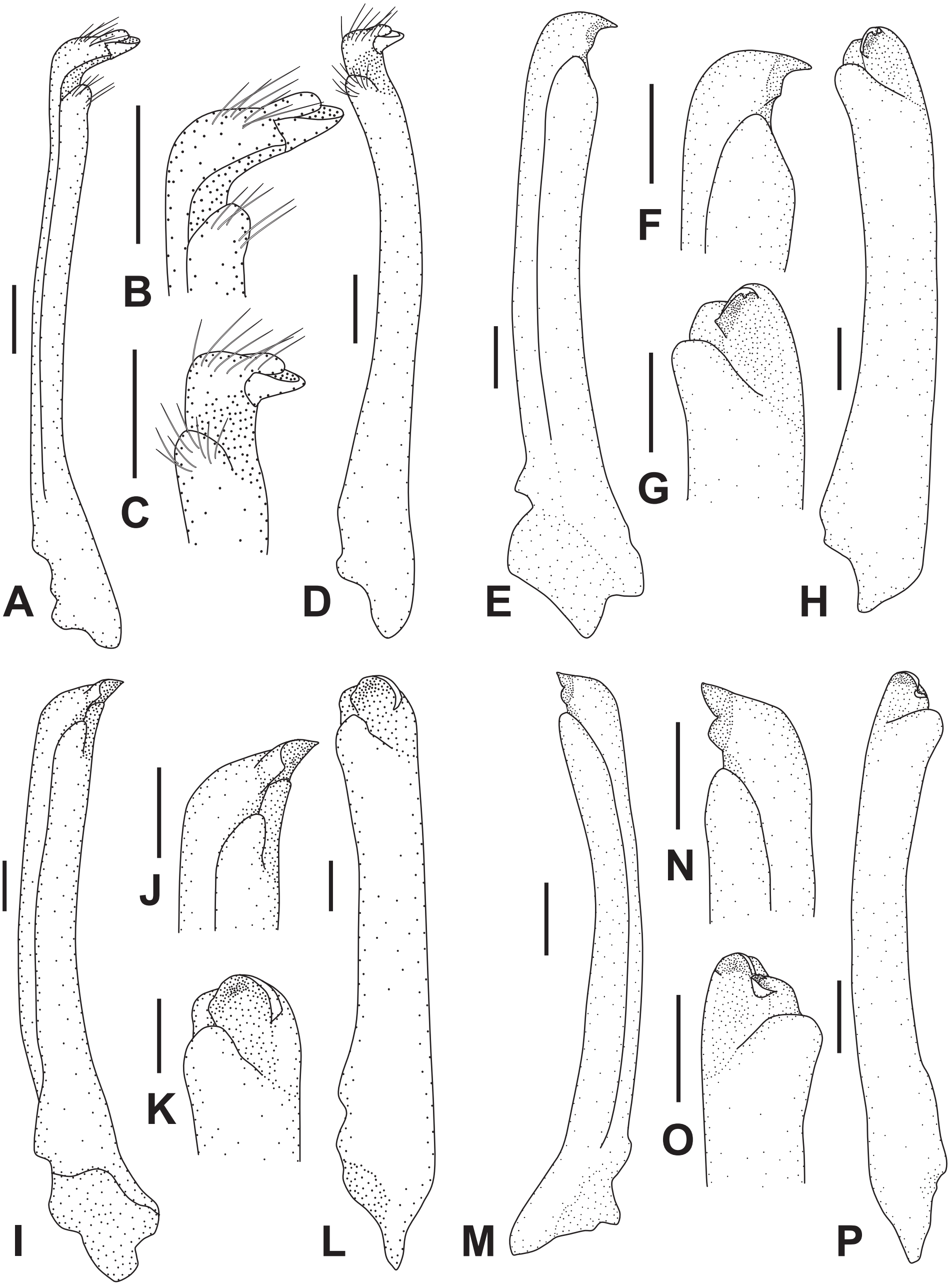

Figure 7. *Metaplax elegans* De Man, 1888 (**A–D** NCHUZOOL 15496, male, 12.7 mm), right G1; *M. longipes* Stimpson, 1858 (**E–H** ZRC 2019.0581, male, 14.9 mm), right G1; *M. sheni* Gordon, 1930 (**I–L** NCHUZOOL 15466, male, 9.9 mm), right G1; and *M. tredecim* Tweedie, 1950 (**M–P** paratype, ZRC 1964.7.14.4-18, 16.2 mm), left G1. Scale bars: 0.5 mm (**A–H, M–P**); 0.2 mm (**I–L**).

As mentioned above, two forms, differing in the number of tubercles or lobes on the infraorbital ridge, were recognized in specimens identified with *M. longipes* by Dai et al. (1986) and Dai and Yang (1991). We confirmed that specimens from the study area, characterized by the possession of about 17 tubercles or lobes closely represent *M. tredecim*. There is little doubt that the material studied by Dai et al. (1986) and Dai and Yang (1991) included two species, *M. longipes* and *M. tredecim*.

Chertoprud et al. (2012) recorded "*M. longipes*" from Nha Phu, southeastern Vietnam. However, given the infraorbital ridge with 17 tubercles and the chelipeds with the length of palm/the length of dactyl ratio about 1.5 (estimated from their plate 47F on page 295), this record is suspected to represent *M. tredecim* instead (see Table 2).

Note on the number of infraorbital tubercles and lobes

As one of the major morphological features for the identification of species of *Metaplax* species, the number of lobes and granules along both infraorbital ridges, which are in all cases sexually dimorphic, differs substantially among species. The following range indicate the number of these lobes and tubercles of both sexes (with the exception of *M. sheni* for which only males were collected), with differences between left and right ridges placed in brackets: in *M. elegans* 46–61 (0–5) for males and 33–42 (0–3) for females, *M. longipes* 7–13 (0–2) for males and 14–22 (0–2) for females, *M. sheni* 16–20 (0–2) for males, and *M. tredecim* 13–20 (0–3) for males and 20–27 (0–3) for females (Table 2; Fig. 8). Tubercle counts overlap slightly in specimens of both sexes in *M. longipes* and *M. tredecim* (13 in males and 20–22 in females) and completely in male specimens of *M. sheni* (16–20) and *M. tredecim* (13–30). Comparing sexes, *M. longipes* and *M. tredecim* counts for males are less than females, but the reverse is true for *M. elegans*. These figures, however, do not show a clear trend in relation to body size (Table 2; Fig. 8).

Molecular analyses

The molecular analysis of the COI marker included 22 specimens of *Metaplax*, with 13 haplotypes (Table 1). The phylogenetic reconstruction (Fig. 9) shows four well-supported clades, which could correspond to the four species treated in this study. It is obvious that only one clade is represented by specimens of *M. longipes* from South China and specimens from the type locality of *M. takahasii* (Danshuei, Taiwan). *Metaplax longipes* and *M. tredecim* are in sister-relation, and the two species and *M. sheni* form a main clade. *Metaplax elegans* is distant from other species of *Metaplax*.

The mean pairwise nucleotide divergence with K2P distances and bp differences of haplotypes of the four species are shown in Table 3. The intraspecific K2P nucleotide divergence of *M. elegans* (≤ 1.86 %) is higher than that of other species (≤ 0.92 %). The interspecific divergences among the four species are ≥ 15.87 %.

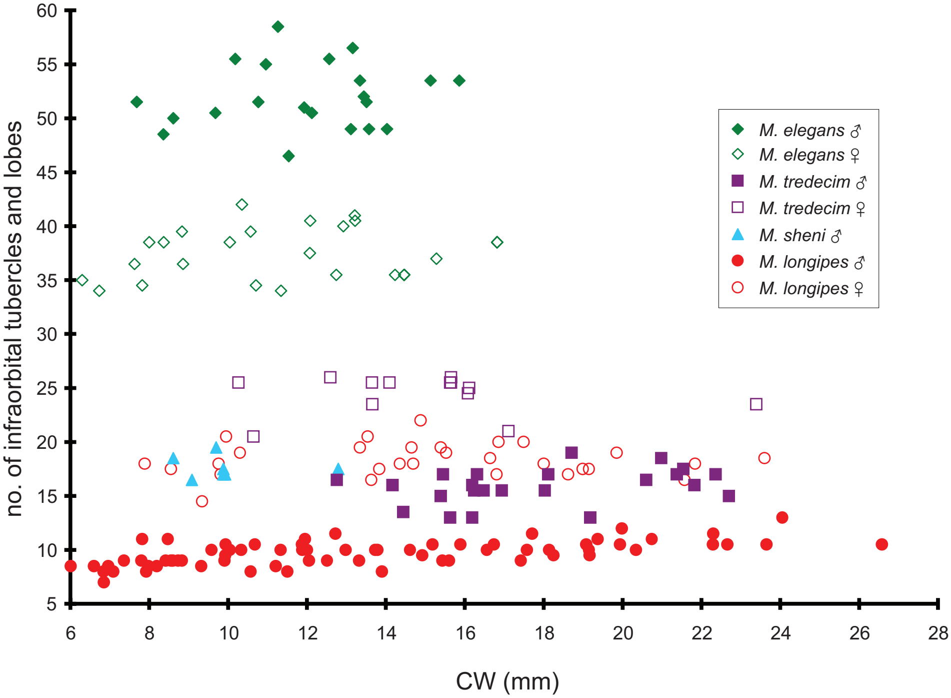

Figure 8. The number of infraorbital tubercles and lobes plotted as a function of carapace width (CW) of *Metaplax elegans* De Man, 1888, *M. longipes* Stimpson, 1858, *M. sheni* Gordon, 1930, and *M. tredecim* Tweedie, 1950.

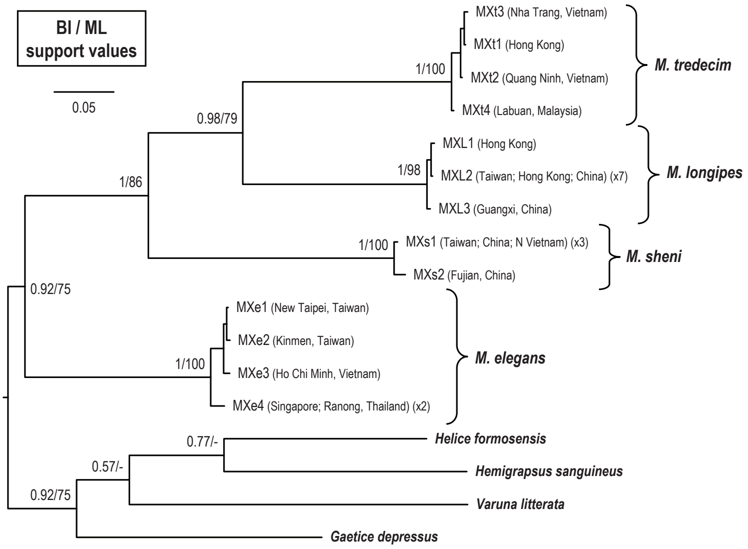

Figure 9. A Bayesian inference (BI) tree for *Metaplax elegans* De Man, 1888, *M. longipes* Stimpson, 1858, *M. sheni* Gordon, 1930, and *M. tredecim* Tweedie, 1950, and the outgroups, based on the cytochrome c oxidase subunit I (COI) gene. Probability values at the nodes represent support values for BI and maximum likelihood (ML). For haplotype names, see Table 1.

Table 3. Matrix of percentage pairwise nucleotide divergence with K2P distance (lower left) and mean number of differences (upper right) based on COI within and between species of *Metaplax* from East Asia and northern Vietnam (see Table 1). Values of range are shown in parentheses.

	Intraspecific		**Interspecific**			
	nucleotide divergence	**Mean nucleotide difference**	***M. elegans***	***M. longipes***	***M. sheni***	***M. tredecim***
M. elegans	1.21 (0–1.86)	7.8 (0–12)		106.47 (104–108)	102 (98–108)	110.35 (107–112)
M. longipes	0.1 (0–0.46)	0.67 (0–3)	18.36 (17.86–18.67)		102 (101–103)	97.75 (93–101)
M. sheni	0.46 (0–0.92)	3 (0–6)	17.5 (16.7–18.7)	17.5 (17.3–17.71)		104.44 (102–106)
M. tredecim	0.48 (0.15–0.92)	3.17 (1–6)	19.12 18.45–19.45)	16.8 (15.87–17.45)	17.99 (17.51–18.3)	

Discussion

In this study, based on morphological and molecular evidences, we resolve the taxonomic confusions and updated the distribution of *Metaplax* species from East Asia and northern Vietnam. The presence of four species, viz., *M. elegans*, *M. longipes*, *M. sheni*, and *M. tredecim* are confirmed, and it is verified that *M. takahasii* is conspecific with *M. longipes*, and thus synonymized.

With regard to the number of infraorbital tubercles and lobes, despite elaborate sexual dimorphism among varunid species, serve as a reliable morphological feature in identifying *Metaplax* species (cf. Table 2), whereas the numbers of *M. elegans* substantially exceed those of congeners (Fig. 8). The numbers of infraorbital tubercles of the *Helice*/*Chasmagnathus* complex (Varunidae) are also used for species identification (K. Sakai et al. 2006), and likewise for species of *Helicana* K. Sakai & Yatsuzuka, 1980, all supported by genetic evidences. However, three species belong to the "*Helice latimera* complex" under *Helice* De Haan, 1833, with varying ranges of tubercle count, were shown to be otherwise (Shih and Suzuki 2008; Ng et al. 2018). The latter case implied *H. latimera* Parisi, 1918; *H. formosensis* Rathbun, 1931; and *H. tientsinensis* Rathbun, 1931 might well belong to a single species, as discussed in Ng et al. (2018). This ambiguity of specific delimitation requires further morphological and developmental investigations.

Phylogenetic relationships in the genus *Metaplax* or among genera of the Varunidae are far from settled. Monophyly of *Metaplax* has not yet been confirmed. Moreover, despite various recent research effort employing even complete mitochondrial sequences, the sister group of *Metaplax* remains unclear (Kitaura et al. 2002; Chen et al. 2019; Liu et al. 2019), which is probably due to the limited genera sampled in phylogenetic analyses.

In our study, the four species of *Metaplax* can be separated by the COI marker with a minimum interspecific distance of 17.5 %, which is higher than that of most other crab species (see Chu et al. 2015). The phylogenetic tree based on COI (Fig. 9) showed *M. longipes* and *M. tredecim* as sister species, and both species form a clade that is sister to *M. sheni*, whereas *M. elegans* is sister to the three as a whole. The phylogenetic relationships of the four species are also consistent with the number of infraorbital tubercles and lobes, i.e., *M. longipes* and *M. tredecim*, have the fewest number of these structures; *M. sheni* has moderate number; and *M. elegans* has the greatest number (Table 2; Fig. 8). This implies the number of infraorbital tubercles is possibly higher in the ancestral form, becoming reduced in successive clades. More species of this genus, however, should be included in the future to test this hypothesis.

Species of *Metaplax* are mainly distributed in the tropical and subtropical continental regions, in muddy and muddy sand habitats, always accompanied by mangroves (Sakai 1939, 1976; Dai et al. 1986; Dai and Yang 1991). It has been suggested that the pattern of geographical distributions agrees with the "continental type" of fiddler crabs, in contrast with the "oceanic type" mainly inhabited on islands (cf. Shih et al. 2010, 2016; Shih 2012). This also explains why no East Asian species of *Metaplax* are recorded from Korea, the main islands of Japan, the Ryukyu islands, and eastern Taiwan. The habitat preferred by species of *Metaplax* is suggested to be related to physiological constraints (e.g., food, temperature, salinity, etc.; Curtis and McGaw 2012; Theuerkauff et al. 2018). Understanding of the population structure may help reveal larval dispersal in the region of East Asia and northern South China Sea (Chan et al. 2007; Shih et al. 2015; Wang et al. 2015; Chai et al. 2017).

The results of this study clarify the biogeographic distribution of three species (Fig. 1). With *M. takahasii* synonymized with *M. longipes*, the distribution of *M. longipes* stretches from western Taiwan (including Matsu and Kinmen) to China (from Jiangsu to Guangxi) and northern Vietnam. *Metaplax sheni* is found in China (Zhejiang and Fujian), Taiwan (Kinmen), Vietnam, and Malay Peninsula (including Singapore); and the known range of *M. tredecim* include South China (Hainan and Hong Kong), Vietnam, and northern Borneo.

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RESEARCH ARTICLE

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A new genus of air-breathing marine slugs from South-East Asia (Gastropoda, Pulmonata, Onchidiidae)

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Abstract

As part of an ongoing effort to revise the taxonomy of air-breathing, marine, onchidiid slugs, a new genus, *Laspionchis* Dayrat & Goulding, **gen. nov.**, is described from the mangroves of South-East Asia. It includes two new species, *Laspionchis boucheti* Dayrat & Goulding, **sp. nov.**, and *Laspionchis bourkei* Dayrat & Goulding, **sp. nov.**, both distributed from the Malacca Strait to the Philippines and Australia. This study is based on extensive field work in South-East Asia, comparative anatomy, and both mitochondrial (COI and 16S) and nuclear (ITS2 and 28S) DNA sequences. The two new species are found in the same habitat (mud surface in mangrove forests) and are externally cryptic but are distinct anatomically. Both species are also strongly supported by DNA sequences. Three cryptic, least-inclusive, reciprocally-monophyletic units within *Laspionchis bourkei* are regarded as subspecies: *L. bourkei bourkei* Dayrat & Goulding, **ssp. nov.**, *L. bourkei lateriensis* Dayrat & Goulding, **ssp. nov.**, and *L. bourkei matangensis* Dayrat & Goulding, **ssp. nov.** The present contribution shows again that species delineation is greatly enhanced by considering comparative anatomy and nuclear DNA sequences in addition to mitochondrial DNA sequences, and that thorough taxonomic revisions are the best and most efficient path to accurate biodiversity knowledge.

Keywords
Biodiversity, biogeography, Euthyneura, integrative taxonomy, revisionary systematics

Introduction

The diversity of invertebrate species in mangrove forests of South-East Asia is still largely unknown, mainly because mangroves have not been explored well enough, which likely has to do with the fact that mangroves are not the most inviting habitats, even for savvy field naturalists: mangroves are extremely muddy, infested with malaria-carrying mosquitoes and pit vipers, and often located in remote areas. Our lack of biodiversity knowledge is a major issue not only because nobody knows exactly how many species live in the mangroves of South-East Asia, but also because mangroves are still being eradicated at a large scale across the entire region. Onchidiid slugs illustrate well this general situation: until recently, nobody knew exactly how many species of onchidiids lived in the mangroves of South-East Asia, even though they are some of the most common and diverse animals in mangroves (Dayrat 2009).

Onchidiids are marine, air-breathing, true slugs. Adult onchidiids live in the intertidal zone and their larvae develop in sea water, although a few species are adapted to high elevation tropical rainforest (Dayrat 2010). They breathe air through a lung and are related to land snails and slugs (Dayrat et al. 2011). They are called 'true' slugs because they lack an internal shell, even a vestigial shell. The only other group of marine, air-breathing, true slugs is the genus *Smeagol* Climo, 1980, which is not closely-related to onchidiids, but rather considered to belong to the Ellobiidae (Dayrat et al. 2011). The terrestrial, air-breathing, true veronicellid slugs are the most-closely related group to the onchidiids (Dayrat et al. 2011).

In the past ten years, our laboratory has worked on a global taxonomic revision of the Onchidiidae, one genus at a time (Dayrat et al. 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c), based on extensive collecting efforts worldwide, especially in South-East Asia where onchidiids have greatly diversified. The application of old generic names, such as *Onchidium* Buchannan, 1800, *Onchidina* Semper, 1882, *Paraoncidium* Labbé, 1934, and *Peronina* Plate, 1893, is now clear, and new genera are also being discovered: *Alionchis* Goulding & Dayrat in Goulding et al. 2018b, *Marmaronchis* Dayrat & Goulding in Dayrat et al. 2018, *Melayonchis* Dayrat & Goulding in Dayrat et al. 2017, *Paromoionchis* Dayrat & Goulding in Dayrat et al. 2019, and *Wallaconchis* Goulding & Dayrat in Goulding et al. 2018a.

In the present contribution, we describe a new genus, *Laspionchis* gen. nov., and two new species: *Laspionchis boucheti* sp. nov., distributed from the Malacca Strait eastwards to the Philippines and Queensland, Australia, and *Laspionchis bourkei* sp. nov., from the Malacca Strait eastwards to the Philippines and the Northern Territory, Australia. Three cryptic, least-inclusive, reciprocally-monophyletic units within *Laspionchis bourkei* are regarded as three subspecies: *L. bourkei bourkei* Dayrat & Goulding, ssp. nov., *L. bourkei lateriensis* Dayrat & Goulding, ssp. nov., and *L. bourkei matangensis* Dayrat & Goulding, ssp. nov. New taxon names are needed because no existing genus-group name applies to the clade described here and no existing species-group name applies to the species and subspecies described here.

The present study follows an integrative approach to taxonomy (Dayrat 2005), which is based on: (1) a comprehensive review of the nomenclature (all available types of onchidiid species were borrowed and re-examined); (2) the field observation of live animals in their natural habitats; (3) comparative anatomy; and (4) analyses of both mitochondrial (COI, 16S) and nuclear (ITS2, 28S) DNA sequences. The new genus described here is characterized by a combination of anatomical characters which is unique (not found in other onchidiid genera), and its monophyly is strongly supported in molecular phylogenetic analyses. Even though both species are cryptic externally, they are strongly supported by DNA sequences and internal anatomy.

Laspionchis slugs live on the surface of the mud in mangrove forests, where they co-occur with many other onchidiid species with a similar appearance. *Laspionchis* slugs are most especially difficult to distinguish externally from *Paromoionchis* slugs, which are found in the same habitats and the same geographical regions (Dayrat et al. 2019).

Materials and methods

Collecting

All specimens were collected by the authors in the last few years. Collecting parties were led by Benoît Dayrat in Brunei Darussalam, Malaysia, Northern Territory (Australia), Philippines, and Singapore, by Tricia Goulding in Queensland (Australia) and Vietnam, and by Munawar Khalil in Indonesia. We often were accompanied by local villagers or fishermen. Sites were accessed by car or by boat. Each site was explored for an average of two hours, but the exact time spent at each site also depended on the time of the low tide, the weather, etc. At each site, photographs were taken to document the kind of mangrove being visited as well as the diverse microhabitats where specimens were collected.

In the field, specimens were individually numbered and photographed in their habitat. At each site, we tried our best to sample as much diversity as possible. In addition to numbering individually the specimens that looked different, we also numbered individually many specimens that looked similar so that we could test for the presence of cryptic diversity. Importantly, a piece of tissue was cut for all specimens individually numbered (for DNA extraction) and the rest of each specimen was relaxed (using magnesium chloride) and fixed (using 10% formalin or 70% ethanol) for comparative anatomy.

Specimens

All available types of Onchidiidae were examined. Many worldwide museum collections were visited (but no *Laspionchis* material was found). Sixty-one specimens of *Laspionchis* are included in this study: 23 specimens of *L. boucheti* and 38 specimens of *L. bourkei*. Each specimen was examined for comparative anatomy and sequenced for molecular phylogenetic analyses. Individual DNA extraction numbers used in the phylogenetic analyses are indicated in the lists of material examined (numbers are between brackets, and a capitalized letter H indicates a holotype), and size (length/width) is indicated in millimeters (mm) for each specimen. All specimens were deposited as vouchers in institutions in the countries of origin.

Museum collection abbreviations

BDMNH	Brunei Museum, Natural History, Brunei Darussalam
ITBZC	Institute of Tropical Biology, Zoology Collection, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam
MTQ	Museum of Tropical Queensland, Townsville, Queensland, Australia
NTM	Museum and Art Gallery of the Northern Territory
PNM	National Museum of the Philippines, Manila, Philippines
UMIZ	Universitas Malikussaleh, North Aceh, Sumatra, Indonesia
USMMC	Universiti Sains Malaysia, Mollusk Collection, Penang, Malaysia
ZRC	Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore

Anatomical preparations and descriptions

Both the external morphology and the internal anatomy were studied. All anatomical observations were made under a dissecting microscope and drawn with a camera lucida. Radulae and male reproductive organs were prepared for scanning electron microscopy (Zeiss SIGMA Field Emission Scanning Electron Microscopy). Radulae were cleaned in 10% NaOH for a week, rinsed in distilled water, briefly cleaned in an ultrasonic water bath (less than a minute), sputter-coated with gold-palladium, and examined by SEM. Soft parts (penis, accessory penial gland, etc.) were dehydrated in ethanol and critical point dried before coating.

The anatomy of *L. boucheti*, the type species, is fully detailed. The written description of the many anatomical features that are virtually identical between species (nervous system, heart, etc.) is given only for the type species to avoid repetition. So, any feature that is only mentioned in *L. boucheti* is identical in the other species. The color of live animals is described in detail for both species in order to demonstrate the overlapping individual variation between species. As expected, differences between species are mostly found in the male copulatory apparatus, which is described and illustrated in detail for each species. Special attention has been paid to illustrating the holotype of each of the species and subspecies, and the plates illustrating habitats also include a picture from type localities.

Intestinal types

Now that the types of intestinal loops have been reported for every species in many genera of onchidiids (Dayrat et al. 2016, 2017, 2018, 2019; Dayrat and Goulding

2017; Goulding et al. 2018a, b, c), it is possible, and actually necessary, to clarify the differences between the various types of intestinal loops. Plate (1893: pl. 8, figs 29–32) first distinguished four types of intestinal loops (types I to IV) and Labbé (1934: 177–178, fig. 3) later added a type V. However, the pattern of intestinal loops varies, both intra-specifically and inter-specifically. The differences between intestinal types are not as sharp as Plate and Labbé assumed they were, and now they must be clarified.

Here we provide a new approach to help reliably determine intestinal types. Because the intestinal loops found in *Laspionchis* are between type I and type II, we focus here on types I and II. This new approach is based on recognizing three different sections in intestinal loops, each section being colored differently: a clockwise loop is colored in blue, a counterclockwise loop in yellow, and a transitional loop between them in green (Fig. 1). For the sake of clarity, Plate's (1893: pl. 8, figs 29, 31) original illustrations of his types I and II are reproduced here (Fig. 1A, C).

The onchidiid types of intestinal loops are defined based on the dorsal pattern of the intestine. The intestine always first appears dorsally on the right side. In a type I (Fig. 1A, B), the intestine starts by forming a clockwise loop (blue loop). This clockwise loop, however, does not form a complete circle and soon transitions into a counterclockwise loop (yellow loop). As a result, the transitional loop (green loop) between the clockwise and counterclockwise loops is oriented to the right, at 3 o'clock (horizontal red arrow). In a type II (Fig. 1C, D), the clockwise loop (blue loop) is longer and rotates more than in a type I and, as a result, the transitional (green) loop is oriented to the left at 9 o'clock (horizontal red arrow). There is, as always, individual variation. Most usually, the orientation of the transitional loop varies within a range of ca. 90 degrees around a mean axis (i.e., 45 degrees on either side of that axis). So, for instance, in *Paromoionchis tumidus* (Semper, 1880), the species that illustrates a typical type II (Fig. 1D), the transitional loop is always oriented to the left but is not always perfectly horizontal (at 9 o'clock); it can be descending (down to approximately 7 o'clock) or ascending (up to approximately 11 o'clock) (Dayrat et al. 2019: fig. 12).

In *Laspionchis* (Fig. 1E–H), the average orientation of the transitional loop is exactly between a typical type I and a typical type II. Indeed, in most *Laspionchis* individuals, the transitional loop is descending vertically at 6 o'clock (Fig. 1F, G). So, intestinal loops of *Laspionchis* slugs cannot be assigned to either type I or type II. Naturally, there is individual variation (Fig. 1E–H): every specimen listed in the material examined was dissected to check its intestinal loops. In some cases, the intestinal loops appear to be of type II, with the transitional loop oriented to the left and descending at approximately 7 o'clock (see the red arrow in Fig. 1E). In some other cases, the intestinal loops appear to be of type I, with the transitional loop oriented to the right and descending at approximately 5 o'clock (see the red arrow in Fig. 1H). In the individuals examined for the present study, the transitional loop is not higher than 5 o'clock on the right (i.e., it is not oriented at 4 or 3 o'clock) and not higher than 7 o'clock on the left (i.e., it is not oriented at 8 or 9 o'clock). So, the intestinal loops of the two known species of *Laspionchis* are exactly between types I and II. Instead of creating a new intestinal type (number VI), the intestinal loops of *Laspionchis* are simply and adequately referred to as "between types I and II."

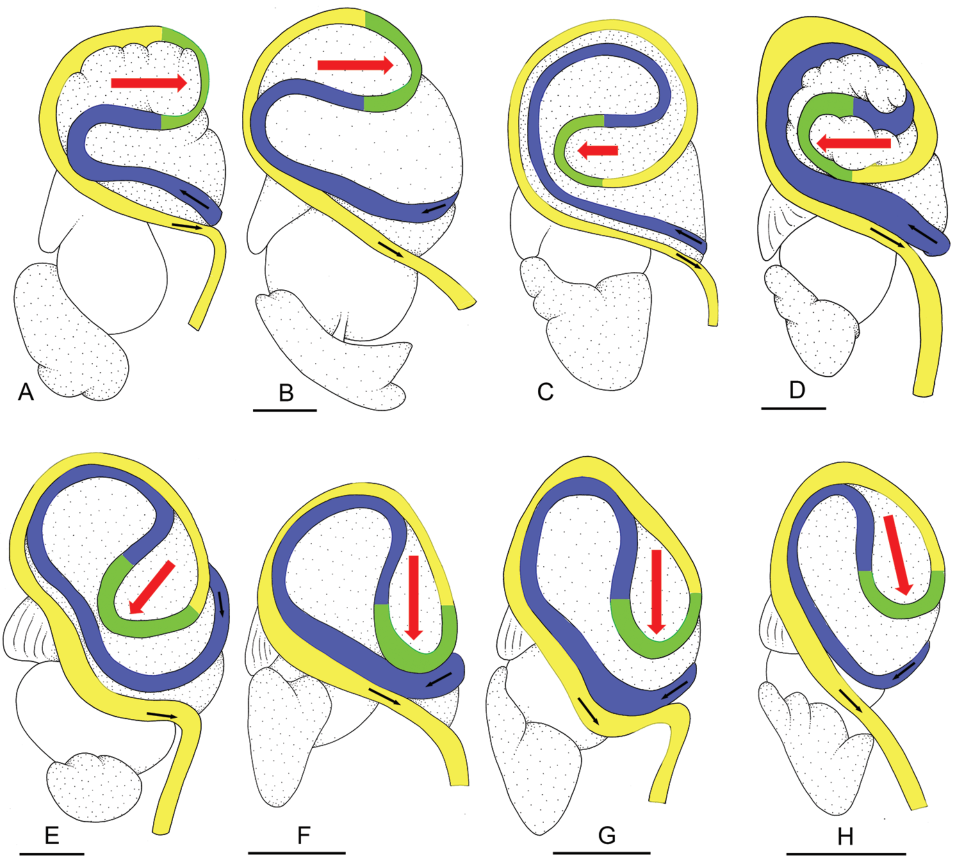

Figure 1. Intestinal loops, dorsal view **A, B** Type I **C, D** Type II **E–H** Between types I and II. Small black arrows show the direction of the intestinal transport. The clockwise loop is in blue. The counterclockwise loop is in yellow. The transitional loop (between clockwise and counterclockwise loops) is in green. Red arrows indicate the orientation of the transitional loop. **A** Type I, with a transitional loop oriented at 3 o'clock, redrawn from Plate (1893: pl. 8, fig. 29) **B** Type I, with a transitional loop oriented at 3 o'clock, *Wallaconchis sinanui* (from Goulding et al. 2018: fig. 8D) **C** Type II, with a transitional loop oriented at 9 o'clock, redrawn from Plate (1893: pl. 8, fig. 31) **D** Type II, with a transitional loop oriented at 9 o'clock, *Paromoionchis tumidus* (from Dayrat et al. 2019: fig. 12A) **E** Between types I and II, with a descending transitional loop at 7 o'clock, holotype, *Laspionchis boucheti*, Australia, Northern Territory, [1688 H] (NTM P.57614) **F** Between types I and II, with a descending transitional loop at 6 o'clock, *L. boucheti*, Australia, Northern Territory, [1681] (NTM P.57612) **G** Between types I and II, with a descending transitional loop at 6 o'clock, *L. boucheti*, Vietnam, [5610] (ITBZC IM 00017) **H** Between types I and II, with a descending transitional loop at 5 o'clock, *L. boucheti*, Australia, Queensland, [2612] (MTQ). Scale bars: 1 mm (**B**), 2 mm (**D, E**), 5 mm (**F–H**).

DNA extraction and PCR amplification

DNA was extracted using a phenol-chloroform extraction protocol with cetyltrimethylammonium bromide (CTAB). The mitochondrial cytochrome *c* oxidase I region (COI) and 16S region were amplified using the following universal primers: LCO1490 (5'-3') GGT CAA CAA ATC ATA AAG ATA TTG G, HCO2198 (5'-3') and TAA ACT TCA

GGG TGA CCA AAR AAY CA (Folmer et al. 1994), 16Sar-L (5'-3') CGC CTG TTT ATC AAA AAC AT (Palumbi 1996), and the modified Palumbi primer 16S 972R (5'-3') CCG GTC TGA ACT CAG ATC ATG T (Dayrat et al. 2011). The nuclear ITS2 region and 28S region were amplified with the following primers: LSU-1 (5'-3') CTA GCT GCG AGA ATT AAT GTG A, LSU-3 (5'-3') and ACT TTC CCT CAC GGT ACT TG (Wade and Mordan 2000), 28SC1 (5'-3') ACC CGC TGA ATT TAA GCA T (Hassouna et al. 1984), and 28SD3 (5'-3') GAC GAT CGA TTT GCA CGT CA (Vonnemann et al. 2005). The 25 µl PCRs for COI and 16S contained 15.8 µl of water, 2.5 µl of 10× PCR Buffer, 1.5 µl of 25 mM $MgCl_2$, 0.5 µl of each 10 µM primer, 2 µl of dNTP Mixture, 0.2 µl (1 unit) of TaKaRa Taq (Code No. R001A), 1 µl of 20 ng/µl template DNA, and 1 µl of 100× BSA (Bovine Serum Albumin). The PCRs for ITS2 used the reagents in the same amounts as COI and 16S, except that water was reduced to 14.8 µl and the amount of 100× BSA was increased to 2 µl. The PCR reaction for 28S included 14.8 µl of water, 2.5 µl of 10× PCR Buffer, 0.5 µl of each 10 µM primer, 1 µl of dNTP Mixture, 5 µl of Q solution (which includes $MgCl_2$) and 0.5 µl of 20 ng/µl template DNA. The thermoprofile used for COI and 16S was: 5 minutes at 94 °C; 30 cycles of 40 seconds at 94 °C, 1 minute at 46 °C, and 1 minute at 72 °C; and a final extension of 10 minutes at 72 °C. The thermoprofile used for ITS2 was: 1 minute at 96 °C; 35 cycles of 30 seconds at 94 °C, 30 seconds at 50 °C, and 1 minute at 72 °C; and a final extension of 10 minutes at 72 °C. The thermoprofile used for 28S was: 4 minutes at 94 °C; 38 cycles of 50 seconds at 94 °C, 1 minute at 52 °C, and 2 minutes 30 seconds at 72 °C; and a final extension of 10 minutes at 72 °C. The PCR products were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) prior to sequencing. Untrimmed sequenced fragments represented approximately 680 bp for COI, 530 bp for 16S, 740 bp for ITS2, and 1000 bp for 28S.

Phylogenetic analyses

Chromatograms were consulted to resolve rare ambiguous base calls. DNA sequences were aligned using Clustal W in MEGA 6 (Tamura et al. 2013). Nineteen onchidiid species outside *Laspionchis* were selected as outgroups from our previous studies (Dayrat et al. 2011, 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c): *Alionchis jailoloensis* Goulding & Dayrat in Goulding et al. 2018b, *Marmaronchis marmoratus* (Lesson, 1831), *Marmaronchis vaigiensis* (Quoy & Gaimard, 1825), *Melayonchis aileenae* Dayrat & Goulding in Dayrat et al. 2017, *Melayonchis eloisae* Dayrat in Dayrat et al. 2017, *Onchidella celtica* (Cuvier in Audouin & Milne-Edwards, 1832), *Onchidella nigricans* (Quoy & Gaimard, 1832), *Onchidina australis* (Semper, 1880), *Onchidium stuxbergi* (Westerlund, 1883), *Onchidium typhae* Buchannan, 1800, *Paromoionchis daemelii* (Semper, 1880), *Paromoionchis tumidus* (Semper, 1880), *Peronia* sp. (Hawaii), *Peronia* sp. (Okinawa), *Peronina tenera* (Stoliczka, 1869), *Peronina zulfigari* Goulding & Dayrat in Goulding et al. 2018c, *Platevindex luteus* (Semper, 1880), *Wallaconchis ater* (Lesson, 1831), and *Wallaconchis sinanui* Goulding & Dayrat in Goulding et al. 2018a. DNA sequences were all deposited in GenBank and vouchers deposited in museum collections (Table 1). The

Table 1. GenBank accession numbers for COI, 16S, ITS2, and 28S DNA sequences. All sequences are new, except the sequences of the outgroups which were obtained from our previous studies (Dayrat et al. 2011, 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c).

Species	Individual (DNA #)	Locality	GenBank COI	GenBank 16S	GenBank ITS2	GenBank 28S
Alionchis jailoloensis	5137	Halmahera, Indonesia	MG953528	MG953538	MG953548	MK122918
Marmaronchis marmoratus	5409	Madang, Papua New Guinea	MK122838	MK122859	MK122893	MK122915
Marmaronchis vaigiensis	1183	Singapore	MK122812	MK122854	MK122877	MK122910
Melayonchis aileenae	970	Peninsular Malaysia	KX240033	KX240057	MK122902	MK125514
Melayonchis eloisae	1011	Singapore	KX240026	KX240050	MK122904	MK125515
Onchidella celtica	5013	France	MG958715	MG958717	MK122906	MK122921
Onchidella nigricans	1524	New South Wales, Australia	MG970878	MG970944	MK122908	MK122923
Onchidina australis	1523	New South Wales, Australia	KX179548	KX179561	MG958719	MG958887
Onchidium stuxbergi	5605	Vietnam	KX179520	KX179537	MG958721	MG958886
Onchidium typhae	965	Peninsular Malaysia	KX179509	KX179525	MG958720	MG958885
Paromoionchis daemelii	1511	New South Wales, Australia	MH055048	MH055129	MH055241	MH055289
Paromoionchis tumidus	1732	Sumatra, Indonesia	MH054951	MH055104	MH055196	MH055268
Peronia sp.	696	Okinawa, Japan	HQ660043	HQ659911	MG958871	MG958883
Peronia sp.	706	Hawaii, USA	HQ660038	HQ659906	MG958722	MG958884
Peronina tenera	960	Peninsular Malaysia	MG958740	MG958796	MG958840	MG958874
Peronina zulfigari	6005	Peninsular Malaysia	MG958775	MG958831	MG958867	MG958882
Platevindex luteus	1001	Singapore	MG958714	MG958716	MG958718	MG958888
Wallaconchis ater	5121	Halmahera, Indonesia	MG970820	MG970911	MG971134	MG971186
Wallaconchis sinanui	2740	Ambon, Indonesia	MG970713	MG970881	MG971093	MG971161
L. boucheti	1004	Singapore	MH619242	MH619303		
	1005	Singapore	MH619243	MH619304	MH619364	MH619413
	1037	Brunei Darussalam	MH619244	MH619305	MH619365	
	1038	Brunei Darussalam	MH619245	MH619306	MH619366	MH619414
	1679	Northern Territory, Australia	MH619246	MH619307		
	1681	Northern Territory, Australia	MH619247	MH619308	MH619368	MH619416
	1685	Northern Territory, Australia	MH619248	MH619309		
	1688 H	Northern Territory, Australia	MH619249	MH619310		
	1729	Sumatra, Indonesia	MH619250	MH619311	MH619369	
	2559	Queensland, Australia	MH619251	MH619312	MH619370	
	2578	Queensland, Australia	MH619252	MH619313	MH619371	
	2593	Queensland, Australia	MH619253	MH619314	MH619372	
	2604	Queensland, Australia	MH619254	MH619315	MH619373	MH619417
	2609	Queensland, Australia	MH619255	MH619316	MH619374	MH619418
	2612	Queensland, Australia	MH619256	MH619317	MH619375	
	2692	Queensland, Australia	MH619257	MH619318	MH619376	MH619419
	2693	Queensland, Australia	MH619258	MH619319		
	3615	Bohol, Philippines	MH619259	MH619320	MH619377	MH619420
	914	Peninsular Malaysia	MH619260	MH619321	MH619378	MH619421
	915	Peninsular Malaysia	MH619261	MH619322	MH619379	
	5520	Peninsular Malaysia	MH619262	MH619323	MH619380	

Species	Individual (DNA #)	Locality	GenBank COI	GenBank 16S	GenBank ITS2	GenBank 28S
L. boucheti	5609	Vietnam	MH619263	MH619324	MH619381	
	5610	Vietnam	MH619264	MH619325	MH619382	MH619422
L. bourkei bourkei	1656	Northern Territory, Australia	MH619290	MH619351		
	1616	Northern Territory, Australia	MH619291	MH619352	MH619402	MH619432
	1617	Northern Territory, Australia	MH619292	MH619353	MH619403	
	1618	Northern Territory, Australia	MH619293	MH619354		
	1621	Northern Territory, Australia	MH619294	MH619355	MH619404	
	1652	Northern Territory, Australia	MH619295	MH619356	MH619405	
	1657 H	Northern Territory, Australia	MH619296	MH619357	MH619406	
	1659	Northern Territory, Australia	MH619297	MH619358	MH619407	
	1666	Northern Territory, Australia	MH619298	MH619359	MH619408	
	1673	Northern Territory, Australia	MH619299	MH619360	MH619409	MH619434
	1692	Northern Territory, Australia	MH619300	MH619361	MH619410	
	1693	Northern Territory, Australia	MH619301	MH619362	MH619411	MH619435
	1694	Northern Territory, Australia	MH619302	MH619363	MH619412	MH619436
L. bourkei lateriensis	2743	Ambon, Indonesia	MH619284	MH619345	MH619396	MH619429
	2753	Ambon, Indonesia	MH619285	MH619346	MH619397	MH619430
	6061	Ambon, Indonesia	MH619286	MH619347	MH619398	
	6063	Ambon, Indonesia	MH619287	MH619348	MH619399	
	6064 H	Ambon, Indonesia	MH619288	MH619349	MH619400	MH619431
	6065	Ambon, Indonesia	MH619289	MH619350	MH619401	
L. bourkei matangensis	978	Singapore	MH619265	MH619326	MH619383	MH619423
	979	Singapore	MH619266	MH619327	MH619384	MH619424
	980	Singapore	MH619267	MH619328		
	983	Singapore	MH619268	MH619329		
	985	Singapore	MH619269	MH619330		
	1783	Sumatra, Indonesia	MH619270	MH619331		
	1784	Sumatra, Indonesia	MH619271	MH619332		
	1785	Sumatra, Indonesia	MH619272	MH619333	MH619385	
	2230	Sulawesi, Indonesia	MH619273	MH619334	MH619386	MH619425
	3343	Bohol, Philippines	MH619274	MH619335	MH619387	MH619426
	3616	Bohol, Philippines	MH619275	MH619336	MH619388	
	5627	Vietnam	MH619276	MH619337	MH619389	MH619427
	5646	Vietnam	MH619277	MH619338	MH619390	
	5958 H	Peninsular Malaysia	MH619278	MH619339	MH619391	MH619428
	5959	Peninsular Malaysia	MH619279	MH619340	MH619392	
	5960	Peninsular Malaysia	MH619280	MH619341	MH619393	
	5961	Peninsular Malaysia	MH619281	MH619342	MH619394	
	5963	Peninsular Malaysia	MH619282	MH619343	MH619395	
	5965	Peninsular Malaysia	MH619283	MH619344		

ends of each alignment were trimmed. Alignments of mitochondrial (COI and 16S) sequences and nuclear (ITS2 and 28S) sequences were concatenated separately, in order to test whether these two data sets support the same relationships. The concatenated mitochondrial alignment included 991 nucleotide positions: 577 (COI) and 414 (16S). The concatenated ITS2 and 28S alignment included 1614 nucleotide positions: 658 (ITS2) and 956 (28S).

Four independent sets of phylogenetic analyses were performed: 1) Maximum Likelihood and Bayesian analyses with concatenated mitochondrial COI and 16S sequences; 2) Maximum Likelihood and Bayesian analyses with concatenated nuclear ITS2 and 28S sequences; 3) Maximum Parsimony analyses with concatenated nuclear ITS2 and 28S sequences; 4) Maximum Parsimony analyses with just nuclear ITS2 sequences. Prior to Maximum Likelihood and Bayesian phylogenetic analyses, the best-fitting evolutionary model was selected for each locus separately using the Model Selection option from Topali v2.5 (Milne et al. 2004): a GTR + G model was independently selected for COI and 16S, and an HKY + G model was independently selected for ITS2 and 28S. Maximum Likelihood analyses were performed using PhyML (Guindon and Gascuel 2003) as implemented in Topali v2.5. Node support was evaluated using bootstrapping with 100 replicates. Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) as implemented in Topali v2.5, with five simultaneous runs of 1.5×10^6 generations each, sample frequency of 100, and burn in of 25% (and posterior probabilities were also calculated). Topali did not detect any issue with respect to convergence. Maximum Parsimony analyses were conducted in PAUP v 4.0 (Swofford 2002), with gaps coded as a fifth character state and 100 bootstrap replicates conducted using a full heuristic search. All analyses were run several times and yielded the same result.

In addition, another set of analyses was performed with only COI sequences: genetic distances between COI sequences were calculated in MEGA 6 as uncorrected p-distances. COI sequences were also translated into amino acid sequences in MEGA using the invertebrate mitochondrial genetic code to check for the presence of stop codons (no stop codon was found).

Results

Molecular phylogenetic analyses

DNA sequences were used to test species limits within *Laspionchis*. The monophyly of *Laspionchis* is strongly supported in all analyses (Figs 2–5). In the analyses based on mitochondrial COI and 16S concatenated sequences, there are four least-inclusive units that are all reciprocally monophyletic: *L. boucheti*, *L. bourkei bourkei*, *L. bourkei lateriensis*, and *L. bourkei matangensis* (Fig. 2). The monophyly of each unit is strongly supported by a bootstrap support of 100 and a posterior

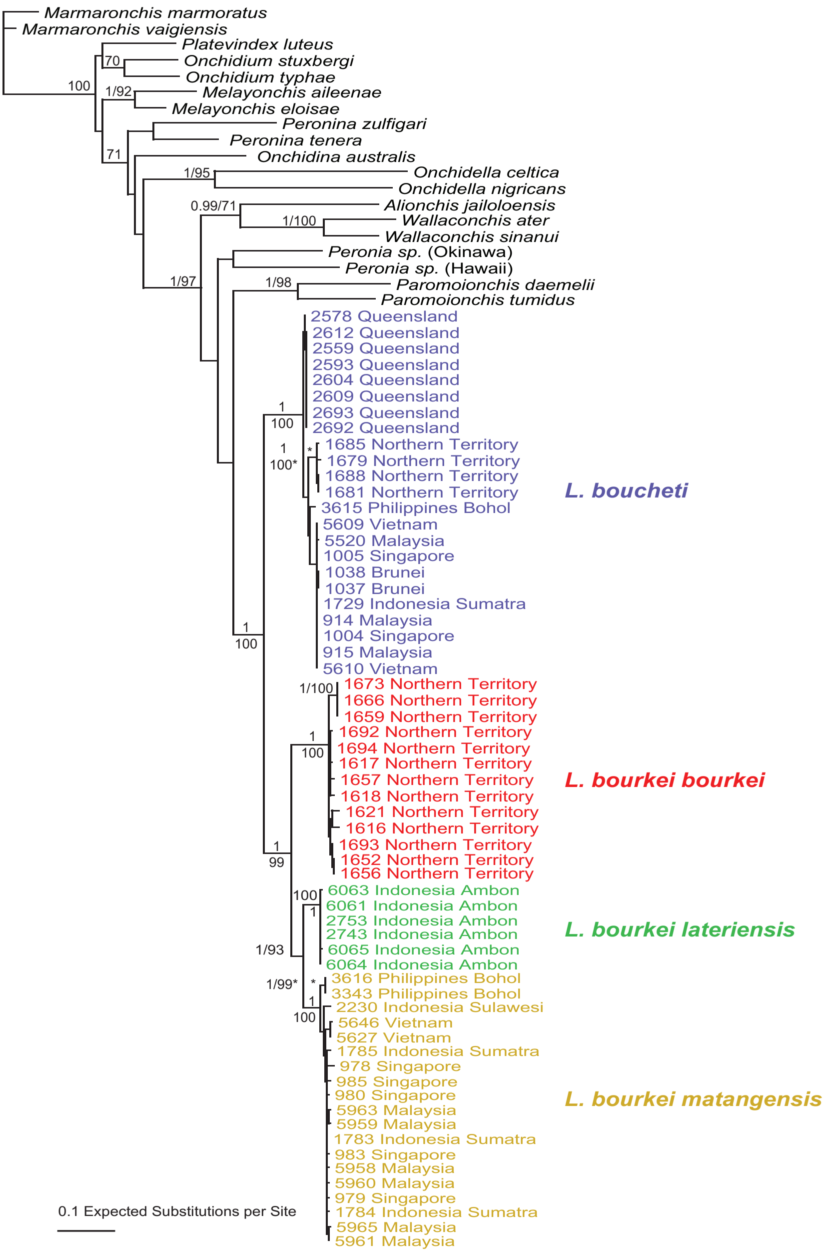

Figure 2. Phylogenetic relationships within *Laspionchis* based on concatenated mitochondrial COI and 16S DNA sequences for 80 individuals (including 19 outgroups). Numbers by the branches are the bootstrap values (maximum likelihood analysis, ML) and the posterior probabilities (Bayesian analysis). Only numbers > 60% (ML) and > 0.9 (Bayesian) are indicated. Numbers for each individual correspond to unique identifiers for DNA extraction. All sequences of *Laspionchis* individuals are new. Sequences of the outgroups are from our previous studies (Dayrat et al. 2011, 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c). Information on specimens can be found in the lists of material examined and in Table 1. The color used for each subspecies is the same as the color used in Figs 3–7.

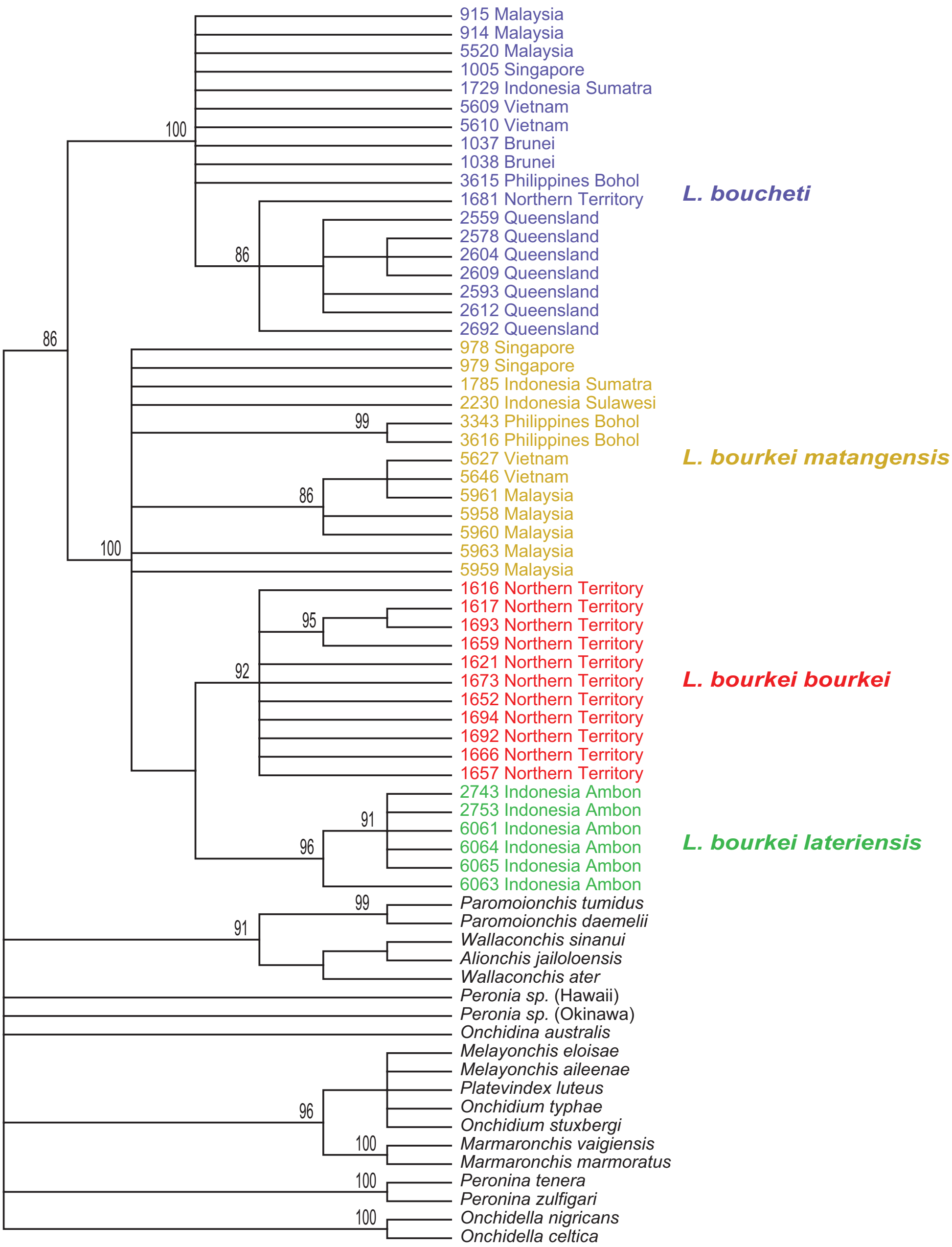

Figure 3. Maximum parsimony consensus tree within *Laspionchis*, performed with ITS2 DNA sequences from 67 individuals (including 19 outgroups). Numbers by the branches are the bootstrap values (only numbers > 70% are indicated). Numbers for each individual correspond to unique identifiers for DNA extraction. All sequences of *Laspionchis* individuals are new. Outgroups sequences are from our previous studies (Dayrat et al. 2011, 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c). Information on specimens can be found in the lists of material examined and in Table 1. The color used for each subspecies is the same as the color used in Figs 2, 4–7.

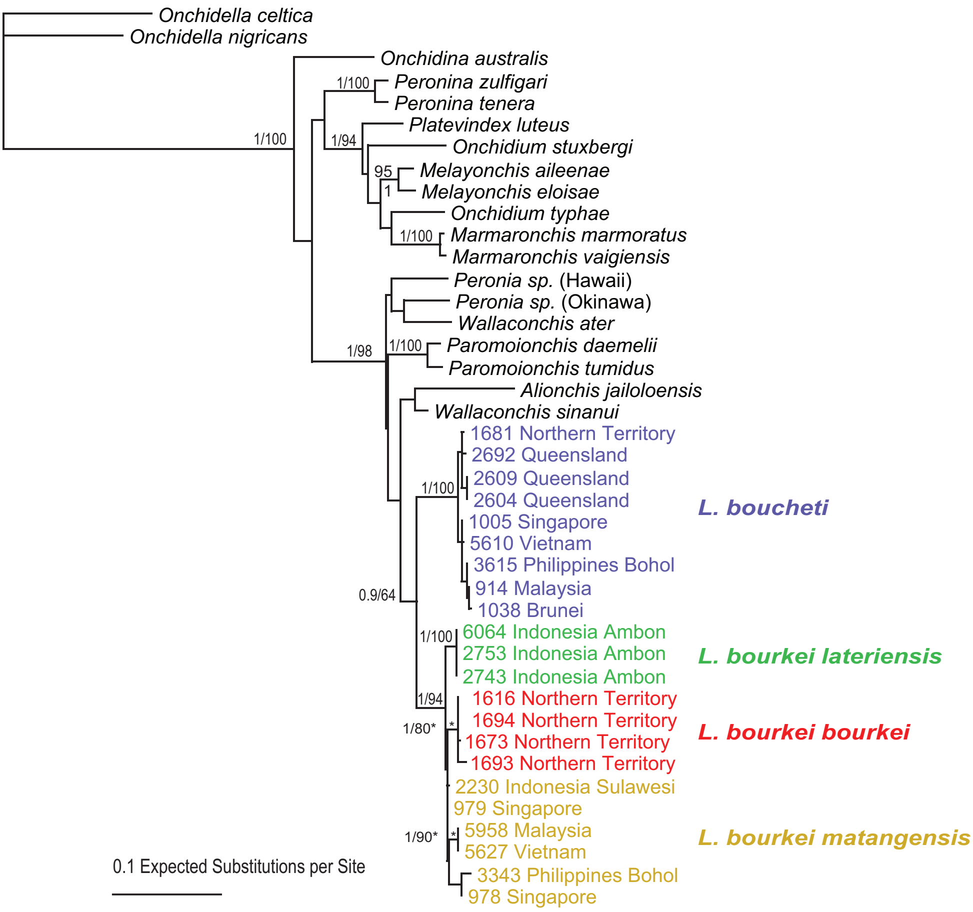

Figure 4. Phylogenetic relationships within *Laspionchis* based on concatenated nuclear ITS2 and 28S DNA sequences for 41 individuals (including 19 outgroups). Numbers by the branches are the bootstrap values (Maximum Likelihood analysis, ML) and the posterior probabilities (Bayesian analysis, B). Only numbers > 60% (ML) and > 0.9 (B) are indicated. Numbers for each individual correspond to unique identifiers for DNA extraction. All sequences of *Laspionchis* individuals are new. Outgroups sequences are from our previous studies (Dayrat et al. 2011, 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c). Information on specimens can be found in the lists of material examined and in Table 1. The color used for each subspecies is the same as the color used in Figs 2, 3, 5–7.

probability of 1. The monophyly of *L. bourkei* is also strongly supported (bootstrap support of 99 and a posterior probability of 1). Analyses with nuclear sequences (ITS2 alone, and concatenated ITS2 and 28S) yielded results similar to the mitochondrial sequences (Figs 3–5). *Laspionchis boucheti*, *L. bourkei*, *L. bourkei bourkei*, and *L. bourkei lateriensis* are strongly supported in all nuclear analyses: the relationships between individuals of *Laspionchis bourkei matangensis* are unresolved in nuclear analyses.

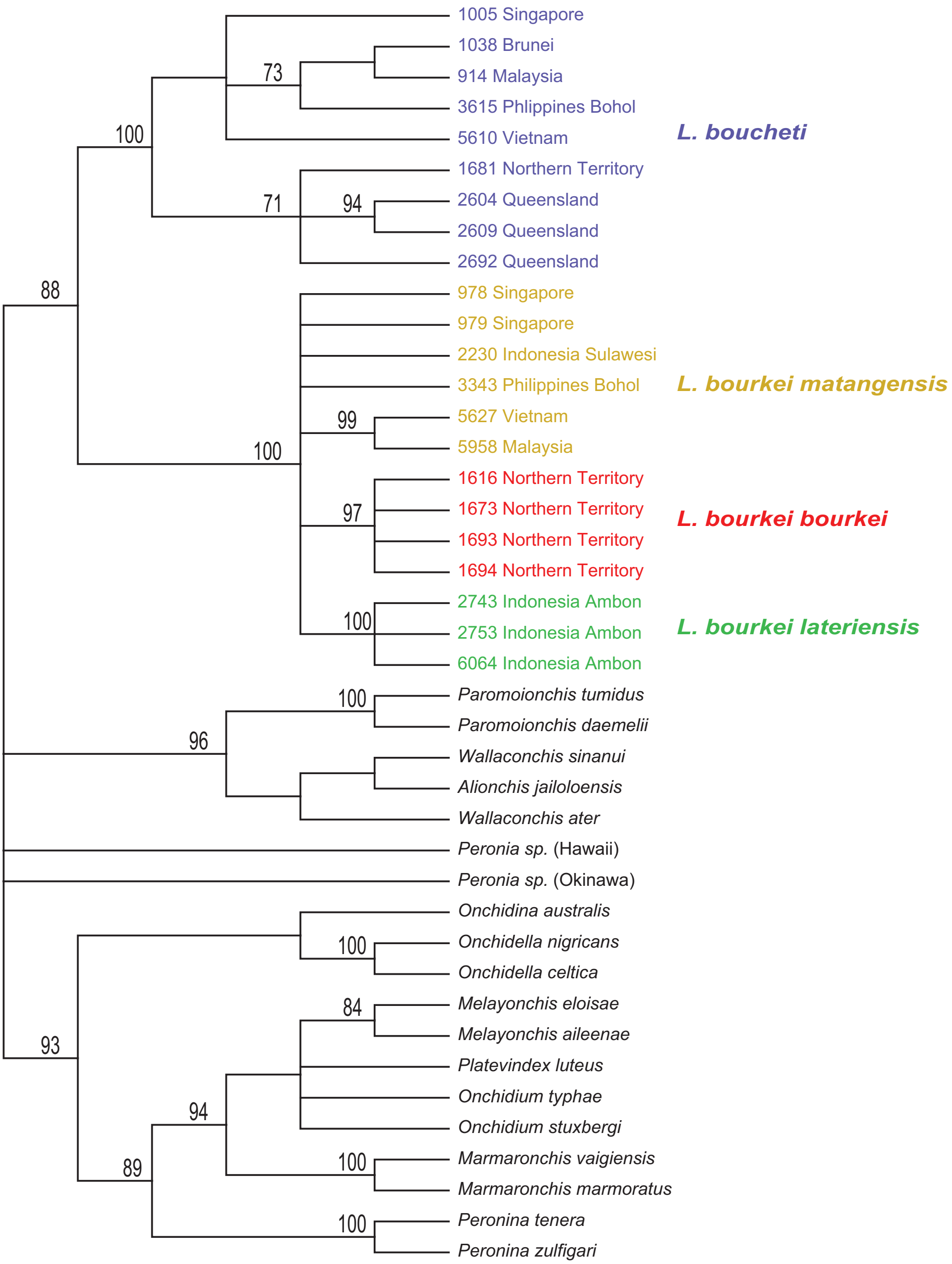

Figure 5. Maximum parsimony consensus tree within *Laspionchis*, performed with concatenated nuclear ITS2 and 28S DNA sequences from 41 individuals (including 19 outgroups). Numbers by the branches are the bootstrap values (only numbers > 70% are indicated). Numbers for each individual correspond to unique identifiers for DNA extraction. All sequences of *Laspionchis* individuals are new. Outgroups sequences are from our previous studies (Dayrat et al. 2011, 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c). Information on specimens can be found in the lists of material examined and in Table 1. The color used for each subspecies is the same as the color used in Figs 2–4, 6, 7.

Pairwise genetic divergences

Pairwise genetic distances (between COI sequences) support the existence of four least-inclusive molecular units of *Laspionchis* (Table 2, Fig. 6). The intra-unit genetic distances are all below 2.5%: below 2.5% within *L. boucheti*, below 2.5% within *L. bourkei matangensis*, below 1.2% within *L. bourkei lateriensis*, and 0% within *L. bourkei lateriensis*. The inter-unit distances vary from 3.9% (the distance between *L. bourkei lateriensis* and *L. bourkei matangensis*) to 10.4% (the distance between *L. boucheti* and *L. bourkei bourkei*). So, overall, the distance gap between *L. boucheti* and *L. bourkei* is between 2.5% and 7.5%, and the distance gap between the three *L. bourkei* units (*L. bourkei bourkei*, *L. bourkei lateriensis*, and *L. bourkei matangensis*) is between 2.5% and 3.9%.

Comparative anatomy

In the field, slugs were numbered individually without being assigned to any particular species because onchidiid species are commonly cryptic externally. As anticipated, *Laspionchis boucheti* and *L. bourkei* are externally cryptic (Table 3). However, *Laspionchis boucheti* differs from *L. bourkei* in internal anatomy, and they cannot be confused: in *L. boucheti*, the long retractor muscle of the penis inserts at the posterior end of the visceral cavity, while the retractor muscle is absent, vestigial, or short (inserting in the first third of the visceral cavity) in *L. bourkei*. Also, additional, distal, retractor muscle fibers are present in *L. boucheti* but absent in *L. bourkei*. However, the three subspecies of *L. bourkei* are hardly distinguishable anatomically (Table 3).

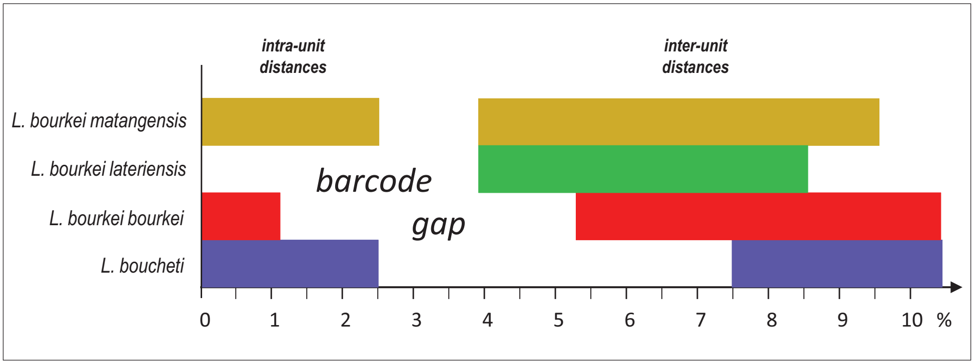

Figure 6. Diagram to help visualize the data on pairwise genetic distances between COI sequences within and between mitochondrial units in *Laspionchis* (see Table 3). Ranges of minimum to maximum distances are indicated (in percentages). For instance, within *L. boucheti*, individual sequences are between 0 and 2.5% divergent; individual sequences between *L. boucheti* and the other units are minimally 7.5% and maximally 10.4% divergent; overall, the distance gap between *L. boucheti* and *L. bourkei* is of 5% (i.e., between 2.5% and 7.5%). The colors are the same as those used in Figs 2–5, 7.

Table 2. Intra- and inter-unit pairwise genetic distances between the four mitochondrial units of *Laspionchis* based on our data set of 61 COI sequences (Table 1). Ranges of minimum to maximum distances are indicated (as percentages): e.g., individual sequences within *L. boucheti* are between 0 and 2.7% divergent, and individual sequences between *L. boucheti* and *L. bourkei bourkei* are minimally 8.6% and maximally 10.4% divergent. Overall, the distance gap between all four mitochondrial units is between 2.5% (the maximum intra-unit distance within *L. boucheti* and within *L. bourkei matangensis*) and 3.9% (the minimum distance between *L. bourkei lateriensis* and *L. bourkei matangensis*). Finally, the distance gap between the two species *L. boucheti* and *L. bourkei* is between 2.5% and 7.5%.

Species	*L. boucheti*	*L. bourkei*		
		bourkei	*lateriensis*	*matangensis*
L. boucheti	0.0–2.5			
L. bourkei bourkei	8.6–10.4	0.0–1.2		
L. bourkei lateriensis	7.5–8.5	6.1–7.8	0.0–0.0	
L. bourkei matangensis	7.5–9.5	5.3–6.1	3.9–5.5	0.0–2.5

Table 3. Summary of traits that can help distinguish the two species of *Laspionchis*. All traits are subject to individual variation. Traits are described in detail in the corresponding species descriptions. Traits are also indicated for the three subspecies of *L. bourkei*.

Species	Retractor muscle (penis)	Retractor muscle (penis) attachment site	Distal muscle fibers	Accessory penial gland spine size (mm)	Penial hooks (µm)	Distribution
L. boucheti	Strong and long	Posterior end of visceral cavity (by the rectum)	yes	0.7 to 1	60 to 160	Peninsular Malaysia, Indonesia (Sumatra), Singapore, Brunei, Vietnam, Philippines (Bohol), Australia (Northern Territory, Queensland)
L. bourkei bourkei	Very short	Anterior third of the visceral cavity	no	0.75 to 1	20 to 35	Australia (Northern Territory)
L. bourkei lateriensis	Absent or vestigial	–	no	0.35 to 0.75	20 to 45	Indonesia (Ambon)
L. bourkei matangensis	Absent or vestigial	–	no	0.43 to 0.57	15 to 40	Peninsular Malaysia, Indonesia (Sulawesi, Sumatra), Singapore, Philippines (Bohol), Vietnam

Species delineation

The new genus described here, *Laspionchis*, is a strongly-supported clade in all molecular analyses (Figs 2–5). It also is characterized by a unique combination of anatomical characters (see below, the Remarks on the genus diagnosis). Two species are recognized here, *Laspionchis boucheti* and *L. bourkei*, which are cryptic externally but distinct anatomically (Table 3). Their reciprocal monophyly is strongly supported by both nuclear and mitochondrial sequences (Figs 2–5) and they are separated by a clear barcode gap (from 2.5% to 7.5%) in genetic distances between COI sequences (Table 2, Fig. 6). In addition, three subspecies are recognized within *Laspionchis bourkei*: *L. bourkei bourkei*, *L. bourkei lateriensis*, and *L. bourkei matangensis*, which are cryptic externally and hardly distinguishable internally (Table 3). Their reciprocal monophyly is strongly supported by mitochondrial sequences as well as by nuclear sequences (Figs 2–5) even though *L. bourkei matangensis* is unresolved in nuclear analyses. All three subspecies of *L. bourkei* are separated by a barcode gap in genetic distances between COI sequences (from 2.5% to 3.9%) which, as expected, is not as large as the gap found between *L. boucheti* and *L. bourkei* (Table 2, Fig. 6). The ranking of the three least-inclusive units within *L. bourkei* as subspecies is discussed in the general discussion.

Systematics and anatomical descriptions

Family Onchidiidae Rafinesque, 1815

Genus *Laspionchis* Dayrat & Goulding, gen. nov.

http://zoobank.org/47CA237B-3E0F-49BF-A866-C551E979A236

Type species. *Laspionchis boucheti*, designated here.

Etymology. Combination of *láspi*, a Greek word meaning mud, and *onchis*, a word derived from the Greek *ὁ ὄγκος* (mass, tumor) and used in the past for onchidiid slugs. *Laspionchis* conveniently refers to those onchidiid species that always live on mud and are covered with a thin layer of mud.

Gender. Gender masculine of *onchis* (ICZN Art. 30.1.1), a word derived from the masculine Greek word *ὁ ὄγκος*.

Diagnosis. Body not flattened. No dorsal gills. Dorsal eyes present on notum. Retractable, central papilla (usually with four dorsal eyes) present, often raised above dorsal surface. Eyes at tip of short ocular tentacles. Male opening below right ocular tentacle (or below it and very slightly to its left). No transversal protuberance on oral lobes. Foot wide. Pneumostome median, on ventral hyponotum. Intestinal loops exactly between types I and II (with a transitional loop on average descending at 6 o'clock). Rectal gland absent. Accessory penial gland present with a hollow spine and a muscular sac. Penis with hooks: numerous, densely arranged next to each other, and pointed.

Remarks. No external diagnostic feature unambiguously distinguishes *Laspionchis* from other onchidiid genera. Externally, *Laspionchis* slugs are especially difficult to distinguish from *Paromoionchis* slugs, which live in the same habitat (mud surface) and are often found together at the exact same sites. Also, for a non-expert, *Laspionchis* slugs could easily be confused with *Peronina* or *Onchidium* slugs, although those are characterized by distinctive, external features. However, *Laspionchis* is characterized by a unique combination of internal and external characters: no dorsal gills, male opening below the right eye tentacle (or below it and very slightly to its left), no rectal gland, intestinal loops between types I and II (i.e., with a transitional loop on average oriented at 6 o'clock), accessory penial gland present with a muscular sac, penis with numerous, pointed hooks densely arranged next to each other. According to our data, any onchidiid slug with this combination of characters belongs to *Laspionchis*.

Intestinal loops between types I and II, with a transitional loop on average oriented at 6 o'clock, could almost be regarded as diagnostic of *Laspionchis* slugs, acknowledging the existence of variation (both intra-specific and inter-specific). Indeed, in *Laspionchis* slugs, the transitional loop is normally oriented at 6 o'clock, even though, strictly speaking, its orientation actually varies between 5 and 7 o'clock (Fig. 1). The intestinal loops of some individuals of other species can sometimes be characterized by a transitional loop oriented within that same range (between 5 and 7 o'clock), such as in species with intestinal loops of type I and a transitional loop oriented from 3 to 6 o'clock (as in *Wallaconchis*, see Goulding et al. 2018a), and in species with intestinal loops of type II and a transitional loop oriented from 6 to 9 o'clock (as in *Paromoionchis*, see Dayrat et al. 2019). However, the important difference here is that a transitional loop oriented at 6 o'clock is the norm in *Laspionchis*, while it is not the norm in those species from other genera.

A new generic name is needed because no existing name applies to the clade described here. Based on the examination of all the type specimens available in Onchidiidae (especially those of all the type species), a careful study of all the original descriptions (especially when no type specimens were available), and our ongoing taxonomic revision of every genus of the family (Dayrat et al. 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c), it appears that there is no generic name of which the type species matches the diagnosis of this genus. For a recent review of the application of all existing generic names of Onchidiidae, see Dayrat et al. (2017: 1861).

Laspionchis boucheti **Dayrat & Goulding, sp. nov.**

http://zoobank.org/41EC4683-63DD-4C15-B3CA-F0FB6E543A33
Figs 8–15

Holotype. AUSTRALIA • holotype, designated here, 30/20 mm [1688 H]; Northern Territory, Darwin, end of the Channel Island Road; 12°33.557'S, 130°52.894'E; 17 Aug. 2012; B Dayrat and party leg.; st 66, sequence of *Sonneratia*, *Rhizophora*, and *Ceriops*; NTM P.57614.

Additional material examined. AUSTRALIA – **Northern Territory** • 2 specimens 25/20 mm [1679], 35/25 mm [1681]; Darwin, near Channel Island Road; 12°34.979'S, 130°55.992'E; 16 Aug. 2012; B Dayrat and party leg.; st 65, sequence of *Sonneratia*, *Rhizophora*, and *Ceriops*; NTM P.57612. • 1 specimen 16/10 mm [1685]; same collection data as for the holotype; NTM P.57613. – **Queensland** • 1 specimen 15/10 mm [2559]; Cairns, Yorkey's Knob; 16°48.558'S, 145°42.768'E; 17 Jun. 2013; TC Goulding and party leg.; st 101, hard, red mud with grasses; MTQ. • 1 specimen 15/9 mm [2578]; Cairns, Yorkey's Knob; 16°48.503'S, 145°42.869'E; 21 Jun. 2013; TC Goulding and party leg.; st 105, hard, red mud with grasses; MTQ. • 1 specimen 18/15 mm [2593]; Townsville, Magnetic Island; 19°09.733'S, 146°48.625'E; 24 Jun. 2013; TC Goulding and party leg.; st 108, very soft mud near creek, with some *Rhizophora* and *Avicennia* trees on sides; MTQ. • 1 specimen 16/15 mm [2604]; Townsville; 19°17.717'S, 146°49.487'E; 25 Jun. 2013; TC Goulding and party leg.; st 110, mangrove of short and dense trees; MTQ. • 2 specimens 21/14 mm [2609], 30/17 mm [2612]; Townsville, Ross River; 19°16.275'S, 146°50.284'E; 26 Jun. 2013; TC Goulding and party leg.; st 111, open forest of young *Avicennia*, very soft mud; MTQ. • 1 specimen 26/20 mm [2692], 30/17 mm [2693]; Mackay, Barnes Creek; 21°07.815'S, 149°11.396'E; 7 Jul. 2013; TC Goulding and party leg.; st 124, soft mud, open area with *Avicennia* and some Rhizophora, wetland restoration area; MTQ. BRUNEI DARUSSALAM • 2 specimens 31/20 mm [1037], and 14/8 mm [1038]; Pulau Pyatan, Teluk Brunei; 04°55.246'N, 115°02.764'E; 27 Jul. 2011; B Dayrat and party leg.; st 32, open mangrove with a few sparse old trees, and large old logs, by the river; BDMNH. INDONESIA – **Sumatra** • 1 specimen 15/10 mm [1729]; Tembilahan; 00°10.243'S, 103°27.982'E; 13 Oct. 2012; M Khalil and party leg.; st 76, mangrove of large *Avicennia* trees, with old logs, soft but solid mud, and *Nypa* on the margin; UMIZ 00112. MALAYSIA – **Peninsular Malaysia** • 1 specimen 20/10 mm [914]; Matang, facing fishermen's village on the other side of river; 04°50.154'N, 100°36.368'E; 20 Jul. 2011; B Dayrat and party leg.; st 29, oldest and open *Rhizophora* forest of tall and beautiful trees, with hard mud, many creeks, and many old logs; USMMC 00054. • 1 specimen 12/8 mm [915]; Matang, off Kuala Sepatang, Crocodile River, Sungai Babi Manpus; 04°49.097'N, 100°37.370'E; 19 Jul. 2011; B Dayrat and party leg.; st 28, old and open *Rhizophora* forest with tall trees, hard mud, creeks, and many old logs; USMMC 00053. • 1 specimen 10/8 mm [5520]; Kuala Sepatang; 04°50.434'N, 100°38.176'E; 19 Jul. 2011; B Dayrat and party leg.; st 27, old forest with tall, old *Rhizophora* trees, high in the tidal zone (ferns), in educational mangrove preserve, at a creek lower in the tidal zone, with mud; USMMC 00052. PHILIPPINES – **Bohol** • 1 specimen 14/11 mm [3615]; Inabanga; 10°00.389'N, 124°03.522'E; 12 Jul. 2014; B Dayrat and party leg.; st 186, old, rehabilitated fish ponds next to a mangrove with some old *Avicennia* but mostly young *Rhizophora* trees; PNM 041252. SINGAPORE • 1 specimen 15/12 mm [1004]; Lim Chu Kang, 01°26.785'N, 103°42.531'E; 5 Apr. 2010; B Dayrat and party leg.; st 9, east of the jetty, open mangrove with medium trees, ending on mudflat outside mangrove with soft mud; ZRC.MOL.10483. • 1 specimen 10/8 mm [1005]; Pasir Ris Park; 01°22.840'N, 103°57.224'E; 30 Mar. 2010; B Dayrat and party leg.; st 5, mangrove forest with rich litter, lobster mounds, and old logs; ZRC.MOL.10482. VIETNAM • 2 specimens 31/20 mm [5609], 39/25 mm [5610]; Can Gio; 10°27.620'N, 106°53.316'E; 17 Jul. 2015; TC Goulding and party leg.; st 231, open mangrove with large *Avicennia* trees, soft mud, some old logs; ITBZC IM 00017.

Distribution (Fig. 7). Australia: Northern Territory, Queensland. Brunei Darussalam. Indonesia: Sumatra. Malaysia: Peninsular Malaysia. Singapore. Philippines: Bohol. Vietnam.

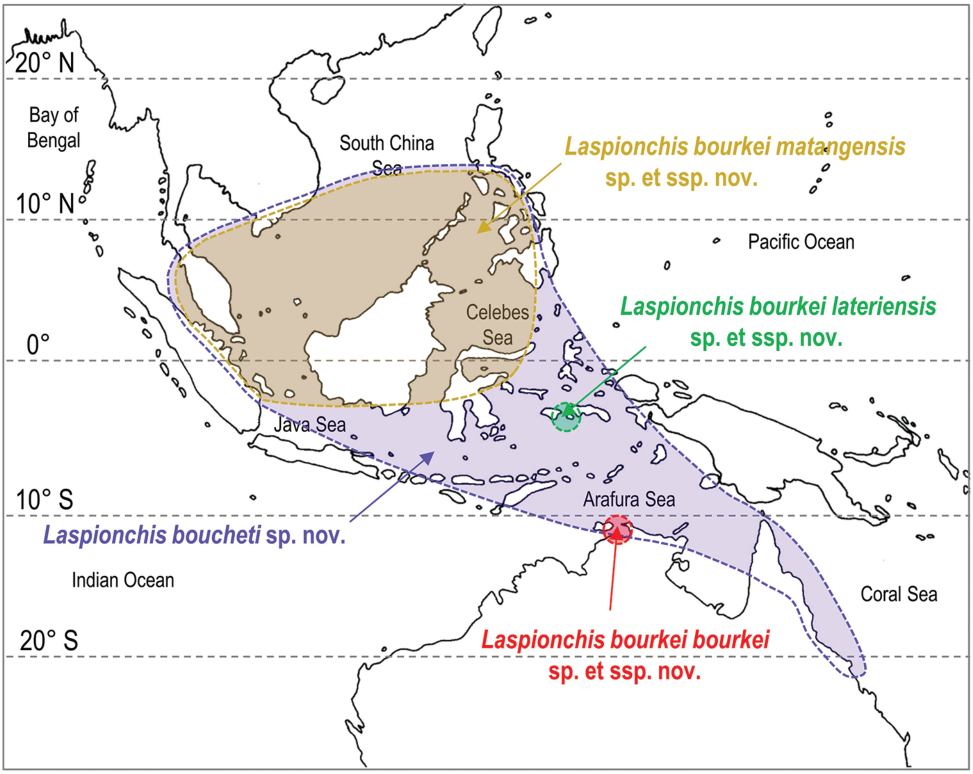

Figure 7. Geographical distribution of the two species of *Laspionchis*. Distinct colors are used for each subspecies of *L. bourkei*. The colors used are the same as those used in the phylogenetic trees (Figs 2–5). Colored areas correspond to hypothetical geographical ranges based on known records.

Habitat (Fig. 8). *Laspionchis boucheti* is found on mud, hard or soft, in open or dense mangrove forests. It is common across its entire distribution range.

Etymology. *Laspionchis boucheti* is dedicated to Philippe Bouchet, professor of Malacology at the Muséum national d'Histoire naturelle, Paris, France, for the training that he generously provided to the first author as a graduate student at the MNHN, years ago, for kindly allowing us to study some material collected during expeditions that he organized (Kavieng, Madagascar, New Caledonia, Papua New Guinea, Vanuatu), and, more broadly, for his unconditional love of snails and slugs, biodiversity exploration, and alpha-taxonomy.

Diagnosis (Table 3). Externally, *Laspionchis boucheti* cannot be distinguished from *L. bourkei*. Internally, however, the insertion of the retractor muscle of the penis (at the posterior end of the visceral cavity) and the presence of additional, distal retractor muscle fibers can help distinguish *L. boucheti* from *L. bourkei*.

Color and morphology of live animals (Figs 9, 10). Live slugs are covered with mud and their dorsal color can hardly be seen. The background of the dorsal notum is brown, light to dark, homogenous or mottled with darker or lighter areas, and,

Figure 8. Habitats, *Laspionchis boucheti*. **A** Australia, Northern Territory, *Sonneratia*, *Rhizophora*, and *Ceriops* mangrove (st 66, type locality) **B** Peninsular Malaysia, *Rhizophora*, hard mud, open space, old forest (st 29) **C** Australia, Northern Territory, *Sonneratia*, *Rhizophora*, and *Ceriops* mangrove (st 65) **D** Australia, Queensland, mangrove of short shrubs and dense trees (st 110) **E** Australia, Queensland, soft mud, open area with *Avicennia*, some *Rhizophora* (st 124).

occasionally, with red areas too. In some slugs, the tip of dorsal papillae (with and without dorsal eyes) can be yellow. The color of the foot is gray (light or dark), yellow, or orange. The hyponotum is light or dark grey, pale yellow or red, sometimes with a

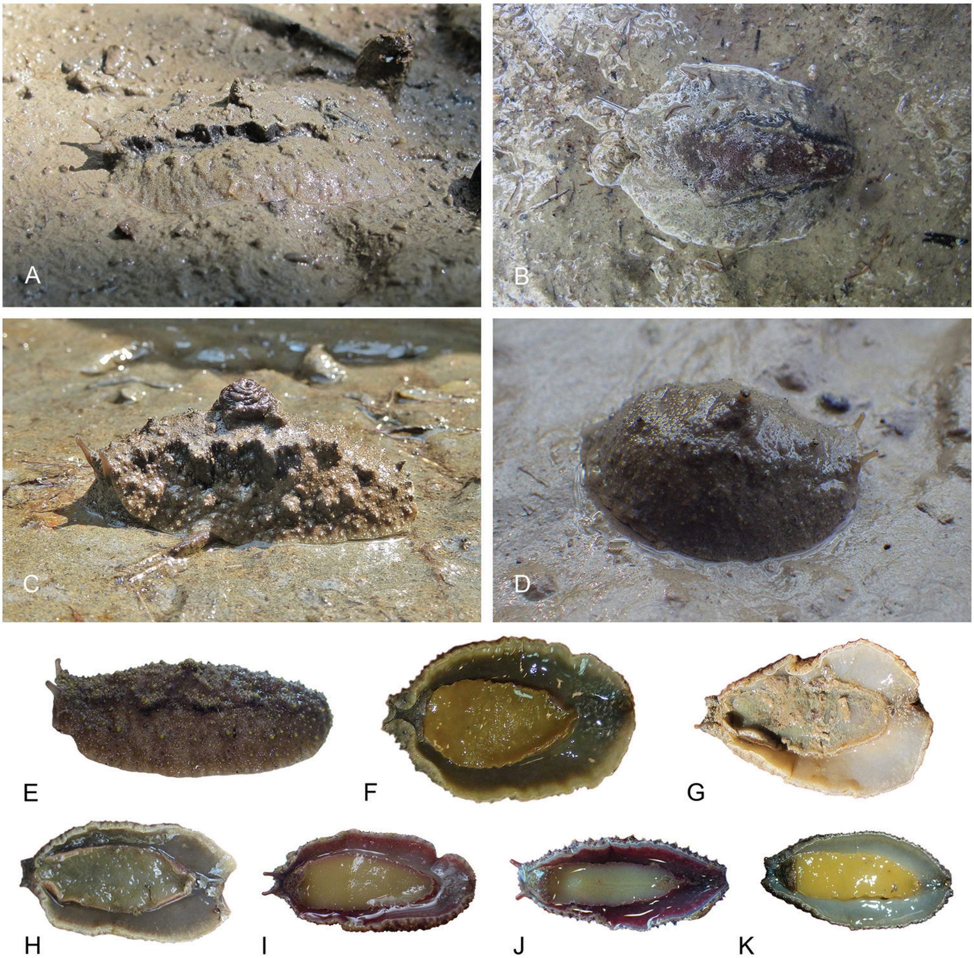

Figure 9. Live animals, *Laspionchis boucheti*. **A** Dorsal view, 35 mm long [1681], Australia, Northern Territory (NTM P.57612) **B** holotype, dorsal view, 30 mm long [1688 H], Australia, Northern Territory (NTM P.57614) **C** dorsal view, 31 mm long [1037], Brunei (BDMNH) **D** dorsal view, 15 mm long [1729], Indonesia, Sumatra (UMIZ 00112) **E** dorsal view, 30 mm long [2612], Australia, Queensland (MTQ) **F** ventral view, same as **C** **G** ventral view, same as **A** **H** ventral view, same as **B** **I** ventral view, 21 mm long [2609], Australia, Queensland (MTQ) **J** ventral view, 26 mm long [2692], Australia, Queensland (MTQ) **K** ventral view, 20 mm long [914], Peninsular Malaysia (USMMC 00054).

lighter whitish margin. The color of the foot and of the hyponotum of an individual can change rapidly, especially when disturbed. The ocular tentacles are brown (variable from light to dark) and short (a few millimeters).

Generally, the dorsal notum of any given slug can rapidly change from almost perfectly smooth to densely covered by many papillae. However, when slugs are not disturbed, the dorsum is usually covered by papillae of various sizes. In some slugs, larger papillae may be arranged in two longitudinal ridges on either side of the median

Figure 10. Live animals, *Laspionchis boucheti*, dorsal view. **A** 39 mm long [5610], Vietnam, Can Gio (ITBZC IM 00017) **B** 31 mm long [5609], Vietnam, Can Gio (ITBZC IM 00017) **C** 15 mm long [2578], Australia, Queensland (MTQ) **D** 16 mm long [1685], Australia, Northern Territory (NTM P.57613).

line, but those ridges can appear and disappear rapidly. Some papillae bear dorsal eyes at their tip (most papillae bear three eyes). The number of papillae with dorsal eyes is variable (between 8 and 12, on average) and they mostly are on the central part of the notum. Their tip can be pale yellow, but not always. A central, much larger papilla, which also bears three dorsal eyes, is entirely retractable within the notum. In addition to the large papillae, the notum is covered by smaller, rounded papillae, which can make it look very granular.

External morphology (Fig. 11A, B). The body is not flattened. The notum is oval. Dorsal gills are absent. The large, central, retractable papilla at the center of the notum can usually only be seen in live animals. In preserved specimens, it is retracted inside the notum. The hyponotum is horizontal. The width of the hyponotum relative to the width of the pedal sole varies among individuals. The width of the hyponotum is approximately half of its total width. In the anterior region, the left and right ocular tentacles are superior to the mouth. Eyes are located at the tip of the ocular tentacles. Inferior to the ocular tentacles, superior to the mouth, the head bears a pair of oral lobes. The latter are smooth, with no transversal protuberance. The male aperture (opening of the copulatory complex) is below the right ocular tentacle (or very slightly to its left in dorsal view). The anus is posterior, medial, close to the edge of the pedal sole. On the right side (to the left in ventral view), a peripodial groove is present at the junction between the pedal sole and the hyponotum, running longitudinally from the

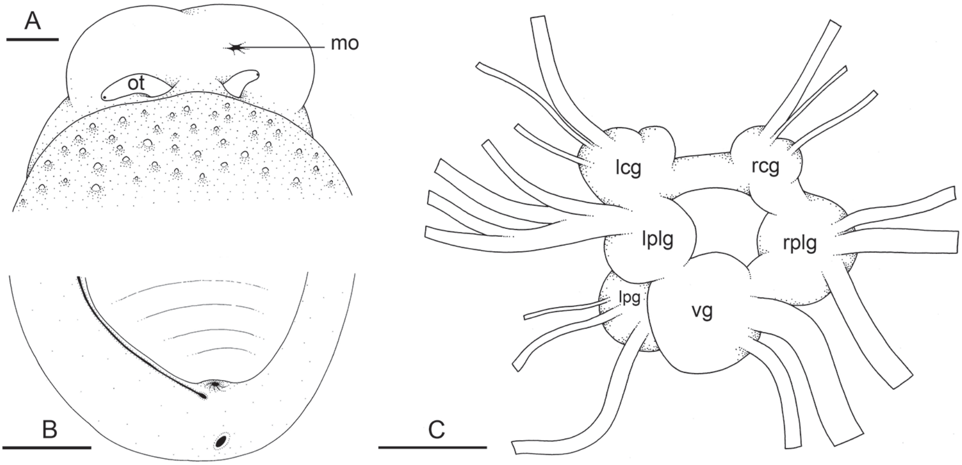

Figure 11. External morphology and nervous system, *Laspionchis boucheti* **A** Australia, Queensland [2693] (MTQ) **B, C** holotype, Australia, Northern Territory [1688 H] (NTM P.57614). **A** Dorsal, anterior view **B** ventral, posterior view **C** nervous system, dorsal view. Scale bars: 2 mm (**A**), 3 mm (**B**), 0.5 mm (**C**). Abbreviations: lcg left cerebral ganglion lpg left pedal ganglion lplg left pleural ganglion mo male opening ot ocular tentacle rcg right cerebral ganglion rplg right pleural ganglion vg visceral ganglion.

buccal area to the posterior end, very close to the anus. The position of the female pore (at the posterior end of the peripodial groove) does not vary much among individuals. The pneumostome is medial. Its position on the hyponotum relative to the notum margin and the edge of the pedal sole varies among individuals but it tends to be closer to the notum margin.

Visceral cavity and pallial complex. The heart, enclosed in the pericardium, is on the right side of the visceral cavity, slightly posterior to the middle. From the anterior ventricle is an anterior vessel supporting several anterior organs such as the buccal mass, the nervous system, and the copulatory complex. The auricle is posterior. The kidney is more or less symmetrical, the right and left parts being equally developed. The kidney is intricately attached to the respiratory complex. The lung is in two left and right, more or less symmetrical, parts.

Digestive system (Figs 12, 13). There are no jaws. The left and right salivary glands, heavily branched, join the buccal mass dorsally, on either side of the esophagus. The radula is between two large postero-lateral muscular masses. Radulae measure up to 2 mm in length. Each radular row contains a rachidian tooth and two half rows of lateral teeth of similar size and shape. Examples of radular formulae are in Table 4. The rachidian teeth are unicuspid: the median cusp is always present; there are two inconspicuous lateral cusps (Fig. 13A). The length of the rachidian teeth (ca. 20 µm) tend to be approximately half the size of the lateral teeth (ca. 50 µm). The lateral aspect of the base of the rachidian teeth is straight, occasionally slightly convex. The half rows of lateral teeth form an angle of 45° with the rachidian axis. With the exception of the few innermost and outermost lateral teeth, the size and shape of the hook of the lateral

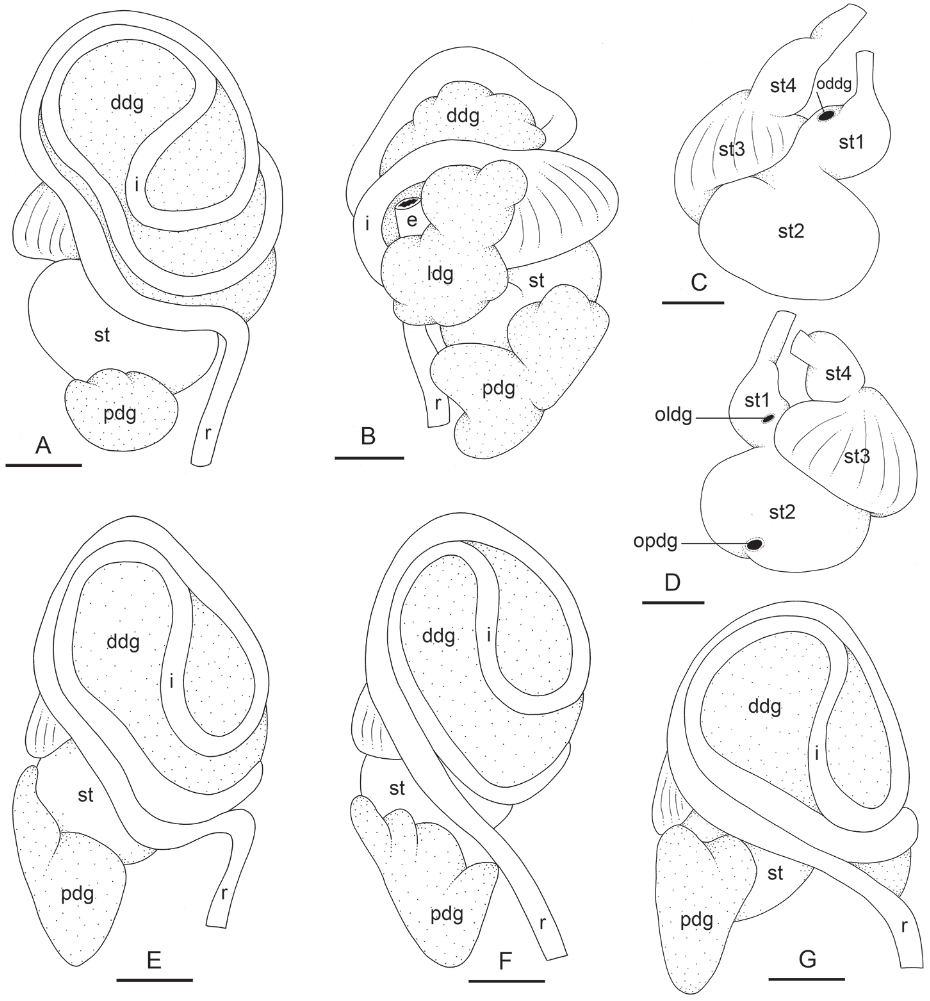

Figure 12. Digestive system, *Laspionchis boucheti*. **A** Holotype, dorsal view, Australia, Northern Territory, [1688 H] (NTM P.57614) **B** ventral view, same as **A C** stomach, dorsal view, same as **A D** stomach, ventral view, same as **A E** dorsal view, Vietnam, [5610] (ITBZC IM 00017) **F** dorsal view, Australia, Queensland, [2612] (MTQ) **G** dorsal view, Australia, Northern Territory, [1681] (NTM P.57612). Scale bars: 2 mm (**A–D**) 3 mm (**E, G**) 2.5 mm (**F**). Abbreviations: ddg dorsal digestive gland e esophagus i intestine ldg lateral digestive gland mo male opening oddg opening of the dorsal digestive gland oldg opening of the lateral digestive gland opdg opening of the posterior digestive gland pdg posterior digestive gland r rectum st stomach st1 stomach chamber 1 st2 stomach chamber 2 st3 stomach chamber 3 st4 stomach chamber 4.

teeth do not vary along the half row, nor do they vary among half rows. The hook of lateral teeth is extended posteriorly by a tail-like structure attaching to the radular membrane and making the hook look longer. The tail-like structure (posterior hook extension, Fig. 13D) is especially obvious in the outermost lateral teeth as its length gradually increases along each half row. The lateral teeth seem to be unicuspid with a

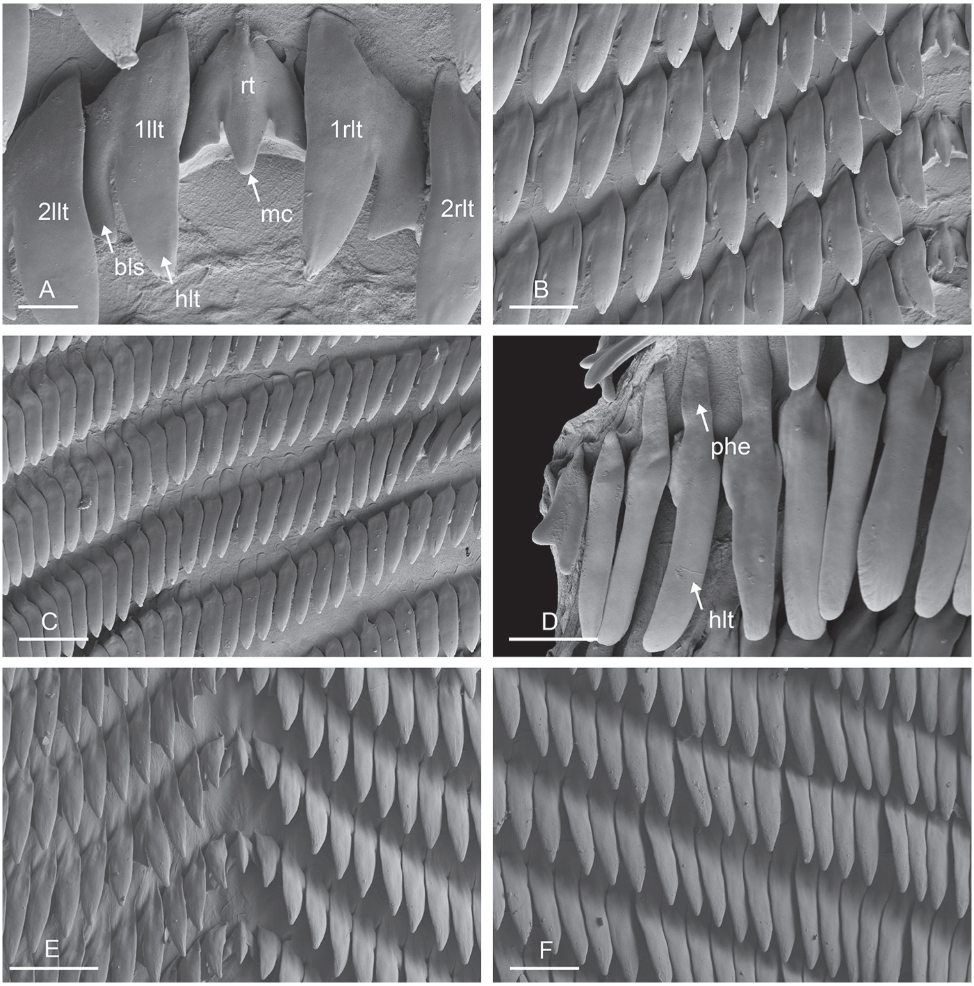

Figure 13. Radula, *Laspionchis boucheti* **A–D** Vietnam [5609] (ITBZC IM 00017) **E, F** holotype, Australia, Northern Territory [1688 H] (NTM P.57614). **A** Rachidian and innermost lateral teeth **B** lateral teeth with rachidian teeth **C** lateral teeth **D** outermost lateral teeth **E** rachidian and lateral teeth **F** lateral teeth. Scale bars: 10 µm (**A**) 30 µm (**B, F**) 50 µm (**C**) 20 µm (**D**) 40 µm (**E**). Abbreviations: 1llt first left lateral tooth 1rlt first right lateral tooth 2llt second left lateral tooth 2rlt second right lateral tooth bls basal lateral spine hlt hook of a lateral tooth mc median cusp phe posterior hook extension rt rachidian tooth.

flattened and curved hook with a rounded or pointed tip, but there also is a pointed spine on the outer lateral expansion of the base (basal lateral spine, Fig. 13A). In most cases, that spine cannot be observed because it is hidden below the hook of the next, outer lateral tooth. It can only be observed when the teeth are not too close (such as in the innermost and outermost regions) or when teeth are placed in an unusual position. The length of the spine decreases along the half row such that outermost teeth may be characterized by reduced or no lateral spine. The inner and outer lateral aspects of the hook of the lateral teeth are straight (i.e., not wavy and not with a protuberance).

Table 4. Radular formulae for the two species of *Laspionchis*, following the format number of rows × number of lateral teeth per left half row – 1 (rachidian tooth)– number of lateral teeth per right half row. Each DNA extraction number corresponds to one individual. The voucher catalog numbers can be shared by several individuals when collected at exactly the same locality (each individual is preserved in its own separate vial with its corresponding DNA number).

Species	Radular formula	Specimen length (mm)	Voucher	DNA extraction number
L. boucheti	43 × 50-1-50	30	NTM P.57614	1688 H
	57 × 90-1-90	31	BDMNH	1037
	55 × 70-1-70	31	ITBZC IM 00017	5609
L. bourkei bourkei	55 × 75-1-75	23	NTM P.57615	1657 H
	50 × 75-1-75	32	NTM P.57618	1666
	45 × 65-1-65	25	NTM P.57619	1692
	43 × 60-1-60	19	NTM P.57618	1673
L. bourkei lateriensis	45 × 65-1-65	17	UMIZ 00115	6064 H
	50 × 50-1-50	18	UMIZ 00116	6063
	45 × 60-1-60	15	UMIZ 00116	6065
	40 × 55-1-55	15	UMIZ 00116	6061
L. bourkei matangensis	42 × 60-1-60	15	USMMC 00055	5958 H
	55 × 70-1-70	25	PNM 041254	3343
	45 × 60-1-60	12	USMMC 00056	5960
	41 × 55-1-55	15	USMMC 00056	5959
	37 × 45-1-45	8	USMMC 00056	5965

The esophagus is narrow and straight, with thin internal folds. The esophagus enters the stomach anteriorly. Only a portion of the posterior aspect of the stomach can be seen in dorsal view because it is partly covered by the lobes of the digestive gland. The dorsal lobe is mainly on the right. The left, lateral lobe is mainly ventral. The posterior lobe covers the posterior aspect of the stomach. The stomach is a U-shaped sac divided into four chambers. The first chamber, which follows the esophagus, receives the ducts of the dorsal and lateral lobes of the digestive gland. The second chamber, posterior, receives the duct of the posterior lobe of the digestive gland. The third chamber is funnel-shaped and lined by ridges internally. The fourth chamber is continuous and externally similar to the third. The intestine is long, narrow, and the intestinal loops are exactly between types I and II, i.e., with a transitional loop on average oriented at 6 o'clock, though the orientation of the transitional loop ranges between 5 and 7 o'clock (Figs 1, 12). There is no rectal gland.

Nervous system (Fig. 11C). The circum-esophageal nerve ring is post-pharyngeal and pre-esophageal. The paired cerebral ganglia are close and the cerebral commissure is short (but its length does vary among individuals). Paired pleural and pedal ganglia are also all distinct. The visceral commissure is very short and the visceral ganglion is more or less median. Cerebro-pleural and pleuro-pedal connectives are short and pleural and cerebral ganglia touch each other on either side. Nerves from the cerebral ganglia innervate the buccal area and the ocular tentacles, and, on the right side, the penial complex. Nerves from the pedal ganglia innervate the foot. Nerves from the pleural ganglia innervate the lateral and dorsal regions of the mantle. Nerves from the visceral ganglia innervate the visceral organs.

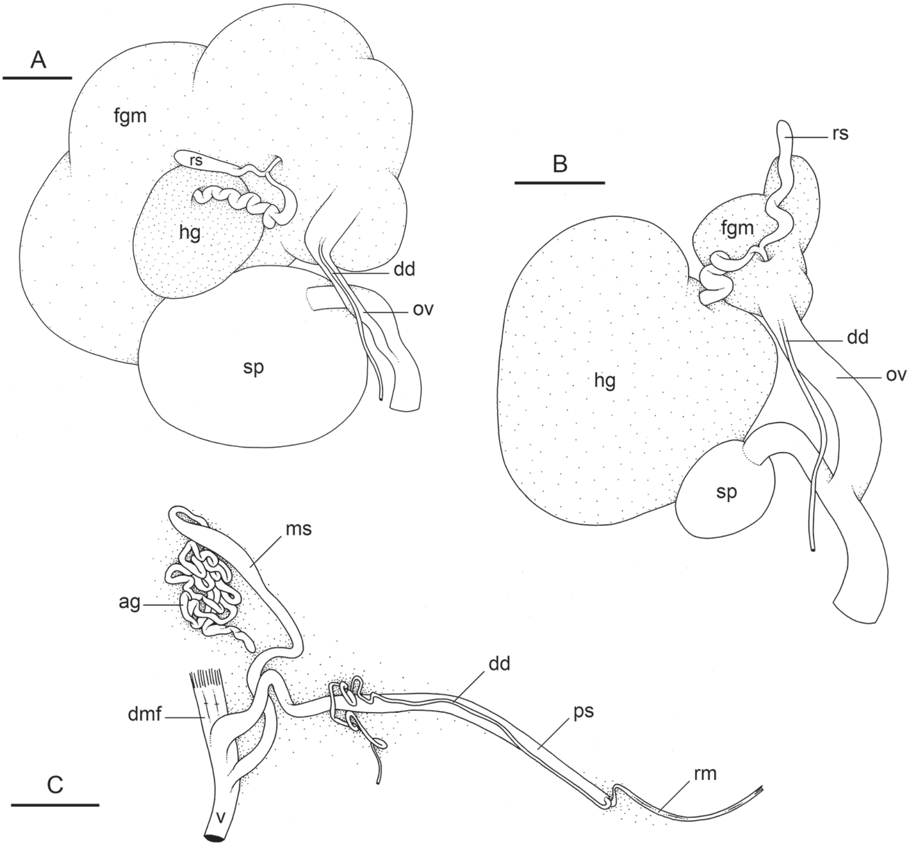

Figure 14. Reproductive system, *Laspionchis boucheti* **A** Brunei [1037] (BDMNH) **B, C** holotype, Australia, Northern Territory [1688 H] (NTM P.57614). **A** Posterior, hermaphroditic (female) reproductive system **B** posterior, hermaphroditic (female) reproductive system **C** anterior, male, copulatory apparatus. Scale bars: 3 mm (**A**) 1 mm (**B**) 2 mm (**C**). Abbreviations: ag accessory penial gland dd deferent duct dmf distal muscle fibers fgm female gland mass hg hermaphroditic gland ms muscular sac ov oviduct ps penial sheath rm retractor muscle rs receptaculum seminis sp spermatheca v vestibule.

Reproductive system (Fig. 14). Sexual maturity is correlated with animal length. Mature individuals have large female organs (with a large female gland mass) and fully-developed male parts. Immature individuals may have inconspicuous (or no) female organs and rudimentary anterior male parts. The hermaphroditic gland is a single mass, joining the spermoviduct through the hermaphroditic duct. There is a narrow receptaculum seminis (caecum) along the hermaphroditic duct. The female gland mass contains various glands (mucus and albumen) which can hardly be separated by dissection and of which the exact connections remain uncertain. The hermaphroditic duct becomes the spermoviduct. Proximally, the spermoviduct is not divided (at least externally) and is embedded within the female gland mass. Distally, the spermoviduct branches into the deferent duct and the oviduct. The free oviduct conveys the eggs up to the female opening and the exosperm from the female opening up to the fertilization chamber. The large, ovate-spherical spermatheca connects to the oviduct through a narrow and short duct. The oviduct is large (larger than the deferent duct) and straight. There is no vaginal gland.

Copulatory apparatus (Figs 14C, 15, 16). The male anterior organs consist of the penial complex (penis, penial sheath, deferent duct, retractor muscle) and the accessory penial gland (flagellum, muscular sac, hollow spine). The penial complex and the accessory penial gland share the same vestibule and the same anterior male opening.

The penial sheath is narrow and elongated. The penial sheath protects the penis for its entire length. The beginning of the retractor muscle marks the separation between the penial sheath (and the penis inside) and the deferent duct. The retractor muscle is strong, shorter than the penial sheath, and inserts at the posterior end of the visceral cavity. In addition, there is a cluster of retractor muscle fibers on the distal part of the penial sheath, near the vestibule. The deferent duct is highly convoluted with many loops. Inside the penial sheath, the penis is a narrow, thin, elongated, hollow tube, with numerous and densely-arranged (next to each other) hooks in its distal part. Penial hooks are pointed and measure from 50 to 100 µm. When the penis is retracted inside the penial sheath, the hooks are inside the tube-like penis; during copulation, the penis is everted like a glove and the hooks are then on the outside.

The accessory penial gland is a long, tube-like flagellum with a proximal dead end. The length of the flagellum of the penial gland varies among individuals but it is always heavily coiled. Near its distal part, the flagellum is enlarged into a muscular sac. Distally, the flagellum ends in a hard, hollow spine protected by a sheath which opens into the vestibule. The hollow spine is narrow, straight, elongated. Its base is conical. Its diameter is ca. 50 µm except at the base where it is larger (ca. 100 µm). The diameter of the opening at the tip measures ca. 30 µm. Its length ranges from 0.7 mm [1037] (BDMNH) to 1 mm [2693] (MTQ). There is no disc separating the spine of the penial gland and the vestibule.

Remarks. A new species name is needed because no existing name applies to the species described here, based on the examination of all the type specimens available in the Onchidiidae, a careful study of all the original descriptions, and our ongoing taxonomic revision of every genus of the family (Dayrat et al. 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c). Several problematic species names, already discussed in detail in our revision of *Paromoionchis* (Dayrat et al. 2019: 68–72), are regarded as nomina dubia for a variety of reasons (the type locality is too vague, the original description is not informative enough, the type material is destroyed or lost). One of those nomina dubia, *Onchidium palaense* Semper, 1880 (type locality in Aibukit, Palau Islands) could belong to *Paromoionchis* or *Laspionchis* but its generic placement cannot be determined. *Onchidium palaense* does not belong to *Onchidium* because several traits mentioned by Semper, such as the absence of a rectal gland and of an accessory penial gland, are incompatible with *Onchidium* (Dayrat et al. 2016). *Onchidium palaense* simply is a nomen dubium which was arbitrarily placed in the genus *Onchidium* and cannot reliably be placed in any of the onchidiid genera.

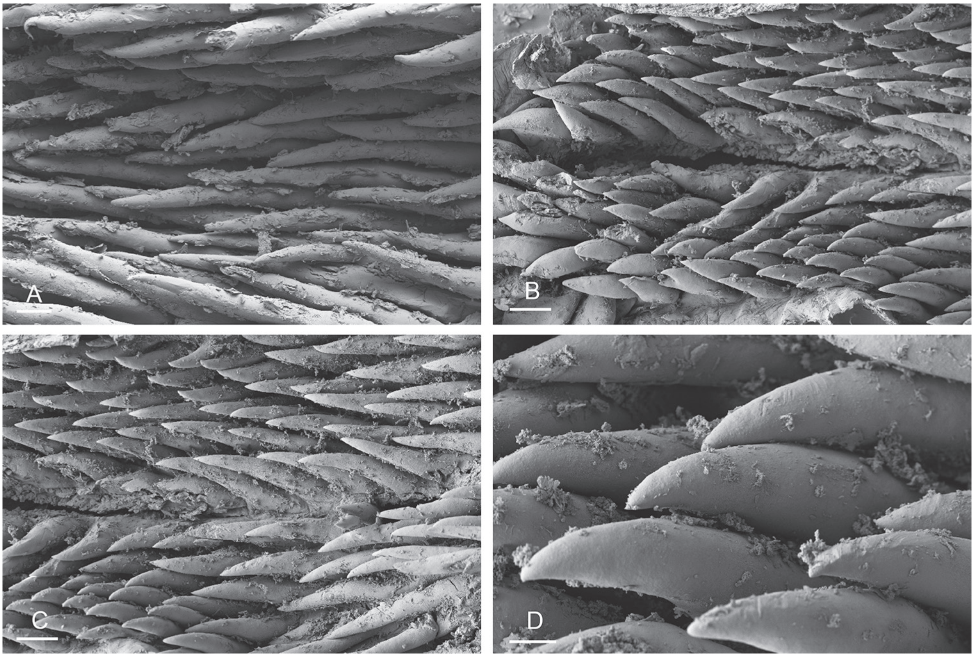

Figure 15. Penial hooks, *Laspionchis boucheti* **A** Brunei [1037] (BDMNH) **B–D** Vietnam [5609] (IT-BZC IM 00017). Scale bars: 20 µm (**A**), 40 µm (**B, C**), 10 µm (**D**).

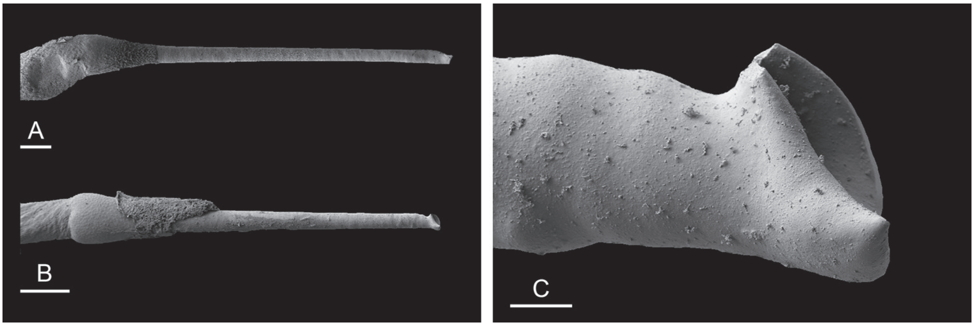

Figure 16. Spine of the accessory penial gland, *Laspionchis boucheti* **A** Australia, Queensland [2693] (MTQ) **B, C** Brunei [1037] (BDMNH). Scale bars: 100 µm (**A, B**), 10 µm (**C**).

Laspionchis bourkei Dayrat & Goulding, sp. nov.

http://zoobank.org/25128A1E-3115-4EF4-9287-537E18D2F955
Figs 17–29

Holotype. AUSTRALIA • holotype, designated here, 23/18 mm [1657 H]; Northern Territory, Darwin; 12°33.228'S, 130°52.580'E; 14 Aug. 2012; B Dayrat and party leg.; st 61, on the right side of the road just before bridge to Channel Island, *Avicennia* mangrove with sandy mud; NTM P.57615.

Figure 17. Habitats, *Laspionchis bourkei* **A–D** *L. bourkei bourkei* Australia, Northern Territory **E** *L. bourkei lateriensis*, Indonesia, Ambon. **A** *Avicennia* on sandy mud (st 61, type locality) **B** same as **A** **C** large *Sonneratia alba*, open forest, soft mud by shore (st 62) **D** *Sonneratia*, *Rhizophora*, and *Ceriops* mangrove (st 66) **E** mudflat beside a mangrove and a creek (st 128, type locality).

Additional material examined. See below for each subspecies.

Distribution (Fig. 7). Australia (Northern Territory) for *L. bourkei bourkei*. Indonesia (Ambon) for *L. bourkei lateriensis*. Indonesia (Sulawesi, Sumatra), Malaysia (Peninsular Malaysia), Singapore, Philippines (Bohol), and Vietnam for *L. bourkei matangensis*.

Habitat (Figs 17, 26). *Laspionchis bourkei* is found on mud, hard or soft, in open or dense mangrove forests. It can be locally common across its entire distribution.

Etymology. *Laspionchis bourkei* is dedicated to Adam Bourke, from Darwin, Northern Territory, Australia, a very knowledgeable mangrove expert and great naturalist, who generously accompanied us in the field around Darwin and showed us good collecting sites.

Diagnosis (Table 3). Externally, *Laspionchis bourkei* cannot be distinguished from *L. boucheti*. Internally, however, the long retractor muscle of the penis inserts at the posterior end of the visceral cavity in *L. boucheti* while the retractor muscle is short (and inserting in the first third of the visceral cavity) in *L. bourkei bourkei* and vestigial or absent in *L. bourkei lateriensis* and *L. bourkei matangensis*. Also, additional, distal, retractor muscle fibers are present in *L. boucheti* but absent in *L. bourkei*.

Color and morphology of live animals (Figs 18, 27). Live slugs are covered with mud and their dorsal color can hardly be seen. The background of the dorsal notum is brown, light to dark, homogenous or mottled with darker or lighter areas. The color of the foot is a mix of gray (light or dark) and yellow, as is the color of the hyponotum. The color of the ventral surface (foot and hyponotum) can change rapidly, especially when slugs are disturbed. The ocular tentacles are brown (variable from light to dark) and short (a few millimeters). The number of papillae with dorsal eyes is variable (between five and ten, on average) and they mostly are on the central part of the notum.

Digestive system (Figs 19, 20, 25). Examples of radular formulae are in Table 4. Radulae measure up to 2.9 mm in length (see below for each subspecies). The intestine is long, narrow, and the intestinal loops are exactly between types I and II, i.e., with a transitional loop on average oriented at 6 o'clock, acknowledging minor individual variation (Figs 1, 19).

Reproductive system (Fig. 21). There is a narrow receptaculum seminis (caecum) along the hermaphroditic duct. The large, ovate-spherical spermatheca connects to the oviduct through a narrow and short duct. The oviduct is straight, slightly larger than the deferent duct or of a similar diameter.

Copulatory apparatus (Figs 22–24, 28, 29). The length of the flagellum of the accessory penial gland varies among individuals but it is always heavily coiled. The hollow spine of the penial gland is narrow, straight, elongated. Its base is conical. Its length varies from 0.35 mm to 1 mm (see below for each subspecies). The penial sheath is narrow and short. The penial sheath protects the penis for its entire length. The beginning of the retractor muscle marks the separation between the penial sheath (and the penis inside) and the deferent duct. The retractor muscle is short (as long as the penial sheath) and inserting in the first third of the visceral cavity, vestigial (and free with no attachment), or absent (see below for each subspecies). There is no additional, distal, retractor muscle fibers. The deferent duct is highly convoluted. Inside the penial sheath, the penis is a narrow, thin, elongated, hollow tube, with numerous and densely-arranged (next to each other) hooks in its distal part. Penial hooks are pointed and measure from 15 to 45 μm (see below for each subspecies). When the penis is retracted inside the penial sheath, the hooks are inside the tube-like penis; during copulation, the penis is everted like a glove and the hooks are then on the outside.

Remarks. A new species name is needed because no existing name applies to the species described here, based on the examination of all the type specimens available

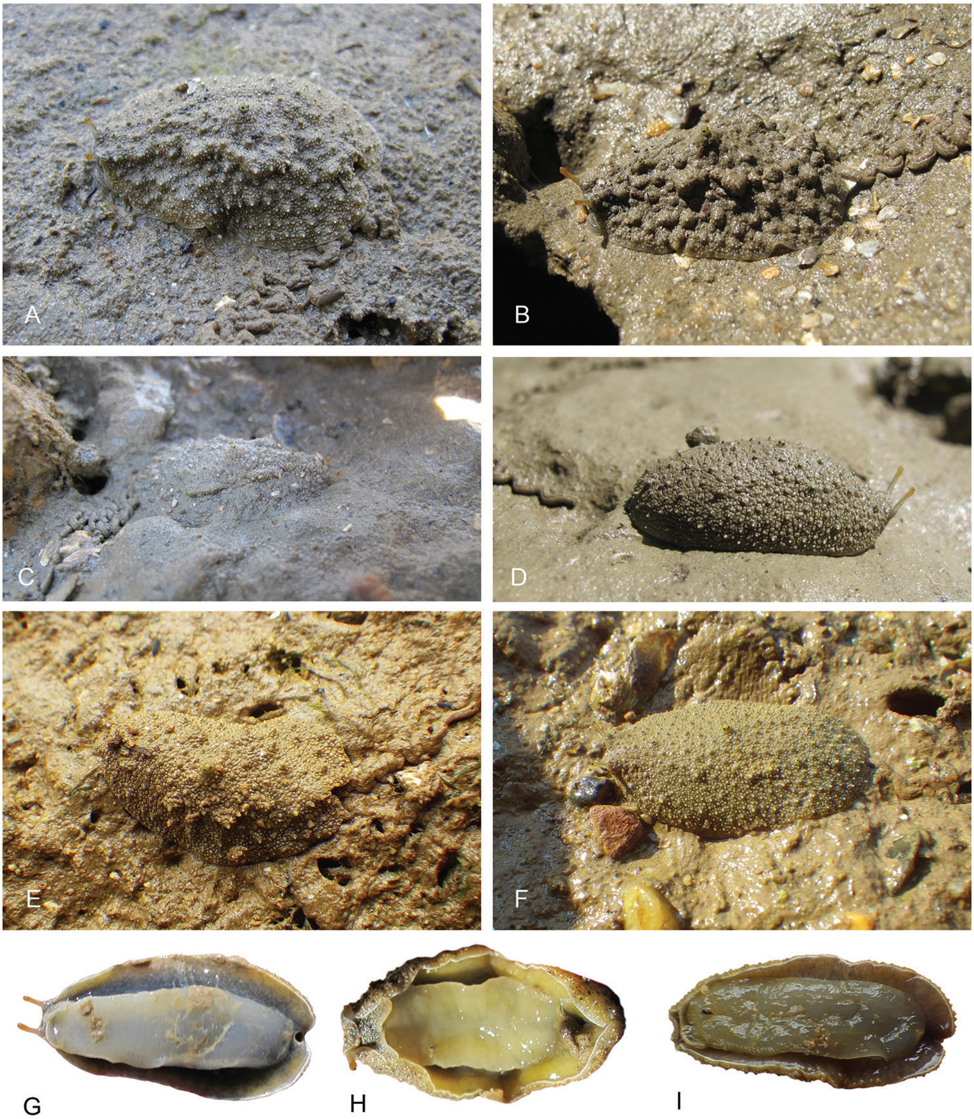

Figure 18. Live animals, *Laspionchis bourkei* **A–D, G, H** *L. bourkei bourkei* Australia, Northern Territory **E, F, I** *L. bourkei lateriensis*, Indonesia, Ambon. **A** Dorsal view, 20 mm long [1618] (NTM P.57617) **B** dorsal view, 21 mm long [1656] (NTM P.57617) **C** holotype, dorsal view, 23 mm long [1657 H] (NTM P.57615) **D** dorsal view, 19 mm long [1673] (NTM P.57618) **E** dorsal view, 12 mm long [2743] (UMIZ 00116) **F** dorsal view, 17 mm long [2753] (UMIZ 00116) **G** ventral view, 19 mm long [1659] (NTM P.57618) **H** ventral view, 23 mm long [1617] (NTM P.57617) **I** ventral view, same as **E**.

in the Onchidiidae, a careful study of all the original descriptions, and our ongoing taxonomic revision of each genus of the family (Dayrat et al. 2016, 2017, 2018, 2019; Dayrat and Goulding 2017; Goulding et al. 2018a, b, c).

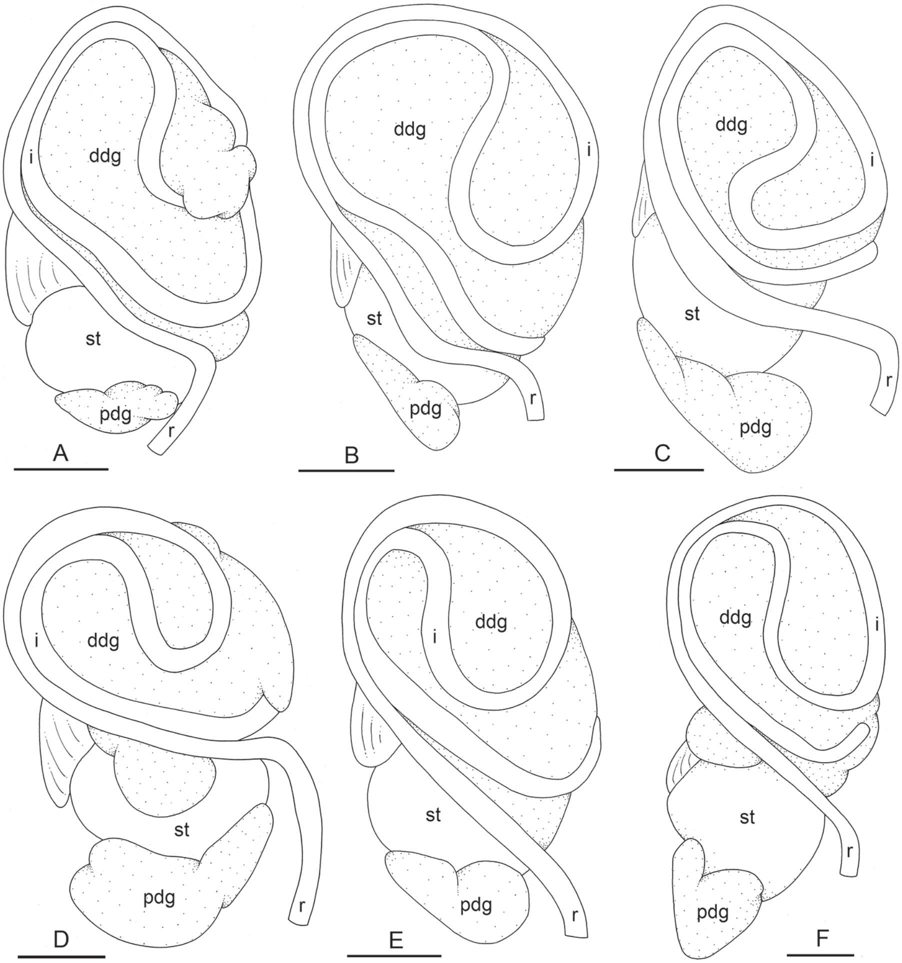

Figure 19. Digestive system, dorsal view, *Laspionchis bourkei* **A–C** *L. bourkei bourkei*, Australia, Northern Territory **D** Holotype, *L. bourkei lateriensis*, Indonesia, Ambon **E, F** *L. bourkei matangensis*, Peninsular Malaysia. **A** [1673] (NTM P.57618) **B** [1666] (NTM P.57618) **C** [1693] (NTM P.57619) **D** [6064 H] (UMIZ 00115) **E** [5959] (USMMC 00056) **F** holotype, [5958 H] (USMMC 00055). Scale bars: 2 mm (**A, C–E**), 2.5 mm (**B**), 1 mm (**F**). Abbreviations: ddg dorsal digestive gland i intestine pdg posterior digestive gland r rectum st stomach.

Laspionchis bourkei is divided in three distinct units of which the reciprocal monophyly is highly-supported in both mitochondrial and nuclear analyses (except for *L. bourkei matangensis*, unresolved using nuclear data). The fact that the three units within *L. bourkei* are distinct taxa means that they should be recognized and named. Even though we could have ranked them as species, we decided to rank them as sub-species for three main reasons.

(1) The three units within *L. bourkei* are cryptic externally and internally. Some minor anatomical differences seem to exist but which can hardly be used for identification (Table 3).

(2) Ranking the three units within *L. bourkei* as subspecies rather than species is more in agreement with the genetic distances observed between *L. boucheti* and *L. bourkei*. Indeed, the distance gap between *L. boucheti* and *L. bourkei* is between 2.5% and 7.5%, while the distance gap between the three *L. bourkei* units is between 2.5% and 3.9%, clearly suggesting that the three *L. bourkei* units are much less divergent (their COI sequences) than *L. boucheti* and *L. bourkei*, supporting their ranking as subspecies. Distance values should not necessarily be compared from one genus to another, but they can be compared between very closely related species.

(3) As of today, the three units within *L. bourkei* are allopatric which means that a doubt remains as to whether the three units are reproductively isolated or not. Overall, the three units within *L. bourkei* probably are relatively young taxa which diverged recently, explaining that they are cryptic internally and that their COI sequences are less divergent than the COI sequences between *L. boucheti* and *L. bourkei*.

Laspionchis bourkei bourkei **Dayrat & Goulding, ssp. nov.**

http://zoobank.org/AEBEFFC9-8AC4-48E1-A37B-5F1E99EFE75D
Figs 17A–D, 18A–D, G, H, 19A–C, 20C–F, 21A, 22A, 23E, F, 24A–D, F

Holotype. The type locality and the holotype of the nominotypical subspecies *L. bourkei bourkei* are the same as those of the nominal species *L. bourkei* (ICZN Arts. 47.1, 61.2, and 72.8).

Additional material examined. AUSTRALIA – **Northern Territory** • 1 specimen 8/4 mm [1616]; Darwin, Lee Point Road, Buffalo Creek; 12°20.460'S, 130°54.600'E; 13 Aug. 2012; B Dayrat and party leg.; st 60, narrow *Rhizophora* mangrove by a river with very dry and hard mud NTM P.57616. • 4 specimens 20/12 mm [1652], 21/13 mm [1656], 23/15 mm [1617], 20/15 mm [1618]; same collection data as for the holotype; NTM P.57617. • 4 specimens 18/10 mm [1621], 19/12 mm [1659], 32/20 mm [1666], 19/12 mm [1673]; Darwin, Talc Head; 12°28.765'S, 130°46.297'E; 15 Aug. 2012; B Dayrat and party leg.; st 62, large and open forest of *Sonneratia alba* with soft mud; NTM P.57618. • 3 specimens 25/18 mm [1692], 22/15 mm [1693], 22/15 mm [1694]; Darwin, end of the Channel Island Road; 12°33.557'S, 130°52.894'E; 17 Aug. 2012; B Dayrat and party leg.; st 66, sequence of *Sonneratia*, *Rhizophora*, and *Ceriops*; NTM P.57619.

Distribution (Fig. 7). Australia (Northern Territory).

Habitat (Fig. 17A–D). Same as the entire species *Laspionchis bourkei* (see above).

Etymology. See above, the species *L. bourkei*.

Diagnosis (Table 3). Externally, the three subspecies of *L. bourkei* cannot be distinguished. Internally, *L. bourkei bourkei* differs from both *L. bourkei lateriensis* and *L. bourkei matangensis*. Indeed, *L. bourkei bourkei* is characterized by a short retractor muscle of the penis which inserts in the anterior third of the visceral cavity while the retractor muscle is vestigial or absent in *L. bourkei lateriensis* and *L. bourkei matangensis*. Also, the spine of the accessory penial gland is on average slightly longer in *L. bourkei bourkei* than in *L. bourkei lateriensis* and *L. bourkei matangensis*.

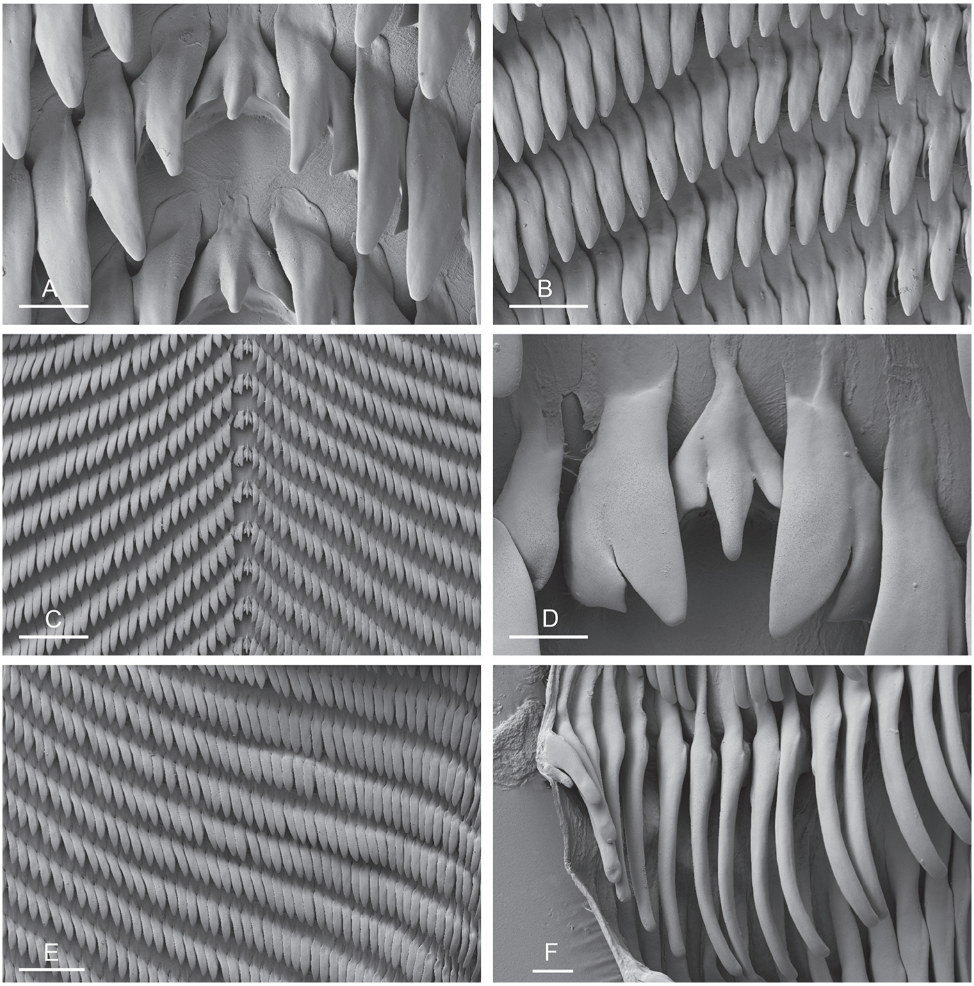

Figure 20. Radula, *Laspionchis bourkei* **A, B** *L. bourkei matangensis*, Peninsular Malaysia [5965] (USMMC 00056) **C–F** Holotype, *L. bourkei bourkei*, Australia, Northern Territory [1657 H] (NTM P.57615). **A** Rachidian and innermost lateral teeth **B** lateral teeth **C** rachidian and lateral teeth **D** rachidian and innermost lateral teeth **E** lateral teeth **F** outermost lateral teeth. Scale bars: 10 µm (**A, D, F**), 30 µm (**B**), 100 µm (**C**), 80 µm (**E**).

Color and morphology of live animals (Fig. 18A–D, G, H). Identical to the species *L. bourkei* (see above).

Digestive system (Figs 19A–C, 20C–F). Identical to the species *L. bourkei* (see above). Examples of radular formulae are in Table 4. Radulae measure up to 2.9 mm in length.

Reproductive system (Fig. 21A). Identical to the species *L. bourkei* (see above).

Copulatory apparatus (Figs 22A, 23E, F, 24A–D, F). Similar to the species *L. bourkei* (see above) acknowledging some minor variations: the length of the spine of the accessory penial gland ranges from 0.75 mm [1657 H] (NTM P.57615) to 1 mm [1666] (NTM P.57618), the retractor muscle is short (as long as the penial sheath) and inserts in the first third of the visceral cavity, and penial hooks measure from 20 to 35 µm.

Remarks. See above, the remarks on the species *Laspionchis bourkei*.

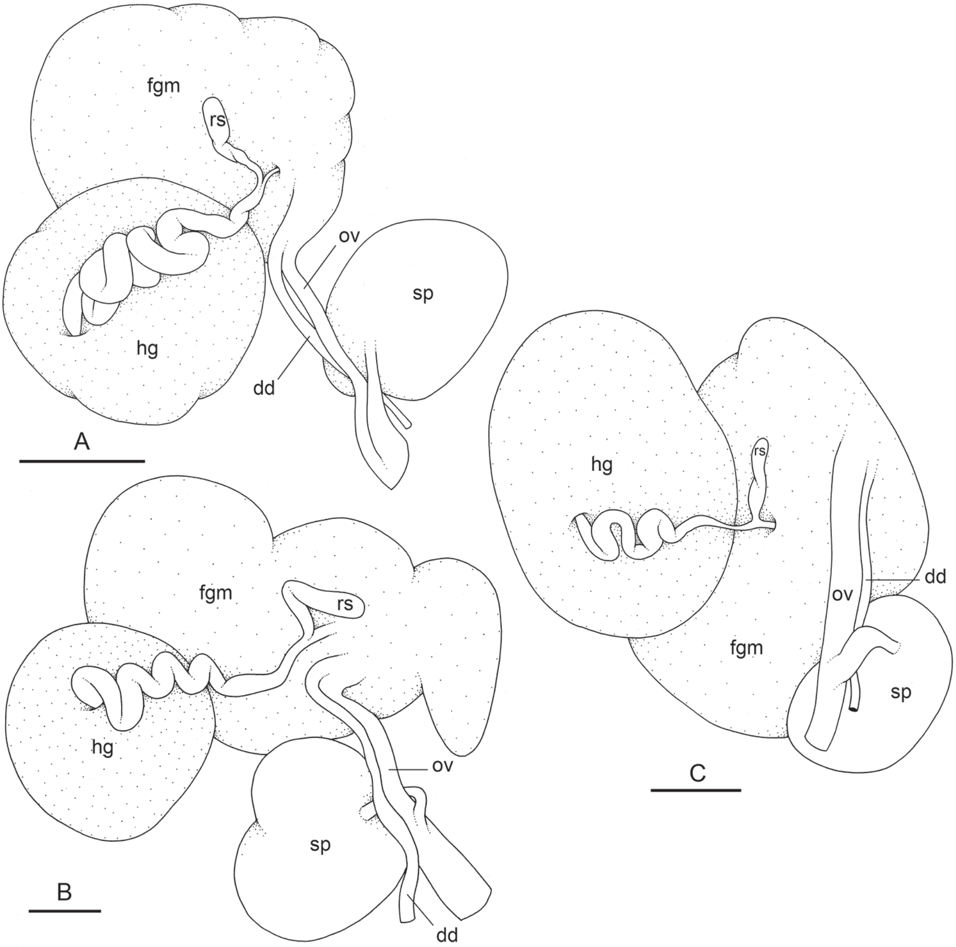

Figure 21. Posterior, hermaphroditic (female) reproductive system, *Laspionchis bourkei*. **A** Holotype, *L. bourkei bourkei*, Australia, Northern Territory, [1657 H] (NTM P.57615) **B** holotype, *L. bourkei lateriensis*, Indonesia, Ambon, [6064 H] (UMIZ 00115) **C** holotype, *L. bourkei matangensis*, Peninsular Malaysia, [5958 H] (USMMC 00055). Scale bars: 2 mm (**A**), 1 mm (**B, C**). Abbreviations: dd deferent duct fgm female gland mass hg hermaphroditic gland ov oviduct rs receptaculum seminis sp spermatheca.

Laspionchis bourkei lateriensis **Dayrat & Goulding, ssp. nov.**

http://zoobank.org/05FE1E0F-0679-4AA0-A40B-21FBE2D1199B
Figs 17E, 18E, F, I, 19D, 21B, 22B, 23A–D, 24E, G, 25

Holotype. INDONESIA • holotype, designated here, 17/16 mm [6064 H]; Ambon, Lateri; 03°38.261'S, 128°14.716'E; 12 Feb. 2014; M Khalil and party leg.; st 128, mudflat next to small creek in the low intertidal of mangrove preserve; UMIZ 00115.

Additional material examined. INDONESIA – **Ambon** • 5 specimens 12/7 mm [2743], 17/10 mm [2753], 15/12 mm [6061], 18/13 mm [6063], 15/12 mm [6065]; same collection data as for the holotype; UMIZ 00116.

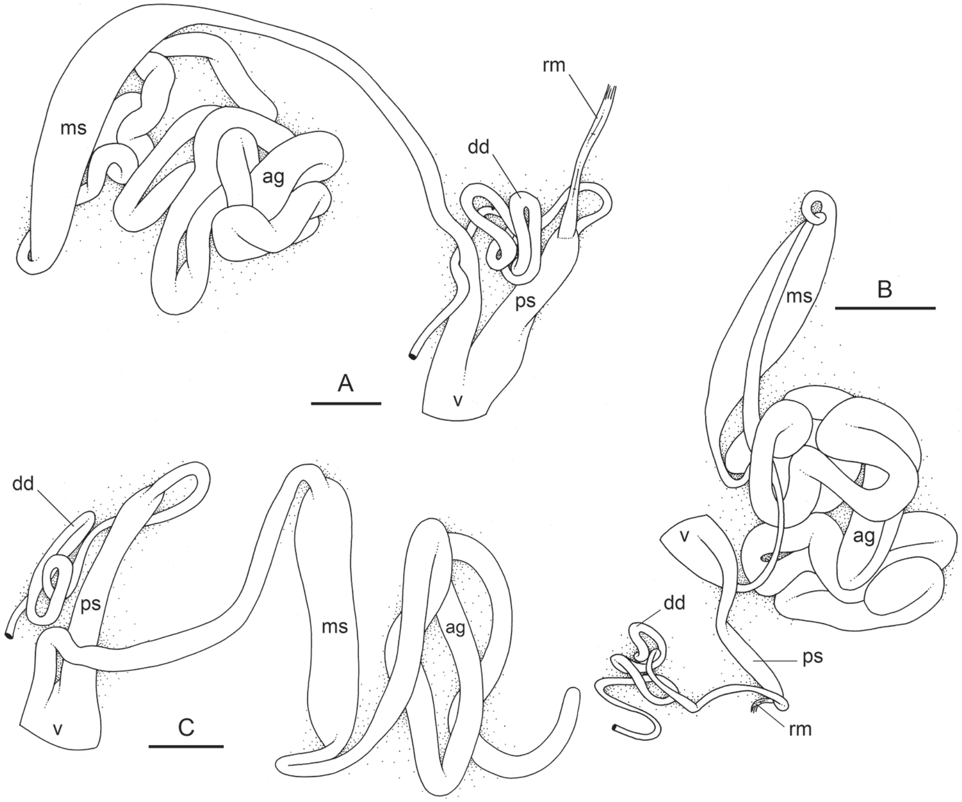

Figure 22. Anterior, male, copulatory apparatus, *Laspionchis bourkei*. **A** Holotype, *L. bourkei bourkei*, Australia, Northern Territory, [1657 H] (NTM P.57615) **B** holotype, *L. bourkei lateriensis*, Indonesia, Ambon, [6064 H] (UMIZ 00115) **C** holotype, *L. bourkei matangensis*, Peninsular Malaysia, [5958 H] (USMMC 00055). Scale bars: 1 mm (**A**), 2 mm (**B**), 0.5 mm (**C**). Abbreviations: ag accessory penial gland dd deferent duct ms muscular sac ps penial sheath rm retractor muscle v vestibule.

Distribution (Fig. 7). Indonesia (Ambon).

Habitat (Fig. 17E). Same as the entire species *Laspionchis bourkei* (see above).

Etymology. The subspecies *Laspionchis bourkei lateriensis* is named after Lateri, in Ambon because the type locality is part of the preserved mangrove of Lateri. The name *lateriensis* is an adjective derived from Lateri and the suffix *-ensis*.

Diagnosis (Table 3). Externally, the three subspecies of *L. bourkei* cannot be distinguished. Internally, *L. bourkei lateriensis* differs from *L. bourkei bourkei* but cannot be distinguished from *L. bourkei matangensis*. All three subspecies, however, are clearly delineated using molecular DNA sequences.

Color and morphology of live animals (Fig. 18E, F, I). Identical to the species *L. bourkei* (see above).

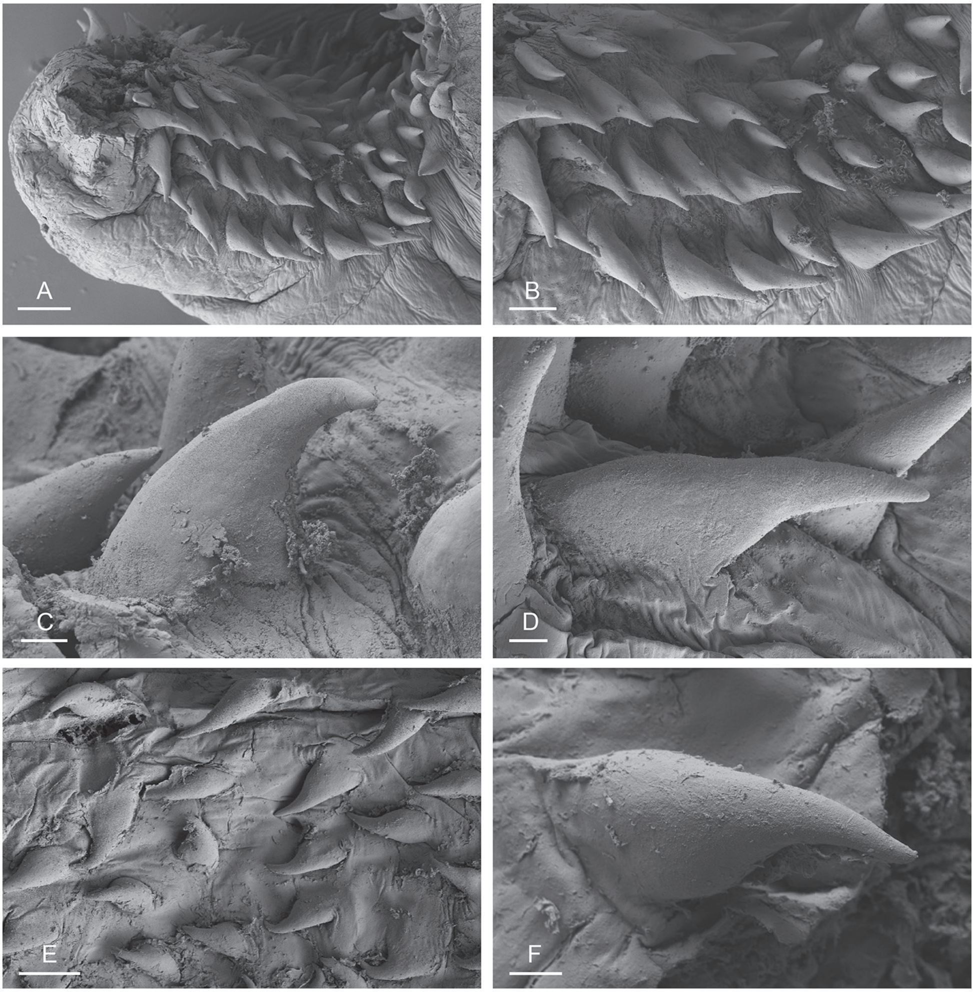

Figure 23. Penial hooks, *Laspionchis bourkei* **A–D** Holotype, *L. bourkei lateriensis*, Indonesia, Ambon [6064 H] (UMIZ 00115) **E, F** *L. bourkei bourkei*, Australia, Northern Territory [1692] (NTM P.57619). Scale bars: 40 µm (**A, E**), 20 µm (**B**), 5 µm (**C, D**), 4 µm (**F**).

Digestive system (Figs 19D, 25). Identical to the species *L. bourkei* (see above). Examples of radular formulae are presented in Table 4. Radulae measure up to 1.7 mm in length.

Reproductive system (Fig. 21B). Identical to the species *L. bourkei* (see above).

Copulatory apparatus (Figs 22B, 23A–D, 24E, G). Similar to the species *L. bourkei* (see above) acknowledging some minor variations: the length of the spine of the accessory penial gland ranges from 0.35 mm [6063] (UMIZ 00116) to 0.75 mm [6064 H] (UMIZ 00115), the retractor muscle is vestigial or absent, and penial hooks measure from 20 to 45 µm.

Remarks. See above, the remarks on the species *Laspionchis bourkei*.

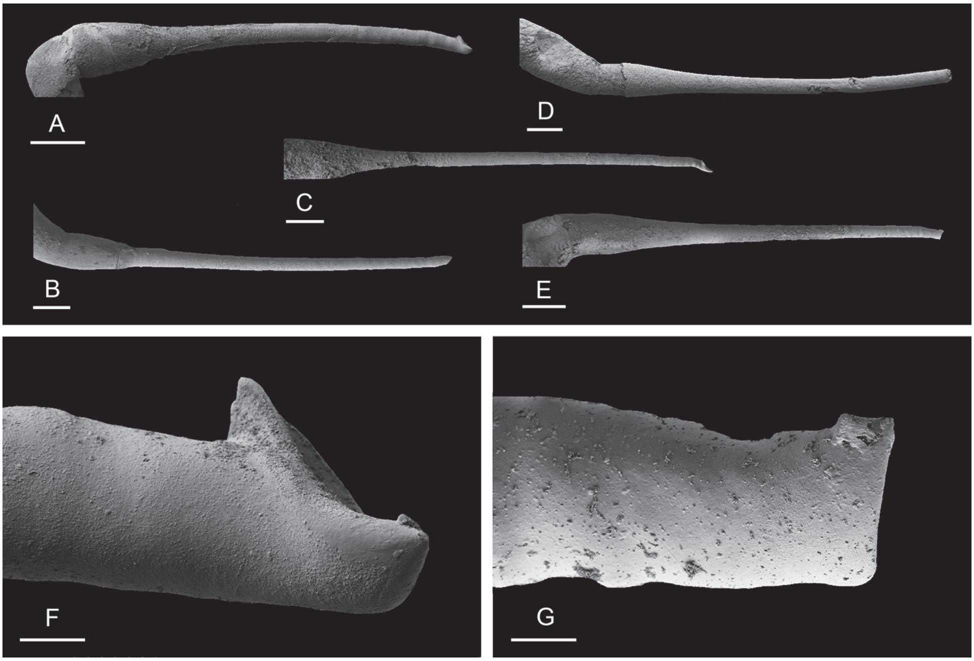

Figure 24. Spine of the accessory penial gland, *Laspionchis bourkei* **A–D, F** *L. bourkei bourkei*, Australia, Northern Territory **E, G** *L. bourkei lateriensis*, Indonesia, Ambon. **A** Holotype, [1657 H] (NTM P.57615) **B** [1666] (NTM P.57618) **C** [1692] (NTM P.57619) **D** [1673] (NTM P.57618) **E** holotype, [6064 H] (UMIZ 00115) **F** same as **A** **G** same as **E**. Scale bars: 100 µm (**A–E**), 10 µm (**F, G**).

Laspionchis bourkei matangensis Dayrat & Goulding, ssp. nov.

http://zoobank.org/05BA0236-4FFB-4F2E-BDB2-58BFECE43FFC
Figs 19E, F, 20A, B, 21C, 22C, 26–29

Holotype. MALAYSIA • holotype, designated here, 15/9 mm [5958 H]; Peninsular Malaysia, Matang, facing fishermen's village on the other side of river; 04°50.217'N, 100°36.826'E; 26 Jul. 2016; B Dayrat and party leg.; st 256, oldest and open *Rhizophora* forest of tall and beautiful trees, with hard mud, many creeks, and many old logs; USMMC 00055.

Additional material examined. INDONESIA – **Sumatra** • 3 specimens 11/7 mm [1783], 10/7 mm [1784], 13/9 [1785]; Tembilahan; 00°10.243'S, 103°27.982'E; 13 Oct. 2012; M Khalil and party leg.; st 76, mangrove of large *Avicennia* trees, with old logs, soft but solid mud, and *Nypa* on the margin; UMIZ 00113. – **Sulawesi** • 1 specimen 12/10 mm [2230]; Bahoi; 01°43.355'N, 125°01.232'E; 12 Mar. 2013; M Khalil and party leg.; st 88, sand, small rocks, pieces of wood outside narrow coastal mangrove; UMIZ 00114. MALAYSIA – **Peninsular Malaysia** • 5 specimens 15/8 mm [5959], 12/9 mm [5960], 15/8 mm [5961], 13/8 mm [5963], 8/5 mm [5965]; same collection data as for the holotype; USMMC 00056. PHILIPPINES – **Bohol** • 1 specimen 25/18 mm [3343]; Mabini; 09°51.532'N, 124°31.685'E; 17 Jul. 2014; B Dayrat

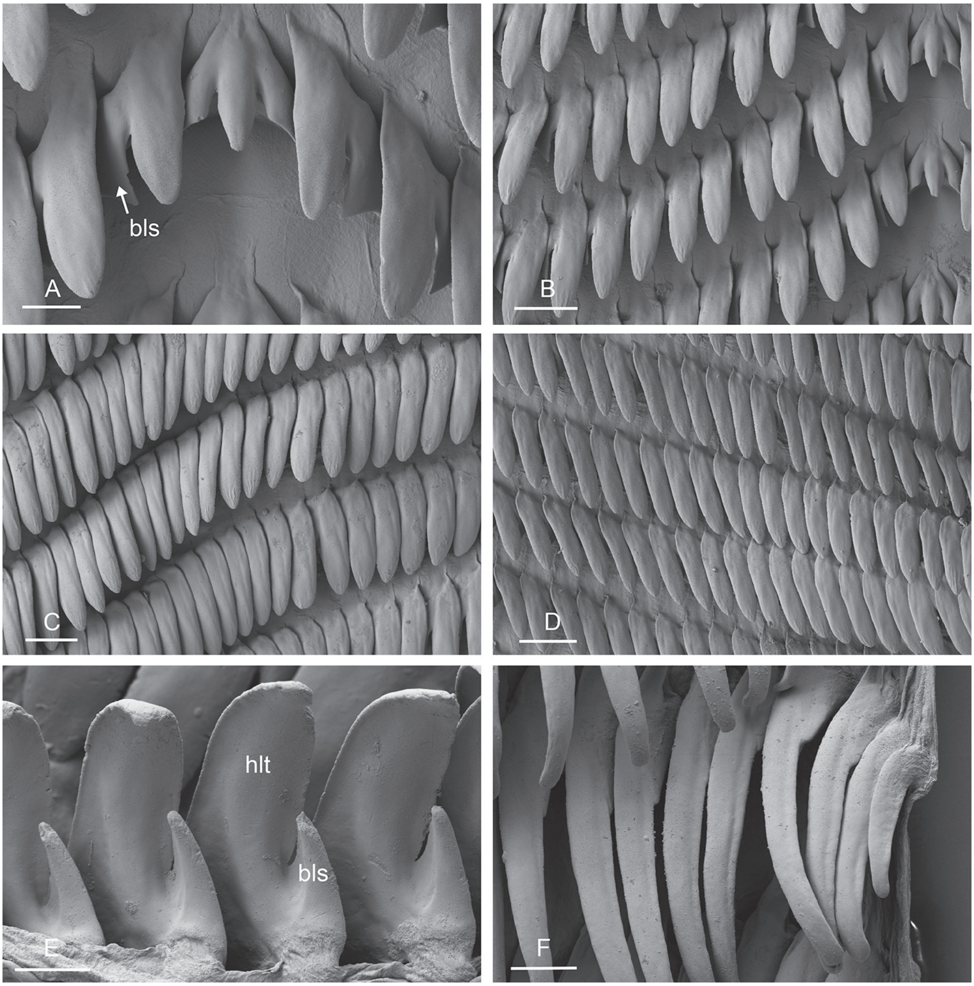

Figure 25. Radula, *Laspionchis bourkei lateriensis*, Indonesia, Ambon **A–D** holotype [6064 H] (UMIZ 00115) **E, F** [6063] (UMIZ 00116). **A** Rachidian and innermost lateral teeth **B** rachidian and innermost lateral teeth **C** lateral teeth **D** lateral teeth **E** lateral teeth (inferior view) **F** outermost lateral teeth. Scale bars: 10 µm (**A, F**), 20 µm (**B, C**), 30 µm (**D**), 8 µm (**E**). Abbreviations: bls basal lateral spine hlt hook of a lateral tooth.

and party leg.; st 194, narrow mangrove on the edge of fish ponds, tall *Rhizophora* and *Avicennia* trees, many old logs; PNM 041254. • 1 specimen 10/7 mm [3616]; Inabanga; 10°00.389'N, 124°03.522'E; 12 Jul. 2014; B Dayrat and party leg.; st 186, old, rehabilitated fish ponds next to a mangrove with some old *Avicennia* but mostly young *Rhizophora* trees; PNM 041253. SINGAPORE • 3 specimens 9/7 mm [978], 9/8 mm [979], 10/8 mm [980]; Lim Chu Kang; 01°26.785'N, 103° 42.531'E; 2 Apr. 2010; B Dayrat and party leg.; st 7, east of the jetty, open mangrove with medium trees, ending on mudflat outside mangrove with soft mud; ZRC.MOL.10485. • 2 specimens 13/8 mm [983], 10/7 mm [985]; Mandai River; 01°26.237'N, 103°45.730'E; 1 Apr. 2010;

Figure 26. Habitats, *Laspionchis bourkei matangensis*. **A, B** Peninsular Malaysia, Matang, old and open *Rhizophora* forest of with hard and soft mud, many creeks, and many old logs (st 256, type locality) **C** Singapore, mud outside mangrove on sun-exposed mudflat (st 7) **D** Philippines, narrow mangrove on the edge of fish ponds, tall *Rhizophora* and *Avicennia* trees, many old logs (st 194) **E** Indonesia, Sumatra, soft but solid mud, big *Avicennia*, a few logs, some *Nypa* on margin, little open space (st 76) **F** Vietnam, open *Avicennia* and *Rhizophora* mangrove with hard mud by a small road and deep mud near water (st 221).

B Dayrat and party leg.; st 6, open mangrove forest with tall trees and soft mud, ending on mudflat outside the mangrove with very soft mud; ZRC.MOL.10484. VIETNAM • 2 specimens 8/4 mm [5627], 4/3 mm [5646]; Can Gio; 10°24.171'N, 106°53.960'E; 10 Jul. 2015; TC Goulding and party leg.; st 221, open *Avicennia* and *Rhizophora* mangrove with hard mud by a small road and deep mud near water; ITBZC IM 00018.

Figure 27. Live specimens, *Laspionchis bourkei matangensis*. **A** Dorsal view, 13 mm long [1785], Indonesia, Sumatra (UMIZ 00113) **B** dorsal view, 10 mm long [1784], Indonesia, Sumatra (UMIZ 00113) **C** dorsal view, 11 mm long [1783], Indonesia, Sumatra (UMIZ 00113) **D** dorsal view, 15 mm long [5961], Peninsular Malaysia, Matang (USMMC 00056) **E** dorsal view, 25 mm long [3343], Philippines, Bohol (PNM 041254) **F** holotype, dorsal view, 15 mm long [5958 H], Peninsular Malaysia, Matang (USMMC 00055) **G** ventral view, 13 mm long [5963], Peninsular Malaysia, Matang (USMMC 00056) **H** ventral view, 8 mm long [5965], Peninsular Malaysia, Matang (USMMC 00056) **I** ventral view, same as **E**.

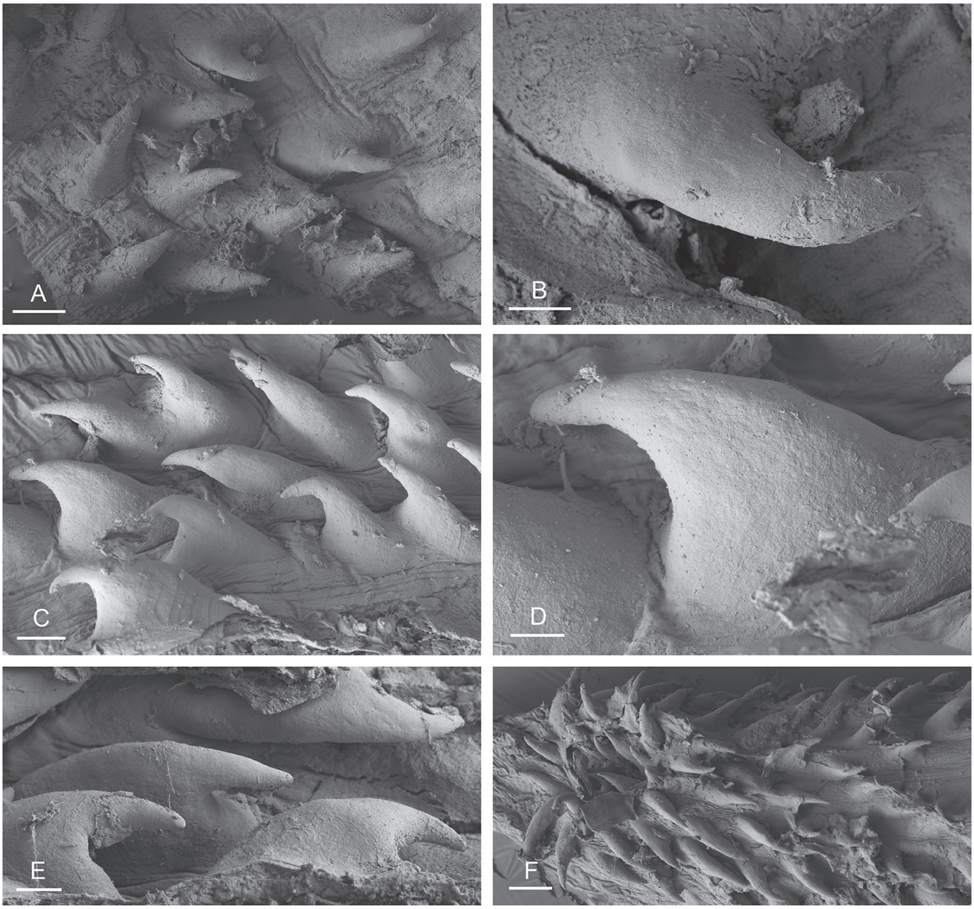

Figure 28. Penial hooks, *Laspionchis bourkei matangensis*, Peninsular Malaysia **A, B** Holotype [5958 H] (USMMC 00055) **C–E** [5959] (USMMC 00056) **F** [5960] (USMMC 00056). Scale bars: 10 µm (**A, C**), 3 µm (**B**), 4 µm (**D**), 5 µm (**E**), 20 µm (**F**).

Distribution (Fig. 7). Indonesia (Sulawesi, Sumatra), Malaysia (Peninsular Malaysia), Singapore, Philippines (Bohol), Vietnam.

Habitat (Fig. 26). Same as the entire species *Laspionchis bourkei* (see above).

Etymology. The subspecies *L. bourkei matangensis* is named after Matang, in Peninsular Malaysia. The type locality is part of the Matang mangrove forest. The name *matangensis* is an adjective derived from Matang and the suffix *-ensis*.

Diagnosis (Table 3). Externally, the three subspecies of *L. bourkei* cannot be distinguished. Internally, *L. bourkei matangensis* differs from *L. bourkei bourkei* but cannot be distinguished from *L. bourkei lateriensis*. All three subspecies, however, are clearly delineated using molecular DNA sequences.

Color and morphology of live animals (Fig. 27). Identical to the species *L. bourkei* (see above).

Digestive system (Figs 19E, F, 20A, B). Identical to the species *L. bourkei* (see above). Examples of radular formulae are in Table 4. Radulae measure up to 2.2 mm in length.

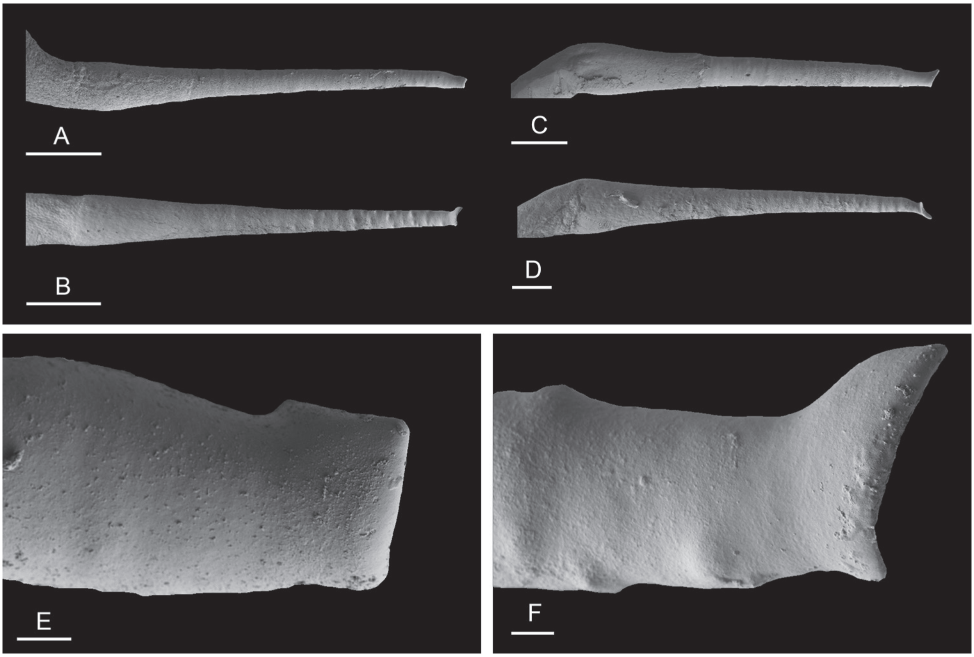

Figure 29. Spine of the accessory penial gland, *Laspionchis bourkei matangensis*. **A** Holotype, Peninsular Malaysia, [5958 H] (USMMC 00055) **B** Peninsular Malaysia, [5959] (USMMC 00056) **C** Philippines, [3343] (PNM 041254) **D** Peninsular Malaysia, [5960] (USMMC 00056) **E** same as **A** **F** same as **B**. Scale bars: 100 µm (**A–C**), 50 µm (**D**), 5 µm (**E, F**).

Reproductive system (Fig. 21C). Identical to the species *L. bourkei* (see above).

Copulatory apparatus (Figs 22C, 28, 29). Similar to the species *L. bourkei* (see above) acknowledging some minor variations: the length of the spine of the accessory penial gland ranges from 0.4 mm [5958 H] (USMMC 00055) to 0.57 mm [3343] (PNM 041254), the retractor muscle is vestigial or absent, and penial hooks measure 15 to 40 µm.

Remarks. See above the remarks on the species *Laspionchis bourkei*.

Discussion

A few preliminary remarks can be made here regarding the types of intestinal loops, even though a more detailed discussion will be provided after our revisions of *Peronia* and *Platevindex* are published (in preparation).

(1) Nearly all onchidiid species are characterized by only one intestinal type. Some intraspecific variation exists, which can be evaluated based on the orientation of the transitional loop. However, the presence of more than one intestinal type in an onchidiid species remains exceptional, such as in *Alionchis jailoloensis* (see Goulding et al. 2018b).

(2) Nearly all onchidiid genera are characterized by only one or two intestinal types. For instance, *Wallaconchis* and *Marmaronchis* are characterized by intestinal loops

of type I; *Onchidina*, *Paromoionchis*, and *Peronina* by type II; *Laspionchis* by loops between types I and II; *Alionchis*, *Melayonchis*, and *Onchidium* by types II and III. *Platevindex* is the only genus characterized by intestinal loops of more than two types (I, II, and III).

(3) Intestinal loops are quite useful to identify genera. For instance, all known species of *Wallaconchis* are characterized by intestinal loops of type I. Therefore, a slug with intestinal loops of type II cannot belong to *Wallaconchis*, unless intestinal loops of type II are found in the future in a new species of *Wallaconchis*. Also, *Laspionchis* slugs are the only ones known so far with an intestinal type between types I and II, with a transitional loop oriented at 6 o'clock (acknowledging individual variation). Therefore, slugs with intestinal loops between types I and II likely belong to *Laspionchis*.

(4) There must be some reasons explaining why intestinal types are not randomly distributed across onchidiid species and genera; however, the exact reasons are still unclear at this stage. Evolutionary history is possibly involved. For instance, the fact that all *Wallaconchis* species are characterized by intestinal loops of type I may be due to the presence of a type I in their common ancestor. Adaptation to different habitats is likely involved as well and will be discussed after our revisions of *Peronia* and *Platevindex* are published (in preparation).

Onchidiids are notoriously difficult to identify, both at the genus and species levels. *Laspionchis* slugs are no exception. They are most readily identified at the genus level using DNA sequences. Externally, they are practically impossible to distinguish from *Paromoionchis* slugs which live in the same habitat (mangrove mud surface) and are often found at exactly the same sites (see Dayrat et al. 2019). The male opening is clearly to the left of the right ocular tentacle in *Paromoionchis*, while it is just below the right ocular tentacle or only slightly to its left in *Laspionchis*, but this character is nearly impossible to check in the field when slugs are alive (because they retract as soon as they are being touched). Animal size can help distinguish *Laspionchis* slugs from *Paromoionchis* slugs in the field. Indeed, the maximum size of *Paromoionchis* slugs – 55 mm in *P. tumidus*, 65 mm in *P. daemelii*, 47 mm in *P. boholensis* Dayrat & Goulding in Dayrat et al. 2019, 48 mm in *P. penangensis* Dayrat & Goulding in Dayrat et al. 2019, and 35 mm in *P. goslineri* Dayrat & Goulding in Dayrat et al. 2019 – is much higher than the maximum size of *Laspionchis* slugs, 31 mm in *L. boucheti* and 32 mm in *L. bourkei*. That being said, animal length needs to be used with caution and it obviously is useless for all individuals less than 30 mm long.

Laspionchis is characterized by a unique combination of external and internal traits: no dorsal gills, male opening below the right eye tentacle (or slightly to its left), no rectal gland, intestinal loops between types I and II, accessory penial gland present with a muscular sac, penis with numerous, pointed hooks densely arranged next to each other. This unique combination of characters of *Laspionchis* is close to that of *Paromoionchis* (Dayrat et al. 2019: 19). However, there are three important differences: in *Paromoionchis*, the male opening is clearly to the left of the right ocular tentacle, the intestinal loops are clearly of type II, and penial hooks (which are present in *P. tumidus* but are absent in the four other *Paromoionchis* species) are sparse (i.e., not densely arranged, next to each other) and not pointed (Dayrat et al. 2019: figs 21, 22).

The two known species of *Laspionchis* are cryptic externally but distinct internally. They are found in exactly the same habitats and cannot be distinguished in the field. However, they can be identified successfully with both DNA sequences and internal anatomy. Species externally cryptic but internally distinct have also been observed in *Paromoionchis*, *Peronina*, and *Wallaconchis* (Dayrat et al. 2019; Goulding et al. 2018a, c). In *Marmaronchis*, species are cryptic externally and internally (Dayrat et al. 2018). In *Onchidium* and *Melayonchis*, species are distinct both externally and internally (Dayrat et al. 2016, 2017). Finally, *Alionchis* and *Onchidina* are monotypic, at least as of today (Dayrat and Goulding 2017; Dayrat et al. 2018).

The two new species described here are widespread and can be locally common. That they are discovered only now is not so surprising. *Laspionchis* is restricted to mangroves and mangroves of South-East Asia have been poorly explored and the biodiversity they host remains poorly known. Also, onchidiid taxonomy has been confused for a long time (Dayrat 2009). Now that onchidiid systematics is finally being revised, new taxa are being discovered (e.g., Dayrat et al. 2017; Goulding et al. 2018a, b, c), contributing to a better knowledge of the diversity of mangrove invertebrates in South-East Asia. It is very possible that additional species of *Laspionchis* will be discovered in the future, within or outside its current distribution. However, the study of the biodiversity of *Laspionchis* remains challenging, mostly because *Laspionchis* slugs are very hard to recognize in the field (they are not distinguishable from *Paromoionchis* slugs) and because *Laspionchis* species are externally cryptic (which means that many individuals looking similar need to be collected and individually numbered).

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Preliminary division of not socially parasitic Greek *Temnothorax* Mayr, 1861 (Hymenoptera, Formicidae) with a description of three new species

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Abstract

The division of Greek members of the genus *Temnothorax* into 17 morphological groups is proposed. *Temnothorax aveli* species group is reviewed with three species: *T. turcicus* (Santschi) (North Aegean Islands, Sterea Ellas, Peloponnese and Thessaly), and two species new to science: *Temnothorax brackoi* **sp. nov.** (Epirus, Ionian Islands, Macedonia, Peloponnese, western Sterea Ellas, Thessaly, and also Dalmatia in Croatia), and *T. messiniaensis* **sp. nov.** (Ionian Islands and Peloponnese); a new species *Temnothorax triangularis* **sp. nov.**, a member of the *Temnothorax nylanderi* species group is also described (Sterea Ellas: Euboea Island).

Keywords

Balkan Peninsula, *Temnothorax aveli* group, *Temnothorax nylanderi* group, taxonomy

Introduction

The Myrmicinae genus *Temnothorax* Mayr, 1861 is one of the most speciose in the Mediterranean Region (sensu Vigna Taglianti et al. 1999). Among 279 Palearctic taxa (Bolton 2019), 194 are known from this region (Borowiec 2014, Radchenko et al. 2015, Salata and Borowiec 2015, Csősz et al. 2015, 2018, Galkowski and Lebas 2016, Galkowski and Cagniant 2017, Sharaf et al. 2017, Catarineu et al. 2017, Salata et al. 2018). Most of the recent research performed on this genus was focused on taxa from European and Anatolian parts of the Mediterranean Region. Those areas are considered as most diverse and species-rich, and each further study confirms this assumption. Greece, located at the joint of European and Anatolian subunits, hosts numerous and various *Temnothorax* taxa and so it is one of the most challenging fields of research. Study on Greek *Temnothorax* is additionally impeded by a complicated taxonomical history of several taxa and a lack of new material. Moreover, recent studies published by Csősz et al. (2015, 2018) revealed that species considered as a widespread represent in fact complexes of cryptic taxa.

As most of *Temnothorax* species are morphologically variable, to determine their distribution range and morphological variability, researchers ought to investigate rich material. During several field trips to various places on Greek islands and the mainland, we collected more than 1000 samples of *Temnothorax* species from 480 localities. The material collected during those expeditions helped us to establish morphological variability within species and divide collected material into several species groups. In most cases, a division into species group is based on species morphology only, and we did not verify if it corresponded with the phylogeny of this genus. This method is a widely accepted tool helping in determining the range of studied material and is commonly practiced (Csősz et al. 2018, Salata and Borowiec 2015, Galkowski and Cagniant 2017, Catarineu et al. 2017). However, we adjusted our species-group definitions to phylogenetical data provided by Prebus (2017) and Csősz et al. (2015).

Some Greek *Temnothorax* have been already included in studies on species groups of *T. nylanderi* (Csősz et al. 2015), *T. interruptus* (Csősz et al. 2018), *T. recedens* (Salata and Borowiec 2015), and *T. muellerianus* (Salata and Borowiec 2015). Below we present our division of *Temnothorax* from Greece, which will be used as a base for further studies and we describe three new species: one of them, *Temnothorax triangularis*, is a member of recently revised *T. nylanderi* species group (Csősz et al. 2015). Two others are a widespread Greek species, members of the *Temnothorax aveli* species group. Because *Temnothorax* taxa known from Crete were revised recently (Salata et al. 2018), and the majority of its endemic species creates single-species groups unknown from other Greek regions, they were excluded from this paper. Also, socially parasitic members of *Temnothorax*, often classified in separate genera *Chalepoxenus* Menozzi and *Myrmoxenus* Ruzsky, are not included in this study (see discussion in Seifert et al. 2016 and Ward et al. 2016).

Materials and methods

Most of the material was sampled between 2007 and 2019 from sites in different parts of Greece. The main method was direct sampling (hand collecting). Individual specimens and nests were collected on the ground, in leaf litter and rock rubble, under stones, on tree trunks, and in dry twigs of herbs. This method was occasionally supplemented by litter sifting and collecting material with an entomological umbrella. All specimens were preserved in 75% EtOH. Study was supported with material deposited in the collection of G. Bračko (Ljubljana, Slovenia). All studied type specimens of taxa mentioned in differential diagnosis or characteristics are listed below.

Examined specimens are housed in the following collections:

BMNH	Natural History Museum, London;
CASC	California Academy of Sciences, San Francisco, California, USA;
DBET	Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, Poland;
MHNG	Museum d'Historie Naturelle, Geneve, Switzerland;
HNHM	Hungarian National History Museum, Budapest, Hungary;
NHMW	Natural History Museum, Vienna, Austria;
MNHW	Museum of Natural History, University of Wrocław, Wrocław, Poland;
MZLS	Museum of Zoology, Lausanne, Switzerland;
NHMB	Naturhistorisches Museum Basel, Switzerland;
PWC	Petr Werner collection, Prague, Czech Republic;
SMNG	Senckenberg Museum für Naturkunde Görlitz, Görlitz, Germany;
ZMHB	Museum für Naturkunde, Zentralin-stitut der Humboldt-Universität, Berlin, Germany.

To determine a distribution range and a morphological variability of the new species we compared them with material collected from other Greek regions. Data concerning samples used in the comparison is provided in series of regional checklists (Borowiec and Salata 2012, 2013, 2017a, b, 2018a, b, c, d, e, Bračko et al. 2016) and we see no reason to repeat this information.

Specimens were compared using standard methods of comparative morphology. All measurements were made in µm using a pin-holding stage, permitting rotations around X, Y, and Z axes. A Nikon SMZ18 stereomicroscope was used at a magnification of ×100 for each character. Photographs were taken using a Nikon SMZ 1500 stereomicroscope, Nikon D5200 camera and Helicon Focus software. All given label data of type specimens are in original spelling, presented in square brackets; a vertical bar (|) separates data on different rows and double vertical bars (||) separate labels. Images of type specimens are available online on AntWeb (www.AntWeb.org) and are accessible using the unique CASENT or FOCOL identifying specimen code. If not stated differently material deposited in Museum of Natural History, University of Wrocław (in permanent deposit in the Department of Biodiversity and Evolutionary Taxonomy).

Pilosity inclination degree applies to this used in Hölldobler and Wilson (1990). The adpressed (0–5°) hairs run parallel, or nearly parallel to the body surface. Decumbent hairs stand 10–15°, subdecumbent hair stands 30°, suberect hairs stand 35–45°, and the erect hairs stand more than 45° from the body surface.

Measurements:

EL	eye length; measured along the maximum vertical diameter of eye;
EW	eye width; measured along the maximum horizontal diameter of eye;
HL	head length; measured in straight line from mid-point of anterior clypeal margin to mid-point of posterior margin in full-face view;
HW	head width; measured in full-face view directly above the eyes;
PEH	petiole height; measured in lateral view, the chord of ventral petiolar profile at node level is the reference line perpendicular to which the maximum height of petiole is measured;
PEL	petiole length; measured in lateral view, from anterior corner of subpetiolar process to dorsocaudal corner of caudal cylinder;
PNW	pronotum width; maximum width of pronotum in dorsal view;
PPH	postpetiole height; measured perpendicularly to a line defined by the linear section of the segment border between dorsal and ventral petiolar sclerite;
PPL	postpetiole length; maximum length of the postpetiole measured in lateral view perpendicular to the straight section of lateral postpetiolar margin;
PPW	postpetiole width; maximum width of postpetiole in dorsal view;
PSL	propodeal spine length; measured from the centre of the propodeal spiracle to the top of the propodeal spine in lateral view;
PW	petiole width; maximum width of petiole in dorsal view;
SDL	spiracle to declivity length; minimum distance from the centre of the propodeal spiracle to the propodeal declivity;
SL	scape length; maximum straight-line length of scape excluding the articular condyle;
WL	mesosoma length; measured as diagonal length from the anterior end of the neck shield to the posterior margin of the propodeal lobe.

Indices:

CI	HW/HL * 100;	**MI**	PNW/WL * 100;
EI1	EW/EL * 100;	**PI**	PL/PH * 100;
EI2	EW/HL * 100;	**PPI**	PPL/PPH * 100;
SI1	SL/HL * 100;	**PSI**	PSL/SDL * 100.
SI2	SL/HW * 100;		

Abbreviations:

g.	gyne;	**m**.	male;	**w**.	worker.

We decided to list all other ant species collected from the same localities as species new to science. In our opinion, it provides valuable information about ecosystem structure and species diversity characteristic for habitats preferred by these species.

Type material of species noted in the comparative diagnoses

Temnothorax affinis (Mayr, 1855), syntype (w.): Oesterreich || Type || *Leptothorax* | *affinis* | Mayr || 29889 || *affinis* | Mayr || GBIF-D/FFoCol | 2006 (ZMHB); syntype (w.): Oesterreich || Type || *Leptothorax* | *affinis* | Mayr || GBIF-D/FoCol | 2007 (ZMBH), available from photo in AntWeb (FOCOL2006);

Temnothorax aveli (Bondroit, 1918), syntype (w.): Lept. aveli | Bondr. || Type || *Leptothorax* | *Aveli* Bondr. | type – Sayat || Sammlung | Dr. F. Santschi | Kairouan || ANTWEB | CASENT0912907 (NHMB), available from photo in AntWeb (CASENT0912907);

Temnothorax artvinensis Seifert, 2006, holotype (w.): ARTVIN – 5km SW Artvin | 100 Mh 1164 | Kiefernwald 50% | Leg. Schulz 27.06.93 TURKEI || Holotype | *Temnothorax* | *artvinense* | SEIFERT || GBIF-D/FoCol | 1361 (SMNG), available from photo in AntWeb (FOCOL1361);

Temnothorax graecus (Forel, 1911), lectotype (w.): *L. bulgaricus* | For. | r. *graecus* | type Forel | Patras … | … (Forel) || Lectotype | *Leptothorax graecus* | Forel, 1911 top specimen | det. A. Schulz & M. Verhaagh 1999 || Typus || r. d. graecus | Forel || Coll. | A. Forel || ANTWEB | CASENT0909017 (MHNG), examined; paralectotype (w.): the same pin as lectotype, bottom specimen (MHNG), examined; paralectotypes (2w.): Typus || L. bulgaricus | For. | r. graecus | Forel | type | Amaroussia | p. Athenes (Forel) || coll. | A. Forel (MHNG), examined.

Temnothorax kemali (Santschi, 1934), syntype (w.): *Leptothorax* | *kemali* Sant | Type || Izmir | 29.VII.33 | Santschi || Type || *Leptothorax* | *kemali* | A. Schulz det || Basel 10 || ANTWEB | CASENT0912952 (NHMB), available from photo in AntWeb (CASENT0912952);

Temnothorax laconicus Csősz, Seifert, Müller, Trindl, Schulz & Heinze, 2015, paratype (w.): *Temnothorax laconicus* || *Temnothorax laconicus* | Csosz et al. 2014 | PARATYPE || PARATYPE || GREECE, GRE 2011-0345 | Taygethos Oros, Street to Profiti | Ilias | 36.968N, ; 22.404E, ; 800 m | leg. A. Schulz, 01.05.2011 || ANTWEB | CASENT0914696 (HNHM), available from photo in AntWeb (CASENT0914696);

Temnothorax lagrecai (Baroni Urbani, 1964), paratype (w.): Caltagirone | Sichel leg. || BOSCO S. PIETRE | 15.IV.62 || TYPUS || ANTWEB | CASENT0919741 (NHMW), available from photo in AntWeb (CASENT0919741)

Temnothorax nigriceps (Mayr, 1855), syntype (w.): *L. tubero* | *nigriceps* | Mt. Tendre | … || Typus || Coll. | A. Forel ||ANTWEB | CASENT0909038 (MHNG), examined;

Temnothorax rabaudi (Bondroit, 1918), paralectotype (w.): *Leptothorax* | *Rabaudi* | Boundr. | type | Gironde || ANTWEB | CASENT0904752 (MSNG), available from photo in AntWeb (CASENT0904752)

Temnothorax sordidulus (Müller, 1923), syntype (w.): No: 175 Types | *L. carinthiacus* | Bernard || TYPE || 20.4.56 | … || Carinthia | Viktring | Holzel leg. || ANTWEB | CASENT0907632 (MZLS), available from photo in AntWeb (CASENT0907632);

Temnothorax tauricus (Ruzsky, 1902), cotypes, (3w.): *L. unifasciatus* | … | r. *tauricus* | Ruzsky | Crimee || Cotypus || v. L. *tauricus* | Ruzsky || 1288 || Coll. Forel || ANTWEB | CASENT0909049 (MHNG), examined; cotypes (3w.): L. unifasciatus | Latr. | tauricus | Ruzsky | Crimee || 1288 || cotype (MHNG), examined.

Taxonomy

Species groups of Greek *Temnothorax*

The number of *Temnothorax* taxa known from Greece is estimated at 59 (including undescribed species) and include recent data published in taxonomic revisions and faunistic papers (Salata and Borowiec 2018). Below we present series of morphological characters that were used in species-groups division. Some of the species groups were defined in former publications and we used those descriptions as a base for our research. This applies to the following cases: *bulgaricus* species group (Radchenko 1995a); *affinis* species group, *tuberum* species group, *corticalis* species group, and *clypeatus* species group (Radchenko 1995b); *nylanderi* species group (Radchenko 1995c, Csősz et al. 2015); *exilis* species group (Cagniant and Espadaler 1997); *interruptus* species group (Csősz et al. 2018); and *recedens* species group (Salata and Borowiec 2015). However, in some cases we adapted those definitions to Greek species and thus they differ from the original definitions. We also list members of each species group known from Greece. Taxa known and described from Crete are not included in this division, as they were recently revised in a separate paper (Salata et al. 2018), and most of them create separate, single species groups, so far known only from this island.

Temnothorax affinis species group: antennae 12-segmented, club not or only slightly darkened, metanotal groove absent, body colouration orange to dark orange with darker head and dark first gastral tergite, propodeal spines very long and thin, straight or only slightly curved, petiole node subangular in profile, head and mesosoma surface moderately sculptured, head completely microsculptured and with more or less developed longitudinal ridges. In Greece only one arboreal species, *T. affinis* (Mayr).

Temnothorax angustulus species group: antennae 12-segmented, club darkened, metanotal groove feebly marked, body colouration mostly brown to almost black, including head, first gastral tergite mostly brown with yellowish brown basal spot, propodeal spines short to long but thin, straight or slightly curved, petiole node angulate in profile, head and mesosoma surface finely sculptured, head only partly microsculptured, central part more or less shiny, longitudinal ridges diffused visible only in ocular area and sides of head. In Greece only one species known from Mediterranean coniferous forests, *T. dessyi* (Menozzi).

Temnothorax anodontoides species group: antennae 12-segmented, club darkened, metanotal groove absent, body colouration brown, dark brown to almost black including head, first gastral tergite completely brown to almost black not or only slightly paler at base, propodeal spines very short with broad base, not longer than basal width, petiole node rounded in profile, head and mesosoma surface very strongly sculptured appear partly rugose, head background microsculptured, whole surface with longitudinal ridges or rugosities. In Greece at least three undescribed species known only from high montane localities.

Temnothorax aveli species group: antennae 12-segmented, completely yellow but club sometimes slightly darkened, metanotal groove absent, body colouration mostly yellow including head, sometimes only gena and femora slightly darkened, first gastral tergite with narrow to broad dark posterior band, always with paler spot at base, propodeal spines short to moderately long, with broad base, petiole node rounded in profile, head and mesosoma surface finely sculptured, head almost uniformly, regularly microsculptured, mostly without longitudinal ridges, sometimes only central part of head with narrow area of diffused microreticulation, but head never appears smooth and shiny – in Greece three, revised below, species mostly known from lowland habitats, especially Mediterranean forests and bushes, nestling inside dry stems of trees and twigs of bushes and large herbs– *T. brackoi* sp. nov., *T. messiniaensis* sp. nov., *T. turcicus* (Santschi, 1934), (?) *T. tauricus* (Ruzsky) (see comments in the redescription of *T. turcicus*).

Comments: Members of the *aveli* species group are morphologically similar to those assigned to the *tuberum* species group. Despite morphological similarities taxa of both groups can be easily distinguished based on nesting and habitat preferences. Species of the *aveli* species group inhabit lowlands and have nests inside dry stems of trees, bushes and herbs. While Greek species of the *tuberum* species group occur in the mountain areas and nest in soil, under moss, in rock crevices or debris.

Temnothorax bulgaricus species group: antennae 12-segmented, club usually distinctly darkened, metanotal groove absent, body colouration mostly yellow, sometimes head partly darkened, first gastral tergite with broad dark posterior band, always with yellow spot at base, propodeal spines reduced to triangular tubercle, petiole node rounded in profile, head in central part more or less smooth and shiny, on sides with longitudinal ridges, sometimes almost whole surface of head smooth and shiny or smooth area reduced to a narrow medial stripe, mesosoma laterally with strong sculpture of longitudinal ridges. In Greece two species associated with humid habitats such as stream valleys or bushes inside dark deciduous forests, *T. bulgaricus* (Forel) and *T. nadigi* (Kutter).

Temnothorax clypeatus species group: antennae 12-segmented, completely yellow, metanotal groove inconspicuous but well-marked, body colouration ochraceous to reddish brown, first gastral tergite with broad dark posterior band, always with pale spot at base, propodeal spines moderate to long, thorn-shaped, petiole with short peduncle, appears high and bulky in profile, head sculpture from complete, with microreticulate background and more or less developed ridges and costae, to mostly smooth and shiny in central part, only on gena and around eyes with longitudinal ridges, mesosoma laterally with distinct microreticulation and strong reticulate and longitudinal sculpture. In Greece one arboreal species associated with deciduous trees, especially large oaks in sunny habitats, *T. clypeatus* (Mayr).

Temnothorax corticalis species group: antennae 12-segmented, completely yellow, metanotal groove absent, body colouration ochraceous to light brown, head always more or less darkened, first gastral tergite with broad dark posterior band, always with pale spot at base, propodeal spines from very short, triangular to moderately long needle-shaped, petiole with very short peduncle, appears triangular in profile with more or less angulate node, head sculpture from complete, with more or less reticulate sculpture but shiny background, occasionally in central part of frons reticulation partly diffused, mesosoma dorsally and laterally with distinct microreticulation and often sides of pronotum with longitudinal ridges. In Greece one arboreal species associated with deciduous forests, *T. corticalis* (Schenck), but occurrence of *T. jailensis* (Arnoldi) is possible.

Temnothorax exilis species group: antennae 12-segmented, club usually distinctly darkened, metanotal groove absent, body colouration extremely variable, from almost completely yellow to black, often mesosoma paler coloured than head and gaster, first gastral tergite in pale forms with dark posterior band, propodeal spines moderate to long, thin, straight, petiole node angulate in profile, head in central part more or less smooth and shiny, on sides usually with longitudinal ridges, sometimes almost whole surface of head smooth and shiny or smooth area reduced to a narrow medial stripe, mesosoma laterally with strong sculpture of longitudinal ridges. Xerothermophilous species associated with rocky, open and arid habitats; in Greece only *T. exilis* (Emery) recorded, but based on a high variability of insular populations the real number of species of this group is difficult to estimate and requires further studies.

Temnothorax flavicornis species group: antennae 11-segmented, unicolourous yellow, metanotal groove present, body colouration almost completely yellow to dark yellow, without distinct contrast between colouration of head and mesosoma, first gastral tergite with dark posterior, propodeal spines from long to very long, claw-shaped, from straight to slightly curved, petiole with moderately long peduncle and node angulate, head always with microreticulate background and more or less developed reticulate or costulate sculpture, along middle of head runs stripe with diffused reticulation, more or less smooth and shiny, mesosoma with microreticulate sculpture, often with distinct ridges or costae. One species associated with various arboreal habitats, *T. flavicornis* (Emery).

Temnothorax graecus species group: antennae 12-segmented, club usually distinctly darkened, metanotal groove absent, body colouration mostly yellow, first gastral tergite with broad dark posterior band, always with yellow spot at base, propodeal spines very short to short, triangular to needle-shaped, petiole node rounded in profile or obtusely angulate, head in central part more or less smooth and shiny, on sides with longitudinal ridges, sometimes almost whole surface of head smooth and shiny or smooth area reduced to a broad medial stripe, mesosoma laterally with moderate sculpture of longitudinal ridges. Associated mostly with moderately humid to arid deciduous forests or mediterranean bushes, collected on rocks and stones; *T. aeolius* (Forel), *T. graecus* (Forel), *T. smyrnensis* (Forel), and at least two undescribed species.

Temnothorax interruptus species group: antennae 12-segmented, club usually distinctly darkened, frontal lobes conspicuously wider than frons, metanotal groove absent or indistinct, body yellow to light brown, gena darker, first gastral tergite with dark posterior band, often interrupted in the middle, propodeal spines very long, thin and curving downwards, petiole node subangulate to obtuse in profile, head with distinct microreticulation and longitudinal ridges, often partly with large reticulate sculpture, mesosoma mostly microreticulate, dorsally and laterally more or less rugose or costulate. In Greece two species associated with open habitats such as rocks and stones overgrown by bushes or limestones on mountain pastures, collected also in deciduous or mixed forests, and occasionally in coniferous forests; *T. morea* Csősz, Salata & Borowiec and *T. strymonensis* Csősz, Salata & Borowiec.

Temnothorax kemali species group: antennae 12-segmented, club usually more or less darkened, occasionally whole antennae yellow, metanotal groove absent, body yellow to orange, gena usually darker, first gastral tergite with dark posterior band, propodeal spines long and thin apically often curving downwards, petiole node subangulate to obtuse in profile, head at least in central part without microreticulation, smooth and shiny, only gena and area around eyes with longitudinal ridges, in some specimens sculpture of sides and central part of head more distinct but area between ridges or costae always smooth and shiny. Species associated with Mediterranean herbs and bushes or dry deciduous and coniferous forests, often nestling inside dry stems of herbs; *T. kemali* (Santschi) and at least one undescribed species.

Temnothorax luteus species group: antennae 12-segmented, club usually more or less darkened, occasionally whole antennae yellow, metanotal groove absent, body yellow to orange, gena sometimes darker, first gastral tergite with dark posterior band, propodeal spines straight, long and thin, petiole node subangulate in profile, head most often without microreticulation, smooth and shiny, only gena and area around eyes with longitudinal ridges. Xerothermophilous species associated with lowland habitats, Mediterranean herbs and bushes; at least one undescribed species.

Temnothorax nylanderi species group: diverse and speciose group, antennae 12-segmented, unicolourous yellow, metanotal groove usually distinct but in some species inconspicuous, body colouration variable, from almost completely yellow to dark brown but usually without distinct contrast between colouration of head and mesosoma, first gastral tergite usually with dark posterior band (except dark species with unicolourous gaster), propodeal spines from very short, triangular to very long, claw-shaped, from straight to distinctly curved, petiole with moderately long peduncle and node from angulate to obtuse in profile, head always with microreticulate background and more or less developed reticulate or costulate sculpture, sometimes along middle of head runs stripe with diffused reticulation, more or less smooth and shiny, mesosoma with microreticulate sculpture, often with distinct ridges or costae. Species associated with various shadowy habitats, nesting in rock, stones, and dry branches inside forests; *T. angulinodis* Csősz, Heinze & Mikó, *T. angustifrons* Csősz, Heinze & Mikó, *T. ariadnae* Csősz, Heinze & Mikó, *T. crasecundus* Seifert & Csősz, *T. crassispinus* (Karavaiev), *T. helenae* Csősz, Heinze & Mikó, *T. laconicus* Csősz, Seifert, Müller, Trindl, Schulz & Heinze, *T. lichtensteini* (Bondroit), *T. lucidus* Csősz, Heinze & Mikó, *T. nylanderi* (Foerster), *T. parvulus* (Schenck), *T. sordidulus* (Müller), *T. subtilis* Csősz, Heinze & Mikó, *T. tergestinus* (Finzi), and *T. triangularis* sp. nov.

Comment: Results presented by Prebus (2017) revealed that the *nylanderi* species group, as defined by Csősz et al. 2015, is paraphyletic and the position of *T. flavicornis* within it is unlikely. Therefore, we decided to exclude this species from this group and consider it as the single representative of the *flavicornis* species group.

Temnothorax recedens species group: antennae 12-segmented, unicolourous yellow to brown, metanotal groove very deep, body colouration variable, from almost completely yellow to dark brown, often mesosoma paler coloured than head and gaster, first gastral tergite in pale forms with dark posterior band, propodeal spines from short, triangular to long and thin, the shape of the needle, straight to slightly curved, petiole with long peduncle and node obtuse in profile, head mostly smooth and shiny, pronotum almost completely shiny, mesonotum and propodeum laterally with microreticulate sculpture, without distinct ridges or costae. Species associated with various arboreal habitats, nesting in rock crevices or under moss; *T. antigoni* (Forel), *T. recedens* (Nylander), *T. rogeri* Emery, and *T. solerii* (Menozzi).

Comment: With great probability this group is more speciose than it is apparent from current data, especially *T. recedens* shows high regional variability and wide ecological variance, which suggests that this taxon is a group of cryptic species

Temnothorax rottenbergi species group: very large species, antennae 12-segmented, club darkened or whole antennae dark, metanotal groove inconspicuous, body completely black or distinctly bicoloured with head and gaster brown to black and mesosoma partly to completely red, propodeal spines very long and strong, apex of spines often curving downwards, petiole with long peduncle and globular node, head with strong reticulate sculpture, mesosoma dorsally and laterally with strong, partly reticulate and partly costate sculpture. Xerothermophilous and alpine species associated with rock and stones on open, sunny habitats such as mountain pastures, grasslands or edges of forests. From Greece *T. rottenbergi* (Emery) and *T. semiruber* (André) were recorded but occurrence of first species needs confirmation.

Temnothorax tuberum species group: diverse group, antennae 12-segmented, club darkened, metanotal groove absent or indistinct, mesosoma colouration variable, from almost completely yellow to ochraceous, head always darker coloured than mesosoma, in extreme case almost black, first gastral tergite always with dark posterior band, propodeal spines from short, triangular to moderately long but never needle-shaped, from straight to slightly curved apically, petiole with moderately long peduncle and node subangulate in profile, head always with microreticulate background and more or less developed reticulate or costulate sculpture, without smooth and shiny areas, mesosoma with microreticulate sculpture, often with distinct ridges or costae – species associated with various habitats, from open and sunny to shadowy arboreal, usually nesting in rocks or stones, most species were noted also on rocks on mountain pastures – *T. melanocephalus* (Emery), *T. nigriceps* (Mayr), *T. tuberum* (Fabricius), *T. unifasciatus* (Latreille), and several undescribed taxa.

Comment: Results presented by Prebus (2017) suggest that the *tuberum* species group (sensu Cagniant and Espadaler 1997) and *unifasciatus* species group (sensu Bernard 1967) are paraphyletic. In both cases species groups were defined based on West-Mediterranean taxa. Only two Greek members of the *tuberum* species group were included in analysis presented by Prebus (2017): *T. nigriceps* and *T. unifasciatus* and they created a separate cluster. Confirmation, if the *tuberum* species group as defined by us here is a natural, monophyletic group requires further studies.

A key to *Temnothorax* species groups known from Greece[*]

1 Metanotal groove present, distinct to inconspicuous **2**

– Metanotal groove absent, mesosoma in lateral view evenly convex .... 7[**]

2 Antennae 11-segmented.................................. ***T. flavicornis* species group**

– Antennae 12-segmented.. **3**

3 Head and mesosoma almost entirely smooth and shiny; metanotal groove very deep; antennal scape very long and thin .... ***T. recedens* species group**

– Head and mesosoma sculptured, sometimes only frons and mesosomal dorsum with reduced or absent sculpture; metanotal groove deep to indistinct; antennal scape short to moderate **4**

4 More or less large species; body completely black or distinctly bicoloured with head and gaster brown to black and mesosoma partly to completely red; propodeal spines very long and sharp, apex of spines often curving downwards; petiole with long and more or less thin peduncle and globular node, head with sparse and thick reticulae and, at least on frons, smooth interspaces...... .... ***T. rottenbergi* species group**

– Moderately sized species; body colouration variable, from almost completely yellow to dark brown but without distinct contrast between colouration of head and mesosoma; propodeal spines short to long, straight or only slightly curved; petiole with peduncle short to moderately long, and node from angulate to obtuse in profile; head with sculpture variable, but always fine and dense, interspaces usually with additional microsculpture **5**

5 Promesonotal suture distinct; body colouration ochraceous to reddish brown; anterior margin of clypeus with distinct notch...... ***T. clypeatus* species group**

– Promesonotal suture absent or indistinct; body yellow to dark brown and never ochraceous or reddish; anterior margin of clypeus without distinct notch **6**

6 Antennal club darkened; metanotal groove feebly marked; body colouration more or less uniform, mostly brown to almost black; first gastral tergite with yellowish brown basal spot; propodeal spines short to long but thin, straight or only slightly curved .... ***T. angustulus* species group**

– Antennal club unicolourous, yellow; metanotal groove usually distinct but in some species inconspicuous; body colouration variable, from almost completely yellow to dark brown but never uniformly coloured (at least legs and antennae brighter); first gastral tergite with dark posterior band or uniformly

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[*] Crete, as an island, creates a well-defined unit and studies performed on *Temnothorax* known from the island were published in a separate paper (Salata et al. 2018). The majority of endemic taxa known from the island create a single-species group and the material collected from other Greek islands and regions adjacent to Crete confirms their uniqueness. Therefore, Cretan endemic species of *Temnothorax* are not included in the key.

[**] Some specimens of the *T. tuberum* species group and *T. interruptus* species group can have very indistinct metanotal grooves. Usually it applies to single specimens within a nest sample.

coloured; propodeal spines from very short, triangular to very long, claw-shaped, always with wide base............................ ***T. nylanderi* species group**

7 Frontal lobes conspicuously wider than frons; first gastral tergite with dark posterior band, often interrupted in the middle; propodeal spines very long, thin and curving downwards........................... ***T. interruptus* species group**

– Frontal lobes never conspicuously wider than frons; dark posterior band on the first gastral tergite never interrupted in the middle or absent; propodeal spines of a different shape **8**

8 Propodeal spines very long and thin and never with wide base.................... **9**

– Propodeal spines very short to moderately long, always with wide base..... **11**

9 Body orange to dark orange with darker head and first gastral tergite with bright spot on its basal part; head and mesosoma surface moderately sculptured, head completely microsculptured and with more or less developed longitudinal ridges .... ***T. affinis* species group**

– Body yellow to orange, gena sometimes darker, first gastral tergite with dark posterior band; head, at least in central part, without microreticulation, smooth and shiny **10**

10 Propodeal spines long and thin, apically often curving downwards; head microsculptured, only its central part with microreticulation reduced or absent .... ***T. kemali* species group**

– Propodeal spines straight, long and thin; head most often without microreticulation, smooth and shiny.................................... ***T. luteus* species group**

11 Body colouration uniform, brown, dark brown to almost black; propodeal spines very short with broad base, not longer than basal width; petiole node rounded in profile; head and mesosoma surface very strongly sculptured; alpine species.. ***T. anodontoides* species group**

– Body bicoloured or entirely yellow; if body uniformly dark brown to black then propodeal spines small to moderately long, and frons and dorsal surface of mesosoma with reduced sculpture or smooth....................................... **12**

12 Propodeal spines indistinct, strongly reduced to triangular tubercles; petiolar node obtuse in profile .... ***T. bulgaricus* species group**

– Propodeal spines short to moderately long, always distinct; petiolar node from obtuse to angulate in profile **13**

13 Head sculpture variable, more or less sparse and fine; at least centre of frons and central part of promesonotal dorsum smooth; propodeal spines moderately long to long; body colouration variable, from almost completely yellow to completely black; also within populations of the same species body colouration variable.. ***T. exilis* species group**

– Head with dense and fine sculpture, frons sometimes with sculpture reduced but never smooth; sculpture on mesosoma never reduced or smooth. If head and dorsum of promesonotum with reduced sculpture, then propodeal spines very small; body colouration from mostly yellow to light brown or bicoloured but constant within a single species..**14**

14 Body distinctly bicoloured, yellow to light brown with head usually darker than mesosoma and gaster with broad dark band......................................**15**

– Body almost entirely yellow to dark yellow; only head sometimes with slightly darker frons or malar area and gaster usually with narrow dark band.....**16**

15 Antennal club never darkened; propodeal spines from very short, triangular to moderately long needle-shaped, petiole with very short pedicel; head with moderately dense reticulate sculpture but smooth background, occasionally in central part of frons reticulation partly diffused .... ***T. corticalis* species group**

– Antennal club darkened; propodeal spines from short, triangular to moderately long but never needle-shaped; petiole with moderately long pedicel; head always with microreticulate background and moderately developed reticulate or costulate sculpture, without smooth and shiny areas.................... .... ***T. tuberum* species group**

16 Propodeal spines short to moderately long with broad base; head and mesosoma surface finely sculptured, head almost uniformly, regularly microsculptured, mostly without longitudinal ridges, sometimes only central part of head with narrow area of diffused microreticulation, but head never appears smooth and shiny; petiolar node angulate in profile .... ***T. aveli* species group**

– Propodeal spines very short to short, triangular to needle-shaped; head in central part more or less smooth and shiny, on sides with longitudinal ridges, sometimes almost whole surface of head smooth and shiny or smooth area reduced to a broad medial stripe; petiolar node rounded to subangular in profile .... ***T. graecus* species group**

Review of Greek species of the *Temnothorax aveli* species group

Temnothorax brackoi sp. nov.

http://zoobank.org/BAAA6DC9-5DB0-4A21-8520-679D413E3BC6
Figs 1, 2, 9, 13

Type material. Holotype: worker (pin), (CASENT0846669): GREECE, Pel. Messinia | 1.8 km E of Saidona, 985 m | 36.88491N, 22.30419E | 20 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02195 (MNHW).

Paratypes: • 11w. (pin), 9w. (EtOH) (CASENT0846607–CASENT0846617): the same nest sample as holotype (DBET); • 11w. (pin), 5w. (EtOH)(CASENT0846618–CASENT0846628): GREECE, Pel. Messinia | 0.8 km SE of Exochori, 535 m, | 36.89582N, 22.27464E | 20 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02184 (DBET, MHNG, CASC, BMNH); • 8w. (pin), 1w. (EtOH) (CASENT0846629–CASENT0846636): GREECE, Pel. Messinia | n. Arachova, 860 m | 37.03922N, 22.21876E | 13 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02029 (DBET); • 2w. (pin) (CASENT0846637–CASENT0846638): GREECE, Pel. Messinia | 1.3 km S of Artemisia, 870 m | 37.08738N, 22.23378E | 17 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02121 (DBET).

Other material. GREECE. Epirus, Preveza: • 4w. (pin) (CASENT0846670–CASENT0846673), 21w. (EtOH): 700 m E of Kanali, oak forest, 39.0638N/20.70621E, 50 m, 2016-08-27, leg. L. Borowiec; • 8w. (pin) (CASENT0846674–CASENT0846681), 14w. (EtOH): n. Kanali, oak forest, 39.05458N/20.70207E, 15 m, 2016-08-28, leg. L. Borowiec; • 27w (EtOH): n. Kanali, pine forest, 39.05388N/20.70118E, 20 m, 2016-08-28, leg. L. Borowiec. **Ionian Islands, Cephalonia**: • 6w. (pin) (CASENT0846682–CASENT0846687): Avithos Lake, area near a small lake in a moist, shaded valley of a small creek, 38.17293N/20.71233E, 278 m, 2014-06-25, leg. L. Borowiec; • 68w. (EtOH): Avithos Lake, shrubs around small lake, 38.17203N/ 20.71107E, 288 m, 2019-06-10, leg. L. Borowiec; • 29w. (EtOH): Katapodata; roadsides with shrubs, 38.23337N/20.64594E, 100 m, 2019-06-10, leg. L. Borowiec; • 4w. (EtOH): 1.5 km NE of Koulourata, mixed forest on shrubs, 38.20667N/ 20.67715E, 273 m, 2019-06-10, leg. L. Borowiec; • 11w. (EtOH): ancient Same; roadsides with shrubs, 38.2522N/20.66423E, 220 m, 2019-06-10, leg. L. Borowiec; • 13w. (EtOH): rd. Skala-Poros; Mediterranean shrubs, 38.12872N/20.79576E, 5 m, 2019-06-12, leg. L. Borowiec. **Ionian Islands, Corfu**: • 6w. (EtOH): Ag. Ilias, deciduous forest, 39.79367N/19.87508E, 191 m, 2013-06-10, leg. L. Borowiec; • 5w. (pin) (CASENT0846688–CASENT0846692), 5w. (EtOH): Akr. Kefali n. Agios Stefanos, frygana on sea coast, 39.75154N/19.63272E, 13 m, 2013-06-05, leg. L. Borowiec; • 4w. (EtOH): E of Nymfes, old olive tree plantation, 39.75047N/19.8057E, 179 m, 2013-06-09, leg. L. Borowiec; • 8w. (pin) (CASENT0846693–CASENT0846700), 1w. (EtOH): Klimatia, deciduous forest, 39.74123N/19.78953E, 311 m, 2013-06-06, leg. L. Borowiec; • 1w. (pin) (CASENT0846701): N of Ag. Stefanos, old olive forest, 39.76338N/19.65213E, 88 m, 2013-06-05, leg. L. Borowiec; • 4w. (pin) (CASENT0846702–CASENT0846705): n. Doukades, old olive tree plantation, 39.70075N/19.75055E, 174 m, 2013-06-08, leg. L. Borowiec; • 3w. (pin) (CASENT0846706–CASENT0846708), 1w. (EtOH): n. Vistonas, old olive tree plantation, 39.68549N/19.71453E, 422 m, 2013-06-08, leg. L. Borowiec; • 20w. (pin) (CASENT0846709–CASENT0846728), 15w. (EtOH): Nymfes, deciduous forest, 39.75478N/19.79535E, 162 m, 2013-06-06, leg. L. Borowiec; • 20w. (EtOH): Old Perithia, old mountain village, 39.76159N/19.87412E, 467 m, 2013-06-10, leg. L. Borowiec; • 3w. (pin) (CASENT0846729–CASENT0846731): Strinilas, deciduous forest, 39.73963N/19.84265E, 632 m, 2013-06-07, leg. L. Borowiec. **Ionian Islands, Lefkada:** • 3w. (pin) (CASENT0846732–CASENT0846734), 2w. (EtOH): Asprogerakata, stream valley with deciduous forest, 38.78278N /20.65262E, 430 m, 2016-09-02, leg. L. Borowiec; • 2w. (pin) (CASENT0846735–CASENT0846736), 8w. (EtOH): Egklouvi, stream valley with deciduous forest, 38.73212N/20.64821E, 690 m, 2016-09-02, leg. L. Borowiec. **Ionian Islands, Zakynthos:** • 4w. (EtOH): 1 km E of Elies, shrubs along roadsides, 37.90173N/20.68787E, 300 m, 2018-05-06, leg. L. Borowiec; •

18w. (EtOH): 1 km N of Exo Chora, mixed forest, 37.81063N/ 20.68459E, 430 m, 2018-05-08, leg. L. Borowiec; • 1w. (pin) (CASENT0846737): 1.2 km N of Ano Vasilikos, roadsides along olive plantation and pasture with oak shrubs, 37.72456N/20.97786E, 30 m, 2018-05-05, leg. L. Borowiec; • 128w. (EtOH): 1.2 km N of Vasilikos, roadsides along olive plantation and pasture with oak shrubs, 37.72456N/20.97786E, 30 m, 2018-05-05, leg. L. Borowiec; • 2w. (EtOH): 1.2 km NE of Anafonitria shrubs around burned forests, 37.85489N/20.64124E, 475 m, 2018-05-10, leg. L. Borowiec; • 1w. (pin) (CASENT0846738), 43w. (EtOH): 1.2 km SW of Ano Vasilikos loc. 1, roadsides along olive plantation, 37.72374N/20.95964E, 72 m, 2018-05-05, leg. L. Borowiec; • 1w. (pin) (CASENT0846739), 25w. (EtOH): 1.2 km SW of Ano Vasilikos loc. 2, roadsides along olive plantation, 37.72325N/20.96123E, 75 m, 2018-05-05, leg. L. Borowiec; • 1w. (pin): 1.2 km SW of Skinaria, limestone hills after burned forests, 37.87694N/20.69272E, 375 m, 2018-05-06, leg. L. Borowiec; • 1w. (pin) (CASENT0846740), 13w. (EtOH): 1.4 km E of Ano Volimes, mixed forest, 37.87594N/20.68525E, 430 m, 2018-05-06, leg. L. Borowiec; • 1w. (EtOH): 1.5 km N of Exo Chora: shrubs around meadow, 37.81446N/ 20.68826E, 460 m, 2018-05-08, leg. L. Borowiec; • 38w. (EtOH): 1.7 km NE of Ano Volimes, shrubs around pastures, 37.88627N/20.68456E, 445 m, 2018-05-06, leg. L. Borowiec; • 3w. (EtOH): 1.8 km SW of Volimes, shrubs along roadsides, 37.86472N/ 20.64234, 350 m, 2018-05-10, leg. L. Borowiec; • 1w. (EtOH): 1.9 km W of Maries, roadsides in burned forests, 37.818N/20.65556E, 290 m, 2018-05-09, leg. L. Borowiec; • 1w. (pin), 30w. (EtOH): 2.5 km NE of Maries, mixed forest, 37.8299N/20.70151E, 475 m, 2018-05-08, leg. L. Borowiec; • 51w. (EtOH): 3.9 km NE of Maries, shrubs around olive plantation, 37.84202N/20.70938E, 370 m, 2018-05-08, leg. L. Borowiec; • 1w. (pin) (CASENT0846741), 16w. (EtOH): 470 m NE of Orthonies, shrubs along roadsides, 37.85435N/20.69843E, 405 m, 2018-05-10, leg. L. Borowiec; • 1w. (EtOH): 500 m S of Apelati, pine forest, 37.66873N/20.81407E, 160 m, 2018-05-07, leg. L. Borowiec; • 1w. (EtOH): 700 m SW of Koroni, maquis, 37.86582N/20.71753E, 290 m, 2018-05-10, leg. L. Borowiec; • 3w. (EtOH): 750 m S of Volimes, shrubs in cypress forest, 37.86762N/20.66191E, 360 m, 2018-05-10, leg. L. Borowiec; • 1w. (pin) (CASENT0846742), 81w. (EtOH): 800 m SE of Xirokastello, roadsides along olive plantation, 37.73491N/20.95139E, 75 m, 2018-05-05, leg. L. Borowiec; • 10w. (EtOH): 880 m S of Orthonies, shrubs in cypress forest, 37.84462N/20.69843E, 390 m, 2018-05-10, leg. L. Borowiec; • 66w. (EtOH): Ag. Georgiou monastery, shrubs along roadsides, 37.85971N/ 20.63646E, 330 m, 2018-05-10, leg. L. Borowiec; • 8w. (EtOH): Ag. Joannis, roadsides with shrubs, 37.72924N/20.94553E, 165 m, 2018-05-05, leg. L. Borowiec; • 6w. (EtOH): Argassi, urban area, 37.76182N/20.92704E, 10 m, 2018-05-04, leg. L. Borowiec; • 1w. (EtOH): Livia Mts. loc. 1, shrubs along roadsides, 37.83018N/20.71619E, 600 m, 2018-05-08, leg. L. Borowiec; • 15w. (EtOH): Vrachionas Mts., mountain pastures with shrubs, 37.81798N/ 20.70621E, 670 m, 2018-05-08, leg. L. Borowiec; • 1w. (EtOH): W of Kampi, pine forest, 37.78161N/20.68078E, 165 m, 2018-05-09, leg. L. Borowiec. **Macedonia, Pieria:** • 4w. (EtOH): Platamonas Castle hill, on stones, soil and herbs, 40.00868N/ 22.59654E, 12 m, 2019-05-11, leg. L. Borowiec; • 1w. (pin) (CASENT0846743): 2 km W of Panteleimonas, roadsides with shrubs, 39.98563N/ 22.59513E, 305 m, 2019-05-15, leg. L. Borowiec; • 20w. (EtOH): road to P. Poroi loc. 1, roadsides with shrubs, 39.97963N/ 22.61563E, 110 m, 2019-05-17, leg. L. Borowiec; • 7w. (EtOH): road to P. Poroi loc. 2, roadsides with shrubs, 39.97627N/ 22.61146E, 185 m, 2019-05-17, leg. L. Borowiec; •81w. (EtOH): road to P. Poroi loc. 3, roadsides with shrubs, 39. 96863N/ 22.60494E, 260 m, 2019-05-17, leg. L. Borowiec. **Peloponnese, Korinthia:** • 16w. (pin) (CASENT0846744–CASENT0846759): n. Evrostina, deciduous forest, 38.07386N/22.39388E, 662 m, 2013-09-01, leg. L. Borowiec. **Peloponnese, Laconia:** • 13w. (pin) (CASENT0846760–CASENT0846772), 2w. (EtOH): Taygetos Mts., 1.5 km SW of Anavryti, coniferous forest, 37.0191N/22.36003E, 990 m, 2016-06-21, leg. L. Borowiec. **Peloponnese, Messinia:** • 1w. (pin) (CASENT0846773): Taygetos Mts., 0.7 km S of Dyrrachio, coniferous forest, 37.1811N/22.2074E, 800 m, 2016-06-22, leg. L. Borowiec; • 5w. (pin) (CASENT0846774–CASENT0846778): Taygetos Mts., 2 km W of Arachova, stream valley with Platanus forest, 37.0357N/22.1978E, 680 m, 2016-06-13, leg. L. Borowiec; • 1w. (pin) (CASENT0846779): Taygetos Mts., Chora Getson, stream valley with deciduous forest, 36.94779N/22.25466E, 615 m, 2016-06-14, leg. L. Borowiec; • 4w. (pin) (CASENT0846780–CASENT0846783): Taygetos Mts., Karveli, rest area with stream, 37.07591N/22.20633E, 600 m, 2016-06-17, leg. L. Borowiec; • 2w. (pin) (CASENT0846784–CASENT0846785): Tetrazi Mts., 0.5 km E of Vastas, stream valley with deciduous forest, 37.36441N/21.99304E, 895 m, 2016-06-19, leg. L. Borowiec; • 1w. (pin) (CASENT0846786), 2w. (EtOH): Tetrazi Mts., Isaris, pine forest, 37.36644N/22.01516E, 790 m, 2016-06-19, leg. L. Borowiec; • 1w. (pin) (CASENT0846787): Tetrazi Mts., Karnasi, stream valley with Platanus forest, 37.31904N/21.99158E, 460 m, 2016-06-19, leg. L. Borowiec. **Sterea Ellas, Aetolia-Acarnania:** • 1w. (EtOH): Psila Alonia, stream valley with Platanus forest, 38.96255N/21.19527E, 62 m, 2016-09-04, leg. L. Borowiec. **Sterea Ellas, Euboea:** • 2w. (EtOH): 1.2 km NW of Gerontas, mediterranean shrubs along roadsides, 38.45885N/23.808E, 405 m, 2018-06-10, leg. L. Borowiec; • 2w. (EtOH): 2.3 km S of Stropones, mixed forest, 38.9933N/23.87807E, 860 m, 2018-06-10, leg. L. Borowiec; • 1w. (EtOH): 300 m NW of Agios, pine forest with mediterranean shrubs, 38.65856N/23.55525E, 600 m, 2018-06-11, leg. L. Borowiec; • 7w. (EtOH): 570 m NW of Drosia, stream valley with mixed forest, 38.61705N/23.59089E, 140 m, 2018-06-11, leg. L. Borowiec. **Thessaly, Larissa:** • 1w. (pin) (CASENT0846788): Mt. Olympus, 5.3 km E of Olympiada, alpine pastures with shrubs, 40.00989N/22.31096E, 800 m, 2017-05-09, leg. L. Borowiec; • 7w. (EtOH): Mt. Ossa, 2.4 km SE of Karitsa, stream valley in deciduous forest, 39.82632N/22.77557E, 425 m, 2017-05-04, leg. L. Borowiec; • 12w. (EtOH): Mt. Ossa, 600 m SE of Karitsa, stream valley in deciduous forest, 39.84009N/22.76983E, 300 m, 2017-05-04, leg. L. Borowiec; • 3w. (pin) (CASENT0846789–CASENT0846791), 44w. (EtOH): Mt. Ossa, Kokkino Nero, stream valley in urban area, 39.83389N/22.79379E, 3 m, 2017-05-10, leg. L. Borowiec. **CROATIA, Podgorje:** • 4w. (pin) (CASENT0846792–

CASENT0846795), vicinity Sibenj, 9 km S Senj, 0–50 m, 3.6.1997, A. Schulz & K. Vock leg.

Locus typicus. Peloponnese, Taygetos Mts.

Differential diagnosis. *Temnothorax brackoi* is most similar to *T. aveli* (Bondroit, 1918), a species widely distributed in the western part of the Mediterranean basin, east to Slovenia. It is similar to *T. aveli* especially in body shape, petiolar structure and unicolour yellow antennae. *Temnothorax aveli* differs in microreticulation of head not as regular as in *T. brackoi*, with slightly shiny surface between sculpture and often with presence of median stripe of diffused microsculpture on frons, while in *T. brackoi* head sculpture is always homogenous, without areas of diffused microreticulation and dull surface between sculpture. From Greek members of *Temnothorax*, *T. brackoi* is most similar to sympatric *T. graecus*, but differs from it in well-developed sculpture on the whole head surface (in *T. graecus* central part of frons is more or less shiny, without microreticulation) and larger, distinctly triangular propodeal spines (in *T. graecus* spines are short, needle-shaped); some specimens of *T. brackoi* can also be confused with species belonging to the *T. tuberum* group, but *T. brackoi* differs from them in low and arched petiolar node and lack of longitudinal striation on frons.

Description. Worker (n = 10): HL: 0.637 ± 0.04 (0.584–0.683); HW: 0.547 ± 0.03 (0.509–0.696); SL: 0.437 ± 0.02 (0.410–0.560); EL: 0.148 ± 0.01 (0.124–0.174); EW: 0.105 ± 0.01 (0.087–0.124); WL: 0.735 ± 0.05 (0.671–0.820); PSL: 0.155 ± 0.015 (0.130–0.174); SDL: 0.111 ± 0.01 (0.096–0.124); PEL: 0.271 ± 0.03 (0.224–0.311); PPL: 0.168 ± 0.01 (0.149–0.186); PEH: 0.208 ± 0.015 (0.186–0.236); PPH: 0.207 ± 0.015 (0.180–0.224); PNW: 0.368 ± 0.02 (0.329–0.401); PLW: 0.170 ± 0.01 (0.149–0.199); PPW: 0.223 ± 0.02 (0.196–0.242); CI: 85.9 ± 1.2 (83.1–87.5); SI1: 68.6 ± 1.6 (66.7–71.3); SI2: 79.9 ± 1.6 (77.1–81.9); MI: 50.0 ± 1.0 (48.4–51.3); EI1: 71.0 ± 5.0 (64.6–78.3); EI2: 16.5 ± 1.4 (14.8–18.75); PI: 130.0 ± 7.9 (120.0–145.5); PPI: 81.4 ± 6.1 (128.2–150.0); PSI: 135.8 ± 12.4 (120.0–155.6).

Colour. Head, antennae, mesosoma, petiole, postpetiole and legs uniformly yellow to dark yellow, in few specimens, frons and femora partly darkened. Gaster yellow, only the first gaster tergite with wide, dark band on its posterior part (Figs 1, 2).
Head. Rectangular, 1.16 times as long as wide, lateral surfaces below and above eyes gently convex, posterior edges convex, occipital margin of head straight or slightly concave (Figs 9, 13). Anterior margin of clypeus slightly convex, medial notch absent. Eyes moderate, oval, 1.4 times as long as wide. Antennal scape short, in lateral view slightly curved, 0.69 times as long as length of the head, in apex gradually widened, its base with small, triangular tooth, funiculus long, club 3-segmented (Fig. 9). Surface of scape with very fine microreticulation, shiny, covered with thin, moderate dense, decumbent to suberect setae. Mandibles rounded with thick sparse, longitudinal striae, shiny. Clypeus shiny with thick, sparse, longitudinal striae, area between striae smooth and shiny. Frontal carinae short, not extending beyond frontal lobes. Antennal fossa deep, with sparse, thin, roundly curved striae and dense reticulation. Frontal lobes narrow, smooth with slight, dense longitudinal striation (Fig. 13). Frons,

Figures 1, 2. Worker of *Temnothorax brackoi* sp. nov. **1** Dorsal **2** Lateral.

vertex and temples with dense, thick, reticulation, sometimes central surface of frons with additional a few thin, longitudinal, interrupted wrinkles, surface between striation smooth; malar area with irregular, thick, reticulation, space between reticulation smooth or with very sparse microreticulation, shiny; genae with sparser, thank of frons, and thick reticulation, shiny. Frons and vertex with erect, pale, short and thick setae (Fig. 13). **Mesosoma.** Elongate, approximately twice as long as wide, slightly arched in profile. Metanotal groove absent. Pronotum convex on sides. Propodeal spines short to moderate, triangular, directed upward, base wide, tips sharp (Fig. 2). Whole surface with dense, reticulation, its dorsal surface and lateral surfaces of pronotum with thick

Figures 3, 4. Worker of *Temnothorax messiniaensis* sp. nov. **3** Dorsal **4** Lateral.

and sparse longitudinal striation or reticulation. Area between thick sculpture shiny, smooth or sometimes with sparse, fait microreticulation. Entire mesosoma bearing erect, pale, short and thick setae (Fig. 2). **Petiole.** In lateral view, with short peduncle, node low, with anterior face flat, and posterior face convex and dorsum convex. Peduncle and petiolar node shiny, with thick, dense reticulation, area between rugae smooth, dorsum with sparser reticulation. Dorsal surface bearing sparse, short, erect setae (Fig. 2). **Postpetiole.** In lateral view, regularly convex, apical half with gently convex sides (Figs 1, 2), on the whole surface shiny, with thick, dense reticulation, dorsum with sparser reticulation; area between rugae smooth. Dorsal surface bearing sparse, short, semierect to erect setae (Fig. 2). **Gaster.** Gaster smooth and shiny, bearing erect, thin, pale setae (Figs 1, 2).

Etymology. Named after Gregor Bračko, a Slovenian myrmecologist, for his significant contributions to the studies on ants of the Balkan Peninsula.

General distribution. Greece: Epirus, Ionian Islands, Peloponnese, western Sterea Ellas, Thessaly; Croatia: Dalmatia.

Biology. Specimens of *T. brackoi* were collected from various localities: sunny and shadowy located on lowlands and highlands (9 - 990 m a.s.l). The species was noted in various habitats, most often on shrubs and trees growing along roadsides and olive plantations, maquis, phrygana, often inside forests (mostly deciduous, occasionally coniferous). We noted also its presence on the leaves of climbing plants growing on walls or trees. Nests were not found, probably like other species of this group, are located inside dry stems of herbs.

The following ant species were recorded in the same areas as *T. brackoi*:

Epirus, Preveza, 700 m E of Kanali: *Aphaenogaster balcanica* (Emery), *A. subterranea* (Latreille), *Camponotus dalmaticus* (Nylander), *C. fallax* (Nylander), *C. lateralis* (Olivier), *C. piceus* (Leach), *C. vagus* (Scopoli), *Cataglyphis nodus* (Brullé), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Lasius lasioides* (Emery), *Messor wasmanni* Krausse, *Myrmecina graminicola* (Latreille), *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. rogeri* Emery; **near Kanali, oak forest:** *Aphaenogaster balcanica* (Emery), *A. subterranea* (Latreille), *Camponotus dalmaticus* (Nylander), *C. fallax* (Nylander), *C. lateralis* (Olivier), *Cataglyphis nodus* (Brullé), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *Lasius lasioides* (Emery), *Pheidole pallidula* (Nylander); **near Kanali, pine forest:** *Temnothorax bulgaricus* (Forel).

Ionian Islands, Cephalonia, Avithos Lake: *Aphaenogaster muelleriana* Wolf, *Camponotus dalmaticus* (Nylander), *C. gestroi* Emery, *C. ionius* Emery, *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota frauenfeldi* (Mayr), *Liometopum microcephalum* (Panzer), *Messor wasmanni* Krausse, *Monomorium monomorium* Bolton, *Myrmecina graminicola* (Latreille), *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Tapinoma festae* Emery, *Temnothorax bulgaricus* (Forel), *T. exilis* (Emery), *T. laconicus* Csősz et al., *Tetramorium kephalosi* Salata & Borowiec; **Katapodata:** *Camponotus dalmaticus* (Nylander), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Plagiolepis pygmaea* (Latreille); **1.5 km NE of Koulourata:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. leviceps* (Emery). *T. messiniaensis* sp. nov.; **ancient Same:** *Camponotus kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Colobopsis truncata* (Spinola), *Crematogaster ionia* Forel, *C. sordidula* (Nylander), *Pheidole balcanica* Seifert, *Temnothorax bulgaricus*

(Forel), *T. messiniaensis* sp. nov.; **road Skala-Poros:** *Camponotus aethiops* (Latreille), *C. gestroi* Emery; *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Temnothorax messiniaensis* sp. nov.

Ionian Islands, Corfu, Ag. Ilias: *Aphaenogaster subterranea* (Latreille), *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *Temnothorax laconicus* Csősz et al.; **Akr. Kefali near Agios Stefanos:** *Aphaenogaster balcanica* (Emery), *Bothriomyrmex communista* Santschi, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. lateralis* (Olivier), *C. piceus* (Leach), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lasius illyricus* Zimmermann, *Lepisiota melas* (Emery), *L. nigra* (Dalla Torre), *Messor wasmanni* Krausse, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Temnothorax laconicus* Csősz et al.; **E of Nymfes:** *Aphaenogaster muelleriana* Wolf, *A. subterranea* (Latreille), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. lateralis* (Olivier), *Crematogaster sordidula* (Nylander), *Dolichoderus quadripunctatus* (Linnaeus), *Formica gagates* Latreille, *Lasius bombycina* Seifert & Galkowski, *L. illyricus* Zimmermann, *Lepisiota frauenfeldi* (Mayr), *Temnothorax laconicus* Csősz et al.; **Klimatia:** *Aphaenogaster balcanica* (Emery), *A. muelleriana* Wolf, *A. subterranea* (Latreille), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. gestroi* Emery, *C. lateralis* (Olivier), *C. heidrunvogtae* Seifert, *C. piceus* (Leach), *Cataglyphis nodus* (Brullé), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Dolichoderus quadripunctatus* (Linnaeus), *Formica gagates* Latreille, *Lasius bombycina* Seifert & Galkowski, *L. illyricus* Zimmermann, *L. lasioides* (Emery), *Lepisiota frauenfeldi* (Mayr), *L. nigra* (Dalla Torre), *Messor ibericus* Santschi, *M. wasmanni* Krausse, *Myrmecina graminicola* (Latreille), *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *P. xene* Stärcke, *Prenolepis nitens* (Mayr), *Temnothorax* cf. *exilis*, *T. laconicus* Csősz et al., *T. rogeri* Emery, *Tetramorium kephalosi* Salata & Borowiec.; **N of Ag. Stefanos:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. fallax* (Nylander), *C. lateralis* (Olivier), *C. piceus* (Leach), *Crematogaster schmidti* (Mayr), *Lasius bombycina* Seifert & Galkowski, *L. illyricus* Zimmermann, *Lepisiota frauenfeldi* (Mayr), *Messor ibericus* Santschi, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Solenopsis* cf. *fugax*, *Temnothorax clypeatus* (Mayr); **near Doukades:** *Camponotus lateralis* (Olivier), *C. piceus* (Leach), *Lasius lasioides* (Emery); **near Vistonas:** *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. piceus* (Leach), *Crematogaster sordidula* (Nylander), *Lepisiota melas* (Emery), *Messor ibericus* Santschi, *M. wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *exilis*, *T. rogeri* Emery, *Tetramorium kephalosi* Salata & Borowiec; **Nymfes:** *Aphaenogaster* cf. *subterranea*, *Temnothorax laconicus* Csősz et al., *T.* cf. *tuberum*, *Tetramorium* cf. *caespitum*; **Corfu, Old Perithia:** *Aphaenogaster balcanica* (Emery), *A. epirotes* (Emery), *A. muelleriana* Wolf, *Bothriomyrmex communista* Santschi, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. gestroi* Emery, *C. lateralis* (Olivier), *C. piceus* (Leach), *Crematogaster schmidti* (Mayr), *Lasius illyricus* Zimmermann, *L. lasioides* (Emery), *Lepisiota frauenfeldi* (Mayr), *L. melas* (Emery), *Messor ibericus* Santschi, *M. wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Tapinoma erraticum* (Latreille), *Temnothorax affinis* (Mayr), *T.* cf. *exilis*, *T. laconicus* Csősz et al., *T.* cf. *nigriceps*, *T. rogeri* Emery, *Tetramorium* cf. *caespitum*, *T. kephalosi* Salata & Borowiec; **Strinilas:** *Aphaenogaster balcanica* (Emery), *A. epirotes* (Emery), *Bothriomyrmex communista* Santschi, *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *Formica gagates* Latreille, *Lasius lasioides* (Emery), *Plagiolepis perperamus* Salata et al., *P. pygmaea* (Latreille), *Ponera coarctata* (Latreille), *Solenopsis* cf. *fugax*, *Tapinoma erraticum* (Latreille), *T. laconicus* Csősz et al., *Tetramorium kephalosi* Salata & Borowiec, *T.* cf. *punctatum*;

Ionian Islands, Lefkada, Asprogerakata: *Aphaenogaster balcanica* (Emery), *A. muelleriana* Wolf, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Pheidole* cf. *pallidula*, *Prenolepis nitens* (Mayr), *Tetramorium kephalosi* Salata & Borowiec; **Egklouvi:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Temnothorax lichtensteini* (Bondroit), *T. rogeri* Emery;

Ionian Islands, Zakynthos, 1 km E of Elies: *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *Messor wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *tergestinus*; **1 km N of Exo Chora:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota frauenfeldi* (Mayr), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. rogeri* Emery, *T.* cf. *tergestinus*; **1.2 km N of Ano Vasilikos:** *Temnothorax exilis* (Emery); **1.2 km N of Vasilikos:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota frauenfeldi* (Mayr), *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *T. graecus* (Forel), *T. rogeri* Emery, *T.* cf. *tergestinus*; **1.2 km NE of Anafonitria:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *T. muellerianus* (Finzi), *T.* cf. *tergestinus*; **1.2 km SW of Ano Vasilikos loc. 1:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota frauenfeldi* (Mayr), *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery); **1.2 km SW of Ano Vasilikos loc. 2:** *Aphaenogaster balcanica* (Emery), *Aphaenogaster* cf. *epirotes*, *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *C. oertzeni* Forel, *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota melas* (Emery), *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery); **1.2 km SW of Skinaria:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. gestroi* Emery, *C. ionius* Emery, *C. kiesenwetteri* (Roger), *C. oertzeni* Forel, *Crematogaster sordidula* (Nylander), *Lepisiota melas* (Emery), *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *T. rog-*

eri Emery, *T.* cf. *tergestinus*, *Tetramorium diomedeum* Emery, *T. kephalosi* Salata & Borowiec; **1.4 km E of Ano Volimes:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *A. muelleriana* Wolf, *Camponotus dalmaticus* (Nylander), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Temnothorax bulgaricus* (Forel), *T.* cf. *tergestinus*, *Tetramorium kephalosi* Salata & Borowiec; **1.5 km N of Exo Chora:** *Aphaenogaster* cf. *epirotes*, *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Messor wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T.* cf. *tergestinus*, *Tetramorium kephalosi* Salata & Borowiec; **1.7 km NE of Ano Volimes:** *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. gestroi* Emery, *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Pheidole* cf. *pallidula*, *Temnothorax* cf. *tergestinus*; **1.8 km SW of Volimes:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *tergestinus*; **1.9 km W of Maries:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *Crematogaster sordidula* (Nylander), *Lepisiota melas* (Emery), *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *tergestinus*; **2.5 km NE of Maries:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *A. subterraneoides* Emery, *Bothriomyrmex communista* Santschi, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax rogeri* Emery, *Tetramorium kephalosi* Salata & Borowiec; **3.9 km NE of Maries:** *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *Crematogaster schmidti* (Mayr), *Prenolepis nitens* (Mayr), *Temnothorax* cf. *tergestinus*, *Tetramorium diomedeum* Mayr; **470 m NE of Orthonies:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota melas* (Emery), *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. exilis* (Emery), *Temnothorax* cf. *tergestinus*; **500 m S of Apelati:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *Crematogaster ionia* Forel, *C. schmidti* (Mayr), *Messor wasmanni* Krausse, *Myrmecina graminicola* (Latreille), *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *tergestinus*; **700 m SW of Koroni:** *Camponotus aethiops* (Latreille), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Lepisiota melas* (Emery), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *Temnothorax* cf. *tergestinus*; **750 m S of Volimes:** *Camponotus dalmaticus* (Nylander), *C. gestroi* Emery, *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Messor wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. rogeri* Emery, *T.* cf. *tergestinus*; **800 m SE of Xirokastello:** *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *C. oertzeni* Forel, *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *Lepisiota melas* (Emery), *Messor wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *T.* cf. *tergestinus*, *Tetramorium* cf. *punctatum*; **880 m S of Orthonies:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *L. melas* (Emery), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. rogeri* Emery, *T.* cf. *tergestinus*; **Ag. Georgiou monastery:** *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *Crematogaster schmidti* (Mayr), *Plagiolepis pygmaea* (Latreille); **Ag. Joannis:** *Camponotus aethiops* (Latreille), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Pheidole* cf. *pallidula*, *Solenopsis* cf. *lusitanica*, *Temnothorax graecus* (Forel), *T. rogeri* Emery, *T.* cf. *tergestinus*, *Tetramorium kephalosi* Salata & Borowiec; **Argassi:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. oertzeni* Forel, *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lasius alienus* (Foerster), *Lepisiota melas* (Emery), *Messor ibericus* Santschi, *Monomorium monomorium* Bolton, *Nylanderia jaegerskioeldi* (Mayr), *Pheidole* cf. *pallidula*, *Plagiolepis perperamus* Salata et al., *P. pygmaea* (Latreille), *Tapinoma erraticum* (Latreille), *Temnothorax rogeri* Emery, *Tetramorium immigrans* Santschi, *T.* cf. *punctatum*; **Livia Mts. loc. 1:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *Bothriomyrmex communista* Santschi, *Camponotus aethiops* (Latreille), *C. kiesenwetteri* (Roger), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Messor wasmanni* Krausse, *Prenolepis nitens* (Mayr), *Tetramorium* cf. *punctatum*; **Vrachionas Mts.:** *Aphaenogaster balcanica* (Emery), *Bothriomyrmex communista* Santschi, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *Messor wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Temnothorax* cf. *tergestinus*, *Tetramorium* cf. *punctatum*; **s, W of Kampi:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *A. muelleriana* Wolf, *Camponotus aethiops* (Latreille), *Crematogaster schmidti* (Mayr), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Solenopsis* cf. *lusitanica*, *Temnothorax rogeri* Emery.

Macedonia, Pieria, Platamonas Castle hill: *Aphaenogaster epirotes* (Emery), *A. muelleriana* Wolf, *A.* cf. *subterranea*, *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *C. piceus* (Leach), *Crematogaster ionia* Forel, *C. sordidula* (Nylander), *Lasius emarginatus* (Olivier), *L. turcicus* (Santschi), *Messor hellenius* Agosti & Collingwood, *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Temnothorax lichtensteini* (Bondroit); **road to P. Poroi loc. 1:** *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. ionius* Emery, *C. piceus* (Leach), *Chalepoxenus muellerianus* (Finzi), *Crematogaster ionia* Forel, *Dolichoderus quadripunctatus* (Linnaeus), *Formica gagates* Latreille, *Lasius emarginatus* (Olivier), *Messor hellenius* Agosti & Collingwood, *Plagiolepis pygmaea* (Latreille), *Tapinoma erraticum* (Latreille), *Temnothorax* cf. *exilis*, *T. graecus* (Forel), *T. turcicus* (Santschi); **road to P. Poroi loc. 2:** *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. piceus* (Leach), *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus); *Lasius emarginatus* (Olivier), *Lepisiota frauenfeldi* (Mayr), *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Tapinoma erraticum* (Latreille), *Temnothorax graecus* (Forel), *T. turcicus* (Santschi), *Tetramorium kephalosi* Salata & Borowiec; **road to P. Poroi loc. 3:** *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *C. vagus* (Scopoli), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Lasius emarginatus* (Olivier), *Messor hellenius* Agosti & Collingwood, *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Temnothorax bulgaricus* (Forel), *T. morea* Csősz, Salata & Borowiec, *T. turcicus* (Santschi).

Peloponnese, Korinthia, near Evrostina: *Aphaenogaster subterranea* (Latreille), *Camponotus lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Lasius emarginatus* (Olivier), *L. turcicus* (Santschi), *Ponera coarctata* (Latreille), *Temnothorax laconicus* Csősz et al.

Peloponnese, Laconia, Taygetos Mts., 1.5 km SW of Anavryti: *Aphaenogaster balcanica* (Emery), *A. subterranea* (Latreille), *Camponotus dalmaticus* (Nylander), *C. oertzeni* Forel, *C. piceus* (Leach), *Cataglyphis nodus* (Brullé), *Crematogaster ionia* Forel, *Temnothorax laconicus* Csősz et al.

Peloponnese, Messinia, Taygetos Mts., 0.7 km S of Dyrrachio: *Camponotus lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Lasius flavus* (Fabricius), *L. illyricus* Zimmermann, *Temnothorax helenae* Csősz et al.; **Taygetos Mts., 0.8 km SE of Exochori:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *Crematogaster ionia* Forel, *Lepisiota frauenfeldi* (Mayr), *Messor structor* (Latreille), *M. wasmanni* Krausse, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *bulgaricus*, *T. exilis* (Emery), *T. helenae* Csősz et al., *T. recedens* (Nylander), *Tetramorium kephalosi* Salata & Borowiec; **Taygetos Mts., 1.3 km S of Artemisia:** *Aphaenogaster subterranea* (Latreille), *Camponotus boghossiani* Forel, *Crematogaster schmidti* (Mayr), *Lasius* cf. *alienus*, *L. illyricus* Zimmermann, *Myrmecina graminicola* (Latreille), *Plagiolepis pygmaea* (Latreille), *Temnothorax crasecundus* Seifert & Csősz, *T. helenae* Csősz et al.; **Taygetos Mts., 1.8 km E of Saidona:** *Lasius illyricus* Zimmermann, *Plagiolepis pygmaea* (Latreille), *Temnothorax crasecundus* Seifert & Csősz, *Tetramorium* cf. *caespitum*; **Taygetos Mts., 2 km W of Arachova:** *Aphaenogaster* cf. *muelleriana*, *Bothriomyrmex communista* Santschi, *Camponotus boghossiani* Forel, *C. dalmaticus* (Nylander), *C. laconicus* Emery, *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Messor wasmanni* Krausse, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Stigmatomma denticulatum* Roger, *Tapinoma erraticum* (Latreille), *Temnothorax* cf. *bulgaricus*, *T. laconicus* Csősz et al., *T.* cf. *luteus*, *T. rogeri* Emery, *Tetramorium kephalosi* Salata & Borowiec, *Tetramorium* cf. *punctatum*; **Taygetos Mts., Arachova:** *Camponotus aethiops* (Latreille), *C. laconicus* Emery, *C. piceus* (Leach), *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Lasius* cf. *alienus*, *L. illyricus* Zimmermann, *Lepisiota frauenfeldi* (Mayr), *Messor wasmanni* Krausse, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Tapinoma erraticum* (Latreille), *Temnothorax* cf. *bulgaricus*, *T. parvulus* (Schenck); **Taygetos Mts., Chora Getson:** *Aphaenogaster balcanica* (Emery), *A.* cf. *muelleriana*, *Camponotus boghossiani* Forel, *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Lasius* cf. *alienus*, *L. illyricus* Zimmermann, *Lepisiota frauenfeldi* (Mayr), *Pheidole pallidula* (Nylander), *Plagiolepis* *pygmaea* (Latreille), *Temnothorax* cf. *bulgaricus*, *T. laconicus* Csősz et al., *T. recedens* (Nylander), *T. strymonensis* Csősz, Salata & Borowiec; **Taygetos Mts., Karveli:** *Aphaenogaster* cf. *muelleriana*, *Crematogaster schmidti* (Mayr), *Lasius* cf. *alienus*, *L. illyricus* Zimmermann, *Myrmecina graminicola* (Latreille), *Temnothorax helenae* Csősz et al., *T. strymonensis* Csősz, Salata & Borowiec; **Tetrazi Mts., 0.5 km E of Vastas:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. fallax* (Nylander), *C. laconicus* Csősz et al., *C. piceus* (Leach), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *Formica gagates* Latreille, *Lasius illyricus* Zimmermann, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax helenae* Csősz et al., *T. laconicus* Csősz et al., *T. morea* Csősz, Salata & Borowiec, *T. rogeri* Emery; **Tetrazi Mts., Isaris:** *Aphaenogaster balcanica* (Emery), *A. subterranea* (Latreille), *Camponotus dalmaticus* (Nylander), *C. laconicus* Csősz et al., *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Lasius* cf. *alienus*, *L. illyricus* Zimmermann, *Lepisiota frauenfeldi* (Mayr), *Temnothorax* cf. *bulgaricus*, *T. helenae* Csősz et al.,; **Tetrazi Mts., Karnasi:** *Aphaenogaster balcanica* (Emery), *A.* cf. *muelleriana*, *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Lasius illyricus* Zimmermann, *Messor hellenius* Agosti & Collingwood, *Pheidole pallidula* (Nylander), *Temnothorax* cf. *bulgaricus*, *Temnothorax laconicus* Csősz et al., *T. morea* Csősz, Salata & Borowiec, *T. rogeri* Emery, *Tetramorium kephalosi* Salata & Borowiec.

Sterea Ellas, Aetolia-Acarnania, Psila Alonia: *Aphaenogaster balcanica* (Emery), *A.* cf. *muelleriana*, *A. subterranea* (Latreille), *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Lasius illyricus* Zimmermann, *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Solenopsis* cf. *lusitanica*, *Temnothorax crassispinus* (Karavaiev).

Sterea Ellas, Euboea, 1.2 km NW of Gerontas: *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. gestroi* Emery, *C. kiesenwetteri* (Roger), *C. laconicus* Emery, *C. lateralis* (Olivier), *C. piceus* (Leach), *Cataglyphis aenescens* (Nylander), *Crematogaster ionia* Forel, *C. schmidti* (Mayr), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Tapinoma erraticum* (Latreille), *Temnothorax graecus* (Forel), *T. recedens* (Nylander); **2.3 km S of Stropones:** *Camponotus aethiops* (Latreille), *C. piceus* (Leach), *C. vagus* (Scopoli), *Cataglyphis nodus* (Brullé), *Formica fusca* Karavaiev, *Lasius alienus* Förster, *L. flavus* (Fabricius), *Pheidole pallidula* (Nylander), *Temnothorax crasecundus* Seifert & Csősz, *T. helenae* Csősz et al., *T. unifasciatus* (Latreille); **300 m NW of Agios:** *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *Cataglyphis nodus* (Brullé), *Crematogaster ionia* Forel, *Dolichoderus quadripunctatus* (Linnaeus), *Formica fusca* Karavaiev, *Lasius lasioides* (Emery), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax lichtensteini* (Bondroit), *T. parvulus* (Schenck), *T. recedens* (Nylander), *T. unifasciatus* (Latreille); **570 m NW of Drosia:** *Aphaenogaster balcanica* (Emery), *A. subterranea* (Latreille), *Camponotus dalmaticus* (Nylander), *C. ionius* Emery, *C. lateralis* (Olivier), *C. piceus* (Leach), *C. vagus* (Scopoli), *Cataglyphis nodus* (Brullé), *Crematogaster ionia* Forel, *C.*

schmidti (Mayr), *Lepisiota frauenfeldi* (Mayr), *Messor hellenius* Agosti & Collingwood, *Temnothorax bulgaricus* (Forel).

Thessaly, Larissa, Mt. Olympus, 5.3 km E of Olympiada: *Aphaenogaster subterranea* (Latreille), *Camponotus aethiops* (Latreille), *C. lateralis* (Olivier), *C. piceus* (Leach), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Formica cunicularia* Latreille, *F. gagates* Latreille, *Lasius alienus* Förster, *L. emarginatus* (Olivier), *Messor hellenius* Agosti & Collingwood, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Solenopsis* cf. *lusitanica*, *Tapinoma erraticum* (Latreille), *Temnothorax recedens* (Nylander), *Tetramorium kephalosi* Salata & Borowiec, *Tetramorium moravicum* Kratochvil; **Mt. Ossa, 2.4 km SE of Karitsa:** *Aphaenogaster subterranea* (Latreille), *Camponotus lateralis* (Olivier), *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Formica gagates* Latreille, *Lasius emarginatus* (Olivier), *Myrmoxenus gordiagini* Ruzsky, *Plagiolepis pygmaea* (Latreille), *Temnothorax crasecundus* Seifert & Csősz, *T.* cf. *subtilis-helenae*, *T. tauricus* (Ruzsky), *T.* cf. *unifasciatus*, *Tetramorium moravicum* Kratochvil; **Mt. Ossa, 600 m SE of Karitsa:** *Aphaenogaster subterranea* (Latreille), *Camponotus fallax* (Nylander), *Colobopsis truncata* (Spinola), *Lasius emarginatus* (Olivier), *Plagiolepis pygmaea* (Latreille), *Prenolepis nitens* (Mayr), *Temnothorax crasecundus* Seifert & Csősz, *T. lichtensteini* (Bondroit), *T.* cf. *subtilis-helenae*, *T.* cf. *unifasciatus*; **Mt. Ossa, Kokkino Nero:** *Aphaenogaster subterranea* (Latreille), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. lateralis* (Olivier), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *Dolichoderus quadripunctatus* (Linnaeus), *Formica cunicularia* Latreille, *Lasius alienus* Förster, *L. emarginatus* (Olivier), *Messor hellenius* Agosti & Collingwood, *Pheidole pallidula* (Nylander), *Prenolepis nitens* (Mayr), *Temnothorax tauricus* (Ruzsky), *T.* cf. *unifasciatus*, *Tetramorium* cf. *caespitum*.

***Temnothorax messiniaensis* sp. nov.**

http://zoobank.org/0C110D03-C293-4AD1-B179-3A1F53E7491E
Figs 3–6, 10, 12, 14

Type material. Holotype, worker (pin) (CASENT0846796): GREECE, Pel., Messinia | 2 km E of Kalamata, 65 m, | 37.01863N / 22.15626E | 12 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR01997 (MNHW).

Paratypes: • 3Q., 7w. (pin) (CASENT0846639–CASENT0846648): the same nest sample as holotype (DBET, BMNH, CASC, MHNG); • 1w. (pin) (CASENT0846649): GREECE, Pel., Messinia | 1.4 km S of Flesiada, 700 m | 37.08964N, 21.76581E | 16 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02091 (DBET); • 1w. (pin) (CASENT0846650): GREECE, Pel., Messinia | Kalamata, old centre, 60 m | 37.04617N, 22.11691E| 11 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02216 (DBET); • 2w. (pin) (CASENT0846651–CASENT0846652): GREECE, Pel., Messinia | Kalamata, railway park, 8m | 37.03157N, 22.11004E | 11 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR01989

Figures 5, 6. Gyne of *Temnothorax messiniaensis* sp. nov. **5** Dorsal **6** Lateral.

(DBET); • 2w. (pin) (CASENT0846653–CASENT0846654): GREECE, Pel., Messinia | 0.8 km N of Koromilea, 485 m | 37.16272N, 21.84809E| 16 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02103 (DBET); • 1w. (pin) (CASENT0846655): GREECE, Pel., Messinia | 0.8 km SE of Exochori, 535 m | 36.89582N, 22.27464E | 20 VI 2016, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02658 (DBET); • 1w. (pin) (CASENT0846656): GREECE, Pel., Messinia | 0.8 km W of Eleochori, 481 m 37.03838N, 22.17227E | 13 VI 2016, lL. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02005 (DBET).

Other material. Greece. Ionian Islands, Cephalonia: • 7w. (EtOH): Avithos Lake, shrubs around small lake, 38.17203N/ 20.71107E, 288 m, 2019-06-10, leg. L. Borowiec; • 3w. (pin), 9w. (EtOH): 1.6 km SW of Digaleto, small gorge with oaks, 38.16558N, 20.67099E, 564 m, 2019-06-11, leg. L. Borowiec; • 1w. (EtOH): 1.8 km SW of Digaleto, pastures with oaks, 38.16593N, 20.66788E, 580 m, 2019-

06-11, leg. L. Borowiec; • 7w. (EtOH): Kapandriti vicinity, roadsides with herbs, 38.12913N/20.72447E, 320 m, 2019-06-09, leg. L. Borowiec; • 12w. (EtOH): Katapodata; roadsides with shrubs, 38.23337N/20.64594E, 100 m, 2019-06-10, leg. L. Borowiec; • 1w. (pin) (CASENT0846797), 43w. (EtOH): 800 m S of Kateleios, roadsides with bushes, 38.07066N/20.75329E, 20 m, 2019-06-09, leg. L. Borowiec; • 5w. (EtOH): 1.5 km NE of Koulourata, mixed forest on shrubs, 38.20667N/ 20.67715E, 273 m, 2019-06-10, leg. L. Borowiec; • 48w. (EtOH): Kremmidi, roadsides with bushes, 38.09048N/20,74471E, 285 m, 2019-06-09, leg. L. Borowiec; • 7w. (EtOH): Moni Aprilion, hill with oak forest and rocks, 38.26221N/20.66651E, 220 m, 2019-06-10, leg. L. Borowiec; • 48w. (EtOH): 1 km NW of Pastra, roadsides with bushes, 38.10058N/20.7421E, 300 m, 2019-06-09, leg. L. Borowiec; • 4w. (EtOH): 1.7 km NW of Pastra, pastures, on shrubs, 38.1084N/20.74085E, 300 m, 2019-06-09, leg. L. Borowiec; • 6w. (pin) (CASENT0846798–CASENT0846803): n. Peratata, pine forest on a rocky hill, 38.14058N/20.55038E, 211 m, 2014-06-24, leg. L. Borowiec; • 11w. (EtOH): ancient Same; roadsides with shrubs, 38.2522N/20.66423E, 220 m, 2019-06-10, leg. L. Borowiec; • 3w. (pin) (CASENT0846804–CASENT0846806), 6w. (EtOH): Skala vicinity loc. 1, small gorge with mediterranean shrubs, 38.08178N/20.79275E, 40 m, 2019-06-07, leg. L. Borowiec; • 5w. (pin) (CASENT0846807–CASENT0846811), 44w, 1q (EtOH): Skala vicinity loc. 2, small gorge with mediterranean shrubs, nest inside dry branch of shrub, 38.08221N, 20.79504E, 34 m, 2019-06-07, leg. L. Borowiec; • 61w. (EtOH): rd. Skala-Poros; mediterranean shrubs, 38.12872N/20.79576E, 5 m, 2019-06-12, leg. L. Borowiec. **Ionian Islands, Zakynthos:** • 3w. (pin) (CASENT0846812–CASENT0846814), 3w. (EtOH): 1 km N of Exo Chora, mixed forest, 37.81063N/20.68459E, 430 m, 2018-05-08, leg. L. Borowiec; • 1w. (pin) (CASENT0846815): 1.2 km N of Vasilikos, roadsides along olive plantation and pasture with oak shrubs, 37.72456N/ 20.97786E, 30 m, 2018-05-05, leg. L. Borowiec; • 5w. (pin) (CASENT0846816–CASENT0846820), 1w. (EtOH): 1.2 km NE of Anafonitria, shrubs around burned forests, 37.85489N/20.64124E, 475 m, 2018-05-10, leg. L. Borowiec; • 1w. (pin) (CASENT0846821): 1.2 km SE of Loucha, roadsides in cypress forest, 37.78617N/ 20.73706E, 445 m, 2018-05-09, leg. L. Borowiec; • 5w. (pin), 3w. (EtOH): 1.2 km SW of Skinaria, limestone hills after burned forests, 37.87694N/20.69272E, 375 m, 2018-05-06, leg. L. Borowiec; • 2w. (pin) (CASENT0846822–CASENT0846823): 1.4 km S of Lithakia, shrubs along olive plantation, 37.70641N/20.82342E, 225 m, 2018-05-07, leg. L. Borowiec; • 2w. (pin) (CASENT0846824–CASENT0846825): 1.8 km SW of Volimes, shrubs along roadsides, 37.86472N/20.64234E, 350 m, 2018-05-10, leg. L. Borowiec; • 3w. (pin) (CASENT0846826–CASENT0846827), 40w. (EtOH): 1.9 km W of Maries, roadsides in burned forests, 37.818N/20.65556E, 290 m, 2018-05-09, leg. L. Borowiec; • 3w. (pin) (CASENT0846828–CASENT0846830), 19w. (EtOH): 330 m S of Stimies, shrubs around olive plantation, 37.69009N/20.79988E, 245 m, 2018-05-07, leg. L. Borowiec; • 1w. (pin) (CASENT0846831): 470 m NE of Orthonies, shrubs along roadsides, 37.85435N/ 20.69843E, 405 m, 2018-05-10, leg.

L. Borowiec; 1w. (pin) (CASENT0846832): 580 m SW of Lithakia, shrubs along roadsides, 37.71491N/20.8242E, 225 m, 2018-05-07, leg. L. Borowiec; • 3w. (pin) (CASENT0846833–CASENT0846835), 4w. (EtOH): 600 m E of Ag. Leon, shrubs in pine forest, 37.77045N/20.72959E, 600 m, 2018-05-09, leg. L. Borowiec; • 2w. (pin) (CASENT0846836–CASENT0846837), 3w. (EtOH): 700 m SW of Koroni, maquis, 37.86582N/ 20.71753E, 290 m, 2018-05-10, leg. L. Borowiec; • 2w. (pin) (CASENT0846838–CASENT0846839): 800 m SE of Xirokastello, roadsides along olive plantation, 37.73491N/20.95139E, 75 m, 2018-05-05, leg. L. Borowiec; • 3w. (pin) (CASENT0846840–CASENT0846842), 9w. (EtOH): 880 m S of Orthonies, shrubs in cypress forest, 37.84462N/ 20.69843E, 390 m, 2018-05-10, leg. L. Borowiec; • 3w. (pin) (CASENT0846843–CASENT0846845), 2w. (EtOH): Ag. Georgiou monastery, shrubs along roadsides, 37.85971N/20.63646E, 330 m, 2018-05-10, leg. L. Borowiec; • 2w. (pin) (CASENT0846846–CASENT0846847): Ag. Joannis, roadsides with shrubs, 37.72924N/20.94553E, 165 m, 2018-05-05, leg. L. Borowiec; • 1w. (pin) (CASENT0846848): Vrachionas Mts., mountain pastures with shrubs, 37.81798N/20.70621E, 670 m, 2018-05-08, leg. L. Borowiec; **Peloponnese, Achaia:** • 1w (pin) (CASENT0846849): Kalavrita, 710 m, 38.03342N, 22.10456E, leg. C. Lebas; **Peloponnese, Lakonia:** • 1w (pin) (CASENT0846850): Mistra vicinity, 378 m, 37.08115N, 22.36545E, leg. C. Lebas.

Terra typica. Greece, Peloponnese, Messinia.

Differential diagnosis. Both *T. messiniaensis* sp. nov. and *T. turcicus* (Santschi) are characterised by very long propodeal spines, character strongly distinguishing them from *T. brackoi* sp. nov. They differ from species of the *T. interruptus* group in lack of wide frontal lobes and not distinctly triangular propodeal spines; from *T. affinis* they differ in brighter body colouration and shape of propodeal spines (*T. affinis* has propodeal spines thin and never triangular, while *T. messiniaensis* and *T. turcicus* have propodeal spines more triangular, with wider base); from *T. kemali* both new species differ in absence of distinctly arched dorsum of petiole node and more triangular shape of propodeal spines, additionally *T. messiniaensis* differs from *T. kemali* in not reduced sculpture on frons centre and *T. turcicus* differs from *T. kemali* in very thin dark band on posterior part of first gastral tergite. *T. messiniaensis* differs from *T. turcicus* in not reduced sculpture on frons centre and wider dark band on posterior part of first gastral tergite. From specimens of *T. aveli* with long propodeal spines and almost complete microreticulation of head *T. messiniaensis* differs in less convex mesosoma, longer head, usually darkened gena and propodeal spines directed more upwards.

Description of worker (n = 10): HL: 0.619 ± 0.04 (0.565–0.682); HW: 0.515 ± 0.03 (0.471–0.564); SL: 0.428 ± 0.02 (0.388–0.459); EL: 0.134 ± 0.008 (0.129–0.153); EW: 0.102 ± 0.01 (0.094–0.118); WL: 0.723 ± 0.05 (0.624–0.765); PSL: 0.171 ± 0.02 (0.135–0.188); SDL: 0.109 ± 0.02 (0.082–0.159); PEL: 0.263 ± 0.02 (0.224–0.282); PPL: 0.167 ± 0.007 (0.152–0.176); PEH: 0.207 ± 0.009 (0.188–0.281); PPH: 0.195 ± 0.01 (0.176–0.212); PNW: 0.359 ± 0.03 (0.318–0.400); PLW: 0.162 ± 0.01 (0.141–0.176); PPW: 0.208 ± 0.01 (0.188–0.223); CI: 83.4 ± 2.5 (78.9–86.6); SI1: 69.1 ± 2.3 (65.5–72.5); SI2: 82.9 ± 2.4 (78.6–87.6); MI: 49.7 ±

1.6 (47.6–52.3); EI1: 76.4 ± 6.8 (66.7–90.9); EI2: 16.5 ± 1.1 (14.5–18.2); PI: 127.2 ± 4.5 (118.0–133.3); PPI: 85.9 ± 4.1 (77.7–93.3); PSI: 170.8 ± 16.8 (153.8–200.0).

Colour. Head, antennae, mesosoma, petiole, postpetiole and legs uniformly yellow to ochre, often posterior part of frons, gena and femora partly darkened, occasionally also antennal club slightly darkened. Gaster yellow, only the first gaster tergite with wide, dark band on its posterior part (Figs 3, 4). **Head.** Rectangular, but slightly longer than in both Greek congeners, 1.2 times as long as wide, lateral surfaces below and above eyes gently convex, posterior edges convex, occipital margin of head straight or slightly concave (Figs 10, 14). Anterior margin of the clypeus slightly convex, medial notch absent. Eyes moderate, oval, 1.31 times as long as wide. Antennal scape short, in lateral view slightly curved, 0.69 times as long as length of the head, in apex gradually widened, its base with small, triangular tooth, funiculus long, club 3-segmented (Fig. 10). Surface of scape with very fine microreticulation, shiny, covered with thin, moderate dense, decumbent setae. Mandibles rounded with thick sparse, longitudinal striae, shiny. Clypeus shiny with thick, sparse, longitudinal striae, area between striae smooth and shiny. Frontal carinae short, not extending beyond frontal lobes. Antennal fossa deep, with irregular, dense rugosity and sometimes with a few thin, roundly curved striae. Frontal lobes narrow, smooth with slight, dense longitudinal striation (Fig. 14). Frons, vertex and temples with dense, thick, reticulation, central surface of frons with longitudinal reticulation and sometimes with additional thin, longitudinal striation, striae sometimes interrupted, surface between striation smooth; malar area with irregular, thick, reticulation, space between reticulation smooth or with very sparse microreticulation, shiny; genae with sparser, than on frons, and thick reticulation, shiny. Frons and vertex with erect, pale, short and thick setae (Fig. 14). **Mesosoma.** Elongate, approximately twice as long as wide, slightly arched in profile. Metanotal groove absent or slightly marked as in the species from the *Temnothorax angustulus* group. Pronotum convex on sides. Propodeal spines long, directed upward, with base slightly to moderate wider than apex, tips sharp (Fig. 4). Whole surface with dense, reticulation, sometimes its dorsal surface and lateral surfaces of pronotum and mesonotum with additional thick, sparse to moderate dense longitudinal striation or longitudinal reticulation. Area between thick sculpture shiny, smooth or sometimes with sparse, fine microreticulation (Fig. 4). Entire mesosoma bearing erect, pale, short and thick setae (Figs 3, 4). **Petiole.** In lateral view, with short peduncle, node moderate high, with anterior face flat, and posterior face convex and dorsum flat or slightly convex. Peduncle and petiolar node shiny, with thick, dense reticulation and sometimes thick, sparse longitudinal wrinkles, area between rugae smooth, dorsum with sparser reticulation. Dorsal surface bearing sparse, short, erect setae (Fig. 4). **Postpetiole.** In lateral view, regularly convex, apical half with gently convex sides (Figs 3, 4), on the whole surface shiny, with thick, dense reticulation, dorsum with sparser reticulation; area between rugae smooth. Dorsal surface bearing sparse, short, erect setae (Figs 3, 4). **Gaster.** Gaster smooth and shiny, bearing erect, thin, pale setae (Figs 3, 4).

Description of gyne (n = 3): **Colour.** Head in frontal part yellowish brown to brown, gena and temples yellowish, border between dark and pale parts of head diffused. Antennae uniformly yellow. Pronotum yellow, scutum yellow laterally and yellowish brown anteriorly, without distinct borders between darker and paler parts. Mesosterna brown, propodeum yellowish dorsally and gradually darker ventrally, petiole and postpetiole brown dorsally and yellow ventrally. Legs mostly yellow, mid- and hind femora largely brown centrally. Gastral tergites mostly brown, first tergite with large yellowish spot basally, all tergites with yellowish to yellowish brown posterior margin (Figs 5, 6). **Head.** Eyes big, oval [EL / HW: 0.30]. Antennal scape short [SL / HW: 42], not reaching occipital margin of head. Clypeus shiny with distinct, longitudinally carinulae, interstices smooth. Antennal fossa deep, with concentric carinae, interspaces smooth. Frontal lobes moderately wide 0.4 times as wide as head width, microreticulate with thick longitudinal costae. Frons shiny, entire surface longitudinally costate, interstices on sides distinctly microreticulate, in central part microreticulation diffused and surface appears partly smooth and shiny. Area above eyes and sides of head microreticulate and longitudinally costate, interstices appear slightly rugulose. Entire head bearing suberect to erect, pale and thin setae. **Mesosoma.** Pronotum anteriorly with regular microreticulation and on sides microreticulate with thin longitudinal costae. Scutum with dense, regular, thin longitudinal costae and more or less diffused microreticulation between costae, appears slightly shiny. Scutellum mostly with thin longitudinal costae only marrow median part with diffused sculpture, appears shiny (Fig. 5). Metanotum with slight sculpture, rugulose or punctate. Propodeum with distinct sculpture. Propodeal spines medium length [PSL / HW: 0.33], wide at base, triangular, straight, with acute apex. Area above propodeal spines with transverse, thin costae, dorsal surface of spines with longitudinal costae, sides of propodeum with concentric costae only area close to base of spines distinctly mocroreticulate but without costae, interstices between costae distinctly microreticulate, shiny. Area between propodeal spines on sides with longitudinal costae, centrally with distinct microreticulation, area below spines with transverse costae. Anepisternum and katepisternum with longitudinal costae and microreticulated interspaces, only anterior and posterior corners of anepisternum with small smooth and shiny areas, and katepisternum close to ventral margin with diffused costae. Metaepisternum and metakatepisternum, with dense, longitudinal costae and microreticulate interspaces only metakatepisternum close to ventral margin with diffused microreticulation and partly shiny. Dorsal surface of mesosoma with sparse, erect, long, thick and pale setae (Figs 5, 6). **Petiole and postpetiole.** Shiny anteriorly, dorsal and lateral surface microreticulate with sparse longitudinally costae. **Gaster.** Smooth and shiny, first tergite on whole surface and rest of tergites posteriorly bearing moderately dense, long, erect setae and sparse, short adhering setae (Figs 5, 6).

Etymology. Named after the historical Greek land of Messinia (Μεσσηνία), Peloponnese, where specimens from the type series were collected.

General distribution. Greece: southern Ionian Islands and Peloponnese.

Biology. Specimens of *T. messiniaensis* were collected from sunny localities in lowlands and highlands (8 - 670 m a.s.l). The species was noted in various habitats, most often on shrubs growing along roadsides and olive plantations, maquis, phrygana and forests (cypress, deciduous and mixed). We noted also its presence in a park in a centre of a city and, occasionally, in shrubs located in a pine forest located on a rocky hill. Nests were found inside dry stems of various herbs and shrubs. Colonies polygynous.

The following ant species were recorded in the same areas as *Temnothorax messiniaensis*:

For localities on Ionian Islands: Cephalonia: Avithos Lake, Katapodata, 1.5 NE of Koulourata ancient Same, rd. Skala-Poros, Zakynthos: 1 km N of Exo Chora, 1.2 km N of Vasilikos, 1.2 km NE of Anafonitria, 1.2 km SW of Skinaria, 1.8 km SW of Volimes, 1.9 km W of Maries, 470 m NE of Orthonies, 700 m SW of Koroni, 800 m SE of Xirokastello, 880 m S of Orthonies, Ag. Georgiou monastery, Ag. Joannis, Vrachionas Mts. and Peloponnese, Messinia, Taygetos Mts., 0.8 km SE of Exochori see *Temnothorax brackoi*.

Ionian Islands, Cephalonia, 1.6 km SW of Digaleto: *Aphaenogaster balcanica* (Emery), *A. muelleriana* Wolf, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *Crematogaster schmidti* (Mayr), *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. laconicus* Csősz et al., *T. rogeri* Emery, *T. strymonensis* Csősz, Salata & Borowiec; *Tetramorium kephalosi* Salata & Borowiec; **1.8 km SW of Digaleto:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *Crematogaster sordidula* (Nylander), *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Temnothorax laconicus* Csősz et al., *T. rogeri* Emery; **Kapandriti vicinity:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. gestroi* Forel, *C. kiesenwetteri* (Roger), *Colobopsis truncata* (Spinola), *Crematogaster schmidti* (Mayr), *Plagiolepis pygmaea* (Latreille), *Temnothorax leviceps* (Emery); **800 m S of Kateleios:** *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lasius alienus* (Förster), *L. illyricus* Zimmermann, *Liometopum microcephalum* (Panzer), *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Temnothorax graecus* (Forel), *T. leviceps* (Emery), *T.* cf. *unifasciatus*; **Kremmidi:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. gestroi* Forel, *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Pheidole balcanica* Seifert; *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. graecus* (Forel), *T. leviceps* (Emery); **Moni Aprilion:** *Aphaenogaster balcanica* (Emery), *Camponotus kiesenwetteri* (Roger), *Pheidole balcanica* Seifert, *Plagiolepis pygmaea* (Latreille), *Temnothorax rogeri* Emery, *T. strymonensis* Csősz, Salata & Borowiec; **1.7 km NW of Pastra:** *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *Bothriomyrmex communistus* Santschi, *Camponotus dalmaticus* (Nylander), *C. gestroi* Forel, *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota frauenfeldi* (Mayr), *Messor wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Tetramorium kephalosi* Salata & Borowiec; **near Peratata:** *Aphaenogaster balcanica* (Emery), *A. muelleriana* Wolf, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lasius lasioides* (Emery), *Messor ibericus* Santschi, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax clypeatus* (Mayr); **Skala vicinity loc. 1:** *Aphaenogaster balcanica* (Emery), *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *Colobopsis* *truncata* (Spinola), *Crematogaster sordidula* (Nylander), *Plagiolepis pallescens* Forel, *P. pygmaea* (Latreille), *Proformica oculatissima* (Forel) *Temnothorax* cf. *exilis*, *Tetramorium kephalosi* Salata & Borowiec; **Skala vicinity loc. 2:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lasius alienus* (Förster), *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* Emery, *Tetramorium kephalosi* Salata & Borowiec.

Ionian Islands, Zakynthos, 1.2 km SE of Loucha: *Aphaenogaster balcanica* (Emery), *A.* cf. *epirotes*, *Bothriomyrmex communista* Santschi, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. gestroi* Emery, *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *Messor wasmanni* Krausse, *Tapinoma erraticum* (Latreille), *Temnothorax bulgaricus* (Forel), *T. exilis* (Emery), *T. graecus* (Forel), *T. rogeri* Emery, *T.* cf. *tergestinus*, *Tetramorium diomedeum* Emery; **1.4 km S of Lithakia:** *Aphaenogaster balcanica* (Emery), *Camponotus dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lepisiota frauenfeldi* (Mayr), *L. melas* (Emery), *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax rogeri* Emery, *T.* cf. *tergestinus*; **330 m S of Stimies:** *Aphaenogaster balcanica* (Emery), *Bothriomyrmex communista* Santschi, *Camponotus kiesenwetteri* (Roger), *C. oertzeni* Forel, *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Messor wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *T. graecus* (Forel); **580 m SW of Lithakia:** *Aphaenogaster* cf. *epirotes*, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. gestroi* Emery, *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Messor ibericus* Santschi, *M. wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *T.* cf. *tergestinus*, *Tetramorium diomedeum* Emery; **600 m E of Ag. Leon:** *Aphaenogaster balcanica* (Emery), *A. muelleriana* Wolf, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. kiesenwetteri* (Roger), *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *Lepisiota frauenfeldi* (Mayr), *L. melas* (Emery), *Messor ibericus* Santschi, *M. wasmanni* Krausse, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. rogeri* Emery, *T.* cf. *tergestinus*, *Tetramorium kephalosi* Salata & Borowiec.

Peloponnese, Messinia, 2 km E of Kalamata: *Aphaenogaster balcanica* (Emery), *A.* cf. *muelleriana*, *Camponotus gestroi* Emery, *C. ionius* Emery, *C. kiesenwetteri* (Roger), *C. laconicus* Emery, *C. lateralis* (Olivier), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Formica clara* Forel, *Lasius illyricus* Zimmermann, *L. lasioides* (Emery), *L. neglectus* Van Loon, Boomsma & Andrasfalvy, *Lepisiota frauenfeldi* (Mayr), *Messor wasmanni* Krausse, *Nylanderia jaegerskioeldi* (Mayr), *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax graecus* (Forel), *T.* cf. *luteus*, *T. recedens* (Nylander), *T. rogeri* Emery, *Tetramorium* cf. *caespitum*; **Egaleo Mts., 1.4 km S of Flesiada:** *Aphaenogaster balcanica* (Emery), *A.* cf. *muelleriana*, *Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *Crematogaster schmidti* (Mayr), *C. sordidula* (Nylander), *Lasius lasioides* (Emery), *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Tapinoma erraticum* (Latreille), *Temnothorax exilis* (Emery), *T. laconicus* Csősz et al., *T. morea* Csősz, Salata & Borowiec; **Kalamata, old centre:** *Aphaenogaster balcanica* (Emery), *Crematogaster schmidti* (Mayr), *Formica clara* Forel, *Lepisiota frauenfeldi* (Mayr), *L. melas* (Emery), *Messor hellenius* Agosti & Collingwood, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Solenopsis* cf. *lusitanica*, *Temnothorax graecus* (Forel), *Tetramorium* cf. *hungaricum*, *Trichomyrmex perplexus* Radchenko; **Kalamata, railway park:** *Camponotus* lateralis (Olivier), *Crematogaster schmidti* (Mayr), *Formica clara* Forel, *Lasius illyricus* Zimmermann, *L. lasioides* (Emery), *L. neglectus* Van Loon, Boomsma & Andrasfalvy, *Messor wasmanni* Krausse, *Pheidole indica* Mayr, *Plagiolepis pygmaea* (Latreille), *Temnothorax graecus* (Forel), *Tetramorium* cf. *caespitum*; **Kondovounia Mts., 0.8 km N of Koromilea:** *Aphaenogaster balcanica* (Emery), *A.* cf. *muelleriana*, *Camponotus dalmaticus* (Nylander), *C. gestroi* Emery, *C. laconicus* Emery, *C. lateralis* (Olivier), *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Lasius lasioides* (Emery), *Lepisiota frauenfeldi* (Mayr), *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *bulgaricus*, *T. exilis* (Emery), *T. laconicus* Csősz et al., *T. morea* Csősz, Salata & Borowiec, *T. recedens* (Nylander); **Taygetos Mts., 0.8 km W of Eleochori:** *Aphaenogaster balcanica* (Emery), *Camponotus gestroi* Emery, *C. ionius* Emery, *C. kiesenwetteri* (Roger), *Lepisiota nigra* (Dalla Torre), *Messor wasmanni* Krausse, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *Temnothorax exilis* (Emery), *Tetramorium kephalosi* Salata & Borowiec.

***Temnothorax turcicus* (Santschi, 1934)**

Figs 7, 8, 11, 15

Leptothorax turcicus Santschi, 1934: 278.

Type material. Syntype, worker (pin): • [TURKEY]: Izmir | 29.VII.33, Santschi || Type || Sammlung | Dr. F. Santschi | Kairouan | ANTWEB | CASENT0913009 (NHMB).

Other material. Greece. North Aegean, Lesbos: • 2w. (pin) (CASENT0846851–CASENT0846852): n. Ahladeri, 39.15958N/26.29292E, 9 m, 2015-06-10, leg. L. Borowiec. **Macedonia, Chalkidiki:** • 12w. (pin) (CASENT0846853–CASENT0846864): Holomontas, Taxiarhis vicinity, mountain deciduous forest, in leaf litter, 40.4N/ 23.51666E, 594 m, 2009-08-30, leg. L. Borowiec; • 2w. (pin) (CASENT0846865–CASENT0846866): Holomontas, Stagira, on wall in deciduous forest, 40.52896N/23.74872E, 539 m, 2009-09-03, leg. L. Borowiec; • 5w. (pin) (CASENT0846867–CASENT0846871): Holomontas, Stagira-Neochori road, on wall in deciduous forest, 40.51666N/23.7E, 512 m, 2009-09-03, leg. L. Borowiec. **Macedonia, Kavalas:** • 1w. (pin) (CASENT0846872): Nestos river near Komnina, 41.169N/ 24.6966E, 100 m, 1999-10-10, leg. E. Nikolakakis. **Macedonia, Pieria:** • 5w. (pin) (CASENT0846873–CASENT0846877), 2w. (EtOH): road to P. Poroi loc. 1, roadsides with shrubs, 39.97963N/ 22.61563E, 110 m,

Figures 7, 8. Worker of *Temnothorax turcicus* (Santschi) **7** Dorsal **8** Lateral.

2019-05-17, leg. L. Borowiec; • 6w. (EtOH): road to P. Poroi loc. 2, roadsides with shrubs, 39. 97627N/ 22.61146E, 185 m, 2019-05-17, leg. L. Borowiec; •14w. (pin) (CASENT0846878–CASENT0846891), 40w. (EtOH): road to P. Poroi loc. 3, roadsides with shrubs, 39. 96863N/ 22.60494E, 260 m, 2019-05-17, leg. L. Borowiec. **Peloponnese, Arcadia:** • 1w. (pin) (CASENT0846892): 3.2 km NW Polidroso, 1000 m, 37.19874N/2257603E, 1000, 2016-06-18, leg. L. Borowiec. **Peloponnese, La-**

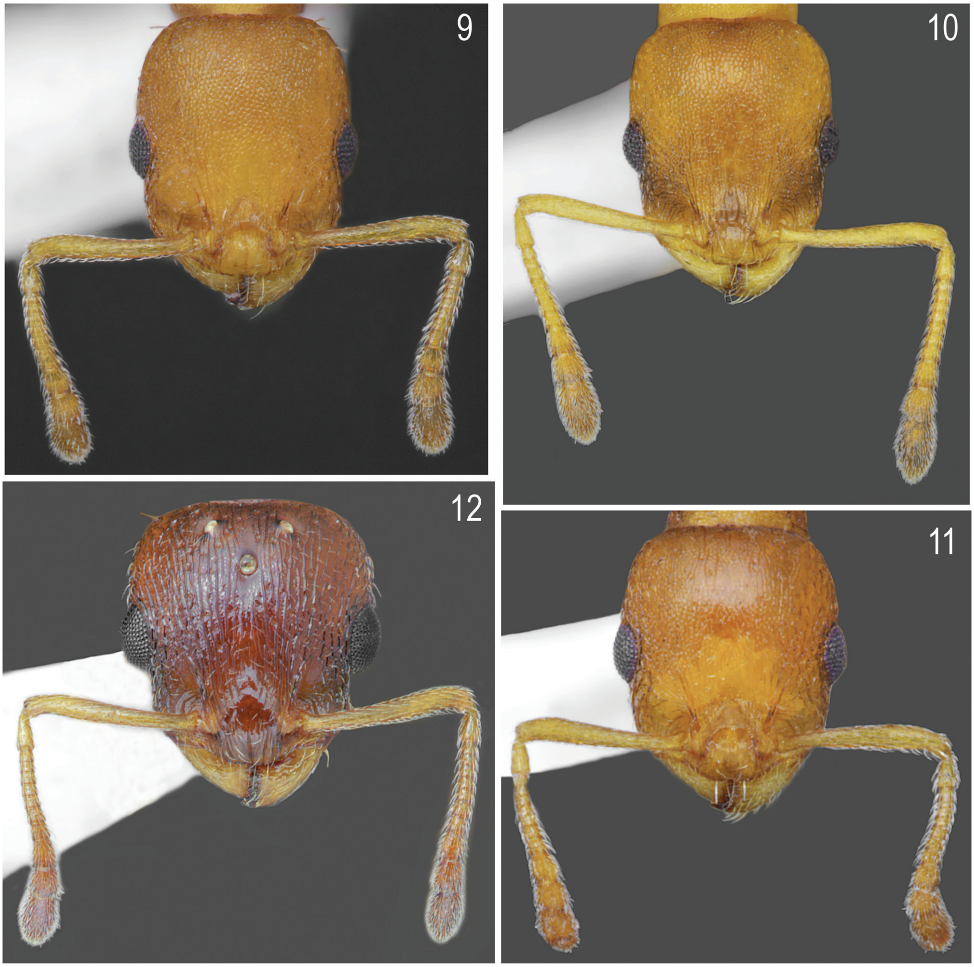
conia: • 6w. (pin) (CASENT0846893–CASENT0846898): Parnon Mts., 5 km NE of Karies, 37.324N/ 22.538E, 1000 m, 2000-04-29, leg. A. Schulz & K. Vock (3w DBET, 3w PW). **Sterea Ellas, Euboea:** • 1w. (pin) (CASENT0846899): 1 km NE of Amfithea, 38.5519N/23.79546, 200 m, 2018-06-10, leg. L. Borowiec. **Thessaly, Larissa:** • 1w. (pin) (CASENT0846900): Kato Olimbos Mts, 6.1 km S of Kalipefki, 39.91322N/22.4641E, 855 m, 2017-05-09, leg. L. Borowiec.

Terra typica. Greece, Thessaly, Mt. Ossa.

Differential diagnosis. Differentiation from *T. kemali*, *T. brackoi* and *T. messiniaensis* – see differential diagnosis in *T. messiniaensis*. *Temnothorax turcicus* differs from specimens of *T. aveli* with long propodeal spines in thin dark band on first gastral tergite, head not darker from mesosoma, mesosoma less convex in profile and propodeal spines directed slightly more upwards; from *T. lagrecai* (Baroni Urbani, 1964), species described and known only from Sicily, differs in petiolar node dorsum flat or slightly convex and distinctly bigger mesosoma size - ML 0.595 ± 0.50 (0.517–0.680) vs. ML = 0.779 ± 0.05 (0.677–0.832).

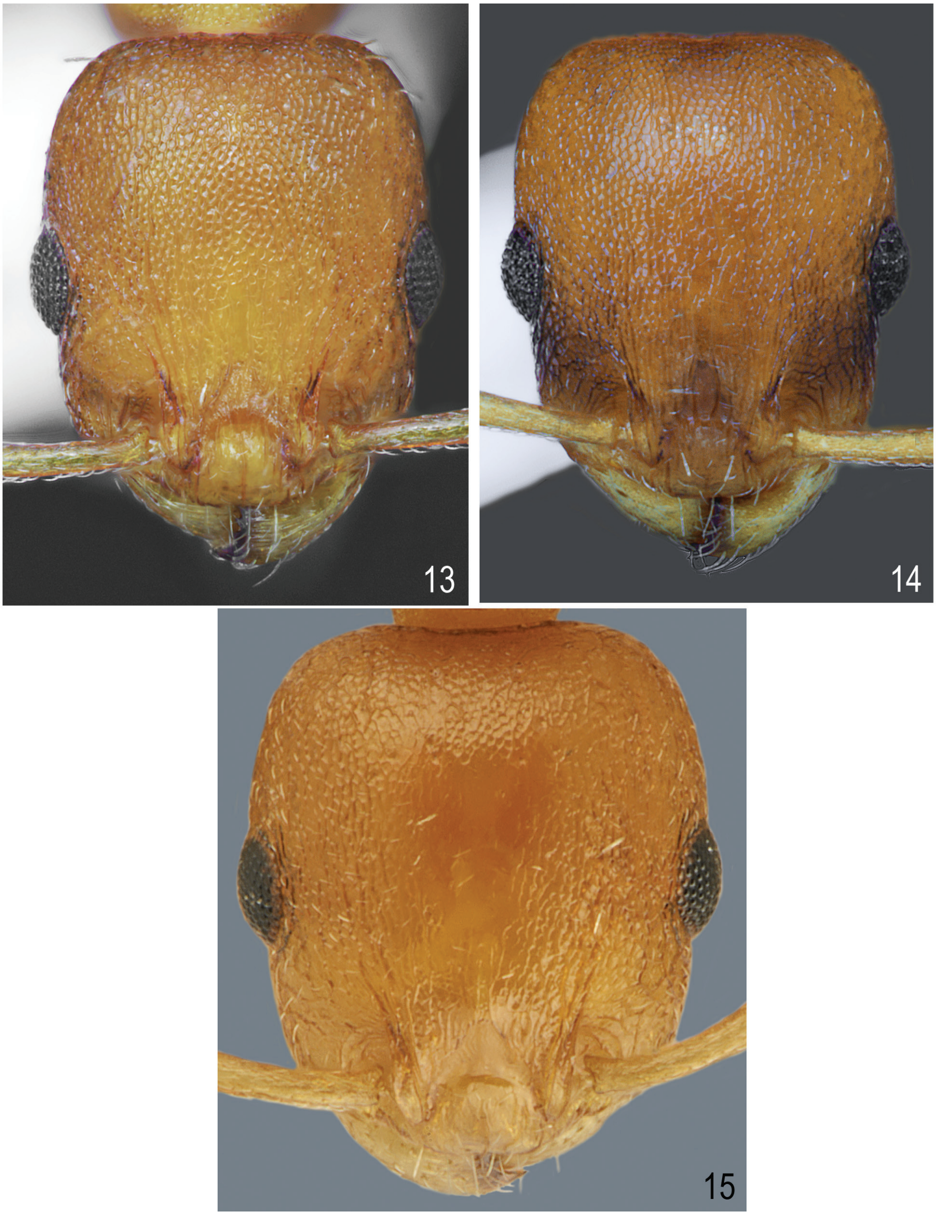
Redescription. Worker (n = 10): HL: 0.670 ± 0.03 (0.614–0.696); HW: 0.573 ± 0.03 (0.522–0.596); SL: 0.465 ± 0.02 (0.431–0.484); EL: 0.158 ± 0.009 (0.149–0.174); EW: 0.118 ± 0.01 (0.102–0.137); WL: 0.779 ± 0.05 (0.677–0.832); PSL: 0.192 ± 0.005 (0.186–0.199); SDL: 0.118 ± 0.007 (0.106–0.124); PEL: 0.271 ± 0.015 (0.248–0.286); PPL: 0.173 ± 0.01 (0.149–0.186); PEH: 0.206 ± 0.015 (0.186–0.230); PPH: 0.205 ± 0.02 (0.174–0.236); PNW: 0.394 ± 0.02 (0.360–0.422); PLW: 0.169 ± 0.007 (0.161–0.180); PPW: 0.219 ± 0.01 (0.199–0.230); CI: 85.6 ± 1.2 (83.6–86.8); SI1: 69.4 ± 0.6 (68.5–70.2); SI2: 81.1 ± 1.3 (79.2–82.7); MI: 50.7 ± 1.3 (49.3–53.2); EI1: 75.3 ± 8.8 (66.1–91.7); EI2: 17.7 ± 1.3 (16.4–20.0); PI: 132.3 ± 9.2 (120.0–148.4); PPI: 84.8 ± 4.5 (75.8–88.9); PSI: 162.7 ± 7.5 (155.0–176.5).

Colour. Whole body uniformly yellow to dark yellow, sometimes club in darker yellow colouration. Gaster yellow, only the first gaster tergite with very thin, dark band on its posterior part (Figs 7, 8). **Head.** Oval, 1.16 times as long as wide, lateral surfaces below and above eyes gently convex, posterior edges convex, occipital margin of head straight or slightly convex (Fig. 15). Anterior margin of clypeus slightly convex, medial notch absent. Eyes moderate, oval, 1.34 times as long as wide. Antennal scape short, in lateral view slightly curved, 0.69 times as long as length of the head, in apex gradually widened, its base with small, triangular tooth, funiculus long, club 3 segmented (Figs 12, 15). Surface of scape with very fine microreticulation, shiny, covered with thin, moderate dense, decumbent setae. Mandibles rounded with thick sparse, longitudinal striae, shiny. Clypeus shiny with thick, sparse, longitudinal striae, area between striae smooth and shiny. Frontal carinae short, not extending beyond frontal lobes. Antennal fossa deep, with irregular, dense to sparse, thick rugosity and sometimes with a few thin, roundly curved striae, surface between thick sculpture smooth or with sparse microreticulation. Frontal lobes narrow, smooth with slight, dense longitudinal striation (Fig. 15). Frons, vertex and temples with dense, thick, longitudinal reticulation, central surface of frons and vertex with longitudinal reticulation sparser or reduced, with additional thin, longitudinal striation, striae sometimes interrupted, surface between



Figures 9–12. Head and antennae **9** Worker of *Temnothorax brackoi* sp. nov. **10** Worker of *Temnothorax messiniaensis* sp. nov. **11** Worker of *Temnothorax turcicus* (Santschi) **12** Gyne of *Temnothorax messiniaensis* sp. nov.

striation smooth and shiny; malar area with irregular, thick, reticulation, space between reticulation smooth or with very sparse microreticulation, shiny; genae with sparser, than on frons, and thick reticulation, shiny (Fig. 15). Frons and vertex with erect, pale, short and thick setae. **Mesosoma.** Elongate, 1.98 times as long as wide, slightly arched in profile. Metanotal groove absent. Pronotum convex on sides. Propodeal spines long, directed upward, with base slightly to moderate wider than apex, tips sharp (Fig. 8). Whole surface with dense, reticulation, sometimes its dorsal surface and lateral surfaces of pronotum and mesonotum with additional thick, sparse longitudinal wrinkles. Area between thick sculpture shiny, smooth or sometimes with sparse, fine microreticulation (Fig. 8). Entire mesosoma bearing erect, pale, short and thick setae (Fig. 8). **Petiole.** In lateral view, with short peduncle, node moderate high, with anterior face straight, and



Figures 13–15. Head sculpture **13** Worker of *Temnothorax brackoi* sp. nov. **14** Worker of *Temnothorax messiniaensis* sp. nov. **15** Worker of *Temnothorax turcicus* (Santschi).

posterior face convex and dorsum flat or slightly convex. Peduncle and petiolar node shiny, with thick, dense reticulation, area between rugae smooth, dorsum with sparser reticulation. Dorsal surface bearing sparse, short, erect setae (Fig. 8). **Postpetiole.** In lateral view, regularly convex, apical half with gently convex sides (Fig. 8), on the whole surface shiny, with thick, dense reticulation, dorsum with sparser reticulation; area between rugae smooth. Dorsal surface bearing sparse, short, erect setae. **Gaster.** Gaster smooth and shiny, bearing erect, thin, pale setae (Figs 7, 8).

General distribution. Eastern Austria, Bulgaria, Croatia, Greece: Macedonia, North Aegean Islands, Sterea Ellas, Peloponnese, and Thessaly, Hungary, Slovakia, western Turkey.

Comment. We examined a syntype of *Temnothorax tauricus* (Ruzsky, 1902) preserved in Forel's collection (MHNG) and it appears to be very similar to specimens of *T. turcicus* collected in Greece. The only difference is a slightly darkened antennal club in the syntype specimen of *T. tauricus* (all studied specimens of *T. turcicus* have antennae uniformly yellow). We discussed this issue with Alex Radchenko (Kiev, Ukraine) who confirmed that all 17 syntypes of *T. tauricus* preserved in Karavaiev's collection (Kiev, Ukraine) also have slightly darkened antennal club. In our opinion this difference could be an infraspecific variation. Within nest samples of *Temnothorax messiniaensis*, a member of the *aveli* species group, we observed single specimens with more or less darkened antennal club. *Temnothorax tauricus* was recorded from Caucasus and southern Ukraine but is sympatric with *T. turcicus* in Bulgaria and Greece. *Temnothorax tauricus* have nests in dry stems of herbs, grasses or rarely in soil under stones (Radchenko 2016) and by those preferences reminds species of the *T. aveli* species group. Clarification of taxonomic relation between those two taxa requires further study based on material collected from the whole distribution range of both species. If our supposition on the conspecifity of both taxa is confirmed, then the name *T. tauricus* will have priority over the name *T. turcicus*.

Biology. Specimens collected on shadow localities, from seacoast to 1000 m a.s.l. Foraging workers were observed on herbs in stream valley of tourist resort, valleys with *Platanus* trees, mountain coniferous forest and mountain pastures close to border of coniferous forest. Nests were not found, probably like other species of this group, are located inside dry stems of herbs.

The following ant species were recorded in the same areas as *T. turcicus*:

For localities on **Macedonia**: Pieria, road to P. Poroi loc. 1, Pieria, road to P. Poroi loc. 2, Pieria, road to P. Poroi loc. 2, and **Thessaly, Larissa, Mt. Ossa, Kokkino Nero:** see *Temnothorax brackoi*.

North Aegean, Lesbos, near Ahladeri: *Camponotus dalmaticus* (Nylander), *C. lateralis* (Olivier), *C. sanctus*, *Crematogaster ionia* Forel, *Lasius neglectus* Van Loon, Boomsma & Andrasfalvy, *Monomorium monomorium* Bolton, *Pheidole pallidula* (Nylander), *Plagiolepis perperamus* Salata et al., *Temnothorax bulgaricus* (Forel), *T.* cf. *luteus*, *Tetramorium rhodium* Emery.

Peloponnese, Arcadia, 3.2 km NW Polidroso: *Aphaenogaster* cf. *subterranea, Camponotus aethiops* (Latreille), *C. dalmaticus* (Nylander), *C. nitidescens* Forel, *C. vagus* (Scopoli), *Cataglyphis nodus* (Brullé), *Crematogaster ionia* Forel, *Formica cunicularia* Latreille, *F. fusca* Linnaeus, *Lasius bomycina* Seifert & Galkowski, *L. flavus* (Fabricius), *L. illyricus* Zimmermann, *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax crasecundus* Seifert & Csősz, *T. helenae* Csősz et al., *T. laconicus* Csősz et al., *T.* cf. *unifasciatus*, *Tetramorium* cf. *caespitum*.

Sterea Ellas, Euboea, 1 km NE of Amfithea: *Camponotus lateralis* (Olivier), *Cataglyphis nodus* (Brullé), *Crematogaster schmidti* (Mayr), *Pheidole* cf. *pallidula*, *Plagiolepis pygmaea* (Latreille), *Temnothorax bulgaricus* (Forel), *T. recedens* (Nylander).

Thessaly, Larissa, Kato Olimbos Mts, 6.1 km S of Kalipefki: Aphaenogaster *epirotes* (Emery), *Bothriomyrmex communista* Santschi, *Camponotus aethiops* (Latreille), *C. oertzeni* Forel, *C. piceus* (Leach), *Formica cunicularia* Latreille, *Lasius alienus* Förster, *Messor mcarthuri* Steiner et al., *M. wasmanni* Krausse, *Plagiolepis pygmaea* (Latreille), *Temnothorax* cf. *unifasciatus*, *Tetramorium* cf. *caespitum*.

A key to Greek members of the *T. aveli* species group

1 Propodeal spines short to moderate (PSI<155), triangular, with wide base (Fig. 2); head on whole frontal surface with regular reticulation, without longitudinal striation, with dull background (Fig. 13).......... ***T. brackoi* sp. nov.**

– Propodeal spines long (PSI > 155), thin, with base slightly to moderate wider than base (Figs 4, 8); head regularly reticulate but often with additional thin, longitudinal striation and partly shiny background (Figs 14, 15) **2**

2 Central surface of frons with dense, thick, longitudinal reticulation and sometimes with additional thin, longitudinal striation (Fig. 10); first gaster tergite with wide, dark band on its posterior part (Figs 3–4); gena, mid and hind femora often obscured in middle .... ***T. messiniaensis* sp. nov.**

– Central surface of frons and vertex with longitudinal reticulation sparser than of rest of head or reduced (Fig. 12); first gaster tergite with very thin, dark band on its posterior part (Figs 7, 8); gena, mid and hind femora never obscured in middle .... ***T. turcicus* (Santschi)**

Description of a new species of the *Temnothorax nylanderi* species group

Temnothorax triangularis sp. nov.

http://zoobank.org/4D263567-874F-45D1-870C-E64C9750A9F5
Figs 16–24

Differential diagnosis. *Temnothorax triangularis* belongs to the *T. nylanderi* species group. It differs from most of members of this group in uniformly brown body with darker frons and posterior band of first gastral tergite, and absent or very shallow, inconspicuous metanotal groove. There are four other known species with dark body colouration: *T. laconicus, T. artvinensis*, *T. sordidulus*, and *T. tergestinus*. *Temnothorax triangularis* differs from all of them in extremely shallow metanotal groove and frons lacking reticulation and covered with dense, thick, longitudinal striations, additionally frons centre has sculpture weaker or reduced, it differs also from *T. laconicus* and *T. artvinensis* in shorter, triangular propodeal spines.

Etymology. Named after short, triangular propodeal spines.

Type material. Holotype, worker (pin) (CASENT0846901): GREECE, Sterea Ellas, Eubea | 2.4 km SW of Stropones | 38,60327N/23,87E, 1025 m |10 VI 2018, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-GR02682 (MNHW).

Paratypes, 25w., 1Q.(pin) (CASENT0846661–CASENT0846691): the same nest sample as holotype (DBET, BMNH, CASC, MHNG); 5w. (EtOH): the same locality as holotype, collected in litter (DBET); 11w, 1Q (pin) (CASENT0846692–CASENT0846704): GREECE, Sterea Ellas | Eubea |3.7 km SW of Metochi, 830 m | 38.60402N/23.91683E, | 13 VI 2018, L. Borowiec (DBET).

Terra typica. Euboea, Greece.

Other material. GREECE, Sterea Ellas, Euboea: 2w. (EtOH): 1.5 km SW of Koutourla, 38.62838N/23.92772E, 695 m, 2018-06-13, leg. L. Borowiec; 1w.(pin) (CASENT0846902), 2w. (EtOH): 2.3 km S of Stropones, 38.9933N/23.87807E, 860 m, 2018-06-10, leg. L. Borowiec; 2w. (EtOH): 2.7 km SE of Stropones, 38.59851N/23.9085E, 855 m, 2018-06-13, leg. L. Borowiec; 3w. (EtOH): 2.9 km S of Stropones, 38.59133N/23.88562E, 880 m, 2018-06-13, leg. L. Borowiec; 43w. (EtOH): 3.7 km SW of Metochi, 38.60402N/23.91683E, 830 m, 2018-06-13, leg. L. Borowiec.

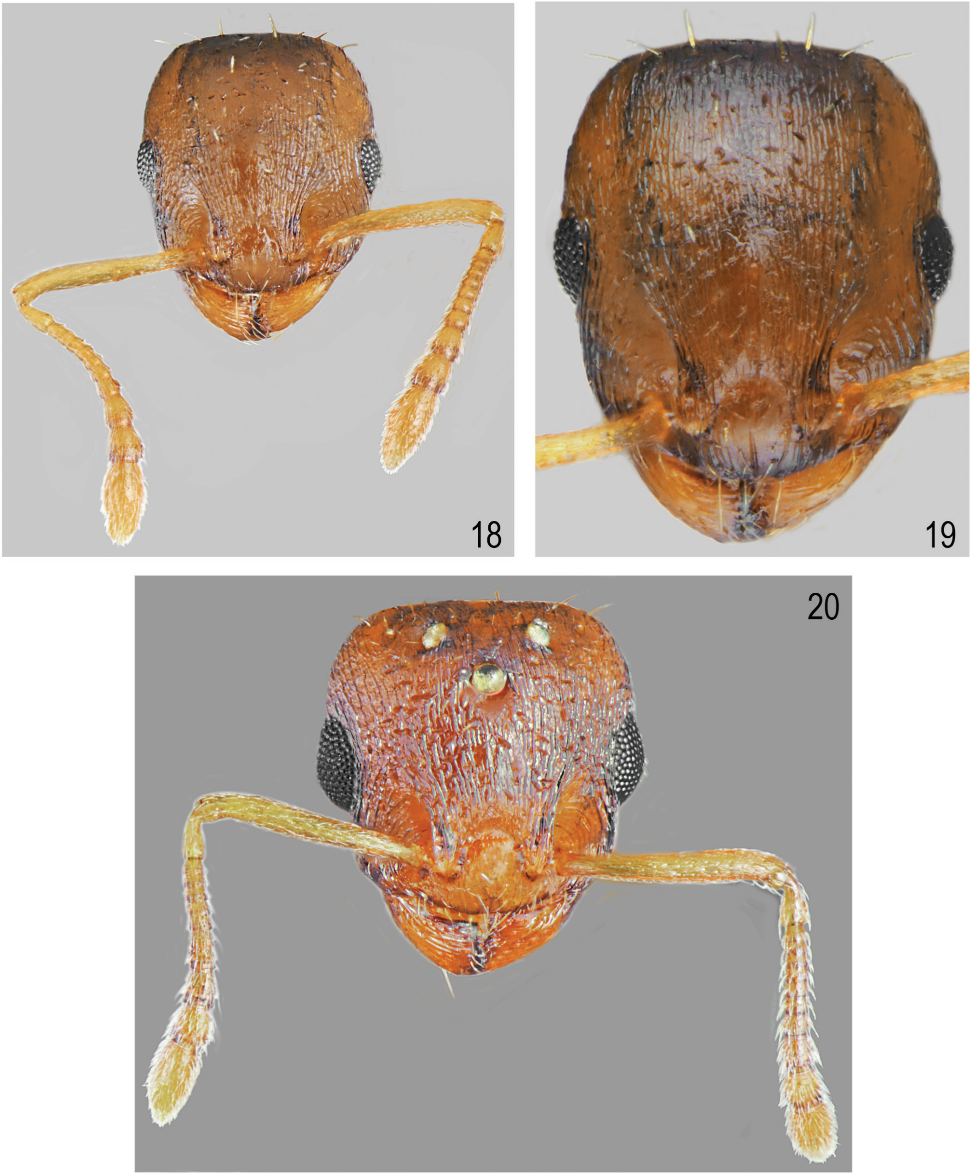
Description of worker (n = 10): HL: 0.684 ± 0.01 (0.671–0.708); HW: 0.603 ± 0.02 (0.578–0.650); SL: 0.504 ± 0.02 (0.484–0.534); EL: 0.140 ± 0.01 (0.124–0.149); EW: 0.105 ± 0.006 (0.093–0.112); WL: 0.769 ± 0.02 (0.742–0.820); PSL: 0.161 ± 0.02 (0.143–0.183); SDL: 0.119 ± 0.006 (0.112–0.130); PEL: 0.294 ± 0.02 (0.273–0.323); PPL: 0.182 ± 0.008 (0.174–0.199); PEH: 0.245 ± 0.01 (0.236–0.270); PPH: 0.231 ± 0.01 (0.217–0.248); PNW: 0.403 ± 0.01 (0.388–0.435); PLW: 0.177 ± 0.009 (0.168–0.199); PPW: 0.241 ± 0.01 (0.230–0.267); CI: 88.2 ± 2.2 (85.3–92.7); SI1: 73.7 ± 1.5 (71.2–76.4); SI2: 83.6 ± 2.4 (78.9–87.5); MI: 52.4 ± 1.2 (50.8–54.7); EI1: 75.0 ± 4.2 (68.8–85.0); EI2: 15.3 ± 1.0 (13.6–16.7); PI: 120.1 ± 5.4 (108.0–128.8); PPI: 79.0 ± 2.8 (75.0–85.7); PSI: 135.8 ± 12.4 (120.0–155.6).

Colour. Whole body uniformly brown to bright brown, sometimes mesosoma and genae brighter. Legs and antennae bright brown to dark yellow, femora in central part darkened (Figs 16, 17). **Head.** Oval, 1.14 times as long as wide, lateral surfaces below and above eyes gently convex, posterior edges convex, occipital margin of head straight or slightly concave (Figs 18, 19). Anterior margin of the clypeus slightly convex, medial notch absent. Eyes small, oval, 1.33 times as long as wide. Antennal scape short, in lateral view slightly curved, 0.73 times as long as length of the head, in apex gradually widened, its base with small, triangular tooth, funiculus long, club 3-segmented (Fig. 18). Surface of scape with very fine microreticulation, shiny, covered with thin, moderate dense, decumbent setae. Mandibles rounded with thick sparse, longitudinal striae, shiny. Clypeus shiny with thick, longitudinal striae, area between striae shiny with few longitudinal wrinkles. Frontal carinae short, not extending beyond frontal lobes. Antennal fossa deep, with sparse, thin roundly curved striae, area between striae with sparse and fine reticulation, shiny. Frontal lobes narrow, smooth with slight, dense longitudinal striation (Figs 18, 19). Space between frontal carinae and vertex with

Figures 16, 17. Worker of *Temnothorax triangularis* sp. nov. **16** Dorsal **17** Lateral.

dense, thick, longitudinal striation, sparser or reduced in the central part, striae sometimes interrupted, surface between striae smooth and shiny; space between frontal carinae ad eyes, temples and malar area with longitudinal, thick reticulation, space between reticulation smooth or with very sparse microreticulation, shiny; genae with very sparse thick reticulation, partly smooth, always shiny. Frons and vertex with erect, pale, short and thick setae. **Mesosoma.** Elongate, 1.9 times as long as wide, slightly arched in profile. Metanotal groove absent or very shallow, inconspicuous. Pronotum convex on sides. Propodeal spines short, triangular, with wide base, directed upward, with angulate tips (Fig. 21), only in fewer than 20% of specimens are propodeal spines moderately long, with wide bases and sharp tips (Fig. 22). Lateral surfaces of pronotum with thick and sparse longitudinal striation or reticulation, its dorsal surface with thick, sparse, irregular reticulation; mesonotum and propodeum on the whole surface with thick, denser than on pronotum, irregular reticulation. Area between thick sculpture shiny, smooth or sometimes with sparse, fine microreticulation (Fig. 17). Entire mesosoma bearing erect, pale, short and thick setae (Fig. 17). **Petiole.** In lateral view, with short peduncle, node high, with anterior face flat to slightly convex, and posterior face and dorsum convex. Peduncle and petiolar node shiny, with thick, dense reticulation, area between rugae smooth, dorsum with sparser reticulation. Dorsal surface bearing sparse, short, erect setae (Figs 21, 22). **Postpetiole.** In lateral view, regularly convex, apical half with gently convex sides (Figs 21, 22), on the whole surface shiny, with thick, dense reticulation, dorsum with sparser reticulation; area between rugae smooth. Dorsal surface bearing sparse, short, semierect to erect setae. **Gaster.** Smooth and shiny, bearing erect, thin, pale setae (Figs 16, 17).

Description of gyne (n = 2). **Colour.** Head brown, temples slightly brighter coloured than frontal parts. Antennae uniformly yellow. Mesosoma, petiole and postpetiole bright brown, legs yellow. First gastral tergite mostly dark brown with yellowish brown spot basally, remaining tergites brown with dark brown posterior margins (Figs 23, 24). **Head.** Eyes big, almost round [EL / HW: 0.26]. Antennal scape short [SL / HW: 0.76], not reaching occipital margin of head. Clypeus smooth and shiny laterally with diffused, longitudinal carinulae. Antennal fossa deep, rugulose with concentric carinae. Frontal lobes wide 0.43 times as wide as head width, rugulose with thick longitudinal costae, interstices microreticulate (Fig. 20). Frons shiny, entire surface longitudinally costate and rugose, interstices distinctly microreticulate. Area above eyes and sides of head rugulose and partly longitudinally costate, only small area behind eyes smooth and shiny. Entire head bearing erect, pale and thin setae. **Mesosoma.** Pronotum with thick rugosities in anterior part, sides with thick rugosity and dense longitudinal costae. Surface between rugosities microreticulate. Scutum with dense, thick longitudinal costae and microreticulation between costae but appears shiny. Scutellum laterally with thick longitudinal costae, to the centre costae partly diffused, along middle smooth and shiny area (Figs 23, 24). Metanotum with fine longitudinal costae and microreticulated background. Propodeum with area above propodeal spines with transverse and on sides with longitudinal costae and microreticulated background. Propodeal spines short [PSL / HW: 0.28], triangular, with wide base, straight and angulate apex. Area between and below propodeal spines with distinct microreticulation tends to form transverse ridges. Anepisternum and katepisternum with gentle, dense longitudinal costae. Metaepisternum and metakatepisternum, with dense, longitudinal costae and shiny area close to ventral margin. Surface between costae microreticulate. Dorsal surface of mesosoma with sparse, erect, long, thick and pale setae (Figs 23, 24). **Petiole and postpetiole.** Microreticulate, the entire surface punctate to rugulose, dorsal surface longitudinally costulate. **Gaster.** Smooth and shiny, bearing sparse, long, erect setae (Figs 23, 24).

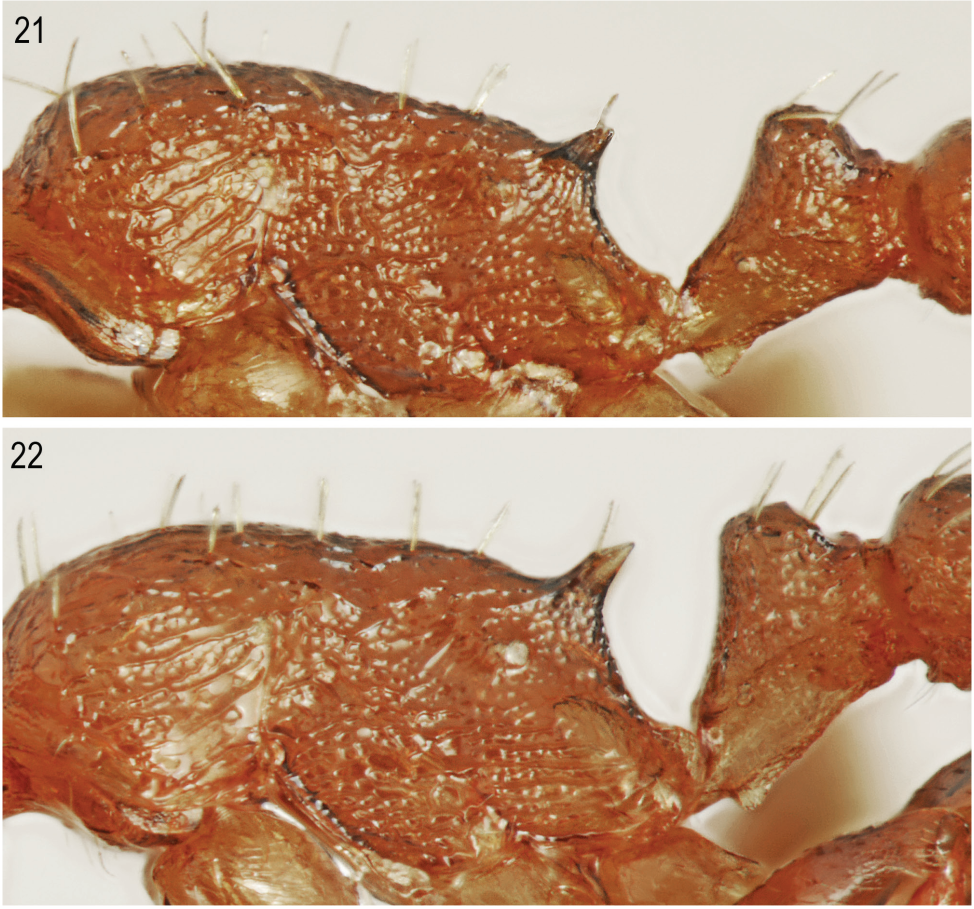


Figures 18–20. *Temnothorax triangularis* sp. nov. **18** Worker head and antennae **19** Worker head sculpture **20** Gyne head and antennae.

General distribution. Greece, Sterea Ellas, Euboea.

Biology. Alpine species. Ants were observed on stones, dry branches, and herbs in coniferous forest, or coniferous forest with an admixture of chestnut. Nests were found in the dry branches of conifers lying on the ground.

The following ant species were recorded in the same areas as *T. triangularis*:



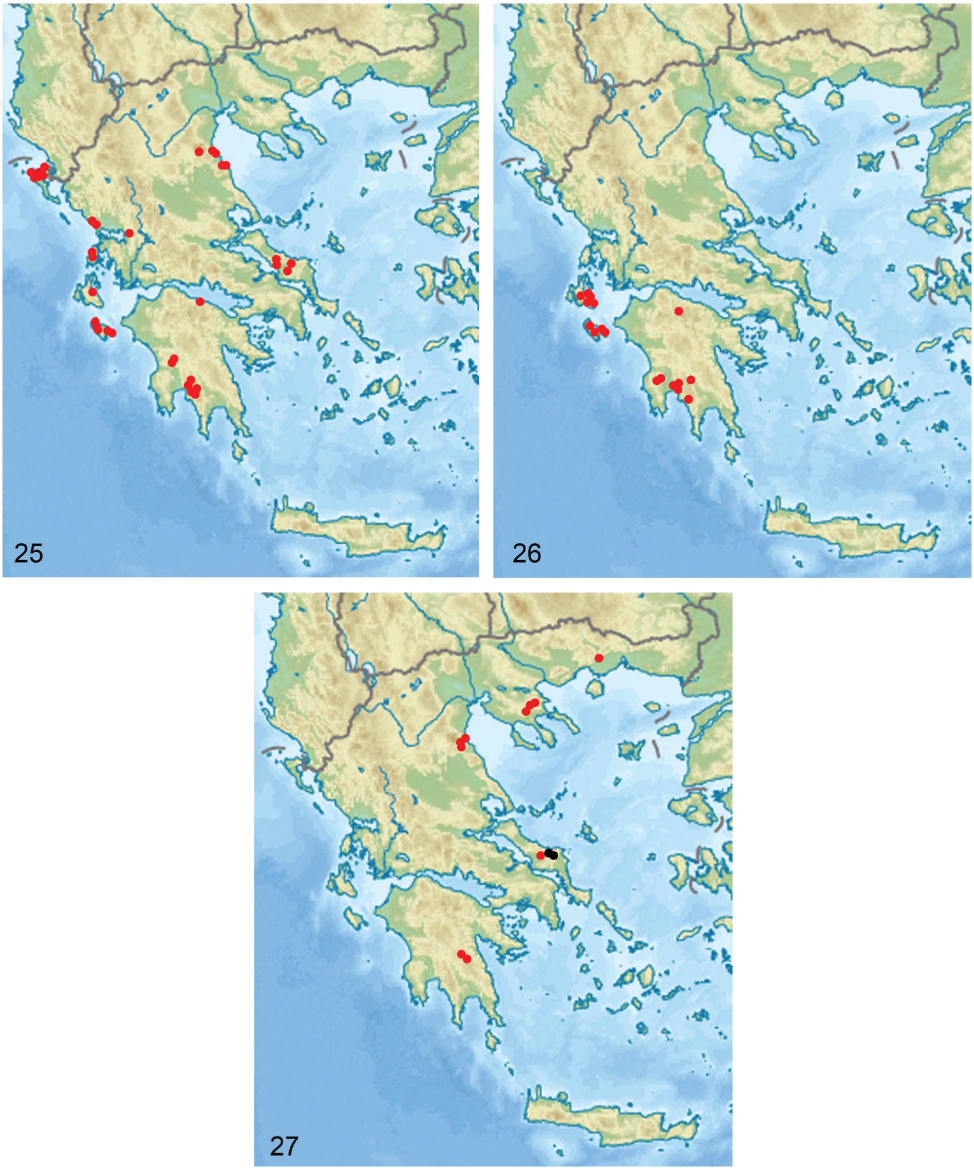
Figures 21, 22. Variation of propodeal spines of *Temnothorax triangularis* sp. nov.

Sterea Ellas, Euboea, 1.5 km SW of Koutourla: *Aphaenogaster subterranea* (Latreille), *Formica fusca* Linnaeus, *Lasius alienus* Förster, *L. brunneus* (Latreille), *L. illyricus* Zimmermann, *Pheidole pallidula* (Nylander), *Plagiolepis pygmaea* (Latreille), *P. pallescens* Forel, *Temnothorax helenae* Csősz et al., *T. lichtensteini* (Bondroit), *T. unifasciatus* (Latreille); **2.3 km S of Stropones:** see *Temnothorax brackoi*; **2.4 km SW of Stropones:** *Camponotus aethiops* (Latreille), *Formica fusca* Linnaeus, *F. sanguinea* Latreille, *Lasius alienus* Förster, *L. illyricus* Zimmermann, *Messor structor* (Latreille), *Temnothorax crasecundus* Seifert & Csősz, *T. unifasciatus* (Latreille), *Tetramorium impurum* (Förster); **2.7 km SE of Stropones:** *Aphaenogaster subterranea* (Latreille), *A.* cf. *subterranea*, *Camponotus aethiops* (Latreille), *C. oertzeni* Forel, *Formica fusca* Linnaeus, *Lasius alienus* Förster, *L. distinguendus* (Emery), *Tapinoma erraticum* (Latreille), *Temnothorax helenae* Csősz et al., *T. unifasciatus* (Latreille);

2.9 km S of Stropones: *Camponotus piceus* (Leach), *C. vagus* (Scopoli), *Lasius brunneus* (Latreille), *L. flavus* (Fabricius), *Myrmica scabrinodis* Nylander, *Temnothorax*

Figures 23, 24. Gyne of *Temnothorax triangularis* sp. nov. **23** Dorsal **24** Lateral.

helenae Csősz et al., *T. unifasciatus* (Latreille); **3.7 km SW of Metochi:** *Aphaenogaster subterranea* (Latreille), *A.* cf. *subterranea, Camponotus fallax* (Nylander), *C. vagus* (Scopoli), *Lasius illyricus* Zimmermann, *Temnothorax crasecundus* Seifert & Csősz, *T. helenae* Csősz et al., *T. unifasciatus* (Latreille).



Figures 25–27. Distribution in Greece **25** *Temnothorax brackoi* sp. nov. **26** *Temnothorax messiniaensis* sp. nov. **27** *T. turcicus* (Santschi) denoted by red circles and *T. triangularis* sp. nov. by black circles.

Note. Although this species has inconspicuous metanotal groove, which tends to disappear in some specimens, we decided to place it in the *Temnothorax nylanderi* group. General body shape, structure of petiole, unicolourous antennae, head and mesosoma sculpture presented by *T. triangularis* are very similar to those observed in large species of the group i.e. *T. nylanderi* (Förster), *T. crassispinus* (Karavaiev) or *T. crasecundus* Seifert

& Csősz. Moreover, an inconspicuous metanotal groove was observed also in some samples of small species of the *nylanderi* group, such as *T. helenae* Csősz, Heinze & Mikó. Usually the depth of metanotal groove is more or less constant within nest samples.

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Pupae of the mega-diverse rove beetle tribe Staphylinini (Coleoptera, Staphylinidae): their traits and systematic significance

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Abstract

This paper is the first comprehensive work on the pupae of Staphylinidae. It is the first-ever attempt to employ the morphological characters of these pupae in phylogenetic analysis. The study shows that the external structures of Staphylinini pupae could be a useful, alternative source for assessing the relationships of certain taxa within a tribe. The paper also includes an illustrated key to the identification of pupae at the subtribe and generic levels (*Abemus*, *Acylophorus*, *Astrapaeus*, *Atanygnathus*, *Bisnius*, *Creophilus*, *Emus*, *Erichsonius*, *Gabrius*, *Hesperus*, *Heterothops*, *Neobisnius*, *Ocypus*, *Ontholestes*, *Philonthus*, *Quedius*, *Quedionuchus*, *Rabigus*, *Staphylinus*, and *Tasgius*) of the tribe Staphylinini, found in Europe. Based on current knowledge of the morphology of pupal stages of Staphylinini species, eight morphological pupal types are presented: Acylophorus, Astrapaeus, Atanygnathus, Erichsonius, Heterothops, Philonthus, Quedius and Staphylinus. The paper also comments on pupal habitat, phenology and morphology in the context of antipredator and environmental adaptations.

Keywords

entomology; morphology; pupae; rove beetles; staphylinids

Introduction

Rove beetles (Staphylinidae) are the largest family of organisms and dominate all ground-based cryptic microhabitats in every habitable landscape of the globe. Among insects, hyper-diverse families like rove beetles are the most difficult to analyse phylogenetically. They display an evolutionary radiation that took place 150–200 million years ago, since the fossil record indicates a notable diversity and abundance of Staphylinidae from at least the Late Jurassic (Solodovnikov et al. 2013). The overall pattern of rove beetle evolution is not well understood, and the phylogenetic system of this family is incomplete. Staphylinini, one of the largest tribes of rove beetles is an exception, however. It has recently been the focus of several phylogenetic studies involving adult morphological data, larval morphological data, the integration of adult morphologies of extinct and extant taxa, DNA sequences, as well as the integration of DNA sequences with adult morphology (e.g., Chani-Posse et al. 2018). Here we attempt to contribute a new set of data to the phylogeny of Staphylinini, relating to their pupae.

The difficulties of collecting and identifying pupae are due to their cryptic biology, and the need to link their morphology with the respective adults explains why little is known about the pupae and, in particular, why they have not been used for phylogenetic purposes.

In comparison with larvae or imagines, the pupae of Staphylinini are far poorer in morphological characters of diagnostic significance. The identification of pupae to species level is based on a small number of morphological characters revealed by morphometric analysis: the size and proportions of various body parts, the structure of the last abdominal segment, the structure and number of cuticular processes (including their range of variability), body microstructure and spiracular structure. Since rove beetles usually pupate in or near the habitats of their adults and larvae, the pupal biotope also provides a useful clue to their identification. Ecological data of this kind are especially helpful when comparing closely related species living in different habitats.

Knowledge of pupal morphology is fragmentary and varies in detail, depending on the subfamily. To date, pupae of the following subfamilies have been described, at least partially: Oxytelinae (almost 30 species), Steninae (6 species), Aleocharinae (a few species, only 3 in detail) and Paederinae (10 species), as well as Omaliinae, Tachyporinae, Scydmaeninae and Pselaphinae (single species) (e.g., Mank 1923; Hinton 1941; Welch 1966; Weinreich 1968; Żurańska 1973; Staniec 1993a, b, 1997, 2001b; Smoleński 1995; Carlton and Watrous 2009; Staniec et al. 2009a, 2010; Jałoszyński 2012; Zagaja et al. 2014). The most comprehensive knowledge of pupae is available for the subfamily Staphylininae, specifically its tribe Staphylinini, where the pupae of 103 species from 27 genera in 9 subtribes are known, albeit mostly from the Holarctic (Table 1).

In view of the above, the idea arose to compile a summary of existing knowledge of Staphylinini pupae. This is the first such comprehensive review worldwide dealing with Staphylinidae pupae. The main body of the paper is an illustrated key to assist the identification of known pupae of European Staphylinini at the subtribal and generic level. We also attempt to shed light on the potential importance of pupal characters in constructing phylogenetic hypotheses. This is the first attempt at applying the morphological characters of pupae to phylogenetic analysis and testing the hitherto accepted systematics at the generic level.

Table 1. Known pupal stages of Staphylinini species. Symbols: #–species considered in phylogenetic analysis. State of knowledge on morphology: very good-detailed and well-illustrated, descriptions reliable for diagnostics and sufficient for phylogenetic analysis; good-detailed enough descriptions with sufficient illustrations, reliable for diagnostics but not fully reliable for phylogenetic analysis; fair-moderately informative descriptions, maybe with sketchy illustrations (sometimes without), can be used for diagnostics but not for phylogenetic analysis; poor-hardly informative descriptions, mostly without any illustrations or no description with sketchy illustration, can be ambiguous even for diagnostic purposes.

Species	State of knowledge	References
Subtribe Acylophorina		
#*Acylophorus wagenschieberi* Kies.	good	Staniec (2005a)
Subtribe Amblyopinina		
#*Heterothops praevius* Er.	very good	Pietrykowska-Tudruj and Staniec (2006c)
Subtribe Cyrtoquediina		
#*Astrapaeus ulmi* (Rossi)	very good	Paulian (1941), Pietrykowska-Tudruj et al. (2014b)
Subtribe Erichsoniina		
Erichsonius alumnus Frank	fair	Schmidt (1996)
#*E. cinerascens* (Grav.)	very good	Pietrykowska-Tudruj and Staniec (2006c)
E. pusio (Horn)	poor	Schmidt (1994b)
Subtribe Philonthina		
Belonuchus rufipennis (Fabr.)	good	Mank (1923), Silvestri (1945)
Bisnius cephalotes Grav.	poor	Xambeu (1907)
#*B. fimetarius* (Grav.)	very good	Pietrykowska-Tudruj and Staniec (2011)
B. nitidulus (Grav.)	very good	Staniec and Kitowski (2004)
B. sordidus Grav.	poor	Xambeu (1907)
Cafius canescens (Mäklin)	poor	James et al. (1971)
C. lithocharinus (LeConte)	poor	James et al. (1971)
C. luteipennis Horn	poor	James et al. (1971)
C. seminitens Horn	poor	James et al. (1971)
Gabrius osseticus (Kolenati)	good	Pietrykowska-Tudruj and Staniec (2011)
G. astutus (Er.)	very good	Pietrykowska-Tudruj and Staniec (2010)
G. splendidulus (Grav.)	very good	Pietrykowska-Tudruj and Staniec (2007)
#*G. appendiculatus* Sharp	very good	Pietrykowska-Tudruj et al. (2014a)
#*Hesperus rufipennis* (Grav.)	good	Staniec (2004a)
Neobisnius sobrinus (Er.)	fair	Schmidt (1994a)
#*N. villosulus* (Steph.)	very good	Pietrykowska-Tudruj and Staniec (2007)
Philonthus albipes (Grav.)	good	Staniec (2002)
P. atratus (Grav.)	good	Pietrykowska-Tudruj and Staniec (2011)
P. carbonarius (Grav.)	good	Pietrykowska-Tudruj and Staniec (2011)
P. chopardi Cameron	poor	Tawfik et al. (1976a)
P. cognatus Steph.	good	Szujecki (1965)
P. corvinus Er.	good	Staniec (2003a)
P. cruentatus Gmelin	fair	Hunter et al. (1989)
P. cyanipennis Fab.	poor	Mank (1923)
P. debilis (Grav.)	good	Pietrykowska-Tudruj and Staniec (2011)
#*P. decorus* (Grav.)	very good	Verhoeff (1918), Pietrykowska-Tudruj and Staniec (2011)
P. flavolimbatus Er.	fair	Hunter et al. (1989)
P. fumarius (Grav.)	good	Staniec and Pietrykowska-Tudruj (2008b)
P. laminatus Creutzer	poor	Xambeu (1907, 1910)
P. lepidus (Grav.)	good	Staniec and Kitowski (2004)
P. longicornis Steph.	fair	Mank (1923), Tawfik et al. (1976b)
P. micans (Grav.)	good	Staniec (2003a)
P. monivagus Heer	poor	Xambeu (1900, 1907)
P. natalensis Boheman	?	Prins (1984)

Species	State of knowledge	References
P. nigrita (Grav.)	very good	Staniec (2001a), Staniec and Pietrykowska-Tudruj (2008a)
P. nitidus (Fabr.)	poor	Verhoeff 1920 (1919)
P. politus (L.)	very good	Pietrykowska-Tudruj and Staniec (2010)
P. punctus (Grav.)	good	Staniec (2003a)
P. quisquiliarius (Gyll.)	good	Hafez (1939), Staniec (2001a)
P. rectangulus Sharp	very good	Staniec (2004b)
P. rubripennis Steph.	very good	Staniec and Pietrykowska-Tudruj (2007)
P. sanamus Tott.	fair	Byrne (1993)
P. sanguinolentus (Grav.)	poor	Xambeu (1907, 1910)
P. sericans Grav.	poor	Mank (1923)
P. splendens Fabr.	poor	Xambeu (1894–97, 1907)
P. succicola Thoms.	good	Staniec (1999b, 2004b)
P. tenuicornis Rey	good	Staniec and Pietrykowska (2005a)
P. turbidus Er.	fair	Tawfik et al. (1976c)
P. umbratilis (Grav.)	good	Staniec and Kitowski (2004)
P. varians Payk.	very good	Xambeu (1907), Staniec (2002)
#*Rabigus tenuis* (Fabr.)	very good	Staniec and Pietrykowska-Tudruj (2008c)
Remus sericeus Holme	fair	Paulian (1941)
Subtribe Quediina		
#*Quedionuchus plagiatus* Mann.	good	Saalas (1917), Staniec (1996)
Quedius abietum Kies.	poor	Xambeu (1900)
Q. brevicornis (Thom.)	good	Drugmand (1988), Staniec (2003b)
Q. brevis Er.	very good	Pietrykowska-Tudruj and Staniec (2006b)
Q. capucinus (Grav.)	poor	Voris (1939b)
Q. cruentus (Ol.)	good	Staniec and Pietrykowska (2005b)
Q. curtipennis Bernh.	good	Outerelo (1978)
#*Q. cinctus* (Payk.)	very good	Pietrykowska-Tudruj and Staniec (2010)
Q. dilatatus (Fabr.)	poor	Strassen (1957), Vorst and Heijerman (2015)
Q. fulgidus Fabricius	poor	Xambeu (1910)
Q. fuliginosus (Grav.)	good	Staniec (1999a)
#*Q. fumatus* (Steph.)	good	Staniec (1999a)
Q. humeralis Steph.	good	Staniec (1999a)
Q. levicollis Brullé	poor	Waterhouse (1836), Lesne (1890)
#*Q. microps* (Grav.)	very good	Pietrykowska-Tudruj and Staniec (2006b)
Q. mesomelinus (Marsh.)	good	Staniec (1999a)
Q. molochinus (Grav.)	poor	Voris (1939b)
Q. ochripennis Ménétriés	fair	Falcoz (1914), Xambeu (1899, 1910), Beier and Strouhal (1928)
Q. umbrinus Er.	poor	Mjöberg (1906)
Q. scintillans Grav.	poor	Perris (1853)
Q. semiobscurus Marsh.	poor	Xambeu (1910)
Q. spelaeus spelaeus Horn.	good	Moseley et al. (2006)
Subtribe Staphylinina		
#*Abemus chloropterus* (Panz.)	fair	Boháč (1982)
#*Creophilus maxillosus* (L.)	poor	Dajoz and Caussanel (1968), Voris (1939a), present study
Emus hirtus (L.)	poor	present study
Hadropinus fossor Sharp	fair	Shibata (1965)
Ocypus aeneocephalus (De Geer)	poor	Boháč (1982)
#*O. fulvipennis* (Er.)	good	Staniec et al. (2009b)
O. fuscatus (Grav.)	fair	Boháč (1982)
O. italicus (Arag.)	poor	Boháč (1987)
O. nitens Schrank	good	Verhoeff (1918), Boháč (1982), present study
O. olens (O. F. Müll.)	poor	Orth et al. (1976)
#*Ontholestes murinus* (L.)	good	Staniec (2004b)

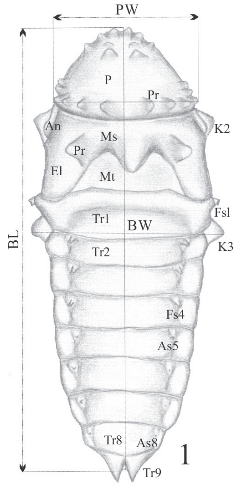
Species	State of knowledge	References
O. cingulatus (Grav.)	poor	Voris (1939a)
#*Platydracus tomentosus* (Grav.)	fair	Voris (1939a), Schmidt (1994b)
P. cinnamopterus (Grav.)	?	Lesage (1977)
P. comes (LeConte)	poor	Voris (1939a)
P. maculosus (Grav.)	poor	Voris (1939a)
P. viridanus Horn	poor	Voris (1939a)
Staphylinus caesareus Ced.	fair	Boháč (1982)
#*S. erythropterus* L.	very good	Szujecki (1960), Boháč (1982), Pietrykowska-Tudruj and Staniec (2012)
#*Tasgius melanarius* (Herr)	very good	Staniec and Pietrykowska (2005b)
Subtribe Tanygnathina		
#*Atanygnathus terminalis* (Er.)	very good	Staniec (2005b)
Subtribe Xanthopygina		
Smilax deneinephyto Chatzimanolis	poor	Eidmann (1937)
Triacrus dilatus Nordm.	poor	Wasmann (1902)

Material and methods

Description of pupal morphology and key to subtribes and genera of the tribe Staphylinini

The diagnostic characters given in this paper were established generally on the basis of current knowledge of the pupal stage in Staphylinini. The key covers 8 subtribes and 20 genera (highlighted in Table 1 by an asterisk) of pupae found in Europe. Most of the data and all the drawings have been taken from papers by the present authors, published between 1996–2014. Some information relating to *Abemus* and in part to: *Creophilus*, *Ocypus*, *Philonthus*, *Platydracus*, *Quedius* and *Staphylinus*, has been taken from papers by other authors (e.g., Szujecki 1965; Dajoz and Caussanel 1968; Orth et al. 1976; Boháč 1982, 1987; Schmidt 1994b; Moseley et al. 2006; Vorst and Heijerman 2015). The paper also includes new information on the pupa of *Emus hirtus* (L.) which was hitherto unknown, and photographs and notes supplementing existing descriptions of the pupae of nine genera, represented by species: *Acylophorus wagenschieberi* Kies., *Creophilus maxillosus* (L.), *Gabrius appendiculatus* Sharp, *Ocypus fulvipennis* (Er.), *Quedius microps* (Grav.), *Rabigus tenuis* (Fabr.) and *Staphylinus erythropterus* L. The photographs were taken with an Olympus DP72 digital camera mounted to an Olympus SZX16 compound microscope (Fig. 7) or with a VEGA3 TESCAN SEM (Figs 2a, 2b, 6b, 13a, 14a), and corrected using CorelDRAW Graphics Suite X6.

Material that was here examined for the first time includes one pupa of *C. maxillosus* (male) and one exuvium of *E. hirtus*, obtained from the collection of the Zoological Museum of the University of Copenhagen, Denmark (**NHMD**, the Natural History Museum of Denmark). The pupae of these four species, as well as others previously described by the authors, are deposited in the collection of the Department of Zoology, Marie Curie Skłodowska University, Lublin, Poland.



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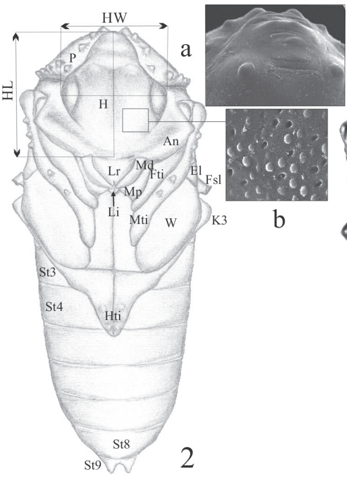

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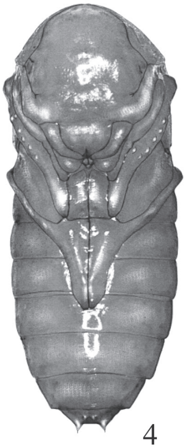
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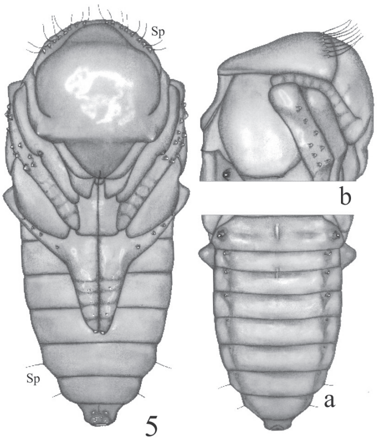

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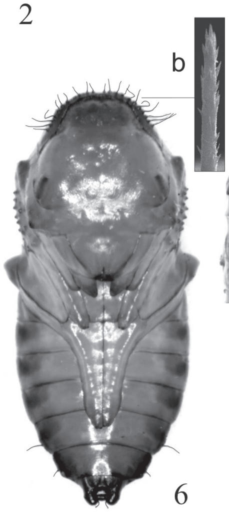

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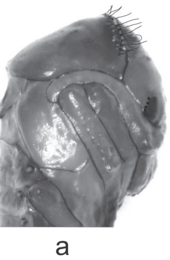

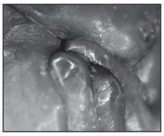
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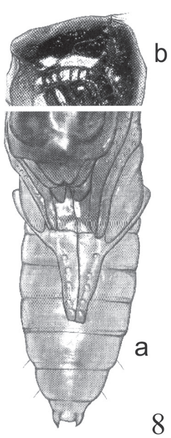

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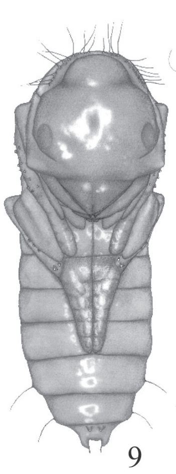
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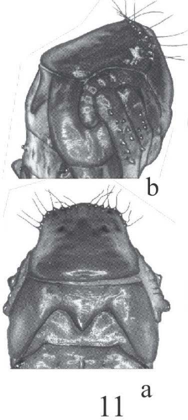

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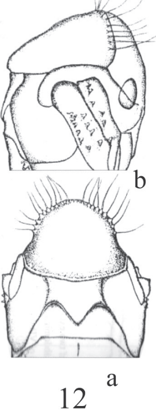

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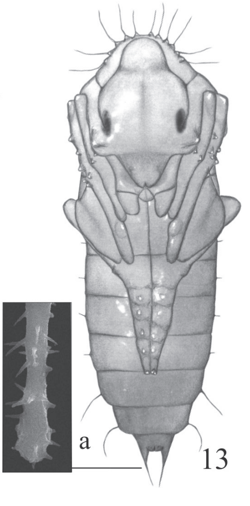

13

Terminology, measurements and abbreviations

The terminology follows Staniec (1999a, b, 2002) and Pietrykowska-Tudruj and Staniec (2011). Measurements and their abbreviations are after Pietrykowska-Tudruj and Staniec (2012) and Pietrykowska-Tudruj et al. (2014b) marked on Figures 1, 2, 5, 18, 26, and 32 as follows: **BL** body length, **BW** body width, **HW** head width, **HL** head length, **PW** pronotum width, Abbreviations of the body parts as follows: **A** accessory, **An** antenna, **As** atrophied spiracle, **El** elytra, **Fs** functional spiracle, **Fti** fore tibia, **H** head, **Hti** hind tibia, **K** knee, **Li** labium, **Lr** labrum, **Md** mandible, **Mp** maxillary palp, **Ms** mesonotum, **Mt** metanotum, **Mti** mid tibia, **P** pronotum, **Pr** protuberance, **Sap** spiracular appendage, **S** spine, **Sp** setiform projection, **St** sternite, **Tp** terminal prolongation, **Tr** tergite, **W** wing, **Vp** ventral prolongation.

Phylogenetic analysis

The phylogenetic analysis was carried out at the generic level. A data matrix was assembled in Nexus Data Editor for Windows v. 0.5.0 (Page 2001) that included 29 morphological characters of the well-known pupae from 22 species as the ingroup belonging to 20 genera of Staphylinini from 8 subtribes (Table 4). Each genus is represented by one species, except for *Quedius* (three species from the subgenera *Distichalius*, *Raphirus*, *Microsaurus*). As the pupae of the species from these three subgenera differ in certain morphological characters, they have been included in the data matrix. Some genera (*Belonuchus*, *Cafius*, *Emus*, *Remus*, *Smilax*, *Triacrus*, *Hadropinus*) have not been included because the available morphological data on their pupae are too fragmentary and superficial, and therefore deemed unreliable. The pupa of *Hypnogyra angularis* (Ganglbauer, 1895) from the tribe Xantholinini is added as an outgroup to the tribe Staphylinini (Staniec and Pietrykowska 2005a, Pietrykowska-Tudruj and Staniec 2006a, unpublished data). Inapplicable characters are assigned a gap value ('–') and treated as equivalent to missing data ('?'). The matrix was analysed in TNT (Goloboff et al. 2008) under settings as follows: the 'traditional search' option for the parsimony

Figure 1–13. Pupae; dorsal aspect (**1, 3**), ventral aspect (**2, 4, 5, 6, 9, 10, 13**), lower part in dorsal aspect (**5a**), lower part in ventral aspect (**8a**), upper part in dorsal aspect (**11a, 12a**), upper part in lateral aspect (**5b, 6a, 11b, 12b**), cuticular projection (**2a, 6b, 13a**), microstructure of the head (**2b**) **1–2b** *Acylophorus wagenschieberi* **3, 4** *Astrapaeus ulmi* **5–5b** *Ontholestes murinus* **6–6b** *Staphylinus erythropterus* **7** *Creophilus maxillosus*, tibiae I and II and antenna **8a, b** *Ocypus fulvipennis* **9** *Tasgius melanarius* **10** *Neobisnius villosulus* **11a, b** *Philonthus decorus* **12a, b** *P. succicola* **13, 13a** *Rabigus tenuis*. Abbreviations: An antenna, As atrophied spiracle, BL body length, BW body width, El elytra, Fs functional spiracle, Fti fore tibia, H head, HL head length, Hti hind tibia, HW head width, K knee, Li labium, Lr labrum, Md mandible, Mp maxillary palp, Ms mesonotum, Mt metanotum, Mti mid tibia, P pronotum, Pr protuberance, PW pronotum width, Sp setiform projection, St sternite, Tr tergite, W wing.

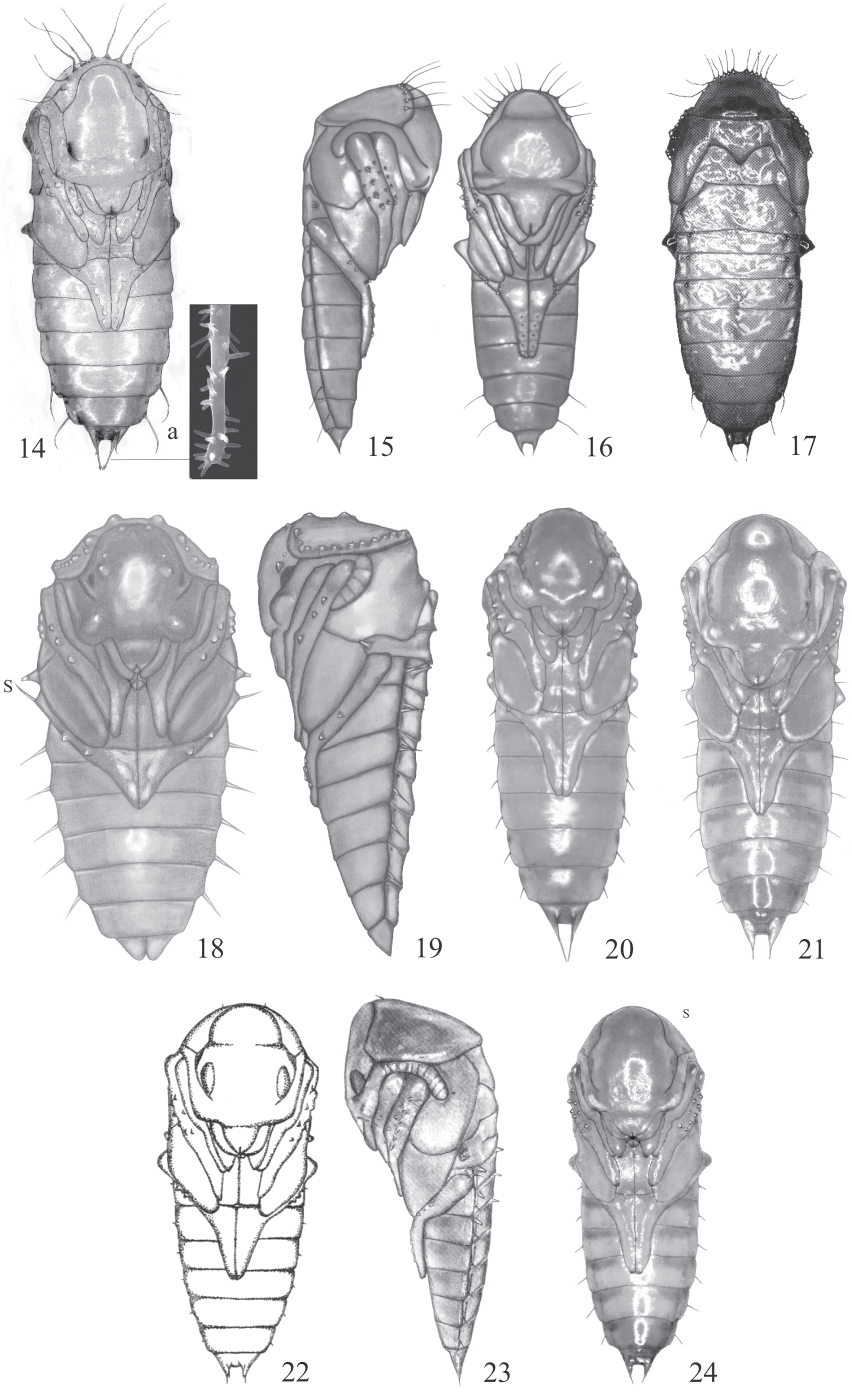

Figure 14–24. Pupae; dorsal aspect (**17**), lateral aspect (**15, 19, 23**), ventral aspect (**14, 16, 18, 20, 21, 22, 24**), accessories of terminal prolongation (**14a**) **14, 14a** *Gabrius appendiculatus* **15, 16** *Hesperus rufipennis* **17** *Bisnius fimetarius* **18, 19** *Atanygnathus terminalis* **20** *Heterothops praevius* **21** *Erichsonius cinerascens* **22** *Quedius fumatus* **23** *Q. cinctus* **24** *Q. microps*.

analysis – 1000 replicates with tree bisection reconnection (TBR) branch swapping and saving 1000 trees per replicate, zero-length branches collapsed, all characters were treated as unordered and equally weighted.

Results

Diagnostic description of pupae of the tribe Staphylinini

Pupa obtect. Body clearly slender, almost cylindrical and weakly sclerotised (e.g., *Neobisnius*), or moderately elongate, slender and moderately sclerotised (e.g., *Erichsonius*, *Gabrius*, *Heterothops*, some species of *Philonthus*), or moderately stocky and well sclerotised (e.g., *Astrapaeus*, *Quedius*) to extremely stocky and strongly sclerotised (e.g., *Atanygnathus*). Colour: almost white or pale yellow shortly after pupation; from dark yellow to reddish brown a few days after pupation; usually almost black just prior to emergence of imago.

Head directed ventrally towards thorax, without any setiform projection or spines, rarely with a few protuberances. Labrum usually V-shaped, exceptionally U-shaped, with short, longitudinal groove running from its anterior margin. Mandibles elongate, usually pointing posteriorly, falcate or almost straight. Maxillae usually moderately long. Antennae curved, rest on knees of fore and mid legs; apex usually protruding beyond knee of mid tibia. Scutiform pronotum widest at the base, usually about as wide as long with 6–32 setiform projections, or a pair of micro spines or 8–26 protuberances, sometimes with no structures. Mesonotum separated from pronotum by a furrow, distinctly wider than long. Metanotum narrower than mesonotum with deeply bisinuate anterior margin. Elytra shortened. Wings protruding to ventral side. Apex of wings protruding at most beyond posterior margin of I (morphologically III), clearly visible abdominal segment. Tibiae and tarsi directed obliquely towards body middle. All tibiae, or only some of them with pointed protuberances. Hind tarsi at most reaching midpoint of V (morphologically VII), clearly visible abdominal segment.

Abdomen with 9 somewhat flattened tergites and 7 convex sternites visible. Abdominal tergite I wider than others and about twice as long as tergite II. Abdominal shape of three kinds: arcuate, with parallel sides or funnel-shaped. Sides of abdomen with: spines on segments II–VIII or II–VII, or setiform projections on segments III–VIII or VII–VIII. Rarely abdomen without any lateral cuticular projections. Last segment usually strongly protruding into two terminal, elongated prolongations, sometimes weakly protruding into two triangular prolongations, exceptionally without prolongations. Terminal sternite with well-marked sexual dimorphism. Gonotheca in female double, in male single. In female pupae, terminal sternite often with a pair of prolongations. Abdominal tergites I–IV with tuberculate, functional spiracles, the first pair usually situated more laterally, most often larger and protruding farther than the others; tergites V–VIII with externally visible, but apparently atrophied spiracles.

Comparison

The following crucial characters distinguish the pupae of the tribe Staphylinini from the tribe Xantholinini within the subfamily Staphylininae for which the pupae are known: abdominal segments divided laterally into ventral and dorsal sclerites (not grown into uniform rings); body with setiform projections, spines or protuberances, apart from the genus *Astrapaeus* which has no cuticular processes (Staniec and Pietrykowska 2005a, Pietrykowska-Tudruj and Staniec 2006a). The combination of characters distinguishing the pupae of Staphylinini within the family Staphylinidae, i.e., the subfamilies Aleocharinae, Omaliinae, Oxyporinae, Oxytelinae, Paederinae, Steninae and Tachyporinae for which the pupae are known, includes: i. exarate pupa; ii. no projections whatsoever on head; iii. short labium; iv. lack of short setae on dorsal and/or ventral part of abdominal sclerites; v. lack of setae on hind margin of prothorax.

Morphological types of pupae of the tribe Staphylinini

Based on current knowledge of the pupal morphology of Staphylinini species, eight morphological types were distinguished: Acylophorus (genus: *Acylophorus*), *Astrapaeus*

Table 2. Characters of the morphological types of pupae of the tribe Staphylinini. Symbols and abbreviations: N number, MS moderately stocky, MSc moderately sclerotized, HS heavily stocky, SSc strongly sclerotised, WS well stocky, WSc well sclerotized, abs absent, pre present, S spine, Sp setiform projection, – no data, for abbreviations of the body parts see Material and methods.

Type of pupa	Body shape/ cuticula	Cuticular processes (Cp)		Protuberance location	Segment IX: Vp ♀ (A)/Tp ♀♂ (A)	Special characteristic
		pronotum	abdomen			
		type: amount/ length/shape/A	type: amount/N of Sg with Cp/ length/ shape/A			
Acylophorus	MS/SSc	abs	abs	H, P, Ms*	pre (-)/ pre (abs)	H rhomboidal, Ist pair of Fs distinctly bigger than the others
Astrapaeus	MS/WSc	abs	abs	Mti, Hti	abs/pre (pre)	Lr U-shaped
Atanygnathus	HS/SSc	abs	S: 12/II–VII/long/ straight/pre	P, Ms**	abs/pre (abs)	Md rounded apically, Mp strongly elongate, Sap of Fs, Ist pair of Fs strongly protruding laterally
Erichsonius	MS/MSc	abs	S: 14/I–III/equal/ straight/pre	P, Mti	pre (usually abs)/ pre (usually abs)	–
Heterothops	MS/MSc	abs	S: 14/II–VIII/ equal/ straight/ pre	H, P, Mti, Hti	pre (pre)/pre (pre)	H small, W short
Philonthus	diverse character	Sp: 6–24 / long/ usually decurved/-	Sp: 12/III–VIII or 4/ VII–VIII/ short III–VI; long VII–VIII/ straight III–VI; curved VII–VIII /pre	Ft, Mti, Hti	pre (pre)/pre (pre)	=
Quedius	MS or WS/ MSc or SSc	abs or pre S: 2/ tiny•/straight/-	S: 14 II–VIII/ equal/ straight/ smooth#.	Mti, Hti	pre (abs #)/pre (abs #)	–
Staphylinus	WS/WSc	Sp: 12–32/ short••/straight or slightly wavy/-	Sp: 4 VII–VIII/ short/ straight or slightly decurved/-	–	all characteristic pre or abs	H relatively wide

*the number of protuberances 2, 26, 3, respectively, **the number of protuberances 7, about 26, 3, respectively, •45–144 µm, ••length less than half of pronotum; #exceptionally in *Q. plagiatus* with accessory (species: *Astrapaeus ulmi* (Rossi)), Atanygnathus (species: *Atanygnathus terminalis* (Er.)), Erichsonius (genus: *Erichsonius*), Heterothops (genus: *Heterothops*), Philonthus (genera: *Bisnius*, *Gabrius*, *Hesperus*, *Neobisnius*, *Philonthus* and *Rabigus*), Quedius (genera: *Quedius* and *Quedionuchus*) and Staphylinus (genera: *Abemus*, *Creophilus*, *Emus*, *Ocypus*, *Ontholestes*, *Staphylinus* and *Tasgius*). These types take into consideration pupae from 20 genera, most of which have been described by the present authors. The diagnosis of the types is presented in Table 2.

Key to pupae of Staphylinini

Key to subtribes of Staphylinini

1 Body without any spines or setiform projections **2**
– Pronotum or/and abdomen with spines or setiform projections **3**
2 Pronotum, head and mesonotum with protuberances (Figs 1, 1A, 2). Abdomen tapering gradually from first to last (IX) segment (Fig. 1) .... **Acylophorina**, genus: *Acylophorus* Nordmann, 1837
– Pronotum without any protuberances (Figs 3, 4). Abdomen arcuate, widening gradually from segment I to V, then tapering to terminal segment .... **Cyrtoquediina**, genus: *Astrapaeus* Gravenhorst, 1802
3 Pronotum with setiform projections located on protuberances at anterior margin (Fig. 28) **4**
– Pronotum without setiform projections, at most with a pair of tiny spines (S) (Figs 22, 23, 29) or globular protuberances (Figs 18–20) **5**
4 Setiform projections on pronotum (Figs 5–9) straight or slightly wavy and short, shorter than half pronotum length. Abdominal segments VII–VIII, each with a pair of usually short, straight or slightly curved setiform projections (Sp) (Figs 5, 6, 8a) .... **Staphylinina**
– Setiform projections on pronotum (Figs 10–17) distinctly wavy and long, at least as long as half pronotum length, lateral projections usually distinctly decurved. Abdominal segments III–VIII or VII–VIII each bearing a pair of setiform projections or (exceptionally) spines .... **Philonthina**
5 Abdominal segments II–VII each with a pair of spines on sides (Figs 18, 19). Functional spiracles of abdominal segments II–IV each with a unique appendage (Fig. 32) .... **Tanygnathina**, genus: *Atanygnathus* Jakobson, 1909
– Abdominal segments II–VIII each with a pair of spines on sides (Figs 22–24). Functional spiracles of abdominal segments II–IV without appendages **6**
6 Pronotum without protuberances .... **Quediina**
– Pronotum with tiny, globular protuberances (Fig. 20) **7**
7 Pronotum at most with 10 protuberances, head large, without protuberances (Fig. 21) .... **Erichsoniina**, genus: *Erichsonius* Fauvel, 1874
– Pronotum with at least 20 protuberances, head small, with 2 protuberances (Fig. 20) .... **Amblyopinina**, genus: *Heterothops* Stephens, 1829

Key to genera of Staphylinini

Subtribe Staphylinina

1 Abdominal segment IX without terminal prolongations (Fig. 25) **2**

– Abdominal segment IX with a pair of short terminal prolongations, each protruding into two recurved accessories (A) (Figs 26–27) **4**

2 Pronotum with 13–16 setiform projections. Head relatively wide (Fig. 5). Antennae short, at most reaching apex of mid tibia (Fig. 5b). Abdomen tapering below segment IV (Fig. 5a). Terminal sternite of female as in Fig. 25. Pupal cocoon as in Fig. 33. BL: 8.63–9.25 mm; BW: 4.00–4.50 mm; HW: 2.70–3.00 mm; PW: 2.80–3.00 mm. Biotope: remains of large animals, excrement and decaying plant matter .... ***Ontholestes* Gangalbauer, 1895**

– Pronotum with more than 16 setiform projections **3**

3 Pronotum with 20 setiform projections. Antennae protruding slightly beyond apex of mid tibia. Abdomen tapering below segment IV. BL: 7.5 mm. Biotopes: old deciduous forests, rotting remains of deciduous trees, moss at the base of trees, leaf litter and decaying plant matter .... ***Abemus* Mulsant & Rey, 1876**

– Pronotum with about 30 setiform projections; two specimens examined. Biotopes: open and wooded areas, excrement, decaying plant matter and carrion... ***Emus* Leach, 1819**

4 Antennae reaching at most to apex of mid tibia (Fig. 7). Body relatively stocky. Pronotum with 22–32 setiform projections at the anterior margin. Hind legs reaching half way along abdominal sternite VI (well visible IV). BL: 11–15 mm. Biotopes: remains of large animals, excrement and decaying plant matter... ***Creophilus* Leach, 1819**

– Antennae protruding at least slightly beyond apex of mid tibia (Figs 6a, 8b). If antenna reaching at most to apex of mid tibia, then pronotum with under 20 setiform projections .... ***Ocypus* Leach, 1819, *Staphylinus* Linnaeus, 1758, *Tasgius* Stephens, 1829**

Subtribe Philonthina

1 Body slender, lateral margins almost parallel (Fig. 10). Colour light yellow, cuticula weakly sclerotised. Head 1.7 times as long as wide. Antennae half as long as elytra. Pronotum 1.5 times as broad as long, with long 7–8 setiform projections. Hind tarsi reaching posterior margin of abdominal sternite V (well visible III). BL: 2.76–3.22 mm; BW: 0.91–1.07 mm. Biotopes: usually moist river and stream banks, under plant debris, slime and stones .... ***Neobisnius* Gangalbauer, 1895**

– Body moderately slender or stocky, abdomen tapering below abdominal segments III or V to terminal prolongations **2**

2 Segments III–VI each with short spines (Fig. 13). Pupal cocoon present (Fig. 34). Antennae nearly two-thirds as long as elytra. Pronotum with 10–12 setiform projections. Mid tarsi protruding distinctly beyond posterior margin of abdominal sternite III (well visible I) (Fig. 13). Spines of segments III–VI smooth, at least 3 x shorter than segment. BL: 3.4–3.8 mm. BW: 1.51–1.72 mm; HW: 0.73–0.82 mm; BW: 0.90–1.00 mm. Biotopes: sunny, moist places, on clayey and loess soil, sparsely covered by grasses or devoid of any vegetation, in plant debris, under stones........***Rabigus*** **Mulsant & Rey, 1876**

– Segments III–VI each with setiform projections (Fig. 13) or without any cuticular projections. Pupal cocoon only exceptionally present **3**

3 Labrum elongated (Figs 15, 16). Mandibles in both sexes long, crossed in apical part. Antennae not reaching midpoint of elytra (Fig. 15); Pronotum with 10–15 setiform projections. Hind tarsi reaching midpoint of abdominal sternite VI (well visible IV) (Fig. 16). Abdomen relatively slender, tapering below segment V. Sternite IX in female without ventral prolongations; terminal abdominal prolongation (Tp) sharpened apically. BL: 6.75–7.25 mm; BW: 2.75–2.95 mm; HW: 1.78–2.08 mm; PW: 1.88–2.13 mm. Biotopes: decaying, deciduous trees...***Hesperus*** **Fauvel, 1874**

– Labrum not elongated (Fig. 14). Mandibles usually short, exceptionally only crossed in male pupa...***Bisnius*** **Stephens, 1829,** ***Gabrius*** **Stephens, 1829,** ***Philonthus*** **Stephens, 1829**

Subtribe Quediina

1 Abdominal spines and apical projections with sparse, tiny protuberances (Fig. 30). Body relatively slender. Antennae protruding slightly beyond apex of mid tibia. Hind tarsi reaching posterior margin of abdominal segments V (well visible III). BL: 5.5–6.0 mm; BW: 2.0–2.2 mm; HW: 1.5–1.6 mm. Biotopes: under bark of *Picea*, *Abies*, *Fagus* and *Acer*.................................... .... ***Quedionuchus*** **Sharp, 1884**

– Abdominal spines and apical projections smooth, without protuberances (Fig. 31).. ***Quedius*** **Stephens, 1829**

Characters for phylogenetic analysis

1. Setiform projections or spines on the body (excluding segment IX): 0. absent, 1. present.
2. Protuberances on head: 0. absent, 1. present.
3. Number on protuberances on head: 0. 2, 1. 7.
4. Labrum, shape in outline: 0. U-shaped, 1. V-shaped.
5. Mandibles, shape of apices: 0. rounded, 1. pointed.

6. Maxillary palps, length: 0. protruding beyond apices of mid legs, 1. not protruding beyond apices of mid legs.
7. Antennae length: 0. at most reaching apices of mid tibiae, 1. protruding beyond apices of mid tibiae.
8. Protuberances on pronotum: 0. absent, 1. present.
9. Number of protuberances on pronotum: 0. about 10, 1. about 25.
10. Setiform projections on pronotum: 0. absent, 1. present.
11. Number of setiform projections on pronotum: 0. four, 1. more than four.
12. Length of setiform projections on pronotum: 0. shorter than half pronotum length, 1. at least half as long as pronotum.
13. Spines on pronotum: 0. absent, 1. present.
14. Protuberances on mesonotum: 0. absent, 1. present.
15. Pairs of tibiae with protuberances: 0. only mid, 1. mid and fore, 2. mid and hind, 3. all pairs.
16. Position of hind tarsi in relation to abdominal segments: 0. not adhering to abdomen, 1. adhering to abdomen.
17. Wing length: 0. at most reaching hind margin of segment III (I visible), 1. protruding beyond hind margin of segment III (I visible).
18. Structure of abdominal segments: 0. tergites and sternites fused into uniform rings, 1. tergites and sternites separate.
19. Abdomen shape: 0. gradually tapering from first to last segment, 1. tapering to last segment only in hind part.
20. Cuticular processes on sides of abdominal segment VII: 0. absent, 1. present.
21. Type of cuticular processes on sides of abdominal segment VII: 0. setiform projections, 1. spines.
22. Length of projections as spines on abdominal segment VII in relation to the width of that segment: 0. tiny, 1. long.
23. Cuticular processes on sides of abdominal segment VIII: 0. absent, 1. present.
24. Segment IX, terminal prolongations: 0. absent, 1. present.
25. Terminal prolongations, apical accessories: 0. absent, 1. present.
26. Apical accessories, shape: 0. straight, 1. curved.
27. Apical accessories, apex: 0. pointed, 1. rounded.
28. First pairs of spiracles, position: 0. in the same longitudinal line as others, 1. protruding laterally much more than others.
29. Appendages at functional spiracles of abdominal segments II–IV: 0. absent, 1. present.

Phylogenetic analysis

The parsimony analysis retrieved 100 most parsimonious trees. The 50% majority rules consensus tree showed the following: i) separation of *Astrapaeus* from a clade of all other Staphylinini; ii) a well-supported clade of *Erichsonius+Heterothops+Atanygnath us+ Acylophorus*; iii) a well-supported clade of Staphylinini propria represented here by the subtribes Philonthina and Staphylinina (Fig. 35).

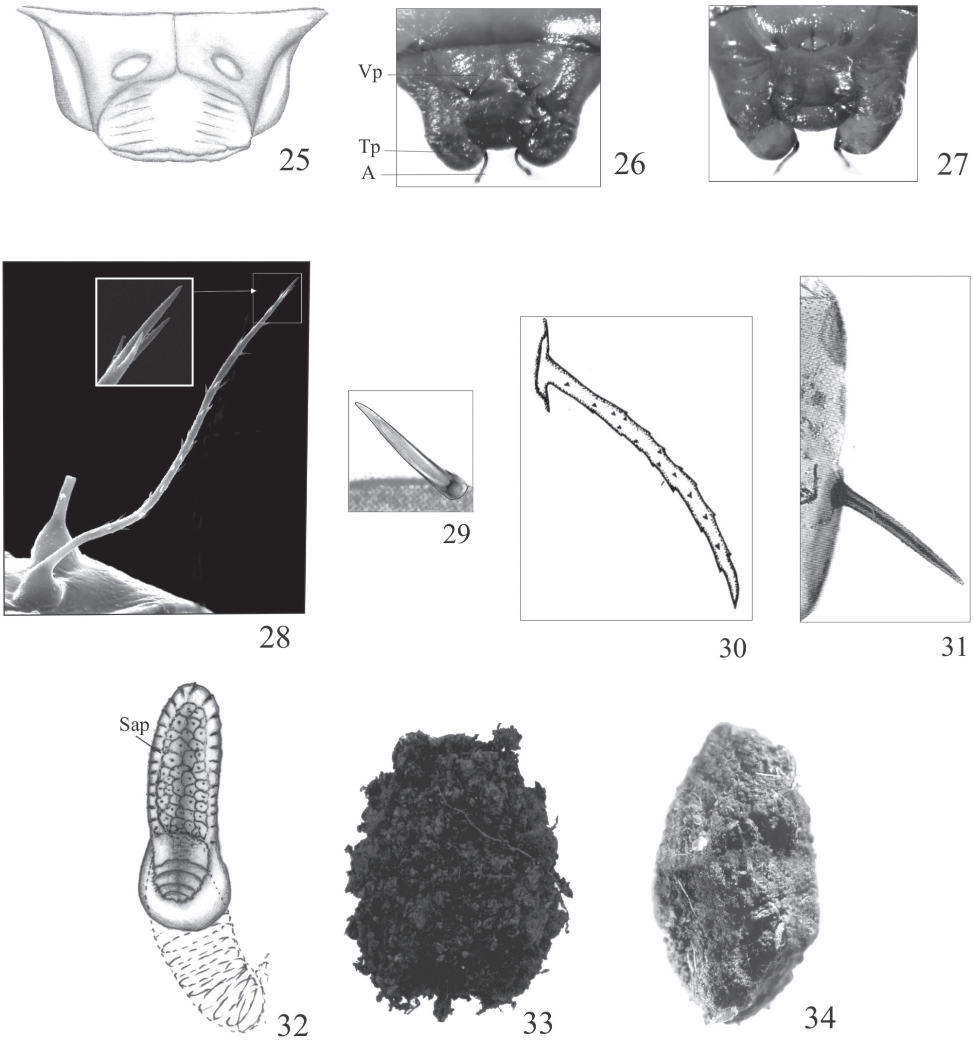

Figure 25–34. Pupae; terminal sternites (**25–27**), setiform projection of pronotum (**28**), spine of pronotum (**29**), spine of abdomen (**30, 31**), functional spiracle (**32**). Cocoon (**33, 34**) **25** *Ontholestes murinus*, female **26, 27** *Staphylinus erythropterus*, female (**26**), male (**27**) **28** *Gabrius appendiculatus* **29** *Quedius cinctus* **30** *Quedionuchus plagiatus* **31** *Quedius cinctus* **32** *Atanygnathus terminalis*, III pair **33** *Ontholestes murinus* **34** *Rabigus tenuis*. Abbreviations: A accessory, S spine, Sap spiracular appendage, Tp terminal prolongation, Vp ventral prolongation.

Discussion

Pupal morphology as an adaptation to environment

The occurrence of obtect pupae in the subfamily Staphylininae (including the tribe Staphylinini) is exceptional compared to other rove beetles and the majority of Coleoptera. The obtect pupa type with a compact body and usually heavily sclerotised cuticle

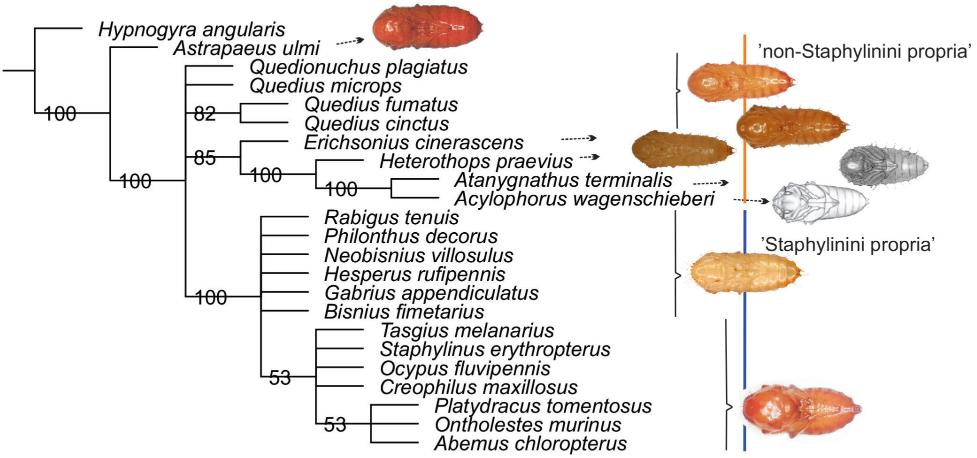

Figure 35. Fifty per cent majority rules consensus tree from a maximum parsimony analysis of 22 species of Staphylinini pupae.

appears to be far more resistant to negative impacts like attack from predators, parasitoids or mechanical damage. Therefore, an obtect pupa is probably a defensive adaptation. Presence of a tough cuticle in a pupa reduces the need for fully-grown larvae to construct a protective pupal cocoon, such as has been reported in a few Staphylinini species (*G. splendidulus* (Grav.), *O. murinus* (L.), *R. tenuis*) (Staniec 2004b; Pietrykowska-Tudruj and Staniec 2007; Staniec and Pietrykowska-Tudruj 2008c). Pupal cocoons are often encountered in representatives of the Aleocharinae, which have free, delicate pupae with weakly sclerotised cuticle (Frank and Thomas 1984; Staniec et al. 2010; Zagaja et al. 2014).

Another form of defensive adaptation is cuticular processes, which occur on various parts of the body (pronotum, abdominal segments) in almost all known pupae of the Staphylinini. They can take several forms: flexible, often arcuate setiform projections (e.g., *Gabrius*, *Philonthus*, *Staphylinus*), stiff spines (e.g., *Quedius*, *Erichsonius*, *Heterothops*) and, more rarely, convex protuberances (e.g., *Acylophorus*). These structures, besides having a defensive function (Hinton 1955), appear to minimise direct contact between the pupal body surface and the surrounding substrate (e.g., soil, leaf litter, plant remains, decaying wood), which probably allows the spiracles to function more efficiently. The number, length and shape of setiform projections or spines are also usually associated with the degree of cuticular sclerotisation and pupal body size. Their presence is particularly important for pupae with a weakly sclerotised cuticle, less resistant to damage. For this reason, such pupae usually bear numerous long, flexible, arcuate (especially on the pronotum) setiform projections (e.g., *G. splendidulus*, *N. villosulus* (Steph.) and most *Philonthus* species). In contrast, pupae with highly sclerotised cuticle bear far fewer such structures on the pronotum (e.g., *Quedius* sp. and *A. terminalis*), and in a few cases, the entire body surface is devoid of them (e.g., *A. wagenschieberi* and *A. ulmi*).

The number of setiform projections on the pronotum is a variable character within a species, the range of which is usually small (e.g., *R. tenuis* – 10–12, *P. nigrita* (Grav.)

– 9–13), but occasionally much larger (e.g., *P. rectangulus* – 13–21, *P. politus* (L.) – 15–22). On small pupae there are usually fewer setiform projections on the pronotum (e.g., *G. splendidulus* – 7–9, *N. villosulus* – 7–8) than on much larger ones (*S. erythropterus* – 19–25, *P. decorus* (Grav.) – 22–24), a fairly obvious observation.

The pupae of some species from very wet habitats (*Atanygnathus terminalis*, *Acylophorus wagenschieberi*) exhibit special adaptations to their environment in the structure and localisation of spiracles (Staniec 2005a, b). They pupate among unsubmerged peat mosses (*Sphagnum*). However, water levels frequently vary in the peat-bogs they inhabit. The first pair of spiracles is thus especially large and protrudes strongly from the body outline, which facilitates gas exchange even at high levels of moisture. In the case of *A. terminalis*, the next three active spiracles additionally bear peculiar phylliform lobes. These probably serve to accumulate a supply of air in case the peat mosses are suddenly flooded or act as an additional respiratory surface (Staniec 2005b).

Some comments on pupation

In the natural environment, larvae and adults of Staphylinini mostly live in the same microhabitats; pupation usually takes place there, too (Table 3). Only in the case of the above-mentioned species from wet microhabitats subject to flooding were pupae found in distinctly drier locations than those inhabited by mobile adults or larvae. These were usually unsubmerged layers of peat mosses, directly adjacent to higher-lying areas of bogs, 2–3 m from small bodies of standing water (Staniec 2005a, b). In the field, pupae of Staphylinini were found from spring to autumn (IV–X), although the pupae of most *Quedius* species were found in spring (IV–V). Among *Quedius*, only species confined to special microhabitats, such as tree hollows, crevices under tree bark or the vicinity of hornets' nests, pupated during the summer (VII) or autumn (X). These phenological observations are in broad agreement with laboratory breeding data. In the laboratory, however, specimens from the same species pupated earlier (mainly V–VI) than in nature (Table 3), probably because of the more stable and warmer conditions (T = 20–24 °C) there, where the pupal stage lasted from 7 to 13 days, depending on the species. Larger species, e.g., *A. ulmi* and *S. erythropterus*, took distinctly longer to pupate than smaller ones, e.g., *R. tenuis*, *P. nigrita* (Table 3) (Staniec and Pietrykowska-Tudruj 2008a, c; Pietrykowska-Tudruj and Staniec 2012; Pietrykowska-Tudruj et al. 2014b).

Phylogenetic potential of pupal characters for testing hypotheses of relationships within Staphylinini

Two major morphological groups were clearly distinguishable among the pupae of Staphylinini: 'Staphylinini propria', represented here by the subtribes Philonthina and Staphylinina only and forming a well-supported clade in our analysis, and 'non-Staphylinini propria', represented by the genera *Acylophorus*, *Astrapaeus*, *Atanygnathus*, *Erichsonius*, *Heterothops* and *Quedius*, whose systematic affiliation at the subtribal level has in recent

Table 3. Data relating to known pupal stages of Staphylinini obtained from field and laboratory observations (Strassen 1957; Szujecki 1960, 1965; Staniec 1996, 1999a, b, 2001a, 2002, 2003a, b, 2004a, b, 2005a, b; Staniec and Kitowski 2004; Staniec and Pietrykowska 2005a, b; Pietrykowska-Tudruj and Staniec 2006b, c, 2007, 2010, 2011, 2012; Staniec and Pietrykowska-Tudruj 2007, 2008a, b, c; Staniec et al. 2009; Pietrykowska-Tudruj et al. 2014a, b; Vorst and Heijerman 2015).

Pupal stages observed	
In natural conditions	
Month/species	Microhabitat
IV/*Q. cinctus*, V/*Q. mesomelinus*, IV–V/*Q. cruentus*, VII/*P. varians*, VIII/*O. murinus*, *P. succicola*, *P. rectangulus*, *P. albipes*	rotting plant remains
V/*Q. fuliginosus*, *Q. fumatus*, *Q. humeralis*	moist leaf litter in woodland
VI/*P. corvinus*	rotting remains of *Carex* sp.
VI–VII/*A. wagenschieberi*, VI–VIII/*A. terminalis*	base of unsubmerged layers of peat mosses
VII/*Q. brevicornis*	tree hollow, in rotting wood
VII/*Q. plagiatus*	under protruding bark on a fir trunk lying on the ground
VIII/*S. erythropterus*	soil surface, under moss
IX/*P. quisquiliarius*	sandy river bank
X/*Q. dilatatus*	substrate under a nest of *Vespa crabro*
In laboratory conditions	
month/species	
II/*Q. brevis*, III/*Q. microps*, IV/*P. politus*, *Q. cruentus*; V/*B. nitidulus*, *P. corvinus*, *E. cinerascens*; VI/*G. splendidulus*, *H. praevius*, *N. villosulus*, *P. fumarius*, *P. micans*, *P. tenuicornis*, *O. fulvipennis*, *T. melanarius*, V–VI/*A. ulmi*, *G. astutus*, *P. cognatus*, *P. nigrita*, *P. punctus*, *P. lepidus*, *R. tenuis*, *S. erythropterus*; V–VII/*G. osseticus*, *P. rubripennis*, VI–VII/*H. rufipennis*, VII/*P. umbratilis*	

Table 4. Character matrix. ? missing data, – inapplicable characters.

Taxon	Character																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Abemus chloropterus	1	0	–	1	1	1	1	0	–	1	1	0	0	0	2	1	1	1	1	1	0	–	1	0	–	–	–	1	0
Acylophorus wagenschieberi	0	1	0	1	1	1	1	1	1	0	–	–	0	1	2	1	0	1	0	0	–	–	0	1	0	–	–	1	0
Astrapaeus ulmi	0	0	–	0	1	1	1	0	–	0	–	–	0	0	2	1	0	1	1	0	–	–	0	1	1	?	?	1	0
Atanygnathus terminalis	1	1	1	1	0	0	1	1	1	0	–	–	0	1	2	1	0	1	0	1	1	1	0	1	0	–	–	1	1
Bisnius fimetarius	1	0	–	1	1	1	1	0	–	1	1	1	0	0	3	1	0	1	1	1	0	–	1	1	1	0	1	1	0
Creophilus maxillosus	1	0	–	1	1	1	0	0	–	1	1	0	0	0	2	1	1	1	1	1	0	–	1	1	0	1	?	?	0
Erichsonius cinerascens	1	0	–	1	1	1	1	1	0	0	–	–	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	1	0
Gabrius appendiculatus	1	0	–	1	1	0	1	0	–	1	1	1	0	0	2	1	1	1	1	1	0	–	1	1	1	0	1	1	0
Hesperus rufipennis	1	0	–	1	1	1	1	0	–	1	1	1	0	0	3	1	0	1	1	1	0	–	1	1	1	0	0	1	0
Heterothops praevius	1	1	0	1	1	1	1	1	1	0	–	–	0	0	2	1	0	1	1	1	?	1	1	1	1	0	0	1	0
Neobisnius villosulus	1	0	–	1	1	1	1	0	–	1	1	1	0	0	3	1	0	1	1	1	0	–	1	1	1	0	1	1	0
Ocypus fulvipennis	1	0	–	1	1	1	1	0	–	1	1	0	0	0	3	1	–	1	1	1	0	–	1	1	1	1	1	1	0
Ontholestes murinus	1	0	–	1	1	1	0	0	–	1	1	0	0	0	3	1	0	1	1	1	0	–	1	0	–	–	–	1	0
Philonthus decorus	1	0	–	1	1	1	1	0	–	1	1	1	0	0	3	1	1	1	1	1	0	–	1	1	1	0	1	1	0
Platydracus tomentosus	1	0	–	1	1	1	0	0	–	1	1	0	0	0	2	1	1	1	1	1	0	–	1	0	–	–	–	1	0
Quedionuchus plagiatus	1	0	–	1	1	?	1	0	–	0	–	–	1	0	?	1	0	1	1	1	1	1	1	1	1	0	?	1	0
Quedius cinctus	1	0	–	1	1	1	1	0	–	0	–	–	1	0	2	1	1	1	1	1	1	1	1	1	1	0	0	1	0
Quedius fumatus	1	0	–	1	1	1	1	0	–	0	–	–	1	0	2	1	1	1	0	1	1	0	1	1	1	0	0	1	0
Quedius microps	1	0	–	1	1	1	1	0	–	0	–	–	0	0	2	1	1	1	1	1	1	1	1	1	1	0	0	1	0
Rabigus tenuis	1	0	–	1	1	1	1	0	–	1	1	1	0	0	3	1	1	1	1	1	0	–	1	1	1	0	1	1	0
Staphylinus erythropterus	1	0	–	1	1	1	1	0	–	1	1	0	0	0	0	1	0	1	1	1	0	–	1	1	1	1	1	1	0
Tasgius melanarius	1	0	–	1	1	1	1	0	–	1	1	0	0	0	2	1	1	1	1	1	0	–	1	1	1	1	1	1	0
Hypnogyra angularis	1	0	–	1	1	1	0	0	–	0	–	–	0	0	0	0	0	0	1	0	–	–	0	1	1	0	?	0	0

years been the topic of much debate and has undergone far-reaching changes (e.g., Solodovnikov and Schomann 2009; Chatzimanolis et al. 2010; Brunke et al. 2016).

The critical characters distinguishing the two groups are: i) the presence of setiform projections on the pronotum in all species of the Staphylinini propria group, but their absence in non-Staphylinini propria; ii) the type of cuticular process on abdominal segment VII – setiform projections in the former clade, but spines in the latter (except for *Acylophorus*, which has no cuticular processes). Among the non-Staphylinini propria, a clade was recovered with species whose pupae bear protuberances on the body (the genera: *Acylophorus*, *Atanygnathus*, *Heterothops* and *Erichsonius*). These protuberances may be situated on the head, pronotum and mesonotum (*Acylophorus*, *Atanygnathus*), only on the head and pronotum (*Heterothops*), or only on the pronotum (*Erichsonius*). Pupae of *Quedius* do not possess any protuberances.

Since subtribal classification within the non-Staphylinini propria has undergone substantial changes in recent years. We discuss below the phylogenetic potential of the external pupal structures of some taxa in the light of such taxonomic revolutions.

Pupa of *Astrapaeus*

There are practically no cuticular structures on the pupa of *A. ulmi*. The cuticular surface is devoid of any visible processes or protuberances (not including those on the legs of all pupae of the Staphylinini and the tiny accessories on terminal prolongations), which makes this species unique among the known pupae of the Staphylinini. Phylogenetic research based on adult and larval morphology, including fossil taxa, suggests that the monotypic genus *Astrapaeus* is not related to the subtribe Quediina (its traditional placement) but is a member of a rather isolated and basal lineage within Staphylinini (Solodovnikov and Schomann 2009; Solodovnikov 2012; Brunke and Solodovnikov 2013; Solodovnikov et al. 2013; Pietrykowska-Tudruj et al. 2014b). Based on molecular and morphological evidence, *Astrapaeus* is now included in subtribe Cyrtoquediina Brunke et al. 2016. Within Cyrtoquediina, a subtribe comprising species with mostly isolated distributions in the Neotropical, Oriental or Palaearctic regions (e.g., *Bolitogyrus*, *Cyrtoquedius*, *Parisanopus*, *Sedolinus*), only *Astrapaeus* occurs in and is restricted to Europe. The pupal characters of *Astrapaeus* support the isolated position of *A. ulmi* (and potentially other Cyrtoquediina) within the tribe, and outside the Quediina sensu Brunke et al. 2016. However, given the lack of data on the pupae of other members of the subtribe Cyrtoquediina, it is it is difficult to tell which morphological features of *Astrapaeus* are representative of the subtribe versus just genus level.

Pupa of *Erichsonius*

Within *Erichsonius*, a genus including more than 160 species distributed over almost all the world, the pupal stage is known for just three: the Nearctic *E. alumnus* Frank and *E. pusio* (Horn) (Schmidt 1996) and the Palearctic *E. cinerascens* (Grav.) (Pietrykowska-Tudruj and Staniec 2006c). Until the end of the 20th century, this genus was placed the subtribe Philonthina. But many recent phylogenetic analyses of adults utilising morphological and molecular data have indicated that the original placement of *Erichsonius* was incorrect (e.g., Brunke and Solodovnikov 2013; Chani-Posse 2013; Brunke et al. 2016). Initially, the genus was withdrawn from Philonthina and allocated to the Anisolinina grade within Staphylinini propria. However, the latest analyses show that *Erichsonius* is monophyletic and forms a separate subtribe Erichsoniina (Brunke et al. 2016, Chani-Posse et al. 2018).

The pupa of *Erichsonius* possesses a series of characters clearly distinguishing it from species classified among Staphylinini propria. They are: i) a lack of setiform projections on the pronotum; ii) the presence of protuberances on the pronotum; iii) cuticular processes on the abdominal segments in the form of spines. At the same time, these characters are shared with species of four genera of non-Staphylinini propria, i.e., *Atanygnathus* and *Heterothops* (all characters), *Acylophorus* (characters i and ii) and *Quedius* (characters i and iii). The results of our analyses suggest *Erichsonius* is distinguished from all other non-Staphylinini propria with known pupae by the number of protuberances on the pronotum. Pupae of *Erichsonius* have few protuberances (10 at most), whereas they are more numerous (more than 10) on the pupae of other taxa. Since the pupal stage is unknown in many other genera of Staphylinini and *Erichsonius* species, it is hard to assess the extent to which the number of protuberances is consistent within and unique to the genus. Given the present state of knowledge of pupae, we can regard it as unique to *Erichsonius*, and therefore evidence in favour of the recently erected subtribe Erichsoniina (Brunke et al. 2016).

Pupa of *Heterothops*

Within *Heterothops*, a globally distributed genus with 149 described species, the pupal stage is known only in *H. praevius* (Herman 2001; Pietrykowska-Tudruj and Staniec 2006c). This poorly defined genus was moved from the conventional subtribe Quediina and initially included in the large lineage Tanygnathinina sensu Solodovnikov and Schomann 2009; later it was placed in the subtribe Amblyopinina, containing fauna mainly from the Neotropical and Australian regions (Solodovnikov and Schomann 2009; Assing and Schülke 2012; Solodovnikov 2012).

Our analyses have demonstrated that the pupa of *Heterothops* has many characters in common with *Atanygnathus*. They are: processes on the head and pronotum, spines on abdominal segments II–VII, broad elytra, short hind leg tibiae (not reaching the lateral margin of the body), protuberances on the mid and hind legs, and long antennae. There are not many characters (not present in Staphylinini propria) shared between *Heterothops* (*H*) and *Quedius* (*Q*) (spines on abdominal segments and protuberances on the mid and hind tibiae), whereas there are many differences: head size (in proportion to the rest of the body) (small – *H*, large – *Q*), protuberances on the head and pronotum (present – *H*, absent – *Q*), antenna length (long – *H*, short – *Q*), width of elytra (wide – *H*, narrow – *Q*). In the light of current knowledge of Staphylinini pupae, one can state unequivocally that the morphology of *Heterothops* pupae supports the separation of this genus from the subtribe Quediina. There are several recent studies based on adult characters, or in combination with DNA that have confirmed its placement within the subtribe Amblyopinina (e.g., Brunke et al. 2016; Chani-Posse et al. 2018; Brunke et al. 2019). Among the 17 genera forming this group, only the pupa of *Heterothops*, the single taxon in this group which occurs beyond the southern hemisphere, is known (Brunke et al. 2016).

The present study has shown that the external structures of Staphylinini pupae could be a useful, alternative source of evidence for resolving the relationships of some higher taxa within the tribe. However, much more descriptive work is needed – mainly expanding the data matrix to include new species/genera and compiling new morphological data. Unfortunately, the pupae of many species of phylogenetic interest will probably remain unknown owing to the great difficulties with their collection and identification.

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